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Vectors for pulmonary gene therapy

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ABSTRACT

The success of gene transfer in preclinical animal models and proof of principle clinical studies has made gene therapy an attractive concept for disease treatment. A variety of diseases affecting the lung are candidates for gene therapy. Delivery of genes to the lungs seems to be straightforward, because of the easy accessibility of epithelial cells via the airways. However, efficient delivery and expression of the therapeutic transgene at levels sufficient to result in phenotypic correction of the diseased state have proven elusive. This review presents a brief summary about current status and future prospects in the development of viral and non-viral strategies for pulmonary gene therapy.

1. Introduction

The lung is an important target organ for gene therapy of many acute and chronic diseases including acute respiratory distress syndrome (ARDS), cancer, asthma, emphysema, cystic fibrosis (CF), alpha 1-antitrypsin deficiency and surfactant protein B (SP-B) deficiency. Gene therapy is particularly attractive for diseases that currently do not have satisfactory treatment options, and is probably more easily applied for monogenetic disorders than for complex diseases like asthma or cancer. The lung is a complex organ and can be roughly divided into the conducting large and small airways (trachea, bronchi, bronchioles), and the parenchyma (gas-exchanging alveolar cells) (Breeze and Wheeldon, 1977). The requirement of gene transfer into individual cell types is dictated by the target disease. In pulmonary gene therapy, the delivery of nucleic acid cargo is limited by the pulmonary architecture, clearance mechanisms, immune activation and the presence of respiratory mucus (Gill et al., 2004). Besides these barriers, basic issues like efficiency of gene delivery, duration of transgene expression, and the toxicity of the gene delivery vectors themselves, are subjects of intense research. Phase I/II clinical trials have already demonstrated the feasibility of lung gene transfer and, with the development of more efficient gene transfer agents, it is hoped that therapeutically viable gene therapies will soon be available (Davis and Cooper, 2007). Numerous viral and non-viral vectors have been developed for lung gene transfer. However, there are a number of limitations associated with the use of both of these vectors (Zhang and Godbey, 2006; Mctaggart and Al-Rubeai, 2002; Gorecki, 2001). Clinical trials with viral vectors such as adenoviruses and adeno-associated viruses have shown that they are unsuited for repeated dosing, as the immune response reduces the effectiveness of each subsequent dose (Lee et al., 2005). Non-viral approaches, such as cationic liposomes or cationic polymers, appear to be more suited for repeated dosing, but have been less effective overall, when compared to viral counterparts. Further, non-degradable polymers like polyethylenimine bear the risk of accumulation in the body, in particular after repeated dosing. Therefore increasing amount of attention is being paid to the development of nontoxic, biodegradable (Luten et al., 2008) and sequence-defined polymers (Schaffert and Wagner, 2008). This review gives an overview of viral and non-viral vectors applied to pulmonary gene therapy.

2. Viral vectors for pulmonary gene therapy

A variety of viral gene transfer vectors have been evaluated in the lung. Major research in pulmonary gene therapy has focused on retroviral (including lentiviral), adenoviral (Ad) and adeno-associated viral (AAV) vectors. Many of these viral vectors are efficient at transducing different lung cells but they all induce an immunological response to some degree and may have safety risks, such as insertional mutagenesis (Hacein-Bey-Abina et al., 2003). Furthermore, their capacity is limited and they are unsuited for repeated dosing (Davis and Cooper, 2007).

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A retrovirus is an RNA virus that is replicated in a host cell via the enzyme reverse transcriptase to produce DNA from its RNA genome. The DNA is then incorporated into the host's genome by an integrase enzyme. The virus thereafter replicates as part of the host cell's DNA. Retroviral vectors are capable of long-term gene expression following genomic integration, but have limited applicability because they fail to efficiently transduce the non-dividing, terminally differentiated cells that make up the bulk of the lung (Wang et al., 1998). The development of lentiviral vectors, a subclass of retrovirus that can transduce terminally differentiated cells, has overcome some of the limitations of the earlier used retroviral vectors (Trono, 2000). Two major types of lentiviral vectors have been tested in airway delivery models including those engineered from human immunodeficiency virus (HIV) (Goldman et al., 1997; Buckley et al., 2008) and feline immunodeficiency virus (Wang et al., 1999).

