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Review

Liposomal nanomedicines as anticancer therapeutics: Beyond targeting tumor cells

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ABSTRACT

Tumor cells have long been the primary target cell type of liposomes for anticancer therapy. At present, it appears that tumor growth and metastasis is facilitated by interactions between tumor cells and supporting cells. These supporting cells consist of adaptive and innate immune cells, endothelial cells, pericytes, fibroblasts, stromal and mesenchymal cells. Insight into the activity of these cells and communication between these cells has provided new tactics for targeting alternative cell types in tumor treatment and offered new drug classes that could be used to modulate the activity of these supporting cells. Here, we provide an overview of liposomal systems that have been designed to target supporting cells in tumor tissue and therapeutic results of these systems.

Contents

1. Introduction

Cancer is a disease that is notoriously difficult to treat. Cytotoxic drugs involved in treatment are designed to kill tumor cells but generally also display unwanted toxicities as they lack tumor cell selectivity (Gardner and Fernandes, 2004). Liposomal tumor targeting research has typically focused on increasing the specificity of these drugs for the malignant tissue. These efforts culminated in the approval of liposomal doxorubicin (Doxil®) (Abraham et al., 2005), initially for treatment of Kaposi's sarcoma but presently also used in ovarian cancer and multiple myeloma (Perez-Lopez et al., 2007; Ludwig et al., 2007). Despite the advantageous characteristics of the Doxil-formulation, new dose-limiting toxicities are seen (such as hand and foot syndrome (O'brien, 2008)) and development of doxorubicin-resistance can occur. This is caused by the inherent genetic instability of cancer cells, inducing heterogeneity. This heterogeneity, in turn, provides a source of cell variants that are responsible for drug resistance selection in a Darwinian manner (Vineis and Berwick, 2006).

In an attempt to circumvent these difficulties, recent research attention has focused on other cell types present in a tumor. These cell types are usually innate and adaptive immune cells, cells that form blood and lymphatic vessels, fibroblasts, and mesenchymal cells. These supporting cell types can promote cancer development, once the tumor cells have mastered ways to recruit them and ways

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to modulate their activity in such a way that tumor cell survival and metastasis is enhanced (Gadea and Joyce, 2006; Noonan et al., 2008). Advantages of a change of treatment focus from tumor cells to supporting cells include the fact that the treatment is less dependent on the tissue of origin of the tumor, as tumors share similar pathophysiological activities of supporting cell types for proliferation. In addition, these supporting cells are, unlike tumor cells, genetically stable which reduces the chance of occurrence of resistance. Moreover, modulation of the activity of these cells can usually be achieved with drugs with a milder side-effect profile. The aim of this contribution is to discuss the tumor-supporting cell types that could be possible targets for liposomal nanomedicines.

2. Intratumoral cell types other than tumor cells supporting tumor growth

In 1863, Virchow described the presence of macrophages in tumors (Virchow, 1863). He postulated that the infiltrate was caused by an inflammatory reaction, which was related to or causing cancer. Later, however, the natural role of macrophages as defenders against invaders, seemed to suggest that macrophages – or more in general the components of the innate immune system – are able to identify the malignant tissue, infiltrate it and attempt to eliminate it (Weiss, 1976; Mauel, 1976). Recently, evidence is accumulating that Virchow's suggestion may be closer to reality. It appears that the innate immune response in concert with the adaptive immune response plays an important role in carcinogenesis and is strongly involved in angiogenesis, tumor cell invasion and metastasis (Fig. 1) (De Visser et al., 2006; Condeelis and Pollard, 2006; Aggarwal et al., 2006; Balkwill et al., 2005).

Generally, the innate immune cells become activated as a result of a local disruption of homeostasis. Macrophages and mast cells acutely react to this local imbalance by releasing mediators to recruit and activate additional immune cells, in order to try to fight the disturbance and to activate the adaptive immune system. As a consequence, the immune cells coordinate angiogenesis and extracellular matrix production for tissue repair.

However, when homeostasis is chronically affected (as in cancer) the continuous activation of the immune cells promotes tumor growth. In particular, the release of cytokines, growth factors and enzymes that remodel the extracellular matrix contribute to cancer progression. At the same time the cross-talk between adaptive and innate immune system becomes distorted due to continuous stimulation of certain inflammatory pathways. These processes contribute to enhanced tumor cell survival, angiogenesis and metastasis.

Angiogenesis (the outgrowth of new blood vessels from pre-existing vasculature) is an important process in tumor progression as it satisfies the growing tumor and metastases with their increasing need for oxygen and nutrients (Kerbel, 2008; Carmeliet, 2005). The disturbance of oxygen homeostasis in the growing tumor leads to a hypoxic milieu which provides a trigger for recruitment and activation of macrophages and mast cells. These cells activate a number of genetic programs to improve oxygenation of the tissue by promoting new blood vessel formation. This results in the so-called 'angiogenic switch'. Normal quiescent blood vessels are primarily composed of two different cell types: endothelial cells and pericytes (Armulik et al., 2005; Gerhardt and Semb, 2008). Coverage of the endothelial tubule by pericytes is thought to be important in the maintenance of the quiescent state. For new blood vessel formation to occur, the pericyte-coating of the pre-existing tubule must first dissociate. Then the surrounding extracellular matrix should be degraded followed by extravascular fibrin deposition. Next, the endothelial cells are able to respond to pro-angiogenic signals with proliferation, migration and new tubule formation. After that, remodeling occurs to prune vessels to fit the needs of the tissue. Coordinated regulation of pro- and anti-angiogenic factors is necessary for each stage to ensure the development of a functional vessel.

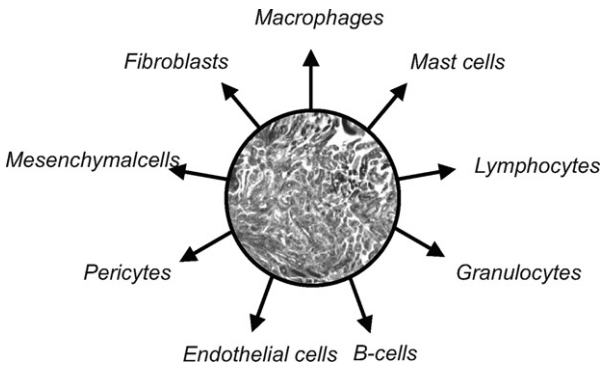

Fig. 1. Supporting cell types present in tumor tissue.

