Gene Therapy (2003) 10, 1–4

www.nature.com/gt

Cardiac gene therapy

True promise fulfilled

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Gene Therapy (2003) **10,** 1–1. doi:10.1038/sj.gt.3301915

Gene therapy is often depicted as representing 'just another drug delivery strategy'. However, there are a growing number of examples of a new and much more fundamental applicability: altering a cell phenotype or structure. In the latest example, published in *Nature*, Miake *et al*[1] present exciting results that suggest a possible alternative to electronic pacemakers.

Most gene therapy trials are in essence examining a genetic means of administering drug therapy. This applies to a diverse range of therapeutic studies, including genetic treatments of cystic fibrosis and hemophilia and the delivering of cytotoxic agents to cancer cells. Gene therapy in these studies may provide more selective or more potent delivery techniques compared to standard drug delivery approaches, but basically the aim and the outcome are the same.

In contrast, the new *Nature* study[1] is one of a growing number of attempts to use gene therapy in a way that is fundamentally different from drug-based therapies. The common aim of this new generation of studies is to actually alter a cell phenotype or structure. In the case of the Miake study, the aim was to create the 'pacemaker' phenotype, usually only present in a select few pacemaker cells, in normal heart cells.

Pacemaker cells are characterized by spontaneous cycles of depolarization and repolarization, which create an electrical impulse transmittable to adjoining heart cells. These cycles are mediated by the opening and closing of transmembrane ion channels in the cells. The authors of the new study hypothesized that one of the potassium channels that is intensely expressed in nonpacemaker heart cells represses pacemaker activity. To test this, they suppressed this potassium channel, encoded by the Kir 2 gene family, in target cells in guinea-pigs by transducing them with a modified, 'dominant negative' version of Kir2.1. Transduced heart cells thus lacked normal function of the potassium channel encoded by Kir2. This strategy worked as predicted, creating spontaneous 'pacemaker' activity in the transduced heart cells.

The structural modification of the target cell in the *Nature* study is a successful fulfillment, one of the true promises of gene therapy: 'getting at' intracellular signaling or structural machinery in a way that would be difficult or impossible with conventional drug treatments. Targeting the cellular phenotype in this way would appear to be a strategy uniquely applicable through gene therapy. Similarly, in recent times gene therapy has been used to modify structurally a diverse range of other cellular proteins including transcription factors, intracellular proteins, and membrane receptors.[2–4]

Realistically, there are many other factors that need to be considered before biological pacemakers can replace electronic ones. Given recent evidence that the Kir 2 potassium channel plays a role in Andersen syndrome (a rare inherited disorder associated with long QT and ventricular arrhythmias), it may not be the ideal protein to target for gene therapy. Other transmembrane channels could provide alternative targets through which this strategy could be successfully applied. Another question that would need to be considered is, would a biological pacemaker need to be located in a specific position in the heart to be effective? Conventional electronic pacemakers in use today need merely to be in contact with some aspect of the atrium/or the ventricle. If a certain position were a requirement, the therapeutic vector could easily be precisely delivered with standard electrophysiology catheter techniques. Finally, the sustainability of such pacemaker cells would need to be reasonably verified before individuals with life-threatening dependence on such pacemaker activity could reliably be treated by this gene therapy.

Despite these minor caveats, the therapeutic first described by Miake *et al* is an impressive accomplishment. Aside from its therapeutic applications, this work clearly demonstrates the latent presence of the 'pacemaker phenotype' in normal heart cells. It also highlights the importance of the Kir 2 potassium channel in determining pacemaker activity in cardiac tissue. Future studies with adenovirus and/or chronic expression vectors are needed to move toward the more effective and persistent effect we would need to create a biological pacemaker. Clinical trials would even be possible in the foreseeable future. In many endeavors, once the gate is cracked open, the floodwaters often pour through. Conceivably, this and similar breaches will provide such an opportunity for delivering the true promise of gene therapy. ■

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Immunotherapy

Oral route to Holy Grail?

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Gene Therapy (2003) **10,** 1–2. doi:10.1038/sj.gt.3301906

The increasing severity of the global AIDS crisis means the need for methods of preventing or treating the disease is similarly increasing. An effective HIV vaccine is the 'Holy Grail' that researchers seek. Now, work by Kenji Okuda and co-workers shows that a surprisingly simple gene therapy approach is a promising future vaccination strategy.

Human immunodeficiency viruses type 1 and 2 (HIV-1, HIV-2), retroviruses belonging to the *Lentivirus* genus, cause acquired immunodeficiency syndrome (AIDS).[1] These viruses are, arguably, currently responsible for more human misery than any other disease: 40 million people are living with HIV-AIDS, more than 50% in the sub-Saharan African region.[2]

People become infected with HIV when the mucosas of the mouth, rectum or vagina are exposed to infected body fluids (during sex or breast feeding) or when such fluids are injected. Subsequently, often years after the initial infection, the virus starts to destroy the $CD4^+$ T cells that are a vital part of the body's immune system. The depleted immune system causes a raft of negative symptoms, and opens the body to other infections and cancers. Tragically, those infected invariably die.[3]

The advent of highly active antiretroviral therapy (HAART) has improved the lot of HIV patients. HAART reduces the symptoms and secondary infections caused by AIDS, and also prolongs survival. It is, however, very far from being a cure. HAART is expensive, must be continued throughout the patient's life, has negative side-effects, and can fail due to resistance of the virus.[4] The ideal solution, as it has been for so many other diseases, would be a vaccine. However, attempts to develop such a vaccine have been less than successful.[5]

The new study, published in September's issue of *Human Gene Therapy*, describes a novel approach for inducing an HIV-specific immune response. The Okuda group

vaccinated mice with a single oral dose of a recombinant adeno-associated virus vector expressing the *env* gene from HIV-1 (AAV/HIV*env* vector).[6] The vaccination induced a strong immune response, both locally (in the mucosa) and systemically.

