Cardiothoracic Transplantation

Cell transplantation preserves matrix homeostasis: A novel paracrine mechanism

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Objectives: Cell transplantation prevents chamber dilatation, but the underlying molecular mechanisms remain undefined. Structural cardiac remodeling involves matrix degradation from an imbalance of matrix metalloproteinases (MMP) relative to endogenous tissue inhibitors of metalloproteinases (TIMP). We aimed to determine the capacity of cell transplantation to alter extracellular matrix in the failing heart and, in so doing, identify novel paracrine molecular mediators underlying the beneficial effects of cell transplantation on chamber dilatation.

Methods: Smooth muscle cells were transplanted to the dilating left ventricle of cardiomyopathic hamsters (CTX, n = 15) compared with age-matched media-injected cardiomyopathic (CON, n = 15) and normal hamsters (n = 7). After 5 weeks, left ventricular volume was measured by computerized planimetry. Fibrillar collagen was examined by confocal microscopy. Matrix homeostasis was quantified by measuring MMP/TIMP expression/activity relative to myocardial collagen synthesis (^{14}C-proline uptake).

Results: Left ventricular dilatation was attenuated in CTX hearts ($P = .02$). CTX restored perimysial collagen fiber content and architecture to normal levels. TIMP-2 and TIMP-3 expression were enhanced in CTX (TIMP-2, 195% ± 42% of CON, $P = .02$; TIMP-3, 118% ± 3% of CON, $P = .002$), and correspondingly, gelatinase MMP-2 activity was reduced ($P < .05$). The TIMP:MMP ratio was increased in CTX hearts (*TIMP-2* to *MMP-2*, 410% ± 134% of CON, $P = .04$, and *TIMP-3* to *MMP-9*, 205% ± 47% of CON, $P = .03$), reflecting a reduced capacity for matrix degradation. Collagen synthesis was equivalent (CTX vs CON), suggesting that restored matrix architecture was a function of attenuated matrix degradation.

Conclusions: These data provide the first evidence that cell transplantation limits ventricular dilatation in the failing heart through a paracrine-mediated mechanism that preserves extracellular matrix homeostasis.

The evolving clinical reality that cell transplantation can limit cardiac dilatation and the progression of heart failure is overshadowed by an absence of molecular and cellular mechanisms underlying the beneficial effects of cell engraftment. An improved understanding of the host tissue response to cell implan-

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Abbreviations and Acronyms
ECM   = extracellular matrix
LV   = left ventricle
MMP   = matrix metalloproteinase
SEM   = standard error of mean
SMC   = smooth muscle cell
TIMP   = tissue inhibitor of metalloproteinase
VSMC   = vascular smooth muscle cell

tation may optimize the development of this technique for its evolving therapeutic use.

Muscle cell transplantation in failing hearts has consistent and predictable benefits on limiting left ventricular dilatation and dysfunction, irrespective of the donor tissue source (cardiac, skeletal, or smooth muscle).[1-3] Despite a relatively small mass of transplanted cells and substantial cell losses after injection into the heart, maladaptive cardiac remodeling is somehow profoundly attenuated. This intriguing phenomenon indicates that the continued presence of the cells themselves may not be necessary to improve cardiac function and contradicts the belief that the cells themselves contribute to useful contractile work. Accordingly, it has been proposed that the beneficial effects of cell transplantation may be a consequence of newly expressed bioactive mediators promoted by the process of cell engraftment. However, the bioactive peptides that mediate this proposed paracrine effect of transplanted cells remain elusive.

In the failing heart, dysregulation of extracellular matrix (ECM) plays a central role in maladaptive cardiac remodeling and the transition to overt congestive heart failure, largely by an enhanced activity of matrix metalloproteinases (MMPs), a family of enzymes that degrade key matrix components.[4-7] Activation of matrix proteases in the injured heart results in matrix scaffold degradation and resultant chamber thinning, dilatation, and a poorly coordinated contraction. Tissue inhibitors of metalloproteinases (TIMPs) are complex endogenous biomolecules capable of inactivating the enzymes that degrade matrix components.[8] However, TIMPs are relatively deficient in the failing heart.[9,10] We previously determined that a reduction in TIMP expression paralleled matrix disruption and left ventricle (LV) dilatation in hamster cardiomyopathy.[9] We later demonstrated in mutant mice that reduced TIMP expression accelerated matrix degradation and induced a progressive dilated cardiomyopathy similar to human heart failure.[11] In this study, we hypothesize that muscle cell transplantation enhances myocardial TIMP expression and reorganizes damaged matrix elements resulting in attenuated chamber dilatation.

Methods

Experimental Animals

The Animal Care Committee of the Toronto General Research Institute approved all procedures performed on animals. All experiments were performed according to the "Guide to the Care and Use of Experimental Animals of the Canadian Council on Animal Care." The Syrian hamster with a genetic dilated cardiomyopathy (BIO 53.58 hamster, Bio Breeders Inc, Fitchburg, Mass) was used. Age-matched normal F1-β hamsters served as nonfailing controls (Charles River Canada Inc, Quebec, Canada). Donor muscle cells were freshly isolated from 4-week-old hamsters. Cardiomyopathic hamsters at 20 weeks of age (predilatation phase) were used as cell transplant recipients.

Cell Processing and Transplantation

Vascular smooth muscle cells (VSMCs) were isolated as previously described.[12] A volume of 0.04 mL of cell suspension or culture medium alone was then transplanted into each heart to deliver 4 million cells. Recipient hamsters were anesthetized and transplanted using a single injection on the surface of the anterior LV free wall. The surgical method was performed as previously described.[12] All hamsters were euthanized by anesthetic overdose at 5 weeks from the time of cell transplantation. In the first group of experiments, the anterior free wall of the LV was snap-frozen in liquid nitrogen and stored at −80°C for enzyme analysis. In a separate group of hamsters, hearts were arrested in diastole and pressure-fixed at 30 mm Hg in formalin and examined for ventricular geometry by planimetry and stained for collagen content and architecture as described next.

