Tissue Barriers 2, e29528; March 2014; © 2014 Landes Bioscience

Barriers to drug delivery in solid tumors

Shravan Kumar Sriraman, Bhawani Aryasomayajula and Vladimir P Torchilin*

Center for Pharmaceutical Biotechnology and Nanomedicine; Northeastern University; Boston, MA USA

Keywords: MPS, nanoparticle, drug delivery, biological barriers, cancer, tumors, EPR, multifunctional nanoparticles

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; bGFG, 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.[1] 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.[2] 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.[3] 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.[4] 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.

*Correspondence to: Vladimir P Torchilin; Email: v.torchilin@neu.edu
Submitted: 05/02/2014; Revised: 06/06/2014; Accepted: 06/09/2014
Citation: Sriraman SK, Aryasomayajula B, Torchilin VP. Barriers to drug delivery in solid tumors. Tissue Barriers 2014; 2:e29528; http://dx.doi.org/10.4161/tisb.29528

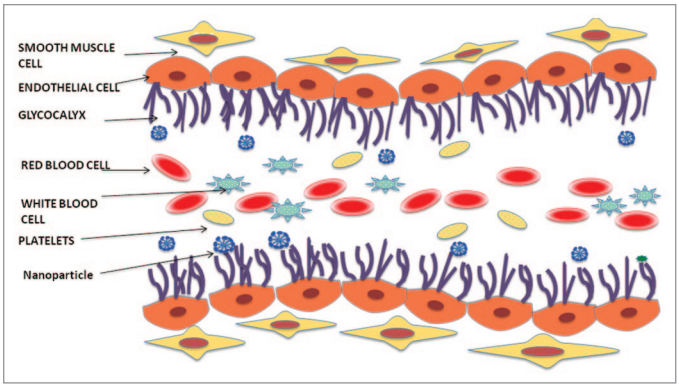

Figure 1. Hemodynamics of blood flow.

Biological Barriers

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'.[5] 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.[6] 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*.[7]

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 residual macrophages.[8] 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,[9] shape,[10] charge[11] and surface heterogeneity.[12] 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.[13] 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.[14] 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.[15] 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.[16] 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, Caracciolo *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.[17] 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.[18] One of the most common approaches involves the use of polyethylene glycol (PEG).[19,20] Over the past decade, our group has repeatedly demonstrated the advantages of using PEGylated NPs.[21-23] 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.[24] Surface-coating of lipid emulsions with monoleate-modified PEG was also shown to significantly enhance the plasma concentration of the loaded breviscapine.[25] The coating of PLGA NPs with poloxamer 188 was also shown to evade uptake by macrophages.[26]

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[27] and along with the glycocalyx, a proteoglycan layer, serves to control the permeability of solutes and macromolecules across blood vessels.[28] The glycocalyx 'coat' imparts a negative charge to the endothelial cell membrane and has been implicated in increased interactions with cationic particles.[29] 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 vessel wall.[30] 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.[31-33]

Enhanced permeability and retention effect

Although 'leakage' of molecules through the vascular endothelium may occur through trans- and para-cellular pathways,[34] 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.[35] 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,[36-38] liposomes[39-41] and dendrimers[42] among others. However, a number of limitations still exist, linked to the heterogeneity of tumors which can prevent the efficient extravasation of NPs.[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.[44] The targeting with transferrin allowed for an 8-fold decrease in IC_{50} values *in vitro* while in an *in vivo* 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.[45] Kuang *et al.* have also employed the use of transferrin receptor-specific T7 peptide to deliver RNA[46] showing that this strategy is not just specific to the delivery of small molecule drugs.[47,48] Apart from transferrin-based strategies, various groups have also shown that targeting the LDL[49,50] as well as glucose transporter receptors[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.[52] These changes in the tumor vasculature have been quantified in a study by Liotta *et al.*[53] Tumors also show increased levels of growth factors like vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) among others.[54,55] These growth factors significantly increase permeability of macromolecules by modulating the sub-endothelial structures.[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 upto 10 μm in a variety of tumor models.[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.[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).[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 polycyanoacrylate NPs were developed and successfully evaluated as a delivery vehicle for TNF-α.[60] In order to further minimize TNF-α associated toxicity, Corti *et al*. had coupled this with a tumor vasculature-specific cyclic CNGRC peptide.[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.[62,63] Interestingly, combination treatments did not show a marked increase in cytotoxicity *in vitro* whereas *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.[64] Netti and coworkers have studied the impeding role of the ECM to the passive diffusion of macromolecules like ImmunoglobulinG (IgG) through the interstitium.[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

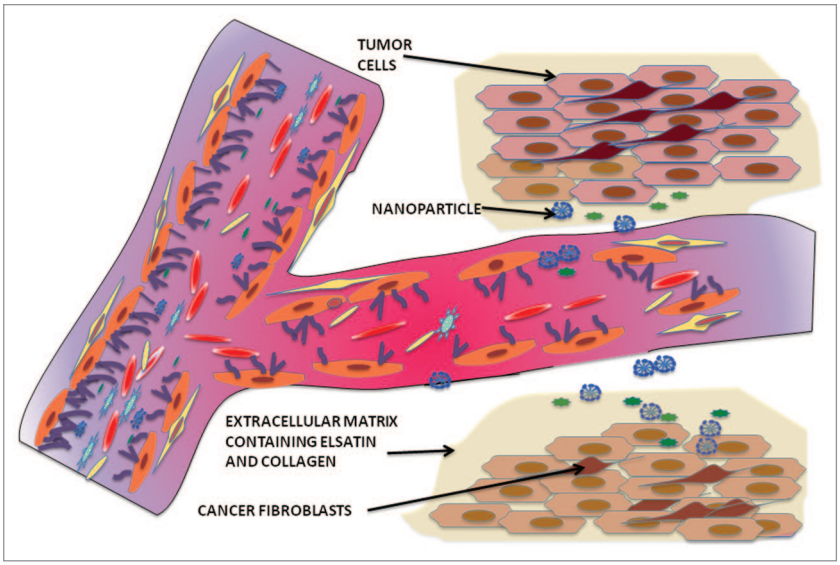

Figure 2. Extravasation of nanoparticles from systemic circulation into the tumor interstitium.

collagen-proteoglycan-linked matrices, which were lacking in the other two tumor lines. Studies by Graff *et al*.[66] as well as Pun *et al*.[67] 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.[68] In a novel approach by Kuhn *et al*., superparamagnetic NPs were cross-linked with collagenase.[69] 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.[70] 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.[71] 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.[72] 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.[73] 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).[74,75] 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.[76] Their specific inhibition therefore, is of significant therapeutic value.[77,78] 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).[79] Chetty 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.[80] 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.[81] 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.[82] Zarrabi and coworkers showed that peptides targeting a PEX domain on MMP-14, a membrane anchored MMP, were able to significantly inhibit tumor dissemination.[83] Administration of pigment-epithelium-derived factor has been shown to significantly reduce MMP-9 expression levels allowing for effective inhibition of gliomas.[84] For more information on the early clinical studies, drawbacks and current approaches to MMP inhibition, see references.[85-87] 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.[23] 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.[88] 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.[89]

