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EGF receptor-targeted nanocarriers for enhanced cancer treatment

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Abstract

The ‘nanomedicine’ approach has revolutionized cancer therapy by enabling the packaging of therapeutic agents within engineered nanovehicles that can specifically accumulate within the tumor stroma and then be internalized within cancer cells, to render site-selective action while minimizing nonspecific uptake and harmful side effects. While the specific accumulation within the tumor stroma is rendered by the ability of the nanovehicles to passively permeate through the tumor’s leaky vasculature, the cellular internalization is often achieved by exploiting receptor-mediated active endocytotic mechanisms using receptor-specific ligand decoration on the vehicle surface. To this end, a highly important receptor found in several cancers is the EGF receptor, which has been implicated in tumor aggression and proliferation. In this context, we provide a comprehensive review of the various approaches of ligand decorations on nanovehicles for active targeting to EGF receptors, and discuss their pros and cons towards optimizing the design of EGF receptor-targeted nanomedicine systems.

Keywords

antibody; antibody fragment; aptamer; cancer nanomedicine; EGF; EGF receptor targeting; peptide

Cancer is a devastating disease that accounts for over half a million deaths in the USA every year [101]. Conventional treatments of cancer with surgery, radiotherapy, chemotherapy and immunotherapy are successful at treating many malignancies, but they often cause detrimental side effects such as trauma, systemic toxicity, immunosuppression, functional debilitation and cosmetic damage. These side effects are due to the invasiveness of treatment procedures, lack of tumor selectivity of therapeutic action and indiscriminate systemic distribution of drugs. In order to alleviate these issues, a significant amount of research has been focused on ‘targeted cancer therapies’ that enable tumor eradication while sparing healthy tissues. Refinement of conventional treatment strategies to achieve ‘targeted’ effects include image-guided surgical procedures and focused radiotherapy treatments, as well as

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tumor-localized administration of chemotherapeutic agents [1,2]. However, many of these strategies are highly complicated and expensive, and several kinds of cancer are not easily accessible for localized therapies. Hence there is significant clinical interest in treatment strategies where the therapeutic agents can still be administered parenterally (e.g., intravenous injection) and yet the agent can accumulate preferentially in the tumor from systemic circulation to render site-selective action. The advent of 'nano medicine' technologies, where therapeutic agents can be packaged within nanoparticulate vehicles, injected intravenously and allowed to be taken up preferentially within the tumors via passive permeation and active internalization mechanisms, have opened the doors for innovative targeted therapies for cancers. Since its inception two decades ago, the nanomedicine technologies have evolved tremendously as an interdisciplinary field involving materials engineering and particle designs, biomolecular strategies of target identification and corresponding ligand development, and combining diagnostic and therapeutic cargo in delivery vehicles (the 'theranostic' approach). Some of these technologies have progressed into actual clinical applications and several are currently in clinical trials [3-6]. In this regard, we review one of the most promising nanomedcine approaches in targeted cancer therapy, namely, the utilization of EGF receptor (EGFR) targeting to enhance delivery of therapeutic and diagnostic agents to EGFR-overexpressing cancers.

Passive & active mechanisms of nanomedicine delivery

Significant research has been carried out in the past few decades on nanoparticle-mediated delivery of drugs and imaging agents to cancer. The driving force of this research was the establishment of the enhanced permeation and retention (EPR) mechanism, first reported by Maeda *et al.* [7-9]. According to this model, drugs modified with macromolecular carriers (e.g., polymers) or encapsulated in nanoparticulate vehicles are able to resist renal clearance, have increased plasma half-life, can passively diffuse into the tumor tissue due to the hyper-permeable state of tumor-associated vasculature and stay retained in the tumor tissue due to compromised lymphatic drainage [7]. The retained nanovehicles can then act as drug release depots, and depending on their composition and charge they can also be internalized within tumor cells via membrane-mediated passive processes over time. Making use of this mechanism has led to two of the most significant antitumor nanomedicine formulations, namely Doxil® (doxorubicin, formulated in liposomes, US FDA approved in 1995) and Abraxane® (paclitaxel, formulated in albumin nanoparticles, FDA approved in 2006) [102,103]. Utilizing the passive mechanisms of EPR still remains a critical design parameter of nanoparticle-mediated cargo delivery to tumors.

Although the EPR mechanism facilitates accumulation of therapeutic cargo within the tumor tissue, it does not necessarily ensure delivery of the cargo within the tumor cell. For nanovehicles that are accumulated via the EPR mechanism, the cellular internalization will be dependent upon spatio-temporal membrane-mediated processes [10]. These processes may not occur in a controlled and consistent manner, and over time there may be a build-up of vehicles within the tumor stroma resulting in a reverse gradient of vehicle permeation. Owing to such possibilities, researchers have looked into incorporating additional mechanisms in the nanovehicles to facilitate tumor cell-specific internalization. One of the most promising strategies in this context is receptor-mediated endocytosis [11]. Tumor cells are known to upregulate a variety of receptors on their surface and the binding of innate ligands to these receptors promote a multitude of signaling cascades that help tumor growth and proliferation, angiogenesis, survival in different oxygen levels and pH conditions, apoptosis resistance and metastasis [12]. Many of these receptors are 'internalizing' receptors; that is, following ligand binding the ligand–receptor complex is actively endocytosed. Hence, directing nanovehicles surface-modified with ligand motifs specific to

such receptors provides a promising way to exploit the receptor-mediated active endocytosis mechanisms to achieve intracellular delivery of the nanovehicle cargo. Endosomal or lysosomal uptake of the drug-loaded nanovehicles through these active mechanisms is usually followed by intracellular disassembly or degradation of the vehicles and release of the drugs for enhanced therapeutic action. This mechanism of 'active targeting' has been investigated for various receptors. To this end, EGFRs have been identified as one of the most promising receptors for targeting several types of cancers.

