Local Gene Delivery for Cancer Therapy

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Abstract: Gene therapy is an emerging technique with widespread applications in treatment of cardiovascular diseases, monogenic disorder, infectious diseases, and especially cancers. The major challenge for gene therapy is to deliver therapeutic genes to target tissues. Although various gene delivery vectors such as harmless viruses and micro/nano-particles have been developed (*i.e.* commonly system delivery), concerns remain for the transfection efficiency and stability of those working copies in these vectors. Local gene delivery such as intratumoral infusion, electroporation and implants offers significantly enhanced transfection efficiency with decreased toxicity compared to system delivery and has been broadly used in clinics. In this paper, we reviewed the local gene delivery methods and discussed their distinctive advantages and potential challenges in cancer treatment.

Keywords: Cancer therapy; drug-eluting implants; electrogene; intratumoral; local gene therapy; magnetic; tumor-tropism delivery; ultrasound.

1. INTRODUCTION

Cancer, the product of multiple-step gene alterations, is still the main cause of mortality and morbidity in the world [1]. Although this situation has been significantly improved by conventional therapies such as chemotherapy and radiotherapy [1-3], these therapies lack selectivity for cell killing, and the clinical efficacy is limited by their non-target effect in normal tissues. Advances in molecular biology, such as DNA sequencing [4], cancer genome studies [5], RNA interference [6], have improved our understanding on the mechanisms of neoplasia and enabled the identification of tumor signal pathways and specific targets [4]. These advances have led to the development of tumor-specific therapy such as anti-VEGF antibody for anti-angiogenesis [7], cytokines for immune therapy [8] and small interfering RNA for inhibiting cellular pathways [6]. Among them, gene therapy holds great promise for cancer treatment where genes specific for the target cancers (*e.g.*, STAT-3 [9], TRAIL [10, 11], IFN-β [12]) are introduced into target tissues to slow or reverse the progress of diseases. For example, more than 1600 gene therapy methods have been translated to clinical trials and 64.5% of them are related to cancer diseases since 1990 [13].

Although gene therapy offers high selectivity and minimized systemic side-effects, its broad clinical application has not been achieved yet due to several remaining challenges including limited efficiency, safety and stability [14]. To address these challenges, various systemic gene delivery methods have been developed such as virus vectors [13-15], plasmids [13], and nano/microparticles [16]. However, broad translation of these systemic gene delivery methods to clinical practices has not been achieved [14, 15, 17], mainly due to the lack of targeting delivery to tumor, low gene transduction efficiency, potential immunoreactions and rapid elimination after systemic administration [14, 15, 17]. Therefore, there is still an unmet need for a suitable gene delivery method.

Local gene delivery holds great potential to overcome the challenges associated with systemic gene delivery by directly releasing therapeutic genes into specific tissues. In this method, gene materials and vectors are spatially confined to the diseased region offering increased treatment efficiency and decreased toxicity of treatment. For example, around 40% of Phase III trials on cancer gene therapy were related to local gene delivery in the last five years [13]. With recent advances in delivery technologies and smart delivery-vector design, more promising local delivery systems have been developed for cancer gene therapy, such as in situ-forming implants, magnetofection, tumor-tropism vectors. In this review, we focused on the current progress of local gene delivery methods. We were trying to explore some clues for further improvements for local delivery systems with aim to increase the possibility of method transformation from bench to bedside for cancer gene therapy.

2. CONVENTIONAL TECHNOLOGIES FOR LOCAL GENE DELIVERY

Conventional technologies for local gene delivery have been widely used in preclinical and clinical researches, such as intratumoral delivery, electrogene therapy and drug-eluting implants [18-20]. Compared to systemic delivery, they don't need strict request on drug property and dosage form, while they can improve drug stability, avoid system

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clearance, increase local efficiency and minimize the toxicity. However, these technologies are limited with therapeutic ranges [21-23], *e.g.*, electroporation is now only available to superficial tumors [24]. In this section, we present recent progress and challenges of these conventional technologies.

2.1. Intratumoral Delivery

As a common treatment strategy for solid tumors, intratumoral delivery belongs to minimally invasive methods, a new competitive sub-specialty within various fields of surgery [25]. It aims to directly infuse chemotherapeutic agents or gene materials into the malignant region, commonly using intratumoral infusion/injection or peritumoral inoculation [26]. The intratumoral method is easy to practice and can significantly increase the local exposure with limited systemic toxicity. They can also produce the least possible damage to structures and achieve the same results by open or more invasive surgery [27]. Intratumoral delivery has been used in treatment of bladder [28], liver [29] and pancreas tumor [30]. Many novel clinical trials of intratumoral gene infusion are also undergoing [13].

