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Transvascular transport of nanocarriers for tumor delivery

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Nanocarriers (NCs) play a crucial role in delivering theranostic agents to tumors, making them a pivotal focus of research. However, the persistently low delivery efficiency of engineered NCs has been a significant challenge in the advancement of nanomedicine, stirring considerable debate. Transvascular transport is a critical pathway for NC delivery from vessels to tumors, yet a comprehensive understanding of the interactions between NCs and vascular systems remains elusive. In recent years, considerable efforts have been invested in elucidating the transvascular transport mechanisms of NCs, leading to promising advancements in tumor delivery and theranostics. In this context, we highlight various delivery mechanisms, including the enhanced permeability and retention effect, cooperative immune-driven effect, active transcytosis, and cell/bacteria-mediated delivery. Furthermore, we explore corresponding strategies aimed at enhancing transvascular transport of NCs for efficient tumor delivery. These approaches offer intriguing solutions spanning physicochemical, biological, and pharmacological domains to improve delivery and therapeutic outcomes. Additionally, we propose a forward-looking delivery framework that relies on advanced tumor/vessel models, high-throughput NC libraries, nano-bio interaction datasets, and artificial intelligence, which aims to guide the design of next-generation carriers and implementation strategies for optimized delivery.

The 2023 Nobel Prize in Physiology/Medicine brought great excitement to nanomedicine fields by recognizing the innovation of mRNA vaccines. During the COVID-19 pandemic, engineered nanocarriers (NCs) enabled the successful application of mRNA vaccines[1]. For intravenous administration, one goal of nanomedicine is to overcome multi-biological barriers in vivo for improving targeted delivery and theranostic efficacy[2,3]. For tumor delivery, NCs will encounter a series of biological barriers during blood circulation[4], vascular extravasation[5], tumor penetration[6], cellular internalization and retention[7]. Among them, the vascular extravasation is a highly critical pathway for NC delivery into tumors, therefore the efficient NC delivery largely depends on tumor vascular systems[8]. In 1986, the enhanced permeability and retention (EPR) effect was postulated to explain the augment vascular extravasation and improved tumor delivery of NCs[9,10]. However, after 30 years of efforts, the clinical translation of such devised NCs according to the EPR effect is still low[11,12]. While there is evidence about the EPR effect in small animals such as mice, in fact, only a few nanomedicines are approved for clinical trials in humans[13]. Additionally, the statistical result from

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literature indicated that less than 1% of intravenously injected NCs can reach the tumor site in vivo[14]. Based on these disappointing facts, the availability of the EPR effect to tumor delivery of NCs has been seriously questioned in recent years[15,16].

The basement membrane surrounding tumor vessels is an important but overlooked biological barrier for NC delivery based on the EPR effect[17]. After passing through the vascular endothelial gap, NCs may be trapped in the subendothelial space due to the dense basement membrane, which would hinder vascular extravasation of NCs into tumors. On the other hand, a research result showed that most of NCs are transvascularized into solid tumors by active transcytosis which may be a dominant mechanism for vascular extravasation[18]. In addition, several cells and bacteria were employed as Trojan Horses to carry NCs for improved transvascular transport and tumor delivery by their intrinsic inflammatory responsiveness or tumor-tropism or tumor-colonization properties[19,20]. These important discoveries of these mechanisms stimulated us to revisit the common approaches for tumor delivery of NCs.

Amidst the burgeoning understanding of cancer nanomedicine, pivotal discussions are underway regarding core concepts and effective approaches for the tumor delivery of NCs, with the aim of charting pathways toward enhanced clinical translation success. Firstly, the intricacy and diversity inherent in tumors and vessels necessitate patient stratification based on precise biomarkers. The patient stratification facilitates the customization of NC formulations, thereby optimizing personalized theranostics. Furthermore, in the realm of preclinical research, there is a pressing need to develop advanced tumor and vessel models that faithfully mimic human physiology, thereby superseding traditional animal models. Contemporary efforts in cancer nanomedicine extend beyond more NC formulation adjustments, now encompassing fundamental investigations into tumor biology and nano-bio interactions. Driven by the collection and analysis of nano-bio interaction data, a forward-looking guidance framework should be established to inform the design of NCs tailored for precise theranostics. In this review, we first compared the differences in the passive EPR effect and active transcytosis, and then summarized a variety of recent strategies for promoting vascular extravasation of NCs. Likewise, we also delve into the strategies of cell/bacterial-mediated delivery to bypass existing bottlenecks of transvascular transport for efficient NC delivery into tumors. Finally, the future and challenges of nanomedicine in clinical translation and applications are discussed. Noteworthy technological advancements poised to bolster the clinical translation of nanomedicines include organ-on-chip model, artificial intelligence (AI), high-throughput screening methodologies, and adept big data management practices. These innovations collectively hold promise in ushering nanomedicine from the realms of research to transformative clinical realities.

Passive EPR effect *versus* active transcytosis

Exploring the mechanism of NC delivery from vessel to tumor (i.e., vascular extravasation) is very important because this is a critical but incompletely understood pathway[21]. In general, vascular extravasation of NCs in tumor region can be mediated by passive EPR effect. The enhanced permeability arises from the large endothelial gaps or fenestrations in tumor vessels that allow the passage of NCs, and the enhanced retention results from a collapse of lymphatic drainage system where NCs cannot be transported away[10,22]. According to this mechanism, extensive efforts have been devoted to augment vascular extravasation by optimizing the physicochemical properties of engineered NCs, such as size/shape[23], composition[24], surface charge and modification[25], as well as flexibility (Fig. 1a)[26]. Besides, the increased vascular extravasation of NCs can also be achieved by regulating the microenvironments of tumor vessels. Administration of anti-vascular endothelial growth factors (e.g., anti-VEGF or anti-VEGFR-2) can normalize vessels, thereby further augmenting the transvascular flux of NCs by the increased blood supply and decreased tumor interstitial fluid pressure (Fig. 1b)[27]. Vascular normalization can improve the extravasation of small sized NCs, however may impede the leakiness of larger NCs due to the reduced vascular permeability. Conversely, the vascular mediators, such as nitric oxide or angiotensin II, are able to improve vascular permeability for more effective extravasation of NCs (Fig. 1c)[28]. Likewise, exogenous assistance technologies including laser, magnetic, radiation and ultrasound offer encouraging tools to cause bursts or temporary pores in tumor vessels by photo-/magneto-thermal effect[29,30], radiant energy[31], or ultrasonic targeted microbubble destruction (UTMD)[32] for boosting vascular extravasation of NCs (Fig. 1d). The latest insights indicate that the EPR effect will also vary with different stages of tumor development and treatment[33]. In general, the EPR effect is relatively significant in the early stage of tumors.

