HHS Public Access

Author manuscript

Chem Rev. Author manuscript; available in PMC 2015 January 22.

Published in final edited form as:

Chem Rev. 2014 January 22; 114(2): 1020–1081. doi:10.1021/cr400166n.

Combinatorial Peptide Libraries: Mining for Cell-Binding Peptides

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1. INTRODUCTION

The surface of a cell represents a collection of macromolecules, which provides the cell with a unique cellular landscape specific to the type and state of the cell. Ligands that discriminate between subtle differences in cell surface phenotypes have utility in a wide variety of research and clinical applications. In particular, cell-binding ligands that can deliver biologically active cargo to a specific cell type or a diseased cell are highly sought. While the concept of the magic bullet drug was introduced by Paul Erlich over a century ago, the scientific community has yet to fully realize this goal.[1] This stems primarily from hurdles in obtaining high-affinity cell-binding ligands with the necessary discriminating power. The difficulty of the problem is realized by considering that the human body contains 210 distinct cell types, not including diseased cells, and is composed of ~10^{14} cells. Furthermore, once isolated, the ligand must be able to be prepared in large quantities, must be amenable to chemical modification for optimal in vivo biodistribution, and must be able to be tailored to suit a variety of clinical applications.

As antibodies typically have high affinity and specificity for their targets, they have garnered attention as cell-targeting agents. Monoclonal antibodies (mAbs) can be generated against differentially expressed cell surface features, and the number of FDA-approved mAbs that bind to cell surface antigens continues to grow.[2] mAb therapies are used to treat a variety of diseases. However, most of the clinically approved therapeutic mAbs are not conjugated to drugs or toxins and therefore fall into the category of molecularly targeted therapies. Such antibodies function passively by either blocking the activity of receptors or activating the immune system to destroy the antibody target.[3] Only a few clinically approved mAbs carry a deliverable. For example, two radiolabeled antibodies, Zevalin (ibritumomab tiuxetan) and Bexxar (iodine-131 tositumomab), are approved in the United States; both are anti-CD20 antibodies used for select patients with non-Hodgkin's lymphoma. The only clinically approved antibody–drug conjugate in the United States is Adcetris (brentuximab vedotin). Approved in 2011, Adcetris is an anti-CD30 antibody

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The authors declare no competing financial interests.

conjugated to the highly toxic microtubule-disrupting agent monomethyl auristatin E and is utilized for the treatment of Hodgkin's lymphoma (HL) or systemic anaplastic large-cell lymphoma (sALCL). Mylotarg, a calicheamicin anti-CD33 antibody conjugate, was recently removed from the market after 10 years in the clinic for failing to show efficacy.

Despite their successes, mAbs have limitations, especially in their ability to serve as delivery vehicles. Significantly, chemically modifying antibodies is challenging, and production costs are substantial. Additionally, nonspecific clearance of antibodies by the reticuloendothelial system can lead to accumulation of conjugated drugs or toxins in unwanted sites such as the liver and bone, damaging these organs.[4,5] Recently, concerns have risen over post-translational modifications on mAbs, especially glycosylation, which can trigger severe hypersensitivity reactions. Due to their long in vivo half-lives, intact mAbs are not well suited for molecular imaging techniques, requiring the use of antibody fragments (Fab's). Of the approved mAb therapies, only 11 different cell surface biomarkers are targeted. This is a minute fraction of the cell surface repertoire.

Peptides are an attractive alternative to antibody-targeting therapies. Unlike antibodies, peptides are easy to synthesize in large quantities,[6] and their smaller size improves tissue penetration while preventing nonspecific uptake by the reticuloendothelial system. Additionally, peptides can be chemically modified to alter affinity, charge, hydrophobicity, stability, and solubility and can be optimized for in vivo use through reiterative modifications. Importantly, peptides can display antibody-like affinities for their receptors. The biological half-life of peptides is well matched with that of many clinically used radionuclides, making them attractive probes for molecular imaging. Several naturally occurring peptides have been used as delivery agents. For example, reproductive hormone peptides and their derivatives are useful for tumor targeting, due to overexpression of their receptors on many cancer cells.[7,8] However, relying on known peptidic ligands limits the types of cells that can be targeted. For this reason, chemists and biologists have turned to diverse peptide libraries to select additional peptides that bind to specific cell types.

2. SCOPE OF THIS REVIEW

This review focuses on methods of selecting cell-targeting ligands from peptide libraries and the downstream use of these peptides. It includes the use of different types of peptide libraries and different selection methods. To highlight the utility of the selected ligands, we have not limited our discussion to a single cell type or disease state. Additionally, we have not merely concentrated on a single application in which these peptides can be used but have presented a broad overview of different applications. We focused on peptides isolated within the past five years but have also included peptides that have been widely used and merit discussion. It is our intention to present a complete compilation of cell-targeting peptides, but due to the scope of the field, we apologize if a peptide has been inadvertently missed. We have not included peptides that bind to nonmammalian cells, the use of naturally occurring peptide-targeting ligands, or studies using directed libraries based on known peptide sequences. Cell-penetrating peptides are not discussed as these peptides do not deliver cargo in a cell-specific fashion. These topics have been reviewed elsewhere.[7–10]

3. PEPTIDE LIBRARIES USED TO SELECT CELL-BINDING PEPTIDES

Peptide libraries used to select cell-binding peptides can be divided into two main categories: biological libraries and chemical libraries (Figure 1, Table 1). Biological libraries have a genotype, or DNA sequence encoding the peptide sequence, that is linked to the phenotype, or expression of the peptide, as part of the library's normal structure. This genotype–phenotype link was first demonstrated for bacteriophage display,[11] which still remains the major type of combinatorial library in use for the isolation of cell-binding peptides. Other types of biological libraries include bacterial, ribosome, mRNA, yeast, cDNA, retrovirus, baculovirus, and mammalian cell display. While all of these library types are promising, only phage and bacterial display have been used to isolate mammalian-cell-binding peptides. Among the numerous types of nonbiological combinatorial libraries, one-bead one-compound (OBOC) libraries and positional scanning synthetic peptide combinatorial libraries (PS-SPCLs) are the two types that have been used to isolate peptides that bind to cells. The generation of each type of library is a review within itself, and others have reviewed this topic for each type of library.[12–16] However, a brief description of the different peptide libraries is included below as the library type affects both the method of selection that can be used and the characteristics of the isolated peptides.

3.1. Phage Display Libraries

Bacteriophage (phage) is a single-stranded DNA virus that infects bacteria and is widely used to generate biological ligand libraries, known as phage display. The field of phage display began with George Smith's discovery in 1985 that foreign peptide sequences can be inserted into coat proteins of filamentous phage without altering phage function.[11] DNA sequences encoding a unique peptide are inserted into the DNA for a phage coat protein such that, as the phage assembles, it expresses the protein–peptide fusions and incorporates them into the normal phage structure. The result is a phage that displays a unique peptide on the surface of one of its coat proteins, allowing this peptide to direct phage binding to a target of interest. Phage manipulation has allowed for display of numerous ligand types, including peptides, antibodies, and receptors.[14,17,18] Moreover, phage coat proteins are accommodating and allow display of both linear and cyclic, cysteine disulfide-linked peptides. Peptide phage libraries usually have a diversity of 10^8–10^{10} different phage displaying different peptide sequences. Importantly, phage manipulation is relatively straightforward; phage are easy to grow and amplify by infecting the bacteria *Escherichia coli*. Additionally, the unique peptide encoded by a phage is easily determined using DNA sequencing.

3.1.1. Types of Phage Display Libraries—Nonlytic filamentous phages, which assemble in and secrete from their bacterial hosts without bacterial cell lysis, are commonly used for library construction. The filamentous phage is a flexible rod composed of five capsid protein types encasing a large, circular single strand of DNA (Figure 2A).[14] The majority of the phage body is comprised of ~2700 copies of the major coat protein pVIII, encoded by a single pVIII gene. Both ends of the phage body are composed of minor coat proteins; one end displays five copies of the minor coat proteins pIII and pVI, while the other end displays five copies of the minor coat proteins pVII and pIX. While all of the

phage proteins can accommodate a foreign peptide sequence, peptides are generally displayed at the N-terminus of the pIII or pVIII proteins.[12] If display of the peptide must present a free carboxy terminus, inverted pVIII proteins have been developed.[19,20] The filamentous phage M13 and the closely related fd filamentous phage are most commonly used for polypeptide display[11] due to their ease of replication and their ability to accommodate large pieces of foreign DNA. M13 phage displaying peptides from the N-terminus of their pIII protein are widely used due to their commercial availability. The fd filamentous phage is also commonly used for pIII and pVIII display, largely due to vectors generated by George Smith's laboratory.[21–23]

Peptides can be displayed such that every copy of the coat protein displays a peptide. Alternatively, the peptide-displaying coat protein can exist as a hybrid with the normal, wild-type coat protein.[14] pIII peptide libraries typically express the peptide on every copy of the pIII protein, resulting in a multivalent presentation of the peptide on the tip of the phage particle.[21,24–26] However, pIII mediates phage binding to the F pilus of an *E. coli* and initiates infection of the bacterium, which is required for library amplification. Large fusion proteins can disrupt this process, requiring the use of a pIII hybrid phage; the wild-type pIII initiates phage binding to *E. coli*, while the pIII–peptide fusion presents the randomized library. As the pVIII coat protein exists in many copies, pVIII display libraries are frequently used in the hybrid form in which only a fraction of the pVIII protein expresses peptides.[23] However, some groups have used a "landscape" display in which all of the pVIII proteins express peptides.[22]

Although less common, lytic phage that lyse their bacterial hosts as they exit are also used for phage display of peptides. The lytic phage structure is very different from the filamentous phage structure; lytic phage have an icosahedral head and a short tail (Figure 2B). The T7 lytic phage species is typically used for phage display. The outer shell of the T7 phage head is comprised of the 10A and 10B capsid proteins, at a total of 415 proteins per head.[27] Peptide sequences are typically displayed as C-terminal fusions of the 10B capsid protein. In the wild-type phage, approximately 10% of the total capsid protein is the 10B form.[28] However, the T7 phage can be modified to express different amounts of 10B versus 10A protein, displaying peptide sequences in 1–415 copies.[29]

The diversity of phage-displayed peptide libraries is generated at the DNA level.[30] Most libraries use an NNK construction in which N = A, C, G, or T and K = G or T. This construction produces all 20 amino acids and minimizes stop codons. The one possible stop codon, TAG, is suppressed in certain *E. coli* strains. However, this construction does not result in all amino acids being represented equally. For example, the amino acids leucine, arginine, and serine are represented three times, whereas most amino acids are represented only one time. Therefore, these amino acids can be over-represented in peptides isolated from these libraries. To overcome this problem, the random DNA insert can be synthesized using trinucleotide blocks.[31] This eliminates stop codons, amino acid bias, and rare codons.

Important consequences arise from the choice of phage library. As multiple copies of a peptide are displayed on the phage particle, the selection process can rely on multivalent binding. This is especially true for pIII libraries in which the peptides are displayed on the

tip of the phage. Peptides isolated from these libraries often have poor affinities when synthesized as monomeric peptides. However, multimerizing these peptides on a scaffold often rescues their affinity. The use of hybrid libraries or low-copy-number T7 phage can bias the selection toward peptides that have higher affinities as monomers. In addition, the "completeness" of diversity must be considered. As the length of the peptide increases, the representation of all possible sequences in the library decreases (20^n). For cell-binding peptides, most of the focus has been on the longer peptide libraries (12–20-mers) at the sacrifice of library completeness. Surprisingly, this has not significantly impacted the isolation of cell-binding peptides; there is almost always a cell-binding peptide in the library. However, the isolated peptide may not be the optimal sequence and may need to undergo further optimization. Additionally, identification of consensus sequences among phage clones is unlikely as nearest neighbors may simply not exist.

3.1.2. Advantages and Disadvantages of Phage Display—There are a number of advantages to using a biological peptide library such as phage display (Table 1). Phage display libraries are inexpensive, commercially available, and easy to amplify and reuse by simply allowing the phage to replicate in bacteria. In addition, they can be aliquoted and stored at −80 °C for years. Importantly, phage can accommodate different peptide sequences, including cyclic peptides, and typical libraries display from 10^8 to 10^9 different peptide sequences. Peptide selection can occur in vitro, ex vivo, or in vivo, as described in more detail in section 4. Phage libraries are tolerant of a variety of selection conditions and can endure harsh washing conditions. Despite these many advantages, phage display is not without disadvantages. First, as for any biological library, amino acid and sequence biases will exist in the library, resulting in a decrease in library diversity.[16,32] This results from differences in the synthesis and packaging of different peptide sequences that are fused to the phage coat protein as well as differences in the efficiency of *E. coli* infection of different clones. Small differences in growth rates during the amplification of the phage pool dramatically affect diversity, although approaches such as amplification on agarose plates or in monodispersed droplets can minimize these problems. T7 libraries exhibit less bias than filamentous phage libraries but are not completely free of this problem.[33] Second, while phage are ideal for displaying linear peptides and simple cyclic peptides, they cannot accommodate more complicated chemical structures, such as branched or bicyclic compounds, and they are typically limited to naturally occurring amino acids.[18] D-Amino acids are traditionally difficult to incorporate, [18] although a recent study demonstrated the ability to incorporate D-amino acids into phage libraries.[34]

3.2. Bacterial Display Libraries

3.2.1. Types of Bacterial Display Libraries—Peptide libraries can also be displayed on the surface of bacteria. As *E. coli* is easy to manipulate and grows quickly, it is ideal for display libraries.[35] Typical libraries can incorporate up to 10^{11} different peptides.[35] Once libraries are made, they are amplified by growth in typical bacterial liquid culture, and specific clones can be isolated by plating the bacteria on agar.[36] *E. coli* libraries are made by genetically incorporating peptides into the membrane flagella and fimbriae proteins. While a variety of different bacterial proteins can be used for these libraries,[35] peptide libraries have been incorporated into FliTrx, OmpA, CPX, and invasin proteins for creation of cell-binding

peptide libraries. For all of these display formats, the peptide can be fused to the N- or C-terminus of the bacterial protein or inserted into the middle of the protein (Figure 3).[35] Several reviews describe the use of bacterial display.[35–40]

Insertional libraries, where the peptide library is inserted into the middle of the bacterial membrane protein such that it forms a loop that sticks out of the membrane with the N- and C-termini of the membrane protein inside the bacterium, include the FliTrx and OmpA libraries. The FliTrx library is particularly convenient as it is commercially available. In this type of library, developed by McCoy and colleagues, peptides are inserted into the active site of the *E. coli* thioredoxin protein and the entire peptide–thioredoxin protein fusion is subsequently inserted into the *E. coli* flagellin protein.[41] Thioredoxin is used because the active site forms a disulfide bond constrained loop that can accommodate foreign sequences and still fold properly. As the flagellin protein is the major component of the bacterium's flagella, the peptide–thioredoxin–flagellin fusion protein is displayed on the cell surface as a partially functional flagellum.[41] The unique peptide is displayed in a disulfide bond constrained loop that extends out of the body of the thioredoxin protein, allowing it to bind selectively to the target protein or cell type.[41,42] An advantage of this form of peptide display is that the isolated cyclic peptides are structurally constrained and are active outside the context of the thioredoxin protein.

N-terminal libraries, such as the CPX library, involve fusion of the peptide to the N-terminus of an outer membrane protein. The CPX library involves a rearrangement of the OmpX outer membrane protein such that its N- and C-termini stick outside the cell membrane.[43] This allows for peptide fusion at the OmpX N-terminus, C-terminus, or both,[43] although most applications have used the N-terminus of the protein for peptide display. C-terminal libraries have also been used, including the invasin library. This library was created by expressing a modified form of the invasin protein from the pathogenic bacteria *Yersinia pseudotuberculosis* in nonpathogenic *E. coli*.[44] Invasin is a bacterial membrane protein that binds to integrins, allowing the *Y. pseudotuberculosis* to penetrate mammalian cells. Replacing its integrin-binding C-terminus with a random peptide library and subsequent transformation into *E. coli* results in *E. coli* displaying invasin–peptide fusions at the outer membrane.

3.2.2. Advantages and Disadvantages of Bacterial Display—Bacterial display libraries have many positive characteristics (Table 1). *E. coli* grows quickly and is easy to manipulate both genetically and physically.[35] Significantly, unlike phage libraries, which require both phage and bacteria, bacterial libraries only have one component: the bacteria. This allows for easy library growth and amplification using typical bacterial liquid culture and for the selection of specific clones by plating the bacteria on agar.[36] Another major advantage of bacterial libraries is the ability to use fluorescence-activated cell sorting (FACS) for library screening, allowing for quantification of clone binding.[37] This screening is relatively straightforward as the bacteria can be modified to incorporate a fluorescent label such as green fluorescent protein (GFP).[37] However, the screening process is then limited to the rate of the flow cytometer, which can significantly slow the selection process.[36] Other disadvantges include the complexity of the bacterial surface, which may interfere with binding of the displayed peptide.[36] Additionally, while typical *E. coli* libraries can

incorporate up to 10^{11} different peptides,[35] other bacteria can only incorporate a library size of approximately 10^5.[36] Significantly, bacterial display libraries can only be screened in vitro or ex vivo; they cannot be used for in vivo screening due to bacterial sepsis that would occur in the animal.

3.3. OBOC Libraries

3.3.1. Synthesis of OBOC Libraries—OBOC libraries are combinatorial peptide libraries synthesized on 80–100 μm beads such that each bead displays approximately 10^{13} copies of a single peptide.[18,45] The OBOC approach using the "split-mix" synthesis method (Figure 4) was first described by Lam et al. in 1991[46] and has previously been reviewed in detail.[18,45,47] Libraries containing α-amino acids are both easy to synthesize using standard solid-phase peptide chemistry and easy to sequence by Edman degradation using automated protein sequencing.[18] However, Edman degradation requires a free N-terminus, so libraries with more complex peptide structures, such as cyclic or branched peptides or peptides containing β- or γ-amino acids, necessitate inclusion of a chemical tag in the bead structure.[18] These chemical tags can be incorporated into the interior of the bead so as to not interfere with binding of the library peptides to targets of interest and then subsequently sequenced using either Edman microsequencing or mass spectrometry.[18] Lam and co-workers recently described two novel methods for generating beads with interior tags, termed bilayer beads,[48,49] and have recently reviewed these methods.[18] OBOC libraries have also been used to select multimeric ligands; Denmeade and co-workers synthesized OBOC dimer libraries for the selection of dimeric peptides.[50,51] It should be noted that the surface density of the displayed peptide results in a high local concentration of the ligand (~100 mM on a TentaGel bead), which can lead to the selection of low-affinity peptides.[52] To avoid this problem, the selection can be performed in the presence of competing proteins or the stringency of the washes can be increased. Alternatively, the peptide density on the bead can be reduced, but this requires bilayer bead encoding as there is insufficient peptide on the bead for sequencing.[53] Alternatively, the beads can be spatially separated.

3.3.2. Advantages and Disadvantages of OBOC Libraries—Similar to phage display, synthetic chemistry OBOC libraries are relatively inexpensive and easy to generate. OBOC libraries can display up to 10^8 different peptides, although most OBOC libraries are smaller in size (Table 1).[18] However, unlike phage display, OBOC libraries are not constrained to natural amino acids and can include both unnatural and D-amino acids, in addition to secondary structures not tolerated by the phage.[18] In fact, completely unnatural peptoid libraries have been used to select cell-binding ligands.[54] As unnatural and D-amino acids are less susceptible to proteases and peptidases than natural L-amino acids, OBOC libraries have the potential to rapidly identify stable peptide sequences. Incorporation of post-translational modifications, such as glycosylation and phosphorylation, can be incorporated into the library design as well.[55,56] The design of OBOC libraries also makes them ideal for use in optimization of known ligands. Peptides previously isolated by phage display or other methods can be used as lead compounds for OBOC library construction, allowing for rapid generation of optimized peptides with higher affinity or specificity.[18] Additionally, these libraries are ideally suited for screening for peptides that induce particular cellular phenotypes, such as the induction of apoptosis.[57] Finally, the process

from library synthesis to sequencing positive hits can potentially be automated.[58] Despite these advantages, OBOC libraries are not as widely used as biological peptide libraries. Although the recent advances in creating bilayer beads has made it easier to identify the peptides displayed by the beads, the required techniques are more involved than the DNA sequencing used to identify phage or bacterial library peptide sequences. Importantly, OBOC libraries cannot be used to select specifically for peptides that internalize into cells in vitro or for in vivo selection due to the large size of the beads.

3.4. PS-SPCLs

3.4.1. Synthesis of PS-SPCLs—Positional scanning synthetic peptide combinatorial libraries are generated by making individual synthetic peptide combinatorial libraries with one amino acid held constant while the remaining amino acids are varied.[59–61] The peptide sequence is then scanned by creating additional unique combinatorial libraries, each holding a different amino acid constant. For a tetrapeptide positional scanning library, this results in four distinct library subsets such that each library subset holds one of the amino acid positions constant. These library mixtures are represented by the designations $_{O1}$XXX, XO_2X, XXO_3X, and XXO_4. For each library, the O denotes the position that is held constant with 1 of the 20 amino acids, while the X represents any amino acid (Figure 5).[62] Mixture 1 consists of all peptides with a first amino acid of “A”, while mixture 2 is all peptides with a first amino acid of “C”. Each of mixtures 3–20 displays 1 of the remaining 18 amino acids in the first amino acid position. The next library subset, mixtures 21–40, contains 1 of the 20 amino acids held constant in the second library position. This scanning is continued until each of the tetrapeptide positions has its own pool of libraries.

3.4.2. Advantages and Disadvantages of PS-SPCLs—Advantages and disadvantages of PS-SPCLs are listed in Table 1. As the PS-SPCLs can be used in solution, they are adaptable to almost any selection technique.[59] They can be incubated with cells or receptors, typically in a high-throughput fashion such as in a 96-well plate or a microarray, and numerous readouts for binding exist. For example, screens can be made for binding versus competitor fluorescently tagged natural ligands (looking for loss of fluorescence)[63,64] or using biotinylated peptide and streptavidin–horseradish peroxidase (HRP) as a detection reagent.[65] PS-SPCLs are relatively easy and inexpensive to synthesize in large numbers. However, this approach depends upon the idea that each amino acid contributes individually to binding to the target of interest, which may make it difficult to determine ideal peptide sequences when multiple peptide motifs exist for the given target.[66] Additionally, PS-SPCL selections typically require multiple rounds of peptide generation and testing for binding. After the ideal amino acids at each peptide position are determined, all possible combinations of peptides using these ideal amino acids must be generated and further tested for binding. Finally, unlike the other peptide libraries discussed previously, PS-SPCLs require spatial resolution of each minilibrary. While PS-SPCLs are not used as frequently for the initial isolation of cell-targeting peptides, they are an excellent way to optimize lead peptides isolated from a phage-displayed or bacterial peptide library.

4. USING PEPTIDE LIBRARIES TO ISOLATE CELL-BINDING PEPTIDES

4.1. Peptide Isolation by Phage Display Libraries

Peptide phage libraries were initially used to isolate ligands against known target proteins.[11,12,14] This in vitro approach met with great success and allowed selection of peptide ligands for receptors without known naturally occurring ligands. Significantly, two papers published in 1996 expanded the field of phage display to include unbiased selection methods. For the first time, phage libraries were used to isolate peptides specific for given cell types without prior knowledge of the cellular receptor. Pasqualini and Rhouslahti pioneered in vivo phage display by intravenously injecting phage libraries into mice.[67] After isolating organs of interest and recovering bound phage, they obtained peptides specific for the vasculature of the organs. In the second paper, Johnston and co-workers pioneered in vitro phage display against whole cells, by using cultured cells as the target and specifically selecting for peptides that could bind and internalize into the cells.[68] The ligands isolated in both studies preferentially bound their target cell types over other nontarget cells. Importantly, these studies also demonstrated the feasibility of selecting peptides against receptors present in their native cellular conformations. Since these seminal papers, both in vitro selection against known target proteins and in vitro or in vivo unbiased selection against cells or tissues have been used extensively to isolate cell-binding peptides.

4.1.1. Panning against Known Targets—During the peptide selection process, commonly known as biopanning (panning), random phage-displayed peptide libraries are incubated with a target protein of interest to select for phage displaying peptides that specifically bind the protein (Figure 6). Typically, the target receptor is immobilized on a solid support before addition of the phage library.[14] After the receptor binds and captures specific phage, the unbound phage are washed away and discarded before elution of the bound phage. The resultant phage population is enriched for binding phage and is amplified by infection in *E. coli*. Subsequent panning rounds are then repeated approximately 3–5 times, until specific phage clones emerge. After the final round of selection, the DNA of the resulting phage clones is sequenced to determine their peptide content and reveal the candidate receptor-specific peptides. If desired, the panning process can also include negative selections in which the phage library is incubated with a control protein or cellular lysate. Performing this selection prior to the selection against the target protein allows for narrowing of the phage library to exclude peptides that bind nonspecifically to other, nontarget proteins.

While this process has been extremely successful for isolating protein-binding peptides, there are challenges in performing the selection on purified cell surface receptors. First, the primary challenge is simply isolating soluble and active membrane protein to use as bait. Membrane-bound proteins are notoriously difficult to work with due to their hydrophobic transmembrane domains and instability when removed from the lipid bilayer. Second, there is no guarantee that the isolated peptide will bind to the extracellular domain of the bait protein; ligands that bind to the transmembrane or intracellular domain of the protein are useless for cell targeting. The extracellular domain of the protein can be used as bait if the protein is modular, but this is not always possible. Third, by panning on purified protein, the

other biological information contained within the cellular membrane is lost. Cell surface proteins interact with other proteins, undergo multiple post-translational modifications, and can be contained in microdomains on the cell surface, affecting the cell surface density and activity of the receptor; the purified protein is not an accurate portrayal of the receptor in its biological context. In summary, isolation of a peptide that binds to a cell surface protein by in vitro panning does not guarantee that the peptide will be a functional cell-targeting ligand.

To overcome the problems of using purified protein, the target protein can be overexpressed in a cell line.[69,70] The receptor-overexpressing cells are employed for the selection process using the parental cell line for a negative selection. This approach has the advantage that the receptor is kept in a more relevant cellular context and overcomes the need for purification of the bait protein. However, it is dependent on a stringent negative selection and has not been used widely.

4.1.2. Unbiased Panning against Cells or Tissues in Vitro—Unbiased panning of phage display peptide libraries can be performed in vitro against specific cells, ex vivo against cells isolated from animal models or human patients, or in vivo against animal or human tissues. Johnston and co-workers first described phage display for the isolation of peptides binding to whole cells in vitro.[68] Although whole cells are heterogeneous targets, isolated peptides typically have high cellular specificity, binding selectively to the cells they were isolated against and not to other related cell types. To help ensure cell specificity, negative selections against related cell types or against other normal cells can be used to exclude peptides that bind to all cell surfaces nonspecifically. However, such selections are often unnecessary as selection against the target cell type alone is generally sufficient to yield highly specific ligands.

In vitro or ex vivo panning against cells of interest involves a protocol similar to that used for in vitro panning against known target proteins except that the bait is now a viable cell. Both cultured cell lines and primary cells used ex vivo are amenable to the process. Random peptide phage libraries are incubated with the cell type of interest for a defined period of time before the cells are washed to remove both extracellular and weakly bound phage (Figure 6). At this point, either surface-bound or internalized phage can be chosen for further amplification. If the goal is to isolate surface-bound phage, the phage are eluted and allowed to infect *E. coli* for phage amplification for further rounds of panning. If the goal is to isolate internalized phage, surface-bound phage are removed by low-pH washes or treatment with a protease. Alternatively, the mixture can be centrifuged through a nonmiscible organic phage to separate unbound phage from the cell-associated phage. This separation process has been termed BASIL (biopanning and rapid analysis of selective interactive ligands).[71] The cells are then lysed, and associated phage are used to infect *E. coli*. This panning process is then repeated approximately 4–6 times, until the ratio of input phage (total amount of phage originally incubated with the cells) to output phage (amount of bound or internalized phage) stagnates. As phage do not have tropism for mammalian cells and the only modified portion of the phage library is the unique peptide motif, the identified peptide should be responsible for mediating binding to the cell type of interest.

There are several advantages to unbiased phage panning on whole cells as opposed to panning on target proteins alone.[72] First, cell receptors remain in their native membrane states—at their normal expression level, in their normal location, and with their normal membrane neighbors. As discussed above, it is impossible to recreate these same conditions for purified receptors. Second, selection can be tailored to isolate either surface-bound or internalized peptides. Selection against purified proteins only allows isolation of peptides that bind to the protein. Third, the selection is completely unbiased and can be performed without prior knowledge of cellular receptors, making it ideal for cells about which little is known. By contrast, panning on isolated proteins obviously requires prior knowledge of which receptors make good targets. Finally, due to the unbiased nature of the cell-panning approach, peptide identification and subsequent receptor identification can lead to the discovery of important cellular targets that were previously unknown.[73,74]

Cell-based biopanning is highly versatile. It has been used for cells from multiple species and can be performed on adherent and nonadherent cells. The process does not require extensive instrumentation and relies on common laboratory techniques. However, cell-based biopanning requires approximately 10^6 cells, limiting the types of cells that can be panned on ex vivo. For example, fine-needle biopsy yields only ~10 000 cells, and biopanning on β-cells of the pancreas required the sacrifice of six rats per round of panning simply to obtain enough cells.[75] Recently, a phage-displayed peptide has been selected on live cells in a microfluidic device.[76] This approach uses fewer cells (10^2–10^4), and the flow of fluids through the chamber is more efficient in removing nonbinding phage. Additionally, fewer cells are lost in the wash process, thus minimizing the risk of losing phage clones. This may represent a more efficient manner for selection of peptides for cell binding.

4.1.3. In Vivo Panning—In vivo phage display was first described by Pasqualini and Rhouslahti as a means to select vasculature-specific peptides.[67] Typically, a random peptide phage library is injected into the tail vein of mice or rats and allowed a brief (5–15 min) circulation time. Phage recovery must occur relatively quickly after injection to maintain phage infectivity. The animals are then sacrificed and the desired tissues collected and homogenized. Phage isolated from these homogenates are then infected into *E. coli* for library amplification so that the panning process can be repeated. Typically, 3–5 pannings are sufficient to isolate target-specific peptides. As vasculature targets are more readily accessible and do not require tissue penetration, the vast majority of peptides selected in this manner target vasculature in the organ of interest and not the organ itself. While a longer phage incubation time in vivo aids in tissue penetration, phage infectivity also decreases during circulation. Several groups have circumvented this problem by first performing in vitro panning to narrow the phage library followed by subsequent in vivo panning. Other groups have performed in vivo panning by injection of the phage at sites closer to the organ of interest.

There are several advantages to in vivo phage panning. Just as with in vitro panning on cells, the approach keeps receptors in their native context, is entirely unbiased, requires no prior knowledge of a cellular receptor, and has the potential to identify new cellular targets. However, unlike any of the other selection methods, phage isolated by in vivo panning are inherently able to reach their target in vivo. They come with the assurance that the receptor

they target is both accessible from the bloodstream and able to bind its ligand in sufficient quantity for detection. By contrast, peptides isolated from whole cells do not come with this guarantee as the receptor they target may not be readily accessible from the bloodstream or may have a different cellular localization in vivo compared to in vitro.

A potential disadvantage of in vivo panning in mice or rats is that any peptides identified as homing to organs or vasculature are binding to mouse or rat tissue. Even in human xenograft tumor models, the tumor vasculature is derived from the mouse. Thus, isolated peptides may not bind the corresponding human vasculature. To translate these peptides to clinically relevant ligands, the target receptors will have to be examined in human tissue and the peptide sequences may have to be further optimized to bind their human counterparts. To bypass these problems, a few groups have turned to panning in humans. Arap and Pasqualini were the first to perform in vivo phage display in human patients.[77] A patient with the B-cell cancer Waldenström macroglobulinemia was injected intravenously with phage and tissue collected from five areas of the body—bone marrow, fat, skeletal muscle, prostate, and skin—for identification of specific tripeptide motifs.

4.1.4. Next-Generation Sequencing in Phage Display Selections—Regardless of the method of phage library panning, amplification is used between each round to enrich for bound clones. At each round, there is the risk of introducing more biological bias and a collapse of library diversity. This can result in the loss of binders and increases the risk of identifying false positives. There has recently been a push to use next-generation sequencing to identify positive clones in earlier rounds of panning.[19,78–83] This approach has the advantage that greater than 10^6 reads can be obtained in a single run so that repeat sequences or consensus sequences can be identified after 1–2 rounds of panning. Deep sequencing can also be used to follow the diversity of libraries before the start of panning and at each round. This is particularly useful in detecting clones that have a competitive growth advantage that are amplified throughout the panning process but do not necessarily bind the target.[82] Standard sequencing, in which a small sampling of clones is selected for characterization, cannot detect sequence repeats until the library diversity collapses onto a few sequences. For example, the commercially available Ph.D-7 phage-displayed peptide library was panned against KS483 cells differentiated into osteoblasts.[78] Using the Illumina platform, phage were sequenced prior to the start of the experiment and after each round of biopanning. As expected, the library converges at each round of panning. However, of the 10 most abundant peptide sequences seen in round 4, 8 of these peptides are also in the top 10 after round 1. This suggests that binding ligands can be identified after 1–2 rounds of panning as opposed to 4–6 rounds. Additionally, false-positive clones were easily ruled out by sequencing the starting library after a single amplification in which the library is not panned on the target. As this is a relatively new technique, it is not obvious if deep sequencing will improve the quality of peptides isolated from phage-displayed libraries or will simply expedite the process.

4.2. Peptide Isolation by Bacterial Display Libraries

To date, all cell-binding peptides identified by bacterial display have been isolated using unbiased selection approaches. Targets include both cultured cells and freshly isolated

murine or human cells. There are two methods of bacterial display: panning,[41] similar to the method used with phage libraries, and a quantitative method employing FACS.[84] Bacterial library panning involves incubating the cells with the bacterial library followed by extensive washing to remove unbound bacteria. Bound bacteria are then recovered by vortexing or centrifugation and regrown for repeated panning rounds. After the final round of selection, the DNA of the isolated bacterial clones is sequenced to reveal the identity of the cell-binding peptide. FACS screening for peptide selection is performed by first expressing the bacterial peptide libraries in *E. coli* that also express a GFP variant.[84] After incubation of the fluorescent bacterial library with target cells and extensive washing to remove unbound bacteria, the cells are sorted by FACS according to GFP fluorescence. Cells with bacteria bound to them should carry a GFP signal that specifically allows for their isolation. As FACS quantifies the fluorescent signal, target cells can be sorted on the basis of the number of bacterial clones they bind. The consequence of this sorting is the isolated bacterial clones have high affinity and/or bind an abundant cell surface marker. The specific peptides displayed by the isolated bacterial clones are then determined using DNA sequencing.

4.3. Peptide Isolation by OBOC Libraries

Much like phage libraries, OBOC libraries have been used to select for peptides against both purified targets and intact cells. OBOC libraries were originally used to select ligands against target proteins.[46] Then, in 1996, the same year that Pasqualini and Rhouslahti first described in vivo phage display[67] and that Johnston and co-workers pioneered unbiased in vitro phage display against cultured cells,[68] Pennington, Lam, and Cress first described the use of OBOC libraries for unbiased screening against live cells.[85]

4.3.1. Selection against Known Targets—Soluble target proteins can be screened for binding to OBOC libraries using several different approaches, all of which rely on the ability to selectively distinguish protein-bound beads. The protein of interest can be labeled with a tag (i.e., fluorescent or colorimetric dye, biotin, enzyme, radionuclide, epitope tag, etc.) that allows for detecting the protein-bound beads. Most commonly, an enzyme-linked colorimetric assay is employed. [45,46,86] This selection is relatively simple and involves the reaction of alkaline phosphatase and the colorimetric substrate bromochloroindolyl phosphate (BCIP). The protein target can be directly labeled with alkaline phosphatase, or if a primary antibody against the protein is available, an alkaline phosphatase (AP) secondary antibody can be employed. After incubation of the protein of interest with the OBOC library and unbound protein has been washed away, treatment with BCIP turns beads that have captured the AP-labeled target turquoise.[86] The peptide content of isolated positive beads is then determined using Edman's sequencing or mass spectrometry. The success of the screen is dependent on the stringency of the selection conditions and effective negative screens. Unlike phage display and bacterial peptide libraries, there is no amplification step. Thus, false-positive ligands are not diluted or lost during subsequent selection steps. This makes the screening conditions particularly important.

4.3.2. Unbiased Selection against Cells—Screening of OBOC libraries against cells in culture or against cells isolated for ex vivo screening is relatively straightforward.[18] The OBOC peptide library is incubated with the cell type of interest. Positive "hits" are

visualized by microscopy as beads covered in cells. These positive beads can then be selected and removed using a micropipet. As with phage display, negative selections can also be used to identify peptides that bind the cells nonspecifically. Lam and co-workers have employed two different negative screening methods to exclude cells that bind to the beads nonspecifically.[18] The first negative screening method involves a screen against the cell type of interest. Beads which capture cells are isolated and treated with guanidium chloride so that the beads can be reused for incubation with a normal, control cell type. Beads binding both the target and control cell types are then discarded. In the second negative screening method, the target cells are fluorescently labeled and then mixed together with both control cells and the bead library. The control cells can be labeled with a different fluorophore or remain untreated. Beads bound to both target cells and control cells are then discarded, and beads that are only bound to the fluorescent target cells are considered hits. Just as with in vitro phage panning on cells, this approach keeps receptors in their native context, is entirely unbiased, requires no prior knowledge of a cellular receptor, and has the potential to identify new cellular targets.

4.4. Peptide Isolation by PS-SPCLs

PS-SPCLs have also been used to select for peptides against both known targets and against unbiased cellular targets. As the PS-SPCLs can be used in solution, they are adaptable to almost any selection technique. They are typically incubated with cells or receptors in a high-throughput fashion, such as in a 96-well plate or a microarray, and the readout for binding is varied. Screens can be made for binding versus competitor natural ligands that are fluorescently tagged (looking for loss of fluorescence)[64,87] or can use biotinylated peptide and streptavidin–HRP as a detection reagent.[65] Other screens have used specific cellular effects as the readout. Once all of the scanning library subsets are screened using the assay of choice to identify the “best” amino acid at each peptide position, additional peptides are synthesized using all of the possible combinations of the best amino acids. These peptides are then used in the same selection process as the original library to identify which amino acid sequences best target the protein or cell type of interest. This screening process requires multiple rounds of peptide synthesis and testing, yet optimized peptides are discovered, and no DNA or peptide sequencing is needed to determine the peptide composition.

5. ISOLATION OF DISEASE-SPECIFIC OR ORGAN-SPECIFIC PEPTIDES

Peptide libraries have been mined to isolate cell-targeting ligands for many different cell types and disease states. This has resulted in an arsenal of peptides that can be used for delivery of different functional cargos. This section of this review is broken down by cell types and highlights how the different types of peptide libraries can be used to isolate highly specific cell-binding ligands. We have focused on peptides isolated in the past five years and seminal cell-targeting peptides that have been used widely or are of high importance. Only those peptides that have subsequently been shown to bind their target receptor in the context of cells are included. Tables 2–17 list peptide sequences shown to bind different cell types and highlight their use.

5.1. Isolation of Cancer-Specific Peptides

Cancer is the leading cause of death for people under 85 in the United States and accounts for one in four total deaths.[88] Due to this large clinical problem, the majority of all cell-binding peptides have been isolated against cancer-specific biomarkers, cancer cells, and tumors. Cell-targeting peptides can be used for both therapeutic and diagnostic applications. This field has been driven by the limitations of current chemotherapeutics which have a narrow therapeutic window. As such, drugs are generally given at the maximally tolerated dose, not the maximally effective dose. The ability to deliver a therapeutic to tumor cells while avoiding normal tissues can improve antitumor efficacy while decreasing off-target effects. Additionally, drugs which have been considered too toxic for systemic use may now be viable options. Moreover, as personalized medicine becomes a reality, there is an increasing need for molecular imaging agents that stratify patients by molecular subclasses to guide therapeutic decisions. Cell-binding peptides can be employed to deliver imaging agents to assess expression of particular cancer biomarkers.

Cancer-targeting peptides can be broken down into two main categories: cancer-cell-specific (or tumor-cell-specific) peptides and tumor-vasculature-specific peptides. Both the tumor cells and the vasculature that feeds the tumor are important targets as both contribute to tumor growth and viability. Phage panning on whole cells tends to isolate peptides that are tumor-specific, while in vivo panning generally isolates vasculature-binding ligands. However, there is overlap between the vasculature and tumor cell surface profiles; as a result, some vasculature-targeting peptides bind to the associated tumor cells as well.

5.1.1. Cancer-Specific Peptides Isolated from Phage Libraries

5.1.1.1. Cancer-Specific Peptides Isolated by Panning on Purified Tumor Biomarkers: Panning against purified cancer antigens was one of the first uses of phage-displayed peptide libraries. A suitable cell surface biomarker for targeting must be abundant on the cancer cell but have negligible expression on normal cells. Additionally, the cellular receptor must not be shed as soluble forms will act as a sink for the targeting peptide. Endothelial cell biomarkers of the neovasculature found in tumors can also serve as cancer-specific targets. With this in mind, numerous cancer-specific receptors have been used as bait for panning (Table 2). Peptides have been selected against 19 different target proteins. Isolated peptides range in size from 6 to 20 amino acids, are linear and cyclic, and have little sequence similarity with each other. Additionally, most peptides have no sequence similarity with the native protein ligand of the targeted receptor. A variety of different receptor targets have been used as bait, but receptor tyrosine kinases are over-represented due to their importance in cancer. Significantly, 12 of the peptides have been shown to target tumors in vivo.

It should be noted that not all cell surface markers are proteins. Phage display has been employed to isolate peptides that bind to galactose β1–3 *N*-acetylgalactosamine, a disaccharide also known as Thomsen–Friedenreich (TF) antigen. TF antigen is found on the majority of human carcinomas but is hidden on normal cells, making it a promising target. Several TF antigen-binding peptides have been isolated, and most contain the sequence WYAW/FSP. More recently, phage display selections have isolated ligands for phospholipids. Phosphatidylserine (PS) is a phospholipid that is normally restricted to the

inner leaflet of the cell membrane lipid bilayer. However, under stress conditions such as hypoxia and high levels of reactive oxygen species (ROS), PS flips and is exposed on the cell surface. Similarly, PS is found on the surface of cells undergoing apoptosis.[89] Tumor cells and the associated tumor endothelial cells that form the vasculature have been shown to expose PS, and drug or radiation treatment increases PS flipping to the outer leaflet. The cyclic peptide CLSYYPSYC was isolated by panning a heptamer phage-displayed peptide library on PS and was shown to home to a xenograft tumor after a single treatment with the drug camptothecin,[90] yet caution must be taken when isolating peptides against hydrophobic targets; numerous other PS-binding peptides have been isolated with varied sequences,[91–93] and the specificity of this heptamer has not been fully established. Nonetheless, these reports show the promise of isolating peptides that target nonproteinaceous cell surface biomarkers.

5.1.1.2. Cancer-Specific Peptides Selected by Whole-Cell Panning: Numerous tumor-targeting peptides have been isolated using in vitro panning against cultured cells, as listed in Table 3. All selections were continued for multiple panning rounds until emergence of convergent peptide sequences occurred, resulting in the isolation of a few dominant peptide sequences per panning. Isolated peptides range in size from 7 to 20 amino acids and are both linear and cyclic. The variety of libraries and protocols used for panning and the diversity of cell lines used as targets have resulted in a diverse group of peptides with very little sequence similarity. Additionally, even when the receptor target is the same, there is no sequence overlap between peptides isolated on purified protein and cells. Again, this likely stems from differences in library design and panning protocols. A number of these peptides internalize into their cell types of interest, indicating that they might be useful for drug delivery. Although these peptides were isolated in vitro, many have been shown to target tumors in vivo.

In addition to panning on cells maintained in culture, isolated primary cells can be employed. Table 4 lists peptides identified by ex vivo panning on either isolated tumor cells or whole tumors. Significantly, laser capture microdissection (LCM) has recently been used to isolate cancer cells for ex vivo panning. LCM allows separation of cancer cells from other cell types residing in the tumor to ensure that the peptides isolated are binding the cancer cells. Kubo et al. used LCM to isolate colon cancer cells from patients for panning.[233] Two of the four isolated peptides have been shown to target tumors in vivo.

Not included in Tables 3 and 4 are studies that selected for peptide motifs instead of convergent sequences. Instead of panning against a single cell line and selecting for the best binding sequences, these studies focused on generating consensus peptide motifs that bind to a panel of cell lines. In one such study, Arap and Pasqualini and co-workers used the NCI-60, a panel of human cancer cells encompassing different histological types and grades, for peptide selection.[234] This panel includes cell lines from kidney cancer, breast cancer, colon cancer, lung cancer, prostate cancer, ovarian cancer, tumors of the nervous system, melanoma, leukemia, and lymphoma. It is a particularly important group of cells to study as protein expression across the cell lines has been extensively examined. A group of 38 tripeptide motifs were identified that bound broadly across the panel of cells and clustered with cell lines known to express the same receptors. Similarly, Shukla and Krag used ex vivo panning on whole breast tumors from patients and examined motifs present among the

isolated phage clones.[235] Panning for peptide motifs is more likely to help profile cell surfaces and expand knowledge about similarities and differences between cancer cells and less likely to generate high-affinity peptides.

5.1.1.3. Cancer-Specific Peptides Isolated by in Vivo Panning: Table 5 lists peptides identified by in vivo panning in animals. The majority of peptides isolated by in vivo panning in animals bind to the tumor vasculature and not the tumor cells, although some of the peptides bind selectively to tumor cells or to both the vasculature and tumor cells. Tumor-vasculature-specific peptides are identified in the table with a **V**, tumor-cell-specific peptides with a **T**, and peptides that bind both vasculature and tumor cells with a **T/V**. On the basis of the variety of peptides isolated from different tumor models, tumor vasculature is shaped by the specific tumor type. Isolated peptides can even distinguish between drug- or radiation-treated tumor vasculature and their untreated tumor counter-parts. For example, peptides have been isolated that can distinguish between the vasculature of untreated tumors compared to tumors treated with the monoclonal antibody VEGF inhibitor bevacizumab[241] or tumors responding to treatment with the small-molecule receptor tyrosine kinase inhibitor sunitinib.[242] Hallahan and colleagues also recently identified a peptide that binds specifically to the tumor vasculature of tumors treated with both radiation and the VEGF receptor tyrosine kinase inhibitor SU11248.[243] This demonstrates the remarkable discriminating power of the peptides isolated by panning.

The selection pressures involved with in vivo panning are very different from those placed on the system during an in vitro panning. As such, in vitro and in vivo panning can lead to the isolation of different peptides, even when the same library and cell line are employed. For example, the same laboratory used a 12-mer library to pan against the CL1-5 NSCLC cell line in vitro and against CL1-5 tumor xenographs in vivo.[229,244] Two different peptides were isolated; the peptide TDSILRSYDWTY (SP5-2) was isolated by in vitro panning on the cell line, and the peptide SVSVGMKPSPRP (SP5-52) was identified from the in vivo panning experiment. SP5-52 targets the tumor vasculature, while SP5-2 penetrates the tumor, resulting in diffuse binding throughout the tumor mass. This emphasizes how differing selection methods can identify ligands with different biological characteristics. However, the peptide SVSVGMKPSPRP has been suggested to be a nonspecific peptide that is isolated from the NEB 12-mer library most likely due to preferential propagation of this phage clone.[207,245] The peptide sequence has been selected on DNA,[246] multiple semiconductor surfaces,[247] phosphatidylserine,[92] and numerous cell types.[248,249] Further studies are required to determine the true specificity of this peptide.

One problem with the use of in vivo panning in animals is that the isolated peptides are binding to mouse or rat and not human vasculature. Additional testing is required to determine whether they also bind human vasculature. One study bypassed the problem of selecting peptides against mouse vasculature by using mice injected with human endothelium for in vivo panning.[250] Mice were injected with tumor endothelial cells derived from human renal carcinomas mixed with Matrigel so that they formed human tumor vessels that grew into the murine vessels. In vivo panning in these mice resulted in peptides that targeted human tumor endothelium and not mouse tumor endothelium.

The first in vivo panning in a human was performed by Arap and Pasqualini and involved the identification of tripeptide motifs specific for different organs.[77] A patient with the B-cell cancer Waldenström macroglobulinemia was injected intravenously with phage and tissue collected from five areas of the body—bone marrow, fat, skeletal muscle, prostate, and skin—for identification of specific tripeptide motifs. One extended peptide motif was validated as evidence of the specific nature of these motifs. A phage bearing the peptide CGRRAGGSC was isolated from the prostate and shown to recognize the vasculature of human prostate tissue specimens. The CGRRAGGSC peptide binds to IL-11Rα,[77,251] which increases in expression during the progression of cancer.[252] This peptide is currently in phase I clinical studies for patients with prostate cancer.[253] Recently, the Krag laboratory performed a study in which patients with different types of stage IV cancer were intravenously injected with phage libraries for panning followed by biopsy and then reinfusion of the library for further panning and biopsy steps.[254] One peptide phage clone isolated from a patient with stage IV melanoma subsequently bound ex vivo to tumor cells from the same patient, but not to tumor cells from other patients. This peptide phage clone did not bind to human melanoctyes and bound only slightly to one of six melanoma cell lines tested. Thus, the peptide appears to be specific for the tumor from which it was isolated.

Selections can also be biased to identify peptides binding to other nonvasculature components of the tumor. Using in vitro or ex vivo phage display in combination with in vivo panning can help this process. Rhouslahti's group recently used sequential ex vivo and in vivo panning on a mouse xenograft MDA-MB-435 breast cancer model to identify peptides specific for both tumor lymphatics and tumor cells.[255–258] The peptides were isolated by using ex vivo panning on cell suspensions of MDA-MB-435 tumors that were depleted of endothelial cells, biasing toward nonvasculature-targeting peptides.[255] Subsequent in vivo panning with this phage population led to selection of the cyclic nonamer peptide LyP-1.[255] LyP-1 has been shown to translocate to the nucleus upon binding and to home to lymphatic vessels in melanoma, breast, prostate, skin, and osteosarcoma tumors while not binding either tumor vasculature or normal tissue.[255–258] Other lymphatic-homing peptides have been identified, as delineated in Table 5. This is significant as tumor lymphatic vessels serve as drainage systems, and some evidence suggests that they are the route through which tumor cells escape to form metastases.

5.1.2. Cancer-Specific Peptides Isolated from Bacterial Libraries—Table 6 lists cancer-specific peptides identified from screening bacterial libraries against either protein or cellular targets. Peptides have been selected against seven cell lines in vitro and against one cell line ex vivo. These peptides range in size from 5 to 18 amino acids, and the majority of the peptides are disulfide-constrained and cyclic. Three of the peptides target tumors in vivo. Compared to phage display, a larger number of clones are isolated and fewer high-frequency clones observed. Consensus sequences are often observed. The library sizes used in these selections are in the 10^8–10^9 range, comparable to the diversity found in phage display libraries. As such, diversity size cannot account for the selection of multiple binding clones from bacterial libraries compared to the convergence on a particular sequence during phage display panning. More likely, fewer bacterial clones are lost during the selection process,

and there is less amplification bias. The selection of multiple clones does increase the time required for validation and characterization of the peptide sequences. This is especially true when it comes to assessing the binding activity of the free peptides. For example, in a selection on HepG2 cells using the FliTrx peptide library, 700 clones were recovered for characterization.[323] Only 28% of the clones contained the whole fusion protein, and of the remaining 200 clones, only 10 had higher binding on HepG2 cells than a control cell line. However, isolation of multiple binding peptides can provide a panel of binding ligands which can be used to profile the tumor cell surface. Case in point, using a suite of 24 cell-binding bacterial clones, a signature for luminal and basal breast cancers has been generated.[324] While this binding array has not been tested on a large set of patient samples, it demonstrates the potential of peptide ligands to serve as diagnostic classifiers.

5.1.3. Cancer-Specific Peptides Isolated from OBOC Libraries—While a few peptides have been selected against purified cell surface cancer biomarkers using OBOC libraries,[329] these chemical libraries have been more widely exploited for selections on whole cells (Table 7). This may due in part to the ease with which positive beads can be isolated by visual inspection. Biased selections can be performed in which the target receptor is overexpressed and the selection occurs within the context of whole cells. For example, the cyclic peptide cGRGDdvc was isolated from OBOC screening using $\alpha_v\beta_3$ integrin-transfected K562 leukemia cells.[330] The parental K562 cell line was utilized for negative selections to remove ligands that bound to receptors other than $\alpha_v\beta_3$. However, most OBOC screens have been performed on cell lines without overexpression of a specific receptor. Nonetheless, all of the peptides isolated from OBOC libraries for which receptors have been identified bind integrin receptors. Much of this is likely due to library synthesis as many of the libraries are designed with a bias toward sequences that are known to bind integrins. It should be noted that receptors were identified by competition experiments using panels of integrin antibodies. As such, it cannot be ruled out that the peptides bind other cellular targets as well. Unlike peptides isolated from phage-displayed libraries, the ligands identified from OBOC libraries frequently bind multiple cancer cell lines. This is not surprising considering that integrins are often upregulated in cancer.[331] The vast majority of peptides selected using OBOC libraries contain D-amino acids, other unnatural amino acids, or both. As unnatural amino acids are more resistant to peptidase cleavage, these amino acids may have better stability in vivo. To date, six of the OBOC-selected peptides have been shown to target tumors in vivo.