Bevacizumab (Avastin), the anti-VEGF antibody approved by U.S. Food and Drug Administration (FDA), is capable of inhibiting angiogenesis and tumor growth through preventing the binding between VEGF and their receptors [7]. But the antibody based drugs are much more expensive and needs multiple injections as compared to chemodrugs [7]. Instead, Watanabe *et al.* [21] developed an AAV-virus gene system for expression of anti-VEGF antibody. They applied this system to lung cancer via intrapleural delivery for lung cancer and got sufficient sustained expression of anti-VEGF antibodies after one single administration. The enhanced and sustained antibodies expression led to reduced metastatic tumor volume and prolonged survival in murine model. The intrapleural administration of AAV-anti-VEGF resulted in sustained and high level of gene expression in lung, no distribution in spleen and liver, indicating less non-target effect and toxicity. Furthermore, clinical experience shows that pleural as the location for gene delivery has little risk of gene or virus-induced inflammation [31]. Taken together, intrapleural delivery is a novel alternative approach of gene therapy for lung cancer.

Similarly, interferon-β (IF-β) has great anti-tumor effect against many malignancies, but the secondary toxicity and short half-time limit its clinical use via system delivery [32]. Shih *et al.* [20] used intravitreal injection of AAV-IF-β for retinoblastoma treatment and observed that AAV-IF-β had potential anti-tumor effect *in vivo* for xenograft. The authors didn't observe detectable spread of the virus outside the eyes. Meijer *et al.* [33] tried to inhibit brain tumor growth by intracerebroventricular delivery of AAV-IF-β. There are no efficient treatments currently available for glioblastomas and both systemic and local drug delivery are limited by the brain-blood-barrier, high interstitial fluid pressure and altered vasculature [34], and the tumors are unaccessible for common administration methods. Intraventricular (ICV) delivery of AAV-IF-β holds great promise to address this problem since it can genetically modify normal brain parenchyma to express IF-β as the reservoir. IF-β can reach high-level around tumors according to the distribution and cerebral spinal fluid flow, resulting in improved survival and inhibited tumor invasive growth. Above all, local gene delivery by tissue-specific intratumoral infusion is an alternative approach for cytokine therapy.

Different from above *in vivo* approach, ex vivo gene delivery provides another option for local cancer immunotherapy [35, 36]. In this approach, autogeneic cells may be isolated, cultured and transduced of gene vectors, so that they are then introduced into a targeted tissue [37]. JM Peron *et al.* [38] genetically modified syngenic fibroblasts to secrete IL-12 using retroviral vector. Transfected fibroblasts then were intrahepatically injected into orthotopic liver tumor of BALB/c mice. During the process, they could improve gene transfer efficiency, enrich transduced cells, assess expression efficiency and exactly tailor therapeutic dose for injection. Like paracrine secretion, direct expression of IL-12 into liver carcinoma induced notable inhibition of tumor growth. Results showed that this effect was associated with activated innate immunity and especially macrophages. And there was no significant treatment-related toxicity. These findings were also confirmed in following researches of murine pancreatic peritoneal carcinomatosis [39]. As expected, relative clinical trials are ready to begin [40].

Intratumoral delivery is effective and widely used, especially for superficial and solid tumors. It is also the basic technology for development of subsequent delivery systems, such as local electrotransfection, implants, ultrasound microbubbles [22, 41]. In spite of its widespread applications, there are several challenges associated with intratumoral delivery, such as invasion and systemic toxicity. These challenges can be addressed through improvement in two aspects, *i.e.*, delivery vehicles and administration route. Previous vehicles can be improved through following areas: 1) engineering virus vectors to enhance transfection efficiency; 2) improving formulations to avoid vectors dissemination, for instance, add poloxamers to increase local viscosity to restrict diffusion [42]; 3) according to therapeutic genes, choosing the particular vectors or delivery regions to utilize immune system, for example, avoid immune response for antibody-gene therapy, but excite local immunity to enhance cytokine therapy. For different tissue structures, specific delivery routes can be designed corresponding to local environments, such as intra-arterial infusion for liver cancer [43] and endoscopic injection for gastrointestinal cancers [44]. We expect intratumoral delivery to be a great strategy for more efficient cancer gene therapy and facilitate rapid developments of novel derivative technologies.

2.2. Electrogene Therapy

For non-viral gene therapy, the efficacy is limited by poor gene convection and diffusion in tumor due to the high interstitial fluid pressure [45] and low transduction of genes into target cells. To increase the transduction efficiency, electric field-mediated gene delivery (electrogene) has been developed and studied both *in vitro* and *in vivo* [45-47]. In this delivery method, cell membrane permeability is increased by an external, pulsed electric field, and then the pass of traditionally non-permeant molecules was increased [22]. Compared to other methods, electrogene, as a non-viral physical method, is safe and does not provoke immune re-

sponses [48]. For example, Chuang *et al.* [48] utilized electroporation to delivery pIL-12 gene in situ for treatment of beagles with the canine transmissible venereal tumor (CTVT). They found that 0.1 mg pIL-12 significantly inhibited the tumor growth and eventually led to complete curing of tumor, without detectable toxicity. Several preclinical and clinical trials are undergoing for evaluating their antitumor efficacy [13, 49, 50].

