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Commentary

Gene targeting in ischemic heart disease and failure: Translational and clinical studies

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ABSTRACT

Alternative and innovative targeted strategies hold relevance in improving the current treatments for ischemic heart disease (IHD). One potential treatment modality, gene targeting, may provide a unique alternative to current IHD therapies. The principal function of gene targeting in IHD is to augment the expression of an endogenous gene through amplification of an exogenous gene, delivered by a plasmid or a viral vector to enhance myocardial perfusion, and limit the long-term sequelae. The initial clinical studies of gene targeting in IHD were focused upon induction of angiogenic factors and the outcomes were equivocal. Nevertheless, significant advancements have been made in viral vectors, mode of delivery, and potentially relevant targets for IHD. Several of these advancements, particularly with a focus on translational large animal studies, are the focus of this review. The development of novel vectors with prolonged transduction efficiency and minimal inflammation, coupled with hybrid perfusion-mapping delivery devices, and improving the safety of vector use and efficacy of gene systems are but a few of the exciting progresses that are likely to proceed to clinical studies in the near future.

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1. Introduction

Ischemic heart disease (IHD) can be defined as that which arises from inadequate myocardial perfusion, which can be acute and transient in nature, such as that associated with an acute coronary syndrome and myocardial infarction (MI), or chronic suboptimal perfusion, which can occur with disease states such as ischemic dilated cardiomyopathy. In either case, IHD is associated with significant changes in the structure and function of both the vascular and myocardial compartments, which is continuous and progressive. While there has been significant and appropriate focus upon the vascular compartment with IHD in terms of thrombolytics, coronary stents, and revascularization, as well as antiplatelet and lipid lowering therapies, much less progress has been made in pharmacological treatments, which directly affect myocardial structure in this context. IHD is invariably associated with changes in LV geometry and myocardial composition, generically termed LV remodeling, and is a significant and independent predictor for morbidity and mortality [1–4]. For example, while early recovery following an MI is excellent, LV remodeling often occurs and is a structural milestone in the progression to heart failure (HF) [4]. Current pharmacological therapy in the post-MI setting is primarily focused upon the interruption of neurohormonal pathways, which includes the systemic delivery of beta-adrenergic and angiotensin-II receptor system inhibitors [5]. However, the post-MI remodeling process can continue unabated in patients receiving current standard of care pharmacotherapy. One potential therapeutic approach would be to target the remodeling myocardium directly, such as that of gene targeting. In this approach, targeting specific biological systems that are relevant to adverse LV remodeling and progressive dysfunction can be achieved and therefore provide a unique alternative or adjunct to current IHD therapies. Gene targeting involves the overexpression or inhibition of a specific gene or translational modifier in order to address the underlying mechanisms that cause LV remodeling and/or dysfunction in IHD. There have been significant advancements in the approaches for expressing a specific gene of interest into the myocardium, many of which have progressed to early clinical trials. The overall goal of this review is to examine some issues regarding gene targeting relevant to myocardial delivery and examine specific examples

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of pathways and systems that have been manipulated by these delivery approaches in the context of LV remodeling and HF. There is a large body of translational and clinical research regarding gene targeting and vascular remodeling, particularly as it applies to vascular stents, grafts, and restenosis [6–8]. The focus of this review will not be on these applications per se, but rather on those delivery systems and targets that have been used to affect LV myocardial structure and/or function, with a particular emphasis on those studies targeting LV remodeling and dysfunction with IHD.

2. Expression vectors in gene targeting and relevance to myocardial delivery

To effectively promote successful gene transfer and subsequent expression in a host cell, careful consideration must be given to the choice of DNA delivery vector. The two vector types most commonly examined for translational and clinical studies are naked DNA plasmids and viral vectors. In choosing a plasmid or viral vector, it is important to consider the type of cell that is being altered and the longevity of expression needed to induce an effect in the targeted host cells while limiting the side effects that can occur. In terms of LV remodeling and IHD, the targeted cells may be of a transient nature, such as inflammatory cells, but most often are targeting two distinct cell types: the cardiocyte and the fibroblast. It must be emphasized that significant efforts are underway to modify the vascular smooth muscle cell and endothelial cells of the coronary and systemic vasculature, but this will not be the focus of this review. Rather, potential delivery systems and vectors that would allow for targeting the LV myocardial cells (cardiocytes, fibroblasts) will be briefly reviewed in terms of advantages and disadvantages, with a summary provided in Table 1.

