# Purification and Spatial-Temporal Analysis of Hepatic and Hematopoietic Stem Cell Niches That Develop Into Structural Proliferative Units in the Mammalian Liver

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Early organogenesis implies the assembly and gain of function of primordial stem cells at patterning. We have postulated and show here that distinct stem cells and niche-forming cells arise and develop together as organ-specific, structural-proliferative units in mouse and human liver.

First, a vasculohemogenic, pulsatil meshwork was identified around nascent hepatic cords at day 8.75-9.5 of gestation (E8.75-E9.5) in mouse liver explant cultures. Colony assays mapped hepatic stem cells and primitive hemogenic forerunners, clearly two days before definitive hemopoietic stem cells (day35 LTC-IC/CAFC assay) colonised the liver from E11.5. Organogenic competence required intimate association of mesodermal (desmin/GFAP$^+$), hepato-cholangiogenic (CK19$^+$, c-met$^+$/CD49f$^+$, connexin-43$^+$), and hematopoietic stem/progenitor cells into compact, node-like niches. Homologous stem cell niches were identified, purified, and characterized in human embryonic and fetal liver.

Video microscopy revealed that purified, single niches developed autonomously into complex hepatic units, and at low seeding density they evolved an interactive, contractil network. The organization and integrity of self-bounded units was maintained synergistically by distinct cell lineages emitting stimulatory and/or veto morphogen signaling factors (HGF, SHH, BMP4, SDF-1, and ephrinB2/EphB4). Unexpectedly, inhibition of SHH signaling by cyclopamine upregulated EphB4/ephrinB2 expression, increased vascular sprouting, hematopoiesis, and abrogated net border formation around hepatic lobules and subunits.

Thus, in addition to its inductive effect, the cardiac mesoderm plays guiding and constitutive roles and complements the emerging hepatic cords with a vasculogenic stroma at the prospective Glisson's capsule. The results show for the first time that aggregation of hepatic, hematopoietic, and mesangiogenic stem cells into individual niches is an absolute requirement of development and numerical amplification of organotypic proliferative units in the mammalian liver.

**References**

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