Recently, the contribution of passive EPR effect to tumor delivery of NCs has been mired in controversy due to several negative clinical trials. In 2023, it was found that the last line of defense for NC extravasation into tumors is not the vascular endothelial barrier, but a dense basement membrane surrounding the endothelium[17]. The basement membrane seriously hinders vascular extravasation of NCs by their entrapment in subendothelial void after crossing endothelial gap, forming a perivascular NC pool (Fig. 1e). Although collagen hydrolases are capable of degrading the basement membrane to release the trapped NCs[34,35], this may also lead to irreversible destruction of basement membrane and increase the risk of cancer metastasis. Furthermore, a cooperative immune-driven strategy was developed, utilizing laser-mediated hyperthermia to induce inflammation which will recruit platelets and neutrophils (NEs) into NC pool. Through NE migration including endothelium adhesion, crawling and diapedesis, a dynamic window is temporarily created in basement membrane, thereby triggering NC pool eruptions and extravasation. The investigation not only explains the controversy over the EPR effect but also provides a different mechanism for the transvascular transport of NCs. In future research, it is worth considering whether the basement membrane uniformly surrounds the endothelium or exhibits the heterogeneity in different tumor vessels or vessel locations. In addition, previous work has already revealed that the EPR effect displays high heterogeneity in different species and tumor types[36], which is not a general principle. There is indication, that the delivery is partly falsely claimed to be by the EPR effect, but instead is achieved by active transcytosis. A very recent work discovered that the frequency of endothelial gaps in tumor vessels is quite low (only 0.048% of vascular wall surface area) and thus insufficient for enhancing tumor delivery of NCs, contrary to the EPR effect[18]. Meanwhile, the results in mouse models and human specimens suggested that most of NCs are transported into solid tumors by active transcytosis of endothelial cells.

For now, both the passive EPR effect and active transcytosis are controversial in the community[37,38]. The heterogeneity between tumors and vessels affects the pathway of vascular extravasation of NCs. In 2023, a research work demonstrated that the EPR effect is still dominant for the tumors with high vascular permeability, while active transcytosis is the main mechanism of low-permeability tumor vessels[39]. Through ingenious design, they developed protein-based NCs for active transendothelial transport in low-permeability vessels by pinocytosis-mediated endocytosis and exocytosis, enabling the improved tumor delivery. Pinocytosis-mediated endocytosis is carried out through the extension of actin-stabilized plasma membranes to phagocytosis NCs, and then forming the macropinosome-based vesicles for their intracellular transfer[7]. As previously mentioned, the tumor delivery efficiency of NCs designed based on the EPR effect is low. The strategies based on active transcytosis or combined EPR effect and active transcytosis can overcome blood-tumor barrier to improve tumor delivery efficiency of NCs, and even promote the crossing of blood-brain barrier to increase NC accumulation in glioma. With such insights into tumor vascular extravasation of NCs, the

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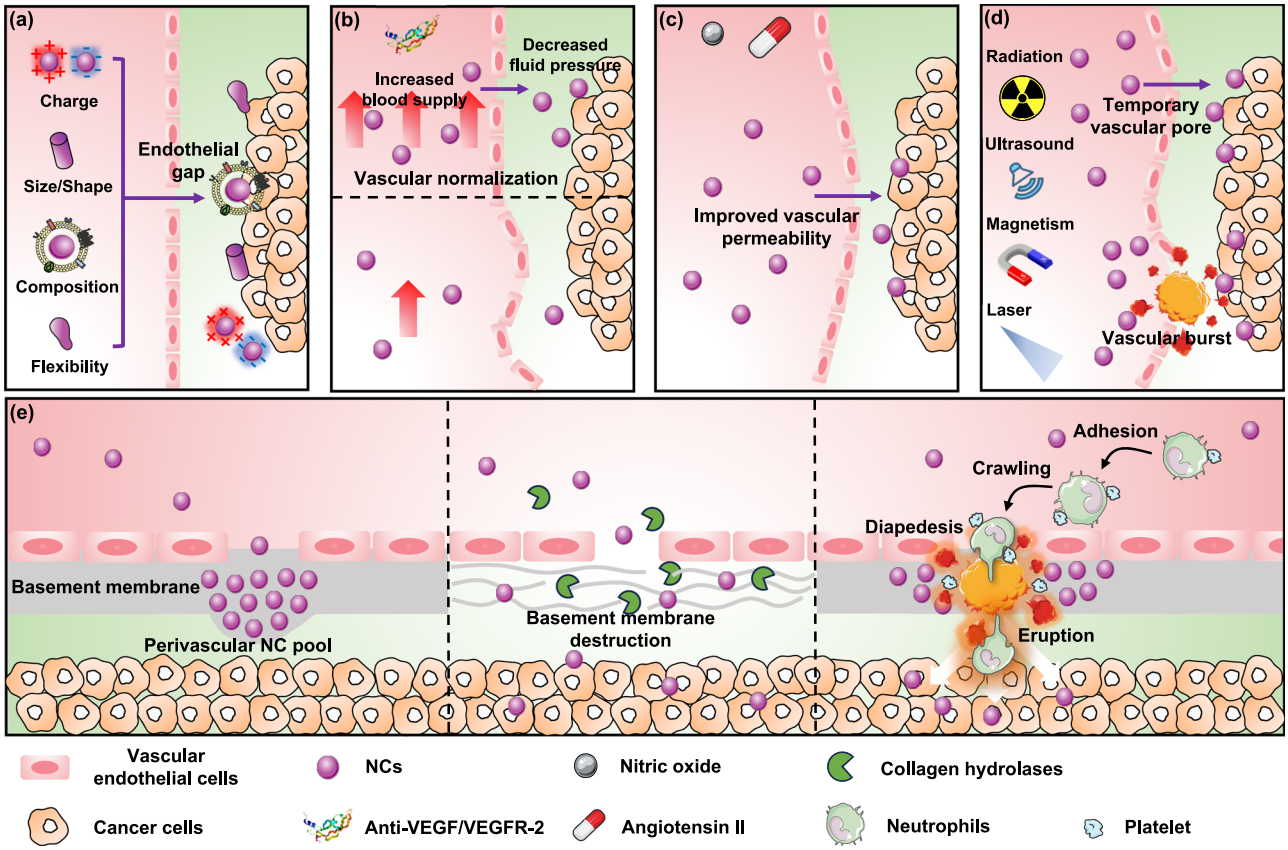

Fig. 1 | Strategies for improved vascular extravasation of nanocarriers (NCs) based on the EPR effect. a Optimization of physicochemical properties of NCs; **b** Administration of anti-vascular endothelial growth factors for vascular normalization; **c** Administration of vascular mediators for increasing vascular permeability; **d** Exogenous assistance technologies for causing bursts or temporary pores of vessels. **e** After crossing the vascular endothelial cells, the NCs are trapped in the subendothelial void by basement membrane surrounding vessels, forming a perivascular NC pool that hinders their extravasation. Collagen hydrolase-mediated basement membrane destruction is used to increase NC extravasation. Another strategy—cooperative immune-driven strategy is proposed for increasing NC extravasation. Neutrophils bind to activated platelets (as recruitment beacons), subsequently migrating into the interior of NC pool through the cascade event including neutrophil adhesion on endothelium, crawling along endothelium, and diapedesis into NC pool. The neutrophil migration process can open the basement membrane barrier of NC pool and lead to the explosive pool eruption, thereby propelling the NCs deeper into tumors.

criteria and strategy for the design of NCs might require some rethought, i.e. focus could be shifted from only considering NCs for passive EPR-mediated delivery to constructing NCs for augmented active transcytosis[21].

