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Review

Engineered nanomedicines with enhanced tumor penetration

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ABSTRACT

Nanomedicine has been extensively explored to enhance the efficacy of chemotherapy with modest therapeutic efficacy in the clinic, owing to various factors. A primary factor is inefficient tumor penetration caused by specific tumor microenvironments, such as insufficient blood supply, high-density tumor cells and extracellular matrix, and increased interstitial fluid pressure. To date, several strategies, including the modulation of tumor microenvironments and optimization of nanoparticle properties, have been reported to improve the tumor penetration of nanomedicines, but these traditional strategies still have limitations. Recently, with unique strategies like tumor-penetrating peptide-mediated transcellular transport, the multifunctional transformable nanoparticles have emerged as an advanced generation of nanomedicine with superior tumor penetration capabilities. In this review, the latest development and limitations of nanomedicines are summarized, and prospects for improving tumor penetration are discussed.

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Introduction

Nanoparticle-based cancer treatment has been in development for nearly half a century and has tremendously advanced the delivery of chemotherapeutic drugs and various new therapeutic agents, including molecular targeting agents, peptides, proteins, and genes [1–3]. However, many biological barriers still hinder the transport of nanomedicines, and considerable research efforts are being made to overcome these barriers. Nanomedicines have prolonged drug circulation and reduced drug toxicity in the cases of several nanoformulated drugs approved by the US Food and Drug Administration, such as doxorubicin liposome (DOXIL®), albumin-bound paclitaxel (PTX; Abraxane®), and irinotecan liposome (Onivyde®). Nanomedicines also enhance drug accumulation at the desired tumor sites through passive and/or active targeting [4–6]. However, the clinical results of these targeted nanomedicines have so far been modest, and some like BIND-014 have failed in clinical trials. Of the many proposed hypotheses to explain the current predicament of nanomedicines, poor tumor penetration is dominant.

The poor penetration of nanomedicines at the tumor sites is mainly attributed to tumor microenvironments and is also related to the properties of nanoparticles. First, the heterogeneous blood supply, which is sufficient at the tumor periphery but diminished in the tumor center, requires that nanoparticles travel increased distances to the tumor center [7]. Second, interstitial fluid pressure (IFP) is elevated from the tumor periphery to the tumor center, hindering nanoparticle diffusion to deep tumor sites after extravasation from peripheral blood vessels [8]. Moreover, the dense extracellular matrix (ECM) further impedes the transport of nanoparticles through the small pores within the matrix [9–11]. In addition to these biological barriers, the unique properties of nanoparticles compared with those of small molecules lead to more significant challenges. For example, nanosized delivery systems are less amenable to extravasation and diffusion after their accumulation at the tumor periphery. Moreover, there are complicated and even contradictory relations between nanoparticle properties and *in vivo* tumor penetration at several steps of the drug delivery process. Overall, the poor tumor penetration of nanomedicines is a consequence of both the tumor microenvironments and the inherent properties of nanoparticles.

Many efforts have been made to improve the tumor penetration of nanomedicines, primarily by modulating tumor microenvironments and optimizing nanoparticle properties (Fig. 1) [12–15]. However, these strategies are limited by the complex tumor microenvironments and delivery cascades. On one hand, although modulating the blood vessels or the tumor ECM alters tumor microenvironments and enables better nanoparticle distribution and diffusion, changes caused by external forces might destroy or affect the tumor microenvironments, ultimately hindering repeated treatment or even causing tumor metastasis. On the other hand, the ideal sizes, shapes, and charges of nanoparticles can be optimized *via* screening to enhance tumor penetration. However, these optimized properties usually depend on tumor types and must be further evaluated as they may affect or even obstruct other drug delivery processes. To overcome these limitations, many tailored nanoplatforms with penetration-assisted ligands or transformable properties, such as shrinkable size or reversible charge, have been explored. This review focuses on strategies to facilitate tumor transport of nanoparticles with a particular emphasis on rationally designed nanoplatforms.

Primary challenges for tumor penetration of nanoplatforms

The challenges for tumor penetration of nanoparticles are due to tumor microenvironments as well as the physical and chemical properties of nanoparticles themselves (Fig. 2).

Nanoparticle transport inside tumors mainly depends on the vasculature and the distribution of blood vessels [12,16,17]. Owing to the rapid proliferation of tumor cells, the burdens of oxygen and nutrition supply not only accelerate the formation of new blood vessels but also induce the irregular vasculature and heterogeneity of these blood vessels at the tumor sites [18]. Such irregular and heterogenous vessels lead to decreased and heterogeneous tumor blood flow, further hindering the tumor penetration of nanoparticles [19]. First, decreased blood flow limits nanoparticle perfusion at the tumor site [20,21]. Recent research showed that the enhanced permeability and retention (EPR) effect of nanoparticles was mediated by vascular bursts, which were also related to blood flow and tumor density [16]. Second, in the center of the tumor tissue, proliferating cancer cells exert substantial stress and compress both blood and lymphatic vessels, leading to vessel collapse [22] and causing functional blood and lymphatic vessels to be concentrated at the tumor periphery but scarce in the tumor center [23]. The distribution of vessels is heterogeneous over the distance from the tumor periphery to the center, which can be up to hundreds of micrometers, further exacerbating the poor penetration of nanoparticles.

After extravasation from the blood vessels to interstitial tumor spaces, dense ECM and elevated IFP further hinder nanoparticle transport [10,11,24]. The ECM is formed by proteins, glycoproteins, proteoglycans, and polysaccharides produced by epithelial, endothelial, and other stromal cells. Pores within the ECM are typically narrow, less than hundreds of nanometers, and block nanoparticle transport through steric restriction and electrostatic interactions [25–27]. In healthy tissues, IFP is nearly 0 mm Hg, whereas solid tumors typically show IFP values of 5–40 mm Hg, reaching 75−130 mm Hg 75 in some types of tumors [8,28–30]. This elevated IFP significantly slows the diffusion of large nanoparticles and even forces nanoparticles back into the blood supply [29].

Additionally, the tumor ECM contributes to the binding site barrier (BSB) for some ligand-modified nanoparticles [31,32]. The BSB was initially in the case of delivery of antibodies, which could be trapped by cells peripheral to the blood vessels, hindering their penetration into tumors [33]. The BSB has also been shown to restrict nanoparticle diffusion, preventing nanoparticles from reaching sites deep within the tumors, possibly owing to the nanoparticles being trapped by the tumor ECM or captured by stromal cells near the blood vessels [34].

Nanoparticles have unique properties that further complicate their delivery to tumors. A significant characteristic of nanoparticles is their size. Although tumor vessels are leakier than healthy blood vessels, vessel pores are permeable to nanoparticles only at certain sites, but not throughout the vessels [35], causing variation in nanoparticle extravasation and distribution [16]. The tumor sites with few eruptions can be treated only by nanoparticles penetrating from other sites, significantly increasing the distance and therefore the difficulty of transport. In addition, owing to the modification of functional ligands, nanoparticles are more readily trapped by the ECM, prohibiting their diffusion in interstitial tumor spaces. In summary, owing to their unique properties, the transport of nanoparticles from well-perfused to poorly-perfused sites are further hampered by narrow pores within the ECM and surface interactions of the nanoparticles with the surrounding environments [36].

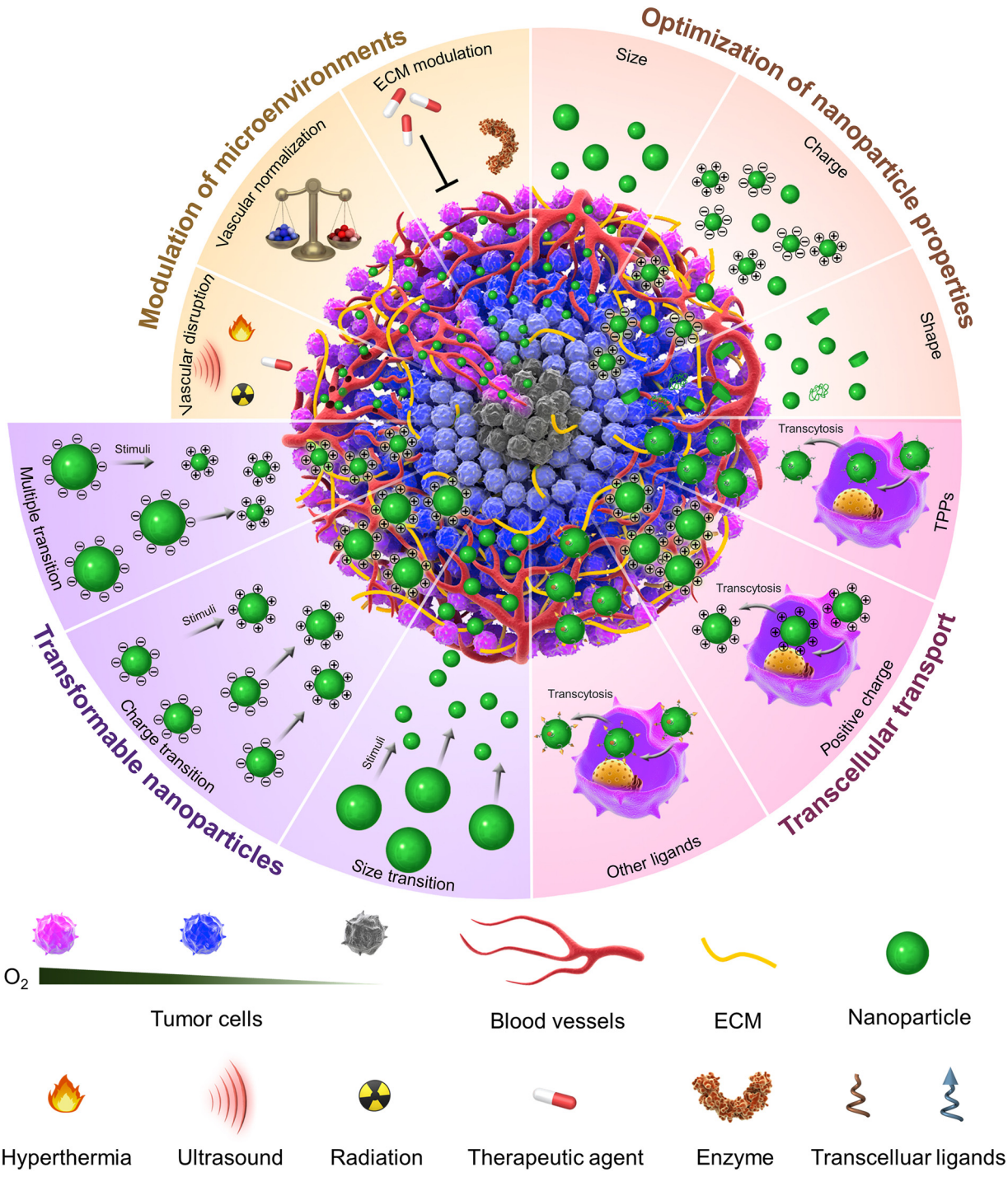

Fig. 1. Transitional and advanced strategies for improved tumor penetration. (1) Modulation of tumor microenvironments by physical and physiological methods. (2) Optimization of nanoparticle properties, such as size, shape, and surface modification. (3) Surface modification to improve transcellular transport. (4) Integration of multiple functions into transformable nanoparticles.

