Searching for Understanding With the Cellular Lining of Life

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stem cell therapy for the prevention and treatment of myocardial dysfunction in response to ischemic injury has progressed from preclinical models to clinical populations in relatively rapid fashion. Whereas some have argued the progress is too rapid because of the lack of a defined mechanism and the identification of an optimal cell type, others have suggested that the potential efficacy, combined with the apparent safety and increasing prevalence of chronic heart disease, requires that we move forward in clinical populations.1

In general, adult stem cell populations have lacked the ability to differentiate into cardiac myocytes that beat spontaneously, suggesting that the improvements seen in preclinical and clinical studies using these stem cell populations, including mesenchymal stem cells (MSCs),2,3 bone marrow mononuclear stem cells,5,6 multipotent adult progenitor cells,8 hematopoietic stem cells,7 and others, are not attributable to replacement of damaged myocardium with new contractile tissue but rather to the release of paracrine factors that induce myocardial repair. With that said, how these paracrine factors lead to improved cardiac function, especially at time remote from acute ischemic injury, remains unclear.

Finally, whether there is the potential for an "off-the-shelf" cell product that can be implemented in an allogeneic strategy that will allow for the availability of therapy at any time and, in particular, when the patient presents with an acute ischemic event is still an open question. Two cell populations, MSCs and multipotent adult progenitor cells, are currently undergoing clinical study in allogeneic strategies; however, the mechanism associated with the immunoprivileged state of these cells and whether this immunoprivilege with extend to cardiac differentiation remain important unknowns.

In this issue of the Circulation Research, Tsuji et al present interesting data that address many of these questions.8 In this report, the authors focus on the human amniotic membrane as a source of mesenchymal stem cells. They hypothesize that MSCs from the membrane, which are known to have the ability to transdifferentiate into multiple cell types, could yield cardiac myocytes, and, by virtue of their role in maintaining the fetus, cells from the membrane could have immunomodulatory properties.

Tsuji et al demonstrate that MSCs isolated from different human amniotic membrane-derived mesenchymal cells (hAMCs) can expand in culture with 20 to 30 population doublings. hAMCs are similar to MSCs in that they are CD90+, CD105+, CD34−, and CD117−. The authors further observe that that the hAMCs at baseline exhibit significant expression of oct4 and cardiac proteins including cardiac troponin, but after coculture with murine fetal cardiac myocytes, there is a broader expression of cardiac proteins and downregulation of oct4. The authors nicely demonstrate that the cardiac myocytes generated from hAMCs have pacemaker like activity and cardiac specific action potentials. Interestingly, the authors further demonstrate that the transdifferentiation in coculture does not require hAMC contact.

The studies in the article by Tsuji et al progress to demonstrate that hAMC transplantation into the infarct border zone of immunocompetent and immunoincompetent rats 2 weeks after acute myocardial infarction results in the presence of hAMCs 4 weeks later, suggesting that in a xenogenic transplant model hAMCs are not rejected. Immunofluorescent studies further demonstrate that the surviving hAMCs have differentiated into cardiac myocytes and appear to have intercalated within surviving cardiac myocytes within the infarct border zone.

Because the hAMCs are derived from the amniotic sack, Tsuji et al investigate the immunomodulatory pathways activated during fetal development and are able to demonstrate that the survival of hAMCs in the immunocompetent xenogenic model is associated with an increase in human leukocyte antigen (HLA)-G expression, low expression of major histocompatibility complex (MHC) I, and, ultimately, the activation of FOXP3+ regulatory T cells. Perhaps not surprisingly, the immunoprivilege of the hAMCs is time-dependent. Initially, the cells appear to survive because of the combination of lack of MHC I expression, which blocks T cell–mediated cell loss, and upregulation of HLA-G, which inhibits natural killer cell activity. Because differentiated hAMCs do not express HLA-G, the long-term survival of the hAMC cardiac myocytes in the xenogenic model is hypothesized to be attributable to the activation of the FOXP3 regulatory T cells that inhibit hAMCs mediated T-cell destruction. Interleukin 10 and progesterone are shown to increase HLA-G expression and hAMCs derived cardiac myocyte number in vivo; however, whether these observations are attributable to increased hAMC differentiation or enhanced survival appears to be in question, although the latter seems more likely. With that said, it is interesting to...
Tsuji et al. demonstrate that injection of ≈1 to 2 million hAMCs 2 weeks after acute myocardial infarction leads to improvement in cardiac function, whereas the untreated hearts continue to decline in function, resulting in a ≈10% absolute increase in fractional shortening 2 weeks after cell transplantation (4 weeks after acute myocardial infarction).