5.1.4. Cancer-Specific Peptides Isolated from PS-SPCLs—Cancer-specific peptides have been isolated from PS-SPCLs, yet to date, only one of the peptides isolated from PS-SPCLs has been shown to target tumors in vivo.[65,344] PS-SPCLs are ideal for assays screening for cellular effects induced by peptides (Table 8). Kang and co-workers used integrin microarrays to select peptides that inhibit angiogenesis.[345,346] They first used an $\alpha_v\beta_3$ microarray and a PS-SPCL to screen for peptides that interfered with the $\alpha_v\beta_3$–vitronectin interaction. Fluorescently labeled vitronectin was mixed with the peptide library before addition to the microarray. Library peptides that inhibited the receptor–vitronectin interaction were thus visible by a reduced fluorescent signal. This resulted in the selection of

two peptides, HGDVHK and HSDVHK. In a similar fashion, they used an $\alpha_5\beta_1$ microarray and a PS-SPCL to screen for peptides that inhibit $\alpha_5\beta_1$–fibronectin interactions.[64]

5.1.5. Common Peptide Motifs Selected from Different Peptide Libraries—The variety of libraries and protocols used for panning and the assortment of cell lines used as targets have resulted in a diverse group of peptides with very little sequence similarity. However, several peptide motifs have been isolated repeatedly from different panning experiments. Most commonly, peptides containing the integrin-binding motif RGD have been found by panning on purified integrins, on whole cells, and in vivo.[351] The specificity of the peptide for a particular integrin varies depending on the flanking sequence around the RGD.[73,111,115,180,352,353] High-affinity binding to integrins is achieved with only three amino acids; any library with a diversity of 8.0×10^3 is statistically likely to contain the RGD binding motif. For this reason and the fact that integrins are often overexpressed in cancer cells and tumor vasculature, it is not surprising that RGD sequences are often isolated. Similarly, numerous NGR-containing peptides have been found over the years.[280,289] This peptide motif binds to aminopeptidase N (CD13) but can also undergo a spontaneous deamination to form *iso*DGR, a ligand for $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrins.[354,355]

5.2. Isolation of Cardiac- and Muscle-Binding Peptides

Cardiovascular disease is the leading cause of death in the world.[149] The primary forms of cardiovascular disease include coronary heart disease, congenital heart disease, stroke, aortic aneurysm, and hypertensive heart disease. The assessment of cardiovascular disease risk as well as the treatment of cardiac disease will benefit from the generation of new reagents that specifically localize to heart tissue or distinct vascular beds. Targeted delivery would increase the therapeutic window of drugs effecting ion channels or contractility and potentially open avenues for gene therapy to rescue damaged tissue.[356] For example, administration of vascular endothelial growth factor (VEGF) may improve revascularization in ischemic heart tissue, but concerns about side effects observed with systemic administration have limited its use.[357] In addition, targeted delivery could help overcome problems with present diagnostic and treatment protocols. Contrast media presently used in cardiac catheterization and related techniques have undesirable side effects, require administration directly into the local vasculature, and are quickly flushed from the tissue bed. Agents that localize at the specific tissue could enhance the observation of changes over time and help identify the transition between normal and diseased tissue. Peptides have the potential to serve as cardiac-targeting agents for new drugs and diagnostics. Table 9 lists cardiac-specific peptides identified by in vitro, ex vivo, and in vivo phage panning. As the heart is essentially a large muscle containing many blood vessels, most cardiac-specific peptides fall into one of two categories: cardiomyocyte-binding (heart-muscle-binding) peptides or cardiac-vasculature-binding peptides. Surprisingly, other peptide libraries have not been employed to identify cardiac-specific peptides.

Peptides specific for cardiomyocytes have primarily been targeted toward normal myocardium, Duchenne muscular dystrophy (DMD) myocardium, or ischemic myocardium. The peptides targeting the normal myocardium include the CTP peptide, isolated by a combination of in vitro and in vivo panning. This peptide binds to normal murine

cardiomyoctyes both in vitro and in vivo.[358] Six different peptides all targeting normal murine cardiac endothelium were identified by a combination of ex vivo and in vivo panning, and receptors for most of the peptides were identified using a bacterial two-hybrid system.[359] While phage displaying all six of these peptides targeted cardiac endothelium in vivo, only one peptide, the CRPPR peptide, has been used for in vivo targeting outside the context of the phage.[359,360] Peptides isolated against ischemic myocardium, characterized by reduced blood supply to the heart, include the CSTSMLKAC peptide isolated from myocardium of rats given an ischemic injury.[361] A dye-labeled version of this peptide demonstrated in vivo specificity for the ischemic left ventricle.

DMD is a fatal X-linked disease involving progressive muscle degeneration.[362] The disease is also associated with dilated cardiomyopathy, and most of those affected die in their late teens or early twenties from pneumonia complicated by cardiac problems.[362] One recent study used in vivo phage panning in an *mdx* mouse model of DMD to isolate peptides specific for the heart and quadriceps.[363] The peptide SKTFNTHPQSTP isolated from quadriceps demonstrated specific homing to both the heart and quadriceps when injected in a dye-labeled form into the tail vein of *mdx* mice.

Peptides specific for cardiac vasculature have been isolated for various disease states: hypertension/stroke, atherosclerotic plaques/inflammation, and aging. Atherosclerosis is the most significant contributor to cardiovascular disease and is a progressive disease driven by inflammation that leads to accumulation of plaque in large arteries and can end with artery rupture and thrombosis.[364] As atherosclerosis begins with endothelial cell changes brought about by activation of inflammatory pathways,[364] targeting these endothelial cells has the potential to enrich the available therapeutic and diagnostic options. The peptide CVHSPNKKC, specific for vascular cell adhesion molecule-1 (VCAM-1), was isolated from murine cardiac endothelial cells in culture.[365] This peptide shows specificity for VCAM-1-expressing endothelial cells and atherosclerotic lesions in vivo and has been used for a variety of ex vivo and in vivo techniques (described in more detail later). The atherosclerotic-plaque-specific peptide CRKRLDRNC was identified after an ex vivo panning on cell suspensions from human atherosclerotic plaques and appears to bind the IL-4R expressed on the endothelial cells, macrophages, and vascular smooth muscle cells of the plaques.[366] Significantly, this peptide was able to home to atherosclerotic plaques in mice and to bind to human tissue sections of atherosclerotic tissue. Peptides specific for vasculature of different ages have also been identified. One peptide bound cardiac vasculature of young (3 month old) mice and not that of aging (18 month old) mice,[367] while another peptide demonstrated specificity for the aging vasculature.[368] Again, phage display panning proves to have the ability to isolate highly specific ligands that can discriminate between healthy and diseased states.

5.3. Isolation of Immune-Cell-Binding Peptides

Peptides that target specific cell types involved in the immune systems have several key uses, including antigen delivery for vaccine development, delivery of immunosuppressant agents for the treatment of autoimmune diseases, and redirection of an immune response to eradicate diseased cells. Identification of immune-cell-specific peptides is challenging. First,

there are numerous subclasses of immune cells, and they share overlapping cell surface markers. Furthermore, these subclasses have high plasticity in which the cells can exist in numerous states of activation or differentiation. Second, several cells of the immune system are inherently phagocytic; background uptake of nontargeted ligands can be high. However, the demands on specificity may not be as great for immune cells as for other cell types. In many cases the cargo is not toxic to other cell types. Unlike delivery of a chemotherapeutic or toxin, uptake of a proteinaceous antigen in a nontarget cell is unlikely to cause harm. Instead, targeting is performed to enhance an immune response compared to a nontargeted antigen. Additionally, the inherent amplification of an immune signal lowers the amount of antigen that needs to be delivered. Finally, many cell surface receptors on immune cells have been identified, and antibody reagents for these markers are available; biased selection for a specific cell surface marker can be performed readily. Immune-cell-specific peptides have been identified using in vitro, ex vivo, and in vivo phage panning and from PS-SPCLs (Table 10).

Dendritic cells are antigen-presenting cells that reside in peripheral tissues such as skin acting like guards on the lookout for molecules of foreign material, or antigens.[389] The dendritic cells capture these antigens and process them into small peptides while transforming into mature dendritic cells that migrate toward peripheral lymphoid organs. There mature dendritic cells present these peptides to naïve T cells by displaying peptide–MHC protein complexes on their cell surface, initiating an adaptive immune response against the origin of the antigen. Dendritic cells also activate B-cells to produce antigen-specific antibodies (humoral response) and further activate natural killer (NK) cells and natural killer T (NTK) cells. Due to their ability to activate all of these different immune pathways, dendritic cells are ideal targets for vaccine development. Subsequently, several dendritic-cell-specific peptides have been isolated for the purposes of specifically delivering antigens to dendritic cells or transducing genes into dendritic cells. Most peptides have been selected to bind a known DC marker. One such peptide, the CGRWSGWPADLC peptide, was isolated against the dendritic-cell-specific marker CD11c/CD18[390] and was later used for vaccination.[391] A TNF-α-competing (TNF-α = tumor necrosis factor-α) peptide, CYTYQGKLC, was isolated by in vitro panning against the murine dendritic cell line JAWSII, using TNF-α to elute bound phage.[392] The peptide was shown to specifically deliver genes to dendritic cells (details later). In an unbiased selection, McGuire et al. also identified a novel dendritic-cell-targeting peptide, XS52.1, by in vitro panning on the mouse dendritic cell line XS52, derived from mouse skin.[393] A multimeric tetramer version of the peptide specifically bound to XS52 cells in vitro and was further used for gene delivery in vivo.

Dendritic cells fall into the category of leukocytes, or white blood cells, which comprise a category consisting of several other cell types. Several peptides have been isolated against other types of leukocytes, including the leukocyte-binding peptide WAWVWLTETAV isolated against the FcγRIIA receptor expressed by neutrophils and mononuclear phagocytes. [394] Importantly, this peptide bound primary mononuclear (PMN) cells and monocytes isolated from human peripheral blood.

Another immune cell type for which cell-binding peptides have been isolated is the microfold, or M, cell. M cells are antigen-sampling cells located in Peyer's patches of intestinal epithelium. The intestines are lined with a single layer of epithelium for protection, making it difficult for most things to pass through, or out, of the gut.[395] However, M cells sample the antigens in the gut and transport them a short distance to a binding pocket in which immune cells such as lymphocytes or macrophages dock.[395] Thus, the M cells can carry antigens out of the gut to immune cells to facilitate an immune response, making M cells desirable targets for orally available vaccines.[395] While the oral vaccine will inherently reach the intestines after administration, subsequent delivery of the vaccine to M cells is variable.[395] Identification of ligands that could direct the oral vaccine to M cells would allow for their specific delivery to immune cells for initiation of an immune response. In vivo phage display performed by injecting phage directly into a loop of rat intestines identified the P8 and P25 peptides as specific for M cells.[396] These peptides were able to specifically bind M cells in vivo when injected into mouse intestine. Importantly, although isolated from rat M cells, these peptides also bound to tissue sections of human intestine as evidenced by immunohistochemistry. Kim et al. used in vitro panning against human M-like cells to identify the Co1 peptide.[397] The Co1 peptide was able to bind both human M-like cells and M cells in vivo in mice. As described earlier, not all cellular features are proteins, and an M-cell-targeting peptide was isolated by panning against immobilized ganglioside GM1 (monosialotetrahexosylganglioside). [398] While this peptide can deliver an antigen to M cells in vivo, it is important to note that GM1 is not restricted to M cells.

5.4. Isolation of Pancreatic-Islet-Cell-Binding Peptides

Diabetes is the seventh leading cause of death in the United States and affects 8.3% of the population, or 25.8 million people. There is no cure for diabetes, and complications of the disease can be serious. There are two major types of diabetes: type 1 and type 2. Although the two types of diabetes differ with regard to their origins, both involve the β cells that produce and secrete insulin. The ability to image β cell mass and β cell function as well as delivery of genes and therapeutics to β cells remains a major goal of the biomedical field. β cells comprise only ~2% of the pancreas and are localized in discrete regions known as the islets of Langerhans; thus, the demand for specificity and sensitivity of detection is great. Additionally, few well-characterized β cell lines are available. Only a handful of islet-specific peptides have been identified. Table 11 lists islet-specific peptides identified by in vitro and in vivo phage panning. The use of phage display to isolate islet-specific antibodies and peptides has recently been reviewed elsewhere. [407]

Ex vivo panning against freshly isolated rat pancreatic islets resulted in selection of two islet-binding peptides termed RIP1 and RIP2.[75] While both peptide-displaying phage associate with isolated rat islets, the RIP1 phage showed 8-fold higher association with the Ins832/13 β cell line in vitro than the RIP2 phage. Thus, it is assumed that the RIP1 phage binds β cells, while the RIP2 phage may bind some other islet component. Although the peptides have not been tested outside the context of the phage, phage displaying both peptides were able to reach and bind islets in vivo as evidenced by immunohistochemistry on pancreatic sections from rats injected with phage. While the RIP1 phage demonstrated specificity for islets, with some expected clearance in other organs, the RIP2 phage

accumulated nonspecifically throughout the rat. Therefore, the RIP1 phage was chosen for further imaging studies, as described later. Importantly, this phage bound only to the islets of normal rats and not to those of a model diabetic rat, suggesting that the RIP1 peptide in the context of the phage can distinguish between normally functioning β cells and dysfunctional β cells.

Peptides specific to islet vasculature have also been identified. In vivo panning in normal mice followed by laser pressure catapult microdissection to specifically isolate pancreatic islets identified the vasculature-binding peptides CVSNPRWKC and CHVLWSTRC.[408] Phage were injected intravenously, and immunohistochemistry of pancreas tissue sections was used to assess phage binding. While both phage clones bound normal islet vasculature, they bound more impressively to angiogenic vasculature of islet tumors from RIP-Tag2 mice.

Another study used ex vivo panning on cell suspensions from both angiogenic islets and solid tumors from RIP1-Tag2 mice followed by in vivo panning in the same mice to look for phage homing to the angiogenic islets or tumors.[315] As RIP1-Tag2 mice spontaneously develop angiogenic islets that progress into solid tumors, 12 week old mice that have a combination of angiogenic islets and tumors are a good model for isolating stage-specific peptides. While six specific phage were initially isolated, only the RGR, RSR, and KAA phage were used as synthetic peptides for further study. Dye-labeled versions of the peptides were injected intravenously into RIP1-Tag2 mice, and in vivo homing was verified by fluorescent images of pancreatic sections. While all three peptides colocalized with both the endothelial and pericyte cells of islet vasculature, they each bound different stages. RGR bound both angiogenic and tumor vasculature, RSR bound only angiogenic vasculature, and KAA bound only solid tumor vasculature. None of them bound normal islet vasculature. This finding highlights the exquisite differences between the vasculature at different stages of tumor development, making it possible to specifically target the vasculature of tumors at a specific stage. Importantly, the RGR, RSR, and KAA peptides are the only islet-cell-binding peptides that have been tested outside the context of the phage. Further use of these peptides for specific delivery of imaging agents or drugs could allow very precise delivery to different types of islet vasculature.

5.5. Isolation of Adipose-Cell-Binding Peptides

Obesity is a significant problem in the United States, with approximately 33% of the adult population classified as obese.[411] For this reason, there is interest in identifying peptides that bind to adipose cells. Table 12 lists adipose-specific peptides identified by in vitro, ex vivo, and in vivo phage panning. To the best of our knowledge, only four adipose-binding peptides have been identified.

Adipose stromal cells are precursors to the cells of white adipose tissue.[412] Two adipose stromal cell peptides were isolated from two separate pannings.[413] Ex vivo panning against adipose stromal cells isolated from white adipose tissue after patient liposuction identified the CMLAGWIPC peptide named hPep. A second in vitro panning against the mouse preadipocyte cell line 3T3-L1 identified the CWLGEWLGC peptide named mPep. Both peptides bound to cultured adipose stromal cells, and the integrin $\alpha_5\beta_1$ was identified as the

receptor for both peptides. Significantly, mPep was used for in vitro therapy, and hPep was shown to have cellular effects (details later). Two other adipose-stromal-cell-binding peptides were isolated using in vivo phage display coupled with FACS.[412] After in vivo phage injection and isolation of white adipose tissue, FACS was used to sort for adipose stromal cells. The peptides CSWKYWFGEC (WAT7) and CGQWLGNWLC were isolated from this selection.

In addition, a peptide specific for white fat vasculature was identified from an in vivo screen in an obese mouse model, leptin-deficient ($Lep^{ob/ob}$) mice.[414] Although identified from an obese mouse model that is not representative of human obesity, the peptide also homed to the vasculature of white fat in normal wild-type mice that better represent the human condition. Significantly, this CKGGRAKDC peptide was able to specifically decrease amounts of white fat in mice and prevent obesity in these mice as well as in Old World monkeys[415] (details later). The receptor for the peptide was identified as prohibitin.

5.6. Isolation of Brain-Specific Peptides

Table 13 lists brain-specific peptides identified by in vitro and in vivo phage panning and from PS-SPCLs. Treatment and diagnosis of all brain-related diseases is greatly hampered by the blood–brain barrier. The blood–brain barrier is composed primarily of brain capillary endothelial cells and is located at the junction of the blood and brain.[420] True to its name, it is a barrier that is extremely difficult to pass physically. Very few drugs are able to penetrate this barrier, including peptides, proteins, antibodies, genes, and more than 98% of small molecules.[421] Significantly, none of the medium- or large-sized pharmaceutical companies are focused on targeting the blood–brain barrier. There is a clear need for ligands that can specifically home to and deliver cargo to this barrier. With the goal of identifying blood–brain-barrier-specific peptides, in vivo phage display was performed using an in situ brain perfusion of the phage library into normal male C57Bl/6 mice.[422] Two of the isolated phage clones (GLA and GYR) demonstrated specificity for the cultured human brain endothelial cell line hCMEC/D3. Importantly, in situ perfusion of both phage into the mouse brain demonstrated specific homing to the brain as compared to that of a control peptide phage. Another study used in vivo phage display to isolate the brain-specific TGN peptide in an effort to find a ligand that can bypass the barrier.[206] Interestingly, this study used a 24 h time point for phage isolation during panning as opposed to the usually short 5–15 min circulation time. Significantly, the TGN peptide homed to the mouse brain in vivo. Another method that allows for bypassing the blood–brain barrier is to deliver agents intranasally. Recently, a phage library was panned in vivo in rats by intranasal administration to identify phage clones that reach the cerebrum.[423] The clone containing the peptide ACTT-PHAWLCG homes to the olfactory nerve and further into the brain.

Several peptides specific for brain vasculature have also been identified. An in vivo panning isolated four peptides, all of which were shown to home to the brain in vivo in the context of the phage.[67] Additionally, one peptide, the CLSSRLDAC peptide, was able to mediate brain-specific homing when conjugated to the surface of a red blood cell. Peptides that target other areas of the brain include the APY, KYL, and VTM peptides isolated against the EphA4 receptor that is expressed in the learning and memory portions of the brain—the

hippocampus and cortex[248]—and the SLNDWIDWSEPH, SHSLMTSSSVWT, SHTTPGKNNDPF, and NYARDPLMSLPQ peptides isolated by ex vivo panning against mouse cerebellar granule neurons.[424]

5.7. Isolation of Liver- and Kidney-Binding Peptides

5.7.1. Liver-Cell-Binding Peptides—Isolation of liver-specific targeting peptides by in vivo phage display has been largely ignored since most intravenously injected phage accumulate nonspecifically in the liver.[67] However, treatment and diagnosis of hepatitis and other chronic liver diseases could benefit from the availability of liver-specific agents. A new type of T7 phage library modified to prevent clearance by the immune system, resulting in subsequent nonspecific liver accumulation, was recently used to identify hepatocyte-binding peptides.[430] In vivo panning was performed in mice with normal immune systems, resulting in selection of liver-specific peptides. Three peptides in the context of the phage were subsequently able to home to hepatocytes in vivo after intravenous injection in mice (Table 14). Importantly, two of the phage displaying hepatocyte-specific peptides were tested for accumulation in other organs and were shown to maintain liver specificity. These results suggest that the liver may still be a viable target for cell-binding peptides.

5.7.2. Kidney-Cell-Binding Peptides—The kidney is involved in a diverse number of diseases. While many peptides nonspecifically accumulate in the kidney, little attention has been focused on isolating specific kidney-targeting ligands. In vivo panning in rats looking for kidney targeting resulted in the isolation of two peptides, termed HTT and HIT (Table 14).[431] Rats predosed with a control (no peptide) phage and then injected with the peptide phages demonstrated specific phage targeting to the kidney with some nonspecific liver accumulation similar to that of the control phage. These peptides were then incorporated into a modified adenovirus for specific gene delivery to the kidneys as described in detail later.[432] A kidney-vasculature-specific peptide, CLPVASC, was also identified from an in vivo screen in mice and has been shown to home to the kidney on the phage platform.[67] Two peptides, ELRGDMAAL and ELRGDRAHW, were isolated by ex vivo panning on microdissected intact cortical collecting ducts (CCDs) from rat kidneys and one peptide, KMGGTNHPE, was isolated by ex vivo panning on microdissected intact proximal convoluted tubules (PCTs) from rat kidneys.[177]

5.8. Isolation of Stem-Cell-Binding Peptides

Stem cells have the capacity to differentiate into any cell type, and their potential for regenerative medicine has spurred research in this area. Despite this potential, few stem-cell-binding peptides have been identified (Table 15). However, of those peptides identified, many distinguish between pluripotent stem cells and differentiated cells.

Peptides that bind stem cells have the potential to recruit such cells to sites of injury and may stimulate their differentiation. For example, the peptide FAQRVPP was selected for binding to murine-derived neural stem cells from a pIII phage-displayed peptide library.[433] This peptide was synthesized as a fusion with a self-assembling peptide and acetylated at the amino terminus. The resultant fusion peptide increased the rate of recovery for rats subjected to spinal cord damage when injected at the site of injury.

Neural stem cells are the multipotent progenitor cells that generate the major cell types of the nervous system. As several diseases such as Parkinson's, Alzheimer's, and Huntington's involve neurodegeneration,[434] ligands that could bind to neural stem cells may have the potential to help deliver therapeutics for these diseases. Additionally, diseases such as epilepsy and depression are connected to altered neurogenesis.[435,436] Two neural stem-cell-binding peptides, the QTRFLLH and VPTQSSG peptides, were identified from an ex vivo panning against neural stem cells isolated from mouse hippocampus.[428] When conjugated to adenoviruses and injected into the brain, these peptides mediated specific binding to neural stem cells.

5.9. Isolation of Vasculature- and Endothelial-Cell-Binding Peptides

Although vasculature flows to all organs, the vasculature varies from organ to organ, with different structure, function, and protein expression profiles.[447] The use of in vivo phage display to isolate vasculature-homing ligands has highlighted this diversity.[67,253,448,449] Significantly, the vasculature also changes during different disease states.[253] Others have reviewed the heterogeneity of the vasculature and the discovery of vasculature-specific peptides.[253,449,450] In addition to the vasculature-specific peptides isolated from all of the organs and tumors already described in the subsections above, peptides specific for the vasculature of the lung, skin, pancreas, intestine, uterus, adrenal gland, retina, prostate, breast, and thymus have been isolated.[448,451–455] Table 16 lists the vasculature- and endothelial-cell-targeting peptides isolated by in vitro, ex vivo, and in vivo phage panning and from a PS-SPCL.

Phage display has been widely used to isolate peptides that bind different vasculature beds with high specificity. For example, the lung-vasculature-specific peptide GFE-1, isolated by in vivo panning, demonstrates specificity for normal lung vasculature over lung tumor vasculature when in the context of the phage.[456] Similarly, the prostate-vasculature-specific peptide SMSIARL homes only to prostate vasculature and not prostate tumor vasculature in mice.[451] Such distinctions point to the molecular changes evident between normal and tumor vasculature and to the ability of phage panning to isolate ligands that recognize these changes. On the other hand, some peptides, such as the CPGPEGAGC peptide isolated from in vivo homing to breast vasculature, bind to both normal breast vasculature and the vasculature of breast tumors in a mouse model of breast cancer.[453]

Arap and Pasqualini and co-workers demonstrated the ability to simultaneously pan for peptides specific for different organs.[452] During an in vivo panning in mice, six different organs (muscle, bowel, uterus, kidney, pancreas, and brain) were simultaneously isolated and screened for peptide binding. While the peptides were not taken to convergence, tripeptide motifs were identified for each organ using statistics. In an effort to identify the cellular receptors for these peptide motifs, the motifs were BLAST searched for homology to known biological protein ligands. Tripeptide motifs isolated from pancreatic vasculature showed homology to ligands of the prolactin-like protein receptor (PLPR). The narrowed phage library from pancreatic panning rounds 2 and 3 was used for in vitro panning against PRLR-overexpressing COS-1 cells. The peptide CRVASVLPC was subsequently identified

as a PRLR-binding peptide and was shown to home to pancreatic vasculature and islets in vivo when in the context of the phage.

5.10. Isolation of Epithelial-Cell-Binding Peptides

In addition to those already discussed, numerous other epithelial-cell-binding peptides have been isolated by in vitro and in vivo phage panning and from bacterial library selection. These peptides have generally been selected for an application specific to a particular cell type. These remaining peptides are highlighted in Table 17. Particularly interesting among this group of peptides are those that mediate transcytosis. During transcytosis, a receptor and its cargo are internalized in a polarized cell at one plasma membrane and transported by vesicles across the cell to the opposite plasma membrane.[488] Using an approach to identify peptides that allow for transcytosis across the intestinal mucosal barrier, an in vivo panning against rats by oral administration of phage was recently performed.[489] The liver, lung, spleen, and kidney were isolated, and the sequence CSKSSDYQC was identified from all of them. The phage displaying this peptide was also able to bind small intestinal mucosal tissue in vitro. Importantly, this phage accumulated more strongly than a control phage in the liver, lung, spleen, and kidney after oral administration. Immunofluorescence from 30 min after oral administration of the phage showed the phage accumulating in goblet cells. Thus, if the synthetic peptide works as well as the phage, it has the potential to be used for oral delivery of drug conjugates. The YPRLLTP phage clone, isolated by selecting for phage in the spleen after in vivo gavage of a phage library into rats, also demonstrated the ability to transcytose the gastrointestinal mucosal barrier.[490]

6. PEPTIDES WITH CELLULAR EFFECTS

Often peptides used for specific targeting of diagnostic and therapeutic agents are considered only as delivery agents and not as ligands that can induce cellular effects.[72] However, given that these peptides are binding to receptors or other components on the cell surface, they have the potential to induce the effects of these receptors. PS-SPCLs are particularly well suited for isolating peptides with cellular effects due to their ability to be used with almost any readout assay, and the majority of peptides isolated using these libraries induce specific effects. However, there is no guarantee that peptides isolated by other methods will not trigger cellular signaling or other cellular behaviors. Not surprisingly, many of the isolated peptides affect cell adhesion and migration. In some cases, peptide binding produces a beneficial outcome, such as a reduction in migration of cancer cells away from the primary tumor. However, if a peptide stimulates growth, it would be unusable as a tumor-targeting agent. Peptide function has been noted in each table of selected peptides. This section highlights examples of peptides that affect four cellular behaviors: (1) adhesion, migration, and invasion, (2) angiogenesis, (3) proliferation, and (4) viability.

6.1. Peptides That Modulate Adhesion, Migration, and Invasion

Ligand binding to cell surface receptors can induce a wide variety of cellular effects, including adhesion, migration, and invasion.[72] This has been most widely studied on tumor or tumor vasculature. Several cancer-specific peptides have demonstrated induction of these pathways.[110,126,140,154,160,163,169,170,251,252] Not surprisingly, peptides that bind integrins

are often found to inhibit adhesion and migration as this family of receptors are critical for such functions, yet many other ligands are able to block cellular migration. For example, a peptide isolated from phage display against MMP-9 inhibited migration of various tumor cells (fibrosarcoma, melanoma, ovarian carcinoma, and Kaposi's sarcoma cells) in vitro and inhibited Kaposi's sarcoma tumor growth when ip injected in vivo.[126] Similarly, a peptide selected from GC9811-P gastric cancer cells prevented cell adhesion and invasion in vitro and reduced peritoneal nodules in vivo.[170] Another peptide selected against metastatic gastric cancer cells inhibited adhesion and migration in vitro and reduced liver metastasis incidence in vivo.[169]

Peptides that bind normal vasculature can also prevent metastasis. The lung-vasculature-specific peptide GFE-1, known to bind membrane dipeptidase (MDP), was able to inhibit metastases in vivo when co-injected with tumor cells.[456] Mice were injected intravenously either with C8161 human melanoma cells plus the GFE-1 peptide or with the cells plus a control peptide. Mouse lung weight was measured as an indicator of metastasis, and the mice given the GFE-1 peptide had a median weight only 12% above the weight of normal lungs. Significantly, the mice injected with tumor cells plus the control peptide had a median lung weight 88% larger than normal lung weight.

Cardiac-specific peptides also have the potential to induce cellular effects. One such peptide, the VCAM-1-specific peptide CVHSPNKKC, which is known to bind to VCAM-1-expressing cardiac endothelial cells, is able to block VCAM-1-mediated leukocyte–endothelial cell interactions in vitro.[365] Murine cardiac endothelial cells activated with TNF-α were preincubated with either the CVHSPNKKC peptide or an anti-VCAM-1 antibody before addition of strain-matched mononuclear cells. The peptide was more efficient at inhibiting leukocyte–endothelial cell interactions than the anti-VCAM-1 antibody, blocking 68% of interactions compared to 52% for the antibody. This finding is particularly interesting as VCAM-1-induced recruitment of monocytes and leukocytes to the cardiac endothelium is an important step during the development of atherosclerosis.

Cell-binding peptides do not always reduce cell adhesion and/or migration. A PS-SPCL screen was used to identify peptides that stimulate arachidonic acid release from neutrophil-like cells. These peptides were later demonstrated to stimulate chemotactic migration of human neutrophils.[405] Similarly, the HFYLPM and MFYLPM peptides, isolated from a PS-SPCL screen for the ability to induce superoxide generation from human monocytes, were also able to induce chemotactic migration of the cells.[406]

6.2. Peptides That Modulate Angiogenesis

Cell-binding peptides can stimulate or inhibit angiogenesis. As angiogenesis is a critical step in tumorigenesis and antiangiogenics are an important class of antitumor drugs, peptides that control this process have been widely sought. One such peptide isolated from a phage display selection against the IL-6 receptor was shown to inhibit angiogenesis in vitro and to inhibit IL-6-mediated tumor growth in a cervical xenograft in vivo.[110] Two $\alpha_v\beta_3$-binding peptides, HGDVHK and HSDVHK, were isolated from a PS-SPCL screen for peptides that interfered with the $\alpha_v\beta_3$–vitronectin interaction.[63] These peptides were subsequently found to inhibit both bFGF-induced human umbilical vein endothelial cell (HUVEC) migration in

vitro and bFGF-induced blood vessel formation in a chorioallantoic membrane (CAM) angiogenesis assay. An $\alpha_5\beta_1$ microarray and a PS-SPCL screen were employed to isolate peptides that inhibit $\alpha_5\beta_1$–fibronectin interactions.[64] One isolated peptide, VILVLF, subsequently inhibited proliferation of HUVECs and inhibited angiogenesis in a bFGF-induced CAM angiogenesis assay. As $\alpha_5\beta_1$ is expressed at high levels in both angiogenic endothelial cells and several cancer types, this peptide may have future utility for antiangiogenic therapy.

Peptides can also stimulate angiogenesis. The peptide SFKLRY-NH_2, isolated by in vitro PS-SPCL screening against the MS-1 cell line, increases angiogenesis as evidenced by its ability to increase proliferation and migration of HUVECs, leading to organization of the HUVECs into tubular formations.[486] Additionally, this peptide was able to induce blood vessel sprouting of aortic rings from rats. Mechanistic studies demonstrated that the peptide induces an increase in intracellular Ca^{2+} via a pertussis-sensitive G protein/phospholipase C-mediated pathway and increases the expression of VEGF, resulting in a pro-angiogenic phenotype. The peptide also exhibits antioxidant activity.[505] Importantly, the peptide accelerates wound healing.

6.3. Peptides That Modulate Cellular Proliferation

To date, most peptides isolated against cancer cells reduce cell growth instead of inducing a protumor phenotype. As an example, Matsuo et al. used a unique strategy to specifically screen for a peptide with antitumor activity.[160] After in vitro phage display against melanoma B16F10-Nex2 cells, 50 isolated phage were incubated with the cells for 72 h before MTT viability studies. Out of the 50 phage, 7 significantly decreased cell viability, and out of those 7, a peptide with antitumor activity emerged. This peptide was able to inhibit cell invasion and adhesion in vitro and prevent metastasis in vivo (Figure 7).

In some cases, peptides selected against cell surface receptors can avoid untoward progrowth cell signaling that occurs when using the native ligand. For example, although only 53 amino acids long, the native EGF ligand cannot be used for targeting because of its role in stimulating EGFR activation. Additionally, EGF binding triggers rapid uptake and degradation of EGFR, resulting in a reduction of the target on the cell surface. By comparison, GE11, an EGFR-binding peptide, does not initiate EGFR signaling. While it is internalized, EGFR is recycled back to the cell surface, making it available to carry in another GE11 peptide (and its cargo). As such, it is the better choice of ligand when targeting EGFR-expressing cells.

On the other hand, the neural-stem-cell-specific peptide CGLPYSSVC (isolated by in vitro panning against murine neural stem cells) increased cellular proliferation when added to the media of either neural stem cells in culture or of freshly isolated neural progenitor cells from the adult mouse brain.[427] This peptide's ability to increase neurogenesis could therefore be explored for the treatment of diseases known to involve loss of neurons, such as Parkinson's, Alzheimer's, and Huntington's diseases.[434]

6.4. Peptides That Stimulate Apoptosis

Although many peptides reduce cellular proliferation rates, few induce cell death unless conjugated to a cytotoxic agent.[256,506] However, the tumor-lymphatic-targeting peptide LyP-1 accumulates in the nucleus and specifically induces apoptosis in cells which have affinity for this peptide.[255,256] Importantly, in vivo treatment with LyP-1 results in a reduction of tumor growth, a decrease in tumor lymphatic endothelium, and an increase in apoptotic tumor cells. The mechanism of LyP-1-mediated apoptosis has not been elucidated. The receptor for the peptide has been identified as p32/gC1qR, a mitochondrial protein which is also found on the cell surface of tumor cells. Interestingly, p32/gC1qR mediates ARF-induced apoptosis.[507] However, the relationship between peptide binding and internalization and the role of p32/gC1qR in apoptosis has yet to be determined.

7. USE OF PEPTIDES FOR THERAPY

Due to their high cell specificity and ease of manipulation, cell-targeting peptides have been exploited for the delivery of therapeutic agents. While some peptides are biologically active, most peptides require attachment to a drug or other agent to exhibit the desired therapeutic effect. Peptides can deliver small-molecule drugs or biologics. The therapeutic applications for individual peptides have been noted in Tables 2–17 according to their use. In this section we discuss approaches to targeted drug delivery and highlight a few of the successes in developing peptide-targeted therapies. Delivery of small-molecule drugs and therapeutic biologics is covered as well as the use of targeting peptides in immunotherapies. While many proof-of-concept studies have been performed to demonstrate delivery, only studies that address a therapeutic application are discussed in the following subsections.

7.1. Peptides for Small-Molecule Drug Delivery

Peptides as drug-targeting agents can widen the therapeutic window by both increasing drug efficacy and decreasing unwanted side effects. This is of particular importance for drugs with high toxicity profiles, such as many chemotherapeutics. Additionally, peptides that mediate cellular uptake can improve uptake of molecules that do not cross the cell membrane. Peptides are used for drug targeting by either directly conjugating the drug to the peptide or by conjugating the peptide to a drug-carrier platform.[508] The ability to be used in multiple platforms again highlights the strengths of peptides as targeting agents. By contrast, using antibodies for drug delivery is hindered by difficulties in chemical conjugation to their cargo. Additionally, antibody size adds ~20 nm to the size of a nanoparticle, which can dramatically affect biodistribution. Before discussion of the successes in peptide-guided drug delivery, it is important to consider the differences between direct drug conjugates and peptide-modified drug carriers.

7.1.1. Approaches for Small-Molecule Delivery: Direct Conjugates vs Drug Carriers—Peptides can be conjugated to a variety of drugs for specific delivery to the desired cell type. These peptide–small-molecule conjugates require chemical groups on both the peptide and the drug that are compatible for conjugation. Additionally, the drug is generally inactive while conjugated to the peptide, meaning that the covalent bond between the peptide and drug must break and release the drug to achieve any therapeutic effects; i.e.,

they are prodrugs. Ideally, the peptide–drug linkage remains stable under normal bodily conditions, releasing the drug once the conjugate has reached its target. Often acid-labile linkers are employed; these linkers are intact at a pH of 7 but hydrolyze and release under acidic conditions, such as those of the endosome and lysosome. Other types of drug linkers can also be used, such as esters or carbamates;[509,510] these linkers are also stable under normal conditions and release after exposure to the high levels of esterases within cells. Similarly, protease cleavage sites can be employed, such as a valine–citrulline linker that is cleaved by the lysosomal cysteine protease cathespin B. The cleavage efficiency by proteases is often diminished by the presence of the drug molecule immediately adjacent to the cleavage site. To overcome this problem, self-immolative linkers have been designed, in which a release site (or trigger site) is placed between the linker and the targeting moiety.[511] Upon cleavage, a reactive intermediate forms which then spontaneously decomposes to release free, active drug. Balancing conjugate stability in circulation with rapid release at the target site can be difficult. Premature or inefficient drug release has resulted in poor efficacy for many peptide–drug conjugates. Common drug linkers that have been used to covalently attach peptides to drugs are shown in Figure 8.

It should be noted that most conjugates are based on the premise that the conjugate will eventually traffic to the lysosome where the low pH and high protease content release the drug. However, it is unlikely that all peptides isolated from the various libraries will direct lysosomal accumulation, and different uptake mechanisms can affect downstream peptide trafficking within the cell. In fact, clatherin-mediated endocytosis, cavelolae-mediated endocytosis, and internalization by macropinocytosis have been observed for different cell-targeting peptides.[118,157,214] This stresses the need to characterize the mechanism of cellular uptake and the resultant localization of the peptide before embarking on the synthesis of peptide–drug conjugates.

Both the primary advantages and disadvantages of peptide–drug conjugates lie in their small size. While this small size allows the conjugates better vasculature escape and penetration through tissues,[512] it also increases their blood clearance rate. Particles below a molecular weight of 40 000 tend to undergo rapid renal filtration and excretion from the body.[513] As most peptide–drug conjugates fall below this molecular weight cutoff, they are only therapeutically beneficial if they accumulate in the desired tissue at effective levels relatively quickly, before the majority of the conjugate is cleared from the body. However, reaching therapeutic levels is possible due to the high specificity and affinity of direct conjugates. Additionally, such conjugates tend to have low background uptake. A significant disadvantage is that peptide–drug conjugates deliver only one drug molecule per targeting event. Thus, to reach an effective dose, the compound must be highly effective at low cellular concentrations or the targeted receptor must be abundant and undergo efficient internalization.

On the other hand, peptides can be conjugated to drug carriers. Most drug carriers fall into one of three categories—polymer scaffolds, micelles, or liposomes—although a variety of other nanoparticles have been created. Liposomes are the most widely used drug carrier for peptide-targeted therapy, and the vast majority of peptides isolated from phage display

libraries that have been conjugated to nanoparticles for drug delivery have been conjugated to liposomes.

Liposomes are spherical nanoparticles formed by lipids self-assembling into a bilayer.[514] As liposomes are formed from phospholipids and cholesterol that are already normal components of the human body, they are naturally biodegradable. [514] The inner compartment of a liposome is an aqueous phase that can encapsulate hydrophilic agents. However, hydrophobic drugs can also incorporate into the hydrophobic portions of the lipid membrane.[515] Early liposome formulations suffered from rapid blood clearance rates due to engulfment by the cells of the reticuloendothelial system, in particular Kupffer cells in the liver. The reticuloendothelial system is the primary means by which foreign macromolecules are eliminated from the body and consists mostly of macrophages in the liver, spleen, and lymph nodes. However, coating the outer lipid membrane with polyethylene glycol (PEG) increases the circulation time of the liposomes by reducing interaction of the liposomes with serum proteins and thus delays clearance by the reticuloendoethial system.[516] Several liposome formulations are already clinically approved.[517,518]

Liposomal formulations are advantageous for a variety of reasons. The large hydrophilic interior of the liposome allows for the encapsulation of thousands of drug molecules, giving a peptide-targeted liposome a much higher peptide:drug ratio than peptide–drug direct conjugates with only a 1:1 peptide:drug ratio. Additionally, hydrophilic drugs can be loaded into liposomes in their natural state and do not require chemical modification for conjugation to the peptide; there is also the potential to encapsulate multiple drugs within the same liposome. The long in vivo circulation time enjoyed by PEGylated liposomes also means that the peptides will have longer to deliver their drug cargo to the tumor than will peptides directly conjugated to a therapeutic. However, liposomes also suffer from several disadvantages. In particular, the larger size (80–200 nm) of liposomes prevents them from penetrating tissue as well as smaller conjugates and can make it more difficult for them to escape from the vasculature.[519]

Although liposomes are the most extensively used drug carriers for peptide-targeted delivery, polymer scaffolds and micelles are also important drug carriers. Polymer scaffolds are created by attachment of water-soluble polymers to the drug.[520] Much like peptide–drug direct conjugates, these drug–polymer scaffolds also require chemically reactive groups and should remain intact until cell internalization, at which point the bond between the polymer and drug breaks, allowing for the drug to release and exert its effects.[520] However, unlike peptide–drug direct conjugates, drug–polymer scaffolds have longer in vivo circulation times due to the increased size of the polymer scaffold. Additionally, the size of drug–polymer conjugates can be tailored to reduce renal clearance. A major disadvantage to these conjugates is that it can be difficult to control both the length of the polymers and the amount of drug conjugated to the scaffold. However, polymer scaffolds have progressed extensively through the drug pipeline, with polymers conjugated to paclitaxel, camptothecin, doxorubicin, carboplatin, and 1,2-diaminocyclohexane (DACH)–platinate all in clinical trials.[520] Despite these successes, few library-selected peptides have been conjugated to polymer scaffolds.

Micelles are formed by amphiphilic copolymers, which have both a hydrophilic component and a hydrophobic component. [521] Upon exposure to an aqueous solution, the insoluble hydrophobic portions of the polymer aggregate into a core structure, with the hydrophilic polymers forming a shell that surrounds the core. The hydrophobic core of micelles allows them to encapsulate hydrophobic drugs. These self-forming nanoparticles are typically 50–100 nm in size.[522] Micelles have many of the same advantages of liposomes, as they are able to incorporate unmodified drugs and can encapsulate multiple agents at once at a fairly high concentration of drug per micelle. As micelles tend to be smaller than liposomes, they cannot incorporate as many drug molecules as the larger nanoparticles and have intermediate circulation times. Their smaller size may allow for better escape from the vasculature and better tumor penetration. To remain intact as nanoparticles, micelles must maintain a critical micelle concentration (cmc).[522] The cmc is the concentration of individual amphiphilic copolymers required for the micelle structure to form. Below the cmc, micelles dissociate into the individual copolymers, releasing the entrapped drug. However, micelles can often be designed to remain intact in vivo long enough to reach their target.[522] Several micelle formulations are currently in clinical trials.[523]

7.1.2. Peptide Conjugates Used for Small-Molecule Drug Delivery—Cancer therapies have a narrow therapeutic window as they affect both normal and cancerous cells; most are administered at their maximally tolerated dose and not at their maximally effective dose due to their toxic side effects. Thus, targeting chemotherapeutics specifically to tumors would be of benefit, and small-molecule drug delivery has focused on this disease. Few peptides selected from OBOC libraries, bacterial display libraries, and PS-SPCLs have been used for in vivo therapy in animals.[337,342] Peptides selected from phage display libraries, on the other hand, have been used extensively for in vivo therapy.

Several different chemotherapeutic drugs have been directly conjugated to cancer-specific peptides, including doxorubicin, [112,158,166,293,321,524] paclitaxel,[118,157,158,224] and a vitamin E analogue.[184] While most of these conjugates have only been used for in vitro toxicity studies, several have also been shown to inhibit tumor growth in vivo. The peptide LTVSPWY, isolated from panning a phage display library against cultured SKBR3 breast cancer cells, inhibited breast tumor growth in transgenic mice when conjugated to the proapoptotic vitamin E analogue α-tocopheryl succinate.[184] Additionally, three different peptides conjugated to doxorubicin have been used to inhibit tumor growth in mice. The NGR and RGD-4C peptides, both isolated from in vivo phage panning in mice bearing MDA-MB-435 breast xenografts, inhibited MDA-MB-435 tumor growth when conjugated to doxorubicin.[112] Of note, both of these peptides target the tumor vasculature and not the tumor cells. However, a peptide specific for tumor cells has also proven effective for drug targeting in vivo. The tumor-cell-targeting A54 peptide, isolated from in vivo panning in mice bearing BEL-7402 hepatocellular carcinoma xenografts, also inhibited tumor growth when conjugated to doxorubicin.[321] More recently, a derivative of the EphA2-targeting peptide YSA has been utilized to deliver paclitaxel to prostate and renal carcinomas in vivo.[118] The parental peptide was modified by replacement of the amino-terminal L-tyrosine with D-tyrosine and by substitution of L-norleucine and L-homoserine for the two methionine residues (dYNH), resulting in a peptide with dramatically improved serum stability while

retaining a low nanomolar affinity for EphA2. This peptide was conjugated to paclitaxel via a carbamate linker to the 2′-hydroxyl of the drug. The conjugate mediated internalization and localized within the lysosome. Treatment with the dYNH–paclitaxel conjugate resulted in a reduction of tumor growth in a subcutaneous prostate tumor model. The conjugate was significantly more effective than free paclitaxel or a scrambled peptide control conjugate. The same conjugate also inhibited growth of an orthotopic renal carcinoma tumor but was no better than free drug.

7.1.3. Peptide-Guided Nanoparticles Used for Small-Molecule Drug Delivery—

Most peptides conjugated to liposomes for targeted drug delivery have been conjugated to liposomal forms of doxorubicin. Seventeen different peptides isolated from phage display libraries have been conjugated to liposomal doxorubicin for either in vitro inhibition of cancer cell growth[102,168,259,299,427] or in vivo inhibition of tumor growth in rodents.[128,155,181,229,244,249,267,282,283,299] All of the targeted peptide–liposomal doxorubicin nanoparticles save one inhibited tumor growth better than liposomes without the targeting peptides. The liposomal studies involving one peptide are particularly worth noting. A peptide derivative of the tumorvasculature-targeting NGR peptide (GNGRG) was conjugated to liposomal doxorubicin.[112] NGR–liposomal doxorubicin inhibited the growth of orthotopic neuroblastoma xenografts in mice, leading to tumor eradication. By comparison, treatment with control peptide–liposomal doxorubicin did not alter tumor growth compared to that in control non-drugtreated mice.[282] This therapeutic efficacy was the result of greater accumulation of the peptide-targeted liposomes in the tumor.[282] NGR–liposomes have been primed for potential future clinical trials by preparation using good manufacturing practices (GMPs),[525] and these liposomes have been shown to increase survival in orthotopic mouse lung, ovarian, and neuroblastoma xenografts.[283]

Despite the many advantages of micelles, fewer libraryselected peptides have been conjugated to this class of nanoparticles. The lung-cancer-targeting peptide H2009.1 has been conjugated to paclitaxel micelles and to doxorubicin/ superparamagnetic iron oxide (SPIO) micelles.[226] Both selectively kill cancer cells in vitro, but neither platform has yet met success in vivo. Recently, the ovarian-cancer-targeting peptide OA02 was used to deliver paclitaxel-loaded micelles.[337] Importantly, this micellar formulation showed improved antitumor efficacy compared to that of nontargeted micelles. These successes suggest that peptide-targeted micelles should be further explored.

Although less commonly used, a variety of other nanoparticles have been used for peptide-targeted drug delivery. These include nanoworms,[103,276] particles created from a phage coat protein,[156] nab-paclitaxel,[265,274,275,301] microbubbles,[300] and polyester-based particles.[320] In particular, nab-paclitaxel, a nanoparticle formed by albumin-coated paclitaxel, has been conjugated to several peptides for tumor inhibition in rodents.[265,274,275,301] As an untargeted form of nab-paclitaxel, Abraxane, is approved for clinical use, these nanoparticles are an attractive drug-targeting candidate. More recently, the EphB4-binding peptide, referred to as TNYL-RAW, was cyclized and conjugated to hollow gold nanospheres.[124] These nanoparticles were loaded with doxorubicin and used to target EphB4-positive tumors in vivo. Irradiation with a near-infrared laser resulted in a dual mode of action: photothermal ablation of the tumor and release of doxorubicin within the tumor environment. Treatment

with the TNYL-RAW nanospheres followed by near-infrared irradiation was more effective than the same regimen using nontargeted nanospheres or targeted nanospheres not loaded with doxorubicin. Impressively, complete regression of subcutaneous Hey ovarian tumors was observed in 75% of mice treated with both TNYL-RAW nanospheres and near-infrared irradiation.

An interesting method recently developed involves keeping peptides in the context of the pVIII phage protein for incorporation into nanoparticles.[526] Following selection, the isolated phage is disrupted with chlorate buffer so that the peptide–pVIII phage protein conjugate can be isolated and purified. Because the pVIII protein functions as a membrane protein, it is able to incorporate into both liposomes and micelles. Peptide–pVIII fusion proteins insert into preformed liposomes during a short incubation time, making this procedure fairly simple. Peptides selected against both PC3 prostate carcinoma cells and MCF7 breast cancer cells have been used in this method to incorporate peptide–pVIII proteins into doxorubicin liposomes for in vitro cytotoxicity studies.[168,189] The MCF-7 peptide–pVIII conjugates have also been incorporated into drug-loaded micelles for in vitro cytotoxicity studies.[191]

7.2. Peptide Delivery of Biologics

As the field of biologic therapy expands, so does the need to deliver such molecules. Proteins and peptides, either naturally occurring or designed de novo, can have potent biological activities. However, they do not cross the cell membrane. As such they are limited to cell surface targets. Using targeting peptides that mediate both cell-specific binding and internalization can overcome this limitation, opening new therapeutic avenues. Additionally, many peptides and proteins are generated in a local environment; systemic administration results in undesirable outcomes. Redirecting the peptide/protein to its local site of action can mitigate such side effects. This subsection highlights a few examples of successful delivery of therapeutic peptides and proteins.

7.2.1. Delivery of Therapeutic Peptides—Targeting peptides can also be conjugated to other peptides with known cellular activities. By far, the peptide most widely used in this context is the proapoptotic peptide $(KLAKLAK)_2$. Although inactive outside cells, the $(KLAKLAK)_2$ peptide exerts intracellular toxicity by disrupting the mitochondria, leading to subsequent cell death. As $(KLAKLAK)_2$ does not cross the cellular membrane unless attached to an internalizing cell-targeting peptide, it is nontoxic to other cells. This peptide is often synthesized using protease-resistant D-amino acids, increasing its chances of reaching a tumor as an intact active peptide. Numerous cancer-targeting peptides have been fused to the proapoptotic peptide for either in vitro killing of cancer cells[104,172,214,215,252,277] or in vivo inhibition of tumor growth in rodents.[104,277]

The $(KLAKLAK)_2$ peptide is also being developed as an antiobesity therapy. The white-fat-vasculature-specific peptide CKGGRAKDC, isolated from an in vivo screen in the obese leptin-deficient ($Lep^{ob/ob}$) mouse model, was used to ablate white fat in mice.[414] This CKGGRAKDC peptide was linked to the D-enantiomer of the proapoptotic $(KLAKLAK)_2$ peptide and injected subcutaneously into wild-type mice forced into obesity by a high-calorie diet. After a month of daily treatment, mice treated with the CKGGRAKDC-

GG-$_D$(KLAKLAK)$_2$ peptide conjugate were an average of 15 g lighter than control mice treated with unconjugated CKGGRAKDC and $_D$(KLAKLAK)$_2$. Immunohistochemistry revealed specific apoptosis in the white fat vasculature of the conjugate-treated mice and not in that of control-treated mice. Significantly, fat ablation also led to increased metabolism and reversal of insulin resistance in conjugate-treated mice. Similar results were observed in obese rhesus macaques and baboons.[415] The only observed side effect was mild renal degeneration, which was reversible.