Overall, the factors resulting in poor penetration include both biological barriers of tumors and properties of nanoparticles themselves. Many studies have focused on modulating the tumor microenvironments to alleviate biological barriers or optimizing nanoparticle properties to improve tumor penetration.

Traditional strategies to improve tumor penetration of nanosystems

Traditional strategies to overcome the limitations of nanoparticle transport at the tumor sites can be divided into two major

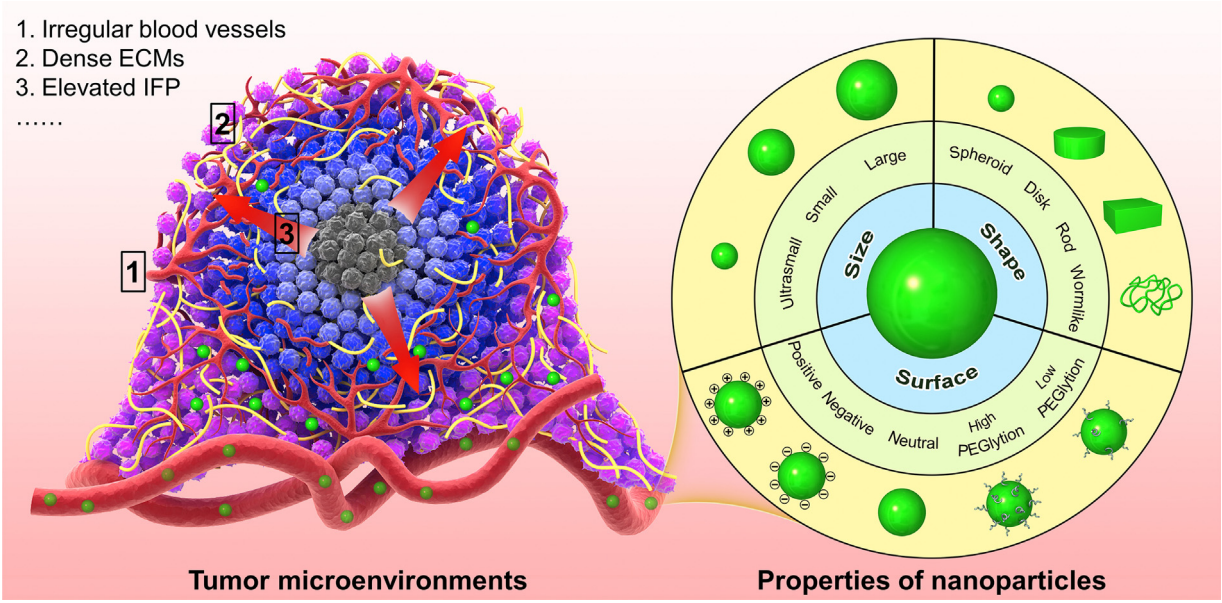

Fig. 2. Multiple challenges for tumor penetration. Irregular blood vessels, heterogeneous blood supply, dense ECM, and elevated IFP contribute to the decreased penetration of nanoparticles. Furthermore, physical and chemical properties of nanoparticles, such as size, shape, and surface charge also have significant effects on the transport of nanoparticles at the tumor sites.

groups: the modulation of tumor microenvironments and the optimization of nanoparticle properties. Although both of these strategies mitigate the poor tumor penetration of nanoparticles, their utility is limited by the complex and interrelated tumor microenvironments and delivery cascades.

Modulations of tumor microenvironments

Vascular disruption

Vascular disruption involves enhancing the blood vessel permeability to facilitate the extravasation of nanoparticles. Usually, the blood vessels can be easily disrupted using physical forces, such as hyperthermia, radiation, or ultrasound, or by using physiological agents like a vascular disrupting agent (VDA) (Fig. 3A).

Hyperthermia requires raising the temperature of the tumor site to 40–43 °C, and has been widely applied in combination with radiotherapy and chemotherapy [37–42]. Previous studies have shown that hyperthermia enhanced the perfusion and extravasation of nanoparticles at the tumor sites because of increased blood flow and damaged vasculature [43,44]. In most early studies, hyperthermia was induced by directly heating the tumor sites with water baths, heating coils, or microwaves, which have relatively low specificity [45]. Nanotechnology integrates heating and therapy functions into one platform. The best-explored system based on this concept is nanoparticle-based photothermal therapy (PTT). Various polymers are used to package or conjugate near-infrared probes or to coat photothermal inorganic nanoparticles [46–49], enabling these nanoparticles to penetrate more deeply into sites distant from the blood vessels when exposed to the near-infrared laser. However, the temperatures applied in PTT are usually higher than 43 °C, which lead to severe damage to the blood vessels and even their collapse. This damage blocks blood flow, hindering the transport of more nanoparticles to the same site [50].

Local radiation has also been shown to improve nanoparticle penetration by several mechanisms. Early clinical data showed that the co-administration of high-dose radiation promoted the intratumoral accumulation of liposomal doxorubicin (DOX) in patients with various tumors [51,52]. Increased blood flow and vascular permeability are possible explanations for this enhanced delivery. Both large single doses and fractionated radiation induced the apoptosis of endothelial cells, leading to the collapse of vessels and facilitating vascular permeability to nanoparticles [53,54]. Moreover, lower-dose radiation of 5 Gy also induced tumor-associated macrophage-mediated vascular burst and further enhanced nanotherapeutic delivery in the tumors [55]. However, vessel damage exacerbates the hypoxia of tumor microenvironments, potentially contributing to drug resistance and tumor recurrence [56].

Ultrasound, a common diagnostic method, has also been applied in cancer therapy [57]. Various exposure conditions (*e.g.*, frequency, pressure, and duration) lead to thermal, cavitational, and acoustic radiation force effects, which promote nanoparticle penetration [58–60]. One approach to enhance the transport of therapeutic nanoparticles is the co-administration of nano/microbubbles and therapeutic nanoparticles, followed by therapeutic ultrasound [61–64]. Although this strategy somewhat improved drug delivery, it was not effective *in vivo*, owing to the short lifetimes of nano/microbubbles and the requirement for the therapeutic nanoparticles to co-localize with the cavitation nuclei. To address this problem, a variety of "all-in-one" acoustically active delivery vectors have been explored. One example of these rationally designed structures is the "nanoparticle–microbubble pendant", in which therapeutic or imaging agents are loaded into nanovehicles, such as liposomes and polyplexes. These nanoparticles could then be conjugated to the surface of microbubbles through coupling reactions or using biotin-avidin affinity [65–69]. Ultrasound causes the microbubbles to collapse, releasing the pendant liposomes. The temporarily increased capillary permeability allows the nanoparticles to penetrate and distribute throughout deeper tumor sites. Another common strategy is to encapsulate therapeutic or imaging agents in the nano/microbubbles formed by lipids or polymers [70–72]. In contrast to lipid-based nano/microbubbles, polymer-based nano/microbubbles encapsulate both hydrophobic and hydrophilic agents and have much higher loading capacity and stability, which offers advantages for clinical applications [73–76]. However, previous studies showed that ultrasound caused substantial damage to small blood vessels at the tumor sites, which might hinder repeated drug administration [77].

VDAs cause the rapid and selective shutdown of established tumor vasculature, leading to subsequent tumor cell death [78].