In this study, Tsuji et al. convincingly demonstrate that the hAMCs can differentiate into cardiac myocytes and that, even after differentiation, these cells survive in vivo. Yet one must admit that the ultimate mechanism resulting in the increase in cardiac function remains elusive. Laser confocal microscopy of the hearts 2 weeks after transplantation of green fluorescent protein (GFP) hAMCs demonstrates the presence of GFP cardiac myocytes, but these cells are scattered throughout the infarct border zone, intercalated in between native cardiac myocytes that survive the infarct. There is also a modest but significant decrease in the area of myocardial scar in response to hAMC engraftment. Notably lacking in these images is the presence of sheets or volumes of new cardiac myocytes replacing the injured myocardium, suggesting the lack of true regeneration of the transmural contractile tissue lost because ischemic injury.

Ultimately then, if replacement of the scar with contractile tissue does not occur, what is the mechanism of benefit and how might we be able to achieve true regeneration, particularly at a time that is remote from acute myocardial infarction, when simply improving cardiac myocyte survival is not a sufficient or pervasive mechanism? Taking the latter issue first, it would seem likely that the replacement of scar with contractile tissue will require the implementation of scaffold- ing on which contractile networks can be grown. At least in the beginning, it would appear that this scaffold may need to be implanted using a surgical approach. Although this seems like a worthy approach, the recent findings demonstrating the lack of benefit of surgical ventricular remodeling in the STICH trial needs to be acknowledged.10

With respect to the mechanism of benefit, the preponderance of the data would suggest that the benefit is associated with optimal remodeling of the ventricle independent of the regeneration of cardiac myocytes.8 More specifically, improvement in the viability of the infarct border zone, the site of the weakest link in the chain. This is not a revolutionary concept, but one that is at times forgotten when those in the field turn to counting cells and debating phenotype, instead of focusing on the biology and physiology at hand. In an organ like the kidney or liver, distributed systems with discrete functional units, perhaps it is all about the number of cells and functional units. The heart, with its interconnect contractile network, however, is not unlike a chain in that it can only be as strong as its weakest link.

It is interesting to note that in this study by Tsuji et al., the authors did not observe an increase in vascular density as is commonly seen in the majority,2,4,11–13 although not all14 of the preclinical cell therapy studies to date. The lack of increase in vascular density following the transplantation of hAMCs could suggest that intercalation of functional contractile cells within the infarct border zone can lead to improvement in cardiac remodeling perhaps by modifying the workload of the surviving cardiac myocytes (Figure). Conversely, cells or paracrine factors that increase vascular density but do not increase the number of contractile cells could improve cardiac function by improving the metabolic performance of the surviving cardiac myocytes (Figure). Ultimately, the same mechanism is in play with both of these strategies: improved functional performance of the infarct...
border zone leads to improved left ventricular remodeling and contractile performance.

In support of this concept is a prior study from our laboratory in which we focused on the importance of remodeling the infarct border zone. In this study, we delivered GATA4 to the cardiac myocytes in the infarct border zone 1 month after acute myocardial infarction using a chimeric cell penetrating peptide strategy.\(^1\) The focal delivery of GATA4 to the border zone led to cardiac myocyte hypertrophy of the border zone cardiac myocytes and no change in the vascular density. This local hypertrophy led to an improvement in cardiac strain in the infarct border zone and global remodeling of the heart in the absence of the generation of new contractile tissue.\(^2\) This global remodeling led to improved strain in the noninfarct zone, ultimately improving cardiac function in the absence of any evidence of contractile activity in the infarct zone. The preclinical and clinical data to date, including this important study by Tsuji et al, support the concept that further understanding the biology and physiology of the infarct border zone, along with strategies designed to augment its function, is what will ultimately lead to improvement in patient outcomes.

In summary, Tsuji et al should be congratulated for their careful work, which has brought forward a cell type that may offer the real potential for off-the-shelf cardiac myocyte-based therapy. Their findings further add to our understanding of the mechanisms associated with immunoprivilege, which is critical as we move forward with allogeneic cell therapy. Their findings further add to our understanding of the infarct border zone, along with strategies designed to augment its function, is what will ultimately lead to improvement in patient outcomes.

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