<u>Review Article</u>

RECENT DEVELOPMENT OF TARGETED GENE DELIVERY SYSTEMS INTO TUMORS: NEW AVENUES FOR CANCER THERAPY

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ABSTRACT

Enormous research in the area of gene delivery has been conducted worldwide, in particular for cancer gene therapy application for nearly past two decades. Numerous novel therapies are in development for targeting tumors cells but cancer gene therapy has not yet been indicated in clinical practice. The focus of present review is on recent developments highlighting the advantages and the limitations of various types of gene delivery systems (viral & non viral vectors) used in cancer gene therapy. Amongst the non viral systems, Rexin-G (Epeius Biotechnologies), recently approved to be an investigational drug by the U.S. FDA, for the treatment of all solid chemo-resistant malignancies, and granted Orphan Drug Status for pancreas cancer, osteosarcoma, and soft tissue sarcoma, as well as fast track status for pancreas cancer is a landmark technology for targetted delivery. Other recent additions to gene delivery systems have been discussed at length.

Keywords: Rexin-G, Gene delivery.

INTRODUCTION

Diseases like tumor are difficult to cure and they require newer better approaches as treatment tools. Gene therapy is one such novel medical approach. Several viral, non-viral, or bacterial vectors (or carriers) for gene transfer have been developed for either in vivo or ex vivo/in vitro use. Inactivated retroviruses, adenoviruses, adeno-associated viruses and herpes simplex viruses are some important viral vectors, characterized in laboratory studies and clinical trials. Virus vectors show relatively high transfection efficiency, but also have some clinical safety concerns [1, 2].

Non-viral methods of gene delivery, including synthetic polymers, liposomes, and electroporation, generally show low transfection efficiency but have very low pathogenicity [3,4], bacterial vectors which display high transfection efficiency as therapeutic genes, but have clinical safety problems such as inflammatory or immune responses [5] and target cell-specific delivery or gene activation are other important techniques for safe and efficient gene therapy. The latter category is especially crucial if gene therapy is applied to tumors. Gene delivery system has critical issues like inability to specifically recognize and target tumor cells and to distinguish them from normal cells, especially in the same tissue or organ.

The most commonly used gene delivery systems (viral and non-viral) for cancer treatment and the recent developments in this area are the focus of this article. Emphasis is placed on Rexin-G, is a landmark technology for targetted delivery and has been approved for the treatment of all solid chemo-resistant tumors in the Philippines, and has been granted Orphan Drug Status for (i) pancreas cancer, (ii) osteosarcoma, and (iii) soft tissue sarcoma, as well as Fast Track Status for pancreas cancer by the U.S. FDA.[6] . Other recent additions to gene delivery systems have been discussed at length.

Table 1: Vectors (carriers) used for tumor-targeted gene delivery

	Type of vector	Advantages	Disadvantages
Viral vector	Retrovirus vector	High transfection efficiency	Clinical safety issues
	Adenovirus vector		Low tumor targeting efficiency.:Require a tumor-specific ligand or promoter
	Adeno-associated virus vector		Reduction in efficiency due to repeated administration
	Herpes simplex virus vector		
	Lentivirus vector		
Non-viral Vector	Synthesized polymers Biopolymers (e.g.,chitosan), Liposomes, Peptides	Low pathogenicity Mass production	• Low transfection efficiency • Low tumor targeting

Literature Search

A literature search was performed using the PubMed database, science direct, willey intersience limited to documents published in the English language. Searches were not date-restricted. Search terms included free text words and combinations of the following Mesh terms: [Targeted delivery], [Rexin-G], [Viral vector and non-viral vector], [Retroviruses], [Gene Therapy], [Genetic Vectors] and [Clinical trial]. The 'Gene Therapy Clinical Trial Worldwide' website was also used to search for clinical trials.

Principles of gene therapy: Selection of a gene, a vector and a management strategy.

Selection of the gene

Mutant gene correction

The principles of gene selection strategies are illustrated in Figure 1. In the case of inherited monogenic diseases, the aim of gene therapy is to transfer and express the defective gene. Cancer gene therapy is more complex as it results from sequential genetic and epigenetic alterations, affecting oncogenes, tumour-suppressor genes and microRNAs, so the approach is to restore tumour-suppressor gene expression or to inhibit oncogene expression. About 11% of transferred genes in gene therapy clinical trials are tumour-suppressor genes and many trials have been performed in cancer gene therapy using the p53 gene, mostly including patients with lung or head and neck cancers [7]

Suicide genes

The aim of suicide gene therapy is to enable, selectively, the transfected cell to transform a prodrug into a toxic metabolite, resulting in cell death. The most widely described suicide gene is the herpes simplex virus thymidine kinase (HSV-tk) gene. HSV-tk can phosphorylate ganciclovir, which is a poor substrate for mammalian thymidine kinases. Ganciclovir can therefore be transformed into ganciclovir triphosphate, which is cytotoxic to the transfected cell, resulting in cell death.[8] This cell death can also affect neighbouring cells that do not express HSV-tk. This phenomenon is called a local

bystander effect, as opposed to a bystander effect that can be observed in distant, nontransduced tumour sites.[9] This distant bystander effect involves the immune system.

