Barriers to drug delivery in solid tumors

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Abbreviations: NP, Nanoparticle; PEG, Polyethyleneglycol; IV, Intravenously; MPS, Mononuclear phagocyte system; apoE, Apolipoprotein E; PLGA, poly(lactic-co-glycolic acid); Red blood cells, RBCs; White blood cells, WBCs; LDL, Low-density lipoprotein; IC, Inhibitory concentration; EPR, Enhanced permeability and retention effect; VEGF, Vascular endothelial growth factor; PDGF, Platelet-derived growth factor; TNF, Tumor necrosis factor; PIGF, Placental growth factor; bFGF, Basic fibroblast growth factor; ECM, Extra-cellular matrix; MMP, Matrix metalloproteinase’s; TIMP, Tissue inhibitor of metalloproteinase’s; IFP, Interstitial fluid pressure; CPP, Cell-penetrating peptide

Over the last decade, significant progress has been made in the field of drug delivery. The advent of engineered nanoparticles has allowed us to circumvent the initial limitations to drug delivery such as pharmacokinetics and solubility. However, in spite of significant advances to tumor targeting, an effective treatment strategy for malignant tumors still remains elusive. Tumors possess distinct physiological features which allow them to resist traditional treatment approaches. This combined with the complexity of the biological system presents significant hurdles to the site-specific delivery of therapeutic drugs. One of the key features of engineered nanoparticles is that these can be tailored to execute specific functions. With this review, we hope to provide the reader with a clear understanding and knowledge of biological barriers and the methods to exploit these characteristics to design multifunctional nanocarriers, effect useful dosing regimens and subsequently improve therapeutic outcomes in the clinic.

Introduction

Cancer accounts for the deaths of millions of people worldwide. It occurs when normal cells acquire a series of critical mutations leading to their uncontrolled cell growth. The fact that cancer originates from an organisms own cells makes it harder to selectively treat. Current clinical approaches are based on the systemic administration of chemotherapeutics drugs. These therapies are limited by solubility and pharmacokinetic factors on account of their physio-chemical properties, as well as fraught with toxicity issues as they generally target any rapidly dividing cells in the body such as those of the hair, skin, spleen and liver among others. Therefore, delivery of these anti-cancer agents with the use of nanoparticles (NPs) helps overcome some of these disastrous side-effects. But it was found that only a small fraction of the administered dose ends up reaching the target site to have its intended effect. This may result in further complications as tumors when exposed to limiting amounts of drug, develop resistance. As a consequence, subsequent dosing regimens will then need to be significantly higher in order to elicit any therapeutic response. It is thus evident that current treatment modalities have significant scope for improvement. In order to arrive at the appropriate solutions we must first seek to identify and understand the problems. The complexity of the in vivo system thus inflicts multiple biological barriers which impedes NP drug delivery to solid tumors. In current anti-cancer therapies, NPs are generally administered intravenously (IV). This route is fast, reliable and allows complete distribution via the systemic circulation. Once in circulation, the NPs face a number of challenges. They may be opsonized by blood proteins following which they can be recognized by the cells of the mononuclear phagocyte system (MPS) and cleared from circulation. The NP population which has evaded clearance by the MPS now needs to extravasate out of circulation effectively past the endothelial lining toward the tumor microspace. Effective extravasation thus represents the second barrier followed by the penultimate barrier, the tumor interstitium. Here the NP encounters the smooth muscle cells, extra-cellular matrix, pericytes, cancer associated fibroblasts etc. in addition to various physiological factors such as low pH, low oxygenation and high interstitial fluid pressure. Once the NPs have extravasated out of systemic circulation, past the tumor microspace etc. the tumor cell membrane and intracellular machinery represents the final barrier the NPs have to get past for the effective intracellular delivery of drug cargo. The design of multifunctional NPs layered with specific attributes in order to sequentially execute functions to cross these biological barriers one at a time is thus imperative. This review presents in detail not only the various biological barriers but also the latest advancements in biomedical nanotechnology and the strategies used by the scientific community to overcome them.

