REVIEW

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Gene delivery for lung cancer using nonviral gene vectors

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Multiple options for the treatment of lung cancer have often been described in the past, including surgery, chemotherapy and radiation, but the therapeutic effect is typically transient and mostly absent with advanced disease. New approaches to the treatment of lung cancer are urgently needed. Gene therapy has been widely proposed as a novel strategy to improve therapy. Although progress has been made using viral vectors, rapid advances in transfection technologies employing nonviral vectors, together with their relatively low toxicity, suggest that nonviral vectors may have significant potential for clinical applications. This paper briefly reviews general principles of gene delivery with emphasis on recent developments in the arena of lung cancer using nonviral vectors (naked DNA, polycationic polymers, cationic liposomes). Employing gene transfer techniques to achieve therapeutically useful levels of expression of therapeutic genes in the lung could provide a new strategy for treatment of lung cancer.

1. Introduction

Lung cancer is still considered the leading cause of cancer-related deaths all over the world. Despite improvements in cancer diagnosis and therapy, the present 5-year survival rate of 14% is only slightly higher than the survival rate of 8% in the early 1960s (Dincer et al. 2005). Although progress has been made, with advances in surgery, chemotherapy and radiation, the majority of patients diagnosed with lung cancer ultimately surrender themselves to despire. A major problem with the present treatment modalities is the lack of tumor specificity giving rise to dose-limiting toxicity and side effects. Gene therapy constitutes an experimental approach gaining increased attention as a putative future cancer therapeutic strategy (Wagner et al. 1990). Gene therapy for lung cancer has attracted a great deal of attention since the first report of successful gene delivery 15 years ago (Simoes et al. 2005). The key technological impediment to successful gene therapy is vector optimization. Gene transfer vectors can be divided into two categories: viral (e.g. retrovirus, adenovirus, adeno-associated virus, vaccinia virus etc.) and non-viral (e.g. naked DNA, polycationic polymers, cationic liposomes) agents. Viral methods of gene delivery are efficient, but they suffer from several drawbacks, including a need for packaging cell lines (Lollo et al. 2000), problems with safety, such as mutation (Marshall 1995; Romano 2005), carcinogenesis (Powell et al. 1999; Check 2003), and the elicitation of an immune response that render transgene expression transient (Freimuth 2003; Lefesvre et al. 1996; Chen et al. 2003; Jooss and Chirmule 2003; Zaiss et al. 2002). In addition, viral vectors are limited by the size of the foreign gene that can be inserted into the viral genome (Labhasetwar et al. 1999). In light of these concerns, nonviral gene transfer systems seem to be more applicable in that they are less toxic, less immunogenic, easier to prepare, they could be ideal methods for *in vivo* gene therapy (Schmidt-Wolf and Schmidt-Wolf 2003; Makiya and Mitsuru 2002), meanwhile, they can be efficient for systematic delivery. Recent advances in vector technology have made nonviral gene therapy approaches particularly appealing for the disease with high mortality such as lung cancer. In this article, we will review the major nonviral vectors that are currently used in gene therapy for lung cancer.

2. Naked DNA

The simplest approach to nonviral delivery systems is direct gene transfer with naked plasmid DNA. Plasmids are able to transfect a broad range of cell types, they are easily produced in a large scale, the size of the gene insert may be large, and plasmids are very safe. However, transfection efficiency with plasmids is generally low and expression is only transient (van der Wouden et al. 2004), thus, attempts in lung cancer gene therapy have been made to enhance the efficiency via the application of jet-injection and electroporation. Lewis-lung carcinoma bearing mice received five jet injections into the tumor at a pressure of 3.0 bar, delivering 3–5 microl plasmid DNA (1 mg DNA/μl in water) per single jet injection. Analysis of tumor cryosections revealed moderate LacZ or GFP expression at 48 h and strong reporter gene expression 72 h and 96 h after jet injection. The simultaneous jet injection of the TNF-alpha and LacZ carrying vectors demonstrated efficient expression and secretion of both the cytokine, as well as LacZ expression within the tumor 24 h, 48 h, 72 h, 96 h and 120 h after jet injection (Walther et al. 2001).