Enhanced active transcytosis

Admittedly, little is known about the mechanism details of active transcytosis, especially what kind of characteristics of NCs may be more likely to trigger the transcytosis of endothelial cells. There is a series of previous works reporting transcytosis for the crossing of biological barriers[40]. The transcytosis of NCs is mainly based on caveolae-dependent endocytosis and exocytosis of vascular endothelial cells[7]. By invaginating the caveolin-coated plasma membrane, the caveolin-stabilized vesicle (i.e., caveolae) is formed for the endocytosis of NCs into endothelial cells. Subsequently, based on the caveolae-based shuttle mechanism, the NCs cross the vascular barrier. It has been reported that the surface engineering strategy of NCs (e.g., modification with specific ligands) is beneficial for promoting caveolae-dependent transcytosis by the interactions with the receptors on endothelium. Concerning delivery, active transcytosis triggered by ligand-receptor binding has been investigated to facilitate tumor vascular extravasation of NCs (Fig. 2a). For instance, albumin-bound drugs can induce caveolae-dependent transcytosis of vascular endothelial cells through the binding of the albumin and glycoprotein receptor on endothelial cells[41]. Moreover, tumor-penetrating peptides (e.g., iRGD) can be conjugated to NCs and then bind to α_v integrins on tumor vascular endothelium[42]. Subsequently, the bound iRGD is cleaved by a protease into CendR fragments that can bind to over-expressed transmembrane glycoprotein of neuropilin-1 to induce caveolae-dependent transcytosis across vascular endothelial cells[43], realizing direct delivery of NCs into tumors. Compared to iRGD-conjugated NCs, the co-administration of free iRGD with NCs shows higher transcytosis capacity as more receptors can be bound by free iRGD[44].

For ligand-receptor triggered transcytosis, the interferences with other cell surface receptors will result in off-target delivery, thereby reducing tumor delivery specificity and causing side effects[45]. Therefore, it is critical to achieve a high level of specific endothelial receptor expression on tumor vessels (Fig. 2a). In 2023, a spatiotemporal controllable aided strategy was proposed[46], which is based on the use of low-dose X-ray irradiation to precisely regulate the expression of P-selectin in vascular endothelial cells of brain tumors. Then, fucoidan-based NCs can target highly expressed P-selectin on the activated endothelial cells, triggering caveolae-dependent transcytosis and overcoming the blood-brain barrier, resulting in more efficient and safe delivery to intracranial tumors[47]. The experimental data shows that the NCs localization in brain tumors in the experimental group increased more than threefold compared with the control group. Although active transcytosis of NCs does not significantly enhance drug delivery in brain tumors by orders of magnitude, it may have the potential to facilitate delivery across the blood-brain barrier[46].

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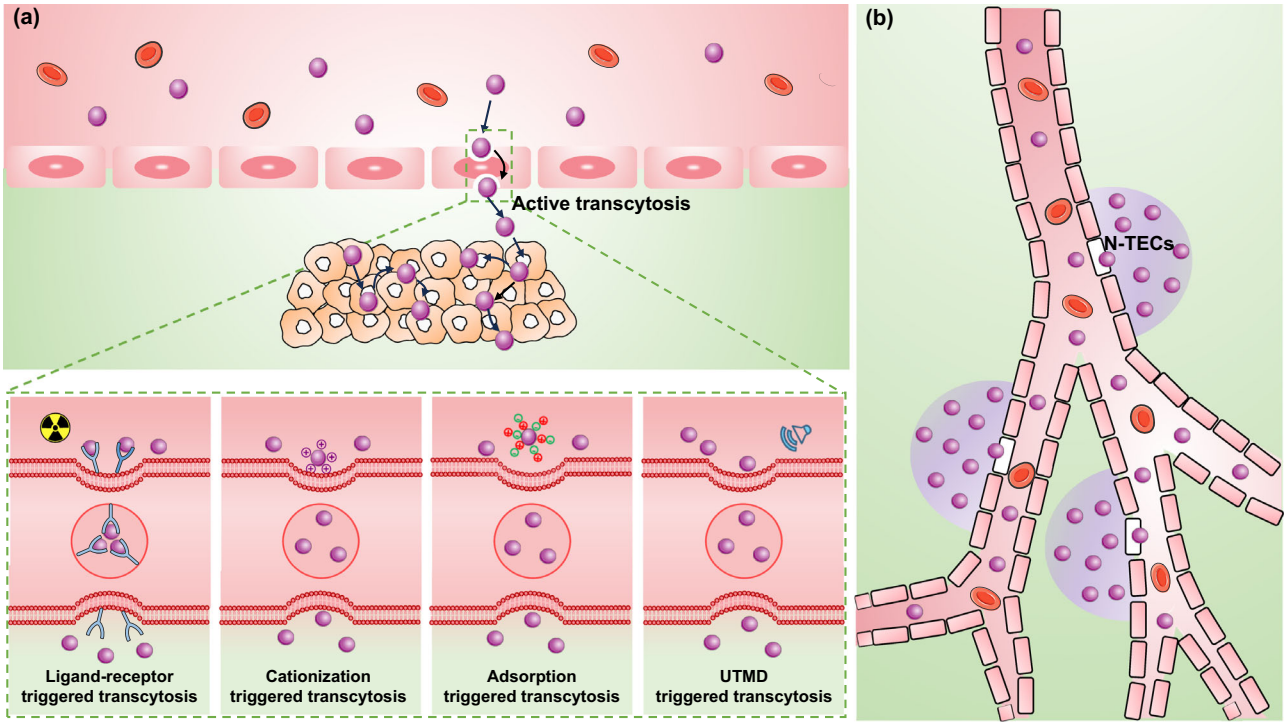

Fig. 2 | Mechanism and heterogeneity of active transcytosis. a Strategies for enhancing active transcytosis to facilitate the vascular extravasation of NCs, involving ligand-receptor triggered transcytosis (irradiation for precise regulation of receptor expression on endothelial cells), cationization triggered transcytosis, adsorption triggered transcytosis, and ultrasonic targeted microbubble destruction (UTMD) triggered transcytosis. **b** Location of special nanoparticle transport endothelial cells (N-TECs), rather than all endothelial cells, determines the vascular extravasation of NCs into tumors (purple area) and affects the distribution heterogeneity of NCs in tumor area, particularly with limited access in the area distant from N-TECs.

