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Cellular therapy promotes endogenous stem cell repair

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Abstract

Cellular transplantation for cardiac repair has emerged as an exciting treatment option for patients with myocardial infarction (MI) and heart failure. Animal models of post-infarction left ventricular remodeling have demonstrated an improvement in left ventricular (LV) function, decrease in scar size, and amelioration of adverse cardiac remodeling after stem cell transplantation. These beneficial effects occur despite minimal engraftment and negligible differentiation of transplanted cells. Evidence of the heart capability to self-renew continues to mount; however, the extent to which this occurs is still unclear. Although there is a specific population of cardiac stem cells capable of differentiating into cardiomyocytes, they alone are not capable of fully regenerating tissue damaged by MI. Therefore, paracrine mechanisms may be responsible for activating endogenous stem cells to promote regeneration and prevent apoptosis. These structural beneficial effects may reduce regional wall stresses, consequently leading to long-term host myocardium gene/protein expression changes, which may subsequently result in improvement in LV function.

Keywords

apoptosis; heart; miRNA; cell therapy; stem cells

Introduction

Some non-mammalian animals, such as zebrafish, have the ability to completely regenerate their hearts after injury (Poss et al. 2002). It has long been the accepted dogma that the adult human heart is not only incapable of regeneration, but that it is a post-mitotic organ (Rubart and Field 2006). This ideology has been challenged more recently with new evidence demonstrating that adult human hearts do indeed contain a population of resident cardiac progenitor cells, which promote cardiomyocyte renewal (Messina et al. 2004; Laug-witz et al. 2005; Bearzi et al. 2007; Smith et al. 2007). However, the extent of cardiac regeneration via endogenous mechanisms in the face of myocardial infarction is not sufficient, as a large proportion of patients develop left ventricular (LV) dysfunction post-infarction. Stem cell transplantation has emerged as a novel therapy for myocardial damage. Although it is a consistent observation in the literature that cellular transplantation improves LV contractile function, the cell engraftment rate a few weeks after the transplantation is usually very low (<0.1%) and an even smaller number of these transdifferentiate into cardiomyocytes (Wollert et al. 2004; Assmus et al. 2006; Janssens et al. 2006; Noiseux et al. 2006; Schächinger et al. 2006; Iso et al. 2007; Zeng et al. 2007). With so few transplanted cells contributing to the repopulation of damaged myocardium, a paracrine mechanism to

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facilitate endogenous cardiomyocyte regeneration has been proposed (Gnecchi et al. 2005, 2006). We have demonstrated that bone marrow derived progenitor cell transplantation in a porcine model of post-infarction LV remodeling leads to an increase in vascular density, which is likely mediated by pro-angiogenic factors such as vascular endothelial growth factor (VEGF) (Zeng et al. 2007; Wang et al. 2009). There may also be a pro-survival milieu released by stem cells that also results in the sparing of native cardiomyocytes that would have succumbed to apoptosis following the initial insult (Zeng et al. 2007; Wang et al. 2009). Currently, we hypothesize that the beneficial effects of stem cell transplantation on the heart stems from transient engraftment of stem cells, leading to either direct action of these factors or changes in the host myocardial gene expression. Subsequently, these changes enhance the stability of the cardiac progenitor cell pool, which then can more effectively participate in endogenous cardiac repair. The mechanism of these pathways that promote endogenous cardiac repair still remain to be elucidated.

Early on it was shown that the young feline heart has a population of small mono-nucleated ventricular myocytes that incorporate bromodeoxyuridine (BRDU), express markers of cell cycle activity (Ki67), and have telomerase activity (Chen et al. 2007). Later, Bolli et al. demonstrated that coronary infusion of c-kit⁺ cardiac progenitor cells in a rat model of chronic MI was associated with functional improvement. They further showed that the improvement was related to the stimulation of endogenous cardiac progenitor cells (Tang et al. 2010). More recently, human myocyte regeneration from endogenous cardiac progenitors has been shown to range all the way from sparse to nearly complete cardiac regeneration, multiple times throughout a normal life span (Bergmann et al. 2009; Kajstura et al. 2010). Even though the evidence for cardiomyocyte regeneration, at some level, seems well supported, why are they unable to mediate cardiac repair on their own (Bearzi et al. 2007)? What relationship is there between the transplanted stem cells and the changes in host gene expression that results in an environment supportive of endogenous regeneration? Further investigation into these important mechanisms will allow further improvements in stem cell therapies, as well as the design of pharmacogenetic approaches to myocardial regeneration.

