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Review

Improving anti-angiogenic therapy via selective delivery of cationic liposomes to tumour vasculature

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Abstract

In the past three decades, two very important findings regarding tumour vasculature have been made. Firstly, it has been known a solid tumour has to establish an adequate blood supply to grow beyond a critical mass. Secondly, it has been proven that the tumour vasculature is relatively more aberrant, dynamic and permeable than healthy host tissue. This review discusses the potential of delivering therapeutic nucleic acids to tumour vasculature using cationic liposomes, vehicles recently demonstrated to be selectively delivered to tumour vasculature.

Keywords: Cancer; Tumour; Vasculature; Liposome; Lipoplex

1. General characteristics of solid tumours hindering effective therapy

When initially diagnosed, most tumours are well advanced. Fidler (1991) stated that a 1 g, 1 cm^3 neoplasia (the smallest size clinically detectable) contains approximately a billion cells. An eradication of even 99.9% of these cells would still leave a million viable cells for expansion. For complete cure, all cells need to be destroyed. Additionally, in nearly 50% of the patients, surgical excision of the primary tumour is not curative due to metastasis, the process whereby mutated cells spread from the primary site via the bloodstream to establish neoplasms in secondary sites such as the liver or lung (Fidler and Ellis, 1994). Often, metastases are undetectable due to their small size (<5 mm in diameter) and may persist in a dormant state for years following removal of the primary tumour (Meltzer, 1990). The greatest obstacle to success of therapy is the heterogeneous composition of tumours. Individual cells within a tumour vary in terms of genetic, biochemical, immunological and biological characteristics (Dass et al., 1998). These differences may involve cell-surface receptors, enzymes, karyotypes, cell morphologies, cell cycling times, sensitivities to various therapeutic agents, tissue architecture and metastatic potential. Such heterogeneity reduces the ability of both surgery and therapeutic agents to kill all neoplastic cells.

Additionally, in certain solid tumours, such as those of the colon, kidney and adrenal glands, overexpression of the *p*-glycoprotein gene causes tumour cells to acquire drug-resistance to therapeutic agents such as cisplatin (Tsuruo and Tomida, 1995). Drug-resistance is due to the *p*-glycoprotein excreting cytotoxic drugs from the cell via its ATPase action (Hamada and Tsuruo, 1988). Dense packing of

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tumour cells constitutes a further form of hindrance to movement of anticancer agents from the bloodstream into tumour interstitium (Thorpe and Burrows, 1995). On the other hand, lesser cell density equates to greater space occupation by interstitium, an extracellular matrix component of the tumour that may pose a great obstacle to the transport of drug molecules from the tumour microvasculature to the target cancer cells (Krol et al., 1999; Stohrer et al., 2000). In addition, tumour vasculature varies in density and composition in different zones within the same neoplasm (Monsky et al., 1999; Ramanujan et al., 2000). Furthermore, solid tumours tend to lack an efficient lymphatic drainage system resulting in an elevated interstitial pressure within the tumour stroma (Leu et al., 2000). Increased interstitial pressure as well as the rapid and aberrant nature of tumour cell growth are believed to be largely responsible for the compression and occlusion of blood vessels characteristic of solid tumours.

2. Neovascularisation

At the early avascular stage, supply of nutrients and oxygen to, and removal of wastes from, a solid tumour is performed entirely by diffusion through the surrounding host tissue. This limits the neoplasm to a certain volume, since for further growth to occur, extensive vascularisation is needed. Most tumours in humans remain restricted in size for months to years until a certain subset of cells acquires an angiogenic phenotype (Gastl et al., 1997). Patients usually have to choose between living with a benign pea-sized tumour that may or may not become life-threatening, or sometimes fairly invasive surgery to eradicate the growth. The initial step in neovascularisation is formation of a primary vascular network, consisting of tubes of nascent endothelial cells (Dass and Su, 2000). Subsequently, vascular smooth muscle cells (VSMCs) are recruited to the endothelium to form a multilayered vessel wall. During maturation and further development of the vessel, the VSMCs serve as biosynthetic, proliferative and contractile components of the vascular wall.

The tumour induces its own blood supply within a relatively short period of time. This is exemplified by the fact that whilst turnover rates for vascular endothelial cells (VECs) are normally in the order of months or even years (Denekamp, 1984), VECs supplying the tumour degrade the overlying basement membrane and proximal extracellular matrix, migrate directionally, undergo mitosis, and organise themselves into functional capillaries within a matter of days (Polverini, 1995). According to Folkman (1992), solid tumour VECs divide at a rate 50- to 200-fold greater than normal VECs. In the clinical context, patient survival decreases once a highly dense tumour vasculature bed is established (Hillen et al., 1997).

Since a blood supply is crucial for growth, tumour cells have the ability to recruit new blood vessels in various ways. Firstly, they synthesise angiogenic factors that activate VECs to divide and grow towards the tumour (Polverini, 1995). They also stimulate production of cytokines which attract various immune cells including macrophages (Polverini and Leibovich, 1984) which in turn secrete angiogenic factors (Plate et al., 1994). Thirdly, tumour cells block production of, or overcome inhibition due to, anti-angiogenesis factors (Rastinejad et al., 1989). Fourthly, they synthesise enzymes that release angiogenic (Briozzo et al., 1991) and other (Taipale and Keski-Oja, 1997) growth factors sequestered in the extracellular matrix. Finally, tumour cells stimulate adjacent normal tissues to synthesise enzymes such as collagenase (van den Hooff, 1991) that can be activated to promote angiogenesis.

In addition to all these, angiogenic VECs have distinctive receptors (Qu et al., 1995), adhesion molecules (Brooks et al., 1994), oligosaccharides (Augustin et al., 1995), and other cell-surface proteins (Thorpe and Burrows, 1995). Stimulation, growth and maintenance of angiogenic VECs, whether supplying a tumour or in response to normal physiological (example ovulation) or physical injury (wound), is in response to a myriad of growth factors such as basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF). The tissue surrounding the VECs also determine the extent and rate at which these dynamic vessels grow and extend.

3. Tumour vasculature abnormalities

In the normal adult, only 0.01% of VECs are undergoing mitosis, while in the tumour vasculature, up to 25% of VECs are dividing (Brien et al., 1989). Tumour blood vessels are typically 3–10 times more

permeable than normal vasculature (Weindel et al., 1994; Yuan et al., 1995). A hypoxic state in the tumour increases vascular permeability (Olesen, 1986) and, provides a potent stimulus for neovascularisation (Adair et al., 1990). However, as highlighted by Jain (1987), not all tumour blood vessels are leaky and permeability may vary both spatially and temporally within the same tumour.

Like other types of endothelium, cells of tumour venules contain in their cytoplasm numerous vesicles, ranging from 50 to 70 nm in diameter, in addition to larger diameter vacuoles (Kohn et al., 1992). These vesicles, called vesiculo-vacuolar organelles (VVOs), join up with each other to form transendothelial cell channels allowing extravasation of macromolecules from the vessel lumen into tumour interstitium. Interestingly, even though the frequency of VVOs per length of vessel is not significantly different between tumour and normal venules, extravasation is greater in tumour vessels. This suggests a discrepant regulatory mechanism for VVO function in normal compared to neoplastic endothelium.

