Chapter 2
Nanotechnology for Cancer Treatment: Possibilities and Limitations

Joseph W. Nichols and You Han Bae

Abstract Drug delivery to solid tumors is one of the seminal challenges to developing more effective cancer therapies. A well-designed drug delivery system can potentially improve the efficacy of a treatment by enhancing drug accumulation in the tumor and combining synergistic effects into a single package. It may also reduce negative side effects by limiting drug access to sensitive noncancerous tissue. The most common drug delivery design is to package small molecule drugs with a nanoparticle. Nanotechnology provides a versatile platform onto which many functions can be added. Nanoparticles are widely considered to have superior biodistribution and efficacy when compared to free drug particles, but this expectation has not matched clinical results. One reason for the disappointing clinical outcomes of nano-sized drug carriers is the numerous barriers to drug delivery encountered by the nanoparticle on route from the administration site to tumor interior. These barriers are encountered along the entire delivery pathway and can severely limit the total effective amount of drug in the tumor.

Introduction

Interest in nanomedicine and drug delivery has increased exponentially in the last several decades. As with many newly developed technologies, the ability to manipulate matter at the nanoscale to create unique structures has generated creativity,

J.W. Nichols
Department of Bioengineering, College of Engineering,
University of Utah, Salt Lake City, UT, USA

Y.H. Bae (✉)
Department of Pharmaceutics and Pharmaceutical Chemistry,
College of Pharmacy, University of Utah, 30 S 2000 E, Salt Lake City,
Utah 84112, USA
e-mail: you.bae@utah.edu

enthusiasm, and a burst of funding. Biology may hold the most intriguing prospects for nanotechnology as it allows access to those scales at which most biological functions take place. The field of nanomedicine has indeed seen an increase of activity and continues to grow, as seen in the rise of both patents and publications [1, 2]. This rise represents a growth of research encompassing many facets of medicine including biomaterials, active implants, in vivo imaging, in vitro diagnostics, therapeutic materials, and gene and drug delivery.

Worldwide funding for all nanotechnology is expected to exceed $1 trillion by 2015 and, perhaps more importantly, market revenues for nanotechnology is thought to be close to $3 trillion worldwide [3, 4]. Nanomedicine research is also receiving a growing amount of funding, with public funding research reaching nearly $1 billion in the United States, $600 million in Japan, and $400 million in Germany. The level of funding for nanomedicine is indicative of the tremendous enthusiasm for the field. Recently, as much as 50 % of biomedical advances were estimated be related to nanotechnology [3].

The field of nanomedicine was originally conceived with fantastic visions of future capabilities. Nobel Prize winning physicist Richard Feynmann envisioned building nanorobots by employing manufacturing robotics to make another series of robotics at a smaller scale and following this sequence in series until the near atomic scale is reached [5, 6]. This concept was later seized upon and expanded to envision submarine-like nanomachines capable of independently performing numerous tasks, from supplementing immune function to eradicating cancer. Theoretically such machines could protect and prolong life by rebuilding damaged tissues, repairing virus-damaged cells, supporting or reconstructing damaged limbs and organs, and even reversing aging [7].

Though such visions of nanotechnology are clearly many decades, if not centuries, away (if physically possible at all), nanomedicine has found many applications and is still rightly hailed as potentially revolutionary. Nanotechnology has been applied to diabetes research for glucose sensors and nano-pancreases [8]; to tuberculosis and other respiratory diseases [9, 10]; in neurological diseases such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis [11]; for hemophilia [12]; to bone healing and osteoporosis [13]; and even for hair growth [14].

Perhaps the most explored application of nanomedicine is in cancer chemotherapy methods. Compared to regular chemotherapeutic treatments, nanoparticle drug carriers are presumed to have improved tumor specificity, fewer side effects, improved efficacy, and more flexibility in treating the highly diverse cancer types. These advantages, in conjunction with the seemingly limitless versatility of nanoparticles in both composition and surface chemistry, have led to an explosion of designs [15–18]. These designs attempt to address the various challenges facing drug delivery to solid tumors, which are present from the point of initial blood contact until the drug action occurs within the tumors. Effective therapy is further challenged by the development of drug resistance mechanisms and intratumoral heterogeneity.

Improving the efficacy of cancer therapy requires that the drug carrier adequately address the challenges of drug delivery so that sufficient drug can be brought against the tumor to eradicate it fully without causing excessive toxicity in the patient. Unfortunately, although the drug delivery designs currently coming out of labs

around the world have shown abundant potential in literature and preclinical studies, they have largely failed to make a significant impact in the clinic. Of the thousands of patents and publications filed in the field of cancer therapy, only a few carriers have found their way on the market in the United States. Doxil® and Abraxane® are two of the most successful drug carriers. Both are simple in concept and design and showed little to no improved efficacy compared with traditional chemotherapy [2, 19, 20].

New and dramatically improved therapies are needed if we are to meet the growing challenge of cancer in the future. In the US, cancer rates are expected to increase nearly four times as fast as population growth through 2030 [21]. This growth is largely attributed to changing demographics and an aging population that has benefitted greatly from the lifesaving and life-extending advances in other fields of medicine, but is now at greater risk of cancer. This places a greater burden on cancer researchers to design new treatments to extend and improve life for this growing group. Many designs are being tested, but the translation to the clinic is failing. Not only must new solutions to drug therapy be found, but improved methods of testing these solutions must be developed.

