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Targeting Tumors with Small Molecule Peptides

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Abstract

Chemotherapeutic treatment of cancers is a challenging endeavor, hindered by poor selectivity towards tumorous tissues over healthy ones. Preferentially delivering a given drug to tumor sites necessitates the use of targeting elements, of which there are a wide range in development. In this Review, we highlight recent examples of peptide-based targeting ligands that have been exploited to selectively deliver a chemotherapeutic payload to specific tumor-associated sites such as the vasculature, lymphatics, or cell surface. The advantages and limitations of such approaches will be discussed with a view to potential future development. Additionally, we will also examine how peptide-based ligands can be used diagnostically in the detection and characterization of cancers through their incorporation into imaging agents.

Keywords

Tumor targeting; cancer; peptides; ligands

1. RATIONALE BEHIND USING SMALL MOLECULE PEPTIDES FOR TARGETED THERAPIES

Cancer is a complex and deadly series of diseases [1–3]. In many cases, surgery and radiation therapy are deficient in their attempts to provide a positive, long-term prognosis, especially for instances where the primary tumor has metastasized and spread to multiple parts of the body [4]. Conventional anticancer drugs rely on a sufficiently long half-life in the blood stream to accumulate in cancerous tissues, exerting their desired biological effect through disruption of cell processes that are essential for cellular replication, such as DNA transcription [5, 6], cellular division [7] and metabolism [8]. Many anticancer drugs, however, possess no inherent means to distinguish healthy and diseased tissues, and it is

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CONFLICT OF INTEREST

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only the unregulated, more rapid cell growth within tumors that results in a greater extent of action and thus gives the appearance of preferential targeting. Unfortunately, healthy cells that also grow rapidly, such as endothelial cells and leucocytes, are more strongly affected, leading to the notorious side-effects of chemotherapy, such as nausea, hair loss and a compromised immune system [9]. These unwanted cellular interactions, combined with the development of multi-drug resistance (MDR) [10] by cancer cells, necessitate the development of new therapies that specifically target tumors and cancerous tissues [11]. One strategy is the use of delivery vehicles, such as nanoparticles, that due to their size and other physicochemical characteristics are able to accumulate in tumors by traversing gaps in the endothelial cells lining the vascular wall [12, 13]. The utilization of this "leaky" vasculature surrounding tumors to deliver drugs is known as the enhanced permeability and retention (EPR) effect [14] and represents a *passive* targeting strategy (Fig. 1). These EPR-based therapies, though important, still possess several inherent limitations; for example, the same leaky vasculature responsible for the absorption of the therapeutics by solid tumors also leads to a non-uniform distribution of the drug [15]. Concerns about its effectiveness for the treatment of human metastatic cancers also exist [16, 17]. A major focus in recent years has been the development of "smart" drugs, which actively target tumors through the interaction of phenotype-specific ligands that are conjugated to the drug (or the drug-carrying delivery vector) [18, 19]. By binding with the corresponding receptors on the tumor and/or in its microenvironment, a greater build-up of drugs at the tumor site can be achieved than would otherwise be possible using a passive strategy (Fig. 1). Additionally, binding to certain receptors may help trigger internalization and potentially help avoid any drug resistance mechanisms that have arisen [20]. There is evidence to suggest that this internalization may play the major role in improving drug efficacy when compared with non-targeted counterparts [21, 22], rather than through accumulation alone, but at this stage it is unclear if this is a general trend or specific to certain tumors and/or targets.

Cells in the body possess a large number of protein-based receptors on their membrane surface that are responsible for communication between the intra- and extracellular environment and among different cells. These receptors bind signaling molecules (cytokines, hormones, and growth factors for instance) that then triggers some changes in the cell function. Cancer cells often express a great number and variety of receptors on their surface, particularly those that will facilitate important biological functions associated with tumor growth [23], migration [24], invasion and metastasis [25, 26]. In theory, this would make the tumor cell surface an attractive target for both therapy and imaging, but in practice it is difficult as many of the cells may not be accessible due to the heterogeneity of the tumor mass [15, 27]. Furthermore, cultured cells that are utilized for screening purposes may not possess the same characteristics as the corresponding *in vivo* tumor cells, *i.e.* they may lose tissue-specific characteristics or abnormally express molecules that *in vivo* cells would not. A more attractive proposition is the targeting of endothelial cells in the tumor vasculature rather than the tumor cells themselves [28–30]. This approach possesses several advantages, the most notable of which being the readily accessible nature of vasculature endothelial cells to intravenously introduced agents. Extensive studies have indicated that cancerous endothelial cells display a distinct pattern of surface receptors that is a product of their tissue type and microenvironment [31]. This differential expression of receptors therefore

presents a viable strategy for the specific targeting of tumor endothelial cells over healthy endothelial cells. The challenge remains in discovering potential targets and optimizing suitable targeting ligands that can exploit them.

To target a specific receptor it is necessary to in some way mimic its natural ligand. One of the most effective means to do so is through the use of monoclonal antibodies (mAbs), which are possibly the closest entities to Paul Erlich's "magic bullet" [32] that have been developed thus far. Naked mAbs, to which no chemotherapeutic is attached, can be used to target and block surface receptors involved in cell proliferation [33], *e.g.* trastuzumab (Herceptin®, Genentech/Roche) for the HER2 protein [34, 35] and Cetuximab (Erbitux®, Bristol-Myers Squib) for the EGFR protein [36, 37]. Furthermore, the use of mAbs has been shown to elicit an antitumor response from both the innate and adaptive immune system through their extra- and intracellular modes of action [38]. Utilizing the specificity of mAbs, the conjugation of a drug should in theory deliver the drug to only those cells which possess the target protein, though the effectiveness of this largely depends on the method and site(s) of conjugation and the tumor microenvironment [39]. As targeting elements, mAbs are not without issues of their own as they are expensive to produce, can be heterogeneous, often elicit an allergic response in the patient, and can have side-effects related to the targeted antigen. One targeting strategy of particularly great interest, and the focus of this review, is the use of small molecule peptides that, unlike mAbs, can be used in combination with drug delivery vehicles. Compared with mAbs, peptides have many important advantages: facile synthesis and design, lower risk of allergic response, their drug conjugates have better tumor penetrating properties, and an antigenic target is not required [40, 41]. Furthermore, they are naturally degraded by cellular catabolism. A major disadvantage to peptides, however, is their short half-life in plasma (on the order of minutes), suffering from both rapid degradation and renal clearance that limits their use as drugs outright and as targeting elements when conjugated to small molecular drugs. Fortunately, these otherwise adverse attributes are largely negated when the peptide is affixed to a larger entity such as a delivery vehicle, and chemical modification strategies exist that can prolong their circulation as smaller entities [42]. As a whole, peptides possess immense potential in the development of increasingly specific and advanced cancer therapies. We will venture to outline engineered systems that are harnessed by such therapies.

The rational design of synthetically engineered peptides for drug delivery applications is dependent upon finding amino acid sequences that can "home in" on the ligand of interest [43]. One of the most common methods employed to find homing peptides is the use of phage display libraries [44]. The phage display technique records how phages containing proteins on their surface interact with other ligands and biomarkers, thus shedding light on new protein- and peptide-based interactions [45]. This knowledge has proven invaluable in the discovery of ligands that selectively bind to specific tumor types and environments. A common strategy that employs this information is the development of antibodies with domains that preferentially bind to extracellular receptors of certain cancer phenotypes [46]. In addition, efforts in computational biology and the construction of phage display libraries have elucidated a host of information on the primary sequence and structure of these target ligands; as such, it has been possible to more easily synthesize small-molecule peptides that can preferentially bind to target tissues in the same manner as their larger antibody

or protein counterparts [47]. These tumor-homing small-molecule peptides have served the field of drug delivery in various ways, the most common being the surface decoration of a cargo-carrying entity, such as dendrimers, liposomes, or microbubbles, with targeting peptides [48]. Alternatively, it has also been found that peptides conjugated directly to a therapeutic agent can function in a similarly selective manner (instances of which will be documented here). This review will seek to expound upon current approaches in the engineering of these peptides as the basis of cancer-targeting drug therapies.

2. HOMING PEPTIDES THAT TARGET THE TUMOR VASCULATURE

A critical process in tumor growth is the formation of new blood vessels, through angiogenesis, signaling the transition of tumors from a benign to malignant state [31]. Since angiogenesis is a process that strongly distinguishes cancerous cells from healthy ones [31], it is an attractive target for guided drug delivery strategies. Conveniently, cancer cells and tumor endothelial cells express their own distinct sets of receptors on their surfaces that vary significantly from normal vasculature and other healthy cells; this makes tumor vasculature a more realistic target for ligand-mediated delivery strategies [49]. Targeting tumor vasculature is also advantageous in that tumor endothelial cells are less drug resistant than more genetically unstable tumor cells; in addition, they are highly accessible to any intravenously delivered therapeutic [50]. An almost ubiquitous strategy to target the tumor vasculature is the use of integrin-binding peptides, with peptides based on the RGD motif a common sight in reports [51]. Given the wide breadth of literature available on this motif, we will instead focus on other strategies and direct the reader to a number of excellent reviews on the subject [52–56]. Table 1 contains a non-exhaustive summary of peptide ligands and their targets (where known) that have been used to target tumor vasculature.

