Drug delivery: Beyond active tumour targeting

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Abstract

Despite improvements in our understanding of cancer and the concept of personalised medicine, cancer is still a major cause of death. It is established that solid tumours are highly heterogeneous, with a complex tumour microenvironment. Indeed, the tumour microenvironment is made up of a collection of immune cells, cancer-activated fibroblasts, and endothelial cells and in some cases a dense extracellular matrix. Accumulating evidence shows that the tumour microenvironment is a major barrier for the effective delivery of therapeutic drugs to tumour cells. Importantly, nanotechnology has come to the forefront as highly effective delivery vehicles for therapeutic agents. This perspective will discuss how nanomedicine can be used to target and deliver therapeutic drugs specifically to tumour cells. Moreover, emerging opportunities to modulate the tumour microenvironment and increase the delivery and efficacy of chemotherapy agents to solid tumours will be highlighted.

From the Clinical Editor: Improving drug delivery to treatment resistant tumors is a major target of many nanomedicine-based applications. This comprehensive review discusses the currently available and emerging opportunities, in addition to discussing tumor microenvironment modulation to facilitate efficient delivery.

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Conventional chemotherapeutics have a greater effect towards cancer cells as compared to normal cells (also known as therapeutic index), however they can cause severe side effects related to their inability to exclusively target cancer cells. Unfortunately, systemic delivery of chemotherapeutics can be highly toxic to certain subpopulations of normal cells including cells in the stomach, bowel, mouth, hair follicles and blood thereby causing side effects such as nausea, diarrhoea, weight loss, mouth sores, hair loss, low blood counts and a compromised immune system. One strategy to circumvent this lack of specificity has been the development of second generation “molecularly targeted” chemotherapeutic agents. However, similar to their first generation counterparts, many of these second generation drugs are hydrophobic making formulation for systemic delivery difficult; additionally, they suffer from rapid clearance, nonspecific biodistribution, and rapid degradation and have thus largely failed in their quest for enhanced efficacy combined with reduced systemic toxicity.\textsuperscript{1-3}

In the past few decades, nanomedicine, the exploitation of the unique properties of nanoscale and nanostructured materials in medical applications has been explored extensively as a promising strategy in the advancement of anticancer therapies with the ability to overcome many of the limitations common to chemotherapeutic agents.\textsuperscript{2,4} Nanoparticles have the potential to improve the biodistribution of chemotherapy drugs by protecting them from degradation, delivering them directly to the tumour site and/or preventing them from affecting healthy tissues. Nanomedicine has seen the incarnation of a handful of nanoparticle–chemotherapy drug formulations approved for clinical use, the most well-known being Doxil (doxorubicin long circulating liposomes) and Abraxane (albumin-taxol nanoparticles), with several additional formulations currently in clinical trials.\textsuperscript{2,6} The majority of these formulations have not necessarily resulted in enhanced efficacy, but have made definite improvements in reducing toxic side effects and increasing maximum tolerated dosages.\textsuperscript{3}
Nanoparticles ranging in size from 1 to 1000 nm have been designed as drug delivery vehicles from a wide variety of materials including lipid based amphiphiles \(7-9\) (liposomes, hexosomes, cubosomes), metallics \(1,10,11\) (iron oxide, gold), carbon nanotubes, \(1\) mesoporous silicates, \(12\) or polymers \(1,2,13\) (polymer based micelles, drug carriers, dendrimers) depicted in Figure 1. These systems are designed such that chemotherapeutics can be either physically encapsulated within or chemically conjugated to the nanoparticle.

Figure 1. Different types of nanocarriers for drug delivery. (A) Lipid based systems: composed of amphiphiles which self-assemble into liquid crystalline phases which can be dispersed into nanosized particles. Hydrophobic drugs can be encapsulated into hydrophobic regions. (B) Metallic nanoparticles: commonly gold or iron oxide based systems which can be surface modified and encapsulate drugs. (C) Nanotubes: carbon cylinders composed of benzene rings capable of drug encapsulation. (D) Polymeric nanocarriers: drugs can be encapsulated in or conjugated to random coil polymers. (E) Polymeric micelles: amphiphilic block copolymers that self-assemble in aqueous solution into a core shell structure. The hydrophobic core can be loaded with hydrophobic drugs whilst the hydrophilic shell makes the system water soluble and stabilises the core. (F) Dendrimers: composed of multiple highly branches monomers emerging from a central core. Drugs can be conjugated or complexed to these systems. Delivery devices listed are non-exclusive and are not to scale.