Challenges to Drug Delivery

Nanoparticles come in an almost infinite variety of sizes, shapes, and compositions, with more diversity of form and function to be found in the ability to modify the surface in myriad ways [22] (see Fig. 2.1). This versatility allows the nanoparticle to become a blank canvas, reflecting the creativity and skill of the researcher and exhibiting a wide range of unique behaviors. Nanoparticles can be designed with multiple functionalities to aid cancer therapies. Targeting moieties can be grafted onto the surface to aid cell uptake [23]. Synergistic drug types can be loaded into the same carrier to improve overall efficacy and combat the development of multiple drug resistance (MDR) [24, 25]. Imaging and therapy can be combined, allowing the progress of a treatment to be monitored in real time and aid clinicians in making appropriate treatment decisions [26]. Nanoparticles can even be designed to respond to outside stimuli, giving doctors the ability to very specifically target the release of drug or other therapeutic effects to a specific region in the body [27–29]. This versatility, however, can be more than matched by the impressive mechanisms the body and tumor employ to guard against potentially dangerous substances.

The body possesses numerous defense mechanisms to protect itself against foreign substances including viruses, bacteria, protein toxins, and other chemicals [30–32]. Nanoparticles are no exception and are actively cleared by the body [33]. The body has multiple strategies to prevent or mitigate tissue damage and maintain cell viability. These defense mechanisms are in place at every level of organization, systemic, organ, tissue, cellular, and intracellular (see Fig. 2.2). These barriers can be accentuated by the development of MDR phenotypes. MDR is associated with poor clinical outcomes and can apply to a wide variety of drugs. Heterogeneity
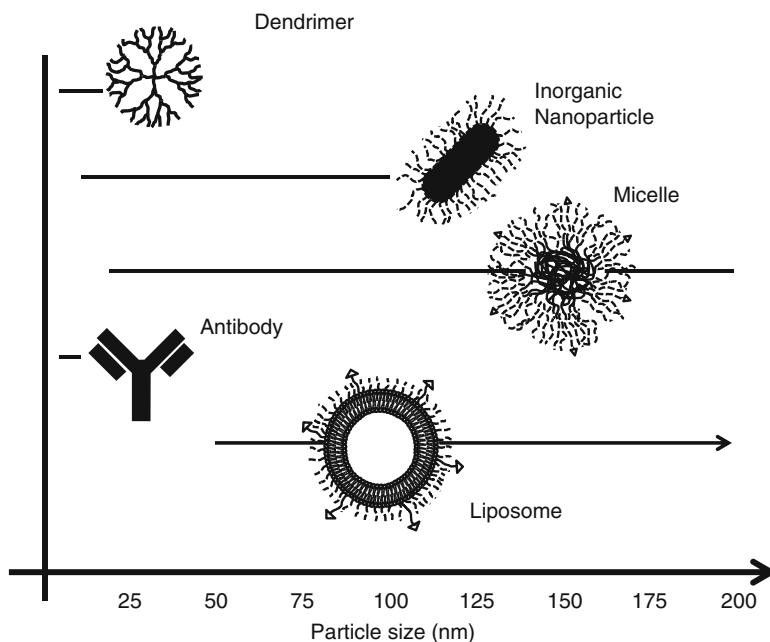
Fig. 2.1 Size of representative nanoparticles. There is a vast range of sizes and compositions of nanoparticles. This diversity gives researchers a great deal of versatility in designing drug delivery strategies

within the tumor presents a final challenge to successful cancer therapy. Tumor heterogeneity encompasses the genotypic variances among cancerous cells as well as the diversity of cell types within the tumor ecosystem that can make the tumor a more robust and resilient organ than a more isotropic model would suggest.

Most chemotherapeutic drugs are cytotoxic agents which have specific targets for action inside a cancerous cell [34]. These drugs are typically introduced into the body intravenously and must then complete a perilous journey through the circulatory system until it can encounter and enter the tumor. While in circulation the drug or drug carrier must avoid the many routes of clearance used by the body to protect against foreign substances, including renal clearance, liver metabolism, and the mononuclear phagocyte system (MPS). Long lasting particles may circulate long enough to encounter the tumor microvasculature and some of those may successfully diffuse out of the blood vessels to enter the tumor interstitial space. The drug that has made it to this point must then diffuse through the tumor, encounter a target cell, cross the lipid bilayer membrane, and finally localize in the cellular compartment relevant to the drug's mechanism of action in sufficient concentrations to cause cell death. The vast majority of drug administered to the patient does not complete all phases of this journey and becomes at best ineffective, and at worst toxic to the patient [35].
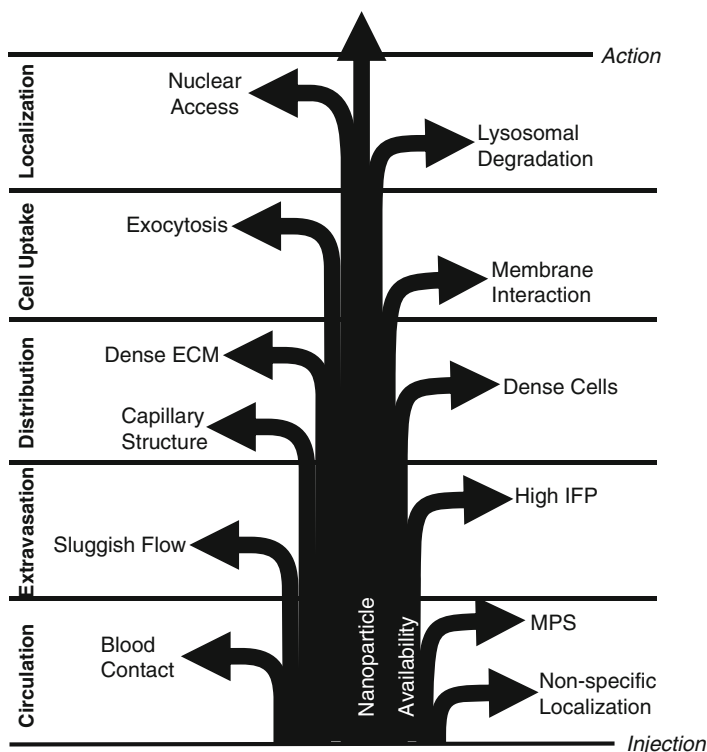
Fig. 2.2 Barriers to drug access. The journey of a nanoparticle from the intravenous injection site to the site of action in the tumor includes numerous obstacles. Each of these obstacles reduces the total quantity of nanoparticles potentially capable of treating the tumor. Successfully treating the tumor requires that enough of the drug navigates all obstacles to kill the tumor cells