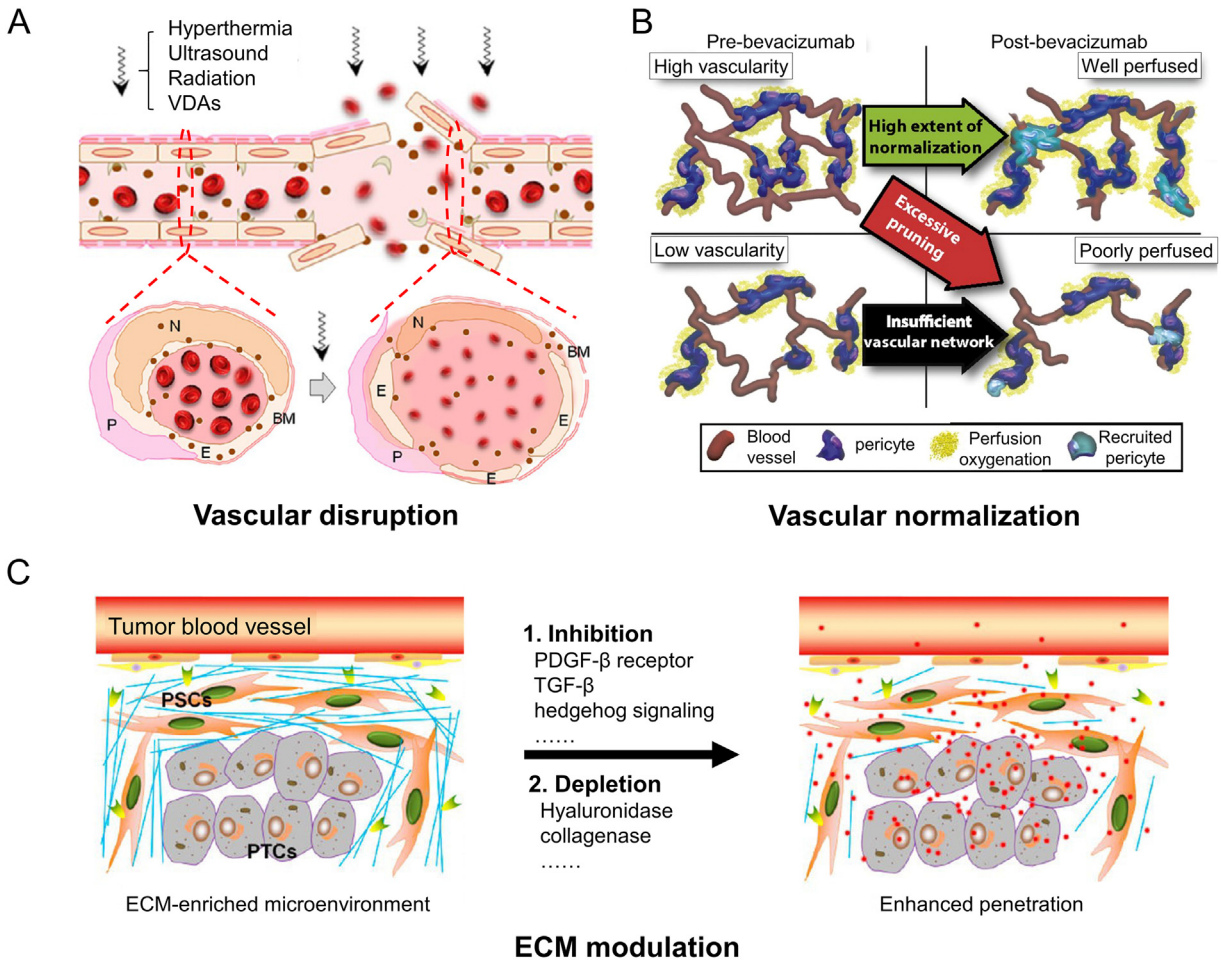

Fig. 3. Modulation of tumor microenvironments for enhanced penetration. (A) Disruption of blood vessels through physical or physiological methods [85]. E, endothelium; P, pericyte; BM, basement membrane; N, endothelial nucleus. Reprinted with permission from Ref. [85]. Copyright 2015, American Chemical Society. (B) Outcomes of vascular normalization by BVB in breast cancer were significantly related to vascular density [86]. (C) Modulation of ECM by inhibiting signaling pathways or using enzymes to deplete ECM [87]. Reprinted with permission from Ref. [87]. Copyright 2017, American Chemical Society.

5,6-Dimethylxanthenone-4-acetic acid was shown to increase the permeability of tumor vessels through the induction of tumor necrosis factor-α, further improving nanoparticle penetration [79]. Combretastatin A-4 phosphate was also reported to enhance the accumulation and optimize the distribution of both liposomes and inorganic nanoparticles [80,81]. In addition to these traditional VDAs, depletion of tumor-associated platelets by antiplatelet antibody also caused vascular disruption. Platelets prevent hemorrhage and maintain the integrity of the tumor vasculature [82]. A high degree of systemic platelet depletion significantly improved the penetration of anticancer drugs [83]. Tumor-targeted delivery of R300, an antiplatelet antibody, facilitated vascular breaches and enhanced penetration of nanotherapeutics [84]. Nevertheless, VDA administration ultimately causes the blood supply to be impeded and tumor vessels to collapse, which is similar with physical forces.

Physical or physiological forces as external modulators temporarily increase capillary permeability and decrease ECM density, thereby promoting the penetration of nanoparticles. However, the damaged blood vessels resulting from these forces ultimately block the blood supply and may even lead to the blood vessel collapse, significantly impairing further treatment.

Vascular normalization

Vascular normalization is a strategy for improving drug delivery that is attracting increasing attention. In this strategy, the blood vessel networks are normalized, and the blood supply is restored, resulting in increased perfusion and optimized distribution of therapeutic agents at the tumor site [88].

Abnormal vessel formation in the tumors is mediated by an imbalance of proangiogenic and antiangiogenic factors [89,90]. Proangiogenic factors are typically overexpressed in the tumors, causing the rapid formation of dysfunctional vascular networks. Balancing the proangiogenic and antiangiogenic factors at the tumor sites normalize the vascular networks [91]. Anti-angiogenic agents and especially anti-vascular endothelial growth factor agents are the most frequently applied approaches in both research and clinical settings [92]. Treatment with anti-angiogenic agents leads to significant changes in the functions and architectures of existing the blood vessels at the tumor site, including inhibition of immature vessels, increased vessel pericyte coverage, and even decreased IFP [93,94]. Many studies have reported that vascular normalization significantly improves the overall distribution and penetration of small agents and macromolecules [88,95,96]. This strategy is, therefore, regarded as a promising alternative in cases where the EPR effect fails in the human body.

Although the normalization of blood vessels enhances the penetration of small therapeutic agents and macromolecules, the improvement of nanoparticle penetration largely depends on the sizes of nanoparticles and the vascular densities of tumors. Vascular normalization may compromise the transvascular transport of very large nanoparticles, owing to the decreased pore size of the vascular walls [97]. It has been reported that only nanoparticles

smaller than 12 nm, similar to the size of antibodies, can be efficiently transported through the normalized blood vessels [98]. A more recent study suggested that the nanoparticle size threshold might be as large as about 40 nm, but most synthetic nanoparticles under investigation still exceed this size limitation [99,100]. Moreover, the initial vascular density in the tumors also significantly affects the outcomes of subsequent chemotherapy (Fig. 3B) [86]. Tumors with high vascular density and recruited pericytes have been shown to respond to bevacizumab (BVB) combined with chemotherapy better than those with low vascular density. As a result, manipulating tumors for the enhanced delivery of anticancer nanotherapeutics will require further studies.

Modulation of extracellular matrix

After extravasation, the ECM is another major obstacle blocking the deep penetration of nanoparticles. The inhibition of ECM formation and the degradation of existing ECM have been widely implemented to modulate the ECM (Fig. 3C) [101]. Moreover, tumor stromal cells, the primary producers of ECM, are considered to be alternative upstream targets for ECM modulation [102,103].

ECM synthesis can be blocked by molecular targeting drugs that inhibit platelet-derived growth factor-β (PDGF-β) receptor, transforming growth factor-β (TGF-β), or hedgehog signaling [104–107]. PDGF-β receptor inhibitors reduce IFP through inhibition of pericytes and stromal cells, increasing the transcapillary transport of nanoparticles in the tumors [104]. TGF-β inhibitors, which inhibit the differentiation of pericyte coverage of endothelial cells even at a low dose, are the most popular agents co-administrated with therapeutic nanoparticles [108–110]. Moreover, TGF-β inhibitors regulate the tumor ECM by decreasing collagen I (Col I) content, facilitating the penetration of nanoparticles [111]. Inhibition of hedgehog signaling disrupts the desmoplastic stroma and vascular density in poorly perfused pancreatic tumors, which ultimately improves the delivery of small chemotherapy drugs [107]. However, the effects of these inhibitors depend dramatically on tumor types. Whereas the inhibitors showed significant improvement of tumor penetration in a BXPC3 model, they had a modest effect in a CT26 model, owing to the different vasculature phenotypes of the two models [112]. Hedgehog signaling has been demonstrated in about 30% of human cancers, including basal cell carcinoma, medulloblastoma, melanoma, breast, prostate, lung, pancreatic, cervical, and ovarian cancer [113]. Therefore, inhibition of signaling pathways might work only in specific types of tumors.

Depletion of existing stromal barriers is another common strategy to modulate the tumor ECM [10]. The ECM contains many types of stromal barriers, including hyaluronic acid (HA) and Col I. Degradation of the ECM decreases IFP and frictional resistance, facilitating nanoparticle transport farther away from the blood vessels. HA, as a critical component of the ECM, has been reported to be highly expressed in a wide range of human tumors [114–116]. Hyaluronidase is widely used to degrade HA at the tumor sites. Intratumoral injection of hyaluronidase reduces IFP and improves the penetration of small-molecule drugs, antibodies, and nanoparticles [117–119]. Another major component of the tumor ECM is Col I, which forms a dense network by linking tumor cells to the cell matrix through cell−cell interactions [120–122]. Depletion of Col I weakens this collagen network and improve the penetration of nanotherapeutics. Pre-treatment with an angiotensin-II receptor antagonist, Iosartan or collagenase, promoted the penetration of macromolecules, liposomes, and polymer-based nanospheres [123–126]. However, collagen degradation was highly correlated with the invasion and metastasis of tumor cells [127–129]. Small fragments of HA degraded by hyaluronidase have also been shown to promote angiogenesis [130,131]. Therefore, ECM degradation should be carefully controlled to maintain the balance of tumor progression.

Re-education or depletion of stromal cells is an alternative strategy for ECM modulation from the upstream pathway [132]. Gold nanoparticles (Au NPs) were reported to be an ideal platform for efficient delivery of therapeutic drugs or small interfering ribonucleic acid (siRNA) for modulation of activated pancreatic stellate cells (PSCs) without any induction of tumor metastasis [133]. Moreover, Au NPs caused the transformation of PSCs and quiescence of activated cancer-associated fibroblasts (CAFs) [134–136]. Re-education or depletion of stromal cells including PSCs and CAFs inhibited ECM hyperplasia, promoting drug penetration to tumors and therapeutic effects. Moreover, it has not yet been reported that this strategy promotes metastasis, revealing the modulation of stromal cells might be a mild but effective route for modulation of ECM.

Optimization of physical properties of nanoparticles

As the *in vivo* behavior of nanoparticles is directly related to their physical properties, such as size, charge, and shape, many studies investigate the ideal properties of nanoparticles for optimization of their tumor penetration. Although nanoparticles with optimized properties show enhanced penetration, their penetration is usually restricted by other factors involved in drug delivery, such as circulation and accumulation.

Optimization of size

The size of nanoparticle is one of the most critical factors determining tumor penetration, owing to the diffusion resistance caused by the narrow gaps in the blood vessels, tumor ECM, and even the density of tumor cells [35]. In general, the penetration depth of nanoparticles after extravasation in the tumor tissue is primarily mediated by the diffusion balance [137]. However, *in vivo* penetration is more complicated because nanoparticle size also affects other factors, such as blood circulation and tumor accumulation [139].

The diffusion balance mediated by the penetration and elimination of nanoparticles after extravasation is correlated with nanoparticle size. First, the transport of different nanoparticles across biological barriers is controlled by different mechanisms according to their size distribution, including a paracellular passage for nanoparticles smaller than 50 nm, endocytosis uptake for nanoparticles smaller than 500 nm, and lymphatic uptake for nanoparticles smaller than 5,000 nm [140]. Although small nanoparticles penetrate deeper than large ones because of their low diffusion scales, their penetration is limited by high rates of elimination, owing to low retention at the tumor site [137]. As a result, the balance between penetration and clearance ultimately determines the depth, to which the extravasated nanoparticles can be transported. In *in vivo* studies, other factors affecting the fates of nanoparticles before extravasation, namely circulation and accumulation, should be taken into consideration to understand the overall delivery effect. For instance, circulation time is also influenced by nanoparticle size, to some extent, *via* multiple mechanisms. Relatively large nanoparticles (> 200 nm) have been reported to be quickly cleared by the complement system and subsequent reticuloendothelial system [141,142]. Tiny nanoparticles (< 5 nm) could be rapidly removed by renal excretion and glomerular filtration [143,144]. Passive tumor accumulation through the EPR effect is also directly related to nanoparticle size [145,146]. The observed differences in the EPR effect may be due to the different sizes of the nanoparticles crossing the vessels by two different mechanisms. Larger nanoparticles escape circulation when the blood vessels erupt, after which the vents are then closed after the eruption, thus inhibiting diffusion back into circulation [16]. How-

ever, smaller nanoparticles, which quickly diffuse from the blood vessels to perivascular sites, diffuse rapidly back into circulation. Circulation and accumulation are preconditions for subsequent tumor penetration. Optimization of nanoparticle size for *in vivo* delivery must integrate all factors affecting the fates of nanoparticles after administration.