Immunoblotting

The relative abundance of MMPs and TIMPs was examined in LV myocardial extracts using standard immunoblotting procedures as previously described.[9]

Matrix Metalloproteinase Gelatin Zymography

Gelatinase activity (MMP-2 and MMP-9) was semiquantified by gelatin zymography as previously described.[13]

Collagen Synthesis

Collagen synthesis in LV myocardium was then determined using the method of Strauss and colleagues as previously described.[9] The rate of collagen synthesis was determined by the rate of ^{14}C-proline incorporation into myocardial collagen.

Confocal Microscopy

Formalin-treated heart sections were imaged using a laser scanning confocal system (BioRad MRC 1024, Bio-Rad Laboratories, Hercules, Calif) after treatment with 0.2% phosphomolybdic acid (pH 1.8-2.2) and 0.1% picrosirius red to selectively stain collagens. Collagen content and fiber architecture were assessed as previously described.[9]
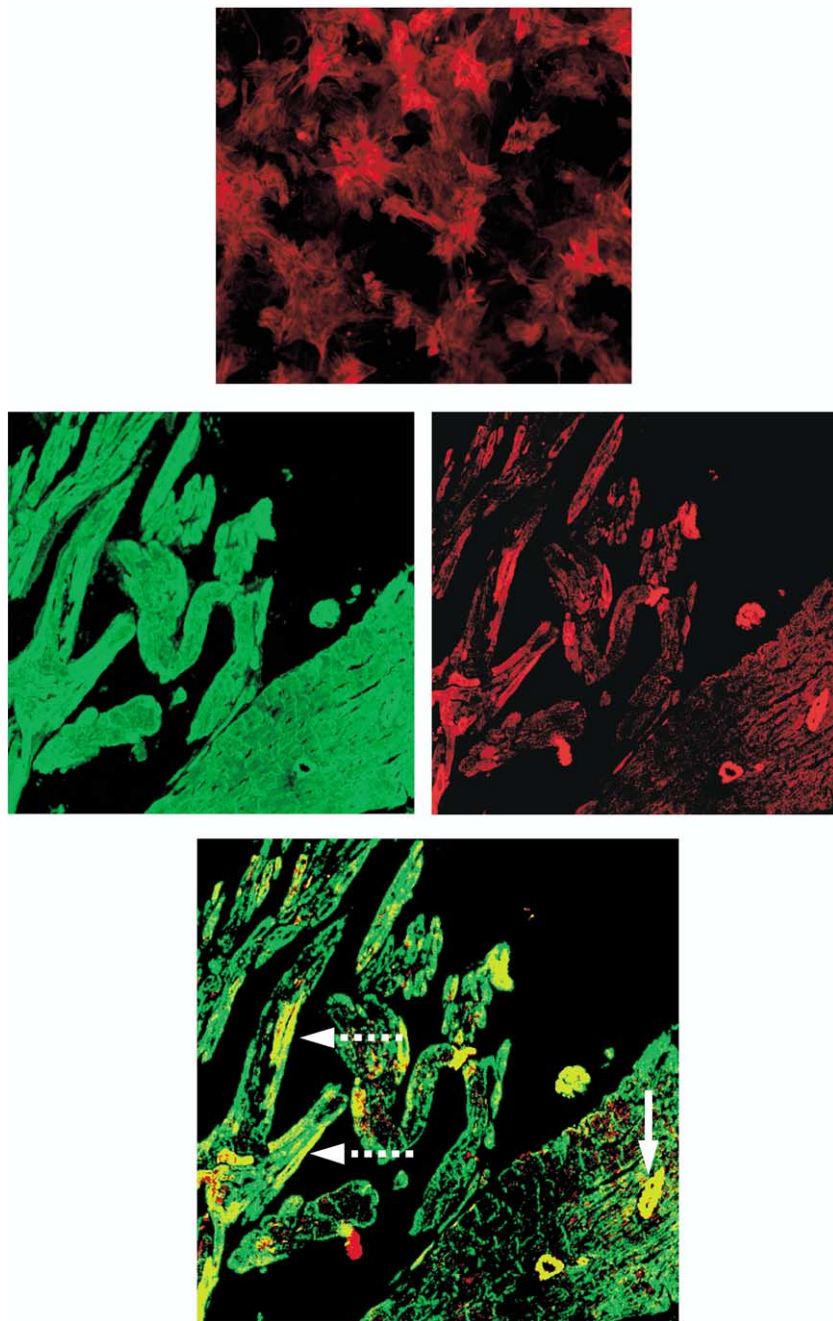
Figure 1. Cell transplantation and smooth muscle cell (SMC) engraftment. Top, Vascular smooth muscle cells (VSMCs) in culture expressed SMC-specific actin (red) confirming an SMC phenotype and expression of contractile proteins. Middle, Engraftment of VSMCs in the left ventricle (LV) of dilated cardiomyopathic hamsters was observed only in cell-transplanted hearts and was made possible with immunostaining (desmin in green, *left*; SMC-specific actin in red, *right*) and confocal microscopy. Bottom, In the merged image, engrafted VSMCs were identified within viable myocardial regions as elongated rod-shaped cells (*dotted arrows*) distinct from adjacent blood vessel walls (*solid arrows*) and cardiomyocytes (400× magnification).

Left Ventricular Volume

The left ventricular volume was measured using image analysis of pressure-fixed heart specimens as previously described.[12]

Statistical Analysis

Results are presented as mean ± standard error of mean (SEM). Comparison between groups was performed by 1-way analysis of variance. If the F ratio was significant, pairwise tests of individual group means were compared using the Student-Newman-Keuls test. All statistical procedures were performed using the SAS software system (SAS Institute, Cary, NC). The critical α-level for these analyses was set at $P < .05$.