Targeted gene therapy

The goal of targeted gene therapy is to increase the specificity and efficiency of gene transfer, there by improving therapeutic outcome and reducing undesirable side effects. Understanding the molecular mechanisms that underlie the disease and identifying the differences between normal and diseased cells is at the core of targeted gene therapy. For example, to target tumor tissues, the cancer cells must preferably express a molecule that should not be present on healthy tissue [10]. Ideally, such a molecule, also called target molecule, should be able to interact with a specific antibody or ligand and it should not be subject to variations or mutations within a single patient or between different patients [11]. Once a suitable target molecule has been identified, a gene delivery vehicle has to be developed to induce gene expression only in the cells of interest. This can be achieved by either transductional or transcriptional targeting (Figure 1). In transductional targeting, the natural interaction of the delivery vehicle is modified so that the gene is only delivered to the cells of interest. This requires detailed knowledge of tissue-specific receptors and vehicle biology. In transcriptional targeting, tissue-specific expression is achieved by placing the gene under the control of cell-specific promoters and/or enhancers. The latter approach is hampered by the fact that only a limited number of specific promoters with acceptable activity have been identified so far.
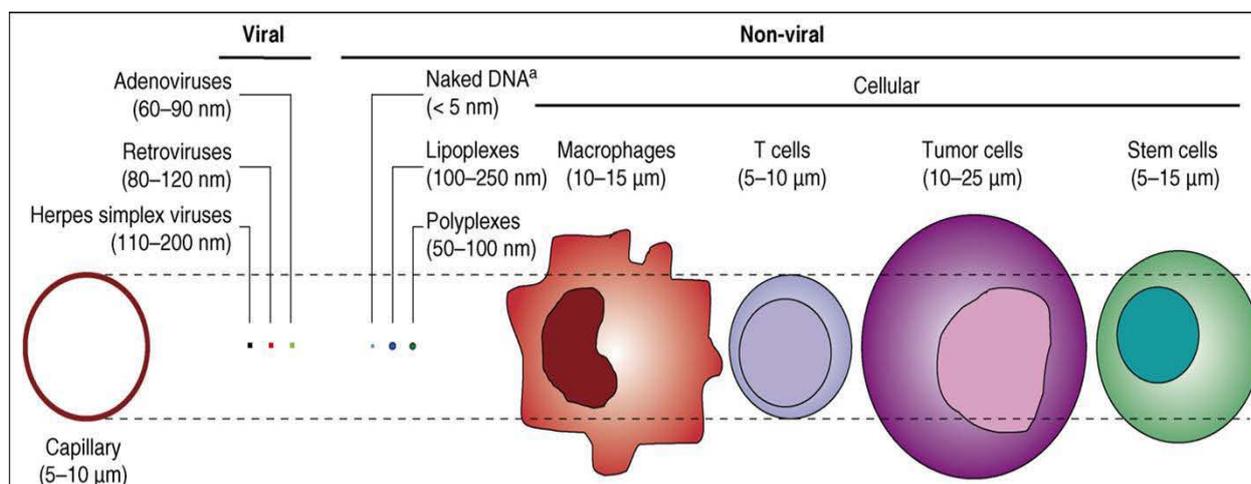

Fig. 1: Targeting strategies to achieve EC-specific transcription

Abbreviations: BM, basement membrane; EC, endothelial cell; ECM, extracellular matrix

In transcriptional targeting (left), tissue-specific expression is achieved by placing the gene under the control of cell-specific promoters and/or enhancers. In transductional targeting (right), the natural interaction of the delivery vehicle is modified so that the gene is only delivered to the cells of interest. This can be achieved by using adaptor molecules that can block the natural interaction between the ligand of the vehicle and a cellular receptor, while they facilitate the interaction with a specific receptor on the target cell, by blocking the natural interaction between ligand of the vehicle and a cellular receptor with, for example, an antibody, or by expressing and/or conjugating a new ligand specific for the target cell.