The increased tumour vessel permeability complements both discontinuous basement membrane in capillaries and gaps induced between VECs of venules by VEGF (Senger et al., 1983). According to Lance Liotta, tumour cells induce adjoining endothelial cells to retract, creating spaces through which malignant cells escape the vascular system (Stetler-Stevenson et al., 1993). In addition, tumour vasculature is usually composed of abnormal vascular elements including sinusoidal vessels, large capillaries and blood channels with discontinuous endothelium (Baillie et al., 1995; Murray and Carmichael, 1995). In comparison to blood vessels supplying normal tissue, vessels supplying tumour tissue tend to be arranged in irregular arrays and have dilated lumens (Kan et al., 1993). In addition, arteriovenous shunts and trifurcations not normally observed in normal tissue are found in tumour vasculature (Jain, 1996). Tumour microvessels are tortuous and do not respond to vasoactive stimuli (Willmott et al., 1991). These aberrant features of tumour vasculature lead to turbulent blood flow with frequent stasis and sudden changes in flow direction (Rak et al., 1995).

As a tumour grows, its effective capillary density decreases, thereby limiting further growth (Tomisaki et al., 1996). Mitosis of neoplastic cells decreases with increasing distance from the nearest capillary in solid tumours (Folkman, 1992). This results in a typical central zone of necrosis surrounded by a layer of viable, proliferating tumour cells with accompanying proliferating VECs (Plate and Mennel, 1995). Due to the relative insufficiency of blood flow to all areas of a tumour, establishment of pockets of hypoxia is common in solid tumours. Hypoxic conditions equate to a highly reducing environment and hence the pH of tumours tends to be low relative to surrounding normal tissues (Dewhirst et al., 1995). This acidity is compounded by an inadequate removal of hydrogen ions by the usually restricted tumour blood supply. Hypoxia, in turn, may act as an inducer for increased vasculature permeability and neovascularisation.

4. Cancer genotherapy—promise and obstacles

Results appearing in the mid-1990s from clinical trials with the various forms of genotherapy highlighted major deficiencies in this form of therapy. A look at the clinical results, primarily in patients with very advanced cancers that were refractory to conventional therapies, revealed that genotherapy had the potential to be effective in inducing tumour regression with a concomitant acceptable low toxicity. Half of all gene therapy clinical trials to date are for cancer. One aspect of genotherapy, that has traditionally plaqued other forms of therapies such as radio- and chemotherapy, the targeting of genetic medicine to neoplastic tissue, has to be addressed adequately. Not only does targeting genetic medicine to tumours prevent side-effects to normal cells, it maximises gene dosage to their cancerous counterparts. Discussed below are the prospects for targeting vectors to tumours via the vascular route.

In general, a major concern in targeting therapeutic agents to tumours is the inherent heterogeneity of tumours. The growth of human cancer cells and vascularisation of xenograft tumours in immunocompromised mice varies from one host to another. Superimposed on this is the fact that the vascular supply to a tumour is not consistent throughout. There are sites that are well-vascularised, while other regions tend to be poorly supplied with a vascular bed. Delivery of an agent into the feeding artery would not equate to exposure of all areas of the tumour equally in a lot of cases. Blood is transported away

from the centre of the tumour towards the periphery, which leaves a poorly-perfused area in the tumour core (Jain, 1996). This tumour core is where drug delivery in particularly is ineffective. Combined with this is the phenomenon of temporal inhomogeneities in blood flow through the various vessels within a tumour (Mollica et al., 2003).

Another form of hindrance is the biological and physiological barriers to intravascular delivery. Firstly, an anticancer agent that is administered via the blood supply encounters the vastness of the vascular space and the possibility of non-specific interactions with the numerous components in blood, including opsonising proteins and lipoproteins, depending on how it is transported. While gross systemic distribution may be counteracted by delivery through an artery upstream of a tumour, additional mechanisms of tumour cell-specific targeting are needed to enhance the targeting effect. Secondly, the agent has to extravasate into the tumour interstitium. Here, exploitation of the tumour vascular bed abnormalities (e.g. greater permeability) may aid selective delivery into the tumour tissue.

Perhaps the greatest obstruction is transport through the tumour interstitial space. Due to a reduced lymphatic fluid drainage in most tumours, fluid pressure tends to build up, and thus further extravasation of fluid and carried agents is hindered. Necrotic regions within a tumour would act to trap and rapidly degrade nucleic acid constructs due to cellular nucleases and other enzymes being released from dying cells. Lastly, entry of genetic constructs into tumour cells is necessary for the therapeutic effect. Furthermore, in the case of therapeutic genes, the construct has to gain entry into the nucleus for the transcriptional process to set in motion expression of the therapeutic protein. Failure to satisfy any of these steps equates into a non-therapeutic result.

There are properties that the nucleic acid agent has to have as it travels towards the tumour via the bloodstream. Even if delivered into the tumour directly, these features are desirable. Firstly, the agent must be resistant against metabolism and degradation. Secondly, it should avoid non-specific binding to proteins and other biomolecules. For instance, binding of proteins to plasmids affects their transfection rate. Finally, the vector must not elicit an immunologic response. This is quite relevant when using viral vectors as traditionally, administration of adenoviral vectors has lead to inflammation. Discussed below is the use of lipoplexes, a safer alternative to viral vectors, for delivery of genetic constructs to solid tumours. While the above features of tumours may seem discouraging to cancer therapists, the fact that a tumour's survival critically depends on its blood supply provides a common mechanism for the destruction of solid tumours in general.

5. Lipoplexes and tumour genotherapy

Since the initial formulation of vesicles in the laboratory of Alec Bangham for the purpose of studying membrane diffusion (Bangham et al., 1965), liposomes have come a long way to become one of the more versatile delivery vehicles for a variety of therapeutic agents for cancer and other disorders. Of all the various liposomes available, cationic liposomes (CLs) are the most commonly used for transfer of DNA and other nucleic acids in cultured cells and in vivo. These vesicles can be formulated using any of the various conventional protocols outlined by Szoka and Papahadjopoulos (1980). While most CLs are available commercially, an increasing number of laboratories are both making and using CLs using standard procedures such as ethanol injection.

Lipoplexes are formed by the interaction of anionic nucleic acids binding to the surface of cationic liposomes eventually forming multilamellar lipid–nucleic acid complexes. In the case of DNA, the nucleic acid molecules persist glued to lipidic molecules with a lipid bilayer surrounding the compacted nucleolipidic particles in one of several different moieties: (a) a cylindrical form, where the DNA is coated by a curved lipid bilayer; (b) a flat lamellar form, where DNA is sandwiched between lipid layers; and (c) a form where DNA is condensed as parallel helices between lipid bilayers (Dass and Burton, 1999). These discrepant observations may be attributed to the lipidic composition of the vesicles, the manner in which the complexes are formed, the lipid:nucleic acid ratio, the size of the nucleic acid construct, and the technique used to treat and visualise these complexes. In addition to electrostatic attraction, hydrophobic interactions are believed to aid complex formation between lipids and nucleic acids (Wong et al., 1996).

