Expert Opinion

Recent advances in tumor vasculature targeting using liposomal drug delivery systems

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Tumor vessels possess unique physiological features that might be exploited for improved drug delivery. The targeting of liposomal anticancer drugs to tumor vasculature is increasingly recognized as an effective strategy to obtain superior therapeutic efficacy with limited host toxicity compared with conventional treatments. This review introduces recent advances in the field of liposomal targeting of tumor vasculature, along with new approaches that can be used in the design and optimization of liposomal delivery systems. In addition, cationic liposome is focused on as a promising carrier for achieving efficient vascular targeting. The clinical implications are discussed of several approaches using a single liposomal anticancer drug formulation: dual targeting, vascular targeting (targeting tumor endothelial cells) and tumor targeting (targeting tumor cells).

Keywords: angiogenesis, anti-angiogenic therapy, anticancer drugs, dual targeting, immunoliposomes, PEG-coated cationic liposome, vascular targeting

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1. Introduction

Many physiological barriers are known to hinder the effective delivery of drugs to tumors [1,2]. The restricted availability of drugs to tumor cells is due, at least in part, to properties of tumor tissue such as irregular vasculature, variable permeability of blood vessels and high interstitial fluid pressure [3,4]. In addition, in certain solid tumors, overexpression of the *p*-glycoprotein gene provokes drug resistance to certain therapeutic agents such as doxorubicin and cisplatin [5]. One way to increase the therapeutic index of drugs would be by specifically delivering the drugs to tumor tissues by means of a nanocarrier system (drug delivery system) [6,7]. However, to achieve the targeting of cells in a solid tumor, a nanocarrier system must first cross the vasculature and then travel through the interstitium in the tumor tissue [8]. This process hinders the effective travel of nanocarrier systems. Therefore, instead of targeting the tumor cells with a nanocarrier system, vascular targeting can be used to overcome the aforementioned obstacles.

Vascular targeting with anticancer drugs is generally considered to have several advantages over targeting tumor cells. For example, normal endothelial cells are quiescent and, therefore, side effects to the non-targeted endothelium are expected to be at a minimum [9]. Proliferating endothelial cells in solid tumors share similar phenotypes among different tumors. This makes vascular targeting applicable to a wide variety of tumor types [10]. Furthermore, endothelial cells are genetically stable (unlike tumor cells) and thus reduce the likelihood of developing drug resistance [11]. Finally, as the vasculature of a tumor occupies a relatively small area in comparison with the tumor interstitium and because most anticancer agents are applied intravenously, tumor vessels are more accessible to circulating

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chemotherapeutics compared with cancer cells. Accordingly, with the use of a nanocarrier system, an equivalent dose of chemotherapeutic agents is expected to induce higher therapeutic efficacy and lower toxicity than what is achieved with conventional treatment using free anticancer drugs.

2. Tumor vasculature

2.1 Tumor-induced angiogenesis

Tumor-induced angiogenesis, the formation of neovessels from pre-existing ones, is critical for the support of tumor growth and progression not only by providing nutrients, oxygen, growth factors and other substances to tumor cells, but also by allowing metastatic cells into circulation [12,13]. Tumor angiogenesis is not a singular process; at least two types of angiogenesis process are believed to contribute to vessel growth in tumors [14]. One involves the stimulation of new blood vessel capillaries to sprout in the vasculature of the neighboring mature host [15]. The other involves the recruitment of circulating endothelial precursor cells from the bone marrow to promote neovascularization [16,17]. Tumor angiogenesis is mainly triggered by growth factors in the microenvironment, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and matrix metalloproteinases (MMPs) [18,19]. These factors are generally produced by tumors cells, by surrounding tissue and/or by infiltrating macrophages. Suppression of the angiogenesis process leads to eradication of primary tumor cells and suppression of metastasis through the disruption of the metastatic pathway, which makes it a promising strategy for the treatment of solid tumors (anti-angiogenic therapy) [20,21].

2.2 Vascular structure of solid tumors

In comparison with blood vessels supplying normal tissues, subtle differences in the structure of tumor vessels make them unique. Tumor vessels tend to arrange in irregular arrays and have dilated lumens [22]. Compared with the walls of normal vessels, the walls of tumor vessels have fenestrations, discontinuous or absent basement membranes, fewer pericytes, and a lack of perivascular smooth muscle. These characteristics make tumor vessels more leaky than normal vessels [23,24]. Tumor vessels are also characterized by the overexpression of several specific surface receptors, or antigens, and by negatively charged macromolecules such as glycoproteins, anionic phospholipids and proteoglycans [25-27]. These unique physiological features of tumor vasculature have been exploited extensively for both tumor (passive) targeting and vascular (active) targeting by nanocarrier systems. The porous nature of tumor vasculature enabled the preferential accumulation of macromolecules and polymeric drugs in tumor tissues (tumor targeting) because of their enhanced vascular permeability compared with normal tissue [28]. This phenomenon is known as the enhanced permeability and retention effect (EPR effect) [29].

3. Vascular targeting strategies

Endothelial cells lining tumor blood vessels overexpress specific cell surface antigens, which are absent or barely detectable in established or quiescent normal blood vessels [30]. These unique characteristics of tumor endothelial cells can be exploited to achieve active vascular targeting of nanocarrier systems. This is accomplished by coupling specific molecules, such as antibodies, specific peptides, growth factors, or a cationic charge, to the surface of nanocarrier systems.

3.1 Vascular targeting with liposomes

There are many nanocarrier systems, such as micelles and nanospheres. In this review, however, the liposomal drug delivery system is focused on as a promising carrier for delivering small molecular therapeutics (anticancer agents) to tumor vasculature. Many approaches have been applied to enhance the targeting efficiency of liposomes to tumor endothelial cells. In the following section, some of the successfully applied approaches for achieving vascular targeting by means of liposome-based carriers are introduced.

3.1.1 Ligand-targeted liposome

Ligand (antibody or peptide)-mediated targeting of liposomal anticancer drugs to cell surface antigens expressed selectively or overexpressed on tumor vasculature is considered as an effective strategy for increasing the overall therapeutic efficiency of anticancer drugs [31]. These ligand-targeted liposomes utilize targeting moieties coupled to the liposome surface to deliver selectively the drug-liposome payload to the desired site of action. Targeting moieties may include whole antibody molecules, antibody fragments or synthetic ligands such as peptides.

3.1.1.1 Liposomal vascular targeting with antibodies

Immunoliposomes, with antibodies conjugated either to the surface of liposomes or to the distal end of polyethylene glycol (PEG) on the liposomes, have been used to achieve increased therapeutic efficacy of encapsulated anticancer drugs with decreasing side effects. Relatively few antibody molecules per liposome (20 – 40 μg/μmol phospholipid) are necessary to deliver selectively high cargo of drugs by immunoliposomes to target cells *in vivo*, even though the liposomes were coated with PEG [32]. High antibody densities (i.e., 140 μg/μmol phospholipid) on the liposome resulted in rapid clearance of the immunoliposome from blood circulation [32]. In addition, the selection of antibody, which is against internalizable receptor or antigens on the target cell, is important to obtain efficient therapeutic efficacy of immunoliposomal drugs. [33]. The coupling of multiple targeting molecules on the surface of individual liposomes can increase the binding avidity for the target antigens [31].

Targeting ligand used in the formulation of immunoliposomes may be a whole antibody or antibody fragments (Fab′ or scFv) [34]. An advantage of using whole monoclonal

antibody is their higher binding avidity owing to the presence of two or more binding domains on the same molecule. In addition, whole antibody molecules may have some stability advantages during preparation and storage over antibody fragments. However, on the down side, owing to the presence of an Fc domain in the whole antibody, Fc-mediated mechanisms are responsible for initiating immunogenic responses and for rapid clearance from the circulation [35]. Therefore, instead, smaller antibody fragments (Fab′ or scFv), which lack Fc domain, are commonly used to reduce immunogenicity of the immunoliposomes as well as to increase their circulation time.

Many studies have reported the targeting of immunoliposomes to tumor endothelial cells. The main targeted receptors/antigens in these studies were endoglins (CD105) [34], vascular endothelial growth factor receptor 2 [36], intercellular adhesion molecule 1 [37], E-selectin [38], membrane type-1 matrix metalloproteinase (MT1-MMP) [39,40] and vascular cell adhesion molecule 1 (VCAM-1) [35].