MicroRNA and small interfering RNA (RNAi) are hot topics in past decade, and has potential application for cancer therapy [51]. However, the poor stability and delivery efficiency of RNAi are barriers for their practical applications. Recently, Vidic *et al.* [19] developed a microRNA gene delivery procedure mediated by electroporation for K-ras mutant colorectal adenocarcinoma and obtained significantly improved antitumor effectiveness *in vitro* and *in vivo*. All these show electrogene therapy is a promising treatment modality for cancer and a powerful adjuvant for other delivery systems.

But further studies are necessary to understand the mechanism of electropermeabilisation and its effect, improve the electrogene equipment for variant tissues, optimize electrical parameters and create new therapeutic strategy of electroporation. There also exist concerns about the changes of cell structure and gene expression in different electric fields, which is directly related to *in vivo* safety and efficacy. Due to the different physiochemical properties of tissues and plasmid constructions, the deep understanding of electropermeabilisation will greatly help to optimize the electrode design, electrical pulse parameters, administration routes and time interval between electroporation and genes injection for individual tumors. We expect these optimizations will increase local transduction efficiency and offer exciting possibilities of future expansion for internal tumor treatments. For example, combination of microelectrodes and endoscopic injection or intra-arterial catheter may be non-invasive and provide controlled electrogene therapy for liver or gastrointestinal tumors. Possibly durative low electric field intensity may promote the plasmid motion in cell interstitium for local cytoplasmic aggregation [45], while nanosecond pulsed electric field with high transient intensity may induce efficient permeation through both cell membrane and nuclear envelope to improve gene expression [24]. Further development of the technologies will permit extensive application of this treatment for more cancers or other diseases.

2.3. Drug-Eluting Polymer Implants

The concentration and persistence of gene materials at target site are the key point for success. Commonly, plasmid and virus vectors are rapidly cleared and repeated delivery is required for intratumoral infusion. But locally humoral and cellular immunity may decrease subsequent efficacies after the first injection [52, 53], Therefore three-dimensional (3D) macroscopic, polymer scaffolds are alternative for regional cancer therapies. As the reservoir, polymers can separate genes from environment, and avoid their degradation and inactivation. Meanwhile, polymers can retain gene materials locally and release them in a sustained manner.

Kangasniemi *et al.* [54] studied the utility of silica gel monoliths for delivering adenovirus vectors. Nearly linear release was observed *in vivo* and virus in silica matrix maintained the biochemical activity in the whole process. More excitingly, virus vectors were able to keep the infective property for weeks at 37℃ and months at 4℃, which was helpful for clinical practice and storage. After implantation into orthotopic pancreatic cancer tumors *in vivo*, gene-silica gel therapy extended doubly the survival of mice without detectable side-effects. Interestingly sustained release also slower antibody formation for adenovirus, which might be useful to facilitate re-administration, peritumoral and intratumoral viral dissemination.

As pre-formed polymer implant, silica-gel needs surgical procedures for local embedding and the elimination of chemical polymers *in vivo* remains unsolved. Hatefi *et al.* [55] designed an injectable in situ-forming implant for adenoviral gene delivery to solid tumor using silk-elastin like protein polymer (SELPs, a genetically engineered biomaterial). Robust hydrogels (sol-to-gel transition) were formed at body temperature. The liquid mixture (matrix and viruses) were injected into the target site with a needle, and then self-solidified into an "implant" *in vivo* [55]. *In vitro* studies indicated that viruses retained release from SELP hydrogel over a period of 4 weeks while preserving their bioactivity. In three xenograft tumor models, adenoviruses showed a regional and long-term expression. This system is helpful to extend application of implants for more internal tumors.

Currently, implants may be an ideal adjunctive modality after cancer surgical treatment [23], but relative designs or modalities need to optimize to achieve safe, effective and long-term therapeutic effects. With the developments of intratumoral methods, more minimally invasive ways are introduced for implant placements. For example, a small cannula is regarded as the adjunctive tool with straightforward access to target site via a minor incision in the skin instead of open surgical procedures. Also we may use computed tomography (CT) or magnetic resonance imaging (MRI) to visually guide the precise implantation without extra wounds. More importantly, a desired release and distribution in tissues is essential for successful treatment. Therefore, we may choose probable polymers and preparation methods to modify gene release, taken into account about local environments, natural diffusion, polymer degradation and implant erosion. We may further use external stimulus for controlled drug release and active tissue penetration after implants placements. Two prominent examples are the use of magnetic fields and ultrasound (see below sections). All these may result in exciting benefits and minimal risk for implant treatment of solid tumors.