Viral methods

Biological gene delivery systems (viral vectors)

Viruses for gene therapy can be classified into two major groups based on their ability to integrate their genetic material into the genome of the host: (i) integrating viruses (e.g. retrovirus and lentivirus), which induce stable expression of the trans gene and are transmitted to all the progeny of transduced cells; and (ii) non-integrating viruses (e.g. adenovirus and herpes virus), which only induce transient expression. In the latter case, it might be necessary to repeat viral administration, which increases the risk of eliciting an immune response against the virus. In anti-angiogenesis cancer therapy, three types of viruses are most commonly used:

a)   Adenoviruses,

b)   Retroviruses and

c)   Herpes simplex viruses.

a)   Adenoviruses

Adenoviruses are double-stranded DNA viruses that can infect both dividing and non-dividing cells [12,13]. The wild type viruses can cause benign respiratory infections in humans [14]. The defective competent adenoviral vectors were first generated by substituting the viral E1 gene with a therapeutic gene. More efficient gene carriers were obtained by altering more genes in the viral genome such as E2 gene [15]. The removal of the whole coding sequence of the viral genome resulted in better gene carriers in terms of their capacity [16]. Transfection with adenoviruses is transient since the DNA genome does not permanently integrate into the host cell genetic material [17].Therefore, repetitive administration of the adenoviral vectors is needed to obtain the desired therapeutic outcomes. Adenoviral vectors have been widely used for cancer therapy applications [18, 19, 20]. It has been shown that adenovirus-mediated gene transfer is more efficient in immune deficient animals [21]. Both cellular and humoral immune responses limit the in vivo efficiency of these gene carriers [22, 23]. Therefore, co-administration of immunosuppressive agents may increase the adenoviral transduction ability. Such strategy may not be attractive for cancer therapy since the immune response can be utilized for tumor destruction. On the other hand, it was hypothesized that systemic immunity can reduce the toxic effects of these vectors [24]. In fact, adenoviral vectors were accounted for the first reported death in clinical gene therapy trails [25]. Surprisingly, the pre-immunization of mice bearing cancerous tissues with the null vector increased the mortality rate between high-dose treated animals in comparison with non-immunized animals. Positive results (less toxic effects), however, were observed for moderate dosing [26]. It was suggested that adenovirus/ antibody immune complex at high doses will induce complement activation which may lead substantially to systemic lethal inflammatory reactions (which does not occur in moderate dosing). Similar to retroviral vectors, conditionally

replicative adenoviruses were also successfully developed for selective cancer gene therapy [27, 28]. In one study, adenoviruses were developed to replicate selectively in wild type p53 (wt p53)-deficient tumor cells. This was achieved by gene deletion of the E1B viral protein, which binds naturally to wt p53 allowing viral propagation in wt p53 cells [29]

b) Herpes simplex viruses

Herpes simplex viruses (HSVs) are flexible and effective vehicles for introducing and expressing foreign genes in mammalian cells both in vivo and in vitro. For anti-angiogenesis therapy, the oncolytic properties of HSV are often used [30]. However, infections of tumors by oncolytic HSV results in tumor reduction but not tumor regression. Similar to retroviruses, HSVs can be retargeted by pseudotyping, genetic modification and transcriptional targeting [31]. Reinblatt et al. [32] and Pin et al. [33] used HSV to express soluble VEGF receptors under the control of a multimerized hypoxia response element. When PC1 xenograft tumors were treated in hypoxic conditions, there was a much higher expression level of soluble VEGF receptor than under normoxic conditions. Besides smaller tumor volumes, in hypoxic conditions there was a higher capillary reduction [32,33]. In a study of Mullen et al. [34], the murine endostatin gene was incorporated into the HSV genome. The produced endostatin could inhibit angiogenesis in a human HT29 colon carcinoma model.

c) Retrovirus

Retrovirus carriers are developed by replacing the vital viral genes with therapeutic ones. The ability of retroviral vectors to successfully deliver foreign genetic materials was first described in 1981 [35, 36]. Retroviruses are small RNA viruses with DNA intermediate, which integrates into the host genome producing the viral proteins (gag, pol and env), which are removed when developing the gene delivery carrier.