EGFR as a target

EGFR, a 170-kDa glycoprotein member of the ErbB family, consists of an extracellular N-terminal ligand-binding domain, a hydrophobic transmembrane region and an intracellular C-terminal tyrosine kinase (TK) domain. The ligand-binding domain can bind ligands from the endogenous EGF ligand family, which results in receptor homo- or hetero-dimerization, leading to receptor internalization (primarily via clathrin-mediated pathways), as well as cytoplasmic TK domain activity. As can be seen in Figure 1, this activates various signaling pathways that inhibit apoptosis, promote cell proliferation, trigger angiogenesis and enhance tumor survival and metastatic potential [13]. EGFR upregulation has been implicated in the aggressiveness of several cancers as seen in Table 1 [14]. As EGFR is implicated in cancer progression and poor prognosis, several anti-EGFR treatment strategies have been clinically approved in recent years (e.g., receptor-blocking monoclonal antibodies such as cetuximab and small molecule TK inhibitors such as erlotinib) [15,16]. In parallel to these direct EGFR-inhibition therapies, significant research efforts have been focused on utilizing EGFR-binding ligands for decorating nanovehicle surfaces to achieve tumor cell-specific delivery and internalization [15,17]. The following sections present a review of the various approaches of actively targeting nanovehicles to EGFRs. We categorize our discussions according to the targeting motif used to achieve EGFR-specific binding of the nanovehicles.

EGFR targeting using full antibodies

Most of the current research on EGFR-targeted nanoparticles involves antibodies and antibody fragments due to the FDA approval of antibody immunotherapies such as cetuximab and trastuzumab. Decoration of these motifs on nanoparticles or direct conjugation of them to bioactive molecules are being utilized for multifunctional purposes including imaging, photothermal ablation, drug delivery and radiofrequency ablation.

EGFR-mediated delivery of chemotherapeutics such as gemcitabine, carmustine, paclitaxel and doxorubicin has been heavily studied *in vitro* and in preclinical animal models yielding largely successful results; although none have received FDA approval [18-21]. Kuo *et al.* utilized anti-EGFR antibodies conjugated to cationic solid lipid nanoparticles for delivery of carmustine, an alkylating agent used in the Gliadel® wafer, to achieve 80% cell death on human glioblastoma cells [18]. Due to their current use in FDA-approved drug formulations, liposomes are a popular model nanoparticle for drug delivery applications. EGFR-targeting antibodies were conjugated to pH-sensitive liposomes by Kim *et al.* to investigate the antitumor activity of gemcitabine in a non-small-cell lung carcinoma animal model [22]. It was found that an overall stagnation in tumor volume could be achieved with this formulation, but the tumor could not be eradicated during the time frame studied (Figure 2A). Polymeric nanoparticles are also a popular platform for conjugating anti bodies, due to the ease of utilizing maleimide and amide chemistries for bioconjugation, the ease of scale-up and the advantage of tuning biodegradation. A variety of polymers have been studied for EGFR targeting including poly(lactic acid-*co*-lysine), poly(ethylene glycol-*co*-caprolactone) and poly(lactic acid-*co*-glycolic acid), all of which have shown promising results *in vitro* but largely have not been studied clinically [23-26]. Carbon nanodiamonds are another class of

nanoparticles that have been studied for cancer drug delivery by modification with EGFR-targeting antibodies [27]. Zhang *et al.* used carbon nanodiamonds to deliver paclitaxel to EGFR-overexpressing breast cancer cells and achieved 60% cell death *in vitro* [27].

Due to their physical characteristics, inorganic particles with anti-EGFR antibodies are largely studied for imaging purposes. A large body of work has been carried out in recent years with gold nanoparticles, which use thiol association to add EGFR targeting ability for imaging of cancer. Promising results have been shown both *in vitro* in model cell lines such as A431 cells, as well as *in vivo* in rodent models. There is also interest in using these nanoparticles for molecular imaging of live cells, as demonstrated by Curry *et al.* and Durr *et al.* [28,29]. Gold nanoparticles are usually used for imaging and ablation studies, but cyclodextrin-covered gold nanoparticles targeted with anti-EGFR antibodies were used by Park *et al.* for drug delivery of β-lapachone for glutathione-mediated release to cancer cells [30]. It was found that the drug release could be tuned based on the concentration of glutathione in the cells. Multifunctional nanoparticles using combinations such as gold with iron oxide coatings, quantum dot/magnetite hybrids, and silica-coated polystyrenes loaded with ferric oxide and quantum dots have been developed to create targeted contrast agents that preferentially accumulate in EGFR-overexpressing tumors, for diagnostic and guided therapy purposes [31-36]. Gold is also a popular nanoparticle of choice in the area of photothermal and radiofrequency ablation due to its ability to cause tissue heating when excited by certain wavelengths of electromagnetic radiation. Several studies including those performed by El-Sayed *et al.* and Melancon *et al.* have shown the ability to cause almost 100% cell death *in vitro* through the use of these antibody-targeted gold nanoparticle systems [37-39]. The pertinent challenge of tumor ablation is the tissue heating and subsequent damage caused to nearby healthy tissue. Current research is focused on resolving these challenges. The previously described works all involve the use of generic anti-EGFR antibodies produced in rabbits, rats and other animals.

