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Stimuli-Responsive Programmed Specific Targeting in Nanomedicine

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Abstract

Both passive targeting and actively enhanced cellular internalization play significant roles in tumor-targeted therapy. Programmed specific targeting, as a novel targeting strategy that exploits stimuli-responsive structures, expects that nanocarriers show high stability during blood circulation for efficient passive targeting, then respond to tumor internal or external stimuli and transform into more cell-interactive forms upon arrival at the tumor tissue for enhanced cellular internalization. In this Perspective, we introduce recent advances in the design and development of stimuli-responsive programmed specific targeting nanomedicines, which are based on switchable surface charge, activatable targeting molecules, and variable coatings, to combine the advantages of passive targeting and actively enhanced cellular internalization.

Graphical abstract

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Chemotherapy is an effective clinical cancer treatment, but it often also kills normal healthy cells and causes severe adverse effects to patients,¹ mainly due to nonspecific tissue biodistribution of chemotherapeutic drugs. Moreover, because of the systemic distribution, only a small portion of drugs reaches tumor tissues, leading to relatively low drug efficacy.² Nanocarrier-based chemotherapeutic drug-delivery systems have received much attention to address these issues, as such formulations offer an opportunity to target tumor tissue passively.³

Passive targeting is a consequence of the enhanced permeability and retention (EPR) effect, which is thought to exploit the unique characteristics (*i.e.*, leaky tumor vasculature and poor lymphatic drainage) of many rapidly growing solid tumors in order to promote the accumulation of nanocarriers in tumor tissue.⁴ The particle size, surface charge, and surface modification of nanocarriers are particularly important factors in passive targeting. Nanocarriers sized within the 10–100 nm range can realize efficient extravasation, as well as reduced liver capture and renal filtration.^{5–8} Neutral or negative surface charge and polyethylene glycol (PEG) coating may also facilitate tumor accumulation of nanocarriers by protecting the nanocarriers against the attack of plasma proteins and opsonization by the reticuloendothelial system (RES) in order to prolong their circulation time.^{9–11} However, after reaching tumor tissues, the nanocarriers must be able to enter tumor cells efficiently to achieve intracellular drug delivery because many of the commonly used antitumor drugs, such as doxorubicin and paclitaxel, act on intracellular targets (DNA and microtubules).^{12,13} A positively charged surface or ligand modification may be helpful to enhance cellular internalization of nanocarriers due to their effective binding to cell membranes by strong electrostatic interactions or specific ligand-receptor interactions.^{14,15}

An ideal nanocarrier for drug delivery should simultaneously achieve high tumor accumulation and enhanced cellular internalization. To this end, the designed nanocarriers should be able to change their structures in different environments. Programmed specific

targeting strategies, which are based on stimuli-responsive materials, promise to achieve this goal. A programmed targeting strategy expects the nanocarriers to show high stability during blood circulation for efficient passive targeting, then to respond to tumor internal or external stimuli, and transform into a more cell-interactive form upon arrival at tumor tissues for enhanced cellular internalization (Figure 1). In this Perspective, we briefly introduce recent advances in the field of stimuli-responsive, programmed specific targeting nanomedicines.

Switchable Surface Charge

While circulating in the blood, nanocarriers show a neutral or negative surface charge. However, when they are exposed to the tumor microenvironment, nanocarriers switch to having a positive surface charge (Figure 1A). The cationic nanocarrier then electrostatically interacts with the negatively charged cell membrane, thus enhancing cellular internalization. These nanocarriers are usually based on surface groups that undergo charge-switch in response to the variation of environmental pH. The pH of healthy tissue is approximately 7.4, whereas the pH of the extracellular environment in a solid tumor is lower than 7.0.¹⁶ In one example, a negatively charged molecule—2,3-dimethylmaleic anhydride (DMMMA)—was reacted with an amino group to shield positive charges.¹⁷ The as-prepared nanocarriers show a negative surface charge at physiological pH. At tumor extracellular pH of ~6.8, the amide bonds between DMMAs and amino groups are cleaved in the acidic environment. Both the detachment of DMMAs and the exposure of protonated amino groups lead to the reversion of surface charge.