In a recent study, a retroviral vector was encapsulated with genetic segment bearing both IL-12 and herpes simplex virus thymidine kinase (HSV-tk) genes [37]. While the former provoke antitumor immune respond [38,39], the latter is a suicide gene that activates the prodrug ganciclovir (GCV) [40,41]. The combined gene delivery resulted in three- to four-fold reduction in tumor size in comparison with single IL-12 gene treatment [37]. It is important to note that multiple gene delivery via retroviral vectors is rarely applied due to their limited encapsulation capacity. Most of retroviruses, however, infect actively dividing cells during mitosis [42,43]. Despite the fact that this feature might protect the normal tissues and provide natural targeting to the tumor, all tumors contain non-dividing cells in the resting phase G0. Such cells can escape the therapy. Lenti-viruses such as human immunodeficiency virus (HIV) and their vectors can, however, infect non-proliferating cells [42, 44]. Transfection efficiency was 10 times higher in ovarian cancer cells when lentiviruses were used than when retroviral vectors were used, Indraccolo et al (2002). Tumor regression was observed in more than 40% of treated mice after intra-tumor injection of lentivirus expressing the HIV-1 vpr gene, Pang et al (2001), capable of cell cycle arrest induction. The usage of lentiviruses, however, has a major drawback because of the original serious clinical consequences of these viruses. In this context, new retroviral vectors namely replication-competent retroviruses were developed and engineered to replicate specifically in the targeted neoplastic tissues; thus, increasing the vectors' transduction non-toxic ability [47, 48]

Especially Designed-Viral methods

Rexin-G: Retroviral Expression Vector Bearing an Inhibitory Construct of the Cyclin G1 Gene

Rexin-G is a matrix (collagen)-targeted retroviral vector encoding an N-terminal deletion mutant form of human cyclin G1 under the control of a hybrid long-terminal repeat/cytomegalovirus (CMV) promoter [49]; in some literature it is referred to as Mx-dnG1 (matrix-targeted vector encoding cytocidal mutant cyclin G1) [50]. In addition, the vector contains the neomycin resistance gene, driven by the SV40 early promoter, which allows selection of virus-

producing cells during manufacture. The Rexin-G vector is produced by transient co-transfection of 293T cells (HEK293 cells transformed with the SV40 large T antigen) with three plasmids simultaneously: the pdnG1/C-REX therapeutic plasmid construct contains the deletion mutant of the human cyclin G1 gene (amino acids 41 to 249) driven by the CMV immediate-early promoter, packaging sequences, and the bacterial neomycin resistance gene under the control of an internal SV40 early promoter; the Mx (Bv1/pCAEP) plasmid, which encodes the collagen targeting viral envelope component of the Moloney murine leukemia virus (MuLV), contains a CMV-driven modified amphotropic 4070A envelope protein wherein the collagen binding portion of the vWF peptide was inserted into an engineered PstI site in the N-terminal region of the 4070A envelope coding sequence; and the third plasmid, pCgpn, contains the MuLV gag-pol elements driven by the CMV immediate-early promoter [49]. The anti-cancer agent Rexin-G combines a proprietary tumor-targeted gene delivery system with a genetically engineered cell-cycle control gene (i.e. a designer anti-cancer gene) that inhibits cyclin G1 function and is thereby lethal to cancer cells and tumor-associated vasculature (i.e., anti-angiogenesis). When administered by intravenous infusions, Rexin-G has been shown to arrest cancer growth and to eradicate primary and metastatic tumors, demonstrating clinical and quality-of-life benefits. By selectively targeting cancer cells and their attendant blood supply, while sparing normal cells and tissues, Rexin-G exhibits profound single-agent efficacy against a broad spectrum of otherwise intractable cancers, without eliciting systemic side effects that are characteristically associated with standard chemotherapies.

How Rexin-G works

Each nanoparticle of Rexin-G is ~100 nanometers in diameter; yet despite its small size, it is a highly complex structure. Each component—the envelope, matrix, capsid, enzymes, and genetic material has its purpose, and in concert they enable Rexin-G to deliver a lethal payload of genetic medicine where it is needed most. Designed with 4 levels of safety: (i) stealth vector enables repeated infusions, (ii) action is limited to proliferative/dividing cells only, (iii) a pivotal growth-associated gene is blocked, and (iv) tumor targeting sequesters the nanoparticles into tumors; and 3 levels of efficacy: (i) cell cycle gene target provides broad-spectrum anti-cancer activity, and (ii) potent anti-angiogenic activity, while (iii) tumor targeting leads to high local concentrations inside the tumors. The delivery of the therapeutic payload by the nanoparticles is "pathotropic," meaning it is specifically targeted to diseased tissues. Pathotropic

Targeting allows Rexin-G to seek out, accumulate in, and destroy tumors regardless of their location in the body, thereby reducing tumor burden, preventing disease progression, prolonging survival, and enhancing the cancer patient's overall quality-of-life.

How Rexin-G is Delivered

Rexin-G is administered by simple intravenous infusion (fig.3). As the nanoparticles are distributed by the general circulation, the exquisite targeting function serves to partition the genetic medicine into the tumors, thereby removing it from the blood stream.