Since its FDA-approval for EGFR immunotherapy in 2004, researchers have made use of cetuximab, the chimeric monoclonal EGFR-specific antibody for creating EGFR-targeted nanoparticles. A variety of nanoparticles including gold, liposomes, carbon nanovectors, polymeric nanoparticles and dendrimers have been modified with cetuximab, and delivery of drugs such as gemcitabine and methotrexate with these various targeted systems have shown comparable results of approximately 80–100% cell death *in vitro* [40-43]. Human serum albumin nanoparticles have also been studied for simultaneous targeted delivery and therapy using cetuximab (Figure 2B) [44]. Cetuximab has also been utilized to develop contrast agents for imaging by decorating inorganic nanoparticles such as supermagnetic iron oxide (SPIO), silica and gold. Liu *et al.* were able to use cetuximab-conjugated SPIO nanoparticles to develop a T_2-weighted MRI sequence targeted to nasopharygeal carcinoma [45]. Reuveni *et al.* have demonstrated the use of cetuximab-decorated gold nanoparticles to obtain contrast-enhanced x-ray computed tomography imaging of an EGFR-upregulating human head and neck carcinoma xenograft in mice, as can be seen in Figure 2D [46]. Cherukuri *et al.* used cetuximab to develop anti-EGFR-targeted gold nanoparticles for radiofrequency ablation of pancreatic and colorectal adenocarcinomas [47]. Melancon *et al.* combined these two concepts to develop cetuximab-conjugated SPIO particles coated with gold to create multifunctional nanoshells for magnetic resonance-guided photothermal ablation of head and neck cancers (Figure 2C) [39]. Similarly, Liao *et al.* synthesized cetuximab conjugated polymeric nanoparticles loaded with doxorubicin and SPIO for targeted therapy and imaging of A431 cells [21].

While it has shown promise in EGFR-targeted delivery of antitumor and imaging agents, antibody targeting suffers from several disadvantages that must be overcome to optimize the drug delivery benefits. Antibodies are very expensive because they must be raised in

animals and then humanized to render them safe for clinical use. However, even after this humanization process, antibodies can pose immunogencitiy issues in some patients. An additional disadvantage is the large size of antibodies, which limits the number of copies that can be decorated on a nanocarrier. This limitation can potentially lead to suboptimal levels of targeting.

EGFR targeting using antibody fragments

Due to the aforementioned size issues of using full antibodies, there have been several attempts to investigate the efficacy of using antibody fragments as targeting moieties. Antibodies are comprised of several regions with some being more important for specific receptor binding than others. Several researchers have utilized single chains of the variable antibody fragment (ScFv) to conjugate onto nanoparticles in order to facilitate recognition of the EGFRs upregulated on cancer cells [48,49]. Mamot *et al.* have carried out several studies using modified cetuximab fragments that target both wild-type EGFR and the constitutively active variant EGFRvIII [50]. They conjugated these fragments to liposomes and found that the nanoparticles were internalized within 15 min when incubated with glioma, epidermoid carcinoma and NR-6 cells stably transfected with EGFRvIII. However, this binding and subsequent uptake was not seen with non-EGFR-overexpressing cell lines such as MCF-7 and the parental NR-6 line. Additionally, they were able to deliver several drugs (doxorubicin, vinorelbine, epirubicin or methotrexate) and achieve up to 80% cell death *in vitro*. While close to 100% cell death was seen *in vitro* with free drug, the lack of targeting could not ensure this same result in an *in vivo* situation. When animal models were studied, significant tumor reduction was seen following treatment with the EGFR-/EGFRvIII-targeted liposomes. Peng *et al.* used ScFv fragments derived from generic anti-EGFR antibodies for conjugation to heparain nanoparticles for delivery of cisplatin and were able to achieve 80% cell death *in vitro* (Figure 3A) [48]. Further research would need to be carried out *in vivo* to determine the efficacy of this system. EGFR ScFvs have also been used by the same group with quantum dots and iron oxide particles for targeted imaging [49]. Another analogous EGFR-targeting motif is the single-domain antibody (denoted sdAb) developed by Ablynx, Belgium, which consists of a single monomeric variable antibody fragment. Called a Nanobody®, it is an order of magnitude smaller than full antibodies and even smaller than ScFv, thus reducing the steric issues seen when using full antibodies. Talelli *et al.* have used this Nanobody along with crosslinked thermosensitive polymeric micelles to form a targeted drug delivery system that showed 100% cell death *in vitro* along with excellent cell binding and uptake, as can be seen in Figure 3B [51,52].

EGF-based targeting

In parallel to antibody-mediated EGFR targeting, researchers have also delved into the possibility of using the native ligand, EGF, for EGFR targeting. EGF is a 6-kDa protein made from 53 amino acid residues. Its small size compared with antibodies makes it an attractive choice of targeting moiety for nanoparticle systems. Tseng *et al.* conjugated EGF to aerosol administrations of gelatin nanoparticles and were able to show specific accumulation in orthotopic lung adenocarcinomas in severe combined immunodeficiency mice after 24 h compared with unmodified nanoparticles (Figure 4A) [53,54]. Both murine and human EGF have been conjugated to nanoparticles for both drug delivery and targeted imaging applications with promising results. Shimada *et al.* conjugated EGF to polymeric lipid-based nanoparticles for the delivery of paclitaxel and were able to show significant growth inhibition *in vivo*; although it should be noted the overall tumor volume still increased over time compared with controls (Figure 4B) [55]. Conjugation with high-density lipoprotein-mimicking nanoparticles, poly(ethylene glycol) (PEG) poly(e-caprolactone) (PCL) micelles and iron oxide nanoparticles have shown promise *in vitro* for both drug

delivery and photothermal ablation; although little *in vivo* work has been carried out with these systems [55-59]. One study of note, carried out by Sandoval *et al.*, was able to show that murine EGF-conjugated lipid nanoparticles loaded with gemcitabine were able to cause significant reduction in tumor volume *in vivo* even after treatment was stopped [19]. It is not clear, however, if this was due solely to the EGF targeting. Studies of multifunctional nanoparticles conjugated with native EGF have also been performed to allow for concurrent delivery of drugs and imaging agents. Tam *et al.* created a hybrid gold nanoparticle/phospholipid system modified with EGF for molecular imaging and surface-enhanced raman spectroscopy of EGFR-upregulated cancers [60]. Fonge *et al.* made indium-loaded EGF-conjugated polymeric micelles for targeted auger electron radiotherapy and were able to cause 100% cell death *in vitro* [56].

