[Frontiers in Bioscience 19, 171-180, January 1, 2014]

Bone marrow stromal cells in the pathogenesis of acute myeloid leukemia

Olga Blau[1]

[1]*Charite University School of Medicine, Labor Berlin, Department of Hematology and Oncology, Berlin, Germany*

TABLE OF CONTENTS

1. ABSTRACT

Acute myeloid leukemia (AML) is clonal disorder affecting pluripotent stem cell and characterized by ineffective hematopoiesis. Genetic abnormalities in a progenitor cells is thought to lead to uncontrolled growth of leukemia cells. In addition, in the last years, it has been clearly recognized that the hematopoietic microenvironment (HM) plays an important role in the pathogenesis of AML. The HM can regulate hematopoiesis by interacting directly with HC and/or by secreting regulatory molecules that exert a positive or negative influence on the growth of HC. Stromal elements are important in the homing of immature HC or hematopoietic stem cells. Several studies propose that important quantitative and functional alterations are present in the BMSC of AML patients. AML may arise in the setting of an abnormal HM, resulting in the generation of multiple populations with varying initiation event. Dysfunction of HM may contribute to leukemia by supplying abundant growth factors that promote proliferation and/or inhibit apoptosis. Recent discoveries utilizing mouse models showed that genetic alteration in cells of HM can induce AML.

2. INTRODUCTION

Acute myeloid leukemia (AML) arises from a series of genetic abnormalities in a stem or progenitor cell that lead to uncontrolled growth. It is have been demonstrated that HM have specific roles in regulation of hematopoiesis including hematopoietic stem cells (HSC). The hematopoietic microenvironment (HM) consists of a complex structure of both non-hematopoietic and hematopoietic cells, extracellular matrix as well as soluble and membrane bound factors that cooperate to support normal hematopoiesis. HM encompass a variety of cell types, including osteoblasts, osteoclasts, endothelial cells, perivascular reticular cells, and mesenchymal stromal cells, all of which are critical for the regulation of HSC maintenance and localization (1-5).

The key component of the HM is bone marrow stromal cells (BMSC). Data from the past few decades have implicated the HM in the pathogenesis of hematologic malignancies (6). Functional relationship between leukemic cells and the bone marrow (BM) microenvironment is a distinct feature of AML. Several studies have provided evidence suggesting that proliferation, survival, and drug resistance of AML can be modulated by BMSC within the

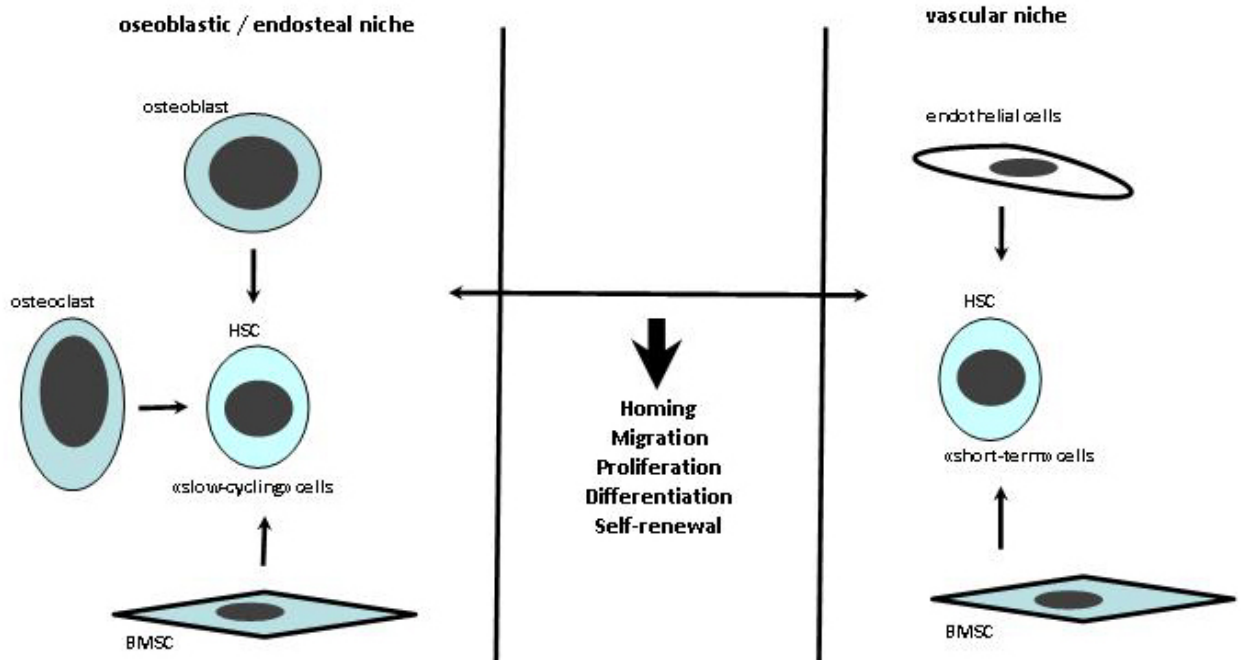

Figure 1. Mechanism of normal stem cell interaction within the niches. The normal hematopoietic stem cells (HSC) reside in the both niches. In the osteoblastic niche, osteoblasts, osteoclasts, and bone marrow stromal cells (BMSC) provide a microenvironment for HSC. In the vascular niche BMSC facilitate migration, homing, proliferation, and differentiation of HSC. Both niches work together. Coordination between the osteoblastic and vascular niches regulates HSC selfrenewal, proliferation, differentiation and mobilization in and out of the BM.

BM microenvironment (7). Direct contact between AML cells and BMSC triggers a pleiotropic spectrum of proliferative and/or anti-apoptotic signalling pathways (8). Therefore, in addition to therapies that directly target AML, interruption of leukemia cell and BMSC interactions should be considered when designing anti-AML therapeutic strategies.

3. THE ROLES OF BONE MARROW MICROENVIRONMENT IN NORMAL HEMATOPOIESIS

It was known as early as the 1960s, based on experiments on mice, that normal hematopoiesis could not occur without a supportive environment (9). *In vitro* studies of the HM over the last several decades have mostly relied on the long-term marrow culture system, first reported by Dexter (10). HSCs development is tightly regulated by microenvironment through production of cytokines, chemokines, and intracellular signals initiated by cellular adhesion.