Given the importance of angiogenesis in tumor progression, it is desirable to develop peptide-based therapies that can inhibit this process and, therefore, prevent further tumor growth and metastasis [57]. One example of an angiogenesis-specific ligand was developed by Travassos *et al.* [58, 59]; using phage display, the group discovered a new melanoma-homing peptide termed peptide C, and conjugated it to a bioactive, anti-angiogenic peptide sequence (Fig. 2a). The resultant peptide was found to effectively slow tumor progression when systemically injected in mice; “proangiogenic” structures were reported to be reduced by 40 percent [59]. This newly created peptide sequence also binds the human sonic hedgehog protein, a protein prevalent in multiple tumor microenvironments, including melanoma [60]. The use of anti-angiogenic peptides, as it happens, is a valid strategy in the treatments of many types of high-risk tumors. One important example is given by Cohn *et al.*, who show promising work in tumor-homing peptides called SPARC peptides (Secreted Protein, Acidic and Rich in Cysteine) that inhibit angiogenesis in neuroblastoma tumors [61]. Although its exact inhibitive mechanism is not fully understood, SPARC is able to interfere with a variety of growth factors, such as VEGF, PDGF, and bFGF, and reduce their ability to stimulate angiogenesis [62]. The same group then synthesized various peptides mimicking conserved SPARC domains, and found that one, named FSEC (Fig. 2b), not only potently inhibited angiogenesis but also inhibited neuroblastoma progression in a preclinical model [61].

Targets for angiogenesis are not limited to endothelial cells, as proteins in the extracellular matrix such as collagen IV play an important role in the angiogenic process, and are therefore suitable candidates for tumor-targeting therapies [63, 64]. Essler *et al.* have discovered a tumor-homing peptide through phage library screening that specifically binds to collagen IV after it has been cleaved by matrix metalloproteinase 2 (MMP-2) [65]. This peptide, of sequence TLTYTWS, was found to block angiogenesis through the targeting of a molecular mechanism necessary for it to occur: the cleavage of the basement membrane by matrix metalloproteinases [66, 67]. This ability to selectively target different types of biomarkers, from overexpressed receptors to by-products of a molecular mechanism, demonstrates the versatility of using peptides as targeting entities.

Angiogenesis is not the only novel characteristic of the tumor microenvironment. Lymphatic vessels in tumors also possess specialized markers that differentiate them from their non-tumorigenic counterparts. Studies have shown that it is even possible to differentiate tumor types in terms of their lymphatic vasculature [68, 69]. In fact, the physical characteristics of a tumor's lymphatics such as size, number, and expressed growth factors have been shown to be an important factor in tumor metastatic capability [70]. Consequently, metastasis may be combated by targeting not just tumor angiogenesis but also the lymphatic structure of tumors as well. This strategy was employed by Ruoslahti *et al.*, who demonstrated the potential of a lytic peptide to reduce metastasis by targeting tumor lymphatics (Fig. 2c) [71]. The peptide in question, termed LyP-1 (CGNKRTRGC), was shown to preferentially accumulate in breast cancer xenografts following intravenous administration. In addition, LyP-1 was found to localize in hypoxic areas within the tumors, induce tumor cell apoptosis, destroy tumor lymphatics, and inhibit tumor growth [71].

Ruoslahti and coworkers later determined that LyP-1 binds to p32 (or gC1qR) [72], a cell surface protein that is overexpressed on tumor cells and stroma, and undergoes proteolysis to produce a *C*-terminal KRTR motif. It was this feature that was found to be responsible for the activity of LyP-1 [73], with a significant number of peptides possessing the consensus motif, R/KXXR/K, and similar cell internalization characteristics found by phage display. This tissue penetration motif was termed the C-end rule or CendR ("sender") motif, and it is it's interaction with neuropilin-1 (NRP-1) that triggers the activation of a transport pathway through both the vascular wall and tumorous tissues. It was postulated that this CendR tissue transport pathway may exist to facilitate the transfer of nutrients that are far from blood vessels or in some form of distress, *i.e.* hypoxic [74]. Overexpression of NRP-1 in tumors may be one way in which tumors exploit physiological pathways to promote their continued growth. The CendR pathway may also have been hijacked by viruses (*e.g.* Ebola [75], Crimean-Congo hemorrhagic fever [76]), microbial toxins (*e.g.* anthrax [77]) and venoms (melittin in bee venom [78], for example) to aid with cellular entry and spreading through tissues, as the CendR motif is a recurring theme in associated peptides/proteins.

Utilizing this knowledge of the CendR pathway, Ruoslahti's group developed the tumor penetrating peptide, iRGD (internalizing-RGD, CRGDKGPDC) [79], in which the RGD motif targets overexpressed integrins in the tumor vasculature, with subsequent proteolytic cleavage revealing the CendR motif (-RGDK) that then enables tumor penetration. It was demonstrated that conjugation of the iRGD peptide to the clinically relevant therapeutic

Abraxane (albumin-bound paclitaxel, Celgene) resulted in an eight-fold increase in the tumor concentration of the drug after intravenous injection when compared to Abraxane alone [79]. Remarkably, it was found that simply co-administering a drug or drug carrier with standalone iRGD resulted in increased tumor concentration of the therapeutic and enhanced their efficacy [80]. For instance, little difference between the effectiveness of Abraxane was found when co-administered with or conjugated to iRGD. The reasoning behind this is that the CendR pathway is a bulk transport pathway and allows greater systemic accessibility for other payloads to pass through at the same time. The obvious appeal of this observation is that it would remove the need for chemical conjugation, thereby greatly simplifying the preparation process. More recently, another tumor-penetrating peptide was developed in a de novo fashion, replacing the RGD targeting sequence of iRGD with the aminopeptidase N targeting NGR motif [28] to give iNGR (CRNGRGPDC) [81]. Similar to iRGD, iNGR can be coadministered with or conjugated to the therapeutic in order to enhance efficacy. The significance of iNGR, however, lies in its creation from known sequence elements that, in theory, points to the possibility of harnessing any targeting sequence provided that a ‘cryptic’ CendR motif can be incorporated and effectively unmasked upon proteolytic processing.

It should be noted that while the approaches described above have demonstrated great utility, it is important to understand the limitations that this tumor vasculature targeting strategy possesses. One such limitation is that there are many pathways involved in angiogenesis; targeting one in particular could force the tumor cells to adapt and use alternative pathways, resulting in resistance to drugs that seek to exploit the targeted pathway [29]. The heterogeneity of cancerous tissues, as well as their highly irregular vasculature, also raises difficulties for drug delivery strategies that operate by targeting angiogenesis or tumor lymphatics [82]. As the distance from the vasculature increases, there is a steep drop off in the availability of oxygen that can result in hypoxic regions (Fig. 1). In such environments, tumor cells can undergo drastic changes that allow them to avoid death and continue proliferating [83, 84]. Destruction of cancerous tissues nearer the vasculature can result in increased oxygenation that then promotes further tumor growth and invasion [85]. Furthermore, tumor cells that existed at the boundary between the oxygen-rich and poor regions can become drug resistant, as they may have been exposed to concentrations of the drug that were insufficient to induce apoptosis [86]. Tumor penetrating peptides, such as those developed by Ruoslahti, may provide a long-term answer to these challenges, though much work remains to be done.

3. HOMING PEPTIDES THAT TARGET SPECIFIC CANCERS

Just as the tumor microenvironment itself contains overexpressed receptors that become key identifiers, particular tumor types exhibit significant overexpression of specific extracellular receptors (*e.g.* growth factor receptors). As a result, monoclonal antibodies have been utilized as targeting moieties due to their high specificity for their target proteins, with a number of antibody-conjugated drugs (ADCs) already approved for clinical use [39], *e.g.* Brentuximab vedotin [112, 113] (Adcetris®, Seattle Genetics) and Adotrastuzumab emtansine [114] (Kadcyla® or T-DM1, Genentech/Roche). Tumor-homing peptides specific for certain cancers operate around the same concept, with the promise of overcoming

some of the shortcomings of their monoclonal antibody counterparts: peptides have a comparatively facile synthesis, lower production cost, and increased efficacy in tumor targeting, penetration and internalization [41, 115]. It is the goal of many current research efforts to identify and develop peptides that can mimic the targeting function of antibodies in a more direct and cost-effective way. This section will seek to discuss the utilization of peptide-based therapies that target specific cancers on the basis of their affinity for certain cancer-specific extracellular biomarkers.