TARGETED DELIVERY SYSTEM

Simple Intravenous Infusion

Fig. 3: Rexin-G is administered by simple intravenous infusion

While the half-life of the active nanoparticle is rather limited, the therapeutic gene is efficiently delivered to the cancer cells and associated vasculature where the mechanism of action induces

active cell death (apoptosis)—which is accompanied histologically by focal necrosis, anti-angiogenesis, and cell degeneration within the regressing tumors. Capable, by design, of delivering the therapeutic gene to dividing cells only, Rexin-G spares normal blood vessels, tissues, and organs, thus focusing the biologic effects and improving safety. With the validation of its overall-safety in formal clinical trials, Rexin-G has been re-formulated to higher-potency, whereby tumor control may be gained by simple infusions administered three to five times a week.

Rexin-G Clinical Development

Following landmark clinical trials, Rexin-G received both Expanded Access and Accelerated Approvals, and was formally approved by the Philippine FDA as a safe and effective treatment for all solid tumors that are refractory to standard chemotherapies; thus, it is currently available at several advanced medical centers in Manila. In the United States, Rexin-G has completed its initial Phase I Safety testing followed by Adaptive Phase I/II and Confirmatory Phase II studies, receiving FDA Orphan Drug Status for pancreatic cancer, soft tissue sarcoma, and osteosarcoma, respectively, followed by FDA Fast Track Designation for second-line therapy for pancreas cancer (in 2009). Currently, Pivotal Phase II/III studies of Rexin-G for (i) second-line therapy, (ii) firstline therapy, and (iii) adjuvant therapy are planned to expedite formal approval in the U.S.[51].

The first phase I study of Rexin-G was performed in three stage IV pancreatic cancer patients [52]. An intrapatient dose escalation of Rexin-G vector (administered daily as an intravenous infusion over 1 to 3 h) was performed as follows: 4.5 x 109, 9.0 x 109 and 1.4 x 1010 cfu were administered on days 1 to 6, 7 to 8, and 9 to 10, respectively. Patients were given a week to rest before receiving 1.4 x 1010 cfu on days 18 to 27. The first patient, who had experienced recurrence of previously resected pancreatic cancer despite prior gemcitabine treatment, entered the study with disease at the original primary site and metastases to the supraclavicular and abdominal lymph nodes. The patient received a total of three 10-day treatment cycles of Rexin-G (a cumulative dose of 3 x 1011 cfu), each separated by 1 week. The volumes of the two supraclavicular lymph node tumors eventually decreased by 33 and 62%, An abdominal magnetic resonance imaging (MRI) scan at day 28 demonstrated 40 to 50% necrosis of the primary tumor, and a significant decrease in the size of a para-aortic lymph node. An MRI scan on day 54 showed no further change. The level of serum carbohydrate antigen 19-9 (CA19-9; a diagnostic marker for pancreatic cancer) decreased from 1200 to 584 U. Unfortunately, a computed tomography (CT) scan on day 101 showed a significant increase in the size of the primary tumor and the supraclavicular lymph nodes. The patient was alive with progressive disease on day 189 [52].

The second patient experienced progressive, locally advanced pancreatic cancer despite prior radiotherapy and chemotherapy with fluorouracil and gemcitabine [52]. The patient received two treatment cycles of Rexin-G, a total cumulative dose of 1.8 x 1011 cfu. An abdominal CT scan on day 28 showed a 47% decrease in tumor volume, and a follow-up scan on day 103 showed no change. The patient then received monthly gemcitabine. The patient was alive with stable disease on day 154. The third patient presented with metastatic pancreatic cancer with numerous metastases to the liver. Rexin-G was administered at a dose of 4.5 x 109 cfu/day for 6 days (total cumulative dose of 2.7 x 1010 cfu), followed by 8 weekly doses of gemcitabine (1000 mg/m2). An abdominal CT scan on day 62 demonstrated a 30% decrease in primary tumor volume, and an 89% regression in the volume of the largest liver nodule. The number of liver nodules decreased from 18 to 5. The patient was alive with stable disease on day 133 [52]. A second phase I study is ongoing in patients with metastatic colorectal cancer, who will receive a hepatic arterial infusion of the Rexin-G retroviral vector once a day on days 1 to 5 at doses of 3 x 109, 6 x 109 and 1 x 1010 cfu [49]. The objectives are to evaluate the safety/toxicity of hepatic arterial administration of Rexin-G, evaluate the pharmacodynamics of hepatic arterial infusion of Rexin-G administered as hepatic arterial infusion, to obtain preliminary data on molecular markers of tumor response, and to identify an antitumor response to hepatic artery-administered Rexin-G.The three patients who received Rexin-

G in the above phase I trial experienced no significant toxicity. No bone marrow suppression, significant alterations in liver and kidney function, nausea and vomiting, mucositis or hair loss were observed. Brief febrile episodes were the only adverse events associated with the vector infusions [49].

