Bone marrow malignancies as paradigms of dysfunctional cell adhesion mechanisms

Cachaço Ana Sofia [1], Salvador Daniela [2], Dias Sérgio [3]

1. Angiogenesis Laboratory, Centro de Investigação de Patobiologia Molecular (CIPM), Instituto Português de Oncologia de Lisboa Francisco Gentil (IPOFG), Lisboa, Portugal. 2. CEDOC, Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisboa, Portugal. 3. Instituto Gulbenkian de Ciência, Oeiras, Portugal.

Correspondence: Sérgio Dias. Address：Angiogenesis Laboratory, Centro de Investigação de Patobiologia Molecular (CIPM), Instituto Português de Oncologia de Lisboa Francisco Gentil (IPOFG), R. Prof. Lima Basto, 1099-023 Lisboa, Portugal. Telephone: 351-217-229-818. Fax: 351-217-229-895. E-mail: sergidias@ipolisboa.min-saude.pt

Abstract

In adult bone marrow (BM), hematopoietic stem/progenitor cells (HSPCs) reside in micro-environments which provide instructions for self-renewal, survival, proliferation, differentiation and migration. Adequate response to such complex signals implies communication between HSCs, BM stroma and extracellular matrix molecules (ECM). This is achieved mainly through adhesion molecules. Malignant hematopoietic cells also interact with the BM microenvironment, which provides them with proliferative and survival advantages. Most of the studies on haematological diseases describe cell-ECM interactions as key mechanisms in tumor progression, while genetic alterations of HSC are considered major initiators of the malignant process. However, accumulating evidence suggests that an altered BM microenvironment provides anomalous cell adhesion signals, facilitating tumor initiation. Myeloproliferative and myelodysplastic syndromes are good examples of haematological disorders where alterations in BM microenvironment may play an important role in disease initiation. This review discusses the role for adhesion signals in regulating the BM microenvironment in normalcy and disease.

Key words

1 Adhesion signaling between hematopoietic cells and their microenvironment regulates hematopoiesis

Hematopoiesis is an extremely well regulated process, ensuring the normal production of all blood cells. In adult bone marrow (BM), hematopoietic stem/progenitor cells (HSPCs) reside in particular microenvironments, known as stem cell niches, which provide them with critical instructions to self-renewal, proliferation, differentiation, homing, migration and survival (about BM niches see, as examples, the reviews [1-8]. HSPCs are believed to be located near bone surfaces (osteoblastic niche) or associated with the sinusoidal endothelium (the vascular niche); the molecular signals generated by these two niches have been extensively studied (in particular for the osteoblastic niche). Osteoblasts produce important

signaling molecules like osteopontin and angiopoietin that interact with their receptors on HSPCs, keeping these in a quiescent state [9-11]. On the other hand, the vascular niche is considered to promote proliferation and further differentiation of HSPCs [12]; it produces FGF-4 and chemokines such as SDF-1 [7] that recruits the HSPCs from the osteoblastic to vascular niche. Recently, with new imaging approaches, it is becoming evident that endosteal and vascular compartments may not be mutually exclusive in terms of their role on HSPC fate [13, 14].