Results

Smooth Muscle Cell Phenotype and Engraftment

Engraftment of VSMCs in the LV of dilated cardiomyopathic hamsters was observed only in cell-transplanted hearts and was made possible using immunostaining and confocal microscopy (Figure 1). Engrafted VSMCs were
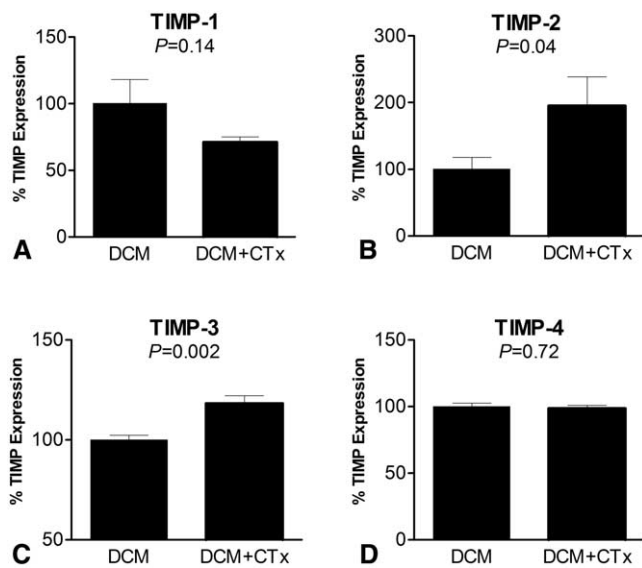
Figure 2. Differential TIMP expression and cell transplantation. Engraftment of VSMCs in the LV of dilated cardiomyopathic hamsters altered the expression of TIMP species. TIMP-2 and TIMP-3 expression was significantly enhanced and the pathologic increases in TIMP-1 were limited, although only a trend was observed. TIMP-4, a TIMP species not usually expressed by SMCs, was unchanged after cell transplantation. *TIMP,* Tissue inhibitor of metalloproteinase; *DCM,* media-injected dilated cardiomyopathic hamsters (n = 8); *DCM+CTx,* cell-transplanted dilated cardiomyopathic hamsters (n = 11). Bars represent mean ± standard error of mean (SEM).
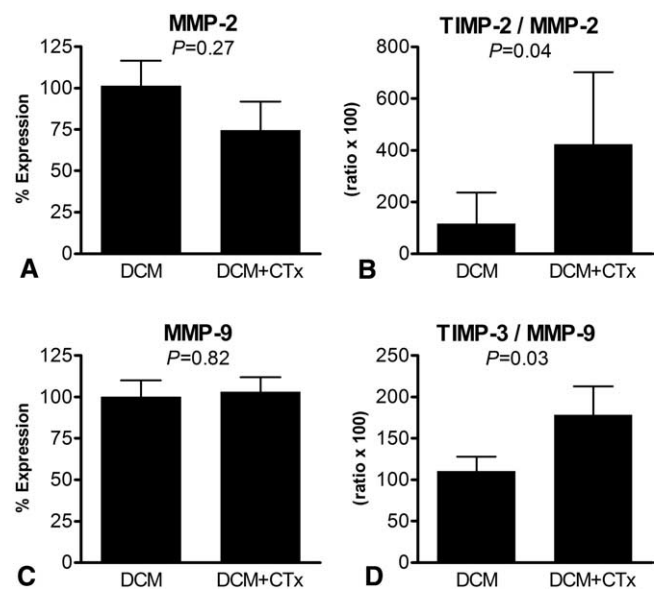
Figure 3. MMP expression and TIMP:MMP expression ratio. Cell transplantation did not significantly influence the expression of MMP-2 and MMP-9 after cell transplantation at 5 weeks. However, the ratio of TIMP-2 to MMP-2 and TIMP-3 to MMP-9 expression was increased in cell-transplanted hearts indicating a reduced capacity for matrix degradation. Bars represent mean ± SEM. *TIMP,* Tissue inhibitor of metalloproteinase; *MMP,* matrix metalloproteinase; *DCM,* media-injected dilated cardiomyopathic hamsters; *DCM+CTx,* cell-transplanted dilated cardiomyopathic hamsters.

identified within viable myocardial regions as elongated rod-shaped cells distinct from adjacent blood vessel walls and cardiomyocytes. The engrafted cells appeared to orient and align in parallel to adjacent cardiomyocytes. The positive immunostaining of the engrafted cells with smooth muscle cell (SMC)-specific actin suggests that the engrafted cells were in a contractile phenotype similar to those in the quiescent medial layer of the adjacent blood vessel walls.

Differential Tissue Metalloproteinase Expression

Engraftment of VSMCs by cell transplantation in the LV altered the expression and content of TIMPs in the failing heart (Figure 2). Specifically, TIMP-1 expression was reduced, but not significantly, and expression of both TIMP-2 and TIMP-3 was significantly increased. Notably, TIMP-3 expression was restored toward normal levels, whereas TIMP-2 expression was increased approximately 2-fold. These data indicate that cell transplantation with VSMCs can enhance the expression of TIMP-2 and TIMP-3 in hamster cardiomyopathy.

Matrix Metalloproteinase Expression

Cell transplantation did not significantly alter the expression of MMP-2 and MMP-9 in hamster cardiomyopathy (Figure 3). However, TIMP to MMP stoichiometry was altered. Specifically, the ratio of TIMP-3 to MMP-9 expression was increased in cell-transplanted hearts (*P* = .03), and the ratio of TIMP-2 to MMP-2 was increased more than 3-fold in cell-transplanted hearts (*P* = .04).

Matrix Metalloproteinase Gelatinase Activity

MMP-2 activity was significantly reduced in cell-transplanted cardiomyopathic myocardium compared with media-injected cardiomyopathic controls (Figure 4). Similarly, MMP-9 activity was reduced in cell-transplanted hearts, but the difference was not statistically significant (*P* = .1).