Electroporation, the controlled electric fields to facilitate cell permeabilization, was first described in the 1960s (Li and Ma 2001), and it has been firstly applied to introduce genes into respiratory epithelial cells in 1988 (Iannuzzi et al.1988). To increase the levels of pulmonary gene transfer by nonviral vectors, Dean et al. have adopted electroporation protocols for use in the lung, the results provide evidence that electroporation is a safe and effective means for introducing naked DNA into the lung and form the basis for future studies on targeted pulmonary gene therapy (Dean et al. 2003). Similarly, there have been reports of lung cancer therapy via gene gun (Nishitani et al. 2000) or combination of nuclease inhibitor (Glasspool-Malone et al. 2002), resulting in high gene expression.

3. Polycationic polymers

Even if it is successful, there are several limitations of using naked plasmid DNA as a gene delivery vehicle, such as the poor entry into the cell (Kawabata et al. 1995), lack of effective transport into the nucleus (Kamiya et al. 2001), and the number and proportion of tumor cells that can be transducted by plasmids is low (Vile and Russel 1994). Furthermore, it is highly prone to tissue clearance and totally inefficient for systematic delivery (El-Aneed 2004). A variety of novel synthetic vectors are emerging for in vivo testing to improve the efficiency of gene delivery, including DNA complexed with polycationic polymers such as poly-L-lysine, polyethyleneimine, chitosan.

3.1. Polyethyleneimine

Polyethyleneimine (PEI), a cationic polymer, is a vector that has attracted much attention since it was first shown to be effective both in tissue culture and *in vivo* (Gautam et al. 2000). The most prominent feature of PEI is its high cationic charge density. Every third atom of PEI is a nitrogen atom capable of protonation. This leads to an extremely high cationic charge density of 20–25 microequivalents per gram (Neu et al. 2005). Abdallah et al. (1996) have shown that the transfection efficiencies of PEI in vivo were influenced by the molecular weights, and found that PEI with a reported molecular weight of 25 kD yielded higher transfection efficiency than PEIs of higher reported molecular weights. PEI has also been used to efficiently deliver DNA to tumors *in vivo* (Gautam et al. 2000). Despite the fact that PEI was reported to be relatively effective in cancer therapy *in vivo* by intravenous injection (Kim et al. 2004), intratumoral injection (Wolschek et al. 2002) or micropump administration (Coll et al. 1999), gene delivery through inhalation may provide a means of treatment for a wide range of pulmonary disorders because it can reach large surface areas and avoid risks associated with other systemic administration methods (Kim et al. 2004).

The mice bearing osteosarcoma lung metastases were treated twice weekly for 2 weeks with aerosolized PEI containing the murine IL-12 gene (PEI:IL-12; 600 μl PEI and 2 mg IL-12), aerosol therapy for 2 weeks resulted in high expression of both the p35 and p40 subunits of IL-12 in the lungs but not in the livers of mice. Peak IL-12 mRNA expression was seen 24 h after a single aerosol PEI:IL-12 treatment. This expression gradually decreased with continued detection for up to 7 days. IL-12 protein was not detectable in plasma even after 6 weeks of aerosol therapy. The number of lung metastases in mice treated with aerosol PEI:IL-12 was decreased significantly (Jia et al. 2003).

