Gene Therapy for Repair of Cardiac Fibrosis
A Long Way to Tipperary

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Because of the increase in life expectancy and a high survival rate after myocardial infarction, cardiac fibrosis is becoming one of the most important problems in cardiology. It is characterized by excessive accumulation of fibrillar collagen in the extracellular space, either because of a loss of cardiomyocytes (replacement fibrosis) and/or as an interstitial response to various chronic cardiovascular diseases such as hypertension, myocarditis, and congestive heart failure (reactive fibrosis). In humans, cardiac fibrosis is universal in the aging heart. Activated fibroblasts play a pivotal role in the formation and maintenance of fibrous tissue by the production of various extracellular-matrix proteins, including collagen and fibronectin. It is now recognized that even in areas with long-standing fibrosis, such as postinfarction scars, fibroblasts remain metabolically active. Their activity is regulated by various autocrine and paracrine factors, such as angiotensin II, aldosterone, endothelins, cytokines, and growth factors. The renin-angiotensin-aldosterone system is considered to be of major importance for the regulation of cardiac fibrosis. Increased tissue levels of angiotensin II have shown to promote, and ACE inhibitors are able to delay the development of, cardiac fibrosis.

Interstitial fibrosis reduces the electrical coupling between cardiac myocytes because fibroblasts produce smaller or larger collagenous septa, which electrically insulate cardiac cells or muscle bundles. As a result, the normal myocardial architecture becomes disrupted and is transformed into a pathological substrate characterized by the presence of multiple insulating barriers, which force the depolarization wave to spread nonuniformly. As predicted by computer simulations and demonstrated in cell cultures, in such a medium, long local conduction delays occur that may slow the effective conduction velocity to very low values. Unlike a decrease of the rapid sodium current, which will result in conduction failure when conduction velocity is depressed to approxi-
cells underwent myogenic differentiation, as evidenced by the appearance of cross-striation and immunostaining of sarcomeric myosin heavy chain. In a quarter of these cells, intracellular Ca\textsuperscript{2+} transients could be elicited by external field stimulation. As expected, when cells are not electrically coupled, adjacent cells showed different stimulus thresholds for induction of these Ca\textsuperscript{2+} transients. After concurrent expression of gap junction protein connexin43, the percentage of cells in which Ca\textsuperscript{2+} transients could be evoked dropped dramatically (by a factor of 7), and now Ca\textsuperscript{2+} transients could be elicited in only 3% to 4% of genetically modified fibroblasts. Microinjection of Lucifer yellow, which is able to pass connexin43 channels, showed dye transfer in 4 of 37 cell pairs. Transfer was not observed after injection of rhodamine-dextran, a dye that cannot pass gap junctions. After concurrent forced myogenesis and connexin43 expression, in 8 of 54 cell pairs, the stimulus threshold for induction of Ca\textsuperscript{2+} transients did become identical, suggesting that these pairs were electrically coupled. From these observations, the authors arrive at the following conclusions. (1) Forced myogenesis in fibroblasts results in the expression of ion channels required for membrane depolarization. (2) Fibroblasts can be genetically modified to produce excitable cells capable of electrical coupling. (3) These findings strengthen the prospect of gene-based repair of cardiac conduction defects in patients. For the reasons given below, we believe that these conclusions are still speculative and for the moment should be regarded as working hypotheses.