Voinea *et al.* [35] designed anti-VCAM-1 coupled liposomes to target tumor vasculature. The target molecule on tumor endothelial cells is an immunoglobulin-like transmembrane glycoprotein (VCAM-1). The expression of VCAM-1 is inducible and virtually absent on normal human vasculature [41]. A robust vascular VCAM-1 expression has been observed mainly in cases of leukemia and lymphoma and, to a varying extent, in a variety of solid tumors such as lung cancer, breast cancer, renal cell carcinoma and gastric cancer [42]. However, the expression also has been observed on other types of cell, such as bone marrow cells, follicular dendritic cells, fibroblasts and epithelial cells of the kidney [43,44]. From *in vitro* study, Voinea *et al.* showed that liposomes coupled with monoclonal antibody, anti-VCAM-1, bound selectively and specifically to activated tumor endothelial cells, and that some of them were taken up by tumor endothelial cells by means of clathrin-mediated endocytosis. Unfortunately, *in vivo* efficiency has not yet been evaluated.

Endoglin (CD105) is an accessory protein of the transforming growth factor-β (TGF-β) receptor complex. Elevated levels of endoglin were detected in the vasculature of different tumors, for example, prostate cancer, breast cancer and melanoma [45,46]. Thus, endoglin represents an attractive molecule for the targeting of tumor vasculature.

Volkel *et al.* [34] developed immunoliposomes targeting proliferating endothelial cells by chemically coupling a single-chain Fv fragment (scFv A5) directed against human endoglin to the liposomal surface. These immunoliposomes showed specific and efficient binding to proliferating endothelial cells and improved cytotoxicity towards endothelial cells *in vitro*. However, *in vivo* study showed that such immunoliposomes were rapidly cleared from circulation with a half-life of 3 min. This drastic reduction in circulation time was mainly due to the lack of PEGylation and negative influence of the functionalized (coupling) lipids and the coupling chemistry on the *in vivo* behavior of the liposomes [47,48]. These limitations might be overcome by PEGylation and a reduced number of coupling lipids.

MT1-MMP, one of the key proteins for angiogenesis in tumor vessels, was found to be overexpressed by tumor angiogenic vessels as well as by tumor cells [39]. The utility of monoclonal antibodies against MT1-MMP as a targeting ligand of liposomal anticancer drugs has been addressed [40]. Fab′ fragments of the antibody were coupled to the distal end of the PEG of doxorubicin (DXR)-encapsulating liposomes (DXR-SL), and, consequently, developed DXR-sterically stabilized immunoliposomes (DXR-SIL[anti-MT1-MMP(Fab′)]) [39,40]. In *in vivo* study, DXR-SIL[anti-MT1-MMP(Fab′)] significantly suppressed tumor growth compared with DXR-SL. Interestingly, no significant difference in the accumulation of DXR-SIL[anti-MT1-MMP(Fab′)] versus DXR-SL at the tumor site was observed. The higher therapeutic efficacy of DXR-SIL [anti-MT1-MMP(Fab′)] was therefore attributed to more drug being delivered to angiogenic blood vessels, which produced a greater degree of cell kill than targeting individual tumor cells. In addition, tumor endothelial cells are more accessible to circulating DXR-SIL[anti-MT1-MMP(Fab′)] and problems related to poor extravasation and tumor tissue penetration can be avoided. A similar result was obtained by Kirpotin *et al.* [49], who have demonstrated that DXR-containing anti-HER2 Fab′ immunoliposomes show a superior antitumor activity compared with DXR-containing non-targeted liposomes against HER2-overexpressing breast cancer cells, although the intra-tumoral accumulation levels of DXR-containing immunoliposomes and DXR-containing non-targeted liposomes were similar. They attributed such enhanced antitumor efficacy of DXR-containing immunoliposomes to the rapid internalization of the immunoliposomes with subsequent specific intracellular drug delivery to the target cells. On the other hand, non-targeted liposomes were localized in the extracellular areas of tumor stroma and tissue macrophages.

3.1.1.2 *Liposomal targeting using peptides*

Biopanning experiments using phage display have yielded several peptide motifs that show preferential binding to angiogenic tumor vasculature. The coupling of such peptides to liposomes has been shown to enhance the accumulation of liposomes at angiogenic sites.

The vascular targetability of liposomes coupled with peptides has been investigated using several experimental animal models. These peptides were Arg-Gly-Asp (RGD) motifs binding to $\alpha_v\beta_3$ integrins [50,51], Asn-Gly-Arg (NGR) motifs binding to aminopeptidase N [52], Cys-Arg-Glu-Lys-Ala (CREKA) binding to fibrinogen or fibrin [53], or Gly-Pro-Leu-Pro-Leu-Arg (GPLPLR) binding to membrane type-1 matrix metalloproteinase [54].

Schiffelers *et al.* [50] demonstrated that coupling of RGD peptides to the surface of PEG-coated liposomes enhanced the binding, with subsequent internalization, of liposomes by activated endothelial cells *in vitro*. In addition, by loading DXR in such RGD liposomes, the liposomes showed a

superior activity in suppressing *in vivo* tumor growth when compared with DXR-containing PEG-coated liposomes.

A peptide containing the NGR motif, which recognizes a tumor-specific isoform of CD13, was identified [55]. By coupling this peptide to anticancer compounds, selective delivery of the anticancer drug to tumor vessels could be achieved. Hence, Pastorino *et al.* [56] coupled NGR peptides to PEG-coated liposomes loaded with DXR and examined their efficiency in an *in vivo* orthotopic neuroblastoma xenograft model. Targeting of PEG-coated liposomes with an NGR peptide caused dramatic vascular damage by encapsulated DXR, resulting in a significant antitumor effect. In addition, pharmacokinetic analysis revealed that coupling of the NGR peptide on the PEG-coated liposomes did not reduce the blood circulation time of the liposomes. Compared with whole IgGs, this is an advantage of peptides when modifying the surface of liposomes.

A peptide substrate of MT1-MMP, with the sequence Gly-Pro-Leu-Pro-Leu-Arg, was used for the potential development of a liposomal formulation actively targeting angiogenic endothelial cells. Kondo *et al.* [54] demonstrated that GPLPLR-modified liposomes showed high binding ability to human umbilical vein endothelial cells (HUVECs) *in vitro*. In *in vivo* animal models, such peptide-modified liposomes accumulated in the tumor about fourfold more effectively than did unmodified liposomes. In addition, peptide-modified liposomes containing the anticancer drug 5′-*O*-dipalmitoylphosphatidyl 2′-*C*-cyano-2′-deoxy-1-β-D-*arabino*-pentofurano-sylcytosine (DPP-CNDAC) was found to suppress *in vivo* tumor growth significantly more effectively compared with unmodified liposomal DPP-CNDAC.

Biopanning experiments using phage display produced a peptide containing an Ala-Pro-Arg-Pro-Gly (APRPG) motif that selectively binds to tumor neovasculature [57]. Maeda *et al.* [58] addressed the use of APRPG peptide-modified liposomes to deliver adriamycin selectively to tumor vasculature. They demonstrated that adriamycin-containing PEGylated liposomes modified with APRPG caused more efficient tumor growth suppression than adriamycin-containing PEGylated liposomes in tumor-bearing mice. This antitumor activity of adriamycin-containing PEGylated liposomes modified with APRPG was mainly due to the selective delivery of the encapsulated drug to tumor angiogenic vessels.

3.1.1.3 *Pharmacokinetics of ligand-targeted liposomes*

Encapsulation of anticancer drugs in a liposomal drug delivery system can result in substantial changes in the pharmacokinetics and biodistribution of the drug [31]. Until it is released from the liposome, the drug adopts the pharmacokinetics of the liposome. Therefore, great attention should be paid during the development of ligand-targeted liposomes to ensure that the targeting moiety does not potentially compromise the pharmacokinetics of the liposome.

