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Strategies for advancing cancer nanomedicine

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Abstract

Cancer nanomedicines approved so far minimize toxicity, but their efficacy is often limited by physiological barriers posed by the tumour microenvironment. Here, we discuss how these barriers can be overcome through innovative nanomedicine design and through creative manipulation of the tumour microenvironment.

In 1986, two independent studies identified intriguing mechanisms for selective delivery of macromolecules to tumours — tumour vessels are more permeable to large molecules than many normal vessels¹, and tumours retain large molecules because of poor clearance². The underlying causes have since been elucidated as physiological abnormalities of tumours. Tumour vessels form under excessive pro-angiogenic signalling without physiological anti-angiogenic signalling, making them immature and leakier than vessels in surrounding healthy tissue^{3–6}. This leakiness is generally a result of large intercellular openings between endothelial cells rather than transcellular fenestrations^{7,8}, and these openings exist because of impaired recruitment of pericytes (support cells) to tumour vessels⁵. The poor clearance results from lymphatic dysfunction in tumours⁹. Intratumoural lymphatics are compressed to the point of collapse¹⁰ as a consequence of physical forces arising from cancer and stromal cell proliferation and transmitted by the tumour interstitial matrix^{11,12}. Clearance still occurs through blood vessels and peritumoural lymphatics¹³, but at a sluggish rate. In the decades since the original phenomenological discoveries, this enhanced permeability and retention (EPR) effect has been demonstrated as a key pharmacokinetic feature of nanomedicines, providing these agents with selective delivery properties that reduce their toxicity in normal tissues¹⁴.

Unfortunately, the promise of nanomedicine for cancer treatment has not been realized in patients. Although direct evidence for the EPR effect in humans is lacking, ample data

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demonstrate that the same pathophysiology that gives rise to the EPR effect in animal models also characterizes tumours in humans. Indeed, human tumour vessels are immature and hyperpermeable^{3,4}, and human tumour lymphatics are dysfunctional¹³. As a result, all human tumour types characterized so far display elevated interstitial fluid pressure¹⁵ — largely confirming the existence of the EPR effect in patients. Yet while the three Food and Drug Administration-approved nanomedicines for cancer — PEGylated liposomal doxorubicin (Doxil/Caelyx), liposomal daunorubicin (DaunoXome) and nanoparticle albumin-bound paclitaxel (Abraxane) — reduce toxicity compared with conventional chemotherapeutics, these nanomedicines only modestly improve the overall survival of patients (Supplementary Table 1)¹⁶. Thus the question is not whether the EPR effect works in humans, but whether it is sufficient to significantly enhance survival of cancer patients.

The human tumour microenvironment is remarkably abnormal, and it poses several barriers to nanomedicine delivery that cannot be overcome by the EPR effect alone (reviewed in refs 3,17). In tumours, perfusion (that is, blood supply) is low, blood flow is unevenly distributed, vessel permeability is heterogeneous and the microenvironment is often dense — all of which combine to limit the supply, penetration and distribution of nanomedicines in tumours. In this Commentary, we discuss how these barriers can be overcome through innovative nanomedicine design and through use of adjuncts to modulate the tumour microenvironment.

Tailoring materials to specific tumours

The properties of the tumour microenvironment vary widely based on tumour type, location and stage of progression. Yet nanomedicine has taken a ‘one-size-fits-all’ approach thus far, in which each nanomedicine is used clinically in multiple cancer types (Supplementary Table 1). Meanwhile cancer care is undergoing a bioinformatics revolution; gene and transcript data for major cancer types and subtypes have been mapped and are being used to design targeted therapies that are tailored to each patient. To advance cancer nanomedicine, a similar approach must be taken to optimize drug-carrier design for the specific physiological properties of each tumour microenvironment.