3.1. Bone marrow niches

HSCs live in a highly specialized complex microenvironment, also known as a niche (11,12). Two distinct microenvironmental niches defined: “osteoblastic (endosteal)” and “vascular” niches (Figure 1) (13). The osteoblastic niche is localized at the inner surface of the bone cavity and provides a microenvironment for long term HSC which are capable of contributing to hematopoiesis as quiescent or slow-cycling cells (1,2,13). The vascular niche consists of sinusoidal endothelial cells lining blood vessels and promotes proliferation and differentiation of actively cycling, short term HSC (14,15). Recent studies suggest that these niches work together. Coordination between the osteoblastic and vascular niches regulates HSC selfrenewal, proliferation, differentiation and mobilization in and out of the BM. HSCs leave the osteoblastic niche, mobilize to the vascular niche, and enter the blood vessel. They subsequently may undergo transendothelial migration from the peripheral circulation and return first to the vascular niche and then to the osteoblastic niche (16,17). Within the niche, there are critical bidirectional signals that ensure the regulation of normal HSCs (1) and maintenance of the quiescent long term HSC pool (18). The quiescent fraction of immunophenotypically defined HSCs has been previously demonstrated to correlate with long term repopulating ability of BM (14) and loss of this fraction is associated with inability to sustain serial transplantation, the most stringent *in vivo* assay of self-renewal (18).

3.2. Bone marrow stromal cells

The BMSC are currently described as mesenchymal stem cells are termed multipotent mesenchymal stromal cells, while the term mesenchymal stem cell should be reserved for a subset of these cells that demonstrate stem cell activity by clearly stated criteria (19). BMSC are primitive cells originating from the mesodermal germ layer and were classically described to give rise to connective tissues, skeletal muscle cells, and cells of the vascular system. Friedenstein and colleagues (20) first described BMSC as fibroblast-like cells that could

be isolated from BM via inherent adherence to plastic in culture. He defined a population of cells as multipotential stromal precursor cells that were spindle-shaped and clonogenic in culture conditions, defining them as colony-forming unit fibroblasts. BMSC, in the traditional view, should refer to stem cells that are also capable of producing blood cells; however, blood cells are actually derived from a distinct cell population called the hematopoietic stem cells. This allows classified BMSC as nonhematopoietic, multipotential stem cells that are capable of differentiating into mesenchymal and non-mesenchymal cell lineages (21). These cells were able to differentiate into adipocytes, chondrocytes, osteocytes, and myoblasts, both *in vitro* and *in vivo*. In addition, it has also been demonstrated that MSCs are capable of differentiating into cardiomyocytes, neurons, and astrocytes *in vitro* and *in vivo* (22-25). By generating functionally distinct cell types and structures, BMSC play a crucial role in supporting hematopoiesis as key components of the HM (4).

Phenotypically BMSC express a number of markers, none of which are specific only to MSC. It is generally agreed that adult human BMSC do not express the hematopoietic markers CD45, CD34, CD14, or CD11. They also do not express the costimulatory molecules CD80, CD86, or CD40 or the adhesion molecules CD31, CD18, or CD56, but they can express CD105 (SH2), CD73 (SH3/4), CD44, CD90 (Thy-1), CD71, and Stro-1 as well as the adhesion molecules CD106, CD166, intercellular adhesion molecule, and CD29 (21,26). Although there are no unique cell surface markers for the identification of BMSC, minimal criteria to define human BMSC have been published. According to such criteria, BMSC must be plastic-adherent; and have to express CD105, CD90 and CD73; they must lack expression of CD45, CD34 and CD14; and they must show *in vitro* differentiation capabilities into osteoblasts, adipocytes and chondroblasts (19,21). This *in vitro* system has allowed for the dissection of the components of the microenvironment and the study of the complex contact dependent and contact independent interactions that occur between the stromal compartment and hematopoietic stem cells that regulate stem cell fate decisions.

3.3. Interaction between HSCs and HM

Normal hematopoiesis requires complex bidirectional interactions between the HM and HSCs. The HM can regulate hematopoiesis by interacting directly with HC and/or by secreting regulatory molecules that exert a positive or negative influence on the growth of HC. These interactions influence HSC self-renewal. HM controls the formation of blood cells through the production and secretion of cytokines, chemokines, and intracellular signals initiated by cellular adhesion (12). Chemokines are a large superfamily of small glycoproteins that are required in a various series of biological processes, including leukocyte trafficking, hematopoiesis, angiogenesis, and organogenesis. BMSC have the ability to migrate into tissues from the circulation, possibly in response to signals that are upregulated under injury conditions. Although the mechanisms by which BMSC are recruited to tissues and cross the endothelial cell layer are not yet fully understood, it is probable that chemokines and their receptors are involved, as they are important factors known to control cell migration (21).

Receptors including VLA- (very late antigen-) 4, CXCR4, and CD44, play a critical role in normal stem cell homing. VLA4 is the $\alpha 4\beta 1$ integrin that mediates adhesion to alternatively spliced fibronectin and cellular vascular cell adhesion molecule-1 (VCAM1). CXCR4 is a chemokine receptor for stromal derived factor-1 (SDF-1) also known as CXCL12. CD44 is a hyaluronic acid receptor that is an E selectin ligand expressed by hematopoietic stem cells known as HCELL when properly glycosylated (27).

CXCL12/stromal cell-derived factor-1alpha (SDF-1α) and its receptor CXCR4 are involved in homing of HSC into BM (5, 28-30). Perivascular reticular cells secrete much higher levels of CXCL12 than other constitutive sources of CXCL12, such as osteoblasts, fibroblasts, and endothelial cells (3). These reticular cells, defined as CXCL12-abundant reticular cells, may serve as a transit pathway for shuttling HSC between the osteoblastic and vascular niches, where essential but different maintenance signals are provided (13).

The molecular interactions between HC and BMSC involve ligand-receptor relationship between adhesion molecules on the surface of HC and stromal cells or between such molecules on the cells surface with specific domains within certain extracellular matrix molecules. BM engraftment involves subsequent cell-to-cell interactions through the BMSC-produced complex extracellular matrix (ECM) (31,32). Vascular cell-adhesion molecule-1 (VCAM-1) or fibronectin is critical for adhesion to the BMSC (33,34). One very important type of interaction between the BMSC and the HSC is the synthesis and presentation by BMSC of hematopoietic growth factors. Interactions of HSC with stromal elements of BM play a role in the egress of mature blood cells from the BM (21).

4. BMSC ALTERATIONS IN AML

Several studies have provided evidence suggesting that proliferation, survival, and drug resistance of AML can be modulated by BMSC within the BM microenvironment (7,12). There is substantial evidence suggesting that leukemia stem cells (LSC) interaction with the BM niche is essential for leukemia survival, resistance to treatment and disease progression. Macanas-Pirard *et al* (35) suggest that the BMSC protect APL cells from tharapy-induced apoptosis both *in vitro* and *in vivo* and that this effect is mediated by soluble factors released by the BMSC (Figure 2). Recently, Zeng *et al* (8) using a mouse model showed, that multiple survival signalling pathways, were up-regulated in primary AML cells cocultured with BMSC.