Apart from the strategy of ligand-receptor binding, cationization triggered transcytosis has also been developed for enhanced tumor delivery[48]. Once contacting tumor vascular endothelium, the overexpressed γ-glutamyl transpeptidase on endothelial cells induces charge cationization of γ-glutamyl transpeptidase-responsive polymers to facilitate caveolae-dependent endocytosis and transcytosis (Fig. 2a), leading to transendothelial and transcellular transport, as well as distribution throughout solid tumors[49]. Moreover, another strategy regarding adsorption triggered transcytosis was proposed (Fig. 2a)[50]. In this work, a polyzwitterion-based NC with protein non-stickiness and appropriate cellular affinity (i.e., reversible cell membrane binding ability) was designed. During blood circulation, non-stickiness towards proteins prolonged NC circulation time, subsequently NCs can bind reversibly to vascular endothelial cells and cancer cells due to their weak interaction with phospholipids. Compared to normal vascular endothelial cells, the adsorption of NCs on more active tumor vascular endothelial cells triggers rapid endocytosis and subsequent transcytosis. Adsorption triggered transcytosis is completed by caveolae-dependent endocytosis and macropinocytosis pathways for promoting the transendothelial and transcellular transport in tumors.

Moreover, some reports have implied that exogenous UTMD technology can not only temporarily increase vascular permeability and endothelial gaps[51], but also promote the clathrin-dependent transcytosis of NCs (Fig. 2a)[52]. The clathrin-dependent transcytosis is the process of invaginating the plasma membrane to form vesicles through conformational changes in motor proteins, and utilizing intracellular actin to achieve cytoplasmic transport of vesicles and transvascular transport of NCs[7]. The increasing evidence shows that active transcytosis plays a key role in transvascular transport and tumor delivery of NCs[50,53]. To more effectively grasp the mechanism of active transcytosis, a deeper understanding of the interactions at the interface between NCs and vascular endothelium would be helpful.

Moreover, recent research has revealed the transvascular transport of NCs is not universally facilitated by all endothelial cells, but rather by a specific subset comprising approximately 21% termed nanoparticle transport endothelial cells (N-TECs). These N-TECs exhibit an uneven distribution along tumor vessels (Fig. 2b)[54]. Serving as gatekeepers, N-TECs exert significant influence over the vascular extravasation of NCs, their distribution, and their access to tumor regions. Gene expression profiling demonstrated that N-TECs present more genes related to vascular permeability and transport compared to other endothelial cells. From a fundamental standpoint, it is imperative to delve deeper into the molecular pathways governing transcytosis and elucidate the biological role of N-TECs. For instance, investigating whether additional endothelial cells can be induced to adopt the N-TEC phenotype is crucial. In terms of practical applications, attention should be directed towards discerning whether N-TECs can be manipulated to enhance NC extravasation into solid tumors and to explore their interactions with specific cancer or immune cells. Looking ahead, significant improvements in the efficiency of NC delivery to tumors could be achieved by optimizing the mechanism of active transcytosis, potentially by orders of magnitude. Such advancements would greatly enhance the clinical translation of nanomedicines by bolstering theranostic efficacy and mitigating side effects.

Cell/bacterial-mediated delivery

Cell-based systems

Over the past few years, a transpiring strategy is to exploit multiple types of living cells (e.g., red blood cells, monocytes, macrophages (MAs), NEs, T cells, mesenchymal and neural stem cells) to bypass the long-standing bottleneck of vascular barrier[55–57]. Each type of cell exhibits distinct advantages and preferred application scenarios[3]. Red blood cells bearing the surface self-marker CD47 endows them with excellent stealth property against the immune systems, thus obviously

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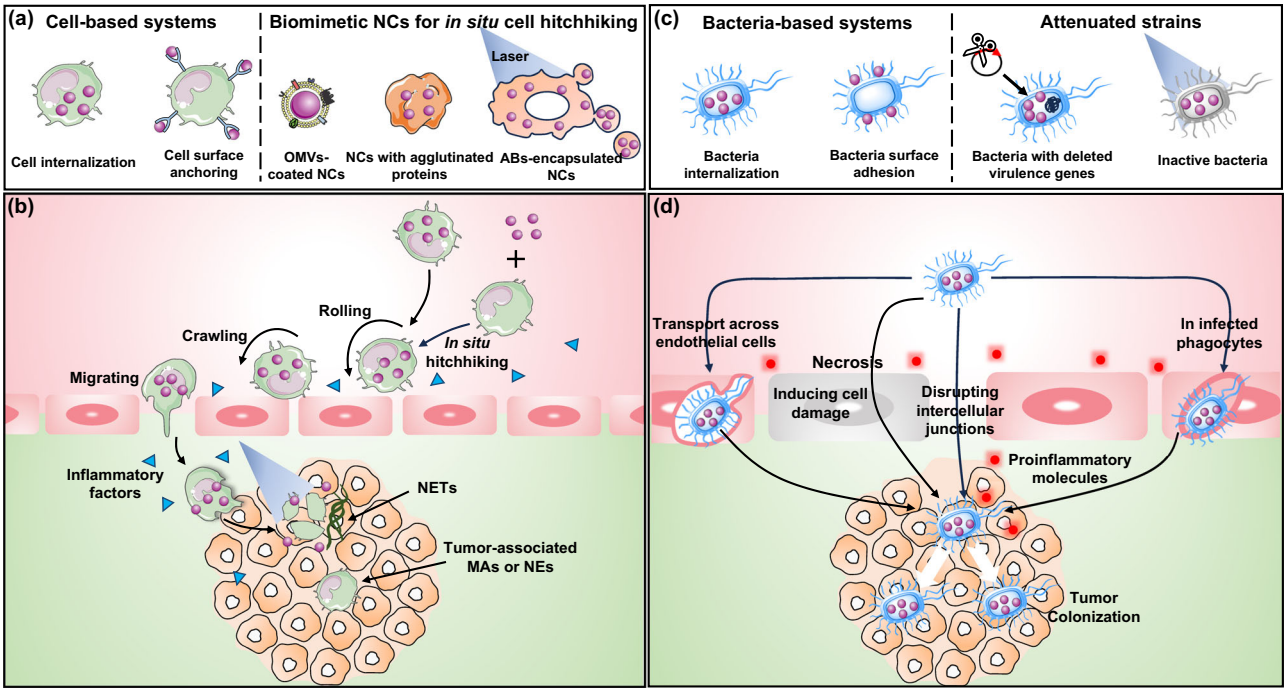