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.[90] 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).[91] In a recent study, Lunt and coworkers had experimentally determined the IFP in murine fibrocarcinoma as well as human cervical carcinoma models.[92] 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.[93] As it is evident that IFP is a direct consequence of angiogenesis, targeting the latter represents a simple approach to circumvent this barrier.[94] Paclitaxel treatment has been shown to be effective in reducing IFP values in the clinic.[95] VEGF blockade to inhibit angiogenesis is another promising strategy to aid in drug penetration against the pressure gradient.[96] Treatment with Imatinib, a PDGF receptor-β inhibitor, led to decreased VEGF expression and subsequently decreased IFP.[97] 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.[98] Vascular disrupting agents such as combretastatin and ZD6126, a tubulin-binding agent, have also successfully been used to reduce IFP.[99,100]

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.[101] 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.[102] Hypoxic tumors demonstrate increased expression of chemokine ligand 28 (CCL28) which is implicated in angiogenesis and evasion of immune cell detection.[103] Hypoxia can also be used as a diagnostic marker for late-stage tumors as they are associated with more malignant phenotypes.[104] 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.[105] 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.[106] Targeting 4T1 mammary as well as MDAMB-231 lung metastatic tumor's hypoxia machinery with the use carboxic anhydrase inhibitors represents another novel approach.[107] More recently, our group developed a novel hypoxia-sensitive NP for the tumoral delivery of siRNA which demonstrated enhanced downregulation of GFP-expressing tumors.[108] In another very interesting study, Bettegowda and coworkers have used anaerobic bacteria to overcome this hypoxic barrier and re-sensitize the cells to radiation therapy.[109] The lower extracellular pH characteristic of tumors, may also affect the permeability of drugs.[110] 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.[111] A study by Vukovic *et al*. demonstrated the weakened cytotoxic effects of paclitaxel, mitoxantrone and topotecan at an extracellular pH of 6.5.[112] Similar studies were performed on other chemotherapeutic agents like anthracyclines.[113] 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,[114] pH-sensitive liposomes for gene delivery,[115] micelles[116] as well as polyethyleneimine-based NPs for DNA delivery.[117] For more information see reviews.[89,118]

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.[119] 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.[120] 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.[16] The effect of size on intracellular entry was studied in detail by Rejman and coworkers.[121] 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, folic acid, glucose transporters, integrins, and LDL among others and the active-targeting of NPs is a promising approach to facilitate their internalization.[122] 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.[122-124] 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.[125] 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.[126-128]

Vesicular and organellar 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, endoplastic 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.[129] 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.[130] However a majority of these pathways may result in

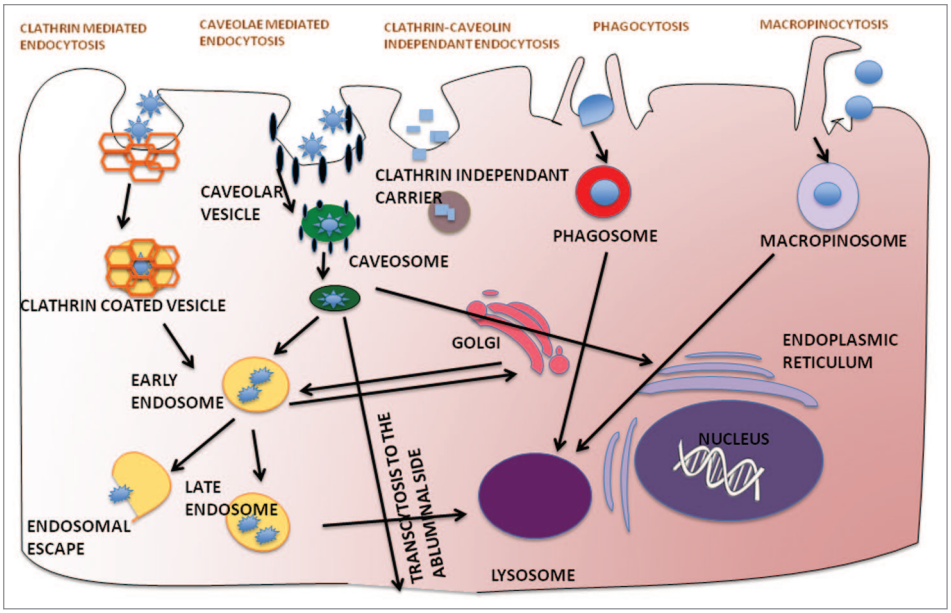

Figure 3. The various mechanisms of cellular internalization of nanoparticles via clathrin-mediated endocytosis, caveolin-mediated endocytosis, clathrin-caveolin independent endocytosis, phagocytosis and macropinocytosis and their subsequent intracellular trafficking

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.[131] 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 dispelling their contents into the cytoplasm.[132] As the endosome matures from early to late and subsequently lysososome, 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.[133,134] DOPE was also conjugated to low molecular weight polyethyleneimine to enhance endosomal escape and subsequent delivery of DNA as well as siRNA.[135,136] For more information on these, see references.[137,138] 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.[139] Once in the lysososome the ceramide is able to permeabilize the membrane and induce caspase-dependant apoptosis. Similarly, as folic acid is essential at sites of nucleotide synthesis, its targeting of liposomal doxorubicin allows for nuclear accumulation of the drug as it subsequently intercalates with DNA and induces apoptosis.[140]

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.[141] 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.[142] Various small molecule P-gp inhibitors such as tariquidar, benzyl dihydropyridines 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.[143-146] As P-gp is also expressed in the BBB, Patil *et al.* have demonstrated the use of targeted tariquidar and paclitaxel using NPs to minimize drug toxicity.[147] Recent work shows that this P-gp expression is regulated by the MDR gene family and induced by a variety of transcription factors such as NF-κB.[148] Kovalchuk and coworkers have also showed that transfection of cancer cells with a microRNA was able to re-sensitize the cells to the primary treatment modality.[149] Co-delivery of anti-survivin siRNA with doxorubicin was also shown to successfully overcome resistance to doxorubicin in human breast cancer cells.[150]

