

# Stem Cell Plasticity: Tools for Investigation and Repair

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Traditionally, the epithelial lining of the gastrointestinal tract has been one of the few organs about which there has been little argument concerning its nature as a stem cell-based lineage system. However, the detailed mechanisms of how these cells function is not as fully elaborated as those for hematopoietic stem cells of the bone marrow. Indeed, only functional and positional assays for these cells have been useful investigatively, and it is only recently that a few antigens have been tentatively identified as markers of “stem cell-ness.”

What is meant by the term “stem cell” in this context? Classically, it is defined as a proliferative cell that necessarily produces at least one stem cell when dividing (i.e., self renewal) and gives rise to more differentiated daughter cells that then populate the various cells that line the rest of the intestinal crypt and villous in the small intestine (i.e., pluripotency). In the gastrointestinal tract, Potten et al. have further extended the definition to include the ability to alter its self-maintenance probability to ensure expansion of stem cell numbers in the event of severe injury, so called “clonogenic capacity.” While markers for these cells have been lacking, careful labeling and tracking studies have indicated that the stem cell of the intestinal crypts can be identified precisely by cell position counted from the base of the crypt. Moreover, since all cells of the crypt lining derive from the stem cells, their relative positions toward the top of the crypt or villous relate to their age and to their differentiation state. These differentiated cells fall into common subtypes (columnar cells, mucin-secreting/goblet cells, endocrine cells and, in the small intestine, Paneth cells) and less common subtypes (caveolated cells and M cells). The subepithelial myofibroblasts of the intestinal crypts form a syncytium, which lines the crypts and extends through the lamina propria, merging with pericytes of the blood vessels. These cells influence or even regulate stem cell behavior, by secreted factors (e.g., hepatocyte growth factor, TGF- $\beta$  keratinocyte growth factor) and by direct cell-cell contact.

## MULTIORGAN PLASTICITY

These comparatively simple concepts for epithelial stem cell function in the gastrointestinal tract have been

complicated in recent years, however, by the finding that cells from the circulation, at least in part bone marrow derived, can engraft as epithelial cells and as myofibroblasts. While the latter possibility still preserves the mesoderm-to-mesoderm standard paradigm, albeit within different organs, the derivation of endodermal tissues from mesoderm upends longstanding dogma. The first demonstration of esophagus, stomach, small and large intestinal epithelial cells deriving from hematopoietic stem cells comes from our own work in mice in which a single hematopoietic stem cell, isolated by functional assay, was able to reconstitute bone marrow, epithelia of the liver, lung, gastrointestinal tract, epidermis and skin adnexa (i.e., mesoderm, endoderm and ectoderm) in mice. Levels of engraftment in the animals were less than 2.5%. Subsequently, Korbiling et al. and Okamoto et al., examining tissues from women who received therapeutic bone marrow transplants from men, were able to identify Y chromosomes in their enterocytes. The levels of engraftment were as high as 5 and 7 percent, respectively. Thus, as we have suggested for other organs, there appear to be intraorgan and extraorgan stem cells, both of which are able to contribute to repair after injury and, perhaps, to normal cell turnover. The level of contribution is low or absent in the absence of injury. If injury occurs, depending on the type and extent of the injury, the extraorgan stem cells can contribute to a greater degree. Interestingly, both bone marrow stromal cells and hematopoietic stem cells have also been shown in rodents and humans to contribute to generation of myofibroblasts. However, no study as yet has looked at both the myofibroblasts and the epithelial cells in the same tissues to assess their relationship. It thus remains unclear if these two compartments engraft in a coordinated process.

