What Is the Oncologic Risk of Stem Cell Treatment for Heart Disease?

Konstantinos E. Hatzistergos, Arnon Blum, Tan A. Ince, James M. Grichnik, Joshua M. Hare

Every therapy has toxic and therapeutic windows, and defining the side effects of any new therapeutic modality is the first order of business in the development of a treatment. With transformative therapies such as cell-based approaches, treatment side effects can be unpredictable and unanticipated. In some cases, experimental data raise serious concerns that must be appropriately managed. In the face of the promise and enthusiasm for cell-based therapy for heart disease, results from rodent experiments have consistently raised the specter of a dreaded side effect—can the use of stem cells lead to cancer, either directly or through promotion of existing early-stage neoplasms?

Mesenchymal stem cells (MSCs) are a multipotent immunotolerant cell source that can be readily expanded into therapeutic quantities from a variety of tissues such as the bone marrow, cord blood, and fat, and as such their use in cell-based therapeutic strategies holds great promise.1 With regard to heart disease, accumulating preclinical2–11 and clinical studies12–15 demonstrate that MSC transplantation may be salutary for both acute myocardial infarction and cardiomyopathy with an acceptable risk profile.

The translational development of cell-based therapy has required rigorous large-animal experimentation, recognizing inherent limitations with rodent experimentation. In this context, more than 500 large animals (swine, canine, and sheep) have been tested to assess the safety and efficacy of MSC therapeutics for treating heart disease with the results demonstrating that MSC transplantation is a safe and durable approach that may be more effective than bone marrow mononuclear cells.16 Importantly, large-animal work provides a phenotyping opportunity not available in rodents, and rigorous cardiac MRI (CMR) and histological analysis in porcine hearts supported the regenerative effects subsequently demonstrated in the adult human heart.12,15 The mechanism of action appears multifaceted, involving direct differentiation of MSCs into cardiomyocytes and vessels,2,11 but to a greater extent, stimulation of the hearts’ own cardiac stem cells to form new cardiac muscle.11

**Chromosomal Instability and the Risk of Neoplastic Transformation**

In the face of these exciting preclinical and clinical findings, a series of concerning reports demonstrate that murine MSCs are prone to chromosomal abnormalities and promote tumor or ectopic tissue formation. The key reports include those of Miura et al,17 Breitbach et al,18 Foudah et al,19 and in the current issue of *Circulation Research* that of Jeong and colleagues.20 Miura et al showed that murine MSCs, after numerous passages, obtained unlimited population doublings and underwent malignant transformation; passage 65 MSCs injected into mice formed fibrosarcomas in multiple organs, including the pericardium. Raising additional concerns, Breitbach et al reported that murine MSCs and whole bone marrow led to unwanted tissue differentiation in the form of extensive bone formation in infarcted mouse hearts. Foudah et al also reported that rat MSCs (rMSCs) exhibited genomic instability and tumorigenicity in culture. However, “considering the apparent genomic stability reported for in vitro cultured human MSCs (hMSCs),” they concluded that “these findings underline the fact that rMSCs may not in fact be a good model for effectively exploring the full clinical therapeutic potential of hMSCs.”

The new report by Jeong et al extends these findings by showing that murine MSCs exhibit genetic instabilities even at low passages and lead to massive tumor formation in the heart and hindlimbs of mice. Chromosomal analysis revealed that culturing of these otherwise normal appearing, tumorigenic mouse MSCs caused multiple chromosomal abnormalities, including fusion, fragmentation, and ring formation. Considering the rarity (∼0.017%) of primary cardiac neoplasms in the human heart,21 the aggressiveness and size of the tumors that Jeong et al describe in ∼33% of the MSC-injected mouse hearts (a 2,000-fold increase in tumorigenic frequency) highlight the importance of potential interspecies variability in translational research. Nevertheless, these reports must be taken very seriously.

With regard to the molecular underpinnings of transformation, an increased susceptibility of rodent versus human cells is described. Rangarajan et al22 demonstrated that whereas perturbation of 6 signaling pathways in human fibroblasts was required for tumorigenic transformation, mouse fibroblasts required only 2 (p53 and Raf). Considering that a typical random mutation rate is 10⁻⁶ to 10⁻⁷ per gene per somatic cell division, having 6 pathways mutated substantially lowers the probability and provides a potential mechanism for the greatly increased resilience of cultured human cells in...
comparison with rodents. Thus, taking into account the caveat that these studies were performed in fibroblasts and not MSCs, per se, there appears to be a molecular basis for a decreased vulnerability of human cells to molecular transformation during culture expansion.

