An anaerobic home for the stem cell proteome

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The paper by Unwin and colleagues in this issue of Blood gives us a first glimpse into the stem cell proteome that points to a key role of anaerobic metabolism and oxidative protection in the maintenance of the stem cell state.

Extensive transcriptional profiling of hematopoietic stem cells (HSCs) has yielded a variety of molecular signatures that are as different as they are similar. Despite all this transcriptional profiling, there have been relatively few insights into how a stem cell really maintains its stem cell nature. Unwin and colleagues used a sophisticated mass spectrometric method with isobaric covalent modification of peptides for relative quantification (iTRAQ) that allowed the relative quantification of hundreds of proteins in 4 samples simultaneously. Cell lysates were prepared from lineage-negative, Sca-1-positive, cKit-positive (LSK +), and LSK-negative (LSK −) cells. LSK + cells are substantially enriched for long-term repopulating stem cells compared with their LSK − counterparts. Individual lysates were proteolytically digested, and the resulting peptides were labeled with one of 4 isobaric isotope-coded tags. After all 4 samples were mixed, the peptides were separated by extensive 2-dimensional liquid chromatography online to a mass spectrometer. These analyses were repeated 3 times using about $1 \times 10^6$ LSK + and LSK − cells for each labeling. Microarray analyses were also performed in triplicate with similarly isolated cells. Careful statistical analyses yielded 145 differentially expressed proteins between LSK + and LSK − cells from a total of 668 relatively quantitated proteins. Comparative analyses of the differentially expressed proteins with the microarray data revealed that less than half (45.3%) showed the same trend in expression, while the remaining 55% showed no change at the transcriptome level. These data reveal that posttranslational controls are important regulators of stem cell properties. A coupling of the 2 methodologies is suggested in order to achieve a complete stem cell profile in systems biology. Of course, these benchmarks will be difficult to achieve with more highly purified, rare stem cell populations than with LSK + cells, because the cell numbers are just too limiting. Nevertheless, given the rapid advances in these methodologies, it is highly likely that this goal will be achieved in the future. Perhaps the most interesting aspect of these data is that the HSC proteome is well adapted to the relatively hypoxic environment of the endosteum, the proposed stem cell niche. The most highly represented metabolic pathway was glycolysis; 8 out of 11 enzymes in the pathway were more highly expressed in LSK + cells. In particular, anaerobic glycolysis was indicated, as LSK + cells showed a greater production of lactate. Coupled with the preponderance of glycolytic enzymes were proteins involved in protection against DNA damage from oxidative stress. These data support the observation that stem cells lose their self-renewal ability if they cannot overcome oxidative damage. The emerging picture of the HSC proteome forces us to ponder a broader role of the metabolome in the maintenance of the stem cell state. It also suggests an additional feature of the stem cell niche, as a sheltering environment that provides protection from damaging systemic stimuli.

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induce AML by itself, requiring a second event. In mice, the use of a transduced WT1 transgene into transplanted BM cells with AML1-ETO induces AML. The speculation that this is primarily related to drug resistance, because chemotherapy agents induce apoptosis in susceptible cells, may be true; however, we will have to ask why that is the case, since this particular gene is also expressed in many other malignancies, including lymphoid malignancies, which are quite sensitive to chemotherapeutic agents.

The authors of this paper have very elegantly demonstrated in a murine cell line that the WT1 gene suppresses the expression of cyclin E, and that they have identified that the A1 gene (BFL1 in humans) in the family of bcl-2 is implicated in differentiation and resistance to apoptosis in hematopoietic cells. If WT1 does indeed regulate different bcl-2 family members, causing different effects depending on the cell line being investigated, the function of A1 might be related to differentiation as well as to apoptosis. The multiple reports that correlate high levels of expression of WT1 in AML with poor prognoses may suggest that there is a relationship between drug resistance and the regulation of WT1 to the bcl-2 family members such as A1.

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HEMOSTASIS

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ICAM-2 and PECAM-1: 2 steps in leukocyte transmigration

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In this issue of Blood, Huang and colleagues show that ICAM-2 mediates the transendothelial migration of neutrophils in vivo, that it can function independently of PECAM-1, and that ICAM-2, like PECAM-1, participates in the diapedesis process in a cytokine-specific manner.

Diapedesis describes the movement of leukocytes through the intact blood vessel wall. For more than 15 years adhesion molecules and chemotactic factors that allow leukocytes to interact with the endothelium at sites of leukocyte exit have been systematically identified and analyzed. Thus, the adhesion and signaling cascade that controls leukocyte capturing is well understood. Yet, the molecular mechanism by which leukocytes actually overcome the barrier of the blood vessel wall is largely unknown.

Two completely different routes have been described. Leukocytes move through endothelial junctions between neighboring cells, and they can use an alternative transcellular route through endothelial cells. Although in vitro and in vivo evidence for the latter pathway exist, the transcellular route seems to be used with low efficiency. Whatever relevance it has in vivo, it is remarkable that more and more endothelial membrane proteins that are involved in this process turn out to be located at endothelial cell contacts. Platelet endothelial cell adhesion molecule-1 (PECAM-1) was the first such protein shown to mediate neutrophil transmigration in vitro and in vivo.

In this issue of Blood, Huang and colleagues use intravital microscopy to show that intercellular adhesion molecule-2 (ICAM-2) is a second membrane protein at endothelial cell contacts that mediates transmigration of neutrophils in vivo (see figure). While a role in the transmigration process had been implicated before from in vitro studies, the current paper is the first that analyzes this directly in vivo and that compares 2 membrane proteins, ICAM-2 and PECAM-1, in this process in vivo. The authors found that a monoclonal antibody (mAb) against ICAM-2 strongly inhibited neutrophil recruitment into inflamed peritoneum beyond the inhibitory effect found in PECAM-1–deficient mice, suggesting that both proteins act at different steps in the diapedesis process. Interestingly, blocking ICAM-2 reduced neutrophil extravasation in the peritonitis as well as in the cremaster model to the same low levels in wild-type (wt) and in PECAM-1–deficient mice. Thus, PECAM-1 had no role in ICAM-2–independent leukocyte transmigration. Together, these results suggest that ICAM-2 and PECAM-1 act in the same molecular pathway, possibly in sequential steps.

In addition, the authors found that ICAM-2 is only relevant for leukocyte recruitment if the
Why target the *WT1* gene?

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