Mesenchymal stem cells are naturally recruited into tumor tissue in response to chemotactic factors. The mesenchymal cells play a role in the formation of mature blood vessels. They express angiopoetin-1, which binds to Tie-2 receptors expressed on the endothelial cells. This binding is thought to help in pericyte recruitment, vessel sprouting and vessel stabilization. After recruitment the mesenchymal cells can differentiate into smooth-muscle cell-like pericytes, which cover the vascular tree. A special role in pericyte recruitment plays the platelet-derived growth factor. This factor is excreted by the angiogenic endothelial cell and function as a chemo-attractant for pericytes, which after associating with endothelial cells stabilize the newly formed blood vessels.

In addition, macrophages in the pro-inflammatory milieu actively remodel the extracellular matrix increasing the ability of tumor cells to migrate. As macrophages communicate with tumor cells and induce tumor cell chemotaxis, this further promotes their migration. Both processes contribute to intravasation (the ability of tumor cells to enter the blood stream). The increased mobility of tumor cells is an important factor in the most lethal characteristics of malignant tumors: invasive growth and metastasis.

A cell type that is normally involved in the resolution of inflammatory processes is the fibroblast. Fibroblasts organize the inflammatory infiltrate and have important functions in the outcome of the inflammatory process (Flavell et al., 2008; Filer et al., 2006). Therefore, they may constitute an equally attractive target as immune cells in anti-inflammatory therapies. Fibroblasts appear to be an important source of anti-inflammatory factors and therefore stimulation of secretion of these factors may be an attractive strategy.

Taken together a number of cell types that contribute to tumor growth could be attractive for liposomal targeting. The following sections focus on the various cell types in the tumor that have been targeted with liposomes.

3. Targeting liposomes to supporting cell types

3.1. Immune cells

Macrophages play a pivotal role in the tumor inflammatory process. It appears that tumor-associated macrophages (TAM) have a distinct phenotype form 'classically activated' macrophages. Classically activated macrophages (e.g. by lipopolysaccharide) produce pro-inflammatory cytokines, like interleukin-1β, tumor necrosis factor-α, and interleukin-12, show enhanced expression of major

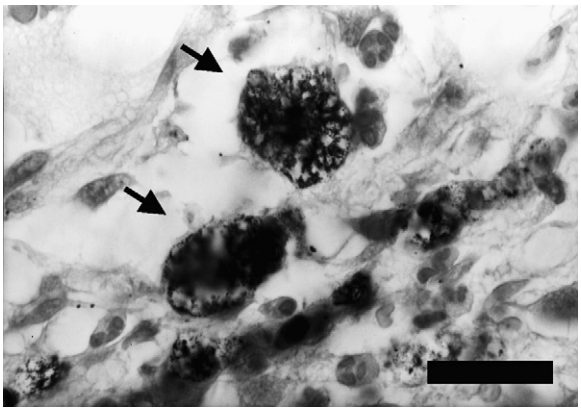

Fig. 2. Uptake of colloidal gold-labeled 100 nm sterically stabilized liposomes by TAM is visualized after silver-enhancement. Bar is 20 μm.

histocompatibility complex-II, CD80 and CD86 important for antigen presentation, enhanced endocytosis, and an enhanced ability to degrade internalized material.

TAM are typically active in angiogenesis, tissue remodeling and repair (Sica et al., 2002). In addition, TAM show certain characteristics that are distinct from classically activated macrophages, such as low production of radicals and pro-inflammatory cytokines and high levels of surface expression of scavenger receptor A and mannose receptor (Martinez et al., 2008). TAM are also considered to have limited capacity to act as antigen presenting cells.

To target TAM, the natural tendency of these cells to endocytose foreign material can be exploited. Although designed to avoid uptake by macrophages, even sterically stabilized liposomes ultimately end up to a significant extent in TAM (Fig. 2).

By loading the liposomes with clodronate, a bisphosphonate that is selectively toxic to macrophages, TAM can be depleted from tumor tissue. Studies with clodronate-liposomes in murine tumor models report marked effects on tumor growth and angiogenesis after liposomal clodronate treatment (Banciu et al., 2008; Zeisberger et al., 2006; Miselis et al., 2008). Our studies show that liposomal clodronate treatment induces a strong reduction of the intratumoral levels of the majority of pro-angiogenic factors that are primarily produced by TAM. Interestingly, treatment also strongly reduced the tumor levels of two anti-angiogenic factors tissue inhibitor of matrix metalloproteinease-1 and -2 that are produced by TAM. Thus, besides tumor growth promoting effects (through pro-angiogenic factors), TAM exert antitumor effects through the production of anti-angiogenic proteins, underlining the dual role of TAM in tumor growth. TAM have a net tumor growth promoting role as their depletion results in a net reduction of tumor growth rate.

Apart from depleting the TAM, milder approaches to alter the pro-angiogenic phenotype use liposomes loaded with immunosuppressive agents (such as prednisolone). A study by Banciu et al. (2006) showed that PEG-liposomal prednisolone strongly reduced pro-angiogenic protein levels whereas levels of anti-angiogenic proteins were hardly affected (in marked contrast to liposomal clodronate where the levels of both pro- and anti-angiogenic factors were reduced). Apparently the milder, prednisolone-based, TAM-activity downregulation strategy offers the advantage of reduction of specifically pro-angiogenic factors (Fig. 3).