Other biologically active peptides can be delivered as well. The cardiac-endothelium-specific CRPPR peptide[359] was coupled to the antioxidant peptide gp91ds for antioxidant therapy.[360] Gp91ds inhibits the assembly of NAD(P)H oxidase, preventing its production of the reactive oxygen species superoxide.[527] As superoxide reacts with nitric oxide, lowering its bioavailable levels, blood vessels no longer relax normally and blood pressure eventually rises.[527] Thus, antioxidant therapy with gp91ds should lower blood pressure if the gp91ds is able to reach its biological target in the cardiac endothelium. The CRPPR peptide–gp91ds conjugate was administered to hypertensive and stroke-prone rats through a subcutaneous osmotic minipump. The rats experienced an increase in nitric oxide availability and a decrease in systolic blood pressure consistent with specific delivery of the gp91ds to the cardiac endothelium.[360] Although this is unlikely to translate into a therapy available for patients due to the large amount of peptide needed to reduce blood pressure, it is an interesting lead for the development of future therapies.[360]

7.2.2. Delivery of Therapeutic Proteins—Targeting peptides can deliver therapeutic proteins when created as peptide–therapeutic protein fusion proteins. Unlike the chemical conjugation used to make peptide–small-molecule and peptide–peptide conjugates, most of these conjugates are created genetically. Peptides selected from phage-displayed libraries have been fused to a variety of proteins, including toxic shock syndrome toxin 1,[154] vascular endothelial cell growth inhibitor,[129] the kringle 5 fragment of human plasminogen,[130] a fragment of tumstatin (tum-5),[292] interferon α (INFα2a),[290,291] and interleukin-2 (IL-2).[316] Additionally, three peptides have been conjugated to either TNF-α or a mutant version of the same protein.[136,261,284,285,287,288] Significantly, all of these peptide–protein fusions have been used for in vivo therapeutic experiments in rodent cancer models, leading to either inhibition of tumor growth or increased survival. Of particular interest is one of the TNF-α conjugates. A shortened version of the NGR peptide, CNGRCG, expressed as a fusion protein with TNF-α is currently in clinical trials.[528] Due to the success of phase I and II trials for this conjugate, it was granted “orphan drug” status in both the European Union and the United States for the treatment of malignant pleural mesothelioma (MPM).[528] Phase III trials for NGR–TNF in MPM patients are ongoing.

7.3. Peptides Used for Immunization

As dendritic cells (DCs) are antigen-sampling and -presenting cells that can initiate both adaptive and humoral immune responses, they are attractive targets for targeted delivery of antigens. In the most common approach to DC-based vaccines, DCs are cultured from blood or bone marrow progenitor cells in the presence of cytokines, pulsed with the appropriate tumor antigen, and injected back into the patient. This is a complicated and expensive

process.[529] However, several recent studies used antibodies, antibody fragments, or other ligands that bind to dendritic cells to specifically deliver antigens in vivo.[529–534] Accordingly, peptides can be used to specifically target antigens in vivo. Faham and Altin used the previously isolated dendritic-cell-binding peptide CGRWSGWPADLC[390] to specifically target dendritic cells in mice in vivo.[391] The peptide was first attached to a liposome carrying OVA as a model antigen. Mice vaccinated with peptide–OVA–liposomes by intravenous injection produced OVA-specific antibodies unlike mice vaccinated with control OVA–liposomes. Additionally, the peptide was grafted onto plasma membrane vehicles derived from B16-OVA cells, a metastatic murine melanoma cell line that secretes OVA. Vaccinating mice with these peptide membrane vehicles before introducing B16-OVA cells into the mice significantly reduced the number of lung metastases (Figure 9). Additionally, mice bearing existing B16-OVA tumors that underwent subsequent vaccination with the peptide membrane vehicles experienced considerable tumor growth inhibition, with four out of five treated mice experiencing complete remission by day 30. The dendritic-cell-specific peptide XS52.1, isolated from in vitro panning against the mouse dendritic cell line XS52, was also isolated with the intent of improving vaccination.[393] Although use of the free peptide to elicit an immune response has not been tested, the peptide displayed on the phage was able to induce an immune response. Mice injected intradermally with XS52.1 phage and not those injected with a control peptide-displaying phage developed antiphage serum antibodies. Further studies need to be performed with the synthetic peptide to verify that the peptide itself is able to deliver antigens and elicit an immune response.

The antigen-sampling M cells of the intestinal epithelium sample antigens in the gut and transport them a short distance to a binding pocket in which immune cells such as lymphocytes or macrophages dock.[395] Thus, the M cells can carry antigens out of the gut to immune cells to facilitate an immune response.[395] Due to these traits, M cells are desirable targets of orally available vaccines.[395] Peptides that could direct the oral vaccine to M cells would allow for specific delivery of the vaccine to immune cells for initiation of an immune response. The Co1 peptide identified from in vitro panning against human M-like cells was used to target a model oral vaccine in mice.[397] When the Co1 peptide was fused to the fluorescent protein EGFP and given orally to mice, it was able to bind M cells and transport across the intestine as evidenced by fluorescent imaging of Peyer’s patch tissue sections at different times post peptide–EGFP injection. Significantly, after six weeks of oral administration of Co1–EGFP in mice, the mice developed both serum IgG and fecal IgA antibodies against the EGFP, indicating that the vaccine induced an immune response. Importantly, Co1–EGFP induced antibody production that was 2–3-fold higher than that induced by EGFP alone. Since Co1 has also been shown to bind (and was isolated against) human M-like cells in culture, this peptide may translate well into the clinic. Similar immune activation was observed when using the M-cell-targeting peptide CL3 to deliver EGFP, suggesting that targeting M cells is a potential route to develop oral vaccines.[398]

8. PEPTIDES USED TO DELIVER OLIGONUCLEOTIDES

Delivery of genes, siRNA, and miRNA to cells requires carriers that protect the oligonucleotides during transport and facilitate delivery of the highly charged molecules

across the cell membrane. Again, cell-targeting peptides have been used to deliver DNA and RNA to cells. Bacteriophage phage clones that internalize into mammalian cells can deliver DNA; however, the transfection efficiency is low.[182,535–540] To overcome low transfection efficiencies, targeting peptides have been spliced onto eukaryotic viral vectors which have high transfection efficiencies or have been incorporated into chemical gene delivery systems. Numerous cell-targeting peptides have been used to transduce specific cells as noted in Tables 2–17. In this section we point out several studies that have either led to in vivo delivery of an oligonucleotide and/or shown a functional biological outcome.

8.1. Engineered Viruses for Delivery of Oligonucleotides

Viral vectors have high transfection efficiencies. However, removal of the native viral tropism must occur to redirect the gene transfer to the targeted cell type.[541–543] Most efforts have focused on grafting cell-targeting peptides onto engineered adenovirus as noted below. Recently, peptides have been inserted into adeno-associated virus (AAV) for targeted gene delivery.[544] Because it has been difficult to maintain peptide targeting function in the context of the AAV,[544] new AAV peptide libraries have been made so that peptides can be isolated directly in the context of the virus in which they will be used.[545,546] These AAV libraries were recently reviewed elsewhere and are not covered in this review as the selected peptides have not been tested outside the context of AAV.[544]

Gene therapy with cardioprotective genes targeted to the cardiac vasculature has the potential to prevent coronary heart disease and to improve the side effects caused by ischemia after heart attacks (myocardial infarction). One recent study isolated the novel peptide DDTRHWG against the cardiac endothelium of hypertensive rats and inserted this peptide into the H1 loop of a modified adenovirus serotype 5 vector (Ad5), termed Ad5/19p, created for its reduced liver tropism.[369] A peptide-directed virus expressing *LacZ* was able to specifically transduce cardiac endothelium in vivo, with no virus detected in the liver. This proof of principle gene transduction suggests that this same peptide–adenovirus vector may be used to deliver a gene directed at improving cardiac vasculature function.

As diseases such as epilepsy and depression are connected to altered neurogenesis,[435,436] gene delivery to neural stem cells has the potential for therapeutic effects. The neural-stem-cellbinding peptides QTRFLLH and VPTQSSG were conjugated to AdGFPL adenovirus, which is mutated to ablate natural tropism and contains the GFP gene.[428] AdGFPL.QTRFLLH and AdGFPL.VPTQSSG were injected into the mouse brain and shown to specifically transduce neural stem cells in the dentate gyrus, as detected by GFP fluorescence in brain tissue sections. This suggests that these peptides could be used to deliver therapeutic genes to the neural stem cells. However, it will be important to verify that these peptides still bind human neural stem cells.

The kidney-specific peptides HTT and HIT were isolated by in vivo panning in rats.[431] Expressing both peptides in the HI loop of the Ad19p adenovirus previously shown to have reduced hepatic specificity[547] allowed for specific peptidemediated gene delivery to the kidneys.[431] Rats were injected with Ad19p-HTT, Ad19p-HIT, or the control Ad19p, all containing the model gene *LacZ*. All rats were sacrificed 5 days later, and kidney sections were visualized by immunohistochemistry for β-galactosidase expression. Unlike the control

vectors, both the HTT and HIT vectors specifically transduced cells in the kidney, resulting in kidney β-galactosidase expression. Ad19p-HTT-treated rats had β-galactosidase expression in the epithelial cells of the kidney tubules, while Ad19p-HIT-treated rats had β-galactosidase expression within the cells of the glomerulus. Importantly, β-galactosidase was not detected in other nontarget organs, including the liver. These results suggest that the HTT and HIT peptides can be used for specific kidney gene targeting when incorporated into the Ad19p adenovirus.

Arap and Pasqualini took an innovative approach in which the features of the viral vectors were incorporated into an fd-tet bacteriophage.[548–550] Specifically, a mammalian transgene cassette from AAV was inserted into a noncoding region of the bacteriophage genome. This vector, referred to as AAVP, has been utilized to deliver several genes to different target cell types. The RGD-4C peptide that binds $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrins has been utilized to create chimeric AAVP virus that facilitates functional gene transfer specifically to tumor vasculature and adjacent tumor cells.[551] For example, delivery and expression of the *tHSVtk* gene results in expression of herpes simplex virus type-1 thymidine kinase selectively in the targeted tumor. Remarkably, no significant gene expression is observed in the liver. The TNF-α gene has been delivered to tumors by the same approach, resulting in TNF-α expression and significant reduction in tumor size.[552] As a result, RGDdirected AAVP carrying *TNF*-α is in preclinical development.[553]

The mPep peptide isolated by panning against the mouse preadipocyte cell line 3T3-L1 was used to specifically deliver the *Ucp1* gene to 3T3-L1 cells.[413] As Ucp1 has antiobesity properties and can inhibit lipogenesis,[554] it was added to an AAVP vector displaying the mPep peptide.[413] mPep-AAVP-Ucp1 specifically targeted 3T3-L1 cells in vitro, and 7 days after transduction the cells failed to differentiate into adipocytes. By contrast, nontargeted AAVP-Ucp1 was unable to transduce 3T3-L1 cells, and the cells differentiated into adipocytes.

8.2. Chemical-Based Systems for Targeted Delivery of Oligonucleotides

Oligonucleotides can be condensed on polycation polymers and delivered to cells using a targeting peptide. These chemical-based systems are easier to synthesize than the corresponding viral gene delivery systems and do not suffer from inherent immunogenicity. While their transfection efficiency is lower than that of viral delivery, synthetic gene delivery systems are progressing as viable options for in vivo treatment. For example, a peptide selected against cells overexpressing Tie2 is able to specifically deliver DNA condensed on a poly(ethylenimine) construct to mice bearing SPC-A1 lung cancer.[462] This construct was used to deliver WT p53, resulting in a significant decrease in tumor size due to restoration of p53's tumor suppressor activity.

The EGFR-targeting peptide GE11 was conjugated to linear poly(ethylenimine) to deliver the sodium iodide symporter (NIS) gene for ^{131}I therapy of liver cancer in mice.[555] NIS is the protein that allows the thyroid to accumulate iodide, and its expression in thyroid cancer allows for the treatment of thyroid cancer using radioiodine, the most effective radiotherapy currently in clinical use. Liver-tumor-bearing mice injected intravenously with the polyplex

and then given ^{131}I 24 h later experienced significant tumor growth delay compared to mice with control treatments.

Gene delivery has not been limited to cancer cells. Nam et al. used the previously identified cardiomyocyte-specific peptide PCM.1[213] to specifically deliver cardioprotective siRNA to H9C2 rat cardiac myocytes in vitro.[371] The PCM.1 peptide was conjugated to a bioreducible polymer, poly(CBA-DAH), and complexed with *Fas* siRNA, a known inhibitor of cardiomyocyte apoptosis. The PCM–polymer–siRNA complex knocked down *Fas* expression in hypoxic H9C2 cells, reducing cell apoptosis. As hypoxia is a side effect of ischemic myocardium, in vivo studies with this complex may provide interesting therapeutic leads for the disease.

The dendritic-cell-specific peptide XS52.1, isolated from in vitro panning against the mouse dendritic cell line XS52, was also used to specifically deliver a gene in vivo.[393] The XS52.1 peptide was incorporated into a liposome containing a luciferase gene plasmid and injected into the skin of mice. Immunofluorescence of cell suspensions from lymph nodes isolated 24 h later demonstrated XS52.1-mediated specific delivery of the luciferase gene to the dendritic cells of the skin. Quantification of luciferase expression demonstrated 10-fold more luciferase in dendritic cells targeted with XS52.1–liposomes as opposed to cells given control peptide or no peptide liposomes. This study suggests that the XS52.1 peptide could be used to deliver biologically active genes to the dendritic cells of the skin.

9. USE OF PEPTIDES FOR DIAGNOSTIC APPLICATIONS

Due to the high specificity of targeting peptides, they can be adapted for diagnostic applications in the same manner in which antibodies are currently used. The advantages of using peptides over antibodies for in vitro assays are minimal; as such, most efforts have focused on developing peptides for in vivo diagnostic imaging. In particular, there is a surge of peptides being used as molecular imaging probes. The next sections will highlight recent advances in translating selected cell-binding peptides into useful diagnostics.

9.1. Peptides Used for in Vitro Diagnostics

Peptides can serve as antibody replacements for immunohistochemistry, and since peptides can easily be labeled with a variety of fluorophores, using them in multiplexed assays is possible. However, as the molecular target for most of the selected peptides is unknown, they are unlikely to replace antibodies for pathological classifications. Rather, the ability of these peptides to bind to intact cells in a highly specific fashion makes them ideal for isolation of the target cell from a background of other cells. This is especially important when the cell of interest is a minority population, such as a circulating tumor cell. Several peptides have been used to isolate tumor cells from whole blood. The cDGWGPNc peptide was isolated from an OBOC library screen against multiple ovarian cancer cell lines. When placed on the surface of polystyrene beads, cDGWGPNc is able to bind ovarian cancer cells mixed into blood.[335] The pA peptide, selected from an OBOC screen against A549 non-small-cell lung cancer cells, was able to pull down cancer cells from the pleural fluid of a patient with lung adenocarcinoma.[338] The QMARIPKRLARH peptide, selected from an OBOC screen against LNCaP cells, also bound LNCaP cells in whole blood.[50] The A20

peptide, selected for binding to a B-cell lymphoma cell line from a phage-displayed library, can enrich for the cancerous cells out of a background of normal Bcells. [212]

Recently, Lam and co-workers described an interesting method with diagnostic potential for OBOC peptides involving what they term “rainbow beads”.[556] After the synthesis of peptides on TentaGel resin beads using standard Fmoc library synthesis, the beads were dyed using commercially available organic dyes. Subsequent washing, drying, and soaking resulted in distinctly colored beads that displayed a given peptide of interest. Using this method, a group of six different peptides and peptidomimetics were synthesized on dye-labeled beads such that each peptide or peptidomimetic had its own distinct bead color. The beads were then mixed and incubated with different cancer cell lines to examine the ability of each peptide to bind to each cell line. Peptide specificity for the cell lines was examined using a standard inverted microscope with white light. The different peptides were easily distinguishable by their different bead colors, and cell specificity for peptides was determined by looking for colored beads that bound cells. This method has the potential to quickly determine peptide specificity for different cell types and can be multiplexed. Similarly, multiplexed assays to determine the binding profile of multiple peptides on a single sample can be performed using peptides conjugated to quantum dots with different emission wavelengths.

Although not diagnostic, another example of using peptides to isolate cancer cells was provided by McDonald and colleagues.[119,120] The EphA2-receptor-specific peptide YSA was conjugated to superparamagentic nanoparticles and used to remove ovarian cancer cells in vitro from peritoneal fluids removed by paracentesis. After incubation with the peptide–nanoparticles, a magnet was used to isolate the nanoparticles along with their bound cancer cells, and the remaining peritoneal fluid was drawn off and reintroduced into the mouse. This resulted in tumor growth inhibition and increased survival times compared to those in control mice treated identically without the peptide–nanoparticles.

9.2. Peptides Used for in Vivo Diagnostics

Imaging agents can be incorporated into cell-targeting peptides with relative ease. Peptides have been used for different imaging platforms, including optical, positron emission tomography (PET), single positron emission tomography (SPECT), and magnetic resonance imaging (MRI). These imaging probes have the potential to detect diseases at early stages, locate residual or metastatic disease, and/or provide molecular profiles of cells. Examples of selected peptides used for optical imaging, PET, and MRI are discussed below. Of note, the use of cancerspecific peptides isolated by phage display for molecular imaging has been reviewed elsewhere.[557]

9.2.1. Peptides Used for Optical Imaging—Progress is being made in the development of near-infrared red reagents,[558] yet whole-body fluorescence imaging in humans is currently not feasible due to high background fluorescence, poor light penetration, and inherent light scattering.[559] However, it is a valuable research tool in animal models to determine biodistribution of targeting peptides and/or optimization of peptides for improved targeting. Peptides have been attached to a variety of dyes or

fluorescent nanoparticles, such as quantum dots, for in vivo optical fluorescence imaging.[305,560,561] The numerous peptides that have been used for optical imaging in animals are noted in the tables; peptides selected from phage display, OBOC, and PS-SPCL libraries are represented.

Optical imaging can also be used in conjunction with other diagnostic tools. For example, ex vivo phage display was used to isolate a 6-mer peptide specific for fresh human colon adenomas. This peptide was fluorescently labeled and administered topically to patients undergoing colonoscopy.[236] Dysplastic regions of the colon could be distinguished from normal tissue with a sensitivity and specificity of 80% using confocal microendoscopy. Another study used in vivo phage display to isolate a peptide specific for mouse colon dysplasia. A fluorescently labeled version of this peptide bound colonic dysplasia in mice and was easily detected using wide-field endoscopy.[317] Although this dysplasia-specific peptide was isolated from a mouse model of the disease, preliminary experiments demonstrate specific binding to human colon dysplastic adenomas over normal colon tissue. Phase I clinical trials have started to determine the utility of this approach in both colon and esophageal cancers. These successes highlight the potential clinical use of peptides for the early diagnosis of cancer. This also points to the possibility of using labeled peptides during surgery to distinguish tumor borders in real time.

Hallahan and co-workers demonstrated use of their TIP-1-receptor-binding peptide HVGGSSV to optically image and improve treatment for irradiated tumors.[265,320] A theranosic agent was synthesized in which the peptide was conjugated to an AlexaFluor 750–nab-paclitaxel (nanoparticle albumin-bound paclitaxel) platform for treatment and near-infrared imaging. In a similar study, a peptide isolated from irradiated glioma cells showed specific near-infrared imaging when labeled with AlexaFluor 750 and delayed tumor growth when conjugated to a paclitaxel-containing nanoparticle.[320]

Near-infrared imaging has also been employed for heart imaging. The CTP peptide, isolated by a combination of in vitro and in vivo panning against normal murine cardiomyoctyes, was conjugated to commercially available 40 nm sized neutravidin–fluorospheres which fluoresce in the near-infrared range.[358] Intracardiac injection in mice demonstrated heartspecific imaging for the CTP–fluorospheres as compared to control peptide–fluorospheres (Figure 10). Specificity after intravenous injection of the peptide–nanoparticles remains to be determined.

The brain-specific TGN peptide was isolated in an effort to bypass the blood–brain barrier. This peptide was conjugated to ~100 nm sized PEG–poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles loaded with the near-infrared dye DiR.[206] After tail vein injection into mice, these particles homed to the brain as evidenced by live animal fluorescence imaging. Ex vivo imaging of organs after the mice were sacrificed revealed that control nanoparticles without peptide primarily accumulated in the liver and spleen and did not accumulate in the brain. The targeted TGN nanoparticles had significantly reduced liver and spleen clearance compared to the control nanoparticles.

9.2.2. Peptides Used for Molecular Positron Emission Tomography—PET can detect molecules down to picomolar concentrations and is considered one of the most sensitive molecular imaging techniques.[562] As such it is an ideal platform for molecular imaging. The low spatial resolution of PET has been compensated for by the generation of PET/CT scanners that combine the sensitivity of PET with the anatomical resolution of computed tomography (CT). PET radioisotopes can be incorporated directly into targeting peptides (i.e., ^{18}F and ^{131}I), or chelators can be conjugated to the peptides and loaded with radiometals (i.e., ^{64}Cu and ^{68}Ga). SPECT is another imaging modality that can utilize cell-targeting peptides for the delivery of radioisotopes (^{99}Tc, ^{111}In, and ^{123}I). Peptidebased imaging probes are anticipated to increase the sensitivity of detection while providing molecular information without the need for biopsy. However, as targeting peptides exhibit restricted binding profiles, it is reasonable to be cautious about their use as early detection agents. This is especially true for the cancer-binding peptides which have affinity for discrete subsets of tumors. Instead, they are more likely to find utility in providing molecular information about a tumor before or during treatment as well as in detecting micrometastases. Information obtained from PET imaging could aid in determining which ligand(s) should be employed for therapeutic targeting for an individual patient. Of importance, PET imaging can also provide the biodistribution information which is critical in preclinical optimization of cell-targeting peptides.[563]

Compared to antibodies, peptides are more amenable to the reaction conditions required for chemical modifications and labeling.[564,565] Unlike antibodies, the in vivo half-life of the peptides is well matched to the half-life of most commonly used PET radionuclides. Most peptide-based PET imaging has been performed using naturally occurring peptidyl ligands such as bombesin and somatostatin.[566–568] Several RGD-containing peptides, including the RGD-4C peptide,[296,569] have been used to image $\alpha_v\beta_3$ in angiogenic vasculature.[570,571] RGD peptides are in early clinical trials as molecular PET imaging agents for angiogenesis.[572–574]

Development of molecular agents for PET requires a fine balance between clearance rates, tumor retention, and nonspecific uptake in other tissues. Radiolabeled peptides must have certain characteristics to work well in vivo: they must have high affinity for their target, exhibit low nonspecific binding, and be stable in serum, maintaining the peptide integrity and retention of the radioisotope. Additionally, the peptide-based imaging agent must clear rapidly from the plasma and excrete in a manner that does not interfere with imaging.[575] Kidney accumulation of radiolabeled peptides is a significant problem.[576] While different mechanisms have been proposed for this accumulation, the current consensus is that particles below 20–30 kDa in size are filtered by the glomerulus and then reabsorbed by the proximal renal tubules.[575,577] Small changes in the peptide sequence, linker, chelator, and isotope can dramatically affect the biodistribution. The empirical process involved in optimizing PET agents is time-consuming and costly. Nonetheless, cell-binding ligands isolated from peptide libraries are emerging as molecular imaging agents.

Many of the cancer-specific peptides have been used for molecular PET and SPECT imaging. For example, a tetrameric, PEGylated version of the plectin-1-binding peptide KTLLPTP was used for SPECT imaging of orthotopic pancreatic tumors in mice.[74]

Importantly, preinvasive lesions as well as liver metastases were detected. This imaging probe could also distinguish pancreatic cancers from chronic pancreatitis and may be an effective probe to determine whether a patient is a candidate for biopsy. Peptides can also be used to determine receptor expression in a tumor. For example, the EphB4-binding TNYL peptide was labeled with 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) and loaded with ^{64}Cu.[125] This imaging probe was able to distinguish between EphB4-positive and -negative tumors by small-animal PET/CT (Figure 11).

In an extraordinary feat, the Sutcliffe laboratory combined screening of an OBOC peptide library with high-throughput imaging to develop molecular imaging agents for the cancer biomarker $\alpha_v\beta_6$.[578] By screening a limited OBOC peptide library, a total of 55 peptides were identified. This set of peptides were labeled with 4-[^{19}F]fluorobenzoic acid and tested for affinity to $\alpha_v\beta_6$ as well as specificity for $\alpha_v\beta_6$ compared to other integrins. On the basis of the in vitro results, 42 peptides were labeled with 4-[^{18}F]fluorobenzoic acid for analysis by PET. Within 11 days, all 42 peptides had been tested, and 4 peptides displayed favorable biodistribution profiles. Surprisingly, little correlation between in vitro affinity and integrin specificity and in vivo tumor accumulation and specificity was observed. Thus, this combination of high-throughput peptide selection and imaging can identify good imaging agents that would otherwise be discarded. However, this approach requires easy access to a source of ^{18}F, large quantities of time on a small-animal PET/CT system, and significant numbers of animals; these resources are expensive and not widely available.

Molecular PET is not limited to cancer imaging. The cardiacendothelium-specific CRPPR peptide[359] was coupled to the surface of PEGylated liposomes incorporating ^{18}F-labled lipid for PET imaging.[370] Mice injected with the liposomes were imaged by PET for 90 min. The CRPPR-targeted liposomes accumulated in the heart with lower accumulation in the liver, spleen, and bladder. Nontargeted liposomes primarily stayed in circulation, resulting in some signal in the heart as well. In another example, an ^{18}F-labeled tetrameric version of the VCAM-1-binding peptide VINP-28 detected inflammatory atherosclerosis by dynamic PET imaging.[379] This probe may find utility in detecting small atherosclerotic plaques.

As properly functioning pancreatic islet β cells are crucial for glucose regulation and prevention and management of diabetes, a great deal of attention has been placed on techniques that could noninvasively monitor β cell mass and function.[409] The RIP1 peptide isolated by ex vivo panning against freshly isolated rat pancreatic islets is selective for the β cells of normal rats and does not home to the β cells of a rat diabetic model.[75] Thus, it could potentially be used to image properly functioning, normal β cells. While the peptide has not been tested outside the context of the phage, ^{124}I-labeled phage was able to specifically target the pancreas in vivo with efficient clearance from other organs.[409] The [^{124}I]RIP1 phage was injected into the tail vain of rats and imaged by PET 4 h later. Although the RIP1 phage appears to accumulate in the pancreas, further studies with radiolabeled RIP1 peptide need to be done to confirm the utility of this peptide for normal β cell imaging.

9.2.3. Peptides Used for Molecular Magnetic Resonance Imaging—MRI yields high-resolution images, but current contrast agents suffer from a lack of sensitivity. Few molecular MR probes have been reported because it is challenging to target enough T1

contrast agent, such as gadoteridol, to the desired cell type to achieve a reasonable signal. For this reason, many have turned to iron oxide particles, which are a highly sensitive T2 agent that darkens the signal in regions in which the nanoparticles accumulate.[579] Several nontargeted iron oxide nanoparticles (IONPs) are FDA-approved. [580]

A plectin-1-binding peptide that homes to pancreatic ductal adenocarcinomas has been coupled to fluorescent cross-linked iron oxide nanoparticles.[561] These peptide–nanoparticles home to pancreatic ductal adenocarcinoma as determined by both intravital fluorescence microscopy and ex vivo MRI. Using the Huisgen cycloaddition, better known as click chemistry, the LyP-1 peptide has been attached to fluorescently labeled, dextran-encapsulated iron oxide particles.[306] The total amount of nontargeted and targeted nanoparticle in the tumor was found to be the same, yet the targeted particle penetrated within the tumor while the naked nanoparticle remained localized around the tumor blood vessels. In both of these examples, the peptides home to the appropriate tumors in animals as assessed by ex vivo fluorescent imaging, but in vivo MR imaging was not performed.

To facilitate conjugation of peptides to iron oxide nanoparticles, a one-step procedure for the surface functionalization of SPIO with a targeting peptide has been developed.[581] The hydrophobic surfactants on the SPIO nanoparticles can be displaced through ligand exchange with a peptide containing a C-terminal polyethylene glycol-tethered cysteine residue. The resulting SPIO particles are biocompatible and demonstrate high T2 relaxivity. Attachment of the $\alpha_v\beta_6$-binding peptide H2009.1 resulted in specific targeting of $\alpha_v\beta_6$-expressing lung cancer cells as demonstrated by in vitro MR imaging and Prussian blue staining. This surface chemistry may expand the use of SPIO for MR imaging, but in vivo compatibility still needs to be determined.

The VCAM-1-specific CVHSPNKKC peptide has been used for a variety of in vivo imaging techniques for both cardiac endothelium in atherosclerotic lesions and for imaging of VCAM-1-expressing endothelial cells in other areas of inflammation.[365] The peptide was conjugated to cross-linked iron oxide nanoparticles labeled with Cy5.5 dye. These magnetofluorescent nanoparticles were then used to image both the vasculature of an inflamed ear and the atherosclerotic lesions in the $ApoE^{-/-}$ mouse model of atherosclerosis. Acute inflammation was created in mouse ears by site-specific injection of TNF-α, causing VCAM-1 upregulation on the ear vasculature. Intravenous injection of peptide–nanoparticles led to specific accumulation of the nanoparticles in the inflamed ear vasculature, as evident by fluorescent images from intravital confocal microscopy (Figure 12A). The iron oxide component of the targeted nanoparticles also allowed for their use in MR imaging of atherosclerotic lesions. $ApoE^{-/-}$ mice with atherosclerotic lesions were injected intravenously with the peptide–nanoparticles and the lesions imaged by MRI. Specific darkening of the atherosclerotic lesions was evident in mice given the peptide–nanoparticles, consistent with accumulation of iron oxide in these areas. Areas of the aortic wall with thickening specifically accumulated the targeted nanoparticles, and this accumulation was verified by both ex vivo MRI and ex vivo fluorescent images of excised aortas (Figure 12B). Importantly, the targeted peptide nanoparticles did not accumulate in aorta of wild-type mice without atherosclerotic lesions, and nontargeted nanoparticles did

not accumulate in the atherosclerotic areas of the aorta, as evidenced by both ex vivo MRI and ex vivo fluorescent imaging of excised aortas.

10. TRANSLATION OF TARGETING PEPTIDES FROM LIBRARY SCAFFOLD TO FUNCTIONAL SYNTHETIC PEPTIDES

Despite the ease of identifying cell-binding ligands from peptide libraries, these approaches fell out of favor with some researchers in the community. Specifically, many peptides have had poor affinities when synthesized as free peptides; maintaining the affinity and activity of peptides selected from peptide libraries outside the context of their original scaffold has been an impediment. Many reports utilized monomeric peptides, and this review has highlighted many of them. However, the affinities of numerous peptides are in the micromolar range, which is unsuitable for most clinical applications. This is especially true for peptides selected from phage or OBOC libraries. In both cases, the peptides are displayed in multiple copies on their scaffold; thus, the peptides may bind to their cellular target via a multivalent interaction. The increased affinity due to multivalent binding is lost when the peptides are used in their monomeric forms. Additionally, many endocytotic processes are initiated by receptor multimerization at the cell surface. If internalization of the peptide is desired, the free peptide must facilitate this interaction on the cell surface.

Several different methods have been used to synthesize multimeric peptides to improve affinity (Figure 13). Multimerization of the cell-targeting peptides on a trilysine core is a useful scaffold for retaining the peptide activity outside the context of the phage. The trilysine scaffold mimics the presentation of the peptides on the pIII protein of the phage in both valency and the orientation of the displayed peptides. Additionally, the trilysine core can be modified with a variety of different moieties (drugs, drug carriers, metal chelators, dyes, biotin, and so on) without affecting peptide binding. While these multimeric peptides can be synthesized by linear Fmoc chemistry, we reported a convergent synthesis involving a chemoselective reaction of a cysteine with a maleimide-capped trilysine core.[223] This chemistry is facile and is not restricted to laboratories with expertise in synthetic peptide chemistry. We and others have found the tetrameric trilysine framework to be a general platform for cell-targeting peptides selected from phage-displayed peptide libraries.[212,218,220,393,582] Tetramerization of a peptide increases its affinity for its target cell 25–100-fold when compared to that of the monomeric peptide, indicating the importance of multivalent binding. For example, we have synthesized a tetrameric peptide that binds $\alpha_v\beta_6$-expressing cells. The monomeric peptide binds H2009 adenocarcinoma NSCLC cells with a half-maximal binding affinity of 9.2 nM, while the tetrameric peptide increases affinity to 11 pM. This tetrameric peptide has affinities that are competitive with those of antibodies. An additional benefit to tetramerization of the peptides on this core is an increase in serum stability due to a reduced susceptibility to protease degradation.[582–585] Although tetrameric peptides are most commonly used, dimeric and trimeric peptides can be synthesized by a similar strategy.[223]

Another useful scaffold for displaying peptides is a pentavalent dendritic wedge.[308,586,587] The AB5 synthon allows for the attachment of five peptides via native chemical ligation or oxime formation. As anticipated, a synergistic boost in affinity is observed due to

multivalent binding to the target. Like the trilysine core, the focal point on the dendron can be modified to carry different cargoes, and linkers can be placed between the AB5 core and the targeting peptide as necessary. Several peptides have been placed on the wedge scaffold, including the CREKA and LyP-1 peptides.

It has been assumed that conjugation of multiple copies of a ligand to the surface of a nanoparticle will impart multivalent binding and improve affinity of the ligand for its target,[588] yet these platforms are unlikely to display the peptides in an optimal multimeric conformation. Additionally, increasing the copy number of the ligand on the nanoparticle to improve the effects of multivalent binding can result in increased nonspecific binding. We recently reported the effects of peptide valency, density, and affinity on nanoparticle delivery and therapeutic efficacy, using the $\alpha_v\beta_6$-binding H2009.1 peptide as a model phage-selected peptide and liposomal doxorubicin as a model nanoparticle.[227] Liposomes displaying the higher affinity multivalent H2009.1 tetrameric peptide demonstrated 5–10-fold higher drug delivery than liposomes displaying the lower affinity monomeric H2009.1 peptide, even when the same number of peptide subunits were displayed on the liposome. Liposomal targeting also increased with increasing concentrations of H2009.1 tetrameric peptide on the liposome surface. Thus, both the multivalent peptide and the multivalent liposome scaffold worked together to increase targeting to $\alpha_v\beta_6$-expressing cells.

It should be noted that the boost in affinity for these multimeric peptide platforms is dependent on the receptor density on the surface of cells; the greater the receptor density, the more likely a significant synergistic improvement in affinity will be observed for multimeric peptide platforms.[587] Additionally, the optimal valency may not necessarily be 4–5 peptide branches due to the spacing of the receptors on the cell surface. This stresses the importance of optimizing peptide affinity in the context of whole cells where the native receptor landscape is maintained.

11. PERSPECTIVES AND FUTURE CHALLENGES

11.1. Identification of Cellular Receptors

A major challenge in the field is the identification of the cellular targets of peptides selected from unbiased selections on whole cells and from in vivo selections. This is particularly striking when one realizes how few receptors have been identified for the peptides listed in Tables 2–17. Although these ligands can be used without knowledge of their cellular receptors, receptor identification remains a high priority. First, receptor identification can provide knowledge about the cell surface profile and how it differs between different cell types or states. Second, once identified, new ligands can be generated for the receptor. While peptides might be appropriate for some applications, antibodies, aptamers, peptoids, or small molecules may be a better choice for others. Third, FDA approval of any targeting ligand without knowledge of its cellular target will be difficult.

Approaches for receptor identification have focused on biochemical affinity purification and/or protein cross-linking followed by mass spectrometric identification of the isolated protein species.[561,589,590] The low success rate is partly due to the inherent nature of membrane proteins. They are present in low abundance, and solubility is an issue. This

makes affinity purification and mass spectrometric identification difficult. It is important to remember that the cell surface has a topography in which proteins can multimerize with binding partners or cluster within microdomains. This surface landscape can contribute to the specificity of the peptidic ligands. In other words, peptide cell specificity may not arise from absolute receptor protein expression levels but from an arrangement of the receptors on the cell. This spatial information can be lost upon the preparation of membrane protein for affinity purification and is not borne out in mRNA levels. It is also important to note that while the assumption in the field has been that the peptides bind to protein receptors, they may instead be binding sugar moieties of glycoproteins or glycolipids or to phospholipids.

Protein databases can be searched for sequence similarity to the peptide. This has yielded candidate receptors for a few isolated peptides.[591–593] For instance, homology of the lung-cancer-binding peptide H2009.1 to the GH viral coat protein of the foot and mouth disease virus led to the identification of $\alpha_v\beta_6$ as the cellular receptor for this peptide.[592] However, most phage-displayed peptide libraries are chemically synthesized and do not originate from biological sources. Furthermore, the complete sequence coverage of the longer peptides is limited. As such, the probability of the peptide sequence matching a biologically derived sequence is statistically low. Furthermore, many matches do not provide biological insight into the potential receptors. In summary, new techniques are needed to identify the receptor partners for the selected ligands. A combination of cell biology, proteomic, and genomic approaches will be needed to tackle this difficult problem. Finally, a note of caution: Many receptors have been repeatedly identified for different peptide ligands. For example, vimentin and gp78 have been identified repetitively. While these may indeed be the peptides' binding partners, any initial protein identification needs to be followed by biochemical and molecular biology studies for confirmation.

11.2. Management of the Ever-Growing Peptide Sequence Data

As can be seen from this review, there are numerous peptides selected from different libraries. Furthermore, this list is not comprehensive and does not include peptides selected against antibodies, intracellular targets, inorganic materials, or small organic molecules. Collecting these data is not trivial and becomes more complicated as the field continues to grow, and individual laboratory databases are often incomplete. Recently, several Web-based databases have been created to allow researchers to search for previously isolated peptides. The TumorHoPe database is focused on tumor-homing peptides and is manually curated.[594] The MimoDB database is broader and not limited to tumor-targeting peptides. This database allows the user to search by sequence, target, library type, and structure.[595] Finally, PepBank contains peptide sequences obtained from text mining of MEDLINE abstracts as well as some manually curated sequences.[596] The database has been modified, allowing users to vote in an effort to improve peptide classification.[597] PepBank is particularly useful in identifying nonspecific phage clones that are repetitively selected from a particular library.[207] It is currently the largest of the peptide databases. Both MimoDB and PepBank have features that allow for BLAST searches of the databases. With these resources available, researchers can determine if a peptide has been previously selected or has sequence similarity to another selected peptide. However, these resources are only as powerful as the completeness of the peptide repository. A random sampling of peptides from

Tables 2–17 found many sequences missing from the PepBank database. As such, central data storage of peptide sequences still needs refinement.

11.3. Physiological Challenges in Cell-Targeted Delivery of Biologically Active Cargo

While the selection process focuses on the molecular interactions between a peptide and its cellular receptor, there are numerous physiological parameters that can hinder the efficacy of targeted therapies.[598] Many of the pharmacokinetic and pharmacodynamic parameters can be optimized by modification of the peptide, as is touched upon in the next subsection. However, even upon reaching the targeted region, delivery of biologically active cargo faces difficulties, such as efficient escape from the vasculature, available access of the receptors, penetration of the peptide to reach a majority of the cells, and release of the cargo in an active form. Vascular targets which are exposed to the bloodstream are more accessible, and many of the successes in targeted therapies involve endothelial receptors.[291,599]

Solid tumors present their own set of hurdles that must be dealt with when considering targeted therapy. Primarily, widespread distribution of the drug throughout the tumor such that the majority of tumor cells are eradicated is difficult.[600] Tumors possess a poorly organized vasculature with chaotic branching structures. As a result, tumor cells can be up to 100 μm from a functional blood vessel. Once the drug conjugate escapes from the vasculature, there remain long interstitial transport distances to reach cells in certain areas of the tumor, and this transport within the tumor relies primarily on diffusion. Diffusion rates and distances are limited by the dense extracellular matrix and tumor size. Additionally, tumors have dysfunctional lymphatic systems, causing them to have high interstitial pressure; this results in the convection of fluids from the interior of the tumor to its periphery and to surrounding tissues. Finally, a binding site barrier may limit diffusion into the tumor. High-affinity ligands can bind to the first target cells they encounter, and then, due to a rapid internalization and/or a slow off rate from the receptor, the ligand is no longer available to travel further into the tumor.[601,602] All together, it is difficult to reach the less vascularized regions of the tumor, and much of the drug remains perivascular. Clearly the magnitude of these challenges will be tumor, receptor, and ligand specific.

Although the challenges are substantial, peptide-targeted tumor therapies are still very promising and may reduce several of these delivery problems. Peptides are smaller than currently used antibodies and are expected to diffuse more rapidly into the tumor. The affinity can be modulated by controlling the valency and chemical composition of the peptide, thus minimizing the binding site barrier. Moreover, peptides that improve tumor penetration have recently been reported, [274,275,278,279,309] and this approach has been recently reviewed.[603] Clearly nonsolid tumors such as leukemias and lymphomas do not suffer from these issues, and neither do micrometastases. Additionally, targeting peptides can deliver agents, such as radioisotopes, that have bystander effects; surrounding tumor cells receive ionizing radiation even if they did not directly bind the peptide. Finally, therapeutic approaches to reduce interstitial pressure and/or normalize the tumor vasculature have been show to improve distribution of drugs and nanoparticles throughout a tumor.

A word of caution about targeting larger drug carrier molecules to tumors is required.[604] Tumors also possess a leaky vasculature which allows for large particles to extravasate from

the vasculature. This coupled with a dysfunctional lymphatic system results in retention of nanoparticles 50–400 nm in size in the tumor.[605] This effect, known as the enhanced permeability and retention (EPR) effect, results in an inherent passive targeting and can complicate our understanding of the active targeting of nanoparticle drug carriers. Mathematical modeling of tumor targeting suggests that passive targeting is the driving force for tumor accumulation of nanoparticles.[606,607] While increased efficacy of targeted liposomal drugs is often observed over nontargeted liposomal formulation in many cases, the reason for this is debatable. In some cases, especially in vasculature targeting, it appears that more liposomes accumulate in the tumor when targeted.[608,609] In others, it appears that the targeting ligand does not increase the liposomal delivery to the tumor, but it facilitates cellular uptake of the drug as well as increases distribution of the liposome throughout the tumor.[610,611]

11.4. Misperceptions about Peptides as Therapeutics

Perhaps one of the biggest challenges to overcome is the misperception that peptides are not viable therapeutics. Traditionally, pharmaceutical companies have avoided peptides in preference for small molecules, yet there are more than 60 peptide therapeutics approved for clinical use worldwide, 5 of which have sales over $1 billion.[612–618] Therapeutic peptides range from 2 to 41 amino acids in length and have varied indications. Furthermore, the number of peptides entering clinical trials has risen to almost 200. To counteract the negative image of peptides as drugs, the Peptide Therapeutic Foundation (www.peptidetherapeutics.org) was founded to promote the development of peptide therapeutics.

The predominate drawbacks attributed to peptides are (1) short biological half-lives due to proteolysis, (2) rapid renal filtration, (3) lack of oral availability, and (4) costs. Proteolysis can be overcome by protection of the free termini, cyclization of the peptide, addition of non-natural amino acids, and elimination of specific cleavage sites. A variety of approaches have been taken to overcome rapid glomerular filtration by the kidneys, including PEGylation, glycosylation, protein conjugation, and hydrophobic depoting. Conjugation of peptides to serum albumin is another approach for extending the peptide half-life in vivo.[619] Albumin-binding peptides have been isolated by phage display panning, opening the possibility for a completely peptidic targeting agent with an extended circulation time.[620] Methods to improve peptide circulation times in vivo have been reviewed elsewhere.[614,621,622] Indeed, peptides are generally not orally bioavailable, and most are administered by injection. However, there have been substantial advances in the development of peptide delivery systems, including controlled release polymeric formulations and osmotic pumps, which can reduce injections from daily to monthly.[614,623] Progress is also being made in transdermal, nasal, pulmonary, and oral delivery of peptides as well.[624] Finally, the cost of large-scale peptide synthesis has dampened the enthusiasm for the development of peptidic drugs, especially considering the large doses that are likely to be required,[625] yet improvements in synthetic methodology and the use of native chemical ligation to synthesize longer peptides have reduced this concern. Peptides are now synthesized in kilogram quantities at costs of ~$1 per gram per amino acid. While more

expensive than small-molecule drugs, this cost is substantially lower than that of monoclonal antibody production.

Peptides isolated from phage-displayed peptide libraries are now entering the market. In 2008 the FDA approved Romiplostim (AMG 531, Nplate) for the treatment of thrombocytopenia in patients with immune thrombocytopenia purpura.[626–628] The lead peptide was identified by panning a phage display library on the thrombopoietin receptor.[629] Romiplostim presents four copies of the peptide on an Fc fragment which extends the peptide's in vivo half-life. A similar peptibody scaffold is used in AMG-386 (trebananib), Amgen's antiangiogenic therapeutic. In this case, the peptide prevents binding of angiopoietin-1 and angiopoietin-2 to Tie2.[630] AMG-386 is in phase III clinical trials for treatment of a variety of cancer types. The drug is well tolerated,[631] and positive signs of efficacy were observed in ovarian cancer patients when used in combination with standard chemotherapeutics.[632] Peginesatide (formerly Hematide), is an erythropoietin mimetic peptide selected from a phage-displayed library at Amgen.[633,634] The peptide is displayed as a PEGylated dimer to increase its affinity and biological half-life. FDA approval of Peginesatide for the treatment of anemia associated with chronic kidney disease was granted in 2012.

Several of the more recently isolated peptides discussed in this review are in clinical trials. The CD13- and $\alpha_v\beta_3$-binding peptide NGR has been fused to human tumor necrosis factor (hTNF)[635] or truncated tissue factor (tTF).[636] NGR–hTNF is in phase III clinical trials for mesothelioma and in phase II trials for a number of solid tumors. Modified adenovirus which displays the RGD-4C peptide on its fiber knob and carries the genes encoding for HSV-TK and the somatostatin receptor has undergone a phase I study.[637] Similarly, the fat-targeting peptide CKGGRAKDC linked to the D-enantiomer of the proapoptotic $(KLAKLAK)_2$ peptide entered phase I trial in early 2012 under the name Adipotide.

In summary, these successes indicate that peptides isolated from peptide libraries can translate into useful therapeutics. It is important to note that it took Romiplostim 10 years to go from initial peptide selection to FDA approval. More recently isolated peptides are likely in early stages of preclinical development.

11.5. Conclusions

The number of cell-targeting peptides has expanded greatly in the past 10 years. This expansion has come about primarily from combinatorial peptide libraries and new selection methods. While phage display methods have been the predominant library type used, other peptide library formats have also been used to isolate cell-targeting agents. Cell-targeting peptides can have affinities comparable to those of clinically used antibodies and represent a new tool for directing targeting therapies and imaging agents. The section protocols are robust, allowing for a wide variety of cell types to be targeted. However, it is important to remember that the isolated ligands are rarely the optimal peptide sequence and should be considered lead compounds, a notion that is often overlooked, yet by blending biology and chemistry, peptide leads can be optimized for affinity, specificity, activity, stability, solubility, and biodistribution. These optimized and validated peptides are anticipated to

have broad clinical applications. A number of peptides identified from peptide libraries are currently in clinical trials, and more are anticipated to follow.

ACKNOWLEDGMENTS

Work in K.C.B.'s laboratory is supported by the Welch Foundation (I1622) and the National Institutes of Health (Grants 1R01EB014244-01A1 and 1R01CA164447-01A1). B.P.G. was supported by a fellowship from the Cancer Research and Prevention Institute of Texas (Grant RP 101496).

Biographies

Bethany Powell Gray received her B.S. in Chemistry from Abilene Christian University (ACU) in 2005. While at ACU, she began organic synthesis work under the direction of Dr. Perry Reeves. In 2006 she joined Dr. Kathlynn Brown's laboratory at the University of Texas Southwestern Medical Center and began using phage display selected peptides for drug delivery. She received her Ph.D. in Biological Chemistry in August 2012 and recently began postdoctoral work in Dr. Bruce Sullenger's laboratory at Duke University Medical Center.

Kathlynn C. Brown is Assistant Professor of Internal Medicine and a member of the Simmons Cancer Center at the University of Texas Southwestern Medical Center. Dr. Brown obtained her Ph.D. in organic chemistry at the University of Texas at Austin, during which time she received fellowships from the Mahler Memorial Foundation and the Organic Division of the American Chemical Society. She continued her training at the University of California at San Francisco, where she was a Damon Runyon Walter Winchell Postdoctoral Fellow. She has utilized her multidisciplinary expertise in organic chemistry, peptide chemistry, biochemistry, and molecular biology to address challenges in biomedical research. Her laboratory was among the early adopters of cell-based biopanning of phage-displayed peptide libraries. Using this approach, she has developed a suite of high-affinity peptides that target a variety of different cell types. Her research team is currently focusing on the development of peptides that target non-small-cell lung cancer for use as delivery vehicles for drugs, nanoparticles, and toxins. Additionally, the peptides are being utilized as molecular imaging agents for diagnosis and classification of lung tumors.