2.1. Plasmid gene delivery

Gene targeting using plasmid vectors was primarily developed to diminish the immunogenic side effects that exist with viral mediated gene expression [9,10]. Plasmids are circular, double-stranded, naked DNA molecules that vary in size from 1 kilobase (kb) to greater than 1000 kb. A plasmid can hold 1–10 kb of the targeted gene of interest, but the longer the foreign DNA is in kb, the more difficult the plasmid is to transfect efficaciously into a host cell. To effectively deliver exogenous genes using plasmid DNA, multiple extracellular and cellular obstacles must be overcome, which include avoiding the degradation by nucleases in the extracellular space [11], difficulty crossing the cell membrane [9], and degradation by nucleases and lysosomes in the cytoplasm of the host cell [10]. All of these impediments contribute to low transfection efficiency and must be factored into vector choice. One advancement in the non-viral approach is the use of cationic liposomes. When mixed with the negatively charged plasmid DNA, the cationic liposome surrounds the plasmid DNA and facilitates interaction with the negatively charged glycoproteins and proteoglycans on the host cell surface [12]. The liposome complexed with the plasmid DNA can then be internalized through the active process of endocytosis, but it can also enter the host cell through passive processes, such as pinocytosis. Typically, plasmids remain extrachromosomal within the nucleus and do not integrate into the genome of the host cell, and therefore have a short period of transduction.

2.2. Viral gene delivery

The major advantage of viral vectors over plasmid DNA alone is the higher transduction efficiency because of the ability of a virus to actively deliver DNA across the host cell membrane and into the nucleus. A previous large animal study compared gene expression of LacZ in the myocardium delivered by a viral vector versus a plasmid–liposome complex [13]. The study demonstrated a 100-fold increase in gene expression when using the virus [13]. Thus, the understanding of the complex structural features and mechanisms of action of various viral vectors remains an important consideration when trying to overexpress exogenous genes. Of the viral vectors that have been studied previously, the most widely used in gene targeting are the replication-deficient adenoviruses (AdV), the adeno-associated viruses (AAV), and the retroviruses.

2.2.1. Adenovirus

The AdV is a non-enveloped virus with an icosahedral capsid that surrounds a linear, double-stranded DNA genome. The capsid has 12 capsomers, which consist of a spherical base and a long fiber that may help in binding host cell membrane receptors. The genome ranges from 26 to 40 kb in length, and it is comprised of two major transcription regions flanked by inverted terminal repeats. One of these major transcription regions contains four transcription units, E1, E2, E3, and E4, which are important for transcription of the viral genome and viral replication. In creating AdV vectors for gene targeting, the E1 and portions of the E3 regions are deleted to render the virus replication-deficient. Importantly, these deletions, along with other non-essential viral DNA components, provide space to allow up to approximately 36 kb of foreign exogenous DNA to be

Table 1
Vectors and systems for gene targeting and myocardial delivery.

		Advantages	Disadvantages
Non-Viral	Plasmids	Simplified construction, versatility of expression cassette size, favorable biosafety risk profiles	Low transduction efficiency, transient expression
	Liposomes	Allow for targeting at the cell surface, ultrasound manipulation possible, favorable biosafety risk profiles	More complex construction, usually requires competent blood flow, transient expression
Viral	Replication-deficient adenoviruses	Large DNA packaging possible, enters both dividing and non-dividing cells, well suited for myocardial delivery	Serotype immunogenicity, potential cytotoxicity, transient expression
	Adeno-associated virus	Greater stability than adenovirus, enters both dividing and non-dividing cells, well suited for myocardial delivery, longer periods of expression, reduced immunogenicity	DNA insertion possible causing oncogenic potential, smaller DNA packaging
	Lentivirus	Integrates into host DNA for long-term expression, minimal immunogenicity	Smaller DNA packaging, high tropism for dividing cells, insertional mutagenesis