Collagen Synthesis

Collagen synthesis was elevated in cardiomyopathic myocardium compared with normal nonfailing controls (*P* = .001, Figure 4). Collagen synthesis was increased to a similar extent in both cell-transplanted and control hearts (*P* = .76, Figure 4).
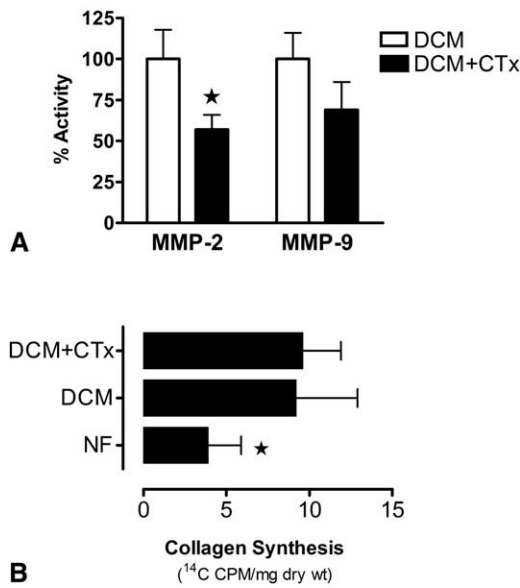
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Figure 4. Collagen degradation and synthesis. Top, Matrix-degrading capacity was inferred by gelatinase MMP activity as assessed by gelatin zymography. MMP-2 activity was attenuated in the cell-transplanted hearts ($P = .04$, n = 7) and MMP-9 activity was reduced, but the difference was not statistically significant ($P = .1$). Bottom, Collagen synthesis was assessed by radiolabeled proline uptake in ex vivo myocardial tissue segments. Collagen synthesis was elevated in cardiomyopathic hamster myocardium compared with normal nonfailing hamster myocardium but was not significantly different between cell-transplanted and media-injected cardiomyopathic groups. Bars represent mean ± SEM. *$P < .05$. *TIMP,* Tissue inhibitor of metalloproteinase; *DCM,* media-injected dilated cardiomyopathic hamsters (n = 8); *DCM+CTx,* cell-transplanted dilated cardiomyopathic hamsters (n = 11); *NF,* nonfailing normal hamsters (n = 6).
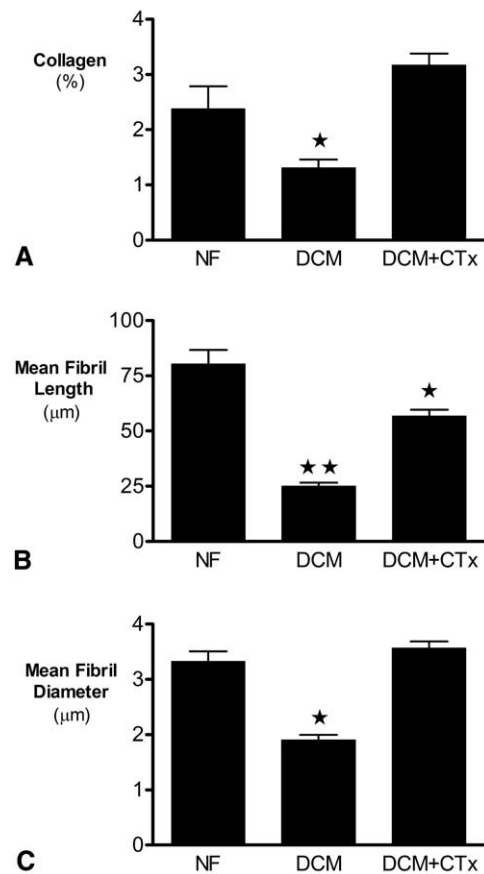

Figure 5. Fibrillar collagen abundance and architecture. Cell transplantation using VSMCs prevented the characteristic loss of collagen content and fiber architecture in viable regions of LV myocardium as assessed by confocal microscopy and a blinded computerized image analysis. Bars represent mean ± SEM. *$P < .05$. **$P < .05$ for DCM versus DCM+CTx. *DCM,* Media-injected dilated cardiomyopathic hamsters (n = 4); *DCM+CTx,* cell-transplanted dilated cardiomyopathic hamsters (n = 4); *NF,* nonfailing normal hamsters (n = 4).

Fibrillar Collagen Content and Fiber Architecture

We previously documented that hamster cardiomyopathy is associated with reduced perimysial collagen content with fiber fragmentation and thinning consistent with accelerated matrix degradation.[9] In viable regions of LV myocardium, confocal microscopy and a blinded computerized image analysis determined that the reduced regional collagen content characteristic of hamster cardiomyopathy was prevented by cell transplantation at 5 weeks compared with media-injected controls (Figure 5). In addition, the length and diameter of perimysial collagen fibers in viable regions of myocardium were assessed in cell-transplanted hearts and compared with age-matched media-injected controls. Notably, cell transplantation limited the characteristic reduction in collagen fiber length and diameter observed in hamster cardiomyopathy (Figure 5). The appearance of the perimysial

collagen fibers in the cell-transplanted hearts was similar to that in nonfailing normal hamsters (Figure 6).

Left Ventricular Chamber Volume

LV dilatation is a hallmark of maladaptive structural cardiac remodeling and a strong predictor of late mortality in patients with ventricular dysfunction.[14,15] LV geometry was assessed by computerized planimetry of heart sections. The VSMC-transplanted chamber volumes (76 ± 14 μL) were smaller than media-injected controls (97 ± 16 μL, $P = .02$) and approached those of age-matched normal hearts (60 ± 6 μL). These data indicate that VSMC cell transplantation limited ventricular dilatation, data that are consistent with our prior observations.[2,12,16]

Discussion

The cellular and molecular mechanisms that mediate the functional benefits after cell transplantation observed in the failing heart are not clear. An active process whereby the engrafted cells replace lost contractile elements and contract synchronously with the host myocardium may occur. Leobon and colleagues[17] recently demonstrated that skeletal myoblasts are capable of contraction after implantation into ischemic heart tissue, but the implanted cells were not coupled to native heart cells. Although transplanted cells may indeed contract in host myocardium under specific conditions, it remains to be determined whether the active contraction of implanted cells is responsible for the improvements of systolic function observed after cell transplantation. The number of cells that ultimately survive and engraft in the host myocardium after direct injection is limited and extremely small relative to total cardiac mass.[18] Given the consistent observation of reduced cell survival after cell transplantation, active contraction of such a limited number of engrafted cells as the primary mechanism of improved contractile function is unlikely. Similarly, active contraction cannot account for the beneficial effects on cardiac structure and function observed after transplantation of SMCs, which are incapable of repetitive rapid contractions.