Although EGF is an attractive choice of targeting motif for cancer-selective delivery, commercially available EGF is often from murine sources, which can cause antigenicity issues. EGF can also be found in human platelets, macrophages and plasma, but purification from human sources is both expensive, time-consuming and may pose immunogenicity risks.

Aptamer-based targeting

Aptamers are a class of functional oligonucleotides developed by artificial combinatorial methodologies that can bind a wide variety of specific targets [61]. Cancer cell-specific aptamers can be used to functionalize nanoparticles for more effective drug delivery. The most prominent work involving nanoparticle conjugation to EGFR-targeting aptamers has been performed by Li *et al.* through their work with gold nanoparticles. They found and then utilized the 80-residue aptamer, J18, to target EGFR on A431 cells and found excellent internalization via receptor-mediated endocytosis [62]. Although this field is promising, currently there is only limited information involving aptamers for nanoparticle-mediated EGFR targeting.

Peptide-based targeting

Perhaps the most promising and new area of research in the context of EGFR-targeting involves several recently developed EGFR-specific low-molecular-weight peptides. In recent years, several EGFR-specific peptides have become popular in research, including ‘D4’ and ‘GE1’ [63-65]. D4 is a novel peptide ligand developed by Song *et al.* that was designed based on the crystal structure of EGFR and is known to bind to a surface pocket of EGFR [66]. D4 was conjugated to liposomes and successful binding experiments on EGFR-overexpressing cells were carried out. However, only fluorescently labeled D4 was investigated *in vivo* and not the D4-targeted liposomes. The EGFR-specific peptide GE11 was developed by Li *et al.* via phage display technique [67]. It is a 12-residue peptide with a dissociation constant of approximately 22 nM, which suggests lower affinity for EGFR than the native ligand EGF, but provides the advantage of lower mitogenic activity, relatively cheaper synthesis and scale-up, and minimum immunogenicty. GE11 has been conjugated to a variety of nanoparticles, including gold nanoparticles, liposomes, polymeric micelles and gelatin nanoparticles. Song *et al.* conjugated GE11 to doxorubicin-loaded liposomes and found that they had similar pharmacological potency to free doxorubicin [68]. Small animal *in vivo* fluorescence images of the doxorubicin-loaded liposomes also revealed that the targeted liposomes were frequently internalized in the tumor mass during the first 12 h before dissipating, while the nontargeted liposomes reached lower levels of internalization during this time [68]. Magadala *et al.* investigated the conjugation of several EGF peptides, including GE11, to gelatin nanoparticles for the purposes of gene delivery to EGFR-overexpressing cells [69]. They were able to show enhanced uptake and therapeutic efficacy

in EGFR-overexpressing pancreatic cells. The same research group also developed GE11 conjugated to the polymer blend nanocarriers made from poly(lactic-co-glycolic acid), PEG and PCL for drug delivery of paclitaxel and lonidamine [70-72]. First, the nanoparticles were tested in several human cell lines and it was found that targeting was successful in EGFR-overexpressing cell lines, particularly after induction of hypoxia. It was subsequently found that nanoparticle dosing led to approximately 90–95% cell death in multidrug-resistant hypoxic cell lines as well as, normoxic cell lines [71]. Biodistribution, pharmacokinetics and therapeutic efficacy were then completed in an orthotopic multidrug-resistant breast cancer mouse model. It was found that maximum tumor accumulation of targeted nanoparticles occurred 3 h after administration and the formulation exhibited superior tumor accumulation compared with free drug and nontargeted nanoparticles, particularly when it came to the lonidamine formulation (Figure 5B$_{I\text{-}II}$). It was also found that the combination paclitaxel-/lonidamine-targeted nanoparticles were able to decrease tumor volume over a 28-day time span, as well as alter the drug resistance phenotype of the tumor xenografts (Figure 5B$_{III}$) [73]. Our laboratory has used the GE11 peptide to create nanoformulations of the photosensitizer Pc 4 for targeted PDT of EGFR-overexpressing cancers. We have demonstrated that GE11 targeting allows for increased drug uptake at lower incubation times compared with analogous nontargeted formulations and up to 80% cell death *in vitro* (Figure 5A) [73,74].

Conclusion

There has been great interest in EGFR-targeted immunotherapies for alternative and adjuvant treatment strategies of cancer. There are several approaches to direct active targeting including the use of antibodies, endogenous ligands and short-chain peptides. Nanoparticle-based treatment strategies harness the EGFR specificity of these active targeting moieties to create targeted intravenous carriers for drugs and contrast agents.