Tumour location and cancer type

Once injected into the bloodstream, nanoparticles must disseminate by means of each tissue’s blood supply, penetrate across microvascular walls and then distribute throughout the extravascular tissue^{18,19} (Fig. 1a). Each microvasculature has a characteristic pore-size distribution²⁰. These pore sizes provide each microvessel with a particular permselectivity, quantified by a ‘cutoff size’ — that is, the largest particle that can penetrate from the blood, across the vessel walls, into the tissue. Furthermore, these vessel-wall pore sizes determine how rapidly particles that are not excluded can penetrate into tumours, quantified by the effective permeability — a measure of particle mass flux across the vessel wall¹⁹. These parameters for normal microvasculature vary substantially by tissue (reviewed in ref. 21), and each tumour type has its own range⁸. Also, because tumour blood vessels are derived from normal microvessels, tumour vessels are influenced by characteristics of the

Supplementary information is available in the [online version of the paper](#).

surrounding tissue^{3,5}. Tumour vessels are more permeable to larger particles than most normal microvessels^{22,23}, but the exact pore cutoff size varies with organ site. For example, the pore cutoff size for a breast or pancreatic tumour may be ~50–60 nm versus ~5 nm for normal breast or pancreatic tissue^{24–26}, whereas in a brain tumour it might be ~7 nm versus less than 1 nm for normal brain tissue⁸. Simply because a particle can extravasate into a tumour does not mean that the extravasation is uniform from all tumour vessels. Cutoff sizes only indicate the largest particle that penetrates anywhere in the tumour, and large particles generally penetrate tumours heterogeneously compared with smaller particles²³ (Fig. 1b,c). Thus, the effective vascular permeability of small particles does not correlate with cutoff size⁸. This disconnection occurs because the vascular pore-size distribution within a single tumour can vary by orders of magnitude, with most of the pores actually being much smaller than the pore cutoff size⁷. As a result, the pore-size heterogeneity of a specific normal tissue and a tumour growing in it are critical considerations for nanomedicine design.

Matching cancer and particle type

Nanomedicines must penetrate the vessel walls and tissue matrix of tumours to reach target cells^{18,19} (Fig. 1a). These can be modelled as a series of interconnected pores with variable cross-sections. The pore sizes vary by cancer type such that one nanomedicine may not be optimal for all diseases. Tumour penetration of nanoparticles is slowed by concerted steric, hydrodynamic and electrostatic hindrances in these pores^{27,28}. Therefore, smaller particles penetrate tumours more rapidly and uniformly than larger particles^{22,24,29}, and smaller particles carrying drugs are more effective against tumours than larger particles^{25,30,31} (see Supplementary Table 2). On the other hand, very small particles (less than 11 nm) are eliminated more quickly by renal and hepatobiliary clearance^{32,33}, and larger particles are efficiently cleared by the mononuclear phagocytic system (MPS), often called the reticuloendothelial system, in many tissues^{34–36}. Thus the choice of nanomedicine size for a cancer type involves finding a balance between maximizing tumour penetration (by reducing nanomedicine size) while minimizing both toxicity to normal tissue and clearance by the MPS (by increasing nanomedicine size).

Meanwhile non-spherical, for example rod-like or disc-shaped, nanoparticles can penetrate tumours more rapidly³⁷ and accumulate at higher levels than size-matched spheres^{38,39}, apparently because of enhanced penetration through the pores as a result of the shortest dimension of the particle³⁷ (see Supplementary Table 3 for additional references). Interestingly, the advantage of non-spherical particles holds for tumours with smaller vessel-pore-sizes but is lost in tumours with large pore sizes⁴⁰. Moreover, particles that are very small in their shortest dimension (for example, the diameter of a rod) also clear more rapidly by renal filtration⁴¹, and particles that are large in their longest dimensions (for example, the length of a rod or face of a disc) are cleared more efficiently by the MPS⁴² unless they are flexible⁴³. Therefore optimizing nanomedicine shape may require tuning the shortest dimension for a balance between maximizing penetration and minimizing renal clearance, while modulating the longest dimensions to minimize clearance through the MPS.

Modulating surface chemistry can have complex effects on delivery to tumours and needs to be elucidated further. Cationic nanoparticles may penetrate tumours more than neutral or

anionic particles⁴⁴ as a result of attractive electrostatic forces between cationic particles and anionic endothelial glycocalyx⁴⁵. Yet neutral or anionic particles distribute throughout tumours more effectively than cationic particles⁴⁶ because of minimized binding to anionic matrix molecules²⁸. Thus, the ideal charge for maximal tumour accumulation varies greatly with tumour type^{46,47} (see Supplementary Table 4 for additional references). Finally, modulating surface antigen display can have major effects on MPS clearance. Decorating nanoparticles with poly(ethylene glycol)^{48,49} or minimal self-antigen⁵⁰, for example, greatly decreases interactions with MPS cells to reduce clearance.

