Genes, Cells, and miRNAs

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Abstract

Novel methods to treat HF include gene, cell and microRNA delivery. There have been few gene therapy trials in HF. The CUPID I and II trials with AAV1.SERCA2a showed unconvincing results. The STOP-HF trial using direct intra-myocardial injection of a non-integrating plasmid vector carrying stromal cell-derived factor-1 showed no clinical effects and the AC6 gene transfer trial using IC infusion of escalating doses of Ad5.hAC6 was similarly negative. Despite high hopes stem cell trials to date in HF have not shown convincing clinical benefits. More recent clinical trials in this area have investigated the injection of cell stimulating paracrine factors (peptides, small molecules, hormone-like molecules) without actual cell delivery. Micro-RNA's (miRNAs) are small non-coding RNA strands, comprising 19-25 nucleotides, that have a distinct signalling role and a various patterns of expression in ischaemic myocardium, hypertrophy, cardiomyopathies, and overt HF. They act at the post-transcriptional regulation level. Numerous novel miRNAs have been discovered, and their in-depth role has been characterised. miRNAs can serve as therapeutic substances or therapeutic targets in a range of cardiovascular diseases. Clinical trials are likely in the near future.

Keywords: heart failure; gene therapy; cell therapy; micoRNA

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Introduction

Cardiac gene therapies

Recently, novel methods have emerged that have widened the opportunities to employ a gene therapeutic approach in the treatment of cardiovascular diseases. To optimally employ such strategies an in-depth knowledge of the transcriptome background of the major cardiovascular diseases will be required. Genetic abnormalities in a wide range of cardiovascular disease are well-known and well-described, but a full description of these are beyond the scope of this chapter (e.g. hypertrophic cardiomyopathy or familial hypercholesterolemia).

The cornerstone of future human applications of gene therapy is an efficient and safe vector for gene delivery. The ideal vector should be highly efficient, preferably non-viral, and non-integrating into the host genome. The first human clinical trials employing gene therapy in the cardiovascular field were performed with an adenoviral vector (AAV). Non-viral vectors, however, generally have demonstrated low-efficacy. Recent methods have targeted plasmid-based techniques.[1] Recently Penny and Hammond have reviewed gene based therapy for heart failure (HF) by searching PubMed and ClinicalTrials.gov (without language restrictions) for clinical trials published between January 1990 and October 2016, using the search terms "gene transfer" (and "gene therapy") and "heart failure" and related terms.[2] They found multiple preclinical and some early clinical investigations, but only 4 randomised clinical trials utilising a gene therapeutic approach for HF. These trials mainly used exogenous transfer of a therapeutic gene product with the aim of improving either vascular or myocyte function in the failing hearts, either with intracoronary delivery of a virus vector or direct endocardial injection of a plasmid.

Although there has been a high degree of scientific interest in the field and attention in particular directed at the CUPID I and II trials which aimed to modify myocardial sarcoplasmic reticulum calcium-ATPase (SERCA) in an effort to improve myocardial contractility via an increase in intracellular calcium concentration in the cardiomyocytes of the failing heart, emerging clinical evidence to date has been inconsistent. The phase 2 CUPID trial in 39 HF patients (24 active, 15 placebo) used escalating doses of intra-coronary adeno-associated virus coding for sarcoplasmic reticulum calcium ATPase codes (AAV1.SERCA2a).[3] It showed nominally significant effects on LVEDV and cardiovascular events, which given its small size could not be considered evidence of benefit, especially when the larger CUPID-2 study (in 243 HFrEF patients) comparing high dose AAV1.SERCA2a to placebo showed no clinical or pathophysiological benefit.[4]

The STOP-HF trial used direct intra-myocardial injection of a non-integrating plasmid vector carrying stromal cell-derived factor-1 in an attempt to enhance the activation of cardiac resident stem cells within the failing myocardium. In a trial of 93 ischaemic HF patients no effect on either symptoms or 6 min corridor walk test distance was demonstrated.[5]

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The AC6 gene transfer trial used IC infusion of escalating doses of adenovirus-5 encoding adenylyl cyclase 6 (Ad5.hAC6) in 42 HFrEF patients versus placebo in 14.[6] It showed no between group differences in either LVEF or exercise tolerance but a difference at 4 weeks in LV peak –dP/dt, but not in LV peak +dP/dt. Thus whilst the possibility of haemodynamic benefits has been established no consistent trial outcomes have been achieved to date by gene therapy in HF patients.