The engineering of peptides that can home in on specific cancers is predicated upon the knowledge of which biomarkers to target. Thus, ideal targets for tumor-homing therapies are those that are overexpressed or abundant in cancerous tissues, but *not* in healthy organs, providing the basis for differentiating cancer cells from their benign neighbors. As such, it is important that the appropriate markers be selected in order for the “smart” therapeutics to maintain the selectivity that distinguishes them from their unmodified counterparts. One of the most promising targets for the guided delivery of anticancer drugs is the luteinizing hormone-releasing hormone (LHRH) receptor; this G-protein coupled receptor has been found to be significantly overexpressed in a wide variety of cancer types, including prostate, ovarian, breast, pancreas, colorectal, kidney, and bladder cancers [116]. In addition to its significant up-regulation in cancer cells, the LHRH receptor is a particularly attractive candidate for guided drug delivery strategies because its expression levels in healthy organs is incredibly low. This provides a strong motivation to pursue LHRH agonist and antagonist-based therapies.

The overexpression of LHRH receptors is greatest in human prostate cancer, wherein it is reported that the hormone receptor is present in 86% of the cases studied [117]. The importance of LHRH in treating prostate cancer is also underscored by the morbidity and mortality for which the disease is responsible; as it stands, there is a great need in the treatment of prostate cancer for more versatile and effective treatments. In particular, cases wherein the cancer has proven to be castration-resistant have resulted in a remarkably poor prognosis, with an expected survival rate of less than nineteen months [118]. It has been shown that in such instances a lack of viable treatment options exists [119]. Given its prevalence in the majority of prostate cancer cases, LHRH receptor has become a promising avenue for the development of more effective treatments. In addition to its high up-regulation, the LHRH receptor has been demonstrated to maintain a steady expression level even after prolonged exposure to LHRH agonists [120]. While its specific role in the progression of cancers is still not clearly understood, its mere presence allows the use of LHRH receptor targeting moieties in cutting-edge therapies [120, 121].

In the creation of LHRH receptor-targeted strategies, the most straight forward homing peptide that can be utilized is the corresponding antigen itself. LHRH is the relatively short polypeptide, pyroE-HWTSYGLRPG-NH_2, which lends itself to facile synthesis and possesses the same advantages as other small-molecule compounds. Indeed, Minko *et al.* have shown that the use of LHRH as a targeting moiety in a drug delivery system (DDS) is sufficient to selectively target tumor cells and therefore increase the relative potency of the chemotherapeutic camptothecin (CPT) [122]. In their design, a LHRH receptor-targeted DDS was constructed by conjugating free CPT to a modified LHRH peptide *via* a

polyethylene glycol (PEG) linker. Compared to the free drug, this new compound displayed increased efficacy by reducing tumor size in mice while decreasing harmful side effects to healthy organs [122]. Using modified LHRH peptides as targeting moieties has also shown great promise in clinical trials when conjugated to the chemotherapy drug doxorubicin (DOX); the compound AEZS-108 (also called AN-152) consists of a cytotoxic analog of LHRH that is conjugated to a molecule of DOX (Fig. 3a) and was shown to achieve disease stabilization in 90% of prostate cancer patients during Phase I trials [123]. It is currently being developed as a chemotherapy treatment option for a variety of cancer types [124, 125]. AEZS-108 exerts its cytotoxic effects by directing the DOX moiety to LHRH receptors, where the compound undergoes receptor-mediated endocytosis [126]. This contrasts with the passive diffusion across the plasma membrane that allows free DOX to internalize into cells [127]. The promise of AEZS-108 lies in its potential to provide a viable therapy to patients with castration-resistant prostate cancer (CRPC). Rick *et al.* have explored this potential in the treatment of DU-145 castration-resistant prostate tumors in nude mice, demonstrating that the peptide-drug conjugate had notably greater efficacy than unconjugated DOX or free LHRH analog alone in the inhibition of tumor growth [126]. The group's results indicate that this formulation of doxorubicin allows for a delayed rate of intracellular uptake that changes DOX's mechanism of action to additionally affect other extranuclear targets. These findings illustrate both the utility and importance of homing peptides in the development of therapies with poor prognoses.

The LHRH receptor is but one of several important biomarkers that allow for targeted therapy. Another well-known cellular component that is overexpressed in several types of cancers is the human epidermal growth factor receptor 2 (HER2/neu or HER2). HER2 is a receptor tyrosine kinase of the EGFR family and a known oncogene that plays a role in several cancer types, including breast, gastric, ovarian, and prostate cancers [128]. In particular, HER2 has become an important biomarker in the diagnosis and treatment of human breast cancer, being overexpressed in approximately 30% of cases [129]. This plasma membrane-bound protein takes part in the transduction of multiple signaling pathways that, when uncontrolled, are characteristic of cancerous phenotypes [130]. Patients with HER2-positive cancers are associated with more aggressive disease and generally poorer prognoses; in light of this, it is desirable to develop HER2-specific therapies that can effectively treat these more threatening occurrences [129]. It has been demonstrated that the intracellular inhibition of HER2 in breast cancer cells where it is overexpressed promotes apoptosis [131]; thus, it can be reasoned that HER2 may prove to be a logical target for other guided therapies.

HER2 does not have any known corresponding binding ligand; however, cases have been documented that demonstrate the successful creation of artificial ligands which target the receptor [128, 132]. In line with these efforts, a monoclonal antibody known as Trastuzumab that interferes with the HER2 receptor has been developed as a first-line treatment against breast cancer [133]. Unfortunately, Trastuzumab-based therapies are susceptible to the same problems encountered by other therapeutic antibodies and antibody-targeted drugs, such as poor tumor and tissue penetration and inherent or acquired mechanisms of resistance [134]. Once again, peptide-based therapies offer significant advantages due to their small size (and consequently excellent tissue penetration), facile synthesis, and flexibility as

targeting ligands. Murali *et al*. successfully created an anti-HER2/neu peptide (AHNP, -FCGDGFYACYMDV-) that acts as a mimetic for the full-chain antibody Trastuzumab. AHNP has been demonstrated to bind tightly to HER2 receptors and encourage apoptosis of tumor cells on an order comparable to its monoclonal antibody counterpart [135]. Treatment of HER2-positive cancers with AHNP also exhibited increased efficacy when used in conjunction with radiation or other chemotherapeutic agents such as doxorubicin [135]. The promise of this antibody-mimicking peptide has also allowed for its use as a targeting moiety for HER2-positive cancer cells, providing a versatile platform for the delivery of a variety of therapeutic cargo [136, 137]. Other artificial HER2-binding ligands have also emerged, including the peptide LTVSPWY, which was developed using phage display methods by Shadidi and Sioud [138]. They demonstrated the HER2-homing capability of the peptide by conjugation to an antisense nucleotide that was shown to be delivered preferentially to HER2-positive cancer cells [138]. This tumor-homing peptide also readily mediates delivery of pro-apoptotic agents which are otherwise non-selective in their cellular uptake. Neuzil *et al*. have conjugated LTVSPWY to the pro-apoptotic α-tocopheryl succinate (α-TOS) (Fig. 3b), which they found to effectively induce rapid apoptosis and slow growth in HER2-positive breast tumors [139]. This ability to enhance uptake of otherwise poorly or non-selective therapeutics in a rapid and biomarker concentration-dependent manner is at the heart of this shift in chemotherapy-based strategies that peptide-based therapies are leading.

Another biomarker of great interest to the field of peptide-based drug delivery is the hormone somatostatin, a cyclic polypeptide that is an important regulator of the endocrine system [140]. By binding with G-protein coupled somatostatin receptors, it inhibits the secretion of various secondary hormones depending on the location of the receptor, *e.g*. insulin release in the pancreas [141]. Somatostatin occurs naturally in two active forms consisting of 14 and 28 amino acids, respectively. Somatostatin is of key interest because the class of receptors through which it exerts its effects is overexpressed in a large number of tumor types, particularly neuroendocrine tumors [142]. In particular, somatostatin receptor 2 (SSTR2) was found to exist at high densities in 14 out of 16 common tumor cell lines, including breast, prostate, lung, pancreatic, ovarian, cervical cancers, leukemia and neuroblastoma [143]. Such ubiquity fosters a high level of interest in SSTRs for those attempting to develop novel peptide-based drug delivery systems.