To tailor nanomedicines to specific cancer types we must understand how particles of varied size, shape and surface chemistry interact with each type of tumour microenvironment. We need to test how these parameters affect the penetration of and clearance from tumours in each tissue of the body, both in primary and metastatic settings. Most importantly, we must gain an understanding of how these physicochemical properties affect nanomedicine delivery in human tumours. These studies will require the development of highly configurable probes for *in vivo* imaging in animal models and in patients. Developing this understanding of nanoparticle–microenvironment interactions is a crucial step towards realizing the full potential of cancer nanomedicines.

Targeting the tumour microenvironment

Targeted nanomedicine — the use of a targeting ligand for tumour-abundant receptors and/or drug release specifically in tumours — could greatly enhance the efficacy and safety of chemotherapy. The potential of this approach is demonstrated by the approval of an antibody–drug conjugate that is extraordinarily effective in patients⁵¹. The use of a targeting ligand (that is, active targeting) can improve chemotherapy retention in tumours or, similar to antibody–drug conjugates, can increase specific drug uptake in targeted cancer cells¹⁴. Meanwhile, engineering nanomedicines to release drugs only in the tumour microenvironment can ensure that chemotherapeutics are only active in tumours⁵². Together, these targeting approaches can greatly enhance drug biodistribution to tumours, going beyond the passive EPR effect.

Challenges for a targeted approach

There are many challenges for targeted nanomedicine (reviewed in ref. 53), some of which are posed by the tumour microenvironment itself. The distribution — via blood — of drugs throughout a tumour suffers from heterogeneous blood flow⁵⁴ caused by collapse- or hyperpermeability-induced flow stasis of as many as 80% of vessels in tumours^{10,55,56}. Tumours therefore exhibit large intervascular distances and correspondingly long interstitial path lengths that a drug must traverse to reach its target cells⁵⁷ (Fig. 1d). These paths are blocked by dense matrix and cells^{58–60}, such that nanoparticle distribution away from blood vessels is greatly hindered^{23,61,62} (Fig. 1c). Furthermore, there is a ‘bindingsite barrier’ due to this long, slow transport, where targeting ligands bind to the first receptors they encounter upon leaving the blood⁶³, which may even be on normal cells⁶⁴, and targeted nanoparticles may hardly reach cells that are distant from vessels^{65,66} (Fig. 2a). Free drugs also suffer from a binding-site barrier of specific or non-specific binding sites and sequestering by tissue components (for example, nucleic acids, endosomes, organelles, matrix molecules,

cytoskeletal components)⁶⁷. As a result, chemotherapeutics may distribute less than 100 μm away from their source of release by nanoparticles in the vessels or tissue^{67–69}. Furthermore, the structure of tumour tissues varies widely such that the first cells a nanomedicine encounters on extravasation are often not cancer cells (Fig. 2a). Blood vessels are mostly situated among cancer cells in some cancers (such as kidney, brain, liver, thyroid, ovarian, and head and neck cancers), whereas vessels are surrounded by stroma — non-cancer cells and matrix — in other cancers (such as breast, pancreatic, colorectal and non-small-cell lung cancers)⁷⁰. In these desmoplastic (that is, stroma-rich) tumours, nanomedicines must penetrate up to hundreds of micrometres through stroma to reach cancer cells.

Importance of tumour microenvironment properties

To overcome the binding-site barrier, distribution and binding rates must be carefully balanced⁷¹. Fast-diffusing particles (for example smaller, non-spherical particles) and low-affinity ligand–receptor pairs may therefore be required for targeting to provide nanomedicines an advantage⁶⁵. Targeting of mutant or truncated receptors unique to cancer cells, rather than receptors that are merely overexpressed on cancer cells⁶⁵, would also enable evasion of the binding-site barrier. To prevent released chemotherapeutics from being limited by the binding-site barrier, nanomedicines should release drugs only once they are distributed away from blood vessels. The tumour microenvironment generally becomes more acidic and hypoxic farther away from blood vessels⁷², and this physiology can result in the expression of specific enzymes in these regions (Fig. 2b). Nanomedicines that release active drug and/or smaller drug carriers in a ‘smart’ manner, with kinetics that are modulated by these tumour microenvironment characteristics, could enhance both tumour localization and intratumour distribution of chemotherapeutics^{52,73–76}.