BM microenvironment comprises not only stromal cells (including osteoblasts, endothelial cells, fibroblasts, etc), but also soluble factors produced by stromal and hematopoietic cells, and the extracellular matrix (ECM) that surrounds all BM cells. ECM is an intricate network of proteins (e.g. collagens), glycoproteins (e.g. fibronectin, laminins), glycosaminoglycans (e.g. hyaluronan) and proteoglycans (e.g. syndecans) which turnover is tightly controlled by adjacent cells (figure 1). Thus, to acquire proper information from the surrounding milieu, hematopoietic cells need to establish physical contacts through adhesion molecules expressed in a regulated fashion at cell surfaces. Such molecules can establish connections between cells and the ECM or mediate cell-cell contact. Integrins are the main receptors for ECM molecules, although others like transmembrane proteoglycans (syndecans) can function as co-receptors for the matrix. In the hematopoietic system, integrins also function as cell-cell adhesion molecules, binding to members of the immunoglobulin superfamily, like VCAM-1 (vascular cell adhesion molecule 1) or ICAM-1 (inter-cellular adhesion molecule 1). Cadherins are cell-cell adhesion proteins which established homophilic interaction between adjacent cells. Selectins exert special adhesion proprieties between leukocytes or platelets and the endothelial cells. Another group of adhesion molecules within BM comprises the sialomucins (CD34, CD43, CD45-RA, PSGL-1, CD164 and PCLP-1) and although their receptors are not completely known, the signals they deliver potently inhibit hematopoiesis [15, 16]. Adhesion molecules participate in a range of signal transduction processes involving not only cell adhesion, but also migration, proliferation and apoptosis. Additionally, other signaling molecules like growth factors and cytokines can modulate cell adhesion, just as cell adhesion signaling can regulate soluble factors receptors [17]. For instance, under physiological levels of IL3 and stem cell factor (SCF), adherence of HSPC to fibronectin via β1 integrins prevents S-phase entrance, whereas supraphysiological concentrations of these cytokines prevent p27(KIP1) elevation and override the integrin-mediated inhibition of proliferation [18]. In fact, signals produced by cytokines and chemokines regulate via an outside-in mechanism the affinity and avidity of integrins, thus providing a further level of control [17]. The chemoattractor stromal cell derived factor-1α (SDF-1α) affects the function of β1 and β2 integrins, allowing the homing of HSPC into the BM [19, 20]. Also well known is the crosstalk between growth factor receptors and integrins. Recent works on angiogenesis and tissue repair demonstrated that VEGF binding domains of fibronectin are required for promoting the specific association between the fibronectin receptor integrin α5β1 with the VEGF receptor, Flk-1 enhancing these biological processes (as examples see [21-23])

In this review we will give special attention to the interaction between hematopoietic cells and the ECM, in particular, via integrin receptors. Such option is based on the fact that perturbations on integrin-ECM ligation occur very often in BM disorders but their importance on tumorigenesis is still not understood.

Far from being exhaustive, table 1 shows some reported cell adhesion events occurring within adult BM. For each hematopoietic cell type, the interaction between a specific receptor (e.g. integrin), expressed on its surface, and the corresponding ECM ligand or another receptor on a stromal cell, modulates hematopoietic cell behavior. Data from human or mouse models were used to compile this table. The majority of studies referred here employs *in vitro* approaches such as long-term BM cultures and are not always in agreement. Although a characterization of ECM molecules distribution within BM has been made by immunofluorescence labeling more than 10 years ago [24], is still not understood if, *in vivo*, there is a preferential localization of different hematopoietic cells within certain ECM niches.

Table 1. Cell-cell and cell-ECM adhesion events that modulate hematopoiesis in bone marrow

BM cell type	Reported receptor	ECM ligand or cell counter-receptor	Function	References
HSPCs	VLA-4 VLA-5	FN FN	Proliferation regulation, survival	[25-27]
	VLA-6	LN-8, LN-10/11	Migration	[28]
	VLA-4 β1 integrins	FN LN	Regulation of multi-drug resistant genes	[29]
	VLA-4 β1 integrins	VCAM-1/FN LN/FN	Homing, engraftment	[29-32]
	n.d	Heparan sulphate	Determines cytokines and ECM molecules localization within stem cells niche	[33-35]
	n.d	Tenascin	Migration inhibition	[36]
	n.d	Osteopontin	Proliferation inhibition	[37]
	CD164	Lectins	Proliferation inhibition	[38, 39]
Megakaryocytes	VLA-4	FN	Adhesion of CFU-MK and immature MKs	[40, 41]
	VLA-4	FN	MK growth	[42]
	VLA-5	FN	On mature MKs	[40, 41]
	VLA-2	Collagen	On mature polyploid MKs	[40, 41]
	VLA-6 αIIbβ3	LN Fibrinogen	Early and late MK differentiation	[40, 41]
	VLA-4 β1 integrins	VCAM-1 FN	Promotion of platelet formation	[43, 44]
	VLA-2	Collagen I	Inhibition of platelet formation	[44]
Erythrocytes	Emp	Emp	Erythropoiesis (apoptosis inhibition, erythroblast nuclear extrusion)	[45, 46]
	ICAM-4	αv integrins	Erythropoiesis (erythroblast-central macrophage interaction)	[47, 48]
	VLA-4	VCAM-1/FN	Erythropoiesis (erythroblast-central macrophage interaction)	[49]
	VLA-6	LN-10/11	Adhesion of erythroid progenitors	[28]
Myeloid cells	VLA-6	LN-10/11	Adhesion of myelonocytic progenitors and myeloid cell lines	[28]
	n.d	Collagen	Myelopoiesis inhibition	[50]
	VLA-4	FN	Development of myeloid lineages	[50]
	VLA-4	n.d.	Leukocyte development and traffic	[51]
Lymphoid cells	VLA-4 VLA-5	VCAM-1/FN FN	B-cell development	[52-57]
	VLA-4	VCAM-1	T-cell development	[58, 55]