Although somatostatin has a short half-life in the body [143], synthetic analogs of higher stability have been successfully created which can still effectively bind SSTRs with high affinity *via* a conserved sequence of amino acids [144, 145]. These somatostatin analogs have become useful tumor-localization agents, and analogs such as Lanreotide (Fig. 3c) and Octreotide (Fig. 3d) have also demonstrated antitumor effects in and of themselves [146]. These peptides have also been recognized for their potential as tumor-homing conjugates for chemotherapy agents; the use of Octreotide as a carrier of cytotoxins such as doxorubicin has been reported [147]. By simply pairing cytotoxic drugs with the analog Octreotide, significant tumor growth inhibition and general toxicity reduction in healthy organs can be achieved [148]. Inspired by these successes, Coy *et al*. developed a novel cytotoxic analog called JF-10-81 by conjugating camptothecin to the SST analog JF-07-69 *via* a degradable carbamate linker [149]. While selectively targeting SSTR2 and retaining

its high binding affinity, this analog is rapidly internalized by tumor cells and inhibits proliferation and growth. This was demonstrated in neuroblastoma, pancreatic, leukemia, pancreatic carcinoid, and prostate tumors; this analog has also demonstrated the capacity to overcome multidrug resistance, inducing potent inhibitory effects in CPT-resistant BON pancreatic carcinoid tumors [149]. Other promising SST-analog-drug conjugates currently in development have displayed success in enhancing the efficacy of small-molecule drugs as well [150, 151].

Peptides may also play an important role in the targeting of many other overexpressed receptors, and even intracellular targets, in treating cancer [152, 153]. Table 2 lists many more examples of cancer-specific targeting peptides, but an in-depth summary of all biomarkers utilized in targeted cancer therapy is beyond the scope of this review. The emphasis here has been on some of the main successes in peptide-based targeting strategies that have shown potential to alter the core tenets of cancer treatment. The advantages that tumor-targeting peptides offer over traditional chemotherapy give them significant potential to change the face of a field that currently has a great need for both increased specificity and efficacy of treatment.

4. TARGETING BRAIN TUMORS

Targeting tumors that form within the brain or central nervous system poses further challenges for effective therapies [199, 200]; an unfortunate happenstance given the most common form of primary brain tumor is the aggressive (WHO grade IV) diffuse glioma, glioblastoma multiforme (GBM) [201]. With a median survival time of 2–3 years (less for the more aggressive forms) [202], it is imperative that advances be made to enable more effective treatments that, if not curative, at least further extend survival time and allow a higher quality of life. The difficulty in treating gliomas arises from the unique environment of the brain, which has evolved to possess a protective barrier that prevents large proteins and bacteria from escaping the blood vessels and affecting the brain. The blood-brain barrier (BBB) results from the very tight junctions formed by the endothelial cells that prevent the diffusion of anything but small or gaseous molecules (water or carbon dioxide) and small (<400 Da) lipophilic molecules [203]. Additional protection against the latter is afforded by a range of efflux transporters expressed in the brain endothelium, the most abundant being the P-glycoprotein (P-gp), a protein that is commonly overexpressed in multidrug resistant cancers [204]. It should be noted that there has been some debate over whether the BBB is actually a hindrance in the treatment of brain tumors as, like other cancers, gliomas have irregular and leaky vasculature that disrupts the BBB in the tumor microenvironment, creating what is named the blood-brain-tumor barrier (BBTB) [199]. However, there is evidence to support that since it is a localized effect and gliomas readily metastasize to areas where the BBB remains intact, the BBB does in fact play a major role in the resistance of gliomas to conventional chemotherapies [205]. Regardless of the extent to which either barrier exists at the tumor site, it is clear that both present challenges to delivering a drug to brain tumors that must be addressed.

While the BBB exists to protect the brain by excluding small molecules, peptides, proteins and larger species, at that same time it is required that some of these entities can actually

cross the barrier as they are essential for the brain to function properly. The brain needs fuel, for example, and so glucose must be able to be pass across the BBB, a task accomplished by glucose transporter proteins [206]. Accordingly, there is a wide range of active transporter proteins that line the brain endothelium and facilitate the import (and export) of vital metabolites and proteins. Exploiting these transport systems through selection of appropriate ligands should, therefore, allow the transport of a conjugated drug or drug carrier across the BBB *via* receptor-mediated endo or transcytosis.

One family of receptors that have attracted great interest is the lipoprotein receptor-related proteins (LRPs), particularly those for low-density lipoproteins (LDLs) such as LRP-1. Béliveau and coworkers developed a family of peptides based on the Kunitz domain of aprotinin [207], a 6.5 kDA protease inhibitor that is a ligand for LRPs 1 and 2. These peptides, termed Angiopeps, demonstrated a greater transcytosis capacity than aprotinin, with Angiopep-2 (TFFYGGSRGKRNNFKTEEY) selected for further studies. One such study was the conjugation of three paclitaxel (Taxol, PTX) molecules to the *N*-terminal and lysine side-chain amines of Angiopep-2 (Fig. 4a) [208]. This peptide–drug conjugate, ANG1005, exhibited a 15% increase in life span in an intracerebral glioblastoma murine model [208] and is currently in Phase II clinical trials (ID NCT01967810), having successfully completed Phase I trials (as GRN1005) [209]. Xin *et al*. have also utilized Angiopep-2 to target their PEG-PCL nanoparticle drug delivery system to glioblastomas [210]. They demonstrated a potential dual targeting effect of the conjugated peptide that allows a PTX-loaded nanoparticle to cross the BBB and preferentially accumulate in the vicinity of glioma cells through recognition of overexpressed LPR-1 on their surface. This increased accumulation was confirmed through *in vivo* fluorescence imaging.

Transferrin is an endogenous iron transport protein that is involved in maintaining iron concentrations within the brain [211]. Zhang *et al*. took advantage of the receptor for this protein using a dual targeting approach to deliver paclitaxel-loaded micelles to U87 MG glioblastomas in a mouse model [212]. Transferrin was grafted to a micelle loaded with a c[RGDfK] peptide-modified PTX (Fig. 4b). In circulation, the attached transferrin allows the micelles to cross the BBB where the micelle breaks down to release the modified drug. The cyclic RGD peptide ensures the drug exhibits a greater selectivity for gliomas over healthy tissues due to the overexpression of integrin proteins in the former [213]. *In vivo* studies showed a 26% increase in mean survival time when using the dual targeting strategy (43 days) over the free drug control and saline controls (34 and 35 days, respectively).

While the above approach to targeting the transferrin receptor relied on the use of the endogenous protein, Lee *et al*. used phage display to develop a small peptide, HAIYPRH (T7), which could effectively bind to the transferrin receptor [214]. Fusion of this peptide with the green fluorescent protein showed that this ligand could facilitate efficient internalization. Jiang and coworkers utilized this peptide to enable their doxorubicin-loaded PAMAM dendrimers to cross the BBB [215], demonstrating a significant improvement in tumor growth inhibition *in vivo* when compared to the non-targeted delivery vectors and saline.

Though not an example of cancer targeting, Son *et al.* exploited the nicotinic acetylcholine receptor (nAchR) on neuronal cells to deliver a polymer/DNA across the BBB and into neurons [216]. To accomplish this an nAchR-targeting RVG peptide (YTIWMPENPRPGTPCDIFTNSRGKfRASNG) was conjugated to the polyplex *via* reducible disulfide linkages in order to transport a gene sequence encoding luciferase into neuronal cells. Fluorescence imaging confirmed that transfection of the luciferase gene was successful, exhibiting fluorescence only when the RVG peptide was attached to the polymer/DNA complex. The potential of this approach to significantly improve targeted transfection for gene therapies is evident and will hopefully lead to and inspire other successful treatments in the future.

5. TARGETING PEPTIDES IN IMAGING AND THERANOSTICS

As we have seen, peptides possess the potential to play a powerful role in the treatment of cancer: they can act as delivery vectors, targeting moieties, and cell- and tumor-penetrating agents. One final aspect of peptide utility to be discussed herein is their role in the imaging and detection of cancer [217, 218]. This follows the logical progression that the effective treatment of disease is linked to, and informed by, the power to image the physiological area under treatment. Imaging methods such as PET, CT, and MRI have become the standard for approaching cancer cases, relying on the use of contrast agents to differentiate the various structures in the body. This section will seek to understand how peptides play a role in these tools to enhance current imaging capabilities.

In recent years, it has been shown that contrast agents can be targeted specifically to tumors by employing similar strategies used to deliver therapeutics. This has included the functionalization of various nanoparticles as intracellular contrast agents *via* conjugation to a peptidic targeting or cell penetrating moiety [219]. One powerful example of this was demonstrated by Chen *et al.*, who employed iron oxide (IO) nanoparticles as a molecular platform for simultaneous targeted PET and MRI imaging through the addition of functional peptide ligands (Fig. 5a) [220]. Polyaspartic acid-coated IO particles were conjugated with two functional groups: macrocyclic chelating agent 1,4,7,10-tetraazacyclododecane-*N*,*N*',*N*'',*N*'''-tetraacetic acid (DOTA), and the Arginine-Glycine-Aspartate (RGD) peptide. This multifaceted nanoimaging agent contains three capabilities: the DOTA group chelates radioisotopes, such as [64] Cu for PET detection; the RGD peptide is a tumor-homing moiety that targets $\alpha_v\beta_3$ integrins overexpressed in tumor vasculature; the IO-nanoparticle core serves as a contrast agent for MRI imaging. These nanoparticles were found to localize specifically in $\alpha_v\beta_3$ integrin-positive tumors *in vivo* (Fig. 5b) [220]; theoretically, these multimodal probes may allow for early clinical tumor detection with high sensitivity. This innovative design is representative of the potential for tumor-homing and tumor-penetrating peptides to change the way that cancer is imaged.