Several design parameters must be considered in order to develop effective targeted nanoparticle systems including drug loading and drug release characteristics of the base nanoparticles themselves. Discussion of the nanoparticle characteristics is outside the scope of this review, the focus of which is on the choice of the EGFR-targeting moiety. The targeting moiety is very important to the functionality of the system with size and affinity being two of the most important design criteria contributing to the success of the system. Table 2 shows the methods of targeting discussed in this review along with the corresponding available molecular weights and dissociation constant values that influence the thermodynamics and kinetics of targeting. The molecular weight (hence size) of the targeting component is important since it affects the density of nanoparticle surface decoration, and in effect this influences the subsequent ligand/receptor binding interactions by virtue of affinity and avidity. Antibodies have been a very attractive targeting option due to their very high affinity, but their large size leads to steric hindrance issues towards multivalent decoration on nanoparticles. In addition to the size obstacle, antibodies also pose the issues of possible immunogencitiy. As antibodies are grown in other species, they must undergo the very costly process of protein sequence modification to yield chimeric or humanized antibodies in order to minimize immunogenicity risks. Such processes make antibody-based targeting technologies highly expensive. The size-related limitations with antibodies can be partly reduced with the use of antibody fragments, but these fragments may have receptor affinities a lot lower than antibodies. Furthermore, because they are developed in ways similar to full antibodies, these fragments may still pose the issues of immunogencitiy and cost. An alternative targeting moiety is the endogenous ligand of EGFR, specifically EGF, which has a very low dissociation constant with respect to EGFR (hence, high binding affinity) and is significantly smaller than both antibodies and antibody fragments. However, isolating and purifying EGF from murine or human sources can make

the targeting technology highly expensive. Also, since physiologically and pathologically EGF binding to EGFR leads to several downstream signaling cascades to promote cell proliferation and survival, targeting EGFR-overexpressing cancer cells with EGF-decorated nanocarriers may potentially trigger signaling processes that amplify cancer growth, thus offsetting the therapeutic effects of the nanocarrier-delivered payload. Another attractive alternative for EGFR-targeting is the use of EGFR-specific peptides, due to their small size (<2000 Da), low immunogenicity, ease of synthesis and low cost of scale-up. To this end, the D4 and GE11 peptides have been used quite extensively in recent years for development of EGFR-targeted nanoparticles for cancer-specific drug delivery. When compared with antibodies and native ligands, these peptides may have lower affinity to EGFR at a single molecule level, but the cumulative effect of multivalent modification (avidity) may compensate for this to achieve effective targeting of cancer cells expressing a high level of EGFRs on the surface. It can be rationalized that the ideal EGFR-targeted nanocarrier system for cancer-specific delivery will be a result of optimizing particle stability, drug loading and release characteristics, and the cell-targeting capability and internalization efficacy achieved via optimum ligand density.

Future perspective

Although a variety of EGFR-targeted nanoparticle-based therapeutic approaches have shown promising results *in vitro* and in pre-clinical models *in vivo*, several important parameters need to be determined and optimized to ensure successful translation of these technologies to clinical application. Ongoing and future work in the field need to correlate the extent of receptor expression to the amount of ligands needed for effective targeting of the nanoparticles in sufficient quantities in order to provide a sufficient dose of the therapeutic agent. It is envisioned that continued research in this area will also enable identification of tumors where EGFR-targeted nanoparticle approaches (active targeting) can provide additional advantages over nontargeted nanoparticles (EPR-based passive targeting). This information is highly critical, because although several cancers are known to have upregulation of EGFRs, the level of upregulation may or may not be sufficient to warrant additional benefit from active targeting over and above passive accumulation. Furthermore, cancers with high EGFR expressions are often found to have widespread hypoxic regions that become barriers for nanoparticles to permeate and penetrate throughout the tumor volume effectively. Therefore, in such cases even if the particles themselves may have excellent EGFR-targeting capability, getting enough particles to penetrate throughout the tumor volume can be a challenge, and this will be a critical component of ongoing and future research. In parallel, further development in the field will integrate the packaging of therapeutic agents and imaging probes (the nanotheragnostic approach) within optimized EGFR-targeted vehicles, to possibly enable image-guided therapy and image-assisted treatment evaluation for candidate EGFR-overexpressing cancers.

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Executive summary

- Ligand-mediated EGF receptor (EGFR) targeting of nanovehicles is an effective way for cancer cell-selective delivery of vehicle-encapsulated bioactive agents.
- EGFR-specific antibodies, antibody fragments, native EGF, aptamers and peptides are the various ligand categories that can be used for nanoparticle decoration to achieve active EGFR targeting.
- Compared with antibodies and their derivatives, native ligands or aptamers, using peptides for EGFR targeting of nanoparticles can provide several advantages, such as reduced immunogenic risks, reduced cost of development and scale-up, and increased control over particle decoration density.
- It is necessary to determine the optimum ligand density necessary for sufficient targeting such that a sufficient number of drug-loaded nanoparticles are internalized to release their therapeutic payload intracellularly for significant treatment efficacy.
- The focus of ongoing and future work in the field will not only be to determine the best EGFR-targeting ligand from a translational perspective, but also to correlate and optimize ligand decoration of nanoparticles to the level of EGFR upregulation on target cancers.
- Future research in the field will help further establish a quantitative metric to assess whether EGFR-based active targeting of the nanovehicles can provide additional advantages over enhanced permeability and retention-mediated passive accumulation, especially in the context of variability in EGFR upregulation levels in various cancers.