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 *in vivo* 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 tranverse all these hurdles is fast becoming a distinct possibility.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144:646-74; PMID:21376230; http://dx.doi.org/10.1016/j.cell.2011.02.013
2. Chrastina A, Massey KA, Schnitzer JE. Overcoming in vivo barriers to targeted nanodelivery. Wiley Interdiscip Rev Nanomed Nanobiotechnol 2011; 3:421-37; PMID:21538941; http://dx.doi.org/10.1002/wnan.143
3. Rabanel JM, Aoun V, Elkin I, Mokhtar M, Hildgen P. Drug-loaded nanocarriers: passive targeting and crossing of biological barriers. Curr Med Chem 2012; 19:3070-102; PMID:22612696; http://dx.doi.org/10.2174/092986712800784702
4. Torchilin VP. Multifunctional nanocarriers. Adv Drug Deliv Rev 2012; 64:302-15; PMID:17092599; http://dx.doi.org/10.1016/j.addr.2012.09.031
5. Nel AE, Mädler L, Velegol D, Xia T, Hoek EM, Somasundaran P, Klaessig F, Castranova V, Thompson M. Understanding biophysicochemical interactions at the nano-bio interface. Nat Mater 2009; 8:543-57; PMID:19525947; http://dx.doi.org/10.1038/nmat2442
6. Aggarwal P, Hall JB, McLeland CB, Dobrovolskaia MA, McNeil SE. Nanoparticle interaction with plasma proteins as it relates to particle biodistribution, biocompatibility and therapeutic efficacy. Adv Drug Deliv Rev 2009; 61:428-37; PMID:19376175; http://dx.doi.org/10.1016/j.addr.2009.03.009
7. Cedervall T, Lynch I, Lindman S, Berggård T, Thulin E, Nilsson H, Dawson KA, Linse S. Understanding the nanoparticle-protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles. Proc Natl Acad Sci U S A 2007; 104:2050-5; PMID:17267609; http://dx.doi.org/10.1073/pnas.0608582104
8. Hume DA. The mononuclear phagocyte system. Curr Opin Immunol 2006; 18:49-53; PMID:16338128; http://dx.doi.org/10.1016/j.coi.2005.11.008
9. Harashima H, Sakata K, Funato K, Kiwada H. Enhanced hepatic uptake of liposomes through complement activation depending on the size of liposomes. Pharm Res 1994; 11:402-6; PMID:8008707; http://dx.doi.org/10.1023/A:1018965121222
10. Decuzzi P, Godin B, Tanaka T, Lee SY, Chiappini C, Liu X, Ferrari M. Size and shape effects in the biodistribution of intravascularly injected particles. J Control Release. 2010 Feb 15;141(3):320-7
11. He C, Hu Y, Yin L, Tang C, Yin C. Effects of particle size and surface charge on cellular uptake and biodistribution of polymeric nanoparticles. Biomaterials 2010; 31:3657-66; PMID:20138662; http://dx.doi.org/10.1016/j.biomaterials.2010.01.065
12. Kulkarni SA, Feng SS. Effects of particle size and surface modification on cellular uptake and biodistribution of polymeric nanoparticles for drug delivery. Pharm Res 2013; 30:2512-22; PMID:23314933; http://dx.doi.org/10.1007/s11095-012-0958-3
13. Lunov O, Syrovets T, Loos C, Beil J, Delacher M, Tron K, Nienhaus GU, Musyanovych A, Mailänder V, Landfester K, et al. Differential uptake of functionalized polystyrene nanoparticles by human macrophages and a monocytic cell line. ACS Nano 2011; 5:1657-69; PMID:21344890; http://dx.doi.org/10.1021/nn2000756
14. Yan X, Kuipers F, Havekes LM, Havinga R, Dontje B, Poelstra K, Scherphof GL, Kamps JA. The role of apolipoprotein E in the elimination of liposomes from blood by hepatocytes in the mouse. Biochem Biophys Res Commun 2005; 328:57-62; PMID:15670750; http://dx.doi.org/10.1016/j.bbrc.2004.12.137
15. Xiao K, Li Y, Luo J, Lee JS, Xiao W, Gonik AM, Agarwal RG, Lam KS. The effect of surface charge on in vivo biodistribution of PEG-oligocholic acid based micellar nanoparticles. Biomaterials 2011; 32:3435-46; PMID:21295849; http://dx.doi.org/10.1016/j.biomaterials.2011.01.021
16. Fleischer CC, Kumar U, Payne CK. Cellular Binding of Anionic Nanoparticles is Inhibited by Serum Proteins Independent of Nanoparticle Composition. Biomater Sci 2013; 1:975-82; PMID:23956836; http://dx.doi.org/10.1039/c3bm60121h
17. Caracciolo G, Pozzi D, Capriotti AL, Cavaliere C, Laganà A. Effect of DOPE and cholesterol on the protein adsorption onto lipid nanoparticles. J Nanopart Res 2013; 15.3:1-11
18. Storm G, Belliot SO, Daemen T, Lasic DD. Surface modification of nanoparticles to oppose uptake by the mononuclear phagocyte system. Adv Drug Deliv Rev 1995; 17:31-48; http://dx.doi.org/10.1016/0169-409X(95)00039-A
19. Torchilin V, Papisov M. Why do polyethylene glycol-coated liposomes circulate so long?: Molecular mechanism of liposome steric protection with polyethylene glycol: Role of polymer chain flexibility. J Liposome Res 1994; 4:725-39; http://dx.doi.org/10.3109/08982109409037068
20. Vonarbourg A, Passirani C, Saulnier P, Simard P, Leroux JC, Benoit JP. Evaluation of pegylated lipid nanocapsules versus complement system activation and macrophage uptake. J Biomed Mater Res A 2006; 78A:620-8; PMID:16779767; http://dx.doi.org/10.1002/jbm.a.30711
21. Apte A, Koren E, Koshkaryev A, Torchilin VP. Doxorubicin in TAT peptide-modified multifunctional immunoliposomes demonstrates increased activity against both drug-sensitive and drug-resistant ovarian cancer models. Cancer Biol Ther 2014; 15:69-80; PMID:24145298; http://dx.doi.org/10.4161/cbt.26609
22. Etzerodt A, Maniecki MB, Graversen JH, Moller HJ, Torchilin VP, Moestrup SK. Efficient intracellular drug-targeting of macrophages using stealth liposomes directed to the hemoglobin scavenger receptor CD163. J Control Release. 2012 May 30;160(1):72-80
23. Zhu L, Kate P, Torchilin VP. Matrix metalloprotease 2-responsive multifunctional liposomal nanocarrier for enhanced tumor targeting. ACS Nano 2012; 6:3491-8; PMID:22409425; http://dx.doi.org/10.1021/nn300524f
24. Parveen S, Sahoo SK. Long circulating chitosan/PEG blended PLGA nanoparticle for tumor drug delivery. Eur J Pharmacol 2011; 670:372-83; PMID:21951969; http://dx.doi.org/10.1016/j.ejphar.2011.09.023
25. Xiong F, Xiong C, Yao J, Chen X, Gu N. Preparation, characterization and evaluation of breviscapine lipid emulsions coated with monooleate-PEG-COOH. Int J Pharm 2011; 421:275-82; PMID:22001842; http://dx.doi.org/10.1016/j.ijpharm.2011.10.008
26. Jain D, Athawale R, Bajaj A, Shrikhande S, Goel PN, Gude RP. Studies on stabilization mechanism and stealth effect of poloxamer 188 onto PLGA nanoparticles. Colloids Surf B Biointerfaces 2013; 109:59-67; PMID:23608470; http://dx.doi.org/10.1016/j.colsurfb.2013.03.027
27. Malik AB, Lynch JJ, Cooper JA. Endothelial barrier function. J Invest Dermatol 1989; 93(Suppl):62S-7S; PMID:2546995; http://dx.doi.org/10.1111/1523-1747.ep12581072
28. Rehm M, Zahler S, Lötsch M, Welsch U, Conzen P, Jacob M, Becker BF. Endothelial glycocalyx as an additional barrier determining extravasation of 6% hydroxyethyl starch or 5% albumin solutions in the coronary vascular bed. Anesthesiology 2004; 100:1211-23; PMID:15114220; http://dx.doi.org/10.1097/00000542-200405000-00025
29. Dull RO, Dinavahi R, Schwartz L, Humphries DE, Berry D, Sasisekharan R, Garcia JG. Lung endothelial heparan sulfates mediate cationic peptide-induced barrier dysfunction: a new role for the glycocalyx. Am J Physiol Lung Cell Mol Physiol 2003; 285:L986-95; PMID:12754183
30. Aarts PA, van den Broek SA, Prins GW, Kuiken GD, Sixma JJ, Heethaar RM. Blood platelets are concentrated near the wall and red blood cells, in the center in flowing blood. Arteriosclerosis 1988; 8:819-24; PMID:3196226; http://dx.doi.org/10.1161/01.ATV.8.6.819
31. Charoenphol P, Onyskiw PJ, Carrasco-Teja M, Eniola-Adefeso O. Particle-cell dynamics in human blood flow: implications for vascular-targeted drug delivery. J Biomech 2012; 45:2822-8; PMID:23010218; http://dx.doi.org/10.1016/j.jbiomech.2012.08.035
32. Charoenphol P, Huang RB, Eniola-Adefeso O. Potential role of size and hemodynamics in the efficacy of vascular-targeted spherical drug carriers. Biomaterials 2010; 31:1392-402; PMID:19954839; http://dx.doi.org/10.1016/j.biomaterials.2009.11.007
33. Charoenphol P, Mocherla S, Bouis D, Namdee K, Pinsky DJ, Eniola-Adefeso O. Targeting therapeutics to the vascular wall in atherosclerosis--carrier size matters. Atherosclerosis 2011; 217:364-70; PMID:21601207; http://dx.doi.org/10.1016/j.atherosclerosis.2011.04.016
34. Armstrong SM, Khajoee V, Wang C, Wang T, Tigdi J, Yin J, Kuebler WM, Gillrie M, Davis SP, Ho M, et al. Co-regulation of transcellular and paracellular leak across microvascular endothelium by dynamin and Rac. Am J Pathol 2012; 180:1308-23; PMID:22203054; http://dx.doi.org/10.1016/j.ajpath.2011.12.002
35. Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. Cancer Res 1986; 46:6387-92; PMID:2946403
36. Riehle RD, Cornea S, Degterev A, Torchilin V. Micellar formulations of pro-apoptotic DM-PIT-1 analogs and TRAIL in vitro and in vivo. Drug Deliv 2013; 20:78-85; PMID:23495715; http://dx.doi.org/10.3109/10717544.2013.766780
37. Sawant RR, Jhaveri AM, Koshkaryev A, Qureshi F, Torchilin VP. The effect of dual ligand-targeted micelles on the delivery and efficacy of poorly soluble drug for cancer therapy. J Drug Target 2013; 21:630-8; PMID:23594094; http://dx.doi.org/10.3109/1061186X.2013.789032
38. Sawant RR, Torchilin VP. Enhanced cytotoxicity of TATp-bearing paclitaxel-loaded micelles in vitro and in vivo. Int J Pharm 2009; 374:114-8; PMID:19446767; http://dx.doi.org/10.1016/j.ijpharm.2009.02.022
39. Biswas S, Deshpande PP, Perche F, Dodwadkar NS, Sane SD, Torchilin VP. Octa-arginine-modified pegylated liposomal doxorubicin: an effective treatment strategy for non-small cell lung cancer. Cancer Lett 2013; 335:191-200; PMID:23419527; http://dx.doi.org/10.1016/j.canlet.2013.02.020
40. Elbayoumi TA, Torchilin VP. Tumor-specific antibody-mediated targeted delivery of Doxil reduces the manifestation of auricular erythema side effect in mice. Int J Pharm 2008; 357:272-9; PMID:18329201; http://dx.doi.org/10.1016/j.ijpharm.2008.01.041
41. Sawant RR, Vaze OS, Wang T, D'Souza GG, Rockwell K, Gada K, Khaw BA, Torchilin VP. Palmitoyl ascorbate liposomes and free ascorbic acid: comparison of anticancer therapeutic effects upon parenteral administration. Pharm Res 2012; 29:375-83; PMID:21845505; http://dx.doi.org/10.1007/s11095-011-0557-8
42. Biswas S, Deshpande PP, Navarro G, Dodwadkar NS, Torchilin VP. Lipid modified triblock PAMAM-based nanocarriers for siRNA drug co-delivery. Biomaterials 2013; 34:1289-301; PMID:23137395; http://dx.doi.org/10.1016/j.biomaterials.2012.10.024