There is emerging evidence that extrinsic components mediated by the microenvironment play a pivotal role in survival and drug resistance of LSC. It is believed that environment-mediated drug resistance is a

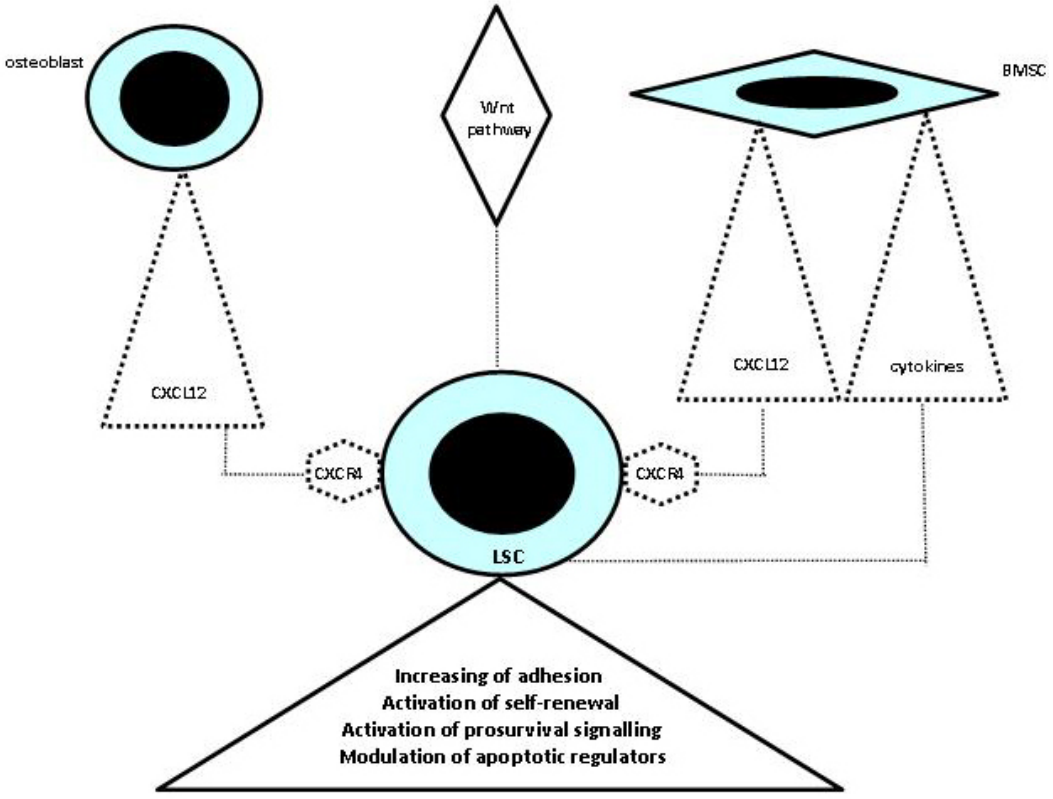

Figure 2. Mechanism of leukemic stem cell (LSC) interaction within the niches. Osteoblasts provide a source of stromal cell derived factor CXCL12 (SDF1a), which induce the migration of CXCR4-expressing LSC towards the osteoblastic niche. The interaction between CXCL12 and its receptor CXCL4 on the LSC contributes to their homing. Bone marrow stromal cells (BMSC) also secreted CXCL12, as well as cytokines, which induce cell proliferation, activate prosurvival signaling cascades, and modulate the expression of the antiapoptotic molecules. Activation of the self-renewal pathway (Wnt) may result tin enhanced LSC survival and may be mediated through the niche (18).

transient state whereby LSCs are protected through signals from the niche, which eventually leads to the selection of secondary genetic changes and outgrowth of cells that acquired multiple mechanisms of pharmacologic resistance (36).

4.1. Leukemic microenvironment

The molecular mechanisms for maintaining quiescence of normal stem cells may also facilitate LSC survival. Whereas LSC share certain features of self-renewal and differentiation with HSC, LSC differ in their deregulated proliferation and ability to invade and spread. LSC exhibit the capacity for long-term self-renewal (37-39) within the BM microenvironment, which is required for maintenance of the malignant clone (40). LSCs are able to generate leukemic blasts, and the leukemic clone is organized as a hierarchy (2). LSC may steal the homeostatic mechanisms, take refuge within the HM during chemotherapy, and consequently contribute to eventual disease relapse (39,40). Consecutively, LSC are believed to arise through transforming events targeting HSC, which allow growth-independent survival and proliferation. BMSC are capable of promoting the growth, survival and drug resistance of leukemic cells by providing the necessary cytokines and cell contact-mediated signals to LSC (42,43). One of the key initial steps of leukemia-stroma interactions *in vivo* is homing and subsequent adhesion of LSC to the protective areas of BM microenvironment. The interaction between CXCL12 and its receptor CXCR4 on LSC contributes to their homing to the BM microenvironment. CXCR4 levels are significantly elevated in leukemic cells from patients with AML, and CXCR4 expression is associated with poor outcome (30,44,45).

Resent data indicate that, the microenvironment may have a role in determining the lineage commitment of acute leukemia. Wei *et al* (46) showed that MLL-AF9–transduced cord blood cells on transplantation into immunodeficient mice generated AML, ALL, or biphenotypic leukemia, depending on the mouse strain or cytokine medium, hence demonstrating the influence of microenvironmental cues for lineage differentiation.

Suppression of normal hematopoiesis is observed frequently in leukemia patients with relatively low tumor burden, which does not necessarily reflect occupancy of BM by LSC. It has been demonstrated that LSC are capable

of distorting normal BM niches and creates a tumor microenvironment (47). These findings indicate that the alteration of signalling mechanisms of BM niches used by normal HSC homing may be hijacked by LSC.

There is increasing evidence that HM alterations may be important and pathogenic in leukemia leading to enhanced stem cell mobilization and the creation of alternate niches (48). Recent data indicate that, in parallel with leukemogenic events in the hematopoietic system, the niche is converted into an environment with dominant signals favoring cell proliferation and growth. In some cases, a combination of these events may be required (49). Therefore, LSC may receive the support of a BM niche for their survival and may in turn influence deregulation of the BM niche by their dominant proliferation-promoting signals.