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The mononuclear phagocyte system

In order for a NP or drug vehicle to reach its target and have its intended effect, it first needs to be stable in systemic circulation. The blood contains a variety of proteins like albumin, fibrinogen and globulin as well as other complement system proteins. Once the NP enters the systemic circulation, these serum proteins can adsorb onto their surface forming a ‘protein corona’. The formation of this particle-protein corona is dynamic and is controlled by a number of biological, physical and chemical interactions at a molecular level. The NP-protein complex is a key determinant of the subsequent fate of the NP in vivo and therefore understanding the extent of their interactions is crucial to their effective design. Cedervall et al. have very elegantly demonstrated a number of methods to study these NP-protein interactions and how these translate to responses in vivo.

The process of protein adsorption onto the particle surface is termed as opsonization and is usually followed by phagocytosis by the cells of the MPS like circulating as well as resident macrophages. Together, these two processes form the main mechanism for the elimination of NPs from the blood. The process of opsonization mainly depends on the physio-chemical characteristics of the NP like size, shape, charge and surface heterogeneity. Recently, Lunov et al. showed that the carboxy-functionalization of the NP surface enhanced its phagocytosis by macrophages while the amino-functionalization allowed for enhanced dynamin-dependent endocytosis by the PMA-differentiated monocytic THP-1 cells. Using apolipoproteinE (apoE) knockout mouse models, Yan et al. were able to demonstrate that the uptake of neutrally-charged liposomes was almost exclusively apoE-mediated while that of the negatively charged ones was not. But cationic or neutrally charged NPs are not the only ones susceptible to enhanced serum protein interactions. To specifically study the effect of surface charge on MPS uptake, Xiao et al. were able to demonstrate that NPs with high negative or positive charge were taken up by murine macrophages in vitro as well as in vivo, leading to higher accumulation of NPs in the liver. More recently, it was shown that the cellular binding of a variety of anionic NPs like quantum dots, citrate-modified gold NPs and low-density lipo-protein particles were significantly inhibited by extracellular serum proteins. It was suggested that this was due to the fact that the protein-NP complex would compete for the same receptors as the free extracellular proteins present thereby reducing their binding efficiencies. Similarly, Caraccioilo et al. had showed that though substitution of cationic lipids like DOTAP with neutral lipids like dioleoylphosphatidylethanolamine (DOPE) and cholesterol helped reduce the binding of fibrinogens, it increased the surface adsorption of other extracellular proteins like albumin and apolipoproteins by DOPE and immunoglobulins as well as complement proteins by cholesterol. In order to circumvent this MPS barrier, a variety of approaches have been used. One strategy has been to modify the NP surface with polymers in order to effectively increase its blood circulation time. One of the most common approaches involves the use of polyethylene glycol (PEG). Over the past decade, our group has repeatedly demonstrated the advantages of using PEGylated NPs. Recently, Parveen et al. showed that coating paclitaxel-loaded poly(lactic-co-glycolic acid) (PLGA) NPs with an optimal combination of chitosan and PEG dramatically prolonged blood circulation times while reducing the sequestration of NPs in the liver. Surface-coating of lipid emulsions with monoleate-modified PEG was also shown to significantly enhance the plasma concentration of the loaded breviscapine. The coating of PLGA NPs with poloxamer 188 was also shown to evade uptake by macrophages.

Nanoparticle extravasation

Once past the MPS barrier, the NP still has multiple hurdles to overcome before it is able to reach its target site. The vascular endothelial layer is one of its most significant barriers and represents a semi-permeable layer of cells which lines the inner walls of the blood vessels and along with the glycocalyx, a proteoglycan layer, serves to control the permeability of solutes and macromolecules across blood vessels. The glycocalyx ‘coat’ imparts a negative charge to the endothelial cell membrane and has been implicated in increased interactions with cationic particles. This could potentially sequester NPs thus preventing them from further extravasation into the tumor microspace.

