

Extra Views

Metamorphosis from Bone Marrow Derived Primitive Stem Cells to Functional Liver Cells

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ABSTRACT

Both stem cell plasticity and cell fusion have been implicated as physiological responses to tissue injury. It remains the ultimate goal for the future to understand the regulatory control of each during regeneration. In our recent paper by Jang et al. we demonstrate the repair of damaged liver by bone marrow derived stem cells (SCs) in response to microenvironmental cues. Within 48 hrs after transplantation or co-culture, conversion of SCs into liver cells was observed. Fusion was ruled out as a major mechanism of this functional regeneration. Direct differentiation of SCs into liver epithelial cells may be clinically useful. However, if plasticity or fusion results in abnormal genetic changes they could be harmful. Before proceeding with therapeutic applications, the consequences of cellular therapy accompanying both plasticity and fusion must be examined in multiple animal models. Functional repair should also be demonstrated prior to treatment in patients.

INTRODUCTION AND HISTORICAL PERSPECTIVE

Hematopoietic SCs (HSCs) differentiate into all blood cells. There is extraordinary expansion from the relatively rare HSCs into more mature cells. Two hypotheses were proposed in the 1970's to explain the underlying mechanisms responsible for hematopoietic differentiation. The first, namely HSC differentiation/proliferation were regulated by intrinsic genetic programming.¹ The alternative mechanism was that extrinsic signals found in the hematopoietic microenvironment could trigger HSC maturation.² The debate, heated at times because many of the microenvironmental factors were yet to be discovered or proven functional in vivo, has evolved into recognition that both hypotheses have elements of the truth; both intrinsic and extrinsic signals regulate differentiation.

Thirty years later similar hypotheses have been evoked based upon the observation that bone marrow derived cells, SCs in particular, can convert into cells of a different germ layer tissue such as regenerating liver. History will repeat itself, i.e., both extrinsic and intrinsic signals will play roles in tissue repair. Current thinking suggests that SCs are attached physically to structures within the host tissue.³ The extrinsic signaling hypothesis would implicate SC residence within this specialized niche. Circulating factors or other cells within the niche stimulate the release of the SCs from their attachment point and trigger one or more signaling cascades which are responsible for differentiation. If the external signals are directed by the injury stimulus (the instructive hypothesis) the events which follow (i.e., homing to injured tissue, gene expression changes, morphological alteration and functional repair) should result in conversion. Alternatively, fusion of SCs with the injured tissue could set in motion major expansion of the fused cells resulting in efficient repair. Since SCs are so rare the frequency of fusion with this cell type would not be advantageous unless the fused cells could expand rapidly. It is likely fusion would occur more frequently with progeny of SCs not the SC itself. Stable fusion would permit gene expression common to the tissue undergoing repair but injury signals could still be necessary to initiate frequent fusion events.

Evidence for both mechanisms continues to be reported. During the last decade there have been many papers which have demonstrated the conversion of adult marrow cells (reviewed in refs. 4–6) and more specifically the conversion of SCs into cells of another tissue type.^{7–12} Experimental evidence supports the hypotheses that SCs can be induced to change phenotype in response to microenvironmental cues^{12,13} or cell fusion.^{14–17}

INJURY SIGNALS ARE NECESSARY FOR BOTH CONVERSION AND FUSION IN LIVER

Conversion of marrow into liver has been demonstrated in both animals¹⁸⁻²¹ and humans.¹¹ Liver tissue responds to injury by rapid regeneration^{22,23} and repair by marrow derived cells has been shown to be either by direct differentiation^{12,13} or cell fusion.^{17,24} Human HSCs from cord blood were transplanted into NOD/SCID mice; after engraftment the mice were exposed to a hepatotoxin and human cells in the liver were shown to express albumin.²¹ We have shown that within two days SCs express multiple epithelial and liver specific markers (i.e., 14 proteins or mRNA transcripts) in response to liver damage.²⁵ This rapid conversion is quite remarkable but likely requires the recognition of and response to the injury stimuli. The severity of injury may play a role in the rapidity and frequency of SC conversion. In fact, we show that irradiation alone as an injury signal in liver is much less effective for repair than a hepatotoxin alone which in turn is less effective than toxin plus irradiation.²⁵ This would imply that the degree of organ damage and multiple signals provided by the injury are essential for repair followed by a physiological activation to express genes otherwise not expressed. HSCs due to their quiescent nature do not expand in vivo for up to 28 days when replacing mature cells of the peripheral blood.²⁶ However in specific response to the injury signals SCs become tetraploid, characteristic of hepatocytes but not blood cells within two days in vitro and in vivo.²⁵