Many *in vitro* and *in vivo* studies have been performed to identify the optimal range of nanoparticle sizes for penetration [137,139,147–153]. Different optimal size ranges were revealed for different nanoparticle systems from 2 and 6 nm for ultrasmall Au NPs [149] to 30 nm for poly(ethylene glycol)-*b*-poly(lactic acid) (PEG-*b*-PLA) micelle [147], 50 nm for larger Au NP or monodisperse drug–silica nanoconjugate (Fig. 4A) [137,154], and up to 70 nm for poly(lactic-*co*-glycolic acid) (PLGA) nanoparticle [155]. Size selection is still debated and not broadly accepted, possibly owing to the various limitations of the strategy of size optimization. First, size is only one of the critical factors that contribute to tumor penetration. As different nanoparticles are made of different materials and contain different components, the optimal size range from one study is only meaningful to one specific type of nanoparticles. Second, tumor types and animal species also play essential roles in determining the extent of tumor penetration. A broad range of nanoparticles with larger sizes show good penetration in relatively loose tumor types, while smaller nanoparticles exhibit good permeability in both dense and loose tumor types [147]. In addition, when considering other delivery cascades, it is challenging to integrate all the optimal nanoparticle properties for different drug delivery cascades, as the ideal sizes for penetration might not be the best suited for circulation and accumulation. For example, although polymer nanoparticle of 100 nm in diameter showed 10 times higher accumulation than nanoparticle of 30 nm in diameter, there were no apparent improvements to tumor therapy because of the relatively poor penetration of the 100 nm nanoparticle after accumulation [139]. Therefore, optimizing nanoparticle size may be a feasible approach for screening materials for clinical applications, but further studies should focus on efficient and reliable methods to find suitable nanoparticle sizes with improved tumor therapies.

Optimization of shape

Spherical nanoparticles are currently among the most widely used platforms for drug delivery. However, the shape of nanoparticles also affects their *in vivo* fates, including their circulation, accumulation, penetration, and cell uptake [156–158]. Apart from spherical nanoparticles, nanoparticles with other shapes, including nanorods, nanotubes, nanodisks, nanocages, and worm-like or filamentous micelles, have been investigated to improve nanoparticle transport within the tumors [159].

Different shapes of nanoparticles have different penetration capabilities and pass through gaps between endothelial cells or dense ECM and tumor cells differently, contributing to their different penetration capabilities [160]. However, the effect of shape on penetration also varies among different tumor models and nanoparticles constructed of different materials. The detection toward three-dimensional (3D) spheroid model *in vitro* showed that nanodisk of 325 nm in diameter and 100 nm in height fabricated by the nanoscale geometry of Jet and Flash Imprint Lithography of PEG hydrogel had better penetration behavior than nanorod of 100 nm in diameter and 400 nm in length [150]. Another study showed that nanorod of 15 nm in diameter and 54 nm in length constructed of CdSe/CdS quantum dot (QD) moved more effectively from perivascular regions to deep tumor sites in orthotopic E0771 mammary tumor than did spherical nanoparticle of 35 nm in diameter [161]. In the *in vivo* evaluation of Au NP, gold nanorod (Au NR), and gold nanocage demonstrated much better penetration to the tumor center than nanosphere and nanodisk with similar diameters (maximum of 50 nm) in a murine EMT6 breast cancer model [162]. Moreover, the transport of nanorods is also related to their aspect ratios (ARs). Nanorods with an AR of 3.5 were reported to have better penetration capability compared with that with AR of 7 or 16.5 [163]. These studies were performed in defined types of tumors, and the conclusions of the studies might have been different if they had been performed in other types of tumors. For example, single-walled carbon nanotube (SWCNT) of 2–3 nm in diameter and 200 nm in length penetrated more efficiently than spherical QD of about 20 nm in diameter in U87MG tumor, but SWCNT showed less permeability in LS174T tumor (Fig. 4B) [138].

Unlike the rigid structures formed by inorganic or cross-linked hydrogels, nanoparticles self-assembled from soft polymers form flexible and variable structures, such as worm-like or filamentous micelles (filomicelles) [164–166]. In general, filomicelles show better efficacy than spherical nanoparticles in terms of tumor inhibition, perhaps owing to prolonged circulation and enhanced tumor penetration [165,167–172]. Under blood flow conditions, longer filomicelles are extended by the flow, reducing the capture of cells during circulation and increasing the possibility of their passing through gaps between endothelial cells [156]. By contrast, spheres or short filamentous micelles spend less time in circulation because they are more readily captured by cells. Another study reported that the arginine-glycine-aspartate (Arg-Gly-Asp, RGD)-modified wormlike micelle formed by methoxy poly(ethylene glycol)-*block*-poly(2-diisopropyl methacrylate) even passed through the blood–brain barrier for deep penetration [166].

Overall, most reported non-spherical nanoparticles have shown better penetration ability than their spherical counterparts. The advantage of non-spherical nanoparticles could be attributed to various factors. First, the extravasation of these nanoparticles is influenced mainly by their shapes, which might result from the different margination dynamics between spherical and non-spherical ones [173]. Non-spherical nanoparticles exhibit more complex dynamics, such as tumbling and rolling behaviors than spherical ones. These behaviors result in a more significant marginal number compared to spherical nanoparticles, adding to the number of these nanoparticles interacted with the vessel walls and thus enhancing the possibility of extravasation [174]. Second, the ARs of non-spherical nanoparticles can be adjusted to improve their transport through pores and porous media within the endothelial cells and tumor ECM [175].

However, the conclusions drawn from the above studies were dependent on the types of nanoparticle matrices and tumors. In addition, nanoparticle shape affects blood circulation, tumor accumulation, cell adhesion, cell uptake of nanoparticles, and nanoparticle distribution in the tumors [176–179]. For example, Au NRs exhibited more efficient uptake by macrophages than gold nanospheres, which might lead to rapid nanoparticle clearance after injection [180]. As a result, optimizing nanoparticle shape depends on the different biological cascades that occur during delivery.

Optimization of surface properties

As reported previously [181], surface properties, such as hydrophobicity, charge, and ligand modification, greatly influence the biological properties of nanoparticles. Surface properties of nanoparticles dictate the interactions between nanoparticles and the blood vessels or tumor ECM, which then affect their transport at the tumor sites. As with size and shape, the surface properties of nanoparticles influence their *in vivo* fates in complex ways, with different conclusions reported in different systems (Fig. 4C).

The hydrophilicity and hydrophobicity of nanoparticles can be easily modulated by the loading density of PEG or PEG alternatives on the surface of nanoparticles. Usually, the hydrophilicity of nanoparticle surface upregulates with increased loading density

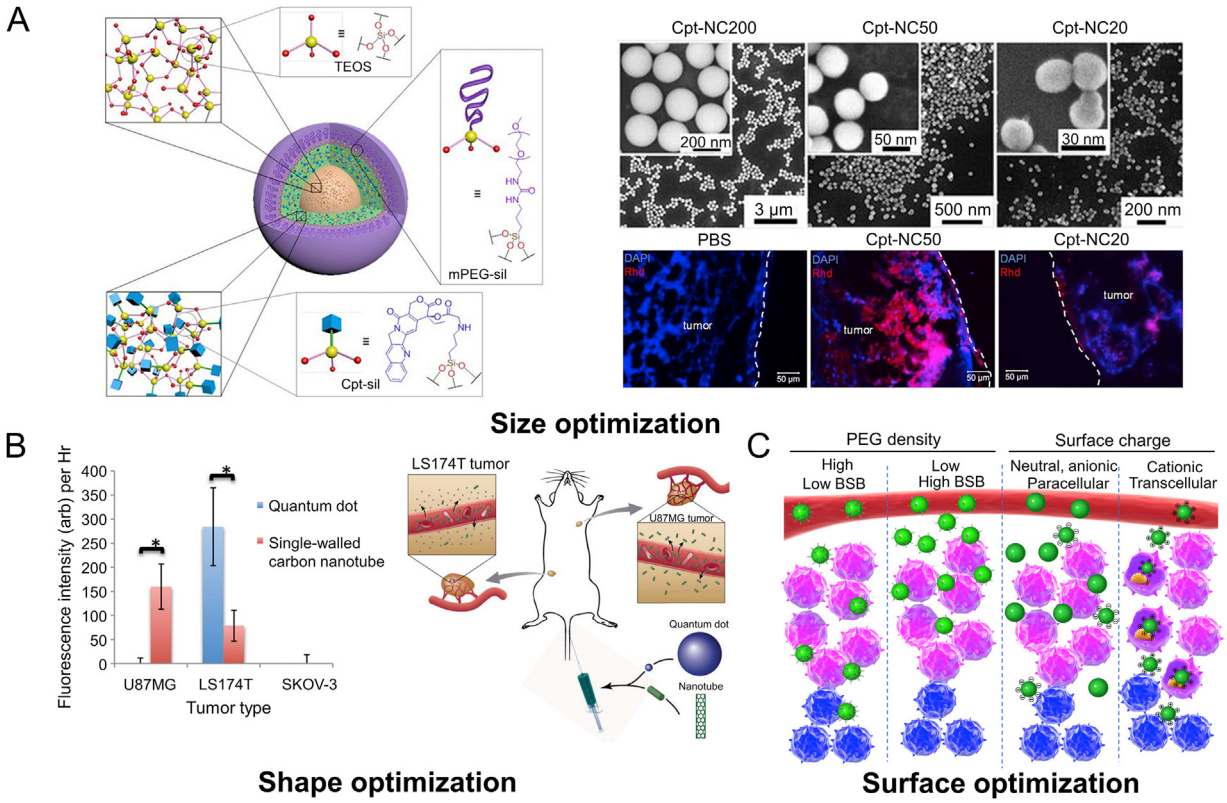

Fig. 4. Optimization of nanoparticle properties. (A) Silica nanoparticle of 50 nm in diameter exhibited better penetration than nanoparticles of 20 and 200 nm in diameter [137]. (B) Nanoparticles with different shapes showed different penetration behaviors in various types of tumors [138]. Reprinted with permission from Ref. [138]. Copyright 2012, American Chemical Society. (C) Surface properties of nanoparticles determined the transport pathways at the tumor sites.

of PEG. PEG density is commonly controlled by changing the PEG length or by adjusting the PEG content. Increasing the density of PEG was reported to facilitate the tumor penetration of nanoparticles, as evidenced by the fact that PEG-*b*-PLA nanoparticle had almost twice the penetration distance of PLA or PLGA one [182]. More importantly, polystyrene nanoparticles highly conjugated with PEG (nine chains per 100 nm^2) showed more effective penetration through the blood–brain barrier than the nanoparticles with lower content of PEG [183]. The enhanced penetration ability was due to adequate PEG shielding, which prevented adhesion between nanoparticles and extracellular spaces and also slowed cell uptake by external tumor cells, thus allowing the nanoparticle to penetrate more deeply. However, high PEG content might influence ligand function [184]. For instance, a PEG density of about 50% decreased the targeting ability of RGD-modified liposome, compared with liposome with 10% PEG loading [185]. Moreover, high PEG loading greatly hinders cell uptake of nanoparticles by tumor cells, diminishing their penetration ability [186].