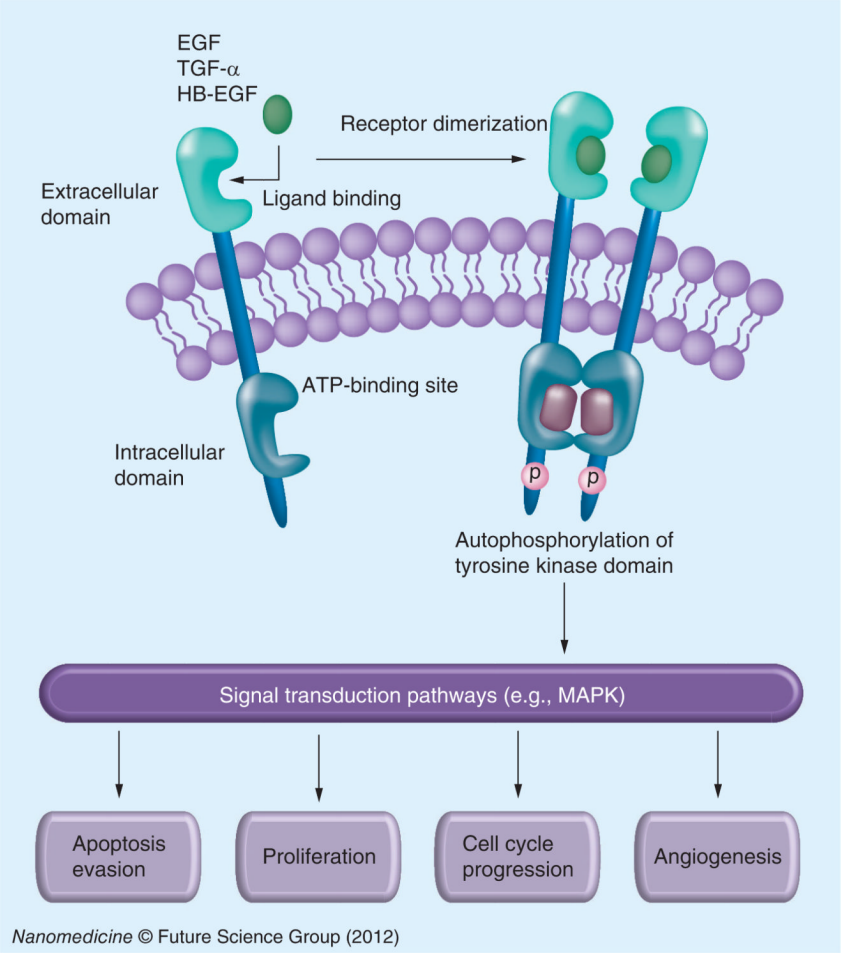

Figure 1. Role of EGF receptors in cancer
HB: Heparin binding.

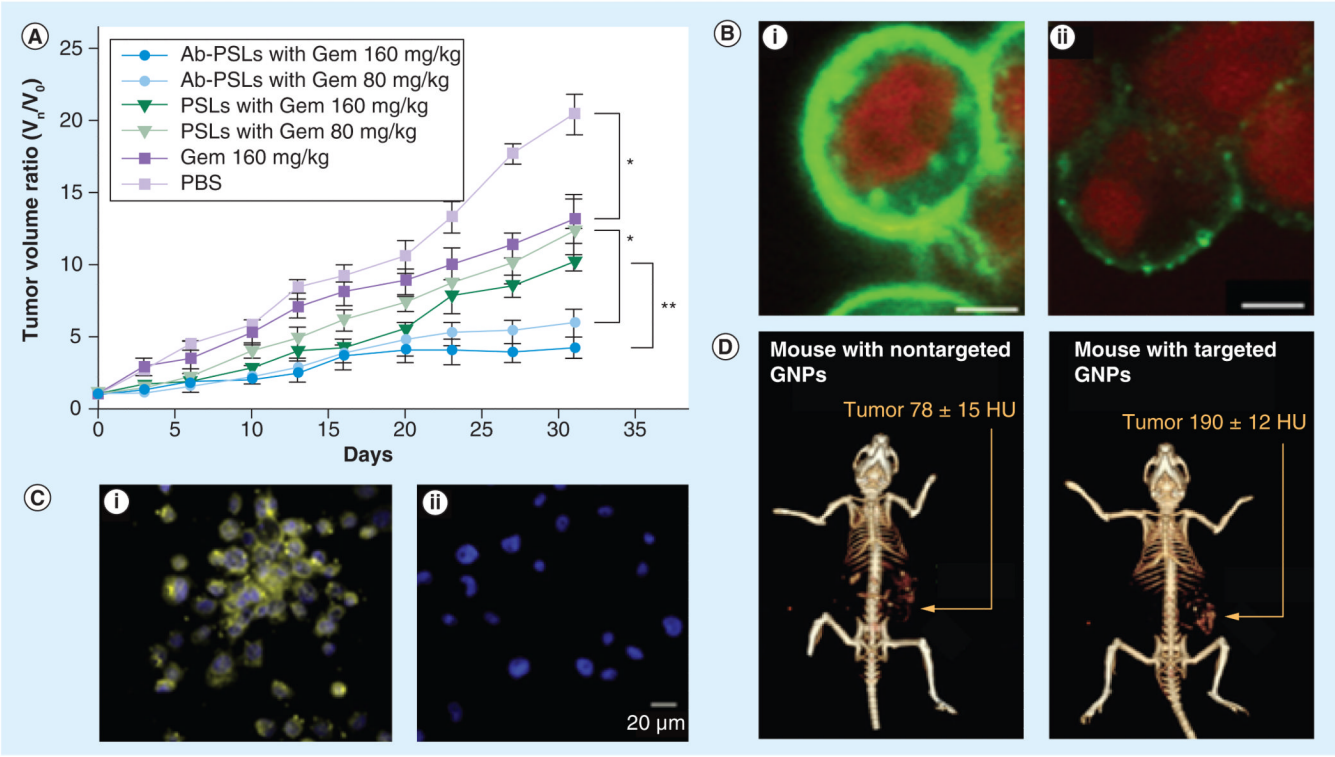

Figure 2. Representative examples of enhanced nanoparticle targeting to EGF receptors using particle decoration with anti-EGF receptor antibodies

(A) Tumor volume analysis of tumor-bearing mice after treatment with various formulations of Gem including Ab-PSLs, PSLs and free drug (Gem) showed that Ab-PSLs rendered a significant reduction in tumor growth compared with the control formulations [26]. **(B)** Confocal imaging of Ab-PSL nanoparticles **(Bi)** shows significantly higher targeting and internalization (increased green fluorescence of particles inside the target cells) compared with analogous nontargeted formulation **(Bii)**. **(C)** Light-scattering images of GNPs indicate higher uptake of antibody-decorated particles **(Ci)** compared with naked particles **(Cii)**. **(D)** Rendered contrast-enhanced x-ray computed tomography imaging of a tumor targeted by anti-EGF receptor antibody GNPs compared with nontargeted GNPs.

