Be Active or Not: the Relative Contribution of Active and Passive Tumor Targeting of Nanomaterials

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Abstract

Malignant tumor (cancer) remains as one of the deadliest diseases throughout the world, despite its overall mortality drops. Nanomaterials (NMs) have been widely studied as diagnostic and/or therapeutic agents for tumors. A feature of NMs, compared to small molecules, is that NMs can be concentrated passively in tumors through enhanced permeability and retention (EPR) effect. In the meantime, NMs can be engineered to target toward tumor specific markers in an active manner, e.g., receptor-mediated targeting. The relative contribution of the EPR effect and the receptor-mediated targeting to NM accumulation in tumor tissues has not been clearly defined yet. Here, we tackle this fundamental issue by reviewing previous studies. First, we summarize the current knowledge on these two tumor targeting strategies of NMs, and on how NMs arrive to tumors from blood circulation. We then demonstrate that contribution of the active and passive effects to total accumulation of NMs in tumors varies with time. Over time, the receptor-mediated targeting contributes more than the EPR effect with a ratio of 3 in the case of urokinase-type plasminogen activator receptor (uPAR)-mediated targeting and human serum albumin (HSA)-mediated EPR effect. Therefore, this review highlights the dynamics of active and passive targeting of NMs on their accumulation at tumor sites, and is valuable for future design of NMs in cancer diagnosis and treatment.

Key words: nanomaterial; nanoparticle; enhanced permeability and retention effect; receptor-mediated tumor targeting; urokinase-type plasminogen activator receptor; amino-terminal fragment.

Introduction

The past decades have witnessed continuous advances in tumor diagnosis and therapies; however, malignant tumor (cancer) remains as one of the deadliest diseases throughout the world. More than 1.6 million new cancer cases and over 0.6 million cancer deaths are estimated to occur in the United States alone in 2017, despite the overall mortality drops [1].

Nanomaterials (NMs), including nanoparticles (NPs), micelles, dendrimers and liposomes, have emerged as a novel class of diagnostic probes and/or therapeutic drugs for tumors [2-4]. The nano-sized dimension renders NMs with unique physicochemical properties, such as optical properties and high ratio of surface area to volume. In addition, NMs can be engineered to be responsive to environmental conditions, such as pH value, redox potential or temperature, for controlled release of imaging agents or drugs. Additional properties can be further engineered to NMs, such as biocompatibility, bioavailability, and tumor selectivity [5-8].

Doxil® and Abraxane® represent two successful clinical applications of NMs in cancer nanomedicine. Doxil® is a polyethylene glycol coated (pegylated) liposome-encapsulated doxorubicin (DOX), and was approved by the US Food and Drug Administration
(FDA) for treatment of AIDS-related Kaposi’s sarcoma, ovarian cancer and multiple myeloma [9]. DOX is a highly potent drug used in tumor chemotherapy, but has poor tumor specificity and is toxic to normal tissues, especially cardiomyocytes [10, 11]. Compared to free DOX, Doxil® is concentrated preferentially in tumors and has a better therapeutic index [12, 13]. Abraxane® is albumin-bound paclitaxel NP and is approved by the FDA for the treatment of breast cancer [14], non-small-cell lung cancer [15] and other solid tumors [16, 17]. The formulation of paclitaxel with albumin into NPs renders water solubility of hydrophobic paclitaxel and avoids the use of the solubilizing agent Cremophor which often causes hypersensitivity reactions [18]. Abraxane® is specially accumulated at tumor sites, which reduces its cytotoxicity to normal tissues and thus increases its maximum tolerated dose. Some other NM formulations, including dendrimers [19, 20], quantum dots (QDs) [21-24], metal NPs (e.g., magnetic iron oxide [25] and gold [26]), are under study for cancer nanomedicine [27].

NMs can target to tumors either in a passive manner through the enhanced permeability and retention (EPR) effect or in an active manner by receptor-mediated targeting [28-31]. The relative contribution of the EPR effect and receptor-mediated targeting to NM accumulation in tumor is not fully defined. In this review, we first describe how NMs arrive at tumor sites from blood circulation in vivo. Next we briefly describe the EPR effect and receptor-mediated targeting. Finally, we try to estimate the relative contribution of the EPR effect and receptor-mediated targeting of NMs by summarizing the results reported in the literature.