REFERENCES

1. Strebhardt K, Ullrich A. Nat. Rev. Cancer. 2008; 8:473. [PubMed: 18469827]

2. Reichert JM. mAbs. 2013; 5:1. [PubMed: 23254906]
3. Reichert JM, Valge-Archer VE. Nat. Rev. Drug Discovery. 2007; 6:349.
4. Tabrizi MA, Tseng C-ML, Roskos LK. Drug DiscoveryToday. 2006; 11:81.
5. Neumeister P, Eibl M, Zinke-Cerwenka W, Scarpatetti M, Sill H, Linkesch W. Ann. Hematol. 2001; 80:119. [PubMed: 11261323]
6. Bray BL. Nat. Rev. Drug Discovery. 2003; 2:587.
7. Nagy A, Schally AV. Curr. Pharm. Des. 2005; 11:1167. [PubMed: 15853664]
8. Zhang X, Xu C. Curr. Pharm. Biotechnol. 2011; 12:1144. [PubMed: 21470143]
9. Schally AV, Engel J, Emons G, Block N, Pinski J. Curr.Drug Delivery. 2011; 8:11.
10. Milletti F. Drug Discovery Today. 2012; 17:850. [PubMed: 22465171]
11. Smith GP. Science. 1985; 228:1315. [PubMed: 4001944]
12. Kehoe JW, Kay BK. Chem. Rev. 2005; 105:4056. [PubMed: 16277371]
13. Hill HR, Stockley PG. Mol. Microbiol. 1996; 20:685. [PubMed: 8793867]
14. Smith GP, Petrenko VA. Chem. Rev. 1997; 97:391. [PubMed: 11848876]
15. Cabilly S. Mol. Biotechnol. 1999; 12:143. [PubMed: 10596371]
16. Fukunaga K, Taki M. J. Nucleic Acids. 2012; 2012:9.
17. Barbas CF, Kang AS, Lerner RA, Benkovic SJ. Proc.Natl. Acad. Sci. U.S.A. 1991; 88:7978. [PubMed: 1896445]
18. Aina OH, Liu R, Sutcliffe JL, Marik J, Pan CX, Lam KS. Mol. Pharmaceutics. 2007; 4:631.
19. Mclaughlin M, Sidhu SS. Methods Enzymol. 2013:327. [PubMed: 23422437]
20. Held HA, Sidhu SS. J. Mol. Biol. 2004; 340:587. [PubMed: 15210356]
21. Scott JK, Smith GP. Science. 1990; 249:386. [PubMed: 1696028]
22. Kuzmicheva GA, Jayanna PK, Sorokulova IB, Petrenko VA. Protein Eng. Des. Sel. 2009; 22:9. [PubMed: 18988692]
23. Zhong G, Smith GP, Berry J, Brunham RC. J. Biol. Chem. 1994; 269:24183. [PubMed: 7523368]
24. Cwirla SE, Peters EA, Barrett RW, Dower WJ. Proc.Natl. Acad. Sci. U.S.A. 1990; 87:6378. [PubMed: 2201029]
25. Mazzucchelli L, Burritt JB, Jesaitis AJ, Nusrat A, Liang TW, Gewirtz AT, Schnell FJ, Parkos CA. Blood. 1999; 93:1738. [PubMed: 10029604]
26. Saldanha R, Molloy M, Bdeir K, Cines D, Song X, Uitto P, Weinreb P, Violette S, Baker MS. J. Proteome Res. 2007; 6:1016. [PubMed: 17330942]
27. Steven, AC.; Trus, BL. Electron Microscopy of Proteins. Vol. 5. New York: Academic Press; 1986. p. 2
28. Condron BG, Atkins JF, Gesteland RF. J. Bacteriol. 1991; 173:6998. [PubMed: 1938901]
29. Xiao N, Cheng D, Wang Y, Chen L, Liu X, Dou S, Liu G, Liang M, Hnatowich DJ, Rusckowski M. Cancer Biol. Ther. 2011; 11:22. [PubMed: 20980835]
30. Lindner T, Kolmar H, Haberkorn U, Mier W. Molecules. 2011; 16:1625. [PubMed: 21326140]
31. Krumpe LRH, Schumacher KM, Mcmahon JB, Makowski L, Mori T. BMC Biotechnol. 2007; 7:65. [PubMed: 17919322]
32. Derda R, Tang S, Li SC, Ng S, Matochko W, Jafari M. Molecules. 2011; 16:1776. [PubMed: 21339712]
33. Krumpe LRH, Atkinson AJ, Smythers GW, Kandel A, Schumacher KM, Mcmahon JB, Makowski L, Mori T. Proteomics. 2006; 6:4210. [PubMed: 16819727]
34. Liu CC, Mack AV, Tsao ML, Mills JH, Lee HS, Choe H, Farzan M, Schultz PG, Smider VV. Proc. Natl. Acad.Sci. U.S.A. 2008; 105:17688. [PubMed: 19004806]
35. Daugherty PS. Curr. Opin. Struct. Biol. 2007; 17:474. [PubMed: 17728126]
36. Georgiou G, Stathopoulos C, Daugherty PS, Nayak AR, Iverson BL, Curtiss R. Nat. Biotechnol. 1997; 15:29. [PubMed: 9035102]
37. Chen W, Georgiou G. Biotechnol. Bioeng. 2002; 79:496. [PubMed: 12209821]
38. Jostock T, Dubel S. Comb. Chem. High Throughput Screening. 2005; 8:127.
39. Lee SY, Choi JH, Xu Z. Trends Biotechnol. 2003; 21:45. [PubMed: 12480350]

40. Samuelson P, Gunneriusson E, Nygren PA, Stahl S. J.Biotechnol. 2002; 96:129. [PubMed: 12039531]
41. Lu Z, Murray KS, Van Cleave V, Lavallie ER, Stahl ML, Mccoy JM. Biotechnology. 1995; 13:366. [PubMed: 9634778]
42. Bessette PH, Rice JJ, Daugherty PS. Protein Eng. Des. Sel. 2004; 17:731. [PubMed: 15531628]
43. Rice JJ, Schohn A, Bessette PH, Boulware KT, Daugherty PS. Protein Sci. 2006; 15:825. [PubMed: 16600968]
44. Nakajima H, Shimbara N, Shimonishi Y, Mimori T, Niwa S, Saya H. Gene. 2000; 260:121. [PubMed: 11137298]
45. Lam KS, Lebl M, Krchnak V. Chem. Rev. 1997; 97:411. [PubMed: 11848877]
46. Lam KS, Salmon SE, Hersh EM, Hruby VJ, Kazmierski WM, Knapp RJ. Nature. 1991; 354:82. [PubMed: 1944576]
47. Lam KS, Liu R, Miyamoto S, Lehman AL, Tuscano JM. Acc. Chem. Res. 2003; 36:370. [PubMed: 12809522]
48. Liu R, Marik J, Lam KS. J. Am. Chem. Soc. 2002; 124:7678. [PubMed: 12083920]
49. Wang X, Peng L, Liu R, Gill SS, Lam KS. J. Comb. Chem. 2005; 7:197. [PubMed: 15762747]
50. Aggarwal S, Janssen S, Wadkins RM, Harden JL, Denmeade SR. Biomaterials. 2005; 26:6077. [PubMed: 15907998]
51. Aggarwal S, Harden JL, Denmeade SR. Bioconjugate Chem. 2006; 17:335.
52. Chen X, Tan PH, Zhang Y, Pei D. J. Comb. Chem. 2009; 11:604. [PubMed: 19397369]
53. Wang X, Peng L, Liu R, Xu B, Lam KS. J. Pept. Res. 2005; 65:130. [PubMed: 15686543]
54. Udugamasooriya DG, Dineen SP, Brekken RA, Kodadek T. J. Am. Chem. Soc. 2008; 130:5744. [PubMed: 18386897]
55. André S, Maljaars CEP, Halkes KM, Gabius HJ, Kamerling JP. Bioorg. Med. Chem. 2007; 17:793.
56. Hintersteiner M, Knox A, Mudd G, Auer M. J. Chem. Biol. 2012; 5:63. [PubMed: 23284589]
57. Kumaresan PR, Wang Y, Saunders M, Maeda Y, Liu R, Wang X, Lam KS. ACS Comb. Sci. 2011; 13:259. [PubMed: 21302937]
58. Cha J, Lim J, Zheng Y, Tan S, Ang YL, Oon J, Ang MW, Ling J, Bode M, Lee SS. J. Lab. Autom. 2012; 17:186. [PubMed: 22357565]
59. Dooley CT, Houghten RA. Methods Mol. Biol. 1998; 87:13. [PubMed: 9523255]
60. Dooley CT, Houghten RA. Life Sci. 1993; 52:1509. [PubMed: 8387136]
61. Pinilla C, Appel JR, Blanc P, Houghten RA. Biotechniques. 1992; 13:901. [PubMed: 1476743]
62. Rothman RB, Baumann MH, Dersch CM, Appel J, Houghten RA. Synapse. 1999; 33:239. [PubMed: 10420171]
63. Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Kherman P, Kaye FJ, Lindeman N, Boggon TJ, Naoko K, Sasaki H, Jujji Y, Eck MJ, Sellers WR, Johnson BE, Meyerson M. Science. 2004; 304:1497. [PubMed: 15118125]
64. Kim EY, Bang JY, Chang SI, Kang IC. Biochem. Biophys.Res. Commun. 2008; 377:1288. [PubMed: 18996355]
65. Denholt CL, Hansen PR, Pedersen N, Poulsen HS, Gillings N, Kjaer A. Biopolymers. 2009; 91:201. [PubMed: 19107925]
66. Liu R, Enstrom AM, Lam KS. Exp. Hematol. 2003; 31:11. [PubMed: 12543103]
67. Pasqualini R, Ruoslahti E. Nature. 1996; 380:364. [PubMed: 8598934]
68. Barry MA, Dower WJ, Johnston SA. Nat. Med. 1996; 2:299. [PubMed: 8612228]
69. Goodson RJ, Doyle MV, Kaufman SE, Rosenberg S. Proc. Natl. Acad. Sci. U.S.A. 1994; 91:7129. [PubMed: 8041758]
70. Zhao P, Grabinski T, Gao C, Skinner RS, Giambernardi T, Su Y, Hudson E, Resau J, Gross M, Vande Woude GF, Hay R, Cao B. Clin. Cancer Res. 2007; 13:6049. [PubMed: 17947467]
71. Giordano RJ, Cardo-Vila M, Lahdenranta J, Pasqualini R, Arap W. Nat. Med. 2001; 7:1249. [PubMed: 11689892]
72. Brown KC. Curr. Pharm. Des. 2010; 16:1040. [PubMed: 20030617]

73. Elayadi AN, Samli KN, Prudkin L, Liu YH, Bian A, Xie XJ, Wistuba Ii, Roth JA, Mcguire MJ, Brown KC. CancerRes. 2007; 67:5889.
74. Bausch D, Thomas S, Mino-Kenudson M, Fernandez-Del CC, Bauer TW, Williams M, Warshaw AL, Thayer SP, Kelly KA. Clin. Cancer Res. 2011; 17:302. [PubMed: 21098698]
75. Samli KN, Mcguire MJ, Newgard CB, Johnston SA, Brown KC. Diabetes. 2005; 54:2103. [PubMed: 15983211]
76. Wang J, Liu Y, Teesalu T, Sugahara KN, Kotamrajua VR, Adams JD, Ferguson BS, Gong Q, Oh SS, Csordas AT, Cho M, Ruoslahti E, Xiao Y, Soh HT. Proc. Natl. Acad. Sci. U.S.A. 2011; 108:6909. [PubMed: 21486998]
77. Arap W, Kolonin MG, Trepel M, Lahdenranta J, Cardo-Vila M, Giordano RJ, Mintz PJ, Ardelt PU, Yao VJ, Vidal CI, Chen L, Flamm A, Valtanen H, Weavind LM, Hicks ME, Pollock RE, Botz GH, Bucana CD, Koivunen E, Cahill D, Troncoso P, Baggerly KA, Pentz RD, Do KA, Logothetis CJ, Pasqualini R. Nat. Med. 2002; 8:121. [PubMed: 11821895]
78. ’t Hoen PAC, Jirka SMG, Ten Broeke BR, Schultes EA, Aguilera B, Pang KH, Heemskerk H, Aartsma-Rus A, VanOmmen GJ, Den Dunnen JT. Anal. Biochem. 2012; 421:622. [PubMed: 22178910]
79. Dias-Neto E, Nunes DN, Giordano RJ, Sun J, Botz GH, Yang K, Setubal JC, Pasqualini R, Arap W. PLoS One. 2009; 4:e8338. [PubMed: 20020040]
80. Ernst A, Gfeller D, Kan Z, Seshagiri S, Kim PM, Bader GD, Sidhu SS. Mol. Biosyst. 2010; 6:1782. [PubMed: 20714644]
81. Ernst A, Sazinsky SL, Hui S, Currell B, Dharsee M, Seshagiri S, Bader GD, Sidhu SS. Sci. Signaling. 2009; 2:ra50.
82. Matochko WL, Chu K, Jin B, Lee SW, Whitesides GM, Derda R. Methods. 2012; 58:47. [PubMed: 22819855]
83. Ryvkin A, Ashkenazy H, Smelyanski L, Kaplan G, Penn O, Weiss-Ottolenghi Y, Privman E, Ngam PB, Woodward JE, May GD, Bell C, Pupko T, Gershoni JM, Ensoli B. PLoS One. 2012; 7:1.
84. Dane KY, Chan LA, Rice JJ, Daugherty PS. J. Immunol.Methods. 2006; 309:120. [PubMed: 16448666]
85. Pennington ME, Lam KS, Cress AE. Mol. Diversity. 1996; 2:19.
86. Aina OH, Sroka TC, Chen ML, Lam KS. Biopolymers. 2002; 66:184. [PubMed: 12385037]
87. Lee Y, Kang DK, Chang SI, Han MH, Kang IC. J.Biomol. Screening. 2004; 9:687.
88. Siegel R, Desantis C, Virgo K, Stein K, Mariotto A, Smith T, Cooper D, Gansler T, Lerro C, Fedewa S, Lin C, Leach C, Cannady RS, Cho H, Scoppa S, Hachey M, Kirch R, Jemal A, Ward E. Ca-Cancer J. Clin. 2012; 62:220. [PubMed: 22700443]
89. Chaurio RA, Janko C, Muñoz LE, Frey B, Herrmann M, Gaipl US. Molecules. 2009; 14:4892. [PubMed: 20032867]
90. Thapa N, Kim S, So IS, Lee BH, Kwon IC, Choi K, Kim IS. J. Cell. Mol. Med. 2008; 12:1649. [PubMed: 18363834]
91. Burtea C, Laurent S, Lancelot E, Ballet S, Murariu O, Rousseaux O, Port M, Vander Elst L, Corot C, Muller RN. Mol.Pharmaceutics. 2009; 6:1903.
92. Shao R, Xiong C, Wen X, Gelovani J, Li C. Mol. Imaging. 2007; 6:417. [PubMed: 18053412]
93. Laumonier C, Segers J, Laurent S, Michel A, Coppeé F, Belayew A, Elst LV, Muller RN. J. Biomol. Screening. 2006; 11:537.
94. Pero SC, Shukla GS, Armstrong AL, Peterson D, Fuller SP, Godin K, Kingsley-Richards SL, Weaver DL, Bond J, Krag DN. Int. J. Cancer. 2004; 111:951. [PubMed: 15300809]
95. Shukla GS, Krag DN. Protein Eng. Des. Sel. 2010; 23:431. [PubMed: 20219829]
96. Karasseva NG, Glinsky VV, Chen NX, Komatireddy R, Quinn TP. J. Protein Chem. 2002; 21:287. [PubMed: 12168699]
97. Kumar SR, Quinn TP, Deutscher SL. Clin. Cancer Res. 2007; 13:6070. [PubMed: 17947470]
98. Deutscher SL, Figueroa SD, Kumar SR. J. Labelled Compd.Radiopharm. 2009; 52:583.
99. Kumar SR, Gallazzi FA, Ferdani R, Anderson CJ, Quinn TP, Deutscher SL. Cancer Biother. Radiopharm. 2010; 25:693. [PubMed: 21204764]

100. Houimel M, Schneider P, Terskikh A, Mach JP. Int. J.Cancer. 2001; 92:748. [PubMed: 11340582]
101. Li Z, Zhao R, Wu X, Sun Y, Yao M, Li J, Xu Y, Gu J. FASEB J. 2005; 19:1978. [PubMed: 16319141]
102. Song S, Liu D, Peng J, Sun Y, Li Z, Gu JR, Xu Y. Int. J.Pharm. 2008; 363:155. [PubMed: 18692120]
103. Cheng Y, Meyers JD, Agnes RS, Doane TL, Kenney ME, Broome AM, Burda C, Basilion JP. Small. 2011:2301. [PubMed: 21630446]
104. Kohno M, Horibe T, Haramoto M, Yano Y, Ohara K, Nakajima O, Matsuzaki K, Kawakami K. Eur. J. Cancer. 2011; 47:773. [PubMed: 21112771]
105. Abourbeh G, Shir A, Mishani E, Ogris M, Rödl W, Wagner E, Levitzki A. IUBMB Life. 2012; 64:324. [PubMed: 22362419]
106. Dejesus O. Curr. Radiopharm. 2012; 5:15. [PubMed: 21864245]
107. Schafer A, Pahnke A, Schaffert D, Van Weerden W, DeRidder C, Rodl W, Vetter A, Spitzweg C, R K, Wagner E, Ogris M. Hum. Gene Ther. 2011; 22:1463. [PubMed: 21644815]
108. Ohno SI, Takanashi M, Sudo K, Ueda S, Ishikawa A, Matsuyama N, Fujita K, Mizutani T, Ohgi T, Ochiya T, Gotoh N, Kuroda M. Mol. Ther. 2012; 21:185. [PubMed: 23032975]
109. Mickler FM, Möckl L, Ruthardt N, Ogris M, Wagner E, Bräuchle C. Nano Lett. 2012; 12:3417. [PubMed: 22632479]
110. Su JL, Lai KP, Chen CA, Yang CY, Chen PS, Chang CC, Chou CH, Hu CL, Kuo ML, Hsieh CY, Wei LH. Cancer Res. 2005; 65:4827. [PubMed: 15930303]
111. Koivunen E, Wang B, Ruoslahti E. Biotechnology. 1995; 13:265. [PubMed: 9634769]
112. Arap W, Pasqualini R, Ruoslahti E. Science. 1998; 279:377. [PubMed: 9430587]
113. Koivunen E, Gay DA, Ruoslahti E. J. Biol. Chem. 1993; 268:20205. [PubMed: 7690752]
114. Murayama O, Nishida H, Sekiguchi K. J. Biochem. 1996; 120:445. [PubMed: 8889832]
115. Kraft S, Diedenbach B, Mehta R, Jonczyk A, Luckenbach GA, Goodman SL. J. Biol. Chem. 1999; 274:1979. [PubMed: 9890954]
116. Pameijer CRJ, Navanjo A, Meechoovet B, Wagner JR, Aguilar B, Wright CL, Chang WC, Brown CE, Jensen MC. Cancer Gene Ther. 2007; 14:91. [PubMed: 17024231]
117. Koolpe M, Dail M, Pasquale EB. J. Biol. Chem. 2002; 277:46974. [PubMed: 12351647]
118. Wang S, Noberini R, Stebbins JL, Das S, Zhang Z, Wu B, Mitra S, Billet S, Fernandez A, Bhowmick NA, Kitada S, Pasquale EB, Fisher PB, Pellecchia M. Clin. Cancer Res. 2013; 19:128. [PubMed: 23155185]
119. Scarberry KE, Dickerson EB, Mcdonald JF, Zhang ZJ. J. Am. Chem. Soc. 2008; 130:10258. [PubMed: 18611005]
120. Scarberry KE, Mezencev R, Mcdonald JF. Nanomedicine. 2011; 6:69. [PubMed: 21182419]
121. Blackburn WH, Dickerson EB, Smith MH, Mcdonald JF, Lyon LA. Bioconjugate Chem. 2009; 20:960.
122. Van Geer MA, Bakker CT, Koizumi N, Mizuguchi H, Wesseling JG, Oude Elferink RP, Bosma PJ. World J.Gastroenterol. 2009; 15:2754. [PubMed: 19522026]
123. Koolpe M, Burgess R, Dail M, Pasquale EB. J. Biol. Chem. 2005; 280:17301. [PubMed: 15722342]
124. You J, Zhang R, Xiong C, Zhong M, Melancon M, Gupta S, Nick AM, Sood AK, Li C. Cancer Res. 2012; 72:4777. [PubMed: 22865457]
125. Xiong C, Huang M, Zhang R, Song S, Lu W, Flores L, Gelovani J, Li C. J. Nucl. Med. 2011; 52:241. [PubMed: 21233177]
126. Koivunen E, Arap W, Valtanen H, Rainisalo A, Medina OP, Heikkila P, Kantor C, Gahmberg CG, Salo T, Konttinen YT, Sorsa T, Ruoslahti E, Pasqualini R. Nat. Biotechnol. 1999; 17:768. [PubMed: 10429241]
127. Medina OP, Soderlund T, Laakkonen LJ, Tuominen EK, Koivunen E, Kinnunen PK. Cancer Res. 2001; 61:3978. [PubMed: 11358815]

128. Penate Medina O, Haikola M, Tahtinen M, Simpura I, Kaukinen S, Valtanen H, Zhu Y, Kuosmanen S, Cao W, Reunanen J, Nurminen T, Saris PE, Smith-Jones P, Bradbury M, Larson S, Kairemo K. J. Drug Delivery. 2011; 2011:160515.
129. Cai J, Wei R, Cheng J. J. Biomed. Biotechnol. 2008; 2008:564969. [PubMed: 18769489]
130. Zou Y, Chen Y, Jiang Y, Gao J, Gu J. Cancer Res. 2007; 67:7295. [PubMed: 17671198]
131. Medina OP, Kairemo K, Valtanen H, Kangasniemi A, Kaukinen S, Ahonen I, Permi P, Annila A, Sneck M, Holopainen JM, Karonen SL, Kinnunen PK, Koivunen E. Anticancer Res. 2005; 25:33. [PubMed: 15816516]
132. Sprague JE, Li WP, Liang K, Achilefu S, Anderson CJ. Nucl. Med. Biol. 2006; 33:227. [PubMed: 16546677]
133. Heikkila P, Suojanen J, Pirila E, Vaananen A, Koivunen E, Sorsa T, Salo T. Int. J. Cancer. 2006; 118:2202. [PubMed: 16331606]
134. Rusckowski M, Gupta S, Liu G, Dou S, Hnatowich DJ. Cancer Biother. Radiopharm. 2007; 22:564. [PubMed: 17803452]
135. Chen L, Wang Y, Cheng D, Dou S, Liu X, Liu G, Hnatowich DJ, Rusckowski M. Nucl. Med. Commun. 2011; 32:920. [PubMed: 21876403]
136. Chen L, Wang Y, Liu X, Dou S, Liu G, Hnatowich DJ, Rusckowski M. Cancer Lett. 2008; 272:122. [PubMed: 18723274]
137. Devemy E, Blaschuk OW. Peptides. 2009; 30:1539. [PubMed: 19465078]
138. Devemy E, Blaschuk OW. Peptides. 2008; 29:1853. [PubMed: 18655820]
139. Askoxylakis V, Garcia-Boy R, Rana S, Kramer S, Hebling U, Mier W, Altmann A, Markert A, Debus J, Haberkorn U. PLoSOne. 2010; 5:e15962.
140. Zou J, Glinsky VV, Landon LA, Matthews L, Deutscher SL. Carcinogenesis. 2005; 26:309. [PubMed: 15528216]
141. Kumar SR, Deutscher SL. J. Nucl. Med. 2008; 49:796. [PubMed: 18413389]
142. Deutscher SL, Figueroa SD, Kumar SR. Nucl. Med. Biol. 2009; 36:137. [PubMed: 19217525]
143. Aggarwal S, Singh P, Topaloglu O, Isaacs JT, Denmeade SR. Cancer Res. 2006; 66:9171. [PubMed: 16982760]
144. Sun J, Zhang C, Li X. J. Peking Univ. Health Sci. 2008; 40:457.
145. Qin X, Wan Y, Li M, Xue X, Wu S, Zhang C, You Y, Wang W, Jiang C, Liu Y, Zhu W, Ran Y, Zhang Z, Han W, Zhang Y. J. Biochem. 2007; 142:79. [PubMed: 17517791]
146. Peletskaya EN, Glinsky VV, Glinsky GV, Deutscher SL, Quinn TP. J. Mol. Biol. 1997; 270:374. [PubMed: 9237904]
147. Landon LA, Peletskaya EN, Glinsky VV, Karasseva N, Quinn TP, Deutscher SL. J. Protein Chem. 2003; 22:193. [PubMed: 12760424]
148. Landon LA, Zou J, Deutscher SL. Mol. Diversity. 2004; 8:35.
149. Kumar SR, Gallazzi FA, Quinn TP, Deutscher SL. J.Nucl. Med. 2011; 52:1819. [PubMed: 21984800]
150. Glinsky VV, Huflejt ME, Glinsky GV, Deutscher SL, Quinn TP. Cancer Res. 2000; 60:2584. [PubMed: 10825125]
151. Kelly KA, Setlur SR, Ross R, Anbazhagan R, Waterman P, Rubin MA, Weissleder R. Cancer Res. 2008; 68:2286. [PubMed: 18381435]
152. Du B, Qian M, Zhou Z, Wang P, Wang L, Zhang X, Wu M, Zhang P, Mei B. Biochem. Biophys. Res. Commun. 2006; 342:956. [PubMed: 16598852]
153. Zhan T, Li P, Bi S, Dong B, Song H, Hui R, Wang L. J.Nanosci. Nanotechnol. 2012; 12:7198. [PubMed: 23035452]
154. Jiang YQ, Wang HR, Li HP, Hao HJ, Zheng YL, Gu J. Mol. Med. 2006; 12:81. [PubMed: 16953561]
155. Lo A, Lin CT, Wu HC. Mol. Cancer Ther. 2008; 7:579. [PubMed: 18347144]
156. Ashley CE, Carnes EC, Phillips GK, Durfee PN, Buley MD, Lino CA, Padilla DP, Phillips B, Carter MB, Willman CL, Brinker CJ, Caldeira Jdo C, Chackerian B, Wharton W, Peabody DS. ACS Nano. 2011; 5:5729. [PubMed: 21615170]

157. Kim Y, Lillo AM, Steiniger SC, Liu Y, Ballatore C, Anichini A, Mortarini R, Kaufmann GF, Zhou B, Felding-Habermann B, Janda KD. Biochemistry. 2006; 45:9434. [PubMed: 16878978]
158. Yoneda Y, Steiniger SC, Capkova K, Mee JM, Liu Y, Kaufmann GF, Janda KD. Bioorg. Med. Chem. Lett. 2008; 18:1632. [PubMed: 18243696]
159. Eriksson F, Culp WD, Massey R, Egevad L, Garland D, Persson MA, Pisa P. Cancer Immunol. Immunother. 2007; 56:677. [PubMed: 16967280]
160. Matsuo AL, Tanaka AS, Juliano MA, Rodrigues EG, Travassos LR. J. Mol. Med. 2010; 88:1255. [PubMed: 20802991]
161. Staquicini FI, Tandle A, Libutti SK, Sun J, Zigler M, Bar-Eli M, Aliperti F, Perez EC, Gershenwald JE, Mariano M, Pasqualini R, Arap W, Lopes JD. Cancer Res. 2008; 68:8419. [PubMed: 18922915]
162. Huang C, Liu XY, Rehemtulla A, Lawrence TS. Int. J.Radiat. Oncol. Biol. Phys. 2005; 62:1497. [PubMed: 16029812]
163. Romanov VI, Durand DB, Petrenko VA. Prostate. 2001; 47:239. [PubMed: 11398171]
164. Zitzmann S, Mier W, Schad A, Kinscherf R, Askoxylakis V, Kramer S, Altmann A, Eisenhut M, Haberkorn U. Clin. CancerRes. 2005; 11:139.
165. Askoxylakis V, Zitzmann-Kolbe S, Zoller F, Altmann A, Markert A, Rana S, Marr A, Mier W, Debus J, Haberkorn U. Molecules. 2011; 16:1559. [PubMed: 21321528]
166. Min K, Jo H, Song K, Cho M, Chun YS, Jon S, Kim WJ, Ban C. Biomaterials. 2011; 32:2124. [PubMed: 21147500]
167. Jayanna PK, Bedi D, Deinnocentes P, Bird RC, Petrenko VA. Protein Eng. Des. Sel. 2010; 23:423. [PubMed: 20185523]
168. Jayanna PK, Bedi D, Gillespie JW, Deinnocentes P, Wang T, Torchilin VP, Bird RC, Petrenko VA. Nanomedicine. 2010; 6:538. [PubMed: 20138246]
169. Hu S, Guo X, Xie H, Du Y, Pan Y, Shi Y, Wang J, Hong L, Han S, Zhang D, Huang D, Zhang K, Bai F, Jiang H, Zhai H, Nie Y, Wu K, Fan D. Biochem. Biophys. Res. Commun. 2006; 341:964. [PubMed: 16458253]
170. Bai F, Liang J, Wang J, Shi Y, Zhang K, Liang S, Hong L, Zhai H, Lu Y, Han Y, Yin F, Wu K, Fan D. J. Mol. Med. 2007; 85:169. [PubMed: 17043801]
171. Liang S, Lin T, Ding J, Pan Y, Dang D, Guo C, Zhi M, Zhao P, Sun L, Hong L, Shi Y, Yao L, Liu J, Wu K, Fan D. J.Mol. Med. 2006; 84:764. [PubMed: 16763842]
172. Kelly KA, Jones DA. Neoplasia. 2003; 5:437. [PubMed: 14670181]
173. Kelly K, Alencar H, Funovics M, Mahmood U, Weissleder R. Cancer Res. 2004; 64:6247. [PubMed: 15342411]
174. Rasmussen UB, Schreiber V, Schultz H, Mischler F, Schughart K. Cancer Gene Ther. 2002; 9:606. [PubMed: 12082461]
175. Rittner K, Schreiber V, Erbs P, Lusky M. Cancer Gene Ther. 2007; 14:509. [PubMed: 17318198]
176. Zhang Y, Chen J, Hu Z, Hu D, Pan Y, Ou S, Liu G, Yin X, Zhao J, Ren L, Wang J. J. Biomol. Screening. 2007; 12:429.
177. Odermatt A, Audige A, Frick C, Vogt B, Frey BM, Frey FJ, Mazzucchelli L. J. Am. Soc. Nephrol. 2001; 12:308. [PubMed: 11158220]
178. Hong FD, Clayman GL. Cancer Res. 2000; 60:6551. [PubMed: 11118031]
179. Bao L, Gorin MA, Zhang M, Ventura AC, Pomerantz WC, Merajver SD, Teknos TN, Mapp AK, Pan Q. CancerRes. 2009; 69:5829.
180. Nothelfer EM, Zitzmann-Kolbe S, Garcia-Boy R, Kramer S, Herold-Mende C, Altmann A, Eisenhut M, Mier W, Haberkorn U. J. Nucl. Med. 2009; 50:426. [PubMed: 19223422]
181. Lee TY, Wu HC, Tseng YL, Lin CT. Cancer Res. 2004; 64:8002. [PubMed: 15520208]
182. Ivanenkov VV, Felici F, Menon AG. Biochim. Biophys.Acta,Gene Struct. Expression. 1999; 1448:463.
183. Shadidi M, Sioud M. FASEB J. 2003; 17:256. [PubMed: 12490548]

184. Wang XF, Birringer M, Dong LF, Veprek P, Low P, Swettenham E, Stantic M, Yuan LH, Zobalova R, Wu K, Ledvina M, Ralph SJ, Neuzil J. Cancer Res. 2007; 67:3337. [PubMed: 17409443]

185. Orbán E, Manea M, Marquadt A, Bánóczi Z, CsíK G, Fellinger E, Bősze S, Hudecz F. Bioconjugate Chem. 2011; 22:2154.

186. Luo H, Yang J, Jin H, Huang C, Fu J, Yang F, Gong H, Zeng S, Luo Q, Zhang Z. FASEB J. 2011; 25:1865. [PubMed: 21350116]

187. Jie L, Cai L, Wang L, Ying X, Yu R, Zhang M, Du Y. Int. J. Nanomed. 2012; 7:3981.

188. Haglund, E.; Seale-Goldsmith, M.; Dhawan, D.; Stewart, J.; Ramos-Vara, J.; Cooper, C.; Reece, L.; Husk, T.; Bergstrom, D.; Knapp, D.; Leary, J. Colloidal Quantum Dots for BiomedicalApplications III, Proceedings of SPIE. Osinski, M.; Jovin, T.; Yamamoto, K., editors. Vol. Vol. 6866. Bellingham, WA: SPIE; 2008.

189. Wang T, D'souza GG, Bedi D, Fagbohun OA, Potturi LP, Papahadjopoulos-Sternberg B, Petrenko VA, Torchilin VP. Nanomedicine. 2010; 5:563. [PubMed: 20528452]

190. Fagbohun OA, Bedi D, Grabchenko NI, Deinnocentes PA, Bird RC, Petrenko VA. Protein Eng. Des. Sel. 2012; 25:271. [PubMed: 22490956]

191. Wang T, Petrenko VA, Torchilin VP. Mol. Pharmaceutics. 2010; 7:1007.

192. Bedi D, Musacchio T, Fagbohun OA, Gillespie JW, Deinnocentes P, Bird RC, Bookbinder L, Torchilin VP, Petrenko VA. Nanomedicine. 2011; 7:315. [PubMed: 21050894]

193. Zhang J, Spring H, Schwab M. Cancer Lett. 2001; 171:153. [PubMed: 11520599]

194. Shahin M, Ahmed S, Kaur K, Lavasanifar A. Biomaterials. 2011; 32:5123. [PubMed: 21501865]

195. Askoxylakis V, Zitzmann S, Mier W, Graham K, Kramer S, Von Wegner F, Fink RH, Schwab M, Eisenhut M, Haberkorn U. Clin. Cancer Res. 2005; 11:6705. [PubMed: 16166451]

196. Askoxylakis V, Mier W, Zitzmann S, Ehemann V, Zhang J, Kramer S, Beck C, Schwab M, Eisenhut M, Haberkorn U. J.Nucl. Med. 2006; 47:981. [PubMed: 16741308]

197. Samoylova TI, Petrenko VA, Morrison NE, Globa LP, Baker HJ, Cox NR. Mol. Cancer Ther. 2003; 2:1129. [PubMed: 14617786]

198. Spear MA, Breakefield XO, Beltzer J, Schuback D, Weissleder R, Pardo FS, Ladner R. Cancer Gene Ther. 2001; 8:506. [PubMed: 11498772]

199. Wu C, Lo SL, Boulaire J, Hong ML, Beh HM, Leung DS, Wang S. J. Controlled Release. 2008; 130:140.

200. Ho IA, Hui KM, Lam PY. Peptides. 2010; 31:644. [PubMed: 20026365]

201. Ho IA, Lam PY, Hui KM. Hum. Gene Ther. 2004; 15:719. [PubMed: 15319030]

202. Beck S, Jin X, Yin J, Kim SH, Lee NK, Oh SY, Jin X, Kim MK, Kim EB, Son JS, Kim SC, Nam DH, Kim S-H, Kang SK, Kim H, Choi YJ. Biomaterials. 2011; 32:8518. [PubMed: 21880363]

203. Loi M, Di Paolo D, Soster M, Brignole C, Bartolini A, Emionite L, Sun J, Becherini P, Curnis F, Petretto A, Sani M, Gori A, Milanese M, Gambini C, Longhi R, Cilli M, Allen TM, Bussolino F, Arap W, Pasqualini R, Corti A, Ponzoni M, Marchiò S, Pastorino F. J. Controlled Release. 2013; 170:233.

204. Robinson P, Stuber D, Deryckere F, Tedbury P, Lagrange M, Orfanoudakis G. J. Mol. Recognit. 2005; 18:175. [PubMed: 15384175]

205. Morpurgo M, Kirschner M, Radu A. J. Biochem. Biophys.Methods. 2002; 52:31. [PubMed: 12121752]

206. Zhang L, Yin G, Yan D, Wei Y, Ma C, Huang Z, Liao X, Yao Y, Chen X, Hao B. Biotechnol. Lett. 2011; 33:1729. [PubMed: 21544611]

207. Vodnik M, Zager U, Strukelj B, Lunder M. Molecules. 2011; 16:790. [PubMed: 21248664]

208. Bockmann M, Drosten M, Putzer BM. J. Gene Med. 2005; 7:179. [PubMed: 15508130]

209. Zitzmann S, Kramer S, Mier W, Hebling U, Altmann A, Rother A, Berndorff D, Eisenhut M, Haberkorn U. J. Nucl. Med. 2007; 48:965. [PubMed: 17504878]

210. Witt H, Hajdin K, Iljin K, Greiner O, Niggli FK, Schafer BW, Bernasconi M. Int. J. Cancer. 2009; 124:2026. [PubMed: 19123480]

211. Sun X, Niu G, Yan Y, Yang M, Chen K, Ma Y, Chan N, Shen B, Chen X. Clin. Cancer Res. 2010; 16:4268. [PubMed: 20570932]
212. Mcguire MJ, Samli KN, Chang YC, Brown KC. Exp.Hematol. 2006; 34:443. [PubMed: 16569591]
213. Mcguire MJ, Samli KN, Johnston SA, Brown KC. J.Mol. Biol. 2004; 342:171. [PubMed: 15313615]
214. Nishimura S, Takahashi S, Kamikatahira H, Kuroki Y, Jaalouk DE, O'brien S, Koivunen E, Arap W, Pasqualini R, Nakayama H, Kuniyasu A. J. Biol. Chem. 2008; 283:11752. [PubMed: 18292083]
215. Karjalainen K, Jaalouk DE, Bueso-Ramos CE, Zurita AJ, Kuniyasu A, Eckhardt BL, Marini FC, Lichtiger B, O'brien S, Kantarjian HM, Cortes JE, Koivunen E, Arap W, Pasqualini R. Blood. 2011; 117:920. [PubMed: 21063027]
216. Choi JH, Lee WK, Han SH, Ha S, Ahn SM, Kang JS, Choi YJ, Yun CH. Int. Immunopharmacol. 2008; 8:852. [PubMed: 18442789]
217. Jager S, Jahnke A, Wilmes T, Adebahr S, Vogtle FN, Delima-Hahn E, Pfeifer D, Berg T, Lubbert M, Trepel M. Leukemia. 2007; 21:411. [PubMed: 17252013]
218. Oyama T, Sykes KF, Samli KN, Minna JD, Johnston SA, Brown KC. Cancer Lett. 2003; 202:219. [PubMed: 14643452]
219. Zhou X, Chang YC, Oyama T, Mcguire MJ, Brown KC. J. Am. Chem. Soc. 2004; 126:15656. [PubMed: 15571383]
220. Oyama T, Rombel IT, Samli KN, Zhou X, Brown KC. Biosens. Bioelectron. 2006; 21:1867. [PubMed: 16386888]
221. Zang L, Shi L, Guo J, Pan Q, Wu W, Pan X, Wang J. Cancer Lett. 2009; 281:64. [PubMed: 19327883]
222. Tu X, Zang L, Lan D, Liang W. Mol. Med. Rep. 2009; 2:1005. [PubMed: 21475935]
223. Li S, Mcguire MJ, Lin M, Liu YH, Oyama T, Sun X, Brown KC. Mol. Cancer Ther. 2009; 8:1239. [PubMed: 19435868]
224. Li S, Gray BP, Mcguire MJ, Brown KC. Bioorg. Med.Chem. 2011; 19:5480. [PubMed: 21868241]
225. Guan H, Mcguire MJ, Li S, Brown KC. BioconjugateChem. 2008; 19:1813.
226. Guthi JS, Yang SG, Huang G, Li S, Khemtong C, Kessinger CW, Peyton M, Minna JD, Brown KC, Gao J. Mol.Pharmaceutics. 2010; 7:32.
227. Gray BP, Li S, Brown KC. Bioconjugate Chem. 2013; 24:85.
228. Huang G, Zhang C, Li S, Khemtong C, Yang SG, Tian R, Minna JD, Brown KC, Gao J. J. Mater. Chem. 2009; 19:6367. [PubMed: 20505790]
229. Chang DK, Lin CT, Wu CH, Wu HC. PLoS One. 2009; 4:e4171. [PubMed: 19137069]
230. Florea BI, Molenaar TJ, Bot I, Michon IN, Kuiper J, Van Berkel TJ, Junginger HE, Biessen EA, Borchard G. J. DrugTargeting. 2003; 11:383.
231. He X, Na MH, Kim JS, Lee GY, Park JY, Hoffman AS, Nam JO, Han SE, Sim GY, Oh YK, Kim IS, Lee B-H. Mol. Pharmaceutics. 2011; 8:430.
232. Li M, Anastassiades CP, Joshi B, Komarck CM, Piraka C, Elmunzer BJ, Turgeon DK, Johnson TD, Appelman H, Beer DG, Wang TD. Gastroenterology. 2010; 139:1472. [PubMed: 20637198]
233. Kubo N, Akita N, Shimizu A, Kitahara H, Parker AL, Miyagawa S. J. Drug Targeting. 2008; 16:396.
234. Kolonin MG, Bover L, Sun J, Zurita AJ, Do KA, Lahdenranta J, Cardo-Vila M, Giordano RJ, Jaalouk DE, Ozawa MG, Moya CA, Souza GR, Staquicini FI, Kunyiasu A, Scudiero DA, Holbeck SL, Sausville EA, Arap W, Pasqualini R. Cancer Res. 2006; 66:34. [PubMed: 16397212]
235. Shukla GS, Krag DN. Oncol. Rep. 2005; 13:757. [PubMed: 15756454]
236. Hsiung PL, Hardy J, Friedland S, Soetikno R, Du CB, Wu AP, Sahbaie P, Crawford JM, Lowe AW, Contag CH, Wang TD. Nat. Med. 2008; 14:454. [PubMed: 18345013]
237. Lee SM, Lee EJ, Hong HY, Kwon MK, Kwon TH, Choi JY, Park RW, Kwon TG, Yoo ES, Yoon GS, Kim IS, Ruoslahti E, Lee BH. Mol. Cancer Res. 2007; 5:11. [PubMed: 17259343]

238. Jia X, Yu Q, Zhang Z, Yang X. OncoTargets Ther. 2012; 5:85.
239. Kelly KA, Bardeesy N, Anbazhagan R, Gurumurthy S, Berger J, Alencar H, Depinho RA, Mahmood U, Weissleder R. PLoS Med. 2008; 5:e85. [PubMed: 18416599]
240. Zhang W, Sui Y, Budha A, Zheng J, Sun X, Hou Y, Wang T, Lu S. World J. Gastroenterol. 2012; 18:2053. [PubMed: 22563192]
241. Cao Q, Liu S, Niu G, Chen K, Yan Y, Liu Z, Chen X. Amino Acids. 2010; 41:1103. [PubMed: 20232090]
242. Passarella RJ, Zhou L, Phillips JG, Wu H, Hallahan DE, Diaz R. Clin. Cancer Res. 2009; 15:6421. [PubMed: 19825959]
243. Han Z, Fu A, Wang H, Diaz R, Geng L, Onishko H, Hallahan DE. Nat. Med. 2008; 14:343. [PubMed: 18297085]
244. Chang DK, Chiu CY, Kuo SY, Lin WC, Lo A, Wang YP, Li PC, Wu HC. J. Biol. Chem. 2009; 284:12905. [PubMed: 19276080]
245. Estephan E, Dao J, Saab M, Panayotov I, Martin M, Larroque C, Gergely C, Cuisinier FJG, Levallois B. Biomed.Tech. 2012; 57:481.
246. Wölcke J, Weinhold E. Nucleosides, Nucleotides Nucleic Acids. 2001; 20:1239. [PubMed: 11562993]
247. Estephan E, Larroque C, Bec N, Martineau P, Cuisinier FJG, Cloitre T, Gergely C. Biotechnol. Bioeng. 2009; 104:1121. [PubMed: 19634182]
248. Murai KK, Nguyen LN, Koolpe M, Mclennan R, Krull CE, Pasquale EB. Mol. Cell. Neurosci. 2003; 24:1000. [PubMed: 14697664]
249. Lee TY, Lin CT, Kuo SY, Chang DK, Wu HC. Cancer Res. 2007; 67:10958. [PubMed: 18006841]
250. Bussolati B, Grange C, Tei L, Deregibus MC, Ercolani M, Aime S, Camussi G. J. Mol. Med. 2007; 85:897. [PubMed: 17384922]
251. Cardo-Vila M, Zurita AJ, Giordano RJ, Sun J, Rangel R, Guzman-Rojas L, Anobom CD, Valente AP, Almeida FC, Lahdenranta J, Kolonin MG, Arap W, Pasqualini R. PLoS One. 2008; 3:e3452. [PubMed: 18941632]
252. Zurita AJ, Troncoso P, Cardo-Vila M, Logothetis CJ, Pasqualini R, Arap W. Cancer Res. 2004; 64:435. [PubMed: 14744752]
253. Pasqualini R, Moeller BJ, Arap W. Semin. Thromb.Hemostasis. 2010; 36:343.
254. Krag DN, Shukla GS, Shen GP, Pero S, Ashikaga T, Fuller S, Weaver DL, Burdette-Radoux S, Thomas C. Cancer Res. 2006; 66:7724. [PubMed: 16885375]
255. Laakkonen P, Porkka K, Hoffman JA, Ruoslahti E. Nat.Med. 2002; 8:751. [PubMed: 12053175]
256. Laakkonen P, Akerman ME, Biliran H, Yang M, Ferrer F, Karpanen T, Hoffman RM, Ruoslahti E. Proc. Natl. Acad. Sci.U.S.A. 2004; 101:9381. [PubMed: 15197262]
257. Zhang L, Giraudo E, Hoffman JA, Hanahan D, Ruoslahti E. Cancer Res. 2006; 66:5696. [PubMed: 16740707]
258. Laakkonen P, Zhang L, Ruoslahti E. Ann. N. Y. Acad. Sci. 2008; 1131:37. [PubMed: 18519957]
259. Akita N, Maruta F, Seymour LW, Kerr DJ, Parker AL, Asai T, Oku N, Nakayama J, Miyagawa S. Cancer Sci. 2006; 97:1075. [PubMed: 16984380]
260. Zhi M, Wu KC, Dong L, Hao ZM, Deng TZ, Hong L, Liang SH, Zhao PT, Qiao TD, Wang Y, Xu X, Fan DM. Cancer Biol. Ther. 2004; 3:1232. [PubMed: 15492500]
261. Chen B, Cao S, Zhang Y, Wang X, Liu J, Hui X, Wan Y, Du W, Wang L, Wu K, Fan D. BMC Cell Biol. 2009; 10:63. [PubMed: 19740430]
262. Hui X, Han Y, Liang S, Liu Z, Liu J, Hong L, Zhao L, He L, Cao S, Chen B, Yan K, Jin B, Chai N, Wang J, Wu K, Fan D. J. Controlled Release. 2008; 131:86.
263. Chen K, Sun X, Niu G, Ma Y, Yap LP, Hui X, Wu K, Fan D, Conti PS, Chen X. Mol. Imaging Biol. 2011; 14:96. [PubMed: 21360213]
264. Chen K, Yap LP, Park R, Hui X, Wu K, Fan D, Chen X, Conti PS. Amino Acids. 2011; 42:1329. [PubMed: 21212998]

265. Hariri G, Yan H, Wang H, Han Z, Hallahan DE. Clin.Cancer Res. 2010; 16:4968. [PubMed: 20802016]
266. Wang H, Yan H, Fu A, Han M, Hallahan D, Han Z. PLoS One. 2010; 5:e12051. [PubMed: 20711449]
267. Lowery A, Onishko H, Hallahan DE, Han Z. J. ControlledRelease. 2011; 150:117.
268. Hariri G, Wellons MS, Morris WH 3rd, Lukehart CM, Hallahan DE. Ann. Biomed. Eng. 2011; 39:946. [PubMed: 21132370]
269. Reference deleted on revision.
270. Liu J, Chu L, Wang Y, Duan Y, Feng L, Yang C, Wang L, Kong D. Int. J. Nanomed. 2011; 6:59.
271. Sun L, Chu T, Wang Y, Wang X. Acta Biochim. Biophys.Sin. 2007; 39:624. [PubMed: 17687498]
272. Newton JR, Kelly KA, Mahmood U, Weissleder R, Deutscher SL. Neoplasia. 2006; 8:772. [PubMed: 16984734]
273. Newton-Northup JR, Figueroa SD, Deutscher SL. Comb.Chem. High Throughput Screening. 2011; 14:9.
274. Sugahara KN, Teesalu T, Karmali PP, Kotamraju VR, Agemy L, Girard OM, Hanahan D, Mattrey RF, Ruoslahti E. Cancer Cell. 2009; 16:510. [PubMed: 19962669]
275. Sugahara KN, Teesalu T, Karmali PP, Kotamraju VR, Agemy L, Greenwald DR, Ruoslahti E. Science. 2010; 328:1031. [PubMed: 20378772]
276. Agemy L, Friedmann-Morvinski D, Kotamraju VR, Roth L, Sugahara KN, Girard OM, Mattrey RF, Verma IM, Ruoslahti E. Proc. Natl. Acad. Sci. U.S.A. 2011; 108:17450. [PubMed: 21969599]
277. Mintz PJ, Cardo-Vila M, Ozawa MG, Hajitou A, Rangel R, Guzman-Rojas L, Christianson DR, Arap MA, Giordano RJ, Souza GR, Easley J, Salameh A, Oliviero S, Brentani RR, Koivunen E, Arap W, Pasqualini R. Proc. Natl. Acad. Sci. U.S.A. 2009; 106:2182. [PubMed: 19168626]
278. Teesalu T, Sugahara KN, Kotamraju VR, Ruoslahti E. Proc. Natl. Acad. Sci. U.S.A. 2009; 106:16157. [PubMed: 19805273]
279. Haspel N, Zanuy D, Nussinov R, Teesalu T, Ruoslahti E, Aleman C. Biochemistry. 2011; 50:1755. [PubMed: 21247217]
280. Corti A, Curnis F, Arap W, Pasqualini R. Blood. 2008; 112:2628. [PubMed: 18574027]
281. Pasqualini R, Koivunen E, Kain R, Lahdenranta J, Sakamoto M, Stryhn A, Ashmun RA, Shapiro LH, Arap W, Ruoslahti E. Cancer Res. 2000; 60:722. [PubMed: 10676659]
282. Pastorino F, Brignole C, Marimpietri D, Cilli M, Gambini C, Ribatti D, Longhi R, Allen TM, Corti A, Ponzoni M. CancerRes. 2003; 63:7400.
283. Pastorino F, Di Paolo D, Piccardi F, Nico B, Ribatti D, Daga A, Baio G, Neumaier CE, Brignole C, Loi M, Marimpietri D, Pagnan G, Cilli M, Lepekhin EA, Garde SV, Longhi R, Corti A, Allen TM, Wu JJ, Ponzoni M. Clin. Cancer Res. 2008; 14:7320. [PubMed: 19010847]
284. Curnis F, Sacchi A, Borgna L, Magni F, Gasparri A, Corti A. Nat. Biotechnol. 2000; 18:1185. [PubMed: 11062439]
285. Curnis F, Sacchi A, Corti A. J. Clin. Invest. 2002; 110:475. [PubMed: 12189241]
286. Zarovni N, Monaco L, Corti A. Hum. Gene Ther. 2004; 15:373. [PubMed: 15053862]
287. Sacchi A, Gasparri A, Curnis F, Bellone M, Corti A. Cancer Res. 2004; 64:7150. [PubMed: 15466213]
288. Sacchi A, Gasparri A, Gallo-Stampino C, Toma S, Curnis F, Corti A. Clin. Cancer Res. 2006; 12:175. [PubMed: 16397040]
289. Corti A, Pastorino F, Curnis F, Arap W, Ponzoni M, Pasqualini R. Med. Res. Rev. 2012; 32:1078. [PubMed: 21287572]
290. Meng J, Yan Z, Wu J, Li L, Xue X, Li M, Li W, Hao Q, Wan Y, Qin X, Zhang C, You Y, Han W, Zhang Y. Cytotherapy. 2007; 9:60. [PubMed: 17354103]
291. Zhang B, Gao B, Dong S, Zhang Y, Wu Y. Regul. Toxicol.Pharmacol. 2011; 60:73. [PubMed: 21338646]
292. Meng J, Ma N, Yan Z, Han W, Zhang Y. J. Biochem. 2006; 140:299. [PubMed: 16845126]

293. Kim JW, Lee HS. Int. J. Mol. Med. 2004; 14:529. [PubMed: 15375578]
294. Hajitou A, Trepel M, Lilley CE, Soghomonyan S, Alauddin MM, Marini FC 3rd, Restel BH, Ozawa MG, Moya CA, Rangel R, Sun Y, Zaoui K, Schmidt M, Von Kalle C, Weitzman MD, Gelovani JG, Pasqualini R, Arap W. Cell. 2006; 125:385. [PubMed: 16630824]
295. Fueyo J, Alemany R, Gomez-Manzano C, Fuller GN, Khan A, Conrad CA, Liu TJ, Jiang H, Lemoine MG, Suzuki K, Sawaya R, Curiel DT, Yung WK, Lang FF. J. Natl. CancerInst. 2003; 95:652.
296. Line B, Mitra A, Nan A, Ghandehari H. J. Nucl. Med. 2005; 46:1552. [PubMed: 16157540]
297. Fogal V, Zhang L, Krajewski S, Ruoslahti E. Cancer Res. 2008; 68:7210. [PubMed: 18757437]
298. Herringson TP, Altin JG. Int. J. Pharm. 2011; 411:206. [PubMed: 21443937]
299. Park JH, Von Maltzahn G, Xu MJ, Fogal V, Kotamraju VR, Ruoslahti E, Bhatia SN, Sailor MJ. Proc. Natl. Acad. Sci.U.S.A. 2010; 107:981. [PubMed: 20080556]
300. Staquicini FI, Ozawa MG, Moya CA, Driessen WH, Barbu EM, Nishimori H, Soghomonyan S, Flores LG 2nd, Liang X, Paolillo V, Alauddin MM, Basilion JP, Furnari FB, Bogler O, Lang FF, Aldape KD, Fuller GN, Hook M, Gelovani JG, Sidman RL, Cavenee WK, Pasqualini R, Arap W. J. Clin. Invest. 2011; 121:161. [PubMed: 21183793]
301. Karmali PP, Kotamraju VR, Kastantin M, Black M, Missirlis D, Tirrell M, Ruoslahti E. Nanomedicine. 2009; 5:73. [PubMed: 18829396]
302. Zhang F, Niu G, Lin X, Jacobson O, Ma Y, Eden HS, He Y, Lu G, Chen X. Amino Acids. 2011; 42:2343. [PubMed: 21769497]
303. Makela AR, Matilainen H, White DJ, Ruoslahti E, Oker-Blom C. J. Virol. 2006; 80:6603. [PubMed: 16775347]
304. Makela AR, Enback J, Laakkonen JP, Vihinen-Ranta M, Laakkonen P, Oker-Blom C. J. Gene Med. 2008; 10:1019. [PubMed: 18655234]
305. Akerman ME, Chan WC, Laakkonen P, Bhatia SN, Ruoslahti E. Proc. Natl. Acad. Sci. U.S.A. 2002; 99:12617. [PubMed: 12235356]
306. Von Maltzahn G, Ren Y, Park JH, Min DH, Kotamraju VR, Jayakumar J, Fogal V, Sailor MJ, Ruoslahti E, Bhatia SN. Bioconjugate Chem. 2008; 19:1570.
307. Sharma, G.; Karmali, P.; Ramirez, M.; Xie, H.; Kotamraju, VR.; Ruoslahti, E.; Smith, JW. NSTI-Nanotech. Vol. 3. Anaheim, CA; Austin, TX: Nano Science and Technology Insitute (NSTI); 2010. p. 382
308. Lempens EH, Merkx M, Tirrell M, Meijer EW. Bioconjugate Chem. 2011; 22:397.
309. Hamzah J, Kotamraju VR, Seo JW, Agemy L, Fogal V, Mahakian LM, Peters D, Roth L, Gagnon MKJ, Ferrara KW, Ruoslahti E. Proc. Natl. Acad. Sci. U.S.A. 2011; 108:7154. [PubMed: 21482787]
310. Uchida M, Kosuge H, Terashima M, Willits DA, Liepold LO, Young MJ, Mcconnell MV, Douglas T. ACS Nano. 2011; 5:2493. [PubMed: 21391720]
311. Böckmann M, Drosten M, Pützer BM. J. Gene Med. 2005; 7:179. [PubMed: 15508130]
312. Hoffman JA, Giraudo E, Singh M, Zhang L, Inoue M, Porkka K, Hanahan D, Ruoslahti E. Cancer Cell. 2003; 4:383. [PubMed: 14667505]
313. Agemy L, Sugahara KN, Kotamraju VR, Gujraty K, Girard OM, Kono Y, Mattrey RF, Park JH, Sailor MJ, Jimenez AI, Cativiela C, Zanuy D, Sayago FJ, Aleman C, Nussinov R, Ruoslahti E. Blood. 2010; 116:2847. [PubMed: 20587786]
314. Numata K, Reagan MR, Goldstein RH, Rosenblatt M, Kaplan DL. Bioconjugate Chem. 2011; 22:1605.
315. Joyce JA, Laakkonen P, Bernasconi M, Bergers G, Ruoslahti E, Hanahan D. Cancer Cell. 2003; 4:393. [PubMed: 14667506]
316. Hamzah J, Nelson D, Moldenhauer G, Arnold B, Hammerling GJ, Ganss R. J. Clin. Invest. 2008; 118:1691. [PubMed: 18398504]
317. Miller SJ, Joshi BP, Feng Y, Gaustad A, Fearon ER, Wang TD. PLoS One. 2011; 6:e17384. [PubMed: 21408169]
318. Elahi SF, Miller SJ, Joshi B, Wang TD. Biomed. Opt.Express. 2011; 2:981. [PubMed: 21483619]