Abbreviations. CFU-MK=colony-forming unit-megakaryocyte; Emp=erythroblast macrophage protein, FN=fibronectin; HSPCs=Hematopoietic stem/progenitor cells; ICAM-4=inter-cellular adhesion molecule 4; LN=laminin; MKs=megakaryocytes; VCAM-1=vascular cell adhesion molecule 1; VLA-2=very late antigen 2, α2β1 integrin; VLA-4=very late antigen 4, α4β1 integrin; VLA-5=very late antigen 5, α5β1 integrin; VLA-6=very late antigen 6, α6β1 integrin; n.d.=Not determined on that particular study

Table 2. Cell adhesion interactions between malignant cells and stromal cells/ECM and their potential importance in hematological malignancies

Group	Cell receptor-ligand expression*	Reported effect on leukemic cells	References
AML	VLA-4, -5-FN, VLA-6-LN, LFA-1-ICAMs	Adhesion, survival	[67- 72]
	Altered function/affinity of VLA-4, -5-FN	AML blasts release intocirculation	[70, 73]
	VLA-4-LN	Protection against chemotherapy	[64, 74]
	Expression of different CD44 isoforms	Adhesion, proliferation	[75, 76]
	CD44-hyaluronan	Proliferation inhibition and terminal differentiation	[77- 80]
CML	VLA-4, -5 inactivation	Expansion and premature release of blasts from BM	[81- 86]
	Expression of different CD44 isoforms	Early differentiation-associated changes in CML CFU-GM	[87]
	β integrins-FN	Drug resistance	[88, 89]
ALL	VLA-4-VCAM-1, VLA-5-FN	Adhesion, proliferation and migration	[68- 95]
	Increased CD44	Adhesion	[96, 97]
CLL	VLA-4-VCAM-1; β2integrins	Endothelial extravasation	[98, 99]
	VLA-4-VCAM-1/FN, β2integrins	Apoptosis inhibition, disease relapse	[100-102]
	Increased CD44	Protection from apoptosis; poor prognosis	[103-105]
MM	LFA-1-ICAM-1	Cell aggregation, proliferation	[106, 107]
	VLA-4-VCAM-1/FN, VLA-5-FN, VLA-6-LN	Transendothelial migration, retention of MM cells in BM	[108-111]
	VLA-5 down-regulation	Exit of MM cells to extramedullar places (at later stages)	[112]
	VLA-4-FN	Drug resistance	[113-115]
	CD44 (different isoforms)/ RHAMM-hyaluronan	Adhesion and migration	[116-120]
	Syndecan-1	Multifunctional regulator of MM microenvironment	[121-123]

Abbreviations. ALL=acute lymphoblastic leukemia; AML=acute myeloid leukemia; CFU-GM=colony-forming units granulocyte-macrophage; CLL=chronic lymphocytic leukemia; CML=chronic myelogenous leukaemia; FN=fibronectin; ICAM=inter-cellular adhesion molecule; LFA-1=lymphocyte function-associated antigen -1, αLβ2integrin; LFA-3= lymphocyte function-associated antigen - 3; LN=laminin; MM=multiple myeloma; NCAM=neural cell adhesion molecule; VLA-3= very late antigen 3, α3β1 integrin; VLA-4=very late antigen 4, α4β1 integrin; VLA-5 = very late antigen 5, α5β1integrin; VLA-6=very late activated-6, α6 β1integrin

