Resynchronization of Separated Rat Cardiomyocyte Fields With Genetically Modified Human Ventricular Scar Fibroblasts

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Background—Nonresponse to cardiac resynchronization therapy is associated with the presence of slow or nonconducting scar tissue. Genetic modification of scar tissue, aimed at improving conduction, may be a novel approach to achieve effective resynchronization. Therefore, the feasibility of resynchronization with genetically modified human ventricular scar fibroblasts was studied in a coculture model.

Methods and Results—An in vitro model was used to study the effects of forced expression of the myocardin (MyoC) gene in human ventricular scar fibroblasts (hVSFs) on resynchronization of 2 rat cardiomyocyte fields separated by a strip of hVSFs. Furthermore, the effects of MyoC expression on the capacity of hVSFs to serve as pacing sites were studied. MyoC-dependent gene activation in hVSFs was examined by gene and immunocytochemical analysis. Forced MyoC expression in hVSFs decreased dyssynchrony, expressed as the activation delay between 2 cardiomyocyte fields (control hVSFs 27.6 ± 0.2 ms [n = 11] versus MyoC-hVSFs 3.6 ± 0.3 ms [n = 11] at day 8, \( P < 0.01 \)). Also, MyoC-hVSFs could be stimulated electrically, which resulted in simultaneous activation of the 2 adjacent cardiomyocyte fields. Forced MyoC expression in hVSFs led to the expression of various connexin and cardiac ion channel genes. Intracellular measurements of MyoC-hVSFs coupled to surrounding cardiomyocytes showed strongly improved action potential conduction.

Conclusions—Forced MyoC gene expression in hVSFs allowed electrical stimulation of these cells and conferred the ability to conduct an electrical impulse at high velocity, which resulted in resynchronization of 2 separated cardiomyocyte fields. Both phenomena appear mediated mainly by MyoC-dependent activation of genes that encode connexins, strongly enforcing intercellular electrical coupling. (Circulation. 2007;116:2018-2028.)

Key Words: electrical stimulation ■ electrophysiology ■ cell culture ■ gene therapy ■ fibroblasts

Myocardial infarction results in replacement of well-coupled electrically active cardiomyocytes by scar tissue, which contains primarily nonexcitable fibroblasts and an electrically insulating extracellular matrix.1 Consequently, coordinated impulse propagation across scarred myocardium is impaired owing to local conduction block and slow conduction.2 This impairment leads to inefficient and inhomogeneous electrical and mechanical activation of different parts of the myocardium, which results in increased left ventricular dyssynchrony and diminished left ventricular contractile performance.3,4 Ultimately, this may result in clinical symptoms of heart failure.

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Cardiac resynchronization therapy (CRT) is a successful treatment modality for patients with drug-refractory heart failure and significant left ventricular dyssynchrony.5-7 However, \( \approx 30\% \) of patients do not respond. Recently, among other causes, an ischemic cause of heart failure was considered as a predictor of nonresponsiveness to CRT.8 More specifically, the extent of both viable and scarred myocardium,9 as well as the location of scar tissue,10 was shown to be predictive for the response to CRT. Importantly, scar burden appears to be involved in both the cause of ventricular dyssynchrony and the lack of response to CRT.

Recently, we demonstrated that overexpression of the gene that encodes the transcription factor myocardin (MyoC) induces the synthesis of cardiac proteins in human nonmuscle cell types,11 including cardiac ion channels and connexins (Cxs).12 Because these proteins are essential in cardiac action potential (AP) transmission and are absent or only present at low levels in native human ventricular scar fibroblasts...
(hVSFs), it was hypothesized that genetic modification of scar tissue cells with a recombinant MyoC gene might be a novel approach to treat conduction abnormalities. In the present study, the potential of MyoC-transduced hVSFs to resynchronize and stimulate cardiac tissue in a standardized in vitro model was investigated.

Methods

Model of Experimental Resynchronization

All animal experiments were approved by the Institutional Animal Experiments Committee and comply with the “Guide for the Care and Use of Laboratory Animals” of the US National Institutes of Health.