Systemic Barriers

One of the primary purposes of drug carriers is to solubilize and protect its drug cargo from clearance and degradation until it reaches its target site. For most nanoparticles the first challenge to that purpose comes immediately on blood contact after administration. Nanoparticles have a very high ratio of surface to bulk atoms, which tends to result in high surface energies and unusual behaviors [36]. These behaviors include aggregation which can impact the polydispersity and biodistribution of the particles. The high surface energies may also result in strong binding of blood proteins to the nanoparticle surface [37]. These bound proteins can serve as a signal for MPS macrophages to engulf circulating nanoparticles, causing them to accumulate outside the tumor [36, 38]. MPS, also known as the reticular endothelial system (RES), is a system of tissue embedded macrophages that clear foreign substances from the blood and tissues. It is most prominent in the white blood cell rich spleen which sees a significant portion of administered nanoparticles in most studies. The liver also collects a very large quantity of nanoparticles due

both to MPS activity and to the unique porous sinusoid structures in the liver which help it to filter and clean blood [32]. Nanoparticles are generally large enough to avoid renal clearance [39].

Currently, the predominant strategy to minimize protein adsorption and MPS uptake is to densely graft a hydrophilic polymer, most commonly poly (ethylene glycol) (PEG), to the surface of the nanoparticle in a process known as PEGylation. The grafted polymer extends from the surface and forms a brush-like barrier that limits access to the nanoparticle surface and slows the rate of binding. PEGylation can significantly slow the kinetics of protein binding and MPS clearance of nanoparticles [35], allowing the circulation time of most nanoparticles to increase by several orders of magnitude compared to unmodified nanoparticles [33, 38].

Long circulating drug carriers are expected to show improved biodistribution and increased intratumoral accumulation in comparison to conventional treatments. This expectation is driven by the enhanced permeability and retention (EPR) of nanoparticles in the tumor, and when EPR leads to improved efficacy of treatment it is known as the EPR effect. EPR is actually a result of two separate phenomena, enhanced permeability and enhanced retention. Both are related to physiological abnormalities resulting from the rapid growth of the tumor and the way it modifies the local microenvironment.

Rapid and uncontrolled cell growth is one of the chief hallmarks of cancer [40]. The division and growth of cells within a confined space can cause cells to become very tightly packed and the resulting compressive stress can crush native blood and lymph vessels [41]. Lacking intact blood vessels the tumors must rely on simple diffusion to deliver oxygen and nutrients and to remove waste from the tumor center. Once the tumor diameter reaches approximately one millimeter, hypoxic conditions become dominant in the core causing hypoxic cells to release factors promoting angiogenesis [42]. Angiogenesis proceeds rapidly to supply the tumor resulting in tortuous, chaotic, and disorganized vasculature. The vessel walls of the newly formed vasculature are similarly disorganized, leaving large gaps or fenestrations through which large particles such as proteins and nanoparticles can diffuse [43–45]. The ability for nanoparticles to diffuse into the tumor more readily than in normal tissues with organized, coherent vascularization is the primary mechanism for the enhanced permeability of EPR.

The enhanced retention of nanoparticles in a solid tumor results largely from the destruction of lymph vessels due to solid compressive stress [41]. Without functioning lymph vessels, fluid must flow out the periphery of the tumor before it can be cleared. The rate of flow to the tumor exterior can be slowed by the hydraulic resistance from the tightly packed cells and dense collagen matrix. This fluid retention has several effects related to drug delivery that will be discussed later, but among them is the tumor's limited ability to clear drug carriers from the tissue [46].

EPR provides what is perhaps the primary advantage to cancer nanotherapies over traditional chemotherapy. The discovery of EPR in the mid-1980s brought a great deal of attention to nanotechnology in search of the next major breakthrough in cancer treatment [47]. Nanotechnology seemed to hold the promise of improved treatment efficacy, combined with a means of controlling or even eliminating non-specific toxicity [48].

EPR is a form of microenvironmental targeting, attacking the pathogenic tumor lymph and blood vessels to achieve tumor specificity [49]. This reliance on the tumor microenvironment to deliver effective drug doses can be problematic for treating potentially metastatic cancers. Metastasized cells or colonies too small to have created a microenvironment are unlikely to be affected by nanotherapies making adjuvant therapy with traditional chemotherapeutic drug cocktails necessary to prevent cancer spreading and relapse. Nanotechnology is thus unlikely to form the basis of a stand-alone cancer therapy.

Long circulation and EPR do not guarantee the drug carriers will reach the tumor site. Most tumors are only a few centimeters in diameter, a small fraction of the total size of the patient [50]. The administered drug is carried indiscriminately throughout the body via the circulatory system, meaning that a given drug particle will rarely encounter the tumor much less extravasate into it, and even under the best of circumstances only a tiny fraction of the injected dose will enter into the tumor where it can be effective. Whatever drug does not enter the tumor does not contribute to the drug's efficacy, but instead causes the dangerous side effects for which chemotherapy is famous. Loading the drug into a carrier can help limit drug access to certain tissues and improve the overall toxicity profile, but the overwhelming majority of the injected drug is incorporated into tissues other than the tumor [19].