43. Prabhakar U, Maeda H, Jain RK, Sevick-Muraca EM, Zamboni W, Farokhzad OC, Barry ST, Gabizon A, Grodzinski P, Blakey DC. Challenges and key considerations of the enhanced permeability and retention effect for nanomedicine drug delivery in oncology. Cancer Res 2013; 73:2412-7; PMID:23423979; http://dx.doi.org/10.1158/0008-5472.CAN-12-4561
44. Cui Y, Xu Q, Chow PK, Wang D, Wang CH. Transferrin-conjugated magnetic silica PLGA nanoparticles loaded with doxorubicin and paclitaxel for brain glioma treatment. Biomaterials 2013; 34:8511-20; PMID:23932498; http://dx.doi.org/10.1016/j.biomaterials.2013.07.075
45. Miao D, Jiang M, Liu Z, Gu G, Hu Q, Kang T, Song Q, Yao L, Li W, Gao X, et al. Co-administration of dual-targeting nanoparticles with penetration enhancement peptide for antiglioblastoma therapy. Mol Pharm 2014; 11:90-101; PMID:24295590; http://dx.doi.org/10.1021/mp400189j
46. Kuang Y, An S, Guo Y, Huang S, Shao K, Liu Y, Li J, Ma H, Jiang C. T7 peptide-functionalized nanoparticles utilizing RNA interference for glioma dual targeting. Int J Pharm 2013; 454:11-20; PMID:23867728; http://dx.doi.org/10.1016/j.ijpharm.2013.07.019
47. Gao JQ, Lv Q, Li LM, Tang XJ, Li FZ, Hu YL, Han M. Glioma targeting and blood-brain barrier penetration by dual-targeting doxorubincin liposomes. Biomaterials 2013; 34:5628-39; PMID:23628475; http://dx.doi.org/10.1016/j.biomaterials.2013.03.097
48. Lv Q, Li LM, Han M, Tang XJ, Yao JN, Ying XY, Li FZ, Gao JQ. Characteristics of sequential targeting of brain glioma for transferrin-modified cisplatin liposome. Int J Pharm 2013; 444:1-9; PMID:23347891; http://dx.doi.org/10.1016/j.ijpharm.2013.01.025
49. Gao X, Qian J, Zheng S, Xiong Y, Man J, Cao B, Wang L, Ju S, Li C. Up-regulating blood brain barrier permeability of nanoparticles via multivalent effect. Pharm Res 2013; 30:2538-48; PMID:23494145; http://dx.doi.org/10.1007/s11095-013-1004-9
50. Zhang B, Sun X, Mei H, Wang Y, Liao Z, Chen J, Zhang Q, Hu Y, Pang Z, Jiang X. LDLR-mediated peptide-22-conjugated nanoparticles for dual-targeting therapy of brain glioma. Biomaterials 2013; 34:9171-82; PMID:24008043; http://dx.doi.org/10.1016/j.biomaterials.2013.08.039
51. Ying X, Wen H, Lu WL, Du J, Guo J, Tian W, Men Y, Zhang Y, Li RJ, Yang TY, et al. Dual-targeting daunorubicin liposomes improve the therapeutic efficacy of brain glioma in animals. J Control Release. 2010 Jan 25;141(2):183-92
52. Folkman J, Watson K, Ingber D, Hanahan D. Induction of angiogenesis during the transition from hyperplasia to neoplasia. Nature 1989; 339:58-61; PMID:2469964; http://dx.doi.org/10.1038/339058a0
53. Liotta LA, Kleinerman J, Saidel GM. Quantitative relationships of intravascular tumor cells, tumor vessels, and pulmonary metastases following tumor implantation. Cancer Res 1974; 34:997-1004; PMID:4841969
54. Chen W, Tang T, Eastham-Anderson J, Dunlap D, Alicke B, Nannini M, Gould S, Yauch R, Modrusan Z, DuPree KJ, et al. Canonical hedgehog signaling augments tumor angiogenesis by induction of VEGF-A in stromal perivascular cells. Proc Natl Acad Sci U S A 2011; 108:9589-94; PMID:21597001; http://dx.doi.org/10.1073/pnas.1017945108
55. Kerbel RS. Tumor angiogenesis. N Engl J Med 2008; 358:2039-49; PMID:18463380; http://dx.doi.org/10.1056/NEJMra0706596
56. Stan RV, Tse D, Deharvengt SJ, Smits NC, Xu Y, Luciano MR, McGarry CL, Buitendijk M, Nemani KV, Elgueta R, et al. The diaphragms of fenestrated endothelia: gatekeepers of vascular permeability and blood composition. Dev Cell 2012; 23:1203-18; PMID:23237953; http://dx.doi.org/10.1016/j.devcel.2012.11.003
57. Li L, ten Hagen TL, Bolkestein M, Gasselhuber A, Yatvin J, van Rhoon GC, Eggermont AM, Haemmerich D, Koning GA. Improved intratumoral nanoparticle extravasation and penetration by mild hyperthermia. J Control Release. 2013 Apr 28;167(2):130-7
58. Liu P, Zhang A, Xu Y, Xu LX. Study of non-uniform nanoparticle liposome extravasation in tumour. International journal of hyperthermia: the official journal of European Society for Hyperthermic Oncology. North American Hyperthermia Group 2005; 21:259-70; http://dx.doi.org/10.1080/02656730500068643
59. Monsky WL, Fukumura D, Gohongi T, Ancukiewcz M, Weich HA, Torchilin VP, Yuan F, Jain RK. Augmentation of transvascular transport of macromolecules and nanoparticles in tumors using vascular endothelial growth factor. Cancer Res 1999; 59:4129-35; PMID:10463618
60. Li Y-P, Pei Y-Y, Zhou Z-H, Zhang X-Y, Gu Z-H, Ding J, Zhou J-J, Gao X-J. PEGylated polycyanoacrylate nanoparticles as tumor necrosis factor-α carriers. J Control Release 2001; 71:287-96; PMID:11295221; http://dx.doi.org/10.1016/S0168-3659(01)00235-8
61. Corti A, Ponzoni M. Tumor vascular targeting with tumor necrosis factor alpha and chemotherapeutic drugs. Ann N Y Acad Sci 2004; 1028:104-12; PMID:15650236; http://dx.doi.org/10.1196/annals.1322.011
62. Curnis F, Sacchi A, Corti A. Improving chemotherapeutic drug penetration in tumors by vascular targeting and barrier alteration. J Clin Invest 2002; 110:475-82; PMID:12189241; http://dx.doi.org/10.1172/JCI0215223
63. Sacchi A, Gasparri A, Gallo-Stampino C, Toma S, Curnis F, Corti A. Synergistic antitumor activity of cisplatin, paclitaxel, and gemcitabine with tumor vasculature-targeted tumor necrosis factor-alpha. Clin Cancer Res. 2006 Jan 1;12(1):175-82