Demonstration of Sca-1⁺ positive cardiac stem cells in the adult heart

Our laboratory has previously shown that Sca-1⁺/CD31⁻ cells exist in the adult mouse heart (10.8% ± 2.4% of the cardiomyocyte-free cardiac cell population) (Wang et al. 2006). In-vitro culture of isolated Sca-1⁺/CD31⁻ leads to the formation of numerous microtubular structures by day 3. The Sca-1⁺/CD31⁻ cells also have the ability to differentiate into cardiomyocytes after induction with Dickkopf-related protein 1 (DKK-1), dimethyl sulfoxide (DMSO), bone morphogenetic protein 2 (BMP-2), fibroblast growth factor 4 (FGF-4), FGF-8, and 5-azacytidine, during a 14 d culture period (Fig. 1). This protocol induced the expression of GATA-4 and homeobox protein NKx2.5. Co-culture of these cells with neonatal rat cardiomyocytes showed further differentiation, as evidenced by the expression of troponin T and phospholamban, suggesting that the stem-cell microenvironment plays an important role in determining cardiomyocyte differentiation.

Sca-1⁺ positive cardiac stem cells increase in number and home to injured myocardium after MI

Seven days after MI, the Sca-1⁺/CD31⁻ cell fraction of the cardiomyocyte-free cardiac cell population increased from 10.8% ± 2.4% to 21.8% ± 1.6% ($p < 0.01$) (Fig. 2) (Wang et al. 2006). Interestingly, this increase in the population of cardiac stem cells after infarction was a transient effect, as the number of these stem cells returned to baseline values at 14 and 21 days after MI. The majority of these cells were found in the periscar region, reflecting their innate ability to home to the damaged area. The question as to whether these cells could

represent bone marrow and circulating blood cells that migrate to the heart after MI was raised; however, we did not find any increase in Sca-1⁺/CD31⁻ cells at an early or late stage after MI in the bone marrow or peripheral circulation, suggesting an endogenous cardiac origin for these cells. The regeneration efficacy of these endogenous stem cells may be limited, given they are not able to prevent the adverse remodeling and depressed ejection fraction (EF) after MI.

Mobilization of endogenous stem cells after myocardial infarction by SDF-1 α

Since endogenous stem cells have a limited capacity to ameliorate adverse remodeling, we sought other mechanisms to activate and mobilize this pool of stem cells. A PEGylated fibrin patch bound with stromal cell derived factor-1 α (SDF-1 α) was placed on the surface of the infarct area after MI was created in a mouse model by left anterior descending artery (LAD) ligation (Zhang et al. 2007). In-vitro ELISA results showed that the SDF-1 α was released from the patch constantly for up to 10 days. The percentage of c-kit⁺ stem cells within the infarct area at 2 weeks was significantly higher in the SDF-1 α -treated animals compared with the controls ($11.20\% \pm 1.7\%$ compared with $4.22\% \pm 0.96\%$, $p < 0.05$), which was maintained for up to one month after MI. This study did not exclude the possibility that the mobilized stem cells may have been hematopoietic stem cells from the bone marrow, and the source of the mobilized cells remains unclear. However, this study demonstrated that a chemotactic strategy with SDF-1 increased mobilization and homing of these c-kit⁺ endogenous stem cells, which subsequently improved LV function, compared with the controls.

Stem cell transplantation in large animal models of MI promotes endogenous repair

A number of bone marrow derived progenitor cell transplantation studies in a swine model of post infarction remodeling in our laboratory have demonstrated an improvement in LV ejection fraction after cell transplantation (Zeng et al. 2007; Wang et al. 2009; Jameel et al. 2010). There is also an improvement in myocardial energetics, with an increase in PCr/ATP ratio in the peri-infarct area after cell transplantation. This is associated with a decrease in scar size, which is apparent at 4 months after cell transplantation (Jameel et al. 2010). However, stem cell engraftment levels are negligible at 4 months, and there were no engrafted stem cells visualized. An even smaller percentage of the engrafted stem cells demonstrated cardiomyocyte differentiation. Thus, the role of exogenous stem cells in regeneration of new cardiomyocytes is minimal at best.