A common molecule used in cationic liposome synthesis is the neutral lipid dioleoylphosphatidyl ethanolamine (DOPE). Phil Felgner correctly hypothesised that the role of DOPE is to facilitate membrane fusion or aid in the destabilisation of the plasmalemma or endosome (Felgner et al., 1994). In addition, helper lipids such as DOPE are required to stabilise the cationic liposome suspension as cationic lipids repel each other (Dass et al., 1999, 2000c). Dan Lasic proposed that liposomes formulated without neutral lipid(s) have inferior rates of transfection (Lasic and Pearlman, 1996), whilst varying rates of transfection may result from varying ratios of cationic:neutral lipid used to formulate the liposomes (Farhood et al., 1995). Success of cationic liposome-mediated DNA transfer is, however, dependent on numerous factors (Dass and Burton, 1999). A major issue is the ability of the CLs to activate the complement pathways in vivo, and thereby become prone to opsonisation and phagocytosis (Chonn et al., 1991). Reduced surface charge and absence of phagocytosis internalisation pathways in differentiated cells may also decrease CL uptake (Matsui et al., 1997). Robert Debs demonstrated that cell-surface proteoglycans are also believed to aid lipoplex uptake both in cell culture and more importantly, in vivo (Mounkes et al., 1998). This plethora of factors may explain the inherent variability of lipofection especially in vivo (Wheeler et al., 1996; Dass et al., 1997). Nevertheless, the fact that lipoplexes may be administered in vivo via the vascular system highlights the usefulness of these gene transfer vehicles.

One basic problem with lipoplexes is toxicity (Dass, 2002a). This is normally closely associated with the charge ratio of the cationic lipid species in the formulation and the charge of the anionic nucleic acid-based drug. Higher charge ratios are generally more toxic to a variety of cell types, including cancer cell lines (Dass et al., 2002a,b). Thus, administration of lipoplexes has to be as close to the target site as possible to minimise side-effects. Size is another issue, as larger complexes tend to get entrapped in the first microvascular bed encountered. For instance, tail vein injections in mice of large lipoplexes will lead to entrapment in the lung microvasculature. Size range may be controlled by forming the lipoplexes using a slow procedure rather than a rapid bulk phase, where exposed plasmids may interact with adjacent liposomes (Dass et al., 1999; Oberle et al., 2000). Naturally, the charge ratio plays an important role in the homogeneity of complexes formed (Almofti et al., 2003). Regardless of all these issues, the fact that these delivery agents have been used in numerous preclinical studies (Dass, 2002b), administered by various routes, and tested in phases I and II clinical trials (Dass, 2002c), attests to their versatility and applicability.

6. Considerations for lipoplex gene transfer into tumour interstitium

In many types of tumours, the vascular bed is well-developed, in some cases, better than in normal tissues (Jain, 1996). For such tumours, liposomal delivery of genetic material may hold great promise, since large liposomes are retained in the first capillary bed they encounter. For instance, tail vein injections result in retention of the majority of liposomal material in the lung vasculature. Small unilamellar vesicles (SUVs; diameter ≤ 100 nm) escape the capillary bed and are predominantly taken up by the organs of the reticuloendothelial system (RES), the liver and spleen. Upstream intra-arterial delivery of cationic large unilamellar vesicles (LUVs, diameter > 1 μm) rather than intermediate lamellar vesicles (IUVs) may be more feasible. Thus, targeting may be achieved by delivering therapeutic genetic constructs as close as possible to the site via a catheter (Dass et al., 1997, 2000c). This would ensure a maximum dose of therapeutic DNA since enzymatic degradation, interaction of the nucleic acid with the biological surroundings such as the vessel wall, and dilution in the blood would be minimised.

As stated above, the tumour vasculature is more permeable compared to normal tissue vasculature. In addition, neovascularisation of tumours usually lead to newly formed vessels that are leaky due to fragile basement membranes (Liotta et al., 1976). Tumour cells in culture and in vivo secrete VEGF, which apart from increasing the permeability of tumour vessels, aids in the accumulation of excess fluid commonly associated with tumours (Brock et al., 1991). Increased permeability would facilitate movement of lipoplexes from vessels into tumour interstitium. However, in reality, the high interstitial pressure (Jain, 1987) within tumours act as one of the major barriers to convective extravasation of molecules into a tumour interstitium.

If lipoplexes are targeted to the tumour vascular bed using such devices as microspheres (Dass and Burton, 2002) or microplexes (Dass and Burton, 2003), then possible side-effects of therapeutic genes (such as *p53*) or antisense strands (such as those against c-*myc*) should be reduced. Highly selective delivery is important particularly when dealing with the vascular system, since generation of new tissue, such as that involved in wound healing and the menstrual cycle, is dependent on vessel regeneration. Once a tumour's blood supply is curbed or completely inhibited, the tumour itself should be eradicated.

It has also been noted that uptake of antisense strands is much faster in leukaemic human cell lines than in normal cells from the same patient (Calabretta et al., 1991; Zhao et al., 1996). Normal brain cells of rats do not permit entry of plasmids as much as brain tumour (glioma) cells (Nishi et al., 1996). A transplanted tumour line in the kidneys of rats shows greater expression of a foreign gene delivered free than the normal kidney parenchyma (Dass et al., 2000c). Such a mechanism may exist because of the greater division rate of mutated cells. Dividing cells undergo breakages in their cellular and nuclear membranes, thereby allowing genes easier access to the nucleus. Alternatively, it may be explained by a more demanding blood supply to the tumour. Regardless of the mechanism, these findings have great implications for vascular-based gene delivery to solid tumours.

One limiting factor to effective tumour lipofection is that cancerous cells often occupy less than 50% of a tumour, with 1–10% of the volume being made up by the vasculature (Jain, 1996). The rest of the tumour volume consists predominantly of the collagen-rich interstitium. To reach a tumour cell, the active agent must traverse the endothelial barrier and through the often thicker interstitial matrix. Additionally, as mentioned before, pressure in the tumour stroma is higher than within blood vessels (Boucher et al., 1996). Hence, movement of large molecules such as DNA through vessels occurs mainly by diffusion (Jain, 1996). However, in regions of the tumour where interstitial pressure is low, movement of large molecules occurs via convective transport caused by 'solvent drag'.

It must be borne in mind that tumours seen clinically contain well-supplied rapidly growing regions interspersed with poorly perfused, often necrotic areas (Murray and Carmichael, 1995). In solid tumour tissue, blood vessels become tortuous, with variable intercapillary distances and compression and occlusion of lumens. Insufficient perfusion results eventually in necrosis in certain areas and also hypoxic areas containing otherwise viable tumour cells. This heterogeneity poses a problem to any sort of drug delivery whether it is administered at a distance or by direct injection into the tumour. Ideally, the drug has to reach the periphery of the tumour, a region that is characterised by vigorous cellular turnover.

