

EDITORIAL

Gene Therapy of Cardiac Arrhythmias

Sherif Mokhtar

Cairo University, Faculty of Medicine, ICU Department

(Heart Mirror J 2008; 2(2): 46-48)

Astounding advances in Molecular Biology have opened up novel opportunities to address the persistent problem of cardiac arrhythmias. In rare instances, such opportunities have already begun to be realized as in the congenital long QT syndromes. The identification of the culprit genes and their functional alterations has enabled the elaboration of rational gene-specific therapeutic strategies. But molecular approaches alone will never solve the problem of arrhythmogenesis. A concerted effort is needed at various levels of integration, and molecular and cellular scientists need to break down the problem into simple elements. Electrophysiologists need to gain an appreciation for the vast potential, as well as the limitations, of Molecular Genetic Approaches to arrhythmogenesis. Problems that are more specific to gene therapy for cardiac arrhythmias are exemplified by, but not limited to our lack of understanding of the molecular mechanisms of many arrhythmias and the spatial complexity of expression of ion channels.

Gene therapy entails transfer of genes to a target somatic cell or an organ to treat or prevent a disease. The first gene therapy trial in 1990 ushered in an era of excitement and in some cases profound disappointment. Currently most of the applications of gene therapy are directed towards cancer and related illnesses while cardiovascular disease constitutes a significant minority of clinical trials. The principal disease targets for gene therapy in the cardiovascular system are atherosclerosis, including peripheral vascular disease, and heart failure. Cardiac arrhythmias represent a promising but a less well-developed target for gene-based therapeutic strategies.

A central mandate of gene therapy is the successful delivery of the nucleic acid to the target tissue. A number of gene delivery systems have been developed that utilize viral- and non-viral-based methods; the latter includes both physical and chemical agents, as well as cell-based therapy. Viruses are ideal vectors because they have evolved the ability to efficiently deliver nucleic acid (i.e., viral genome) and avoid immune surveillance. Each of the viral vector systems has its advantages and disadvantages. The most commonly used viral vectors in clinical gene therapy trials are the Retroviruses (1, 2). These small RNA viruses often require dividing cells for entry into the cell nucleus and integration into the cell genome. Certain retroviruses, such as the Lentiviruses [e.g., human

immunodeficiency virus (HIV)], can infect and integrate into the genome of non dividing cells (3). But, integration into the genome has the potential for disruption of essential genes and malignant transformation. Disadvantages of Retroviral vector systems include the relatively short half-life of the viruses, difficulty in manufacturing the concentrations necessary for in vivo myocardial or vascular gene transfer, the need for actively dividing target cells (with the exception of lentiviruses), and public reluctance toward any therapy using an HIV-like systems (1,3).

Replication-Deficient Adenoviruses (4) are the most commonly used for cardiovascular applications. They have the advantage of being able to infect non- dividing cells and not integrating into the genome, but the disadvantages of transient expression of the gene product and both cell-mediated and humoral immune responses limit gene expression. Adeno-Associated Virus (AAV) (3) is a single-stranded DNA virus of the parvovirus family that requires a helper virus for replication. The advantages are ability to infect non-dividing cells, integrate into the host genome, and evading the host immune system, thus producing efficient and long-term gene expression. Disadvantages include the requirement for co-infection with a helper virus, such as adenovirus or herpes simplex virus, the limited size of genes that can be packaged and the complex process required for virus production with requirement for helper viruses or plasmids to accomplish virus amplification.

Non Viral Gene Delivery Systems (1,3) are physicochemical methods to facilitate gene transfer. They include Liposome Mediated Delivery, Gene Guns, and DNA conjugates to polycations. Methods that involve the delivery of DNA alone either by physical or chemical methods are less pathogenic but also less efficient in genetic transduction of most tissues, including the heart. The advantages are relative ease of manufacturing and greater public acceptance. Non-viral vectors share many of the viral vector problems as longevity of expression, incitement of an immune response, variable and limited efficiency compared to viral vectors. However, they avoid any of the complications associated with integration of genes into the host genome.

Cell therapy is an indirect way of delivering genes and proteins to the heart. Hughes, 2002 (5) the feasibility of

myoblast transplantation has been demonstrated in humans. Myoblast cells transplanted to the heart appear to form gap junctions with cardiac myocytes in animal models. Engrafted myoblasts improve LV performance, although systolic function of the exogenous cells is uncertain. The electrophysiologic implications of cell therapy in the human heart have yet to be determined.

