Liver Stem Cells: The Fall and Rise of Tissue Biology

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The first reports of marrow-derived hepatocytes are only a few years old,[1-4] and both information and controversy have since proliferated. Considerable experimental literature has been published (reviewed in Alison et al.[5]) as have a few human, largely confirmatory studies,[6-8] the newest of which, by Ng et al., appears in this issue of HEPATOLOGY.[9] The first studies focused on the most dramatic, dogma-bursting findings: marrow-derived hepatocytes and cholangiocytes. This recent study hints at broader developments, the focus not on marrow-derived hepatobiliary cells alone, but also on other cell compartments, in particular, monocytes and endothelium.

In part, the importance of a broader inquiry is an understanding that movement of cells from one organ to another is part of complex, orchestrated processes at a tissue level, rather than relying solely on cellular or molecular mechanisms. This is a reversal of a trend spanning centuries, now starting to show its age, of progressive, analytical dissection of organisms into smaller and smaller components. Surface observation of the body gave way to examination of the interior, then gross anatomy yielded to microanatomy and histology, then to cellular and, eventually, triumphantly, to molecular biology. With the mapping of the human genome, the successes of ever-finer dissection are clear.

However, a bias has risen where scientific data is best expressed as products of molecular analysis. A seldom discussed aspect of reports of *in vivo* cell plasticity is the reliance on photomicrographs as essential data. These images were not merely supporting players or attempts to secure a spot on the journal's cover (though I'll be the first to admit of the latter's success), they were the whole point. They showed transplanted, "transdifferentiated" cells engrafted structurally and functionally into tissue, pointing to a renewed focus on tissue biology, for which cellular and molecular biology would now be the supporting players.

Early criticisms of plasticity research were raised by cell and molecular biologists insisting on "proof" that pertained more to their own fields, not to phenomena at a tissue level. For example, early calls for demonstration of clonality, robustness, and functionality to prove *in vivo* plasticity[10,11] were applications of criteria important for examining cells in culture, but with less relevance to tissue biological processes. *Ex vivo* demonstration of plasticity required clonality and robustness of effects to rule out phenomena arising from low-level contaminant cell populations. Function is most important for *ex vivo* cell typing, because without tissue integration, markers alone are unreliable. But *in vivo*, at a tissular level, small numbers of cells may play roles that are important far beyond their number; functional and structural organization counts as much as quantity (if not more so); and integration into and maintenance of tissue structure is, in fact, a demonstration of function.

What is meant by "tissue biology?" Biological investigation of the interactions of diverse cellular compartments, arrayed in normal or pathologically altered, microanatomic juxtapositions, in 3 spatial dimensions and over time. As complicated as this sounds, it is not more than burgeoning studies in proteomics, for example. Indeed, the last few months have seen many exciting studies from investigators taking such a tissue-level approach. For example, stromal derived factor 1 (SDF-1), once thought to be produced only by marrow stromal cells as a homing mechanism for $CXCR4^+$, marrow-directed hematopoietic stem cells, was shown by Hatch et al. to be also produced by hepatobiliary cells in severely injured liver, perhaps as an attractant for circulating hepatic progenitors.[12] Kollet et al., in an extraordinarily broad, yet finely detailed study, have recently shown that interplay of SDF-1, matrix metalloproteinase 2 (MMP-2), MMP-9, and hepatocyte growth factor are responsible for recruiting $CD34^+$ circulating cells to the liver in response to injury.[13] Whereas SDF-1 is produced by the injured or regenerating hepatic epithelia, the other factors are produced by stellate cells.

Prior studies also showed that biliary cells in normal as well as reactive tissue are c-kit^+[14,15] and that stem cell factor (SCF), the ligand for c-kit, is produced by stellate cells.[16] Now, Simpson et al. have shown that SCF production rises after acute acetaminophen injury, where blocking with anti-SCF antibodies can lead to increased lethality.[17] Administration of SCF will not only rescue the

Abbreviations: SDF, stromal derived factor; MMP, matrix metalloproteinase; SCF, stem cell factor; FAH, fumarylacetoacetate.

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SCF-blocked animals, but also attenuate liver injury in normally lethal doses. These findings can, in part, be explained again by the interplay between stellate and hepatobiliary cells in the injured liver. Indeed, it seems that the proximal branches of the biliary tree are integrally paired with stellate cells to form a functional, and perhaps anatomical, "stem cell niche," as has been described in other organs.