* In some situations, only the malignant cell receptor is referred and not its ligand

2 Dysregulation of adhesion signaling between hematopoietic cells and their microenvironment is involved in hematological disorders

Malignant hematopoietic cells are known to express particular cell adhesion repertoires that provide them with proliferative and survival advantages within the BM microenvironment. Specific niche composition provides ideal

conditions for some leukemic cells to escape from chemotherapy-induced apoptosis and acquire drug-resistance. On this subject, several reviews can be consulted, for instance: [59- 61]. Most of the studies on leukemias and other hematological diseases refer integrins and their ligation with ECM or stromal cells as key mechanisms involved in tumor progression; well identified genetic hits occurring on HSCs would be the major beginners of the oncogenic process (see, as examples [62, 63]). Presently, the acceptance that the BM microenvironment is important in supporting leukemia stem cells survival has conducted to the rational development of therapies that target microenvironment molecules [59, 60]. For instance, the inhibition of VLA-4 integrin - fibronectin ligation in in acute myeloid leukemia (AML) patients increases their sensibility to chemotherapy [64]. Also, adhesion molecules profiles of hematological patients are presently being considering, since variations among them may have important clinical consequences.

In table 2 we summarize major cell adhesion interactions that occur between BM malignant cells and ECM or stromal cells, contributing to hematological diseases. In some situations, like in multiple myeloma, perturbations in cell-ECM adhesion take place, like dysregulation of integrin function or upregulation of different ECM receptors, leading to aberrant behaviors and clonal expansion of the malignant cells. In other cases, like in some leukemias, the same receptor-ECM ligation occurs in normal and in malignant cells, but malignant cells acquire proliferative and survival advantages upon normal cells, replacing them. This suggests that such advantages may be supported by modifications in BM microenvironment that normal cells are not able to cope with, using the common adhesion pathways. In agreement with this, two early *in vitro* studies showed that leukemia-derived stromal cells could not sustain appropriate maturation of normal HSPCs [65, 66].

3 Role of BM microenvironment in the onset of tumorigenesis – two examples from non-malignant disorders

There are growing evidences that an altered microenvironment may not only support tumor progression, but can also facilitate tumor initiation providing anomalous cell adhesion signaling. Few recent works on epithelial cancers suggest that ECM can not only subsidize but also initiate the oncogenic conversion of epithelial cells [124]. One possible cause for such tumor-driven ECM alterations may be irreversible damages (repeated traumas, fibrosis, inflammation, etc) in stroma or other sources of ECM. Some hematological disorders are characterized by an altered stroma, in particular an accumulation of ECM molecules, like in primary or idiopatic myelofibrosis (PMF). Additionally, fibrosis can also occur as a secondary event in some leukemias, which is associated with bad prognosis. In an interesting review, Dührsen and Hossfeld [125] define two mechanisms for hematological tumorigenesis: "malignancy-induced microenvironment" and "malignancy-inducing microenvironment". In the first case, neoplastic hematopoietic cells induce alterations in BM microenvironment that became permissive to malignant clone expansion. In the second case, the first event includes a stroma lesion which restrains normal hematopoietic cell regulation, leading to the emergence of a neoplastic cell population. In accordance, perturbations in BM niche signaling in mouse models were shown to mimic human diseases [126, 127].

In this section we will provide two examples of hematological disorders where microenvironment, and adhesion signaling in particular, may have an important role in the initiation of the malignant phenotype. That is particular evident and has been extensively studied for PMF, a myeloproliferative syndrome (MPS), and also for myelodysplasic syndromes (MDS).