3. PHYSICAL GUIDANCE

Virus associated pathogenesis and systemic dissemination [56] may be difficult to completely avoid in above conventional delivery systems. Recently, more emphasis has been put on developing non-viral vectors, which offer the advantages of controllable plasmid DNA production, lower cost, lower immunogenicity and toxicity. But the challenges of specific delivery and non-target effect remain. To address these, various guided delivery methods based on external physical energies (*e.g.*, magnetic field, ultrasound, light) have been developed to indirectly drive non-viral carriers to

target sites and enhance regional cell permeability to improve therapeutic efficiency. The special carriers for delivery could be the reservoirs and protect undesired degradation of DNA/RNA materials. Moreover, administration of vectors into deep tissues can also be achieved through the direction of external energies. As a stimulus, physical energies can also retain particles near tumors without dissemination, and solely trigger releases at target sites. More importantly, magnetic/ultrasound associated equipments are widely used in clinical diagnosis, which are practical, noninvasive and safe. Accordingly, physical guidance delivery has great potential for local gene therapy.

3.1. Ultrasound System

Ultrasound has been widely used in biomedical research and clinical applications [57]. Ultrasound can enhance cell-membrane permeability for the delivery of naked DNA and drug particles [58], possibly due to the creation of transient pores on cells (sonoporation) [59]. But exorbitant-intensity or uncontrollable ultrasound is also destructive for normal tissue. As the echo-contrast agent, gas-encapsulated microbubble/nanobubbles can significantly decrease the ultrasound intensity for *in vivo* application [60]. Further, these nanobubbles can also be simultaneously designed as carriers for gene materials, offering two attracting features. Firstly, in the low-intensity ultrasound field, acoustic pressure impels nanobubbles to move along the ultrasound beam axis without destructing the vehicle integrity. This facilitates the accumulation of nanobubbles at the target site [61], where high-intensity ultrasound will be then applied to induce nanobubbles collapsing, drug release and sonoporation [58, 60]. All these lead to increased delivery-specificity and transfection efficacy of gene materials. Therefore, ultrasound/nanobubble is an alternative modality for gene therapy of acoustically accessible tissues.

Recently, bubble liposomes (liposomes entrapping an ultrasound imaging gas) have been designed as gene delivery carriers into tumors [58]. The gene transfection efficiency by bubbles depended on ultrasound intensity and exposure time. Compared with commercially Lipofectamine 2000, bubble liposomes/ultrasound can induce greater transfection efficiency of luciferase DNA plasmids into mouse ascites tumor cells and solid tumor tissue *in vivo*. In addition, *in vivo* luciferase imaging and hemolysis assay suggested that this gene delivery system can achieve tumor specific delivery. [62]. This system has also been assessed for IL-12 plasmid DNA in cancer gene therapy [62]. After repeated delivery, sufficient IL-12 production triggered immune responses and dramatically suppressed tumor growth.

In previous researches, only fixed intensity of ultrasound was used for bubbles delivery [62-64]. While, alternate ultrasound (dual-intensity) offers high regional concentration and specific gene release, and thus may be a better choice for local gene delivery [65] Fig. (**1**). Studies based on bladder show that bubbles should be firstly in contact with target cells for the efficient delivery, which agreed with observations in vascular endothelium [66]. Ultrasound image and HE-stained results directly demonstrated that lower acoustic pressure can guide the bubbles to move and accumulate at designed areas, Fig. (**1**). In addition, although the transfection efficiency can be increased with repeated exposure to high-intensity ultrasound, multiple sets of dual-intensity ultrasound are more powerful. The assumed mechanism is as follows. Only partial bubbles collapse under high-intensity ultrasound in each pulse, and subsequent sets of low-intensity ultrasound result in further accumulation of remaining nanobubbles to targets sites. The aggregation of bubbles further strengthens sonoporation. Consequently, alternant exposure of dual intensity ultrasound tautologically enhanced gene permeability into the bladder wall. All these indicated appropriate combination of ultrasound intensities would be an attractive method to facilitate high localization and efficiency of cancer gene delivery.

Ultrasound system is especially attractive for real-time imaging and monitoring of delivery location and release for cancer treatment. But inherent problems also need to be addressed. For current bubbles, the poor stability and big particle size directly limit their *in vivo* circulation, distribution and tumor permeability under appropriate ultrasound intensity. More smart nanobubbles are urgently needed to offer better ability through tissue interstitium, and also increase transduction efficiency for a low gene dosage. On the other hand, the potentially harmful effects induced by cavitation under high US intensity should be avoided. To decrease the US intensity, we can possibly screen for stronger echo-contrast agents, or design new ultrasound devices, such as multi-needles, focal plane US field. Meanwhile, improvements of therapeutic strategy for ultrasound may provide unexpected results, such as above dual-intensity combination, ultrasound related hyperthermia for heat-sensitive delivery.