Further animal models and translational studies have aimed to improve the efficiency of gene delivery by methods such as magnetic beads attached to the AAV vector which can drive the homing of therapeutic substances using external magnetic fields[7] or by the use of exogenous cells (e.g. mesenchymal stem cells) as vectors of therapeutic genes in a complex regenerative product. Other gene therapeutic approaches that may offer utility, at least in ischaemic HF, are factors which promote angiogenesis such as VEGF, angiopoietin, and hypoxia-inducible factor alpha-1.

Generally, the studies conducted to date for cardiac gene transfer have shown no major safety signals of concern, in terms of major adverse events related to the gene transfer process. Arrhythmias and mild troponin rises were more commonly seen in those subjects receiving direct endomyocardial injections. Higher doses of gene delivery and more efficient non-viral transfection methods may be needed in larger clinical trials to show clinical benefit in patients with HF.

Stem cells and endogenous cellular responses

Another therapeutic area that has received a lot of scientific interest is that of stem cell therapy for cardiovascular diseases. Despite the high hopes in terms of a possible successful regeneration of the heart, trials to date have not shown convincing clinical benefits. The human heart comprises billions of cells with distinct functions (electro-mechanical conduction, pacemaker function, contraction) and characteristics, such as atrial and ventricular myocytes, Purkinje cells, sinus node cells, cardiofibroblasts, paracrine cells, to name but a few, which are extremely hard to develop in a dish.

The first clinical investigations in this field used cardiac and peripheral myoblasts, which are now not considered sufficiently safe or feasible for larger scale clinical use. Cardiac resident stem cells possess a low capacity for self-renewal, and thus can rarely efficiently regenerate the muscle at the site of a myocardial infarction. Therefore, the majority of clinical studies have been performed with mesenchymal stem cells. A large number of studies have been conducted, and we have learned that: 1) the most efficient delivery route is that of intra-myocardial injection, 2) the homing of the cell into the myocardial tissue remains a limiting factor; 3) tracking the implanted cells is key to establishing the effectiveness of injections; 4) a gold-standard assessment of left ventricular function (such as cardiac magnetic resonance imaging) is warranted in clinical trials; 5) mesenchymal stem cells seem incapable of differentiating to mature cardiomyocytes, rather instead developing only into cardiac precursor cells; 6) the major regenerative potential lies in the paracrine effects of mesenchymal stem cells.[8]

The most recent clinical trials in this area have investigated the injection of cell stimulating paracrine factors (peptides, small molecules, hormone-like molecules) without actual cell delivery.[9] The rationale is that human embryonic stem cells and human induced pluripotent stem cells can differentiate into mature cardiovascular cells from such a pluripotent state if they can be stimulated in-situ. In this context, a number of differentiation protocols exist to develop specific cell types including endothelial cells, cardiomyocytes, and smooth muscle cells. Embryonic stem cell-derived products would need immunosuppressive therapy, in a manner analagous to allogenic heart transplantation. Currently, one clinical trial is running in France, where embryonic stem cell-derived cardiomyocytes are being implanted in the epicardial sheet during coronary-artery-bypass grafting procedures.[10] Human induced pluripotent stem cells are reprogrammed from adult somatic cells, thus there is an option to deliver the patients specific new cardiovascular cells. Initial clinical trials are underway, building on the experience from other indications, such as macular degeneration treatments, pioneered with induced pluripotent stem cells derived epithelial cells. Pluripotent stem cells can give rise to a form of cardiovascular tissue engineering and they can act as a source of cells to repopulate 3D printed biomaterials, which in particular may be attractive for paediatric congenital heart repair procedures.