319. Li ZJ, Wu WKK, Ng SSM, Yu L, Li HT, Wong CCM, Wu YC, Zhang L, Ren SX, Sun XG, Chan KM, Cho CH. J. Controlled Release. 2010; 148:292.
320. Passarella RJ, Spratt DE, Van Der Ende AE, Phillips JG, Wu H, Sathiyakumar V, Zhou L, Hallahan DE, Harth E, Diaz R. Cancer Res. 2010; 70:4550. [PubMed: 20484031]
321. Du B, Han H, Wang Z, Kuang L, Wang L, Yu L, Wu M, Zhou Z, Qian M. Mol. Cancer Res. 2010; 8:135. [PubMed: 20145035]
322. Wang W, Ke S, Kwon S, Yallampalli S, Cameron AG, Adams KE, Mawad ME, Sevick-Muraca EM. Bioconjugate Chem. 2007; 18:397.
323. Li W, Lei P, Yu B, Wu S, Peng J, Zhao X, Zhu H, Kirschfink M, Shen G. Acta Biochim. Biophys. Sin. 2008; 40:443. [PubMed: 18465030]
324. Dane KY, Gottstein C, Daugherty PS. Mol. Cancer Res. 2009; 8:1312.
325. Yang W, Luo D, Wang S, Wang R, Chen R, Liu Y, Zhu T, Ma X, Liu R, Xu G, Meng L, Lu Y, Zhou J, Ma D. Clin.Cancer Res. 2008; 14:5494. [PubMed: 18765541]
326. Zitzmann S, Kramer S, Mier W, Mahmut M, Fleig J, Altmann A, Eisenhut M, Haberkorn U. J. Nucl. Med. 2005; 46:782. [PubMed: 15872351]
327. Brown CK, Modzelewski RA, Johnson CS, Wong MK. Ann. Surg. Oncol. 2000; 7:743. [PubMed: 11129422]
328. Weller GE, Wong MK, Modzelewski RA, Lu E, Klibanov AL, Wagner WR, Villanueva FS. Cancer Res. 2005; 65:533. [PubMed: 15695396]
329. Lam KS, Lou Q, Zhao ZG, Smith J, Chen ML, Pleshko E, Salmon SE. Biomed. Pept. Proteins Nucleic Acids. 1995; 1:205. [PubMed: 9346854]
330. Xiao W, Wang Y, Lau EY, Luo J, Yao N, Shi C, Meza L, Tseng H, Maeda Y, Kumaresan P, Liu R, Lightstone FC, Takada Y, Lam KS. Mol. Cancer Ther. 2010; 9:2714. [PubMed: 20858725]
331. Rathinam R, Alahari S. Cancer Metastasis Rev. 2010; 29:223. [PubMed: 20112053]
332. Yao N, Xiao W, Wang X, Marik J, Park SH, Takada Y, Lam KS. J. Med. Chem. 2009; 52:126. [PubMed: 19055415]
333. Xiao W, Yao N, Peng L, Liu R, Lam KS. Eur. J. Nucl.Med. Mol. Imaging. 2009; 36:94. [PubMed: 18712382]
334. Park SI, Renil M, Vikstrom B, Amro N, Song LW, Xu BL, Lam KS. Pept. Res. Ther. 2002; 8:171.
335. Aina OH, Marik J, Liu R, Lau DH, Lam KS. Mol.Cancer Ther. 2005; 4:806. [PubMed: 15897245]
336. Aina OH, Marik J, Gandour-Edwards R, Lam KS. Mol.Imaging. 2005; 4:439. [PubMed: 16285906]
337. Xiao K, Li Y, Lee JS, Gonik AM, Dong T, Fung G, Sanchez E, Xing L, Cheng HR, Luo J, Lam KS. Cancer Res. 2012; 72:2100. [PubMed: 22396491]
338. Lau D, Guo L, Liu R, Marik J, Lam K. Lung Cancer. 2006; 52:291. [PubMed: 16635537]
339. Mikawa M, Wang H, Guo L, Liu R, Marik J, Takada Y, Lam K, Lau D. Mol. Cancer Ther. 2004; 3:1329. [PubMed: 15486200]
340. Deroock IB, Pennington ME, Sroka TC, Lam KS, Bowden GT, Bair EL, Cress AE. Cancer Res. 2001; 61:3308. [PubMed: 11309285]
341. Sroka TC, Pennington ME, Cress AE. Carcinogenesis. 2006; 27:1748. [PubMed: 16537560]
342. Nair RR, Emmons MF, Cress AE, Argilagos RF, Lam K, Kerr WT, Wang HG, Dalton WS, Hazlehurst LA. Mol.Cancer Ther. 2009; 8:2441. [PubMed: 19671765]
343. Zhang H, Aina OH, Lam KS, De Vere White R, Evans C, Henderson P, Lara PN, Wang X, Bassuk JA, Pan CX. Urol.Oncol. 2012; 30:635. [PubMed: 20888272]
344. Denholt CL, Binderup T, Stockhausen MT, Poulsen HS, Spang-Thomsen M, Hansen PR, Gillings N, Kjaer A. Nucl.Med. Biol. 2011; 38:509. [PubMed: 21531288]
345. Choi Y, Kim E, Lee Y, Han MH, Kang IC. Proteomics. 2010; 10:72. [PubMed: 19882657]
346. Bang JY, Kim EY, Kang DK, Chang SI, Han MH, Baek KH, Kang IC. Mol. Cell. Proteomics. 2011; 10:M110 005264. [PubMed: 21558493]

347. Baek SH, Seo JK, Chae CB, Suh PG, Ryu SH. J.Biol. Chem. 1996; 271:8170. [PubMed: 8626507]
348. Le Y, Gong W, Li B, Dunlop NM, Shen W, Su SB, Ye RD, Wang JM. J. Immunol. 1999; 163:6777. [PubMed: 10586077]
349. Christophe T, Karlsson A, Dugave C, Rabiet MJ, Boulay F, Dahlgren C. J. Biol. Chem. 2001; 276:21585. [PubMed: 11285256]
350. Seo JK, Choi SY, Kim Y, Baek SH, Kim KT, Chae CB, Lambeth JD, Suh PG, Ryu SH. J. Immunol. 1997; 158:1895. [PubMed: 9029131]
351. Ruoslahti E. Annu. Rev. Cell Dev. Biol. 1996; 12:697. [PubMed: 8970741]
352. Koivunen E, Gay DA, Ruoslahti E. J. Biol. Chem. 1993; 268:20205. [PubMed: 7690752]
353. Koivunen E, Wang B, Ruoslahti E. J. Cell Biol. 1994; 124:373. [PubMed: 7507494]
354. Curnis F, Cattaneo A, Longhi R, Sacchi A, Gasparri AM, Pastorino F, Di Matteo P, Traversari C, Bachi A, Ponzoni M, Rizzardi GP, Corti A. J. Biol. Chem. 2010; 285:9114. [PubMed: 20064928]
355. Curnis F, Longhi R, Crippa L, Cattaneo A, Dondossola E, Bachi A, Corti A. J. Biol. Chem. 2006; 281:36466. [PubMed: 17015452]
356. Galagudza, M. Novel Strategies in Ischemic Heart Disease. Lakshmanadoss, U., editor. Rijeka, Croatia: InTech; 2012. p. 253
357. Scott RC, Rosano JM, Ivanov Z, Wang B, Chong PL-G, Issekutz AC, Crabbe DL, Kiani MF. FASEB J. 2009; 23:3361. [PubMed: 19535683]
358. Zahid M, Phillips BE, Albers SM, Giannoukakis N, Watkins SC, Robbins PD. PLoS One. 2010; 5:e12252. [PubMed: 20808875]
359. Zhang L, Hoffman JA, Ruoslahti E. Circulation. 2005; 112:1601. [PubMed: 16144998]
360. Greig JA, Shirley R, Graham D, Denby L, Dominiczak AF, Work LM, Baker AH. J. Cardiovasc. Pharmacol. 2010; 56:642. [PubMed: 20838228]
361. Kanki S, Jaalouk DE, Lee S, Yu AY, Gannon J, Lee RT. J. Mol. Cell. Cardiol. 2011; 50:841. [PubMed: 21316369]
362. Emery AE. Lancet. 2002; 359:687. [PubMed: 11879882]
363. Seow Y, Yin H, Wood MJ. Peptides. 2010; 31:1873. [PubMed: 20621144]
364. Libby P. Nature. 2002; 420:868. [PubMed: 12490960]
365. Kelly KA, Allport JR, Tsourkas A, Shinde-Patil VR, Josephson L, Weissleder R. Circ. Res. 2005; 96:327. [PubMed: 15653572]
366. Hong HY, Lee HY, Kwak W, Yoo J, Na MH, So IS, Kwon TH, Park HS, Huh S, Oh GT, Kwon IC, Kim IS, Lee BH. J. Cell. Mol. Med. 2008; 12:2003. [PubMed: 19012727]
367. Cai D, Xaymardan M, Holm JM, Zheng J, Kizer JR, Edelberg JM. Am. J. Physiol. Heart Circ. Physiol. 2003; 285:H463. [PubMed: 12730063]
368. Cai D, Holm JM, Duignan IJ, Zheng J, Xaymardan M, Chin A, Ballard VL, Bella JN, Edelberg JM. Physiol. Genomics. 2006; 24:191. [PubMed: 16352696]
369. Nicol CG, Denby L, Lopez-Franco O, Masson R, Halliday CA, Nicklin SA, Kritz A, Work LM, Baker AH. FEBS Lett. 2009; 583:2100. [PubMed: 19481546]
370. Zhang H, Kusunose J, Kheirolomoom A, Seo JW, Qi J, Watson KD, Lindfors HA, Ruoslahti E, Sutcliffe JL, Ferrara KW. Biomaterials. 2008; 29:1976. [PubMed: 18255141]
371. Nam HY, Mcginn A, Kim PH, Kim SW, Bull DA. Biomaterials. 2010; 31:8081. [PubMed: 20674007]
372. Li Z, Fan J, Zhao W, Jin L, Ma L. J. Pept. Sci. 2011; 17:771. [PubMed: 22033953]
373. Samoylova TI, Smith BF. Muscle Nerve. 1999; 22:460. [PubMed: 10204780]
374. Yu CY, Yuan Z, Cao Z, Wang B, Qiao C, Li J, Xiao X. Gene Ther. 2009; 16:953. [PubMed: 19474807]
375. Wang Q, Yin H, Camelliti P, Betts C, Moulton H, Lee H, Saleh AF, Gait MJ, Wood MJ. J. Gene Med. 2010; 12:354. [PubMed: 20235089]
376. Yin H, Lu Q, Wood M. Mol. Ther. 2008; 16:38. [PubMed: 17968354]
377. Kelly KA, Nahrendorf M, Yu AM, Reynolds F, Weissleder R. Mol. Imaging Biol. 2006; 8:201. [PubMed: 16791746]

378. Nahrendorf M, Jaffer FA, Kelly KA, Sosnovik DE, Aikawa E, Libby P, Weissleder R. Circulation. 2006; 114:1504. [PubMed: 17000904]
379. Nahrendorf M, Keliher E, Panizzi P, Zhang H, Hembrador S, Figueiredo JL, Aikawa E, Kelly K, Libby P, Weissleder R. JACC: Cardiovasc. Imaging. 2009; 2:1213. [PubMed: 19833312]
380. Liu C, Bhattacharjee G, Boisvert W, Dilley R, Edgington T. Am. J. Pathol. 2003; 163:1859. [PubMed: 14578186]
381. White K, Buning H, Kritz A, Janicki H, Mcvey J, Perabo L, Murphy G, Odenthal M, Work LM, Hallek M, Nicklin SA, Baker AH. Gene Ther. 2008; 15:443. [PubMed: 18004401]
382. Houston P, Goodman J, Lewis A, Campbell CJ, Braddock M. FEBS Lett. 2001; 492:73. [PubMed: 11248240]
383. Kim JH, Bae SM, Na MH, Shin H, Yang YJ, Min KH, Choi KY, Kim K, Park RW, Kwon IC, Lee BH, Hoffman AS, Kim IS. J. Controlled Release. 2012; 157:493.
384. Wu XL, Kim JH, Koo H, Bae SM, Shin H, Kim MS, Lee BH, Park RW, Kim IS, Choi K, Kwon IC, Kim K, Lee DS. Bioconjugate Chem. 2010; 21:208.
385. Park K, Hong HY, Moon HJ, Lee BH, Kim IS, Kwon IC, Rhee K. J. Controlled Release. 2008; 128:217.
386. Lee GY, Kim JH, Oh GT, Lee BH, Kwon IC, Kim IS. J. Controlled Release. 2011; 155:211.
387. Michon IN, Hauer AD, Von Der Thusen JH, Molenaar TJ, Van Berkel TJ, Biessen EA, Kuiper J. Biochim. Biophys. Acta. 2002; 1591:87. [PubMed: 12183059]
388. Work LM, Nicklin SA, Brain NJ, Dishart KL, VonSeggern DJ, Hallek M, Buning H, Baker AH. Mol. Ther. 2004; 9:198. [PubMed: 14759804]
389. Banchereau J, Palucka AK. Nat. Rev. Immunol. 2005; 5:296. [PubMed: 15803149]
390. Frick C, Odermatt A, Zen K, Mandell KJ, Edens H, Portmann R, Mazzucchelli L, Jaye DL, Parkos CA. Eur. J.Immunol. 2005; 35:3610. [PubMed: 16252253]
391. Faham A, Altin JG. Int. J. Cancer. 2010; 129:1391. [PubMed: 21128234]
392. Chamarthy SP, Jia L, Kovacs JR, Anderson KR, Shen H, Firestine SM, Meng WS. Mol. Immunol. 2004; 41:741. [PubMed: 15234553]
393. Mcguire MJ, Sykes KF, Samli KN, Timares L, Barry MA, Stemke-Hale K, Tagliaferri F, Logan M, Jansa K, Takashima A, Brown KC, Johnston SA. DNA Cell Biol. 2004; 23:742. [PubMed: 15585132]
394. Berntzen G, Andersen JT, Ustgard K, Michaelsen TE, Mousavi SA, Qian JD, Kristiansen PE, Lauvrak V, Sandlie I. J.Biol. Chem. 2009; 284:1126. [PubMed: 18957413]
395. Clark MA, Jepson MA, Hirst BH. Adv. Drug Delivery Rev. 2001; 50:81.
396. Higgins LM, Lambkin I, Donnelly G, Byrne D, Wilson C, Dee J, Smith M, O'mahony DJ. Pharm. Res. 2004; 21:695. [PubMed: 15139528]
397. Kim SH, Seo KW, Kim J, Lee KY, Jang YS. J.Immunol. 2010; 185:5787. [PubMed: 20952686]
398. Kim SH, Lee KY, Kim J, Park SM, Park BK, Jang YS. Mol. Cell. 2006; 21:244.
399. Richards JL, Abend JR, Miller ML, Chakraborty-Sett S, Dewhurst S, Whetter LE. Eur. J. Biochem. 2003; 270:2287. [PubMed: 12752448]
400. Herringson TP, Altin JG. J. Controlled Release. 2009; 139:229.
401. Kelly KA, Clemons PA, Yu AM, Weissleder R. Mol.Imaging. 2006; 5:24. [PubMed: 16779967]
402. Kim SH, Jung DI, Yang IY, Kim J, Lee KY, Nochi T, Kiyono H, Jang YS. Eur. J. Immunol. 2011; 41:3219. [PubMed: 21887786]
403. Yoo MK, Kang SK, Choi JH, Park IK, Na HS, Lee HC, Kim EB, Lee NK, Nah JW, Choi YJ, Cho CS. Biomaterials. 2010; 31:7738. [PubMed: 20656343]
404. Fievez V, Plapied L, Plaideau C, Legendre D, Des Rieux A, Pourcelle V, Freichels H, Jerome C, Marchand J, Preat V, Schneider YJ. Int. J. Pharm. 2010; 394:35. [PubMed: 20417702]
405. Bae YS, Park EY, Kim Y, He R, Ye RD, Kwak JY, Suh PG, Ryu SH. Biochem. Pharmacol. 2003; 66:1841. [PubMed: 14563494]
406. Bae YS, Bae H, Kim Y, Lee TG, Suh PG, Ryu SH. Blood. 2001; 97:2854. [PubMed: 11313281]
407. Ueberberg S, Schneider S. Regul. Pept. 2010; 160:1. [PubMed: 19958795]

408. Yao VJ, Ozawa MG, Trepel M, Arap W, Mcdonald DM, Pasqualini R. Am. J. Pathol. 2005; 166:625. [PubMed: 15681844]

409. Lin M, Lubag A, Mcguire MJ, Seliounine SY, Tsyganov EN, Antich PP, Sherry AD, Brown KC, Sun X. Front. Biosci. 2008; 13:4558. [PubMed: 18508529]

410. Blevins KS, Jeong JH, Ou M, Brumbach JH, Kim SW. J. Controlled Release. 2012; 158:115.

411. Flegal KM, Carroll MD, Ogden CL, Curtin LR. JAMA,J. Am. Med. Assoc. 2010; 303:235.

412. Daquinag AC, Zhang Y, Amaya-Manzanares F, Simmons PJ, Kolonin MG. Cell Stem Cell. 2011; 9:74. [PubMed: 21683670]

413. Nie J, Chang B, Traktuev DO, Sun J, March K, Chan L, Sage EH, Pasqualini R, Arap W, Kolonin MG. Stem Cells. 2008; 26:2735. [PubMed: 18583538]

414. Kolonin MG, Saha PK, Chan L, Pasqualini R, Arap W. Nat. Med. 2004; 10:625. [PubMed: 15133506]

415. Barnhart KF, Christianson DR, Hanley PW, Driessen WHP, Bernacky BJ, Baze WB, Wen S, Tian M, Ma J, Kolonin MG, Saha PK, Do KA, Hulvat JF, Gelovani JG, Chan L, Arap W, Pasqualini R. Sci. Transl. Med. 2011; 3:108.

416. Hossen MN, Kajimoto K, Akita H, Hyodo M, Ishitsuka T, Harashima H. J. Controlled Release. 2010; 147:261.

417. Staquicini FI, Cardo-Vila M, Kolonin MG, Trepel M, Edwards JK, Nunes DN, Sergeeva A, Efstathiou E, Sun J, Almeida NF, Tu SM, Botz GH, Wallace MJ, O'connell DJ, Krajewski S, Gershenwald JE, Molldrem JJ, Flamm AL, Koivunen E, Pentz RD, Dias-Neto E, Setubal JC, Cahill DJ, Troncoso P, Do KA, Logothetis CJ, Sidman RL, Pasqualini R, Arap W. Proc. Natl. Acad. Sci. U.S.A. 2011; 108:18637. [PubMed: 22049339]

418. Kim DH, Woods SC, Seeley RJ. Diabetes. 2010; 59:907. [PubMed: 20103704]

419. Liu J, Wang L, Zhang A, Di W, Zhang X, Wu L, Yu J, Zha J, Lv S, Cheng P, Hu M, Li Y, Qi H, Ding G, Zhong Y. Endocr. J. 2011; 58:199. [PubMed: 21325744]

420. De Boer AG, Gaillard PJ. Annu. Rev. Pharmacol. Toxicol. 2007; 47:323. [PubMed: 16961459]

421. Pardridge WM. Drug Discovery Today. 2007; 12:54. [PubMed: 17198973]

422. Van Rooy I, Cakir-Tascioglu S, Couraud PO, Romero IA, Weksler B, Storm G, Hennink WE, Schiffelers RM, Mastrobattista E. Pharm. Res. 2010; 27:673. [PubMed: 20162339]

423. Wan XM, Chen YP, Xu WR, Yang WJ, Wen LP. Peptides. 2009; 30:343. [PubMed: 19007831]

424. Hou ST, Dove M, Anderson E, Zhang J, Mackenzie CR. J. Neurosci. Methods. 2004; 138:39. [PubMed: 15325109]

425. Fabes J, Anderson P, Brennan C, Bolsover S. Eur. J.Neurosci. 2007; 26:2496. [PubMed: 17970742]

426. Galimberti I, Bednarek E, Donato F, Caroni P. Neuron. 2010; 65:627. [PubMed: 20223199]

427. Staquicini FI, Dias-Neto E, Li J, Snyder EY, Sidman RL, Pasqualini R, Arap W. Proc. Natl. Acad. Sci. U.S.A. 2009; 106:2903. [PubMed: 19193855]

428. Schmidt A, Haas SJ, Hildebrandt S, Scheibe J, Eckhoff B, Racek T, Kempermann G, Wree A, Putzer BM. Stem Cells. 2007; 25:2910. [PubMed: 17641242]

429. Hildebrandt S, Schmidt A, Stoll A, Schmitt O, Kohling R, Wree A, Haas SJ, Putzer BM. Brain Struct. Funct. 2010; 215:105. [PubMed: 20652310]

430. Ludtke JJ, Sololoff AV, Wong SC, Zhang G, Wolff JA. Drug Delivery. 2007; 14:357. [PubMed: 17701525]

431. Denby L, Work LM, Seggern DJ, Wu E, Mcvey JH, Nicklin SA, Baker AH. Mol. Ther. 2007; 15:1647. [PubMed: 17551506]

432. Diaconu I, Denby L, Pesonen S, Cerullo V, Bauerschmitz GJ, Guse K, Rajecki M, Dias JD, Taari K, Kanerva A, Baker AH, Hemminki A. Hum. Gene Ther. 2009; 20:611. [PubMed: 19239383]

433. Gelain F, Cigognini D, Caprini A, Silva D, Colleoni B, Donega M, Antonini S, Cohen BE, Vescovi A. Nanoscale. 2012; 4:2946. [PubMed: 22476090]

434. Winner B, Kohl Z, Gage FH. Eur. J. Neurosci. 2011; 33:1139. [PubMed: 21395858]

435. Parent JM. Epilepsy Res. 2002; 50:179. [PubMed: 12151127]

436. Kempermann G, Kronenberg G. Biol. Psychiatry. 2003; 54:499. [PubMed: 12946878]

437. Sun J, Zhang C, Liu G, Liu H, Zhou C, Lu Y, Zhou C, Yuan L, Li X. Clin. Exp. Metastasis. 2012; 29:185. [PubMed: 22228571]
438. Morita Y, Mamiya K, Yamamura S, Tamiya E. Biotechnol.Prog. 2006; 22:974. [PubMed: 16889372]
439. Zhao S, Zhao W, Ma L. Peptides. 2010; 31:2027. [PubMed: 20713104]
440. Lu S, Xu X, Zhao W, Wu W, Yuan H, Shen H, Zhou C, Li LS, Ma L. PLoS One. 2010; 5:e12075. [PubMed: 20711469]
441. Zhao W, Yuan H, Xu X, Ma L. J. Biomol. Screening. 2010; 15:687.
442. Caprini A, Silva D, Zanoni I, Cunha C, Volontè C, Vescovi A, Gelain F. New Biotechnol. 2013; 30:552.
443. Shao Z, Zhang X, Pi Y, Wang X, Jia Z, Zhu J, Dai L, Chen W, Yin L, Chen H, Zhou C, Ao Y. Biomaterials. 2012; 33:3375. [PubMed: 22322196]
444. Cui Y, Kim SN, Jones SE, Wissler LL, Naik RR, Mcalpine MC. Nano Lett. 2010; 10:4559. [PubMed: 20942387]
445. Little LE, Dane KY, Daugherty PS, Healy KE, Schaffer DV. Biomaterials. 2011; 32:1484. [PubMed: 21129772]
446. Nowakowski GS, Dooner MS, Valinski HM, Mihaliak AM, Quesenberry PJ, Becker PS. Stem Cells. 2004; 22:1030. [PubMed: 15536193]
447. Pasqualini R, Arap W, Mcdonald DM. Trends Mol. Med. 2002; 8:563. [PubMed: 12470989]
448. Rajotte D, Arap W, Hagedorn M, Koivunen E, Pasqualini R, Ruoslahti E. J. Clin. Invest. 1998; 102:430. [PubMed: 9664085]
449. Trepel M, Arap W, Pasqualini R. Curr. Opin. Chem. Biol. 2002; 6:399. [PubMed: 12023122]
450. Giordano RJ, Edwards JK, Tuder RM, Arap W, Pasqualini R. Proc. Am. Thorac. Soc. 2009; 6:411. [PubMed: 19687212]
451. Arap W, Haedicke W, Bernasconi M, Kain R, Rajotte D, Krajewski S, Ellerby HM, Bredesen DE, Pasqualini R, Ruoslahti E. Proc. Natl. Acad. Sci. U.S.A. 2002; 99:1527. [PubMed: 11830668]
452. Kolonin MG, Sun J, Do KA, Vidal CI, Ji Y, Baggerly KA, Pasqualini R, Arap W. FASEB J. 2006; 20:979. [PubMed: 16581960]
453. Essler M, Ruoslahti E. Proc. Natl. Acad. Sci. U.S.A. 2002; 99:2252. [PubMed: 11854520]
454. Lee NK, Kim M, Choi JH, Kim EB, Lee HG, Kang SK, Choi YJ. Peptides. 2010; 31:2247. [PubMed: 20863866]
455. Yang Y, Zizheng W, Tongxin D. J. Biomol. Screening. 2008; 13:968.
456. Oh Y, Mohiuddin I, Sun Y, Putnam JB Jr, Hong WK, Arap W, Pasqualini R. Chest. 2005; 128:596S. [PubMed: 16373855]
457. Mai J, Song S, Rui M, Liu D, Ding Q, Peng J, Xu Y. J.Controlled Release. 2009; 139:174.
458. Paradis-Bleau C, Lloyd A, Sanschagrin F, Clarke T, Blewett A, Bugg T, Levesque R. BMC Biochem. 2008; 9:33. [PubMed: 19099588]
459. Carter DM, Gagnon JN, Damlaj M, Mandava S, Makowski L, Rodi DJ, Pawelek PD, Coulton JW. J. Mol. Biol. 2006; 357:236. [PubMed: 16414071]
460. Park JP, Cropek DM, Banta S. Biotechnol. Bioeng. 2010; 105:678. [PubMed: 19891006]
461. Chung WJ, Kwon KY, Song J, Lee SW. Langmuir. 2011; 27:7620. [PubMed: 21291244]
462. Wu X, Li Z, Yao M, Wang H, Qu S, Chen X, Li J, Sun Y, Xu Y, Gu J. Acta Biochim. Biophys. Sin. 2008; 40:217. [PubMed: 18330476]
463. Nicklin SA, White SJ, Watkins SJ, Hawkins RE, Baker AH. Circulation. 2000; 102:231. [PubMed: 10889136]
464. Nicklin SA, Buening H, Dishart KL, De Alwis M, Girod A, Hacker U, Thrasher AJ, Ali RR, Hallek M, Baker AH. Mol.Ther. 2001; 4:174. [PubMed: 11545607]
465. Nicklin SA, Von Seggern DJ, Work LM, Pek DC, Dominiczak AF, Nemerow GR, Baker AH. Mol. Ther. 2001; 4:534. [PubMed: 11735337]
466. Parker AL, Fisher KD, Oupicky D, Read ML, Nicklin SA, Baker AH, Seymour LW. J. Drug Targeting. 2005; 13:39.

467. Nicklin SA, Dishart KL, Buening H, Reynolds PN, Hallek M, Nemerow GR, Von Seggern DJ, Baker AH. CancerLett. 2003; 201:165.
468. White SJ, Nicklin SA, Buning H, Brosnan MJ, Leike K, Papadakis ED, Hallek M, Baker AH. Circulation. 2004; 109:513. [PubMed: 14732747]
469. Nicklin SA, White SJ, Nicol CG, Von Seggern DJ, Baker AH. J. Gene Med. 2004; 6:300. [PubMed: 15026991]
470. Hardy B, Raiter A, Weiss C, Kaplan B, Tenenbaum A, Battler A. Peptides. 2007; 28:691. [PubMed: 17187899]
471. Glaser-Gabay L, Raiter A, Battler A, Hardy B. Microvasc.Res. 2011; 82:221. [PubMed: 21803052]
472. Maruta F, Parker AL, Fisher KD, Murray PG, Kerr DJ, Seymour LW. J. Drug Targeting. 2003; 11:53.
473. Giordano RJ, Lahdenranta J, Zhen L, Chukwueke U, Petrache I, Langley RR, Fidler IJ, Pasqualini R, Tuder RM, Arap W. J. Biol. Chem. 2008; 283:29447. [PubMed: 18718906]
474. Morris CJ, Smith MW, Griffiths PC, Mckeown NB, Gumbleton M. J. Controlled Release. 2011; 151:83.
475. Veleva AN, Cooper SL, Patterson C. Biotechnol. Bioeng. 2007; 98:306. [PubMed: 17657770]
476. Veleva AN, Nepal DB, Frederick CB, Schwab J, Lockyer P, Yuan H, Lalush DS, Patterson C. Molecules. 2011; 16:900. [PubMed: 21258297]
477. Trepel M, Grifman M, Weitzman MD, Pasqualini R. Hum. Gene Ther. 2000; 11:1971. [PubMed: 11020797]
478. Yan Z, Lu L, Shi J, Bao C, Han W, Wu Y, Zhang Y. Appl. Biochem. Biotechnol. 2006; 133:149. [PubMed: 16702607]
479. Rajotte D, Ruoslahti E. J. Biol. Chem. 1999; 274:11593. [PubMed: 10206967]
480. Jarvinen TA, Ruoslahti E. Am. J. Pathol. 2007; 171:702. [PubMed: 17600129]
481. Jarvinen TA, Ruoslahti E. Proc. Natl. Acad. Sci. U.S.A. 2010; 107:21671. [PubMed: 21106754]
482. Urakami T, Järvinen TaH, Toba M, Sawada J, Ambalavanan N, Mann D, Mcmurtry I, Oka M, Ruoslahti E, Komatsu M. Am. J. Pathol. 2011; 178:2489. [PubMed: 21549345]
483. Obeid M. Mol. Cancer Ther. 2009; 8:2693. [PubMed: 19755516]
484. Oku N, Asai T, Watanabe K, Kuromi K, Nagatsuka M, Kurohane K, Kikkawa H, Ogino K, Tanaka M, Ishikawa D, Tsukada H, Momose M, Nakayama J, Taki T. Oncogene. 2002; 21:2662. [PubMed: 11965539]
485. Asai T, Nagatsuka M, Kuromi K, Yamakawa S, Kurohane K, Ogino K, Tanaka M, Taki T, Oku N. FEBS Lett. 2002; 510:206. [PubMed: 11801255]
486. Lee CH, Lee MS, Kim SJ, Je YT, Ryu SH, Lee T. Peptides. 2009; 30:409. [PubMed: 18992291]
487. Lee SJ, Park SG, Chung HM, Choi JS, Kim DD, Sung JH. Int. J. Pept. Res. Ther. 2009; 15:281.
488. Okamoto CT. Adv. Drug Delivery Rev. 1998; 29:215.
489. Kang SK, Woo JH, Kim MK, Woo SS, Choi JH, Lee HG, Lee NK, Choi YJ. J. Biotechnol. 2008; 135:210. [PubMed: 18440083]
490. Duerr DM, White SJ, Schluesener HJ. J. Virol. Methods. 2004; 116:177. [PubMed: 14738985]
491. Braathen R, Sandvik A, Berntzen G, Hammerschmidt S, Fleckenstein B, Sandlie I, Brandtzaeg P, Johansen FE, Lauvrak V. J. Biol. Chem. 2006; 281:7075. [PubMed: 16423833]
492. Herman RE, Makienko EG, Prieve MG, Fuller M, Houston ME Jr, Johnson PH. J. Biomol. Screening. 2007; 12:1092.
493. Jost PJ, Harbottle RP, Knight A, Miller AD, Coutelle C, Schneider H. FEBS Lett. 2001; 489:263. [PubMed: 11165262]
494. White AF, Mazur M, Sorscher EJ, Zinn KR, Ponnazhagan S. Hum. Gene Ther. 2008; 19:1407. [PubMed: 18778196]
495. Vaysse L, Burgelin I, Merlio JP, Arveiler B. Biochim.Biophys. Acta. 2000; 1475:369. [PubMed: 10913838]
496. Writer MJ, Marshall B, Pilkington-Miksa MA, Barker SE, Jacobsen M, Kritz A, Bell PC, Lester DH, Tabor AB, Hailes HC, Klein N, Hart SL. J. Drug Targeting. 2004; 12:185.

497. Irvine SA, Meng QH, Afzal F, Ho J, Wong JB, Hailes HC, Tabor AB, Mcewan JR, Hart SL. Mol. Ther. 2008; 16:508. [PubMed: 18180778]
498. Wong JB, Grosse S, Tabor AB, Hart SL, Hailes HC. Mol. Biosyst. 2008; 4:532. [PubMed: 18493650]
499. Tagalakis AD, Mcanulty RJ, Devaney J, Bottoms SE, Wong JB, Elbs M, Writer MJ, Hailes HC, Tabor AB, O'callaghan C, Jaffe A, Hart SL. Mol. Ther. 2008; 16:907. [PubMed: 18388925]
500. Manunta MDI, Mcanulty RJ, Tagalakis AD, Bottoms SE, Campbell F, Hailes HC, Tabor AB, Laurent GJ, O'callaghan C, Hart SL. PLoS One. 2011; 6:e26768. [PubMed: 22046351]
501. Costantini TW, Putnam JG, Sawada R, Baird A, Loomis WH, Eliceiri BP, Bansal V, Coimbra R. Surgery. 2009; 146:206. [PubMed: 19628075]
502. Kumar S, Sahdev P, Perumal O, Tummala H. Mol.Pharmaceutics. 2012; 9:1320.
503. Ardelt PU, Wood CG, Chen L, Mintz PJ, Moya C, Arap MA, Wright KC, Pasqualini R, Arap W. J. Urol. 2003; 169:1535. [PubMed: 12629410]
504. Chen Y, Shen Y, Guo X, Zhang C, Yang W, Ma M, Liu S, Zhang M, Wen LP. Nat. Biotechnol. 2006; 24:455. [PubMed: 16565728]
505. Lee SJ, Park S, Chung HM, Choi JS, Kim DD, Sung JH. Int. J. Pept. Res. Ther. 2009; 15:281.
506. Li B, Russell SJ, Compaan DM, Totpal K, Marsters SA, Ashkenazi A, Cochran AG, Hymowitz SG, Sidhu SS. J. Mol.Biol. 2006; 361:522. [PubMed: 16859704]
507. Itahana K, Zhang Y. Cancer Cell. 2008; 13:542. [PubMed: 18538737]
508. Prokop A, Davidson JM. J. Pharm. Sci. 2008; 97:3518. [PubMed: 18200527]
509. Van Hensbergen Y, Broxterman HJ, Elderkamp YW, Lankelma J, Beers JC, Heijn M, Boven E, Hoekman K, Pinedo HM. Biochem. Pharmacol. 2002; 63:897. [PubMed: 11911842]
510. Meyer-Losic F, Quinonero J, Dubois V, Alluis B, Dechambre M, Michel M, Cailler F, Fernandez AM, Trouet A, Kearsey J. J. Med. Chem. 2006; 49:6908. [PubMed: 17154520]
511. Kratz F, Müller IA, Ryppa C, Warnecke A. ChemMedChem. 2008; 3:20. [PubMed: 17963208]
512. Jain RK. J. Controlled Release. 1998; 53:49.
513. Torchilin VP, Lukyanov AN. Drug Discovery Today. 2003; 8:259. [PubMed: 12623240]
514. Allen TM, Cheng WW, Hare JI, Laginha KM. Anticancer Agents Med. Chem. 2006; 6:513. [PubMed: 17100556]
515. Torchilin VP. Nat. Rev. Drug Discovery. 2005; 4:145.
516. Owens DE 3rd, Peppas NA. Int. J. Pharm. 2006; 307:93. [PubMed: 16303268]
517. Chang HI, Yeh MK. Int. J. Nanomed. 2012; 2:49.
518. Duggan S, Keating G. Drugs. 2011; 71:2531. [PubMed: 22141391]
519. Yuan F, Leunig M, Huang SK, Berk DA, Papahadjopoulos D, Jain RK. Cancer Res. 1994; 54:3352. [PubMed: 8012948]
520. Vicent MJ, Duncan R. Trends Biotechnol. 2006; 24:39. [PubMed: 16307811]
521. Sutton D, Nasongkla N, Blanco E, Gao J. Pharm. Res. 2007; 24:1029. [PubMed: 17385025]
522. Torchilin VP. J. Controlled Release. 2001; 73:137.
523. Matsumura Y, Kataoka K. Cancer Sci. 2009; 100:572. [PubMed: 19462526]
524. Zhou X, Chang YC, Oyama T, Mcguire MJ, Brown KC. J. Am. Chem. Soc. 2004; 126:15656. [PubMed: 15571383]
525. Garde SV, Forte AJ, Ge M, Lepekhin EA, Panchal CJ, Rabbani SA, Wu JJ. Anti-Cancer Drugs. 2007; 18:1189. [PubMed: 17893520]
526. Jayanna PK, Torchilin VP, Petrenko VA. Nanomedicine. 2009; 5:83. [PubMed: 18838343]
527. Rey FE, Cifuentes ME, Kiarash A, Quinn MT, Pagano PJ. Circ. Res. 2001; 89:408. [PubMed: 11532901]
528. Corti A, Pastorino F, Curnis F, Arap W, Ponzoni M, Pasqualini R. Med. Res. Rev. 2012; 32:1078. [PubMed: 21287572]
529. Van Broekhoven CL, Parish CR, Demangel C, Britton WJ, Altin JG. Cancer Res. 2004; 64:4357. [PubMed: 15205352]
530. Reddy ST, Swartz MA, Hubbell JA. Trends Immunol. 2006; 27:573. [PubMed: 17049307]

531. Proudfoot O, Apostolopoulos V, Pietersz GA. Mol.Pharmaceutics. 2007; 4:58.
532. Van Broekhoven CL, Altin JG. Int. J. Cancer. 2002; 98:63. [PubMed: 11857387]
533. Bonifaz LC, Bonnyay DP, Charalambous A, Darguste DI, Fujii S, Soares H, Brimnes MK, Moltedo B, Moran TM, Steinman RM. J. Exp. Med. 2004; 199:815. [PubMed: 15024047]
534. Castro FV, Tutt AL, White AL, Teeling JL, James S, French RR, Glennie MJ. Eur. J. Immunol. 2008; 38:2263. [PubMed: 18651710]
535. Larocca D, Burg MA, Jensen-Pergakes K, Ravey EP, Gonzalez AM, Baird A. Curr. Pharm. Biotechnol. 2002; 3:45. [PubMed: 11883506]
536. Larocca D, Jensen-Pergakes K, Burg MA, Baird A. Mol.Ther. 2001; 3:476. [PubMed: 11319907]
537. Larocca D, Jensen-Pergakes K, Burg MA, Baird A. Methods Mol. Biol. 2002; 185:393. [PubMed: 11769003]
538. Larocca D, Kassner PD, Witte A, Ladner RC, Pierce GF, Baird A. FASEB J. 1999; 13:727. [PubMed: 10094933]
539. Larocca D, Witte A, Johnson W, Pierce GF, Baird A. Hum. Gene Ther. 1998; 9:2393. [PubMed: 9829538]
540. Poul MA, Marks JD. J. Mol. Biol. 1999; 288:203. [PubMed: 10329137]
541. Trepel M, Grifman M, Weitzman M, Pasqualini R. Hum.Gene Ther. 2000; 11:1971. [PubMed: 11020797]
542. Makela A, Matilainen H, White D, Ruoslahti E, Oker-Blom C. J. Virol. 2006; 80:6603. [PubMed: 16775347]
543. Mizuguchi H, Hayakawa Y. Hum. Gene Ther. 2004; 15:1034. [PubMed: 15610604]
544. Michelfelder S, Trepel M. Adv. Genet. 2009; 67:29. [PubMed: 19914449]
545. Muller OJ, Kaul F, Weitzman MD, Pasqualini R, Arap W, Kleinschmidt JA, Trepel M. Nat. Biotechnol. 2003; 21:1040. [PubMed: 12897791]
546. Perabo L, Buning H, Kofler DM, Ried MU, Girod A, Wendtner CM, Enssle J, Hallek M. Mol. Ther. 2003; 8:151. [PubMed: 12842438]
547. Denby L, Work LM, Graham D, Hsu C, Von Seggern DJ, Nicklin SA, Baker AH. Hum. Gene Ther. 2004; 15:1054. [PubMed: 15610606]
548. Hajitou A, Lev D, Hannay J, Korchin B, Staquicini F, Soghomonyan S, Alauddin M, Benjamin R, Pollock R, Gelovani J, Pasqualini R, Arap W. Proc. Natl. Acad. Sci. U.S.A. 2008; 105:4471. [PubMed: 18337507]
549. Hajitou A, Rangel R, Trepel M, Soghomonyan S, Gelovani J, Alauddin M, Pasqualini R, Arap W. Nat. Protoc. 2007; 2:523. [PubMed: 17406616]
550. Hajitou A, Trepel M, Lilley C, Soghomonyan S, Alauddin M, Marini F, Restel B, Ozawa M, Moya C, Rangle R, Sun Y, Zaoui K, Schmidt M, Von Kalle C, Weitzman M, Gelovani J, Pasqualini R, Arap W. Cell. 2006; 125:385. [PubMed: 16630824]
551. Soghomonyan S, Hajitou A, Rangel R, Trepel M, Pasqualini R, Arap W, Gelovani J, Alauddin M. Nat. Protoc. 2007; 2:416. [PubMed: 17406603]
552. Tandle A, Hanna E, Lorang D, Hajitou A, Moya CA, Pasqualini R, Arap W, Adem A, Starker E, Hewitt S, Libutti SK. Cancer. 2009; 115:128. [PubMed: 19090007]
553. Paoloni MC, Tandle A, Mazcko C, Hanna E, Kachala S, Leblanc A, Newman S, Vail D, Henry C, Thamm D, Sorenmo K, Hajitou A, Pasqualini R, Arap W, Khanna C, Libutti SK. PLoS One. 2009; 4:1.
554. Hansen JB, Kristiansen K. Biochem. J. 2006; 398:153. [PubMed: 16898874]
555. Klutz K, Schaffert D, Willhauck MJ, Grunwald GK, Haase R, Wunderlich N, Zach C, Gildehaus FJ, Senekowitsch-Schmidtke R, Goke B, Wagner E, Ogris M, Spitzweg C. Mol.Ther. 2011; 19:676. [PubMed: 21245850]
556. Luo J, Zhang H, Xiao W, Kumaresan PR, Shi C, Pan CX, Aina OH, Lam KS. J. Comb. Chem. 2008; 10:599. [PubMed: 18558750]
557. Deutscher SL. Chem. Rev. 2010; 110:3196. [PubMed: 20170129]
558. Becker A, Hessenius C, Licha K, Ebert B, Sukowski U, Semmler W, Weiedenmann B, Grotzinger C. Nat. Biotechnol. 2001; 19:327. [PubMed: 11283589]

559. Pierce M, Javier D, Richards-Kortum R. Int. J. Cancer. 2008; 123:1979. [PubMed: 18712733]
560. Park JH, Von Maltzahn G, Ruoslahti E, Bhatia S, Sailor M. Angew. Chem. Int. Ed. 2008; 47:7284.
561. Kelly K, Bardeesy N, Anbazhagan R, Gurumurthy S, Berger J, Alencar H, Depinho R, Mahmood U, Weissleder R. PLoS Med. 2008; 5:657.
562. Ametamey SM, Honer M, Schubiger PA. Chem. Rev. 2008; 108:1501. [PubMed: 18426240]
563. Van Dongen G, Visser G, Lub-De Hooge M, De Vreis E, Perk L. Oncologist. 2007; 12:1379. [PubMed: 18165614]
564. Okarvi SM. Cancer Treat. Rev. 2008; 34:13. [PubMed: 17870245]
565. Okarvi SM. Med. Res. Rev. 2004; 24:357. [PubMed: 14994368]
566. Reubi J, Maecke H. J. Nucl. Med. 2008; 49:1735. [PubMed: 18927341]
567. Wangler C, Buchmann I, Eisenhut M, Haberkorn U, Mier W. Protein Pept. Lett. 2007; 14:273. [PubMed: 17346233]
568. Lee S, Xie J, Chen X. Biochemistry. 2010; 49:1364. [PubMed: 20102226]
569. Wang H, Chen K, Cai W, Li Z, He L, Kashefi A, Chen X. Mol. Cancer Ther. 2008; 7:1044. [PubMed: 18483294]
570. Liu S. Mol. Pharmaceutics. 2006; 3:472.
571. Dijkgraaf I, Beer AJ, Wester HJ. Front. Biosci. 2009; 14:887.
572. Kenny L, Coombes R, Oulie I, Contractor K, Miller M, Spinks T, Mcparkland B, Cohen P, Hui A, Palmieri C, Osman S, Glaser M, Turton D, Al-Nahhas A, Aboagye E. J. Nucl. Med. 2008; 49:879. [PubMed: 18483090]
573. Zhao D, Jin X, Li F, Liang J, Lin Y. J. Nucl. Med. 2012; 53:1872. [PubMed: 23071350]
574. Zhu Z, Miao W, Li Q, Dai H, Ma Q, Wang F, Yang A, Jia B, Jing X, Liu S, Shi J, Liu Z, Zhao Z, Wang F, Li F. J. Nucl.Med. 2012; 53:716. [PubMed: 22499615]
575. Aloj L, Morelli G. Curr. Pharm. Des. 2004; 10:3009. [PubMed: 15379665]
576. Vegt E, De Jong M, Wetzels J, Masereeuw R, Melis M, Oyen W, Gotthardt M, Boerman O. J. Nucl. Med. 2010; 51:1049. [PubMed: 20554737]
577. Gotthardt M, Eerd-Vismale J, Oyen W, De Jong M, Zhang H, Rolleman E, Maecke H, Behe M, Boerman O. J. Nucl.Med. 2007:596. [PubMed: 17401097]
578. Gagnon M, Hausner S, Marik J, Abbey C, Marshall J, Sutcliffe J. Proc. Natl. Acad. Sci. U.S.A. 2009; 106:17904. [PubMed: 19815497]
579. Laurent S, Forge D, Port M, Roch A, Robic C, Elst L, Muller R. Chem. Rev. 2008; 108:2064. [PubMed: 18543879]
580. Corot C, Robert P, Idee JM, Port M. Adv. Drug DeliveryRev. 2006; 58:1471.
581. Huang G, Zhang C, Li S, Khemtong C, Yang SG, Tian R, Brown KC, Gao J. J. Mater. Chem. 2009; 19:6367. [PubMed: 20505790]
582. Falciani C, Lozzi L, Pini A, Bracci L. Chem. Biol. 2005; 12:417. [PubMed: 15850978]
583. Bracci L, Falciani C, Lelli B, Lozzi L, Runci Y, Pini A, De Montis MG, Tagliamonte A, Neri P. J. Biol. Chem. 2003; 278:46590. [PubMed: 12972419]
584. Falciani C, Lozzi L, Pini A, Corti F, Fabbrini M, Bernini A, Lelli B, Niccolai N, Bracci L. Chem. Biol. Drug Des. 2007; 69:216. [PubMed: 17441908]
585. Pini A, Falciani C, Bracci L. Curr. Protein Pept. Sci. 2008; 9:468. [PubMed: 18855698]
586. Bastings MMC, Helms BA, Van Baal I, Hackeng TM, Merkx M, Meijer EW. J. Am. Chem. Soc. 2011; 133:6636. [PubMed: 21473586]
587. Helms BA, Reulen SWA, Nijhuis S, De Graaf-Heuvelmans PTHM, Merkx M, Meijer EW. J. Am. Chem. Soc. 2009; 131:11683. [PubMed: 19642697]
588. Mammen M, Choi SK, Whitesides GM. Angew. Chem. Int. Ed. 1998; 37:2755.
589. Kim Y, Lillo AM, Steiniger SCJ, Liu Y, Ballatore C, Anichini A, Mortarini R, Kaufmann GF, Zhou B, Felding-Habermann B, Janda KD. Biochemistry. 2006; 45:9434. [PubMed: 16878978]
590. Reynolds F, Panneer N, Tutino CM, Michael W, Skrabal WR, Moskaluk C, Kelly KA. PLoS One. 2011; 6:1.

591. Joyce J, Laakkonen P, Bernasconi M, Bergers G, Ruoslahti E, Hanahan D. Cancer Cell. 2003; 4:393. [PubMed: 14667506]

592. See ref 73.

593. Kolonin MG, Bover L, Sun J, Zurita AJ, Do KA, Lahdenranta J, Cardo-Vila M, Giordano RJ, Jaalouk DE, Ozawa MG, Moya CA, Souza GR, Staquicini FI, Kunyiasu A, Scudiero DA, Holbeck SL, Sausville EA, Arap W, Pasqualini R. Cancer Res. 2006; 66:34. [PubMed: 16397212]

594. Kapoor P, Singh H, Gautam A, Chaudhary K, Kumar R, Raghava GPS. PLoS One. 2012; 7:e35187. [PubMed: 22523575]

595. Ru B, Huang J, Dai P, Li S, Xia Z, Ding H, Lin H, Guo F, Wang X. Molecules. 2010; 15:8279. [PubMed: 21079566]

596. Shtatland T, Guettler D, Kossodo M, Pivovarov M, Weissleder R. BMC Bioinf. 2007; 8:280.

597. Duchrow T, Shtatland T, Guettler D, Pivovarov M, Kramer S, Weissleder R. BMC Bioinf. 2009; 10:317.

598. Jain RK. Adv. Drug Delivery Rev. 2012; 64:353.

599. Reardon D, Nabors L, Stupp R, Mikkelsen T. Expert Opin.Invest. Drugs. 2008; 17:1225.

600. Minchinton AI, Tannock IF. Nat. Rev. Cancer. 2006; 6:583. [PubMed: 16862189]

601. Rudnick SI, Lou J, Shaller CC, Tang Y, Klein-Szanto AJP, Weiner LM, Marks JD, Adams GP. Cancer Res. 2011; 71:2250. [PubMed: 21406401]

602. Rudnick SI, Adams GP. Cancer Biother. Radiopharm. 2009; 24:155. [PubMed: 19409036]

603. Ruoslahti E. Adv. Mater. 2012; 24:3747. [PubMed: 22550056]

604. Bae YH, Park K. J. Controlled Release. 2011; 153:198.

605. Jain RK. Adv. Drug Delivery Rev. 2001; 46:149.

606. Thurber G, Schmidt M, Wittrup K. Adv. Drug Delivery Rev. 2008; 60:1421.

607. Thurber G, Schmidt M, Wittrup K. Trends Pharmacol. Sci. 2008; 29:57. [PubMed: 18179828]

608. Pastorino F, Brignole C, Marimpietri D, Cilli M, Gambini C, Ribatti D, Longhi R, Allen T, Corti A, Ponzoni M. Cancer Res. 2003; 63:7400. [PubMed: 14612539]

609. Chang DK, Lin CT, Wu CH, Wu HC. PLoS ONE. 2009; 4:e4171. [PubMed: 19137069]

610. Kirpotin D, Drummond D, Shao Y, Shalaby R, Hong K, Nielsen U, Marks J, Benz CC, Park JW. Cancer Res. 2006; 66:6732. [PubMed: 16818648]

611. Mamot C, Drummond D, Noble C, Kallab V, Guo Z, Hong K, Kirpotin D, Park JW. Cancer Res. 2005; 65:11631. [PubMed: 16357174]

612. Glaser, V. Genetic Engineering and Biotechnology News. Vol. Vol. 29. New Rochelle, NY: GEN Publishing; 2009.

613. Ladner R, Sato A, Gorzelany J, De Souza M. DrugDiscovery Today. 2004; 9:525.

614. Nestor, J. Comprehensive Medicinal Chemistry II. Taylor, J.; Triggle, D., editors. Vol. Vol.2. Oxford, U.K.: Elsevier Ltd.; 2007. p. 573

615. Otvos, L. Peptide-Based Drug Design. Otvos, L., editor. Vol. Vol. 494. New York: Humana Press; 2008. p. 1

616. Vlieghe P, Lisowski V, Martinez J, Khrestchatisky M. DrugDiscovery Today. 2010; 15:40.

617. Albericio F, Kruger HG. Future Med. Chem. 2012; 4:1527. [PubMed: 22917241]

618. Ahrens VM, Bellmann-Sickert K, Beck-Sickinger AG. Future Med. Chem. 2012; 4:1567. [PubMed: 22917246]

619. Kratz F. J. Controlled Release. 2008; 132:171.

620. Dennis MS, Zhang M, Meng YG, Kadkhodayan M, Kirchhofer D, Combs D, Damico LA. J. Biol. Chem. 2002; 277:35035. [PubMed: 12119302]

621. Mcgregor D. Curr. Opin. Pharmacol. 2008; 8:616. [PubMed: 18602024]

622. Lu Y, Yang J, Sega E. AAPS J. 2006; 8:E466. [PubMed: 17025264]

623. Malik D, Baboota S, Ahuja A, Hasan S, Ali J. Curr. DrugDelivery. 2007; 4:141.

624. Antosova Z, Mackova M, Kral V, Macek T. TrendsBiotechnol. 2009; 27:628.

625. Bray BL. Nat. Rev. Drug Discovery. 2003; 2:587.

626. Cines D, Yasothan U, Kirkpatrick P. Nat. Rev. DrugDiscovery. 2008; 7:887.

627. Frampton J, Lyseng-Williamson K. Drugs. 2009; 69:307. [PubMed: 19275274]

628. Bussel J, Kuter D, George J, Mcmillan R, Aledort L, Conklin G, Lichtin A, Lyons R, Nieva J, Wasser J, Wiznitzer I, Kelly R, Chen CF, Nichol J. N. Engl. J. Med. 2006; 355:1672. [PubMed: 17050891]

629. Cwirla SE, Balasubramanian P, Duffin DJ, Wagstrom CR, Gates CM, Singer SC, Davis AM, Tansik RL, Mattheakis LC, Boytos CM, Schatz PJ, Baccanari DP, Wrighton NC, Barrett RW, Dower WJ. Science. 1997; 276:1696. [PubMed: 9180079]

630. Oliner J, Min H, Leal J, Yu D, Rao S, You E, Tang X, Kim H, Meyer S, Han SJ, Hawkins N, Rosenfeld R, Davy E, Graham K, Jacobsen F, Stevenson S, Ho J, Chen Q, Hartmann T, Michaels M, Kelley M, Li L, Sitney K, Martin F, Sun JR, Zhang N, Lu J, Estrada J, Kumar R, Coxon A, Kaufman S, Pretorius J, Scully S, Cattley R, Payton M, Coats S, Nguyen L, Desilva B, Ndifor A, Hayward I, Radinsky R, Boone T, Kendall R. Cancer Cell. 2004; 6:507. [PubMed: 15542434]

631. Hebst R, Hong D, Chap L, Kurzrock R, Jackson E, Silverman J, Rasmussen E, Sun YN, Zhong D, Hwang Y, Evelhoch J, Oliner J, Le N, Rosen L. J. Clin. Oncol. 2009; 27:3557. [PubMed: 19546406]

632. Karlan BY, Oza AM, Richardson GE, Provencher DM, Hansen VL, Buck M, Chambers SK, Ghatage P, Pippitt CH, Brown JV, Covens A, Nagarkar RV, Davy M, Leath CA, Nguyen H, Stepan DE, Weinreich DM, Tassoudji M, Sun Y-N, Vergote IB. J. Clin. Oncol. 2012; 30:362. [PubMed: 22184370]

633. Wrighton NC, Farrell F, Cchang R, Kashyap A, Barbone F, Mulcahy L, Hjohnson D, Barrett RW, Jolliffe L, Dower WJ. Science. 1996; 273:458. [PubMed: 8662529]

634. Wrighton NC, Balasubramanian P, Barbone F, Kashyap A, Farrell F, Jolliffe L, Barrett RW, Dower WJ. Nat. Biotechnol. 1997; 15:1261. [PubMed: 9359108]

635. Gregorc V, Santoro A, Bennicelli E, Punt C, Citterio G, Timmer-Bonte J, Caligaris Cappio F, Lambiase A, Bordignon C, Van Herpen C. Br. J. Cancer. 2009; 101:219. [PubMed: 19568235]

636. Bieker R, Kessler T, Schwoppe C, Padro T, Persigehi T, Bremer C, Dreischaluck J, Kolkmeyer A, Heindel W, Mesters R, Berdel W. Blood. 2009; 113:5019. [PubMed: 19179306]

637. Kim KH, Dmitriev I, O'malley JP, Wang M, Saddekni S, You Z, Preuss MA, Harris RD, Aurigemma R, Siegal GP, Zinn KR, Curiel DT, Alvarez RD. Clin. Cancer Res. 2012; 18:3440. [PubMed: 22510347]

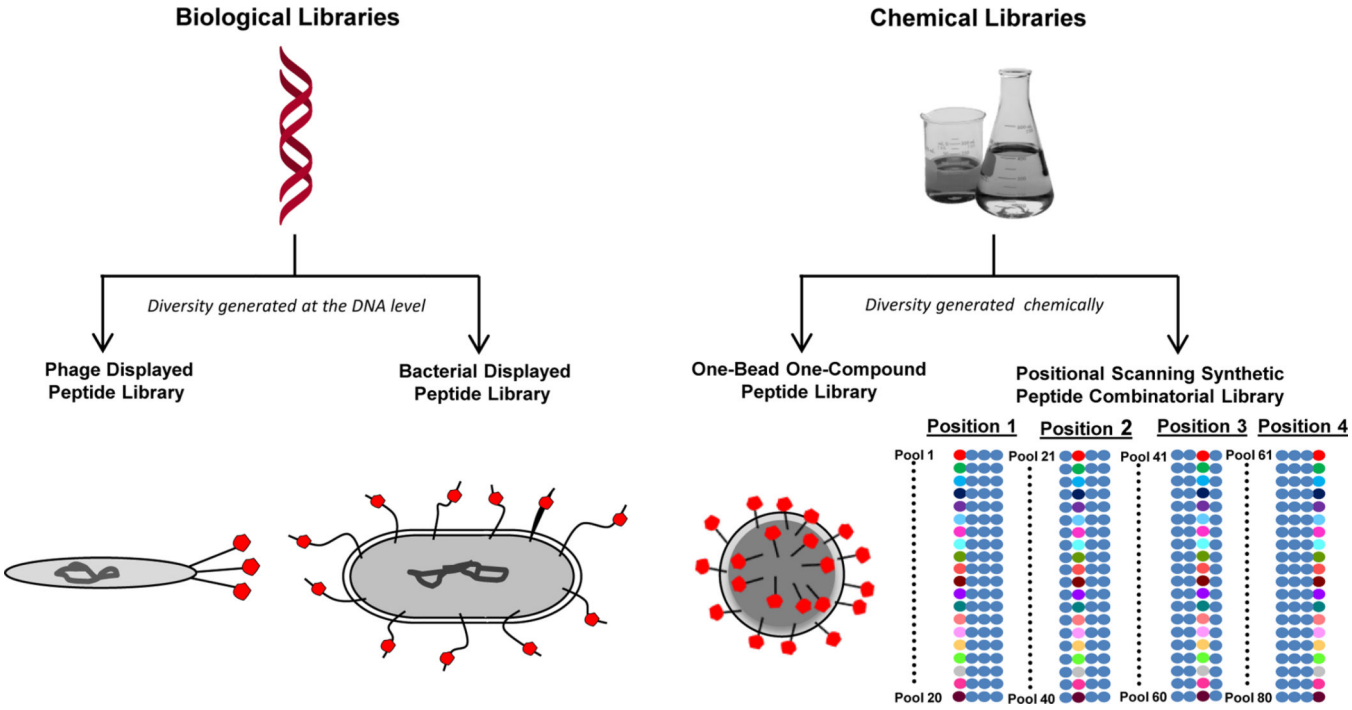

Figure 1.

