Cardiomyocyte-mediated contact programs human mesenchymal stem cells to express cardiogenic phenotype

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Background: Intercellular crosstalk and cellular plasticity are key factors in embryogenesis and organogenesis. The microenvironment plays a critical role in directing the progression of stem cells into differentiated cells. We hypothesized that intercellular interaction between adult human mesenchymal stem cells and adult human cardiomyocytes would induce stem cells to acquire the phenotypical characteristics of cardiomyocytes, and we tested the role that direct cell-to-cell contact plays in directing this differentiation process. Human mesenchymal stem cells were cultured in the presence of human cardiomyocytes ("coculture") or in the presence of media conditioned by separate cultures of human cardiomyocytes ("conditioned media").

Methods: Human cardiomyocytes were labeled with chloromethyl derivatives of fluorescein diacetate. In the coculture experiments, human mesenchymal stem cells and human cardiomyocytes were mixed at a 1:1 ratio in smooth muscle 2 media and seeded at a cell density of 10,000 cells/cm^2. Cells were cocultured in an incubator at 37°C for 48 hours. Subsequently, fluorescence-activated cell sorting was used to extract the differentiating human mesenchymal stem cells. In the conditioned media experiments, human mesenchymal stem cells were incubated in media previously conditioned by cardiomyocytes, in the presence and absence of serum (±serum). The conditioned media was changed 3 times, at intervals of 48 hours. Total RNA was isolated and reverse transcriptase-polymerase chain reaction was performed for expression of contractile proteins and cardiac specific genes. Immunostaining against myosin heavy chain, β-actin troponin-T, and troponin-I was performed.

Results: Fluorescence-activated cell sorting analysis identified 66% of the human mesenchymal stem cells in the G1 phase. Differentiated hMSCs from the coculture experiments expressed myosin heavy chain, β-actin, and troponin-T by reverse transcriptase–polymerase chain reaction. Immunostaining was also positive against myosin heavy chain and troponin-T. In contrast, only β-actin expression was observed in the human mesenchymal stem cells incubated with conditioned media ± serum.

Conclusion: In addition to soluble signaling molecules, direct cell-to-cell contact is obligatory in relaying the external cues of the microenvironment controlling the differentiation of adult stem cells to cardiomyocytes. These data indicate that human mesenchymal stem cells are plastic and can be reprogrammed into a cardiomyogenic lineage that may be used in cell-based therapy for treating heart failure.

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Abstract was accepted for oral presentation at the American Heart Association's Scientific Sessions, Chicago, Ill, November 17-20, 2002.

This study was supported in part by State of Pennsylvania (#11700014), Defense Advanced Research Agency (DARPA), and Grant-in-Aid from Cardiovascular Institute of Philadelphia, Pa.

Received for publication Sept 30, 2002; revisions requested Oct 25, 2002; revisions received Nov 9, 2002; accepted for publication Dec 3, 2002.

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J Thorac Cardiovasc Surg 2003;126:124-32

0022-5223/2003 $30.00 + 0

doi:10.1016/S0022-5223(03)00074-6

Cell transplantation is being explored as an alternative therapy for treating patients with end-stage heart failure. Fetal cardiomyocytes,[1,2] skeletal myoblasts,[3-5] immortalized cell lines,[6] fibroblasts,[7] smooth muscle cells,[8] and hematopoietic stem cells[9] have been transplanted into host myocardium. Although this implantation was associated with improved cardiac function,[10,11] evidence for normal electromechanical coupling between the implanted cells and host cardi-