Maruyama *et al.* [59] demonstrated that Fab′-coupled immunoliposomes showed a sixfold increase in circulation half-lifes compared with whole antibody-coupled immunoliposomes, even though the density of Fab′ molecules was ~ 10-fold higher than that of whole antibody on the surface of liposomes. They attributed the rapid clearance of whole antibody-coupled immunoliposomes to Fc-mediated mechanisms.

Pastorino *et al.* [56] demonstrated that although NGR-targeted PEGylated liposomes showed a preferential accumulation into spleen, ~ 10 – 20 times higher than that of non-targeted PEGylated liposome, the liposomes coupled to NGR peptide have good stability and long circulation times after intravenous injection, ~ 30% of the liposomes remaining in the blood 24 h after liposomal administration.

The reader is referred to an interesting review on ligand-targeted liposomes for further detailed description of this area [31].

3.1.2 *Liposomal targeting using a surface charge (cationic liposomes)*

A possible role of proteoglycans in transfection by lipoplexes (complex of cationic liposome with plasmid DNA) has been suggested by Mislick and Baldeschwieler [60] and Mounkes *et al.* [61]. Hoekstra and collaborators [62] provided evidence for the role of integrin β1 in the uptake of SAINT-2 (2(*N*-methyl-4(dioleyl)methylpyridiniumchloride))/DOPE (dioleoyl phosphatidyl ethanolamine) and lipofectamine lipoplexes by epithelial cells. However, the role of these proteoglycans in uptake or binding enhancement was not investigated further.

As mentioned earlier, tumor endothelial cells are known to overexpress negatively charged cell surface molecules, for example, glycoproteins, anionic phospholipids and proteoglycans [25,26]. Tumor endothelial cells are preferred as targeting sites for cationic liposomes to achieve selective delivery of anticancer agents.

3.1.2.1 *In vivo toxicity and behavior of cationic liposomes*

The capability of cationic liposomes to target endothelial cells of tumor vasculature has been documented extensively. However, a concomitant examination of the toxic effects of such drug delivery is lacking, especially in *in vivo* applications. It is assumed that the toxicity of cationic liposomes arises mainly from the incorporated cationic lipids in their composition. Hagerstrand *et al.* [63] and Kitagawa *et al.* [64] demonstrated that at high cationic lipid concentration, the liposomes cause the lysis of erythrocytes, whereas at lower concentration, the liposomes induce a variety of plasma membrane alterations that probably result in a decrease in the mechanical strength of erythrocytes.

Immune responses to cationic lipids that are frequently used for formulating cationic liposomes are much less well understood. However, Zelphati *et al.* [65] have shown that a complement system can be activated by cationic lipids. Complement activation could result in complement components binding to the liposomes (no-PEGylation) and targeting them to receptors for complement components that are found in the lung [66] or Kupffer cells in liver; this could also

explain the rapid clearance of cationic liposomes from the blood and uptake into the lung and subsequently liver. Also, Chonn *et al.* [67] demonstrated a direct correlation between the amount of plasma protein bound to cationic liposomes (no-PEGylation) and their rate of elimination from systemic circulation. Such studies led to the realization that reducing the binding of opsonizing plasma proteins to liposomes could improve their biodistribution. This was accomplished by inclusion of PEG to increase liposome stability in blood and reduce rapid clearance. Moreover, as many blood components are negatively charged, intravascular administration of cationic liposomes might result in aggregation with serum components and/or microemboli formation. Consequently, tissue ischemia may be problematic [68].

3.1.2.2 The importance of PEGylation for in vivo use of cationic liposome

Following intravenous injection, cationic liposomes easily made 'aggregations' with negatively charged blood components such as serum proteins and blood cells. Therefore, the liposome was rapidly eliminated from the circulation by the 'first-pass' organs, such as the lungs, liver and spleen [69]. This transient scavenging of cationic liposomes in such organs might decrease their systemic availability and, hence, reduce the amount of therapeutic agent delivered to tumor angiogenic vessels.

PEGylation, the inclusion of a large molecular mass polymer, polyethylene glycol, to the liposome surface, is considered to be an efficient approach to avoid the interaction of conventional liposomes with serum proteins and cells of the mononuclear phagocyte system (MPS) in the liver and spleen, and, thus, prolongs their plasma circulation time [70,71]. In fact, the coating of cationic liposomes with PEG tended to disallow aggregate formation [72,73].

The efficient shielding effect imparted by the inclusion of a PEG lipid at the surface of the liposome is believed to be due to the formation of a physical barrier, a hydration zone, around liposomes. This zone of exclusion diminishes liposome–protein interaction, and thus enhances its long-term circulation characteristics. However, the concentration of PEG lipid incorporated into liposomes was found to be critical for long-term circulation properties of the liposomes. The inclusion of > 15 mol% of PEG was found to cause unfavorable structural changes in the liposome bilayer, causing rapid clearance of liposomes from circulation. For this reason, ~ 5 – 10 mol% of PEG lipid is included in the preparation of PEGylated liposomes. This amount has been found to be sufficient to delay the cell recognition of MPS, resulting in prolonged liposomal circulation time. Levchenko *et al.* [74] demonstrated that PEG lipid concentrations ≥ 6 mol% will shield the electric surface potential of cationic liposomes whereas higher concentrations (≥ 15 mol%) completely abolish the effect of charged groups on the liposome surface. It is worth noting that the partial coating of cationic liposomes with PEG delays liposome clearance from blood but not at the expense of interaction and uptake by tumor endothelial cells [75]. Campbell *et al.* [76] reported that although PEGylation of cationic liposomes lowered the ζ-potential of cationic liposomes, it slightly affected the interaction with tumor angiogenic vessels *in vivo*. Furthermore, they showed that the inclusion of PEG lipid at the surface of liposomes imparts long-circulating characteristics of cationic liposomes, thus enhancing the therapeutic efficiency of an encapsulated anticancer agent.

3.1.2.3 Pharmacokinetics of cationic liposomes

In spite of numerous publications about *in vivo* fate of neutral and negatively charged liposomes, those concerning cationic liposomes have been scarce. Cationic liposomes have been reported to interact strongly with blood components and may even induce aggregate formation in the circulation. This leads to an extensive opsonization and therefore cationic liposomes are rapidly cleared by macrophages of the MPS, particularly Kupffer cells in the liver and spleen macrophages [69]. Ishiwata *et al.* [77] have investigated the *in vivo* fate of cationic liposomes composed of O,O′-ditetradecanoyl-*N*-(α-trimethyl ammonio acetyl)diethanolamine chloride (DC-6-14)/DOPE/cholesterol (CHOL) (4:3:3, molar ratio). They demonstrated that > 60% of injected dose was found in the lung at 3 min post-injection. Thereafter, the amount of these liposomes in the lung decreased and that in the liver coincidently increased. However, only low levels of liposome were observed in the plasma, spleen and kidney in this period. They suggested that intravenously injected cationic liposomes form aggregates with blood cells and these aggregates were trapped in lung capillaries temporally, and as the liposomes left the lung, aggregate breakage might occur. Then, blood cells and liposomes were gradually redistributed to the bloodstream and liver, respectively.

Many studies have highlighted the effect of the molar percent of cationic lipid incorporated into liposomes and the presence of PEG on the pharmacokinetic and biodistribution of cationic liposomes [77,78]. Generally, PEGylated cationic liposomes showed enhanced pharmacokinetic profile with prolonged circulation time over non-PEGylated cationic liposomes. In addition, incorporating a high mol% of cationic lipid in the composition of liposomes was found to hinder the *in vivo* targetability of cationic liposomes as a result of enhancing the blood clearance of the liposomes. Stuart *et al.* [78] have demonstrated that in the absence of PEGylation (1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*n*-[methoxy(polyethylene glycol)-2000 [$mPEG_{2000}$-DSPE]), cationic liposomes consisting of 5 – 50 mol% cationic lipid (1-oleoyl-2-[6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]hexanoyl]-3-trimethylammonium propane [DOTAP]) were rapidly cleared from circulation, resulting in < 5% of liposomes present in blood at 24 h post-injection. Also, for liposomes containing 50 mol% cationic lipid (a DOTAP/hydrogenated soya phosphatidylcholine [HSPC] in a molar ratio of 1:1), inclusion of $mPEG_{2000}$-DSPE did not

increase the blood levels of liposomes. However, when the cationic lipid was reduced to 20 mol%, inclusion of 5 mol% $mPEG_{2000}$-DSPE significantly increased the blood levels of liposomes. At ≤ 10 mol% cationic lipid, $mPEG_{2000}$-DSPE had its maximum protective effect on circulation times; there was no significant difference between blood levels of PEG-containing liposomes at 0, 5 and 10 mol% cationic lipid. These results indicate that $mPEG_{2000}$-DSPE can increase the circulation times of liposomes containing DOTAP, particularly when ≤ 20 mol% DOTAP is used.