Peptide libraries used for the selection of cell-binding peptides. Biological and chemical peptide libraries have been used to isolate cell-specific peptides. For phage and bacterial display, the diversity is generated at the DNA level and there is an inherent genotype–phenotype connection. For one-bead one-compound and positional scanning synthetic peptide libraries, the diversity is generated chemically and is based on the use of a collection of monomers. The resultant peptides are displayed in red for clarity. The PS-SPCL schematic illustrates the pools of peptide libraries generated for a tetrameric peptide where each of the 20 amino acids is a unique colored circle and the mixture of 20 amino acids is shown in blue.

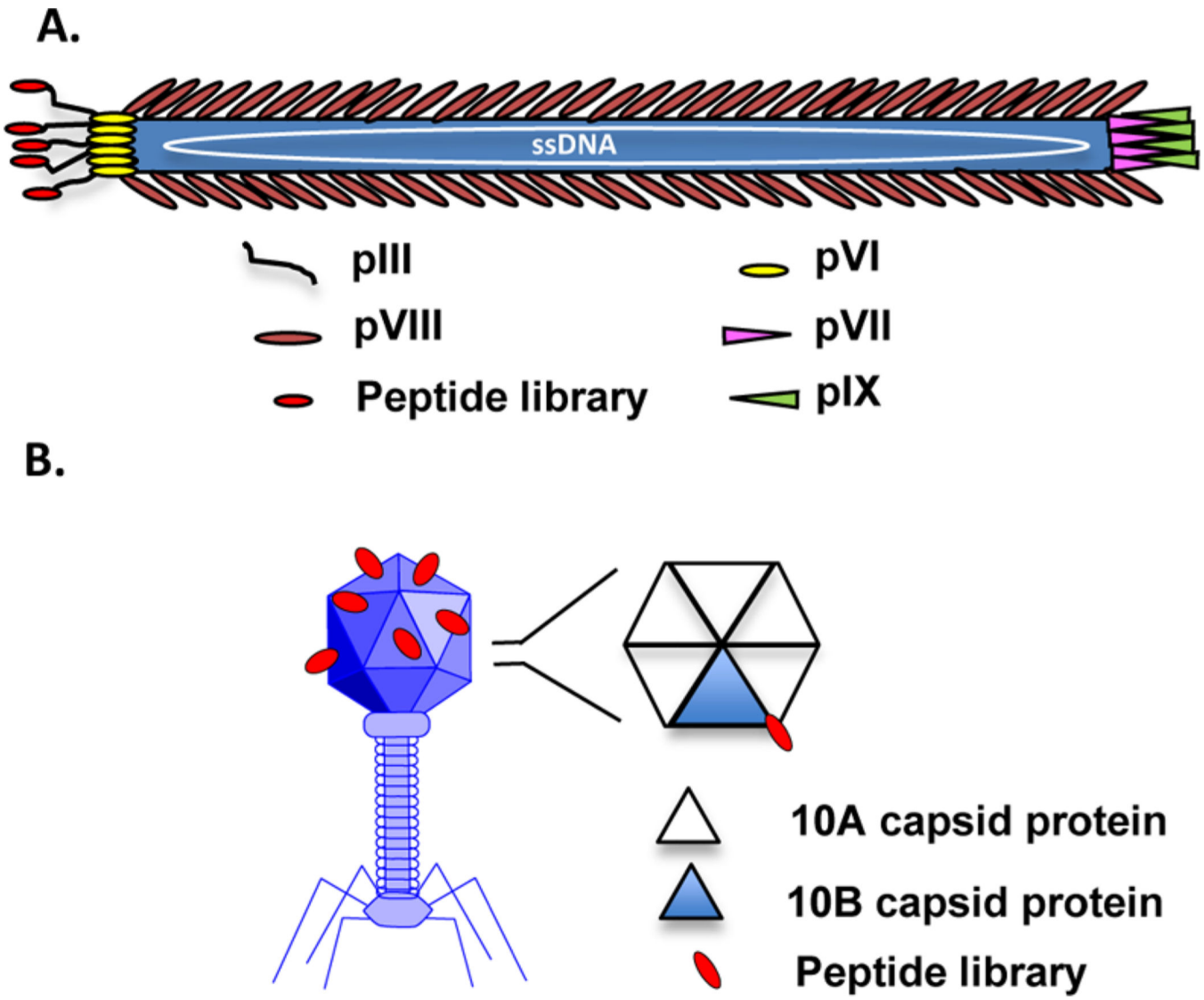

Figure 2.

Filamentous and lytic phage structures. (A) Schematic representation of fd filamentous phage. The random peptide is shown fused to the amino terminus of the pIII coat protein. (B) Representative T7 lytic phage structure. The T7 phage head is comprised of the 10A and 10B capsid proteins arranged as hexamer or pentamer units at a total of 415 proteins per head. A graphical representation of the hexamer capsid unit is shown with a random peptide (red) fused to the 10B protein (blue triangle). T7 phage can be modified to express varying ratios of 10B to 10A protein, displaying peptide sequences in 1–415 copies.

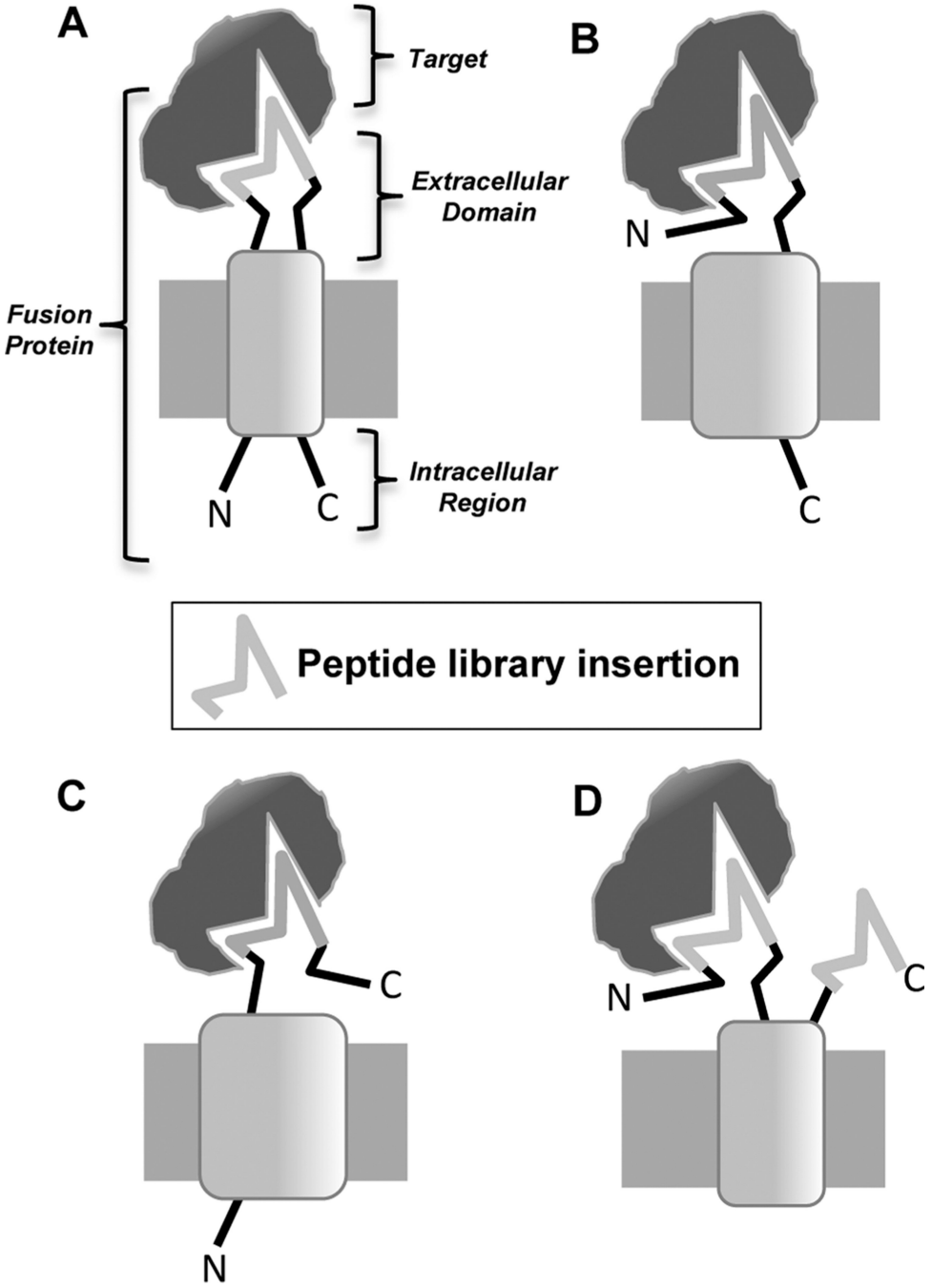

Figure 3.

Different types of bacterial display peptide libraries. Peptide libraries, including FliTrx, OmpA, CPX, and invasin libraries, have been incorporated into bacterial membrane proteins at different locations as shown in the four panels. (A) Peptide library insertion into the middle of the membrane protein. (B) Display of the peptide library at the N-terminus of the membrane protein. (C) Display of the peptide library at the C-terminus of the membrane protein. (D) Display of the peptide library through a combination of N- and C-terminal display.

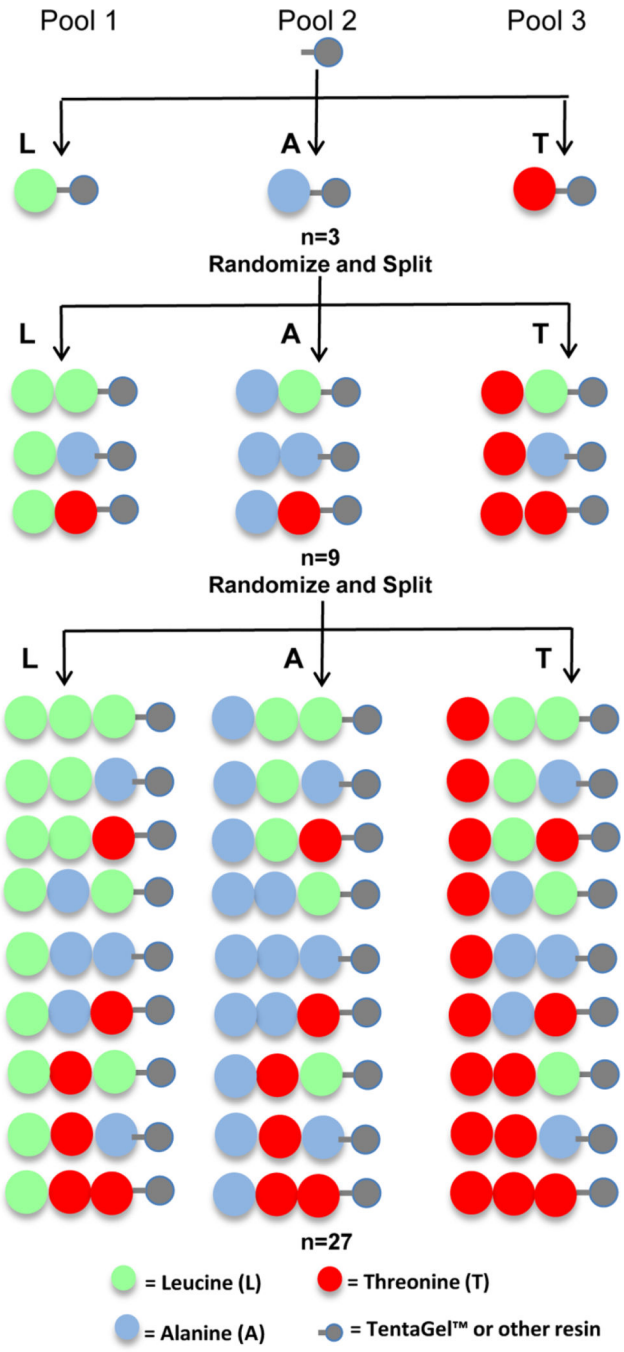

Figure 4.

OBOC peptide library generation using “split-mix” synthesis. An example of split-mix synthesis for tripeptides composed of leucine (L), alanine (A), and threonine (T) is shown. The beads are divided into three different pools, one pool for conjugation to each of the amino acids using standard solid-phase synthesis. Pool 1 is coupled to L, pool 2 to A, and pool 3 to T. The beads from all pools are combined and randomly split into three new pools before a second round of amino acid conjugation. As before, pool 1 beads are coupled to L, pool 2 beads to A, and pool 3 beads to T. Finally, the pools are mixed and randomly sorted

again for another round of amino acid conjugation. This results in a library of bead-bound peptides composed of every combination of the 3 amino acids, totaling 27 different peptide sequences (3^3).

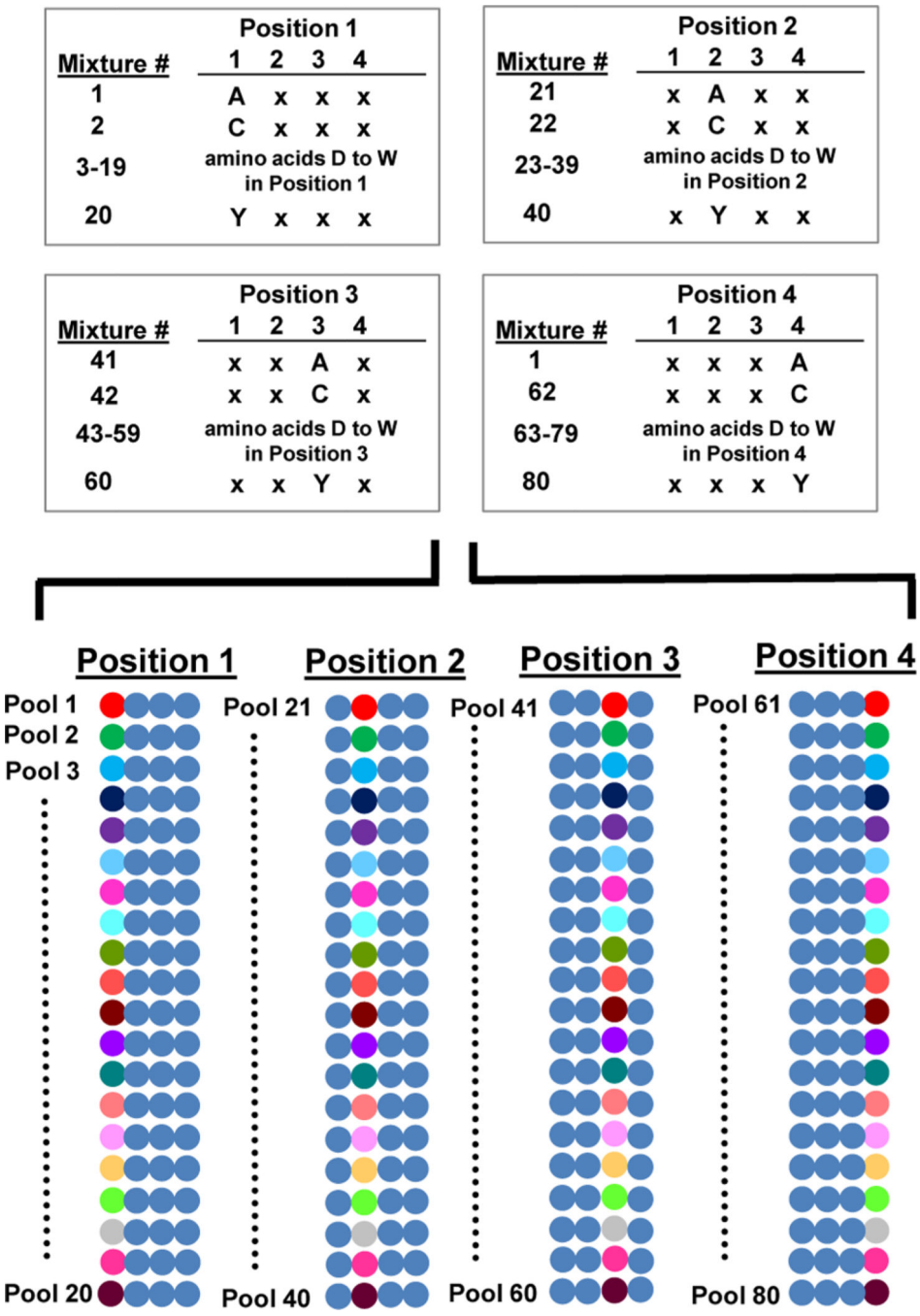

Figure 5.

Design of a PS-SPCL library. Mixture 1 consists of all peptides with a first amino acid of "A", while mixture 2 is all peptides with a first amino acid of "C". Each of mixtures 3–20 displays 1 of the remaining 18 amino acids in the first amino acid position. The next library subset, mixtures 21–40, contains 1 of the 20 amino acids held constant in the second library position. This scanning is continued until each of the tetrapeptide positions has its own pool of libraries. This is represented graphically with each amino acid being represented by a unique colored circle and a mixture of the 20 amino acids being represented as a blue circle.

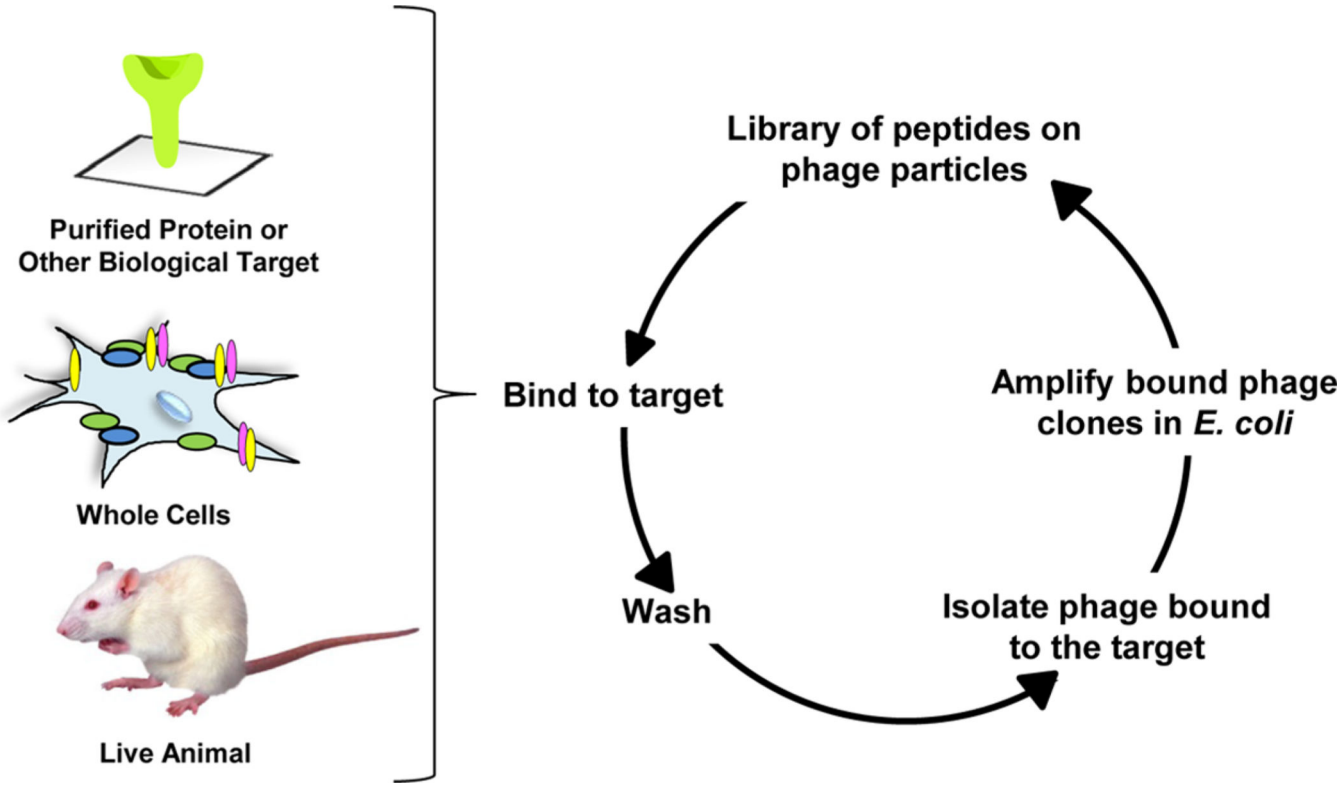

Figure 6.

Panning of phage-displayed peptide libraries. In each case, the phage library is bound to the target, which can be a purified protein, viable cells, or an animal. Nonbinding clones are removed by stringent washes, and phage associated with the target are amplified in *E. coli*. The process is repeated, enriching for binding peptides at each round.

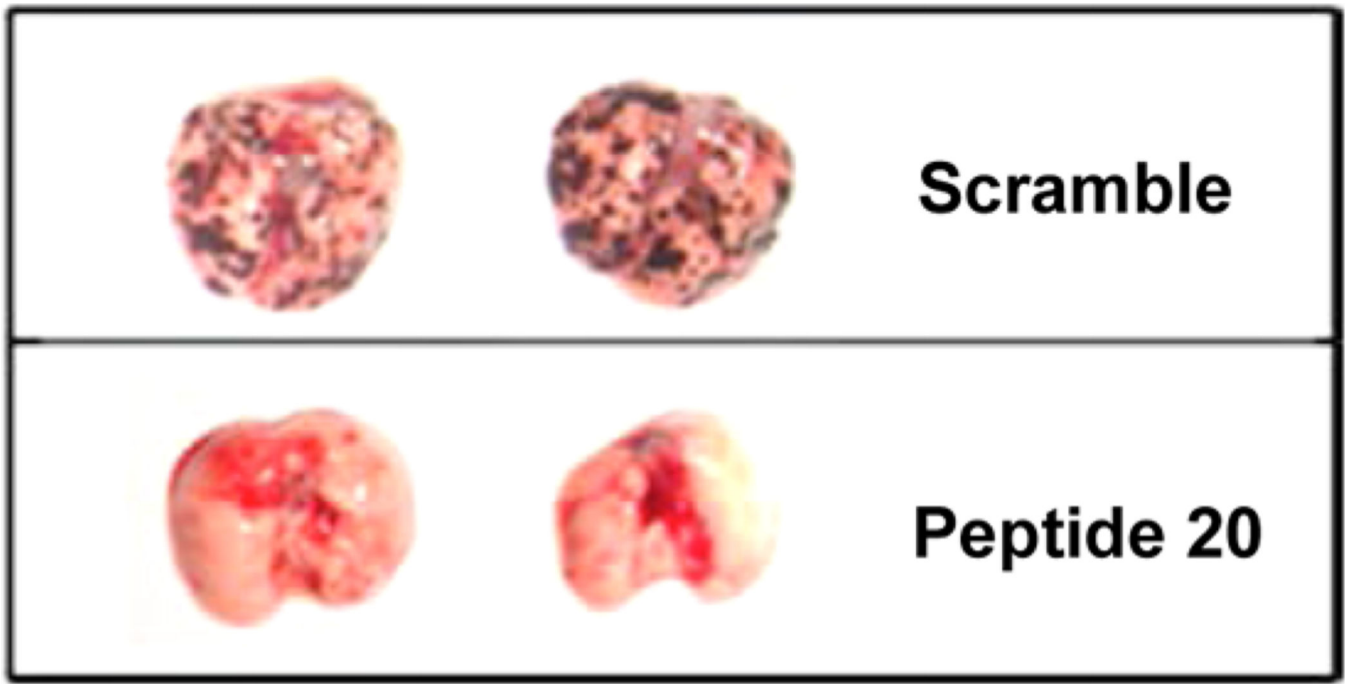

Figure 7.

A phage-display-selected peptide inhibits tumor metastasis. B16F10-Nex 2 cells were injected intravenously into mice, and the mice were treated via an intraperitoneal injection with peptide 20 (CSSRTMHHC), a scrambled control peptide, or buffer. Images of the lungs show a reduction in metastatic nodules (brown spots) in the animals treated with peptide 20. Reprinted with permission from ref 160. Copyright 2010 Springer-Verlag.

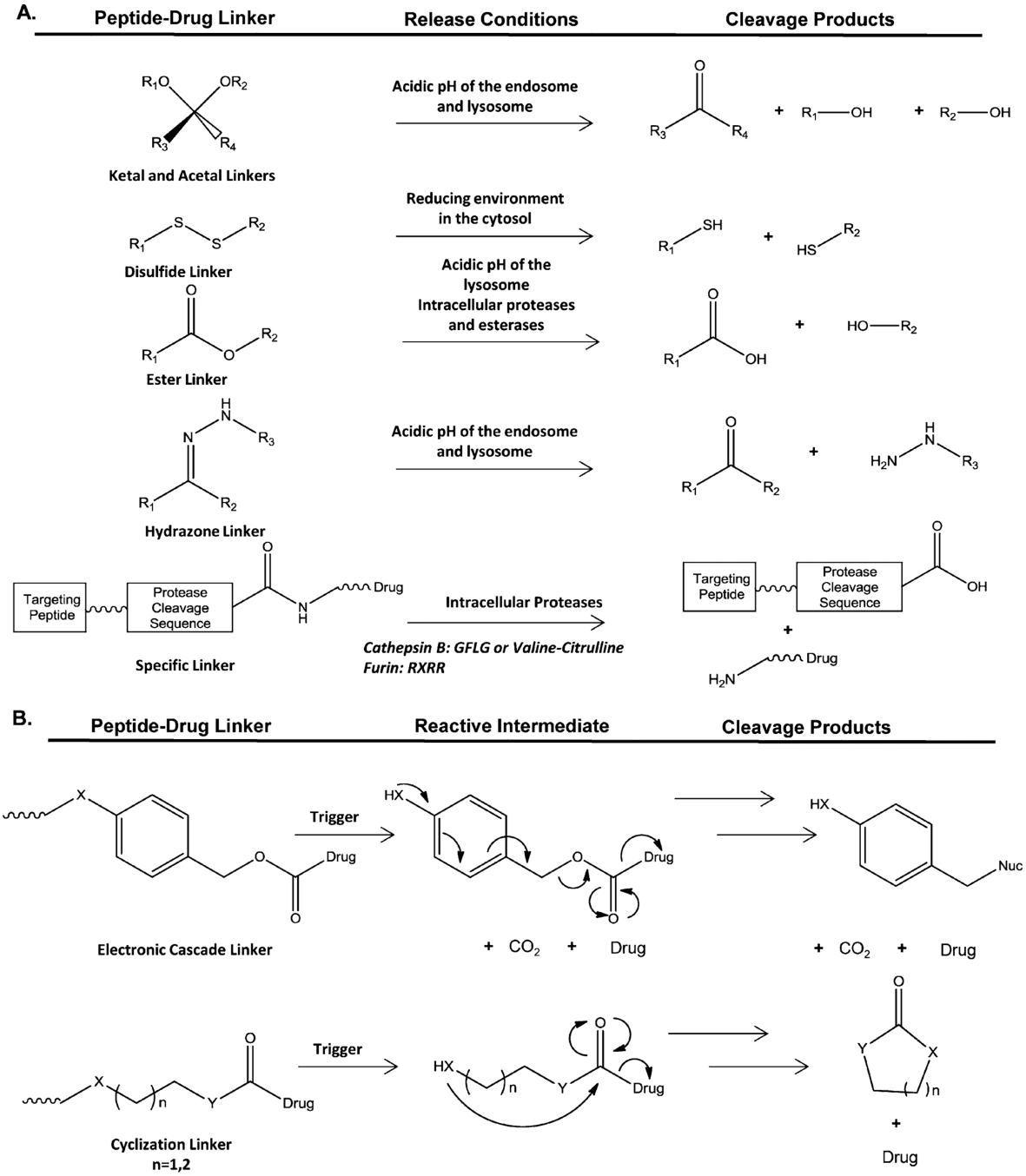

Figure 8.

Structures of common cleavable linkers used to attach drugs to targeting peptides. (A) Cleavable linkers are shown along with the conditions which release the drug from the peptide carrier. The acetal, ketal, and hydrazone linkers require incorporation of reactive moieties not found within the 20 naturally occurring amino acids. (B) Two types of commonly used self-immolative linkers are shown along with the mechanism in which drug is released from the carrier. The release is initiated by a trigger, such as a cleavage reaction shown in panel A, where X = O, S, or NH and Y = CH_2, NR, or O. The drug serves as a

leaving group and is typically attached as an ester or amide. In both cases, the peptide is represented as a squiggly line.

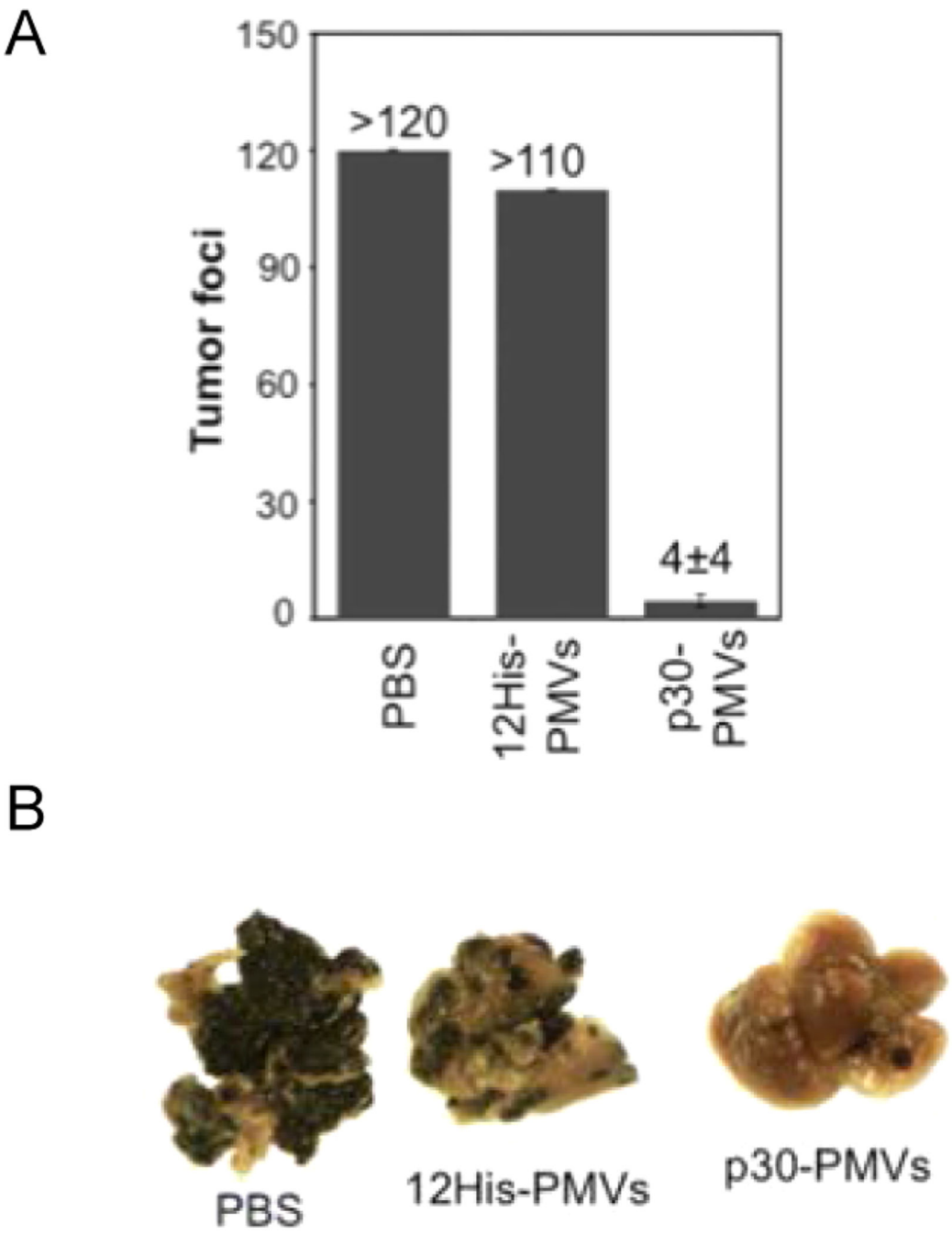

Figure 9.

Vaccination with plasma membrane vehicles modified with the dendritic-cell-specific peptide CGRWSGWPADLC (p30) leads to antitumor activity. Naïve mice were injected iv with B16-OVA cells on day 0. At days 2, 8, and 14 different groups of mice (five mice per group) were vaccinated with PBS or B16-OVA-derived plasma membrane vehicles modified with the control peptide 12His or the dendritic-cell-specific peptide p30. At day 21, the lungs were removed from the mice, and tumor foci were counted via microscopy. (A) Bars indicate the mean number of tumor foci for each vaccination group, and this

number is indicated above each bar. (B) Representative lung images from each vaccination group. Reprinted with permission from ref 391. Copyright 2010 Union for International Cancer Control (UICC).

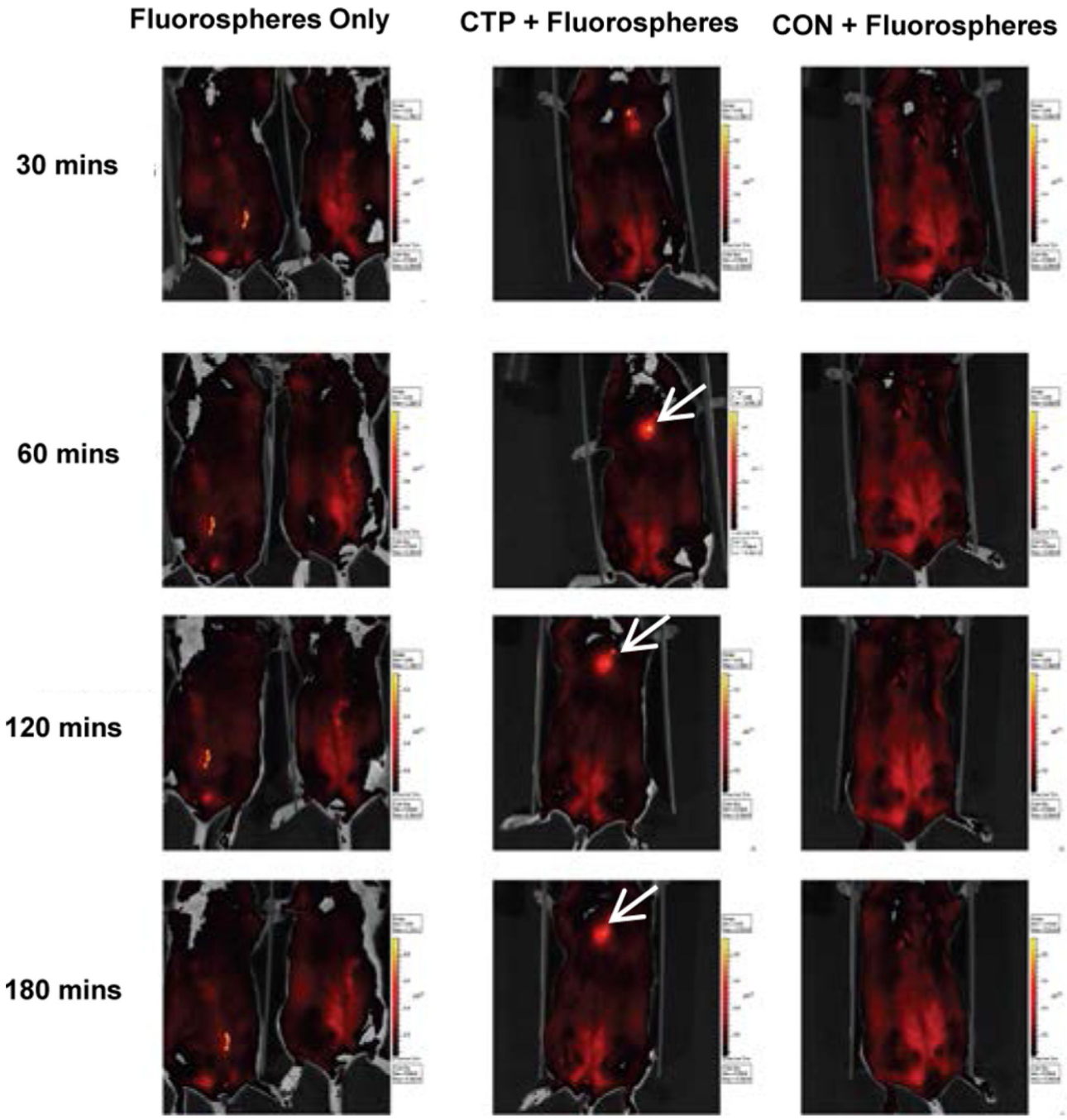

Figure 10.

The CTP peptide allows for specific cardiac imaging. Mice were imaged by in vivo fluorescent imaging following intracardiac injection of fluorospheres alone, CTP peptide–fluorospheres, or control peptide–fluorospheres. Shown are representative images from the three mice per group at different time points postinjection. Heart accumulation, indicated by the arrow, is only observed for the CTP peptide–fluorospheres. Reprinted with permission from ref 358. Copyright 2010 Public Library of Science (PLoS).

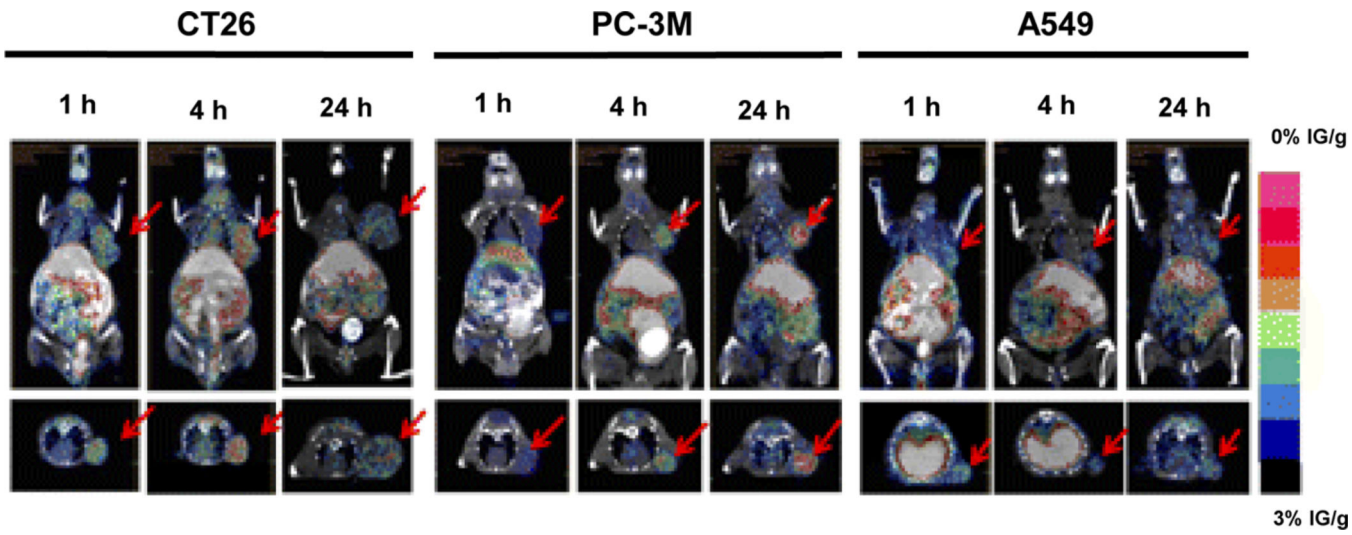

Figure 11.

Small-animal PET/CT imaging of EphB4-positive tumors. The EphB4-binding peptide TNYLFSPNGPIARAW was conjugated to DOTA and loaded with ^{64}Cu. Signal is observed at 4 h in CT-26 and PC-3M but not in the EphB4-negative A549 tumors. Reprinted with permission from ref 125. Copyright 2011 Society of Nuclear Medicine, Inc.

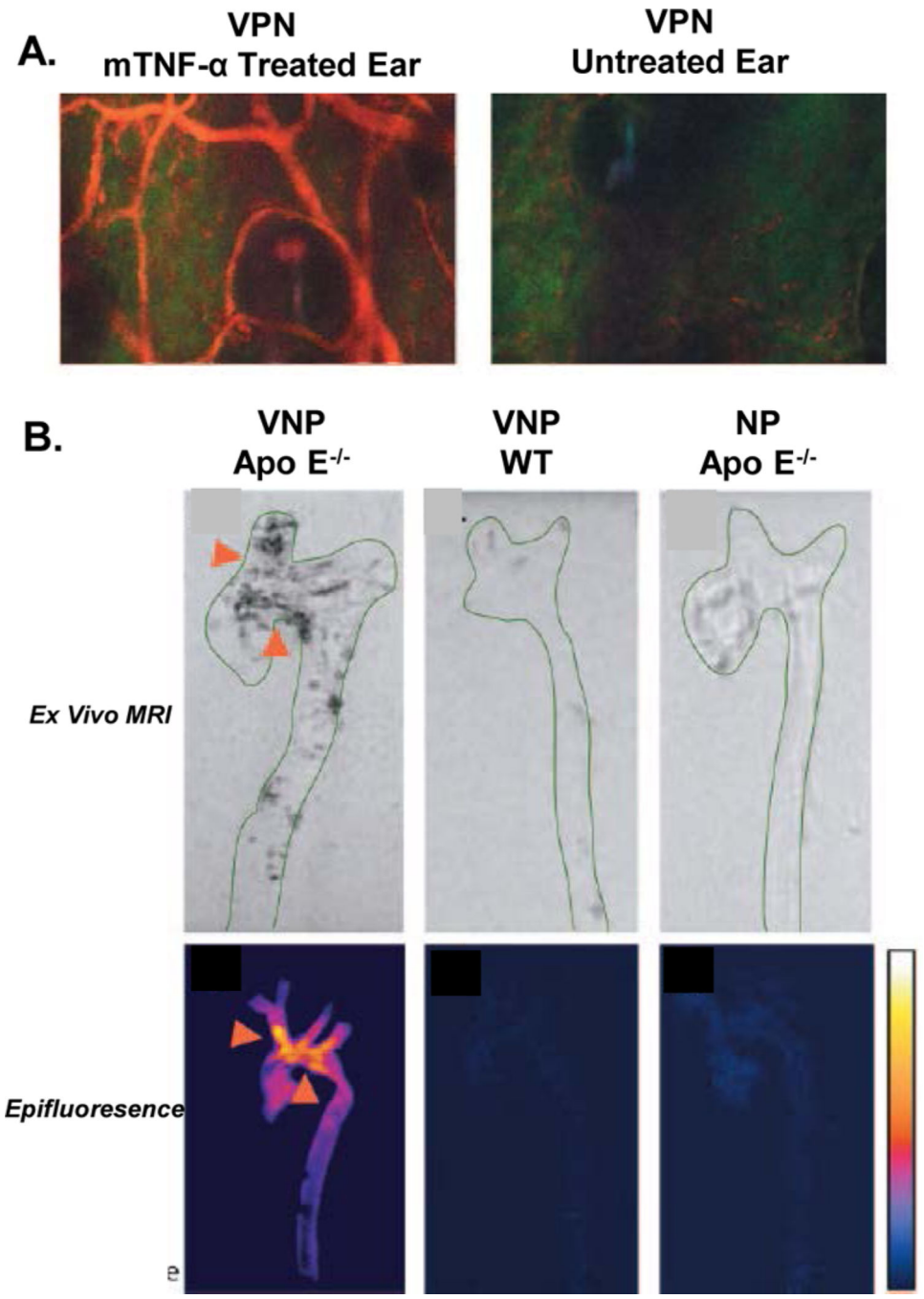

Figure 12.

VCAM-1-specific peptide homes to areas of inflammation and atherosclerotic deposits. (A) The VCAM-1-binding peptide CVHSPNKKCGGSKGK was coupled to a magnetofluorescent nanoparticle (VPN). TNF-α-induced inflammation was induced in one ear of a mouse, while the other was left untreated. Intravital microscopy shows clear accumulation of VPN (red) in the inflamed ear at 4 h, while no binding is observed in the normal control ear. (B) Ex vivo imaging by MR and macroscopic fluorescence at 24 h post intravenous injection detects binding of VPN in animals with atherosclerotic plaques (Apo

$E^{-/-}$ mice). No signal is detected in wild-type animals with no plaque development or when a nontargeted nanoparticle is used. The high accumulation of VPN in the aortic arch is indicated by the arrows. Adapted with permission from ref 365. Copyright 2005 American Heart Association Inc.

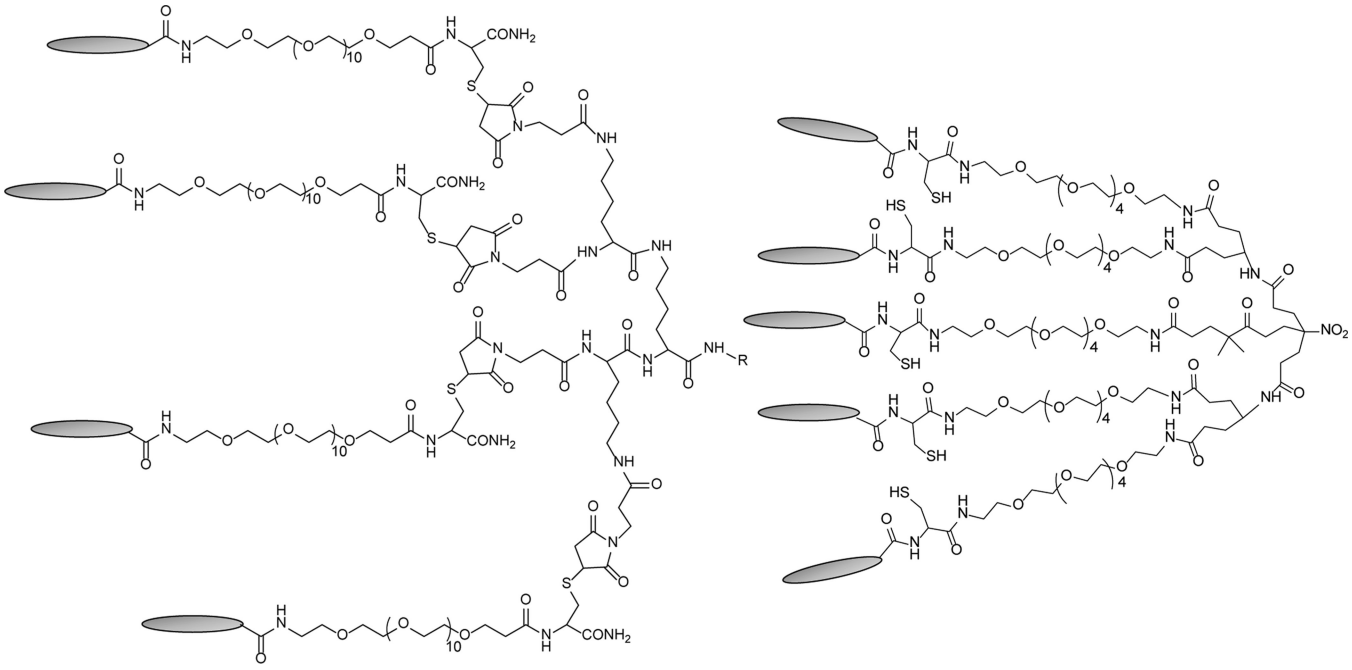

Figure 13.

Structures of two multimeric peptide scaffolds. Multimeric presentation of the targeting peptides mimics the valency and orientation of the phage particle. The tetrameric peptide based on a trilysine core is shown to the left and the pentavalent dendritic wedge on the right. The targeting peptide is shown as a gray oval. A variety of chemical moieties have been attached to the trilysine core structure, indicated by “R” in the figure.