Autologous mesenchymal stem cell transplantation in a porcine model of MI, using a patch for delivery, resulted in a significant increase in the LV systolic thickening fraction in the infarct zone at 20 days after MI (Liu et al. 2004). In this particular study, the fibrin patch scaffold that entrapped millions of stem cells was placed on top of the area of infarct. The engraftment rate with patch was 10%; however, despite the high stem cell engraftment, only a small fraction of these cells expressed markers of cardiomyocyte differentiation. Thus, even with a higher engraftment level, the improvement in the systolic thickening fraction in the infarct area cannot be fully explained by de novo differentiation of the transplanted cells. However, the decrease in scar size and the increase in thickening fraction in the infarct area is clear evidence of myocardial repair within this injured area. Histology from the peri-infarct area demonstrated a higher proportion of cardiomyocytes in the peri-infarct by comparison with the controls (Fig. 3). Although these could represent spared native

cardiomyocytes, it is very possible they are the result of new cardiomyocyte formation from endogenous cardiac stem cells.

In another study, we transplanted allogeneic bone marrow derived stem cells in a porcine model of post-infarction LV remodeling by direct intramyocardial injection in the peri-infarct region (Zeng et al. 2007). While this also resulted in improvements in EF, infarct zone thickening, and myocardial energetics, only 0.35% of the transplanted cells engrafted and of these only 2% demonstrated markers suggestive of cardiomyocyte differentiation. Histological sections from the infarct zones demonstrated patches of cardiomyocytes within the scar area, again suggesting activation of endogenous repair mechanisms and subsequent mobilization and de-novo differentiation of endogenous stem cells to cardiomyocytes in the infarct and peri-infarct regions (Fig. 4).

In another model of ischemia/reperfusion in pigs, the results of a transcatheter injection of bone marrow derived stem cells in the peri-infarct region were very consistent with our previous studies: an improvement in EF and myocardial energetics was seen with only 0.55% engraftment and only 2% of transplanted cells co-staining positive for cardiomyocyte markers (Wang et al. 2009). Histology also indicated cardiomyocytes in the infarct and peri-scar areas, which again may represent spared native myocytes or differentiation of endogenous cardiac stem cells.

Perhaps the most robust data for endogenous regeneration and paracrine mechanisms was a recent study in which we followed animals for 4 months after direct intramyocardial injection of bone marrow derived stem cells in a porcine model of MI (Jameel et al. 2010). No engrafted cells were detectable at 4 months. However, the unique feature of this study was the serial cardiac MRI measurements, which demonstrated that the scar size at 10 days post-MI was similar in cell transplanted (Tx hearts) and control groups (approximately 10%), but decreased through the course of follow-up measurements in the cell-treated animals, and was significantly lower in the transplanted group at 4 months ($4.6\% \pm 1\%$ compared with $8.6\% \pm 2.4\%$, $p < 0.05$). This would suggest that the coronary ligation established an initial scar area, which then reduced in size over the follow-up period. If the primary mechanism involved was sparing native cardiomyocytes, the effect should have been seen at the initial time point. On the other hand, stimulation of endogenous cardiac stem cells would lead to the formation of new cardiomyocytes and over a period, this myocardial regeneration may lead to a reduction in scar size. Interestingly, even in the control group there was a slight, non-significant decrease in scar size, which could be hypothesized to be the endogenous repair mechanisms, which were further stimulated or enhanced by the paracrine effect of the transplanted cells.

Similarly, in a recent study, Hatzistergos et al. (2010) theorized that bone marrow derived cells have a cell-autonomous effect on the host myocardium that stimulates endogenous repair. To test this they transendocardially injected male porcine mesenchymal stem cells (MSCs) that were green fluorescent protein (GFP) labeled into the infarct and border zones in female pigs, 3 days post-MI. The animals were followed with serial cardiac magnetic resonance imagery (MRI) to evaluate the scar size and heart function, and even as early as 4 days after MI, the absolute size of MI was reduced by $8.3\% \pm 1.8\%$ (%LV mass) in animals receiving MSCs (0% in controls, $p = 0.018$), and $10.9\% \pm 5.1\%$ at 8 weeks post-MI by comparison with only $2.3\% \pm 1.3\%$ ($p = 0.002$) in the controls. Similarly, LV function improved significantly by 2 weeks in the treated group, and persisted for 8 weeks. Histological examination indicated that the MSCs were predominantly localized to the border zone and infarct zone and had differentiated to both vascular and cardiac lineages. At 2 weeks they also found chimeric clusters in the border and infarct zones that had Y-chromosome immature MSC and endogenous cardiac stem cells (CSCs). The endogenous