**NMs from blood circulation to tumors**

For in vivo application, NMs are typically delivered or redistributed to tumors through blood circulation in vascular systems. In normal tissues, molecular exchange across vasculature takes place primarily in capillaries, which consist of a layer of endothelial cells and occasional connective tissue (Figure 1) [32, 33]. Molecules smaller than 3 nm, such as water, gases, salts, sugars and certain metabolites pass capillary endothelial cells freely, largely by diffusion through the space between adjacent capillary endothelium (intercellular cleft, Figure 1) or transcytosis [33, 34]. Molecules larger than 3 nm cannot pass through endothelium freely and only a small amount of macromolecules, such as albumin, immunoglobulins (Igs) and other plasma proteins, are found to extravasate from circulation into normal tissues [32, 34].

Under some circumstances like inflammation, large molecules can exit vasculature in quantity. This occurs primarily in post-capillary venules [35, 36]. The transcellular passage of large molecules may be through enlarged intercellular cleft induced by vascular permeabilizing factors [34]. Another potential mechanism of extravasation is through the vesiculo-vacuolar organelle (VVO). The venular endothelial cells are cuboidal and characterized by clusters of interconnected vesicles and vacuoles in their cytoplasm, distinctive from capillary endothelial cells. These intracellular vesicles and vacuoles together form VVOs [37-39]. The VVOs are linked to the plasma membrane by stomata that are normally closed by thin diaphragms [40]. When exposed to vascular permeabilizing factors, stromal diaphragms are pulled apart mechanically and VVOs are open, allowing transcellular passage of large molecules [41]. The vascular permeabilizing factors include vascular permeability factor (VPF)/vascular endothelial growth factor (VEGF) [42-46], histamine [35, 36], serotonin [35] and platelet activating factor. A single exposure to any of these permeability factors results in a rapid (~20-30 min) hyperpermeability.

Solid tumors stimulate the formation of new blood vessels (neovasculature) in order to absorb excessive nutrients and proliferate quickly [47]. Extensive angiogenesis and high vascular density are
hallmarks of solid tumors [48]. The neovasculature is quite different from normal vasculature both in form and in architecture. The vascular basement membranes (VBM)s of neovasculature are easily degraded by various proteases (the matrix metalloproteases and the plasminogen activator) [49], allowing the detachment of pericytes from vascular endothelial cells. The endothelial cells, no longer restrained by VBM or pericytes, become thin as their lumens expand in response to intravascular pressure [50]. In addition, the endothelial cells are poorly aligned with wide fenestrations; both smooth muscle layer and innervation are malformed or even absent, and functional receptors for some modulators, e.g., angiotensin II (AT-II), are missing [51]. These characteristics ultimately lead to a highly leaky vasculature. Large molecules thus can extravasate out of these blood vessels and into tumors as a result of transvascular osmotic pressure [39]. This increased vascular permeability is chronic and can be utilized to deliver NMs to solid tumors. In the meantime, it is worth to mention that this tumor related permeability is dynamic instead of static [52].

Once reaching tumor sites, NMs need to traverse into intracellular space (endocytosis or cellular trafficking) to take effect. This is a critical event affecting the efficacy and specificity of NMs, and has been widely studied or reviewed [53-56].

**The EPR effect of NMs**

A term of “enhanced permeability and retention (EPR) effect” was firstly proposed by Matsumura and Maeda in 1986 to describe the preferential accumulation of macromolecules in tumor, which has leaky capillaries, and at the same time, poor lymphatic drainage [57]. Size or molecular weight of the macromolecule is a key parameter determining the EPR effect in solid tumors (Figure 2). Typically, molecules with sizes ranging from 10 to 200 nm or 40 to 800 kilodaltons (kDa) in mass exhibit a strong EPR effect [58-60]. Such macromolecules include plasma proteins and NMs [61, 62]. Small molecules tend to diffuse freely in and out of tumor blood vessels because of their small sizes, and thus do not accumulate in tumors as much as macromolecules do over time (Figure 2) [63]. Besides the size, other properties, such as shape and surface chemistry, also affect the EPR effect of NMs, which are discussed in recent reviews [31, 64] and will not be elaborated here.