Although the functional characteristics of the cells themselves may be important, such as the ability to contract, it is possible that some of the beneficial effects of cell transplantation may be the result of attenuated ventricular dilatation through a reorganization of the ECM surrounding the engrafted cells. In theory, because myocardial matrix disruption can decrease systolic performance without changing myocyte contractility,[19] engrafted cells could improve overall cardiac function without themselves contributing to contractile function and may do so by restoring deficient interstitial matrix components lost in the process of heart failure. The use of cell engraftment to restore the content and spatial organization of key ECM components in the failing heart is conceivable, although this concept has never been formally evaluated before this study. We hypothesized that muscle cell transplantation may provide its benefits on structural cardiac remodeling by limiting matrix degradation, perhaps by regulating the MMP-TIMP axis, the central mechanism that controls matrix remodeling in the failing heart.

To eliminate the possibility of host cell contraction resulting in beneficial effects, we used VSMCs for cell transplantation, which are incapable of rapid contraction commensurate with the cardiac cycle. Prior studies have determined that SMC transplantation results in consistent and similar beneficial effects on preserving cardiac structure and function compared with cardiac or skeletal muscle cell sources.[1-3,20] Compared with skeletal myoblasts and bone marrow progenitor cells, VSMC transplantation has not
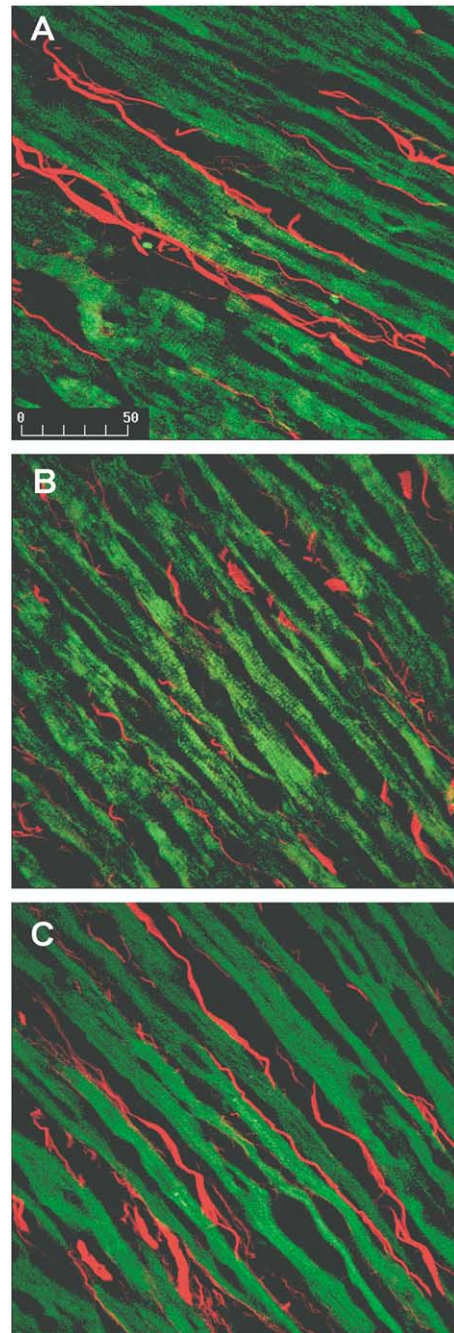
Figure 6. Confocal micrographs of fibrillar collagen network. Cell transplantation using VSMCs prevented the characteristic loss of collagen content in viable regions of LV myocardium and prevented perimysial fiber thinning and fragmentation compared with media-injected controls. All assessments were performed in age-matched hamsters at 25 weeks of age in normal (A), dilated cardiomyopathic (B), or cell-transplanted dilated cardiomyopathic hamsters (C). Bar = 50 μm.

been examined in human clinical trials to date. However, SMCs may offer some unique benefits over other cell types for clinical cell transplantation such as ease of harvest, rapid in vitro expansion, and a capacity for hyperplasia and hypertrophy in response to hemodynamic stimuli. In addition, VSMCs are capable of secreting angiogenic factors and ECM components, characteristics that may enhance cell engraftment and survival after implantation However, these proposed advantages are currently speculative and remain to be proven by further studies.

Allaire and colleagues[21] recently tested the hypothesis that the presence or absence of VSMCs modulates ECM destruction by proteases and the spreading of inflammation to the aortic media, which represent early events in the formation of aneurysms. VSMCs were seeded onto decellularized aortas and then implanted in the abdominal aorta of rats. The presence of VSMCs in the arterial wall significantly enhanced the expression of TIMPs, specifically TIMP-2 and TIMP-3, resulting in reduced inflammation. In addition, the enhanced TIMP expression prevented proteolytic ECM degradation and, in so doing, maintained vessel wall integrity. It follows that VSMCs secrete TIMPs and exert a profound paracrine effect that participates in vessel wall homeostasis against inflammation and matrix proteolysis. Other studies support this concept.[22,23] For example, Blindt and colleagues[23] determined that VSMCs constitutively express TIMP-2, TIMP-3, and collagen, and the expression of these matrix genes can increase in response to environmental cues. Accordingly, VSMCs actively respond to external stresses and environmental stimuli by secreting bioactive molecules and ECM components. Under normal physiologic conditions, VSMCs maintain local matrix homeostasis by continually producing and secreting TIMPs.[23,24-26] The bioactive properties of the secreted TIMPs limit MMP activity promoting structural matrix stability.