CSCs were c-kit⁺ and lacked GFP, indicating that they did not arise from the injected MSCs. There was a 20-fold increase in c-kit⁺ cells in the treated group by comparison with the control group at 2 weeks. In-vitro testing of porcine endomyocardial biopsies either co-cultured on GFP-MSCs or without MSCs demonstrated that there was a 6-fold greater increase in GFP c-kit⁺ cells in biopsies that were co-cultured with MSCs, by comparison with the controls. This indicates that the interaction of MSC with the native cardiomyocytes supports the expansion of the local CSC pool to promote endogenous repair.

Similar effects of improved cardiac function with MSCs were also seen in a model of chronic hibernating porcine myocardium in a report by Suzuki et al. (2011). Autologous GFP⁺-MSCs were delivered via intracoronary injection. They found that in animals treated with MSCs there was an improvement in left anterior descending artery (LAD) regional wall thickening at both 2 and 6 weeks after injection compared with the control; however, there was no increase in coronary flow reserve, indicating that functional collaterals did not develop. They also noted that there was no difference in circulating mononuclear cells after MSC injection; however, there was a 4-fold increase in c-kit⁺ and CD133⁺ populations at 3 days through 2 weeks in animals receiving MSCs. The c-kit⁺ population also co-expressed cardiac transcription factors GATA-4 and NKx2.5, which indicates their cardiac commitment. Interestingly, they also demonstrated that MSCs stimulate the native cardiomyocytes to reenter the cardiac cell cycle. Markers of proliferation such as Ki67 were significantly increased in hibernating myocardium in MSC treated animals as well as phospho-histone H3 staining.

Mobilization of endogenous cardiac stem cells by engrafted cells

This is probably one of the most exciting areas of current research and the mechanisms underlying the mobilization of endogenous cardiac stem cells need to be further elucidated. Gene expression studies at 4 months after cell transplantation in a porcine model of MI did suggest differential expression of certain key enzymes in the cell transplanted cohort. We hypothesize that exogenous stem cell transplantation leads to transient engraftment and secretion of factors that leads to host myocardium gene expression changes, which lead to long term beneficial effects after stem cell transplantation. One of the genes that showed an increased expression in the cell transplanted group was *Myocyte Enhancer Factor 2a* (*MEF 2a*), which is the predominant *MEF 2* gene product expressed in the post natal cardiac muscle. MEFs play an important role in myogenesis. This might play a role in the differentiation of endogenous cardiac stem cells into cardio-myocytes after injury.

Cardiomyocyte regeneration levels in the human heart

Reported levels of myocyte renewal vary dramatically, and bring to light several issues surrounding the methodologies utilized. Cardiomyocytes themselves comprise only about 20% of the total cells within the myocardium (Rubart and Field 2006), and the techniques used to restrict data accumulated to this cell population only, appears to vary the results obtained. At the lower extreme, Bergmann et al. (2009) claimed a ca. 1% repopulation rate at the age of 25, which then drops to a rate of 0.45% by the age of 75. Values were determined utilizing the integration of atmospheric ¹⁴C within the nuclei of human cardiomyocytes sorted for the presence of troponin T and troponin I, and then the data was evaluated using a mathematical model based on a constant cellular turnover rate. What they determined was that the heart would only repopulate approximately 45% of its cells over a lifetime. At the other end of the spectrum, Kajstura et al. (2010) examined iododeoxyuridine (IdU) incorporation in the hearts of human cancer patients who received therapeutic administrations of IdU. Utilizing the IdU labeling, along with other myocyte markers, they were able to determine the number of new myocytes that formed in 8 patients after their

treatments (Fig. 5). From this data they extrapolated that the average rate of myocyte regeneration was near 20% a year, or about 0.06% a day.

Cardiac repair appears to be mediated by activation of endogenous cardiac progenitor cells

Typically, engraftment levels following stem cell transplantation for cardiac repair are very low, and just a small percentage of those remaining cells differentiate to the cardiac lineage. The cells that repopulate the damaged myocardium following stem cell transplant must therefore be from an autologous source. Loffredo et al. (2011) used a genetic-lineage-mapping approach capable of identifying dilutions in the cardiomyocyte population following 4-OH-tamoxifen induction in a double-transgenic mouse. They found that following MI, mice treated with c-kit⁺ bone marrow progenitors showed activation of endogenous cardiac progenitor cells. Interestingly, mice treated with MSCs isolated from male mice did not show any activation of the endogenous cardiac progenitor cells, and subsequently did not exhibit any cardiac improvements following cell therapy.

