

STEM CELLS

Blood matters

Tools to study hematopoietic stem cell development, engraftment and interaction with the niche.

Hematopoietic stem cells are the longest-studied stem cells and have been deployed for decades in many therapeutic contexts. Nevertheless, there remain significant lacunae in our understanding of even fundamental aspects of their biology. Two recent studies present tools to further the study of these fascinating cells.

At Harvard Medical School, George Daley and colleagues took on the mammoth task of transcriptionally profiling cells in the mouse embryo undergoing development into definitive hematopoietic stem and progenitor cells (HSPCs), a project that involved the isolation of cells from thousands of staged mouse embryos. The investment of time and resources was justified, explain Shannon McKinney-Freeman—now at St. Jude Children's Hospital in Tennessee—Patrick Cahan and Hu Li, first authors on the paper, because they had determined that a deeper molecular understanding of HSPC development is the only way to solve current problems in deriving fully functional HSPCs from pluripotent stem cells.

The researchers isolated embryonic cell populations from tissues where hematopoietic development is known to occur: the aorta-gonad-mesonephros region, yolk sac, fetal liver and placenta. The markers they used for isolation are known to enrich for functional cell populations (that is, cells able to repopulate the bone marrow after transplant). In parallel, they profiled HSPCs from adult mouse bone marrow as well as HSPCs derived using the best-performing current protocols for differentiation from embryonic stem cells (ESCs).

The resulting expression profiles could be used to cluster the cells into three groups that roughly follow the temporal trajectory of development. The transcriptional profiles of ESC-derived HSPCs resembled more closely those of definitive bone marrow HSPCs

rather than those of more primitive progenitors in the yolk sac as had been suggested by previous studies.

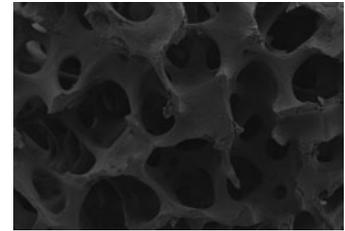
Nevertheless, some differences between bone marrow-derived and ESC-derived HSPCs are visible in the expression profiles: for instance, the latter cells appear to be defective in response to Notch activation. Continued analysis by this and other groups—the data set is freely available to other researchers—will help develop better strategies to make fully functional HSPCs from pluripotent stem cells.

Ontogeny is not the only aspect of HSPC biology that is incompletely understood. The interactions of HSPCs with their niche(s) in the bone marrow as well as their homing and long-term engraftment there—processes that are also key for effective bone marrow transplantation and immunotherapy—are under intense study. Though there are now a few reports of direct *in vivo* imaging of HSPCs, this is typically carried out on the calvarium in the skull. It remains difficult to watch these cells where they (mostly) live.

In part to address this problem, Biju Parekkadan and colleagues, also at Harvard Medical School but working independently, designed a biomaterial scaffold to mimic the structure of bone marrow. If it were able to attract and retain viable hematopoietic stem cells when implanted *in vivo*, they reasoned, the artificial niche could provide a powerful way to study cues important for HSPC viability, engraftment and function.

Parekkadan and colleagues began with hydrogel scaffolds with repeating units of cavities and junctions, such as are found in the porous structure of bone marrow. They coated the scaffolds with collagen and seeded them with stromal cells derived from human bone marrow (BMSCs). They found that BMSCs cultured in the three-dimensional scaffold environment had more potent anti-inflammatory effects than equivalent cells cultured in two dimensions.

But what of the ability to attract and



The structure of trabecular bone marrow, in which hematopoietic stem cells are found. Image courtesy of B. Parekkadan.

nurture HSPCs? To study this, Parekkadan and colleagues implanted the humanized scaffolds subcutaneously in immunocompromised mice (further exploiting this microscopically accessible location by engineering in a dorsal imaging window). They saw that the scaffolds became vascularized, with endothelial cells appearing to take on a sinusoidal phenotype typical of bone marrow.

Four weeks after implant, Parekkadan and colleagues report, endogenous mouse bone marrow progenitor cells could be detected in the scaffolds. Furthermore, directly injected human HSPCs were retained in the scaffold for at least 16 weeks, whereas systemically delivered normal and leukemic cells could be imaged extravasating into the implanted niche a few hours after tail-vein injection. The researchers envision the biomimetic scaffold as a powerful humanized model for *in situ* imaging studies of blood stem cell fate.

Perhaps the day will come when the fruits of such resources are brought together: when an implantable humanized niche is used to study the engraftment of human HSPCs derived from pluripotent stem cells *in vivo*.

Natalie de Souza

RESEARCH PAPERS

McKinney-Freeman, S. *et al.* The transcriptional landscape of hematopoietic stem cell ontogeny. *Cell Stem Cell* **11**, 701–714 (2012).

Lee, J. *et al.* Implantable microenvironments to attract hematopoietic stem/cancer cells. *Proc. Natl. Acad. Sci. USA* **109**, 19638–19643 (2012).