Table 1

Comparison of Different Peptide Libraries

Library Type	Format	Strengths	Weaknesses
Biological Libraries	**Phage display**	• Library sizes of 10^8-10^{11} different peptides • Accommodates large peptide sequences • Ease of library amplification and replication in bacteria • Inexpensive • Libraries are commercially available • Library aliquots can be stored at −80°C for years • Peptide selection can be done on whole cells or in living animals or patients • Phage are amenable to many conditions, including high salt, low pH, and urea containing buffers • Libraries can be constructed and propagated using standard laboratory equipment	• Typically only display natural, L-amino acid peptides although it has recently been shown that unnatural amino acids can be encoded in phage libraries • Complicated structures cannot be incorporated • Clone screening is not quantitative • Two hosts are required, the phage and bacterium • Biological pressures can reduce the diversity of the library
	Bacterial display	• Library sizes of 10^{11} different peptides for *E. coli* libraries • Easily manipulated both genetically and physically • *E. coli* grows quickly • Only one host (the bacterium itself) • Library amplification does not require reinfection • Bacteria can be fluorescently labeled for quantitative and high throughput screening using fluorescence-activated cell sorting (FACS) • Commercially available	• Complex bacterial cell surface can interfere with binding of displayed peptide • Bacteria other than *E. coli* are limited to a library size of 10^5 • Limited to the rate of the flow cytometer for quantitative results • Limited to *in vitro* panning and screening studies • Requires access to a flow cytometer with cell sorting capabilities
Chemical Libraries	**Positional Scanning Synthetic Peptide Combinatorial Library (PS-SPCL)**	• Peptides are their own entity (not bound to support], allowing them to interact in solution for use in any assay • Large number of peptides can be synthesized in a single library	• Libraries are not commercially available • Assumes that each amino acid independently contributes to binding • Requires additional peptide synthesis and

Library Type	Format	Strengths	Weaknesses
		• Library can be aliquoted for use in multiple assays • Not limited to naturally occurring amino acids • Lead peptide sequences can be rapidly optimized	testing after the initial screening
	One-bead one-compound (OBOC)	• Library sizes of 10^6-10^8 compounds • Able to display peptides that contain L-amino acids, D-amino acids, or unnatural amino acids • Peptide selection can be done on whole cells *in vitro* or *ex vivo* • Library synthesis can be completed using standard equipment found in a synthetic chemistry lab	• Peptides are connected to the beads by linkers, with the potential for steric hindrance between the cellular receptor and peptide • Peptide sequences of identified beads have to be determined by Edman sequencing or mass spectrometry, or the beads must be coded • Libraries are not commercially available • Cannot be used to select peptides that internalize *in vitro* • Not available for *in vivo* screening in living animals or patients

Table 2

Cancer-Targeting Peptides Selected in Vitro against Known Protein Targets

Cellular Target	Peptide Sequence[a,b,c,d]	Applications and Notes
HER2/ErbB2	WTGW**C**LNPEESTWGF**C**TGSF[94] (pIII)	***Functional Peptide Activity:*** The peptide inhibits ErbB2 phosphorylation.
	MCGVCLSAQRWT[95] (pIII) SGLWWLGVDILG[95] (pIII)	***Note:*** The peptides were selected as a β-lactamase fusion but neither was tested for activity as free peptides.
	KCCYSL[96] (pIII)	***Imaging Applications:*** Peptide targets HER2 positive tumors *in vivo* and has been employed as a molecular imaging probe.[97–99] MicroSPECT/CT imaging has been performed using ^{111}In-DOTA-peptide and ^{64}Cu-DOTA, NOTA, and CB-TE2A-peptide conjugates.
	MARSGL[100] (pIII) MARAKE[100] (pIII) MSRTMS[100] (pIII)	***Functional Peptide Activity:*** The peptides were fused to a pentameric protein in order to present them in a multimeric fashion. These "peptabodies" bound the extracellular domain of ErbB2 with low nanomolar affinity and bound selectively to ErbB2 expressing cells.
EGFR	YHWYGYTPQNVI[101] (GE11) (pIII)	***Therapeutic Applications:*** The GE11 peptide has been conjugated to doxorubicin and shown to kill EGFR-positive cells *in vitro*.[102] GE11 peptide-Au nanoparticles with phthalocyanine killed cells after irradiation *in vitro*.[103] A GE11-lytic peptide chimera killed cells in culture and inhibited tumor growth in mice.[104] LPEI-PEG-PEI-PEG-GE11 conjugate delivered polyinosine/cytosine to EGFR expressing tumors resulting in a 2-fold increase in the mean survival of the tumor bearing mice.[105] ***Imaging Applications:*** Cy5.5-labeled peptide and Cy5.5-labeled peptide-liposomes have been used to image EGFR positive tumors in mice.[102] The synthesis of [^{64}Cu]Cu-NOTA-Bn-GE11 has been reported but has not been evaluated *in vivo* as a molecular PET agent.[106] Peptide polyplexed with NIS gene specifically transfected cells *in vitro* and homed to EGFR positive tumors in mice, allowing imaging with ^{123}I and inhibition of tumor growth with ^{131}I. ***Oligonucleotide Delivery:*** The peptide has been conjugated to PEI for delivery of a luciferase gene to cells *in vitro* and tumors in mice.[101,107] GE11 modified exosomes delivered miRNA to EGFR-positive tumors in a xenograft breast cancer model.[108] Delivery of let-7a by this method reduced tumor growth. ***Note:*** GE11 undergoes a clatherin mediated endocytosis without activation of EGFR signaling.[109]
IL-6 receptor	LSLITRL[110] (pIII)	***Functional Peptide Activity:*** The peptide itself has anti-angiogenic properties. Injected i.p. into a tumor-bearing mouse, the free peptide inhibited tumor growth.
$\alpha_v\beta_3$	**C**DCRGD**C**F**C**[111,112] (RGD-4C) (pIII)	***Note:*** See Table 5 for a full description of this widely used peptide.
$\alpha_5\beta_1$	GA**C**RGD**C**LGA[113] (pIII)	
$\alpha_6\beta_1$	VSWFSRHRYSPFAVS[114] (pIII) HRWMPHVFAVRQGAS[114] (pIII) FGRIPSPLAYTYSFR[114] (pIII)	
$\alpha_v\beta_6$	RTDLDSLRTYTL[115] (pIII)	***Note:*** The peptide has been utilized as a targeting agent for a chimeric antigen receptor and used to redirect cytotoxic T cells to $\alpha_v\beta_6$-positive ovarian cancer cells in culture.[116]
EphA2	YSAYPDSVPMMS[117] (YSA) (pIII)	***Therapeutic Applications:*** A modified YSA peptide has been used to deliver paclitaxel to prostate and renal tumors *in vivo*.[118] The peptide was conjugated to superparamagentic nanoparticles and used to remove ovarian cancer cells *in vitro* from peritoneal fluid removed by paracentesis, reducing metastasis and increasing survival times in mouse models.[119,120]

Cellular Target	Peptide Sequence[a,b,c,d]	Applications and Notes
		Oligonucleotide Delivery: Peptide functionalized nanogels loaded with EGFR siRNA knocked down EGFR expression *in vitro*.[121] A peptide modified adenovirus with the luciferase gene delivered the gene to cells *in vitro* but was unable to target a pancreatic xenograft tumor in a mouse.[122]
EphB4	TNYLFSPNGPIA[123] (TNYL) (pIII)	***Therapeutic Applications:*** A cyclic version of TNYL was conjugated to a doxorubicin loaded gold nanosphere. A combination of thermal ablation and doxorubicin delivery resulted in tumor regression.[124] ***Imaging Applications:*** The peptide was optimized for binding affinity to EphB4 by the addition of 3 amino acids to the C-terminal side of original peptide (TNYLFSPNGPIARAW, TNYL-RAW). DOTA-labeled TNYL-RAW was used as a molecular PET imaging probe for EphB4-expressing tumors.[125]
MMP-9	**C**TTHWGFTL**C**[126] (CTT) (pIII)	***Therapeutic Applications:*** The CTT peptide conjugated to liposomal doxorubicin increased cell death,[127] and a CTT derivative conjugated to doxorubicin liposomes increased survival in tumor-bearing mice.[128] Conjugation of a vascular endothelial growth inhibitor (VEGI) to the peptide inhibited tumor growth in mice when injected i.p.[129] Fusion of the peptide with kringle 5 fragment of human plasminogen inhibited tumor growth and increased survival in tumor-bearing mice after i.p. injection.[130] ***Imaging Applications:*** The peptide has been used in several different imaging formats.[204, 301–304]. Gamma imaging of tumor-bearing mice given ^{99m}Tc-CTT liposomes encapsulated with ^{125}I-albumin[131] and microPET imaging of tumor-bearing mice given ^{64}Cu-DOTA-CTT[132] have been performed. ***Functional Peptide Activity:*** The peptide itself inhibits cell migration[126] and invasion *in vitro*.[133] When injected adjacent to the tumor or i.p in mouse tumor models, the free peptide inhibited tumor growth and improved survival.[126] A hydrophilic peptide derivative injected via tail vein inhibited tumor growth and improved survival.[133]
TAG-72	FRER**C**DKHPQK**C**TKFL[29] (pVIII hybrid) DPRH**C**QKRVLP**C**PAWL[29] (pVIII hybrid)	***Imaging Applications:*** Peptides were labeled with ^{99m}Tc and used for SPECT/CT imaging of tumors in mice, although the tumor to blood ratio was low for both peptides.
	NPGT**C**KDKWIE**C**LLNG[134] (A3-10) (pVIII hybrid)	***Imaging Applications:*** The A3-10 and A2-6 peptides have been labeled with ^{99m}Tc and used for SPECT imaging.[135] TAG-72 positive tumors are clearly distinguished from tumors that do not express the biomarker.
	GGVS**C**MQTSPV**C**ENNL[136] (A2-6) (pVIII hybrid)	***Functional Peptide Activity:*** Free A2-6 peptide binds TAG-72 positive cells in culture as well as formalin fixed paraffin embedded tumors that are TAG-72 positive.
E-cadherin	SWELYYPLRANL[137] (pIII)	***Functional Peptide Activity:*** Peptide also binds to N-cadherin. Cell adhesion is blocked by free peptide.
N-cadherin	SWTLYTPSGQSK[138] (pIII)	***Functional Peptide Activity:*** Peptide inhibited adhesion and tube formation of HUVECs.
Carbonic anhydrase IX	YNTNHVPLSPKY[139] (CalX-P1) (pIII)	***Note:*** ^{131}I-CAIX-P1 did not show significant accumulation in carbonic anhydrase IX positive tumors *in vivo*.
Galectin-3	ANTP**C**GPYTHD**C**PVKR[140] (G3-C12) (pVIII hybrid) PQNSKIPGPTFLDPH[140] (G3-A9) (pVIII hybrid)	***Imaging Applications:*** G3-C12 peptide has been labeled with ^{111}In and employed as a molecular imaging probe for SPECT/CT & microSPECT/CT imaging of tumors.[141,142]
PSMA	WQPDTAHHWATL[143] (pIII)	***Functional Peptide Activity:*** A dimeric version of the peptide inhibited PSMA peptidase activity.
CD133	LQNAPRS[144] (pIII)	
VEGFR-3	**C**SDSWHYW**C**[145] (pIII)	
Phophatidyl serine	**C**LSYYPSY**C**[90] (pIII)	***Imaging Applications:*** Fluorescein-labeled peptide could detect PS on the cell surface after a single treatment of camptothecin.

Cellular Target	**Peptide Sequence**[a,b,c,d]	**Applications and Notes**
Galactose β1-3 N-acetyl-galactosamine (Thomsen-Friedenreich carbohydrate antigen)	HGRFILPWWYAFSPS[146] (P-30) IVWHRWYAWSPASRI[147,148] (P30-1)	***Imaging Applications:*** P30-1 has been conjugated to NOTA and labeled with ^{64}Cu for PET imaging of TF antigen on MDA-MB-435 tumors.[149] ***Functional Peptide Activity:*** P-30 inhibited aggregation of MDA-MB-435 cells to each other and blocked adhesion to endothelial cells.[150]
Met[e]	YLFSVHWPPLKA[70] (pIII)	***Imaging Applications:*** Radionuclear imaging of tumor-bearing mice injected with ^{125}I-peptide demonstrated low but specific tumor accumulation in a mouse bearing a Met-positive human tumor. ***Functional Peptide Activity:*** The peptide competed with HGF/SF binding to Met receptor.
Hepsin[e]	IPLVVPL[151] (pIII)	***Imaging Applications:*** Peptide was conjugated to fluorescent, cross-linked iron oxide (CLIO) nanoparticle for fluorescence-mediated tomography of tumors in mice.

[a] Cysteine residues that form disulfrde bonds are indicated in bold.

[b] Common peptide names that have been used in the literature are indicated in parenthesis next to the peptide sequence.

[c] The phage display library type is indicated in parenthesis. All phage are filamentous unless noted as T7 phage.

[d] Peptides clustered in the same cell in this table were isolated in the same panning experiment

[e] Indicates that the selection was performed on cells that were transfected to overexpress the target protein.

Table 3

Cancer-Targeting Peptides Selected by Unbiased Panning against Whole Cells

Cancer Type	Cell Line used for Selection[a]	Peptide Sequence[b,c,d,e]	Cellular Receptor	Applications and Notes
Hepatocarcinoma	BEL-7402	TACHQHVRMVRP[152] (pIII)		***Therapeutic Applications:*** The peptide has been employed to deliver gold nanorods to a HepG2 tumor xenograft *in vivo*. Subsequent treatment with a near-infrared laser resulted in photothermal ablation of the tumor.[153]
	SMMC-7721	KSLSRHDHIHHH[154] (pIII)		***Therapeutic Applications:*** A peptide-toxic shock syndrome toxin 1 (TSST-1) fusion protein killed cells *in vitro* and inhibited tumor growth in mice. ***Functional Peptide Activity:*** The peptide inhibited cell migration.
	Mahlavu	SFSIIHTPILPL[155] (pIII)		***Therapeutic Applications:*** Virus-like particles modified with the peptide and loaded with doxorubicin, cisplatin, and 5-fluorouracil, or with an siRNA cocktail against cyclins, or with ricin toxin A-chain all selectively killed cells *in vitro*.[156] Peptide-liposomal doxorubicin inhibited tumor growth in mice.[155]
Melanoma	Me6652/4	**C**TVALPGGYVRV**C**[157] (phagemid, pVII and pIX)	GRP78[157]	***Therapeutic Applications:*** A peptide-taxol conjugate,[157] peptide-doxorubicin prodrug, and peptide-taxol prodrug[158] exhibited cell cytotoxicity *in vitro*.
	B16-F10 (murine)	TRTKLPRLHLQS[159] (pIII)		***Functional Peptide Activity:*** Injection of the phage adjacent to the tumor site inhibited tumor growth in mice.
	B16-F10-Nex2 (murine)	**C**SSRTMHH**C**[160] (pIII)	Cadherins	***Functional Peptide Activity:*** The peptide reduced cell viability and inhibited cell invasion *in vitro*. I.P. injection of the peptide in a tumor bearing mouse decreased metastatic nodules, delayed tumor growth and improved survival.

Cancer Type	Cell Line used for Selection[a]	Peptide Sequence[b,c,d,e]	Cellular Receptor	Applications and Notes
	B16 cells co-cultured with B-1 lymphocytes (murine)	**C**LFMRLAW**C**[161] (pIII)	MUC18	
Prostate	Capan-2 (irradiated)	SHGFSRHSMTLI[162] (pIII)		***Functional Peptide Activity:*** The peptide homed to irradiated tumors *in vivo*. A modest specificity for irradiated Capan-2 tumors over non-irradiated tumors was observed.
	LNCaP	DPRATPGS[163] (pVIII landscape)		***Functional Peptide Activity***: *In vivo*, the peptide increases invasiveness and stimulates MMP-2 production.
	DU-145	FRPNRAQDYNTN[164] (DUP-1) (pIII)		***Therapeutic Applications:*** The peptide has been shown to target tumors *in vivo*.[164,165] A peptide-RNA aptamer- chimera delivered doxorubicin *in vitro* and super paramagnetic iron nanoparticles *in vivo*.[166]
	PC3	DTPYDLTG[167] DTDSHVNL[167] DVVYALSDD[167] (pVIII landscape)		***Therapeutic Applications:*** Landscape phage pVIII coat protein displaying the peptide inserted into liposomal Doxil® specifically killed cells.[168]
	PC-1	GGKRPAR[76] (T7 phage) RIGRPLR[76] (T7 phage)	Neuropilin-1	
Gastric	XGC9811-L4	GRRTRSRRLRRS[169] (pIII)		***Functional Peptide Activity:*** The peptide decreased cell invasion and migration and adherence to Type IV collagen. Cells preincubated with peptide and then implanted orthotopically had decreased incidence of liver metastases.
	GC9811-P	SMSIASPYIALE[170] (pIII)		***Functional Peptide Activity:*** The peptide decreased invasion and adhesion of cells *in vitro*. *In vivo*, i.p delivery of the peptide into mice with peritoneal dissemination models of gastric cancer resulted in reduced metastases to the peritoneum and significantly longer survival.
	HUVEC/SGC701 co-culture	**C**TKNSYLM**C**[171] (GEBP11) (pIII)		***Note:*** Selected to bind to gastric cancer

Cancer Type	Cell Line used for Selection[a]	Peptide Sequence[b,c,d,e]	Cellular Receptor	Applications and Notes
				vasculature, not the actual tumor cells.
Colon	HT29	**C**PIEDRPM**C**[172] (RPMC) (pIII)	$\alpha_5\beta_1$[173]	***Therapeutic Applications:*** The peptide conjugated to $_D$(KLAKLAK)$_2$ selectively killed cells *in vitro*.[172] ***Imaging Applications:*** Gamma imaging of ^{111}In-DOTA-peptide in tumor-bearing mice showed tumor homing.[173] Fluorescence endoscopy of colon cancer in mice using peptide-FITC.[173]
	WiDr	HEWSYLAPYPWF[174] (HEW) (pIII)		***Oligonucleotide Delivery:*** Peptide modified-*lacZ* adenoviruses selectively infected cells *in vitro* but did not home to tumors *in vivo*.[175]
	SW480	VHLGYAT[176] (pIII)		
	T84	**C**QARGDLGKIR**C**[177] (pIII)		
Head and Neck	MDA167Tu	TSPLNIHNGQKL[178] (HN-1) (pIII)		***Therapeutic Applications:*** HN-1 peptide homes to its target tumor *in vivo*.[178] HN-1 peptide was conjugated to an inhibitory peptide that blocks protein kinase C-ε. This chimeric peptide inhibited cell motility, invasion, and proliferation *in vitro* and inhibited tumor growth in mice when injected i.p.[179]
	HNO223	SPRGDLAVLGHKY[180] (HBP-1) (pIII)	$\alpha_v\beta_6$ (suggested)	***Functional Peptide Activity:*** Homes to tumor *in vivo.*
	NPC-TW 04	RLLDTNRPLLPY[181] (pIII)		***Therapeutic Applications:*** Peptide-liposomal doxorubicin inhibited tumor growth in mice.
	Hep-2	**C**RLTGGKGVG**C**[182] (phagemid pvIII hybrid		***Note:*** Hep-2 cells are now known to be contaminated with HeLa cells. This peptide was also isolated on HPV-16 transformed SiHa cells. The cell specificity of this peptide remains in question.
Breast	MDA-MB-231	YQATPARFYTNT[174] (pIII)		***Note:*** Not specific for MDA-MB-231 cells
		CGWMGLEL**C**[174](pIII)		***Note:*** Not specific for MDA-MB-231 cells

Cancer Type	Cell Line used for Selection[a]	Peptide Sequence[b,c,d,e]	Cellular Receptor	Applications and Notes
	SKBR3	LTVSPWY[183] (pIII)	ErbB2[184]	***Therapeutic Applications:*** Conjugation of the peptide to pro-apoptotic α-tocopheryl succinate selectively killed ErbB2 positive cells *in vitro* and reduced initial tumor volumes in a transgenic mouse model of ErbB2-positive breast cancer (i.p. injection).[184] The peptide has been conjugated to daunomycin. This conjugate was selectively internalized into ErbB2 expressing cells, but IC_{50} values did not correlate to ErbB2 levels.[185] ***Imaging Applications:*** A tetrameric far-red fluorescent protein (KatushkaS158A) was used as a scaffold to create an octavalent peptide fluorescent nanoparticle that allowed far-red fluorescent imaging of tumors in mice.[186] Peptide coated magnetic nanoparticles were synthesized but active targeting to ErbB2-positive tumors was modest.[187] Peptide-coated quantum dots targeted ErbB2-positive SKBR3 cells *in vitro*.[188] ***Oligonucleotide Delivery:*** Peptide-antisense oligonucleotide against ErbB2 inhibited ErbB2 gene expression in cells.[183]
		WNLPWYYSVSPT[183] (pIII)		***Note:*** Isolated in a mixed library panning with the LTVSPWY peptide listed above. Binds to a wide panel of cell lines derived from solid tumors.[183]
	MCF-7	DMPGTVLP[189,190] VPTDTDYS[190] VEEGGYIAA[190] DWRGDSMDS[190] (pVIII landscape)	nucleolin[190]	***Therapeutic Applications:*** Landscape phage pVIII coat protein displaying the DMPGTVLP peptide was incorporated into Doxil® for specific cell killing *in vitro*.[189] Similarly, the peptide-coat protein was incorporated into polymeric micelles

Cancer Type	Cell Line used for Selection[a]	Peptide Sequence[b,c,d,e]	Cellular Receptor	Applications and Notes
				loaded with paclitaxel and demonstrated improved efficacy on MCF7 cells *in vitro*.[191] ***Oligonucleotide Delivery:*** Landscape phage pVIII coat protein displaying the DMPGTVLP peptide was incorporated into liposomes loaded with *PRDM14* siRNA and shown to reduce PRDM14 protein expression.[192]
	BT-474	LSVCVRGLLGCG[95] **C**GDRLCRMLWL**C**[95] DRCWSILATSTF[95] **C**GRVGMDVMGG**C**[95] VWGVFGGCSQRP[95] LSVWMQGLSRSL[95] **C**GLGWCGVRWG**C**[95] (phagemid vector that expresses P99 β-lactamase as N-terminal fusion to pIII protein)		
Nervous System	WAC2 (neuroblastoma)	HLQIQPWYPQIS[193] (pIII) VPWMEPAYQRFL[193] (p160) (pIII)		***Therapeutic Applications:*** p 160 peptide-micelles loaded with paclitaxel killed cells *in vitro*.[194] ***Imaging Applications:*** p160 homes to WAC2 tumors *in vivo* as determined by biodistribution of radiolabeled peptide[195,196] but has not been used for imaging.[463,464]
	RG2 (rat glioma)	DSTKSGNM[197] DYDMTKNT[197] DLTKSTAP[197] ESRGDSYA[197] (pVIII landscape)		
	U87-MG (glioma)	M**C**PKHPLG**C**[198] (pIII)		
		VTWTPQAWFQWV[199] (VTW) (pIII)	GP130 (suggested)	***Note:*** The peptide can deliver active β-galactosidase into U87MG cells.
	Gli36 (glioma)	LLADTTHHRPWT[200] (pIII)		
	Mixture of dGli36, SF767, U87MG, U251MG, and U373MG (glioma)	LWATFPPRPPWL[201] (pIII)		***Oligonucleotide Delivery:*** Peptide-(K_{16}) complexed with luciferase DNA transfected SF767 glioma cells.
	Glioma stem cells established from human glioblastoma samples	AQYLNPS[202] (pIII)	Nestin	***Functional Peptide Activity:*** The peptide homes to nestin positive cells within a glioblastoma tumor in subcutaneous and orthotopic models when injected intravenously. This suggests that the

Cancer Type	Cell Line used for Selection[a]	Peptide Sequence[b,c,d,e]	Cellular Receptor	Applications and Notes
				peptide crosses the blood-brain barrier.
	GI-LI-N and HTLA-230 (neuroblastoma)	YEGLISR[203] HSYWLRS[205] WSWPREL[203] ALAAHKL[203] KSFFLSH[203] (pIII)		***Therapeutic Applications:*** Doxorubicin loaded liposomes conjugated to the WSWPREL or ALAAHKL peptides showed a significant anti-tumor response in two different animal models of neuroblastoma compared to non-targeted lipsomes.
Cervical	SiHa (HPV-16 infected)	**C**RLTGGKGVG**C**[204] **C**ADPNSVRAM**C**[204] **C**AAHYRVGPW**C**[204] (phagemid pVIII hybrid)		
	HeLa	**C**SSGKPLV**C**[205] (pIII) **C**NISRTGT**C**[205] (pIII) **C**NSTELSG**C**[205] (pIII)		***Oligonucleotide Delivery:*** The peptides increase transfection of a luciferase DNA polyplex.
Ovarian Cancer	SK-OV-3	SVSVGMKPSPRP[206] (pIII)		***Note:*** This peptide has been suggested to be a non-specific peptide that is isolated from the NEB 12-mer library most likely due to preferential propagation of this phage clone.[207]
Thyroid Cancer	TT	**C**HTFEPVG**C**[208] (pIII)		
	FRO82-2	EDYELMDLLAYL[209] (FROP-1) (pIII)		***Note:*** The peptide binds to many carcinoma cell lines. Radiolabeled peptide homes to FRO82-2 and MCF tumors in mice.
Rhabdo-myosarcoma	RD	**C**QQSNRGDRKR**C**[210] (RMS-I) **C**MGNKRSAKRP**C**[210] (RMS-II) (T7 phage)	$\alpha_v\beta_3$ (RMS-I)	***Note:*** RMS-II shares sequence similarity to the well-studied Lyp-1 peptide. RMS-II targets tumor vasculature in RD tumor xenografts.
Osteosarcoma	143B	ASGALSPSRLDT[211] (OSP-1) (pIII)	Heparin sulfate proteoglycans (suggested)	***Imaging Applications:*** MicroPET imaging has been performed with ^{18}F-labeled peptide in tumor-bearing mice.
Lymphoma and Leukemia	A20 (murine lymphoma)	SAKTAVSQRVWLPSHRGGEP[212] (A20.1) KSREHVNNSACPSKRITAAL[212] (A20.2) WLSEAGPVVTVRALRGTGSW[212] (PCM.1) (pIII)		***Note:*** The peptides distinguish A20 cells from a mixture of splenocytes. PCM.l was also isolated from a whole cell biopanning on primary cardiomyoctyes.[213]
	(lymphoma)			peptide $_D$(KLAKLAK)$_2$ kills cells *in vitro*.
		CGFYWLRS**C**[215] (p III)	NRP-1	***Therapeutic Applications:*** Peptide-

Cancer Type	Cell Line used for Selection[a]	Peptide Sequence[b,c,d,e]	Cellular Receptor	Applications and Notes
				proapoptotic peptide $_D(KLAKLAK)_2$ kills cells *in vitro*.
	Raji (lymphoma)	**C**TLPHLKM**C**[216] (pIII)	Variable region of human immunoglobulin heavy chain (suggested)	
	Kasumi-1 (leukemia)	**C**PLDIDFY**C**[217] (pIII)	$\alpha_4\beta_1$	
Lung Cancer	H1299 (large cell)	VSQTMRQTAVPLLWFWTGSL[218] (H1299.1)(pIII)		***Therapeutic Applications:*** Peptide-doxorubicin conjugate selectively killed H1299 cells.[219]
		YAAWPASGAWTGTAPCSAGT[220] (H1299.2) (pIII)		
		EHMALTYPFRPP[221] (ZS-1) (pIII)		
		QQMHLMSYAPGP[222] (ZT-1) (pIII)		
	H2009 (adenocarcinoma)	RGDLATLRQLAQEDGVVGVR[218] (H2009.1)(pIII)	$\alpha_v\beta_6$[73]	***Therapeutic Applications:*** The H2009.1 peptide has been shown to home to $\alpha_v\beta_6$-positive tumors in NSCLC xenografts.[223] A peptide-doxorubicin conjugate,[219] a peptide-taxol conjugate,[224] a peptide-polyglutamic acid polymer-doxorubicin conjugate,[225] peptide-displaying micelles loaded with doxorubicin and SPIO,[226] and peptide conjugated to doxorubicin liposomes all selectively kill $\alpha_v\beta_6$-positive cells.[227] ***Imaging Applications:*** H2009.1 has been conjugated to SPIO particles for MRI imaging.[228] These particles target $\alpha_v\beta_6$-positive cells *in vitro* but has not been tested *in vivo* to date.
Lung Cancer	A549 (adenocarcinoma)	MTVCNASQRQAHAQATAVSL[218] (A549.1) (pIII)		
	CL1-5	TDSILRSYDWTY[229] (pIII)		***Therapeutic Applications:*** Peptide-liposomal doxorubicin and liposomal vinorelbine inhibited tumor growth in mice.[229]
	Calu-3 (adenocarcinoma)	**C**DSAFVTVDWGRSMSL**C**[230] (IB1) (pVIII)		***Oligonucleotide Delivery:*** Biotinylated peptide complexed with *luciferase*/PEI transfected Calu-3 cells with luciferase.

Cancer Type	Cell Line used for Selection[a]	Peptide Sequence[b,c,d,e]	Cellular Receptor	Applications and Notes
	H460 (large cell)	**C**SNIDARA**C**[231] (T7)		***Therapeutic Applications:*** The peptide was coupled to liposomal doxorubicin and found to be more effective than free doxorubicin or non-targeted liposomal doxorubicin in reducing tumor growth in a H460 subcutaneous tumor model. ***Imaging Applications:*** Fluorescence imaging of H460 tumors in animals has been accomplished using a FITC conjugate of the peptide.
Barrett's Esophagus	OE33	SNFYMPL[232] (pIII)		

[a] All cells lines are of human origin unless stated otherwise.

[b] Cysteine residues that form disulfide bonds are indicated in bold.

[c] Common peptide names that have been used in the literature are indicated in parenthesis next to the peptide sequence.

[d] The phage display library type is indicated in parenthesis. All phage are filamentous unless noted as T7 phage.

[e] Peptides clustered in the same cell were isolated in the same panning experiment.

Table 4

Cancer-Targeting Peptides Selected by Panning ex Vivo

Cancer Type	Cells or Tumor used for Selection	Peptide Sequence [a,b]	Receptor Identified	Applications and Notes
Colon	Human colonic adenomas	VRPMPLQ[236] (pIII)		***Imaging Applications:*** Topical administration of the peptide labeled with fluorescein was used for fluorescence confocal microendoscope imaging of colonic adenomas in humans.
	Resected human colon tumors	SPTKSNS[233] (pIII)		***Notes:*** Used laser capture microdissection to isolate cells for biopanning.
Bladder	Cells from HT-1376 xenograft in mice	**C**SNRDARR**C**[237] (pIII)		***Notes:*** Peptide homed to HT-1376 xenografts in mice and captured shed bladder cancer cells in the urine of patients with early stage bladder cancer.[237,238]
Pancreatic	Pancreatic ductual carcinomas arising from Kras/p52$^{L/L}$ mice	KTLLPTP[239] (pIII)	Plectin-1[239]	***Imaging Applications:*** The peptide has been conjugated to crosslinked iron oxide-Cy5.5 nanoparticles for intravital confocal microscopy in tumor-bearing mice.[239] The ^{111}In labeled peptide has been used for SPECT/CT imaging in tumor-bearing mice.[74]
Gastric	Freshly harvested human gastric cancer tissue	AADNAKTKSFPV[240] (pIII)		

[a] Cysteine residues that form disulfide bonds are indicated in bold.

[b] The phage display library type is indicated in parentheses. All phage are filamentous.

Table 5

Cancer-Targeting Peptides Selected by Panning in Vivo

Tumor Type[a]	Peptide Sequence[b,c,d,e,f]	Cellular Receptor	Applications and Notes
Gastric (AZ-P7a)	SWKLPPS[259] ([g]) (pIII, **T**)	$\alpha_3\beta_1$ (suggested)	***Therapeutic Applications:*** Peptide-liposomal doxorubicin killed AZ-P7a cells better than unconjugated liposomes *in vitro*.
Human gastric adenocarcinoma (propagated in mice)	**C**GNSNPKS**C**[260] (GX1) (pIII, **V**)		***Therapeutic Applications:*** The free peptide fused to recombinant human tumor necrosis factor alpha (rmhTNFα) induced HUVEC apoptosis *in vitro*.[261] This peptide-rmhTNFα fusion also inhibited SGC7901 tumor growth in mice.[261] ***Imaging Applications:*** $^{99}Tc^{m}$-peptide[261,262] and $^{99}Tc^{m}$-peptide-rmhTNFα[261] successfully detected SGC7901 tumors by SPECT imaging. MicroPET imaging using ^{64}Cu-DOTA-peptide detected U87MG tumors in mice.[263] Near-infrared fluorescence imaging of Cy5.5-peptide in U87MG tumor-bearing mice demonstrated tumor targeting.[264]
Lung (irradiated and SU11248 treated murine Lewis lung carcinoma) & Glioblastoma (GL261, murine)	HVGGSSV[243] ([h]) (T7 phage, **V**)	TIP-1[265,266]	***Therapeutic Applications:*** Peptide-nab-paclitaxel inhibited Lewis lung carcinoma and H460 tumor growth in mice in conjunction with irradiation.[265] Lewis lung carcinoma and H460 tumor growth inhibition was observed when animals were treated with irradiation and peptide liposomal doxorubicin.[267] ***Imaging applications:*** Near-infrared fluorescence imaging using dye labeled peptide has been performed for several tumor types and in various types of tumor-bearing mice treated with irradiation or TKI (tyrosine kinase inhibitors) or both.[243,265–269]
Lung (CL1-5)	SVSVGMKPSPRP[249] (pIII, **V**)		***Therapeutic Applications:*** Peptide

Tumor Type[a]	Peptide Sequence[b,c,d,e,f]	Cellular Receptor	Applications and Notes
			liposomal doxorubicin inhibited CL1-5 tumor growth and increased survival in mice. ***Note:*** This peptide has been suggested to be a non-specific peptide that is isolated from the NEB 12-mer library most likely due to preferential propagation of this phage clone.[207]
Lung (H460)	R**C**PLSHSLI**C**Y[270] (pIII, **T**)		
Oral (SAS)	SVSVGMKPSPRP[249] (pIII, **V**)		***Therapeutic Applications:*** Peptide liposomal doxorubicin inhibited SAS tumor growth and increased survival in mice. ***Note:*** This peptide has been suggested to be a non-specific peptide that is isolated from the NEB 12-mer library most likely due to preferential propagation of this phage clone.[207]
	SNPFSKPYGLTV[244] YPHYSLPGSSTL[244] (pIII, **V**)		***Therapeutic Applications:*** Peptide-liposomal doxorubicin inhibited tumor growth and increased survival in mice with various tumor types compared to non-conjugated liposomal doxorubicin. Similar growth inhibition was observed for both peptides.
Nasopharyngeal Carcinoma (CNE-1)	EDIKPKTSLAFR[271] (pIII, **ND**)		
Prostate (PC-3)	IAGLATPGWSHWLAL[272] (G1) (pIII, **T**)		***Imaging applications:*** ^{111}In-DOTA-Gl peptid ***Functional Peptide Activity:*** The peptide induced apoptosis of PC-3 cells in culture.[273]
	LKGD**C**TQRYVY**C**MKSK[273] (H5) (pVIII hybrid, **T**)		***Imaging applications:*** ^{111}In-DOTA-H5 peptic ***Functional Peptide Activity:*** The peptide induced apoptosis of PC-3 cells in culture.[273]
	CRGDKGPD**C**[274] (iRGD) (T7 phage, **T/V**)	$\alpha_v\beta_3$, $\alpha_v\beta_5$ then cleaved to CRGDK which binds NRP-1	***Therapeutic Applications:*** Co-injection of the peptide with Nab-paclitaxel, trastuzumab, liposomal doxorubicin or doxorubicin inhibited BT474 or 22Rv1 orthotopic tumor growth in mice.[275] CGKRK-D[KLAKLAK]$_2$-iron oxide nanoworms systemically injected into mice with 005 tumors prolonged survival when co-

Tumor Type[a]	Peptide Sequence[b,c,d,e,f]	Cellular Receptor	Applications and Notes
			injected with the peptide.[276] ***Imaging applications:*** The peptide was conjugated to Cy7 for near-infrared imaging of tumors in mice bearing novo pancreatic ductal adenocarcinoma. MRI of 22Rv1 orthotopic xenografts in mice using peptide-linked SPIO nanoworms has been performed.[276] ***Note:*** This peptide has been postulated to have a unique mode of action in which the peptide first binds the target integrin and is then cleaved to expose a NRP-1 binding peptide. The binding of NRP-1 increases penetration of cargo throughout the tumor.
Prostate (TRAMP mice)	**C**REAGRKA**C**[257] (REA) ([i]) (T7 phage, **L**)		***Therapeutic Applications:*** Peptide-$_D$(KLAKLAK)$_2$ conjugate reduced PPC1 orthotopic tumor lymphatic vessels in mice.
Prostate (DU145)	YR**C**TLNSPFFWEDMTHE**C**HA[277] (pIII, **T**)	CRKL	***Therapeutic Applications:*** Peptide-proapoptotic peptide$_D$(KLAKLAK)$_2$ is cytotoxic to DU145 cells *in vitro* and inhibits DU145 tumor growth in mice.
Prostate (PPC-1)[i]	RPARPAR[278] (T7 phage, **T**)	Neuropilin-1 (NRP-1)[278,279]	
Breast (MDA-MB-435)	**C**NGRCVSG**C**AGRC[112,280] (NGR)[j] (pIII, **V**)	Amino-peptidase N (CD13)[281]	***Therapeutic Applications:*** A NGR peptide-doxorubicin conjugate inhibited MDA-MB-435 tumor growth & prolonged survival in mice.[112] Peptide-liposomal doxorubicin inhibited growth of orthotopic neuroblastoma xenografts in mice[282] and increased survival in mouse lung, ovarian, and neuroblastoma xenografts.[283] NRG peptide-TNF (tumor necrosis factor α) inhibited tumor growth[284] and synergistically increased the effects of various chemotherapy drugs on tumor inhibition in mice (for various tumor types).[285–289] Human interferon alpha (hIFN-

Tumor Type[a]	Peptide Sequence[b,c,d,e,f]	Cellular Receptor	Applications and Notes
			α2a)-peptide conjugate[290] and recombinant hIFN-α2a-peptide[291] inhibited tumor growth in mice when injected i.p. in various tumor models. A conjugate of the NRG peptide with a Tum-5-peptide (derived from tumstatin) inhibited S180 tumor growth in mice.[292]
Breast (MDA-MB-435)	**C**DCRGD**C**FC[112] (RGD-4C)[j] (pIII, **T**/**V**)	$\alpha_v\beta_3$,$\alpha_v\beta_5$,$\alpha_5\beta_1$	***Therapeutic Applications:*** A RGD-4C-doxorubicin conjugate inhibited MDA-MB-435 tumor growth and prolonged survival in mice[112] and inhibited MH134 orthotopic tumor growth in mice.[293] A conjugate of RGD-4C-tumor necrosis factor-α (TNF) inhibited MDA-MB-435 orthotopic tumor growth.[136] Conjugation of RGD-4C to adeno-associated virus phage vector (AAVP) allowed for delivery of the *HSVtk* gene. Treatment of DU145, KS1767, and UC3 xenografts in mice and EF43-FGF4 mouse mammary tumors with RGD-4C-AAVP- *HSVtk* and gancilovir inhibited tumor growth.[294] RGD-4C-Delta-24 adenovirus (an adenovirus with anticancer activity) is cytotoxic to a variety of cancer cell lines.[295] Intratumoral injection of RGD-4C-Delta-24 adenovirus into orthotopic U-87 MG tumors in mice increased survival.[295] This conjugate has recently begun Phase I clinical trials. ***Imaging applications:*** Scintigraphic imaging of DU145 tumors in mice using $^{99m}Tc(CO_3)$-RGD-4C and $^{99m}Tc(CO_3)$-HPMA polymer-RGD-4C has been performed; the polymer conjugate was also imaged in PC-3 tumors.[296] MicroPET imaging using ^{64}Cu-DOTA-RGD-4C-TNF-α was able to detect U87MG and MDA-

Tumor Type[a]	Peptide Sequence[b,c,d,e,f]	Cellular Receptor	Applications and Notes
			MG-435 tumors in mice.[136] ***Oligonucleotide Delivery:*** Conjugation of RGD-4C to AAVP resulted in delivery of GFP to KS1767 xenografts in mice & delivery of luciferase to DU145 xenografts as evidenced by bioluminescent imaging.[294] RGD-4C-AAVP delivered the *HSVtk* gene to DU145 xenografts as evidenced by PET imaging with [^{18}F]FEAU.[294]
	CGNKRTRG**C**[255] (LyP-1)[i] (T7 phage, **T/L/M**)	p32/gC1qR[297]	***Therapeutic Applications:*** LyP-1 conjugated to liposomes containing doxorubicin are no more effective in reducing tumor growth than non-targeted liposomes.[298] However, doxorubicin loaded LyP-1 liposomes injected after gold nanorod-mediated heating of tumors caused tumor regression in mice with MDA-MB-435 tumors.[299] Peptide-doxorubicin liposomes killed cells with or without heat treatment *in vitro.* Peptide coupled to microbubbles containing paclitaxel killed cells when treated in combination with ultrasound but this approach was only tested *in vitro*.[300] Dye-labeled–peptide-abraxane inhibited tumor growth in mice.[301] ***Imaging applications:*** Near-infrared fluorescence imaging and *ex vivo* imaging of lymphanogenesis were performed using Cy5.5-peptide injected via the middle phalanges of both upper extremities in 4T1 tumor bearing mice.[302] ***Oligonucleotide Delivery:*** Baculovirus displaying peptide-VSVG protein fusions and carrying the luciferase gene specifically transduced cells *in vitro*.[303,304] ***Functional Peptide Activity:*** The LyP-1 peptide itself induced apoptosis of cancer cell

Tumor Type[a]	Peptide Sequence[b,c,d,e,f]	Cellular Receptor	Applications and Notes
			lines in culture and inhibited MDA-MB-435 tumor growth in mice.[256] ***Note:*** LyP-1 has been shown to home to tumor lymphatics *in vivo* when used in a variety of formats[255,256,298,299,301,302,304–308]. LyP-1 also accumulates in atherosclerotic plaques, primarily in the associated macrophage.[309,310]
Breast (MDA-MB-231 and MCF-7 in mice treated with sunitinib and responding to therapy)[h]	EGEVGLG[242] (T7 phage, **V** after sunitinib treatment)		***Imaging applications:*** A peptide-AlexaFluor 750 conjugate was used for near-infrared imaging of sunitinib treated MDA-MB-435 and MCF7 tumors in mice.
Medullary Thyroid Carcinoma (TT)	**C**HTFEPVG**C**[311] (pIII, **T**)		***Oligonucleotide Delivery:*** Peptide linked to adenovirus vector delivered the GFP gene to cells *in vitro* resulting in GFP expression but this peptide has not been tested *in vivo*.[311]
Basal cell squamous carcinoma from K14-HPV16 mice[i]	CGKRK[312] CDTRL[312] (T7 phage, **V**)		***Therapeutic Applications:*** The CGKRK peptide was conjugated to a clot inducing nanoworm particle. This conjugate induces clotting in prostate tumors in mice, resulting in a reduction of tumor burden.[313] A CGKRK peptide-$_D$[KLAKLAK]$_2$-iron oxide nanoworm decreased the number of blood vessels in bFGF-Matrigel plugs in mice. When systemically injected into mice bearing induced brain tumors, the peptide-conjugate cured most mice.[276] CGKRK Peptide-$_D$[KLAKLAK]$_2$-iron oxide nanoworms co-injected with the iRGD peptide into mice with 005 tumors prolonged survival.[276] ***Oligonucleotide Delivery:*** Baculovirus displaying CGKRK peptide-VSVG protein fusions and carrying the luciferase gene specifically transduced cells *in vitro*,[303] and the CGKRK peptide has been used to transfect cancer cells with a

Tumor Type[a]	Peptide Sequence[b,c,d,e,f]	Cellular Receptor	Applications and Notes
			spider silk derived gene carrier.[314] ***Note:*** Both peptides home to the vasculature of several different tumor types but the CGKRK peptide binds to a broader number of tumor types.
Melanoma (C8161)[i]	**C**LSDGKRK**C**[257] (LSD) (T7 phage, **L**)		***Therapeutic Applications:*** LSD peptide-$_D$(KLAKLAK)$_2$ conjugate reduced C8161 orthotopic tumor lymphatic vessels in mice.
Melanoma (murine B16-F10)[g,i]	TRTKLPRLHLQS[159] (WDC-2) (pIII, **T**)		***Therapeutic Applications:*** Phage inhibited B16-F10 tumor growth when injected adjacent to tumor, but the free peptide was not tested. The anti-tumor effect is likely due to infiltration of neutrophils induced by the phage particle.
RIP1-Tag2 mice Pancreatic islets[i]	CRGRRST[315] (RGR) CRSRKG[315] (RSR) CKAAKNK[315] (KAA) CKGAKAR[315] (KAR) FRVGVADV[315] (VGVA) (T7 phage, **V/T**)	PDGFRβ is a candidate receptor for RGR peptide	***Therapeutic Applications:*** Combination treatment with RGR peptide-anti-CD40 antibody and murine IL-2-peptide fusion protein increased survival in RIP1-Tag2 mice. 80% of mice treated with the combination treatment plus adoptive transfers of anti-Tag CD4$^+$ and CD8$^+$ T cells survived long-term.[316] ***Note:*** KAA and KAR peptides bind to the RIP1-Tag2 tumor vasculature. RSR binds specifically to angiogenic islet vasculature but not to the tumor vasculature. VGVA and RGR bind to both angiogenic and tumor vasculature.[315]
Dysplastic Colon Mucosa/colon adenomas from *CPC;Apc* mice	QPIHPNNM[317] T7 phage, **T**)		***Imaging applications:*** FITC-labeled peptide bound adenomas in *CPC;Apc* mice as viewed by endoscopy[317] and microendoscopy.[318]
Colorectal (Bevacizumab-treated LS174T)	LLADTTHHRPWT[241] (BRP) ([h]) (pIII, bevacizumab-treated **V**)		***Imaging applications:*** BRP peptide-IRDye800 conjugate was used for near-infrared imaging of bevacizumab-treated LS174T tumors in mice. PET imaging using a ^{18}F-BRP-peptide detected LS174T tumors

Tumor Type[a]	Peptide Sequence[b,c,d,e,f]	Cellular Receptor	Applications and Notes
			in mice treated with bevacizumab.[241]
Colorectal (Colon 26 cells orthotopically implanted in BALB/c mice)	**C**TPSPFSH**C**[319] (TCP-1) (pIII phage, **V**)		***Therapeutic Applications:*** The peptide homes to the vasculature of the colon 26 tumor in a mouse but not to normal vasculature. Conjugation of TCP-1 to the proapoptotic peptide $_D$(KLAKLAK)$_2$ conjugate induces apoptosis in the tumor vasculature. ***Note:*** The peptide binds to vasculature of resected human colorectal tumors.
Gliomas (irradiated murine GL261)	GIRLRG[320] (^{h}) (T7 phage, irradiated **V**)	GRP78	***Therapeutic Applications:*** Peptide conjugated to a nanoparticle encapsulating paclitaxel inhibits MDA-MB-231 tumor growth in irradiated mice.[320] ***Imaging applications:*** Peptide-AlexaFluor 750 has been used for near-infrared imaging in mice with irradiated G1261 tumors.[320]
Hepatocarcinoma (BEL-7402)	AGKGTPSLETTP[321] (A54) (pIII, **T**)		***Therapeutic Applications:*** Peptide-doxorubicin conjugate inhibited tumor growth and increased survival in mice with BEL-7402 tumors.[152]
Human Renal Carcinoma Tumor Endothelial Cells	**C**VGNDNSS**C**[250] (pIII, **V**)		***Therapeutic Applications:*** Biotinylated peptide conjugated to saporin induced apoptosis in tumor endothelial cells in mice.
Waldenström macro-globulinemia B cell malignancy (from pancreas of a human patient)	**C**GRRAGGS**C**[77] (pIII, **T**)	IL-11Rα[77,251]	***Therapeutic Applications:*** A peptide-proapoptotic peptide $_D$(KLAKLAK)$_2$ conjugate induced apoptosis in LNCaP and MDA-PCa-2b prostate cell lines *in vitro*.[152] ***Imaging applications:*** ^{111}In-DTPA-IR dye-pe

[a] All tumors are human xenografts unless otherwise noted.

[b] Cysteine residues that form disulfide bonds are indicated in bold.

[c] Common peptide names that have been used in the literature are indicated in parenthesis next to the peptide sequence.

[d] The phage display library type is also indicated in parentheses. All phage are filamentous unless noted as T7 phage.

[e] **T** indicates tumor cell targeting, **V** indicates tumor vasculature targeting, **L** indicates tumor lymphatics homing, and **M** indicates tumor associated macrophage homing. **ND** means it is unclear whether the peptide binds the tumor cells or tumor vasculature.

[f] Intravenous injections used for selection unless otherwise indicated.

[g] Selection used intraperitoneal injections instead of intravenous injections.

[h] Selection used intracardiac injections instead of intravenous injections.

[i] Combination of *ex vivo/in vivo* panning employed.

[j] This peptide has been used in numerous other studies not listed.

Table 6

Cancer-Targeting Peptides Isolated from Bacterial Display Libraries

Cancer Type	Cell Line used for Selection[a]	Peptide Sequence[b,c,d]		Library Type
Prostate Cancer	PC-3M-1E8	NVVRQ[325] (TMTP1, targets *in vivo*)		FliTrx
	PC-3	**C**PGDRGQRRLFSKIEGP**C**[326] (MM-2, targets *in vivo*)		FliTrx
Breast Cancer	ZR-75-1	T**C**VLHRQR**C**LMFTLR[84] I**C**VNIKKSLWA**C**EIR[84]	WARVLLIEGRLIVCE[84] WWDMVSDRYIWKPVK[84]	OmpA
		VP**C**QKRPGWV**C**LW[84] KW**C**VIWSKEG**C**LF[84] SSW**C**MRGQYNKI**C**MW[84] VE**C**YLIRDNL**C**IY[84]	WW**C**LGERVVR**C**AH[84] FY**C**VIERLGV**C**LY[84] RV**C**FLWQDGR**C**VF[84]	CPX
	MDA-MB-231	MS**C**LMNSNSF**C**SI[324] WA**C**LMNMYSF**C**SS[324] LR**C**LTTLDNF**C**TI[324] LI**C**LHRIDRF**C**SV[324] ME**C**LKSMFTY**C**DI[324] LS**C**LYSMYSY**C**DV[324] LW**C**LTDLMGW**C**TV[324] LG**C**LLDVQSW**C**IV[324]	LW**C**LLDLMSW**C**EI[324] LD**C**FRNIYGF**C**NI[324] LK**C**LWEMRGF**C**EI[324] VD**C**LFHTDRF**C**YI[324] WR**C**LMSLETW**C**MV[324] LA**C**LMSLEQW**C**AV[324] WS**C**LWDLSQF**C**NF[324] PS**C**LFNLDSF**C**EF[324]	CPX
	MCF-7	VE**C**DPVRNNF**C**WW[324] LE**C**HRLRTNM**C**FL[324] EW**C**GIVRVGY**C**LG[324] DA**C**GIIHVGY**C**KV[324] RV**C**TWNWSWI**C**KE[324] RM**C**TWNLEWV**C**DL[324] RL**C**VWDWEWL**C**RD[324]	RV**C**TWRMVWV**C**DY[324] NL**C**RGDLEKL**C**MK[324] YA**C**RGDAYYL**C**AT[324] HS**C**RGDMALL**C**WL[324] FA**C**RGDRWVL**C**NS[324] GL**C**VADGRPR**C**LE[324] GW**C**FRDGRPM**C**SY[324]	CPX
	T47D	FW**C**MGDGRPR**C**TG[324] VW**C**YLWKYGY**C**VY[324]	PI**C**RGDRDWR**C**RD[324] GQIWKGEWVKLWRDV[324]	CPX
Hepatoma (Liver)	HepG2	IAVAPGWLWEEE[323] KELCELDSLLRI[323] TRGRPRDVANGH[323]	IRELYSYDDDFG[323] ESLSVDFMGERA[323] QELAPYSWSEKD[323] ARRILKGGGVHT[323]	FliTrx
Murine Squamous Carcinoma	SCC VII[e]	**C**GGRRLGG**C**[327,328] (targets *in vivo*)		FliTrx

[a] All cell lines are of human origin unless otherwise noted.

[b] Cysteine residues that form disulfide bonds are indicated in bold.

[c] Peptides clustered in the same cell in this table were isolated in the same panning experiment.

[d] Peptides that have been confirmed to target the appropriate tumor *in vivo* are noted.

[e] This was an *ex vivo* panning using isolated cells.