Results from the authors' laboratory (in preparation) have shown that, in tumor-bearing mice, the circulation half-life of PEG-coated cationic liposomes consisting of HSPC/CHOl/DC-6-14/$mPEG_{2000}$-DSPE (2:1:0.2:0.2 molar ratio) was similar to that of PEG-coated neutral liposomes consisting of HSPC/CHOl/$mPEG_{2000}$-mDSPE (2:1:0.2 molar ratio), 10.4 ± 0.7 versus 9.4 ± 0.5 h, respectively. However, PEG-coated cationic liposomes showed lower area under the blood concentration versus time curve (AUC) when compared with PEG-coated neutral liposomes. This lower AUC was a result of enhanced blood clearance and tissue distribution of PEG-coated cationic liposomes owing to the presence of liposomal surface positive charge.

3.1.2.4 *Release of drug encapsulated in PEGylated cationic liposomes*

The pharmacokinetics and biodistribution of liposome-encapsulated drugs are controlled by the interplay of two variables: the clearance rate of the liposome and the drug release rate from the liposome in blood circulation [79]. As mentioned previously, the rate of blood clearance of cationic liposomes could easily be manipulated by coating liposomes with ideal percentage of PEG. On the other hand, the drug release rate from the liposome in circulation depends mainly on formulation parameters such as the lipid composition, ζ-potential and the particle size, and on the degree of interaction with blood proteins. Recently, the authors have developed an oxaliplatin (l-OHP)-containing-PEG-coated cationic liposome. *In vitro* stability studies showed that > 60% of the encapsulated l-OHP was retained in the liposome after 24 h incubation in 50% mouse plasma at 37°C. This result might guarantee the *in vivo* efficacy of such l-OHP liposomal formulation [80].

3.1.2.5 *Interaction of cationic liposomes with tumor vascular endothelial cells*

The notion of exploiting accessible anionic sites along with tumor vessels is promising for cancer therapy by means of cationic liposomes containing an anticancer agent. It is a well-known fact that cationic liposomes target the sub-endothelial cells (aortic smooth muscle cells and mesangial cells) and human endothelial cells *in vitro* and tumor-related vasculature *in vivo*, which is a phenomenon not noted with anionic (negatively charged) or electroneutral (zero charge potential) liposomes [81,82]. Campbell *et al.* [76] reported that cationic liposomes, sterically stabilized by including 5 mol% PEG, accumulated extensively in tumor vessels as compared with either PEG-coated neutral or anionic liposomes. Krasnici *et al.* [83] demonstrated that after intravenous application of anionic and neutral liposomes, there was no specific targeting to tumor vasculature in a dorsal skinfold model. By contrast, cationic liposomes had a significantly enhanced accumulation in tumor tissue and tumor vasculature by as much as threefold compared with surrounding tissue within 20 min post-administration. Thus, there is strong evidence that cationic liposomes have the inherent potential to bind selectively to tumor vascular endothelial cells.

Recently, the authors tried to develop PEG-coated cationic liposomes that have *in vivo* long-term circulation properties, as well as selective binding properties, to endothelial cells of tumor vasculature. For this purpose, the dorsal air sac (DAS) model [84,85] was used as a common and reliable method to evaluate the selectivity of PEG-coated cationic liposomes to tumor angiogenic vessels [80]. The DAS model is technically simple and provides a natural environment in which blood vessels and their tumor-induced formation can be studied. In addition, the model takes only ~ 5 days to develop; therefore, it is less time-consuming than the tumor-bearing mouse model, which takes > 10 days. Accordingly, the DAS model was used to screen a nanocarrier system (PEG-coated cationic liposomes) targeting tumor-induced neovasculature and to evaluate the anti-angiogenic efficacy of anticancer agents associated with PEG-coated cationic liposomes.

By using the DAS model, it was possible to obtain a PEG-coated cationic liposome, which accumulated preferentially and selectively in angiogenic vessels induced in the skin. This PEG-coated cationic liposome was composed of the following, in a molar ratio of 2:1:0.2:0.2, respectively: HSPC; CHOL; DC-6-14; and $mPEG_{2000}$-DSPE. The cationic lipid DC-6-14 was used generously to prepare a non-viral vector (cationic liposome) for delivering nucleic acids [86]. The size of the liposome was ~ 250 nm (homogenous size) and the ζ-potential was relatively positive (+11.2 ± 0.7). In addition, the PEG-coated liposome showed no selective accumulation/binding to pre-existing blood vessels in the skin. This indicates an important difference in distribution of liposomes in blood vessels between normal tissues and tumor tissues, which may be exploited while attempting to achieve successful anti-angiogenic therapy. Kalra *et al.* [87] have demonstrated previously that PEG-coated cationic liposomes associate with ~ 27 and 5% of vessel areas in tumors and normal tissues, respectively, in human and murine tumor xenograft mouse models.

The targeting efficiency of cationic liposomes to tumor endothelial cells can also be improved by manipulating the molecular charge. Many reports have revealed that cationic liposomes possessing relatively high cationic charge content showed a preferential binding to tumor vessels. Campbell *et al.* [76] showed that an increase in cationic lipid from 10 to 50 mol% led to a twofold increase in liposomal accumulation in tumor vessels. However, on the down side,

incorporating a high mole per cent of cationic lipid in the composition of liposome was found to enhance liposomal uptake by the cells of MPS, resulting in a rapid clearance of the liposomes from blood circulation. Therefore, optimizing the cationic lipid content incorporated in the liposomal membrane is considered to be crucial for liposome biodistribution *in vivo*.

3.1.2.6 *Selective delivery of anticancer drugs by cationic liposomes to tumor vasculature*

As introduced above, cationic liposomes have been shown to bind selectively to angiogenic tumor endothelial cells after intravenous injection. Therefore, encapsulation of cytotoxic substances in cationic liposomes is considered to be a promising approach for the targeting of tumor vasculature.

Kunstfeld and colleagues [88] demonstrated that paclitaxel encapsulated in cationic liposomes strongly suppressed tumor angiogenesis and inhibited orthotopic melanoma growth in severe combined immunodeficient (SCID) mice. By contrast, free paclitaxel was unable to suppress angiogenesis and tumor growth. Strieth and co-workers [89] utilized A-Mel-3 growing in the dorsal skinfold to demonstrate that intravenous administration of paclitaxel encapsulated in cationic liposomes significantly retarded tumor growth, as compared with treatment using free paclitaxel. They showed that the potent antitumor activity of liposomal paclitaxel resulted from a decrease in tumor vessel density and a reduction in the microcirculatory perfusion index in these animals. Schmitt-Sody *et al.* [90] also emphasized that, with a dorsal skinfold preparation, vascular targeting of paclitaxel was achieved by encapsulating the drug in cationic liposomes. In subcutaneously growing A-Mel-3 tumors, growth was significantly suppressed and the appearance of regional lymph node metastases was significantly delayed by treatment with paclitaxel encapsulated into cationic liposomes in comparison with free paclitaxel. Wu *et al.* [91] addressed the utilization of cationic liposomes to target selectively activated tumor endothelium and showed that cationic liposomes loaded with DXR increased the survival rate of tumor-bearing mice compared with either free DXR or DXR-containing PEG-coated neutral liposomes.

Recently, the authors reported that l-OHP encapsulated in PEG-coated cationic liposomes, which preferentially bound to angiogenic vessels, strongly suppressed tumor angiogenesis in a dorsal air sac mouse model. Neither free oxaliplatin nor oxaliplatin encapsulated in PEG-coated neutral liposomes showed such a strong suppressive effect. The strong anti-angiogenic effect of l-OHP containing PEG-coated cationic liposomes was attributed to the selective delivery of the drug to the angiogenic vessels and its subsequent uptake by endothelial cells [80].