The alternative approach, activation of TAM, has also been explored. The immune stimulatory activity of cationic lipid complexed with nucleic acid has been used to drive macrophages towards more classically activated phenotype, to promote their antitumor effects (Kuramoto et al., 2006, 2008a,b). These studies

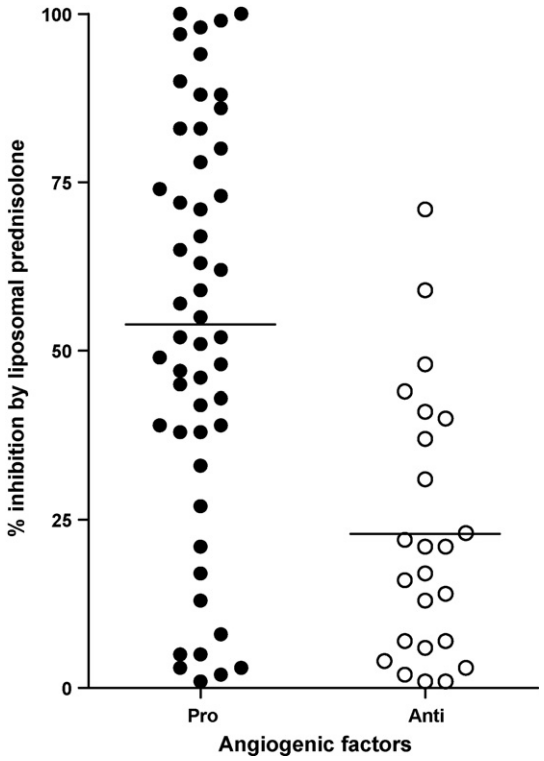

Fig. 3. Inhibition of B16F10-tumor levels of a panel of 17 pro-angiogenic and 8 anti-angiogenic factors measured in triplicate by treatment with liposomal prednisolone (Banciu et al., 2006). Expression of the majority of pro-angiogenic proteins is strongly inhibited, whereas the expression of the majority of anti-angiogenic proteins is not.

attribute the stimulatory activities of, especially, phosphorothioate CpG-containing nucleic acid complexed to cationic lipids, to the enhanced cytokine release of macrophages. Effects on other cell types (such as endothelial cells) cannot be excluded due to non-specific charge-based interactions with negatively charged cell membranes.

Alternatively liposomal targeting can be based on binding to specific receptors that are overexpressed on TAM. The folate receptor, for example, is overexpressed on many tumor cells as well as TAM. In a study in a mouse folate-receptor positive ovarian cancer model, it appeared that folate-targeted liposomes were taken up preferentially by TAM as compared to folate-receptor positive tumor cells. The receptor-mediated uptake was responsible for 50% of the total TAM-accumulation (Turk et al., 2004).

To our knowledge, the other two receptors that have been reported to be upregulated on TAM (i.e. mannose receptor and Scavenger receptor A) have not been used for liposome-based targeting nor have other immune cells in the tumor been the target cell type to downregulate their activity. Nevertheless, there have been some studies attempting to stimulate the immune cells in the tumor. For example, Kim et al. (2002) used sterically stabilized liposomes to deliver the pro-inflammatory cytokine tumor necrosis factor to the site of the tumor. As a result of treatment, tumor growth was strongly inhibited, likely because of sustained recruitment and activation of natural killer cells, macrophages and neutrophils.

3.2. Endothelial cells and pericytes

Proliferating endothelium is different from quiescent endothelium (Nanda and St Croix, 2004; Joyce et al., 2003; Zurita et al., 2003). This is the result of a local shift in balance between pro-angiogenic molecules (such as vascular endothelial growth factor),

and anti-angiogenic molecules (like thrombospondin and tissue inhibitor of metalloproteinases). This shift initiates the first of phase of angiogenesis. Most of the liposomal targeting strategies focus on targeting the endothelial cells, as this cell type plays a pivotal role in the entire process and offers easy accessibility after intravenous administration of the liposomes.

One of the most straightforward methods to target liposomes to neovasculature is the inclusion of positively charged lipids in the composition. An initial study by Mclean et al. (1997) showed that cationic 1-[2-[9-(*Z*)-octadecenoyloxy]]-2-[8](*Z*)-heptadecenyl]-3[hydroxyethyl] imidazolinium chloride (DOTIM):cholesterol liposomes in complex with DNA aggregated intravascularly after intravenous administration in mice. The particles were primarily cleared by macrophages in the liver and spleen but also by certain endothelial cells in specific tissues. Within 5 min, the binding to the endothelium was followed by ingestion and subsequent endosomal/lysosomal degradation. These studies demonstrated that normal endothelium can take up positively charged particles (or aggregates). This finding of cationic liposome-uptake by endothelial cells should be taken into consideration when cationic liposomes are designed for targeting the neovasculature. The specificity of uptake is usually attributed to charge interactions of the positively charged particles with the negatively charged proteoglycans on the endothelial cell surface. Interestingly, in a study where positively charged protamine was administered before cationic liposomes were injected, a 2-fold increase of liposome binding to tumor vaculature was seen, whereas binding to quiesecent endothelium remained low. It was postulated that protamine saturates binding in areas of high affinity uptake outside the tumor or alters the tumor microenvironment to promote endothelium interaction of the liposomes (Eichhorn et al., 2004).

Subsequent experiments in mice bearing a pancreatic islet cell carcinoma demonstrated that cationic liposomes are preferentially taken up by activated angiogenic endothelial cells to an approximately 15–30-fold higher degree as compared to quiescent endothelium in control animals (Thurston et al., 1998). Furthermore, this study also demonstrated that bound liposomes were rapidly internalized as soon as 20 min post-injection. Interestingly, uptake by the angiogenic endothelium was not homogeneous. There were focal 'hotspots' of high liposome uptake, which may be explained by differences in the phase of angiogenesis between different neovascular regions.

