## Modeling the marrow stem cell niche *in vitro*: is proximity the key to reproduction?

In the marrow microenvironment, the proliferation and development of hematopoietic stem cells (HSC) and their progeny takes place in close association with mesenchymal stromal cells (MSC).[1] MSC are an integral part of the marrow microenvironment, providing HSC with attachment sites and secreted or surface-bound growth factors, and thereby regulating hematopoiesis. In a recent issue of Haematologica, Jing *et al*. described *in vitro* interactions between mobilized peripheral blood CD34$^+$ HSC and MSC.[2] The authors described a co-culture system of HSC and MSC, and investigated cell cycle, proliferation, cell division, and immunophenotype of the CD34$^+$ cells over a period of seven days. The authors demonstrate migration of a fraction of the HSC beneath and underneath the MSC in a CXCR4- and integrin-dependent fashion. Based on this *in vitro* migration, they distinguish three different compartments with a differential impact on the fate of HSC, especially on their proliferation and differentiation. More immature HSCs were found beneath and underneath the stromal cell layer, associated with reduced cell proliferation. In contrast, HSC attached to the stromal cell layer or in the supernatant surface displayed a more mature immunophenotype, higher proportions of cells in the G2/M-phase of the cell cycle, and higher rates of cell division. Therefore, Jing *et al*. proposed that the compartment of HSC beneath the MSC represents an *in vitro* equivalent to marrow niches which contain non-cycling, immature HSC *in vivo*, suggesting that close proximity between HSC and MSC favors quiescence and not reproduction.

In 2003, we published a study in the British Journal of Haematology in which we came to some similar, but also some opposite conclusions.[3] First, we established kinetics and mechanism of migration of mobilized peripheral blood CD34$^+$ and cord blood-derived CD34$^+$ HSC beneath MSC, an *in vitro* phenomenon previously termed "pseudoemperipolesis"[4] or cobble stone area-forming cells (CAFC), which are cell clusters within stroma layers that have a cobblestone-like appearance in phase contrast microscopy. We found that cord blood (CB) CD34$^+$ cells had a higher capacity for pseudoemperipolesis than granulocyte-colony-stimulating factor (G-CSF)-mobilized blood (MB) CD34$^+$ cells or acute myeloid leukemia (AML) cells. Also, we defined that this HSC migration beneath MSC was dependent on CXCR4 chemokine receptors and VLA-4 integrins ($\alpha 4\beta 1$ CD49d), a finding now confirmed by Jing *et al.*

Because Jing *et al*. did not mention the literature related to "pseudoemperipolesis" or our study, we think it is worthwhile to point out the experimental differences. In contrast to Jing *et al.*, we found cell cycle progression and significantly increased numbers of cell divisions in PB CD34$^+$ cells in the fraction of cells that had migrated beneath the MSC, but not in the supernatant/adherent fraction (which we collected as one fraction). Our findings appear to be contradictory to those of Jing *et al*. and earlier studies that demonstrated maintenance of primitive hematopoietic cells in a quiescent state within adherent stromal cell layers in the long-term culture (LTC) system.[5,6] We believe that these differences are, at least in part, due to differences in the culture systems, and specifically due to differences in the stromal layers used. We used M2-10B4 cells,[7] a murine hematopoiesis-supporting MSC line, whereas Jing *et al.* used primary MSC from marrow aspirates from volunteers. The photographs displayed in Figure 1 in the paper of Jing *et al*. show a spindle-shaped MSC phenotype, in contrast to the more fried egg-shaped M2-10B4 cells (Figure 1A in our paper). We can assume that different MSC, dependent, for example, upon differentiation and passage number, provide different levels of cytokines and hence different degrees of stimulation of HSC. These differences may, at least in part, explain the differences seen in these two studies. We have recently compared murine and human MSC, both MSC cell lines and primary stromal cells, and standardized conditions to test their capacity to support and protect leukemia cells from cytotoxic drugs.[8] In those systems, we found high levels of stroma-mediated protection with both human and murine MSC, and highly reproducible results in four different laboratories. It could be worthwhile to use a similar approach with side-by-side co-cultures of HSC with different types of MSC at defined ratios to address the question of HSC cycling and differentiation in different HSC fractions (supernatant, adherent, beneath MSC). Such experiments would be a next step to establish a reliable and reproducible *in vitro* model to study the impact of MSC and the marrow niche on hematopoietic progenitors.

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