Opinion Paper

Limitations and niches of the active targeting approach for nanoparticle drug delivery

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Abstract

The active targeting approach has been widely employed to improve nanoparticle drug delivery. Contrary to popular conceptions, attachment of a targeting ligand to a nanopaticle does not alter its biodistribution, but only increases its internalization by target cells. Despite its potential, this strategy has drawbacks that can negate efficacy against tumors. Specifically, compared to non-targeted nanoparticles, a number of active targeting nanoparticles have decreased blood circulation time due to non-specific binding or immunogenicity, reduced tumor penetration, and high susceptibility to lysosomal degradation after internalization. In order to maximize the advantages and overcome the disadvantages, the active targeting approach is best suited for delivering membrane impermeable drugs to targets directly exposed to i.v. injected nanoparticles, such as those in circulation or in the luminal site of tumor vasculatures.

Selective drug delivery to tumors is one of the major applications of nanoparticles. Nanoparticles are able to circulate in the blood for a prolonged period of time, and then preferentially extravasate and accumulate in tumors possessing enlarged endothelial gaps. Unlike in tumor tissues, the endothelial lining in most normal tissues is tight, preventing penetration of macromolecules, including nanoparticles, thus minimizing non-selective nanoparticle distribution to normal tissues.

The active targeting approach was first pursued in the early 1980s by covalently attaching a specific antibody to the surface of a liposome (named immunoliposome) with the goal

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of recognizing a selected antigen on the target cell to enhance targeted delivery (1). This strategy was quickly adopted by investigators in the field to increase drug delivery by nanoparticles, and has become one of the most successful ways of improving the efficacy of nanomedicine, at least in animal models (2, 3). Targeting antibodies or ligands are often selected because of their high specificity and high affinities towards overexpressed antigens on target cells and their ability to trigger receptor-mediated endocytosis after binding. However, the targeting ligands or antibodies do not influence the biodistribution of the nanoparticle: biodistribution still depends on passive targeting to tissues with a leaky vasculature, and targeting ligands only triggers internalization after extravasation into the tumor. This mechanism of drug delivery has been demonstrated with a number of targeted nanoparticles. Kirpotin et al. (4) covalently conjugated a recombinant anti-HER2/Neu monoclonal antibody (MAb) fragment to PEGylated liposomes, and discovered that the MAb fragment did not affect the biodistribution of the liposomes. Both the HER2/Neu-targeted and non-targeted liposomes distributed to the liver and spleen at large doses [~15% and 30%–40% injected dose/g tissue (ID/g), respectively], while the tumor uptake of both was comparably moderate (7%–8% ID/g), despite HER2/Neu being only overexpressed on the tumor. Moreover, the tumor uptake kinetics of the targeted and non-targeted liposomes by the HER2/Neu+ tumor were similar, exhibiting a slow accumulating process that peaked at 24–48 h post injection, followed by prolonged retention for >1 week. These data showed that surface coating of the liposome with anti-HER2/Neu antibody did not improve the specificity of nanoparticle biodistribution and the tumor uptake kinetics, which still followed the passive targeting profiles of the non-targeted nanoparticles. Even though the distribution of targeted and non-targeted liposomes is similar, the internalization of the targeted liposomes by the tumor epithelial cells in the HER2+ tumor was 6-fold higher than that of the non-targeted liposomes. Bartlett el al. (5) developed a multifunctional nanoparticle that targeted the transferrin receptor and encapsulated a ^{64}Cu-labeled siRNA against a reporter gene encoding for luciferase. The biodistrubtion and gene-silencing efficacy of the ^{64}Cu-nanoparticles could be measured in real-time by positron emission tomography (PET) and luciferase bioluminescent imaging, respectively. The authors compared the biodistribution of targeted (transferrin conjugated) and non-targeted (PEGylated) versions of the nanoparticles, and demonstrated that the tissue distribution of these two versions was the same with 2% ID/g detected in

the tumor overexpressing the transferrin receptor. In terms of biodistribution specificity, liver uptake of the targeted nanoparticles was 10-fold higher than tumor uptake, with a broad pattern of distribution to other normal tissues expressing low levels of transferrin receptor. Nevertheless, the targeted nanoparticles displayed ~2-fold enhanced gene silencing activity compared to the non-targeted ones. The results suggest that transferrin coating did not increase the biodistribution specificity of the nanoparticles nor the tumor uptake, but enhanced the internalization and bioavailability of the siRNA, leading to improved efficacy. Li et al. (6) created PEGylated lipid-polycation-DNA (LPD) nanoparticles for siRNA delivery, and conjugated a small molecule ligand, anisamide, to the distal end of PEG that recognized cancer cells overexpressing the sigma receptor. They also demonstrated that the targeted LPD nanoparticles did not increase the tumor uptake compared to the non-targeted PEGylated nanoparticles. While the targeting ligand did not influence the distribution of the nanoparticles, it made a profound difference in the intratumoral localization of the siRNA. The fluorescent siRNA delivered by the anisamide-LPD nanoparticles was detected in the cytosol of the tumor cells 4 h post injection, while the siRNA encapsulated by the non-targeted LPD nanoparticles largely localized in the tumor interstitium. As a result, significant gene-silencing activity and antitumor efficacy were observed only with the targeted LPD nanoparticle treatment. The data from these three independent reports are consistent, and support the hypothesis that surface modification of a nanoparticle with a tumor-targeting ligand does not increase the biodistribution specificity, but only enhances internalization by the target cell after extravasation. The cellular internalization improves bioavailability of the drug carried by the targeted nanoparticles, leading to enhanced efficacy.