Targeting approaches must also be tailored to tumour composition and structure of each cancer type. Non-desmoplastic (stroma-poor) tumours may be amenable to cancer-cell targeting for enhanced cellular drug uptake, whereas the goal should be improved retention through stromal targeting in desmoplastic tumours where nanoparticles cannot reach cancer cells. In either case, cancer cells may not be the best targets because of heterogeneous expression of targeted molecules — a major challenge for all targeted therapies in the clinic. Similarly, targeting blood vessel cells — endothelial cells or pericytes — may aid vascular-disruption therapy and/or enable rapid transcytosis of nanoparticles across tumour vessel walls^{4,19,77}, although heterogeneous expression of vascular targets may compromise their efficacy. Other tumour stromal cells (for example cancer-associated fibroblasts and immune cells) in contrast, can express targetable receptors more homogeneously and may enable improved drug retention⁷⁸.

For targeted nanomedicine to achieve clinical success we must understand the structure and biochemical properties of each tumour’s microenvironment along with the expression and distribution of targetable receptors. We need to test how different targeting ligand–receptor combinations affect the penetration, distribution and clearance from each tumour type. Furthermore, we must develop new mechanisms for triggering drug release specifically in the tumour microenvironment. Fulfilling these requirements will require extensive biochemical and molecular biological characterization, along with the development of

advanced chemical synthesis techniques. Building this knowledge and technology base would greatly enhance our ability to selectively target and treat cancer.

Normalizing the microenvironment

Each barrier to drug delivery results from a pathophysiology that is characteristic of tumours. Repairing, or normalizing, these abnormalities can increase the delivery and efficacy of chemotherapeutics, including nanomedicines. This strategy has been shown to enhance the efficacy of chemotherapy, radiation therapy and immune therapy in patients through a variety of mechanisms³. As many of the same barriers that hinder conventional chemotherapy also affect nanomedicines, normalizing therapies show great promise for combination with nanomedicine. Such therapies are especially attractive because they address barriers that cannot be overcome through nanomedicine design alone.

Several strategies for normalizing the tumour microenvironment have been developed for enhancing cancer therapy (reviewed in ref. 3), and each improves delivery differently. Vascular normalization involves correction of excessive angiogenesis signalling to repair abnormalities in vascular structure and function¹⁵ (Fig. 3a). Vascular normalizing therapies, such as anti-angiogenics, shrink vessel pores²⁴, reduce interstitial fluid pressure³ and increase perfusion⁷⁹. As a result, vascular normalization improves the supply of nanomedicines to tumours while also increasing their effective permeability²⁴, although it preferentially benefits smaller rather than larger nanomedicines^{24,80}. Importantly, vascular normalization can enhance the efficacy of smaller nanomedicines²⁴. Stress normalization is the reduction of physical forces (known as solid stresses) in tumours to decompress tumour blood vessels and restore blood flow in these vessels⁵⁶ (Fig. 3b). Stress normalization involves the targeting of stromal cells and matrix molecules such as collagen and hyaluronan, which contribute to solid stress in tumours¹². Depleting cancer-associated fibroblasts¹², degrading the matrix^{12,26}, or inhibiting cancer-associated fibroblast activity⁵⁶ can reduce solid stress to decompress vessels, improve perfusion, increase drug delivery and enhance chemotherapy efficacy. Because stress normalization improves delivery by increasing perfusion it can be used with all types of therapeutic regardless of size. Finally, matrix normalization therapies modulate matrix structure to increase nanoparticle diffusion rates and improve fluid flow through tumour tissue^{17,58} (Fig. 3c). Degrading the matrix^{58,62}, modifying matrix organization⁸¹, or reducing matrix production^{25,82} can therefore increase nanoparticle distribution. Matrix normalization benefits the delivery of larger nanomedicines the most^{25,56}, as they are more hindered by the matrix⁶¹. Importantly, matrix normalization can enhance the nanomedicine efficacy^{25,82}. Intriguingly, these normalization strategies may be combined for a pronounced improvement in nanomedicine delivery, distribution and efficacy⁸³.

