Status of Therapeutic Gene Transfer to Treat Cardiovascular Disease in Dogs and Cats

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KEYWORDS

• Cardiomyopathy • Animal model • Heart disease • Heart failure • Gene transfer
• Gene therapy

KEY POINTS

- Therapeutic gene delivery is used to treat inherited and acquired heart diseases by targeting a missing or defective gene (inherited disease) or modifying a deranged molecular pathway (acquired disease).
- Preclinical studies in large animal models of heart disease suggest various candidate transgenes may be effective in companion animals with naturally occurring heart disease.
- Multiple clinical trials have been completed or are underway in humans with heart disease with encouraging results.
- A clinical trial in Dobermans with dilated cardiomyopathy and congestive heart failure is planned to begin in 2017.

Therapeutic gene delivery involves introducing recombinant genetic material to a patient to alter levels of the gene product either directly or indirectly. Although most current gene therapy clinical trials focus on cancer and inherited diseases, multiple studies have evaluated the efficacy of gene therapy to abrogate various forms of heart disease. It is a particularly promising modality because the understanding of the molecular changes that occur with heart disease and heart failure has grown. One goal of gene transfer is to express a functional gene when the endogenous gene is inactive. Alternatively, complex diseases, such as end-stage heart failure, are characterized by several molecular abnormalities at the cellular level, many of which can be targeted using gene delivery approaches. Thus, gene delivery may effectively treat inherited and acquired heart diseases.

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There are also variable potential goals of therapy to be considered with gene delivery. For example, the optimal treatment effect might be local (ie, within the myocardium) or systemic (circulating in the blood). Obvious target cells for feline and canine cardiovascular disease are cardiomyocytes; however distal cell types (eg, liver), could be used for protein production and systemic release. Because of advances in molecular cardiology, the molecular and cellular pathways involved in the progression of cardiovascular disease have been elucidated and many potential targets are being studied in animal models and human clinical trials. This article focuses primarily on results from studies in large animal models and human clinical trials.

PACKAGING OF THE GENE

In general, viral vectors are more efficient that nonviral vectors (DNA plasmids or minicircles that are devoid of bacterial sequences) for gene delivery, although various techniques (eg, electroporation, ultrasound-targeted microbubbles) have been used to increase efficiency of nonviral vectors.[1–4] Viral vectors bind to the host cell and introduce their genetic material into the host cell. With rare exceptions, the various vector types are devoid of viral genes and only contain the gene or genes of interest (the therapeutic or candidate gene), often together with other elements, such as promoters. As a result, these viral vectors are rendered replication-deficient and, therefore, are only capable of transferring the therapeutic gene without risk of viral replication and/or lytic infection. However, other factors, such as immune response, remain challenging.[4] Multiple types of viral vectors have been used for this packaging purpose, and each has positive and negative aspects for its use in gene transfer.

To increase transduction (protein production) in the target cells, the transgene is usually delivered with gene regulatory elements: a promoter ± enhancers.[5] The promoter sequence usually lies upstream of the transgene and it controls gene expression. Cardiac-specific promoters are used to focus expression of the transgene in the heart. Cytomegalovirus is a commonly used promoter that is a constitutive viral promoter. Although cytomegalovirus results in robust expression, it is not cardiac specific. Cardiac-specific promoters may be safer if they result in the transgene remaining silent if it is inadvertently delivered to noncardiac tissue. These cardiac-specific promoters (ie, myosin light chain 2v or cardiac troponin T) are cardiac specific, but less potent than cytomegalovirus.[5] An miRNA-regulated cassette that selectively represses gene expression in noncardiac tissue is an alternative approach to minimize off target transduction.[6] Enhancers are other gene regulatory sequences that are added to increase cardiac specificity.

Retroviruses (lentiviruses) are efficient at transferring genetic material to the host cell (transduction); however, their application in cardiovascular disease is more limited given their poor transduction of myocardium.[6] Another concern is that they can integrate randomly into the target cell genome, thereby carrying a risk of triggering "insertional mutagenesis" (if the insertion occurs within the regulatory or coding sequence of an endogenous gene, gene expression can be disrupted or enhanced). If the disrupted gene happens to be one that regulates cell division, uncontrolled cell division (neoplasia) can result. The risk of insertional mutagenesis may be lower in cardiomyocytes because they are terminally differentiated, but it remains a concern.[6]

Adenoviruses are nonintegrating double-stranded DNA vectors that enter cells predominantly through clathrin-mediated endocytosis. In the heart, adenovirus vector transduction is robust, but transient (usually about 1–2 weeks). These vectors are capable of packaging larger genes (>30 kb). The genetic material they carry is not incorporated into the host cell's DNA, but remains free in the nucleus (episomal). It