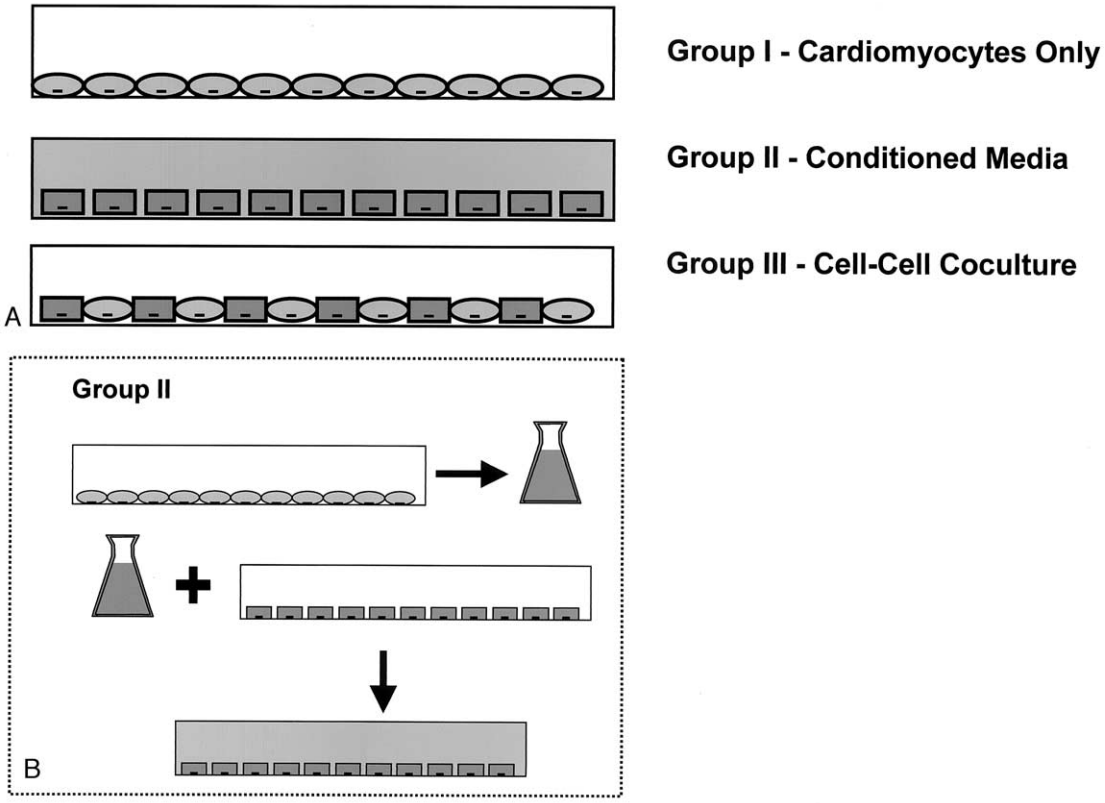

Figure 1. A, Group I cardiomyocytes grown in the tissue culture plates. Group II conditioned media prepared from the cardiomyocytes after 48 hours in culture. Group III coculture of hMSC with cardiomyocytes. B, Conditioned media prepared from the soluble factors released from the cardiomyocytes, in which hMSC were subsequently cultured.

omyocytes has been absent. All reported techniques of cellular cardiomyoplasty (CCM) have limitations and shortcomings, and these depend primarily on the type of cell used for transplantation. Ideally, cells for use in CCM should be pluripotent, possess the capacity to differentiate to the desired cell type under appropriate stimuli, have a limited capacity to multiply, and should be capable of functional integration into the host myocardium.

The adult human mesenchymal stem cell (hMSC) has many of these characteristics, and as such may be highly suitable for CCM. Under appropriate stimuli these stem cells are highly plastic[12] and can differentiate into specialized tissues such as cartilage,[13] osteocytes,[14] adipocytes,[15] chondroblasts,[16] myogenic cells,[17] and cardiac cells.[18] Mesenchymal stem cells treated with azacytidine transdifferentiate into a cardiac phenotype in vitro.[19] Moreover, these stem cells can also differentiate into cardiomyocytes when injected into normal or acutely injured myocardium. However, the signals that are crucial for cardiac-specific lineage are not well known. To improve the clinical utility of cell-based therapy, the signaling pathways that induce the transformation of stem cells into cardiomyocytes need to be identified to achieve successful engraftment.

Potential signals that direct stem cells to differentiate into cardiomyocytes include chemical (soluble) and mechanical (physical) factors. Stem cells differentiate into mature cells based on the signals from the microenvironment. The objective of our study was to determine whether the signals that are produced by mature cardiomyocytes are sufficient to induce stem cells to differentiate into cardiomyocytes and whether both physical contact and soluble factors are required for this process.

Material and Methods

Three experimental groups of cell cultures were used to study the effects of soluble and mechanical factors on the transformation of stem cells into cardiomyocytes. In group I (Figure 1, *A*), adult human cardiomyocytes were cultured alone. In group II (Figure 1, *B*), hMSCs were cultured in media that had been conditioned by separate cultures of cardiomyocytes ("conditioned media"). In group III (Figure 1, *A*), stem cells and cardiomyocytes were cultured together ("coculture"). In each group, cells were studied for the expression of cardiac-specific genes.

Culture of hMSCs

Human mesenchymal stem cells were obtained from a commercial source (BioWhittaker Molecular Applications/Cambrex Inc, East Rutherford, NJ). The cells were originally isolated from the bone marrow of the posterior iliac crest of the pelvic bone of normal healthy volunteers and were positive for SH2, SH3, SH4, CD29, CD44, and CD45 and negative for CD14, CD34, and CD45 as determined by flow cytometric analysis of surface antigens markers. These hMSCs were grown in human mesenchymal stem cell medium containing 440 mL of basal medium, 50 mL of mesenchymal growth supplements (10% fetal bovine serum), 200 mmol/L of L-glutamine, 25 units of penicillin, and 25 μg of streptomycin at 37°C in a CO_2 incubator. At 80% confluence the hMSCs were split and subcultured.