Hemodynamics

The hemodynamics of nanoparticles is also an important parameter that determines their effective extravasation (See Figure 1). More than a decade ago, Aarts et al. had shown that red blood cells tend to flow in the center of blood vessels forcing the platelets out radially causing them to concentrate near the wall. When applied to the field of nanoparticulate drug delivery, this could lead to better extravasation past the endothelia. However, this has not been studied well enough and there is only a handful of pertaining literature available. Enhanced permeability and retention effect

Although ‘leakage’ of molecules through the vascular endothelium may occur through trans- and para-vascular pathways, NPs generally bigger than 5–6 nm would not be able to cross healthy vessels characterized by a continuous endothelium. However, under pathological states like inflammation, infarcts and tumors, the endothelial lining tends to become more permeable leading to ‘gaps’ in the lining. Matsumura and Maeda were the first to show that nanoparticles are able to extravasate through these gaps to reach the tumor space and stay there due to
the poor lymphatic drainage of tumors.\textsuperscript{39} This phenomenon was later termed as the enhanced permeability and retention (EPR) effect and paved the way for the passive targeting of tumors using NPs. Our group has successfully exploited this strategy in order to deliver a wide range of PEGylated nanoparticles like micelles, liposomes,\textsuperscript{36-38} and dendrimers\textsuperscript{42} among others. However, a number of limitations still exist, linked to the heterogeneity of tumors which can prevent the efficient extravasation of NPs.\textsuperscript{43} There seem to be significant differences in the endothelial pore sizes in primary and metastatic tumors as well as within the same tumor type. Targeting and manipulation of the tumor vasculature have emerged as useful strategies to overcome some of these limitations and have been discussed in greater detail below.

Targeting to facilitate extravasation

Though the addition of targeting moieties to NPs has been used extensively to improve intracellular delivery, it can also be used to enhance extravasation across endothelia characterized by tight junctions as is the case with the blood-brain barrier (BBB). Most of these approaches are based on the fact that the brain endothelium as well as glioma cells, overexpress certain cell-surface receptors like the transferrin, glucose transporter and low-density lipoprotein (LDL) receptors. Transferrin-targeted PLGA nanoparticles co-loaded with doxorubicin and paclitaxel have been used to demonstrate enhanced anti-glioma activity.\textsuperscript{34} The targeting with transferrin allowed for an 8-fold decrease in IC\textsubscript{50} values \textit{in vivo} while in \textit{in vitro} murine tumor model, the targeted drug combination showed a 47-fold reduction in tumor volume compared with a 1.3-fold reduction observed with the un-targeted combo. Similarly, Miao et al. demonstrated the use of paclitaxel-loaded NPs targeted with lactoferrin and a tumor-specific peptide as a novel approach for anti-glioma therapy.\textsuperscript{40} Kuang et al. have also employed the use of transferrin receptor-specific T7 peptide to deliver RNA\textsuperscript{46} showing that this strategy is not just specific to the delivery of small molecule drugs.\textsuperscript{47,48} Apart from transferrin-based strategies, various groups have also shown that targeting the LDL\textsuperscript{49,50} as well as glucose transporter receptors\textsuperscript{51} with NPs are equally viable approaches representing high therapeutic potentials.