Viral vectors are more efficient than plasmids entering the nucleus because they can target nuclear receptors with specific viral proteins, thus, many of peptides are derived from viral proteins, have been exploited to boost delivery of plasmids (Gomez-Vargas and Hortelano 2004). Similarly, in order to improve the transfection and targeting efficiency of PEI-based formulations, PEI also have been conjugated to specific viral proteins. It has been shown that an oligopeptide related to the protein transduction domain of HIV-1 TAT was covalently coupled to 25 kDa PEI through PEG resulting in a TAT–PEG–PEI conjugate. Transfection efficiencies of both PEI and TAT–PEG–PEI polyplexes with DNA were studied under *in vitro* conditions (A549) and in mice after intratracheal instillation. While luciferase expression in A549 cells was much lower for-TAT–PEG–PEI (0.2 ng/mg protein) than for PEI (2 ng/mg), significantly higher transfection efficiencies for TAT–PEG–PEI were detected in mice (Kleemann et al. 2005). In addition, conjugation of PEI with ligands such as glycose, has been shown to enhance targeting of specific cell types with a high degree of specificity. There have been reports of the aerosol containing glucosylated PEI and recombinant plasmid pcDNA3.0-phosphatase and tensin homologue deleted on chromosome 10 (PTEN) complex was delivered into K-ras null lung cancer model mice through a nose-only inhalation system, resulting in high expression of PTEN protein in mice lungs (Kim et al. 2004).

3.2. Poly-L-lysine

One of the first polycations characterized as a potential nonviral vector for DNA condensation was poly(L)-lysine (Ogris and Wagner 2002). Meanwhile, poly-L-lysine and its derivates are the most widely reported polypeptides employed for gene delivery. In order to require successful transfection efficiency, poly-L-lysine and its derivatives usually have been used with chloroquine (Joubert et al. 2003), receptor ligands (Wu et al. 2002), or covalently linked to pluronic, both PEG and palmitoyl groups (El-Aneed 2004). Moreover, poly-L-lysine can enhance viral mediated gene transfer efficiency (Nguyen et al. 1997; Schwarzenberger et al. 2001). When complexes of epidermal growth factor/poly-L-lysine conjugate and DNA were incubated with several different lung cancer cell lines, high levels of gene expression resulted when uptake was performed in the presence of the endosomal lysis agent (Cristiano et al. 1996).

3.3. Chitosan

In contrast to the abundance of structurally different synthetic non-viral gene delivery vectors (PEI, PLL, etc.), there is only a small number of polycations of natural origin available (Borchard 2001). Chitosan, a natural-based polymer obtained by alkaline deacetylation of chitin, is nontoxic, biocompatible, and biodegradable (Prabaharan and Mano 2005), which can form poly-electrolyte complexes with DNA (Borchard 2001). Therefore, chitosan and chitosan derivatives may represent potentially safe and efficient cationic carriers for gene delivery.

Gene powders with chitosan are a useful pulmonary gene delivery system, which is a noninvasive and novel option for gene delivery. Chitosan-pDNA powder with an N/P ratio=5 increased the luciferase activity to 2700% of that of the Cytomegalovirus promoter (pCMV-Luc) solution. These results suggest that the addition of chitosan can suppress the degradation of pDNA, and increase the yield

of powders (Hirokazu et al. 2003). Although few gene therapy trials aimed directly at lung cancer using chitosan and its derivatives have been commenced, there is a lot of basic research in this field (Regnstrom et al. 2006; Koping-Hoggard et al. 2005; Koping-Hoggard et al. 2001; Hirokazu et al. 2003). We believe that lung cancer is a very intriguing candidate for gene therapy using the natural-based polymer-chitosan.

4. Cationic liposomes

Liposomes have attracted much attention as potential drug carriers. Liposomes are easy to prepare, highly biocompatible and can be loaded with a broad variety of drugs, DNA and diagnostic agents (Torchilin 2005). However, as a rule, transfection by the use of anionic or neutral liposomes is not very efficient, and requires entrapment of DNA inside vesicles (Li and Ma 2001). On the whole, these liposomes are transfection-efficient when applied *in vitro* and much less efficient being applied *in vivo*. Consequently, cationic liposomes mediated gene transfer and delivery attract great attention as a result of their positive charge on the particle surface ensures their binding to the negatively charged cellular membranes. Cationic lipid-based delivery systems can be efficient for gene delivery if the composition of the DNA-lipid complexes is properly controlled (Liu et al. 1997). However, some authors recently showed that size, not surface charge, is a major determinant of the *in vitro* lipofection efficiency of lipoplex. They considered that area-specific gene expression in lung metastases may be achieved by controlling the physicochemical properties (pDNA to lipid ratio) of the lipoplex (Li et al. 2005). Despite cationic liposomes can be toxic when administered at high doses in cell culture, no toxicity or inflammatory reactions has been reported using liposomes in humans (Templeton and Lasic 1999).