Thus, there are various limiting factors in the delivery of genotherapeutic agents to solid tumours. While the efficacy of the vector is dependent on matters such as the expression level in cells or resistance against endonucleases, it is largely reliant on the route of delivery to the tumour. At present, the vascular route promises to be the best mode of administration since it provides the therapeutic construct access to the rapidly growing regions of the tumour. Whilst the use of three-dimensional tumour models may be useful for basic studies, it may fail in truly portraying the natural architecture of a tumour, including the microvascular bed. The issues pertinent to gene therapy are also relevant to other forms of drug therapy such as chemo- and radiotherapy. The importance of the vascular system in targeting genetic constructs to tumours is in general relevant to all these modes of cancer management.

7. Selective delivery of cationic liposomes to tumour vasculature

Whilst it is known that CLs may deliver nucleic acids to tumour interstitium, a recent finding, highly pertinent to anti-angiogenic gene delivery, is the finding that CLs target quite selectively, the vasculature of tumours. Such effects are not noted with anionic or electroneutral liposomes (Thurston et al., 1998; Campbell et al., 2002). Campbell et al. (2002) found that CLs, stabilised with the addition of a 5 mol% of PEG, accumulated more when CLs were used as opposed to electroneutral liposomes. PEG was used to increase circulation lifetime of the positively charged liposomes, as noted in the laboratory of Vladimir Torchilin (Levchenko et al., 2002). Inclusion of PEGylated lipids in the vesicles also tends to disallow

aggregate formation (Meyer et al., 1998; Shi et al., 2002). Unmodified lipoplexes have a relatively short circulation half-life of <5 min (Campbell et al., 2002). Furthermore, when the mol% of cationic lipid was increased from 10 to 50 mol%, the accumulation in tumour VECs increased twofold.

Campbell et al. (2002) believe that PEG delayed liposome clearance from blood, but not at the expense of interaction and uptake by tumour VECs. In contrast, no change in interstitial accumulation could be detected, which may have been just due to spatial heterogeneity within the tumour tissue. This selective delivery to tumour VECs was noted in two human tumour types (LS174T and MCAIV) and at two study locations (cranial window and dorsal skin fold chamber). Distribution of vesicles in tumour vessels was heterogeneous, and this may have some bearing on whether this technology is sufficient to eradicate a number of tumour VECs equating to a tumour-regressive response. Interestingly, a 50% molar charge on the liposomes significantly increased accumulation in the lungs of mice 24 h post-injection. While this is expected with an intravenous injection, a more detailed effect of time on biodistribution would have conveyed valuable information on how such accumulation varied with time.

Campbell et al. (2002) state that the positive charge on liposomes is required to enhance interaction with the glycoprotein layer of the endothelium. Studies with cationic ferritin molecules (Vincent et al., 1988) have shown that irregular and patchy anionic domains exist along the capillary endothelium in a brain tumour model. A common hypothesis, based on several lines of evidence, is that mammalian cells interact with and internalise cationic macromolecules by endocytosis (Vincent et al., 1988; Thurston et al., 1998), and that this interaction is at least partially mediated by proteoglycans (Mounkes et al., 1998). Furthermore, in the case of mosaic tumour vessels, that is vessels comprised of both VECs and tumour cells themselves, the tumour cells may come in direct contact with cationised macromolecules, including lipoplexes, and uptake may occur in both VECs and neoplastic cells (Chang et al., 2000).

Whether this phenomenon is relevant to other tumour models, and especially clinical tumours, remains to be tested. Campbell et al. (2002) speculate that the sluggish and stunted blood flow in tumour vessels, in contrast to normal continuous flow in most healthy tissues, may enhance the interaction between the anionic sites on the dynamic vasculature of the tumour and the cationic liposomes. One criticism of the work of Campbell et al. (2002) is that only encapsulated doxorubicin was analysed, and moreover, only distribution of the doxorubicin was quantitated, and any efficacious effect of the drug in the tumour models used was not evaluated.

Earlier on, Thurston et al. (1998) demonstrated that in the RIP-Tag2 (expression of the SV40 virus large T antigen (Tag) oncogene is driven by the 5′ flanking region of rat insulin gene including the promoter (RIP)) and the K14-HPV16 (the oncogene from the human papilloma virus (HPV) is driven by a region of the keratin 14 (K14) promoter) tumour models, the quantity of liposomes accumulating in tumour vessels were up to 33-fold than in corresponding vessels in non-tumour-bearing mice. Of the CLs inside tumour VECs, 89% were in multivesicular bodies, 10% in small vesicles, and ∼1% in complex structures composed of multiple interconnecting vesicles, probably VVOs.

Notable was the observation that within 20 min, liposomes appeared on the luminal surface of angiogenic VECs or in vesicular structures within these cells (Thurston et al., 1998). Furthermore, 51% of the CLs accumulating on the tumour VEC surface were associated with fenestrae, although fenestrae constituted a mere 4% of the luminal VEC surface. This may be attributed to the cationic charge on the vesicle surface, since cationic ferritin, but not native ferritin, exhibits ready binding to fenestrae, suggesting the presence of anionic moieties on fenestral diaphragms (Bankston and Milici, 1983; Milici et al., 1985). Thurston et al. (2002) put forward several lines of evidence indicating that extravasation was due to trans-VEC transport rather than via leakage through the basement membranes of tumour endothelium.

The vascular network is naturally highly accessible to intravascularly-administered therapeutic agents. Even agents delivered non-intravascularly, such as through the subcutaneous or intraperitoneal routes, once the agent gains access to the circulatory system, it has potential to target actively proliferating vessels, such as those in a tumour. The vasculature of the tumour also occupies a relatively small area in comparison to the tumour interstitium, thus the doses of anti-angiogenic agents to be delivered in vivo should

theoretically be much less than what needs to be administered for general anticancer chemotherapeutics. Conventional cytotoxic agents that recently have been found to be anti-angiogenic, such as vinblastine and paclitaxel, would need to be injected in much smaller doses, albeit maybe more frequently in keeping with the recently proposed metronomic dosing schedules (Bocci et al., 2002; Kerbel et al., 2002).

It is known that lipoplexes injected intravenously are taken up by VECs in an organ- and vessel-specific pattern in mice (McLean et al., 1997). VECs in the lung and anterior pituitary quite readily accumulate lipoplexes via endocytosis, whilst there is at most a negligible uptake in VECs of the brain and posterior pituitary. McLean et al. (1997) revealed that CLs do accumulate at sites of chronic inflammation (chronic airway inflammation in *Mycoplasma pulmonis*-infected mice). Such accumulation ranged from an average of 15-fold that in corresponding normal vessels, and exceeded up to 100-fold in some hotspots. Interestingly, these researchers showed that either of two commonly used cationic lipids (dioleoyl trimethyl ammonium propane (DOTAP) and dimethyl dioctadecyl ammonium bromide (DDAB)) could be used to have the same tumour VEC-selective effect. Likewise, McLean et al. (1997) found significant accumulation in vessels surrounding large ovarian follicles and in newly established corpora lutea in mice. No accumulation was observed inside follicles, but was confined to the plexus of vessels around large antral follicles. Vessels in rapidly growing corpora lutea had substantial accumulation, while those found in regressing corpora lutea had negligible distribution, if any.