Other Non-viral gene delivery

Non-viral vehicles are suitable with respect to their lack of inducing a specific immune response, simplicity, packaging capacity and potential for large scale production. The simplest form of non-viral gene therapy is direct injection of naked DNA at the site of interest. However, because this approach does not enable active retargeting, it is not discussed here. Besides injection of naked DNA, non-viral gene delivery can be achieved by employing lipoplex formulationsor even whole cells.

a) Lipoplex vehicles

Most non-viral DNA delivery systems are lipid-based vehicles (also called lipoplexes), such as liposomes and micelles. The function of the lipidic component is to protect the DNA from degradation, to stabilize the particles for endocytosis and to promote endosomal release by membrane fusion. For in vivo application, lipoplexes should (i) be able to entrap a high concentration of DNA, (ii) be small, (iii) have a long half-life, (iv) be stable in the serum, (v) be non-immunogenic and (vi) be able to deliver its DNA content specifically into the target cell. We and others have already successfully used the RGD– peptide for targeting liposomal formulations to angiogenic endothelium [53, 54]. RGD–peptides bind to integrin avb3, which is overexpressed on actively proliferating endothelium [55]. Schiffelers et al. [56] used a cyclic RGD– peptide to target lipoplexes loaded with doxorubicin to murine c26 colon carcinoma, which is insensitive for doxorubicin. They demonstrated that tumor growth inhibition is a result of anti-angiogenesis.

b) Cationic liposomes

Cationic liposomes are in most cases the method of choice for in vitro transfections. Owing to their positive charge, they easily bind to the negatively charged DNA, forming a dense complex. When the formulation of the lipids is chosen so that the cationic liposome–DNA complex has a net positive charge, easy cell binding can also be achieved through electrostatic interactions with anionic sulphated proteoglycans associated with the cell membrane [57]. The in vivo gene transfer of cationic liposomes is in general low and depends on the way of administration. Cationic liposomes can be administrated via the respiratory system, intratumoral injection or intravenous injections. Using the first two methods, there is predominantly transgene expression in the first organ and/or tissue the liposomes encounter (e.g. the pulmonary system or the peritoneum). Using intravenous injection, it is difficult to reach expression levels of therapeutic magnitude [58]. To target tumors with cationic liposomes, different kinds of ligand have been used, such as the folate receptor, antibodies and scFvs [59-61]. For anti-angiogenic therapy, most studies rely on the passive targeting capacity of the vehicles together with the expression of angiostatic proteins such as endostatin [62]. Cationic liposomes target angiogenic ECs of solid tumors primarily because of size but also because of a charge-related mechanism. Eichhorn et al. [63] exploited this system by first charging the ECs by injecting the polycation protamine, which increased the selectivity of cationic liposomes in targeting angiogenic microvessels. Mori et al. [64] used cationic liposomes conjugated with inactivated hemagglutinating virus of Japan

c) Coated cationic liposomes

The disadvantage of most lipoplex gene-delivery systems is the poor expression level after in vivo systemic delivery. Coated cationic liposomes (CCLs) fulfill many of the above described requirements for an ideal systemic in vivo gene delivery vehicle. They are small, non-immunogenic, have high DNA entrapment efficiency (>90%) and are stable in the blood circulation (half-life of several hours) [65]. When CCLs are prepared, the negative charge of the DNA is neutralized by cations. These complexes are subsequently 'coated' with neutral lipids. The formulation of the coating can be optimized for the specific application. Without targeting, they interact at very

low levels with cells. By coupling ligands, they can be made specific for several cell types [66]. In a study of Bartsch et al. [67], untargeted CCLs were compared with CCLs that are targeted with poly-anion aconitylated human serum albumin (Aco-HAS). Although the polyethylene glycol (PEG)- stabilized untargeted particles showed long-life circulating properties (half-life of >10 hours), untargeted CCLs hardly bound to liver ECs, whereas targeted CCLs massively and specifically interacted with these cells. Using targeted CCLs, downregulation of ICAM was achieved by delivering antisense oligodeoxynucleotides (ODNs) [67].