Without specific tissue injury HSCs are capable of fusion but at very low frequency²⁷ and in some cases not at all.^{15,28} In a rare genetic metabolic disorder of liver, tyrosinemia type I (the FAH^{-/-} mouse) it was shown that transplanting small numbers of HSCs could correct the chronic disease.¹⁹ Subsequent studies have suggested that the repair was due to cell fusion rather than direct differentiation.^{17,24} Due to the genetic instability in this severe injury model, it is conceivable that the cells would be more prone to fusogenic events over time.^{29,30} Since a high incidence of aneuploidy in the FAH^{-/-} mouse following cell fusion was observed,^{17,24} it is possible in diseases associated with chromosomal instability the consequences of aneuploidy following fusion including malignancy would be dangerous and the risk of cellular therapy should be carefully evaluated.

SC-PLASTICITY VS. MYELOID CELL-FUSION?

The nature of the cells which are the targets for conversion have not been clearly elucidated. Given that many of the earlier studies used undefined bone marrow cells it was difficult to interpret that the target for conversion was any particular cell type let alone the rare SCs. There is the general belief that all SCs are created equal. However, given the extensive differentiative potential of SCs even for mature hematopoietic cells, it is possible that this population is functionally heterogeneous as well. HSCs are not the only SC residing in the bone marrow. The most primitive SC in the marrow may give rise to HSCs and other SCs which contribute to the regeneration of other organs. Within the SC compartment there may be an age structure which represents cells with more or less pluripotency and differentiation potential. For instance, is it possible that SCs have different functions as they mature and lose potential for self renewal and cell fate changes? The SCs isolated from one study may not have the same capacity for differentiation as those isolated from other studies. The cells which we isolate based upon both quiescence and the absence of lineage and progenitor markers may be more primitive than those isolated by phenotype alone.^{26,31,32} SCs isolated on the

basis of quiescence express very few differentiation markers. Moreover, it is possible that the most primitive SCs do not express the transgenes that have been used to detect donor chimerism. Thus, the EGFP marker may not be as ubiquitously expressed as thought previously. Unfortunately, EGFP positivity was used to select HSCs in the study which observed only limited plasticity of epithelial tissue³³ leading those authors to conclude that it is a very rare event. In a recent publication,²⁵ we observe a conversion frequency of SC derived hepatocytes of 7.6% 2 days after transplant which is not a rare event. A less enriched HSC population (i.e., Fr25 lin-cells) show a quantitative reduction in the frequency of conversion and the onset is also delayed by 24 hrs both in vitro and in vivo. It is possible that more mature SCs are less capable of conversion. In our in vitro assay an enriched population of mature macrophages did not convert into cells with a liver phenotype.²⁵ These data all support the hypothesis that conversion may require as a target a rare primitive SC.

Studies of in vivo fusion when examined many months following transplant demonstrated a high fusion rate.^{17,24} Differentiated macrophages may be the fusion partner for liver injury in this tyrosinemia mouse.³⁴ Most in vivo studies demonstrating fusion have utilized the same injury (FAH^{-/-}, tyrosinemia model) which has genetic instability. It therefore will be important to study several potential sources of cells for their repair capacity as well as multiple acute and chronic injury models which are not associated with genetic abnormality.

Evaluation of repair will require finding the appropriate target cell for conversion, careful genetic marking of donor and recipient cell types, and both protein and mRNA analysis of the converted cells. To actually measure the level of repair, a functional analysis of the repaired tissue needs to be demonstrated. The more primitive the population the more likely these cells represent immature phenotypes and consequently they may be more plastic. On the other hand, mature bone marrow cells would be useful in repair if they contribute to the regeneration of liver due to their increased frequency of fusion only as stable and safe heterokaryons.

SHOULD WE START CELLULAR THERAPY IN PATIENTS?

Studies with animal bone marrow cell conversion¹⁸⁻²³ as well as the important retrospective analysis in which human patients demonstrated donor chimerism in non-hematopoietic tissue^{11,28} show great promise for bone marrow transplant therapy to repair organ damage in human disease. However, there have been concerns^{35,36} that the studies which demonstrate plasticity have led to premature clinical trials in diseases which are life threatening (i.e., heart disease). Therefore, the underlying mechanisms of conversion, either direct differentiation induced by microenvironmental influences or cell fusion, as well as the long term complications of each, should be carefully and rigorously studied before proceeding with clinical approaches. Convincing demonstration of conversion with multiple injury models and careful analysis to determine the levels of repair should be pursued pre-clinically by multiple investigators and by phase one safety approaches in the clinical setting. We anticipate many important improvements in the use of SC therapy following additional extensive studies of cell fate changes.

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