MPS are a group of BM clonal diseases with enhanced proliferation and survival of one or more cells types of the myeloid lineage. They are categorized by the presence or absence of the Philadelphia chromosome (t9;22), and include, among

others, PMF, polycythemia vera (PV), essential thrombocythemia (ET) (all Philadelphia chromosome-negative) and chronic myelogenous leukemia (CML - Philadelphia chromosome-positive) [63, 128]. A complete genetic and clinicopathological classification of this group of disorders is out of the scope of this review and can be consulted, for instance, in [63, 129, 130]. In PMF, both initial prefibrotic (not associated with reticulin accumulation) and more advanced myelofibrotic stages show increased abnormal megakaryo- and granulopoiesis; atypical megakaryocytes (MKs) are clustered and medium-sized to giant, with cloud-like, and often hyperchromatic nuclei, a feature not seen in other subtypes of MPS [130-132]. MKs promote fibroblast growth, a mechanism where cell-cell contact mediated by integrins VLA-3 and VLA-5 is implicated [133]. More, the abnormal MKs release specific growth factors (like TGF-β) that enhance ECM production by fibroblasts [134, 135]. In a typical case of PMF, hematopoietic cells in BM are replaced by collagen fibrosis (although other ECM proteins, like fibronectin, are also increased), impairing the patient ability to generate new blood cells; this results in progressive pancytopenia, extramedullary hematopoiesis and splenomegaly. A significant inverse correlation between fibre content and number of HSPCs in BM has been established in PMF [136]. Not only increased production of ECM occurs in PMF but there is also an imbalance between metalloproteases (MMPs) and TIMPs (MMPs inhibitors) that may contribute to BM   niche, probably through perturbations in HSPCs-ECM adhesion, is of outmost importance for disease development [137].

MDS are a heterogenous group of clonal hematopoietic diseases characterized by peripheral cytopenia (despite a normocellular or hypercellular BM) and with a variable probability to progress to AML [138, 139]. In the past decade, it has become apparent that this ineffective hematopoiesis is largely caused by excessive apoptosis of myeloid precursors [140-143]. Recent observations suggest that downregulation of VLA-4 and VLA-5 integrins on HSPCs, correlated with decreased in vitro adhesiveness to fibronectin fragments, can act as a pro-apoptotic mechanism in MDS [144]. It is possible that these alterations in the adhesive proprieties of myeloid progenitors are cell-autonomous and confer cell susceptibility to apoptosis [145]. However, changes in BM stroma, namely on ECM, have already been detected in some MDS patients, suggesting again a role for microenvironment in MDS progression [146, 147]. In a considerable percentage of biopsies, myeloid precursors are found in an abnormal central localization within the BM, instead of being near the osteoblastic niche, a feature associated with poor disease prognosis [148, 149]. Nevertheless, the question remains: Are microenvironment alterations the initial event that dysregulates hematopoiesis in MDS? Studies using normal cord blood cells cultivated with BM stromal cells from patients with childhood MDS show aberrant cell differentiation of precursor cells; here, stroma cells expressed increased levels of thrombospondin-1, collagen -I α2-chain, osteoblast-specific factor-2 and osteonectin, indicating the presence of increased osteoblast content that may contribute to abnormal hematopoiesis in this pathology [150]. In another in vitro study, both fibroblasts and macrophages from MDS BM are functionally abnormal, denoting increased apoptosis and high production of both IL-6 and TNF-α [151]. Like for PMF, megakaryocytic proliferation and differentiation are typically abnormal in patients with MDS [152], being thrombocytopenia one of the first features of this syndrome. Also, the expression of the stem cell marker CD34 on mature MKs from MDS patients is correlated with reduced survival [153]. Other adhesion molecule patterns are modified in HSPCs from MDS and secondary AML, like ICAM-1 (overexpressed) and L-selectin (underexpressed), having prognosis importance [154]. Also, plasma levels of soluble CD44 are significantly elevated in MDS patients as compared to those of healthy donors and are increased in high-risk MDS subtypes [155].

While pro-apoptotic mechanisms are activated in HSPCs in MDS, the evolution to an aggressive AML includes a suppression of apoptosis due to changes in intracellular levels of Bcl-2-family proteins [156, 143]. Nevertheless, important interactions between leukemic cells and BM microenvironment also contribute to AML progression: When natural killer cells and cytotoxic lymphocytes adhere to BM stroma, they inhibit AML blasts clonogenic growth; however, AML cells compete with them for stromal binding sites via β1 (principally VLA-4) and β2 integrins, escaping from