An additional mechanism for MSC-stimulated promotion of neoplasia has been described. MSCs integrate within the tumor-associated stroma in conditions such as breast cancer and sarcomas, and therefore the possibility that human MSCs could also undergo chromosomal transformations that promote growth or increase the metastatic potency of a tumor is being extensively studied. These findings raise the concern that MSCs could track to areas of early malignant transformation in the body and promote or accelerate tumor formation. However, the study by Jeong et al clearly illustrates how these studies were performed in fibroblasts and therefore there are clear biological differences between rodent and human MSCs, such that they may not recapitulate large mammalian biology with regard to regenerative performance. The findings of efficacy of cell-based therapy are consistently reported to be less than those seen in large animals or humans. For example, while human and porcine wild-type MSCs are capable of regenerating scarred myocardium in both direct and indirect manners, mouse and rat MSCs need to be genetically modified for enhanced survival or differentiation capacity to exert similar therapeutic effects. Furthermore, rodent MSCs are postulated to exert their effects largely through paracrine signaling, because their in vivo differentiation into cardiomyocytes has been difficult to demonstrate. For example, murine MSCs express sca-1 abundantly, whereas the human orthologue is yet to be described. Thus rodent and human MSCs differ at multiple levels.

What Is the Risk of Human MSC Transformation?

Unlike the several reports described above, human MSCs demonstrate substantial stability even when cultured ex vivo for long term, and direct evidence for spontaneous laboratory-induced transformations in human MSCs has not been definitively provided.

In an influential report, Rubio and colleagues reported that long-term in vitro culturing of human adipose tissue–derived MSCs over a period of 4 to 5 months could transform them into “mutant stem cells that may seed cancer.” When the transformed human MSCs were transplanted into immunocompromised mice, they generated cancers in nearly all mouse organs—including the heart—within 4 to 6 weeks. Therefore, the authors recommended that MSCs should not be considered safe for clinical testing. At the time when this article was published, we stopped our clinical trials of MSCs for a very careful review of the existing state of knowledge. After a careful review of the available large-animal data at the time and with additional safeguards, the trial was recommended for continuation and was completed. Ironically, this study was subsequently retracted by the authors because they were “unable to reproduce some of the reported spontaneous transformation events and suspect the phenomenon is due to a cross-contamination artifact.” It was later reported that, indeed, the transformed cells in Rubio’s report originated from cross-contamination with the fibrosarcoma cell line HT1080.

In a similar vein, Rosland et al reported that prolonged cultured human MSCs from the bone marrow could frequently undergo spontaneous malignant transformations. Moreover, more rigorous DNA analysis highlighted that their human MSC cultures were also cross-contaminated with human fibrosarcoma or osteosarcoma cell lines. Moreover, in the case of clinical studies, all human MSCs lines are manufactured under certified good manufacturing practice (GMP) facilities and used from passage 1 (autologous transplant) up to passage 5 (heterologous transplant), therefore excluding any risk for long-term culturing or cultured-induced chromosomal instabilities or mutations. Accordingly, Wang and coworkers generated more than 100 human MSC lines, of which 1 yielded a transformed population. Of crucial importance, however, was that this transformed cell was present in the original bone marrow sample and expanded with time in culture, suggesting that it was actually a transformed line isolated from the patient. The tumorigenic population could be clearly distinguished from the MSC population by morphology and demonstrated an abnormal karyotype.

Together, this series of findings very strongly supports the safety of human MSCs in clinical trials, albeit with ongoing and extreme vigilance.

Addressing the Risk of Neoplasia from Cell Therapy

How do we interpret these findings and what is the appropriate response? First, it should be recognized that the risk of neoplasia from stem cells, particularly MSCs, has long been recognized and managed in clinical trial development. In the case of MSCs, long-term studies in porcine models have used whole-body autopsies to establish that MSC-based therapies for heart disease do not bear a major unacceptable risk for ectopic tissue formation. Very importantly, phase I
clinical trials have specifically monitored for unwanted tissue formation, including neoplasia. In both the Osiris sponsored phase I trial and a series of studies led by our group, whole-body computed tomography (CT) has been performed to monitor for this side effect. In addition, patients with increased oncological risk due to underlying comorbidities—such as HIV, hepatitis, hematologic disorders, or history of malignancy—have been excluded by trial design.