In addition to their utility in the modification of radiological contrast agents, peptides can also function as targeting moieties in the development of molecular probes, such as quantum dots (QD). QDs are nanoscale crystals made of semiconductor material that, given a base solubility and biocompatibility, can be labeled with targeting ligands and utilized as fluorescent molecular probes [221, 222]. The potential use of quantum dots for imaging

purposes was first demonstrated by Nie *et al.*, who conjugated QDs to prostate-specific membrane antigen (PSMA) targeting monoclonal antibodies for the detection of prostate cancer [223]. Just as peptides possess advantages over antibodies as drug delivery vectors, the same principles apply to peptidic modification of nanoparticles in molecular probes; in addition, given their smaller size, a greater number of peptides may be conjugated to the surface of a QD than antibodies [224]. Chen *et al.* report the conjugation of $\alpha_v\beta_3$ integrin-targeting RGD peptides to the surface of QD705 (Fig. 5c), a quantum dot with a near-infrared emission maximum [225]. This new imaging agent was found to only bind to, and fluorescently visualize, integrin-positive cells, resulting in successful *in vivo* tumor vasculature imaging (Fig. 5d and e). It is once again evident that the ability of peptides to target imaging compounds selectively and effectively to cancerous sites represents a powerful new tool in cancer detection and consequent treatment.

Pomper and colleagues have developed peptidic urea-based inhibitors that can act as ligands for PSMA, imbuing a capacity for prostate cancer targeting [226]. Prostate-specific membrane antigen is a type II integral membrane glycoprotein that has close correlation with human prostate cancer. PSMA is highly expressed in primary prostate cancer and lymph node metastases [227–229], and recent evidence suggests that PSMA is also expressed in tumor-associated neovasculature [230], making PSMA a potential biomarker for prostate cancer imaging and therapeutics. PSMA has two natural ligands or substrates including *N*-acetyl-aspartylglutamate (NAAG, a neurotransmitter) and poly-γ-glutamated folate. However, these two peptidic ligands are readily degraded by PSMA due to its enzymatic activity [231, 232], preventing their use as targeting ligands for drug delivery and imaging. The synthesized ligands were labeled with ^{125}I, ^{18}F and ^{99m}Tc through different chemistries,[226] and the complexes of these imaging agents with PSMA were studied by X-ray crystallography, revealing that all the ligands invariably bound with a glutarate moiety within the S1′ pocket of the enzyme [233]. The urea linkage that these ligands are based on prevents their PSMA-catalyzed degradation. *In vivo* studies showed that these urea-based reagents exhibited very specific binding to PSMA-overexpressed prostate tumors initiated by PC-3 PIP and LNCaP cell lines, but not to prostate tumors initiated by PC-3 (PSMA negative) and the breast cancer cell line MCF-7 (PSMA negative) [226, 234–236]. These results clearly indicate that these urea-based imaging contrasts have great potential in prostate cancer diagnosis.

Just as selectivity is important in the delivery of drugs exclusively to diseased tissues *via* recognition of certain biomarkers, it is also desirable to use molecular probes to visualize very specific molecular occurrences which play a part in the disease [237]. In recent years, activatable imaging probes that respond to specific biochemical events have been under development [238, 239]. Conventional “molecular beacons” consist of a fluorophore and a quencher connected to a functional peptide; they remain “quenched” until they are modified by a biochemical process, such as cleavage by a protease [240, 241]. A major hurdle for the development of reliable molecular beacons is their relatively short half-life under physiological conditions: for soluble beacon molecules, cleavable linkers designed to activate the fluorescent signal are often exposed to the environment, leading to non-specific proteolytic degradation (and, consequently, a positive signal with no correlation to the target physiological process). To address this problem, Cui *et al.* developed a new form

of amphiphilic, self-assembling supramolecular nanobeacon (NB) that forms core-shell micelles under physiological conditions to protect the enzyme-cleavable segment (Fig. 5f) [242]. The NB molecule contains a fluorescent dye, 5-carboxyfluorescin (5-FAM), and a black hole quencher, BHQ-1, which form a hydrophobic core within the micelles. These molecules are conjugated to the hydrophilic, cell-penetrating TAT peptide to facilitate internalization of the nanobeacon. Once inside the cell and trafficked to the lysosome, the micelles dissociate into monomeric form, wherein the peptide linker, GFLG, that connects the 5-FAM dye to the amphiphile is cleaved specifically by the lysosomal enzyme Cathepsin B [243]. Cathepsin B is overexpressed in tumor cells and is important for tumor growth [244]. The 5-FAM entity thus dissociates from the BHQ-1 and generates a green fluorescent signal when irradiated. Experimental data indicated that the NB molecules not only effectively penetrated cells, but were also effectively activated intracellularly to produce a fluorescent signal (Fig. 5g) [242]. Although the field of peptide-based molecular imaging is still in its infancy, the facile synthesis and “tunability” of these supramolecular nanomaterials is incredibly promising for the future of imaging and diagnostic methods.

An important point to note is the arrangement of this review might suggest that targeted therapeutics and smart imaging and diagnostic methods are compartmentalized issues; the fact of the matter is the two ideas must be pursued in a concerted and holistic way. More simply put, the new concept of “theranostics” is interested in the development of technologies that address a spectrum of medical needs, from diagnosis to treatment [60, 245–252]. Current and future research areas focus on drug delivery systems which contain targeting, imaging, and therapeutic components. Such advances will undoubtedly cause a much needed paradigm shift in the field of cancer treatment.

FUTURE PROSPECTS

Incorporating onto a non-targeting object small molecule peptides capable of recognizing particular tumor types or tumor-relevant vasculature appears to be a logical choice in the construction of targeted drug delivery systems that promise both improved treatment efficacy and reduced side effects. In combination with recent progress in delivery technology that can facilitate release of the therapeutic cargo at only tumor sites, there is great hope that the synergy of the two strategies can realize Paul Erlich’s concept of the “magic bullet” for cancer therapy and diagnostics. However, there are still many hurdles ahead in translating this promising strategy into clinically useful therapies or tools. The major obstacles in this path include the individualized human anatomy and cancer pathophysiology, as well as the characteristic tumor heterogeneity, with the former preventing the development of consensus peptide sequences that would work for all patients diagnosed with the same tumor type, and the latter limiting effective dissemination of the delivered drugs throughout the tumor. The key to future successes in the field may lie in our ability to screen and identify targeting peptides at the individual patient level to develop customized medicines in a timely and cost-effective manner.

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Biography

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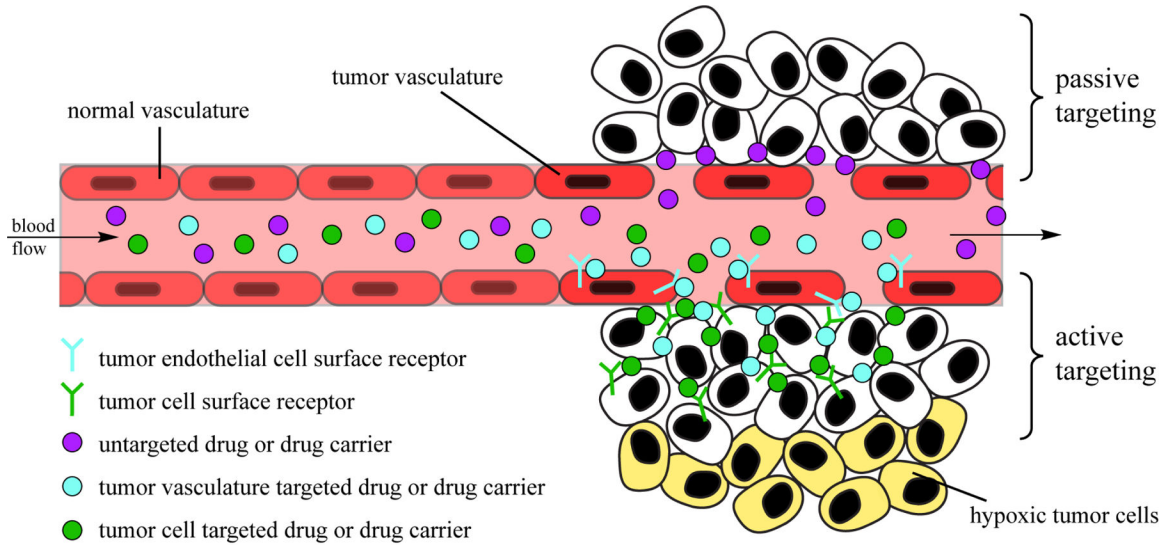

Fig. (1).