Meanwhile, the laboratories of Tania Roskams have published a body of work showing the display of neural/neuroendocrine markers (including muscarinic receptors) on ductular-reactive hepatobiliary cells.[18-21] They have shown that vagotomy blunts ductular reactions[21] and, last month in HEPATOLOGY, in collaboration with the laboratory of Anna Mae Diehl, noted that epinephrine administration can increase progenitor cells and decrease toxic liver injury.[22] Thus the biliary tree and stellate cells of the liver are now further linked to nerves as part of a network of progenitor/stem cell activation.

Additional recent investigations show still more nuance at the tissue level. Hess et al. report reduced blood glucose following streptozotocin-induced diabetes correlating with early pancreatic endothelial engraftment from marrow, not with later β-cell engraftment.[23] The endothelial engraftment appears to potentiate regeneration of the native islets, perhaps superceding the "surprise" of marrow-derived beta cells.[24] Ng et al., in the current study,[9] see only rare endothelial engraftment in human livers. Is this a difference between humans and rodents, or between hepatic and pancreatic tissues? The key point: stem cell engraftment must not only be categorized and counted by cell type, but precise descriptions of temporal and spatial aspects of engraftment are also important.

This idea then highlights a significant problem for tissue biologists studying the liver: the poverty of our knowledge of hepatic microanatomy.[25] Here is a short list of important microanatomic details of which we are largely ignorant, but which are likely to play a role in liver stem cell recruitment and functioning:

- The components of basement membrane of the canals of Hering, a likely intrahepatic stem cell niche
- The relationship of sinusoids, capillaries, or arterioles to the canals of Hering and ductules
- The position of stellate cells in relation to these structures and whether such elements are different from other stellate cells
- The precise anatomic relationship of nerves to these structures, with detailed classification of neurotransmitters and the distribution of neurotransmitter receptors

What of the space of Disse and the lymphatics? What of Kupffer cells, endothelium, and fibroblasts? How might bile salts affect these structures, and is there an arterial-biliary countercurrent exchange mechanism? Our ignorance of microanatomy and the physiological interplay of all the components of the portal and periportal anatomic regions is profound. Garnering this detail is imperative for a full, rapid exploitation of the therapeutic potential of liver stem cells.

A tissue biological approach also sounds a cautionary note regarding overinterpretation of injury-specific experimental findings. A recent and influential example is the use of the fumarylacetoacetate (FAH)-null mouse, a model of tyrosinemia type I, to show the fusion pathway for marrow to hepatocyte plasticity.[26,27] The investigators of one of these studies concluded "that hepatocytes derived from bone marrow arise from cell fusion and not by differentiation of haematopoietic stem cells." However, other studies have shown that fusion does not always play a role in hematopoietic-to-epithelial cell engraftment,[5] but is likely to be one of several active mechanisms.[28]

The discrepancy is explained by a failure to consider the effects at a tissue level on cellular behavior. In the FAH-null mouse, the hepatic lobule is rapidly and widely disrupted, cellular components are unstable, nuclei become aneuploid with frequent carcinogenesis and vasculo-epithelial relationships are distorted or obliterated.[29] An "oval" or progenitor cell response appears to be absent in this model. Since there is now convincing evidence that marrow-derived hepatic progenitors enter the liver through site-specific, receptor-ligand–dependent mechanisms in lesser degrees of injury, it is perhaps not surprising that transdifferentiation events are less prominent in the FAH-null mouse model, in which the microanatomy and cellular integrity is so remarkably disrupted. One might explain the high levels of fusion in the model, instead, by trapping of circulating progenitors in the disrupted sinusoids[30] and mechanical compression of these cells against defective hepatocytes with membrane instabilities. However, despite the specificities of the model, overly general conclusions regarding the mechanisms of all plasticity phenomena were drawn. The moral: specificity of tissue effects of specific models demands careful interpretation without generalization.

This may also reflect on the lower rates of human hepatobiliary engraftment reported by Ng et al. as compared with other reports.[3,6,7] The differences may reflect more stringent detection in this study than in prior publications, but they may also reflect differences in severity and type of injury. Ng et al. report only low-level injury; the highest reported engraftment is in severely injured livers with exuberant ductular reactions.[3] Again, solutions to puzzling differences between studies may lie in what is happening at a tissue level where injury and regeneration

play themselves out with the full orchestra of biological mechanisms.

These are exciting times in hepatology. Given recent surprises, it is clear that we are very far indeed from a complete understanding of liver physiology, although progress accelerates. A richer understanding will be born from the synthesis of molecular, cellular, and tissular insights. Unlike Humpty-Dumpty, the fall of tissue biology was not truly a fall, but a measured descent into ever finer detail; in reassembling the pieces, we now stand at the threshold of exciting and productive endeavors.

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