Results of Preclinical and Clinical Studies
Careful monitoring for adverse effects of MSC-based therapies in preclinical and clinical settings very strongly supports an acceptable safety profile for MSC therapeutics with regard to cancer or ectopic tissue formation. Importantly, our findings are supported by the work of many other laboratories (see the meta-analysis of Van der Spoel et al). By studying more than 150 swine in our laboratory during a 10-year period using different MSC preparations and methods of delivery to the heart, we monitored the safety and efficacy of our treatment using cardiac MRI and whole-body histological analysis. In these studies, tumors (cardiac or otherwise) or ectopic tissue formation have not been observed. These findings contributed to the design and conduct of 4 clinical trials (the TAC-HFT [NCT00768066], POSEIDON [NCT01087996], Provacel [NCT00114452], and PRO-METHEUS [NCT00587990]) that have recruited in the past 5 years more than 125 patients; a major risk of tumor growth has not been detected in these patients. In addition, numerous trials are ongoing for MSCs in multiple disease areas, including but not limited to graft versus host disease, ulcerative colitis, chronic obstructive lung disease, and osteogenesis imperfecta. Ongoing phase I cardiac studies will add substantially to the database of safety information (sustained ventricular arrhythmias, ectopic tissue formation, or sudden unexpected death) and will begin to build the case for efficacy in patients with acute and chronic heart disease. At the same time, more than 700 patients with heart disease have received cell-based therapies with whole bone marrow in the last 10 years at different medical centers worldwide. No indications of tumor outgrowth have yet been reported, substantiating the concept that the risk for primary cancer development following bone marrow–based cellular cardiomyploasty is minimal.

Summary
Accumulating clinical and preclinical trials are adding to the database, supporting the idea that human cell therapy with MSC transplantation is a safe and a reliable procedure for treating heart disease. Long-term rigorous patient monitoring demonstrates the durability and safety of cell-based therapies for heart disease, with no incidence of tumorigenesis. As with any new therapy, extreme vigilance is required to monitor for, manage, and understand the risk of unwanted and desirable side effects. The specter of neoplasia raises major concerns. However, we conclude that the observations in rodent animal models used to study human diseases should be interpreted with caution when assessing safety and efficacy of any new therapeutic modality and that the risk–benefit profile of MSC cell therapy in the rodent is substantially different from that in large mammals. We believe that ongoing trials of MSCs in humans are of acceptable risk, but strongly argue for ongoing vigilance, particularly over the long term.

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Disclosures
Dr Grinchik discloses that he is a major shareholder in DigitalDerm, Inc. and a consultant for both Spectral Image, Inc, and Genentech, Inc. The remaining authors have nothing to disclose.

References


35. Gneecchi M, He H, Liang OD, Melo LG, Morello F, Mu H, Noiseux N, Zhang L, Pratt RE, Ingwall JS, Dzau VJ. Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. Nat Med. 2005;11:367–368.

36. Mirotou M, Zhang Z, Deb A, Zhang L, Gneecchi M, Noiseux N, Mu H, Pachori A, Dzau V. Secreted frizzled related protein 2 (Sfrp2) is the key Akt-mesenchymal stem cell–released paracrine factor mediating myocardial survival and repair. Proc Natl Acad Sci U S A. 2007;104:1643–1648.


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What Is the Oncologic Risk of Stem Cell Treatment for Heart Disease?: Correction

In the Editorial that appears on pages 1300–1303 of the May 27, 2011 issue, author Tan Ince should have appeared as Tan A. Ince. In addition, the article did not contain Dr Ince’s affiliations with the Department of Pathology, Interdisciplinary Stem Cell Institute and Braman Family Breast Cancer Institute, Leonard M. Miller School of Medicine, University of Miami, Miami, Florida.

The publisher regrets this error. The error has been noted and corrected in the online version of the article which is available at http://circres.ahajournals.org/content/108/11/1300.full.

Reference


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