4. Dual targeting approach to both tumor endothelial cells and tumor cells

In previous studies, the combination of vascular targeting agents with anticancer agents, or angiogenesis inhibitors, has led to additive or synergistic activity in experimental solid tumors [92,93]. However, traditional chemotherapeutic anticancer agents kill cancer cells as well as all rapidly growing cells in the body, such as blood and hair cells and cells lining the intestine. This leads to the distressing side effects of chemotherapy, and imposes practical limits on the drug dose and dosing frequency. In the last few years, as has been introduced in this review, liposome-encapsulated anticancer agents have been shown to increase the selective toxicity of the agents in cancer, resulting in improved therapeutic outcome and/or minimized damage to normal tissues such as heart and bone marrow [94,95]. Moreover, ligand-targeted liposomal anticancer agents (immunoliposomes or targeted liposomes) that promote selective binding and internalization of the liposomes into target cells have shown enhanced antitumor effects relative to non-targeted liposomal agents [95]. As has been described, targeting anticancer agents to tumor vasculature by means of a liposomal delivery system has attracted considerable attention for the purpose of increasing the therapeutic index of the agents without increasing the severe side effects [96,97]. The targeting of liposomal anticancer agents directly to tumor cells by targeted liposomal delivery has also attracted much attention [96]. Accordingly, it is easy to imagine that a strategy that targets both the tumor vasculature and the tumor cells by targeted liposomes is more effective than a strategy that targets only tumor vasculature, which can leave a cuff of unaffected tumor cells at the tumor periphery that can subsequently regrow and kill the animals or patients.

Pastorino *et al.* [52] provided the proof-of-principle for the hypothesis that combined administration of liposomal anticancer drugs that target tumor cells and tumor vasculature improves therapeutic effect relative to each therapy used individually. For the targeting of tumor cells, they used anti-GD2 monoclonal antibody against the disialoganglioside GD2 that is widely expressed on cancer cells of neuronal origin, including neuroblastoma, and at low levels, in cerebellum and peripheral nerves [98]. For targeting tumor vasculature, DXR-loaded liposome was modified with NGR peptides that target the angiogenic endothelial cell marker aminopeptidase N [56]. In an orthotopic neuroblastoma xenograft model, the combined formulations GD2-SIL(DXR) (tumor-targeted) and NGR-SL(DXR) (vascular-targeted) showed superior antitumor efficiency over either tumor- or vascular-targeted liposomal formulations. This report was the first proof-of-principle in anticancer therapy by delivery of anticancer agents to both tumor cells and tumor vasculature by means of two different targeting liposomes.

A PEG-coated cationic liposome composed of HSPC: CHOL:DC-6-14:mPEG$_{2000}$-DSPE was developed recently and it was confirmed that this type of cationic liposome is a promising carrier for delivery of encapsulated chemotherapeutic agents to tumor endothelium [80]. In that study, it was also shown that l-OHP encapsulated in such a cationic liposome can exert a potent anti-angiogenic effect in a DAS model [80]. In addition, the authors recently demonstrated

that l-OHP-containing PEG-coated cationic liposomes have a superior antitumor activity over either free l-OHP or l-OHP-containing PEG-coated neutral liposomes in a murine tumor-xenograft model [99]. The superior antitumor activity of l-OHP encapsulated in PEG-coated cationic liposomes was confirmed to result from the dual targeting activity of PEG-coated cationic liposomes against both tumor endothelial cells and tumor cells [99]. This type of dual targeting approach – vascular targeting and tumor targeting with a single liposomal anticancer drug formulation – may have great potential to overcome some of the major shortcomings of conventional strategies.

5. Conclusions

In recent years, researchers have made substantial progress in attempting to achieve specific targeting of drugs to a solid tumor. Among these studies, active targeting of anticancer drugs to solid tumors by means of targeted liposomal delivery systems such as antibody- or peptide-modified liposomes (immunoliposomes) has achieved significant *in vivo* therapeutic efficacy with no severe side effects compared with conventional treatments using free anticancer agents. Moreover, the tumor vascular targeting approach using either immunoliposomes or cationic liposomes is a promising strategy to suppress tumor growth by killing endothelial cells in angiogenic vessels. In clinical applications, however, a multiple target approach, based on a combination of antitumor and antivascular therapies, is frequently used because of insufficient therapeutic activity of antivascular agents in patients when used alone. Accordingly, the dual targeting approach proposed recently – vascular targeting and tumor targeting with a single liposomal anticancer drug formulation – may have potential to overcome some of the major shortcomings of conventional strategies.

6. Expert opinion

Targeting tumor vasculature is considered a rational alternative to tumor cell targeting. In fact, targeting tumor vasculature was been shown to be highly effective in suppressing tumor growth in preclinical animal models [100,101]. There are several advantages of targeting anticancer agents to proliferating endothelial cells in the tumor vasculature rather than targeting directly to tumor cells. First, the acquired drug resistance that results from genetic and epigenetic mechanisms reduces the effectiveness of available drugs [102,103]. Anti-angiogenic therapy has the potential to overcome this problem, or at least to reduce its impact. This therapy targets the tumor vasculature, derived from local and circulating endothelial cells, which are considered genetically stable. Second, the fact that many cancer cells depend on a few endothelial cells for their growth and survival might also amplify the therapeutic effect [104]. Third, anti-angiogenic therapies may also circumvent what may be a major mechanism of intrinsic drug resistance, namely insufficient drug penetration into the interior of a tumor mass because of high interstitial pressure gradients from within the tumors [1]. Fourth, oxygen consumption by neoplastic and endothelial cells, along with poor oxygen delivery, creates hypoxia within tumors. These pathophysiological characteristics of solid tumors compromise the delivery and effectiveness of conventional cytotoxic therapies, as well as molecularly targeted therapies [1,104]. Finally, the therapeutic target is independent of the type of solid tumors; the killing of proliferating endothelial cells in the tumor microenvironment can be effective against a variety of malignancies. However, a strategy that targets both the tumor vasculature and the tumor cells themselves must be more effective than strategies that target only the tumor vasculature because this strategy can leave a cuff of unaffected tumor cells at the tumor periphery that can subsequently regrow and kill the animals [105].

In the last decade, liposomes have been used to increase selective toxicity of anticancer drugs, resulting in improved therapeutic outcomes and/or reduced toxicities [96]. In addition, many studies have shown that the use of immunoliposomes significantly increases the binding to target cells and improves therapeutic efficacy *in vivo* with no severe side effects [95,106]. Thus, on the basis of the concept described above, ligand- or antibody-mediated immunoliposome targeting within the vasculature, where the targets are readily accessible, appears to be a success, resulting in optimistic therapeutic outcomes and reduced toxicity of anticancer agents. Nevertheless, it must be noted that ligands used on the immunoliposomes are commonly directed at receptors/antigens overexpressed on endothelial cells in tumor vasculature. Most *in vivo* target cells are considered to show a lesser or greater degree of heterogeneity in the expression of the target receptors/antigens. In addition, such receptors/antigens recognized by utilized ligands may be expressed not only on the target cells but also on other cells in the body, leading to unintended uptake in these off-target cells. Such heterogeneity of receptors/antigens expression in the targeted endothelial cells may become a problem, resulting in less therapeutic efficacy and/or causes of severe side effects of immunoliposomal anticancer agents. One possible solution would be to use charge interactions between cationic liposomes (positive charge) and endothelial cells (negative charge) in the tumor vasculature. Cationic liposomes can selectively bind to tumor endothelial cells of different origins because all tumor endothelial cells share the common feature of overexpressing negatively charged molecules on their surface. Anionic sites distributed along the tumor vasculature were identical to general locations where cationic liposomes accumulated [76]. However, several studies support the concept that the distribution of anionic sites is patchy and heterogeneous [76]. In studies using mice, the distribution varies among the different vessel types and between young and relatively old mice [107]. In addition, the mechanism(s) by which an anionic surface charge is altered in disease is not

completely understood [108]. Therefore, further studies are required to elucidate how the structural and morphological differentiation of the microvasculature in diseases and in tumors influences the efficacy of vascular targeting with cationic liposomes.