64. Kuppen PJK, van der Eb MM, Jonges LE, Hagenaars M, Hokland ME, Nannmark U, Goldfarb RH, Basse PH, Fleuren GJ, Hoeben RC, et al. Tumor structure and extracellular matrix as a possible barrier for therapeutic approaches using immune cells or adenoviruses in colorectal cancer. Histochem Cell Biol 2001; 115:67-72; PMID:11219610; http://dx.doi.org/10.1007/s004180000224
65. Netti PA, Berk DA, Swartz MA, Grodzinsky AJ, Jain RK. Role of extracellular matrix assembly in interstitial transport in solid tumors. Cancer Res 2000; 60:2497-503; PMID:10811131
66. Graff BA, Vangberg L, Rofstad EK. Quantitative assessment of uptake and distribution of iron oxide particles (NC100150) in human melanoma xenografts by contrast-enhanced MRI. Magn Reson Med. 2004 Apr;51(4):727-35
67. Pun SH, Tack F, Bellocq NC, Cheng J, Grubbs BH, Jensen GS, Davis ME, Brewster M, Janicot M, Janssens B, et al. Targeted delivery of RNA-cleaving DNA enzyme (DNAzyme) to tumor tissue by transferrin-modified, cyclodextrin-based particles. Cancer Biol Ther 2004; 3:641-50; PMID:15136766; http://dx.doi.org/10.4161/cbt.3.7.918
68. Goodman TT, Olive PL, Pun SH. Increased nanoparticle penetration in collagenase-treated multicellular spheroids. Int J Nanomedicine 2007; 2:265-74; PMID:17722554
69. Kuhn SJ, Finch SK, Hallahan DE, Giorgio TD. Proteolytic surface functionalization enhances in vitro magnetic nanoparticle mobility through extracellular matrix. Nano Lett 2006; 6:306-12; PMID:16464055; http://dx.doi.org/10.1021/nl052241g
70. Neeves KB, Sawyer AJ, Foley CP, Saltzman WM, Olbricht WL. Dilation and degradation of the brain extracellular matrix enhances penetration of infused polymer nanoparticles. Brain Res 2007; 1180:121-32; PMID:17920047; http://dx.doi.org/10.1016/j.brainres.2007.08.050
71. Chiang AC, Massagué J. Molecular basis of metastasis. N Engl J Med 2008; 359:2814-23; PMID:19109576; http://dx.doi.org/10.1056/NEJMra0805239
72. Stetler-Stevenson WG, Yu AE. Proteases in invasion: matrix metalloproteinases. Semin Cancer Biol 2001; 11:143-52; PMID:11322833; http://dx.doi.org/10.1006/scbi.2000.0365
73. Jones PA, DeClerck YA. Destruction of extracellular matrices containing glycoproteins, elastin, and collagen by metastatic human tumor cells. Cancer Res 1980; 40:3222-7; PMID:7000340
74. Stetler-Stevenson WG, Liotta LA, Kleiner DE Jr. Extracellular matrix 6: role of matrix metalloproteinases in tumor invasion and metastasis. FASEB J. 1993 Dec;7(15):1434-41
75. Chambers AF, Matrisian LM. Changing views of the role of matrix metalloproteinases in metastasis. J Natl Cancer Inst 1997; 89:1260-70; PMID:9293916; http://dx.doi.org/10.1093/jnci/89.17.1260
76. Wu ZS, Wu Q, Yang JH, Wang HQ, Ding XD, Yang F, Xu XC. Prognostic significance of MMP-9 and TIMP-1 serum and tissue expression in breast cancer. Int J Cancer. 2008 May 1;122(9):2050-6
77. Brown PD, Giavazzi R. Matrix metalloproteinase inhibition: a review of anti-tumour activity. Ann Oncol. 1995 Dec;6(10):967-74
78. Stetler-Stevenson WG. Matrix metalloproteinases in angiogenesis: a moving target for therapeutic intervention. J Clin Invest 1999; 103:1237-41; PMID:10225966; http://dx.doi.org/10.1172/JCI6870
79. Wei S, Kashiwagi M, Kota S, Xie Z, Nagase H, Brew K. Reactive site mutations in tissue inhibitor of metalloproteinase-3 disrupt inhibition of matrix metalloproteinases but not tumor necrosis factor-alpha-converting enzyme. J Biol Chem 2005; 280:32877-82; PMID:16079149; http://dx.doi.org/10.1074/jbc.C500220200
80. Chetty C, Lakka SS, Bhoopathi P, Kunigal S, Geiss R, Rao JS. Tissue inhibitor of metalloproteinase 3 suppresses tumor angiogenesis in matrix metalloproteinase 2-down-regulated lung cancer. Cancer Res 2008; 68:4736-45; PMID:18559520; http://dx.doi.org/10.1158/0008-5472.CAN-07-6612
81. Spurbeck WW, Ng CY, Strom TS, Vanin EF, Davidoff AM. Enforced expression of tissue inhibitor of matrix metalloproteinase-3 affects functional capillary morphogenesis and inhibits tumor growth in a murine tumor model. Blood 2002; 100:3361-8; PMID:12384438; http://dx.doi.org/10.1182/blood.V100.9.3361
82. Song C, Zhu S, Wu C, Kang J. Histone deacetylase (HDAC) 10 suppresses cervical cancer metastasis through inhibition of matrix metalloproteinase (MMP) 2 and 9 expression. J Biol Chem 2013; 288:28021-33; PMID:23897811; http://dx.doi.org/10.1074/jbc.M113.498758
83. Zarrabi K, Dufour A, Li J, Kuscu C, Pulkoski-Gross A, Zhi J, Hu Y, Sampson NS, Zucker S, Cao J. Inhibition of matrix metalloproteinase 14 (MMP-14)-mediated cancer cell migration. J Biol Chem 2011; 286:33167-77; PMID:21795678; http://dx.doi.org/10.1074/jbc.M111.256644
84. Zhang T, Guan M, Xu C, Chen Y, Lu Y. Pigment epithelium-derived factor inhibits glioma cell growth in vitro and in vivo. Life Sci 2007; 81:1256-63; PMID:17919663; http://dx.doi.org/10.1016/j.lfs.2007.08.025
85. Overall CM, López-Otín C. Strategies for MMP inhibition in cancer: innovations for the post-trial era. Nat Rev Cancer 2002; 2:657-72; PMID:12209155; http://dx.doi.org/10.1038/nrc884
86. Talbot DC, Brown PD. Experimental and clinical studies on the use of matrix metalloproteinase inhibitors for the treatment of cancer. Eur J Cancer 1996; 32A:2528-33; PMID:9059343; http://dx.doi.org/10.1016/S0959-8049(96)00398-X