## STEM CELL POSSIBILITIES FOR INFLAMMATORY BOWEL DISEASE

The “new” stem cell plasticity raises far more questions than it answers, and creative engagement of these findings (ever more rapidly) may lead to unexpected possibilities. For example, circulating, marrow-derived stem cells or stromal cells can be easily obtained targets for gene insertion and then might serve as vehicles to deliver

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genes to target organs. As Brittan and Wright have suggested, the possibility that such cells can engraft into inflamed tissue as myofibroblasts that then might locally secrete proteins that can alleviate disease (e.g., TNF-neutralizing antibodies) is very intriguing. Still more bizarre by classic standards are the possibilities inherent by combining plasticity effects with new techniques for developing completely tolerized and tolerant hematopoietic chimeras between a donor and recipient animal (or patient). Alan Flake et al. have developed an approach that first accomplished this in mice. *In utero* hematopoietic stem cell transplantation is a nonablative way to attain mixed hematopoietic chimerism. Then, post-natally, the animal receives non-myeloablative, total body irradiation followed by another bone marrow transplant of hematopoietic stem cells from the original donor. The result: 100% hematopoietic engraftment from the donor population but no rejection of the graft by the recipient and no graft-vs-host disease.

Such experiments might allow for separation of end-organ vs. immunocyte mediated mechanisms of injury in the various animal models of IBD by mixing and matching wild-type immune systems and genetically altered viscera or vice versa. Moreover, if injury occurs in such mismatches, careful assessment of the levels of marrow to intestinal (enterocyte or myofibroblast) engraftment may itself prove illuminating. After all, once the inflammatory cycle of tissue destruction and regeneration begins, the intraorgan stem cell response must of course come into play. A cursory review of the literature suggests that behaviour of the stem cell compartment in IBD is an infrequently investigated area.

These are only examples of what creative application of the newest stem cell and cell plasticity findings might entail. But they certainly make clear that therapeutic possibilities may extend well beyond the “usual” science fiction-sounding speculations from the world of fiction or from the popular press of today.

### Key Questions

- 1) How do intraorgan stem cells for intestinal epithelium respond to chronic, immune-mediated injury as seen in IBD?
- 2) Do circulating stem cells engraft into chronically inflamed intestinal mucosa, and do they do so as enterocytes, myofibroblasts or both?
- 3) Can circulating stem cells be used for gene therapies of IBD?
- 4) Can high-level allogeneic chimerism with complete tolerance in experimental models help to resolve mechanisms relevant to IBD?
- 5) If genetic profiles predictive of IBD can be used to diagnose likely development of the disease in utero, can the above low-risk, in utero transplantation cure IBD in the neonatal period without risk of rejection or graft-vs-host disease?

### KEY REFERENCES

1. Brittan M, Wright NA. Gastrointestinal stem cells. *J Pathol* 2002; 197:492–509.
2. Marshman E, Booth C, Potten CS. The intestinal epithelial stem cell. *Bioessays* 2002;24:91–8.
3. Krause DS, Theise ND, Collector M, et al. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell* 2001;105:369–77.
4. Theise ND, Nimmakayalu M, Gardner R, et al. Liver from bone marrow in humans. *Hepatology* 2000;32:11–6.
5. Theise ND, Badve S, Saxena R, et al. Derivation of hepatocytes from bone marrow cells in mice after radiation-induced myeloablation. *Hepatology* 2000;31:235–40.
6. Korbli M, Katz RL, Khanna A, et al. Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells. *N Engl J Med* 2002;346:738–46.
7. Okamoto R, Yajima T, Yamazaki M, et al. Damaged epithelia regenerated by bone marrow-derived cells in the human gastrointestinal tract. *Nat Med* 2002;8:1011–7.
8. Theise ND, Krause DS. Toward a new paradigm of cell differentiation capacity. *Leukemia* 2002;16:542–8.
9. Peranteau WH, Hayashi S, Hsieh M, et al. High-level allogeneic chimerism achieved by prenatal tolerance induction and postnatal nonmyeloablative bone marrow transplantation. *Blood* 2002;100: 2225–34.
10. Hayashi S, Abdulmalik O, Peranteau WH, et al. Mixed chimerism following in utero hematopoietic stem cell transplantation in murine models of hemoglobinopathy. *Exp Hematol* 2003;31: 176–84.