Adenovirus, a double-stranded DNA virus, that has been extensively used as a non-integrating vector in lung, transduces a wide variety of proliferating and non-proliferating cells (Cao et al., 2004; St George, 2003) and shows tropism for airway cells. The first generation adenoviral vectors (FGAd) with the E1 region deleted have been the most extensively used vector for pulmonary gene transfer. Although once thought to be an ideal vector for lung gene therapy, more than a decade of research has revealed a number of serious shortcomings and the enthusiasm for FGAd has diminished: first, pulmonary delivery of FGAd in small animals, large animals, and humans is inefficient (Grubb et al., 1994; Harvey et al., 1999; Joseph et al., 2001; Perricone et al., 2001; Zuckerman et al., 1999). Second, pulmonary delivery of FGAd resulted in dose-dependent inflammation and pneumonia (Harvey et al., 2002; Simon et al., 1993; Wilmott et al., 1996; Yei et al., 1994). Significant improvement in the safety and efficacy of adenoviral-based vectors came with the development of helper-dependent adenoviral vectors (HDAd), which are deleted of all viral coding sequences (Palmer and Ng, 2005). HDAd vectors are replication defective, retain only a small packaging signal and inverted terminal repeats in order to reduce the host immune response to viral gene products (Hartigan-O'connor et al., 2002). Application of HDAd vectors in animal models of pulmonary diseases would provide vital information for their applicability in the clinic. Unlike the genome integrating retroviruses, adenoviral vectors remain as episomal elements in the nucleus of the host cell and consequently there is a minimal risk of insertional mutagenesis.

An adeno-associated virus (AAV) is a non-pathogenic parvovirus, which needs a helper virus (usually an adenovirus) for proliferation. Major interest in AAV has been due to their lack of pathogenicity, prolonged gene expression and their capability to transduce both dividing and non-dividing cells, and in the absence of a helper virus, to integrate into a specific locus of the host genome at a high frequency (Hamilton et al., 2004). When used as a vector (rAAV), the *rep* and *cap* genes of the virus are replaced by the transgene and its associated regulatory sequences resulting in predominantly episomal persistence (Smith, 2008). Comparison of AAV serotypes 2, 3, 5 and 6 *in vivo* with respect to their airway transduction potential, found AAV6 to be the most efficient (Halbert et al., 2000, 2001). AAV vectors tested in the airways show potential for persistent expression whilst being maintained as an episome, or integrated into the genome (Flotte, 2005; Tal, 2000). In a study, Seiler et al. (Seiler et al., 2006) showed that vectors having AAV type 5 or 6 capsids show high transduction rates in airway epithelial cells, in a range that should be sufficient for treating lung disease.

3. Non-viral methods for DNA transfer

In addition to the above described viral vectors, various non-viral vectors have been developed and applied for gene transfer to the lung. In general, synthetic vectors are thought to circumvent concerns raised by immunogenicity and safety issues of viral vectors, while offering the potential for repeated administration and large-scale production. They have no limitation in DNA size for packing and the possibility of modification with ligands for tissue- or cell-specific targeting. Non-viral gene delivery systems can be divided into three categories: naked DNA delivery, lipid-based (lipoplexes) and polymer-based (polyplexes) delivery. Among these three, lipoplexes and polyplexes have been extensively used in achieving gene transfer to the lungs (Davis and Cooper, 2007). Physical methods like electroporation enable delivery of naked plasmid DNA by physical force such as electricity into target cells. Dean et al. (Dean et al., 2003) showed high-level non-viral gene transfer in the lung of mice by electroporation.