Campbell et al. (2002) investigated the distribution of cationic liposomes that were shielded by a poly(ethylene glycol)-coating in histological slides. Overall tumor uptake appeared not to be affected by charge but intravital microscopy revealed that increasing the charged lipid content from 10 to 50 mol% doubled the degree of localization of PEG-liposomes at tumor endothelium in both LS174T- and McaIV models. The pegylated cationic liposomes were also evaluated therapeutically, loaded with 5 fluoro-uracil and doxorubicin. As a result of the liposome-mediated shift in delivery of doxorubicin towards endothelial cells, tumor growth inhibition of liposomal doxorubicin was improved. The authors suggested that the nature of the preferential interaction of the liposomes with tumor microvessels is based on the slow and irregular tumor blood flow in tortuous and leaky tumor vasculature (Kalra and Campbell, 2006). Thereby, more interactions between cationic liposomes and anionic structures, possibly proteoglycans, on the angiogenic vasculature are enabled compared to normal vessels in which blood flow velocity is higher.

The therapeutic efficacy of cationic liposome-encapsulated paclitaxel has been studied in a number of different rodent tumor models (Strieth et al., 2008, 2004; Schmitt-Sody et al., 2003; Kunstfeld et al., 2003). In general, paclitaxel encapsulated in liposomes inhibited tumor growth and metastasis and thereby improved the survival of the animals. An anti-angiogenic mechanism of action was reflected by a reduced blood vessel density in the tumor rim and reduction of and endothelial cell proliferation. In addition, cationic liposomes (with or without paclitaxel, were also demonstrated to induce an increase of platelet adherence to endothelial cells in tumor microvessels. The binding of platelets reduced tumor capillary blood flow leading to reduced tumor perfusion. After repeated injections, the increased platelet adherence was only observed for paclitaxel-loaded cationic liposomes. Also, only in case of this liposome type, microthrombi were seen in the tumor capillaries. Disturbance of the coagulation cascade within the tumor seems to be an alternative mechanism of action involved in the antitumor activity of this cationic liposomal paclitaxel formulation.

Similar observations were made in a model of Meth-A sarcoma where porphyrins were delivered by cationic liposomes to the mouse tumor vasculature. After laser irradiation of the tumor, neovascular destruction was seen with concomitant reduced tumor growth along with a prolonged survival time of the tumor-bearing mice. Immunohistochemistry was used to confirm that antitumor effects were related to destruction of angiogenic endothelium resulting in tumor cell apoptosis (Takeuchi et al., 2003, 2002).

Finally, the self-assembly of nucleic acids and cationic lipids into lipoplexes has been used to target oligonucleotides and plasmids to (tumor) endothelium. As an example, in a study by Santel et al. (2006) positively charged liposomes containing also fusogenic lipids were complexed with negatively charged siRNA. After intravenous administration, endothelial cell-specific uptake in tumor tissue was seen that could be used to silence expression of endothelium-specific genes, such as those encoding CD31 and Tie2.

Apart from charge-based targeting, specific receptors overexpressed on the angiogenic endothelial cells have been used for tumor targeting. Membrane type 1-matrix metalloproteinase (MT1-MMP) is a membrane-anchored enzyme that is involved in degradation of various extracellular membrane components like collagen types I, II, and III, fibronectin, laminin types 1 and 5, vitronectin, fibrin, and aggrecan. MT1-MMP can activate several pro-matrix metalloproteinases which expands its role in connective tissue remodeling, especially angiogenesis (Barbolina and Stack, 2008; Itoh and Seiki, 2006; Genis et al., 2006; Handsley and Edwards, 2005). Peptides with an RLPLPG-motif have affinity for MT1-MMP and have been used to target PEG-liposomes to the enzyme by coupling them to the liposome surface (Kondo et al., 2004). In vitro, RLPLPG-liposomes showed high binding to human umbilical vein endothelial cells as compared to liposomes lacking the peptide. As the peptide contains an arginine residue, this confers a small positive charge to the liposome surface which may contribute to charge-based interactions in a similar manner as described for the cationic lipid-based targeting. In vivo, RLPLPG-targeted liposomes showed a 4-fold enhanced tumor accumulation as compared to the unmodified liposomes. When the RLPLPG-liposomes were used to target the nucleoside analogue 5′-O-dipalmitoylphosphatidyl 2′-C-cyano-2′-deoxy-1-beta-D-arabino-pentofuranosylcytosine, tumor growth was strongly inhibited.

In a similar set-up, antibody Fab′ fragments against MT1-MMP were coupled to a PEG-liposome-formulation of doxorubicin. The system was evaluated in a HT1080 tumor model, in which also the tumor cells express high levels of MT1-MMP. It was shown that targeted liposomes showed significant suppression of tumor growth compared to the non-targeted formulations. The active targeting of the immunoliposomes is thought to reach both tumor cells and angiogenic endothelial cells and improved cellular uptake of liposomes (Hatakeyama et al., 2007).

Vascular cell adhesion molecule 1 (VCAM-1) is overexpressed upon activation of endothelial cells. But its expression is not confined to endothelial cells alone. VCAM-1 is known to be expressed on macrophages, myoblasts, dendritic cells and tumor cells. It interacts with integrin $\alpha4\beta1$ which facilitates leukocyte extravasation. VCAM-1 expression on tumor endothelium is well documented, together with expression of other adhesion molecules like intercellular adhesion molecule-1 (ICAM-1) and E-selectin.

Chiu et al. (2003) coupled an anti-VCAM-1 antibody to the surface of PEG-liposomes containing phosphatidylserine with the objective to target to tumor vasculature and induce local thrombogenesis. In vitro it was shown that these liposomes bound to interferon-stimulated endothelial cells dependent on the surface antibody density. Also, the authors demonstrated that the use of a PEG-coating that could be shed from the liposome surface allowed unmasking of the phosphatidylserine leading to blood coagulation. Using PEG-lipid anchors of different lengths the rate of PEG-shedding could be modified to prevent premature coagulation. This strategy has up to now not been followed up in vivo.

Antibodies against VCAM-1 coupled to the surface of sterically stabilized liposomes were also evaluated by Gosk et al. (2008) The VCAM-1-targeted liposomes showed binding to activated endothelial cells under static as well as flow-conditions. Tumor targeting was evaluated in mice bearing human Colo 677 xenografts at 30 min and 24 h after intravenous injection. Although overall tumor levels were similar, VCAM-1-antibody liposomes bound to angiogenic tumor vessels already at 30 min after injection, whereas PEG-liposomes without targeting ligand showed a more uniform distribution over the tumor.