Fig. 3 | Cell- and bacterial-based systems for enhanced tumor delivery. **a** Construction of cell-based systems, in which NCs are internalized by cells or are anchored on cellular surface. Preparation of biomimetic NCs for in situ cell hitchhiking based on enhanced cellular phagocytosis. **b** Two strategies for implementing transvascular delivery of cell-based systems (ex vivo preparation and in situ hitchhiking). Cell-based systems are guided by inflammatory factors (blue arrows) to infiltrate the inflamed tumor. Cell-based systems first adhere to activated vascular endothelium, and then roll and crawl along the endothelium, subsequently crossing the endothelial barrier into tumors by cell migration. In tumor region, the neutrophil extracellular traps (NETs) release and laser-induced photothermal ablation can disrupt cell carriers to trigger the release of NCs. **c** Construction of bacteria-based systems by bacterial internalization or surface adhesion of NCs. Through knocking out virulence genes or inactivating with laser to obtain attenuated strains as safe bacteria-based systems. **d** Bacteria-based systems for crossing vascular barrier in four ways: through transport across endothelial cells, by inducing cell damage or disrupting intercellular junctions, and in infected phagocytes. Subsequently, bacteria-based systems can also colonize in tumor due to the unique tumor microenvironments.

prolonging blood circulation time. Monocytes and MAs display diapedesis and chemotaxis toward the tumor microenvironments (e.g., hypoxia), and possess a natural ability to overcome the endothelial barriers, enabling the enhanced tumor delivery. The abundancy of NEs in blood allows them to accommodate large amounts of NCs, facilitating NC loading by phagocytosis or surface anchoring. The inflammatory microenvironments can actively recruit NEs to migrate across the endothelial barriers into inflamed tumors. Moreover, apart from being widely used in targeted immunotherapy of tumors, the main attraction of T cells as carrier is their ability to migrate toward inflammatory or lymphoid organs. Unlike the aforementioned circulating cells, mesenchymal and neural stem cells exhibit certain surface markers that endow them with tumor-tropic properties, thereby significantly improving tumor delivery. Different to the EPR effect and active transcytosis, living cells as Trojan Horses carrying NCs are able to improve transvascular transport and tumor delivery by utilizing cell-intrinsic properties (e.g., inflammatory-tropism, tumor-homing, tumor-infiltration, and immune evasion) (Fig. 3a, b). Circulating cells can adhere to the surface of activated endothelial cells, subsequently rolling and crawling alone vessels, which induces transvascular transport of cell-based systems[58]. Likewise, a large number of immune cells are also present in tumor tissues, called tumor-associated MAs or NEs, which have a positive impact on the continuous recruitment of circulating MA-/NE-based systems.

For the preparation of cell-based systems (Fig. 3a), NCs are taken up by cells due to their phagocytic nature (i.e., NC-internalized cell system)[59], or are anchored on the cell surface by the specific binding (i.e., NC-anchored cell system)[60]. These cell-based systems may exhibit minimal immunogenicity and non-tumorigenicity. Among them, the NC-anchored cell system will still be affected by the exposed NCs, and further internalization of such NCs is inevitable. In addition, cell-based systems can also migrate into tumors and to metastatic cancer cells by responding to tumor-associated chemokines, which will effectively thwart tumor function and metastatic potential[61,62]. Note, that for all these benefits protocols for loading NCs into cells need to be optimized. The endocytosed NCs may in the worst case reduce cell viability, or change cell migratory behavior, which would impair tumor homing[63]. Likewise, NCs will not necessarily reside over extended periods of times in cells, and the respective contributions of exocytosis and proliferation need to be taken into account[64]. The release of NCs from cell-based systems in tumor region can be achieved by the exocytosis, neutrophil extracellular traps (NETs)-mediated cell disruption, or photothermal-mediated cell ablation. Furthermore, NCs may also be degraded in the endosomes/lysosomes of cell carriers[65]. For all those reasons, it is important to optimize the protocols around the expected time that NCs should remain biologically active. Apart from the loading protocols also the cells can be optimized. For example, genetically engineered cells exhibiting chemokine receptors (C-X-C chemokine receptor type 4 and C-C chemokine receptor type 2) and endothelial adhesion molecules (P-selectin glycoprotein ligand-1) are developed for targeted delivery[66]. The endothelial adhesion molecules are typically a type of selectin ligands or integrins that can bind to the overexpressed receptors on vascular endothelium (e.g., P selectin). The endothelial adhesion molecules of engineered cells can induce transient-cell tethering and rolling on the endothelium in disease region, which also promotes them exposure to specific chemokines displayed on endothelial surface, thus further binding to the chemokine receptors of engineered cells to activate a cascade of intracellular signaling responses. Subsequently, these bound cells undergo transvascular migration into the diseased tissue. Recently, some examples

(e.g., neural stem cell-mediated 5-fluorocytosine prodrug) have been approved by the FDA for clinical trials to treat recurrent high-grade gliomas[67].

Tumor development is often accompanied by inflammation, and immune cells will be continuously recruited into inflammation region of tumors[68]. Moreover, the amplification of inflammatory signals after surgery promotes cell-mediated tumor delivery[58]. After surgical tumor resection, the inflammation reaction occurs in brain, accompanied by the release of inflammatory factors, which activate the prepared NC-internalized NEs for migrating across vascular barrier into inflamed brain and improving brain tumor targeting (Fig. 3b)[69]. Compared to drug-loaded cationic liposomes, the use of NC-internalized NEs to deliver drug results in 86-fold higher drug concentrations in the brain. Remarkably, these immune cells are capable of activating the patient's own immune systems to mount the antitumor response through immunotherapy.

Despite all the above examples, the impaired migratory capacity caused by low viability of reinjected living cell systems remains an unsolved difficulty[70]. Currently, a strategy was reported by bovine serum albumin-based NCs in situ hitchhiking activated living cells, which can overcome the limitations of ex vivo preparation of cell-based systems[71]. It was reported that the internalization of bovine serum albumin-based NCs by activated NEs in situ does not affect the mobility and the functions of cells. Some biomimetic NCs are beneficial for specific phagocytosis of cells in situ (Fig. 3a). Recently, a strategy of pathogen-mimicking nano-pathogenoids for hitchhiking NEs in situ was proposed[72]. The bacteria-secreted outer membrane vesicles (OMVs) containing pathogen-associated molecular patterns of native bacteria can used to coat NCs, that are effectively recognized and further internalized by NEs to form cell-based systems in vivo for enhanced delivery in inflamed tumor. Another vectorization strategy was developed to realize in vivo MAs-specific loading of NCs[73]. Cell-derived apoptotic bodies (ABs) as biological vehicles of NCs are readily engulfed by MAs[74,75]. After intravenous injection, ABs-encapsulated NCs can be rapidly phagocytized by MAs during blood circulation, leading to the enhanced tumor delivery by natural migratory and homing capacities of MAs. In a very recent work, a design criteria was reported to induce selective phagocytosis of NCs by NEs in vivo[76]. The interplay between agglutinated protein on NCs and complement proteins raises the potential to develop different approaches to immunotherapy[77]. Encouragingly, these strategies to promote NC phagocytosis by living cells are expected to address the bottleneck of high off-target phenomenon attributed to tumor heterogeneity[19]. Nevertheless, for cell-mediated delivery, the controlled release of NCs from cell carriers before and after their homing to tumors has always been the most challenging issue. Recent research showed that when NEs reach the inflammation region, they can be activated by the inflammatory microenvironments, and further form NETs within hours to facilitate the release of NCs from NEs[69,78]. Moreover, external triggers such as photothermal ablation of cell carriers may be one possible solution for controlled timing of release[79].