Schematic illustration of targeting strategies in drug delivery. Passive targeting is based simply on extravasation of the drug or drug carrier through the leaky vasculature of the tumor. Active targeting is based on the binding of drug or carrier-conjugated ligand to its corresponding receptor on either the tumor endothelial cells (vasculature targeting) or tumor cell surface (tumor targeting).

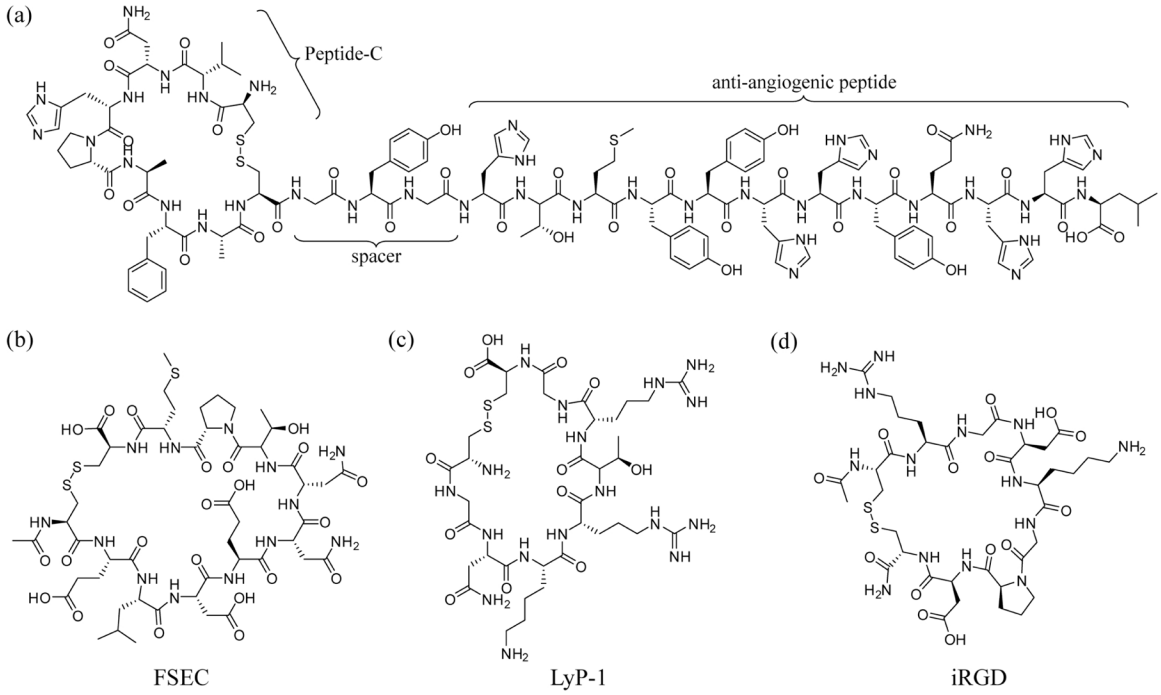

Fig. (2).

Examples of tumor vasculature targeting peptides. (**a**) A Peptide-C based therapeutic that targets melanoma and inhibits angiogenesis through the conjugated anti-angiogenic peptide. (**b**) A shortened peptide mimic of a larger SPARC peptide that has tumor-homing properties. (**c**) LyP-1, a peptide that can be utilized to target a tumor's lymphatic vasculature. (**d**) iRGD, a tumor penetrating peptide.

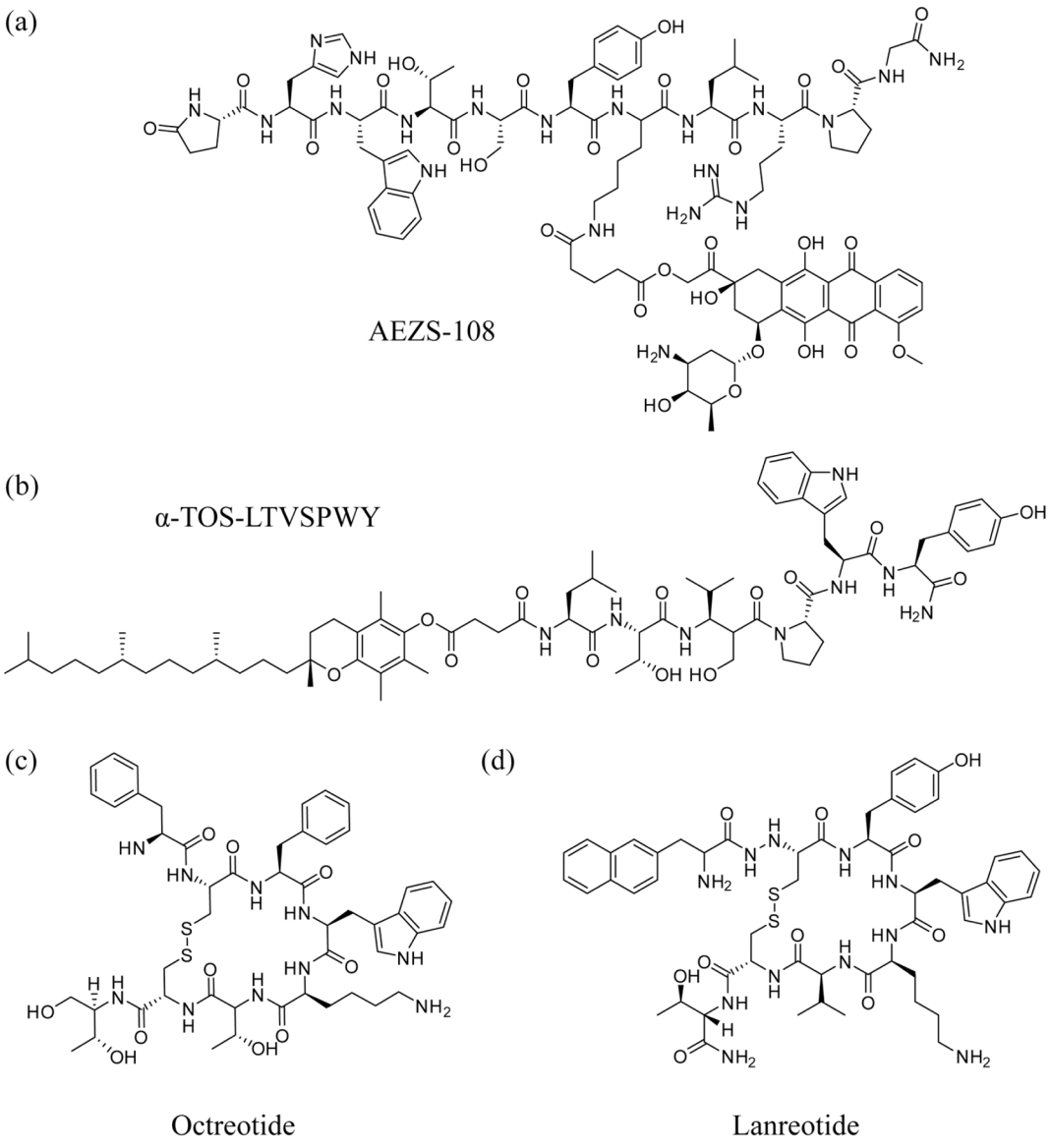

Fig. (3).

Examples of tumor-homing peptides and peptide-drug conjugates. (**a**) AEZS-108, in which the chemotherapeutic doxorubicin is conjugated to a modified LHRH peptide for the treatment of castration-resistant prostate cancer. (**b**) Conjugation of the anti-apoptotic α-TOS to the HER2-targeting peptide, LTVSPWY. (**c**) Octreotide and (**d**) lanreotide, two somatostatin analogues developed as both anti-tumor agents and targeting moieties).