CD105 is a homodimeric transmembrane glycoprotein mainly present on the vascular endothelial cell surface. Its expression is strongly upregulated during angiogenesis in a variety of tumors. Anti-CD105-single chain Fv monoclonal antibody fragments coupled to the liposome surface induced strong binding of the liposomes to CD105-positive endothelial cells in vitro which was followed by internalization. Binding to CD105-negative cells was negligible (Volkel et al., 2004). CD105-targeted liposomes loaded with doxorubicin selectively killed endothelial cells in vitro. Remarkably, in vivo studies in healthy animals showed that the immunoliposomes were cleared at a dramatically increased rate with an approximate half-life of 3 min. Although therapeutic efficacy has not been reported yet, these findings could considerably hamper the use of these CD105-targeted liposomes as there may be insufficient time for interaction with the target cells.

In the drug targeting field, integrins have arguably been most popular for specifically addressing the tumor endothelium cells as reviewed by Temming et al. (2005). For targeting the $\alpha v\beta3$-integrin and $\alpha v\beta5$-integrin, peptides with an RGD-motif act as high affinity ligands. The binding affinity of this motif is enhanced when it is offered in a cyclic form.

A number of studies demonstrated the binding to and subsequent internalization of PEG-liposomes bearing cyclic RGD-peptides on their surface by activated endothelial cells in vitro and in vivo (Mulder et al., 2005; Koning et al., 2004; Janssen et al., 2003). There was a clear correlation between number of peptides coupled to the liposome surface and number of liposomes bound to the endothelial cells. When RGD-liposomes were carrying doxorubicin or ^{10}B, they were cytotoxic to endothelial cells in vitro, whereas non-targeted controls were not, indicating that the cytotoxicity stems from the RGD-mediated targeting effect. In vivo, in a doxorubicin-resistant murine C26-colon carcinoma model, only RGD-liposomal doxorubicin inhibited tumor growth whereas control RAD-liposomes and non-targeted PEG-liposomes failed. As C26-tumor cells were highly resistant to doxorubicin the RGD-targeted liposomes effectuated antitumor effects likely through their cytotoxic action on the angiogenic endothelium (Schiffelers et al., 2003). In a similar set-up 5 fluoro-uracil has been tested (Dubey et al., 2004). In a model of spontaneous lung metastasis, both metastatic activity and angiogenesis was shown to be inhibited stronger by RGD-liposomal 5-fluoro-uracil as compared to free drug, non-targeted liposomes and RAD-controls. The same strategy was used to increase the therapeutic effects of combretastatin, an anti-angiogenic drug. In a B16F10 syngeneic tumor model conventional irradiation was combined with this targeted anti-angiogenic treatment. Only tumors from the group that was irradiated and treated with RGD-liposomal combretastatin did not grow, whereas all monotherapies did not inhibit tumor growth (Pattillo et al., 2005).

The NGR-motif has been shown to promote peptide binding to aminopeptidase N. Aminopeptidase N is a protease expressed preferentially on angiogenic endothelial cells. Pastorino et al. (2003) coupled peptides with the NGR-motif to the surface of sterically stabilized liposomes containing doxorubicin [67]. These liposomes were studied in an orthotopic neuroblastoma xenograft model in severe combined immunodeficient (SCID) mice. The NGR-peptide-targeted-liposomes showed a remarkable 10-fold increased tumor accumulation as compared to conventional sterically stabilized liposomes. The specificity of the liposome binding was demonstrated by co-injection with an excess of free NGR-peptide which abolished tumor accumulation completely. Antitumor efficacy was studied in an adrenal tumor model, where frequent low dosing of NGR-liposomal doxorubicin was shown to result in eradication of tumors.

The APRPG-motif has been used to target tumor vasculature in a series of studies. The motif has been identified by phage display and at present the corresponding endothelial cell target is not known. Liposomes modified with APRPG-peptide bind to human umbilical vein endothelial cells in vitro and to angiogenic endothelial cells in murine tumor models in vivo (Maeda et al., 2004a; Asai et al., 2002; Oku et al., 2002). Tumor accumulation of APRPG-targeted liposomes was comparable to non-targeted liposomes but the intratumoral distribution showed preferential binding to the endothelial cells for the peptide-modified liposomes (Maeda et al., 2004b). APRPG-modified liposomes containing doxorubicin effectively inhibited tumor growth in several murine tumor models (Shimizu et al., 2008; Yonezawa et al., 2007; Maeda et al., 2006; Shimizu et al., 2005). Similar results were obtained with incorporation of the drug 5′-O-dipalmitoylphosphatidyl 2′-C-cyano-2′-deoxy-1-beta-D-arabino-pentofuranosylcytosine (Asai et al., 2008).

4. Future perspectives

Over the past few years, the supporting cell types in the tumor have received increasing attention as target for anticancer liposomal nanomedicines. Still, the majority of supporting cell types have not been considered as yet. Virtually all studies have focused on macrophages and endothelial cells. Despite this limitation, studies with liposomal nanomedicines targeting these two cell types have already shown remarkable antitumor efficacy of a variety of drugs.

To take full advantage of the shift of focus from tumor cells to supporting cells the presence of other cell types in the tumor and the use of drugs other than those applied in conventional cytotoxic chemotherapy should be considered. In this respect, drugs that affect the communication between tumor cells and supporting cells may be particularly interesting.

For this, the major challenge is to develop a comprehensive picture of the cellular interactions in the tumor micro-environment and the key pathways involved in tumor proliferation and metas-

tasis. It is expected that the ability to address multiple cell types in the tumor and to attack different pathways that promote tumor cell survival and metastasis will improve the efficacy of anticancer treatment.