The EPR effect predicts that long circulating nanoparticles should accumulate in higher concentrations in the tumor relative to surrounding tissue, thus improving the overall efficacy of the treatment. There is a great deal of evidence showing this effect in animal models and limited clinical evidence that limited preferential tumor accumulation does take place, but the clinical benefit has yet to be seen [19, 51, 52]. The discrepancy between preclinical and clinical success exposes the inadequacy of the models used to study cancer nanomedicine. While these models are both necessary and useful for designing and testing drug carriers, they are at best flawed representations of reality. Tumor models developed in mice are generally grown much more quickly than naturally occurring tumors, a condition which accentuates the rapid angiogenesis leading to the disorganized vasculature presaging EPR [53, 54]. Additionally, murine model tumors are grown to as much as 10 % of the total mouse weight, compared to a human tumor which generally only grows to a tiny fraction of a percent of body weight. A circulating nanoparticle in a mouse will thus encounter the tumor exponentially more often than in a human and is more likely to extravasate into it.

Extravasation

Due to the relatively small size of a tumor compared to the rest of the patient and the effectiveness of the body at clearing foreign material from the blood, a large portion of nanoparticles will never encounter the tumor and thus have no opportunity to provide a therapeutic effect. Of those nanoparticles that do encounter the tumor vasculature, most pass straight through and back into the larger circulatory system, eventually causing unwanted side effects in distant organs. Moving a drug carrier

from the blood compartment and into the tumor interstitial space is a significant problem for drug delivery researchers.

Blood flow through the tumor can be sluggish and intermittent due to the disorganized, chaotic nature of the hastily formed vasculature and can result in poor or intermittent delivery of blood-borne drug to the vascularized areas [28, 44, 45]. This uneven supply can have an important impact on the spatial distribution of drug in the tumor as a whole, leaving large regions of the tumor untreated or undertreated.

When nanoparticles do pass through the microvasculature, they are expected to diffuse out of the capillary and into the tumor interstitial space via the large fenestrations or openings in the capillary wall [55]. In healthy capillaries, movement across the capillary wall is described by the Starling equation, which expresses the balance of hydrostatic and oncotic pressures across the wall [56]. At the arterial side of the capillary, the hydraulic pressure provided by the heart exceeds the tissue interstitial pressure, which tends to drive bulk fluid flow out of the vessel. Waste-bearing fluid is returned to the venous side by osmosis. The osmotic potential in the blood is generally higher than in the interstitial fluid due to the exclusion of blood proteins such as albumin from the interstitial space.

In cancerous tissue this balance is disrupted as a result of the pathological structure of the vessel walls. The large fenestrae in tumor vasculature are not only permeable to nanoparticles, but to all blood-borne macromolecules including albumin and other large proteins [57]. The free flow of large solutes across the capillary wall results in equal osmotic potentials both inside and outside the capillary. The combination of high osmotic pressure, lack of lymphatic drainage, and high hydraulic resistance in the tumor results in a tumor interstitial fluid pressure (IFP) that approaches the microvascular pressure [41, 58]. With no pressure differentials across the capillary wall, the driving force for bulk fluid exchange is negligible and extravasation out vessel fenestrae must rely almost entirely on diffusion [55]. Extravasation then becomes dependent on the probability that a particle encounters a fenestration by random motion. Once extravasated, there is also no flow gradient to prevent the particle from passing back into the capillary rather than diffuse deeper into the tissue.

Reducing tumor IFP to restore bulk flow across the capillary wall is one potential strategy to improve particle extravasation. One way to accomplish this is to normalize the tumor vasculature [54]. This can be done by blocking the proangiogenic factors released by the tumor, thus slowing the rate of angiogenesis and giving the nascent blood vessels time to organize [44]. Tumor IFP may also be temporarily reduced by degrading the collagen mesh that makes up the tumor extracellular matrix (ECM). The dense collagen mesh gives the tumor a high hydraulic resistance and prevents fluid from draining out. Degrading this mesh may allow more drainage and reduce IFP [59]. Both of these strategies would seem to offset some of the natural advantages of nanotherapies gained from EPR. Normalizing the vasculature to restore normal pressure gradients would reduce the fenestration size in the capillaries and negate the enhanced permeability of nanoparticles to the tumor. Improving tumor drainage may hurt nanoparticle retention in the tumor and also increase metastatic potential as cells are brought outside the tumor mass.

More specific methods of promoting extravasation include attaching the drug carrier to a tumor penetrating peptide such as iRGD [60]. The mechanism of these

peptides is currently unclear, but it appears to improve transcytosis in tumor tissues by binding to α_v integrins on the tumor endothelial cells. Some studies have shown significant improvements in tumor accumulation when using the tumor penetrating peptides compared with controls [61, 62]. Localized hyperthermia may also be used to enhance nanoparticle penetration in a tumor-specific manner by increasing vascular permeability in a targeted area. This method relies on good imaging methods so that doctors can see exactly where to direct heating stimuli [63].

Most efforts to improve extravasation and intratumoral accumulation have been focused on lengthening the particle circulation time, giving circulating particles more opportunity to encounter the tumor. PEGylation has proven to be the most successful method of lengthening circulation time, with coated particles lasting more than 40 fold longer than uncoated particles [64]. However, some studies have indicated that extending circulation time beyond a certain point does not significantly improve treatment efficacy, but does contribute to worsening side effects [65]. This may be due to the limited mobility of extravasated nanoparticles, preventing them from moving away from the fenestrae. These nanoparticles can then become a barrier preventing subsequent nanoparticles from extravasating. Particle extravasation may thus be partially limited by the rate of diffusion away from the fenestrae after passing out of the capillary. Some evidence suggests that extremely long circulation times may also result in greater toxicity than shorter circulating drug carriers [65, 66]. Very long circulation times allow the kinetically slow extravasation of nanoparticles into skin and other tissues to become much more significant, leading to painful side effects such as foot and hand syndrome [66].