3.2. Magnetofection

Magnetofection, magnetically mediated gene delivery, is another promising technique to enhance the introduction of therapeutic genes into the target cells [67]. Generally, nucleic acids, non-viral and viral vectors are reversibly bound to the superparamagnetic particles, which can be guided and retained to the designated area via a high-energy magnetic field. In contrast with other external physical energies, magnetic field is better in anti-disturbance, safety and operability. For instance, bone-related reflection and gas-related attenuation of ultrasound confines its *in vivo* applicability [68], and photodynamic therapy may provoke the production of cytotoxic free radicals. According to changes of electric currents, different magnetic fields (permanent, gradient, oscillating *etc*) are easy to get. These combinations would lead to the nimble locomotion of magnetic particles, and reinforce the penetrativity into deep tissues [69, 70]. Accordingly, magnetic target may be an alternative or complementary tool for site-specific delivery, even by systemic administration See Fig. (**2**).

For successful transition from benchside to the bedside, many efforts have been focused on the design of new magnetic carriers. Namiki *et al.* [68] reported a magnetoliposome formulation for magnetic targeting, termed LipMag. Due to hydrophobic interaction, oleic acid-coated magnet nanocrystal core can self-assemble with cationic lipid shells in the magnetic fluid. After removal of solvents, LipMag was generated as a lipid-coating magnetic nanostructure, and the lipid shell can be the reservoir for nucleic acids. Compared

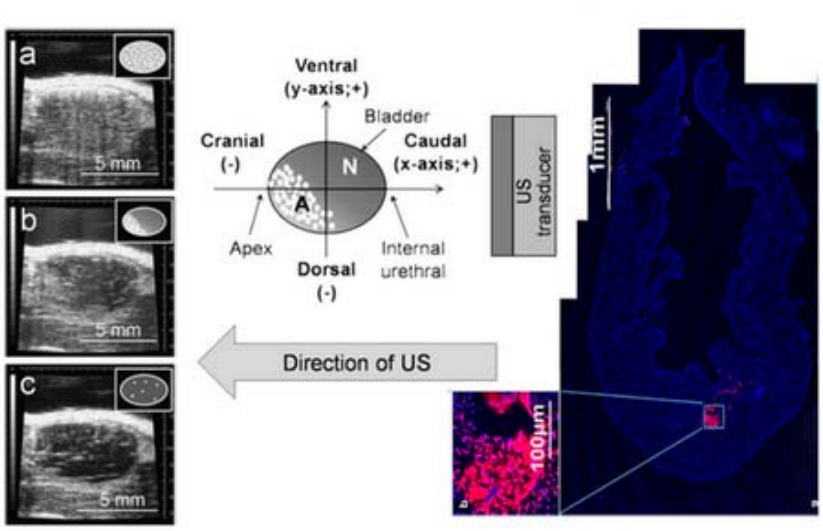

Fig. (1). Illustration of dual-intensity ultrasound for targeting gene delivery. (**a**) the B-mode ultrasound imaging of real-time changes of nanobubbles under alternate ultrasound. At the beginning, nanobubbles distributed throughout the bladder (a, 0s), and then low-intensity ultrasound impelled nanobubbles to aggregate at the designed region (b, 10s). At last, high-intensity ultrasound induced the collapsing of nanbubbles (c, 11s); (**b**) illustration of the experiment; (**c**) the confocal image of the bladder locally transduced by fluorescent genes using nanobubbles and dual-intensity ultrasound. Modified from reference [65].

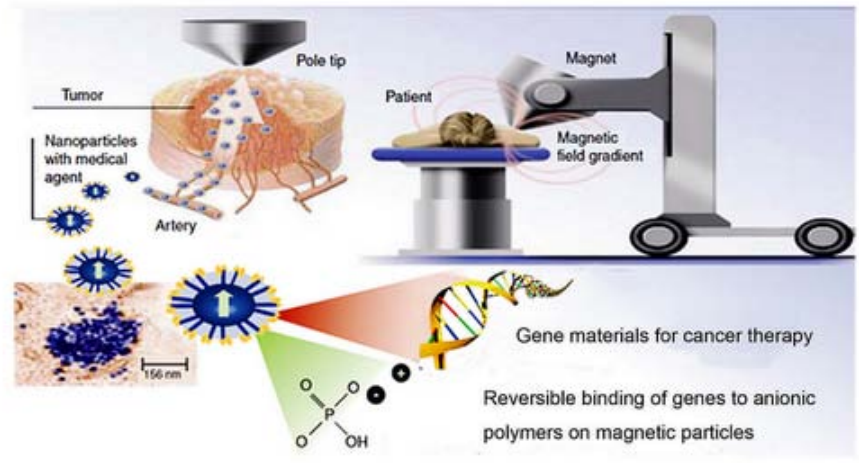

Fig. (2). Magnetofection: under the traction of magnetic field, magnetic carriers migrate into targeting sites and release therapeutic genes. Modified from reference [112].

with commercially polymer-magnetic vector (PolyMag), LipMag displayed a better transfection efficiency and gene silencing effect in 9 of 13 cell lines. Furthermore, with the attraction of the transplantable magnet, LipMag showed great site-specific distribution and anti-tumor effects after intravenous administration to mice bearing tumors. Thus LipMag is worth studying further as a great delivery system.