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References

Abraham, S.A., Waterhouse, D.N., Mayer, L.D., Cullis, P.R., Madden, T.D., Bally, M.B., 2005. The liposomal formulation of doxorubicin. Methods Enzymol. 391, 71–97.
Aggarwal, B.B., Shishodia, S., Sandur, S.K., Pandey, M.K., Sethi, G., 2006. Inflammation and cancer: how hot is the link? Biochem. Pharmacol. 72, 1605–1621.
Armulik, A., Abramsson, A., Betsholtz, C., 2005. Endothelial/pericyte interaction. Circ. Res. 97, 512–523.
Asai, T., Miyazawa, S., Maeda, N., Hatanaka, K., Katanasaka, Y., Shimizu, K., Shuto, S., Oku, N., 2008. Antineovascular therapy with angiogenic vessel-targeted polyethyleneglycol-shielded liposomal DPP-CNDAC. Cancer Sci. 99, 1029–1033.
Asai, T., Shimizu, K., Kondo, M., Kuromi, K., Watanabe, K., Ogino, K., Taki, T., Shuto, S., Matsuda, A., Oku, N., 2002. Anti-neovascular therapy by liposomal DPP-CNDAC targeted to angiogenic vessels. FEBS Lett. 520, 167–170.
Balkwill, F., Charles, K.A., Mantovani, A., 2005. Smoldering and polarized inflammation in the initiation and promotion of malignant disease. Cancer Cell 7, 211–217.
Banciu, M., Schiffelers, R.M., Fens, M.H., Metselaar, J.M., Storm, G., 2006. Anti-angiogenic effects of liposomal prednisolone phosphate on B16 melanoma in mice. J. Control. Release 113, 1–8.
Banciu, M., Metselaar, J.M., Schiffelers, R.M., Storm, G., 2008. Antitumor activity of liposomal prednisolone phosphate depends on the presence of functional tumor-associated macrophages in tumor tissue. Neoplasia 10, 108–117.
Barbolina, M.V., Stack, M.S., 2008. Membrane type 1-matrix metalloproteinase: substrate diversity in pericellular proteolysis. Semin. Cell Dev. Biol. 19, 24–33.
Campbell, R.B., Fukumura, D., Brown, E.B., Mazzola, L.M., Izumi, Y., Jain, R.K., Torchilin, V.P., Munn, L.L., 2002. Cationic charge determines the distribution of liposomes between the vascular and extravascular compartments of tumors. Cancer Res. 62, 6831–6836.
Carmeliet, P., 2005. Angiogenesis in life, disease and medicine. Nature 438, 932–936.
Chiu, G.N., Bally, M.B., Mayer, L.D., 2003. Targeting of antibody conjugated, phosphatidylserine-containing liposomes to vascular cell adhesion molecule 1 for controlled thrombogenesis. Biochim. Biophys. Acta 1613, 115–121.
Condeelis, J., Pollard, J.W., 2006. Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. Cell 124, 263–266.
De Visser, K.E., Eichten, A., Coussens, L.M., 2006. Paradoxical roles of the immune system during cancer development. Nat. Rev. Cancer 6, 24–37.
Dubey, P.K., Mishra, V., Jain, S., Mahor, S., Vyas, S.P., 2004. Liposomes modified with cyclic RGD peptide for tumor targeting. J. Drug Target 12, 257–264.
Eichhorn, M.E., Strieth, S., Krasnici, S., Sauer, B., Teifel, M., Michaelis, U., Naujoks, K., Dellian, M., 2004. Protamine enhances uptake of cationic liposomes in angiogenic microvessels. Angiogenesis 7, 133–141.
Filer, A., Pitzalis, C., Buckley, C.D., 2006. Targeting the stromal microenvironment in chronic inflammation. Curr. Opin. Pharmacol. 6, 393–400.
Flavell, S.J., Hou, T.Z., Lax, S., Filer, A.D., Salmon, M., Buckley, C.D., 2008. Fibroblasts as novel therapeutic targets in chronic inflammation. Br. J. Pharmacol. 153, S241–S246.
Gadea, B.B., Joyce, J.A., 2006. Tumour–host interactions: implications for developing anti-cancer therapies. Expert Rev. Mol. Med. 8, 1–32.
Gardner, S.N., Fernandes, M., 2004. Cytostatic anticancer drug development. J. Exp. Ther. Oncol. 4, 9–18.
Genis, L., Galvez, B.G., Gonzalo, P., Arroyo, A.G., 2006. MT1-MMP: universal or particular player in angiogenesis? Cancer Metastasis Rev. 25, 77–86.
Gerhardt, H., Semb, H., 2008. Pericytes: gatekeepers in tumour cell metastasis? J. Mol. Med. 86, 135–144.
Gosk, S., Moos, T., Gottstein, C., Bendas, G., 2008. VCAM-1 directed immunoliposomes selectively target tumor vasculature in vivo. Biochim. Biophys. Acta 1778, 854–863.
Handsley, M.M., Edwards, D.R., 2005. Metalloproteinases and their inhibitors in tumor angiogenesis. Int. J. Cancer 115, 849–860.
Hatakeyama, H., Akita, H., Ishida, E., Hashimoto, K., Kobayashi, H., Aoki, T., Yasuda, J., Obata, K., Kikuchi, H., Ishida, T., Kiwada, H., Harashima, H., 2007. Tumor targeting of doxorubicin by anti-MT1-MMP antibody-modified PEG liposomes. Int. J. Pharm. 342, 194–200.
Itoh, Y., Seiki, M., 2006. MT1-MMP: a potent modifier of pericellular microenvironment. J. Cell. Physiol. 206, 1–8.
Janssen, A.P., Schiffelers, R.M., Ten Hagen, T.L., Koning, G.A., Schraa, A.J., Kok, R.J., Storm, G., Molema, G., 2003. Peptide-targeted PEG-liposomes in anti-angiogenic therapy. Int. J. Pharm. 254, 55–58.
Joyce, J.A., Laakkonen, P., Bernasconi, M., Bergers, G., Ruoslahti, E., Hanahan, D., 2003. Stage-specific vascular markers revealed by phage display in a mouse model of pancreatic islet tumorigenesis. Cancer Cell 4, 393–403.
Kalra, A.V., Campbell, R.B., 2006. Development of 5-FU and doxorubicin-loaded cationic liposomes against human pancreatic cancer: Implications for tumor vascular targeting. Pharm. Res. 23, 2809–2817.