Intratumoral Distribution

Nanoparticles may have to diffuse relatively huge distances to reach a large portion of tumor cells. The chaotic nature of the vasculature can leave large regions of the tumor underserved and difficult to access, especially for large, relatively immobile nanoparticles [67]. These regions also tend to be hypoxic and select for highly resistant and potentially dangerous cells [68]. Killing these cells may be critical to the long-term success of a therapy. The distance a drug carrier must travel to reach these cells, however, becomes even more daunting in light of the many barriers to oppose the already weak diffusion driving force (see Fig. 2.3).

The difficulty of diffusion through a tumor can be compounded by the dense ECM. The structure of the collagen matrix can limit or halt the movement of large particles [69]. The densely packed cells of the tumor can be another impediment to nanoparticle motion [70]. Cells are very large compared to most nanoparticles; for example, if the nanoparticles were the size of a soccer ball, the cell would be approximately the size of the field. Navigating a mess of such relatively huge obstacles can significantly increase the effective path length the drug carrier must travel to diffuse within the tumor [71]. These physical barriers can be greatly exacerbated by interactions with either the ECM components or the cell membrane [72]. Many particles are designed to interact with markers on the cancer cell membrane to
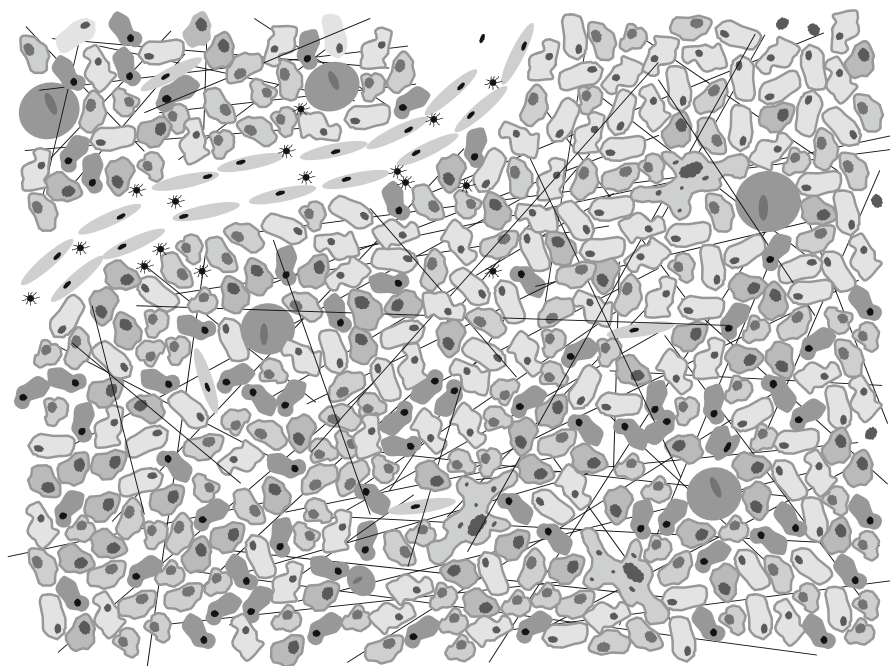
Fig. 2.3 Tumor composition and distribution barriers. Effective therapy requires that the drug carrier extravasate from the blood vessel to the tumor interstitial space and then diffuse throughout the whole tumor. This diffusion is made very difficult by the tense tumor cells and ECM

improve cell uptake and specificity. This may result in the development of a "binding site barrier" in which the drug carriers get caught on the first cells encountered after extravasation and fail to penetrate more deeply [73].

The large size of nanoparticles, relative to small molecule drugs, is a major liability for the intratumoral distribution portion of drug delivery. Improving intratumoral distribution would hugely benefit the efficacy of treatment. Limiting interactions with the ECM may be the most important strategy to improve distribution. Fortunately PEGylation appears to be effective at limiting these interactions and can dramatically increase diffusivity in some circumstances [72]. Even PEG coated particles are much too large to diffuse readily through the tumor environment. Recognizing this, some drug carriers are designed to degrade in the tumor microenvironment, leaving the small drug cargo to diffuse the remainder of the way [74].

Unfortunately, opportunities to increase diffusivity by modifying the nanoparticles are limited leaving many researchers to attempt to modify the tumor microenvironment to be more conducive to particle distribution. One such strategy is coadministration of the nanoparticle with collagenase enzymes to degrade the ECM [75]. Breaking up the collagen matrix should allow more space for diffusion to occur, though this benefit may be somewhat offset by remaining debris [76]. This method also carries the potential risk of metastasis from cells that have become more mobile in the degraded matrix. As discussed above, degrading the collagen

matrix has the added benefit of reducing intratumoral IFP and potentially improving drug extravasation into the tumor [59].

Cancers should not be considered an isotropic mass of identical cells, but may be more accurately thought of as an organ whose primary function is growth and achieves that objective by acting as a parasite on other tissue [77, 78]. As with other organs, the tissue contains both primary cells and cells serving secondary support functions, including epithelial cells, fibroblasts, endothelial cells, perivascular cells, mesenchymal stem cells, and immune cells, all in addition to the primary cancer cell type [78].