Use of a zwitterionic surface with pH-responsive zwitterionic-to-cationic charge conversion is an alternative approach to enable surface charge switch. Aryl acylsulfonamide, a pH-responsive zwitterionic group, is attractive because it shows high sensitivity to weakly acidic tumor pH.¹⁸ At physiological pH, zwitterionic nanoparticles with aryl acylsulfonamide groups show neutral surface charge due to the zwitterionic structure. However, when the pH is lowered, the nanoparticles become positively charged because of the protonation of negatively charged groups. A zwitterionic surface can also be achieved by using mixed self-assembled monolayers.^{19,20} The pK_a value of these zwitterionic surface nanocarriers can be tuned by adjusting the ratio of the positively and negatively charged groups.

Activatable Targeting Molecules

Nanocarriers with a positive cell-penetrating peptide (CPP) or ligand surface modification often cause increased interaction with serum proteins and normal healthy cells, leading to high nonspecific adsorption and accelerated RES clearance. An ideal active targeting strategy would be for the targeting molecules to be inactivated during circulation, but to become activated and interact with tumor targets efficiently after being accumulated in the tumor area.

Uncaged Targeting Molecules

Due to the high specificity of ligand–receptor interactions, ligand binding is abrogated in the presence of caging groups (Figure 1B). The photolabile *o*-nitrobenzyl group with ultraviolet (UV) light-triggered bond breaking was commonly used for this photocaging strategy. On

the basis of this approach, various phototargeted nanocarriers involving caged folate and CPPs were developed.^{21,22} The targeting function of caged ligands was inactivated until the cage was removed by exposure of UV light. The overexpressed specific enzymes (*e.g.*, peptidases, proteinases) in tumor tissues were also exploited for enzyme-mediated activatable targeting. For example, an inactivated polycationic CPP was prepared by conjugating a neutralizing polyanion to it *via* a matrix metalloproteinase (MMP)-cleavable linker.²³ The polyanion disables the targeting of CPP until the linker is cut by MMP. Upon cleavage of the linker, the caging polyanion is released, thus activating the CPP, which can then associate with and enter cells.

Conformation Changeable Targeting Molecules

Some special peptide sequences can respond to certain stimuli and change conformations (Figure 1C). The pH low insertion peptide (pHLIP) is water-soluble at neutral and basic pH. However, in an acidic environment, two Asp residues in the transmembrane region of pHLIP are protonated, which leads to an increase in hydrophobicity. Thus, the pHLIP forms a rigid transmembrane α -helix that spontaneously crosses the lipid membrane into the cell.^{24,25} Therefore, pHLIP modified nanocarriers are promising drug delivery systems to achieve programmed tumor targeting.

Variable Coatings

As mentioned above, modifying nanocarriers with ligands or cationic groups may lead to high nonspecific adsorption and short circulation time. Furthermore, some exposed targeting molecules/ligands are susceptible to degradation by enzymes in the blood.²⁶ Surface coating shielding is regarded as an effective method to address this issue. The targeting molecules are hidden in the inner layer of the nanocarriers. Once the nanocarriers reach the tumor sites, they respond to certain stimuli and expose the targeting molecules/ligands.

Unshielding of Targeting Molecules

Nanocarriers with a sheddable PEG coating have been actively explored for drug delivery because PEG is able to improve the stability and to prolong the circulation time of nanoparticles. Long PEG chains form a neutral surface coating to protect short-chain targeting molecules against the attack of plasma proteins. Various targeting molecules, such as folate, TAT peptide, RGD peptide, or positively charged lysine and quaternary ammonium salt, have been connected to nanocarriers and shielded by PEG chains to obtain smart coating sheddable nanocarriers.^{27–30} In blood circulation and normal tissues, the targeting property is turned off. Once the nanocarriers reach the acidic tumor site by passive targeting, under certain stimuli in the tumor environment the PEG chains are detached in order to turn on the targeting molecules for receptor binding and internalization (Figure 1D).

Another way to protect targeting molecules involves the use of a biodegradable coating that can be rapidly degraded in the tumor environment. Many enzymes that can rapidly degrade certain macromolecules or polypeptides, such as hyaluronidase (HAase), are widely expressed in tumor interstitium.^{31–33} Hyaluronic acid (HA), a negatively charged natural macromolecule, is a suitable coating material for programmed targeting nanocarriers

because it can be chopped quickly in the presence of HAase (Figure 1D).³⁴ In a recent study, HA was coated onto the surface of positively charged CPP-modified liposomes by an electrostatic effect for shielding the positive charge of CPP.³⁵ In the tumor site, the HA coating can be detached by HAase, leading to the CPP- and positive charge-enhanced cellular uptake.