d) Polymer-based vehicles

The biggest benefit of polymer-based gene-delivery systems is their solubility and stability in the serum, making them suitable vehicles for in vivo systemic administration. In most studies, cationic polymers are used, and complexes of these polymers with DNA are also called polyplexes. Similar to lipoplex formulations, the difficulty for polyplexes is to escape from the endosomes after cellular uptake. Therefore, cell transfection requires the codelivery of an endosomes lytic agent such as inactivated adenovirus [67]. As an alternative, polymers such as polyethylenimine (PEI) can be used. PEI itself induces anendosome disruption mechanism by buffering the contents of the endosomes [69], causing an influx of chloride ions [70].This process – called proton motive force – results in a net increase in ion concentration, which causes osmotic swelling and subsequent disruption of the endosomes. Transfection efficiency of polyplexes depends on the length of the polymer. A long polymer is also beneficial for condensing and protection of DNA. In a study by Gautam et al. [71], a significant reduction of B16F10 lung tumor growth was achieved after aerosol delivery of PEI– p53-expressing DNA in the epithelial cells lining the airways.The mean survival of the mice increased by �50%. The p53 transfection induced an upregulation of the antiangiogenesis factor trombospondin-1, a downregulation of VEGF and a decrease in the angiogenic phenotype of the tumors 71]. To achieve targeted uptake of small interfering (si)RNA against VEGF receptor-2 in tumor neovasculature, Schiffelers et al. [72] generated complexes with PEI that were PEGylated with an RGD–peptide ligand. Cell delivery and activity was siRNA-sequence-specific and dependent on the presence of peptide ligand. Intravenous administration in tumor-bearing mice gave selective tumor uptake, siRNA-sequence-specific inhibition of protein expression within the tumor, and inhibition of both tumor angiogenesis and growth rate [72].

e) Cellular vehicles

In cell-based therapy, a cell carries the DNA vector to the site of interest. At present, the ex vivo loading of cells is adequate, whereas tumor-specific homing capacities and in vivo gene-transfer efficiency still need improvement. Obviously, choosing the appropriate cell vehicle is a crucial factor for successful gene delivery [73]. When autologous cells are used, the immune response can be avoided or diminished. Also, the toxic effects of high doses of viral vehicles can be circumvented. Furthermore, in vitro transfection methods can be applied to the carrier cell, which ensure high transfection efficiency. Finally, by transferring DNA constructs, the homing features of a given cell type can be modified (e.g. by expressing specific receptors or ligands).

Human umbilical vein ECs preferentially accumulate at the tumor vasculature [74]. Ojeifo et al. [75] infected HUVECs with a retroviral vehicle containing the LacZgene. Beta-galactosidase-expressing cells were intravenously injected in mice bearing aNIH3T3 murine fibroblast tumor that secreted fibroblast growth factor-1 (FGF-1). The transduced HUVECs accumulated at sites of FGF-1, induced angiogenesis and persisted for at least four weeks [75]. Apart from HUVECs, other cell types such as immune cells, stem cells and even tumor cells have been used. Kershaw et al. [76] used lymphocytes that were genetically modified with a gene construct encoding a single-chain antibody fragment against VEGF receptor-2 (KDR) via retroviruses. The lymphocytes were assessed for their ability to secrete cytokines in response to KDR binding. These authors demonstrated that incubation of these lymphocytes with HUVECs resulted in both target-cell lysis and secretion of cytokines and chemokines [76]. Niederman et al. [77] followed a similar approach

and loaded CD8-positive lymphocytes with a chimeric receptor that consisted of VEGF-coding sequences. The transduced cells possessed an efficient killing specificity for cells that express the VEGF receptor (FLK1). The modified T cells showed a strong inhibition of tumor growth in three in vivo mouse cancer models. Tumor growth was even more inhibited when treatment was combined with the angiogenesis inhibitor TNP-470 [77]. In a study of Davidoff et al. [78], murine bone-marrow cells were transduced with a retroviral vector to deliver a gene that encodes a soluble truncated form of VEGF receptor-2. In transplanted mice, the ECs in the tumor were partly derived from the bone-marrow precursors, and tumor growth was significantly inhibited [78]. De Palma et al. [79] demonstrated that bone-marrow progenitor cells that are loaded with a suicide gene under the control of transcription regulatory elements of TIE-2, marked a hematopoietic population that homed to the tumor [79]. The observation that cells of a given histological cell type bind preferentially to cells of the same histological type made autologous tumor cells good candidates as cell carriers [80]. Even tumor cells of unrelated origin associate with, and adhere to, other primary tumor cells [81]. The obvious disadvantage of tumor cells as carriers is the risk of introducing a new source of neoplasm into the body and the fact that they can only adhere at places where the tumor is in direct contact with the blood. For clinical use, it would be desirable to have an additional mechanism by which the adoptive transferred cells could be killed after fulfilling their functions..