87. Overall CM, Kleifeld O. Towards third generation matrix metalloproteinase inhibitors for cancer therapy. Br J Cancer 2006; 94:941-6; PMID:16538215; http://dx.doi.org/10.1038/sj.bjc.6603043
88. Zhu L, Wang T, Perche F, Taigind A, Torchilin VP. Enhanced anticancer activity of nanopreparation containing an MMP2-sensitive PEG-drug conjugate and cell-penetrating moiety. Proc Natl Acad Sci U S A 2013; 110:17047-52; PMID:24062440; http://dx.doi.org/10.1073/pnas.1304987110
89. Zhu L, Torchilin VP. Stimulus-responsive nanopreparations for tumor targeting. Integr Biol (Camb). 2013 Jan;5(1):96-107
90. Fukumura D, Jain RK. Tumor microenvironment abnormalities: causes, consequences, and strategies to normalize. J Cell Biochem 2007; 101:937-49; PMID:17171643; http://dx.doi.org/10.1002/jcb.21187
91. Junttila MR, de Sauvage FJ. Influence of tumour micro-environment heterogeneity on therapeutic response. Nature 2013; 501:346-54; PMID:24048067; http://dx.doi.org/10.1038/nature12626
92. Lunt SJ, Kalliomaki TM, Brown A, Yang VX, Milosevic M, Hill RP. Interstitial fluid pressure, vascularity and metastasis in ectopic, orthotopic and spontaneous tumours. BMC Cancer 2008; 8:2; PMID:18179711; http://dx.doi.org/10.1186/1471-2407-8-2
93. Simonsen TG, Gaustad JV, Leinaas MN, Rofstad EK. High interstitial fluid pressure is associated with tumor-line specific vascular abnormalities in human melanoma xenografts. PLoS One 2012; 7:e40006; PMID:22768196; http://dx.doi.org/10.1371/journal.pone.0040006
94. Jain RK. Normalizing tumor vasculature with anti-angiogenic therapy: a new paradigm for combination therapy. Nat Med 2001; 7:987-9; PMID:11533692; http://dx.doi.org/10.1038/nm0901-987
95. Taghian AG, Abi-Raad R, Assaad SI, Casty A, Ancukiewicz M, Yeh E, Molokhia P, Attia K, Sullivan T, Kuter I, et al. Paclitaxel decreases the interstitial fluid pressure and improves oxygenation in breast cancers in patients treated with neoadjuvant chemotherapy: clinical implications. J Clin Oncol. 2005 Mar 20;23(9):1951-61
96. Tong RT, Boucher Y, Kozin SV, Winkler F, Hicklin DJ, Jain RK. Vascular normalization by vascular endothelial growth factor receptor 2 blockade induces a pressure gradient across the vasculature and improves drug penetration in tumors. Cancer Res 2004; 64:3731-6; PMID:15172975; http://dx.doi.org/10.1158/0008-5472.CAN-04-0074
97. Vlahovic G, Rabbani ZN, Herndon JE 2nd, Dewhirst MW, Vujaskovic Z. Treatment with Imatinib in NSCLC is associated with decrease of phosphorylated PDGFR-beta and VEGF expression, decrease in interstitial fluid pressure and improvement of oxygenation. Br J Cancer 2006; 95:1013-9; PMID:17003785; http://dx.doi.org/10.1038/sj.bjc.6603366
98. Dickson PV, Hamner JB, Sims TL, Fraga CH, Ng CY, Rajasekeran S, Hagedorn NL, McCarville MB, Stewart CF, Davidoff AM. Bevacizumab-induced transient remodeling of the vasculature in neuroblastoma xenografts results in improved delivery and efficacy of systemically administered chemotherapy. Clin Cancer Res. 2007 Jul 1;13(13):3942-50.
99. Ley CD, Horsman MR, Kristjansen PEG. Early effects of combretastatin-A4 disodium phosphate on tumor perfusion and interstitial fluid pressure. Neoplasia 2007; 9:108-12; PMID:17356706; http://dx.doi.org/10.1593/neo.06733
100. Skliarenko JV, Lunt SJ, Gordon ML, Vitkin A, Milosevic M, Hill RP. Effects of the vascular disrupting agent ZD6126 on interstitial fluid pressure and cell survival in tumors. Cancer Res 2006; 66:2074-80; PMID:16489007; http://dx.doi.org/10.1158/0008-5472.CAN-05-2046
101. Upreti M, Jyoti A, Sethi P. Tumor microenvironment and nanotherapeutics. Transl Cancer Res 2013; 2:309-19; PMID:24634853
102. Cairns R, Papandreou I, Denko N. Overcoming physiologic barriers to cancer treatment by molecularly targeting the tumor microenvironment. Mol Cancer Res 2006; 4:61-70; PMID:16513837; http://dx.doi.org/10.1158/1541-7786.MCR-06-0002
103. Facciabene A, Peng X, Hagemann IS, Balint K, Barchetti A, Wang LP, Gimotty PA, Gilks CB, Lal P, Zhang L, et al. Tumour hypoxia promotes tolerance and angiogenesis via CCL28 and T(reg) cells. Nature 2011; 475:226-30; PMID:21753853; http://dx.doi.org/10.1038/nature10169
104. Kizaka-Kondoh S, Inoue M, Harada H, Hiraoka M. Tumor hypoxia: a target for selective cancer therapy. Cancer Sci 2003; 94:1021-8; PMID:14662015; http://dx.doi.org/10.1111/j.1349-7006.2003.tb01395.x
105. Harada H, Kizaka-Kondoh S, Hiraoka M. Optical imaging of tumor hypoxia and evaluation of efficacy of a hypoxia-targeting drug in living animals. Mol Imaging 2005; 4:182-93; PMID:16194450
106. Cairns RA, Papandreou I, Sutphin PD, Denko NC. Metabolic targeting of hypoxia and HIF1 in solid tumors can enhance cytotoxic chemotherapy. Proc Natl Acad Sci U S A 2007; 104:9445-50; PMID:17517659; http://dx.doi.org/10.1073/pnas.0611662104
107. Lou Y, McDonald PC, Oloumi A, Chia S, Ostlund C, Ahmadi A, Kyle A, Auf dem Keller U, Leung S, Huntsman D, et al. Targeting tumor hypoxia: suppression of breast tumor growth and metastasis by novel carbonic anhydrase IX inhibitors. Cancer Res 2011; 71:3364-76; PMID:21415165; http://dx.doi.org/10.1158/0008-5472.CAN-10-4261
108. Perche F, Biswas S, Wang T, Zhu L, Torchilin VP. Hypoxia-targeted siRNA delivery. Angew Chem Int Ed Engl 2014; 53:3362-6; PMID:24554550; http://dx.doi.org/10.1002/anie.201308368