The ability to harness the innate capacity of the heart to regenerate is an exciting area of research. The discovery of endogenous cardiac stem cells in the post-natal heart leads us to hypothesize that the mechanism of beneficial effects of stem cell transplantation may be related to mobilization and differentiation of endogenous cardiac stem cells that results in the observed decrease in scar size after cell transplantation. The mammalian heart, similar to that of the zebrafish, appears to possess a population of cardiac progenitor cells capable of repopulation, but seems incapable of mediating self-repair to the extent necessary, following MI. Understanding to what extent these cellular therapies effect endogenous cardiac stem cell activation, mobilization, and differentiation is key to improving future cell therapies as well as identifying potential pharmacological targets. The scientific community needs to work together to identify the signaling pathways induced by transplanted cell populations, along with the corresponding myocardial gene and protein expression changes that contribute to the LV chamber function improvement.

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Fig. 1.

Sca-1⁺/CD31⁻ cells were induced to differentiate into cardiomyocyte-like cells. Sca-1⁺/CD31⁻ cells were cultured in the basal medium containing 10 ng/mL Dickkopf-1, 0.75% dimethyl sulfoxide, 10 ng/mL bone morphogenetic protein 2, 100 ng/mL fibroblast growth factor 4 (FGF-4), and 10 ng/mL FGF-8 in combination (with the addition of 10 μ mol/L 5'-azacytizine for the initial 3 days); total time in culture was 14 days. (A)

Immunofluorescence staining of induced Sca-1⁺/CD31⁻ cells showing nuclear expression of NKx2.5-stained (left, red) and DAPI-stained (middle) nuclei. Right, merged view of NKx2.5-stained and DAPI-stained nuclei. (B) GATA-4-stained (left, red) and DAPI-stained (middle) nuclei. Right, merged view of GATA-4-stained and DAPI-stained nuclei. (C)

Sca-1⁺/CD31⁻ cells cocultured with neonatal rat cardiomyocytes were induced to express cardiac specific markers. Top row of photomicrographs shows troponin T (red), DAPI (light blue), X-gal (dark blue), and a merged view of the preceding photomicrographs. Arrowheads point to triple-positive cells. The bottom photomicrograph shows an X-gal-positive cell (blue) costained for phospholamban (brown). DAPI, 4,6-diamidino-2-phenylindol dihydrochloride; NKx2.5, Homeobox protein NKx2.5; X-gal, 5-bromo-4-chloro-3-indolyl- β -D-galactoside. (From Wang et al. 2006, reproduced with permission of Stem Cells, Vol. 24, p. 1783, © 2006 John Wiley and Sons).

Fig. 2.

A significant increase of Sca-1⁺/CD31⁻ cell population after myocardial infarction. (A) Representative fluorescence-activated cell sorting analyses of cardiomyocyte-depleted cell suspensions obtained from a normal heart (left), a normal skeletal muscle (middle), and a heart that was infarcted 7 days prior to cell harvest (right). Significantly more endogenous Sca-1⁺/CD31⁻ cells (right bottom quadrant) are present in normal myocardium than in skeletal muscle. The number of Sca-1⁺/CD31⁻ cells in myocardium increased after myocardial infarction. (B) The time course of the increase of the myocardial Sca-1⁺/CD31⁻ cell population following infarction. There was a significant increase ($p < 0.01$) from the control values for 7 days after infarction. (C) Numerous endogenous Sca-1⁺ cells distributed in the peri-infarct region 7 days after MI. Arrows point to Sca-1 (red) positive cells in upper photomicrograph and double-positive cells in merged picture of the same section stained with DAPI (blue). (D) Representative Western blot demonstrating enhanced Sca-1 protein expression in hearts 7 days after MI (top); same blot reprobed with GAPDH antibody as controls for equal loading (bottom). GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MI, myocardial infarction. (From Wang et al. 2006, reproduced with permission of Stem Cells, Vol. 24, p. 1783, © 2006 John Wiley and Sons).