Surface charge is another crucial factor that determines the interaction of nanoparticles with the tumor ECM or tumor cells. However, the effects of surface charge on tumor penetration of nanoparticles vary among different research systems, and sometimes even conflict. On one hand, cationic nanoparticles target tumor endothelial cells and exhibit higher vascular permeability than neutral or anionic nanoparticles [187–190]. However, cationic nanoparticles easily adhere to tumor ECM, decreasing their effective diffusivity [26]. Therefore, neutral or anionic nanoparticles are theoretically the best for tumor tissue penetration. However, many experimental results oppose the general conclusion outlined above. In comparative research of PEG-*b*-PLA nanoparticles coated with differently charged lipids, cationic nanoparticles showed much better penetration ability in both an *in vitro* 3D tumor spheroid model and an *in vivo* tumor model than did neutral and anionic nanoparticles of comparable sizes [191]. However, in another study, silica nanoparticle with higher anionic charge, −40 mV, penetrated deeper than nanoparticle with smaller anionic charge, −20 mV, in a 3D tumor spheroid model combining murine breast cancer 4T1 cells with stromal 3T3 cells [192]. Similar trends were also observed by other groups [193,194]. Such contradictory results might be caused by the balance of cell adhesion and transcellular transport. Although the positively charged nanoparticles penetrated through the transcellular transport pathway, they were also easily captured or adhered to ECM or stromal cells [195]. Besides, charged nanoparticles are not an optimal choice for *in vivo* applications because these nanoparticles exhibit shorter circulation times and higher liver clearance compared with the neutral ones [196–198].

In summary, although the properties of nanoparticles are closely linked to their biological functions, it remains difficult to pinpoint a broadly accepted optimal size, shape, or charge, for two main reasons. First, the unique features of various nanoparticles and the specific tumor microenvironments of different types of tumor s lead to inconsistent and even contradictory conclusions regarding optimized properties. Second, nanoparticle fates after administration are also extremely complex, and the optimized properties for different delivery cascades are sometimes contradictory, so the optimized features for penetration in one delivery process might not benefit others. In conclusion, optimizing the physical properties of nanoparticles only partially solves the problem of poor nanoparticle penetration.

Advanced strategies to enhance tumor penetration of nanoformulations

Owing to the complexity of delivery cascades, strategies involving modulating tumor microenvironments and optimizing nanoparticle properties both have cons and pros. Modulating tumor microenvironments by disrupting tumor vessels or the surrounding microenvironments temporarily enhances nanoparticle penetration but has disadvantages for subsequent therapeutic administrations. This approach also requires external physical forces or physiological agents, increasing the financial and treatment burdens for patients. Optimizing physical properties of nanoparticles involves compromising on multiple factors because the optimized properties affect different delivery cascades in conflicting ways. For example, although small nanoparticles show better penetration efficacy, they cannot be applied *in vivo* because of their rapid renal clearance. Fortunately, new strategies to solve these problems using tailored nanoparticles are being developed, including enhancing transcellular transport and using transformable nanoparticles.

Enhancement of transcellular transport as a universal strategy

Traditional attempts to improve tumor penetration have mainly focused on enhancing paracellular transport to overcome the barriers of solid tumor ECM. However, the effects of these methods depend primarily on types of nanoparticles and tumors. Transcellular transport, that is, transportation of nanoparticles through cells, might be a universal approach to enhance tumor penetration.

Peptide candidates with C-terminal arginine, or less commonly, lysine, residues in a consensus sequence of R/KXXR/K were successfully identified by screening random peptides for binding to prostate cancer cells *via* phage display [199,200]. Peptides with the C-end rule (CendR) bound neuropilin-1 or neuropilin-2 (NRP-1/2) to target tumor cells, leading to the internalization and subsequent transcellular transport of peptide-modified nanoparticles [201]. Early-stage research has focused on cell-penetrating peptides and their ability to enhance cell membrane penetration. Some of these peptide sequences also follow the CendR [202,203]. However, these peptides showed little tumor specificity and entered any cells, not just tumor cells. The integration of the CendR and the tumor-homing peptide RGD contributed to the construction of the first tumor-specific penetration peptide (TPP), internalizing RGD (iRGD, CRGDKGPDC) [204]. iRGD integrates both tumor targeting and penetration through a multi-step process. The RGD motif first recognizes the tumor sites by binding to α_v integrin upregulated in angiogenic endothelial cells and specific tumor cells (Fig. 5A) [205]. After proteolytic cleavage, the CendR binding motif for NRP-1 is exposed, which facilitated vascular leakage and tumor penetration through transcellular transport [206]. For example, an iRGD-modified PEGylated polyamidoamine (PAMAM) dendrimer conjugated with DOX through an acid-sensitive linkage exhibited improved penetration ability and higher accumulation in brain tumors than an RGD-modified one (Fig. 5B) [207]. Further studies revealed that systemic co-administration of free iRGD improved the tumor penetration of various therapeutic platforms, including DOX, trastuzumab (TZB), and even nanoparticles [208]. The tumor penetration of other TPPs has been explored, including Lyp-1 (CGNKRTRGC), tLyp-1 (CGNKRTR), F3 (KDEPQRRSARLSAKPAPPKPEPKPKKAPAKK), iNGR (CRNGRGPDC), TT1 (CKRGARSTC), CRGRRST, and so forth [209–213].

As TPPs have been widely used to improve the tumor penetration of various nanoparticles in different types of tumors, TPPs are considered to represent a universal strategy for enhancing tumor penetration (Table 1). First, the conjugation or co-administration of TPPs for penetration is not restricted by properties of the nanoparticles themselves. Both organic nanoparticles, such as liposomes, micelles, nanogels, dendrimers, and protein-based nanoparticles, and inorganic nanoparticles including QDs, iron oxide nanoparticles, silica nanoparticles, and bismuth sulfide nanoparticles have been used successfully [208,218,245–249]. One possible reason for this broad applicability of different kinds of nanoparticles after modification with TPP might be that transcellular transport primarily depends on the peptides instead of the nanoparticles themselves. The TPP-mediated penetration has also been observed in many common types of tumors, such as prostate cancer, breast cancer, ovarian cancer, pancreatic cancer, liver cancer, melanoma, and glioblastoma [214,216,218,236,250,251]. More importantly, the TPP modification or co-administration enhanced nanoparticle penetration in metastatic tumors in the lung and brain [219,237,252]. These results revealed that TPPs affected on various cancer types, a finding consistent with the widespread expression of α_v integrin and NRP-1/2 in endothelial cells and/or specific tumor cells [209,253,254]. Overall, TPPs have broad applicability for various nanoparticles and many types of tumors, suggesting the great potential to construct a universal strategy for improving tumor penetration of nanoparticles.

As well as simple conjugation or co-administration of TPPs, rationally designed nanosystems with activatable TPPs have been investigated to optimize nanoparticle penetration at desired sites. A matrix metalloproteinase 2 (MMP-2)-responsive nanocapsule was reported to have enzyme-activated tissue penetration capability (Fig. 5C) [255]. The nanocapsule was prepared by *in situ* surface cross-linking of acrylic acid-decorated liposome, using the acrylated MMP-2 cleavable peptide (GPLGVRGK) as the cross-linker. The cross-linked network serveed as a protective layer to prevent cargo leakage and also functioned as an enzyme-degradable substrate to activate further penetration. After the disassembly of surface coating, the CRGDK peptide was exposed to the surface and able to interact with NRP-1, thus enhancing tumor penetration through transcellular transport. This design also enhanced the blood circulation and tumor accumulation of the nanoparticles compared with unmodified nanoparticles. Another enzyme-responsive prodrug system, also based on MMP-2-mediated activation of tumor penetration, showed better penetration efficiency than traditional co-administration (Fig. 5D) [256]. In this study, DOX was conjugated to the side chain of *N*-(2-hydroxypropyl)-methacrylamide using cathepsin B cleavable peptide (GFLG), while iRGD was conjugated through MMP-2 degradable peptide (PLGLAG). After the accumulation of these polymer–drug conjugate, linker peptides were cleaved and iRGD detached from the polymer to activate the NRP-1-mediated transcellular pathway. Interestingly, the polymer–drug conjugated with a tailored structure exhibited much better penetration ability in a 3D prostate cancer cell spheroid than the group co-administrated with prodrug and iRGD. This strategy might provide an advanced strategy to facilitate the tumor penetration of nanoparticles further.

In addition to the modification of TPPs, the nanoparticles with transcellular transport capability were also designed. A virion-like nanoparticle assembled from tailor-made dendritic arginine-rich peptide prodrug exhibited the enhanced tumor penetration and cell uptake [257]. The dendritic peptide mimicked viral protein transduction domain and disrupted cell membrane. During circulation, the peptide prodrug was modified with an acid-sensitive group, shielding the arginine groups to ensure long circulation half-life. Under acidic conditions at the tumor site, the membrane-disrupting ability of this dendritic peptide was activated, spreading the prodrug among tumor cells. *In vivo* studies in impermeable SKOV3/R tumor showed that the nanoparticle exhibited robust transvascular extravasation and penetrated deeply into the tumor tissue using the transcellular pathway. Other tailored nanopar-

Table 1
Widely reported TPPs in various tumor and nanoparticles.