Unbinding of Targeting Molecules

Another strategy to hide the targeting molecules is to anchor them in the inner layer of the nanocarriers. Upon internal or external stimuli at the tumor site, the targeting molecules are moved from the inner layer to the surface of the nanocarriers (Figure 1E). These nanocarriers are usually based on a micelle structure. For example, poly(L-histidine) (polyHis) is a peptide that is hydrophobic at physiological pH, but becomes hydrophilic at acidic pH due to the protonation of imidazole groups. On this basis, a short polyHis chain has been used as a linker between PEG chains and biotin. The other end of the PEG chain was connected to a relatively long hydrophobic chain, then the synthesized amphiphilic polymer self-assembled to form polymeric micelles.³⁶ At neutral pH, the polyHis chains are hydrophobic, so the biotin molecules stay inside the polymeric micelles and are shielded by the PEG chains. In the acidic tumor area, protonation of imidazole groups led to the hydrophilic conversion of polyHis chains. Thus, the biotin molecules are pushed to the surface of polymeric micelles.

CONCLUSIONS AND PROSPECTS

In this Perspective, we have summarized the different strategies to develop stimuli-responsive programmed specific targeting nanomedicines. These strategies include exploitation of switchable surface charge, activatable targeting molecules, and variable coatings. In general, the targeting molecules in these systems are annulled under physiological condition and then reactivated by certain internal or external stimuli. Most of the reported studies only worked at the cellular level, but *in vivo* tests are rarely performed. There is still a long way to go before any of these systems can be translated into the clinic.

For effective clinical translation of the stimuli-responsive programmed specific targeting nanomedicines, the following aspects should be taken into account: (1) in the physiological environment, the active targeting component should be largely shielded to reduce nonspecific interactions with plasma proteins and RES organs and to maintain high stability in blood circulation; (2) in the tumor environment, the nanoparticles should sensitively respond to the stimuli and change the structure to allow active targeting; (3) the reactivated targeting molecules should retain the original targeting functions and facilitate cellular interaction; (4) the drug release behavior should be controllable and stable in circulation and in the extracellular environment, and the nanoparticles should rapidly release the drug molecules upon entering the cells; and (5) the formulations should be biocompatible and safe to administer. Although still at an early stage, the stimuli-responsive programmed specific targeting strategy appears to be a promising direction to improve tumor targeting efficiency in nanomedicine.