Not only are cationic liposomes useful for delivering genetic constructs to the tumour vasculature, but also causing an antivascular effect with cytotoxic agents. Recently, Kunstfeld et al. (2003) demonstrated that paclitaxel encapsulated in cationic liposomes diminishes tumour angiogenesis and inhibits orthotopic melanoma growth in SCID mice. In contrast, paclitaxel administered in its normal Cremophor EL medium, while showing an inhibitory effect in cell culture, was unable to significantly decrease angiogenesis and tumour growth in vivo. Kunstfeld et al. (2003) speculate further that factors governing constitutive mitosis, such as that in normal physiological processes, are different from that inducing division in angiogenic vessels. However, the question remains whether such delivery also affects vessels involved in reproduction and wound healing.

8. The case for using lipoplexes against tumours at certain sites in the body

Lipoplexes injected intraperitoenally have been noted to transfect intraperitoneal tumours and not into the other organs located in the peritoneal cavity (Aoki et al., 1997; Kikuchi et al., 1999; Reddy et al., 2002). Such selectivity may be due to the fact that organs residing within the peritoneal cavity are covered by both the peritoneum and the underlying connective tissue (Kiyasu et al., 1981; Niedbala et al., 1985). These tissues act as efficient barriers to uptake of lipoplexes into these organs. However, a peritoneal tumour lacks such barriers, exposing the tumour cells to the lipoplexes administered intraperitoneally. Also, since tumour cells have a greater mitotic index and a more demanding blood supply, they are expected to accumulate the lipoplexes at a greater rate.

Lipoplexes injected via the tail vein in mice have been noted to transfect the lungs shortly after administration, and in the liver after 24–48 h (Parker et al., 1997; Niven et al., 1998). One hypothesis for this phenomenon is that in the presence of serum proteins, lipoplexes could form aggregates, which probably would be retained in the first microvascular bed encountered (Audouy et al., 2002). Whilst this poses as a drawback to most cases of nucleic acid delivery, when the tumours are located within the lungs, such as in the case of pulmonary metastases, this may indeed be a great advantage. Jack Roth's laboratory has demonstrated that intravenously-delivered CL–*p53* plasmids complexes inhibited the growth of pulmonary metastases in immunocompromised mice (Ramesh et al., 2001). Furthermore, repeated administration of the plasmids via this route increased gene expression and inhibition of metastases. Pulmonary VECs are noted to take up lipoplexes (Liu et al., 1997; McLean et al., 1997; Uyechi et al., 2001).

Lipoplexes injected via the tail vein in mice have been noted to transfect the liver fairly efficiently, and such selectivity may be exploited against hepatic tumours. However, it is important to tease out whether expression is in hepatocytes or in other cells such as VECs (good for anti-angiogenic approach) or in

Kupffer cells (Sakurai et al., 2002). In any case, delivery to hepatic tumour cells may be quite different to that which occurs in hepatocytes, due mainly to an increased mitotic index in tumour cells and a more demand for blood supply by the neoplasia.

As mentioned earlier, the delivery of agents mediated by CLs may be enhanced by administration directly into the circulation. Rainov et al. (1999) found that when administered via the carotid artery, plasmid expression with CL was enhanced in VECs in the normal brain, but whether this was also occurring in tumour VECs was not determined. Zhu et al. (1993) in Robert Debs laboratory were the first to demonstrate the selective potential of CLs for delivery of agents into the endothelium of various organs, including lung, heart, liver and kidney. Further targeting may be achieved with the use of tissue-specific ligands such as asialofetuin for intravenous delivery of lipoplexes to hepatocytes in vivo (Templeton et al., 1997). Targeting to tumour VECs based on selective expression of receptors in tumour vasculature (Arap et al., 1998, 2002) may also be a possibility with CLs.

Finally, cancer, is not the only disease indication amenable to anti-angiogenic therapy. Patients suffering from other disorders such as haemangiomatosis, diabetic retinopathy, neovascular glaucoma, psoriasis and rheumatoid arthritis may also benefit from an anti-angiogenic intervention. There is certainly a need for appropriate animal disease models paralleling very closely what the symptoms and disease progression is in patients in the clinics for the various ailments. Finally, CLs may not only be used for delivery of nucleic acid constructs, but other agents such as therapeutic proteins or synthetic cytotoxins. While traditionally, these vesicles are used for ferrying negatively charged agents, one challenge is whether it may deliver agents that are either electroneutral or even positively charged.

9. Summary

The relatively permeable vasculature of the tumour has been exploited for selective delivery of therapeutic agents to a tumour. In addition, the tortuous and disarrayed nature of a tumour's microvasculature, greater mitotic index of tumour cells, and a high demand for a blood supply, allows selective delivery of genotherapeutic carriers such as lipoplexes. While lipoplexes enhance the intracellular delivery of nucleic acids, there are various hindrances to optimal delivery of lipoplexes into the tumour interstitium and eventually into targeted tumour cells. Recently, independent labs have found that cationic liposomes target dynamic vasculature such as that of tumours. This has led to the possibility of selective delivery of anti-angiogenic agents against solid tumours and possibly against other diseases caused by aberrant angiogenesis and vasculature.

References

Adair, T.H., Gay, W.J., Montani, J.P., 1990. Growth regulation of the vascular system: evidence for a metabolic hypothesis. Am. J. Physiol. 259, R393–R404.

Almofti, M.R., Harashima, H., Shinohara, Y., Almofti, A., Baba, Y., Kiwada, H., 2003. Cationic liposome-mediated gene delivery: biophysical study and mechanism of internalisation. Arch. Biochem. Biophys. 410, 246–253.

Aoki, K., Yoshida, T., Matsumoto, N., Ide, H., Hosokawa, K., Sugimura, T., Terada, M., 1997. Gene therapy for peritoneal dissemination of pancreatic cancer by liposome-mediated transfer of herpes simplex virus thymidine kinase gene. Hum. Gene Ther. 8, 1105–1113.

Arap, W., Pasqualini, R., Ruoslahti, E., 1998. Cancer treatment by targeted drug delivery to tumor vasculature in a mouse model. Science 279, 377–380.

Arap, W., Haedicke, W., Bernasconi, M., Kain, R., Rajotte, D., Krajewski, S., Ellerby, H.M., Bredesen, D.E., Pasqualini, R., Ruoslahti, E., 2002. Targeting the prostate for destruction through a vascular address. Proc. Natl. Acad. Sci. U.S.A. 99, 1527–1531.

Audouy, S.A., de Leij, L.F., Hoekstra, D., Molema, G., 2002. In vivo characteristics of cationic liposomes as delivery vectors for gene therapy. Pharm. Res. 19, 1599–1605.

Augustin, H.G., Braun, K., Telemenakis, I., Modlich, U., Kuhn, W., 1995. Ovarian angiogenesis. Phenotypic characterization of endothelial cells in a physiological model of blood vessel growth and regression. Am. J. Pathol. 147, 339–351.

Baillie, C.T., Winslet, M.C., Bradley, N.J., 1995. Tumour vasculature: a potential therapeutic target. Br. J. Cancer 72, 257–267.