We assessed the effects of VSMC engraftment in hamster cardiomyopathy on differential TIMP expression and parameters of matrix remodeling. Cell transplantation with VSMCs enhanced the expression of TIMP-2 and TIMP-3. These data are in keeping with a contractile VSMC phenotype, and, indeed, transplanted VSMCs were observed to engraft in the myocardium and express contractile phenotypic markers. Remarkably, both matrix content and its structural integrity were improved in the cell-transplanted hearts, indicating a protective effect against matrix proteolysis. Gelatinase MMP activity was attenuated in cell-transplanted hearts. We previously demonstrated that an imbalance of TIMP-3 relative to MMP-9 directly results in a dilated cardiomyopathy by accelerated matrix degradation.[11] In addition, a restoration of the relative balance of TIMP-3 with MMP-9 was associated with reverse remodeling in human patients after mechanical unloading of the

failing heart.[27] Furthermore, the balance of TIMP-3 with MMP-9 regulates ECM homeostasis and tissue remodeling during development.[28] In the present study, cell transplantation increased the ratio of TIMP-3 to MMP-9 and the ratio of TIMP-2 to MMP-2, indicating a reduced capacity for matrix degradation and preserved myocardial matrix homeostasis.

Important to our observation of the effects of cell transplantation on matrix integrity are the extensive data indicating that SMC transplantation provides significant benefits on preserving LV function in experimental models of cardiac disease, including hamster cardiomyopathy, with effects similar to heart cell transplantation.[2,3,16] We propose the novel concept that SMC transplantation may exert its protective effects on the progression of cardiomyopathy in a paracrine manner by restoring the balance of TIMP expression relative to MMPs and thereby promoting matrix reorganization in the failing heart. Cell transplantation appears to alter matrix homeostasis toward a healthy functional matrix, rather than excessive alteration of the remodeling process resulting in maladaptive fibrosis. Further studies will be required to determine whether this mechanism is responsible for the benefits of other muscle cell types on the remodeling process in the failing heart. In the context of regional myocardial infarction, it is conceivable that cell transplantation could restore damaged matrix elements resulting in reduced scar expansion and attenuated structural remodeling.[29] We recognize that the process of cell engraftment is complex, and that the biologic effects of cell transplantation in the failing heart likely involve multiple cellular and molecular pathways. However, on the basis of these data, the TIMP-MMP axis may have an important contribution to this intriguing process.

Limitations

First, we cannot conclusively establish a cause and effect relationship between the enhanced expression of TIMPs after cell transplantation and the simultaneous reduction in matrix degradation and limitation of ventricular dilatation. Thus, it is possible that the altered TIMPs are secondary to the improved myocardial environment afforded by cell transplantation. However, it is widely acknowledged that the relative balance of MMPs with TIMPs drives the local matrix environment, and in support, we observed improved matrix content and organization after cell transplantation. Second, we cannot establish the source of the enhanced TIMP secretion from these data, although we presume that the implanted cells were the primary donor. Because TIMP-2 expression is not characteristically altered in hamster cardiomyopathy,[9] the enhanced expression of this TIMP species suggests that its enhanced expression was a product of the engrafted cells, which are known to readily produce and secrete TIMP-2,[23] particularly when trans-

planted into a cell-deficient matrix environment.[21] Further studies should establish the cellular source of the elevated TIMP expression. We are actively pursuing these studies by selectively transplanting TIMP-deficient cells into animal models of cardiomyopathy to determine whether the benefits of cell therapy are lost in the absence of TIMP expression. Third, dose-response studies were not used in the current study, and these observations may provide additional mechanistic insights if performed.

Conclusion

The cellular and molecular mechanisms that govern cell engraftment and the subsequent improvements of cardiac function in the failing heart are likely multifactorial, complex, and far from understood. From the perspective that cardiac remodeling determines the clinical progression of heart failure and is emerging as a therapeutic target in heart failure of all causes,[15] the mechanisms underlying cell transplantation are an important area of investigation. These data provide the first evidence that muscle cell transplantation limits ventricular dilatation in the failing heart by preserving ECM homeostasis through a paracrine-mediated mechanism. Gene-enhanced cell transplantation with specific TIMPs may optimize the ability of engrafted cells to attenuate maladaptive remodeling and the progression to heart failure.