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References

1. Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R. Nanocarriers as an Emerging Platform for Cancer Therapy. *Nat Nanotechnol.* 2007; 2:751–760. [PubMed: 18654426]
2. Cheng Z, Al Zaki A, Hui JZ, Muzykantov VR, Tsourkas A. Multifunctional Nanoparticles: Cost Versus Benefit of Adding Targeting and Imaging Capabilities. *Science.* 2012; 338:903–910. [PubMed: 23161990]
3. Petros RA, DeSimone JM. Strategies in the Design of Nanoparticles for Therapeutic Applications. *Nat Rev Drug Discovery.* 2010; 9:615–627. [PubMed: 20616808]
4. Matsumura Y, Maeda H. A New Concept for Macromolecular Therapeutics in Cancer-Chemotherapy-Mechanism of Tumorotropic Accumulation of Proteins and the Antitumor Agent Smance. *Cancer Res.* 1986; 46:6387–6392. [PubMed: 2946403]
5. Danhier F, Feron O, Preat V. To Exploit the Tumor Microenvironment: Passive and Active Tumor Targeting of Nanocarriers for Anti-Cancer Drug Delivery. *J Controlled Release.* 2010; 148:135–146.
6. Unezaki S, Maruyama K, Hosoda JI, Nagae I, Koyanagi Y, Nakata M, Ishida O, Iwatsuru M, Tsuchiya S. Direct Measurement of the Extravasation of Polyethyleneglycol-Coated Liposomes into Solid Tumor Tissue by *In Vivo* Fluorescence Microscopy. *Int J Pharm.* 1996; 144:11–17.
7. Hobbs SK, Monsky WL, Yuan F, Roberts WG, Griffith L, Torchilin VP, Jain RK. Regulation of Transport Pathways in Tumor Vessels: Role of Tumor Type and Microenvironment. *Proc Natl Acad Sci U S A.* 1998; 95:4607–4612. [PubMed: 9539785]
8. Alexis F, Pridgen E, Molnar LK, Farokhzad OC. Factors Affecting the Clearance and Biodistribution of Polymeric Nanoparticles. *Mol Pharmaceutics.* 2008; 5:505–515.
9. Lankveld DP, Rayavarapu RG, Krystek P, Oomen AG, Verharen HW, Van Leeuwen TG, De Jong WH, Manohar S. Blood Clearance and Tissue Distribution of PEGylated and Non-PEGylated Gold Nanorods after Intravenous Administration in Rats. *Nanomedicine.* 2011; 6:339–349. [PubMed: 21385136]
10. Gullotti E, Yeo Y. Extracellularly Activated Nanocarriers: A New Paradigm of Tumor Targeted Drug Delivery. *Mol Pharmaceutics.* 2009; 6:1041–1051.
11. Owens DE, Peppas NA. Opsonization, Biodistribution, and Pharmacokinetics of Polymeric Nanoparticles. *Int J Pharm.* 2006; 307:93–102. [PubMed: 16303268]
12. Sherlock SP, Tabakman SM, Xie L, Dai H. Photothermally Enhanced Drug Delivery by Ultrasmall Multifunctional FeCo/Graphitic Shell Nanocrystals. *ACS Nano.* 2011; 5:1505–1512. [PubMed: 21284398]
13. Chen Q, Liang C, Wang C, Liu Z. An Imagable and Photothermal “Abraxane-Like” Nanodrug for Combination Cancer Therapy to Treat Subcutaneous and Metastatic Breast Tumors. *Adv Mater.* 2015; 27:903–910. [PubMed: 25504416]
14. Gratton SEA, Ropp PA, Pohlhaus PD, Luft JC, Madden VJ, Napier ME, DeSimone JM. The Effect of Particle Design on Cellular Internalization Pathways. *Proc Natl Acad Sci U S A.* 2008; 105:11613–11618. [PubMed: 18697944]
15. Yang X, Grailer JJ, Rowland IJ, Javadi A, Hurley SA, Matson VZ, Steeber DA, Gong S. Multifunctional Stable and pH-Responsive Polymer Vesicles Formed by Heterofunctional Triblock Copolymer for Targeted Anticancer Drug Delivery and Ultrasensitive MR Imaging. *ACS Nano.* 2010; 4:6805–6817. [PubMed: 20958084]
16. Cheng H, Zhu JY, Xu XD, Qiu WX, Lei Q, Han K, Cheng YJ, Zhang XZ. Activable Cell-Penetrating Peptide Conjugated Prodrug for Tumor Targeted Drug Delivery. *ACS Appl Mater Interfaces.* 2015; 7:16061–16069. [PubMed: 26161578]
17. Du JZ, Du XJ, Mao CQ, Wang J. Tailor-Made Dual pH-Sensitive Polymer-Doxorubicin Nanoparticles for Efficient Anticancer Drug Delivery. *J Am Chem Soc.* 2011; 133:17560–17563. [PubMed: 21985458]