Bangham, A.D., Standish, M.M., Watkins, J.C., 1965. Diffusion of univalent ions across the lamellae of swollen phospholipids. J. Mol. Biol. 13, 238–252.

Bankston, P.W., Milici, A.J., 1983. A survey of the binding of polycationic ferritin in several fenestrated capillary beds: indication of heterogeneity in the luminal glycocalyx of fenestral diaphragms. Microvasc. Res. 26, 36–48.

Bocci, G., Nicolaou, K.C., Kerbel, R.S., 2002. Protracted low-dose effects on human endothelial cell proliferation and survival in vitro reveal a selective antiangiogenic window for various chemotherapeutic drugs. Cancer Res. 62, 6938–6943.

Boucher, Y., Leunig, M., Jain, R.K., 1996. Tumor angiogenesis and interstitial hypertension. Cancer Res. 56 (18), 4264–4266.

Brien, S.E., Zagzag, D., Brem, S., 1989. Rapid *in situ* cellular kinetics of intracerebral tumor angiogenesis using a monoclonal antibody to bromodeoxyuridine. Neurosurgery 25, 715–719.

Briozzo, P., Badet, J., Capony, F., Pieri, I., Montcourrier, P., Barritault, D., 1991. MCF7 mammary cancer cells respond to bFGF and internalize it following its release from the extracellular matrix: a permissive role for cathepsin D. Exp. Cell Res. 194, 252–259.

Brock, T.A., Dvorak, H.F., Senger, D.R., 1991. Tumor-secreted vascular permeability factor increases cytosolic Ca^{2+} and von Willebrand factor release in human endothelial cells. Am. J. Pathol. 138, 213–221.

Brooks, P.C., Clark, R.A., Cheresh, D.A., 1994. Requirement of vascular integrin αvβ3 for angiogenesis. Science 264, 569–571.

Calabretta, B., Sims, R.B., Valtieri, M., Caracciolo, D., Szczylik, C., Venturelli, D., Ratajczak, M., Beran, M., Gerwitz, A.M., 1991. Normal and leukemic hematopoietic cells manifest differential sensitivity to inhibitory effects of c-*myb* antisense oligodeoxynucleotides: an in vivo study relevant to bone marrow purging. Proc. Natl. Acad. Sci. U.S.A. 88, 2351–2357.

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.

Chang, Y.S., di Tomaso, E., McDonald, D.M., Jones, R., Jain, R.K., Munn, L.L., 2000. Mosaic blood vessels in tumors: frequency of cancer cells in contact with flowing blood. Proc. Natl. Acad. Sci. U.S.A. 97, 14608–14613.

Chonn, A., Cullis, P., Devine, D., 1991. The role of surface charge in the activation of the classical and alternative pathways of complement by liposomes. J. Immunol. 146, 4234–4241.

Dass, C.R., 2002a. Cytotoxicity issues pertinent to lipoplex-mediated gene therapy in vivo. J. Pharm. Pharmacol. 54, 593–601.

Dass, C.R., 2002b. Liposome-mediated delivery of oligodeoxynucleotides in vivo. Drug Deliv. 9, 169–180.

Dass, C.R., 2002c. Biochemical and biophysical characteristics of lipoplexes pertinent to solid tumour gene therapy. Int. J. Pharm. 241, 1–25.

Dass, C.R., Burton, M.A., 1999. Lipoplexes and tumours. J. Pharm. Pharmacol. 51, 755–770.

Dass, C.R., Burton, M.A., 2002. A model for evaluating selective delivery of plasmid DNA to tumours via the vasculature. Cancer Biother. Radiopharm. 17, 501–505.

Dass, C.R., Burton, M.A., 2003. Modified microplex vector enhances transfection of cells in culture while maintaining tumour-selective gene delivery in-vivo. J. Pharm. Pharmacol. 55, 19–25.

Dass, C.R., Su, T., 2000. Delivery of lipoplexes for genotherapy of solid tumours: role of vascular endothelial cells. J. Pharm. Pharmacol. 52, 1301–1317.

Dass, C.R., Walker, T.L., Burton, M.A., DeCruz, E.E., 1997. Cationic liposomes and gene therapy for solid tumors. Drug Deliv. 4, 151–166.

Dass, C.R., Walker, T.L., Burton, M.A., 1998. Tumour vasculature—summary of recent findings and therapeutic implications. Cancer Forum 22, 149–152.

Dass, C.R., Walker, T.L., Kalle, W.H.J., Burton, M.A., 1999. A microsphere-lipoplex (microplex) vector for targeted gene therapy of cancer. I. Construction and in vitro evaluation. Drug Deliv. 6, 259–270.

Dass, C.R., Saravolac, E.G., Li, Y., Sun, L.Q., 2002a. Cellular uptake, distribution, and stability of 10–23 deoxyribozymes. Antisense Nucl. Acid Drug Dev. 12, 289–299.

Dass, C.R., Walker, T.L., Burton, M.A., 2002b. Liposomes containing cationic dimethyl dioctadecyl ammonium bromide: formulation, quality control, and lipofection efficiency. Drug Deliv. 9, 11–18.

Dass, C.R., Walker, T.L., Kalle, W.H.J., Burton, M.A., 2000c. A microsphere-lipoplex (microplex) vector for targeted gene therapy of cancer. II. In vivo biodistribution study in a solid tumour model. Drug Deliv. 7, 15–20.

Denekamp, J., 1984. Vasculature as a target for tumor therapy. Prog. Appl. Microbiol. 4, 28–38.

Dewhirst, M.W., LaRue, S.M., Gerweck, L., 1995. Tumor physiology and cell kinetics. Semin. Vet. Med. Surg. (Small Animal) 10, 148–157.

Farhood, H., Serbina, N., Huang, L., 1995. The role of dioleoyl phosphatidylethanolamine in cationic liposome mediated gene transfer. Biochim. Biophys. Acta 1235, 289–295.

Felgner, J.H., Kumar, R., Sridhar, C.N., Wheeler, C.J., Tsai, Y.J., Border, R., Ramsey, P., Martin, M., Felgner, P.L., 1994. Enhanced gene delivery and mechanism studies with a novel series of cationic lipid formulations. J. Biol. Chem. 269, 2550–2561.

Fidler, I.J., 1991. Orthotopic implantation of human colon carcinoma into nude mice provides a valuable model for the biology and therapy of metastasis. Cancer Metast. Rev. 10, 229–243.

Fidler, I.J., Ellis, L.M., 1994. The implications of angiogenesis for the biology and therapy of cancer metastases. Cell 79, 185–188.

Folkman, J., 1992. The role of angiogenesis in tumor growth. Semin. Cancer Biol. 3, 65–71.

Gastl, G., Hermann, T., Steurer, M., Zmija, J., Gunsilius, E., Unger, C., Kraft, A., 1997. Angiogenesis as a target for tumor treatment. Oncology 54, 177–184.

Hamada, H., Tsuruo, T., 1988. Characterization of the ATPase activity of the 170- to 180-kilodalton membrane glycoprotein associated with multidrug resistance: the 170- to 180-kilodalton membrane glycoprotein is an ATPase. J. Biol. Chem. 263, 1454–1458.

