

Human Hepatic Stem Cells

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Human hepatic stem cells (hHpSCs) have long been assumed to be hepatoblasts, cells identifiable by their signature features of α -fetoprotein (AFP) expression and by their bipotency, giving rise to both biliary and hepatocytic lineages. Cells expressing AFP are rare in normal adult livers (<0.01%) except in response to severe liver injury or disease. This has given rise to the dogma that adult livers do not have stem cells and that all regenerative responses are from mature parenchymal cells. We show that there are hHpSCs in livers of all donor ages and remaining at a relatively constant percentage, 0.5-2.5%, of the total parenchymal cell population. The higher percentages (above 1.5%) occur in livers subjected to ischemia that selectively kills the mature liver cells but not the progenitors. Viable hHpSCs can be isolated from cadaveric livers for a few hours after death in livers of most donors and for up to 8 hours after death from neonatal livers, comprised largely of progenitors (and therefore most of the cells being tolerant of ischemia). The hHpSCs *in vivo* are located in ductal plates in fetal and neonatal livers and in Canals of Hering in pediatric and adult livers, are $\sim 9 \mu\text{m}$ in diameter, and can be isolated by immunoselection for epithelial cell adhesion molecule (EpCAM) or neural cell adhesion molecule (NCAM). They express cytokeratins 8, 18 and 19, CD133/1, CD44H, claudin 3, Indian and Sonic hedgehog, and telomerase, weakly express albumin, and are negative for ICAM-1 and markers for adult liver cells (e.g. P450s), hemopoietic cells (e.g. CD34, CD45, CD38, glycophorin A), mesenchymal cells (VEGFr, desmin, α -smooth muscle actin, CD146, Von Willebrand factor) and, surprisingly, for alpha-fetoprotein (AFP). Under specific conditions, the hHpSCs give rise to hepatoblasts that overlap entirely with hHpSCs for their antigenic and biochemical profiles with several notable exceptions: hepatoblasts do not express NCAM and claudin 3 and express strongly ICAM1, P450A7 and AFP. The findings by immunohistochemistry and flow cytometry were confirmed by analyses utilizing quantitative RT-PCR and Western blots for 21 genes; the studies indicate that there are distinct phenotypes for hHpSCs, hepatoblasts, and adult hepatocytes. Wholly defined, serum-free conditions for *ex vivo* maintenance of hHpSCs (and for hepatoblasts) have been defined and that permit self-replication of hHpSCs through more than 150 population doublings at rates of a doubling every ~ 36 hours. Viability and expansion of the hHpSC colonies are dependent on paracrine signaling from "companion" cells found in close association with the colonies and comprised of hepatic stellate cell precursors and angioblasts. If transferred to STO feeders, hHpSCs slow in doubling times to greater than 2 weeks and give rise to differentiated cell outgrowths from the edges of the colonies. The outgrowths are hepatoblasts, recognizable by cord-like morphology interspersed by clear channels, presumptive biliary canaliculi, and by acquisition of a gene expression profile typical of hepatoblasts. Transplantation of freshly isolated EpCAM+ cells or of long-term colonies of hHpSCs into SCID/nod mice resulted in mature liver tissue with expression of human-specific proteins. The hHpSCs are candidates for liver cell therapies.

Representative Papers (of 22) on Identification and Regulation of Rodent Hepatic Progenitors:

- Sigal SH, et al. Characterization and enrichment of fetal rat hepatoblasts by immunoadsorption ("panning") and fluorescence activated cell sorting. *Hepatology* 19:999-1006, 1994.
- Sigal SH, et al. Demonstration of differentiation in hepatocyte progenitor cells using dipeptidyl peptidase IV deficient mutant rats. *Cell Mol Bio Res* 41:39-47, 1995.
- Sigal SH, et al Evidence of a terminal differentiation process in the rat liver. *Differentiation* 59:34-42, 1995.
- Sigal SH, Partial hepatectomy-induced polyploidy attenuates hepatocyte replication and activates cell aging events. *Am.J. Physiol.* 276 (Gastrointest.Liver Physiol.) 39:G1260-G1272, 1999.
- Kubota H and Reid LM. Clonogenic hepatoblasts, common precursors for hepatocytic and biliary lineages, are lacking classical major histocompatibility complex class I antigen. *PNAS USA* 97 (22):12132-12137, 2000
- Kubota H, Yao H, and Reid, LM. Identification and isolation of Vitamin A-containing Hepatic Stellate Cell precursors. *Stem Cells* (in press), 2007

Representative Papers on Identification and Regulation of Human Progenitors

- Kubota, H, Storm, R, and Reid LM. Variant forms of α -fetoprotein transcripts expressed in human hemopoietic progenitors. *J Biol Chem.* 277 (31): 27629-27635, 2002.
- Sicklick, J. et al. Hedgehog signaling in rodents and humans maintains resident hepatic progenitors throughout life. *Amer. J. Phys.* 290:G859-G870, 2005.
- Schmelzer E, Wauthier E, and Reid LM. Phenotype of human hepatic progenitors. *Stem cells.* 64: 1852-1858. 2006
- Turner et al & Reid LM. Human hepatoblasts maintained by hyaluronan hydrogels. *J. Biomedical Biomaterials Research* E-publication was in December, 2006.
- Schmelzer E et al & Reid, LM. Human Hepatic Stem Cells. *J. Experimental Medicine.* (in press), 2007
[A number (5) of other manuscripts have been submitted on our studies on human hepatic stem cell, but these are not yet in press]

Recent Reviews:

- Macdonald J, et al. & Reid LM. Bioartificial Livers. In: *Handbook on Encapsulated Cells and Bioartificial Organs.* pp 254-289. 1999
- R Susick, et al & Reid LM. Hepatic Progenitors and Strategies for Cell Therapies. *Ann.N.Y.Acad.Sci.* 944:398-401, 2001.
- Macdonald JM, et al. & Reid LM. Stem cells and liver lineage biology. In: *Methods for Tissue Engineering.* R. Lanza, editor. Academic Press, NY. 2002.
- Schmelzer E, et al. & Reid LM. Hepatic Stem Cells and the Liver's Maturation Lineages. In: *Tissue Stem Cells: Biology and Applications.* Edited by Chris Potten, R Clarke, J Wilson, and A. Renahan. Publisher: Taylor and Francis, New York. pp. 161-214, 2006
- Cheng N, Yao H, and Reid LM. Hepatic Stem Cells: Lineage Biology and Pluripotency: *Principles of Regenerative Medicine.* A Attala editor Elsevier Press, NY. (In Press) 2007
- McClelland R and Reid LM. Bioartificial livers. In: *Principles of Regenerative Medicine.*,A. Attala, editor. Elsevier Press, NY. (In press) 2007
- Schmelzer E and Reid LM. EpCAM Expression in Normal, Non-pathological Tissues. In: *Frontiers in Biosciences.* (In press) 2007

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