*$p < 0.001$; **$p < 0.003$.

Ab-PSL: Antibody-based EGF receptor-targeted liposome; Gem: Gemcitabine; GNP: Gold nanoparticle; HU: Hounsfield unit; PBS: Phosphate-buffered saline; PSL: Nontargeted liposome.

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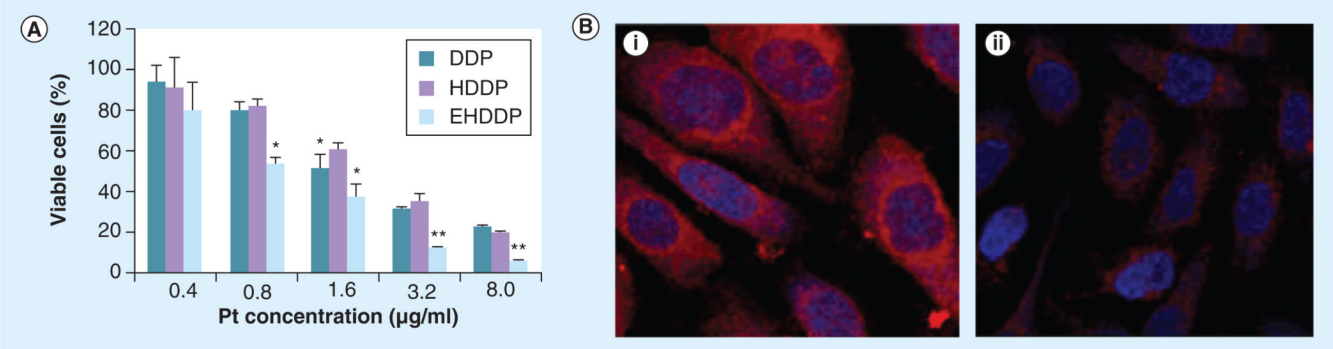

Figure 3. Representative examples of enhanced nanoparticle targeting to EGF receptors using nanoparticle decoration with anti-EGF receptor antibody fragments

(A) Cell viability assay shows a significant decrease in cell viability following treatment with varying DDP concentrations delivered via anti-EGF receptor fragment-labeled nanoparticles, compared with nontargeted nanoparticles or free drug. **(Bi)** Anti-EGF receptor fragment-labeled red fluorescent polymeric micelles undergo higher internalization compared with **(Bii)** the nontargeted formulation.

*$p < 0.05$; **$p < 0.01$.

DDP: Cisplatin; EHDDP: EGF receptor-targeted–heparin–cisplatin; HDDP: Heparin–cisplatin; Pt: Platinum.

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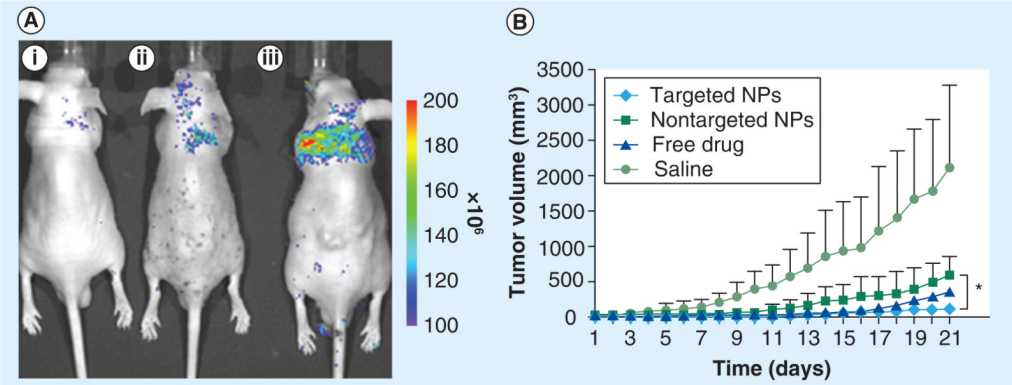

Figure 4. Enhanced nanoparticle targeting to EGF receptors using nanoparticle decoration with native EGF ligand

(A) *In vivo* fluorescence images of orthotopic lung cancer-bearing mice following treatment with **(Ai)** phosphate-buffered saline, **(Aii)** nontargeted NPs and **(Aiii)** EGF-decorated NPs, showed that the EGF receptor-targeted formulation undergoes significantly higher localization at the tumor site. **(B)** Antitumor effects of paclitaxel delivered by EGF-decorated (targeted) versus nontargeted NPs showed that the targeted formulation cause significantly greater tumor suppression compared with the nontargeted formulation.
*$p < 0.001$.
NP: Nanoparticle.
Adapted with permission from [53,55].
For color images see online at www.futuremedicine.com/doi/10.2217/nnm.12.160.