TPP type	Drug	Nanoplatform	Type of tumor	Reference
iRGD (CRGDK/RGPD/EC)	Nab-PTX/DOX/DOX liposome/TZB	–	22Rv1 human prostate cancer and BT474 human breast cancer xenograft	[204,208]
iRGD (CRGDKGPDC)	Gemcitabine (GEM)	–	Pancreatic adenocarcinoma cancer	[214]
iRGD	Anti-EGFR	poly(ethylene glycol)-*blcok*-poly(ε-caprolactone) (PEG-*b*-PCL)-coumarin-6-NP	BGC-823 human gastric adenocarcinoma xenograft	[215]
iRGD	DOX, sorafenib (SFB)	Gadoxeticacid (Gd-EOB-DTPA)	HepG2 and Hu-7 human hepatocellular carcinoma xenograft	[216]
iRGD	SFB	Porous silicon nanoparticle	Highly metastatic prostate cancer (PC3-MM2) human prostate cancer xenograft	[217]
iRGD	Mitochondria-targeted peptide	Iron oxide	Glioblastoma	[218]
iRGD	–	Iron oxide	MDA-MB-231BR eGFP-positive, 4T1-BR5 tumor	[219]
iRGD	DOX	–	Lovo-6-luc-1 human colon cancer and MKN45 P gastric cancer xenograft	[220]
iRGD	Cisplatin (CDDP)	CDDP-loaded methoxy poly(ethylene glycol)-*block*-poly(L-glutamic acid)	A549 human non-small cell lung carcinoma xenograft	[221]
iRGD	GEM	–	A549 human non-small cell lung carcinoma xenograft	[222]
iRGD	DOX	DOX-Au NPs modified on GNP (DOX-AuNPs-GNP)	4T1 mouse mammary cancer	[223]
iRGD	PTX	Poly(ε-caprolactone)-*block*-poly(*N*-vinylpyrrolidone) nanoparticle	H22 murine liver cancer	[224]
iRGD	Thymopentin fused to iRGD	–	MCF7 human breast cancer xenograft	[225]
iRGD	DOX	Chitosan−poly(*N*-3-acrylamidophenylboronic acid) nanoparticle	H22 murine liver cancer	[226]
iRGD	DOX liposome	Liposome	B16 mouse melanoma	[227]
iRGD	DOX multilamellar liposome	Crosslinked multilamellar liposomal vesicle	4T1 mouse mammary cancer	[228]
iRGD	PTX	PEG-*b*-PLA	C6 rat glioma	[229]
iRGD	Oncolytic virus	–	A549 human non-small cell lung carcinoma xenograft	[230]
iRGD	PTX liposome	Liposome	B16 mouse melanoma	[231]
iRGD	PTX and surviving siRNA	Polymer micelle	A549 human non-small cell lung carcinoma xenograft	[232]
iRGD	DOX	Nanosized membrane vesicle	MDA-MB-231 human breast cancer xenograft	[233]
iRGD	Salinomycin	DSPE-PEG2000 micelle	HepG2 human liver cancer xenograft	[234]
iRGD	Indocyanine green	Liposome	4T1 mouse mammary cancer	[235]
iRGD	Liposomal DOX	Liposome	B16 mouse melanoma	[236]
iRGD	PTX	PTX nanodot	4T1 mouse mammary cancer	[237]
iRGD	CDDP and DOX	Polysaccharide-based nanoparticle	HeLa human cervical cancer	[238]
RPARPAR	–	Neutravidin	PPC-1 human prostate carcinoma xenograft	[199]
iNGR (CRNGRGPDC)	DOX	Elongated iron oxide nanoparticle	4T1 mammary cancer	[239]
Arg-X-(Arg/Lys) (Arg/Lys)	DOX	DOX-TPP	Rhabdomyosarcoma	[240]
TT1 (CKRGARSTC)/Linear TT1 (AKRGARSTA)	–	Iron oxide nanoparticle	MCF10CA1A breast tumor	[213]
A22p (HTPGN-SNKWKHLQENKKGR-PRR)	Monoclonal antibodies (mAb): Erbitux®, Herceptin®	mAb-A22P	Human cancer cell lines: SK-OV-3(Ovary), A431 (Epidermis), FaDu (Pharynx)	[241]
LyP-1 (CGNKRTRGC)	DOX-loaded liposome	Liposome	Lymph node metastases tumor (MDA-MB-435)	[242]
tLyP-1 (CGNKRTR)	PTX	Lactoferrin-functionalized nanoparticle	C6 rat glioma	[243]
tLyP-1	Tumor targeted nanoparticle	PEG-*b*-PLA	C6 rat glioma (intracranial)	[244]

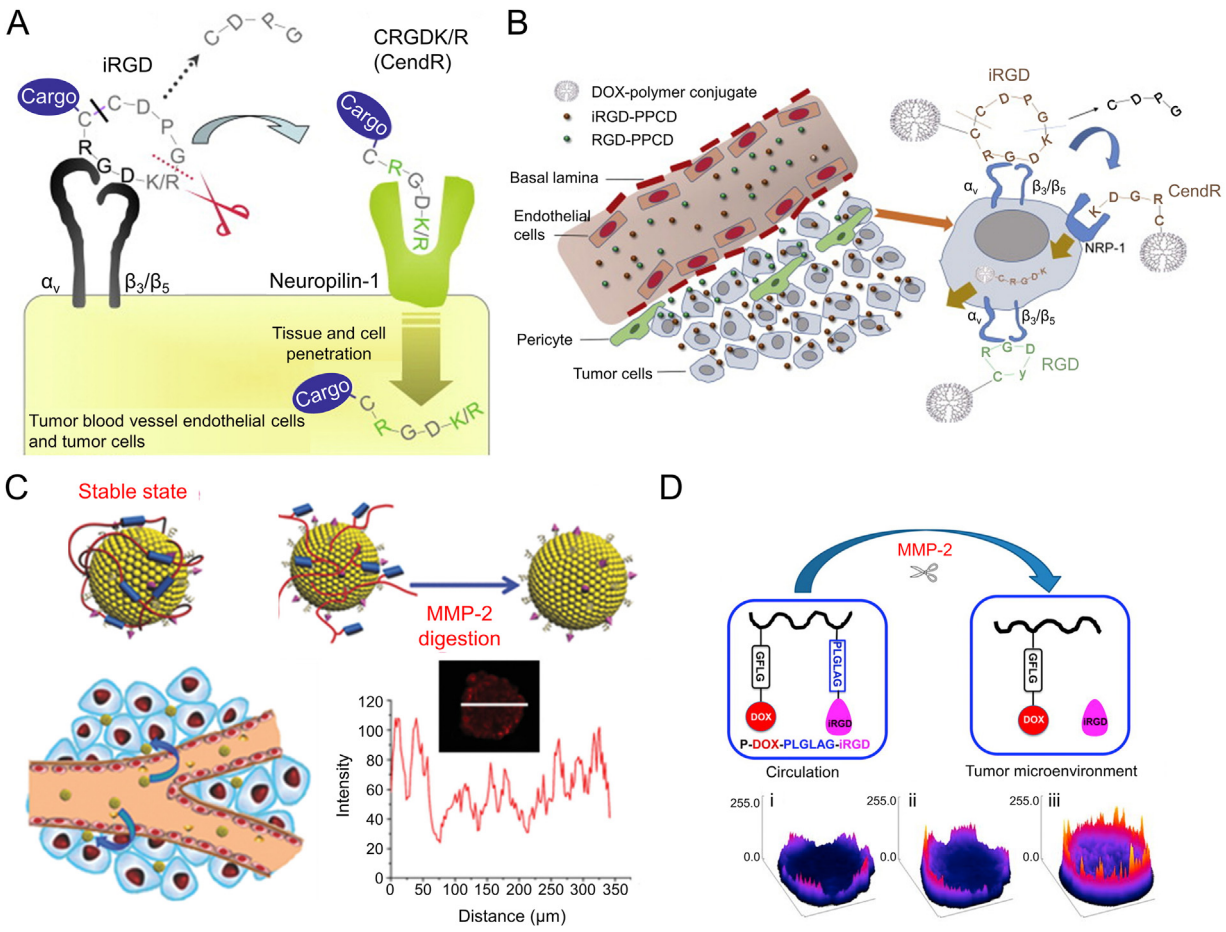

Fig. 5. TPP enhances tumor penetration. (A) Mechanism of TPP-mediated transcellular pathway [204]. Reprinted with permission from Ref. [204]. Copyright 2009, Elsevier. (B) iRGD increased the penetration of dendrimers [207]. Reprinted with permission from Ref. [207], Copyright 2014, Elsevier. (C) Nanocapsules with activatable iRGD [255]. Printed with permission from Ref. [255]. Copyright 2015, John Wiley & Sons. (D) MMP-2-sensitive DOX prodrug with detachable iRGD [256]. Reprinted with permission from Ref. [256]. Copyright 2015, American Chemical Society.

ticles, such as cationic nanoparticles, graphene nanosheets, and boronic acid-rich chitosan nanoparticles, also showed good transcellular transport in other studies [226,258,259].

Overall, the transcellular transport of nanoparticles mediated by peptides or structural design can be applied to a broad range of nanoparticles and types of tumors. Moreover, through tailored design, the TPP-activatable nanosystems can be achieved for tumor-specific penetration. This universal strategy has great potential to solve the problem of poor penetration of nanoparticles.

Transformable nanoparticles integrating multiple functions

As previously discussed, nanoparticle optimization entails compromises, owing to the varied and even opposing requirements of nanoparticles in different delivery cascades. The delivery cascades following nanomedicine administration can be categorized into five steps: circulation, accumulation, penetration, internalization, and drug release [14]. The optimal features of nanoparticles for each of these steps are quite different and may even contradict each other. For example, the penetration capability of nanoparticles increases with decreased sizes, but tiny nanoparticles are quickly cleared and show less tumor accumulation [139]. The PEGylation of nanoparticles enhances tumor penetration by promoting the paracellular pathway but also hinders internalization into tumor cells [260]. Cationic nanoparticles might enhance tumor penetration by facilitating an alternative transcellular pathway, but also have relatively short circulation time compared with neutral or slightly negative ones [196]. In summary, nanoparticle optimization is restricted by the conflicting requirements for nanoparticle properties in different steps of drug delivery. Transformable nanoparticles, which adjust their properties to the requirements of different delivery steps under different conditions, might be a way to integrate the ideal features for different delivery cascades into a single nanoplatform.