Beside simplicity of formulation and operability for magnetic field, the surface characteristics of magnetic carriers is another key point for *in vivo* applications. By modification of nanoparticles, researchers expected to prolong the blood half-life, improve the colloidal stability, enhance the recognition and internalization into targeted cells [69, 71, 72]. Chertok *et al.* [73] designed polyethyleneimine (PEI)-modified iron oxide nanoparticles (GPEI) for brain tumor gene delivery. As the high cationic macromolecule, PEI could condense nucleic acids and facilitate endosomal escape to the cytoplasm through the "proton sponge effect" [74]. In this study, GPEI exhibited high cell penetration ability and low cell toxicity. Combined "passive" GPEI presentation with subsequent "active" magnetic capture after intra-carotid administration, nanoparticles displayed the evident accumulation in brain tumor lesions. In order to overcome the rapid plasma clearance of cationic nanoparticles in systemic deliv-

ery, they also developed "non-covalent surface masking" with a conjugate of polyethylene glycol and low molecular weight heparin [72]. Results displayed a 2-fold increase of nanoparticles in orthotopic brain tumors after the magnetic target. Therefore, the artful design for magnetic delivery particles is critical for effective targeting.

Although significant advances in magnetofection have been made, few have entered the stage of clinical trials and many challenges remain to be overcome. Firstly, we may try to design new magnetic delivery vehicles. For magnetic core materials, we may screen more powerful superparamagnetism, in order to prevent particles' self-agglomeration and optimize magnetic force at a smaller dimensions (nano-size) [75]. This is important for the *in vivo* distribution of drug particles, regional locomotion and tissue penetrability. Better coating materials and methods are also critical for improvements of structural stability, prolonged circulation lifetime, biocompatibility, drug-loading, surface recognition and biodegradability. Besides, we should seek for more effective magnetic targeting strategies, such as dynamic magnetic fields. For example, a time-varying/gradient magnetic field superimposed on the permanent field may induce oscillating locomotion of carriers. The non-linear motion may help to break extracelluar barriers and aid tissue penetration. In addition, the adjustable extra energies may improve particle uptake and transfection efficiency. Thus with developments of interdisciplinary, magnetic system may show greatly potential for specific treatment in the future.

4. TUMOR-TROPISM DELIVERY

Tumor-tropism delivery is a novel strategy for cancer targeting, where bioactive vectors spontaneously trail tumors and selectively accumulate at malignant sites. For example, anaerobic bacterium prefers to congregate at the hypoxic and necrotic area of tumor (1000-10000 fold than normal tissues [76, 77]); Mesenchymal stem cells can be specifically recruited to tumor periphery for generating the stroma [78]. In the past decade, many preclinical and clinical trials have been increasingly reported about such live organisms for local gene delivery or cancer therapy, even by system administration [76, 79, 80]. Compared with foregoing technologies, biotic vectors possess many unique advantages. For instance, they have great tumor-targeting potential, especially to capture small metastasis. No external energies are needed for delivery, and they are non-invasive, safe and controllable for *in vivo* studies. More importantly, bioactive vectors can multiply or differentiate in the supportive tumor microenvironments, and achieve long-term anticancer effects. Thus tumor-tropism gene delivery has become an extraordinary tool for cancer treatment.

4.1. Bacteria-Mediated Delivery

Since 1800s, bacterial therapy has been widely studied for cancer therapy [81]. Many studies showed that several facultative/obligate anaerobic bacteria (called tumor tropism) can autonomously migrate, congregate and enter into tumor cells [82]. To avoid harmful induced by bacteria, researchers have exploited attenuated bacteria as delivery agents for anticancer drugs and vectors for gene therapy. With rapid developments recently, bacteria-mediated gene delivery (bactofection) show a series of advantages. Beside the excellent tumor localization, bacteria can multiply and even disseminate within the tumor by bacterial enzyme-mediated ECM degradation [83]. The approach is also safe and controllable *in vivo*, because applied bacteria can be genetically engineered to attenuate self-toxicity and increase sensitiveness to common antibiotics. In addition, tumor specific microenvironment (hypoxic, specific cytokine or else) can be a stimulus for plasmid corresponding promotors, which leads to precise spatial and temporal delivery control [84, 85]. Now, many novel bacteria-mediated gene delivery systems are still under research.