The EPR effect can be modulated by a number of in vivo factors. EPR augmenting factors include (1) vasoconstrictors to raise the systemic blood pressure [65], e.g., AT-II [66]; (2) free radicals that affect integrity of vascular endothelium, e.g., peroxynitrite [67]; (3) nitric oxide-releasing agents, e.g., nitroglycerin [68]; (4) vascular permeability promoters: bradykinin/kinin [69], prostaglandins, VEGF/VPF and other inflammatory cytokines [70]. These stimulators result in an enhanced vascular permeability and extravasation of macromolecules, and thus increase the EPR effect.

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**Figure 2. Schematic illustration of the enhanced permeability and retention (EPR) effect in tumors.** In tumor tissues (lower panels), endothelial cells are poorly aligned with wide fenestrations, and there is a lack of lymphatic clearance and a smooth muscle layer compared to normal tissues (top panels). Therefore, macromolecules (10 to 200 nm or 40 to 800 kDa) tend to accumulate in tumor tissues much more than in normal tissues. In contrast, small molecules diffuse freely in and out of blood vessels in both normal and tumor tissues due to their small sizes, leading to their low concentrations over time.
There are a number of studies on tumor-targeted NMs mediated by the EPR effect, which were summarized in other reviews [31, 71]. Therefore, only a few representative examples are included in this review. In early 1980s, Maeda and his colleagues prepared a polymer (poly(styrene-comaleic acid)/half-n-butyl ester, SMA) conjugated with a derivative of a DNA-damaging agent (neocarzinostatin, NCS) [72], designated as SMANCS [73]. SMANCS is small in mass (16 kDa) but binds to albumin (67 kDa) in blood circulation [74], leading to an apparent molecular mass of about 83 kDa. The conjugates achieved not only high contrast in tumor imaging when combined with an imaging agent (Lipiodol®), but also a strong anti-tumor therapeutic response [57]. The clinical use of SMANCS was approved in 1993 by the Japanese Government for hepatoma treatment [75]. Doxil® and Abraxane® with ~100 nm in size [76, 77], also have their tumor targeting attributed to the EPR effect.

A few drawbacks, however, exist for the EPR effect-mediated tumor targeting of NMs. The delivery of NMs to tumors through the EPR effect was reported to be inefficient and provides only 20-30% increases in delivery compared with normal organs [64]. This inefficiency is probably because of tumor heterogeneity. Solid tumors are highly dynamic and complex with heterogeneity on size, genomic makeup, vasculature, interstitial pressures and necrotic cores [78-81]. In addition, the EPR effect has modest specificity, because high vascular permeability also occurs under other pathological conditions, e.g., inflammation [28].

Receptor-mediated targeting of NMs

Tumor cells express a profile of surface receptors different from normal cells (Figure 3) [48]. Receptor-mediated targeting of NMs to tumors takes advantage of these specific receptors, which potentially has high safety margin by reducing damage to normal tissues [28, 82-86]. Commonly used tumor surface receptors include epidermal growth factor receptor (EGFR) [87], human epidermal growth factor receptor 2 (HER2) [87], transferrin receptor [88, 89], folate receptor [90, 91], integrins [92] and urokinase-type plasminogen activator (uPA) receptor (uPAR) [93-96]. A complete list of targeted receptors can be found in recent reviews [97, 98].

Figure 3. Schematic illustration of receptor-mediated targeting in tumors. Tumor cells express a profile of surface receptors different from normal cells.
For example, EGFR, a member of the ErbB family of receptor tyrosine kinases, is a widely targeted receptor for clinical applications [99-102]. Aberrant EGFR over-expression was reported in tumors, including lung cancer [103, 104], pancreatic cancer [105], and glioblastoma [106]. Monoclonal anti-EGFR antibodies (mAbs) cetuximab [107-110] and panitumumab [111, 112] are both approved by the FDA, originally for metastatic colorectal cancer. A list of other mAbs (zalutumumab, nimotuzumab and matuzumab) is in clinical development [113, 114]. HER2, another member of the ErbB family, is also successfully explored for tumor targeting [115]. Trastuzumab is effective toward breast cancers where HER2 is over-expressed [116]. Pertuzumab was approved by the FDA for use in combination with trastuzumab in 2012 [117].

uPAR is expressed in a low level on the surfaces of quiescent cells, but is greatly over-expressed on the surfaces of a wide range of invasive tumor cells, and is believed to play a critical role in tumor cell migration, adhesion and tissue remodeling [118-121]. In fact, uPAR has been studied as a promising candidate receptor in tumor targeting therapy [122-127] and/or imaging [128-133]. A peptidyl antagonist of uPAR was successfully used for nuclear imaging of primary tumors and lymph node metastases in 10 patients, and its uptakes were found to correlate with high uPAR expression in excised tumor tissues [134].