Gene therapy is a promising approach for cancer treatment. Research has lead to much progress on the development of novel strategies and transfer vehicles. Nevertheless, gene transfer vehicles that are safe, non-immunogenic, efficacious and tumor selective are still lacking, and several questions have to be addressed before angiogenesis-targeted cancer gene therapy will become a valuable tool in the clinical setting . Viral vehicles are still a topic of new studies. Although much improvement has been made on their gene-transfer mechanisms, further development relies on a better understanding of the biology underlying virus–host interactions. In addition, the in vivo transduction efficiency of viral vehicles has to be improved. A major step forward will include methods to prevent uptake of the vehicles by mononuclear phagocytic system (MPS) in the circulation. Progressions will also arise from the use of replication competent viruses and the development of non-viral vehicles, which represent a simple, cheap and safe alternative to viral vectors. However, barriers such as low transfection efficiency and insufficient distribution to target cells in vivo remain substantial. Two of the most important needs for effective use of lipoplexes are an improved control over DNA release after internalization and an enhanced regulation of gene expression. Additional success will rely on the formulation of customized lipids for specific cell types and applications.

Another important challenge is the use of cellular vehicles for gene transfer. Several recent reports have described the homing of bone-marrow-derived endothelial precursor cells (EPCs) to tumor sites where they contribute to angiogenesis [82, 83]. Such cells would provide an excellent opportunity for angiogenesis-targeted gene therapy. However, the frequency of incorporation of EPCs in the tumor vasculature seems to be low. Although radiation therapy can increase this percentage [84], it remains to be determined whether the numbers of stem cells would be sufficient to yield a therapeutic effect. Besides the development of adequate transfer vehicles and regulation of adequate and therapeutic expression levels of the transgene, the main future challenge is the implementation of specific targeting ligands in the gene transfer vehicles. Over the past decade, we and others have identified such targets, which enable the design of both viral and non-viral vehicles that specifically home to the angiogenic vasculature [85, 86, 87]. Using these molecules in the different gene-transfer vehicles will improve targeting specificity and reduce toxicity. This step might facilitate the translation of angiogenesis-targeted cancer gene therapy to the clinical setting.

Concluding Remarks

Targeted gene delivery has emerged as a promising approach to enhance the efficacy of tumor-selective gene delivery. Because the ECs in the tumor vasculature express specific molecules, they can be

used for selective targeting of gene-transfer vehicles (i.e. viral or non-viral vectors). The targeting of such vectors can be achieved via specific ligands, receptors, (part of) antibodies, peptides or transcriptional control elements. Additional benefits of targeting the tumor vasculature instead of tumor cells are accessibility, genetic stability and the possibility to be effective against more than one cancer type. Furthermore, targeted gene therapy does not exclude other cancer therapies, such as chemotherapy or radiation therapy. Combination therapy might work even better [88]. Despite promising results with different gene-transfer vehicles, there are still limitations that have to be solved before targeted cancer gene therapy can be routinely used in the clinical setting. Nevertheless, it can be expected that future research will bring successful targeted gene-delivery strategies for the treatment of cancer.

Future directions

Gene therapy is a promising approach for cancer treatment. Research has lead to much progress on the development of novel strategies and transfer vehicles. Nevertheless, gene transfer vehicles that are safe, non-immunogenic, efficacious and tumor selective are still lacking, and several questions have to be addressed before angiogenesis-targeted cancer gene therapy will become a valuable tool in the clinical setting.

Viral vehicles are still a topic of new studies. Although much improvement has been made on their gene-transfer mechanisms, further development relies on a better understanding of the biology underlying virus–host interactions. In addition, the in vivo transduction efficiency of viral vehicles has to be improved. A major step forward will include methods to prevent uptake of the vehicles by mononuclear phagocytic system (MPS) in the circulation. Progressions will also arise from the use of replication competent viruses and the development of non-viral vehicles, which represent a simple, cheap and safe alternative to viral vectors. However, barriers such as low transfection efficiency and insufficient distribution to target cells in vivo remain substantial. Two of the most important needs for effective use of lipoplexes are an improved control over DNA release after internalization and an enhanced regulation of gene expression. Additional success will rely on the formulation of customized lipids for specific cell types and applications. Another important challenge is the use of cellular vehicles for gene transfer. Several recent reports have described the homing of bone-marrow-derived endothelial precursor. Besides the development of adequate transfer vehicles and regulation of adequate and therapeutic expression levels of the transgene, the main future challenge is the implementation of specific targeting ligands in the gene transfer vehicles. Over the past decade, we and others have identified such targets, which enable the design of both viral and non-viral vehicles that specifically home to the tumor vasculature. Recently approved nanotech product Rexin-G is expected to improve targeting specificity and reduce toxicity which is associated with existing therapy.

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