Culture of Human Cardiomyocytes

Cultured human cardiomyocytes were obtained from BioWhittaker Inc. The cardiomyocytes were grown in smooth muscle cell media (SM2) containing 500 mL of smooth cell basal medium 0.5 mg/mL of human epithelial growth factor (EGF), 5 mg/mL of insulin, 1 mg/mL of human fibroblast growth factor (FGF), 50 mg/mL gentamicin, 50 mg/mL of amphotericin B, and 5% fetal bovine serum.

Preparation of Conditioned Media

The cardiomyocytes were cultured in T-25 cm^2 flask with 5 mL of SM2 media for 48 hours. The resulting conditioned media was replaced with equal volume of fresh media. Subsequently, the conditioned media was filtered with a 0.22 μm filter and used to feed cultures of hMSCs.

Fluorescent Staining of the Human Cardiomyocytes

Before cardiomyocytes were cocultured with hMSCs, cardiomyocytes were labeled with green-fluorescent fluorescein diacetate (CMFDA, CellTracker, Molecular Probes Inc, Eugene, Ore) and sorted by fluorescent activated cell sorting (FACS). CMFDA is a fluorescent chloromethyl derivative that freely diffuses through the membranes of live cells. Once inside the cell, this mildly thiol-reactive probe undergoes a glutathione S-transferase-mediated reaction to produce a membrane-impermeant glutathione-fluorescent dye adduct. In brief, CMFDA was mixed with prewarmed (37°C) serum-free SM2 media to a final concentration of 10 μmol/L. This concentration of probe was determined to be optimal for staining of cardiomyocytes using serial dilutions. The cardiomyoctes were incubated with the probe for 45 minutes at room temperature. The media was subsequently replaced with fresh serum-free media and incubated for another 45 minutes to ensure complete modification of the probe and then the cells were washed with phosphate-buffered saline solution (PBS) to remove the excess fluorescent label.

Coculture of Human Cardiomyocytes and hMSCs

Labeled human cardiomyocytes and hMSCs were mixed at a ratio of 1:1 in SM2 media, plated at a density of 10,000 cells/cm^2 and incubated in a CO_2 incubator at 37°C for 48 hours. At the end of the experiment the cells were washed with PBS three times and 0.25% trypsin was added to detach the cells from the surface. Cardiomyocytes and treated hMSCs were separated by FACS before analysis.

Culture of hMSCs with Conditioned Media

Media conditioned by cultures of human cardiomyocytes was filtered with a 0.22 μm filter. Conditioned media (5 mL) was used to feed hMSC cultures for 48 hours, at which time it was replaced with fresh conditioned media. Duplicate experiments were conducted in which 10% serum was added to the conditioned media before feeding the hMSCs. At the end of the experiment the cells were washed with PBS three times and 0.25% trypsin was added to detach the cells from the surface.

FACS

After 48 hours of coculture, the differentiated hMSCs and the human cardiomyocytes were trypsinized and centrifuged at 700 rpm for 5 minutes. The cell pellet was suspended in 5 mL of PBS and the hMSCs were sorted and segregated with an 488-nm optical filter. The hMSCs were collected and centrifuged at 500*g* for 10 minutes and processed.

Total RNA Extraction, Reverse Transcriptase-Polymerase Chain Reaction

Total RNA was extracted from untreated hMSCs (negative control), differentiated hMSCs, and human cardiomyocytes (positive control) using an RNeasy Mini isolation kit (Qiagen Inc, Alameda, Calif). Reverse transcriptase-polymerase chain reaction (RT-PCR) was performed to detect expression of myosin heavy chain, β-actin, troponin-T, and troponin-I using specific primers and Superscript One-Step RT-PCR (Invitrogen, Carlsbad, Calif) for cDNA synthesis. Pre-denaturation was performed at 50°C for 30 minutes and 94°C for 2 minutes. PCR amplification was carried out at 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds for a total of 35 cycles and final extension of 1 cycle at 94°C for 7 minutes. The PCR products were size fractionated by 0.7% SeaKem GTG agarose gel electrophoresis. The following primers were used: Myosin heavy chain 5'-GGAGGAGGACAGGAAAAACCT-3' (forward), 5'-CGGCTTCAAGGAAAATTGC-3' (reverse), troponin-T 5'-GGCAGCGGAAGAGGATGCTGAA-3' (forward), 5'-GAGGCACCAAGTTGGGCATGAACGA-3' (reverse), β-actin 5'-CGCACCACTGGCATTGTCAT-3' (forward), 5'-TTCTCCTTGATGTCACGCAC-3' (reverse), and troponin-I 5'-CCCTGCACCQGCCCCAATCAGA-3' (forward), 5'-CGAAGCCCAGCCCGGTCAACT-3' (reverse). Similarly, RT-PCR was performed for expression of the various connexins in the hMSC using specific primers as follows Cx-45 5'-CTATGCAATGCGCTGGAAACAACA-3' (forward), 5'-CCCTGATTTGCTACTGGCAGT -3' (reverse), Cx-40 5' ATGCACTGTGCGCATGCAGGA-3' (forward), 5'-CAGGTGGTAGTGTTCAGCCAG-3' (reverse), Cx-32 5'-CTGCTCTACCCGGGCTATGC-3' (forward), 5'-CTGCTCTACCCGGGCTATGC-3' (reverse), CX-43 5'-GAATTCTGGTTATCATCGTCGGGGAA-3' (forward), 5'-TACCATGCGACCAGTGGTGCGCT-3' (reverse).