Targets applicable to gene therapy could include molecules that alter active membrane properties, such as Ion Channels and Transporters, or those that mediate cell-to-cell communication (i.e., Connexins). The aim of such therapy could be one of the following:

1. Replacement or correction of an abnormal gene, in monogenic disorders of cardiac rhythm, such as (the Long QT Syndrome, Brugada Syndrome or Catecholamine Induced Polymorphic VT).
2. Correction of single gene defects that produce structural heart disease may be antiarrhythmic.
3. Polygenic Structural Heart Disease that forms the substrate strate for arrhythmias such as Scar-mediated Ventricular Tachycardia, or Atrial Fibrillation in diseased atria.
4. Alter the Action Potential Profile by infection with a Channel Gene-Containing Virus e.g. reconstitution of the transient outward current (Ito), which is down regulated in the failing heart.
5. Another gene therapy strategy to alter conduction is Manipulation of Heart or Progenitor Cells ex Vivo, creating designer myocytes or specialized conducting tissue cells to modify cardiac excitability.

A recent example is the creation of "Automatic" Ventricular Myocytes that may serve as "biological pacemakers" (6) similar to the sinoatrial (SA) node, a structure composed of specialized cardiocytes richly innervated by autonomic nerves. Critical to the function of the responsible channel is that the inward Na current carried by the channel activates upon hyperpolarization, hence the designation "Funny Current," or If. There are four isoforms of the gene, labeled HCN1 to HCN4. The predominant isoform in the SA node is HCN4 and in ventricle is HCN2. The avenues available for creating biological pacemakers include manipulating autonomic control, manipulating ion channel number, structure, and/or function in order to create a nidus of pacemaker cells.

Both Embonic and Adult Mesenchymal Stem Cells have been used in attempts to fabricate Biological Pacemakers. Both are stem cells, but the commonality ends there. Embryonic Stem Cells are pluripotent and have the potential to differentiate into any cell type in the body. The general strategy is to direct cells down a lineage that will incorporate PM properties in its own right, couple to adjacent myocytes, and be integrated as a new sinus node cell. Adult Mesenchymal Stem Cells are multipotent and are expected to differentiate only further along mesenchymally derived lineages. The strategy is to use the cells as platforms to carry genes of interest to regions of the heart where the cells would need to couple with adjacent myocytes. The Embryonic Stem Cell is considered more a

tool for tissue engineering, whereas the Mesenchymal Stem Cell is considered more a tool for delivering traditional gene therapy.

Embryonic Stem Cells, pluripotent cells, can differentiate into neuronal, pancreatic islet, hematopoietic progenitor, endothelial and heart cells. As a cardiac lineage they have the property of performing as a syncytium, [facilitated by the presence of the gap junctional proteins connexin (Cx43) and connexin (Cx45)]. Whether in culture or as embryoid bodies, human embryonic stem cells generate action potentials having a range of characteristics from those of pacemaker Cells (low membrane potential, phase 4 depolarization, low upstroke velocities) to those of relatively mature myocardium (high membrane potential, no phase 4 depolarization, rapid upstroke velocities). Human embryonic stem cells provide a rich source of material for regenerating myocardium and initiating electrical activity in heart. Kehat et al recently reported the use of cardiomyocyte cell grafts derived from human embryonic stem cells to create a biological pacemaker.

The cells were implanted into the ventricles of swine with complete heart block, and mapping experiments revealed that pacemaker activity originated at the implantation site (7). The cells were readily available, and there were no objections to, or constraints on, their use for research purposes. These cells are immunoprivileged, that is, they might not elicit the immune response that complicates homologous or heterologous transplantation.

Using Adult Human Mesenchymal Stem Cells in the normal sinus node, cells have a complete complement of ion channel genes, including the pacemaker gene HCN4. The resultant current, If activates upon hyperpolarization, initiating phase 4 depolarization and an action potential that propagates via low-resistance gap junctions to other cells. With platform therapy, the human mesenchymal stem cell has been transfected with an HCN gene. However, the human mesenchymal stem cell does not have a complete complement of channels to permit initiation an action potential. The human mesenchymal stem cell is in close proximity to a ventricular myocyte, which has a complete complement of channels to generate an action potential and actually has HCN2 and can generate if current.

