Recent Progress in Non-viral Gene Delivery

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1 Introduction

Gene therapy provides a unique approach to medicine as it can be adapted towards the treatment of both inherited and acquired diseases. Gene delivery relies upon the encapsulation of a gene of interest, which is then ideally delivered to target cells. After uptake up by endocytosis, the DNA must be released into the cell so that transcription and translation may occur to produce the protein of interest. To achieve successful gene delivery, significant barriers must be overcome at each step of this process in order to optimize gene activity while minimizing the potential for inhibitory inflammatory responses.

Particular interest has been paid in recent years to the development of efficient non-viral vectors. Viral vectors (i.e., retroviruses and adenoviruses) may provide superior gene delivery to target cells compared to their non-viral counterparts, but viral vectors also come with the significantly increased risk of triggering a specific immune response, which under extreme circumstances could result in death (Lehrman 1999; Marshall 1999). Non-viral vectors may trigger an inflammatory response but are not likely to elicit specific recognition, making these types of vectors less hazardous in terms of antigen-specific immune responses. Although non-viral vectors are more appealing in this respect, there are several other factors that must be considered in vector design, including specific cell targeting, optimized uptake, and efficient intracellular release of the vector, in addition to minimizing the immune response. The development of novel non-viral vectors intended to optimize one or more of these aspects of gene delivery will be discussed briefly in this chapter.

2 Delivery of Naked DNA by Physical Methods

Naked plasmid DNA provides a promising mechanism for gene delivery as it is less immunogenic than most non-viral vectors currently used. Complications arise, however, in that naked plasmid DNA has no target-specific recognition and is more susceptible to nuclease degradation than encapsulated DNA. These issues limit the

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quantity of naked DNA that is able to reach the target cells, thereby limiting the efficiency of gene expression. Much effort has been made in recent years to overcome these limitations by developing new methods to effectively deliver naked DNA to target cells.

Delivery of naked DNA has been promoted through the application of electric or magnetic fields to the target areas. Electroporation involves injection of the naked DNA, followed by the application of an electric field over the targeted tissue. The electric field increases the permeability of the cell membranes and allows for increased uptake of the naked DNA into the cells. Compared to injection alone, electroporation has been found to increase gene expression by several orders of magnitude (Ndoye et al. 2004; Wells 2004). Efforts have been made specifically to reduce tissue damage associated with electroporation techniques. Modifications such as the application of low voltages, pulses of high voltage followed by low voltage, and the development of new electrodes have been shown to increase gene uptake and reduce damage compared to previous methods (Bureau et al. 2000, 2004; Liu and Huang 2002; Zhang et al. 2002). Electroporation is particularly useful for delivering DNA to superficial areas, but it requires invasive surgical procedures to deliver DNA to specific organs. Magnetic fields have also been employed as a mechanism to deliver DNA to targeted areas. Magnetofection involves attaching DNA onto a magnetic nanoparticle coated with a cationic polymer (i.e., PEI) (Huth et al. 2004; Scherer et al. 2002). In vitro studies indicated that the presence of a magnetic field increased the rate of transfection from hours to minutes compared to the controls (Scherer et al. 2002).

Laser beam gene transduction (LBST) aids in the delivery of naked DNA by following injection with several short pulses from a laser beam directed towards the targeted area. As described by Ziera et al., delivery by LBST resulted in gene expression that was comparable to that induced by electroporation (Zeira et al. 2003). The advantage of this method compared to electroporation is that histological analysis revealed minimal tissue damage as a result of LBST, whereas tissues treated by electroporation had substantial damage.

Clinical ultrasonic methods have been found to increase gene expression in naked DNA delivery. Cavitation resulting from the ultrasonic waves increases the permeability of the cells, allowing for more efficient gene delivery. High- and low-level ultrasonic waves have been investigated for increasing DNA delivery to a variety of tissues, such as muscle and heart as well as to tumors (Wells 2004). Further studies of gene delivery by sonoporation have found that the presence of microbubbles, which act as contrast agents by reflecting the ultrasonic waves, promote efficient gene expression (Unger et al. 2004). Advances have also been made in target-specific delivery by incorporation of specific ligands onto the surface of the microbubbles.