109. Bettegowda C, Dang LH, Abrams R, Huso DL, Dillehay L, Cheong I, Agrawal N, Borzillary S, McCaffery JM, Watson EL, et al. Overcoming the hypoxic barrier to radiation therapy with anaerobic bacteria. Proc Natl Acad Sci U S A 2003; 100:15083-8; PMID:14657371; http://dx.doi.org/10.1073/pnas.2036598100
110. Raghunand N, Gillies RJ. pH and drug resistance in tumors. Drug Resist Updat. 2000 Feb;3(1):39-47
111. Gerweck LE, Kozin SV, Stocks SJ. The pH partition theory predicts the accumulation and toxicity of doxorubicin in normal and low-pH-adapted cells. Br J Cancer 1999; 79:838-42; PMID:10070878; http://dx.doi.org/10.1038/sj.bjc.6690134
112. Vukovic V, Tannock IF. Influence of low pH on cytotoxicity of paclitaxel, mitoxantrone and topotecan. Br J Cancer 1997; 75:1167-72; PMID:9099966; http://dx.doi.org/10.1038/bjc.1997.201
113. Mahoney BP, Raghunand N, Baggett B, Gillies RJ. Tumor acidity, ion trapping and chemotherapeutics. I. Acid pH affects the distribution of chemotherapeutic agents in vitro. Biochem Pharmacol 2003; 66:1207-18; PMID:14505800; http://dx.doi.org/10.1016/S0006-2952(03)00467-2
114. Koren E, Apte A, Jani A, Torchilin VP. Multifunctional PEGylated 2C5-immunoliposomes containing pH-sensitive bonds and TAT peptide for enhanced tumor cell internalization and cytotoxicity. J Control Release. 2012 Jun 10;160(2):264-73
115. Kale AA, Torchilin VP. Enhanced transfection of tumor cells in vivo using "Smart" pH-sensitive TAT-modified pegylated liposomes. J Drug Target 2007; 15:538-45; PMID:17671900; http://dx.doi.org/10.1080/10611860701498203
116. Wu H, Zhu L, Torchilin VP. pH-sensitive poly(histidine)-PEG/DSPE-PEG co-polymer micelles for cytosolic drug delivery. Biomaterials 2013; 34:1213-22; PMID:23102622; http://dx.doi.org/10.1016/j.biomaterials.2012.08.072
117. Sawant RR, Sriraman SK, Navarro G, Biswas S, Dalvi RA, Torchilin VP. Polyethyleneimine-lipid conjugate-based pH-sensitive micellar carrier for gene delivery. Biomaterials 2012; 33:3942-51; PMID:22365809; http://dx.doi.org/10.1016/j.biomaterials.2011.11.088
118. Sawant RR, Torchilin VP. Liposomes as 'smart'pharmaceutical nanocarriers. Soft Matter 2010; 6:4026-44; http://dx.doi.org/10.1039/b923535n
119. Chen KL, Bothun GD. Nanoparticles meet cell membranes: probing nonspecific interactions using model membranes. Environ Sci Technol 2014; 48:873-80; PMID:24341906; http://dx.doi.org/10.1021/es403864v
120. Verma A, Stellacci F. Effect of surface properties on nanoparticle-cell interactions. Small 2010; 6:12-21; PMID:19844908; http://dx.doi.org/10.1002/smll.200901158
121. Rejman J, Oberle V, Zuhorn IS, Hoekstra D. Size-dependent internalization of particles via the pathways of clathrin- and caveolae-mediated endocytosis. Biochem J 2004; 377:159-69; PMID:14505488; http://dx.doi.org/10.1042/BJ20031253
122. Bhattacharjee H, Balabathula P, Wood GC. Targeted nanoparticulate drug-delivery systems for treatment of solid tumors a review. Ther Deliv. 2010 Nov;1(5):713-34
123. Lu Y, Low PS. Folate-mediated delivery of macromolecular anticancer therapeutic agents. Adv Drug Deliv Rev 2002; 54:675-93; PMID:12204598; http://dx.doi.org/10.1016/S0169-409X(02)00042-X
124. Daniels TR, Bernabeu E, Rodríguez JA, Patel S, Kozman M, Chiappetta DA, Holler E, Ljubimova JY, Helguera G, Penichet ML. The transferrin receptor and the targeted delivery of therapeutic agents against cancer. Biochim Biophys Acta. 2012 Mar;1820(3):291-317
125. Verdurmen WP, Wallbrecher R, Schmidt S, Eilander J, Bovee-Geurts P, Fanghanel S, Burck J, Wadhwani P, Ulrich AS, Brock R. Cell surface clustering of heparan sulfate proteoglycans by amphipathic cell-penetrating peptides does not contribute to uptake. J Control Release. 2013 Aug 28;170(1):83-91
126. Ciechanover A, Schwartz AL, Dautry-Varsat A, Lodish HF. Kinetics of internalization and recycling of transferrin and the transferrin receptor in a human hepatoma cell line. Effect of lysosomotropic agents. J Biol Chem 1983; 258:9681-9; PMID:6309781
127. Zorko M, Langel U. Cell-penetrating peptides: mechanism and kinetics of cargo delivery. Adv Drug Deliv Rev 2005; 57:529-45; PMID:15722162; http://dx.doi.org/10.1016/j.addr.2004.10.010
128. Paulos CM, Reddy JA, Leamon CP, Turk MJ, Low PS. Ligand binding and kinetics of folate receptor recycling in vivo: impact on receptor-mediated drug delivery. Mol Pharmacol 2004; 66:1406-14; PMID:15371560; http://dx.doi.org/10.1124/mol.104.003723
129. Trowbridge IS, Collawn JF, Hopkins CR. Signal-dependent membrane protein trafficking in the endocytic pathway. Annu Rev Cell Biol 1993; 9:129-61; PMID:8280459; http://dx.doi.org/10.1146/annurev.cb.09.110193.001021
130. Sahay G, Alakhova DY, Kabanov AV. Endocytosis of nanomedicines. J Control Release. 2010 Aug 3;145(3):182-95
131. Kim HS, Kim JS, Lee YK, Koo KH, Park YS. An efficient liposomal gene delivery vehicle using Sendai F/HN proteins and protamine. Cancer Gene Ther 2008; 15:214-24; PMID:18259213; http://dx.doi.org/10.1038/sj.cgt.7701121
132. Ting CL, Wang ZG. Interactions of a charged nanoparticle with a lipid membrane: implications for gene delivery. Biophys J 2011; 100:1288-97; PMID:21354402; http://dx.doi.org/10.1016/j.bpj.2010.11.042