Ramesh et al. described an improved extruded DOTAP:cholesterol (DOTAP:Chol) cationic liposome that efficiently delivers therapeutic tumor suppressor genes p53 and FHIT, which are frequently altered in lung cancer, to localized human primary lung cancers and to experimental disseminated metastases. Transgene expression was observed in 25% of tumor cells per tumor in primary tumors and 10% in disseminated tumors. When treated with DOTAP:Chol-p53 and -FHIT complex, significant suppression was observed in both primary and metastatic lung tumor growth. Furthermore, repeated multiple treatments revealed a 2.5-fold increase in gene expression and increased therapeutic efficacy compared to single treatment. Finally, animal survival experiments revealed prolonged survival when treated with liposome-p53 DNA complex (Ramesh et al. 2001). Aid components, such as protein (Yanagihara and Cheng 1999; Sorgi et al. 1997; Vaysse et al. 2002), or certain amino acids (Li et al. 2005), can be used to enhance gene efficiency and promote selective targeting to the lung. Current cationic liposomes are able to achieve high transfection ratios and are notable for concentrated biodistribution to the pulmonary system when given intravenously (Dow et al. 2005; Li et al. 2005; Ito et al. 2004), percutaneously (Saito et al. 2000), intratracheally (Zou et al. 2000), etc. Moverover, with the development of novel cationic lipids, such as pyridinium cationic lipids (Ilies et al. 2005), the application of cationic liposomes in lung cancer become more and more promising. Lipid based-nanoparticles are also used for gene therapy for lung cancer.

The use of DOTAP:cholesterol (DOTAP:Chol) nanoparticles coupled to gene has been reported. Ramesh et al. demonstrated that DC (DOTAP:Chol) nanoparticles effectively deliver tumor suppressor genes to primary and disseminated lung tumors. They evaluated nanoparticle-mediated delivery of the human mda-7/IL-24 gene to primary and disseminated lung tumors *in vivo*. They demonstrate that DOTAP:Chol efficiently delivers the mda-7/IL-24 gene to human lung tumor xenografts, resulting in suppression of tumor growth (Ramesh et al. 2004). Although DOTAP:Chol nanoparticles complexed to DNA (DNA-nanoparticles) are efficient vectors for systemic therapy, induction of an inflammatory response in a dose-dependent fashion has also been observed thereby limiting its use (Gopalan et al. 2004). Gopalan et al. demonstrated that systemic administration of DNA-nanoparticles induced multiple signaling molecules both *in vitro* and *in vivo* that are associated with inflammation. Use of small molecule inhibitors against the signaling molecules resulted in their suppression and thereby reduced inflammation without affecting transgene expression (Gopalan et al. 2004).

5. Conclusion

Gene therapy has the potential to become an important modality for treating lung cancer. Consequently, recent preclinical studies of lung cancer have reported promising results. It is encouraging to confirm that the efficacy of nonviral vectors has now improved to achieve cell levels that would be curative in human patients. Nevertheless, improved gene delivery systems would be necessary before efficient and safe nonviral gene therapy of lung cancer is realized. The effect of the physical and chemical properties of the various nonviral delivery systems on the efficiency of each step must also be adequately understood because vector optimization to improve the efficiency of one step in the process may be detrimental to the effectiveness of another (Wiethoef and Middaugh 2003). As a result, the safety and clear expression mechanism is very important to the nonviral gene therapy trial for lung cancer in the future.

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