Conclusions

Cancer nanomedicine has been a story of incremental improvements of patient survival so far as a consequence of a reliance on the EPR effect, which may be insufficient to overcome barriers to nanomedicines in tumours. We propose that nanomedicines should be specifically tailored to the characteristics of the tumour microenvironment in each cancer and metastasis

type such that there is a nanomedicine specialized to the challenges of each disease. Advanced targeting mechanisms and tumour-selective drug-release kinetics, which can be achieved through careful consideration of these microenvironment properties, must also be implemented. Finally, nanomedicines should be combined with therapies that manipulate the tumour microenvironment through normalization approaches, which can overcome barriers that advanced design of nanomedicines cannot. Through these strategies we believe that the nanomedicines of the future could be far more effective cancer drugs than those of the present.

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Figure 1.

The transport barriers to drug delivery in tumours. **a**, Drugs enter a tumour through its blood supply. These drugs — ranging in size from a few ångströms to roughly 100 nm — must then cross the blood vessel walls (that is, extravasate) to penetrate into tissues. As convection is negligible in large regions of tumours, these drugs must then diffuse through the extravascular space. As the drugs distribute through the extravascular space they may eventually reach their target cancer cells. **b**, In tumours, the pores of blood vessel walls range from roughly 1 nm up to hundreds of nanometres in diameter. As a consequence of this spatially non-uniform permeability, nanoparticles extravasate only from some regions of tumour vessels, and when they do, they penetrate only a few cell layers into tumours. **c**, Matrix molecules such as collagen can form a barrier that limits nanomedicine distribution through the interstitial space. These matrix molecules create pores of roughly 10 nm to hundreds of nanometres and can sterically block particles from regions of tumours or can

impede these particles through hydrodynamic and electrostatic interactions. **d**, The blood supply to tumours is spatially and temporally heterogeneous. Blood flow in some regions of tumours is quite brisk, while impaired in other regions because of vascular compression and excessive leakiness. As few as 20% of the blood vessels in a tumour may actually carry blood flow, making the distances a drug must travel to reach cancer cells up to hundreds of micrometres. Figure reproduced with permission from: **b**, ref. 23, © 1994 AACR; **c**, ref. 62, © 2006 AACR; **d**, ref. 56, © 2013 NPG.

Figure 2.

The complex structure of the tumour microenvironment. **a**, Tumours may be ‘desmoplastic’ (rich in stromal cells and extracellular matrix) or ‘cellular’ (largely composed of cancer cells). Targeted nanomedicines or their drug cargo can bind, specifically or non-specifically, to components of both tumour classes including cells and matrix molecules, which can retard their movement into tumours. The nature of this binding-site barrier therefore varies by cancer type. **b**, The properties of the microenvironment change with increasing depth away from functional blood vessels. Oxygen levels decrease with depth, and this problem is exacerbated by low perfusion, resulting in hypoxia. Excessive carbon dioxide and lactic acid production lead to low pH away from blood vessels. As a consequence of increasing cancer-cell density and hypoxia, the concentrations of various enzymes also vary with depth away from vessels. These factors can be exploited to trigger targeted drug release deep in tumours.

Figure 3.

Normalizing the tumour microenvironment to improve drug delivery and efficacy. Drug-delivery barriers resulting from tumour pathophysiology cannot all be overcome by nanomedicine design. Nanomedicines can be combined with therapies that normalize these physiological abnormalities for enhanced anti-tumour efficacy. **a**, Vascular normalization repairs blood vessels, making them more mature, more homogenous and less leaky. This lowers interstitial fluid pressure which restores a transvascular fluid pressure difference that results in improved blood flow and convective nanoparticle penetration in tumours. **b**, Stress

normalization reduces solid stress, the mechanical stress that compresses tumour blood vessels, to restore perfusion throughout tumours. This increases the supply of drugs, such as nanomedicines, throughout tumours. **c**, Matrix normalization modulates the structure of matrix molecules such as collagen, reducing their hindrance to nanomedicine distribution. This results in a more uniform distribution of nanomedicines in tumours. Depending on the extent of matrix depletion and reorganization, matrix normalization can potentially also normalize solid stress and lead to increased perfusion in addition to improved matrix penetration.