Receptor-mediated NMs have been widely demonstrated for their tumor targeting efficacies in vitro. The representative examples are listed in Table 1. The gold NPs with the average size of 35 nm conjugated with an anti-EGFR antibody were shown to specifically bind to cancer cells with 6-fold greater affinity than to the noncancerous cells [135]. The cytotoxicity of a drug formulated in NPs decorated with trastuzumab targeting at HER2 (around 300 nm) was found to be 12.7-fold higher than that of the bare ones without the mAb [136]. The cytotoxic paclitaxel embedded in transferrin-conjugated NPs (about 220 nm) targeting at transferrin receptor was shown a 3-fold higher uptake by human prostate cancer cells than the unconjugated ones [137]. An NM construct was prepared by conjugating magnetic iron oxide NPs with the amino-terminal fragment (ATF) of uPA, which is a highly potent (~0.2 nM dissociation constant) peptide targeting to uPAR [138]. As a result, these conjugated NPs (66 nm) exhibited 7-fold higher accumulation in pancreatic cancer cells than unconjugated ones [139].

Two parameters are critical for the success of receptor-mediated targeting strategy. One is the abundance of tumor surface receptors compared to that in normal tissues. Such abundance typically undergoes active regulation at transcriptional/translational levels or through receptor endocytosis. Genetic mutations may alter receptor structures and/or functions, and thus lower or disrupt the efficacies of specific diagnostic agents or therapeutic drugs, leading to drug resistance [84, 140-142]. The other parameter is the targeting agents for the receptors. The potency, specificity, molecular display, pharmacodynamics, pharmacokinetics and safety profiles should be taken into account for the choice of targeting agents. The common targeting agents include small molecules, aptamers, peptides, proteins, and antibodies, which can be roughly classified into two categories based on size/molecular weight with small organic molecules and mAbs as representative member (Table 2). The molecular mass of small organic molecules tends to be below 500 Daltons as

| Targeted Receptor | Targeting Ligand | NM | Cell Type | Outcome | Reference |
|-------------------|------------------|----|-----------|---------|-----------|
| EGFR              | Antibody         | Gold NPs | HaCaT HOC 313 clone 8 HSC 3 | Targeting NM showed 6-fold higher affinity for tumor than non-tumor cells | [135] |
|                   |                  |           | SK-BR-3   | Targeting NM showed 12.7-fold higher cytotoxicity than non-targeting NM | [136] |
| Transferrin       | Transferrin      | PLGA NPs | PC3       | Targeting NM showed 3-fold higher uptake than non-targeting NM | [137] |
| uPAR              | ATF              | IONPs    | MIA PaCa-2 | Targeting NM showed 7-fold higher accumulation than non-targeting NM | [139] |

HaCaT: nonmalignant epithelial cell line; HOC 313 clone 8/HSC 3: malignant oral epithelial cell lines; PLGA/MMT NPs: poly(D,L-lactide-co-glycolide)/montmorillonite nanoparticles; SK-BR-3: breast cancer cell line; PC3: prostate cancer cell line; IONPs: magnetic iron oxide nanoparticles; MIA PaCa-2: pancreatic cancer cell line.
summarized by Lipinski’s rule of five [143, 144]. Though generally used in clinical practice, small molecular agents bear one inherent concern on their target specificity. mAbs have high molecular mass, e.g., ~160 kDa for IgG, and belong to NMs in term of their sizes (a hydrodynamic radius of 7-12 nm for an IgG molecule). They tend to have strong potencies and high specificities to their targets, but have potential antigenicity and poor oral bioavailability. The conventional administration route for mAbs is through intravenous or subcutaneous delivery [145]. The importance of these two parameters for a successful target has been clarified in a recent review [146].

Estimation of the relative contribution between the EPR effect and receptor-mediated targeting of NMs

As both tumor-targeting strategies have their pros and cons (Table 3), a combination of these two strategies may add value. Supplementing the EPR effect of NMs with receptor-mediated targeting may reduce the effect of tumor heterogeneity as well as enhance selectivity and efficacy of NMs against solid tumors. Despite the prevalent development of such tumor-targeting NMs, a question remains: what are the relative contribution between the EPR effect and receptor-mediated effects to tumor targeting in vivo?