Despite the enhanced efficacy demonstrated by many targeted nanoparticles, the active targeting approach also faces three major limitations: (a) immunogenicity or non-specificity of the targeting ligand leading to accelerated blood clearance; (b) further impaired tumor penetration compared to the non-targeted nanoparticles; (c) receptor-mediated endocytosis and subsequent lysosomal digestion resulting in a major dose loss by the lysosomal digestion. Harding et al. (7) demonstrated the propensity of targeted nanoparticles to generate an immune response. The authors produced immunoliposomes by linking mouse anti-human epidermal growth factor receptor (EGFR) IgG (C225) onto a liposomal nanoparticle, and demonstrated that the C225-immunoliposomes exhibited long circulation times in rats upon initial injection [mean residence time (MRT)=8.5 h, clearance (Cl)=0.2 mL/h]. However, the subsequent injections of the immunoliposomes into the same animals resulted in accelerated clearance (MRT≤0.7 h; Cl≥7 mL/h), which was accompanied by a significant increase in anti-C225 titers triggered by the constant human region of C225. Cheng and Allen (8) compared the pharmacokinetics of immunoliposomes prepared with whole MAb, Fab' fragments and single chain Fv (scFv), and demonstrated that the blood clearance of the Fab'-liposomes was similar to that of the non-targeted liposomes (0.08–0.1 mL/h), while the MAb-liposomes and scFv-liposomes exhibited enhanced clearance

(0.41 and 0.12 mL/h, respectively). Additionally, the MAb-liposomes and scFv-liposomes showed a ~2-fold increase in uptake by the mononuclear phagocyte system (MPS) in the liver and spleen. The increased MPS uptake was due to the Fc region of the MAb and the poly-His and the c-myc tags in the scFv construct. Chen et al. (9) developed a glycan ligand [BPC]NeuAc that bound with CD22 overexpressed on B cell lymphoma, and attached this ligand to the liposomal surface for targeted delivery of doxorubicin. This glycan ligand was not specific in that it cross-reacted with CD169 on mouse macrophage, leading to ~97% clearance of the injected [BPC]NeuAc-liposomes from the blood in 5 h compared to <10% clearance with the non-targeted liposomes. This phenomenon has also been reported with folate, the most widely used small molecule targeting ligand for drug delivery (10–12). Gabizon et al. (10, 11) and McNeeley et al. (12) independently demonstrated that the folate-liposomes displayed increased blood clearance compared to the non-targeted liposomes ($t_{1/2}$=6.7 vs. 18.1 h), with an up to 2-fold increase in liver uptake. The enhanced clearance of the folate-liposomes by the liver could be attributed to their interactions with folate receptors in the liver (FR), or the binding of the plasma folate binding protein to the liposomes, which facilitates opsonisation and particle removal by the MPS. Again, the biodistribution of liposomes to the FR+ solid tumors was not improved by folate conjugation, and both laboratories reported no difference in antitumor efficacy between the folate-liposomes and non-targeted liposomes. The second major drawback of the active targeting approach is that the targeted nanoparticles display further reduced tumor penetration compared to non-targeted nanoparticles. Even though macromolecules such as polymers, antibodies, and nanoparticles preferentially accumulate in tumors over normal tissues, once they reach the tumor, they exhibit decreased tumor penetration due to decreased rate of diffusion and suppressed convective movement within tumors. When targeted nanoparticles bind to their target after extravasation, their mobility within the tissue will be further decreased, leading to increased heterogeneity in intratumoral distribution of nanoparticles and consequently, uneven exposure to chemotherapy, which can lead to tumor recurrence and drug resistance. Lee et al. (13) showed that the tumor penetration of the 25-nm EGF-micelles targeting the EGFR was significantly reduced compared to its non-targeted counterparts; the mean distance travelled from the blood vessel was 34 μm for the EGF-micelles, compared with 46 μm for the non-targeted micelles. Similar results were obtained with the 60-nm micelles; the mean distance travelled from the blood vessel for the EGF-micelles was 14 μm compared to 20 μm for its non-targeted counterparts. Finally, receptor-mediated endocytosis of the nanoparticle often results in acid-mediated and enzyme-mediated degradation of encapsulated drugs (particularly biological agents such as proteins, peptides, and nucleic acids) in the endosome and lysosome, resulting in loss of activity. Collins and Huang (14) utilized immunoliposomes to deliver a toxin fragment of ricin, which was less cell membrane permeable than the full length ricin and required cytosolic delivery. They demonstrated that a large portion of the toxin fragment was trapped and degraded in the lysosome

after cellular internalization, and the cytotoxicity was completely abolished.