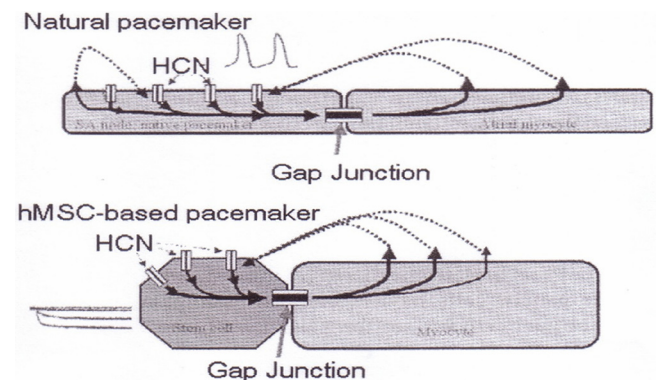


Figure 1: Rationale for human mesenchymal stem cell (hMSC)-based pacemaker.
Top: Sinoatrial node myocyte coupled to an atrial myocyte via gap junctions.

It is hypothesized that when myocyte and stem cells communicate via gap junctions, the high membrane potential of the myocyte initiates HCN channel opening in the stem cell and the occurrence of a pacemaker potential. The action would generate current flow from stem cell to myocyte depolarizing the latter. When the myocyte reaches threshold potential, it would initiate an action potential whose positive voltages would turn off the pacemaker current. The action potential also would propagate to other cells in the myocardial syncytium, as per usual. Coupling between myocyte and human mesenchymal stem cell resulted in effective pacemaker function was demonstrated in vitro. Potapova I. et al. (8)

There remain some obstacles that need to be overcome. With the human Embryonic Stem Cell, the investigator has available cells with the potential to become any form of myocyte with every ion channel that characterizes myocytes, and the investigator has the additional option to over-express specific genes. The final piece of the platform-building puzzle is transfecting the human mesenchymal stem cells with a pacemaker gene. Electroporation resulted in a transfection efficacy that now approaches 50%. There are still concerns regarding their maintenance as stem cells vs their differentiation into other cell types. It is important that the cells not evolve into unwanted cell lineages, as we would not want to see cartilage or lakes of hematopoietic cells in the heart. There are also concerns regarding delivery systems. Whether administered via a needle/electrode combination operating through a catheter, a hollow-lumen steerable catheter, or a combination of both, the ability to place constructs in the proper region without inducing trauma remains a challenge. The design of systems in which biological and electronic pacemakers can be used to complement each other's operation as hybrid therapy also is challenging. At this point, there is a means of loading human mesenchymal stem cells with the appropriate gene. The human mesenchymal stem cell so loaded can generate an If-like current that responded to cesium and to autonomic modulation much like native If. The era of biological pacemakers may not be very far. There may be a chance that we see phase I trials beginning in 3 to 5 years. This is not outside the realm of possibility. As is

always the case, each setback ensures delay. However, if we are given the combination of a long lifetime and enthusiastic and informed investigation, a biological pacemaker sinus node will come true.

REFERENCES

1. Su C, Na M, Chen J, et al. Gene-viral cancer therapy using dual-regulated oncolytic adenovirus with antiangiogenesis gene for increased efficacy. *Mol Cancer Res* 2008; 6(4):568-75.
2. Li Q, Guo Y, Tan W, et al. Gene therapy with iNOS provides long-term protection against myocardial infarction without adverse functional consequences. *Am J Physiol Heart Circ Physiol* 2006; 290(2):H584-9.
3. Lyon AR, Sato M, Hajjar RJ, et al. Gene therapy: Targeting the myocardium. *Heart* 2008; 94(1):89-99.
4. Echavarría M. Adenoviruses in immunocompromised hosts. *Clin Microbiol Rev* 2008; 21(4):704-15.
5. Hughes GC, Biswas SS, Yin B, et al. A comparison of mechanical and laser transmural revascularization for induction of angiogenesis and arteriogenesis in chronically ischemic myocardium. *J Am Coll Cardiol* 2002; 39(7):1220-8.
6. Coppen SR, Fukushima S, Shintani Y, et al. A factor underlying late-phase arrhythmogenicity after cell therapy to the heart: Global downregulation of connexin43 in the host myocardium after skeletal myoblast transplantation. *Circulation* 2008; 118(14 Suppl):S138-44.
7. Kehat I, Gepstein A, Spira A, et al. High-resolution electrophysiological assessment of human embryonic stem cell-derived cardiomyocytes: A novel in vitro model for the study of conduction. *Circ Res* 2002; 91(8):659-61.
8. Potapova I, Plotnikov A, Lu Z, et al. Human mesenchymal stem cells as a gene delivery system to create cardiac pacemakers. *Circ Res* 2004; 94(7):952-9.

Corresponding Author:

Sherif Mokhtar, MD
ICU Department, Faculty of medicine, Cairo University