Isolation, Culturing, and Preparation of Cardiomyocytes and Myocardial Scar Fibroblasts

Cardiomyocytes were dissociated from ventricles of 2-day-old male neonatal Wistar rats and grown in culture medium supplemented with 5% horse serum, penicillin (100 U/mL), and streptomycin (100 µg/mL; all from Invitrogen, Carlsbad, Calif), as described previously. The hVSFs were isolated from human myocardial scar tissue of 8 different patients who underwent surgical reconstruction of the left ventricle. Each sample was dissected into small pieces, covered by glass coverslips, and cultured in porcine gelatin-coated culture dishes containing Dulbecco’s modified Eagle’s medium supplemented with 100 µM penicillin, 100 µg/mL streptomycin, and 10% fetal bovine serum (Invitrogen). Outgrowth of cells was first seen after 2 days of culture, and after another 3 days, coverslips and tissue pieces were removed, with the hVSFs left attached to the culture dish. Next, hVSFs were trypsinized, counted, and replated at 70% confluence. Cultures were refreshed every 3 days, and passage numbers 4 to 8 were used for further experiments. Importantly, culturing of the hVSFs on poly-d-lysine plates as described by Smith et al did not give rise to cardiospheres, which makes contamination of the hVSFs with cardiac progenitor cells unlikely. Before experiments, hVSFs were transduced with vesicular stomatitis virus G protein–puromycin–lentivirus vectors encoding either human MyoC and enhanced green fluorescent protein (eGFP; MyoC-hVSFs) or β-galactosidase and eGFP (LacZ-hVSFs) as control. For this purpose, hVSFs were seeded at a density of 2 × 10⁴ cells/cm² in 10-cm² culture dishes and infected in the presence of 20 µg/mL diethylaminoethyl-dextran sulfate (Pharmacia, Uppsala, Sweden), at a multiplicity of infection of 8 HeLa cell–transducing units per cell. After 4 hours of incubation, the cells were washed 3 times and cultured for 5 to 7 days before being seeded in microelectrode array culture dishes and before analysis of ion channel and connexin gene expression. The nucleotide sequences of the shuttle plasmids used to generate the lentivirus vectors are deposited in GenBank under the accession numbers EF205034 and EF205035.

Microelectrode Arrays

Cardiomyocytes were cultured in microelectrode array culture dishes (MEAs, Multichannel Systems, Reutlingen, Germany). Before plating, MEAs were glow-discharged and coated with collagen to improve adhesion of cells. Electrical activation maps were generated after 2 days of culture to confirm the presence of a synchronously beating monolayer. After 3 days of culture, the monolayer was divided into 2 fields of cardiomyocytes by an acellular channel either (A) 250 to 300 µm or (B) 300 to 350 µm wide, which crossed the entire diameter of the culture dish. The acellular channel was generated by 2 preprogrammed linear laser dissections with a PALM Microlaser System, which included PALM RoboSoftware version 4.0 (Microlaser Technologies GmbH, Bernried, Germany). Removal of the strip of monolayer between the 2 laser dissection lines created an acellular channel that electrically separated the 2 cardiomyocyte fields. Cells were applied in a channel-crossing pattern after we ensured that no cells or cell debris was present in the channel and after we confirmed the presence of a conduction block between the 2 cardiomyocyte fields. Application of cells was achieved with a pipette mounted in a micromanipulator and a light microscope (20× magnification). To study electrical dysynchrony (group A), the channel was filled with 3 × 10⁴ eGFP-labeled LacZ-hVSFs, 3 × 10⁴ eGFP-labeled MyoC-hVSFs, or 3 × 10⁴ cardiomyocytes. In an additional set of control experiments, no cells were applied. To investigate electrical stimulation (group B), 5 × 10⁴ labeled cells were applied to the channel to reach confluence. After 24 hours, the culture medium was refreshed to remove nonattached cells. In group A, the effect of cell transplantation on resynchronization was assessed for 8 days. In group B, resynchronization driven by electrical stimulation of the transplanted cells was investigated over the same period of time.