Immunostaining

The differentiated hMSCs were sorted by FACS and cytospun onto slides. The cells were fixed with acetone/methanol 50%/50% vol/vol for 5 minutes at room temperature and repeated twice. The slides were air-dried overnight and bathed in 2 mL of PBS for 10 minutes. The slides were incubated with monoclonal immunoglobulin G (IgG) primary antibody for 45 minutes at room temperature. Primary antibodies were specific for β-myosin heavy chain

(MyHC) (A4.1025, 1:10 DSHB, University of Iowa), sarcomeric myosin (MF-20, 1:100 DSHB, University of Iowa), cardiac troponin-T (CT-3 1:100 DSHB, University of Iowa), and troponin-I. The cells were washed with PBS and then incubated with secondary antibody (fluorescein isothiocyanate-conjugated Affinipure goat anti-mouse IgG) diluted 1:200 for 45 minutes at room temperature. Fluorescence imaging was performed using Olympus AX-70 microscope and Open Labs Software.

Results

When initially plated, adult human mesenchymal stem cells appeared rounded in shape. After 24 hours of plating, the cells were adherent, elongated, and spindle-shaped (Figure 2, *a*). During mitosis the cells regained a rounded appearance and remained loosely attached until division was complete. At this phase of the cell cycle the cells flattened and elongated. The hMSCs were subcultured when they reach 80% confluence.

The cardiomyocytes had a rod-shaped morphology and were arranged in a syncytial fashion as shown (Figure 2, *b*). Their particular phenotype lineage was established by immunostaining against myosin heavy chain and troponin-T.

FACS analysis of cocultured hMSCs determined that 66% of the cells were in G1 phase. The remaining cells were in S phase (21%) and G2 phase (13%).

In the coculture experiments, immunohistochemistry revealed an absence of staining for fast myosin (MF-1) in both cultured cardiomyocytes (Figure 3, *a*) and differentiating hMSCs (Figure 3, *b*) and positive staining against sarcomeric myosin (MF-20), β-MyHC, CT-3, and troponin-I in cardiomyocytes (Figure 3, *c, e, g*, and *i*, respectively) and in transformed hMSCs (Figure 3, *d, f,* and *h*, respectively). There was no staining against troponin-I protein at 2 days of coculture (Figure 3, *j*). In addition, RT-PCR revealed the expression of myosin heavy chain, β-actin, and cardiac troponin-T in cell-to-cell contact coculture (Figure 4). Untreated hMSCs and human cardiomyocytes were used as negative and positive controls, respectively. In the conditioned media experiments, β-actin expression was noted in the hMSCs exposed to cardiomyocyte-conditioned media, both in the presence and absence of serum. There was no expression of the β-MyHC, troponin-I, or troponin-T in hMSCs treated with the conditioned media (Table 1).

Importantly, the expression of gap-junction proteins (Cx 40, Cx-43, Cx-45, Cx-32) was identified in untreated cultured hMSCs. This finding is particularly encouraging since gap junctions are critical to establishment of cell-to-cell electrochemical coupling (Figure 5 and Table 2).

Discussion

These studies demonstrate that adult human mesenchymal stem cells have the potential to differentiate into cardiomyocytes under the appropriate microenvironment. Under coculture conditions, when there was direct contact between cardiomyocytes and hMSCs, the hMSCs begin to express the cardiac-specific proteins myosin heavy chain, β-actin, and troponin-T. Strikingly, only β-actin was expressed when the hMSCs were cultured with conditioned media, either in the presence or absence of serum, when there was no physical contact between the cardiomyocytes and the hMSCs.

Elements of the microenvironment provide the critical signals to direct and control differentiation of human mesenchymal stem cells to a cardiac lineage. The potential factors involved are numerous but may be broadly characterized as either chemical (soluble) or physical (mechanical). In these experiments, we demonstrated that the soluble factors alone were not sufficient to induce differentiation of hMSCs into cardiomyocytes and that physical contact between the cardiomyocytes and hMSCs is requisite. In addition, the cell density may be critically important since the expression of cardiac specific proteins occurred when car-