In the study by Kizana et al., no direct electrophysiological measurements were made. Action potentials were not recorded, and the proposed transmembrane currents were not demonstrated by patch-clamp techniques. Instead, intracellular Ca\textsuperscript{2+} transients measured by loading the cells with a fluorescent calcium dye were used as a surrogate of cellular excitability. Because of the limited knowledge about the magnitude of possible transmembrane Ca\textsuperscript{2+} currents and intracellular Ca\textsuperscript{2+} release in the cell lines used, it is questionable whether induction of intracellular Ca\textsuperscript{2+} transients by electrical field stimulation can be taken as proof that these cells actually were excitable. The term “electrical excitation” is generally used to indicate a self-regenerative depolarization of the cell membrane as a result of activation of voltage-gated ion channels. Often, but not necessarily, ionic transmembrane currents trigger intracellular events such as release of Ca\textsuperscript{2+} ions from intracellular stores. However, intracellular Ca\textsuperscript{2+} release is not occurring necessarily or exclusively through depolarization of the cell membrane. For example, it has recently been shown that sinoatrial pacemaker cells do not require membrane depolarization for ryanodine receptor–induced Ca\textsuperscript{2+} release.\textsuperscript{23} Even in the case of field stimulation, propagating intracellular Ca\textsuperscript{2+} waves may occur without excitation of the cell membrane.\textsuperscript{24} In skeletal muscle as well, subthreshold depolarizations (to −60 mV) may already induce Ca\textsuperscript{2+} release from the sarcoplasmic reticulum.\textsuperscript{25} This is of interest because, in the experiments of Kizana et al., a skeletal myogenic determination factor was used to induce myogenesis. To rule out the possibility that in modified fibroblasts, intracellular Ca\textsuperscript{2+} transients are evoked without actually exciting the cell membrane, the membrane potential of these fibroblasts needs to be recorded.

Also, the evidence provided for the evolution of electrical coupling between genetically modified fibroblasts is indirect, and no quantitative information is available about the properties of the newly formed gap junctions. To judge the efficacy and significance of electrical coupling, additional studies are needed to establish the specific electrical conductance between pairs of genetically modified fibroblasts. Another question that arises is whether genetic modification is necessary for fibroblasts to form “electrical bridges” between myocytes. Since 1969, it has been known that in cell cultures, spontaneous discharge of two distant cardiac cells can be effectively synchronized by interposition of various types of nonmyocytes.\textsuperscript{26} In two more recent studies (in cocultures of myocytes and fibroblasts\textsuperscript{20} and in intact rabbit sinoatrial node\textsuperscript{21}), it was shown that, without genetic modification, fibroblasts are able to form functional gap junctions either with each other or with cardiomyocytes. The type of connexin (Cx40, Cx43, or Cx45) used to make homologous or heterologous electrical connections may differ. In the study by Gaudesius et al.,\textsuperscript{20} fibroblasts were able to bridge electrical gaps between cardiac myocytes up to distances of 300 μm. Whether fibroblasts can also form electrical bridges in working myocardium (normal or fibrotic) is currently unknown. Genetic modification of fibroblasts might be useful not only to increase the likelihood of gap junction formation but also, more importantly, to increase the efficacy of electrical coupling. This would not only limit long conduction delays, expected to be arrhythmogenic, but would also increase the distance of nonexcitable gaps that might be bridged by fibroblasts.

Notwithstanding the above limitations, the prospect of genetic modification of cardiac fibroblasts is fascinating and exerts a natural and strong attraction for most of us. It is exciting to imagine that a single injection of genetically modified cells into the coronary arteries would restore and maintain sinus rhythm by reverting electrically diseased atria back into a well-coupled syncytium of myocytes and fibroblasts. However, the problems that have to be conquered are formidable. Although in situ modification of cardiac cells and injection of differentiated stem cells have been shown to be feasible,\textsuperscript{27} reports about improved cardiac function are still anecdotal, and a clear demonstration of electrical and mechanical integration of “seeded” cells with the surrounding myocardium is lacking. In a recent study, transplanted skeletal myoblasts did not become electrically coupled with the surrounding myocardium and therefore can hardly be expected to improve cardiac conduction and contraction.\textsuperscript{28} But even if such strategies as explored in the study by Kizana et al.\textsuperscript{22} may enable effective manipulation of electrical coupling of endogenous and exogenous (non)myocytes, there remains a considerable risk of proarrhythmia. One of the major electrophysiological determinants of cardiac arrhythmias is spatial heterogeneity in electrophysiological properties. If the upstroke and duration of the action potential of implanted cells differ considerably from surrounding native cells, cardiac arrhythmias may be promoted rather than suppressed. To make a difference, the amount of modified or injected cells...
must be considerable and the induced genetic changes must last for a long time. Indications for gene therapy should be identified, and techniques to accurately target gene transfer to specific areas and to keep the expression of transfected genes under control must be developed. Although it will be “a long way to Tipperary,” tackling these problems is certainly one of the greatest challenges of present-day cardiac electrophysiology.

References

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