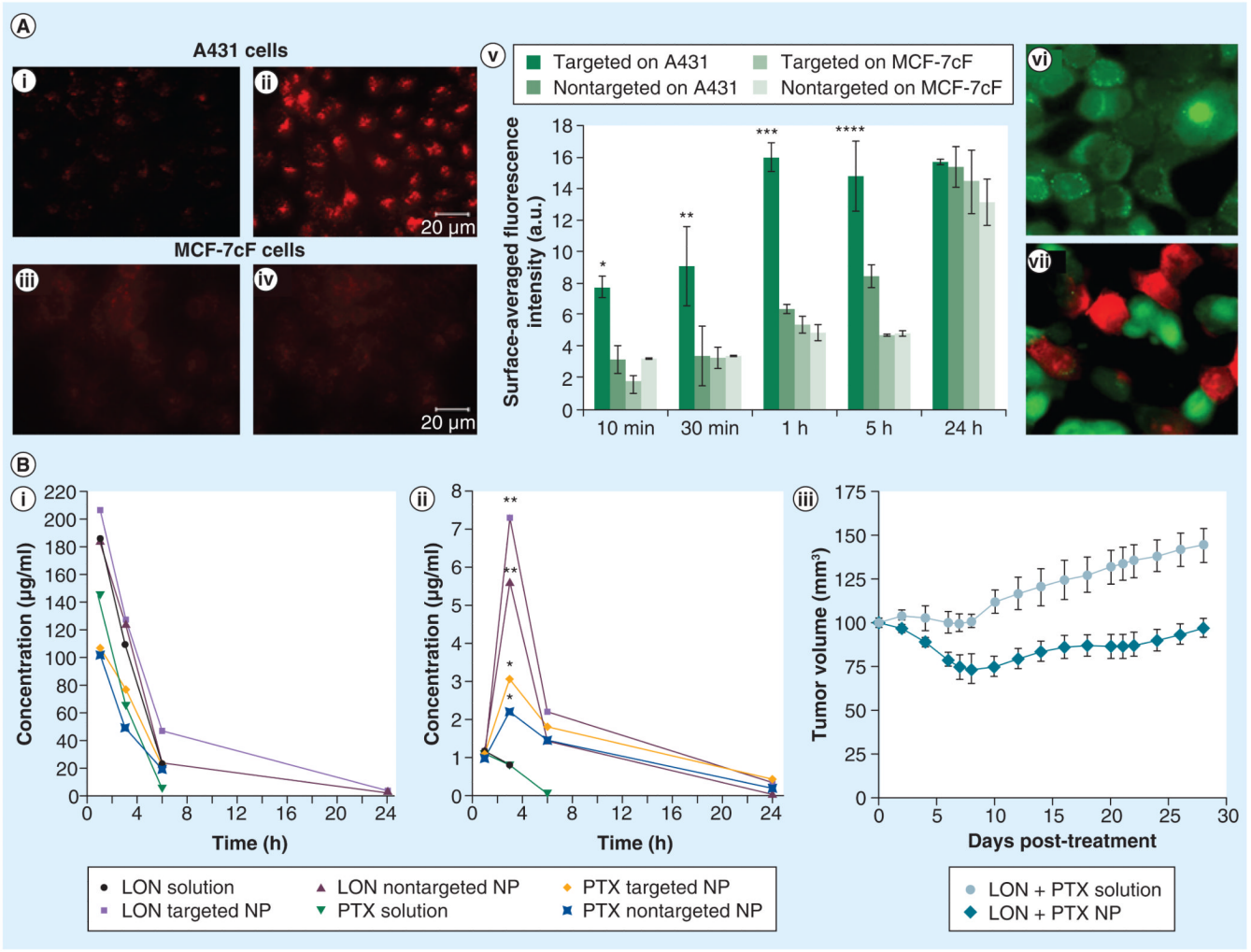

Figure 5. Representative examples of enhanced nanoparticle targeting to EGF receptors using EGF receptor-selective peptides

(Ai–iv) Fluorescence images indicate that peptide-decorated EGF receptor (EGFR)-targeted micelles undergo significantly higher internalization in **(Aii)** EGFR-overexpressing A431 cells compared with the same formulation in **(Aiv)** EGFR-deficient MCF-7c3 cells, while **(Ai & iii)** the nontargeted formulation undergoes very little internalization in either cell line at the incubation time points studied. **(Av)** Quantitative assessment of drug uptake following incubation with EGFR-targeted versus nontargeted formulations shows significantly higher uptake with the targeted formulation along with subsequent higher cell death as seen through live/dead fluorescence images **(Avii)** (green: live; red: dead), compared with the nontargeted formulation **(Avi)**. **(B)** Biodistribution data in the **(Bi)** plasma and **(Bii)** tumor of peptide-decorated EGFR-targeted polymer blend NPs delivering LON and PTX show much higher tumor-specific uptake and reduced nonspecific uptake compared with nontargeted formulations. **(Biii)** NP formulations significantly improved tumor suppression compared with free drug.

*$p < 0.05$; **$p < 0.01$.

LON: Loninadine; NP: Nanoparticle; PTX: Paclitaxel.

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Table 1

EGF receptor expression and estimated deaths in the USA for various EGF receptor-overexpressing cancers

Tumor type	Tumors overexpressing EGF receptor (%) [75]	Estimated deaths per year in the USA [76]
Head and neck	80–100	11,500
Renal	50–90	13,500
Non-small-cell lung cancer	40–80	128,000
Glioma	40–63	14,000
Ovarian	35–70	15,500
Pancreatic	30–50	37,000
Colon	25–77	52,000

Table 2

Molecular weight and dissociation constant data of various EGF receptor-based targeting moieties

Targeting moiety	Molecular weight (Da)	Dissociation constant (nM)	Ref.
Cetuximab for wt EGFR	152,000	0.20	[77,78]
Cetuximab for EGFRvIII	152,000	0.38	[79]
Anti-EGFRvIII antibodies	150,000	2.00–6.00	[80,81]
Anti-EGFR antibody	~160,000	0.20	[82]
ScFv EGFR (antibody fragment)	25–28,000	3.36	[49]
ScFv C10 (antibody fragment)	26,000	264	[83]
Nanobody® (antibody fragment)	12–15,000	5.00–20.00	[84]
EGF	6000	1.00–2.00	[85]
EGF peptide (D4)	685	Data not published	[66]
EGF peptide (GE11)	1540	22.00	[67]
Aptamer (J18)	8–25,000	7.00	[62]

EGFR: EGF receptor; wt: Wild-type.