The targeting of tumor vasculature has shown potential in preclinical studies. From a clinical point of view, however, the situation is much more complicated. Tumor vessels vary significantly in the density, surface area, degree of permeability and angiogenic potential of each solid tumor. Accordingly, it seems that a single therapeutic approach with an anti-angiogenic agent cannot be efficient in suppressing tumor growth in a broad range of solid malignancies. In contemporary combination therapy, cytotoxic agents and anti-angiogenic agents are frequently used in the clinical setting and have improved the overall antitumor response *in vivo* [109]. However, preclinical and clinical studies have indicated that the toxicity profile of such combinations differs from that of conventional single chemotherapy, thus ruling out additive toxicity as a main limitation of combination chemotherapy [110,111]. Therefore, the dual targeting approach, vascular targeting and tumor targeting with a single liposomal anticancer drug formulation, may have great potential to overcome some major limitations of conventional anticancer chemotherapy in the clinical setting.

Recently, endothelial progenitor cells in the adult bone marrow have shown their ability to circulate in the peripheral blood and incorporate into new blood vessels, contributing to tumor angiogenesis [112-114]. A therapeutic strategy based on targeting endothelial progenitor cells, in combination with other tumor- and vascular-targeted therapies, may achieve further increased therapeutic efficacy over the dual targeting approach. If such cells highly expressed negatively charged macromolecules such as proteoglycans on their surface, similar to the endothelial cells in tumor angiogenic vessels, those cells in blood circulation were considered to be one of the targets for the PEG-coated cationic liposome containing anticancer drugs. To elucidate this possibility, the interaction of PEG-coated cationic liposome with endothelial progenitor cells is worth further investigation.

Nevertheless, despite the great success achieved with cationic liposomes targeted to tumor vasculature in preclinical studies, the ultimate utilization of cationic liposomes in the clinical setting is still limited by the toxicity of the cationic lipid used and by optimization of the overall positive charge of the carrier. Therefore, further development and characterization of cationic liposomes to complete the dual targeting approach is essential to the improvement of their clinical efficiency.

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Declaration of interest

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Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Jain RK. The next frontier of molecular medicine: delivery of therapeutics. Nat Med 1998;4:655-7
2. Pluen A, Boucher Y, Ramanujan S, et al. Role of tumor-host interactions in interstitial diffusion of macromolecules: cranial vs. subcutaneous tumors. Proc Natl Acad Sci USA 2001;98:4628-33
3. Jain RK. Physiological barriers to delivery of monoclonal antibodies and other macromolecules in tumors. Cancer Res 1990;50(Suppl 3):814s-9s
4. Netti PA, Baxter LT, Boucher Y, et al. Time-dependent behavior of interstitial fluid pressure in solid tumors: implications for drug delivery. Cancer Res 1995;55:5451-8
5. Tsuruo T, Tomida A. Multidrug resistance. Anticancer Drugs 1995;6:213-8
6. Peer D, Karp JM, Hong S, et al. Nanocarriers as an emerging platform for cancer therapy. Nat Nanotechnol 2007;2:751-60
7. Lammers T, Hennink WE, Storm G. Tumour-targeted nanomedicines: principles and practice. Br J Cancer 2008;99:392-7
8. Lu Y, Yang J, Sega E. Issues related to targeted delivery of proteins and peptides. AAPS J 2006;8:E466-78
9. Bergers G, Benjamin LE. Tumorigenesis and the angiogenic switch. Nat Rev Cancer 2003;3:401-10
10. Griffioen AW, Barendsz-Janson AF, Mayo KH, Hillen HF. Angiogenesis, a target for tumor therapy. J Lab Clin Med 1998;132:363-8
11. Pezzolo A, Parodi F, Corrias MV, et al. Tumor origin of endothelial cells in human neuroblastoma. J Clin Oncol 2007;25:376-83
12. Shimizu K, Oku N. Cancer anti-angiogenic therapy. Biol Pharm Bull 2004;27:599-605
13. Ye J, Li Y, Hamasaki T, et al. Inhibitory effect of electrolyzed reduced water on tumor angiogenesis. Biol Pharm Bull 2008;31:19-26
14. Thurston G, McLean JW, Rizen M, et al. Cationic liposomes target angiogenic endothelial cells in tumors and chronic inflammation in mice. J Clin Invest 1998;101:1401-13
15. Pandya NM, Dhalla NS, Santani DD. Angiogenesis–a new target for future therapy. Vascul Pharmacol 2006;44:265-74
16. Staton CA, Stribbling SM, Tazzyman S, et al. Current methods for assaying angiogenesis in vitro and in vivo. Int J Exp Pathol 2004;85:233-48
17. Hussain S, Slevin M, Matou S, et al. Anti-angiogenic activity of sesterterpenes; natural product inhibitors of FGF-2-induced angiogenesis. Angiogenesis 2008;11:245-56
18. Shibusa T, Shijubo N, Abe S. Tumor angiogenesis and vascular endothelial growth factor expression in stage I lung adenocarcinoma. Clin Cancer Res 1998;4:1483-7
19. Rosen LS. Clinical experience with angiogenesis signaling inhibitors: focus on vascular endothelial growth factor (VEGF) blockers. Cancer Control 2002;9(Suppl 2):36-44
20. Folkman J. The role of angiogenesis in tumor growth. Semin Cancer Biol 1992;3:65-71
21. Fong TA, Shawver LK, Sun L, et al. SU5416 is a potent and selective inhibitor of the vascular endothelial growth factor receptor (Flk-1/KDR) that inhibits tyrosine kinase catalysis, tumor vascularization, and growth of multiple tumor types. Cancer Res 1999;59:99-106
22. Kan Z, Ivancev K, Lunderquist A, et al. In vivo microscopy of hepatic tumors in animal models: a dynamic investigation of blood supply to hepatic metastases. Radiology 1993;187:621-6
23. Stewart PA, Hayakawa K, Farrell CL, Del Maestro RF. Quantitative study of microvessel ultrastructure in human peritumoral brain tissue. Evidence for a blood-brain barrier defect. J Neurosurg 1987;67:697-705
24. Yonenaga Y, Mori A, Onodera H, et al. Absence of smooth muscle actin-positive pericyte coverage of tumor vessels correlates with hematogenous metastasis and prognosis of colorectal cancer patients. Oncology 2005;69:159-66
25. Iozzo RV, San Antonio JD. Heparan sulfate proteoglycans: heavy hitters in the angiogenesis arena. J Clin Invest 2001;108:349-55
26. Ran S, Thorpe PE. Phosphatidylserine is a marker of tumor vasculature and a potential target for cancer imaging and therapy. Int J Radiat Oncol Biol Phys 2002;54:1479-84
27. Fears CY, Gladson CL, Woods A. Syndecan-2 is expressed in the microvasculature of gliomas and regulates angiogenic processes in microvascular endothelial cells. J Biol Chem 2006;281:14533-6
28. Seymour LW. Passive tumor targeting of soluble macromolecules and drug conjugates. Crit Rev Ther Drug Carrier Syst 1992;9:135-87
29. 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(12 Pt 1):6387-92
30. Hajitou A, Pasqualini R, Arap W. Vascular targeting: recent advances and therapeutic perspectives. Trends Cardiovasc Med 2006;16:80-8
31. Sapra P, Allen TM. Ligand-targeted liposomal anticancer drugs. Prog Lipid Res 2003;42:439-62

• **The authors summarize some recent advances in the field of ligand-targeted liposomes for the delivery of anticancer drugs.**

32. Allen TM, Brandeis E, Hansen CB, et al. A new strategy for attachment of antibodies to sterically stabilized liposomes resulting in efficient targeting to cancer cells. Biochim Biophys Acta 1995;1237(2):99-108
33. Ahmad I, Longenecker M, Samuel J, Allen TM. Antibody-targeted delivery of doxorubicin entrapped in sterically stabilized liposomes can eradicate lung cancer in mice. Cancer Res 1993;53:1484-8
34. Volkel T, Holig P, Merdan T, et al. Targeting of immunoliposomes to endothelial cells using a single-chain Fv fragment directed against human endoglin (CD105). Biochim Biophys Acta 2004;1663:158-66
35. Voinea M, Manduteanu I, Dragomir E, et al. Immunoliposomes directed toward VCAM-1 interact specifically with activated endothelial cells–a potential tool for specific drug delivery. Pharm Res 2005;22:1906-17
36. Bloemen PG, Henricks PA, van Bloois L, et al. Adhesion molecules: a new target for

immunoliposome-mediated drug delivery. FEBS Lett 1995;357:140-4

37. Spragg DD, Alford DR, Greferath R, et al. Immunotargeting of liposomes to activated vascular endothelial cells: a strategy for site-selective delivery in the cardiovascular system. Proc Natl Acad Sci USA 1997;94:8795-800