Table 7

Cancer-Targeting Peptides Isolated from OBOC Libraries Using Cultured Cells as the Target

Cancer Type	Cell Line used for Selection[a]	Peptide Sequence[b,c,d,e,f]	Cellular Receptor	Applications and Notes
Breast Cancer	MDA-MB-231	**c**dGLGBN**c**[332] (LXY1) **c**dGTyr(3-NO_2)GBN**c** (LXY3)[332]	$\alpha_3\beta_1$	***Imaging applications:*** LXY1 peptide-biotin-streptavidin-Cy5.5 used for near-infrared imaging of U-87MG tumors in mice.[333] LXY3 peptide-biotin-streptavidin-Cy5.5 used for near-infrared imaging of orthotopic MDA-MB-231 tumors. ***Note:*** LXY3 was identified from a focused peptide library based on the LXY1 sequence.
Leukemia	Jurkat	LTGpLDI[334]	$\alpha_4\beta_1$	
Ovarian Cancer	CaOV-3, SKOV-3, OVCAR-3, & ES-2	**c**LDWDLI**c**[335] **c**DGLGDD**c**[335] **c**DGWGPN**c**[335]	α_3 (DGLG peptide)	
	ES-2	**c**dGHCitGPQ**c**[336] (OA02)	α_3	***Therapeutic Applications:*** OA02 peptide displayed on the surface of paclitaxel loaded micelles improves efficacy in two ovarian cancer models compared to the non-targeted paclitaxel or paclitaxel-micelles.[337] ***Imaging applications:*** A OA02 peptide-biotin-streptavidin-Cy5.5 and peptide-Cy5.5 have been used for near-infrared imaging of ES-2 tumors in mice.[336] [^{64}Cu] DOTA-OA02 peptide was used for microPET imaging of ES-2 tumor and SKOV3 metastatic lesion in mice.[18]
	SKOV-3	**c**dG-Cha-G-HCit-Q**c**[18] **c**dG-Chg-G-Hyp-N**c**[18] **c**dG-HCit-GPQ**c**[18] **c**dGIGPQ**c**[18] **c**dGLGQ-Bta-**c**[18] **c**dG-Phe-GP-Cha-**c**[18] **c**dG-Tyr-GI-Pra-**c**[18]		***Note:*** The peptides bind multiple cancer cell lines but most also bind a normal human lung epithelial cell line.
Lung Cancer	A549	**c**NGQGEQ**c**[338] (pA)	$\alpha_3\beta_1$	***Note:*** A consensus sequence of NGXG was observed for most positive hits.
	HI650	**c**NleDNleTHypr**c**[339] (pM2)	$\alpha_4\beta_1$	
Prostate	DU145	LNIVSVNGRHX[85] (RU-1) DNRIRLQAKXX[85] (RX-1)		
		kmviywkag[340] (RZ-3) kikmviswkg[340] (HYD-1)	$\alpha_3\beta_1$[341] (RZ-3) $\alpha_6\beta_1$ and $\alpha_3\beta_1$[341] (HYD-1)	***Functional Peptide Activity:*** Free HYD-1 peptide induced cell death in multiple myeloma cells and inhibited H929 tumor growth in mice when injected i.p.[342] RZ-3 inhibited random haptotaxis of PC3N cells on lamin-5.[341] HYD-1 blocked random haptotaxis and inhibited invasion of PC3N cells on lamin-5.[341] HYD-1 also inhibited adhesion of H929 cells to fibronectin mediated by $\alpha_4\beta_1$.[342]

Cancer Type	Cell Line used for Selection[a]	Peptide Sequence[b,c,d,e,f]	Cellular Receptor	Applications and Notes
	LNCaP	QMARIPKRLARH[50]		***Note:*** The peptide binds to other epithelial derived cancer cell lines and can be used to label LNCaP cells spiked into whole blood.
Bladder Cancer	5637	**c**QDGRMGF**c**[343] (PLZ4)		***Imaging applications:*** Near-infrared imaging of human bladder tumor-bearing mice was performed with a peptide-biotin-SA-Cy5.5 conjugate.

[a] All cell lines are of human origin.

[b] Cysteine residues that form disulfide bonds are indicated in bold.

[c] Lowercase indicates D-amino acids.

[d] Abbreviations for unnatural amino acids are as follows: B = hydroxyproline, Cha = cyclohexylalanine, Chg = α-cyclohexylglycine, HCit = homocitrulline, Hyo = hydroxyproline, Bta = benzothioenylalanine, Phe = 4-methylphenylalanine, Pra = propargylglycine, Tyr = 3-nitrotyrosine, and Nle = norleucine.

[e] Common peptide names that have been used in the literature are indicated in parentheses next to the peptide sequence.

[f] Peptides clustered in the same cell in this table were isolated in the same selection experiment.

Table 8

Cancer-Targeting Peptides Isolated from PS-SPCLs

Protein or Cell Line used for Selection[a]	Peptide Sequence[b,c]	Cellular Receptor	Applications and Notes
NR6 cells transfected to overexpress EGFRvIII (mutation variant III)	H-FALGEA-NH_2[65] H-FALIEA-NH_2[65]	EGFRvIII	***Imaging applications:*** The labeled 4-[^{18}F]fluorobenzoyl-peptide has been used for microPET imaging of NR6M cells in mice.[344]
$\alpha_5\beta_1$ (selected for ability to compete with fibronectin binding)	VILVLF[64] (A5-1)	$\alpha_5\beta_1$	***Functional Peptide Activity:*** The synthetic peptide inhibits proliferation, bFGF-induced migration, bFGF-induced tubular network formation of HUVEC cells and bFGF-induced neovascularization in a chorioallantoic membrane angiogenesis assay.
$\alpha_v\beta_3$ (selected for ability to compete with vitronectin binding)	HGDVHK-NH_2[63] HSDVHK-NH_2[63]	$\alpha_v\beta_3$[63,345]	***Functional Peptide Activity:*** Both peptides inhibit bFGF-induced HUVEC cell migration and bFGF-induced neovascularization in a chorioallantoic membrane angiogenesis assay. HSDVHK inhibits HUVEC proliferation.[346]
U266 B myeloma cells (selected for stimulation of inositol phosphates)	WKYMVM-NH_2[347]	Formyl peptide receptor-like 1[348,349] & N-formyl peptide receptor-like 2[349]	***Functional Peptide Activity:*** The peptide stimulated phosphoinositide hydrolysis and release of $[Ca^{2+}]_i$ from U266, U937 and HL60 cells.[347,350] It also stimulated phosphoinositide hydrolysis and superoxide production in human neutrophils.[350] In human peripheral blood monocytes and neutrophils, the peptide induced migration and Ca^{2+} mobilization.[348]

[a] All cell lines are of human origin.

[b] Common peptide names that have been used in the literature are indicated in parentheses next to the peptide sequence.

[c] Peptides clustered in the same cell in this table were isolated in the same selection experiment.

Table 9

Cardiac- and Muscle-Targeting Peptides

Cell Type and Isolation Method	Peptide Sequence[a,b,c,d,e,f]	Cellular Receptor	Applications and Notes
In vivo panning in normotensive Wistar Kyoto rats, recovering phage from the heart	IPTHIRP[369] (cell type not determined) HPWPTSI[369] (cell type not determined) (pIII)		
In vivo panning in 3 month old C57B61/L mice, recovering phage from the heart	CQAQGQLVC[367] (cardiac endothelium, pSKAN phagemid)	TNF-R1 (suggested)	
In vivo panning in aging (18 mo) C57B61/L mice, recovering phage from the heart	CARRGQAVC[368] (cardiac endothelium, pSKAN phagemid)	Trk B (suggested)	
Dual *ex vivo* & *in vivo* panning: first *ex vivo* panning against cell suspensions from BALB/c murine hearts and then *in vivo* panning in the mice, recovering phage from the heart	CARPAR[359] (cardiac cndorhclium, T7)	Cysteine-rich protein 2 (CRIP2; HLP; ESP-1)	***Therapeutic Applications:*** The CRPPR peptide linked to the antioxidant peptide gp91 ds increased nitric oxide availability and reduced systolic blood pressure in a SHSRP rat model of genetic hypertension.[360] ***Imaging applications:*** The CRPPR peptide was displayed on ^{18}F radiolabeled-liposomes for PET imaging and dynamic PET analysis of hearts in FVB mice.[370]
	CARPAR[359] (cardiac endothelium, T7)	EST (putative)	
	CKRAVR[359] (cardiac endothelium, T7)	Single immunoglobulin interlukin-1 receptor-related protein (SIGIRR: TIR8)	
	CRSTRANPC[359] (cardiac endothelium, T7)	unamed protein product; similar to integral membrane protein CII-3 (MpcII-3)	
	CPKTRRVPC[359] (cardiac endothelium, T7)	*Mus musculus* bladder cancer-associated protein (human) homologue (bc10)	
Combined *in vitro* & *in vivo* screening: panning *in vitro* on rat cardiomyoblast H9C2 cells followed by *in vivo* panning in Balb/c mice, recovering phage from the heart	APWHLSSQYSRT[358] (CTP) (cardiomyocytes, pIII)		***Imaging applications:*** Peptide-biotin labeled neutravidin-fluospheres were used for near-infrared imaging of hearts after intra-cardiac injection in mice. ***Note:*** This peptide has been isolated on numerous targets and it has been suggested to be a nonspecific phage that is isolated due to propagation advantages.[207]
Ex vivo panning against murine primary cardiomyocytes	WLSEAGPVVTVRALRGTGSW[213] (PCM.1) (cardiomyocytes, pIII)		***Oligonucleotide Delivery:*** Peptide conjugated to polymer-*Fas* siRNA polyplex knocked down *Fas* expression and inhibited apoptosis in H9C2 ells.[371]
In vitro panning on pluripotent stem cell derived cardiomyocytes	QPFTTSLTPPAR[372] NNWSSPPQMISR[372] ATFSPPQQSLMM[372] ISTSPPQGSTSS[372] (pluripotent stem cell derived cardiomyocytes, pIII)		
Combined *in vitro* & *in vivo* screening: first screened *in vitro* against murine C2C12 myotubes & then *in vivo* in Balb/C mice, isolating phage from skeletal muscle	ASSLNIA[373] (MSP) (myoblasts and myotubes, pIII)		***Oligonucleotide Delivery:*** Adeno-associated virus modified with the MSP peptide allowed for luciferase and EGFP transduction into C2C12 myotubes *in vitro* and transduced cardiac muscle and other striated muscles with luciferase when injected i.v.[374] The MSP peptide was conjugated to a phosphrodiamidate morpholino antisense oligonucleotide

Cell Type and Isolation Method	Peptide Sequence[a,b,c,d,e,f]	Cellular Receptor	Applications and Notes
			specific for the dystrophin transcript and produced functional truncated dystrophin transcripts in H_2K *mdx* myoblasts.[375] The MSP peptide conjugated to a peptide nucleic acid (PNA) antisense oligonucleotide specific for the dystrophin transcript increased the number of dystrophin-positive muscle fibers when injected into the tibialis anterior muscles of a *mdx* mouse model of muscular dystrophy.[376]
In vitro panning against murine cardiac endothelial cells	CVHSPNKKC[365] (VP) (cardiac endothelium, pIII)	VCAM-1	***Imaging applications:*** A VP peptide-modified magnetofluorescent nanoparticle has been used to image vasculature in the ears of mice with mTNF-α-induced inflammation using intravital confocal microscopy. The nanoparticle also homed to atherosclerotic lesions in cholesterol fed apoE$^{-/-}$ mice.[365]
In vivo panning against rats with ischemia/ reperfusion injury, isolating phage from the ischemic left ventricular tissue	CSTSMLKAC[361] (ischemic cardiomyocytes, pIII)		***Note:*** Recombinant peptide conjugated to SUMO-mCherry localized to ischemic myocardium in mice.
In vivo panning against atherosclerosis lesion-bearing ApoE$^{-/-}$ mice, isolating phage from plaque-loaded segments of the aorta	VHPKQHR[377] (VINP-28) (atherosclerotic plaques, primarily binds endothelial cells but also has affinity for smooth muscle cells and macrophages, pIII)	VCAM-1	***Imaging applications:*** **VINP-28** peptide conjugated to a crosslinked iron oxide-Cy5.5 nanoparticle was used to image vasculature in the ears of mice with mTNF-α-induced inflammation using intravital laser microscpoy. These nanoparticles were also used for MR imaging of atherosclerotic lesions in cholesterol fed ApoE$^{-/-}$ mice; significantly, the peptide-nanoparticle gave no MRI signal change in stain-treated ApoE$^{-/-}$ mice.[378] Dynamic PET/CT imaging of atherosclerotic plaques in ApoE$^{-/-}$ mice has been performed using ^{18}F-labeled tetrameric version of peptidie.[379]
In vivo panning against atherosclerosis lesion-bearing ApoE$^{-/-}$ mice, isolating phage from atherosclerotic plaques from the aorta	CAPGPSKSC[380] (atherosclerotic plaque endothelium, pIII)	Glucose-regulated protein precursor (Grp78)	***Oligonucleotide Delivery:*** Peptide inserted into adeno-associated virus-2 carrying the luciferase gene transduced rat endothelial cells *in vitro*.[381] *In vivo*, peptide inserted into adeno-associated virus-2 carried *eGFP* transgene to atherosclerotic plaques in ApoE$^{-/-}$ mice and not to the aorta of non-diseased mice.[381]
	CNQRHQMSC[380] (atherosclerotic plaque endothelium, pIII)		
	CNHRYMQMC[380] (atherosclerotic plaque endothelium pIII)	Membrane type 1 matrix metalloproteinase[381]	***Oligonucleotide Delivery:*** Peptdie inserted into adeno-associated virus-2 carrying the luciferase gene transduced human, murine, and rat endothelial cells *in vitro* and carried *eGFP* trangene to atherosclerotic plaques in ApoE$^{-/-}$ mice.[381]
	CYNRSDGMC[380] (atherosclerotic plaque endothelium, pIII)		
In vivo panning in LDLr knockout mice on a Western diet, isolating phage from atherosclerotic lesions of vasculature	CLVEAYPGLVRSC[382] (ON2604, pVIII display)		

Cell Type and Isolation Method	Peptide Sequence[a,b,c,d,e,f]	Cellular Receptor	Applications and Notes
Ex vivo panning against cell suspensions from human atherosclerotic plaque tissue	CRKRLDRNC[366] (AP) (atherosclerotic plaques - endothelial cells, macrophages, and smooth muscle cells, T7)	IL-4R	***Therapeutic Applications:*** The AP peptide has found use in tumor targeting. Peptide conjugated to hydrophobically modified glycol chitosan nanoparticles loaded with paclitaxel inhibited H226 and MDA-MB-231 tumor growth in mice. Cy7.5-labeled versions of the naoparticles were used for near-infrared imaging of the same tumor models in mice.[383] AP peptides were placed on pH-sensitive micelles loaded with doxorubicin and shown to inhibit MDA-MB-231 tumor growth in mice.[384] ***Imaging applications:*** AP peptide was conjugated to hydrophobically modified glycol chitosan-Cy5.5 nanoparticles for near-infrared imaging of atherosclerotic plaques in Ldlr$^{-/-}$ mice.[385] The AP peptide was conjugated to pH-sensitive micelles loaded with TRITC and used for near-infrared imaging of MDA-MB-231 tumors in mic.[384]
In vivo panning in hypertensive SHSRP rats, recovering phage from the heart	DDTRHWG[369] (cardiac endothelium) ASAGGPN[369] (cell type not determined) VQASNSN[369] (cell type not determined) (pIII)		***Oligonucleotide Delivery:*** The DDTRHWG peptide cloned into the HI loop of the AD5/19p adenovirus vector expressing *LacZ* is able to transduce cardiac vasculature.
In vitro panning against recombinant human Stabilin-2	CRTLTVRKC[386] (S2P) (atherosclerotic plaques - endothelial cells, macrophages, and smooth muscle cells, T7)	Stabilin-2	***Note:*** Peptide accumulates in atherosclerotic lesions in Ldlr−/− mice. However, stabilin-2 is not specific to plaques but is expressed widely in sinusoidal endothelial cells.
In vitro panning against cultured primary aortic vascular smooth muscle cells followed by *in vivo* panning in mice with endothelial denudation of the carotid artery, isolating phage from the carotid artery	CNIWGVVLSWIGVFPEF[387] CESLWGGLMWTIGLSDC[387] (proliferating VSMCs, pComb8 phagemid)		
Ex vivo panning against primary human saphenous vein smooth muscle cells	EYHHYNK[388] (saphenous vein smooth muscle, pIII)		***Oligonucleotide Delivery:*** Peptide inserted into adeno-associated virus-2 transduced HSVSMCs and HCASMCs with β-galactosidase.
In vivo panning in Duchenne muscular dystrophy *mdx* mice, isolating phage from the heart and quadriceps	SKTFNTHPQSTP[363] (T9) (cardiomyocytes and quadriceps, pIII)		

[a] Cysteine residues that form disulfide bonds are indicated in bold.

[b] Common peptide names that have been used in the literature are indicated in parentheses next to the peptide sequence.

[c] The phage display library type is indicated in parenthesis. All phage are filamentous unless noted as T7 phage.

[d] The targeted cell type is indicated if determined.

[e] Peptides clustered in the same cell in this table were isolated in the same selection experiment. A dotted line between cells indicates that the peptides were isolated in the same panning but are separated for clarity.

[f] Peptides selected to bind diseased heart arc highlighted in blue.

Table 10

Immune-Cell-Targeting Peptides

Isolation Method[a]	Peptide Sequence[b,c,d]	Immune Cell Type	Applications and Notes
In vitro panning against the heterodimeric protein CD11c/CD18 purified from splenic tissue of a patient with hairy cell leukemia	CGRWSGWPADLC[390]	Dendritic Cells	***Immunomodulation:*** C57BL/6 mice vaccinated with peptide-liposomes carrying OVA induced OVA-specific T cell priming and antibody production. The peptide was also engrafted into plasma membrane vesicles derived from B16-OVA cells and used to vaccinate C57BL/6 mice carrying either lung or subcutaneous B16-OVA tumors, inhibiting tumor growth and metastasis in both models[391]
In vitro panning against immobilized CD40-Ig	ATYSEFPGNLKP[399]	Dendritic and B cells	***Oligonucleotide Delivery:*** A bifunctional peptide, created by conjugating this peptide to a peptide that binds the adenoviral knob protein, allowed for specific transduction of adenoviral GFP to human and murine dendritic cells, murine B cell blasts, murine L10A cells, and human B cells.
In vitro panning against the murine dendritic cell line JAWSII. Bound phage were eluted with TNFα to identify TNF-R2 ligands.	CYTYQGKLC[392] (pTNF)	Dendritic Cells	***Oligonucleotide Delivery:*** Both a K_{16}-G_5-peptide fusion and peptide-K_{16} microspheres transduced JAWSII and BMDC with a GFP expressing plasmid[392] Peptide-lipoplexes containing EGFP-siRNA induced silencing of EGFP in EGFP-expressing DC2.4 cells.[400]
In vitro panning against XS52 dendritic cells	GPEDTSRAPENQQ-KTFHRRW[393] (XS52.1)	Dendritic Cells	***Oligonucleotide Delivery:*** Peptide-liposomes containing luciferase plasmid transduced Langerhans cells when injected into mouse skin. ***Immunomodulation:*** The peptide containing phage clone induced a more rapid and robust humoral immune response against phage coat proteins than did control peptide phage clones.[393]
In vitro panning against LPS-activated U937 hematopoietic precursor cells	PTAPTST[401] PSPRSGS[401] PAIAHRT[401]	Macrophages (Activated)	
In vitro panning against immobilized ganglioside GM1	AFHKNSQTTTLY[398](CL1) TNCLQNCSGVHL[398] (CL2) GWKERLSSWNRF[398] (CL3)	M Cells	***Immunomodulation:*** Oral delivery of the CL3 peptide-fused to EGFP induced an EGFP-specific mucosal and systemic immune response in mice (IgG & IgA production, Th2 type response).
In vitro panning against human M-like cell model system (co-	SFHQLPARSPLP[397](Col)	M Cells	***Immunomodulation:*** Peptide-fused EGFP given orally bound M cells and transported to the

Isolation Method[a]	Peptide Sequence[b,c,d]	Immune Cell Type	Applications and Notes
cultures of Caco-2 and Raji cells)			mucosal immune induction site, inducing an EGFP-specific mucosal and systemic immune response in mice (IgG and IgA production, Th2 type response). ***Suggested Receptor:*** C5aR[402]
In vitro panning against human M-like cell model (co-cultures of Caco-2 and Raji cells), selecting for transcytosis	CKSTHPLSC[403] (CKS9)	M Cells	***Functional Peptide Activity:*** Chitosan nanoparticles that display CKS9 localized in the Peyer's patch when injected into a closed ileal loop.
In vitro panning of a T7 phage peptide library against monocultures of Caco-2 cells and co-cultures of Caco-2 and M cells, selecting for phage transcytosis	CTGKSC[404] PAVLG[404] LRVG[404]	M Cells	***Functional Peptide Activity:*** The peptides transcytose nanoparticles across M-like cells in an *in vitro* assay.
In vivo panning in Winstar rats, delivering the phage library into the closed ileal loop and isolating Peyer's patches	LETTCASLCYPS[396] (P8) VPPHPMTYSCQY[396] (P25)	M Cells	***Functional Peptide Activity:*** The D- analog of P8 delivers polystyrene beads to M cells and Peyer's patch when injected locally into the intestine.
In vitro panning against immobilized FcγRIIA-R134-GST	WAWVWLTETAV[394]	Leukocytes	
Ex vivo panning against freshly isolated human neutrophils	FGPNLTGRW[25] CKDGLFLGSWLC[25] DLVTSKLQV[25]	Polymorphonuclear leukocytes	
In vitro screening against neutrophil-like differentiated HL60 cells screening for arachidonic acid release using PS-SPCL	RKYHVM-NH_2[405] RKYYYM-NH_2[405] MKYYKM-NH_2[405] MKYYYM-NH_2[405]	Leukocytes	***Functional Peptide Activity:*** The peptides stimulate superoxide production and induce chemotactic migration. ***Suggested Receptor:*** FPRL1
Ex vivo screening against human monocytes using PS-SPCL, screening for superoxide anion generation	HFYLPM[406] HFYLPm[406] MFYLPM[406]	Monocytes	***Functional Peptide Activity:*** The peptides induce superoxide production and cause an increase in intracellular Ca^{2+} rise.

[a] All libraries are pill phage libraries unless otherwise noted.

[b] Cysteine residues that form disulfide bonds are indicated in bold.

[c] Common peptide names that have been used in the literature are indicated in parentheses next to the peptide sequence.

[d] Peptides clustered in the same cell in this table were isolated in the same selection experiment.

Table 11

Islet-Targeting Peptides

Isolation method[a]	Peptide Sequence[b,c,d]	Target Cell	Applications and Notes
Ex vivo panning against freshly isolated rat pancreatic islets	LSGTPERSGQAVKVKLKAIP[75] (RIP1) GAWEAVRDRIAEWGSWGIPS[75] (RIP2)	β-cells (RIP1) Non-specific binding (RIP2)	***Note:*** Radiolabeled RIP1 phage was shown to home to islets *in vivo*.[409]
In vivo panning in male C57BL/6 mice followed by laser pressure catapult microdissection (LPCM) of pancreatic islet sections	CVSNPRWKC[408] CHVLWSTRC[408]	vasculature of islets of Langerhans	***Oligonucleotide Delivery:*** Peptide-polymer carrying luciferase DNA transduced MS1 cells in culture.[410] ***Note:*** EphA4 and EphA2 have been suggested as the receptors.
Ex vivo panning on cell suspensions from solid tumors of RIP1-Tag2 mice and then *in vivo* panning identifying phage homing to tumors using a T7 phage library	CRGRRST[315] (RGR) CRSRKG[315] (RSR) CKAAKNK[315] (KAA) CKGAKAR[315] (KAR) FRVGVADV[315] (VGVA)	RGR: hyperplastic & tumor vasculature of pancreatic islets (endothelial cells & pericytes) RSR: hyperplastic vasculature of pancreatic islets (endothelial cells & pericytes) KAA: tumor vasculature of pancreatic islets (endothelial cells & pericytes) KAR: tumor pancreatic islets VGVA: hyperplastic & tumor pancreatic islets	***Therapeutic Applications:*** Combination treatment with RGR peptide-anti-CD40 antibody and murine IL-2-RGR peptide fusion protein increased survival in RIP1-Tag5 mice, and 80% of mice treated with the combination treatment plus adoptive transfers of anti-Tag $CD4^+$ and $CD8^+$ T cells survived long-term.[316] ***Note*:** PDGFRβ has been suggested as the receptor for the RGR peptide.
Ex vivo panning on cell suspensions from angiogenic islets of RIP1-Tag2 mice and then *in vivo* panning isolating phage that homed to angiogenic islets	CEYQLDVE[315] (EYQ)	hyperplastic pancreatic islets	

[a] All libraries are pIII phage libraries unless otherwise noted.

[b] Cysteine residues that form disulfide bonds are indicated in bold.

[c] Common peptide names that have been used in the literature are indicated in parentheses next to the peptide sequence.

[d] Peptides clustered in the same cell in this table were isolated in the same selection experiment.

Table 12

Adipose-Targeting Peptides

Isolation method and cell type[a]	**Peptide Sequence**[b,c]	**Receptor Identified**	**Applications and Notes**
In vitro panning against mouse preadipocyte 3T3-L1 cells	CWLGEWLGC[413] (mPep)	$\alpha_5\beta_1$	***Therapeutic Applications:*** Peptide-$_D$(KLAKLAK)$_2$ killed mouse 3T3-L1 preadipocytes that were induced toward adipocyte differentiation *in vitro*. ***Oligonucleotide Delivery*:** When the peptide was displayed on a chimeric prokaryotic-eukaryotic AAVP vector carrying the *Ucp 1* gene, it transduced 3T3-L1 preadipocytes, preventing them from differentiating into adipocytes.
Ex vivo panning against adipose stromal cells isolated from white adipose tissue after patient liposuction	CMLAGWIPC[413] (hPep)	$\alpha_5\beta_1$	***Functional Peptide Activity*:** Free peptide increased cell migration of human adipose stem cells *in vitro*.
In vivo panning against white fat in obese leptin-deficient *Lep*$^{ob/ob}$ mice using a T7 phage peptide library	CKGGRAKDC[414] (binds white fat vasculature)	Prohibitin[414,416,417]	***Therapeutic Applications:*** Subcutaneous injection of peptide-GG-$_D$(KLAKLAK)$_2$ into obese mice[414,418] or rats[418] reversed obesity and reduced white fat mass. Injection of a peptide-BVT.2733 (an 11β-HSD1 inhibitor) conjugate into obese mice reversed obesity, reduced adipose tissue mass and size, and decreased plasma glucose tolerance.[419] Subcutaneous injection of peptide-GG-$_D$(KLAKLAK)$_2$ into obese rhesus macaques monkeys decreased white fat mass, total body weight, BMI, abdominal circumference, and insulin resistance.[415] ***Note:*** The peptide also targets to prohibitin-positive tumors.[472]
In vivo panning (T7 library) against white adipose tissue in mice followed by FACS to isolate adipose stromal cells	CSWKYWFGEC[412] (WAT7)	Truncated decorin lacking glycanation site (ΔDCN)	

[a] All libraries are pIII phage libraries unless otherwise noted.

[b] Cysteine residues that form disulfide bonds are indicated in bold.

[c] Common peptide names that have been used in the literature are indicated in parentheses next to the peptide sequence.

Table 13

Brain-Targeting Peptides

Isolation Method[a]	Peptide Sequence[b,c,d,e]	Applications and Notes
In vitro panning against EphA4 extracellular domain fused to human Fc	APYCVYRGSWSC[248] (APY) KYLPYWPVLSSL[248] (KYL) VTMEAINLAFPG[248] (VTM)	***Functional Peptide Activity*:** All three peptides block binding of ephrin-A3 to EphA4 but do not activate the receptor. The free peptides disrupted migration of neural crest cells in chick trunk explants.[248] The KYL peptide inhibited EphA4-Fc mediated collapse of E17 rat neocortical axonal growth cones and infusion of KYL into spinal cord lesions in rats prevented retrograde degeneration of the corticospinal tract and improved corticospinal tract function.[425] When KYL was applied to hippocampal slices from *Thyl-mGFP*^{sil} mice, the peptide increased the number of terminal aborizations, reduced remodeling at the most plastic terminal aborizations in mossy fibers and disrupted terminal aborization topography.[426]
In vitro panning against EphA5 extracellular domain fused to human Fc	SLRDTYMRAEVL[248] WDCNGPYCHWLG[248] WTFPVLWDDKHP[248]	***Note*:** WTFPVLWDDKHP binds both EphA5 and EphA6.
In vitro panning against EphA7 extracellular domain fused to human Fc	WASHAPYWPHPP[248] SVSVGMKPSPRP[248] KHLPFYPHPTSP[248]	***Note:*** These peptides bind multiple members of the EphA receptor family. SVSVGMKPSPRP has been suggested to be a non-specific peptide that is isolated from the NEB 12-mer library most likely due to preferential propagation of this phage clone.[207]
In vitro panning against a prototypical clone of murine neural stem cells (C17.2)	CGLPYSSVC[427]	***Functional Peptide Activity:*** The peptide induced proliferation of C17.2 neural stem cells and primary neural progenitors isolated from adult mouse brain. ***Note*:** The receptor for the peptide is laminin γ1.
In vitro panning against cultured mouse cerebellar granule neurons (CGNs)	SLNDWIDWSEPH[424] SHSLMTSSSVWT[424] SHTTPGKNNDPF[424] NYARDPLMSLPQ[424]	
Ex vivo panning against cultures of primary neurospheres isolated from hippocampus of adult C57BL/6 mice	QTRFLLH[428] VPTQSSG[428]	***Oligonucleotide Delivery*:** The QTRFLLH and VPTQSSG peptides were linked to adenovirus carrying RFP. Both constructs infected hippocampal stem cells after injection into brains of pNestin-GFP transgenic or C57BL/6 mice, but the QTRFLLH peptide was limited to the precursor cells of the hippocampal dentate gyrus. A QTRFLLH-adenovirus construct carrying GFP infected hippocampal stem cells after injection into brains of Wistar rats. This allowed for monitoring of stem cell differentiation.[429]
Ex vivo selection for the ability to inhibit binding of the cocaine analog [^{125}I]RTI-55 to the dopamine transporter from rat caudates. This selection used a positional scanning D-amino acid library.	Ac-frrwfc-NH_2[62] Ac-frrwrc-NH_2[62]	***Functional Peptide Activity*:** Microdialysis of the Ac-frrwfc-NH_2 peptide into brains of rats increased extracellular dopamine and serotonin levels.
In vivo panning in mice using a tail vein injection and isolating phage from the brain	TGNYKALHPHNG[206] (TGN)	***Imaging Applications*** The peptide crossed the blood-brain barrier, even when injected via the tail vein, and transported PEG-PLGA nanoparticles containing DiR dye into the brain as determined by near-infrared imaging.
In vivo panning via nasal passageways in rats, isolating phage from the cerebrum	ACTTPHAWLCG[423]	***Note:*** The peptide enters the brain through the olfactory nerve pathway. Phage do not pass through the blood brain barrier when administered intravenously.
In vivo panning in male C57B1/6 mice by *in situ* brain perfusion of phage, isolating phage from the brain	GLAHSFSDFARDFVA[422] (GLA) GYRPVHNIRGHWAPG[422] (GYR)	***Functional Peptide Activity*:** The phage homed to brain endothelium when perfused *in situ* into the brain of a mouse. The free peptide was not tested.

Isolation Method[a]	**Peptide Sequence**[b,c,d,e]	**Applications and Notes**
In vivo panning (T7 phage) in Balb/c mice, using a tail vein injection and isolating phage from the brain	CLSSRLDAC[67] CNSRLHLRC[67] CENWWGDVC[67]	***Functional Peptide Activity*:** The CLSSRLDAC peptide was conjugated to red blood cells resulting in an accumulation of red blood cells in the brain. All phage home to the brain vasculature.
	WRCVLREGPAGGCAWFNRHRL[67]	***Functional Peptide Activity*:** The phage clone binds to the brain vasculature.
In vivo panning in normal mice, using i.v. injection of phage and recovery of phage from the brain	CRTIGPSVC[300]	***Therapeutic Applications:*** A peptide-adeno-associated virus phage vector (AAVP) carrying herpes simplex virus thymidine kinase gene (*HSV-TK*) transduced intracranial glioblastomas in mice after i.v. injection. This led to tumor inhibition after i.p. ganciclovir administration. ***Imaging applications:*** Treatment with an intravenous injection of the above peptide-AAVP allowed for microPET/CT imaging of accumulation in tumors after i.v. injection of [^{18}F]-FEAU. ***Note:*** The peptide binds normal brain and glioblastoma vasculature and parenchyma. The cellular receptor is the Transferrin receptor.

[a] All libraries are pIII phage libraries unless otherwise noted.

[b] Cysteine residues that form disulfide bonds are indicated in bold.

[c] Common peptide names that have been used in the literature are indicated in parentheses next to the peptide sequence.

[d] Peptides clustered in the same cell in this table were isolated in the same selection experiment.

[e] Lowercase letters indicate D-amino acids and Ac = acetylation.

Table 14

Liver- and Kidney-Targeting Peptides

Isolation Method[a]	Peptide Sequence[b,c,d]	Target Cell	Applications and Notes
In vivo panning in ICR mice using a T7 phage library injected i.v., isolating phage from the liver	LRRKIVE[430] TRRQTIK[430] KTHQNSR[430] ANQIRSR[430] GQKLRYT[430] TKQMRKS[430]	hepatocytes hepatocytes hepatocytes sinusoids sinusoids microvilli of hepatocytes	
In vivo panning in Wistar Kyoto rats infusing phage into the femoral vein and selecting for kidney targeting	HTTHREP[431] (HTT) HITSLLS[431] (HIT)	tubular epithelium glomeruli	***Oligonucleotide Delivery*:** The HTT peptide inserted into Ad5/19p adenovirus transduced kidneys with β-galactosidase.[431] The HIT peptide inserted into Ad5/19p adenovirus transduced fresh human renal tumor cells, and normal human kidney cells *in vitro* and *in vivo* with β-galactosidase.[431,432] The HIT peptide labeled adenovirus also transduced ACHN subcutaneous or intraperitoneal tumors in mice at levels similar to or above wild type adenovirus and reduced liver uptake of the virus.[432]
In vivo panning in Balb/c mice, i.v. injecting phage and isolating phage clones from the kidneys	CLPVASC[67]	kidney vasculature	
Ex vivo panning on microdissected intact cortical collecting ducts from rat kidneys	ELRGDMAAL[177] ELRGDRAHW[177]	tubular epithelial cells of the cortical collecting ducts	***Note:*** The peptides are likely to bind an integrin receptor since they contain an RGD motif. The peptides bind preferentially to the basolateral side of the cortical collecting ducts in the kidney. ELRGDMAAL also binds T84 colon carcinoma cells.
Ex vivo panning on microdissected intact proximal convoluted tubules from rat kidneys	KMGGTNHPE[177]	tubular epithelial cells of the proximal convoluted tubules	

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[b] Cysteine residues that form disulfide bonds are indicated in bold.

[c] Common peptide names that have been used in the literature are indicated in parentheses next to the peptide sequence.

[d] Peptides clustered in the same cell in this table were isolated in the same selection experiment.

Table 15

Stem-Cell-Binding Peptides

Isolation method[a]	Peptide Sequence[b,c,d]	Applications and Notes
In vitro panning on the extracellular domain of the cancer stem cell marker CD133	LQNAPRS[144] APSPMIW[144]	***Functional Peptide Activity:*** The LQNAPRS peptide inhibits migration of colon and breast cancer cells. Treatment of cells with the peptide reduced c-Met and STAT3 protein levels.[437]
In vitro whole cell panning on undifferentiated pluripotent P19 cells from a murine teratocarcinoma	ALPSTSSQMPQL[438]	***Note:*** The peptide does not bind to differentiated P19 cells.
In vitro panning on mouse embryonic stem cells	KHMHWHPPALNT[439] (Seq2)	***Note:*** The peptide does not bind to differentiated mouse embryonic stem cells.
In vitro panning on an embryonic stem cell line derived from rhesus macaque	APWHLSSQYSRT[440]	***Note:*** This peptide has been isolated on numerous targets and is suggested to be a nonspecific phage that is isolated due to propagation advantages.[207]
In vitro panning on mouse neural stem cells	**C**GLPYSSV**C**[427]	***Note:*** The peptide shares sequence similarity with netrin-4 and binds laminin γ1.
In vitro panning against neural stem cells derived from rheusus monkey embryonic stem cells	HGEVPRFHAVHL[441]	
In vitro panning on murine neural stem cells	FAQRVPP[433] QHLPRDH[433] SSLSVND[433]	***Functional Peptide Activity:*** The FAQRVPP peptide promotes cell growth and differentiation. Injection of the peptide fused to a self-assembly peptide domain increases the rate of recovery of spinal injury induced in rats.
In vitro panning on undifferentiated murine neural stem cells isolated from the subventricular zone	KLPGWSG[442]	***Functional Peptide Activity:*** The KLPGWSG peptide promotes cell differentiation towards a neuronal state.
Ex vivo panning against human bone-marrow derived mesenchymal stem cells	EPLQLKM[443] (E7)	***Functional Peptide Activity:*** The E7 peptide was conjugated to polycaprolactone meshes and implanted into cartilage defects created in the knee joints of rats followed by generation of microfractures. The E7-mesh recruited more cells with stem cell markers than that of a control-peptide mesh. ***Note:*** E7 was also identified from panning on graphene, calling into question the specificity of the peptide for mesenchymal stem cells.[444]
Bacterial peptide display selection on hippocampal neural stem cells from a rat brain using a CPX library	WWCDMRGDSRCSG[445] DHKFGLVMLNKYAYAG[445] KLCCFDKGYYCMR[445]	***Functional Peptide Activity:*** The peptides mediate cell binding when adsorbed onto a solid support or incorporated into a polymer hydrogel. The cells were able to differentiate into different lineages.
In vivo panning amplifying the phage from mouse bone marrow and *in vitro* panning on murine bone marrow stem cells	STFTKSP[446] NHWASPR[446]	***Functional Peptide Activity:*** The STFTKSP peptide homes to bone marrow *in vivo* and reduces homing of stem cells to the bone marrow.

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[b] Cysteine residues that form disulfide bonds are indicated in bold.

[c] Common peptide names that have been used in the literature are indicated in parentheses next to the peptide sequence.

[d] Peptides clustered in the same cell in this table were isolated in the same selection experiment.

Table 16

Vasculature- and Endothelial-Cell-Specific Peptides

Isolation method[a]	Peptide Sequence[b,c,d]	Cellular Receptor and/or Vasculature Specificity	Applications and Notes
In vitro panning on purified Tie2 protein	TMGFTAPRFPHY[457] (PH1)	Tie2 is widely expressed in the endothelium as well as in many cancer cell lines	***Therapeutic Applications:*** This peptide has been conjugated to cisplatin loaded liposomes for specific cell killing *in vitro*. ***Note:*** This peptide has also been isolated from phage displayed peptide selections against the bacterial Murligase enzyme MurF, which is involved in cell wall biosynthesis,[458] and against the *E. coli* protein TonB.[459] It was also observed in a panning on purified troponin 1,[460] hydroxyapatite,[461] and graphene.[444] This calls into question the specificity of the peptide.
In vitro panning on SMMC772 cells overexpressing Tie2	NSLSNASEFRAPY[462] (GA5)	Tie2 is widely expressed in the endothelium as well as in many cancer cell lines	***Oligonucleotide Delivery*:** The peptide has been conjugated to PET for delivery of the luciferase gene *in vitro*, and the WT p53 gene was delivered by intratumoral injection in mice.
In vitro panning against HUVECs (human umbilical vein endothelial cells)	SIGYPLP[463]		***Oligonucleotide Delivery*:** The peptide has been used in numerous formats, including viral constructs and poylcation complexes, to transduce HUVEC cells with eGFP or β-galactosidase.[463–466] The peptide also mediates transduction in a panel of 6 cancer cell lines.[467]
	MSLTTPPAVARP[468] (MSL) MTPFPTSNEANL[468] (MTP)		***Oligonucleotide Delivery*:** MSL and MTP peptide-modified adeno-associated virus (AAV-2) transduced HUVECs with β-galactosidase and reduced transduction of HepG2 cells.[468] Both peptides were cloned into modified adenovirus and found to transduce HUVECs and HSVECs with EGFP *in vitro* and abdominal aorta/vena cava with β-galactosidase after tail vein injection into mice.[469]
Ex vivo panning against HUVECs under normoxia	QPWLEQAYYSTF[470] (QF) VPWMEPAYQRFL[470] (VL) TLPWLEESYWRP[470] (TR)		***Functional Peptide Activity*:** QF, VL and TR peptides induced proliferation and migration of serum starved endothelial cells *in vitro*. The QF and TR peptides induced endothelial cell tube formation *in vitro* and increased the number of blood vessels when injected into the ears of BALB/c mice. ***Note*:** The TR and VL peptides were isolated from panning of HUVECs under normoxic and hypoxic conditions.

Isolation method[a]	Peptide Sequence[b,c,d]	Cellular Receptor and/or Vasculature Specificity	Applications and Notes
Ex vivo panning against HUVECs under hypoxia for 3hrs	YPHIDSLGHWRR[470] (YR) SAHGTSTGVPWP[470] (SP)		***Functional Peptide Activity*:** YR and SP peptides induced proliferation and migration of serum starved endothelial cells and promoted endothelial cell tube formation under hypoxia. The YR peptide increased the number of blood vessels when injected into the ear of BALB/C mice. ***Note*:** The SP peptide was isolated from panning of HUVECs kept under hypoxic conditions for 3 or 24 hours.
Ex vivo panning against HUVECs under hypoxia for 24hrs	LLADTTHHRPWT[470] (LT) SAHGTSTGVPWP[470] (SP) VPWMEPAYQRFL[470] (VL) TLPWLEESYWRP[470] (TR)	Vimentin is the putative receptor for the SP peptide.[471]	***Functional Peptide Activity*:** The LT, SP, VL, and TR peptides induced proliferation and migration of serum starved endothelial cells. The VL and SP peptides promoted endothelial cell tube formation, although SP only exhibits this effect under hypoxic conditions.[471] The VL peptide increased the number of blood vessels when injected into the ear of BALB/C mice. Intramuscular injections of the SP peptide into ischemic hind limbs of the mice increased blood perfusion and the number of capillaries.[471] ***Note*:** The SP peptide was isolated from panning of HUVECs kept under hypoxic conditions for 3 or 24 hours. The TR and VL peptides were isolated from panning of HUVECs under normoxic and hypoxic conditions.
Ex vivo panning against human umbilical cords, isolating endothelial cells	KPSGLTY[472]	umbilical vein endothelium	
Ex vivo panning against H-2K^b-tsA58 mouse derived lung microvascular endothelial cells	**C**GSPGWVR**C**[473]	lung vasculature	***Therapeutic Applications:*** Peptide-$_D$(KLAKLAK)$_2$ killed lung endothelial cells *in vitro*, and i.p. injection of peptide-$_D$(KLAKLAK)$_2$ into C57B16/6J mice induced apoptosis of alveolar cells.
Ex vivo panning against primary rat lung alveolar epithelial cell cultures	**C**TSGTHPR**C**[474] (LTP-1)	lung vasculature	***Functional Peptide Activity*:** Peptide-dendrimer conjugates demonstrated transport across lung airways to pulmonary vasculature in an *ex vivo* isolated rat perfused lung model.
Ex vivo panning against human blood outgrowth endothelial cells	SVPPRYTLTLQW[475] TPSLEQRTVYAK[475] SPPPSNAGSHHV[475]MPTLTRAPHTAC[475]		
	QFPPKLTNNSML[476]		***Note*:** The phage clone homes to Lewis lung carcinoma tumors *in vivo*.
Combination *ex vivo* and *in vivo* panning using a T7 library in K14-HPV16 mice with	**C**SRPRRSE**C**[312]	neovasculature of dysplastic skin	

Isolation method[a]	Peptide Sequence[b,c,d]	Cellular Receptor and/or Vasculature Specificity	Applications and Notes
dysplastic skin. Phage were isolated from the skin of the ears and chest.			
In vivo panning selecting for pancreas homing followed by *in vitro* panning against both prolactin-like protein receptor (PLPR) overexpressing COS-1 cells and purified recombinant PRLR	**C**RVASVLP**C**[452]	PRL receptor on the vasculature of the pancreas and pancreatic islet cells	
In vivo panning in Balb/c mice, injecting phage via tail vein injection followed by phage isolation from the lungs	**C**GFE**C**VRQ**C**PER**C**[448] (GFE-1) **C**GFELET**C**[448] (GFE-2) **C**TLRDRN**C**[448] **C**IGEVEV**C**[448]	lung vasculature	***Imaging applications:*** GFE-1 delivered a quantum dot to lung epithelium *in vivo*.[305] ***Oligonucleotide Delivery*:** GFE-1 conjugated to a neutralizing antibody that binds adenovirus (Ad5) transduced MDP expressing cells with β-galactosidase and GFP.[477] ***Functional Peptide Activity*:** GFE-1 inhibited metastases *in vivo* when co-injected with C8161 human melanoma cells.[456] GFE-1 delivered IFN-α2a to the lung when expressed as a fusion protein but therapeutic efficacy was not tested.[478] ***Note:*** The receptor for GFE-1 and GFE-2 is membrane dipeptidase (MDP).[479]
In vivo panning in Balb/c nude mice followed by phage isolation from the skin	**C**VAL**C**REA**C**GEG**C**[448]	skin vasculature	***Functional Peptide Activity*:** Peptide—GST fusion protein blocked phage binding *in vivo*.
In vivo panning in rats with a skin wound, using tail vein or intracardiac injections and isolating phage from wounded skin	**C**RKDK**C**[480] (CRK)	Vasculature of wounded skin or tendons, preferentially binds at later stages of wound healing	***Imaging applications*:** Conjugation of CRK to nanoworms (elongated iron oxide nanoparticles) allowed for nanoparticle targeting to tumor vasculature and MRI imaging in an orthotopic prostate tumor model.[313] Accumulation of nanoworms also blocked blood flow to the tumor and induced necrosis.
In vivo panning of a T7 library in rats with a patellar tendon wound, isolating phage from the patellar tendon	**C**ARSKNKD**C**[480] (CAR)	Heparin sulfate & heparin[480]	***Functional Peptide Activity:*** CAR-decorin fusion protein inhibited cell spreading and TGF-β dependent cell proliferation of CHO-K cells.[481] Intravenous injection of CAR-decorin fusion protein into mice bearing thick dorsal skin promoted wound healing and reduced scar formation.[481] The peptide also homed to lung vasculature in a pulmonary arterial hypertension rat model and spread throughout the lung.[482]
In vivo panning in CD-I mice selecting for prostate homing	SMSIARL[451]	prostate vasculature	***Therapeutic Applications:*** Injection of peptide-$_D$(KLAKLAK)$_2$ into mice

Isolation method[a]	Peptide Sequence[b,c,d]	Cellular Receptor and/or Vasculature Specificity	Applications and Notes
			caused destruction of prostate glandular epithelial cells and injection into TRAMP mice increased survival.[451] Treatment with peptide-PP1/GaDD34 inhibitor peptide (LKARKVRFSEKV) followed by treatment with peptide-$_D$(KLAKLAK)$_2$ increased survival of TRAMP mice.[483]
In vivo panning of T7 phage library in normal mice selecting for breast homing	**C**PGPEGAG**C**[453]	Amino-peptidase P in breast vasculature	***Functional Peptide Activity*:** Phage uptake is inhibited by the free peptide.
In vivo panning in female lactating rats isolating phage from the mammary tissue	**C**LHQHNQM**C**[454] (MG1)	Acidic Ribosomal Protein Large P0 (RPLP0)	
In vivo panning using a dorsal air sac model of angiogenic vessels	PRPGAPLAGSWPGTS[484] DRWRPALPVVFPLH[484] ASSSYPLTHWRPWAR[484]		***Therapeutic Applications:*** The peptide fragment APRPG was coupled to a doxorubicin loaded liposome and shown to reduce tumor growth compared to free drug or non-targeted liposome.[484] ***Functional Peptide Activity*:** All three peptides accumulate in angiogenic vasculature in mouse tumor xenografts. PRPGAPLAGSWPGTS binds vasculature in human tumors.[484] ASSSYPLIHWRPWAR suppresses angiogenesis.[485]
In vivo panning in mice selecting for thymus homing	**C**HAQGSAE**C**[455]	thymus vasculature	***Functional Peptide Activity*:** When injected into mice, the peptide down regulated the expression of T cell receptor circles, suggesting inhibition of thymus bioactivity.[455]
Screening of a PS-SPCL against murine endothelial MS-1 cells, selecting for an increase in intracellular calcium	SFKLRY-NH_2[486] (Angio-S)		***Functional Peptide Activity*:** Angio-S induced proliferation, migration, and tube formation of HUVECs as well as sprouting of rat aortic rings. The peptide also protected human dermal fibroblasts and B16 melanoma cells from oxidative stress and increased their survival.[487]

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[b] Cysteine residues that form disulfide bonds are indicated in bold.

[c] Common peptide names that have been used in the literature are indicated in parentheses next to the peptide sequence.

[d] Peptides clustered in the same cell in this table were isolated in the same selection experiment.

Table 17

Epithelium-Targeting Peptides

Isolation method[a]	**Peptide Sequence**[b,c,d]	**Cellular Receptor and/or Tissue Specificity**	**Applications and Notes**
In vitro panning against immobilized human polymeric Ig receptor (pIgR) also called membrane secretory component (SC)	**C**VVWMGFQQV**C**[491] (C9A)	pIgR (SC) on mucosal epithelium	***Functional Peptide Activity*:** The C9A phage clone is translocated through polarized MDCK cells that express the pIgR-receptor.
In vitro panning against human bronchial epithelial (16HBE14o$^-$) cell monolayers treated with EGTA	FDFWITP[492]	bronchial epithelium	***Functional Peptide Activity*:** A cyclic form of the peptide reduced transepithelial electrical resistance of MDCK cells
In vitro panning against human bronchial epithelial (16HBE14o$^-$) cells	**C**THALWHT**C**[493]	CD47 (suggested)[494] on bronchial epithelium	***Oligonucleotide Delivery*:** When cyclized and synthesized with $(K)_{16}$, the peptide delivered a luciferase gene to 16HBE14o- cells.[493] Peptide inserted into modified adeno-associated virus (AAV2) encoding luciferase transduced HEK-293, Calu-3, polarized Calu-3, and CFBE41o- cells.[494] ***Note:*** This peptide sequence was also selected by another group that panned the same library on 16HBE14o$^-$ cells.[492]
In vitro panning against tracheal epithelial cells CFT-2	**C**RFDSLKV**C**[495] **C**GRGDGDV**C**[495]		***Oligonucleotide Delivery*:** When fused to a DNA binding peptide, both peptides were able to transfect various cell types with EGFP.
In vitro panning against human airway epithelial (1HAEo$^-$) cells	**C**LPHKSMP**C**[496] **C**SERSMNF**C**[496] **C**YGLPHKF**C**[496] **C**PSGAARA**C**[496] **C**LQHKSMP**C**[496]	ICAM-1 (suggested)	***Oligonucleotide Delivery*:** All five peptides were individually fused to DNA-binding K_{16} and mixed with lipofectin and luciferase plasmid in order to deliver a luciferase gene to a variety of cell types.[496] Additionally, peptide- K_{16}-luciferase DNA-cationic liposomes transfected rabbit, porcine, and human smooth muscle cells.[497] The **C**SERSMNF**C** peptide has been employed in numerous other formats for gene delivery,[498] including mediating delivery of the cystic fibrosis transmembrane conductance receptor gene (*CFTR*) via intratracheal administration, resulting in CFTR expression m the lungs.[499] Whole body nebulization in mice of peptide-K_{16}*luciferase* or l*acZ* DNA-cationic liposomes transfected lungs and trachea.[500]
In vitro selection against SV40 transformed lung fibroblast VA-13 cells using an invasin displayed bacterial library	CGPSVITSCSIC[44] CGKMLFWGGCRADC[44] **C**SNFLTQRVSM**C**[44] **C**LGPYFMKGMQ**C**[44]		***Note:*** The disulfide bond pattern has not been defined for the peptides containing 3 cysteines.
Ex vivo panning against isolated intestinal mucosal cells from mice with 30% total body surface area steam burn	LTHPQDSPPASA[501]	Intestinal lymphatics (suggested)	***Functional Peptide Activity*:** The peptide delivered Qdots to the gut mucosa when injected into the intestinal lumen.

Isolation method[a]	**Peptide Sequence**[b,c,d]	**Cellular Receptor and/or Tissue Specificity**	**Applications and Notes**
Ex vivo panning for phage that penetrate porcine and mouse skin	LVGVFH[502] (T2)		***Functional Peptide Activity*:** Pretreating the skin of animals with the peptide enhanced penetration of small molecules, including 5-FU, into the *stratum corneum*. The peptide interacts with lipids in the skin resulting in a change of lipid organization.
Ex vivo panning against fresh human urothelium derived cells	GGLSGL[503]	normal urothelium	
	HALE[503]	normal urothelium and RT4 transitional bladder carcinoma cells	
	ISGL[503]	normal urothelium and RT4 and T24 transitional bladder carcinoma cells	
In vivo panning in rats by oral administration of phage, isolating phage from the liver, lung, spleen, and kidney	**C**SKSSDYQ**C**[489]	goblet cells (suggested)	***Note:*** Isolated phage from organs known to retain Ml3 bacteriophage in order to isolate phage that cross the intestinal mucosal barrier.
In vivo panning in rats applying phage by gavage and isolating phage from the spleen	YPRLLTP[490]	Enterocytes and sub mucosal cells in the intestine	***Note:*** Isolated phage from spleen in order to isolate phage that cross the intestinal mucosal barrier.
In vivo panning for phage that penetrate mouse skin and enter the bloodstream	A**C**SSSPSKH**C**G[504] (TD-1)		***Functional Peptide Activity*:** Co-administration of TD-1 with insulin or human growth hormone resulted in transdermal delivery of the protein therapeutics.

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[b] Cysteine residues that form disulfide bonds are indicated in bold.

[c] Common peptide names that have been used in the literature are indicated in parentheses next to the peptide sequence.

[d] Peptides clustered in the same cell in this table were isolated in the same selection experiment.