Electrical Stimulation of Cell Cultures and Assessment of Dyssynchrony

Cultures were stimulated via an external pipette electrode (diameter 60 µm) that produced bipolar rectangular pulses (1.5% threshold, pulse width 10 ms, 1 to 2 Hz) placed ~1 mm above the cell layer. Cultures were stimulated for at least 30 seconds at a fixed location in the center of the upper cardiomyocyte field before recordings were started. Two-dimensional color-coded activation maps and conduction velocities (CVs) were derived as described previously. Electrical recoupling of the 2 cardiomyocyte fields was defined as the presence of a consistent correlation between local electrical activation times recorded at both fields for 30 seconds while the upper cardiomyocyte field was stimulated.

The correlation between electrical activation of the cardiomyocyte fields (local electrical activation time) and mechanical contraction (local mechanical activation time) of the cardiomyocyte fields was assessed by high-frame-rate (60 frames/s) video recordings of the cultures in the MEAs with a microscope equipped with a digital camera (Orca-RE, Hamamatsu Photonics Deutschland GmbH, Herrsching am Ammersee, Germany) and Openlab software (Improvision, Lexington, Mass).

Electrical Stimulation of Transplanted Cells and Assessment of Electrical Capture

The above-mentioned pipette electrode was used to stimulate at the site of hVSFs. The threshold was defined as the minimal current necessary to capture the beating cardiomyocyte culture. Stimulation was performed at twice the threshold. The electrode was placed within 1 mm above the strip of hVSFs under microscopic control with a micromanipulator and with a focused bundle of external light used to delineate the cell strip. Cell strips were stimulated for at least 30 seconds at a fixed location before recordings were started and color-coded activation maps were constructed. Electrical capture was considered to be present if a consistent phase relation between electrical stimulation of the cell strip and subsequent electrical activation of both the cardiomyocyte fields was observed for a period of 30 seconds.

Immunocytotoxic and Genetic Analysis

Immunocytotoxicity

Cocultures of 2 × 10⁴ cardiomyocytes and 5 × 10⁴ eGFP-labeled MyoC-hVSFs or LacZ-hVSFs were stained on day 1 or day 8 with Cx40-, Cx43-, or Cx45-specific antibodies (Sigma, St Louis, Mo) to look for gap junctions. The primary antibodies were detected by goat anti-rabbit IgG conjugated to Alexa Fluor568 (Invitrogen). All antibodies were used at a dilution of 1:200. Staining was quantified in 6 cultures (60 cells per culture at 40× magnification) at each time point (threshold value 50 on a 0 to 255 gray-intensity scale). A fluorescence microscope equipped with a digital camera (Eclipse, Nikon Europe, Badhoevedorp, Netherlands) and dedicated software (Image-Pro Plus, version 4.1.0.0, Media Cybernetics, Silver Spring, Md) were used for data analysis. All cocultures of cardiomyocytes and eGFP-labeled hVSFs were stained with the same solutions, and equal exposure times were used for all recordings.
### Reverse-Transcription Polymerease Chain Reaction

Semi-quantitative reverse-transcription polymerase chain reaction (RT-PCR) analyses were performed as reported previously, including primer pairs that targeted transcripts encoding the α-subunit of the cardiac voltage-gated sodium channel (SCN5A or Nav1.5), the α1C-subunit of the voltage-dependent L-type calcium channel (CACNA1C or Cav1.2), and the inward rectifier potassium channel J3 (KCNJ3, also known as Kir3.1). An overview of all primer pairs used for RT-PCR is given in the Table. As internal controls for the quantity and quality of the RNA specimens, RT-PCR amplifications that targeted transcripts of the housekeeping gene GAPDH were performed in parallel. PCRs performed on cDNA derived from total RNA samples of human atrium, ventricle, vascular smooth muscle, and skeletal muscle served as positive controls; PCRs in which the cDNA was replaced by water were included as negative controls. For comparisons of relative mRNA levels, only PCR samples from within the linear range of amplification were used.