18. Mizuhara T, Saha K, Moyano DF, Kim CS, Yan B, Kim YK, Rotello VM. Acylsulfonamide-Functionalized Zwitterionic Gold Nanoparticles for Enhanced Cellular Uptake at Tumor pH. *Angew Chem, Int Ed.* 2015; 54:6567–6570.
19. Wang S, Teng Z, Huang P, Liu D, Liu Y, Tian Y, Sun J, Li Y, Ju H, Chen X, Lu G. Reversibly Extracellular pH Controlled Cellular Uptake and Photothermal Therapy by PEGylated Mixed-Charge Gold Nanostars. *Small.* 2015; 11:1801–1810. [PubMed: 25565411]
20. Pillai PP, Huda S, Kowalczyk B, Grzybowski BA. Controlled pH Stability and Adjustable Cellular Uptake of Mixed-Charge Nanoparticles. *J Am Chem Soc.* 2013; 135:6392–6395. [PubMed: 23527630]
21. Dvir T, Banghart MR, Timko BP, Langer R, Kohane DS. Photo-Targeted Nanoparticles. *Nano Lett.* 2010; 10:250–254. [PubMed: 19904979]
22. Fan NC, Cheng FY, Ho J-aA, Yeh CS. Photocontrolled Targeted Drug Delivery: Photocaged Biologically Active Folic Acid as a Light-Responsive Tumor-Targeting Molecule. *Angew Chem, Int Ed.* 2012; 51:8806–8810.
23. Olson ES, Jiang T, Aguilera TA, Nguyen QT, Ellies LG, Scadeng M, Tsien RY. Activatable Cell Penetrating Peptides Linked to Nanoparticles as Dual Probes for *In Vivo* Fluorescence and MR Imaging of Proteases. *Proc Natl Acad Sci U S A.* 2010; 107:4311–4316. [PubMed: 20160077]
24. Davies A, Lewis DJ, Watson SP, Thomas SG, Pikramenou Z. pH-Controlled Delivery of Luminescent Europium Coated Nanoparticles into Platelets. *Proc Natl Acad Sci U S A.* 2012; 109:1862–1867. [PubMed: 22308346]
25. Yu M, Guo F, Wang J, Tan F, Li N. Photosensitizer-Loaded pH-Responsive Hollow Gold Nanospheres for Single Light-Induced Photothermal/Photodynamic Therapy. *ACS Appl Mater Interfaces.* 2015; 7:17592–17597. [PubMed: 26248033]
26. Koren E, Apte A, Sawant RR, Grunwald J, Torchilin VP. Cell-Penetrating TAT Peptide in Drug Delivery Systems: Proteolytic Stability Requirements. *Drug Delivery.* 2011; 18:377–384. [PubMed: 21438724]
27. Ding M, Li J, He X, Song N, Tan H, Zhang Y, Zhou L, Gu Q, Deng H, Fu Q. Molecular Engineered Super-Nanodevices: Smart and Safe Delivery of Potent Drugs into Tumors. *Adv Mater.* 2012; 24:3639–3645. [PubMed: 22689222]
28. Xiao D, Jia HZ, Zhang J, Liu CW, Zhuo RX, Zhang XZ. A Dual-Responsive Mesoporous Silica Nanoparticle for Tumor-Triggered Targeting Drug Delivery. *Small.* 2014; 10:591–598. [PubMed: 24106109]
29. Zhu L, Kate P, Torchilin VP. Matrix Metalloprotease 2-Responsive Multifunctional Liposomal Nanocarrier for Enhanced Tumor Targeting. *ACS Nano.* 2012; 6:3491–3498. [PubMed: 22409425]
30. Wang S, Zhang S, Liu J, Liu Z, Su L, Wang H, Chang J. pH-and Reduction-Responsive Polymeric Lipid Vesicles for Enhanced Tumor Cellular Internalization and Triggered Drug Release. *ACS Appl Mater Interfaces.* 2014; 6:10706–10713. [PubMed: 24941446]
31. Stern R, Jedrzejewski MJ. Hyaluronidases: Their Genomics, Structures, and Mechanisms of Action. *Chem Rev.* 2006; 106:818–839. [PubMed: 16522010]
32. Mo R, Jiang T, DiSanto R, Tai W, Gu Z. ATP-Triggered Anticancer Drug Delivery. *Nat Commun.* 2014; 5:3364. [PubMed: 24618921]
33. Stern R. Hyaluronidases in Cancer Biology. *Semin Cancer Biol.* 2008; 18:275–280. [PubMed: 18485730]
34. Jiang T, Mo R, Bellotti A, Zhou J, Gu Z. Gel-Liposome-Mediated Co-Delivery of Anticancer Membrane-Associated Proteins and Small-Molecule Drugs for Enhanced Therapeutic Efficacy. *Adv Funct Mater.* 2014; 24:2295–2304.
35. Jiang T, Zhang Z, Zhang Y, Lv H, Zhou J, Li C, Hou L, Zhang Q. Dual-Functional Liposomes Based on pH-Responsive Cell-Penetrating Peptide and Hyaluronic Acid for Tumor-Targeted Anticancer Drug Delivery. *Biomaterials.* 2012; 33:9246–9258. [PubMed: 23031530]
36. Lee ES, Na K, Bae YH. Super pH-Sensitive Multifunctional Polymeric Micelle. *Nano Lett.* 2005; 5:325–329. [PubMed: 15794620]

Figure 1.

Stimuli-responsive programmed specific targeting nanosystems based on switchable surface charge (A), uncaged targeting molecules (B), conformation changeable targeting molecules (C), unshielded targeting molecules (D), and unbound targeting molecules (E).