Cationic lipids are amphipathic molecules with hydrophobic tail groups and a positively charged head group capable of interacting with the negatively charged backbone of DNA (Marshall et al., 2000) and have been widely tested in the airways. In previous studies, direct intratracheal administration of lipoplexes led to efficient transfection of the mouse airways (Oudrhiri et al., 1997; Guillaume-Gable et al., 1998; Griesenbach et al., 1998). Successful transfection of the lungs *in vivo* has also been observed when the lipoplexes were delivered intravenously (Barron et al., 1999; Li and Huang, 1997). In clinical setting however, cationic cholesterol derivates have been hampered by their relative low transfection efficiency *in vivo* and concerns regarding their proinflammatory activity (Noone et al., 2000; Ruiz et al., 2001). Over the years, it has become clear that unmethylated CpG motifs of DNA are the major inducers of inflammation (Hyde et al., 2008). Although lipoplexes often show high level of transgene expression, following direct administration or injection into target tissues, their non-specific membrane binding usually precludes cell-selective targeting. Moreover, their positively charged surface leads to interactions with plasma proteins and other extracellular proteins, which bind non-specifically to the lipoplexes and inactivate them (Urtti et al., 2000; Ernst et al., 1999; Rosenecker et al., 2003). In this regard, protein-resistant lipoplexes have been developed (Faneca et al., 2004; Papanicolaou et al., 2004; Takahashi et al., 2005).

Polymer-based gene delivery systems like poly(L-lysine) (PLL), polyethylenimine (PEI), biodegradable polycations, polysaccharide-based systems (cyclodextrin, chitosan) or other polycation-based gene delivery systems e.g. poly(2-(dimethylamino)ethyl metacrylate) (pDMAEMA) are widely used in the field of gene delivery (Park et al., 2006). Cationic polymer-based vectors condense DNA, which offers protection from degradation and facilitates release from endosomes. PEI and PLL have been reported to promote gene transfection into lungs. PEI has been extensively tested in animal models and shows transfection up to 5% of pulmonary cells after intravenous administration (Dif et al., 2006). This is due to the strong positive charge of nucleic acid PEI complexes (Bragonzi et al., 2000; Goula et al., 2000) and the fact that after injection into a periphery vein, lungs contain the first capillary bed that must be traversed.

4. Vector delivery to the lungs

In the airways, depending on the route of administration, transfected cells are epithelial cells at the bronchial and/or alveolar levels, as well as, macrophages and endothelial cells (Griesenbach et al., 2004). The choice of the route of administration depends on the vector to be delivered and the cells to be transfected. For example, viral vectors are almost always delivered via the airways for pulmonary gene delivery. Table 1 summarizes the different application routes used with the various viral vectors in mice and human clinical trials. In the case of non-viral vectors, for transfecting airway epithelial cells (nasal and conducting airways) use of

Table 1
Viral vectors for pulmonary gene delivery.

Viral vector	Pseudotype/subtype	Route of administration	References
LVV[a]	Filovirus envelope protein	Intratracheal	Kobinger et al. (2001)
LVV[a]	Baculoviral gp64 glycoprotein	Intranasal	Buckley et al. (2008)
LVV[a]	Vesicular stomatits virus glycoprotein	Intranasal	Buckley et al. (2008)
LVV[a]	F and HN gylcoproteins of Sendai virus	Intranasal	Shirohzu et al. (2004) and Kobayashi et al. (2003)
Adenovirus	Serotype 5	Intratracheal	Zhou et al. (2006) and Zuckerman et al. (1999)
AAV[b]	Types 1 and 5	Intratracheal	Liu et al. (2009)
AAV[b]	Type 6	Intranasal, intratracheal	Limberis et al. (2009)
AAV[b]	Type 9	Intranasal, intratracheal	Limberis and Wilson (2006)
Sendai virus	–	Intranasal	Ferrari et al. (2007)

[a] Lentiviral vector.
[b] Adeno-associated virus.

Table 2
Non-viral vectors for pulmonary gene delivery.

Non-viral vectors	Route of administration	References
Naked plasmid DNA	Intranasal	Zabner et al. (1997)
Electroporation	Intratracheal	Dean et al. (2003) and Zhou et al. (2008)
Lipoplexes	Intravenous	Barron et al. (1999)
	Aerosol	Ruiz et al. (2001)
	Intranasal	Hyde et al. (2000)
	Aerosol	Davies et al. (2008) and Rudolph et al. (2005)
	Intravenous	Goula et al. (2000) and Kihara et al. (2003)
Polyplexes	Intratracheal	Koping-Hoggard et al. (2004), Kukowska-Latallo et al. (2000) and Ziady et al. (2003)
	Intranasal	Ziady et al. (2003)

intravenous route is ineffective. However, if alveolar epithelial cells are the target, intravenous route is the method of choice, though aerosol delivery is also effective. Different routes of application used with non-viral vectors are presented in Table 2.