Role of tumor vasculature

Initially, tumors are dependent on the vasculature of the surrounding host tissues for their blood supply. But as they grow further, they switch into an angiogenic state in order to meet their increasing metabolic demands.\textsuperscript{52} These changes in the tumor vasculature have been quantified in a study by Liotta et al.\textsuperscript{53} Tumors also show increased levels of growth factors like vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) among others.\textsuperscript{54,55} These growth factors significantly increase permeability of macromolecules by modulating the sub-endothelial structures.\textsuperscript{56} But the vascular permeability of tumors is also very heterogeneous in its distribution due to this abnormal angiogenesis and can negate the effects of EPR. Therefore, a number of groups have attempted to manipulate the tumor vasculature by the use of hyperthermia, growth factors, tumor necrosis factor (TNF) etc. in order to facilitate extravasation of NPs into the tumor microspace. Li et al. have shown that local hyperthermia was able to increase the vascular permeability up to 10 µm in a variety of tumor models.\textsuperscript{57} This allowed for increased liposomal extravasation not seen with normothermia. Similarly, Liu et al. were able to demonstrate that the thermally induced extravasation of liposomes led to their increased accumulation in murine mammary carcinomas.\textsuperscript{58} Application of exogenous VEGF was found to increase pore size of human colon carcinoma xenografts allowing for the enhanced extravasation of albumin (7nm) as well as PEGylated liposomes (100–400nm).\textsuperscript{59} Interestingly, no significant difference in pore size was seen on the application of other growth factors like placental growth factor (PIGF) and basic fibroblast growth factor (bFGF). The short half-life and acute toxicity resulting from high dose administration makes systemic treatment with free TNF-α difficult. To circumvent these issues, PEGylated polycyanocrylate NPs were developed and successfully evaluated as a delivery vehicle for TNF-α.\textsuperscript{60} In order to further minimize TNF-α associated toxicity, Corti et al. had coupled this with a tumor vasculature-specific cyclic CNGRC peptide.\textsuperscript{61} This followed by combination therapy with targeted doxorubicin liposomes, demonstrated a marked uptake in neuroblastoma tumors leading to enhanced therapeutic effects. The same group was also able to show that combinatorial treatment of TNF-α with a variety of chemotherapeutic drugs like paclitaxel, cisplatin, gemcitabine and melphalan led to better therapeutic outcomes.\textsuperscript{62,63} Interestingly, combination treatments did not show a marked increase in cytotoxicity \textit{in vitro} whereas \textit{in vivo} an almost synergistic effect was seen which indicates that TNF may be acting on the stromal compartment rather than directly on the tumor itself.

Tumor Microenvironment

After successfully crossing the vascular-endothelial barrier by extravasation, the NP still has to get through the complicated maze that is the tumor microenvironment. The main features of the microenvironment have been investigated in detail here, but their heterogeneous distribution throughout the microspace remains the biggest challenge.

Extracellular matrix

One of the first challenges is to cross the tumor interstitium or extra-cellular matrix (ECM) (See Figure 2). This consists of a cross-linked network of collagen and elastin fibers, proteoglycans and hyaluronic acid. It not only provides structural integrity, but also helps to transport important nutrients as well as oxygen to support cell growth. A highly developed matrix may result in significant resistance to the diffusion of therapeutic particles through the interstitium causing the drug-cargo to be released too far from the tumor space to have its intended effect.\textsuperscript{64} Netti and coworkers have studied the impeding role of the ECM to the passive diffusion of macromolecules like ImmunoglobulinG (IgG) through the interstitium.\textsuperscript{65} For their studies, they used four different tumor lines: human colon adenocarcinoma (LS174T), human glioblastoma (U87), human soft tissue sarcoma (HSTS26T) and a murine mammary carcinoma (MCAIV). They found that IgG had more resistance to diffusion in the U87 and HSTS26T than in the MCAIV and LS174T lines. In lieu of this observation, they found that collagenase treatment improved the diffusivity of IgG almost 2-fold. Subsequent histological studies showed that this difference in diffusivity correlates with the fact the U87 and HSTS26T lines had well-organized
collagen-proteoglycan-linked matrices, which were lacking in the other two tumor lines. Studies by Graff et al. as well as Pun et al. have shown that in many cases NPs are not able to efficiently penetrate and are localized in the peripheral regions of the tumor microspace. Using a multicellular spheroid model, Goodman and coworkers were able to demonstrate the efficiency of collagenase pre-treatment in improving the penetration of NPs. In a novel approach by Kuhn et al., superparamagnetic NPs were cross-linked with collagenase. Application of a magnetic field allowed for increased mobility of the NP while the collagenase served to keep the NP clear of any collagen barriers. Another approach has been to dilate the pores of the ECM by co-infusion of NPs with hyperosmolar mannitol solution or hypertonic buffer solution. Pre-treatment with hyaluronidase was also able to significant enhance NP penetration.