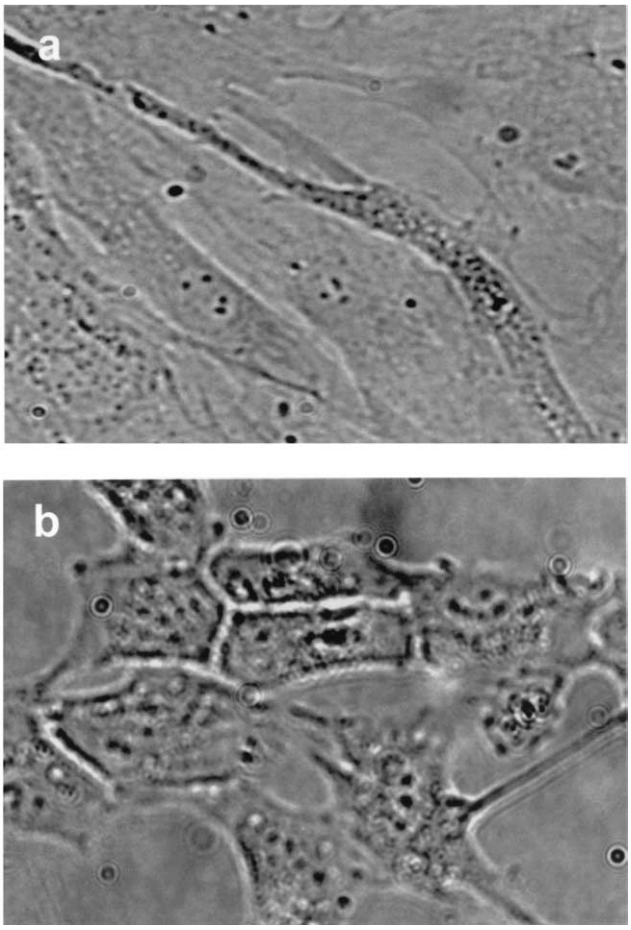

Figure 2. a, Spindle-shaped human mesenchymal stem cells (20×), 24 hours after seeding on cell polystyrene cell culture surface. b, Rod-shaped human cardiomyocytes forming intercellular junctions with neighboring cells.