Beside exogenous cellular therapies, it is well-known that cellular immune responses after major cardiovascular events are activated. These responses include changes in native immune cells which may be modified, boosted or silenced with the therapeutic aim of attenuating cardiovascular inflammation, adverse myocardial remodelling, scarring, and fibrosis.

In the myocardium myocardial resident immune cells exist alongside resident stem cells. These are resident monocytes/macrophages, which are activated and join the circulation in response to stress stimuli and the systemic inflammatory response syndrome (SIRS). In the failing heart, different populations of leukocytes exist, e.g. neutrophils and macrophages. Resident macrophages in the heart usually express CXC-motif-chemokine-ligands, which are reasonable targets in immune-modulation for anti-remodelling and anti-fibrosis treatments in HF. Macrophages after a cardiac event actively secrete a number of substances, which can heavily influence the processes of angiogenesis, remodelling and fibrosis. For instance, secreted proteolytic enzymes (matrix metalloproteinases) influence scarring and myocardial fibrosis. Macrophage-derived VEGF can enhance neo-angiogenesis and can promote the development of collateral vessel networks. Cardiac resident macrophages have an immune memory of residual medullary state, and their secretome pattern is similar. TGF-β becomes highly overexpressed after myocardial events, resulting in excessive fibrous tissue growth, diastolic and systolic dysfunction. Interestingly, the zebra-fish is able to regenerate a sufficient amount of left ventricle after its dissections. In the regenerative process mainly embryo-derived CCR2 negative macrophages play a key important signalling role. For this reason, the regenerative steps of zebrafish heart is a highly attractive process for cardiac researchers. Furthermore, in adult murine heart CCR2 negative embryonic macrophage population is lacking and rather CCR2 blood-derived macrophages are in situ as resident macrophages. Resident macrophages populate around 7-8% of the adult heart, which is a significant number, taken into count those lost after a myocardial infarction. SIRS and inflammatory responses are not only present in failing and

ischaemic hearts but also in heart failure with preserved ejection fraction. Inflammatory responses and enhanced monocyte/macrophage activation has a huge impact on the arterial walls, neo-intima proliferation, and atherosclerosis as well.

Recently, the CANTOS trial,[11] one of the first immune-therapy trials in patients with a history of myocardial infarction and evidence of inflammatory activation, proved that treatment with canakinumab, a neutralising antibody against human IL-1β, was associated with a decrease in the combined end point of nonfatal myocardial infarction, nonfatal stroke, or cardiovascular death, with significance for only one of the three tested doses (the middle dose) versus placebo. However, canakinumab was also associated with a significantly higher incidence of fatal infection compared to placebo. The concept that immune medication can have benefical cardiovascular effects, but the nature of the clinical benefit off-set against its risks have not led to a major uptake of this therapy. The possibility of immune modification for HF remains one of future research effort.

Recent preclinical studies have targeted modification monocyte phenotype from inflammatory to reparative in order to decrease the inflammatory reaction and resultant remodelling. These include immune-modulatory processes, efferocytosis or even gene editing via the CRISPR-cas9 system.

Micro-RNAs

Micro-RNA's (miRNAs) are small non-coding RNA strands, comprising 19-25 nucleotides. They have a distinct signalling role and a various patterns of expression in ischaemic myocardium, hypertrophy, cardiomyopathies, and overt HF. They act at the post-transcriptional regulation level. Numerous novel miRNAs have been discovered, and their in-depth role has been characterised.

miRNAs can serve as therapeutic substances or therapeutic targets in a range of cardiovascular diseases. Furthermore, miRNAs are secreted into body fluid compartments (e.g. pericardial fluid) and into the circulation, thus they may have play a unique biomarker role in cardiovascular diseases. Extracellular miRNAs are usually packed into lipid vesicles. Their circulatory levels change in response to standard HF medication treatment, and in response to the initiation of device therapies.[12] Table 1. shows prominent miRNAs and their role in cardiovascular physiology. Beside cardiac miRNAs, miRNAs of other origins (e.g. liver and other parenchymal tissues) also have an important role in the systemic stress reaction after a cardiovascular event.