Matrix metalloproteinase

However aggressive a tumor maybe, their growth cannot be indefinite and will be limited depending on a number of factors such as the vascular supply, supply of nutrients, hypoxia, physical boundaries of the surrounding space etc. They are able to circumvent these limitations by the process of metastasis where they migrate and colonize distant organs or tissues. The presence of the ECM impedes the migration of metastatic cells. The destruction or partial degradation of this matrix by a battery of enzymes allows the tumor cell to circumvent this barrier. The presence of the ECM can be perceived as a hurdle or as an opportunity to be exploited. Almost two decades ago, Jones and coworkers showed that a variety of metastatic human tumor cell lines demonstrated both elastolytic and collagenolytic activity as well as a plasmin-induced degradation of the other matrix glycoproteins. The proteolysis of the extracellular matrices thus fuels the process of angiogenesis and is mediated by a family of proteases known as matrix metalloproteinases (MMPs). MMPs have been implicated in a variety of late stage metastatic cancers and have been evaluated as diagnostic biomarkers for a variety of malignant tumors. Their specific inhibition therefore, is of significant therapeutic value. Of the MMP family, MMP-2 and MMP-9 are thought to play a more prominent role in tumorigenesis. One of the most common strategies to target MMPs has been the use of tissue inhibitor of metalloproteinases (TIMP). Cheetty and coworkers have showed that downregulating MMP-2 in a murine lung cancer model was able to significantly inhibit angiogenesis by expression of tissue inhibitor of matrix protease-3 (TIMP-3) which prevents endothelial cell proliferation as well as VEGF expression. In a similar study, forced expression of TIMP-3 by retroviral gene delivery led to significant anti-angiogenic effects by the inhibition of capillary morphogenesis in a murine tumor model. Song et al. were also able to show that in a cervical cancer model, histone deacetylase (HDAC) inhibitors downregulate MMP-2 and MMP-9 levels and could subsequently prevent cancer metastasis. Zarrabi and coworkers showed that peptides targeting a PEX domain on MMP-14, a membrane anchored MMP, were able to significantly inhibit tumor dissemination. Administration of pigment-epithelium-derived factor has been shown to significantly reduce MMP-9 expression levels allowing for effective inhibition of gliomas. For more information on the early clinical studies, drawbacks and current approaches to MMP inhibition, see references. Another approach to exploiting MMPs has been the development of MMP-sensitive drug-release systems. Our group had recently demonstrated the use of a novel MMP-2 sensitive multifunctional liposome system. Presence of MMP triggers the shedding of the PEG coat exposing the underlying targeting moieties, a cancer-specific 2C5 antibody as well as a TATp cell-penetrating peptide, thus allowing for enhanced targetability and internalization. More recently, a TATp-targeted micellar carrier containing a MMP-2-sensitive paclitaxel prodrug was developed. This ‘smart’ nanocarrier was able to demonstrate enhanced anti-tumor activity both in vitro and in vivo in an A549 lung cancer model. For more information on such stimulus-sensitive preparations see reference.

Interstitial fluid pressure

Interstitial blood flow is one of the major effectors of nanoparticle distribution in the tumor microspace as the drug is effluxed from the vasculature through the interstitium and finally to the target cells. Drugs and various NPs move through the interstitium by diffusion based on a concentration gradient or by convection based on a pressure gradient. Uneven vascularization typical of tumors causes considerable heterogeneity in vascular blood flow. This combined with a lack of proper lymphatic drainage due to uneven lymphatic vessel distribution results in increased interstitial fluid pressure (IFP). In a recent study, Lunt and coworkers had experimentally determined the IFP in murine fibrocarcinoma as well as human cervical carcinoma models. They were not only able to find substantial variations in IFP values between the different models, but also between tumors.
growing in the same mouse thus demonstrating the extent of IFP heterogeneity. High IFP has also been implicated in increased metastatic frequency as well as reduced sensitivity to radiation. As it is evident that IFP is a direct consequence of angiogenesis, targeting the latter represents a simple approach to circumvent this barrier. Paclitaxel treatment has been shown to be effective in reducing IFP values in the clinic. VEGF blockade to inhibit angiogenesis is another promising strategy to aid in drug penetration against the pressure gradient. Treatment with Imatinib, a PDGF receptor-β inhibitor, led to decreased VEGF expression and subsequently decreased IFP. Similarly, Dickson et al. have shown that pretreatment with Bevacizumab, an anti-VEGF monoclonal antibody, helped to improve the anti-tumor efficacy of systemically administered topotecan in a murine neuroblastoma model. Vascular disrupting agents such as combretastatin and ZD6126, a tubulin-binding agent, have also successfully been used to reduce IFP.