The *Human Gene Therapy* study showed that anti-HIV antibodies (IgG and IgA) were present for 3–5 months after vaccination. Importantly, this means that the oral vaccine induced humoral mucosal immunity. Demonstrable mucosal immunity is crucial when the aim is to protect individuals from pathogens like HIV that are generally acquired via mucosal surfaces.

The researchers also convincingly demonstrated that the vaccine induced a strong HIV-specific cell-mediated immunity. This represents a significant improvement compared to previous vaccines administered by injection into muscle tissue that, typically, did not induce a strong cellular immune response.

However, to really show a vaccine actually works, one must show it induces protective immunity. To do this, the authors challenged immunized mice with a recombinant vaccinia virus expressing HIV-1 *env*. Using a rectal inoculum they observed a decreased viral load (about 2 logs) in immunized animals. These results are encouraging, but, in addition to the surrogate viral challenge used by Okuda and his colleagues, they need to be repeated for HIV itself.

The success of this new approach in mice is exciting because, theroretically, it could be equally successful in humans. We already know that the AAV vector used is safe and effective in humans.[7] This vector is also almost certainly less pathogenic than all vectors presently adopted in animal and human trials.[8] In addition, because the vaccine is a live viral preparation, it is more likely to induce a protective immune response. Now Okuda's group have shown that AAV's resistance to a range of temperatures, proteases and pH variations allows successful oral delivery of an HIV vaccine.

However, we should not crack open the champagne yet. The short duration of the induced immune response in the mouse model suggests that the AAV vector mainly infects rapidly renewing cells of the intestinal lumen. Therefore, in conjunction with the AAV/HIV*env* oral vaccination, we need to boost immunity in other ways. Adoptive immunotherapy with professional antigen-presenting dendritic cells or combined DNA/protein vaccinations with different gene products and schedules are two promising ways this may be achieved.

General use of this approach in humans will also be hampered by the strong anti-AAV immunity induced by the initial vaccination, that curtails the positive effects of subsequent vaccinations.[9] Consequently, AAV vector-based vaccines such as this one may be most effective when used in conjunction with other approaches.

The way to a successful HIV vaccine is paved with difficulties and surely monkeys, and not mice, will have to be used for setting the first standard of protection before human trials. Notwithstanding, the new work by Okuda's group at least represents a promising lead in the quest for the Holy Grail of a vaccine to prevent further misery being inflicted by the plague of AIDS. ■

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Cardiac gene therapy

Pumping up the heart

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Gene Therapy (2003) **10,** 2–3. doi:10.1038/sj.gt.3301910

Numerous studies have shown that gene therapy can transiently forestall heart failure.[1–5] Now, for the first time, Chien and his colleagues have demonstrated that experimental heart failure can persistently be prevented with gene therapy.[1]

If you could give your heart one piece of advice, the most sensible would be to emulate the Energizer bunny – keep the pump going and going. In simple terms, the heart is a regulated pump, each beat providing blood sufficient for the body's demands. While the normal heart has sufficient reserve to maintain the pump function throughout life, the stress of common disorders such as hypertension, atherosclerosis, or diabetes can cause the cardiac pump to deteriorate. The result is heart failure: the faltering pump cannot provide adequate blood flow to other organs and there is an accumulation of fluid in the lungs and lower extremities

The molecular mechanisms underlying heart failure are complex. They involve energy generation and signal transduction pathways, as well as the mechanics of cardiac function.[6,7] The key is contractility. The ability of the heart cell to contract and relax at rest and in response to enhanced demand is controlled at three levels: signaling via activation of G-protein-related receptor pathways, transduction of this signal through cAMP/protein-A-kinase-mediated control of calcium (Ca^{2+}) cycling in the sarcoplasmic reticulum, and transduction of the Ca^{2+} cycling signal to the apparatus that mediates muscle contraction (Figure 1). Each of these processes is complex, and abnormalities in each are linked to heart failure in experimental animals and humans.

The new work from the Chien laboratory, published in August's *Nature Medicine*,[1] focuses on correcting abnormalities in Ca^{2+} cycling to treat heart failure. One such abnormality, clearly linked to heart failure,[2,3,6–8] is a decrease in the activity of an enzyme that regulates diastolic and systolic function, sarcoplasmic reticulum Ca^{2+} ATPase (SERCA). Such a decrease leads to reduced Ca^{2+} cycling and thus decreased contractility

SERCA activity decreases as the level of an inhibitory protein, phospholamban, increases.[7,9] Phosphorylation of phospholamban releases the controlled inhibition of the Ca^{2+} pump, resulting in enhanced cardiac function. In heart failure, the phospholamban regulatory pathway goes awry. Phospholamban is chronically under-phosphorylated, resulting in chronic suppression of SERCA and thus reduced contractility.

Based on these observations, persistent activation of SERCA is an obvious therapeutic strategy for heart failure. But how can this be accomplished? In an attempt to meet this challenge, the Chien laboratory used a gene therapy approach. They delivered a mutant form of phospholamban to the heart in a vector already shown to be effective at transferring and persistently expressing genes in the heart:[10] recombinant adeno-associated virus serotype 2 vector (AAV2). The mutant phospholamban (S16E) has a serine replaced by a glutamate at one of its two phosphorylation sites, mimicking phosphorylation of the serine (ie pseudo-phosphorylation).

Theoretically, delivering an S16E form of phospholamban to heart cells should increase SERCA activity, and thus increase