Fig. 3. Engraftment and differentiation of pMultistem in vivo. Infarcted swine hearts with LacZ-labeled pMultistem injection were harvested and dissected into 10 μ m sections. Dissected samples were treated with zinc fixative, stained for both LacZ (blue) and troponin T (green), or von Willebrand factor (VWF) (red). Nuclei were stained with 4,6-diamidino-2-phenylindol dihydrochloride (DAPI). (A) Intermediate zone (IZ) of a Lac-Z⁺ pMultistem-treated heart demonstrating engraftment and differentiation to cardiomyocytes. The first image on the left, a phase-contrast image (10 $\times$ with 40 $\times$ inset) demonstrates that Lac-Z⁺ cells engrafted in the infarct zone. The fluorescent image corresponds to the same region which is stained for cardiac troponin T. The fluorescent inset shows arrows indicating Lac-Z⁺ cells that coexpress cardiac troponin T. (B) IZ of a Lac-Z⁺ pMultistem cell-treated heart demonstrating engraftment and differentiation to vascular cell types. As in (A), Lac-Z⁺ cells are shown to engraft in the infarct zone. The fluorescent image is stained for VWF and arrows indicating Lac-Z⁺ and VWF costaining. (C) IZ of a Lac-Z⁺ pMultistem cell-treated heart with phase contrast section (left) and corresponding fluorescent image stained for cardiac troponin T (right). Patches of spared myocytes are seen, which was not observed in the untreated hearts. (From Zeng et al 2007, reproduced with permission of Circulation, Vol. 115, p. 1872, © 2012 American Heart Association.

Fig. 4.

(A) Control heart compared with (B) a heart that received transplanted bone marrow stem cells (Tx heart). X-gal staining shows that “blue” cells are homing into the anterior left ventricular wall. (C) Products (560 bp) were only found in patch and peri-scar areas of Tx hearts. Primers were designed for specifically identifying the Ad5RSV-*lacZ* vector sequence. (D) In stem cell culture medium, the blue cells migrated out of the specimens taken from the periscar area. (E) Typical specimen maintained in stem cell culture medium on day 3, before the second change of the medium. The cells migrated out of the specimen and continued dividing. (F) Typical triple staining of hematoxylin (nuclei), cardiac-specific troponin T, and X-gal (β -galactosidase). Striation of myocytes can be clearly observed by staining of cardiac-specific troponin T. The size of this blue cell is demonstrated by the ratio of the blue cell to the nuclei of native myocytes (hematoxylin and eosin). (G) A heart 18 days after the stem cell transplantation; the histological examination showed that blue cells distributed sparsely in peri-scar area, and most of them accumulated in the scar area. (H) Low-power view of periscar (left) and scar area (right). (I) In the peri-scar area, a few large, rod-shaped blue cells were identified (left). High-power view of respective box zone (right panel). Double staining of the sections from the peri-scar areas where the rod-shaped blue cells were identified for β -galactosidase, and cardiac-specific proteins showed that β -galactosidase colocalized with α -actin (J, K), desmin (L, M), phospholamban (N, O), and cardiac-specific troponin T (P, Q). (J, L, N) and (P) and (K, M, O), and (Q) are cross-

sectional and longitudinal sections, respectively. These data suggest these few blue rod-shaped cells derived from exogenous mesenchymal stem cells were likely cardiomyocytes. N, necrosis; M, spared myocardium. (From Liu et al 2004, reproduced with permission of Am. J. Physiol. Heart Circ. Physiol., Vol 287, p. H506, © 2012 the American Physiological Society).

Fig. 5. Iododeoxyuridine (IdU) in human cardiomyocytes. (A) Several myocyte nuclei (arrows) and nonmyocyte nuclei (arrowheads) are labeled with IdU (green). Myocytes are positive for α -sarcomeric actin (α -SA, red). Laminin (white) defines the boundary of myocytes and interstitial cells. (B) Metaphase chromosomes in a dividing myocyte nucleus (upper panel) are labeled by IdU (lower panel). (C) Percentage of IdU-positive myocyte nuclei in each of the 8 patients injected with the thymidine analog. The interval between each IdU administration and patient's death is shown. IdU was not detected in the control hearts. The average degree of myocyte formation in the 8 patients is also shown. (From Kajstura et al. 2010, reproduced with permission of Circulation Research, Vol. 107, p. 306, © American Heart Association).