The use of high pressure to deliver naked DNA has led to several new and modified methods for non-viral gene delivery, including modification of particle bombardment techniques (i.e., gene gun), jet injection, mechanical massage and hydrodynamic injection. Recent efforts have yielded the production of a modified gene gun in which DNA coated on gold particles is introduced into the target tissue by a high-pressure helium blast. Gene expression was increased several orders of magnitude and tissue penetration more than doubled compared to delivery by the conventional gene gun (Dileo et al. 2003b). Jet injection, by contrast, involves the injection of low volumes of DNA solution at high pressures. Gene expression has been observed

at depths of up to 10 mm in targeted tissues after delivery by a high-speed jet injector and transfection efficiencies were similar to those observed using particle bombardment techniques (Walther et al. 2001). DNA delivery by particle bombardment and jet injection are limited to a relatively small target area compared to many other gene delivery methods.

Hydrodynamic injection allows DNA delivery to larger target regions and is not limited to superficial tissue (Zhang et al. 2001, 2004; Maruyama et al. 2002). This method involves high-pressure injection of a large volume of solution containing the DNA of interest. Gene expression was found to increase, particularly in hepatocytes, as a result of defects in the cells resulting from the high-pressure injection (Zhang et al. 2004). A main drawback to this technique is that the large injection volume can lead to side effects, such as high blood pressure and low heart rate, and possibly death. Alternatively, mechanical massage of the liver (MML) has recently been found to successfully deliver naked DNA to hepatocytes in a manner that has the potential for less lethal side effects (Liu et al. 2004a).

3 Cationic Polymer-Based Non-viral Vectors

Encapsulation of naked DNA by cationic polymers to form polyplexes is one of the primary non-viral vector systems that has been examined for the optimization of gene delivery. Generally, the cationic polymer first functions as a condensing agent, to collapse DNA into compact bundles of a size suitable for delivery. After the complex arrives at the target cells, the polymer must promote the release of DNA from the endosomal compartments into the cell. To accomplish these tasks, the cationic polymer must form a strong association with the DNA but release it under the appropriate conditions as well as have the ability to recognize specific cells. Polymers frequently used for DNA encapsulation and delivery include polyethylenimine (PEI), poly-L-lysine, cationic dendrimers, and arginine-rich proteins or peptides (i.e., protamines, HIV-TAT) (Pannier and Shea 2004; Wagner 2004). In recent years, efforts have been directed towards optimizing the polymer to become more efficient at these functions, often by increasing the biocompatibility, primarily through modifications to existing polymers.

Polyethlyenimines (PEI) have evolved into a prominent cationic polymer for gene delivery. PEI condenses plasmid DNA into compact particles and protects them from nuclease degradation during gene delivery (Ferrari et al. 1999). Once encapsulated into the endosome, PEI has the ability to act as a proton sponge, which destabilizes the endosomal compartment and allows release of the DNA into the cytoplasm (Kircheis et al. 2001). The ability to facilitate DNA release into the cell is a major asset, as release into the cytoplasm is one of the main barriers encountered in gene delivery. Recently, PEI has been modified to contain various degrees of branching and chain lengths, as well as by methylation of the polymer to a charged quaternary ammonium derivative. The quaternary ammonium derivative was found to decrease the toxicity of the molecule by up to four-fold (Brownlie et al. 2004).

The formation of novel block copolymers has also led to the development of more efficient, less toxic delivery agents. Polyethylene glycol (PEG) has been linked to PEI and has been shown to decrease toxicity by nearly ten-fold as compared to PEI alone.

By linking the polymers to a more biocompatible molecule (e.g. PEG), the copolymers are less toxic but maintain other positive characteristics originally observed with the cationic polymer alone. PEG has also been linked to poly-L-lysine, polyamidoamine and poly-histidine, among others (Ahn et al. 2004; Miyata et al. 2004; Putnam et al. 2003; Rackstraw et al. 2002). Furthermore, some novel copolymers have also been developed. The copolymer polyhydroxyproline (PHLP) has been shown to maintain sustained gene activity but with reduced toxicity due to the biodegradable nature of the molecule (Li and Huang 2004). The development of copolymers allows for the formation of novel structures that maintain the characteristics of the individual components, which in return allows for the formation of optimized polymers. Advances in the development of novel copolymers have provided promising options for biocompatible and efficient non-viral gene delivery vectors.