For bacterial delivery, common therapeutic genes include prodrug converting enzyme (suicide genes), cytokine, small interfering RNAs (siRNA) and so on [76, 86-88]. Friedlos *et al.* [87] engineered Salmonella typhimurium VNP2009 with prodrug activating enzyme carboxypetidase G2 (CPG2) expression plasmid. *In vitro* studies demonstrated that after expression of CPG2, prodrugs were activated to induce great cytotoxicity in tumor cells and not in hosts. After intravenous administration, bacteria multiplied within tumor xenografts, and steadily expressed high concentration CPG2 (1~6 units/g). Interestingly, bacteria alone can also reduce the growth of xenografts. The presence of prodrugs further enhanced the inhibition effect. Similar results were also observed with Listeria monocytogenes as a cytosolic protein secretion vector for other two prodrug converting enzymes [89]. In these studies, no obvious side-effects have been detected.

Different from protein products, siRNA is more fragile and has more rigorous requirements for delivery vehicles, such as great gene protection, specific tumor targeting, sustained and long-term delivery, efficient transfection etc. [17]. Recent studies indicated that bioengineered bacteria can be used as alternative for local siRNA delivery *in vivo* [86, 88]. Zhang *et al.* [76] developed the attenuated Salmonella typhimurium carrying plasmid-based STAT3-specific siRNA for inhibition of prostate tumor growth. In a similar manner, bacteria selectively homed to tumor and showed time-dependent distribution and aggregation in the tumor over the liver and spleen (after 1000~5000:1 after 5 days). The antitumor effect was also exerted by combination of Salmonella and Stat3 siRNA expression. In addition, tumor metastases were significantly reduced (about 84%). All these results indicate that tumor-targeting bacteria are very promising candidates for local gene delivery into solid tumors.

To maximize the potency of bacteria-based gene therapy for cancer, more detailed researches are required. Firstly, the possible mechanism of bacterial tumor-tropism is urgently required to be elucidated. This will be greatly helpful to seek for more potential tumor-targeting strains, and contrapuntally engineer bacteria vectors. In the future, we expect that bacterial vectors of gene can exactly capture both primary tumor and small metastasis, with no or minimized gene release in normal tissues. The bacteria associated biosafety is also critical for successful clinical translation. Reports showed that even after removal of lethal genes, mice demonstrated 15%-45% mortality in combinational bacteriolytic therapy [90]. Bacterial reverse mutation also has potential risk of *in vivo* toxicity [91]. Therefore, discovery of more efficient attenua-

tion-methods is urgently needed. Another major concern for bacteria based gene therapy is immunological rejection, which limits its repeated delivery and *in vivo* efficacy [82, 85, 91]. New therapeutic strategies are thus needed. For example, oral administration is preferable for alimentary tracts' therapy due to its high safety and efficacy in bacterial delivery [92]. In addition, efficiency for single delivery needs to be further improved, according to better construction of expression plasmids, such as double prokaryotic-eukaryotic expression and hypoxia-inducible expression. All these further optimization of bacterial delivery system would promote the application potentials.

4.2. Cell-based Gene Delivery

Cell-based therapy is the ideal strategy for cancer treatment, because of the low immunogenicity and toxicity. Recent reports demonstrated that mesenchymal stem cells (MSCs) are particularly attractive vehicles for gene delivery [93, 94]. Contrast with conventional cell therapy, MSCs are relatively easy to isolate, able to self-renew and expand *in vitro* and to specially migrate toward and engraft into tumor sites, even the micrometastases [95]. Although the exact mechanism is still not clear, it may be due to the interaction between cell receptors with cytokines and chemokines secreted from tumor microenvironments [96]. Along with the tumor tropism, MSCs can integrate and persist into the tumor stroma [78]. All these indicated that MSCs are be a promising tool for delivery of various biological agents and localized expression and release of functional products, providing the advantages of improved pharmacokinetics, enhanced tumor suppression and minimized toxicity.

The potential of MSCs-mediated gene therapy has also been shown by the results from brain tumors which are refractory to conventional therapy and unaccessible for common vectors [96-101]. Like bacterial delivery, suicide genes are introduced to MSCs therapy. Chang *et al.* [98] developed the cytosine deaminase (CD) –expressing MSCs for brain tumors. *In vitro* studies indicated the system worked via by stander effect and the enzyme activity/level was critical for clinical efficacy. So tumor suppression succeeded *in vivo* only by multiple transplantations and sufficient prodrug supply. This effect was further confirmed by Herpes simplex virus-thymidine kinase (HSV-tk)-expressing MSCs [100], where gap junction intracellular communication capability of AT-MSC with tumors cells was found to play a significant role in the tumor targeting and bystander effect. Thus more detailed works are required for enhancing therapeutic effect for brain tumors.