Hillen, H.F.P., Hak, L.E., Joosten-Achjanie, S.R., Arends, J.-W., 1997. Microvessel density in unknown primary tumors. Int. J. Cancer (Predict Oncol.) 74, 81–85.

Jain, R.K., 1987. Transport of molecules in the tumor interstitium: a review. Cancer Res. 47, 3039–3051.

Jain, R.K., 1996. 1995 Whitaker Lecture: delivery of molecules, particles, and cells to solid tumors. Ann. Biomed. Eng. 24, 457–473.

Kan, Z., Ivancev, K., Lunderquist, A., McCuskey, P.A., Wright, K.C., Wallace, S., McCuskey, R.S., 1993. In vivo microscopy of hepatic tumors in animal models: a dynamic investigation of blood supply to hepatic metastases. Radiology 187, 621–626.

Kerbel, R.S., Klement, G., Pritchard, K.I., Kamen, B., 2002. Continuous low-dose anti-angiogenic/metronomic chemotherapy: from the research laboratory into the oncology clinic. Ann. Oncol. 13, 12–15.

Kikuchi, A., Aoki, Y., Sugaya, S., Serikawa, T., Takakuwa, K., Tanaka, K., Suzuki, N., Kikuchi, H., 1999. Development of novel cationic liposomes for efficient gene transfer into peritoneal disseminated tumor. Hum. Gene Ther. 10, 947–955.

Kiyasu, Y., Kaneshima, S., Koga, S., 1981. Morphogenesis of peritoneal metastasis in human gastric cancer. Cancer Res. 41, 1236–1239.

Kohn, S., Nagy, J.A., Dvorak, H.F., Dvorak, A.M., 1992. Pathways of macromolecular tracer transport across venules and small veins. Lab. Invest. 67, 596–607.

Krol, A., Maresca, J., Dewhirst, M.W., Yuan, F., 1999. Available volume fraction of macromolecules in the extravascular space of a fibrosarcoma: implications for drug delivery. Cancer Res. 59 (16), 4136–4141.

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.

Lasic, D.D., Pearlman, R., 1996. Liposomes and lipidic particles in gene therapy. In: Rosoff, M. (Ed.), Vesicles. Marcel Dekker, Brooklyn, New York, pp. 477–489.

Leu, A.J., Berk, D.A., Lymboussaki, A., Alitalo, K., Jain, R.K., 2000. Absence of functional lymphatics within murine sarcoma: a molecular and functional evaluation. Cancer Res. 60, 4324–4327.

Levchenko, T.S., Rammohan, R., Lukyanov, A.N., Whiteman, K.R., Torchilin, V.P., 2002. Liposome clearance in mice: the effect of a separate and combined presence of surface charge and polymer coating. Int. J. Pharm. 240, 95–102.

Liotta, L.A., Kleinerman, J., Saidel, G.M., 1976. The significance of heterogeneous tumor cell clumps in the metastatic process. Cancer Res. 36, 889–894.

Liu, Y., Mounkes, L.C., Liggitt, H.D., Brown, C.S., Solodin, I., Heath, T.D., Debs, R.J., 1997. Factors influencing the efficiency of cationic liposome-mediated intravenous gene delivery. Nat. Biotechnol. 15, 167–173.

Matsui, H., Johnson, L.G., Randell, S.H., Boucher, R.C., 1997. Loss of binding and entry of liposome–DNA complexes decreases transfection efficiency in differentiated airway epithelial cells. J. Biol. Chem. 272, 1117–1126.

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.

Meltzer, A., 1990. Dormancy and breast cancer. J. Surg. Oncol. 43, 181–188.

Meyer, O., Kirpotin, D., Hong, K., Sternberg, B., Park, J.W., Woodle, M.C., Papahadjopoulos, D., 1998. Cationic liposomes coated with polyethylene glycol as carriers for oligonucleotides. J. Biol. Chem. 273, 15621–15627.

Milici, A.J., L'Hernault, N., Palade, G.E., 1985. Surface densities of diaphragmed fenestrae and transendothelial channels in different murine capillary beds. Circ. Res. 56, 709–717.

Mollica, F., Jain, R.K., Netti, P.A., 2003. A model for temporal heterogeneities of tumor blood flow. Microvasc. Res. 65 (1), 56–60.

Monsky, W.L., Fukumura, D., Gohongi, T., Ancukiewcz, M., Weich, H.A., Torchilin, V.P., Jain, R.K., 1999. Augmentation of transvascular transport of macromolecules and nanoparticles in tumors using vascular endothelial growth factor. Cancer Res. 59, 4129–4135.

Mounkes, L.C., Zhong, W., Cipres-Palacin, G., Heath, T.D., Debs, R., 1998. Proteoglycans mediate cationic liposome–DNA complex-based gene delivery in vitro and in vivo. J. Biol. Chem. 273, 26164–26170.

Murray, J.C., Carmichael, J., 1995. Targeting solid tumours: challenges, disappointments, and opportunities. Adv. Drug Deliv. Rev. 17, 117–127.

Niedbala, M.J., Crickard, K., Bernacki, R.J., 1985. Interactions of human ovarian tumor cells with human mesothelial cells grown on extracellular matrix. An in vitro model system for studying tumor cell adhesion and invasion. Exp. Cell Res. 160, 499–513.

Nishi, T., Yoshizato, K., Yamashiro, S., Takeshima, H., Sato, K., Hamada, K., Kitamura, I., Yoshimura, T., Saya, H., Kuratsu, J., Ushio, Y., 1996. High-efficiency in vivo gene transfer using intraarterial plasmid DNA injection following in vivo electroporation. Cancer Res. 56, 1050–1055.

Niven, R., Pearlman, R., Wedeking, T., Mackeigan, J., Noker, P., Simpson-Herren, L., Smith, J.G., 1998. Biodistribution of radiolabeled lipid–DNA complexes and DNA in mice. J. Pharm. Sci. 87, 1292–1299.

Oberle, V., Bakowsky, U., Zuhorn, I.S., Hoekstra, D., 2000. Lipoplex formation under equilibrium conditions reveals a three-step mechanism. Biophys. J. 79, 1447–1454.

Olesen, S.P., 1986. Rapid increase in blood–brain barrier permeability during severe hypoxia and metabolic inhibition. Brain Res. 368, 24–29.

Parker, S.E., Ducharme, S., Norman, J., Wheeler, C.J., 1997. Tissue distribution of the cytofectin component of a plasmid–DNA/cationic lipid complex following intravenous administration in mice. Hum. Gene Ther. 8, 393–401.

Plate, K.H., Mennel, H.D., 1995. Vascular morphology and angiogenesis in glial tumors. Exp. Toxicol. Pathol. 47, 89–94.

Plate, K.H., Breier, G., Risau, W., 1994. Molecular mechanisms of developmental and tumor angiogenesis. Brain Pathol. 4, 207–218.

Polverini, P.J., 1995. The pathophysiology of angiogenesis. Crit. Rev. Oral Biol. Med. 6, 230–247.