### Whole-Cell Patch-Clamp Measurements

Cocultures of 2×10^6 cardiomyocytes and 5×10^5 eGFP-labeled MyoC-hVSFs or LacZ-hVSFs were used for intracellular measurements at day 1 and day 8 of culture. After identification of hVSFs with fluorescence microscopy, APs in cardiomyocytes and hVSFs were evaluated at days 1 and 8. At the same time points, input conductance (reverse of resistance) was considered as an approximate measure of intercellular coupling under the present study conditions. Functionality of ion channels was assessed in single cells with appropriate voltage-clamp protocols during the 8 days of culture.

### Statistical Analysis

Statistical analysis was performed with SPSS 11.0 for Windows (SPSS Inc, Chicago, Ill). Data were compared with 1-way or 2-factor mixed ANOVA test with Bonferroni correction for multiple comparisons and expressed as mean±SD. Linear correlation analysis was performed by calculating Pearson’s correlation coefficient. Probability values <0.05 were considered statistically significant. Calculation of sample size and power for the experiments was less appropriate because the expected means and SDs were difficult to estimate; however, the pronounced effects of myocardin suggest that only a limited number of experiments would have rendered significant results.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

### Results

#### Myocardin Gene Expression in hVSFs and Resynchronization

Electrical activation of the cardiomyocyte fields (local electrical activation time) was directly related to mechanical contraction (local mechanical activation time) of the cardiomyocyte fields during the entire course of the experiments (Figure 1). During this period, no mechanical activity was observed in the center of cell strips that consisted of LacZ-hVSFs or MyoC-hVSFs, as examined by light microscopy.

After 3 days of culture, only cardiomyocyte monolayers with a high degree of structural and functional homogeneity, as determined by light microscopy and electrophysiological mapping, were included in the study (yield ~60%). By laser dissection, an acellular channel (group A, 230±10 μm; group B, 335±15 μm) was created in the MEAs, which resulted in 2 asynchronously beating cardiomyocyte fields. Asynchronous beating was associated with maximal dyssynchrony (~) between the 2 cardiomyocyte fields. Application of LacZ-hVSFs (n=12) in group A resulted in resynchronization of the 2 cardiomyocyte fields within 24 hours. Resynchronization was associated with a relatively large conduction delay (latency) of 28±1 ms and a low CV of 2.1±0.4 cm/s across the LacZ-hVSFs between the 2 cardiomyocyte fields (Figures 2A, 2E, and 2F). Electrograms derived from the LacZ-hVSF strip showed clear signs of decremental conduction. During follow-up until day 8 of culture, conduction delay and CV did not change significantly (n=11; Figures 3A, 3E, 3F, and 4). Application of MyoC-hVSFs (n=11) in group A also resulted in synchronized beating of the 2 cardiomyocyte fields within 24 hours, but with a smaller conduction delay of 20±1.5 ms.
and a CV of 3±0.7 cm/s (Figures 2B, 2E, and 2F). In contrast to the experiment performed with LacZ-hVSFs, conduction delay between the 2 cardiomyocyte fields coupled by MyoC-hVSFs decreased progressively to 4±1 ms at day 8 (n=11), with a corresponding increase in CV to 18±1.2 cm/s (P<0.01 versus LacZ-hVSFs; Figures 3B, 3E, 3F, and 4). Furthermore, the decremental nature of conduction had disappeared. The acellular controls (n=12) showed asynchrony
between the 2 cardiomyocyte fields during follow-up (Figures 2C, 2E, 3C, and 3E). Application of cardiomyocytes (n=12) resulted in electrical coupling of the 2 cardiomyocyte fields within 1 day, associated with a conduction delay of 3±1 ms and a CV of 20.5±1 cm/s (Figures 2D, 2E, and 2F). No significant changes in conduction delay or CV were observed during follow-up in the cardiomyocyte group (Figures 3D, 3E, 3F, and 4). CV across cardiomyocyte fields was 21.1±1.7 cm/s (n=70), which is comparable to CV across cardiomyocytes applied between cardiomyocyte fields.