Bacteria-based systems

In recent years, some bacteria (e.g., *E. coli*, *S. typhimurium*, *L. monocytogenes*, and *L. lactis*) have also been utilized as Trojan Horses to deliver NCs into tumors, by virtue of the ability of gene editing, tissue infiltration/colonization, and cell invasion[80,81]. Due to the low cultivation cost, high proliferative efficiency, and the natural ability to cross the vascular barrier, *E. coli* is widely used for targeted drug delivery. As another human symbiotic bacteria, *L. lactis* exhibits the tolerance to harsh gastrointestinal environments and the ability to colonize specific intestinal tissues. *S. typhimurium* enables tumor delivery by targeting hypoxic and necrotic areas of tumor, while the attenuated *S. typhimurium* can reduce the risk of septic shock, allowing it with an excellent safety. Unlike other bacteria, *L. monocytogenes* cannot replicate and spread in vivo and is often desirable as vaccine carriers. The bacteria can cross the blood-tumor barrier and even the blood-brain barrier in various ways: through transport across endothelial cells, by inducing cell damage or disrupting intercellular junctions, and in infected phagocytes (Fig. 3c, d)[82]. In general, the intracellular bacteria achieve transvascular transport through the pathways in infected phagocytes. Moreover, the extracellular bacteria can induce transcytosis of endothelial cells after adhesion to promote their delivery, or induce intracellular signaling pathways or produce cytolytic toxins leading to intercellular junction disruption or cell necrosis, thereby crossing the vascular barrier. Similar to cell-mediated delivery, inflammation also plays a central role in the transvascular transport of bacteria. The invasion of bacterial carriers will cause inflammation and result in the secretion of proinflammatory molecules, and this innate immune response can further increase vascular permeability (Fig. 3d)[83]. Meanwhile, bacteria are able to selectively colonize tumors due to the hypoxia and immunosuppressive tumor microenvironments, where bacteria circulating to normal tissues are eliminated, while those accumulated in tumors continue to proliferate[84]. However, the in vivo safety of bacteria-based systems is controversial due to the high proliferation and toxicity of bacteria, such as bacteremia. With the development of synthetic biology, gene engineering can be employed to knock out the major virulence genes to obtain attenuated strains (Fig. 3c)[85]. Additionally, different from pure bacterial carriers which will preserve a lot of residual bacteria after treatment, the residual bacteria can be effectively eliminated from the body after the treatment with bacterial-based systems[86]. A recent work indicated that the mineralized bacterial-based systems, i.e. the fixed bacteria coated with MnO_2 nanoparticles, display more efficient tumor immunotherapy and safer in vivo use compared to pure bacterial carriers[87].

Typically, in bacteria-based systems, the internalization is based on bacteria-specific ATP-binding cassette transporter or surface adhesion of NCs based on electrostatic interactions or covalent bonding (Fig. 3c)[84,88]. The former is a complete Trojan Horses system and can avoid the damage of surface adhered NCs to the bacterial capsule. In 2022, the attenuated facultatively anaerobic bacteria (*S. typhimurium* and *E. coli*) were utilized to uptake indocyanine green-based nanoparticles by the ATP-binding cassette transporter pathway[86]. The prepared bacterial-based system displays the hypoxia-targeting ability to overcome the blood-brain barrier and achieve deep penetration for enhanced glioma treatment. Excitingly, both bacteria and nanoparticles are present in significantly higher amounts in the brain compared to other organs, which may be due to the selective proliferation of bacteria in the unique glioma microenvironments including hypoxia, immunosuppression and biochemical properties. Upon the irradiation of near-infrared laser, the photothermal effect can ablate tumor cells and promote the release of tumor-associated antigens, while it also lyses bacterial cells and facilitates the release of various pathogen-associated molecular patterns, thus eliciting potent antitumor immunity. Besides, anaerobic bacteria possess the capability to specifically colonize the hypoxic tumor region as oxygen concentration gradients guide them to migrate towards hypoxic microenvironments[89].

Although the attenuated bacteria are capable of reducing the risk of septic shock in the host, any retained virulence may cause problems for immunocompromised patients[90]. In 2023, through inactivating with UV irradiation (Fig. 3c), a supposedly safe dead *E. coli*-based system was developed to improve tumor delivery of NCs without potential bacterial toxicity as they retain the intact structure and chemotaxis of *E. coli* while losing the capacity of proliferation and pathogenicity[91]. After intravenous injection, the dead *E. coli*-based system can efficiently deliver nanotherapeutics to the brain for the treatment of bacterial meningitis and glioma. More importantly, the dead *E. coli*-based system exhibits a high biosafety in vivo. Even with high injected dose (~1×10^9 CFU), all mice are still alive after 14 days. As a comparison,

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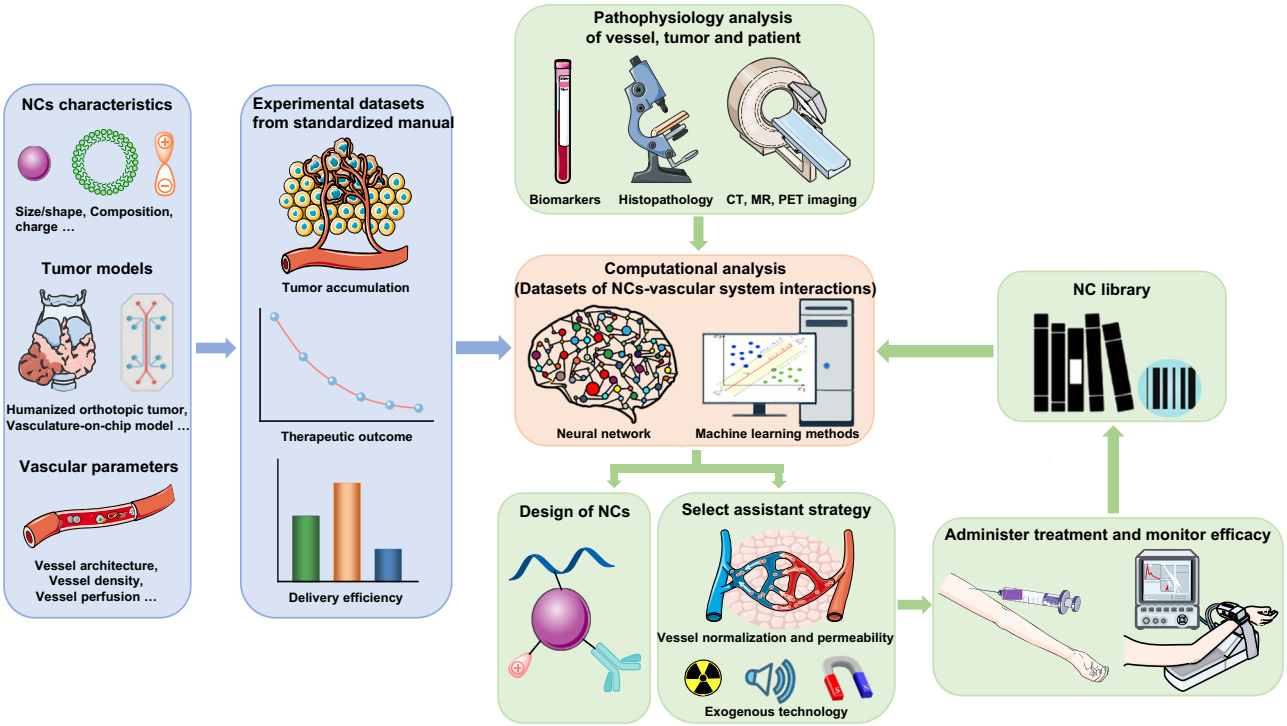