Limitations of miRNAs include their expense and the meticulous process of collection and characterisation required for therapeutic use. Identification of miRNAs in blood samples may be time-limited. Furthermore, sex- and age-related variations are not yet fully characterised. A recent large EU funded project the HOMAGE (Heart Omics in Aging) project aimed to identify age-related variations in biomarkers molecules of cardiac diseases. Population-based variation of these expression patterns will help a better understanding of regulatory and biomarker role of miRNAs and may result in further steps towards their modulation and targeted inhibition with therapeutic intent.[13]

Table 1. Role of cardiac miRNAs in different pathophysiological aspects.

	Pro	Anti
Fibrosis	miR133, miR98	miR21, miR29, miR208, miR199b, miR130, miR29, miR24
Angiogenesis	miR126, miR24,	miR92, miR503, miR519, miR34
Apoptosis	miR21, miR1, miR15, miR320	miR15, miR199, miR30
Atherosclerosis, lipid metabolism	miR145, miR33	miR21, miR126
Hypertrophy	miR212, miR208, miR22	miR132, miR133a miR1, miR378

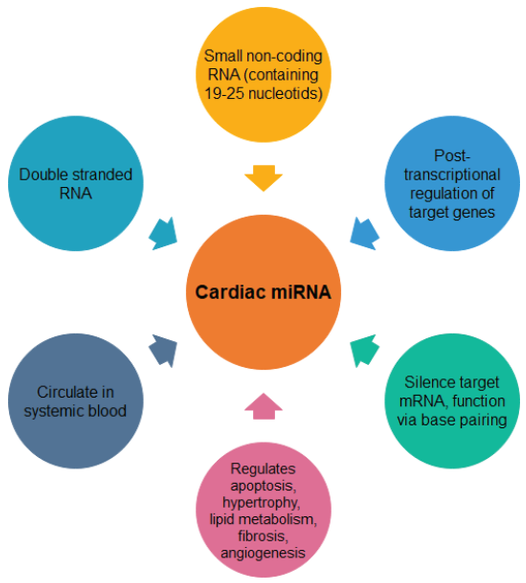

Figure 1. Cardiac miRNAs

Ongoing or recently completed clinical trials have mainly characterised the biomarker role of miRNAs in specific clinical conditions. The MIRRACLE trial investigated the predictive role of the set of circulating miRNAs in non-invasive diagnosis of allograft rejection in a heart transplantation population and validates a predictive role in comparison with endomyocardial biopsies and histology results. Other trials are investigating potential biomarker roles of plasma circulating miRNAs in coronary artery disease or coronary arterial calcification as they have in observational studies in other conditions (e.g. stroke, renal failure).

For therapeutic purposes miRNAs are promising targets. Recently, preclinical translational trials (small and large animals) have showed promising results in ischaemic heart disease, HF and in the arena of cardioprotection. The first-in-human anti-miR trial has just been launched and investigates a safety profile, tolerability and pharmacokinetics of ascending single dose of anti-miR (investigational medicinal product: S95010) in healthy

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male volunteers (NCT03494712).

Future aspects of miRNAs as biomarker or/and therapeutic targets warrant detailed clinical trials to characterise miRNA expression patterns and responses to evidence-based cardiovascular therapies. Furthermore, automated and high-throughput techniques will be essential for reliable processing of miRNAs in clinical practice.[14]

Declarations of interest

The authors declare no conflict of interest.

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