Polyplexes may also be modified by the addition of lipids to form lipopolyplexes. One of the more studied systems incorporates lipid with protamine molecules to encapsulate DNA and form complexes of lipid-protamine-DNA (LPD). LPD vectors have been found to have increased gene activity compared to lipid-based vectors (Li and Huang 1997; Li et al. 1998). These vectors have recently been adapted to contain a target ligand, asialofetuin (AF), which aids in delivery to cells containing a receptor for this molecule, particularly heptatocytes. The incorporation of the target ligand increased gene expression by nearly ten-fold over the lipid-based vector in the absence of ligand and polymer (Arangoa et al. 2003). Additionally, recent studies have shown that modified LPD vectors may be useful in cancer treatment (Dileo et al. 2003a; Whitmore et al. 2001). Thus, it is evident that LPD vectors are multifaceted and that further modifications of these vectors may provide successful gene delivery systems for a wide variety of treatments.

4 Lipid-Based Non-viral Vectors

Liposomes and DNA combine to form complexes referred to as lipoplexes. Since the initial investigations of lipoplexes nearly 20 years ago by Felgner et al., various cationic lipids and combinations of cationic and neutral lipids have been thoroughly examined for their potential as non-viral vectors (Felgner et al. 1987). In recent years, optimization of lipoplexes has focused primarily on improving the targeting of these vectors to specific cells as well as increasing the release of the DNA into the cytoplasm. These modifications in lipoplex composition have significantly improved vector association with specific cells as well as DNA expression by working to overcome several significant barriers limiting successful gene delivery.

Lipoplexes have been designed to target cell types that contain specific receptors or have a higher propensity to recognize certain molecules than other cells. Ligands recognized by the target cells are incorporated into the liposomes. The ability to target cancer cells has been the main focus of numerous studies, as in many cases the cells are not necessarily localized. Both folate and transferrin receptors are known to be over expressed on cancer cells, so vectors have been designed to be recognized by these receptors. By incorporating the ligands, vectors were shown to have an increased affinity for tumor tissue in both instances (Dauty et al. 2002; Xu et al. 2002; Zuber et al. 2003). Further investigation of ligand incorporation may allow for increased specificity for gene delivery, thereby increasing the gene expression observed in the target cells.

Improved targeting of vectors to specific cells increases gene expression, but lipoplex modifications have also been aimed towards increasing DNA release inside the cell. This aim has proven multifaceted, in that by working to modify vectors to breakdown intracellularly, new vectors tend to be more biocompatible and do not elicit as severe an immune response from the recipient. Many liposomes are being engineered such that under a particular condition (e.g. low pH, reducing environments) the vector will become defective and allow DNA to leak out of the complex (Asokan and Cho 2002; Guo and Szoka 2003; Thomas and Tirrell 2000; Yatvin et al. 1980). A primary example is novel lipids designed to contain cysteine residues that can form stable disulfide linkages (i.e. ligand binding) under most conditions, but are sensitive to reducing conditions (Guo and Szoka 2001, 2003; Dauty et al. 2001; Kwok et al. 2003). These vectors may contain a ligand that assists in the delivery of the vector to specific cells then, once the vector is released into the cytoplasm, the environment will reduce the disulfide linkages and allow the DNA to escape. Similar systems relying on the addition of moieties that are sensitive to intracellular conditions, which trigger the release of DNA have also been developed for cationic polymer based vectors (Kwok et al. 2003; McKenzie et al. 2000; Erbacher et al. 1999).

5 Toxicity of Non-viral Vectors

Non-viral vectors, although less lethal than viral vectors, still may elicit a strong, non-specific immune response. Toxicity frequently results from characteristics of the encapsulating polymer or lipid such as the length, saturation, or branching of the polymer (Ahn et al. 2004; Kramer et al. 2004). Efforts to reduce the toxicity of non-viral vectors have largely resulted in efforts to make the vectors more biodegradable and biocompatible. Many of the aforementioned systems (i.e. triggered release with disulfides, PEG copolymers) incorporated more biologically active components into the system, thereby reducing the immune response induced by delivery of the vector. Further efforts to reduce toxicity have involved the incorporation of molecules known to suppress the production of the cytokine, NF-κB. These "safeplexes" were able to maintain low levels of tumor necrosis factor (TNF-α) as compared to lipoplex alone, while achieving comparable levels of gene expression (Liu et al. 2004b). Another method explored by Tan et al. found to significantly reduce toxicity requires the sequential injection of liposome, then DNA as opposed to delivery of the lipoplex. By injecting the liposome prior to the plasmid, cytokine levels (IL-12, TNF-α) were reduced by greater than 80% compared to lipoplex (Tan et al. 2001). Thus, significant advances have been made towards decreasing the toxicity of these non-viral vectors.

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