AML may arise in an abnormal HM, resulting in the generation of multiple populations with varying initiation events. Ninomiya *et al.* (50) modeled the homing, proliferation, and survival sites of human leukemia cells and of cord blood CD34+ cells. The transplanted leukemia cells initially localized on the surface of osteoblasts in the epiphysial region and then expanded to the inner vascular and diaphysial regions. 8 weeks after transplantation, the number of leukemia cells transiently increased by as much as 50%, predominantly in the epiphysial region. After administration of high-dose cytarabine, residual leukemia cells clustered and adhered to the blood vessels as well as to the endosteum, suggesting that LSC receive anti-apoptotic signals not only from osteoblasts but also from vascular endothelium (50). Many of the adhesion molecules, chemokines receptors and signalling cascades that are critical to hematopoietic stem cell (HSC) homing, maintenance and egress from the BM niche are shared by the LSC. LSC home to and engraft within the osteoblast-rich area of the BM, competing for the same microenvironment of normal HSCs.

There are significant data to support mechanism, in which the malignant hematopoietic clone induces reversible functional changes in the HM that result in improved growth conditions for the malignant cells. Gene expression changes occurred in the stroma cell lines, HS5 and HS27a, derived from normal marrow in response to TNFα exposure (51), known to be up-regulated in the bone marrow of patients with MDS. Previous experiments showed that interactions between BMSC and HSC were required for TNFα to trigger apoptosis in HSC (52).

4.2. BMSC alteration in AML

Several studies have proposed that important quantitative and functional alterations occur in BMSC of patients with different hematological disorders (53,54). In some disorders, such as multiple myeloma, MSC show alterations in the expression of some cell adhesion molecules and cytokines, and reduced immunosuppressive efficiency (55,56). Neoplastic plasma cells communicate with the environment through cell/cell contact as well cytokines to induce functional changes that support the malignant population (57,58). In myeloproliferative disorders, has been shown, that megakaryocytes and macrophages play a significant role in the pathogenesis of the fibrotic reaction by secreting PDGF, FGF and TGFα cytokines (59).

Dysfunction of a BM niche may contribute to leukemogenesis by supplying abundant growth factors that promote proliferation and/or inhibit apoptosis (60). BMSC seem to have a relevant role in AML as they prevent spontaneous and induced apoptosis and may attenuate chemotherapy-induced cell death. This possibility has been confirmed by the finding that co-cultivation of a leukemic cell line with the murine stroma cell line MS-5 can block apoptosis (61).

The significance role of the HM in initiation of leukemia has been suggested by studies with mice deficient in phosphatase and tensin homolog (PTEN) (62). PTEN deficiency in both HSC and the HM resulted in myeloproliferation that progressed to overt leukemia/lymphoma. However, inducible PTEN deletion in HSC in the presence of a wild type HM promoted HSC depletion without evidence of myeloproliferation or leukemic development. These results suggest that PTEN deficiency in HSC alone is not sufficient for malignant transformation. Rupec *et al* (63) reported that activation of NF-kB in myelopoietic cells and the absence of its inhibitor IκBα are not sufficient for induction of hypergranulopoiesis, but these changes in the non-hematopoietic compartment, such as fetal liver, resulted in increased numbers of dysplastic hematopoietic cells with progression into secondary AML. These results indicate that non-hematopoietic cells with inactive IκBα can initiate premalignant hematopoietic disorder, conceivably via activation of the Notch pathway. Additional studies indicate the role of Notch signaling in the interactions of HSC and the HM (64) demonstrated that the tumor suppressor Fbxw7, which negatively regulates cyclin E, Notch, and c-Myc protein levels, plays a role in maintaining HSC quiescence and repressing potential oncogenic activity of HSC. Notably, Notch ligand Jagged is expressed by the HSC niche, and Jagged/Notch activation results in increased HSC number and niche expansion (1).

Evidence from research conducted over the last few decades has clearly implicated abnormalities of the marrow microenvironment in the pathophysiology of hematologic malignancies. Marcondes *et al* (65) demonstrated that BMSC derived from patients with myelodysplastic syndrome (MDS), in contrast to that from more advanced stages of disease expressed 14- to 17-fold higher levels of IL-32 mRNA than healthy controls, and this constitutive IL-32 expression promoted apoptosis in LSC, reproducing the inefficient hematopoiesis and extensive apoptosis in BM. These findings indicate that stroma-produced IL-32 could contribute to the pathophysiology of MDS, and serve as a therapeutic target. Furthermore, this modified HM phenotype was reproduced when the BMSC were exposed to TNFα, known to be produced at high levels by MDS cells.

4.3. Genetic changes in the BMSC in AML

Recent discoveries utilizing mouse models have provided the first experimental evidence for genetic changes in the HM contributing to or required for

leukemogenesis. Raaijmakers *et al* (66) using transgenic mice showed that genetic alteration of HM can induce MDS with ineffective hematopoiesis and dysmorphic HCs, and with occasional transformation to AML. The authors used Dicer1 deletion as a means of altering several gene products in subsets of mesenchymal osteolineage cells. Dicer1 is an RNase III endonuclease essential for microRNA biogenesis (67) and RNA processing (68), which regulates haematopoietic cell fate (69). Global repression of microRNA maturation by Dicer1 deletion promotes cellular transformation and tumorigenesis (70). Raaijmakers *et al* (66) show that deletion of Dicer1 in HM cells of mouse may be sufficient to initiate a complex change of homeostasis with similarities to MDS. The authors demonstrated that the ability of HM abnormality to result in the emergence of a clonal neoplasm in a cell type of clearly distinct lineage with distinct secondary genetic changes (66).

Previously, Walkley *et al* (71,72) demonstrated that conditional deletion of the Retinoblastoma gene (RB) in the HM can contribute to the development of pre-leukemic myeloproliferative disease in mice. They showed that this was a result of interactions between myeloid cells and the microenvironment. The defect had to be present in both HC and the microenvironment to initiate disease. Widespread inactivation of RB, a central regulator of the cell cycle and a tumor suppressor, resulted in extramedullary hematopoiesis and myeloproliferative disease in the murine hematopoietic system. However, myeloid-specific loss of RB did not induce myeloproliferative disease or HSC abnormalities. Therefore, the myeloproliferative-like disorder in the RB mutants is the result of perturbed interactions between hematopoietic cells and the HM (71). The final model, reported by the same group, may be the most compelling. In this report, deletion of the Retinoic Acid Receptor γ (RARγ) in mice resulted in a chronic myeloproliferative disorder. Transplant studies revealed that RARγ-hematopoietic cells functioned normally when transplanted into normal mice. However, transplantation of normal hematopoietic cells into the RARγ-microenvironment resulted in a myeloproliferative disorder in the transplanted cells. TNFα was implicated in the pathogenesis of the myeloproliferative disorder as the disease was partially abrogated when TNFα null stem cells were transplanted into the RARγ-microenvironment (72). These studies showed that a defect in HM could be sufficient to generate a myeloproliferative disorder.