Fig. 4 | Dataset collection of nano-bio interactions using interdisciplinary tools. Statistics-based artificial intelligence (AI) analysis provides tools to correlate multiple parameters between nano-bio interactions, such as NC characteristics, tumor/vessel models, vascular parameters, tumor accumulation, delivery efficiency, and therapeutic outcome. A prospective guiding framework for personalizing the design of effective NCs for precise theranostics of specific tumors. First, the pathophysiology of tumor, vessel, and patient is analyzed, as well as the experiment datasets are from standardized manual. Second, the information and delivery goals are used as input of a computer algorithm, which is based on the established datasets of nano-bio interactions. Third, the specification of NC design and the selection of assistant strategy are the output. Fourth, injection of appropriate NCs dose with/without adjuvants is performed along with the clinical performance and therapeutic effects monitoring of NCs. Fifth, clinically validated NCs are incorporated into the library, and fed back to AI for dataset calibration.

when the injected dose of living *E. coli* is only 1×10^6 CFU, all mice are dead after 5 days owing to their severe pathogenicity.

It is worth mentioning that unlike conventional delivery systems, the engineering bacteria with vast gene packaging capacity can also in situ produce active drugs, such as nucleic acids, peptides, proteins, or enzymes for the treatment of tumors[92,93]. Nevertheless, the regulation of expression levels and extracellular delivery of these active drugs has not been effectively addressed. Further investigations are needed to integrate these therapeutically engineered bacteria with NCs for targeted delivery and theranostics of tumors. Moreover, a small amount of literature has shown that the accumulation of bacterial carriers in various organs in vivo (mainly liver and kidney) can be completed cleared within 1-2 weeks, but the systematic description of the whole process of bacterial fate in vivo is missing, which is also an issue that cannot be ignored for further clinical transformation of bacterial carriers.

Challenges of clinical translation

As our understanding of tumor delivery of NCs continues to evolve, innovative NCs have been meticulously engineered to surmount the vascular barrier. Regrettably, many of these studies merely serve as proof-of-concept demonstrations, lacking quantitative comparisons against existing methodologies. Consequently, it is likely that numerous newly developed delivery routes are not inherently more efficient than their predecessors. Furthermore, the bulk of research endeavors tend to focus solely on biomedical applications of NCs from the vantage point of their chemical design, neglecting the practical clinical requirements and engineering challenges associated with material preparation. This glaring disparity between the proliferation of nanoplatforms and their limited clinical translation has sparked intense debate[94]. Moving beyond the strategies discussed above, a change in research culture and advancement in key technologies may usher in a different guiding delivery framework, thereby catalyzing substantive progress in cancer nanomedicine (Fig. 4). Different from previously reported frameworks for designing delivery systems[95], the framework proposed here highlights the importance of assembling high-quality datasets and optimizing NCs-vascular system interactions through the utilization of machine learning and AI tools. Particularly, it emphasizes the need to account for tumor/vessel heterogeneity and patient stratification. Concurrently, this framework advocates for the formulation of standardized guidelines for nanomedicine and the establishment of an NC library to address issues such as poor reproducibility of data results and the repetitive implementation of validation work, respectively.

Tumor/vessel models and patient stratification

The complexity and heterogeneity of human tumors/vessels and preclinical animal models are a major cause of the poor clinical translation of NCs[96]. A recognized obstacle is the lack of tumor models that can recapitulate human cancers for preclinical nanomedicine research[97]. Currently, humanized orthotopic tumors are constructed in animal models to allow the investigation of NCs in more physiologically relevant tumor development, but their complexity hinders testing based on large screening[98]. It is encouraging that the developed organ-on-chip models may simultaneously address these challenges, including preclinical models to recapitulate human biology and tumor heterogeneity, as well as high-throughput testing[99,100]. In a recent work, a vasculature-on-chip model was engineered to dissect the role of flow rate and shear stress in vascular endothelial transcytosis of NCs[101]. In 2023, an AI-assisted single vessel quantitative analysis method (nanoISML) was developed and then quantified more than 67000 individual vessels from 32 tumor models, revealing the highly

heterogeneous transvascular transport of NCs[39]. This method can effectively classify the vascular permeability and assist in the rational design of NCs.

Moreover, patient stratification is also urgently needed to match the heterogeneity of cancer patients with specific NC formulations[102,103]. Some researchers are trying to establish the biomarkers for patient stratification[11,104]. For instance, immunohistochemistry is employed to assess specific or highly expressed biomarkers (e.g., receptors or antigens) on tumors and vessels. Besides, circulating tumor cell analysis can also be exploited for patient stratification. Medical diagnostic technologies, such as magnetic resonance, positron emission tomography, or single-photon emission computed tomography imaging, not only monitor the biomarker expression on tumors and vessels for stratification of metastatic patients, but also obtain the detailed information on the intuitive biodistribution and accumulation of the labeled NCs for screening responsive patients. A recent study proposed a method based on histopathological biomarkers that can predict the NC accumulation in tumors by scoring the density of tumor vessels and tumor-associated macrophages, thus providing a powerful and straightforward protocol for patient stratification in clinical trials[105]. However, the development of more diagnostic biomarkers and tools with precise sensitivity and universality for patient stratification is still a challenge in cancer nanomedicine[104]. It is expected that once the challenge is resolved, the clinical translation rate of nanomedicines will be greatly improved.

Standardization of preclinical research

The clinical translation of nanomedicine has been plagued by different synthesis methods, various physicochemical properties of NCs, inconsistent characterization in vitro/in vivo, and disappointing experimental reproducibility. To unify these operations, a standardized manual of nanomedicines should be drafted[106]. Although the Minimum Information Reporting in Bio-Nano Experimental Literature (MIRIBEL) provides some guidelines[107], there have been claims that it cannot meet the requirements of NC diversity and has therefore received inconsistent opinions[108]. Actually, the current literature, even in the same field, is lacking standardized guidance, which is detrimental to experiment data reproducibility, quantitative comparison, meta-analysis, and modeling.