Kerbel, R.S., 2008. Tumor angiogenesis. N. Engl. J. Med. 358, 2039–2049.
Kim, D.W., Andres, M.L., Miller, G.M., Cao, J.D., Green, L.M., Seynhaeve, A.L., Ten Hagen, T.L., Gridley, D.S., 2002. Immunohistological analysis of immune cell infiltration of a human colon tumor xenograft after treatment with Stealth liposome-encapsulated tumor necrosis factor-alpha and radiation. Int. J. Oncol. 21, 973–979.
Kondo, M., Asai, T., Katanasaka, Y., Sadzuka, Y., Tsukada, H., Ogino, K., Taki, T., Baba, K., Oku, N., 2004. Anti-neovascular therapy by liposomal drug targeted to membrane type-1 matrix metalloproteinase. Int. J. Cancer 108, 301–306.
Koning, G.A., Fretz, M.M., Woroniecka, U., Storm, G., Krijger, G.C., 2004. Targeting liposomes to tumor endothelial cells for neutron capture therapy. Appl. Radiat. Isot. 61, 963–967.
Kunstfeld, R., Wickenhauser, G., Michaelis, U., Teifel, M., Umek, W., Naujoks, K., Wolff, K., Petzelbauer, P., 2003. Paclitaxel encapsulated in cationic liposomes diminishes tumor angiogenesis and melanoma growth in a "humanized" SCID mouse model. J. Invest. Dermatol. 120, 476–482.
Kuramoto, Y., Kawakami, S., Zhou, S., Fukuda, K., Yamashita, F., Hashida, M., 2008a. Efficient peritoneal dissemination treatment obtained by an immunostimulatory phosphorothioate-type CpG DNA/cationic liposome complex in mice. J. Control. Release 126, 274–280.
Kuramoto, Y., Kawakami, S., Zhou, S., Fukuda, K., Yamashita, F., Hashida, M., 2008b. Use of mannosylated cationic liposomes/immunostimulatory CpG DNA complex for effective inhibition of peritoneal dissemination in mice. J. Gene Med. 10, 392–399.
Kuramoto, Y., Nishikawa, M., Hyoudou, K., Yamashita, F., Hashida, M., 2006. Inhibition of peritoneal dissemination of tumor cells by single dosing of phosphodiester CpG oligonucleotide/cationic liposome complex. J. Control. Release 115, 226–233.
Ludwig, H., Strasser-Weippl, K., Schreder, M., Zojer, N., 2007. Advances in the treatment of hematological malignancies: current treatment approaches in multiple myeloma. Ann. Oncol. 18, ix64–70.
Maeda, N., Miyazawa, S., Shimizu, K., Asai, T., Yonezawa, S., Kitazawa, S., Namba, Y., Tsukada, H., Oku, N., 2006. Enhancement of anticancer activity in antineovascular therapy is based on the intratumoral distribution of the active targeting carrier for anticancer drugs. Biol. Pharm. Bull. 29, 1936–1940.
Maeda, N., Takeuchi, Y., Takada, M., Namba, Y., Oku, N., 2004a. Synthesis of angiogenesis-targeted peptide and hydrophobized polyethylene glycol conjugate. Bioorg. Med. Chem. Lett. 14, 1015–1017.
Maeda, N., Takeuchi, Y., Takada, M., Sadzuka, Y., Namba, Y., Oku, N., 2004b. Anti-neovascular therapy by use of tumor neovasculature-targeted long-circulating liposome. J. Control. Release 100, 41–52.
Martinez, F.O., Sica, A., Mantovani, A., Locati, M., 2008. Macrophage activation and polarization. Front. Biosci. 13, 453–461.
Mauel, J., 1976. Activation and cytotoxic activity of macrophages: a short review. Recent Results Cancer Res., 31–40.
Mclean, J.W., Fox, E.A., Baluk, P., Bolton, P.B., Haskell, A., Pearlman, R., Thurston, G., Umemoto, E.Y., Mcdonald, D.M., 1997. Organ-specific endothelial cell uptake of cationic liposome–DNA complexes in mice. Am. J. Physiol. 273, H387–H404.
Miselis, N.R., Wu, Z.J., Van Rooijen, N., Kane, A.B., 2008. Targeting tumor-associated macrophages in an orthotopic murine model of diffuse malignant mesothelioma. Mol. Cancer Ther. 7, 788–799.
Mulder, W.J., Strijkers, G.J., Habets, J.W., Bleeker, E.J., Van Der Schaft, D.W., Storm, G., Koning, G.A., Griffioen, A.W., Nicolay, K., 2005. MR molecular imaging and fluorescence microscopy for identification of activated tumor endothelium using a bimodal lipidic nanoparticle. FASEB J. 19, 2008–2010.
Nanda, A., St Croix, B., 2004. Tumor endothelial markers: new targets for cancer therapy. Curr. Opin. Oncol. 16, 44–49.
Noonan, D.M., De Lerma Barbaro, A., Vannini, N., Mortara, L., Albini, A., 2008. Inflammation, inflammatory cells and angiogenesis: decisions and indecisions. Cancer Metastasis Rev. 27, 31–40.
O'brien, M.E., 2008. Single-agent treatment with pegylated liposomal doxorubicin for metastatic breast cancer. Anticancer Drugs 19, 1–7.
Oku, N., Asai, T., Watanabe, K., Kuromi, K., Nagatsuka, M., Kurohane, K., Kikkawa, H., Ogino, K., Tanaka, M., Ishikawa, D., Tsukada, H., Momose, M., Nakayama, J., Taki, T., 2002. Anti-neovascular therapy using novel peptides homing to angiogenic vessels. Oncogene 21, 2662–2669.
Pastorino, F., Brignole, C., Marimpietri, D., Cilli, M., Gambini, C., Ribatti, D., Longhi, R., Allen, T.M., Corti, A., Ponzoni, M., 2003. Vascular damage and anti-angiogenic effects of tumor vessel-targeted liposomal chemotherapy. Cancer Res. 63, 7400–7409.
Pattillo, C.B., Sari-Sarraf, F., Nallamothu, R., Moore, B.M., Wood, G.C., Kiani, M.F., 2005. Targeting of the antivascular drug combretastatin to irradiated tumors results in tumor growth delay. Pharm. Res. 22, 1117–1120.