Hypoxic core and extracellular pH of tumors

Due to the Warburg effect, the extracellular pH and oxygen concentration decreases as we move away from the vasculature into the tumor space. Acidic pH and low oxygen levels have been shown to impart resistance to certain anti-cancer therapies such as radiation and a variety of chemotherapeutic drugs. Hypoxic tumors demonstrate increased expression of chemokine ligand 28 (CCL28) which is implicated in angiogenesis and evasion of immune cell detection. Hypoxia can also be used as a diagnostic marker for late-stage tumors as they are associated with more malignant phenotypes. Hypoxia-specific targeting of drugs could thus significantly improve therapeutic efficacy. Harada et al. have shown the development of a hypoxia imaging system using a hypoxia-specific luciferase reporter. They subsequently showed that treatment with a hypoxia-sensitive prodrug TOP3, significantly enhanced hypoxic cell-death. Cairns and coworkers have shown that further increasing its hypoxic environment using inhibition of hypoxia inducible factor allows increased mitochondrial metabolism to facilitate enhanced targeting of hypoxia-specific cytotoxins. Targeting 4T1 mammary as well as MDAMB-231 lung metastatic tumor’s hypoxia machinery with the use carbboxic anhydrase inhibitors represents another novel approach. More recently, our group developed a novel hypoxia-sensitive NP for the tumoral delivery of siRNA which demonstrated enhanced downregulation of GFP-expressing tumors. In another very interesting study, Bettegowda and coworkers have shown anaerobic bacteria to overcome this hypoxic barrier and re-sensitize the cells to radiation therapy. The lower extracellular pH characteristic of tumors, may also affect the permeability of drugs. The change in pH may cause the drug to be more polar or charged thus preventing it from crossing biological membranes and exert its cytotoxic effects. A study by Vukovic et al. demonstrated the weakened cytotoxic effects of paclitaxel, mitoxantrone and topotecan at an extracellular pH of 6.5. Similar studies were performed on other chemotherapeutic agents like anthracyclines. Encapsulation of drugs into pH-sensitive NPs represents a novel approach to overcome this barrier. Some examples include 2C5-targeted pH-sensitive liposomes for doxorubicin delivery, pH-sensitive liposomes for gene delivery, micelles as well as polyethyleneimine-based NPs for DNA delivery. For more information see reviews.

Cellular Barriers

Cell membrane as a barrier

Most macromolecular drugs and genes exert their effects intracellularly. It is therefore imperative that their carrier be able to traverse the outer membrane of the cell. This internalization of the NP depends solely on its interaction with the cell membrane. Various models have been used to study these interactions and it has been seen that surface charge, hydrophobicity and size play prominent roles. It is well known that charged particles in general have increased interactions with the membrane while uncharged ones like PEGylated NPs have reduced interactions by virtue of their steric hindrance. This may cause the NP to ‘cluster’ around the membrane preventing further entry of the other NPs. As mentioned earlier in this review, proteins may adsorb onto the NP surface depending on their charge and this may well dictate their entry into the cell by receptor-mediated endocytosis. The effect of size on intracellular entry was studied in detail by Rejman and coworkers. It was seen that particles lesser than 200 nm were preferentially internalized by clathrin-mediated endocytosis, while with increasing particle sizes beyond 200 nm, a shift toward caveolin-mediated endocytosis was observed. It was also noticed that internalization was an energy-dependant process and depleting cholesterol seemed to inhibit particle uptake. Cancer cells overexpress a variety of cell-surface receptors like transferrin, folate acid, glucose transporters, integrins, and LDL among others and the active-targeting of NPs is a promising approach to facilitate their internalization. This concept has been around for a long time now and as numerous reviews have been published to keep abreast of advances, these will not be discussed here. However, just increasing the density of targeting ligands does not necessarily translate to better internalization. Sometimes, the targeting ligands may bind to the peripheral cancer cells with high affinity causing a ‘binding barrier’ which prevents the subsequent NPs from penetrating further. This was also noticed when the use of cell-penetrating peptides (CPPs) targeting heparan sulfate chains resulted in significant clusters which did not correlate with increased NP uptake. This could also be due to the fact that only a finite amount of energy is devoted by the cell for particle uptake. The kinetics of receptor recycling is thus an important parameter to study when designing ligand density and NP dosing regimens so as to not over-saturate the receptors. For more information see references.