Until recently, there has been little evidence to support the role of primary stromal abnormalities in the pathogenesis of hematologic neoplasms. Some independent studies have documented the existence of genomic alterations in the stroma of leukemia patients (73-77). Different groups have shown the extensive variability of the aberrations, such as hypodiploidy, balanced and unbalanced translocations, whole chromosome gains, and deletions. All cytogenetic markers in BMSC never repeated aberrations identified in leukemia cells. These findings suggest enhanced genetic instability of BMSC in leukemia, and indicate the potential involvement of BMSC in the pathophysiology of AML (74,77). Recently, Lopez-Villar *et al* (75) reported the presence of cytogenetic aberrations on BMSC from MDS patients by array-based comparative genomic hybridization and fluorescence *in situ* hybridization, some of them specially linked to a particular MDS subtype, the 5q-syndrome.

These date indicate that there are significant functional abnormalities, genetic aberrations, and epigenetic changes in MSC in leukemia patients. Also of interest are the recent reports of abnormalities in the stroma that lead to malignancies of the hematopoietic compartment. Although historically, hematologic malignancies are thought to arise from a stem or progenitor cell abnormality, there may be groups of patients that have a primary stromal defect leading to the hematologic malignancy. Moreover, although a series of genetic and epigenetic events in a single cell may be necessary for oncogenesis, they may not be sufficient, and a permissive microenvironment has been suggested to be required for frank malignancy to emerge (78).

5. CONCLUSION

Understanding the niche has ramifications beyond simple biological interest. By elucidating the role of the BM microenvironment in the pathogenesis of hematologic tumors, recent studies have provided the framework for identifying and validating novel therapies that target both leukemic cells and cells in their surrounding microenvironment (12). Thus in general, treatment strategies have been focused on the eradication of the stem or progenitor cell from which the malignancy arose.

If primary stromal defects are identified and implicated in the initiation of malignancy, this clearly will have great impact on the treatment strategies offered to patients. By explanation the role of the BMSC in the pathogenesis of AML, recent studies have provided novel therapies that target both leukemic cells and cells of microenvironment. Studies of BMSC can also aid in potentially modifying the relative abundance of normal versus malignant cells in the context of the post chemotherapy setting in AML. The underlying molecular mechanisms implicated in stem cell activation and homing to the niche will provide important insight into the precise mechanisms involved in interactions between leukemic and normal cells that contribute to drug resistance. This understanding will provide a framework for the rational combination of agents in clinical trials to overcome drug resistance and improve patient outcomes. Detection of alterations in BMSC suggests that unstable BMSC may facilitate the expansion of LSC. In view of these data, alterations in BMSC may be a particular mechanism of leukemogenesis. Especially, further understanding of the contribution of the BM niche to the process of leukemogenesis may provide new targets aimed at destroying LSC without adversely affecting normal stem cell.

6. REFERENCES

1. Calvi, L. M., G. B. Adams, K. W. Weibrecht, J. M. Weber, D. P. Olson, M. C. Knight, R. P. Martin, E. Schipani, P. Divieti, F. R. Bringhurst, L. A. Milner, H. M.

Kronenberg, and D. T. Scadden: Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature* 425, 841-846 (2003)

2. Zhang, J., C. Niu, L. Ye, H. Huan, X. He, W. G. Tong, J. Ross, J. Haug, T. Jonson, J. Q. Feng, S. Harris, L. M. Wiedemann, Y. Mishina, and L. Li: Identification of the haematopoietic stem cell niche and control of the niche size. *Nature* 425, 836–841 (2003)

3. Sugiyama, T., H. Kohara, M. Noda, and T. Nagasawa: Maintenance of the hematopoietic stem cell pool by CXCL12–CXCR4 chemokine signaling in bone marrow stromal cell niches. *Immunity* 25, 977–988 (2006)

4. Sacchetti, B., A. Funari, S. Michienzi, S. Di Cesari, S. Piersanti, I. Saggio, E. Tagliafico, S. Ferrari, P. G. Robey, M. Riminucci, and P. Bianco: Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. *Cell* 131, 324–336 (2007)

5. Morrison, S. J., and A. C. Spradling: Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. *Cell* 132, 598–611 (2008)

6. Ramakrishnan, A., and H. J. Deeg: A Novel Role for the Marrow Microenvironment in Initiating and Sustaining Hematopoietic Disease. *Expert Opin Biol Ther* 9, 21–28 (2009)

7. Konopleva, M. Y., and C. T. Jordan: Leukemia stem cells and microenvironment: biology and therapeutic targeting. *J Clin Oncol*, 29, 591-599 (2011)

8. Zeng, Z., Y. X. Shi, T. Tsao, Y. Qiu, S. M. Kornblau, K. A. Baggerly, W. Liu, K. Jessen, Y. Liu, H. Kantarjian, C. Rommel, D. A. Fruman, M. Andreeff, and M. Konopleva: Targeting of mTORC1/2 by the mTOR kinase inhibitor PP242 induces apoptosis in AML cells under conditions mimicking the bone marrow microenvironment. *Blood* 120, 2679-2689 (2012)

9. Russell, E. S.: Hereditary anemias of the mouse: a review for geneticists (Review). *Adv Genetics* 20, 357–459 (1979)

10. Dexter, T. M., T. D. Allen, and L. G. Lajtha: Conditions controlling the proliferation of haemopoietic stem cells *in vitro. J Cell Physiol* 91, 335–344 (1977)

11. Scadden, D. T.: The stem cell niche in health and leukemic disease. *Best Pract Res Clin Haematol* 20, 19-27 (2007)

12. Konopleva, M., Y. Tabe, Z. Zeng, and M. Andreeff: Therapeutic targeting of microenvironmental interactions in leukemia: mechanisms and approaches. *Drug Resist Updat* 12, 103-113 (2009)

13. Perry, J. M., and L. Li: Disrupting the stem cell niche: good seeds in bad soil. *Cell* 129, 1045–1047 (2007)

14. Passegue, E., A. J. Wagers, S. Giuriato, W. C. Anderson, and I. L. Weissman: Global analysis of proliferation and cell cycle gene expression in the regulation of hematopoietic stem and progenitor cell fates. *J Exp Med* 202, 1599-1611 (2005)