The diversity of cell types in the tumor present both a challenge and an opportunity for cancer therapy. The support functions performed by the secondary cells render the tumor more robust than the isotropic model would indicate. However, the tumor also depends on these cells to perform important functions to maintain viability and thus may represent a target for therapy. Targeting the vascular endothelial cells, for example, eliminates the distribution barrier because the cells are immediately accessible from the vasculature. VEGF inhibition slows angiogenesis and may lead to more normal blood vessels capable of distributing drugs [54]. Attacking the blood vessels may also be used as a method to starve the tumor by restricting its blood supply [79].

Tumor associated macrophages (TAMs) also present a potentially inviting target. TAMs may aid drug distribution by collecting drugs then leaking it as it travels through the tumor [80, 81]. They also play a role in some critical functions such as angiogenesis, metastasis, and tumor progression [82, 83]. Therapies targeting secondary cells have shown impressive clinical potential but generally must be administered in conjunction with traditional therapies to effectively combat cancer [79].

Cell Uptake

The lipid bilayer membrane is designed to serve as a selectively permeable barrier to a wide range of substances. Only small, hydrophobic molecules are capable of diffusing through the membrane without assistance from protein channels or active uptake mechanisms. Most small molecule chemotherapeutic drugs diffuse directly across the membrane to access the cytoplasm [84]. Large hydrophilic molecules are not capable of diffusing across the membrane and do not have uncontrolled access to the cell [85].

Drug resistance is among the major problems facing cancer drug delivery, and one of the primary mechanisms of MDR is in the cell membrane. P-glycoprotein (Pgp) is a membrane embedded active pump responsible for removing a wide variety of toxins from cells. It is a member of a broad family of protein pumps known as the ATP-binding cassette (ABC) pump family, which are commonly found in cells frequently exposed to toxic environments such as in the liver, jejunum, and skin [86–88]. Pgp is also significantly upregulated in MDR cancer cells, protecting the cell against a wide variety of cytotoxic drugs. It mops up these substances and then pumps them to the cell exterior, hydrolyzing ATP in the process. Pgp can maintain significant concentration gradients across the membrane, meaning that to

achieve lethal concentrations inside the cells by passive means, unsustainable doses must be used [89, 90].

Bypassing Pgp mediated MDR is critical to treating many of the most lethal cancers. One solution to resolve the Pgp obstacle is to co-deliver the drug with a Pgp modulator. These modulators use various mechanisms to compete or block Pgp activity, allowing small molecule drugs to diffuse more easily across the membrane [91]. Early modulators had problems with specificity, inhibiting other ABC pumps and causing harmful drug interactions. New modulators are promising better specificity and fewer negative reactions, though the safety of these modulators is as yet unproven [92, 93]. The systemic toxicity caused by these inhibitors can limit the maximum tolerated dose of a treatment regime [94].

A nanoparticle may also circumvent the Pgp barrier entering the cell interior intact while carrying the drug. Nanoparticles are not able to enter the cell by diffusion and thus must gain access almost exclusively via an active form of endocytosis [84]. There are several mechanisms by which endocytosis can take place. Pinocytosis is one such mechanism in which the cell randomly samples the surrounding fluid while other methods are generally mediated through particle-membrane interactions and cell receptors. Increasing nanoparticle interaction with those receptors is one method to improve overall cell uptake [95].

Equipping nanoparticles with ligands for cancer-specific cell receptors can theoretically improve the drug internalization rate and is thought to simultaneously enhance specificity, though the claim is controversial [96–99]. Achieving tumor cell specificity requires the presence of cancer-specific markers, which are extremely difficult to find. Cancer is born of our own biology, so nearly all proteins in cancer serve a role somewhere in the body. At a minimum, similar, if not identical, proteins will be present rather abundantly in the body compared to the total expression in the tumor. The cumulative effect of the lower affinity interactions elsewhere in the body may still lead to a great deal of nonspecific toxicity. The relevant interactions also take place on the scale of a few nanometers or less, so the nanoparticle cannot be actively guided to the tumor by receptor-ligand targeting [100].

Targeting strategies are popular in drug delivery research but have thus far failed to provide much clinical benefit. Nearly 30 years of intensive research has yielded only a handful of clinically available nanotechnology based cancer therapies (see Table 2.1). Most of these treatments are antibody therapies, but of the dozen that have gained clinical approval, only trastuzumab is indicated to directly attack the cells of solid tumors [101]. Other clinically approved cancer nanotherapeutic designs are even rarer. The two most popular formulations are Doxil® and Abraxane® which are both FDA approved to treat solid tumors [19, 20]. However, these therapies rely wholly on passive targeting, rather than active receptor-ligand targeting, and are not representative of the complexity of drug carrier designs seen in literature.

The lack of clinical success for these treatments is surprising, given the promising preclinical results. The discrepancy may again be largely due to problems with the models used to test these formulations. Many tests are conducted in two dimensional Petri dish models with cultured cells [95]. While useful in proof-of-concept studies, these models suffer from two important shortcomings. First, they give the drug formulation unhindered access to the cells, free of any physical barriers such

Table 2.1 Cancer nanotherapies currently in clinical use. NYA = Not yet approved in U.S. but in clinical use elsewhere

Antibody Formulations

Trade Name	Formulation	Target	Indication	Approval Date
Rituxan	Rituximab	CD20	Non-hodgkin Lymphoma-Leukemias	1997
Herceptin	Trastuzumab	HER2	Metastatic breast cancer, adjuvant for gastric cancers	1998
Campath	Alemtuzumab	CD52	Leukemia	2001
Zevalin	90Y-ibritumomab	CD20	Non-hodgkin lymphoma	2002
Bexxar	131I-tositumomab	CD20	CD20+ Non-hodgkin lymphoma	2003
Erbitux	Cetuximab	EGFR	Head and neck, some colon; adjuvant with radiation	2004
Avastin	Bevacizumab	VEGF	Metastatic colon and rectal cancers; antiangiogenic	2004
Vectibix	Panitumumab	EGFR	Colon and Rectal cancer with traditional therapy	2006
Arzerra	Ofatumumab	CD20	Chronic lymphocytic leukemia	2009
Yervoy	Ipilimumab	CTLA-4	Melanoma	2011
Adcetris	Brentuximab vedotin	CD30	Anaplastic large cell lymphoma (ALCL), Hodgkin lymphoma	2011
Kadcyla	Trastuzumab emtansine	HER2	Metastatic breast cancer	2013
Mylotarg	Gemtuzumab ozogamicin	CD33	Acute myeloid leukemia (AML)	2000–2010