References

1. Yoo KJ, Li RK, Weisel RD, Mickle DA, Jia ZQ, Kim EJ, et al. Heart cell transplantation improves heart function in dilated cardiomyopathic hamsters. *Circulation.* 2000;102(19 Suppl 3):III204-9.
2. Yoo KJ, Li RK, Weisel RD, Mickle DA, Li G, Yau TM. Autologous smooth muscle cell transplantation improved heart function in dilated cardiomyopathy. *Ann Thorac Surg.* 2000;70:859-65.
3. Li RK, Jia ZQ, Weisel RD, Merante F, Mickle DA. Smooth muscle cell transplantation into myocardial scar tissue improves heart function. *J Mol Cell Cardiol.* 1999;31:513-22.
4. Ducharme A, Frantz S, Aikawa M, Rabkin E, Lindsey M, Rohde LE, et al. Targeted deletion of matrix metalloproteinase-9 attenuates left ventricular enlargement and collagen accumulation after experimental myocardial infarction. *J Clin Invest.* 2000;106:55-62.
5. Kim HE, Dalal SS, Young E, Legato MJ, Weisfeldt ML, D'Armiento J. Disruption of the myocardial extracellular matrix leads to cardiac dysfunction. *J Clin Invest.* 2000;106:857-66.
6. Spinale FG. Matrix metalloproteinases: regulation and dysregulation in the failing heart. *Circ Res.* 2002;90:520-30.
7. Bradham WS, Moe G, Wendt KA, Scott AA, Konig A, Romanova M, et al. TNF-alpha and myocardial matrix metalloproteinases in heart failure: relationship to LV remodeling. *Am J Physiol Heart Circ Physiol.* 2002;282:H1288-95.
8. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circ Res.* 2003;92:827-39.
9. Fedak PW, Altamentova SM, Weisel RD, Nili N, Ohno N, Verma S, et al. Matrix remodeling in experimental and human heart failure: a possible regulatory role for TIMP-3. *Am J Physiol Heart Circ Physiol.* 2003;284:H626-34.
10. Li YY, Feldman AM, Sun Y, McTiernan CF. Differential expression of tissue inhibitors of metalloproteinases in the failing human heart. *Circulation.* 1998;98:1728-34.
11. Fedak PW, Smookler DS, Kassiri Z, Ohno N, Leco KJ, Verma S, et al. TIMP-3 deficiency leads to dilated cardiomyopathy. *Circulation.* 2004;110:2401-9.
12. Ohno N, Fedak PW, Weisel RD, Mickle DA, Fujii T, Li RK. Transplantation of cryopreserved muscle cells in dilated cardiomyopathy: effects on left ventricular geometry and function. *J Thorac Cardiovasc Surg.* 2003;126:1537-48.
13. Fedak PW, de Sa MP, Verma S, Nili N, Kazemian P, Butany J, et al. Vascular matrix remodeling in patients with bicuspid aortic valve malformations: implications for aortic dilatation. *J Thorac Cardiovasc Surg.* 2003;126:797-806.
14. Fedak PW, Verma S, Weisel RD, Li RK. Cardiac remodeling and failure: from molecules to man (Part I). *Cardiovasc Pathol.* 2005;14:1-11.
15. Cohn JN, Ferrari R, Sharpe N. Cardiac remodeling—concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. Behalf of an International Forum on Cardiac Remodeling. *J Am Coll Cardiol.* 2000;35:569-82.
16. Yoo KJ, Li RK, Weisel RD, Mickle DA, Tomita S, Ohno N, et al. Smooth muscle cells transplantation is better than heart cells transplantation for improvement of heart function in dilated cardiomyopathy. *Yonsei Med J.* 2002;43:296-303.
17. Leobon B, Garcin I, Menasche P, Vilquin JT, Audinat E, Charpak S. Myoblasts transplanted into rat infarcted myocardium are functionally isolated from their host. *Proc Natl Acad Sci U S A.* 2003;100:7808-11.
18. Reinecke H, Murry CE. Taking the death toll after cardiomyocyte grafting: a reminder of the importance of quantitative biology. *J Mol Cell Cardiol.* 2002;34:251-3.
19. Baicu CF, Stroud JD, Livesay VA, Hapke E, Holder J, Spinale FG, et al. Changes in extracellular collagen matrix alter myocardial systolic performance. *Am J Physiol Heart Circ Physiol.* 2003;284:H122-32.
20. Sakai T, Li RK, Weisel RD, Mickle DA, Jia ZQ, Tomita S, et al. Fetal cell transplantation: a comparison of three cell types. *J Thorac Cardiovasc Surg.* 1999;118:715-24.
21. Allaire E, Muscatelli-Groux B, Mandet C, Guinault AM, Bruneval P, Desgranges P, et al. Paracrine effect of vascular smooth muscle cells in the prevention of aortic aneurysm formation. *J Vasc Surg.* 2002;36:1018-26.
22. Allaire E, Forough R, Clowes M, Starcher B, Clowes AW. Local overexpression of TIMP-1 prevents aortic aneurysm degeneration and rupture in a rat model. *J Clin Invest.* 1998;102:1413-20.
23. Blindt R, Vogt F, Lamby D, Zeiffer U, Krott N, Hilger-Eversheim K, et al. Characterization of differential gene expression in quiescent and invasive human arterial smooth muscle cells. *J Vasc Res.* 2002;39:340-52.
24. Hu Y, Baker AH, Zou Y, Newby AC, Xu Q. Local gene transfer of tissue inhibitor of metalloproteinase-2 influences vein graft remodeling in a mouse model. *Arterioscler Thromb Vasc Biol.* 2001;21:1275-80.
25. George SJ, Lloyd CT, Angelini GD, Newby AC, Baker AH. Inhibition of late vein graft neointima formation in human and porcine models by adenovirus-mediated overexpression of tissue inhibitor of metalloproteinase-3. *Circulation.* 2000;101:296-304.
26. Cheng L, Mantile G, Pauly R, Nater C, Felici A, Monticone R, et al. Adenovirus-mediated gene transfer of the human tissue inhibitor of metalloproteinase-2 blocks vascular smooth muscle cell invasiveness in vitro and modulates neointimal development in vivo. *Circulation.* 1998;98:2195-201.
27. Li YY, Feng Y, McTiernan CF, Pei W, Moravec CS, Wang P, et al. Downregulation of matrix metalloproteinases and reduction in collagen damage in the failing human heart after support with left ventricular assist devices. *Circulation.* 2001;104:1147-52.
28. Whiteside EJ, Jackson MM, Herington AC, Edwards DR, Harvey MB. Matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-3 are key regulators of extracellular matrix degradation by mouse embryos. *Biol Reprod.* 2001;64:1331-7.
29. Fedak PWM, Weisel RD, Yau TM, Mickle DAG, Li R-K. Cell transplantation, ventricular remodeling, and the extracellular matrix. *J Thorac Cardiovasc Surg.* 2002;123:584-5.

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Discussion

Dr Robert C. Robbins *(Stanford, Calif)*. What is the underlying mechanism of the dilatation in these hamsters? Is it an increased degradation? Is that what causes their heart failure?

Dr Fedak. We previously published data in the *American Journal of Physiology* that implicated the TIMP-MMP axis in the process of hamster dilated cardiomyopathy. Our observations in these hamsters implicated a progressive loss of TIMP expression and excessive MMP-mediated matrix degradation as an important mechanism underlying left ventricular dilatation and failure. These findings were identical in our patients with end-stage dilated cardiomyopathy, suggesting a similar mechanism in human disease.

Dr Robbins. What other cells have you tried in this particular model, a nonischemic model of heart failure?