Myocardin Gene Expression in hVSFs and Electrical Stimulation

In group B, a 330±15-μm-wide acellular channel was present after laser dissection. The pipette electrode was located successfully above the center of the hVSF strip in 7 of 10 experiments. The strip of cells was visually distinguishable throughout follow-up. One day after cell application to the channel, electrical stimulation at the LacZ-hV SFs site did not result in electrical capture (n=15). Also during follow-up (n=11), no electrical capture was observed (Figure 5B1). However, electrical stimulation at the site of MyoC-hV SFs was successful in 2 of 10 experiments at day 1 (n=15), resulting in electrical capture and subsequent activation of both cardiomyocyte fields (Figure 5B2). Interestingly, electrical stimulation at the site of MyoC-hV SFs was successful in 8 of 10 experiments at day 8 (n=10, P<0.001; Figure 5A). After application of cardiomyocytes, electrical stimulation at day 1 (n=16) resulted in electrical capture in 100% of the attempts (Figure 5B3). As expected, during 8 days of follow-up (n=15), the 100% success rate was maintained, which indicates stable activation of the cardiomyocyte fields (Figure 5A).

Immunocytochemical and Genetic Analysis of Myocardin Gene Expression in hVSFs

**Immunocytochemistry**

After day 1 of culture, Cx43 staining was found in the cytoplasm of LacZ-hV SFs and between adjacent LacZ-hV SFs, where it displayed a punctuated pattern that reflected the presence of gap junctions (Figure 6B1). Staining for Cx40 and Cx45 was only weakly present in the cytoplasm, with some Cx40 (Figure 6A1) and Cx45 (Figure 6C1) staining between LacZ-hV SFs or around the nucleus in LacZ-hV SFs,

**Figure 3.** Extracellular electrophysiological recordings at day 8 after application of (A) hV SFs (LacZ-hV SFs), (B) MyoC-hV SFs, (C) no cells, and (D) cardiomyocytes (CMCs). E, Electrical stimulation of 1 CMC field resulted in activation of the distal CMC field after application of LacZ-hV SFs, MyoC-hV SFs, or CMCs. Electrical stimulation of acellular channel cultures resulted in nonresponse, associated with asynchrony throughout follow-up. F, CV across LacZ-hV SFs, MyoC-hV SFs, and CMCs. Conduction block was present in the absence of cells in the channel. LacZ-hV SFs are presented as hV SFs in this Figure. One-way ANOVA with Bonferroni correction for multiple comparisons: "P<0.05 vs MyoC-hV SFs, ""P<0.01 vs CMCs.
respectively. MyoC-hVSFs displayed similar Cx40, Cx43, and Cx45 staining patterns as LacZ-hVSFs at day 1 of culture (Figures 6A3 through 6C3); however, in MyoC-hVSFs, the amount of Cx40 and Cx45 was much higher (3266 and 2733, respectively; P<0.001; Figures 6A5 through 6C5 and 6A6 through 6C6). Comparison of cardiac connexin levels between neonatal rat cardiomyocytes and MyoC-hVSFs at day 8 revealed that Cx43 amounts were >15 times higher in cardiomyocytes (P<0.01), whereas amounts of Cx40 and Cx45 were significantly higher in MyoC-hVSFs (>40 and 10 times, respectively; P<0.001; Figures 6A5 through 6C5 and 6A6 through 6C6).

**RT-PCR Analysis**

Differences in gene expression were studied between untreated hVSFs, LacZ-hVSFs, and MyoC-hVSFs, both at day 1 and at day 8 of culture (Figure 7). No or only minor differences existed in expression of the analyzed genes between hVSFs and LacZ-hVSFs. However, forced expression of MyoC in hVSFs upregulated the transcription of the genes SCN5A and CACNA1C, which encode the voltage-gated fast sodium channel and voltage-gated L-type calcium channel proteins, respectively. In addition, the expression levels of both SCN5A and CACNA1C increased in MyoC-hVSFs from day 1 to day 8 of culture. Furthermore, MyoC transduction led to a downregulation of KCNA4 expression, which was especially noticeable at day 8. No induction of KCNH2 or KCNJ2 was found in MyoC-hVSFs during follow-up. However, forced MyoC expression resulted in the time-dependent expression of the genes KCNJ3 and KCNJ8, both of which encode inward-rectifier potassium channel proteins. In addition, at day 8, MyoC-hVSFs but not LacZ-hVSFs were expressing KCNMB1, which encodes a voltage- and calcium-sensitive potassium channel protein. Analyses of MyoC gene transduction in hVSFs showed the increased expression of ion channels important for excitation17,18 and a significant increase in the expression of connexins, components that are essential for intercellular electrical conduction.