Liposomal Formulations

Trade Name	Formulation	Drug	Indication	Approval Date
Doxil	PEGylated liposome	Doxorubicin	Secondary treatment for ovarian cancer	1995
DaunoXome	Citrate liposome	Daunorubicin	Karposi's sarcoma	1996
DepoCyt	Cytarabine liposomal	Cytarabine	Lymphomatous meningitis, leukemia	1999
Myocet	Non-PEGylated liposome	Doxorubicin	Metastatic breast cancer with cyclophosphamide	NYA

Nanoparticle Formulations

Trade Name	Carrier Type	Drug	Indication	Approval Date
Abraxane	Albumin	Paclitaxel	Secondary treatment for breast cancer	2005
Genexol-PM	Polymeric micelle	Paclitaxel	Metastatic breast cancer	NYA

as those previously discussed. This allows a much greater than normal portion of nanoparticles to come within the nanometer range required for specific interactions. Second, the cultured cells used typically lack the genetic diversity of natural tumors and may thus overpredict the actual presence of the relevant markers. These cultured cell lines are also inoculated into animals to generate tumor models that lack

the genetic diversity of natural tumors and may not realistically reflect the composition of surface receptors or the presence of secondary cell types [102].

Finding a silver bullet for cancer targeting remains an elusive goal. A great deal of research has gone into finding cancer-specific markers to be used as drug targets [103, 104]. These studies have revealed a better understanding of cancer biology, but few new therapies. Part of the difficulty of translating newly discovered markers to new treatments is the intratumoral diversity of marker expression. Her2 is a good example of the genetic diversity of cancer cells. Her2 is a protein receptor overexpressed in some breast cancers and targeted by the antibody trastuzumab under the brand Herceptin®. The American Society of Clinical Oncology has established guidelines used to determine eligibility for Herceptin® treatment. A sample of the tumor is biopsied and stained for Her2 expression and based on the degree of staining the tumor is assigned an immunohistochemistry (IHC) score ranging from 0 to 3+. The highest score (3+) is given to tumors in which 30 % or more of cells strongly stain for Her2 and the tumor is considered Her2 positive (score of 2+) if only 10 % of cells show definite staining [105, 106]. If 30 % staining represents nearly an ideal case in a clinically proven receptor, then any targeted drug formulation should acknowledge that targeting gaps will exist in other receptors as well. Furthermore, samples drawn for biopsy are small and IHC scoring can vary spatially as well as temporally [107].

Other receptors are also used to target cancer in various studies. The folate receptor is a longstanding and popular target for cancer treatment. It is strongly expressed in the pulmonary, endocrine, gastrointestinal, and genitourinary systems as well as tumors derived from those sources [108, 109]. The folate receptor was the target of one of the earliest chemotherapeutic treatments for leukemia [110]. The transferrin receptor is another target commonly overexpressed in tumors, but because it plays an important role in iron transport between blood and tissue, it is found in almost all cells [111]. Though these receptors are considered to be overexpressed in many tumors, overexpression and specificity should not be conflated. These markers are abundant throughout the body, and intratumoral expression can vary both spatially and temporally according to the microenvironmental conditions surrounding the cell.

An alternative to receptor-ligand targeting is to equip the nanoparticles with nonspecific peptides that are exposed only in the appropriate environmental conditions, such as the relatively acidic extracellular pH found in most tumors. TAT is a peptide sequence used by some viruses to penetrate the cell membrane and gain access to the cytoplasm [112]. It works to enhance cell uptake on all cells but can be shielded using pH-sensitive polymers until it reaches the tumor [113]. This method alleviates the problem of intratumoral heterogeneity by targeting the environment rather than the cells individually.

Intracellular Distribution

Gaining access to the cell is still not sufficient to guarantee treatment efficacy. The drug must still be delivered intact to whatever region of the cell it is designed to attack. The first barrier to drug carriers that entered the cell by endocytosis is

avoiding drug degradation by lysosomal digestion. Most active uptake mechanisms include a digestion phase to break down the endocytosed material into usable components and destroy potentially pathogenic substances before the material is given access to the cell [114, 115]. The lysosome is an acidified organelle filled with proteases optimized to function near pH 4.5. Conditions within the lysosome may be harsh enough to degrade or deactivate many drugs, rendering them ineffective against the cancer cell [116].

Avoiding lysosomal degradation may be critical in delivering an effective drug dose to the tumor cell and may be achieved in a number a ways. One strategy is to avoid the lysosome by utilizing endocytotic pathways that do not undergo cellular digestion. Caveolae-mediated endocytosis appears to bypass the lysosomal phase and may be activated by the TAT peptide [117, 118]. Particles uptaken by different pathways may require a strategy to escape the vesicle during the endosomal phase before the lysosome can form. This can be done by releasing the drug from the nanoparticle during the endosomal phase, allowing the small molecule drug to diffuse out into the cytoplasm before the lysosome forms. Drug carriers may also be designed to rupture the endosome and release the contents. The proton sponge effect is a popular strategy to disrupt the endosome and avoid lysosomal degradation [119]. The proton sponge effect works by sequestering excess protons, usually by a polymer such as polyethylenimine (PEI) which contains unsaturated amino groups that can act as a buffer. This forces additional counter ions and water to be pumped into the endosome which may eventually cause it to swell and rupture, releasing the contents directly into the cytoplasm. The reality of the proton sponge effect is still somewhat controversial, but the improved transfection efficiency of PEI-based gene delivery systems provides some evidence of its utility [119].