Nano-bio interaction collection based on interdisciplinary tools

For the development of NCs in tumor delivery and theranostic applications, the current guiding framework is based on a trial-and-error strategy: 1) preparation of NCs, 2) characterization at cell level and animal models, and 3) evaluation of delivery efficiency and theranostic outcomes[95]. The standardized screening of NC myriad is unrealistic. Therefore, we should shift the focus from the preparation of versatile NCs (engineering perspective) to the exploration of basic relationship between NCs and vascular system interactions (biological perspective), which is critical to determine the optimal design of NCs for highly efficient tumor delivery[109,110]. Nevertheless, it is a complex set of interactions defined by multiple property parameters, such as NC characteristics[34,111], injected NC dose[112], tumor types[113], blood velocity[114], vessel architecture[95], vessel density[115], vessel perfusion[116], vessel permeability[53], and immune cell composition[117]. Examples to date have been limited to one or a few interaction between NCs and vascular system.

The integration of AI and big data management is able to facilitate multivariate research and complex relationship analysis to build predictive models for optimizing the design of NCs[118,119]. A prominent example is the exploitation of DNA barcoding and sequencing to achieve the testing data of a large number of NCs in same experimental animal[120,121]. Moreover, a high-throughput screening approach (nanoPRISM) was proposed to systematically evaluate the interactions between 35 different nanoparticle types (including core composition, surface chemistry and size parameters) and hundreds of cancer cell lines[122]. This approach can screen the key factors for cell internalization of NCs, thereby accelerating the rational design of NCs for specific cell types and reducing the requirement for preclinical animal experiments. Although this work only focused on cellular internalization of NCs, it is exciting that this approach can easily be expanded to investigate the relationship between NC properties and transvascular transport. For example, this approach is integrated with organ-on-chip models to simulate transvascular processes and then collect the nano-bio interaction data. Together, the development of these AI technologies will bring the promise of nanomedicine closer to reality. Moreover, such AI technology is supported by the new policies of the FDA[118], and the personalized tumor radiotherapy powered by AI has been clinically successful[123]. Therefore, the high-quality datasets of NCs-vascular system interactions defined by computer technology will provide a forward-thinking and brand-new guiding delivery framework, that is, to design personalized NCs and delivery strategies for specific patients[124,125].

NC library

Finally, an authoritative and publicly available library of nanomedicines should be created to collect the NCs that have been validated in preclinical and clinical trials. The researchers or industries should submit the information regarding NC formulations, tumor/vessel models, and implementation proposals. Furthermore, the independent third-party organizations and governments should validate NC properties, nano-bio interactions, delivery efficiency, and therapeutic efficacy according to the standard nanomedicine guidelines. Finally, the governments should establish a reliable NC library. The library will not only avoid a lot of repetitive verification work, but also guide the design of the next-generation NCs.

Perspectives

In summary, we are gaining a deeper understanding of the opportunities and challenges presented by transvascular transport and tumor delivery of NCs. Different insights into cancer nanomedicine will change the NC design and tumor theranostics, and further promote the clinical translation of NCs. First, the vascular basement membrane, as an insurmountable biological barrier, limits the EPR effect. For this long-neglected barrier, more engineered strategies need to be developed to overcome it. Besides, it is crucial to strike a balance between transvascular transport of NCs and transvascular metastasis of cancer cells, which will occur with the removal of basement membrane. An ideal strategy to overcome the basement membrane barrier is to enhance transvascular delivery while avoiding cancer metastasis. In addition, the EPR effect should not be considered as a dogma in tumor delivery of NCs, and the active transcytosis mechanism may be regarded as an important route for transvascular transport. However, for active transcytosis, a lot of unknown work needs to be explored to reveal the detailed endocytosis and transcytosis process (especially the biological role of N-TECs), as well as the interactions between NCs and vascular systems. Besides, the tumor progression and microenvironments also have an impact on transvascular transport of NCs[3]. For instance, during tumor progression, the extracellular matrix becomes highly unregulated and disorganized, leading to tumor fibrosis or tumor vascularization downregulation. The stromal cells and immunosuppressive environments of tumors play important roles in regulating angiogenesis-related markers and genes. By modulating tumor microenvironments, such as reducing the extracellular matrix, or decreasing the interstitial fluid pressure, or disrupting stromal cells, or optimizing immune cells, the vascular permeability can be improved and the transvascular transport of NCs can be facilitated. Remarkably, cell/bacterial-mediated delivery provides the pathways to cross the vascular barrier and overcome the long-standing bottleneck of NC delivery into tumors. Meanwhile, the detailed research is needed on the unique functions of different types of cells or bacteria as well as

their mechanisms of targeted tumor delivery. These emerging bioinspired strategies may be essential to drive further progress of cancer nanomedicine. When choosing or designing one suitable strategy to improve transvascular delivery, the following four aspects should be considered: 1) the biosafety and clinical accessibility, 2) the precise targeting of tumor vascular endothelium, 3) the efficiency of transvascular transport, and 4) the inhibitory effect of cancer transvascular metastasis.

In addition, the guiding framework for NC delivery into tumors still remains changing. For the dilemma of tumor/vessel complexity and heterogeneity, the protocols for patient stratification are urgently needed to select the beneficial patients for nanotheranostics. Generally, the biomarkers and clinical imaging techniques can be employed to screen for responsive patients, thereby optimizing the therapeutic efficacy of nanomedicines. In preclinical experiments, animal models should be added on humanized orthotopic tumor/vessel models. Moreover, the development of organ-on-chip models can not only obtain preclinical models that are close to human physiology, but also reveal and collect a large amount of data on NCs-vascular system interactions by high-throughput testing. Meanwhile, the integration with machine learning and AI technologies enables the execution of complex multiparameter studies, thereby establishing predictive models to guide the design of NCs. The implementation guidelines of MIRIBEL would in a first attempt able to standardize preclinical research of nanomedicine, and thus facilitating experiment reproducibility, quantitative comparisons, and meta-analyses. Still, in the future a more widely agreed-on guideline would be helpful. Many of these approaches are performed according to a trial-and-error strategy that verifies the effectiveness of the designed NCs through a complicated experimental process. Recently, we have re-examined the focus on tumor delivery of NCs from the engineering to the biological perspective. A forward-looking guiding framework is proposed based on the collection and analysis of complex interactions of NCs-vascular systems by the interdisciplinary tools and developments, including advanced tumor/vessel models, high-throughput NC libraries, big data management, and AI. Once sufficient high-quality nano-bio interaction datasets are established, one can guide the design of improved NC properties and delivery strategies by computer algorithms according to the biological indicators of a specific cancer patient. We can foresee that these in-depth recognitions of NC delivery into tumors can facilitate the transition of nanomedicines from bench to bedside.

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Author contributions

X.L., X.Z., W.P. and A.P. conceived the manuscript format. X.L. and A.P. wrote the initial manuscript. X.L. designed the figures. All authors edited the manuscript.

Competing interests

The authors declare no competing interests.

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