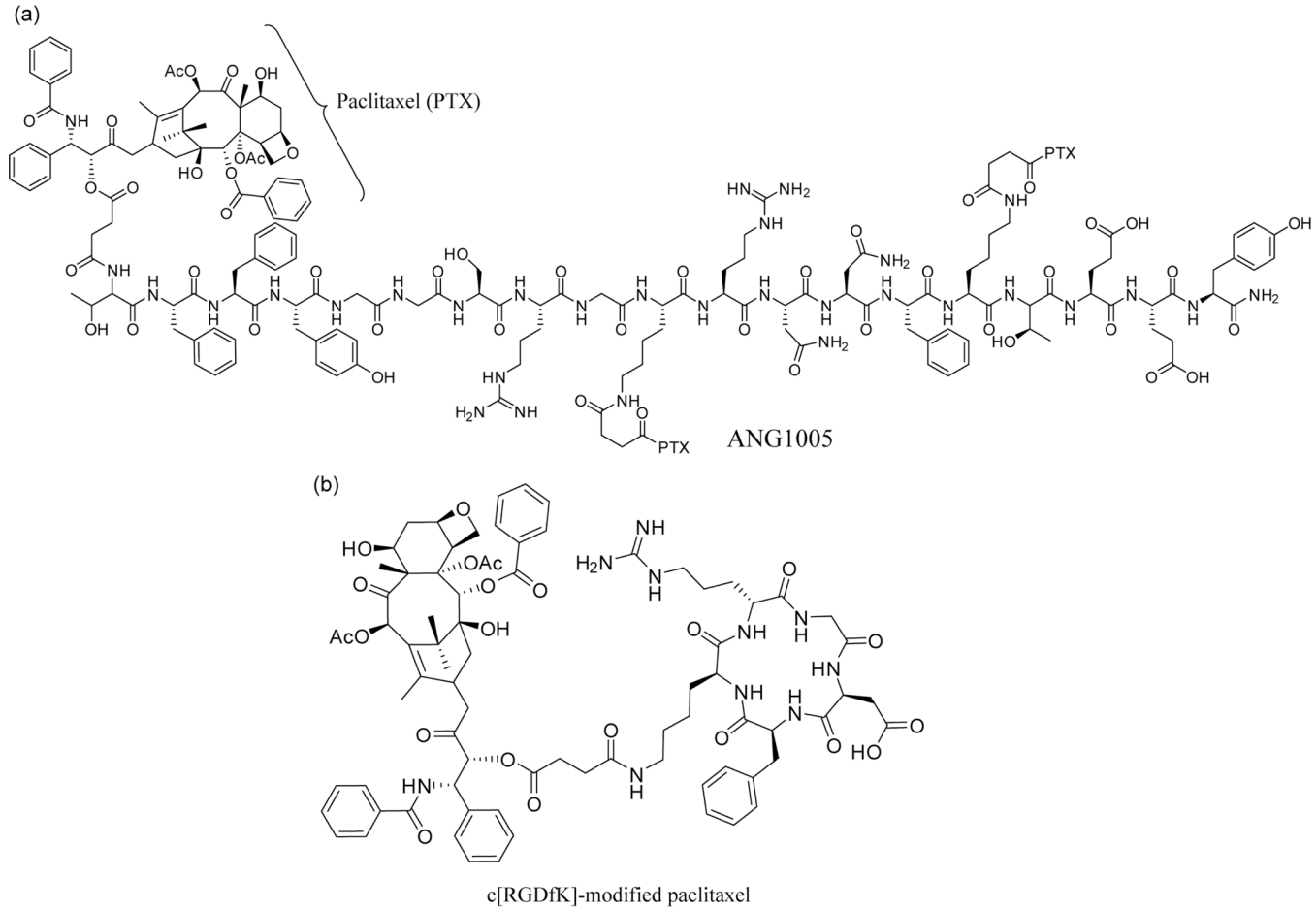

Fig. (4).

Examples of peptide-drug conjugates that are used to target brain tumors. (**a**) Beliveau's PTX-conjugated Angiopep-2 therapeutic, ANG1005. (**b**) c[RGDfK]-modified paclitaxel that is loaded into transferrin-bearing micelles for targeting of gliomas.

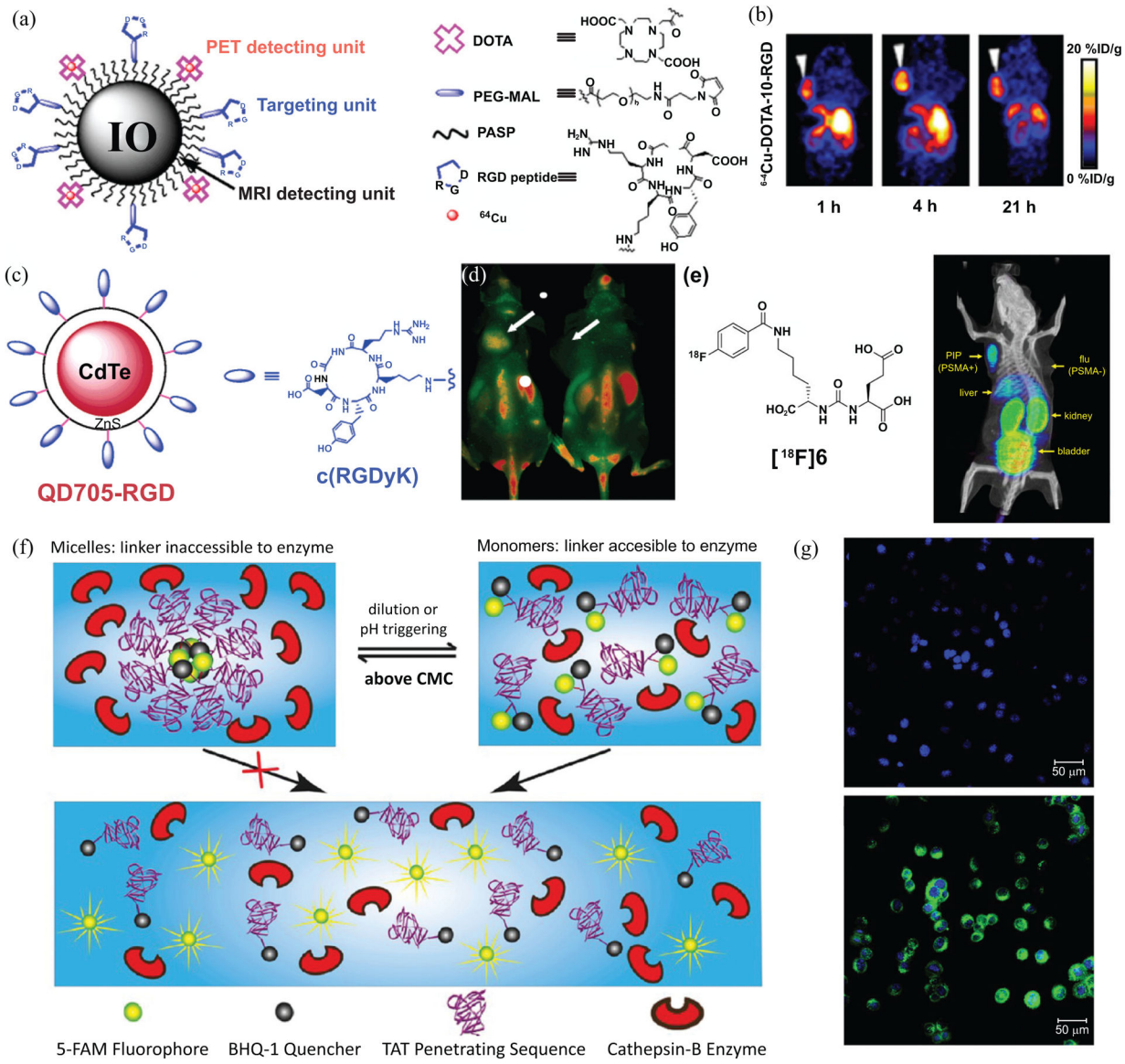

Fig. (5).

Examples of nanoimaging agents that utilize peptides in their design. (**a**) A dual-modality imaging agent developed by Chen *et al.*, possessing an iron oxide nanoparticle core for MRI imaging and conjugated ^{64}Cu chelating DOTA groups for PET imaging. RGD peptide ligands target tumors displaying $\alpha_v\beta_3$ integrins. (**b**) Decay-corrected whole body coronal PET images of nude mouse bearing human U87MG tumor at 1, 4, and 21 h after injection of 3.7 MBq of the ^{64}Cu-containing imaging agent. These images show that it can accumulate in the tumor xenograft (white arrows), with uptake by the liver also indicated. (**c**) A quantum dot (QD) based fluorescence imaging agent, QD705-RGD, developed by Chen *et al.* This agent consists of a CdTe/ZnS with a PEG-polymer coating. The surface was decorated with cyclic-RGD ligands to enable targeting of $\alpha_v\beta_3$ integrins. (**d**) *In vivo* NIR fluorescence image of U87MG tumor-bearing mice 6 h after treatment with 200 pmol of QD705-RGD (left) and QD705 (right), showing how the RGD ligand allows accumulation in the tumor

relative to a control QD imaging agent. Prominent uptake was also seen in the liver, bone marrow and lymph nodes. (**e**) An example of Pomper's urea-based PSMA-targeting ligand with an 18F radiolabel. Preferential uptake is seen in a PSMA+ (PIP) tumor, but not a PSMA− (flu) tumor *via* PET imaging. (**f**) Schematic illustration of Cui's nanobeacon (NB) concept. In the assembled state, the cleavable linker (GFLG) is inaccessible to the Cathepsin B protease and remains intact. Upon breakdown of the nanostructure, triggered by either dilution or a change in pH, the linker becomes accessible and cleavage occurs. The separation of the 5-FAM fluorophore from the BHQ-1 quencher results in a measurable signal that can be used to probe the activity of the protease. (**g**) Fluorescence images of MCF-7 human breast cancer cells incubated with NBs after 0 h (top) and 1.5 h (bottom). The cell nuclei were stained with the blue dye Hoechst 33342. (Images in (**a**) and (**b**) were adapted from ref. [220], (**c**)-(**d**) were adapted from ref. [225], (**e**) from ref. [226], and (**f**)-(**g**) were adapted from ref. [242]).