Perez-Lopez, M.E., Curiel, T., Gomez, J.G., Jorge, M., 2007. Role of pegylated liposomal doxorubicin (Caelyx) in the treatment of relapsing ovarian cancer. Anticancer Drugs 18, 611–617.

Santel, A., Aleku, M., Keil, O., Endruschat, J., Esche, V., Fisch, G., Dames, S., Loffler, K., Fechtner, M., Arnold, W., Giese, K., Klippel, A., Kaufmann, J., 2006. A novel siRNA-lipoplex technology for RNA interference in the mouse vascular endothelium. Gene Ther. 13, 1222–1234.

Schiffelers, R.M., Koning, G.A., Ten Hagen, T.L., Fens, M.H., Schraa, A.J., Janssen, A.P., Kok, R.J., Molema, G., Storm, G., 2003. Anti-tumor efficacy of tumor vasculature-targeted liposomal doxorubicin. J. Control. Release 91, 115–122.

Schmitt-Sody, M., Strieth, S., Krasnici, S., Sauer, B., Schulze, B., Teifel, M., Michaelis, U., Naujoks, K., Dellian, M., 2003. Neovascular targeting therapy: paclitaxel encapsulated in cationic liposomes improves antitumoral efficacy. Clin. Cancer Res. 9, 2335–2341.

Shimizu, K., Asai, T., Fuse, C., Sadzuka, Y., Sonobe, T., Ogino, K., Taki, T., Tanaka, T., Oku, N., 2005. Applicability of anti-neovascular therapy to drug-resistant tumor: suppression of drug-resistant P388 tumor growth with neovessel-targeted liposomal adriamycin. Int. J. Pharm. 296, 133–141.

Shimizu, K., Sawazaki, Y., Tanaka, T., Asai, T., Oku, N., 2008. Chronopharmacologic cancer treatment with an angiogenic vessel-targeted liposomal drug. Biol. Pharm. Bull. 31, 95–98.

Sica, A., Saccani, A., Mantovani, A., 2002. Tumor-associated macrophages: a molecular perspective. Int. Immunopharmacol. 2, 1045–1054.

Strieth, S., Eichhorn, M.E., Sauer, B., Schulze, B., Teifel, M., Michaelis, U., Dellian, M., 2004. Neovascular targeting chemotherapy: encapsulation of paclitaxel in cationic liposomes impairs functional tumor microvasculature. Int. J. Cancer 110, 117–124.

Strieth, S., Nussbaum, C.F., Eichhorn, M.E., Fuhrmann, M., Teifel, M., Michaelis, U., Berghaus, A., Dellian, M., 2008. Tumor-selective vessel occlusions by platelets after vascular targeting chemotherapy using paclitaxel encapsulated in cationic liposomes. Int. J. Cancer 122, 452–460.

Takeuchi, Y., Kurohane, K., Ichikawa, K., Yonezawa, S., Nango, M., Oku, N., 2003. Induction of intensive tumor suppression by antiangiogenic photodynamic therapy using polycation-modified liposomal photosensitizer. Cancer 97, 2027–2034.

Takeuchi, Y., Kurohane, K., Nango, M., Oku, N., 2002. Intensive tumor suppression by anti-angiogenic photodynamic therapy with polycation-modified liposomal photosensitizer. Cell. Mol. Biol. Lett. 7, 301.

Temming, K., Schiffelers, R.M., Molema, G., Kok, R.J., 2005. RGD-based strategies for selective delivery of therapeutics and imaging agents to the tumour vasculature. Drug Resist. Updat. 8, 381–402.

Thurston, G., Mclean, J.W., Rizen, M., Baluk, P., Haskell, A., Murphy, T.J., Hanahan, D., Mcdonald, D.M., 1998. Cationic liposomes target angiogenic endothelial cells in tumors and chronic inflammation in mice. J. Clin. Invest. 101, 1401–1413.

Turk, M.J., Waters, D.J., Low, P.S., 2004. Folate-conjugated liposomes preferentially target macrophages associated with ovarian carcinoma. Cancer Lett. 213, 165–172.

Vineis, P., Berwick, M., 2006. The population dynamics of cancer: a Darwinian perspective. Int. J. Epidemiol. 35, 1151–1159.

Virchow, R., 1863. Die Cellularpathologie in ihrer Begrundung auf physiologische und pathologische Gewebelehre. August Hirschwald Verlag, Berlin.

Volkel, T., Holig, P., Merdan, T., Muller, R., Kontermann, R.E., 2004. Targeting of immunoliposomes to endothelial cells using a single-chain Fv fragment directed against human endoglin (CD105). Biochim. Biophys. Acta 1663, 158–166.

Weiss, D.W., 1976. Neoplastic disease and tumor immunology from the perspective of host–parasite relationships. Natl. Cancer Inst. Monogr. 44, 115–122.

Yonezawa, S., Asai, T., Oku, N., 2007. Effective tumor regression by anti-neovascular therapy in hypovascular orthotopic pancreatic tumor model. J. Control. Release 118, 303–309.

Zeisberger, S.M., Odermatt, B., Marty, C., Zehnder-Fjallman, A.H., Ballmer-Hofer, K., Schwendener, R.A., 2006. Clodronate-liposome-mediated depletion of tumour-associated macrophages: a new and highly effective antiangiogenic therapy approach. Br. J. Cancer 95, 272–281.

Zurita, A.J., Arap, W., Pasqualini, R., 2003. Mapping tumor vascular diversity by screening phage display libraries. J. Control. Release 91, 183–186.