**Intracellular Electrical Recordings**

Intracellular patch-clamp recordings were performed in cocultures of LacZ-hVSFs (n=9) or MyoC-hVSFs (n=9) and cardiomyocytes (n=8) at day 1 and day 8 after culture initiation. Both LacZ-hVSFs and MyoC-hVSFs showed conducted APs. However, at day 1 of culture, MyoC-hVSFs showed larger conducted APs than LacZ-hVSFs (Figure 8A). During 8 days of culture, the maximum diastolic potential became more negative, and the conducted APs of MyoC-hVSFs increased significantly in amplitude and rate of rise and became comparable to APs derived from cardiomyocytes (Figure 8B and 8D1 through 8D4). This is in contrast to the maximum diastolic potentials and conducted APs of LacZ-hVSFs. Importantly, the estimated gap junctional conductance of the MyoC-hVSFs (n=9) increased significantly over time, from 18.1±5 to 80.3±18 nS, in contrast to LacZ-hVSFs (n=9; Figure 8C), which indicates a time-dependent increase in the degree of intercellular coupling after MyoC gene transfer in hVSFs. Surprisingly, inward currents of ion channels typically involved in excitation (SCN5A, CACNA1C) were not recorded in MyoC-hVSFs at day 1 (n=8) or day 8 (n=8) of culture, despite their increased gene expression (Figure 7). These currents were easily measured in control cardiomyocytes (n=12).17

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**Figure 4. CV across CMCs, LacZ-hVSFs (presented as hVSFs), and MyoC-hVSFs, with the associated dyssynchrony between 2 CMC fields. No differences were found in CV or dyssynchrony in the cultures receiving CMCs or LacZ-hVSFs during follow-up. However, in cultures having MyoC-hVSFs, both CV and dyssynchrony were changed significantly in the first 8 days (P<0.01). One-way ANOVA with Bonferroni correction for multiple comparisons: †P<0.01 vs CMCs day 8. One-way repeated-measures ANOVA (with time point as factor) and Bonferroni-corrected: ‡P<0.01.**
Discussion

The key findings of the present study are (1) application of MyoC gene–expressing hVSFs in an acellular channel between 2 cardiomyocyte fields resulted in resynchronization of 2 dyssynchronously beating cardiomyocyte fields, and (2) MyoC gene expression in hVSFs enabled simultaneous activation of the 2 adjacent cardiomyocyte fields by electrical stimulation of these cells, whereas it was not possible to stimulate via control hVSFs. Both phenomena appear to be mediated mainly by the MyoC-dependent activation of genes encoding connexins, strongly enforcing intercellular electrical coupling.

Role of Scar Tissue in Cardiac Dyssynchrony and Resynchronization

Scar tissue formation after myocardial infarction is associated with an accumulating population of electrically inert fibroblasts and extracellular matrix formation. Scar fibroblasts are not only nonexcitable and poorly coupled, thereby creating areas of conduction block and zones of slow conduction, but they also modulate excitability and conduction properties of surrounding cardiomyocytes. Previous studies reported on the limited capacity of cardiac fibroblasts to conduct electrical current over extended distances. As a result of modest expression levels of connexins between these cells and their nonexcitability, conduction is not only limited in distance but also in velocity. Efficient mechanical activation of the myocardium depends on a coordinated and fast spread of electrical activation across well-coupled excitable cardiomyocytes. Therefore, the presence of poorly coupled, electrically inert scar fibroblasts contributes to increased dyssynchrony between ventricular segments.