Polverini, P.J., Leibovich, S.J., 1984. Induction of neovascularization in vivo and endothelial cell proliferation in vitro by tumor-associated macrophages. Lab. Invest. 51, 635–642.

Qu, H., Nagy, J.A., Senger, D.R., Dvorak, H.F., Dvorak, A.M., 1995. Ultrastructural localization of vascular permeability factor/vascular endothelial growth factor (VPF/VEGF) to the abluminal plasma membrane and vesiculovacuolar organelles of tumor microvascular endothelium. J. Histochem. Cytochem. 43, 381–389.

Rainov, N.G., Ikeda, K., Qureshi, N.H., Grover, S., Herrlinger, U., Pechan, P., Chiocca, E.A., Breakefield, X.O., Barnett, F.H., 1999. Intraarterial delivery of adenovirus vectors and

liposome–DNA complexes to experimental brain neoplasms. Hum. Gene Ther. 10, 311–318.

Rak, J.W., St. Croix, B.D., Kerbel, R.S., 1995. Consequences of angiogenesis for tumor progression, metastases and cancer therapy. Anti-Cancer Drugs 6, 3–18.

Ramanujan, S., Koenig, G.C., Padera, T.P., Stoll, B.R., Jain, R.K., 2000. Local imbalance of proangiogenic and antiangiogenic factors: a potential mechanism of focal necrosis and dormancy in tumors. Cancer Res. 60, 1442–1448.

Ramesh, R., Saeki, T., Templeton, N.S., Ji, L., Stephens, L.C., Ito, I., Wilson, D.R., Wu, Z., Branch, C.D., Minna, J.D., Roth, J.A., 2001. Successful treatment of primary and disseminated human lung cancers by systemic delivery of tumor suppresser genes using an improved liposome vector. Mol. Ther. 3, 337–344.

Rastinejad, F., Polverini, P.J., Bouck, N.P., 1989. Regulation of the activity of a new inhibitor of angiogenesis by a cancer suppressor gene. Cell 56, 345–355.

Reddy, J.A., Abburi, C., Hofland, S.J., Howard, S.J., Vlahov, I., Wils, P., Leamon, C.P., 2002. Folate-targeted, cationic liposome-mediated gene transfer into disseminated peritoneal tumors. Gene Ther. 9, 1542–1550.

Sakurai, F., Terada, T., Yasuda, K., Yamashita, F., Takakura, Y., Hashida, M., 2002. The role of tissue macrophages in the induction of proinflammatory cytokine production following intravenous injection of lipoplexes. Gene Ther. 9, 1120–1126.

Senger, D.R., Galli, S.J., Dvorak, A.M., Perruzzi, C.A., Harvey, V.S., Dvorak, H.F., 1983. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science 219, 983–985.

Shi, F., Wasungu, L., Nomden, A., Stuart, M.C., Polushkin, E., Engberts, J.B., Hoekstra, D., 2002. Interference of poly(ethylene glycol)-lipid analogues with cationic-lipid-mediated delivery of oligonucleotides role of lipid exchangeability and non-lamellar transitions. Biochem. J. 366, 333–341.

Stetler-Stevenson, W.G., Aznavoorian, S., Liotta, L.A., 1993. Tumor cell interactions with the extracellular matrix during invasion and metastases. Ann. Rev. Cell Biol. 9, 541–573.

Stohrer, M., Boucher, Y., Stangassinger, M., Jain, R.K., 2000. Oncotic pressure in solid tumors is elevated. Cancer Res. 60, 4251–4255.

Szoka, F., Papahadjopoulos, D., 1980. Comparative properties and methods of preparation of lipid vesicles (liposomes). Ann. Rev. Biophys. Bioeng. 9, 467–508.

Taipale, J., Keski-Oja, J., 1997. Growth factors in the extracellular matrix. FASEB J. 11, 51–59.

Templeton, N.S., Lasic, D.D., Frederik, P.M., Strey, H.H., Roberts, D.D., Pavlakis, G.N., 1997. Improved DNA–liposome complexes for increased systemic delivery and gene expression. Nat. Biotechnol. 15, 647–652.

Thorpe, P.E., Burrows, F.J., 1995. Antibody-directed targeting of the vasculature of solid tumors. Breast Cancer Res. Treat. 36, 237–251.

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.

Tomisaki, S., Ohno, S., Ichiyoshi, Y., Kuwano, H., Maehara, Y., Sugimachi, K., 1996. Microvessel quantification and its possible relation with liver metastasis in colorectal cancer. Cancer 77, 1722–1728.

Tsuruo, T., Tomida, A., 1995. Multidrug resistance. Anti-Cancer Drugs 6, 213–218.

Uyechi, L.S., Gagne, L., Thurston, G., Szoka Jr., F.C., 2001. Mechanism of lipoplex gene delivery in mouse lung: binding and internalization of fluorescent lipid and DNA components. Gene Ther. 8, 828–836.

van den Hooff, A., 1991. The role of stromal cells in tumor metastasis: a new link. Cancer Cells 3, 186–187.

Vincent, S., DePace, D., Finkelstein, S., 1988. Distribution of anionic sites on the capillary endothelium in an experimental brain tumour model. Microcirc. Endothelium Lymphatics 4, 45–67.

Weindel, K., Moringlane, J.R., Marme, D., Weich, H.A., 1994. Detection and quantification of vascular endothelial growth factor/vascular permeability factor in brain tumor tissue and cyst fluid: the key to angiogenesis? Neurosurgery 35, 439–449.

Wheeler, C.J., Felgner, P.L., Tsai, Y.J., Marshall, J., Sukhu, L., Doh, S.G., Hartikka, J., Nietupski, J., Manthorpe, M., Nichols, M., Plewe, M., Liang, X., Norman, J., Smith, A., Cheng, S.H., 1996. A novel cationic lipid greatly enhances plasmid DNA delivery and expression in mouse lung. Proc. Natl. Acad. Sci. U.S.A. 93, 11454–11459.

Willmott, N., Goldberg, J., Anderson, J., Bessent, R., McKillop, J., McArdle, C., 1991. Abnormal vasculature of solid tumours: significance for microsphere-based targeting strategies. Int. J. Rad. Biol. 60, 195–199.

Wong, F.M.P., Reimer, D.L., Bally, M.B., 1996. Cationic lipid binding to DNA: characterization of complex formation. Biochemistry 35, 5756–5763.

Yuan, F., Dellian, M., Fukumura, D., Leunig, M., Berk, D.A., Torchilin, V.P., Jain, R.K., 1995. Vascular permeability in a human tumor xenograft: molecular size-dependence and cut-off size. Cancer Res. 55, 3752–3756.

Zhao, Q., Song, X., Waldschmidt, T., Fisher, E., Krieg, A.M., 1996. Oligonucleotide uptake in human hematopoietic cells is increased in leukemia and is related to cellular activation. Blood 88, 1788–1795.

Zhu, N., Liggitt, D., Liu, Y., Debs, R., 1993. Systemic gene expression after intravenous DNA delivery into adult mice. Science 261, 209–211.