Table 2. Examples of targeting agents for tumor receptor-mediated NMs.

| Tumor targeting agents | Size/Molecular weight | Representative example |
|------------------------|-----------------------|------------------------|
| Small molecules        | 0.3-1 nm/<500 Da       | Folic acid (Folic acid receptor) |
| Peptides               | 1-2 nm/<15 kDa         | RGD (αvβ3)/ATF (uPAR) |
| Proteins               | 5-10 nm/5-150 kDa      | Transferrin (Transferrin receptor) |
| Aptamers               | 5-20 nm/10-20 kDa      | Nucleic acid-based aptamers |
| Antibodies             | 10-20 nm/<180 kDa      | Cetuximab (EGFR)/Trastuzumab (HER2) |

The corresponding receptor targets are listed in the parentheses.

Table 3. The pros and cons of the EPR effect (passive targeting) and receptor-mediated targeting (active targeting) for NM accumulation in tumors.

| Tumor targeting strategy | Pros                  | Cons                      |
|--------------------------|-----------------------|---------------------------|
| Enhanced permeability and retention (EPR or passive targeting) | universal | limited efficacy | modest specificity |
|                          | low cost              |                           |
| Receptor-mediated targeting (Active targeting) | high efficacy | drug resistance |
|                          | high specificity      | high cost                 |
Figure 4. Schematic illustration of the estimation of the relative contribution between the EPR effect and receptor-mediated targeting for NMs. A novel tumor targeting NM was constructed of a recombinant protein of human serum albumin (HSA) fused with a tumor receptor targeting agent (ATF) at the N-terminus of HSA (labeled as ATF-HSA). ATF-HSA exhibits dual targeting modes: Its ATF peptide targets at uPAR over-expressed on tumor surfaces and HSA targets at tumor through the EPR effect. HSA was used as a control, which has only the EPR effect but no receptor targeting effect.

We then measured the fluorescent signals of HSA or ATF-HSA on Hepatoma-22 (H22)-bearing Kunming mice, which express a high level of murine uPAR (Figure 5A) [155]. As seen in Figure 5B, at 6 h and 12 h post-injection of 0.05 mg CPZ/kg of mouse body weight via caudal vein, both HSA and ATF-HSA accumulated at the tumor sites with ~3-fold higher concentrations compared to the non-tumor sites. At these two time points, both ATF-HSA and HSA had almost the same amount at tumor sites. Such tumor accumulation is most likely due to the EPR effect. At 24 h post-injection, ATF-HSA showed a higher amount of tumor accumulation than HSA (~2 folds), with HSA accumulation in tumors remained at about 3-fold comparing to non-tumor accumulation. This difference is likely due to uPAR-mediated targeting, which takes effect following the EPR effect in vivo. At 96 h post-injection, this tumor retention difference was further enlarged and ATF-HSA showed almost 3 times more tumor accumulation compared to HSA, which demonstrated the tumor targeting contribution of the receptor-mediated effect and the EPR effect were 75% and 25%, respectively.

The main conclusions we extracted from these results (Figure 5) are: 1) for once infusion, the tumor accumulation of NMs through the passive targeting (the EPR effect) reaches a plateau (at least 3-fold compared to non-tumor accumulation) at around 24 h, but gradually reduces after 48 h; 2) the tumor accumulation due to the active targeting becomes prominent at 24 h post-injection, and increases continuously up to 96 h; 3) the relative ratio of the active to passive targeting effect depends on time after the NM injection, which is about 1 at 24 h and 3 at 96 h.