In light of the advantages and challenges presented, the niches for the active targeting approach include: (a) delivery of membrane impermeable drugs to increase their cellular bioavailability; (b) carrying nanoparticles to a target on the luminal site of blood vessel; (c) targeting blood-borne diseases such as leukemia. Li et al. (6) and Bartlett et al. (5) demonstrated that the targeted nanoparticles enhanced the siRNA bioavailability to the tumor cells and increased the gene-silencing activity compared to the non-targeted nanoparticles, despite equal doses of siRNA delivered to the tumors. However, a lysosomal escaping mechanism must be incorporated into the nanoparticle formulation to prevent a major dose loss, with common strategies that take advantage of pH sensitivity, ion pairing and proton sponge effect (3, 15). For highly membrane permeable drugs, such as doxorubicin, it is advantageous to utilize a sustained and extracellular release mechanism for prolonged pharmacological activity and increased tumor penetration via diffusion. Targeting ligands that recognize tumor-overexpressed antigens such as HER2, EGFR, FR and transferrin receptors do not direct the biodistribution of nanoparticles because the ligands only encounter the receptors after nanoparticle extravasation into the solid tumor via the slow passive targeting process. On the other hand, a ligand has a rapid and free access to a target on the luminal site of blood vessel compared to one located on the basomembrane or within the tissue. Magnussen et al. (16) compared the tumor targeting kinetics of antibodies that target a luminal receptor ($\alpha_5\beta_1$ integrin) vs. those that target basomembrane antigens (fibrin and fibronectin) in a solid tumor. They reported that the tumor uptake of the $\alpha_5\beta_1$ integrin targeting MAb peaked in 10 min and stayed high for 24 h, while maximal tumor uptake of the basomembrane targeting MAbs and a control IgG required at least 6 h. The results suggest that in order to alter the biodistribution and rapidly focus the nanoparticle to the target tissue, one should consider choosing a target on the tissue vasculature. To this end, Simberg et al. (17) utilized the in vivo phage display technology to identify a targeting peptide (CREKA) that bound to the meshwork of clotted plasma proteins in the luminal site of tumor vasculature. The CREKA-nanoparticles specifically distributed to the tumor in 2–8 h post i.v. injection with minimal non-specific uptake by other tissues. Oh and colleagues (18) demonstrated that the anti-aminopeptidase P antibody specifically targeted nanoparticles to the luminal caveolae of rat lung endothelium. The caveolae then operated as a pump, transporting the antibody-conjugated nanoparticles from the blood across the endothelium into the lung tissue, a process known as transcytosis. An approximately 80% ID/g of delivery was achieved within 30 min with little uptake in other tissues. These two examples demonstrated that targeting luminal receptors can result in rapid distribution of targeted nanoparticles to the target tissue with improved tissue specificity. Furthermore, if the target is within the blood circulation with no endothelial barriers, it will be directly exposed to targeted nanoparticles. Cheng and Allen (8) showed that the antitumor efficacy of the anti-CD19-MAb-liposomes was enhanced compared to the non-targeted liposomes in a B cell lymphoma model, even though MAb-liposomes experienced a 5-fold higher blood clearance than the non-targeted liposomes. Chen et al. reported similar results (9), demonstrating enhanced anti-B cell lymphoma activity of their CD22-targeted liposomes compared to the non-targeted liposomes despite significantly shorter blood circulation. Both studies suggest that the targeted nanoparticles could rapidly bind with the diseased cells in the blood to exert enhanced activity. Gabizon et al. (11) reported that i.v. injection of folate-targeted liposomal doxorubicin did not enhance the antitumor efficacy against a subcutaneous solid tumor model compared to the non-targeted liposomal doxorubicin. However, i.p. administration of folate-targeted liposomal doxorubicin prolonged the survival of mice bearing i.p. lymphoma cells by >2-fold compared to the non-targeted liposomal doxorubicin. The results indicate that targeted nanoparticles will show benefits compared to their non-targeted counterparts if they can freely and directly access their targets. This advantage is negated in solid tumors, because targeted nanoparticles bind with their target only after the extravasation, a process determined by the passive targeting mechanism.

Despite the advantages normally associated with targeted delivery, the active targeting approach does not always improve the delivery and efficacy of nanomedicine, possibly due to a number of shortcomings discussed earlier. In order to maximize the advantages while avoiding the drawbacks, we need to have a fundamental understanding of this approach, and focus our designs on controlling the biodistribution and metabolism of the targeted nanoparticles for enhanced delivery and efficacy. Doing so will improve upon the active targeting systems currently in place, and will be applicable in the niches described in this opinion.

Acknowledgment

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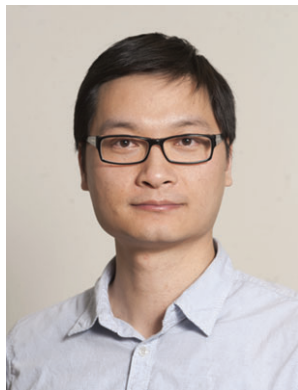
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