Tumour targeting

Direct targeting of solid tumours can occur via both passive accumulation and active targeting, approaches which have been shown to be valid in both preclinical and clinical studies. \(1,3,4,14\)

Passive accumulation exploits the pathophysiological properties of the tumour vasculature which is generally highly disorganised with enlarged gap junctions between endothelial cells and compromised lymphatic drainage allowing for the extravasation of nanocarriers with sizes up to several hundred nanometres. Molecules of this size cannot pass through the tight junctions that exist within the endothelial cell lining of the vessels of healthy tissues \(\text{Figure 2}\). \(1,6,13-15\) Passive accumulation of particles is largely dependent on the ability of a drug nanocarrier to exhibit an increased circulation lifetime resulting in enhanced accumulation at the target site (also referred to as the enhanced permeability and retention effect). Circulation time is dictated by the nanoparticle physicochemical properties (size, charge, biodegradability, solubility, shape, rigidity), which can be easily manipulated in the majority of the delivery systems described. \(1,13\) The most common modification used to evade macrophage capture and increase circulation time is accomplished by making the nanoparticle surface hydrophilic through the addition of polyethylene glycol (PEG) and/or its derivatives to the nanoparticle surface. \(2,5,15,16\) The use of polysaccharides as an alternative to PEG has been promising as similar to PEG, they provide steric protection against nonspecific protein adsorption resulting in nanoparticle stability when in circulation. For instance, Goncalves et al demonstrated increased stability and increased blood circulation time for self-assembled polymer micelles consisting of dextrin vinyl acetate with hexadecane grafted side chains. \(17\) Similarly, galactosylated chitosan-5-fluorouracil nanoparticles exhibited increased stability, circulation time and enhanced efficacy \textit{in vivo} \(18\) with similar results obtained for galactosylated chitosan grafted polyethylenimine/DNA nanoparticle complexes. \(19\) Furthermore, polysaccharides possess functional groups which can be easily conjugated to bioactive molecules such as peptides, antibodies or proteins thereby imparting biofunctionality within the nanoparticle beyond simple drug delivery capabilities. \(17-20\) The majority of the nanoparticle-drug formulations used clinically and in
development rely mainly on passive accumulation at tumours, and thus imparting “stealth” properties through the inclusion of such hydrophilic polymers and biopolymers to increased circulation time is vital to their success.

As a means of increasing recognition of target cells by nanoparticles, active targeting has been implemented. Active targeting utilises target ligands such as peptides or antibodies that bind to molecules specifically expressed or overexpressed on target cells. Thus, active targeting does not actually improve overall accumulation at the tumour site, but rather enhances cellular uptake of the particles following their passive extravasation due to the leaky vasculature.\textsuperscript{2,3,21} Transferrin and folate ligands are two examples of commonly used active targeting moieties in nanomedicine formulations targeting tumours.\textsuperscript{22,23} The only clinically approved actively targeted nanomedicines are antibody-drug conjugates used in the treatment of leukemias and lymphomas.\textsuperscript{3,24} Currently, no actively targeted nanoparticle formulations are approved for clinical use, with only a small number in clinical trials.

Despite the ample evidence and extensive research effort supporting the benefits of both passively and actively targeted nanomedicines in the treatment of cancer, clinically, both strategies have met with only moderate success. This is likely due to the fact that the complexity of the tumour microenvironment (tumour heterogeneity, vascularity, location) is commonly overlooked and will have a major effect on nanoparticle extravasation, accumulation, and penetration into the tumour. The tumour microenvironment is highly heterogeneous in composition with as much as half of its volume occupied by non-cancerous cells and dense extracellular matrix (Figure 3).\textsuperscript{3,14} Furthermore, the hyperpermeable nature of the tumour vasculature, whilst being ideal for allowing nanoparticles to enter into tumour tissue, also allows fluid to leak from the vessel into the tumour microenvironment thereby causing extraordinarily high interstitial pressure throughout the tumour interior.\textsuperscript{3,14,25} The interstitial pressure tends to increase with increasing tumour volume and remain lower in the outermost areas of the tumour. Finally, malignant cells within solid tumours tend to be tightly packed and heterogeneous in nature.\textsuperscript{3,14} Thus, whilst the leaky nature of tumour vessels can promote nanoparticle deposition and accumulation, the microenvironment creates a number of barriers which prevent these delivery systems from effectively accessing tumour cells and thus reaching their full potential as the “magic bullets” of anticancer therapies.