Resynchronization After Transplantation of MyoC-Expressing hVSFs

We demonstrated that transplantation of MyoC-expressing hVSFs in an acellular channel between 2 cardiomyocyte fields resulted in synchronization of the 2 fields, which was accompanied by an increase in CV both at day 1 and 8 of coculture compared with control hVSFs. Previously, Kizana et al. showed that forced expression in dermal fibroblasts of the gene encoding the myogenic transcription factor MyoD resulted in excitability of some of these fibroblasts. However, because MyoD was used, the differentiated fibroblasts (now myotubes) did not express sufficient Cx43 to be electrically coupled to each other. Surprisingly, forced Cx43 expression in these myotubes even reduced the number of excitable cells (to <4%), possibly due to an increased electrotonic load. In the present study, we used hVSFs, and these cells already naturally express Cx43.

Although the electrophysiological mechanisms responsible for resynchronization in the present study remain to be
determined in more detail, increased connexin expression in MyoC-hVSFs ensures improved intercellular coupling, whereas a minor role might be attributed to ion channels. Although both mRNAs and proteins associated with a number of essential ion channels involved in excitation are present in hVSFs after myocardin gene transduction, their functionality could not be shown. However, ion channels are complex structures, and several requirements are needed to ensure functionality. Nevertheless, the pore protein is present, which is at least necessary for the expression of a functional ion channel.

**Figure 6.** Comparison of immunocytochemical analysis of Cx40, Cx43, and Cx45 between LacZ-hVSFs (presented as hVSFs) and MyoC-hVSFs at both day 1 and day 8 after culture and cardiomyocytes after 8 days of culture. Two-factor mixed ANOVA with Bonferroni correction for multiple comparisons: *P*<0.001 vs hVSFs at day 1, **P*<0.05 vs MyoC-hVSFs at day 1; 1-way ANOVA, Bonferroni-corrected: ***P*<0.05 vs cardiomyocytes.

**Figure 7.** Semiquantitative RT-PCR analysis of ion channel gene expression in untreated (U) hVSFs or in hVSFs expressing eGFP and either LacZ (L), or MyoC (M) at day 1 and day 8 after culture. MyoC gene transduction in hVSFs resulted in (increased) expression of SCN5A, CACNA1C, KCNJ3, KCNJ8, and KCNMB1. These genes were exclusively expressed or more expressed at day 8. In contrast, MyoC gene expression resulted in downregulation of KCNA4 expression. Expression of KCNH2 and KCNJ2 was not detectable in MyoC-hVSFs during follow-up. GAPDH and MyoC gene expression were used as reference. The dilution factor of the template DNA is indicated at the bottom of the Figure.
Electrical Capture in hVSFs After Forced MyoC Expression

It was not possible to resynchronize the 2 cardiomyocyte fields at any moment by electrical stimulation of control hVSFs. However, after forced expression of the MyoC gene in hVSFs, electrical capture of the cardiomyocyte fields was observed in 20% of the experiments at day 1 and 80% of the experiments at day 8. In contrast to control hVSFs, which play a rather depressing role in conduction, hVSFs that overexpress MyoC allow rapid AP transmission. We also showed that MyoC-hVSFs were able to conduct the electrical pacing pulse to adjacent cardiomyocyte fields, which led to their electrical and mechanical activation. Hence, relatively high expression levels of connexin appear to be mandatory in successful electrical capture.

In Vitro to In Vivo Translation

Genetic modification of cells requires efficient transfection to ensure optimal expression of the gene of interest. Several vectors are available, but adeno-associated virus (AAV) vectors appear preferable with regard to cardiac gene therapy in vivo, because they demonstrated good tissue penetration and sustained transgene expression.26

In the present study, we show the beneficial effects of myocardin gene overexpression in hVSFs on resynchronization; however, Badorff et al27 showed that a similar overex-