Size and charge appear to be the features most amenable to transformation, and these two features have been widely studied concerning drug delivery optimization. The two typical transition designs for size transition are small and large hybrid nanoparticles and size-shrinkable nanoparticles. The charge transition applied in enhancing tumor penetration is usually from neutral/anionic to cationic. Some nanoplatforms even combined these two transitions together hoping to obtain better tumor penetration. Moreover, the unique microenvironments at the tumor sites, such as mild acidity and overexpressed enzymes, have been exploited to trigger both transitions. As well as endogenous microenvironments, external stimuli, such as light and ultrasound, have also been used in the design of such kinds of nanoparticles [261–267].

Small and large hybrid nanoparticles are usually composed of two nanoparticles with different sizes: a larger nanoparticle for circulation and accumulation, and a smaller nanoparticle for penetration. Smaller nanoparticles can be encapsulated within or conjugated onto larger nanoparticles to form larger hybrid nanoplatforms, which have reduced clearance by the liver capture or renal filtration during circulation. After accumulation at

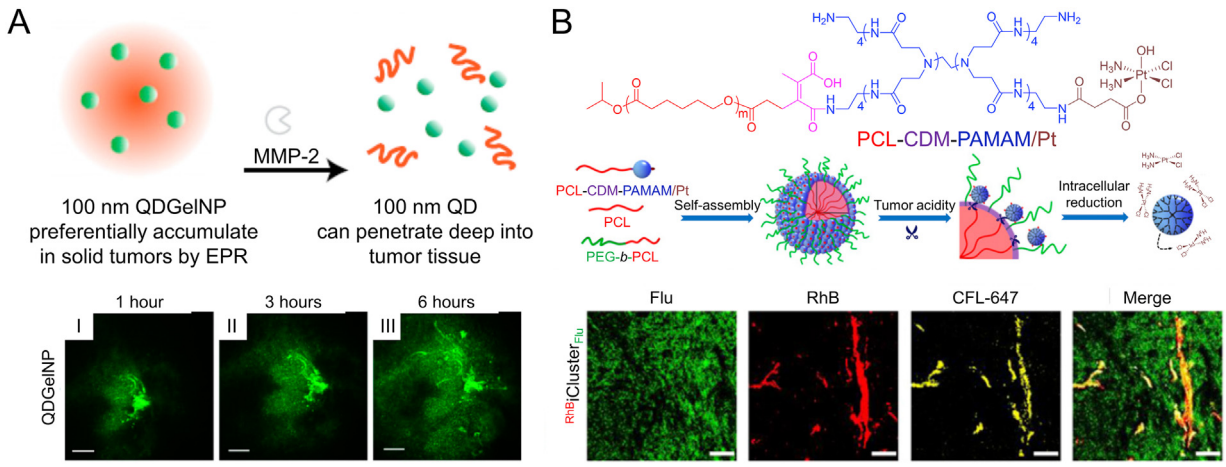

Fig. 6. Size-transformable nanoparticle. (A) MMP-2 catalyzed a size reduction for "small-in-large" hybrid nanoparticle. Small QD loaded into larger GNP was released for further penetration by MMP-2 [268]. (B) "Small-on-large" hybrid nanoparticle with acid-triggered size transformation. Small Flu-labeled PAMAM nanoparticle was conjugated onto larger RhB-labeled PEG-*b*-PCL nanoparticle. Under tumor acidity, the conjugation between small and large nanoparticles was cleaved, and the small one was released for penetration [277].

the tumor sites through the EPR effect or active targeting, these nanoparticles respond to stimuli to detach or release the smaller nanoparticles from the hybrid nanoparticles. One example of "small-in-large" hybrid nanoparticles was reported by Fukumura and coworkers [268]. QD of 10 nm in diameter was encapsulated into 100 nm gelatin nanoparticle (GNP), which could be degraded by MMP-2 overexpressed at the tumor site (Fig. 6A). After degradation of the gelatin scaffold, smaller QD was released from the hybrid nanoparticle and was able to penetrate to deeper tumor tissue *in vitro* and *in vivo*. Many other "small-in-large" hybrid nanoparticles, including PAMAM dendrimer or dendrigraft poly(L-lysine) (PLL) in polymer micelles, polyplex micelles, and liposomes, activated by various stimuli, have been reported to show improved penetration abilities [269–275]. Beyond encapsulating smaller nanoparticles within larger ones, smaller nanoparticles also self-assemble to form larger nanoparticles [276]. For example, amphipathic polymer-conjugated PAMAM dendrimer assembled to form nanoparticle of about 80 nm in size. In the acidic tumor microenvironment, the hybrid nanoparticle exhibited an ultrasensitive transition to smaller nanoparticle, obtaining improved penetration at the tumor sites. "Small-on-large" hybrid nanoparticles are another typical design of hybrid nanoparticles. For example, the platinum prodrug-conjugated PAMAM (PAMAM/Pt) dendrimer was conjugated onto polymer nanoparticle using an acid-cleavable linker (Fig. 6B) [277]. This hybrid nanoparticle exhibited better circulation behavior than free PAMAM/Pt dendrimer. After accumulation at the tumor sites, the PAMAM/Pt dendrimer detached from the hybrid nanoparticle and exhibited better penetration *in vitro* and *in vivo*. Other hybrid nanoparticle with MMP-2-responsiveness has been reported by Gao's group [278–280]. Small DOX-loaded dendrigraft PLL or conjugated Au NP was conjugated to larger GNP. At the tumor site, GNP was degraded by MMP-2, releasing the smaller nanoparticle from the hybrid nanoparticle for enhanced penetration.

Size-shrinkable nanoparticles are another kind of size-transformable nanosystems. Unlike hybrid nanoparticles, the size transition, in this case, is caused by nanoparticle shrinkage instead of the release of smaller nanoparticles. Two typical examples have been reported by Kohane's group [281,282]. Spiropyran-contained monodisperse nanoparticle underwent reversible photoisomerization after ultraviolet (UV) light of 365 nm irradiation, which caused the nanoparticle to shrink in size from about 150 to 40 nm, enhancing the penetration ability of the micelle. Moreover, the release of drug loaded in this nanoparticle was accelerated by the size shrinkage under irradiation. These shrinkable nanoparticles demonstrated good blood circulation and recovered their ability to penetrate tumors after UV light activation.

Charge transition can also be the basis for recoverable tumor penetration of nanoparticles, owing to enhanced transcellular drug delivery [191]. A reversible pH-sensitive nanogel with a polyelectrolyte core was prepared for a sequential intra-intercellular nanoparticle delivery (Fig. 7) [283]. During circulation, the nanogel was negatively charged and efficiently accumulated at the tumor sites. After internalization into cancer cells, the acidic endolysosomal conditions trigger the nanogel to swell, leading to the rapid release of loaded drugs (Fig. 7A). More interestingly, the nanogel could be liberated from dead cells and then infected adjacent cancer cells, resulting in penetration closer to the tumor center *in vitro* (Fig. 7B) and *in vivo* (Fig. 7C). In addition to the pH-activated charge transition, an enzyme-activatable polymer–drug conjugate was reported to augment tumor penetration through γ-glutamyl transpeptidase-activated transcellular transport [284]. After caveolae-mediated endocytosis and transcytosis, the activated transport enabled both transendothelial and transcellular modalities, leading to a relatively uniform tumor distribution. Benefiting from the enhanced penetration, this prodrug system was shown to eradicate small solid tumor of ~100 mm^3 and regress large established tumor of ~500 mm^3.

The integration of size and charge transition into one platform might further promote tumor penetration through both paracellular and transcellular transport. Chen and coworkers reported a rationally designed nanoparticle with both acidity-activated shrinkage and charge transformation for the treatment of xenograft human A549 lung carcinoma (Fig. 8) [285]. The unique shell-stacked nanoparticle (SNP) was formed with an acidity-detachable shell, which shielded the cationic core from clearance during circulation (Fig. 8A). After the tumor pH caused the stacked shell to detach, the size of the nanoparticle decreased from about 145 to 40 nm, and the surface charge reversed from −7.4 to 8.2 mV (Fig. 8B). The combination of smaller size and cationic charge further improved both the extravasation and penetration of the activated nanoparticle compared with a control *in vivo* (Fig. 8C and D). Owing to the enhanced penetration, the loading nanoparticle was able to eradicate A549 tumor xenograft. Moreover, a similar dual-transformable nanosystem was used for siRNA and photosensitizer delivery [286]. A programmed death ligand 1 blockade and mitochondrion-targeting photosensitizer were co-loaded into a nanoparticle assembled by acid-cleavable PEG shell.