38. Kessner S, Krause A, Rothe U, Bendas G. Investigation of the cellular uptake of E-Selectin-targeted immunoliposomes by activated human endothelial cells. Biochim Biophys Acta 2001;1514:177-90

39. Atobe K, Ishida T, Ishida E, et al. In vitro efficacy of a sterically stabilized immunoliposomes targeted to membrane type 1 matrix metalloproteinase (MT1-MMP). Biol Pharm Bull 2007;30:972-8

40. Hatakeyama H, Akita H, Ishida E, et al. Tumor targeting of doxorubicin by anti-MT1-MMP antibody-modified PEG liposomes. Int J Pharm 2007;342:194-200

41. Kuzu I, Bicknell R, Fletcher CD, Gatter KC. Expression of adhesion molecules on the endothelium of normal tissue vessels and vascular tumors. Lab Invest 1993;69:322-8

42. Dienst A, Grunow A, Unruh M, et al. Specific occlusion of murine and human tumor vasculature by VCAM-1-targeted recombinant fusion proteins. J Natl Cancer Inst 2005;97:733-47

43. Ryan DH, Nuccie BL, Abboud CN, Winslow JM. Vascular cell adhesion molecule-1 and the integrin VLA-4 mediate adhesion of human B cell precursors to cultured bone marrow adherent cells. J Clin Invest 1991;88:995-1004

44. Seron D, Cameron JS, Haskard DO. Expression of VCAM-1 in the normal and diseased kidney. Nephrol Dial Transplant 1991;6:917-22

45. Bodey B, Bodey B Jr, Siegel SE, Kaiser HE. Over-expression of endoglin (CD105): a marker of breast carcinoma-induced neo-vascularization. Anticancer Res 1998;18:3621-8

46. Dawn G, MacKie RM. Expression of endoglin in human melanocytic lesions. Clin Exp Dermatol 2002;27:153-6

47. Bendas G, Rothe U, Scherphof GL, Kamps JA. The influence of repeated injections on pharmacokinetics and biodistribution of different types of sterically stabilized immunoliposomes. Biochim Biophys Acta 2003;1609:63-70

48. Ishida T, Harashima H, Kiwada H. Liposome clearance. Biosci Rep 2002;22(2):197-224

49. Kirpotin DB, Drummond DC, Shao Y, et al. Antibody targeting of long-circulating lipidic nanoparticles does not increase tumor localization but does increase internalization in animal models. Cancer Res 2006;66:6732-40

50. Schiffelers RM, Koning GA, ten Hagen TL, et al. Anti-tumor efficacy of tumor vasculature-targeted liposomal doxorubicin. J Control Release 2003;91:115-22

51. Koning GA, Fretz MM, Woroniecka U, et al. Targeting liposomes to tumor endothelial cells for neutron capture therapy. Appl Radiat Isot 2004;61:963-7

• **The authors provided the proof-of-principle for the hypothesis that the dual targeting approach, vascular targeting and tumor cell targeting, could potentially enhance the overall antitumor efficacy of liposomal chemotherapy.**

52. Pastorino F, Brignole C, Di Paolo D, et al. Targeting liposomal chemotherapy via both tumor cell-specific and tumor vasculature-specific ligands potentiates therapeutic efficacy. Cancer Res 2006;66:10073-82

53. Simberg D, Duza T, Park JH, et al. Biomimetic amplification of nanoparticle homing to tumors. Proc Natl Acad Sci USA 2007;104:932-6

54. Kondo M, Asai T, Katanasaka Y, et al. Anti-neovascular therapy by liposomal drug targeted to membrane type-1 matrix metalloproteinase. Int J Cancer 2004;108:301-6

55. Curnis F, Arrigoni G, Sacchi A, et al. Differential binding of drugs containing the NGR motif to CD13 isoforms in tumor vessels, epithelia, and myeloid cells. Cancer Res 2002;62:867-74

56. Pastorino F, Brignole C, Marimpietri D, et al. Vascular damage and anti-angiogenic effects of tumor vessel-targeted liposomal chemotherapy. Cancer Res 2003;63:7400-9

57. Oku N, Asai T, Watanabe K, et al. Anti-neovascular therapy using novel peptides homing to angiogenic vessels. Oncogene 2002;21:2662-9

58. Maeda N, Takeuchi Y, Takada M, et al. Anti-neovascular therapy by use of tumor neovasculature-targeted long-circulating liposome. J Control Release 2004;100:41-52

59. Maruyama K, Takahashi N, Tagawa T, et al. Immunoliposomes bearing polyethyleneglycol-coupled Fab′ fragment show prolonged circulation time and high extravasation into targeted solid tumors in vivo. FEBS Lett 1997;413:177-80

60. Mislick KA, Baldeschwieler JD. Evidence for the role of proteoglycans in cation-mediated gene transfer. Proc Natl Acad Sci USA 1996;93:12349-54

61. Mounkes LC, Zhong W, Cipres-Palacin G, et al. Proteoglycans mediate cationic liposome-DNA complex-based gene delivery in vitro and in vivo. J Biol Chem 1998;273:26164-70

62. Zuhorn IS, Kalicharan D, Robillard GT, Hoekstra D. Adhesion receptors mediate efficient non-viral gene delivery. Mol Ther 2007;15:946-53

63. Hagerstrand H, Iglic A, Bobrowska-Hagerstrand M, et al. Amphiphile-induced vesiculation in aged hereditary spherocytosis erythrocytes indicates normal membrane stability properties under non-starving conditions. Mol Membr Biol 2001;18:221-7

64. Kitagawa S, Kasamaki M, Hyodo M. Cationic vesicles consisting of 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) and phosphatidylcholines and their interaction with erythrocyte membrane. Chem Pharm Bull (Tokyo) 2004;52:451-3

65. Zelphati O, Uyechi LS, Barron LG, Szoka FC Jr. Effect of serum components on the physico-chemical properties of cationic lipid/oligonucleotide complexes and on their interactions with cells. Biochim Biophys Acta 1998;1390:119-33

66. Varsano S, Frolkis I, Ophir D. Expression and distribution of cell-membrane complement regulatory glycoproteins along the human respiratory tract. Am J Respir Crit Care Med 1995;152:1087-93

67. Chonn A, Cullis PR, Devine DV. The role of surface charge in the activation of the classical and alternative pathways of complement by liposomes. J Immunol 1991;146:4234-41

68. Dass CR, Choong PF. Targeting of small molecule anticancer drugs to the tumour

and its vasculature using cationic liposomes: lessons from gene therapy. Cancer Cell Int 2006;6:17

69. Takakura Y, Nishikawa M, Yamashita F, Hashida M. Influence of physicochemical properties on pharmacokinetics of non-viral vectors for gene delivery. J Drug Target 2002;10:99-104

70. Torchilin VP. Polymer-coated long-circulating microparticulate pharmaceuticals. J Microencapsul 1998;15:1-19

71. Allen C, Dos Santos N, Gallagher R, et al. Controlling the physical behavior and biological performance of liposome formulations through use of surface grafted poly(ethylene glycol). Biosci Rep 2002;22:225-50

72. Song LY, Ahkong QF, Rong Q, et al. Characterization of the inhibitory effect of PEG-lipid conjugates on the intracellular delivery of plasmid and antisense DNA mediated by cationic lipid liposomes. Biochim Biophys Acta 2002;1558:1-13

73. Zalipsky S, Brandeis E, Newman MS, Woodle MC. Long circulating, cationic liposomes containing amino-PEG-phosphatidylethanolamine. FEBS Lett 1994;353:71-4

74. Levchenko TS, Rammohan R, Lukyanov AN, et al. Liposome clearance in mice: the effect of a separate and combined presence of surface charge and polymer coating. Int J Pharm 2002;240:95-102