15. Kopp, H. G., S. T. Avecilla, A. T. Hooper, and S. Rafii: The bone marrow vascular niche: Home of HSC differentiation and mobilization. *Physiology* (Bethesda) 20, 349-356 (2005)

16. Lapidot, T., A. Dar A., and O. Kollet: How do stem cells find their way home? *Blood* 106, 1901–1910 (2005)

17. Cancelas, J. A., and D. A. Williams: Stem cell mobilization by beta2-agonists. *Nat Med* 12, 278–279 (2006)

18. Fleming, H. E., V. Janzen, C. Lo Celso, J Guo, K. M. Leahy, H. M. Kronenberg, and D. T Scadden: Wnt signaling in the niche enforces hematopoietic stem cell quiescence and is necessary to preserve self-renewal *in vivo*. *Cell Stem Cell* 2, 274-283 (2008)

19. Horwitz, E. M., K. Le Blanc, M. Dominici, I. Mueller, I. Slaper-Cortenbach, F. C. Marini, R. J. Deans, D. S. Krause, and A. Keating A : Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. *Cytotherapy* 5, 393-395 (2005)

20. Friedenstein, A. J., R. K. Chailakhyan, N. V. Latsinik, A. F. Panasyuk, and I. V. Keiliss-Borok: Stromal cells responsible for transferring the microenvironment of the hemopoietic tissues. Cloning *in vitro* and retransplantation *in vivo*. *Transplantation* 17, 33-40 (1974)

21. Chamberlain, G., J. Fox, B. Ashton, and J. Middleton: Concise Review: Mesenchymal Stem Cells: Their Phenotype, Differentiation Capacity, Immunological Features, and Potential for Homing. *Stem Cells* 25, 2739–2749 (2007)

22. Pittenger, M. F., A. M. Mackay, S. C. Beck, R. K. Jaiswal, R. Douglas, J. D. Mosca, M. A. Moorman, D. W. Simonetti, S. Craig, and D. R. Marshak: Multilineage potential of adult human mesenchymal stem cells. *Science* 284, 143–147 (1999)

23. Jori, F. P., M. A. Napolitano, M. A. Melone, M. Cipollaro, A. Cascino, L. Altucci, G. Peluso, A. Giordano, und U. Galderisi: Molecular pathways involved in neural *in vitro* differentiation of marrow stromal stem cells. *J Cell Biochem* 94, 645–655 (2005)

24. Beyer Nardi, N., and L. da Silva Meirelles: Mesenchymal stem cells: Isolation, *in vitro* expansion and characterization. *Handb Exp Pharmacol* 249–282 (2006)

25. Tokcaer-Keskin, Z., A. R. Akar, F. Ayaloglu-Butun, E. Terzioglu-Kara, S. Durdu, U. Ozyurda, M. Ugur, and K. C. Akcali: Timing of induction of cardiomyocyte

differentiation for *in vitro* cultured mes- enchymal stem cells: A perspective for emergencies. *Can J Physiol Pharmacol* 87, 143–150 (2009)

26. Sordi, V., M. L. Malosio, F. Marchesi, A. Mercalli, R. Melzi, T. Giordano, N. Belmonte, G. Ferrari, B. E. Leone, F. Bertuzzi, G. Zerbinin, P. Allavena, E. Bonifacio, and L. Piemonti: Bone marrow mesenchymal stem cells express a restricted set of functionally active chemokine receptors capable of promoting migration to pancreatic islets. *Blood* 106, 419 – 427 (2005)

27. Becker, P.: Dependence of Acute Myeloid Leukemia on Adhesion within the Bone Marrow Microenvironment. *The Scientific World Journal* 2012, 1-4 (2012)

28. Abkowitz, J. L., A. E. Robinson, S. Kale, M. W. Long, and J. Chen: Mobilization of hematopoietic stem cells during homeostasis and after cytokine exposure. *Blood* 102, 1249–1253 (2003)

29. Broxmeyer, H. E., C. M. Orschell, D. W. Clapp, G. Hangoc, S. Cooper, P. A. Plett, W. C. Liles, X. Li, B. Graham-Evans, T. B. Campbell, G. Galandra, G. Bridger, D. C. Dale, and E. F. Strour: Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist. *J Exp Med* 201, 1307–1318 (2005)

30. Peled, A., and S. Tavor: Role of CXCR4 in the pathogenesis of acute myeloid leukemia. *Theranostics*. 3, 34-39 (2013)

31. Zuckerman, K. S., and M. S. Wicha: Extracellular matrix production by the adherent cells of long-term murine bone marrow cultures. *Blood* 61, 540–547 (1983)

32. Wight, T. N., M. G. Kinsella, A. Keating, and J. W. Singer: Proteoglycans in human long-term bone marrow cultures: biochemical and ultrastructural analyses. *Blood* 67, 1333–1343 (1986)

33. Miyake, K., K. Medina, K. Ishihara, M. Kimoto, R. Auerbach, and P. W. Kincade: A VCAM-like adhesion molecule on murine bone marrow stromal cells mediates binding of lymphocyte precursors in culture. *J Cell Biol* 11, 557–565 (1991)

34. Garcia-Gila, M., E. M. Lopez-Martin, and A. Garcia-Pardo: Adhesion to fibronectin via alpha4 integrin (CD49d) protects B cells from apoptosis induced by serum deprivation but not via IgM or Fas/Apo-1 receptors. *Clin Exp Immunol* 127, 455–462 (2002)

35. Macanas-Pirard, P., A. Leisewitz, R. Broekhuizen, K. Cautivo, F. M. Barriga, F. Leisewitz, V. Gidi, E. Riquelme, V. P. Montecinos, P. Swett, P. Besa, P. Ramirez, M. Ocqueteau, A. M. Kalergis, M. Holt, M. Rettig, J. F. DiPersio, and B. Nervi: Bone marrow stromal cells modulate mouse ENT1 activity and protect leukemia cells from cytarabine induced apoptosis. *PLoS One* 7, 37203 (2012)

36. Meads, M. B., R. A. Gatenby, and W. S. Dalton: Environment-mediated drug resistance: A major contributor to minimal residual disease. *Nat Rev Cancer* 9, 665-674 (2009)

37. Holyoake, T. L., X. Jiang, M. W. Drummond, A. C. Eaves, and C. J. Eaves: Elucidating critical mechanisms of deregulated stem cell turnover in the chronic phase of chronic myeloid leukemia. *Leukemia* 16, 549–558 (2002)

38. Liesveld, J. L., C. T. Jordan, and G. L. Phillips: The hematopoietic stem cell in myelodysplasia. *Stem Cells* 22, 590–599 (2004)