CSP

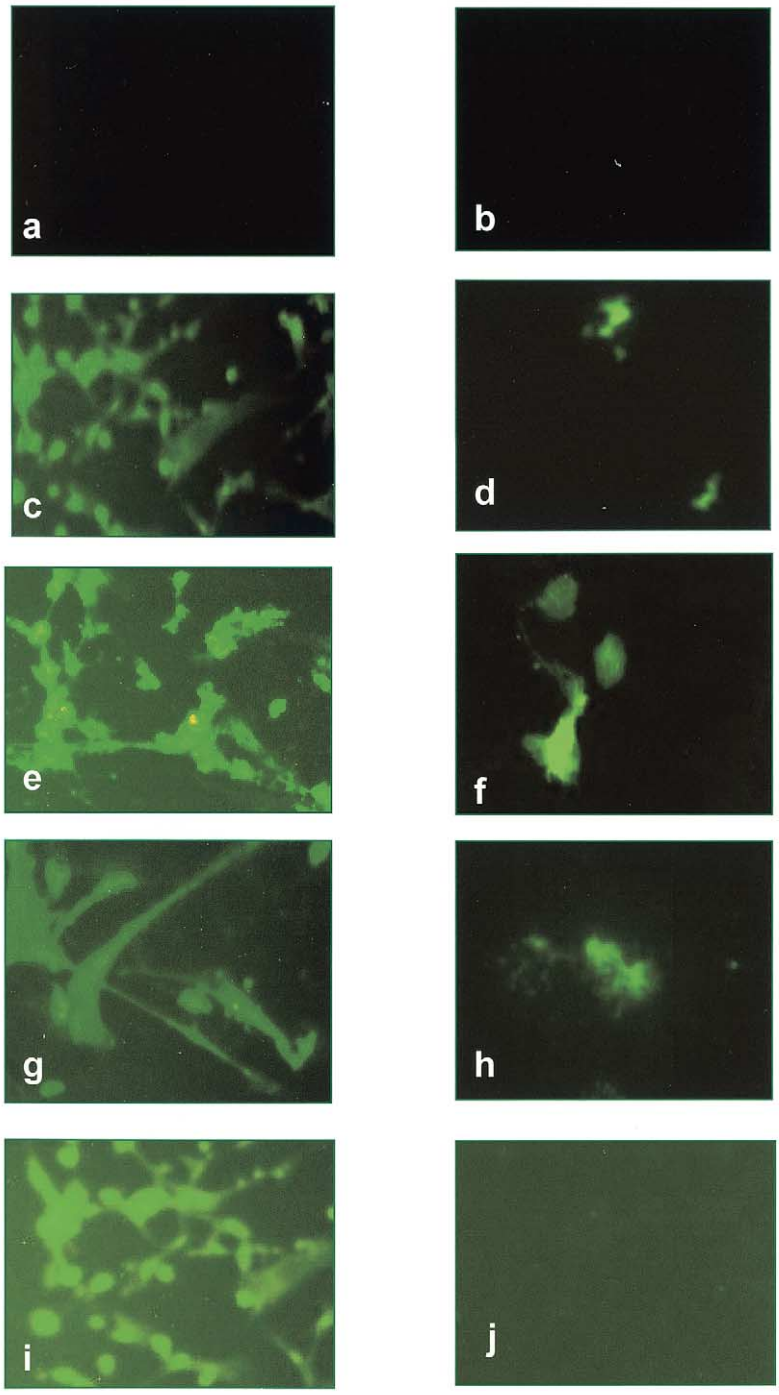

Figure 3. a to h, Left panel shows positive controls, consisting of cardiomyocytes. Right panel shows transformed hMSCs (10×). a, No staining observed against fast myosin (MF-1) in human cardiomyocytes. b, No staining observed against fast myosin (MF-1) in untreated hMSCs (negative control). d, Positive staining for sarcomeric myosin (MF-20) in cocultured hMSCs. f, Positive staining for heavy chain myosin (β-MyHC) in cocultured hMSCs. h, Positive staining for troponin-T in cocultured hMSCs. j, Negative staining against troponin-I. c, e, g, i, Positive staining in cultured cardiomyocytes for MF-20, β-MyHC, troponin-T, and troponin-I, respectively.

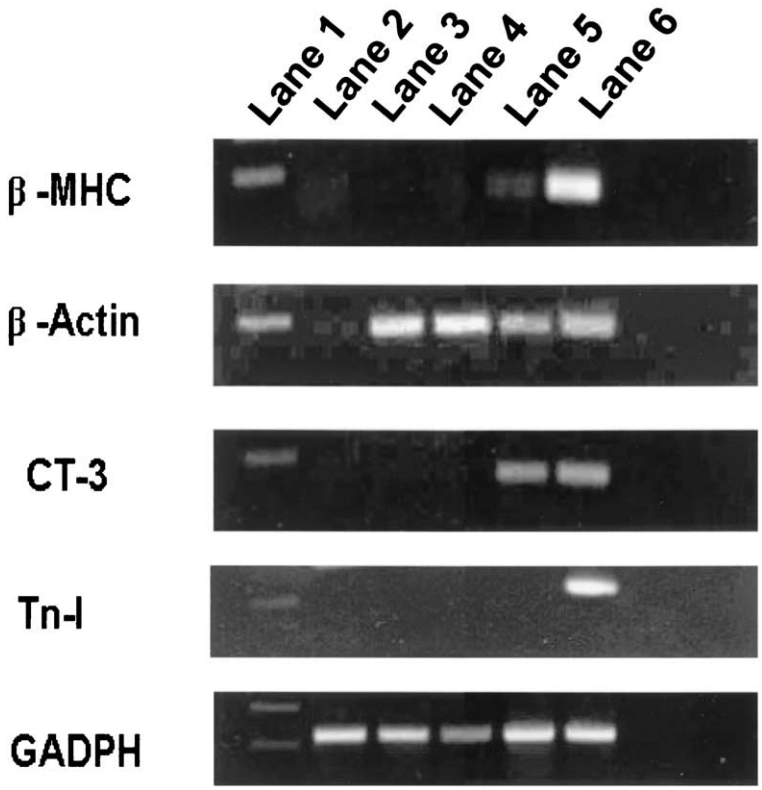

Figure 4. RT-PCR for specific hMSC transformation markers (48-hour coculture). *Lane 1*: 1-kb DNA ladder; *lane 2*: hMSC (negative control); *lane 3*: conditioned media treated with hMSC in the presence of serum; *lane 4* conditioned media treated with hMSC in the absence of serum; *lane 5*: cell-cell contact-dependent coculture; *lane 6*: human cardiomyocytes (positive control). β-MHC, β-actin, and CT-3 expression in cell-cell contact dependent coculture. Expression of β-actin in conditioned media ± serum. *GADPH* is internal control. *Tn-I* is troponin-I expression in cultured cardiomyocytes.

TABLE 1. Group I (cardiomyocytes alone) showed expression of β-myosin heavy chain, β-actin, troponin-T, and troponin-I; Group II (hMSC exposed to conditioned media) showed only expression of β-actin; Group II (cell-cell contact coculture) showed expression of β-myosin heavy chain, β-actin, and troponin-T

	β-MyHHC	β-Actin	Troponin-T	Troponin-I
Group I	+	+	+	+
Group II	−	+	−	−
Group II	+	+	+	−

diomyocytes and hMSCs were cocultured at 1:1 ratio, but were not detected at other plating ratios in preliminary work performed in our laboratory.