Once it is in the cytoplasm, most drugs must proceed to a specific target within the cell. The location of the target depends on the drug type and mechanism of action. Most taxanes act on the microtubules that are ubiquitous in the cytoplasm. Cisplatin and related drugs indiscriminately alkylate proteins and nucleotides, but is most effective in the nucleus. Doxorubicin and its derivatives work by intercalating with DNA and must enter the nucleus to be effective.

Nuclear entry is one of the most formidable challenges to intracellular localization. Nuclear access is typically regulated at the nuclear pore complex (NPC) [120]. As with the cell membrane, the nuclear envelope is soluble to small hydrophobic compounds, but diffusion across the membrane can be limited by the presence of Pgp, giving the nuclear envelope high drug resistivity [121]. Nuclear access through the NPC can be aided by co-delivering the drug with compounds that dilate it from free flowing channels between the two compartments [122]. Mitosis also provides an opportunity for drugs to interact without the nuclear envelope present [123]. The nuclear envelope must disassemble during prophase to allow the chromatids to separate and is reassembled during telophase. Compounds that associate with the DNA during this window may be incorporated into the nucleus upon reassembly.

The individual cell has other mechanisms to protect itself and mitigate damage from cytotoxic compounds that must be considered when designing a drug delivery strategy. Sequestering the drug away from sensitive areas of the cell is one such mechanism. MDR cancer cells may overexpress acidic vesicles which can

concentrate and sequester a variety of slightly basic drugs until it can be metabo-
lized or exocytosed [124]. Lung resistance related proteins (LRP) or vault proteins
are another mechanism for sequestering and exocytosing drugs that are commonly
seen in MDR lung cancers [125].

Cells may also modify certain chemical pathways to mitigate or compensate
for damage done to the cell by a drug. Upregulating pathways that metabolize
toxic substances can speed the breakdown of cytotoxic drugs and mitigate the
damage done to the cell. Glutathione is a key protein in many cells' detoxification
pathway and can be overexpressed in resistant cells [126, 127]. Further resistance
can be conferred by modifying certain pathways to raise the threshold for apopto-
sis and cell death. Pro-apoptotic factors such as p53 are mutated or suppressed,
while pro-survival factors such as Bcl-2 are inhibited [128, 129]. Repair mecha-
nisms can be upregulated to repair damage done by DNA targeting drugs [130].
There are few specifically designed methods with which nanomedicine can com-
bat these resistance mechanisms. Most often the best that can be done is to achieve
sufficiently high intracellular drug concentrations to neutralize the cell in spite of
its resistance.

Conclusion

Cancer is a formidable foe. It is born as a "distorted version of our own selves"
having wriggled free of the remarkable cooperative system of the body to pursue its
own objectives [131]. It takes advantage of the natural defenses by which the body
protects itself against diverse pathogens and dangers. Nanomedicine is a remarkable
tool to approach the difficult task of treating a so elusive disease. The nanoparticle's
large size may confer it with inherent advantages, specifically the ability to target
the tumor vasculature via EPR. Nanoparticles are also extremely diverse, encom-
passing many sizes, shapes, surfaces, and compositions. This versatility gives it the
capability of stretching to accommodate the creativity of the researcher. Our ability
to design and manufacture nanoparticles is continuing to grow and will provide
even more capability in the future.

However, nanomedicine should not be looked on as a panacea or miracle cure,
it carries inherent disadvantages to go along with its advantages. Distribution
through the tumor is severely limited by the relatively large size of the nanopar-
ticle which slows diffusion and can become trapped in the ECM. Nanoparticle
entry into the cell is restricted to specific pathways, often relying on unreliable
interactions between cell receptors and ligands and introducing the nanoparticle
and drug to the lysosome digestion process. Drug carriers are also subject to MPS
clearance and other mechanisms the body uses to clear nonself particles from
blood and tissue.

The sheer quantity of barriers to effective drug delivery turns it into a game of
attrition, in which progressively more particles are sheered away at each obstacle
until little or none is left to treat the tumor. Failure at any point in the drug delivery
pathway may irreparably harm the ability of the drug to sufficiently treat the tumor.

However, attempting to build specific mechanisms to bypass each of these barriers can quickly become cumbersome and overcomplicated. Imagine a bare nanoparticle to which PEG is added to prolong circulation and limit unwanted protein or ECM interactions, tumor penetrating peptides are included to promote extravasation by transcytosis, collagenases are inserted to degrade the collagen matrix, reducing tumor IFP and improving diffusion, targeting moieties are attached to promote cell uptake and improve cancer cell specificity, pH-sensitive polymer with buffering capacity is included to increase environmental sensitivity and aid endosomal escape, and numerous compounds are co-delivered with the drug to inhibit angiogenesis, aid nuclear entry, and limit cellular resistance. Designing a drug delivery system in such a way would quickly become onerous, possibly much too complicated to be effective and certainly expensive.

To reach the promise of nanomedicine, it is necessary to take a step back and look at the problems facing drug delivery as a whole rather than designing around only one or two obstacles. Incremental designs may not be sufficient to accomplish the task of treating cancer effectively. Instead, a revolution in concept is needed; one that incorporates a healthy respect for the complexity of both body and tumor and the ability of each to protect itself from harm.

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