Vascular endothelial growth factor (VEGF), its receptors (VEGFR-1, VEGFR-2) and neuropilin-1 (NRP-1) are expressed at variable levels in bone marrow. NRP-1 expression is higher in fatty bone marrow than in hematopoietic marrow. Adipocytes are responsible for NRP-1 expression suggesting that they may play a role in hematopoiesis by producing NRP-1 or that NRP-1 may regulate adipocyte activity.

Recent evidence suggests that vascular endothelial growth factor (VEGF) is involved in hematopoiesis and in the pathogenesis of hematopoietic malignancies. Neurophilin-1 (NRP1) was recently recognized as an isoform-specific receptor for VEGF165 acting as coreceptor for the VEGFR-2. Our primary goal was to evaluate the expression of NRP-1 in human hematopoietic marrow and to identify cells responsible for its expression in vivo. Key partners of NRP-1 such as VEGF, VEGFR-1 and VEGFR-2 were also studied. Samples from iliac bone (rich in hematopoietic marrow), and from femurs (mostly composed of fatty cells) were from 18 human bone marrow core samples obtained from autopsies performed at the Department of Pathology and from 8 human core biopsies obtained from patients undergoing hip surgery at the Department of Orthopedic Surgery. The total RNA was purified by centrifugation on a cesium chloride cushion and from cell suspensions using the High Pure RNA Isolation kit (Roche Diagnostics, Mannheim, Germany). Reverse transcription-polymerase chain reaction (RT-PCR) was performed under non-competitive conditions in the presence of a synthetic RNA used as internal standard in order to make the procedure quantitative. To evaluate a possible correlation between the hematopoietic activity and the mRNA level of the VEGF isoforms and their receptors, total RNA was isolated from femoral fatty bone marrow and from iliac crest hematopoietic bone marrow collected from the same donor and RT-PCR was performed as above. The hematopoietic activity was evaluated by calculating the relative percentages of the tissue hematopoietic cells versus adipose cells. In all tested specimens, the main isoforms found were VEGF165 and VEGF121 expressed at variable levels. This pattern of expression seems to be specific to bone marrow and is in agreement with the data reporting the RT-PCR analysis of the VEGF121 and VEGF165 in a series of human hematopoietic cell lines. A significant expression of the 189 isoform was observed in femoral bone marrow whereas it was barely detectable in iliac bone marrow (Figure 1A). In most of samples of femoral bone marrow, the total VEGF mRNA was higher than in iliac crest bone marrow.
this could be related to the predominance of adipocytes in the femoral samples. Indeed, it has been shown that VEGF mRNA is upregulated during the conversion of 3T3 preadipocytes to adipocytes. The VEGFR-1, VEGFR-2 and NRP-1 were measured in the same series of samples. VEGFR-1 mRNA levels were quite variable from case to case. VEGFR-1 mRNA was either absent in iliac crest and femoral bone marrow or expressed at the same level in both tissues or expressed only in femoral bone marrow or was expressed at higher level in femoral bone marrow than in iliac crest bone marrow (Figure 1B). Values for VEGFR-2 were available in five samples only; there was no significant difference between femoral and iliac crest marrow (Figure 1C).

VEGFR-2 is essential for the development of hematopoietic stem cells during early embryonic development, it may be redundant in adult bone marrows. Since activation of VEGFR-1 is fully sufficient to rescue hematopoietic stem cell survival in vitro and hematopoietic repopulation in vivo, the presence of VEGFR-2 may be related to the maintenance of bone marrow vasculature. NRP-1 was expressed at higher level in femoral bone marrow than in iliac crest in each donor (Figure 1D) and it seems to be inversely correlated with the hematopoietic activity. The cellular origin of NRP-1 was assessed on isolated cell populations by a floatation/sedimentation procedure. By contrast to sedimented cells (hematopoietic and stromal cells), high levels of NRP-1 mRNA were detected in the adipocytic population (Figure 2A). This was confirmed by in situ hybridization (not shown) and at the protein level by immunohistochemistry (Figure 2B). This is the first report demonstrating neuropilin-1 expression in bone marrow in vivo. The capacity of adipocytes to produce NRP-1, previously suspected to play an interactive role with hematopoietic cells1 suggests that adipocytes may contribute to the regulation of hematopoiesis and/or that NRP-1 may be a novel regulator of adipocyte activity in the bone marrow, possibly as a receptor for VEGF. Although this study does not provide a definitive link between NRP-1, adipocyte function and hematopoiesis, such a relationship may exist and deserves further studies.

Zakia Belaid,* Frederique Hubin,* Chantal Humbert,* Jacques Boniver,* Betty Nusgens,* Marie-Paule Defesne*

*Laboratoire d’Histologie-Cytologie; 
Laboratoire d’Anatomie et Cytologie Pathologiques; 
Laboratoire de Biologie des Tissus Conjonctifs, Tour de Pathologie B23, Université de Liège, Sart Tilman, Liège, Belgium

Acknowledgements: we thank Colige CA, Lambert CA and Munauf C for expert technical assistance. We also thank Prof. P. Gillet and Dr. A. Rodriguez for providing bone marrow biopsies. We also thank Prof. Kolodkine from John Hopkins University, Baltimore USA, for providing us the anti-neuropilin-1 antibody. Funding: this work was supported by the “Belgian Federation Against Cancer”, non-profit organization and the National Foundation for Scientific Research, Belgium. Zakia Belaid and Frederique Hubin are Télèvèe Fellows granted from the National Foundation for Scientific Research. Correspondence: Zakia Belaidz, Laboratoire d’Histologie-Cytologie, Tour de Pathologie B23, Université de Liège, Sart Tilman, B-4000 Liège, Belgium. Phone: international +32.4.366.3403. Fax: international +32.4.366.2419. E-mail: belaid@ulg.ac.be or defesne@ulg.ac.be

References