39. Warner, J. K., J. C. Wang, K. J. Hope, L. Jin, and J. E. Dick: Concepts of human leukemic development. *Oncogene* 23, 7164–7177 (2004)

40. Braun, B. S., and K. Shannon: Targeting Ras in myeloid leukemias. *Clin Cancer Res* 14, 2249–2252 (2008)

41. Lane, S. W., D. T. Scadden, and D. G. Gilliland: The leukemic stem cell niche: current concepts and therapeutic opportunities. *Blood* 114:1150-1157 (2009)

42. Dazzi, F., R. Ramasamy, S. Glennie, S. P. Jones, and I. Roberts: The role of mesenchymal stem cells in haemopoiesis. *Blood Rev* 20, 161–171 (2006)

43. Ramasamy, R., E. W. Lam, I. Soeiro, V. Tisato, D. Bonnet, and F. Dazzi: Mesenchymal stem cells inhibit proliferation and apoptosis of tumor cells: impact on *in vivo* tumor growth. *Leukemia* 21, 304–310 (2007)

44. Kim, H. Y., Y. S. Oh, I. C. Song, S. W. Kim, H. J. Lee, H. J. Yun, S. Kim, and D. Y. Jo: Endogenous stromal cell-derived factor-1 (CXCL12) supports autonomous growth of acute myeloid leukemia cells. *Leuk Res* 37. 566-572 (2013)

45. Konoplev, S., G. Z. Rassidakis, E. Estey, H. Kantarijian, C. I. Liakou, X. Huang, L. Xiao, M. Andreeff, M. Konopleva, and L. J. Medeiros: Overexpression of CXCR4 predicts adverse overall and event-free survival in patients with unmutated FLT3 acute myeloid leukemia with normal karyotype. *Cancer* 109, 1152-1156 (2007)

46. Wei, J., M. Wunderlich, C. Fox C, S. Alvarez, J. C. Cigudosa, J. S. Wilhelm, Y. Zheng, J. A. Cancelas, Y. Gu, M. Jansen, J. F. Dimartino, and J. C. Mulloy: Microenvironment determines lineage fate in a human model of MLL-AF9 leukemia. *Cancer Cell* 13, 483- 495 (2008)

47. Colmone, A., M. Amorim, A. L. Pontier, S. Wang, E. Jablonski, and D. A. Sipkins: Leukemic cells create bone marrow niches that disrupt the behavior of normal hematopoietic pro- genitor cells. *Science* 322, 1861-1865 (2008)

48. Lataillade, J. J., O. Pierre-Louis, H. C. Hasselbalch, G. Uzan, C. Jasmin, M. C. Martyre, and M. C. Le Bousse-Kerdilies, on behalf of the French INSERM and the

European EUMNET Networks on Myelofibrosis: Does primary myelofibrosis involve a defective stem cell niche? From concept to evidence. *Blood* 112, 3026-3035 (2008)

49. Li, L., and W. B. Neaves: Normal stem cells and cancer stem cells: the niche matters. *Cancer Res* 66, 4553–4557 (2006)

50. Ninomiya, M., A. Abe, A. Katsumi, J. Xu, M. Ito, F. Arai, T. Suda, M. Ito, H. Kiyoi, T. Kinoshita, and T. Naoe: Homing, proliferation and survival sites of human leukemia cells *in vivo* in immunodeficient mice. *Leukemia* 21, 136–142 (2007)

51. Stirewalt, D. L., A. J. Mhyre, M. Marcondes, E. Pogosova-Agadjanyan, N. Abbasi, J. P. Radich, and H. J. Deeg : Tumour necrosis factor-induced gene expression in human marrow stroma: clues to the pathophysiology of MDS? *Br J Haematol* 140, 444–453 (2008)

52. Goda, C., T. Kanaji, S. Kanaji, G. Tanaka, K. Arima, S. Ohno, and K. Izuhara: Involvement of IL-32 in activation-induced cell death in T cells. *Int Immunol* 18, 233–240 (2006)

53. Borojevic, R., R. Roela, R. Rodarte, L, S. Thiago, F. S. Pasini, F. M. Conti, M. I. D. Rossi, L. F. L. Reis, L. F. Lopes, and M. M. Brentani: Bone marrow stroma in childhood myelodysplastic syndrome: composition, ability to sustain hematopoiesis *in vitro*, and altered gene expression. *Leuk Res* 28, 831-844 (2004)

54. Flores-Figueroa, E., J. J. Montesinos, P. Flores-Guzman, G. Gutiérrez-Espíndola, R. M. Arana-Trejo, S. Castillo-Medina, A. Pérez-Cabrera, E. Hernández-Estévez, L. Arriaga, and H. Mayani: Functional analysis of myelodysplastic syndromes-derived mesenchymal stem cells. *Leuk Res* 32, 1407–1416 (2008)

55. Wallace, S. R., M. M. Oken, K. L. Lunetta, A. Panoskaltsis-Mortari, and A. M. Masellis: Abnormalities of bone marrow mesenchymal cells in multiple myeloma patients. *Cancer* 91, 1219–1230 (2001)

56. Corre, J., K. Mahtouk, M. Attal, M. Gadelorge, A. Huynh, S. Fleury-Cappellesso, C. Danho, P. Laharrague, B. Klein, T. Rème, and P. Bourin: Bone marrow mesenchymal stem cells are abnormal in multiple myeloma. *Leukemia* 21, 1079–1088 (2007)

57. Mitsiades, C. S., N. S. Mitsiades, N. C. Munshi, P. G. Richardson, and K.C. Anderson: The role of the bone microenvironment in the pathophysiology and therapeutic management of multiple myeloma: interplay of growth factors, their receptors and stromal interactions (Review). *Eur J Cancer* 42, 1564–1573 (2006)

58. Podar, K., P. G. Richardson, T. Hideshima, D. Chauhan, and K. C. Anderson: The malignant clone and the bone marrow environment (Review). *Best Pract Res Clin Haematol* 20, 597–612 (2007)

59. Chagraoui, H., F. Wendling, and W. Vainchenker: Pathogenesis of myelofibrosis with myeloid metaplasia: Insight from mouse models (Review). *Best Pract Res Clin Haematol* 19, 399–412 (2006)

60. Jones, D. L., and A. J. Wagers: No place like home: anatomy and function of the stem cell niche. *Nat Rev Mol Cell Biol* 9, 11–21 (2008)

61. Konopleva, M., S. Konoplev, W. Hu, A. Y. Zaritskey, B. V. Afanasiev, and M. Andreeff: Stromal cells prevent apoptosis of AML cells by up-regulation of antiapoptotic proteins. *Leukemia* 16, 1713–1724 (2002)