Direct delivery of anti-neoplastic cytokines (IL-2, IL-12, IFN-β etc) [102-106] by MSCs is another potential therapy for cancer treatment. Kim *et al.* [99] engineered human umbilical cord blood-derived MSCs to deliver TRAIL via adenoviral transduction. According to *in vitro* coculture and *in vivo* experiments, MSC-TRAIL showed great migratory capacity toward gliomas and pronounced therapeutic efficiency in the intracranial xenograft mouse model. This delivery system was further improved by introducing the tetracycline promoter for expression control and high efficient lentivirus system for transduction [80]. As a result, besides the significant tumor inhibition and survival prolongation, MSCs-TRAIL was localized to metastases regions and eliminated about 38% metastases in the pulmonary metastasis model. Therefore, MSCs are possibly potential vehicles for local gene delivery in the treatment of both primary tumors and their metastases.

In spite of all these promising results, there is a long way from bench to bedside for MSC-mediated delivery. There is a concern about MSC biosafety, since little is known about its *in vivo* biology. For example, it was found that MSCs may initiate or enhance tumor developments and progression [95, 107]. Therefore, it is very important to monitor MSCs before the clinical applications. Innovative, rigorous and normative isolation and culture protocols are still needed to prevent MSCs' transformation during *in vitro* passages [95]. Moreover, as normal and endogenous elements, MSCs are also sensitive to cell damage induced by common viral vectors and therapeutic genes. Till now, only limited genes and vectors are available for cancer treatment. In addition, unlike bacterial delivery, cost-effectiveness needs to be resolved for routine clinical use. Although MSC-based approach is associated with these challenges, it still holds great potential as a treatment modality for incurable invasive cancers.

5. CONCLUSIONS

Effective gene delivery method is still one of the most important challenges for successful gene therapy of cancer and local gene delivery holds the potential to address this challenge. In this review, we summarized current progress for local gene delivery, their applications and potential challenges. Different routes have their respective advantages and limitations (Table **1**). Conventional technologies for local gene delivery are usually simple and effective, but are limited with specified therapy range, such as superficial tumors. Gene delivery methods based on acoustic and magnetic forces could provide precise spatial and temporal control of gene targeting, even to internal cancers. However, further optimizations are required to improve the safety, pharmacokinetic profile or tumor targeting. In tumor-tropism delivery, bacteria and MSC provide potent modalities for incurable and unaccessible tumors, such as gliomas. But this may involve concerns about the tropism mechanisms and biosafety. Therefore, to optimize each element for every technology and beat out the possible mechanism for delivery *in vivo* is extremely urgent, which can provide individual guidance for practical application. With developments of multidisciplinary incorporation, combination therapeutics will be one compelling new strategy. For example, magnetic implants were inserted directly into interesting site, which created a permanent and powerful internal local magnetic field gradient. This showed promising and effective local treatment in long term [108].

Compared to system administration, local gene delivery possesses a number of unique advantages. Gene stability is significantly improved without systemic clearance since gene distribution would be limited into a specific area. Gene transfection efficiency is notably enhanced and long-term gene expression within the tumor can be achieved. Toxicity and immune response can be reduced by local therapy. For different cancer types, even the unaccessible and refractory tumors, individual technology is available to achieve better

Table 1. Advantages and Limitations of Local Gene Delivery Systems

Local delivery Systems	Advantages	Limitations	References
Conventional technologies			
Intratumoral delivery	Simplicity, practicability and safety	Low efficiency; virus dissemination; limited therapeutic range	[20, 21, 23]
Electrogene therapy	High efficiency; low immune responses; specific and repeated deliveries	Limited therapy range; particular equipment; optimization of electrical parameters	[19, 45, 46, 48, 110]
Polymer implants	Protection for genes/vectors; sustained release and long-time therapy	Invasive; limited therapy range; in vivo safety for polymers	[23, 42, 54, 55]
Physical guidance			
Ultrasound system	controlled release; Site-specific delivery; real-time monitoring	Optimization of ultrasound system; potentially harmful effects by high US intensity stability and tissue permeability of micro/nanobubble;	[58, 59, 61, 62, 65]
Magnetofection	Exactly spatial control; better operability; in vivo safety	Little researches about magnetic fields; optimization of magnetic vehicles (magnetic core and coating)	[68-70, 73, 111]
Tumor-tropism vector			
Bactofection	Tumor-tropism; multiply within tumors; high efficiency; in vivo safety	little understand about the tropic mechanism; potential bacteria-associated risk (immunologic rejection; reverse mutation)	[76, 87-89]
MSCs-based delivery	Tumor-tropism; in vivo safety; capturing micrometastases; low immunogenicity and toxicity	Limited vectors and cytokines; cost-effectiveness; little understand about MSCs in vivo (potential enhancement of tumor progression)	[80, 95, 99, 100, 102, 103, 105]

efficacy, such as inhalation for lung [109], intracerebroventricular fusion [33], MSCs for metastases [80]. Therefore, local gene delivery is the booming, efficient, hypotoxic way for cancer therapy. In the future, depending on characteristics of therapeutic genes and cancer environments, suitable choice of local gene delivery strategies may accelerate the successful translation from bench to bedside.

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