During embryogenesis, stem cells differentiate to form specialized organs and tissues. In the adult, some tissues are able to regenerate lost or damaged cells through the differentiation of progenitor cells or resident stem cells. However, cardiomyocytes are not readily replaced with contractile cells when they are lost as a result of myocardial infarction. Cardiomyocytes are in a terminal phase of the cell cycle and do not undergo cellular division. However, the hMSCs progress through the cell cycle and are capable of cellular division. The observation that 66% of the hMSCs were in G1 phase of the cell cycle suggests that prolongation of this phase could play an important role in directing hMSC commitment. Because cardiomyocytes are incapable of division and no readily available source of replacement cells exists, lost cells are partially replaced with scar tissue, while neighboring cardiomyocytes hypertrophy in an effort to restore cardiac function. There is no clear understanding why stem cells that are capable of producing cardiomyocytes in the embryo are incapable of such a task in the adult heart. Recent evidence demonstrates that such replacement may occur to a small degree, but that the rate may be too slow to be functionally significant.[20] The purpose of these experiments was to try to elucidate some of the signals that are involved in the differentiation of mesenchymal stem cells into cardiomyocytes. Through a clear understanding of these processes, we may be able to manipulate stem cells as

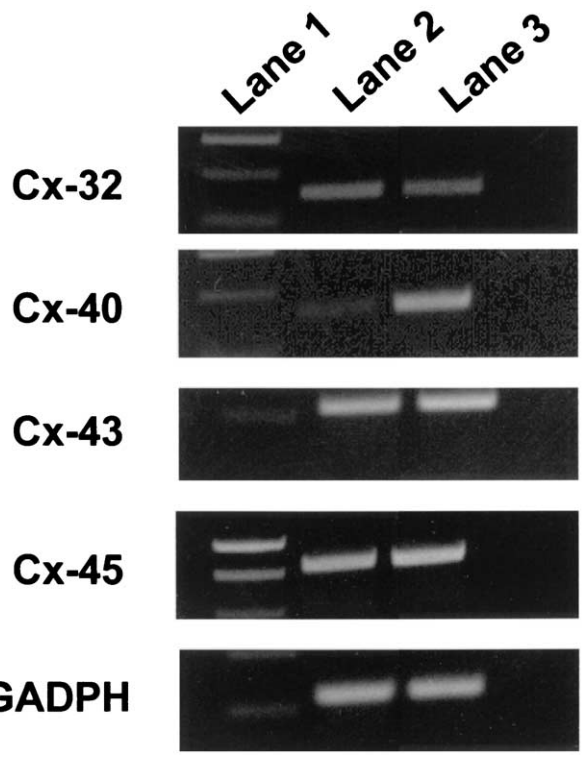

Figure 5. Expression of gap junctions connexin-specific genes (Cx-32, Cx-40, Cx-43, and Cx-45) in hMSC. *Lane 1*: 1-kb DNA ladder; *lane 2*: hMSC expression of specific connexins; *lane 3*: expression of connexin-specific genes in human cardiomyocytes (positive control).

TABLE 2. Expression of connexin-specific genes in untreated hMSC and adult cardiomyocytes

	hMSC	Cardiomyocyte
Cx-32	+	+
Cx-40	+	+
Cx-43	+	+
Cx-45	+	+

an ideal source of cells for cellular cardiomyoplasty or that could be able to direct them to migrate to the myocardium to replenish cardiomyocytes at a rate that can be clinically useful.

Previous work has demonstrated the ability of stem cells to undergo differentiation into cardiomyocytes, but these studies have not examined the mechanisms of the differentiation process. It is clear that the microenvironment of the cells is an important component. If stem cells are implanted into myocardial scar, they differentiate into a variety of nonmyocyte cell types,[21] reflecting the prevailing influence of the myocardial microenvironment. Conversely, if stem cells are implanted into an "optimally" permissive microenvironment, they may selectively differentiate into cardiomyocytes.

Stem cells may also be used to repopulate the heart without direct injection into the myocardium. In mice that have undergone bone marrow transplantation and subsequent regional myocardial infarction, marrow-derived cardiomyocytes and endothelial cells have been found in the peri-infarct region.[9] Host-derived cardiomyocytes have been located in small numbers in transplanted human hearts that have been later excised.[22] These data demonstrate both the ability of adult mesenchymal stem cells to differentiate into cardiomyocytes under the proper conditions and also reflect the pluripotent nature of these stem cells in that they also can form the supporting structures of the heart. Finally, stem cell–derived cardiomyocytes have only been located in hearts subjected to injury, and not under normal conditions. This suggests that the microenvironment of the stem cell is critical in permitting the process differentiation and that the stem cells are responding to some factor(s), either chemical or mechanical, active during these periods of stress or injury.

The processes that regulate cell differentiation are complex, and the interactions between signals are largely unknown. However, it is clear that the microenvironment of the developing cell plays a critical role in determining its ultimate fate. The components of the microenvironment that influence cellular differentiation can be broadly classified as either chemical or mechanical. The chemical signals include cytokines, hormones, ionic gradients, and other soluble factors that are produced by either neighboring or distant cells. The mechanical factors can be equally complex and may include stimulation of receptors through direct contact with neighboring cells or by the components of the extracellular matrix, the influences of cell stretch or other forces, the electrical environment of the cells, and perhaps even other local signals.