Dr Fedak. By using a model of hamster cardiomyopathy, we previously performed cell transplantation with heart cells, skeletal muscle cells, SMCs, and bone marrow cells. All of these cell types improved cardiac structure and function, and did so to a similar extent. Given the consistent benefits of these diverse cell types in both nonischemic and ischemic cardiomyopathy, we entertained the idea that the process of cell engraftment alone may provide beneficial effects on host myocardium, somewhat independent of the cell type. Perhaps when a cell engrafts in the host environment it alters its local environment to maintain its survival. This process may involve the release of paracrine factors that alter angiogenesis, cell growth, and cell survival. Because cells engraft by incorporating into the local interstitial matrix it is likely that the cells must alter this matrix and act to maintain its integrity to survive. Perhaps the reorganization of damaged ECM components during cell engraftment is a simple explanation that can account for the profound attenuation of ventricular dilatation observed with a diverse group of cell types and models of heart failure.

Dr Robbins. What is the signal? Have you found any signal that the cells are releasing that can cause the effects you have observed in your cell signaling?

Dr Fedak. Our data suggest that cell transplantation enhances the expression of specific TIMP proteins in the host myocardium. We previously outlined that cardiac TIMP deficiency is sufficient to induce maladaptive structural cardiac remodeling consistent with a dilated cardiomyopathy. Cell transplantation may restore deficient TIMP species and in so doing improve matrix homeostasis. Improved matrix architecture enhances structural support and prevents progressive LV dilatation and failure. In addition, the ECM is far more than just a structural support system. It really is the signaling interface for the cells. The matrix is where the growth factors are pooled and the cell signals converge. Accordingly, if you improve local matrix you probably also change signaling systems in a profound way, and this may be, at least in part, how the beneficial effects on structural remodeling may be produced by cell transplantation.

Dr G. Alexander Patterson *(St Louis, Mo)*. I believe that there are some data in other cell transplant modeling systems in the transplant arena. There is some work from Hebert's group in Montreal in which they demonstrated a similar paracrine mechanism that I think, if I recall the details, has to do with renal allograft vasculopathy. Surely this isn't a specific thing relevant

to cardiac cell transplantation. Do you have some thoughts on that?

Dr Fedak. Absolutely. One thing we know from matrix biology is that a cell cannot survive if it is not attached to a healthy local matrix. So without a doubt, no matter what type of cell or what organ it is, if the donor cell does not have a healthy matrix to adhere to, it simply won't survive. So if you have successful cell engraftment, by definition, you must have a somewhat healthy matrix. I would propose that if donor cells are going to survive in any host tissue, the donor cells would be best served to actively improve their local environment, particularly by helping to coordinate and maintain matrix homeostasis. Because the matrix is damaged in most disease states, including heart failure of all causes, engrafted cells would likely provide benefits by improving matrix structure and function. Improved matrix homeostasis would have profound effects on overall tissue architecture and function, which is what we see consistently with experimental and perhaps even clinical cell transplantation.

Dr David H. Harpole, Jr *(Durham, NC)*. This is a very elegant study. What I really want to do is take 2 steps back. There has been a tremendous amount of work in the TIMPs and so forth with respect to controlling invasion and tumors. Have you all looked at, first of all, any of the compounds, just directly injecting or putting those in now that you define a mechanism?

And second, there are other ways that invasion takes place that have to do with turning on and off this pathway. Do you think those have a role in this as well?

Dr Fedak. You bring up 2 important issues. One is a question of what we really need to be using as the therapy. Do we really need the cells themselves or would injection of some sort of matrix be of benefit? I think that is an interesting and testable idea with direct clinical implications. If we find that we can restore damaged matrix elements just by injecting an off-the-shelf matrix alone, imagine the clinical possibilities? That is one important question. Certainly we can target TIMP deficiency by TIMP gene-enhanced cell transplantation. Our preliminary data using these enhanced cells are very encouraging. By targeting the paracrine mediators that mediate the benefits of cell transplantation with gene-enhancement of the donor cells, we may best optimize use of this novel therapy.

The second issue is that the TIMPs themselves not only inhibit the MMPs but also have many other important biologic effects. As you mention, the role of TIMPs in cancer invasion is a prime example. TIMPs have been implicated to have direct effects on cell death, growth, migration, and signaling through growth factor activation. Probably what I am presenting here is a simplistic version of what is in reality a very complex phenomenon.

Dr Masashi Komeda *(Kyoto, Japan)*. I enjoyed your talk very much. It is very interesting. We did a study using hepatocyte growth factor slow-release method to dilated cardiomyopathy, 2 of them. One is a spontaneous hypertension rat model, and the other is an antimyosin cardiomyopathy model. In either case, by decreasing too much fibrosis, cardiac function recovered well, and we showed that amount of fibrosis decreased.

From your model, I got the impression that there are good and bad aspects of matrix, and maybe it is in part model-dependent, but maybe we have to get more information about which elements

should be decreased and which should be preserved. I want to hear your opinion about that.

Dr Fedak. Thank you for your question, Dr Komeda. I am familiar with your work. Indeed, matrix remodeling is a complex phenomenon. It is confusing, because increased matrix degradation is often observed in association with increased total amounts of collagen. Matrix alterations are dependent on the mode of injury as well as the timing and location of the analysis being performed. These parameters may show significant differences depending on the stage of the disease. In this study we looked specifically at perimysial collagen fibers, which we believe are the key for structural support. Perimysial collagen content and architecture are disrupted in dilated cardiomyopathy. That is not to say that there isn't increased total collagen in these hearts. Scarring and fibrosis occur, as you mentioned, but that seems to be more of an inflammatory process around blood vessels and areas of focal cell necrosis, as opposed to the healthy and active matrix that we targeted in our analysis using confocal microscopy.

Dr Komeda. There are 2 different aspects of the cardiomyopathy. They are similar in part, but in part different. There is restricted or dilated, purely dilated, or a combination. In your model maybe it is a purely dilated model and not too much diastolic dysfunction.

Dr Fedak. That is correct, it is a model of progressive dilated cardiomyopathy. I agree that our model is primarily that of LV dilatation, compared with other models that have transient compensated hypertrophy phases. However, LV dilatation is the most important predictor of decompensation and death from heart failure. I would argue that in the context of congestive heart failure, the LV dilatation we see in our patients is really the disease itself. I believe that we should target LV dilatation in terms of developing novel surgical approaches, such as cell transplantation and others.

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