Another aspect of nanoparticle delivery that is sometimes overlooked upon translation from \textit{in vitro} to \textit{in vivo} studies is alterations to nanoparticle surface chemistries in response to the proteins which adsorb to the particle surface creating what is termed the “protein corona”. The composition of the protein corona can have an effect on cellular uptake, inflammation, accumulation, degradation and nanoparticle clearance. Furthermore, the composition and conformation of the adsorbed proteins can influence the overall bioactivity of the nanoparticle.\textsuperscript{26} The physicochemical characteristics of nanoparticles such as their chemical composition, hydrophobicity, pH, presence of functional groups, size and shape have all been shown to affect surface protein adsorption. The different role of these physicochemical properties on protein corona composition as well its influence on biological interactions has been reviewed extensively elsewhere.\textsuperscript{26,27}

### Beyond tumour targeting

As the understanding of how the tumour microenvironment prevents nanoparticles from accessing their targets improves, strategies are being developed to bypass these barriers. While the initial research effort into the utilisation of nanoparticles as drug delivery vehicles focused mainly on passive and active targeting of tumours, in more recent years, studies have begun to design
One strategy that has been employed which can circumvent many of the barriers encountered by nanoparticles upon extravasation from the tumour vessels is to target nanoparticles to the tumour vasculature. Tumour blood vessels tend to express or overexpress cell surface and extracellular matrix proteins that are either not present or present only at low levels in normal vessels making them ideal as potential targets. Since the luminal surface of tumour vessels is completely accessible to circulating compounds, nanoparticles targeting the tumour endothelium can bind to their target molecules without the need to penetrate into the tumour to deliver their contents. This feature eliminates the problems associated with passing through the multiple layers between the endothelium and the tumour cells, penetrating the high cell density common within tumours, obstruction from the extracellular matrix, and high interstitial pressure. Tumour vessel targeting has been accomplished through the addition of antibody fragments and peptides which bind to tumour vessel associated extracellular matrix proteins such as the EDB domain of fibronectin and the fibrin-fibronectin complex, as well as peptides designed to bind specifically to receptors and molecules which are highly expressed on tumour endothelial cells such as certain integrin receptors, aminopeptidase-N (CD13) and nucleolin.

While there are some obvious benefits to this strategy, it is not a suitable option for avascular or poorly perfused tumours. Furthermore, chemotherapeutics delivered within the local tumour vessel environment via nanoparticle targeting to tumour vessels can still suffer from poor intratumoral distribution. Studies have shown that chemotherapy drugs themselves generally only penetrate about three to five cell diameters in from the blood vessel, with little to no drug reaching distant tumour cells and this can result in the development of drug resistance.

The ability of nanoparticles to carry their contents deep within the tumour would be of huge benefit to enhancing the efficacy of chemotherapeutics. Certain active tumour vessel targeting peptides have been suggested to possess tumour penetrating properties as well. Ruoslathi et al demonstrated the addition of a cyclic iRGD peptide sequence to the nanoparticle surface resulting in a system which first targets integrin receptors in the tumour vasculature via the RGD motif. Upon binding, the sequence undergoes proteolytic cleavage of the peptide exposing a new binding motif specific for neutrophilin-1 allowing for deep penetration of the tumour tissue. Further studies demonstrated that combining the CendR (the R/KXXR/K peptide motif) tumour penetrating aspects of iRGD with a tumour homing peptide (NGR) to create the iNGR peptide imparted superior tumour homing and penetrating properties. These and other peptides with tumour homing/penetrating properties have been identified via phage display highlighting the need to for a cross disciplinary approach towards the development of advanced nanodelivery systems.
In addition to the discovery of moieties which can home to and assist in tumour penetration, studies have also focused on developing an understanding of how nanoparticle characteristics such as size, shape, and surface properties affect their ability to penetrate into tumours. It has been demonstrated in vivo that only extremely small particles (those with diameters of 20 nm or less) can properly penetrate into tumours. Unfortunately, nanoparticles within this size range will be rapidly cleared through the kidneys and thus, are unlikely to be able to effectively accumulate within the tumour creating a “nanoparticle size paradox” in which larger particles are necessary for increased circulation time whilst small particles are necessary for tumour penetration. Wong et al have attempted to address this paradox through the development of 100 nm “multistage” gelatin quantum dot (QD) nanoparticles designed to be broken down by tumour-associate proteases into smaller 10 nm nanoparticles following extravasation which can then effectively fully penetrate into the tumour. These particles consist of a gelatin core with amino-PEG QDs conjugated to the surface using 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)/N-hydroxysulfosuccinimide (NHS) coupling chemistry. The larger nanoparticle is cleaved when in the presence of MMPs which are involved in many aspects of tumourigenicity and metastasis and thus are present in high abundance within the tumour microenvironment.  

In addition to size, shape and surface charge can also affect tumour penetration. Studies have indicated that particle aspect ratio can play a role in tumour penetration with higher aspect ratio particles (i.e. those that are more cylindrical rather than spherical) exhibiting improved penetration. Similarly, negative surface charge has been shown to be of benefit for enhancing penetration as well. However, the role of nanoparticles characteristics such as shape and size on tumour penetration is still poorly understood and more research is necessary to elucidate how these different properties can be altered to maximise tumour penetration.