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**Figure 8.** Intracellular patch-clamp measurements in LacZ-hVSFs (presented as hVSFs), MyoC-hVSFs, and cardiomyocytes (CMCs) at day 1 and day 8 of culture. Gj indicates gap junctional conductance; MDP, maximum diastolic potential; APA, AP amplitude; dV/dtmax, maximal upstroke velocity; and APD90, AP duration until 90% repolarization. Two-factor mixed ANOVA with Bonferroni correction for multiple comparisons: *P<0.05 vs MyoC-hVSFs, **P<0.05 vs day 1.
pressure in neonatal cardiomyocytes resulted in hypertrophy. Therefore, not only is the efficiency of infection important to obtain an optimal effect, but the site-directed introduction and cell-type–specific expression of the gene is important to prevent a negative outcome. In addition, because the heart forms a functional electrical syncytium that comprises different cell types with different electrophysiological characteristics, it is of importance to study the adaptation of genetically modified cells in relation to properties of the target tissue.

Possible Clinical Implications

Slowing and even blocking of the spread of activation by scarred myocardium is not the only deleterious consequence of myocardial scar formation. In previous clinical studies, we demonstrated the negative effects of scar tissue on the response to CRT.9,10,29 A significantly lower response rate was observed in patients with scars near the pacing electrode (14% versus 81% in patients without detectable scar tissue).10 In the present study, we demonstrated the striking effects of forced MyoC gene expression in hVSFs on electrical conduction. Genetic modification of hVSFs with a recombinant MyoC gene both resulted in resynchronization of 2 adjacent beating cardiomyocyte fields and endowed hVSFs with the ability to serve as a pacing site to capture the cardiac impulse. Although conducted ex vivo, the present study could provide a rationale for the treatment of scar-related myocardial dyssynchrony and contribute to the increased efficiency of CRT in patients with scarred myocardium.

Study Limitations

In the present study, myocardial scar tissue was represented by fibroblasts only. Although this model is sufficient to investigate the concept raised in the present study, it does not fully mimic the composition of myocardial scar tissue in vivo. Excessive extracellular matrix and surviving cardiomyocytes might influence the effects of MyoC expression in the infarcted area. Furthermore, neonatal rat cardiomyocytes were used in the present study. Although the use of human cardiomyocytes is preferable, the difficulties related to the availability of this cell type and their tendency to dedifferentiate during culture prevented the use of human cardiomyocytes.

Conclusions

Forced MyoC expression in hVSFs allowed resynchronization of 2 separated cardiomyocyte fields and establishment of interconnecting tissue for electrical pacing. Both phenomena primarily result from the MyoC-dependent activation of genes that encode connexins, thereby strongly enforcing intercellular electrical coupling.

Disclosures

None.

References


**CLINICAL PERSPECTIVE**

Myocardial scar tissue consists primarily of electrically inert fibroblasts. Consequently, impulse propagation and subsequent mechanical activation are hampered, resulting in increased ventricular dyssynchrony. Cardiac resynchronization therapy is a promising treatment for patients with heart failure and significant dyssynchrony that can improve cardiac function and increase life expectancy. However, \( \approx 30\% \) of patients subjected to cardiac resynchronization therapy do not respond; this nonresponse has been associated with the presence of ventricular scar tissue. Scar burden appears to be involved in both the cause of dyssynchrony and the lack of response to cardiac resynchronization therapy. Modification of scar tissue, favoring electrical conduction, might therefore result in reduction of dyssynchrony and nonresponse. The present study provides a rationale for (1) treatment of scar-related myocardial dyssynchrony and (2) reduction of scar-related nonresponse to cardiac resynchronization therapy. This reduction was accomplished by genetic modification of human ventricular scar fibroblasts by myocardin gene transfer. Myocardin is a cardiac transcription factor known to upregulate expression of cardiac genes, including connexins, which play a crucial role in electrical conduction. The present study shows that forced myocardin gene expression in scar fibroblasts allowed electrical stimulation of these cells and endowed them with the ability to conduct an electrical impulse at high velocity, which resulted in resynchronization of 2 separated cardiomyocyte fields. Genetic modification of myocardial scar tissue may be a promising therapy to resynchronize the infarcted myocardium, thereby improving cardiac function.
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