Table 1.

Peptides that target tumor vasculature and lymphatics.

Peptide Ligand	Cellular Target	Refs.
Targeting Tumor Vasculature		
CDCRGDCFC (RGD-4C)	$\alpha_v \beta_3$, $\alpha_v \beta_5$	[87, 88]
ACDCRGDCFCG	$\alpha_v \beta_3$, $\alpha_v \beta_5$	[89]
CNGRCVSGCAGRC	Aminopeptidase N	[90]
TAASGVRSMH, LTLRWVGLMS	NG2 proteoglycan	[91]
CGSLVRC, CGLSDSC	Vasculature of various tumors	[28]
NRSLKRISNKRIRRK, LRIKRKRRKRKKTRK, NRSTHI	IC-12 rat tracheal tumor	[92]
SMSIARL, VSFLEYR	Mice prostate	[93]
CPGPEGAGC	Aminopeptidase P	[94]
ATWLPPR	VEGF	[95]
RRKRRR	VEGF	[96]
ASSSYPLIHWRPWAR	VEGF	[97]
CTTHWGFTLC	Gelatinase	[98]
Ciso DGRCG	$\alpha_v \beta_3$, $\alpha_v \beta_5$	[99]
cyclo[(3R,6S)DKP-isoDGR], cyclo[(3S,6R)DKP-isoDGR]	$\alpha_v \beta_3$, $\alpha_v \beta_5$	[100]
CTPSPFSHC	Unknown	[101]
CSRPRRSEC, CGKRK, CDTRL	Unknown	[102]
CKAAKNK, CKGAKAR, CRGRRST	First two unknown Last one PDGF-β	[103, 104]
CRGDK/RGPD/EC (iRGD)	$\alpha_v\beta_3$, $\alpha_v\beta_5$, neuropilin-1	[79, 80]
CPRECESIC	Aminopeptidase A	[105]
CGNSNPKSC	Unknown	[106]
SVSVGMKPSPRP	Unknown	[107]
IFLLWQR	Annexin 1	[108]
Targeting Tumor Lymphatics		
CGNKRTRGC (LyP-1)	p32/gC1qR	[72, 109, 110]
CNRRTKAGC, CLSDGKRKC, CREAGRKAC, CAGRRSAYC		[110, 111]

Table 2.

Peptides that target specific cancer types.

Cancer Type	Peptide Ligand	Cellular Target	Refs.
Human lymphoma	KNGPWYAYTGRO, NWAVWXKR, YXXEDLRRR XXPVDHGL	Surface idiotype of SUP-88 human B-cell lympoma	[104, 154]
	LVRSTGQFV, LVSPSGSWT ALRPSGEWL, AIMASGQWL QILASGRWL, RRPSHAMAR DNNRPANSM, LQDRLRFAT PLSGDKSST	Surface idiotype of human chronic lymphocytic leukaemia (CLL)	[155]
	IELLQAR	HL 60 human lymphoma & B-16 mouse melanoma	[156]
	SAKTAVSQRVWLPSHRGGEP, KSREHVNNSACPSKRITAAL, WLSEAGPVVTVRALRGTGSW	A20	[157]
	CAYHRLRRC	Molt-4	[158]
Myeloma	FDDARL, FSDARL, FSDMRL FVDVRL, FTDIRL, FNDYRL FSDTRL, PIHYIF, YIHYIF, RIHYIF	Human multiple myeloma M-protein	[159]
Prostate cancer	CVFXXXYXXC CXFXXXYXYLMC CVXYCXXXXCYVC CVXYCXXXXCWXC	Prostate-specific antigen (PSA)	[160]
	DPRATPGS	LNCaP	[161]
	SHGFSRHSMTLI	Capan-2 (irradiated)	[162]
	FRPNRAQDYNTN	DU-145	[163]
	IAGLATPGWSHWLAL	PC-3	[164]
	CREAGRKAC (REA)	Prostate Tumors from TRAMP mice	[111]
Breast cancer	VPWMEPAYQRFL	MDA-MB435 breast cancer	[165]
	YQATPARFYTNT, CGWMGLELC	MDA-MB-231	[166]
	LTVSPWY WNLPWYYSVSPT	SKBR3	[138]
	CNGRCVSGCAGRC (NGR), CDCRGDCFC (RGD-4C)	MDA-MB-435, Amino-peptidase N & $\alpha_v\beta_3$, $\alpha_5\beta_1$	[28]
Neuroblastoma	HLQIQPWYPQIS VPWMEPAYQRFL	WAC-2 human neuroblastoma	[165]
Head and neck cancer	TSPLNIHNGQKL	MDA Tu167 head and neck cancer lines	[167]
Laryngeal carcinoma	RLTGGKGVG	HEp-2 human laryngeal carcinoma	[168]
	RLLDTNRPLLPY	NPC-TW 04	[169]

Cancer Type	Peptide Ligand	Cellular Target	Refs.
	EDIKPKTSLAFR	Nasopharyngeal carcinoma (CNE-1)	[170]
Hepatocarcinoma	TACHQHVRMVRP	BEL-7402	[171]
	KSLSRHDHIHHH	SMMC-7721	[172]
	SFSIIHTPILPL	Mahlavu	[173]
Melanoma	CTVALPGGYVRVC	GRP78	[174]
	TRTKLPRLHLQS	B16-F10	[175]
	CLSDGKRKC (LSD)	C8161 Melanoma	[111]
Gastric	GRRTRSRRLRRS	XGC9811-L4	[176]
	SMSIASPYIALE	GC9811-P	[177]
	SWKLPPS	AZ-P7a, $\alpha_3\beta_1$	[178]
	CGNSNPKSC	Human gastric adenocarcinoma, $\alpha_3\beta_1$	[179]
Colon	CPIEDRPMC,	HT29	[180]
	HEWSYLAPYPWF, QIDRWFDAVQWL	WiDr	[166]
	VRPMPLQ	Human Colonic adenomas	[181]
	SPTKSNS	Resected human colon tumors	[182]
	VHLGYAT	SW480	[183]
	VGLPEHTQ, ELRGDSLP,		
Glioma	DSTKSGNM, DYDMTKNT, DLTKSTAP, ESRGDSYA,	RG2	[184]
	MCPKHPLGC	U87-MG	[185]
Cervical	CRLTGGKGVGC, CADPNSVRAMC, CAAHYRVGPWC	SiHa	[186]
Medullary Thyroid	CHTFEPVGC	TT	[187]
	SRESPHP	Medullary thyroid carcinoma (RET-C634R transgenic mice)	[188]
Rhabdomyosarcoma	CQQSNRGDRKRC, CMGNKRSAKRPC	$\alpha_v\beta_3$	[138]
Leukemia	CPLDIDFYC	Kasumi-1 $\alpha_4\beta_1$	[189]
	KDEPQRRSARLSAKPAPPKPEPK-PKKAPAKK	Human myeloid leukemia (HL-60)	[190]
Lung cancer	VSQTMRQTAVPLLWFWTGSL, YAAWPASGAWTGTAPCSAGT, EHMALTYPFRPP	H1299 (large cell)	[191–193]
	RGDLATLRQLAQEDGVGVR	H2009 $\alpha_v\beta_6$	[191]

Cancer Type	Peptide Ligand	Cellular Target	Refs.
	MTVCNASQRQAHAQATAVSL	A549	[191]
	TDSILRSYDWTY	CL1-5	[194]
	HVGGSSV	Lewis lung carcinoma (irradiated/SU11248)	[195]
	SVSVGMKPSPRP	Lung (CL1-5)	[107]
Bladder	CSNRDARRC	HT-1376	[196]
Pancreatic	KTLLPTP	Pancreatic ductal carcinomas arising from Kras/p52L/L mice, Plectin-1	[197]
	CRGRRST, CRSRKG, CKAAKNK, CKGAKAR, FRVGVADV	RIP1-Tag2 mice Pancreatic islets PDGFRβ	[103]
Oral	SVSVGMKPSPRP, SNPFSKPYGLTV, WDSNTYTPRPLM	Oral (SAS)	[107, 198]
Squamous carcinoma	CGKRK, CDTRL	K14-HPV16 mice basal cell	[102]