Coculture and conditioned media techniques provide an excellent model to study the signals that influence cellular development and differentiation. Through the use of coculture techniques, stem cells are exposed to many of the physical and chemical signals that are present within the native myocardium, particularly the mechanical signals that are produced through direct cell-to-cell contact between the cardiomyocytes and hMSCs. In contrast, the experiments that use conditioned media provide the soluble factors that are elaborated from the cardiomyocytes without allowing direct cell-to-cell contact, thus separating the effects of the chemical stimuli from the physical. Although the conditioned media contains cytokines and other soluble elements that are elaborated from the cardiomyocytes during normal growth, these are not sufficient to stimulate the stem cells to differentiate into cardiomyocytes. In the conditioned media experiments, the signals that are related to direct contact

between the cell types are lacking. This suggests that the cell-to-cell contact between cardiomyocytes and hMSCs in the coculture scenario is critical in the differentiation process.

Cell-cell interaction is a complex phenomenon, which involves contact between cells through junctional complexes including tight junctions, desmosomes, and gap junctions. For this reason, it is important that the transformed stem cells are capable of expressing the connexins that are critical in the formation of gap junctions. The expression of the gap junction proteins seen in the untreated hMSCs is critical for the complete expression of cardiac phenotype and functional integration into the host tissue. These proteins may be involved in the signal transduction that occurs between the hMSCs and cardiomyocytes during the coculture experiments. Perhaps the connexins have been arranged into dormant channels that open with cell-to-cell contact and aid in the transfer of intracellular signaling molecules.

Cell contact can also result in changes in cell shape due to mechanical stretch imposed by neighboring cells. In addition, homology of cell surface receptors and other proteins involved in cell-cell adhesion between the cardiomyocytes and stem cells could activate the differentiation-associated genes and alter the genotype and phenotype. The intracellular signal transduction pathways may be triggered by transmembrane receptors such as epidermal growth factor or platelet-derived growth factor via autophosphorylation and by binding with ligands that regulate the transmission of mitogen-activated protein kinase signaling pathway or activation of protein kinase C pathway via hydrolysis of phosphoinositol.

Evaluation of the factors required to promote stem cell differentiation is critical in refining the techniques of cellular cardiomyoplasty. Current techniques are inadequate to produce a clinically significant increase in cardiac function for a variety of reasons. When skeletal myoblasts or other noncardiomyocytes are used, there is incomplete electromechanical integration of the implanted cells with the native cardiomyocytes. Although there may be a concomitant improvement in cardiac function, this is likely due to changes in the diastolic properties of the ventricle. As such, maximal benefit can only be obtained if the transplanted cells have the capacity to differentiate into cardiomyocytes that can fully integrate into the myocardium. Another concern with the current techniques of CCM is the low rate of integration of implanted cells. Only a small fraction of the implanted stem cells remain viable in the myocardium after CCM, limiting its therapeutic benefit.

If the signals that are involved in the transformation of stem cells into cardiomyocytes can be understood, then the rate of functional integration of these cells, and thus the success of CCM, can be improved significantly. Because these results suggest that mechanical factors are important in the transformation process, then it is possible that stem cells can be preprogrammed in vitro in an environment that mimics many of these conditions before implantation. By doing this, it may be possible to commit the stem cells to a cardiomyocyte lineage before implantation to increase the therapeutic yield of CCM.

Limitations of This Study

These studies were conducted using an in vitro controlled microenvironment and observed for relatively short period of time (48 hours). Although several myogenic markers were expressed in the stem cells subjected to coculture, β-MyHC, β-actin, and troponin-T are also expressed to varying degrees by skeletal muscle cells. In addition, troponin-I was not seen in the treated stem cells. However, the time frame of the study was relatively short, and it may take upward of 7 days before troponin-I expression may be detectable in transforming cells. Future studies will need to look at this phenomenon for longer periods of time to see the expression of other cardiac proteins. In addition, it is anticipated that electromechanical integration of the hMSCs with the cultured cardiomyocytes will also occur as a late finding, if the cell cultures can be maintained long enough.

Cell fusion[23] between the cardiomyocytes and hMSCs remains a concern when interpreting these observations and those of related studies of gene expression in differentiating stem cells. The fact that troponin-I expression was absent in cocultured hMSCs suggests that fusion is not responsible. A related issue is the chance that separation of the cardiomyocytes and hMSCs was not complete and that RT-PCR amplified genes from the cardiomyocytes that contaminated the hMSC cell population. If this were to have occurred, then the RNA encoding troponin-I would have been seen in the stem cells subjected to coculture.

Conclusion

In addition to soluble signaling molecules, direct cell-to-cell contact is obligatory in relaying the external cues of the microenvironment controlling the differentiation of adult stem cells to cardiomyocytes. These data indicate that hMSCs are plastic and can be reprogrammed in vitro into a cardiomyogenic lineage that may be used in cell-based therapy for treating heart failure.

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