62. Yilmaz, O. H., R. Valdez, B. K. Theisen, W. Guo, D. O. Ferguson, H. Wu, and S. J. Morrison: Pten dependence distinguishes haematopoietic stem cells from leukaemia-initiating cells. *Nature* 44, 475–482 (206)

63. Rupec, R. A., F. Jundt, B. Rebholz, B. Eckelt, G. Weindl, T. Herzinger, M. J. Flaig, S. Moosmann, G. Plewig, B. Dörken, I. Förster, R. Huss, and K. Pfeffer: Stroma-mediated dysregulation of myelopoiesis in mice lacking I kappa B alpha. *Immunity* 22, 479–491 (2005)

64. Matsuoka, S., Y. Oike, I. Onoyama, A. Iwama, F. Arai, K. Takubo, Y. Mashimo, H. Oguro, E. Nitta, K. Ito, K. Miyamoto, H. Yoshiwara, K. Hosokawa, Y. Nakamura, Y. Gomei, H. Iwasaki, Y. Hayashi, Y. Matsuzaki, K. Nakayama, Y. Ikeda, A. Hata, S. Chiba, K. I. Nakayama, and T. Suda: Fbxw7 acts as a critical fail-safe against premature loss of hematopoietic stem cells and development of T-ALL. *Genes Dev* 22, 986–991 (2008)

65. Marcondes, A. M., A. J. Mhyre, D. L. Stirewalt, S. H. Kim, C. A. Dinarello, and H. J. Deeg: Dysregulation of IL-32 in myelodysplastic syndrome and chronic myelomonocytic leukemia modulates apoptosis and impairs NK function. *Proc Natl Acad Sci USA* 105, 2865–2870 (2008)

66. Raaijmakers, M. H., S. Mukherjee, S. Guo, S. Zhang, T. Kobayashi, J. A. Schoonmaker, B. L. Ebert, F. Al-Shahrour, R. P. Hasserjian, E. O. Scadden, Z. Aung, M. Matza, M. Merkenschlager, C. Lin, J. M. Rommens, and D. T. Scadden: Bone progenitor dysfunction induces myelodysplasia and secondary leukemia. *Nature* 464:852-857 (2010)

67. Bartel, D. P.: MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116, 281–297 (2004)

68. Krol, J., A. Fiszer, A. Mykowska A. K. Sobczak, M. de Mezer, and W. J. Kiryzosiak: Ribonuclease dicer cleaves triplet repeat hairpins into shorter repeats that silence specific targets. *Mol Cell* 25, 575–586 (2007)

69. Lu, J., S. Guo, B. L. Ebert, H. Zhang, X. Peng, J. Bosco, J. Pretz, R. Schlanger, J. Y. Wang, R. H. Mak, D. M. Dombkowski, F. I. Preffer, D. T. Scadden, and T. R. Golub: MicroRNA-mediated control of cell fate in

megakaryocyte-erythrocyte progenitors. *Dev Cell* 14, 843–853 (2008)

70. Kumar, M. S., J. Lu, K. L. Mercer, T. R. Golub, and T. Jacks: Impaired microRNA processing enhances cellular transformation and tumorigenesis. *Nature Genet* 39, 673–677 (2007)

71. Walkley, C. R., J. M. Shea, N. A. Sims, L. E. Purton, and S. H. Orkin: Rb regulates interactions between hematopoietic stem cells and their bone marrow microenvironment. *Cell* 129, 1081–1095 (2007)

72. Walkley, C. R., G. H. Olsen, S. Dworkin, S. A. Fabb, J. Swann, G. A. McArthur, S. V. Westmoreland, P. Chambon, D. T. Scadden, and L. E. Purton: A microenvironment-induced myeloproliferative syndrome caused by retinoic acid receptor gamma deficiency. *Cell* 129, 1097–1110 (2007)

73. Flores-Figueroa, E., R. M. Arana-Trejo, G. Gutiérrez-Espíndola, A. Pérez-Cabrera, and H. Mayani: Mesenchymal stem cells in myelodysplastic syndromes: phenotypic and cytogenetic characterization. *Leuk Res* 29, 215-224 (2005)

74. Blau, O., W. K. Hofmann, C. D. Baldus, G. Thiel, V. Serbent, E. Schümann, E. Thiel, and I. W. Blau: Chromosomal aberrations in bone marrow mesenchymal stroma cells from patients with myelodysplastic syndrome and acute myeloblastic leukemia. *Exp Hematol* 35, 221-229 (2007)

75. Lopez-Villar, O., J. L. Garcia, F. M. Sancez-Guijo, C. Robledo, E. M. Villaron, P. Hernandez-Campo, N. Lopez-Holgado, M. Diez-Campelo, M. V. Barbado, J. A. Perez-Simon, J. M. Hernandez-Rivas, J. F. San-Miguel, and M. C. del Canizo: Both expanded and uncultured mesenchymal stem cells from MDS patients are genomically abnormal, showing a specific genetic profile for the 5q- syndrome. *Leukemia* 23, 664-672 (2009)

76. Klaus, M., E. Stavroulaki, M. C. Kastrinaki, P. Fragioudaki, K. Giannikou, M. Psyllaki, C. Pontikoglou, D. Tsoukatou, C. Mamalaki, and H. A. Papadaki: Reserves, Functional, Immunoregulatory, and Cytogenetic Properties of Bone Marrow Mesenchymal Stem Cells in Patients with Myelodysplastic Syndromes. *Stem Cells Dev* 19, 1043-1055 (2010)

77. Blau, O., C. D. Baldus, W. K. Hofmann, G. Thiel, F. Nolte, T. Burmeister, S. Türkmen, O. Benlasfer, E. Schümann, A. Sindram, M. Molkentin, S. Mundlos, U. Keilholz, E. Thiel, and I. W. Blau: Mesenchymal stromal cells of myelodysplastic syndrome and acute myeloid leukemia patients have distinct genetic abnormalities compared with leukemic blasts. *Blood* 118, 5583-5592 (2011)

78. Hanahan, D., and R. A. Weinberg: The hallmarks of cancer. *Cell* 100, 57–70 (2007)

Key Words: Hematopoietic microenvironment, Bone marrow niches, Mesenchymal stromal cells, Acute myeloid leukemia, review

Send correspondence to: Olga Blau, Charite University School of Medicine, Labor Berlin, Department of Hematology and Oncology, Berlin, Germany, Tel: 49-30-405026492, Fax: 49-30-8445-4468, E-mail: olga.blau@charite.de