Several procedures have been used to administer cationic non-viral vectors to mouse airways, including instillation of a liquid bolus (Rudolph et al., 2000; Bragonzi et al., 2000) and aerosol delivery via jet nebulisation (Rudolph et al., 2005; Densmore et al., 2000; Davies et al., 2008). Many reports also focus on the effect of various ligands conjugated to DNA-binding cationic polymers/lipids to enable specific targeting to different cell types, enhance gene delivery and reduce toxicity. Numerous targeting ligands for example small chemical compounds (carbohydrates) (Weiss et al., 2006), drugs (Elfinger et al., 2009) or synthetic peptide (Tagalakis et al., 2008) and proteins (lactoferrin, growth factors or antibodies) (Elfinger et al., 2007; Kloeckner et al., 2006) have been investigated.

Plasmid DNA is the central component of non-viral gene delivery. Choice of plasmid backbone has been shown to influence the transgene expression (level and duration), and immune response post-delivery (due to CpG motifs) (Chen et al., 2004). In other studies choice of promoter has been shown to be the determining factor in achieving prolonged expression in lung tissue (Gill et al., 2001; Yew et al., 2001). Novel non-viral integration-based technologies have been developed such as the phage ϕC31 integrase and Sleeping Beauty transposon that allow either the expression cassette or the desired donor plasmid to be integrated into host chromosomes thus prolonging the expression of the gene in lung tissue (Aneja et al., 2007; Belur et al., 2003).

The first polymeric gene carriers, including PEI and polylysine derivates, have already been tested in clinical trials, focusing on local administration to tumors (PEI in bladder carcinoma) (Sidi et al., 2008), or regional delivery to airway epithelium (PEG-polylysine for cystic fibrosis) (Konstan et al., 2004). Nevertheless, obstacles including low efficiency, polymer polydispersity and poorly understood delivery mechanisms still have to be overcome for polymer-based gene therapy. In conjunction with the use of PEI as a polymer-based gene delivery system, cytotoxicity of PEI is a serious limitation and therefore modifications of PEI may be necessary to reduce toxic properties of PEI (Wen et al., 2009). Another alternative could be to use biocompatible systems, for example, chitosan-poly-(e-caprolactone) based nanoparticles (Haas et al., 2005). In a related study Kumar et al. using cationic SiO_2 nanoparticles showed successful gene transfer in the mouse lung. Moreover very low or no cell toxicity was observed suggesting silica nanoparticles as potential alternatives for pulmonary gene transfer (Ravi Kumar et al., 2004).

The field has pursued numerous strategies to make pulmonary gene therapy more safe, specific and efficient. The development of chemically dynamic polyplexes, including biodegradablility, incorporation of cell targeting ligands, surface shielding together with improved chemistry for syntheses of polymers with uniform size and topology is an important tool to overcome previous problems.

5. Conclusion

Success of gene therapy for pulmonary diseases depends on the development of vectors which are capable of aerosolization, are nontoxic, allow repeated dosing, and are sufficiently efficient for a therapeutic benefit. The requirement for repeated dosing has focused attention on non-viral vectors, and the requirement to minimize toxicity for carrier–DNA complexes has driven investigators to reengineer plasmid DNA to reduce inflammatory sequences. Besides these criteria, it is critical that vector production is reproducible and sufficiently chemically defined as to be of pharmaceutical quality. Modification of the plasmid DNA resulted in vectors which show less toxicitiy and longer duration of transgene expression. These improved vectors appear to be sufficient for partial and possibly therapeutic correction of the genetic defect. Improvements in gene carriers may reduce the required therapeutic doses, thereby reducing dose-related toxicities. Development of biodegradable vectors may circumvent accumulation in the body. For DNA nanoparticles, addition of targeting ligands to the complexes may improve the specificity of gene transfer to airway or alveolar epithelium, may permit lower doses to be effective, and may address structural lung barriers. In summary, pulmonary gene

therapy has been demonstrated using various non-viral and viral vectors but the clinical translation of that knowledge still needs to be attained.

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