Vesicular and organelar barriers

Once internalized, the NP needs to reach its intracellular target to unload its cargo. Endosomal vesicles are responsible for the trafficking of macromolecules to various intracellular sites like golgi, endoplasmic reticulum, nucleus, mitochondria and lysosome among others. This pre-determined trafficking of cargo has been shown to be signal-dependant and forms the basis of intracellular drug delivery. Endocytosis is mediated by a number of pathways and processes such as clathrin-dependant endocytosis, caveolin-dependant endocytosis, macropinocytosis, phagocytosis etc. (See Figure 3) and different NPs are internalized by different pathways. However a majority of these pathways may result in
trafficking to a non-target organelle site as well as the lysosome where the NP is subsequently degraded. This is especially important for the delivery of more labile drugs like genes and peptides. The use of cationic lipids and polymers for intracellular drug delivery by facilitating endosomal escape is well documented. Lipids such as DOTMA and DOTAP as well as branched polymers like polyethyleneimine and PAMAM dendrimers have been used extensively for their ability to fuse with the endosomal membrane thus dispensing their contents into the cytoplasm. As the endosome matures from early to late and subsequently lysosome, it acidifies internally by the action of ATPases. This property has been exploited by a variety of NPs to facilitate endosomal escape into the cytoplasm. Use of fusogenic lipids like DOPE has also proven popular, as these undergo transition from bilayer to hexagonal phase based on pH change facilitating the fusion with endosomal membrane. DOPE was also conjugated to low molecular weight polyethyleneimine to enhance endosomal escape and subsequent delivery of DNA as well as siRNA. For more information on these, see references. Another approach to improve intracellular drug delivery is receptor-mediated endocytosis by active targeting of NPs. Transferrin-targeting of liposomal ceramide allowed for enhanced lysosomal compartmentalization of ceramide. Once in the lysosome the ceramide is able to permeabilize the membrane and induce caspase-dependant apoptosis. Similarly, as folic acid is essential at sites of nucleic acid synthesis, its targeting of liposomal doxorubicin allows for nuclear accumulation of the drug as it subsequently intercalates with DNA and induces apoptosis.

**Drug efflux transporters**

As the journey of the NP nears its end, only a small fraction of the administered dose is available to exert its cytotoxic effects intracellularly. It is due to this that a variety of solid tumors, equip themselves to be able to efflux out these drugs with specialized machinery known as drug-efflux pumps. Overexpression of the drug efflux transporter, P-glycoprotein (P-gp) is one such mechanism and clinical refractoriness to chemotherapy has been extensively correlated with P-gp expression. It has been demonstrated that certain targeted NPs serve to circumvent these membrane bound drug-efflux pumps. Paclitaxel NPs targeted with a tumor vasculature-specific peptide were shown to be a valid strategy. Various small molecule P-gp inhibitors such as tariquidar, benzyl dihydroxypyridines as well as bacterial-derived compounds like H6 have been used in conjunction with anti-cancer drugs like paclitaxel to effectively combat drug-resistant cancers.

**Conclusion**

It is thus evident that the tremendous amount of research that has been performed in the last few decades has allowed us to effectively identify the specific barriers that exist to drug delivery. A lot of progress has also been made in the field of nanoparticulate delivery in order to overcome each of these barriers and a number of novel approaches have been brought to the forefront. It is now clearly evident that the active targeting of long-circulating NPs is not sufficient to translate into clinical success. A review of the recent literature suggests that along with combinatorial treatment regimens, stimulus-sensitive functions need to be accorded to these NPs as well. With the increasing number of multi-functional NPs now entering clinical studies, the development of the ideal ‘smart’ nanocarrier that will be able to traverse all these hurdles is fast becoming a distinct possibility.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.
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