133. Maitani Y, Igarashi S, Sato M, Hattori Y. Cationic liposome (DC-Chol/DOPE=1:2) and a modified ethanol injection method to prepare liposomes, increased gene expression. Int J Pharm 2007; 342:33-9; PMID:17566677; http://dx.doi.org/10.1016/j.ijpharm.2007.04.035

134. Koshkaryev A, Piroyan A, Torchilin VP. Bleomycin in octaarginine-modified fusogenic liposomes results in improved tumor growth inhibition. Cancer Lett 2013; 334:293-301; PMID:22743614; http://dx.doi.org/10.1016/j.canlet.2012.06.008

135. Sawant RR, Sriraman SK, Navarro G, Biswas S, Dalvi RA, Torchilin VP. Polyethyleneimine-lipid conjugate-based pH-sensitive micellar carrier for gene delivery. Biomaterials 2012; 33:3942-51; PMID:22365809; http://dx.doi.org/10.1016/j.biomaterials.2011.11.088

136. Navarro G, Sawant RR, Essex S. Tros de Ilarduya C, Torchilin VP. Phospholipid–polyethylenimine conjugate-based micelle-like nanoparticles for siRNA delivery. Drug Deliv Transl Res. 2011 Feb 1;1(1):25-33

137. Zuhorn IS, Engberts JB, Hoekstra D. Gene delivery by cationic lipid vectors: overcoming cellular barriers. Eur Biophys J 2007; 36:349-62; PMID:17019592; http://dx.doi.org/10.1007/s00249-006-0092-4

138. Whitehead KA, Langer R, Anderson DG. Knocking down barriers: advances in siRNA delivery. Nat Rev Drug Discov 2009; 8:129-38; PMID:19180106; http://dx.doi.org/10.1038/nrd2742

139. Koshkaryev A, Piroyan A, Torchilin VP. Increased apoptosis in cancer cells in vitro and in vivo by ceramides in transferrin-modified liposomes. Cancer Biol Ther 2012; 13:50-60; PMID:22336588; http://dx.doi.org/10.4161/cbt.13.1.18871

140. Goren D, Horowitz AT, Tzemach D, Tarshish M, Zalipsky S, Gabizon A. Nuclear delivery of doxorubicin via folate-targeted liposomes with bypass of multidrug-resistance efflux pump. Clin Cancer Res 2000; 6:1949-57; PMID:10815920

141. Kuwazuru Y, Yoshimura A, Hanada S, Utsunomiya A, Makino T, Ishibashi K, Kodama M, Iwahashi M, Arima T, Akiyama S. Expression of the multidrug transporter, P-glycoprotein, in acute leukemia cells and correlation to clinical drug resistance. Cancer 1990; 66:868-73; PMID:1974821; http://dx.doi.org/10.1002/1097-0142(19900901)66:5<868::AID-CNCR2820660510>3.0.CO;2-Z

142. Bai F, Wang C, Lu Q, Zhao M, Ban FQ, Yu DH, Guan YY, Luan X, Liu YR, Chen HZ, et al. Nanoparticle-mediated drug delivery to tumor neovasculature to combat P-gp expressing multidrug resistant cancer. Biomaterials 2013; 34:6163-74; PMID:23706689; http://dx.doi.org/10.1016/j.biomaterials.2013.04.062

143. Sun YL, Chen JJ, Kumar P, Chen K, Sodani K, Patel A, Chen YL, Chen SD, Jiang WQ, Chen ZS. Reversal of MRP7 (ABCC10)-mediated multidrug resistance by tariquidar. PLoS One 2013; 8:e55576; PMID:23393594; http://dx.doi.org/10.1371/journal.pone.0055576

144. Patel NR, Rathi A, Mongayt D, Torchilin VP. Reversal of multidrug resistance by co-delivery of tariquidar (XR9576) and paclitaxel using long-circulating liposomes. Int J Pharm 2011; 416:296-9; PMID:21703341; http://dx.doi.org/10.1016/j.ijpharm.2011.05.082

145. Baumert C, Günthel M, Krawczyk S, Hemmer M, Wersig T, Langner A, Molnár J, Lage H, Hilgeroth A. Development of small-molecule P-gp inhibitors of the N-benzyl 1,4-dihydropyridine type: novel aspects in SAR and bioanalytical evaluation of multidrug resistance (MDR) reversal properties. Bioorg Med Chem 2013; 21:166-77; PMID:23199479; http://dx.doi.org/10.1016/j.bmc.2012.10.041

146. Zhu H, Liu Z, Tang L, Liu J, Zhou M, Xie F, Wang Z, Wang Y, Shen S, Hu L, et al. Reversal of P-gp and MRP1-mediated multidrug resistance by H6, a gypenoside aglycon from Gynostemma pentaphyllum, in vincristine-resistant human oral cancer (KB/VCR) cells. Eur J Pharmacol 2012; 696:43-53; PMID:23051672; http://dx.doi.org/10.1016/j.ejphar.2012.09.046

147. Patil Y, Sadhukha T, Ma L, Panyam J. Nanoparticle-mediated simultaneous and targeted delivery of paclitaxel and tariquidar overcomes tumor drug resistance. J Control Release. 2009 May 21;136(1):21-9

148. Bentires-Alj M, Barbu V, Fillet M, Chariot A, Relic B, Jacobs N, Gielen J, Merville MP, Bours V. NF-kappaB transcription factor induces drug resistance through MDR1 expression in cancer cells. Oncogene 2003; 22:90-7; PMID:12527911; http://dx.doi.org/10.1038/sj.onc.1206056

149. Kovalchuk O, Filkowski J, Meservy J, Ilnytskyy Y, Tryndyak VP, Chekhun VF, Pogribny IP. Involvement of microRNA-451 in resistance of the MCF-7 breast cancer cells to chemotherapeutic drug doxorubicin. Mol Cancer Ther 2008; 7:2152-9; PMID:18645025; http://dx.doi.org/10.1158/1535-7163.MCT-08-0021

150. Navarro G, Sawant RR, Biswas S, Essex S, Tros de Ilarduya C, Torchilin VP. P-glycoprotein silencing with siRNA delivered by DOPE-modified PEI overcomes doxorubicin resistance in breast cancer cells. Nanomedicine (Lond) 2012; 7:65-78; PMID:22191778; http://dx.doi.org/10.2217/nnm.11.93\