75. Campbell RB, Ying B, Kuesters GM, Hemphill R. Fighting cancer: from the bench to bedside using second generation cationic liposomal therapeutics. J Pharm Sci 2009;98:411-29

76. Campbell RB, Fukumura D, Brown EB, et al. Cationic charge determines the distribution of liposomes between the vascular and extravascular compartments of tumors. Cancer Res 2002;62:6831-6

77. Ishiwata H, Suzuki N, Ando S, et al. Characteristics and biodistribution of cationic liposomes and their DNA complexes. J Control Release 2000;69:139-48

78. Stuart DD, Kao GY, Allen TM. A novel, long-circulating, and functional liposomal formulation of antisense oligodeoxynucleotides targeted against MDR1. Cancer Gene Ther 2000;7:466-75

79. Gabizon A. Liposome circulation time and tumor targeting: implications for cancer chemotherapy. Adv Drug Deliv Rev 1995;16:285-94

80. Abu-Lila A, Suzuki T, Doi Y, et al. Oxaliplatin targeting to angiogenic vessels by PEGylated cationic liposomes suppresses the angiogenesis in a dorsal air sac mouse model. J Control Release 2009;134:18-25

• **In this article, using a dorsal air sac mouse model, the authors investigated a potent anti-angiogenic activity of oxaliplatin on encapsulating it in PEGylated cationic liposomes.**

81. Harigai T, Kondo M, Isozaki M, et al. Preferential binding of polyethylene glycol-coated liposomes containing a novel cationic lipid, TRX-20, to human subendthelial cells via chondroitin sulfate. Pharm Res 2001;18:1284-90

82. Dass CR. Improving anti-angiogenic therapy via selective delivery of cationic liposomes to tumour vasculature. Int J Pharm 2003;267:1-12

83. Krasnici S, Werner A, Eichhorn ME, et al. Effect of the surface charge of liposomes on their uptake by angiogenic tumor vessels. Int J Cancer 2003;105:561-7

84. Oikawa T, Sasaki M, Inose M, et al. Effects of cytogenin, a novel microbial product, on embryonic and tumor cell-induced angiogenic responses in vivo. Anticancer Res 1997;17:1881-6

85. Nakamura M, Katsuki Y, Shibutani Y, Oikawa T. Dienogest, a synthetic steroid, suppresses both embryonic and tumor-cell-induced angiogenesis. Eur J Pharmacol 1999;386:33-40

86. Tagami T, Barichello JM, Kikuchi H, et al. The gene-silencing effect of siRNA in cationic lipoplexes is enhanced by incorporating pDNA in the complex. Int J Pharm 2007;333:62-9

87. Kalra AV, Campbell RB. Development of 5-FU and doxorubicin-loaded cationic liposomes against human pancreatic cancer: Implications for tumor vascular targeting. Pharm Res 2006;23:2809-17

88. Kunstfeld R, Wickenhauser G, Michaelis U, et al. Paclitaxel encapsulated in cationic liposomes diminishes tumor angiogenesis and melanoma growth in a 'humanized' SCID mouse model. J Invest Dermatol 2003;120:476-82

89. Strieth S, Eichhorn ME, Sauer B, et al. Neovascular targeting chemotherapy: encapsulation of paclitaxel in cationic liposomes impairs functional tumor microvasculature. Int J Cancer 2004;110:117-24

90. Schmitt-Sody M, Strieth S, Krasnici S, et al. Neovascular targeting therapy: paclitaxel encapsulated in cationic liposomes improves antitumoral efficacy. Clin Cancer Res 2003;9:2335-41

91. Wu J, Lee A, Lu Y, Lee RJ. Vascular targeting of doxorubicin using cationic liposomes. Int J Pharm 2007;337:329-35

92. Siim BG, Lee AE, Shalal-Zwain S, et al. Marked potentiation of the antitumour activity of chemotherapeutic drugs by the antivascular agent 5,6-dimethylxanthenone-4-acetic acid (DMXAA). Cancer Chemother Pharmacol 2003;51:43-52

93. Siemann DW, Mercer E, Lepler S, Rojiani AM. Vascular targeting agents enhance chemotherapeutic agent activities in solid tumor therapy. Int J Cancer 2002;99:1-6

94. Gabizon A, Catane R, Uziely B, et al. Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene-glycol coated liposomes. Cancer Res 1994;54:987-92

95. Allen TM. Ligand-targeted therapeutics in anticancer therapy. Nat Rev Cancer 2002;2:750-63

96. Gabizon AA, Shmeeda H, Zalipsky S. Pros and cons of the liposome platform in cancer drug targeting. J Liposome Res 2006;16:175-83

97. Gabizon AA. Stealth liposomes and tumor targeting: one step further in the quest for the magic bullet. Clin Cancer Res 2001;7:223-5

98. Schulz G, Cheresh DA, Varki NM, et al. Detection of ganglioside GD2 in tumor tissues and sera of neuroblastoma patients. Cancer Res 1984;44:5914-20

99. Abu Lila AS, Kizuki S, Doi Y, et al. Oxaliplatin encapsulated in PEG-coated cationic liposomes induces significant tumor growth suppression via a dual-targeting approach in a murine solid tumor model. J Control Release 2009;137:8-14

• **The authors tried to give an insight into the probable mechanism of the enhanced antitumor activity of oxaliplatin-containing PEG-coated cationic liposomes and they confirmed that such efficient antitumor activity is strongly related to a dual targeting**

approach against both tumor endothelial cells and tumor cells as well.

100. Ribatti D. The discovery of antiangiogenic molecules: a historical review. Curr Pharm Des 2009;15:345-52

101. Ruegg C, Mutter N. Anti-angiogenic therapies in cancer: achievements and open questions. Bull Cancer 2007;94:753-62

102. Browder T, Butterfield CE, Kraling BM, et al. Antiangiogenic scheduling of chemotherapy improves efficacy against experimental drug-resistant cancer. Cancer Res 2000;60:1878-86

103. Klement G, Baruchel S, Rak J, et al. Continuous low-dose therapy with vinblastine and VEGF receptor-2 antibody induces sustained tumor regression without overt toxicity. J Clin Invest 2000;105:R15-24

104. Jain RK. Normalizing tumor vasculature with anti-angiogenic therapy: a new paradigm for combination therapy. Nat Med 2001;7:987-9

105. Huang X, Molema G, King S, et al. Tumor infarction in mice by antibody-directed targeting of tissue factor to tumor vasculature. Science 1997;275:547-50

106. Maruyama K. PEG-immunoliposome. Biosci Rep 2002;22:251-66

107. Simionescu M, Simionescu N, Santoro F, Palade GE. Differentiated microdomains of the luminal plasmalemma of murine muscle capillaries: segmental variations in young and old animals. J Cell Biol 1985;100:1396-407

108. Hardebo JE, Kahrstrom J. Endothelial negative surface charge areas and blood-brain barrier function. Acta Physiol Scand 1985;125:495-9

109. Ghosh S, Maity P. Augmented antitumor effects of combination therapy with VEGF antibody and cisplatin on murine B16F10 melanoma cells. Int Immunopharmacol 2007;7:1598-608

110. Martinelli M, Bonezzi K, Riccardi E, et al. Sequence dependent antitumour efficacy of the vascular disrupting agent ZD6126 in combination with paclitaxel. Br J Cancer 2007;97:888-94

111. Knox JJ, Hedley D, Oza A, et al. Combining gemcitabine and capecitabine in patients with advanced biliary cancer: a phase II trial. J Clin Oncol 2005;23:2332-8

112. Gao D, Nolan D, McDonnell K, et al. Bone marrow-derived endothelial progenitor cells contribute to the angiogenic switch in tumor growth and metastatic progression. Biochim Biophys Acta 2009; published online 19 May 2009, doi:10.1016/j.bbcan.2009.05.001

113. Chase JL. Clinical use of anti-vascular endothelial growth factor monoclonal antibodies in metastatic colorectal cancer. Pharmacotherapy 2008;28:23S-30S

114. Ding YT, Kumar S, Yu DC. The role of endothelial progenitor cells in tumour vasculogenesis. Pathobiology 2008;75:265-73

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