Pre-treatment of tumours with drug, enzymes or inflammatory mediators or co-delivery of these molecules with nanoparticles has been implicated in increasing the ability of nanoparticles to deeply penetrate into tumours by breaking down the dense ECM barrier and/or increasing interstitial space. A number of studies, the co-delivery of matrix degrading enzymes such as collagenase, gelatinase, and hyaluronidase has been shown to significantly enhance intratumoural transport of nanoparticles thereby greatly improving their efficacy. Similarly, pre-treatment of tumours with the hormone relaxin, which causes changes in collagen structure, resulted in a 2-3 fold increase in the delivery of large macromolecules. Tumour priming with low doses of the chemotherapy drugs paclitaxel and doxorubicin is yet another method which has been successfully used to expand interstitial space and enhance the penetration of larger nanoparticles ranging from 85-200 nm in size. Finally, Chauhan et al showed that administration of the angiotensin inhibitor losartan significantly reduced tumour stromal ECM and hyaluronan production, which in turn decreased the expression of profibrotic signalling and tumour vessel compression. This leads to improved tumour oxygen levels and increased drug delivery to tumours, thereby potentiating chemotherapy and reducing hypoxia in breast and pancreatic cancer mouse models. Given that angiotensin inhibitors are inexpensive and already used in the clinic, their use to modulate the tumour microenvironment to allow for improved drug delivery to tumour cells could become a reality in the near future.

A final area that has been explored for enhancing nanoparticle delivery and efficacy in tumours is by designing systems that can be triggered to release their contents upon application of external stimuli such as heat, light, magnetic fields, or ultrasound. Drug release can be restricted to a specific region by confining the external stimulus within that region. Furthermore, these external stimuli have been demonstrated to help improve the ability of larger nanoparticles to effectively distribute throughout the tumour. The most successful example of this type of system is Thermodox, a temperature sensitive PEGylated liposomal doxorubicin delivery system currently in phase III clinical trials. While this strategy holds a lot of promise, stability of such systems and difficulties associated with effectively and specifically applying loco-regional stimuli have mostly prevented them from clinical success.
As most nanoparticle studies are conducted initially in two-dimensional (2D) cell monolayers where nanocarriers tend to show great promise, it is usually not until these systems are tested in animals or even preclinical trials that their inability to effectively access tumour cells \textit{in vivo} is observed. The complexity present within the tumour microenvironment combined with this poor \textit{in vitro} to \textit{in vivo} correlation highlights the need to employ more advanced \textit{in vitro} models in the prescreening of nanoparticle delivery systems as \textit{blanket in vivo} screening is not feasible. This is due to the fact that not only are \textit{in vivo} models costly, complicated, and time consuming, but also due to the ethical considerations of such undertakings. Tumour spheroids represent the simplest step to employing more advanced 3D \textit{in vitro} systems for nanoparticle prescreening.\textsuperscript{40,41}

Further complexity can then be imparted through the inclusion of stromal and other supporting cells normally present within the tumour microenvironment and/or the inclusion of either natural or synthetic ECM support structures.\textsuperscript{42,43} These 3D tumour spheroid systems can be employed to examine both nanoparticle penetration (Figure 4) and efficacy. One caveat to employing these models, however, centers around the difficulties in effectively adapting existing 2D biological assays to these more complex 3D systems which has been comprehensively reviewed elsewhere.\textsuperscript{44}

With advances in cancer biology, advances in understanding the requirements for improving nanoparticle formulations will follow, thus highlighting the necessity for a cross disciplinary approach to the field of nanomedicine.

\textbf{Conclusion}

The use of nanomedicines in systemic drug delivery has received a lot of attention over the past couple of decades and resulted in a several clinically approved formulations. These systems have been shown to have a number of advantages over conventional chemotherapeutics, however they have not yet reached their full potential as anticancer agents. This is likely due to the fact that until more recently, features of the tumour microenvironment that can create barriers to effective nanoparticle delivery have been largely overlooked. With advances in cancer biology, improvements in understanding how the tumour microenvironment affects nanoparticle delivery and distribution within tumours will provide new opportunities, further highlighting the necessity for a cross disciplinary approach to the field of nanomedicine. Strategies will then be developed to better address and overcome the shortcomings of current delivery systems as the chemistry takes its lead from the field of the targeted disease. Thus, future anticancer therapies using nanomedicine can be envisioned to specifically kill cancer cells within the tumour whilst leaving normal tissue in the body virtually untouched.

\textbf{References}