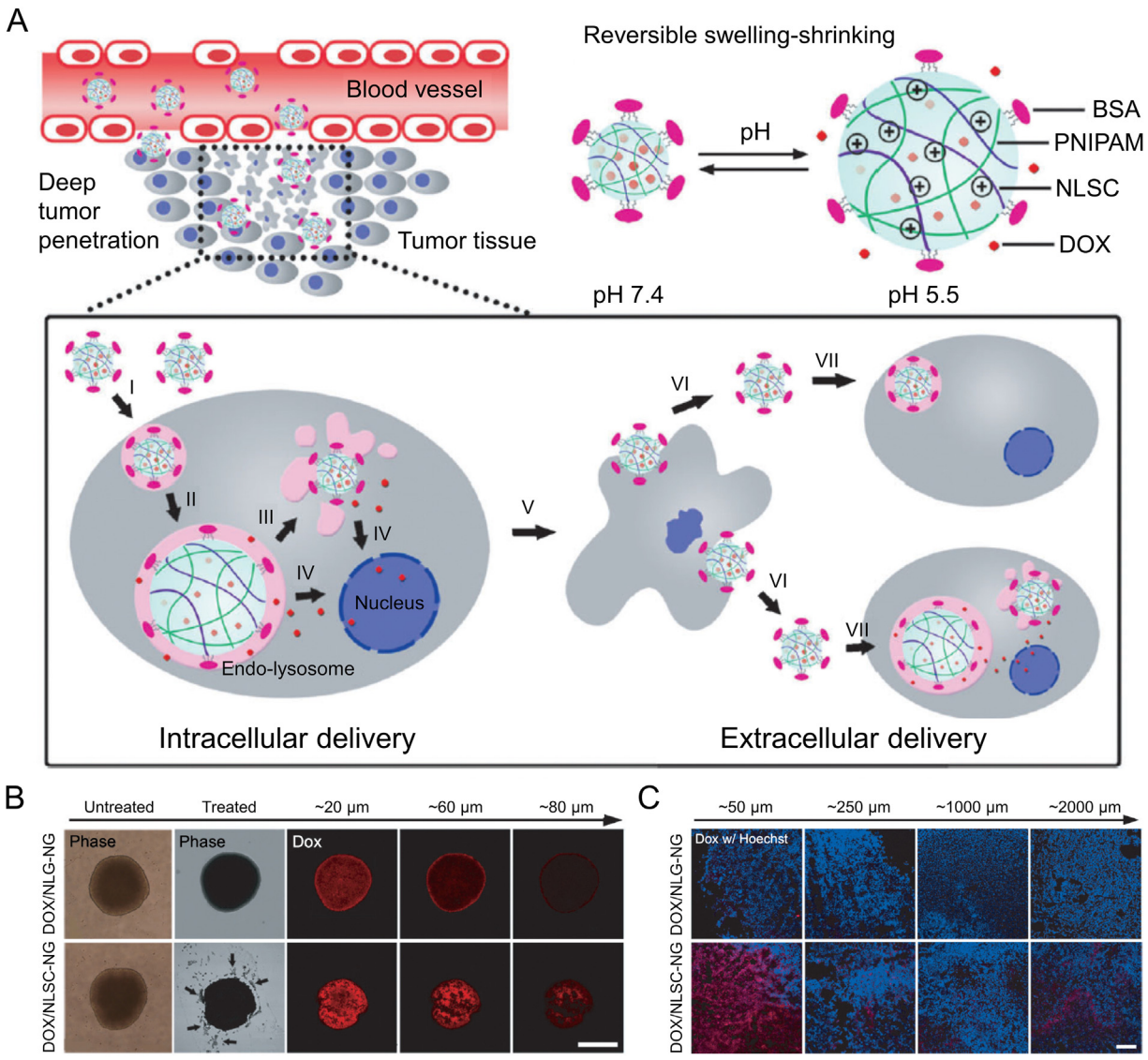

Fig. 7. Charge-transformable nanogel for acidity-activated tumor penetration [283]. (A) Acidity-activated charge transformation enhanced tumor penetration by activating the transcellular pathway under tumor microenvironments. NLSC, *N*-lysinal-*N*'-succinyl chitosan; PNIPAM, poly(*N*-isopropylacrylamide) ; BSA, bovine serum albumin. (B) Charge-transformable nanogel increased *in vitro* penetration in a 3D tumor spheroid model. The arrows indicate the cell fragments shedding from the surface of the tumor spheroid. Scale bar is 400 μm. (C) Charge-transformable nanogel enhanced *in vivo* penetration of DOX in xenografted hepatocellular carcinoma. Scale bar is 200 μm. Reprinted with permission Ref. [283]. Copyright 2014, John Wiley & Sons.

Owing to detachment of PEG shell, the nanoparticle underwent size reduction and charge transition, which further enhanced the photodynamic therapy and checkpoint inhibition. The consequent systemic antitumor immune response inhibited melanoma growth and also reduced the recurrence rate.

The tailored design of transformable nanoparticles integrates various properties into one platform, providing advantages during different drug delivery cascades, and potentially representing a feasible strategy for advanced drug delivery. However, the manufacture of existing transformable nanoparticles is usually complex and uncontrollable. Thus, the clinical application of these platforms is still unclear and need further improvement.

Conclusion and perspectives

Several decades of nanomedicine development have resulted in numerous functional nanotherapeutics for different requirements of drug delivery. Tumor penetration, the most crucial step for delivery cascades of nanotherapeutics, is severely hindered by multiple biological barriers, including abnormal tumor vasculature, dense ECM, and IFP. Moreover, nanoparticle properties, such as size, shape, and charge, have played significant effects on tumor penetration efficiency.

To overcome the penetration limitation at the tumor sites, several traditional strategies have been explored, such as modulation of tumor microenvironments and optimization of nanoparticles. Disrupting the tumor blood vessels and depleting the tumor ECM are two common and direct approaches to increase nanoparticle diffusion at the tumor sites. An indirect approach, normalizing tumor vessels, enhances the permeability of small nanoparticles. However, the effects of modulating tumor microenvironments depend on tumor types and the specific nanoparticles used, and so this cannot be applied as a universal strategy to improve tumor penetration. Moreover, modulating tumor microenvironments may disrupt the balance of tumor microenvironments and cause other serious problems. Optimization of nanoparticles identify the nanoparticle properties required for better penetration. However, effective drug delivery is exceptionally complex

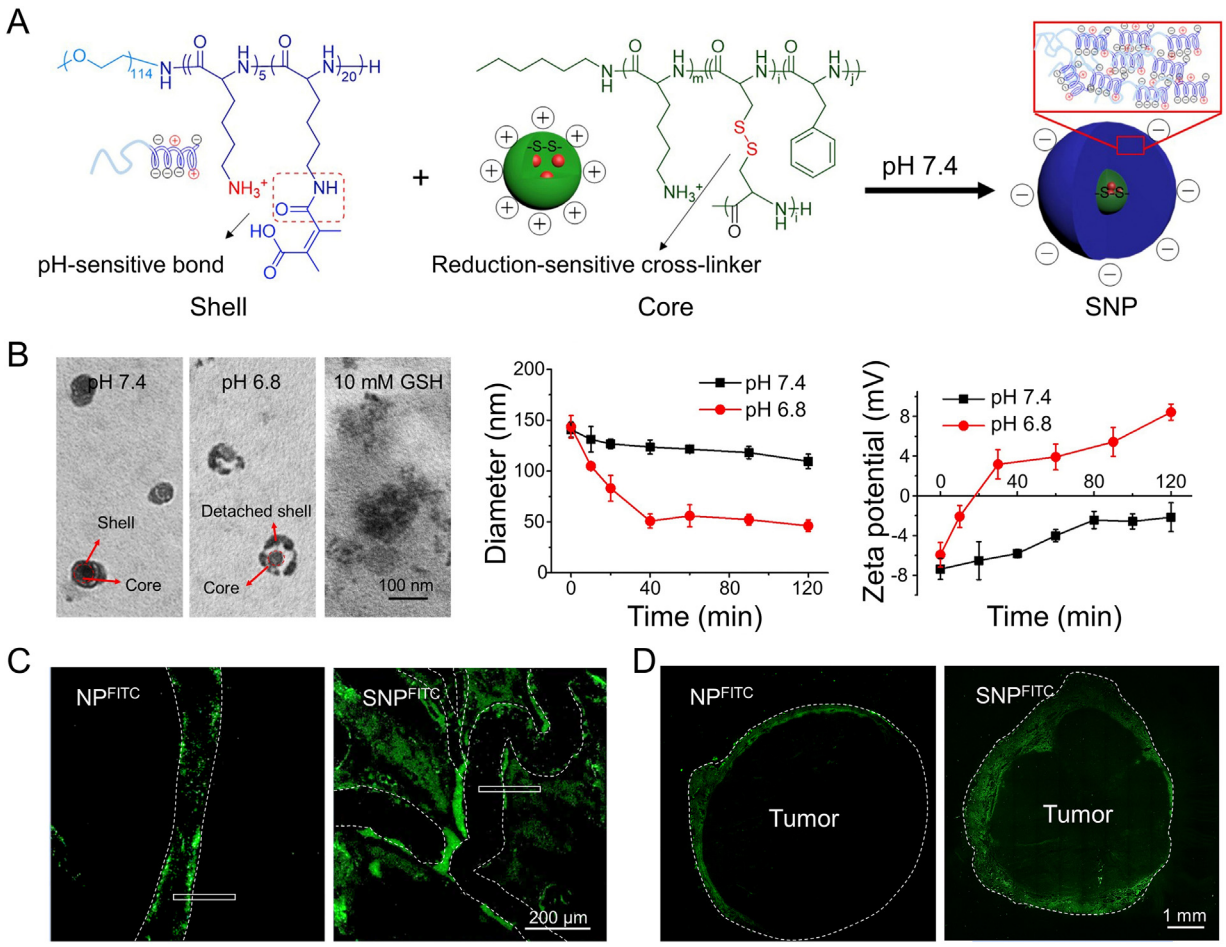

Fig. 8. Dual-transformable nanoparticle in both size and charge [285]. (A) Formation of SNP. (B) Size and charge transition of SNP under pH 6.8. (C) Enhanced extravasation of SNP from tumor vessels compared with control nanoparticle NP. (D) Promoted penetration of SNP from tumor edge to center. Reprinted with permission from Ref. [285]. Copyright 2017, John Wiley & Sons.

and requires the integration of multiple drug delivery cascades, which has conflicting demands with respect to optimal properties. Nanoparticle optimization at best achieves compromises for efficient drug delivery. It is not possible to integrate all the ideal properties into one drug delivery nanoplatform.

To solve the problem of contradictory optimal nanoparticle properties, two promising strategies have been identified. Targeted transcellular drug delivery with an alternative delivery pathway bypasses the biological barriers at the tumor sites, and stimuli-responsive nanoplatforms can be tailored to transform properties when exposed to the tumor microenvironments or external stimuli. Transcellular drug delivery, mediated by TPPs or other ligands, shows broad applicability by multiple types of nanoparticles and tumors and can be regarded as a universal strategy for tumor penetration. Rationally designed transformable nanoparticles allow the integration of multiple functionalities into a single delivery system, overcoming multiple barriers of drug delivery. These two strategies show high potential for enhancing the tumor penetration of nanoparticles.

Although rationally designed nanoplatforms have significantly improved tumor penetration, their clinical applications require further effort. First, more attention should be paid to obtain a thorough understanding of tumor microenvironments, especially factors affecting the penetration of nanoparticles. Second, the manufacture of tailored nanocarriers is usually extremely complex, and their activities and transformations in the body remain unclear. More detailed studies should be carried out to develop more straightforward and more efficient templates and to further investigate the fates of these nanoparticles after *in vivo* administration. Furthermore, although great efforts have been made to improve the tumor penetration of nanomedicines, the reported depth of penetration is still modest because of the complexity of biological barriers. New therapeutic methods, which do not rely on penetration, might represent alternative methods to bypass these barriers. For example, the nanoparticle-based immunotherapy, such as activation of the immune system by co-delivery of immune agonists, tumor-specific antigens, and photothermal agents, leads to tumor regression without the need for deep tumor penetration [287,288]. Therefore, nanoparticle design must be able to accommodate the requirements of different therapeutic agents.

Declaration of Competing Interest

The authors declare no competing interests.

Acknowledgments

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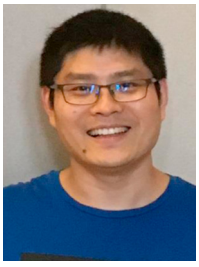

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