To further validate these results, we used murine ATF (mATF) as targeting agent which binds more tightly to surface uPAR of xenografted murine tumors [156]. The imaging probe loaded mATF-HSA and ATF-HSA have nearly identical shapes, charges and molecular masses (82 kDa and 84 kDa, respectively), and are therefore likely to have the identical EPR effect. We observed that the CPZ average concentration of mATF-HSA group in tumor sites was significantly higher (at least 2 times) than that of the ATF-HSA group 6 h post-injection (especially at 12 h and 24 h), demonstrating a higher contribution of receptor-mediated targeting compared to the EPR effect (6-fold, figure not shown) [156].
Figure 5. The relative contribution of the EPR effect and receptor-mediated targeting effect to tumor accumulation of NMs. (A) Specific accumulation of both HSA (top panels) and ATF-HSA (bottom panels) in tumors are shown in the front view of the representative mice and three-dimensional view of tumors 48 h post-injection to H22 tumor-bearing Kunming mice. (B) Dynamics of tumor targeting of HSA (blank bars) and ATF-HSA (shaded bars) on H22 tumor-bearing mice. Average concentrations of fluorescent probe (CPZ) were quantitated by non-invasive fluorescent molecular tomography at the tumor and non-tumor sites at indicated time points after injection to the tumor-bearing mice. Both HSA and ATF-HSA accumulated rapidly at the tumors sites, as seen from the data at 6 h and 12 h, with signals of 3 folds compared to the non-tumor sites. After 24 h injection, ATF-HSA showed higher tumor retention than HSA (about 2 times). At 96 h post-injection, ATF-HSA showed almost 4 times tumor accumulation compared to HSA. The data were averaged from 3 mice in each group at each time point; bars represent standard error of the mean (SEM). *Significantly higher CPZ tumor accumulation for ATF-HSA compared to that of HSA, p < 0.05.

Our estimation of this relative contribution certainly has some limitations. We used a transplanted murine tumor model and the results need to be validated in other tumor models, e.g., in situ nascent tumors. It will be useful to include more time points in active/passive targeting measurements to validate our estimation. Moreover, NMs with different compositions or radii, and additional readout methods, e.g., positron emission tomography or magnetic resonance imaging, need to be explored. The tumor targeting NMs used in our current studies are proteins, which are soft, and this estimation may be different from that for rigid NMs.

To our knowledge, there are various controversial in vivo data on estimation of this relative contribution in literature. In one study, NPs of super-paramagnetic iron oxide (SPIO) were functionalized with tripeptide arginine-glycine-aspartic acid (RGD) targeting to integrin αvβ3 with a size of about 240 nm [157]. Their bio-distributions were detected in mice inoculated subcutaneously with CT26 colon carcinoma cells using electron spin resonance spectroscopy. The mice injected with RGD-functionalized SPIO NPs showed a 2.5-fold higher signal in tumor tissues compared to the mice treated with bare NPs 1 h and 4 h post-injection [157]. This study thus demonstrated the receptor-mediated tumor targeting contributed 2.5-fold more than the EPR effect, which is consistent with our results. In another study, active accumulation of transferrin-coated gold nanoparticles (60 nm) was found to be 5 times faster and approximately 2-fold higher relative to their passive poly(ethylene glycol)-coated counterparts in MDAMB-435 orthotopic tumor xenografts [158]. However, in one study using cyclic RGD-conjugated gold nanorods (about 80 nm), the total tumor accumulation of these so-called targeting NMs was found to be only marginally improved compared to non-targeting ones [159]. Another study using folate as targeting agent showed that the targeting liposomes (in the range of 70 to 90 nm) did not enhance the overall liposome deposition in tumors [160]. A comprehensive analysis of NP delivery to tumors based on studies published between 2005 and 2015 showed that the NMs using active targeting had a slight higher efficiency than those relied on the passive targeting (0.9% versus 0.6%) [161]. Taken together, further studies are definitely needed to address this important issue of active vs. passive targeting, and to gain further mechanism insights.
Perspectives

NMs are widely studied as diagnostic and/or therapeutic agents targeting tumors in a passive manner through the EPR effect, in an active strategy by receptor-mediated targeting, or with a strategy combining these two. However, cancer nanomedicine is under debate recently [162]. It appears that tumor targeting NMs need to be re-examined [163, 164].

Here, our studies showed the relative contribution of active and passive targeting varied with time, and active targeting appeared contributing more than passive targeting over time. The nature of targeting agents and tumor receptors is of course a critical variable determining the ratio between these two targeting effects [159]. The observed dynamics of active and passive targeting also directly affects the efficacies of NMs, which is an important factor to be considered in the design of NMs. As a consequence, further studies on the dynamics, and the underlying mechanisms, including the factors affecting the dynamics, are highly advocated [165].

Abbreviations

ATF: amino-terminal fragment of uPA; CPZ: β-carboxy phthalocyanine zinc; EPR: enhanced permeability and retention; HSA: human serum albumin; NM: nanomaterial; NP: nanoparticle; uPA: urokinase-type plasminogen activator; uPAR: uPA receptor.

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Competing Interests

The authors have declared that no competing interest exists.

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