apoptosis [70, 72, 157]. Finally, in advanced stages, the lost of contact with ECM is necessary for AML blasts to egress to circulation [158].
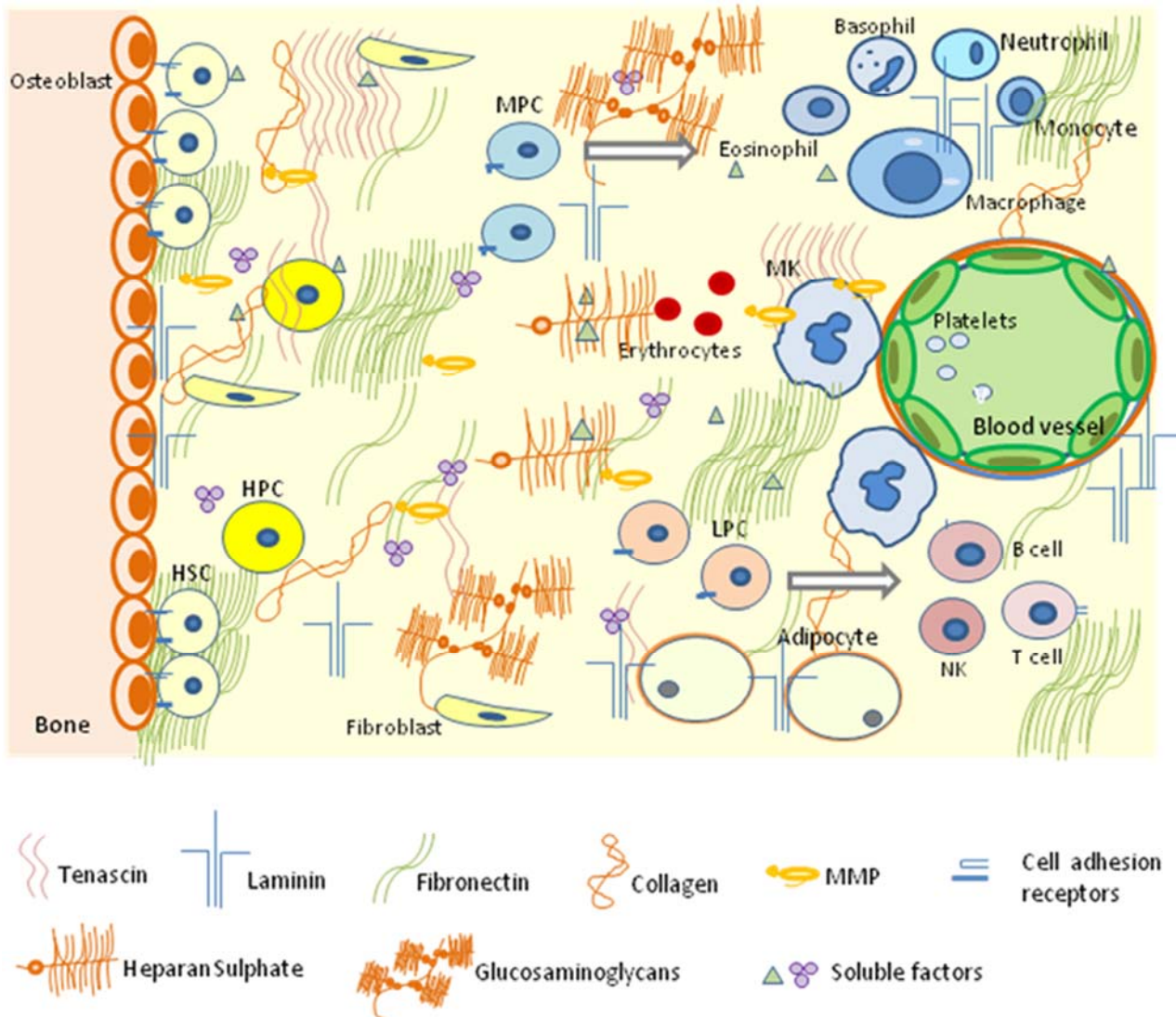

Figure 1. Schematic representation of major steps in hematopoiesis within BM microenvironment. The image represents BM niches: the osteoblastic niche, where quiescent hematopoietic stem cells (HSC) and hematopoietic progenitor cells (HPC) localize, and the vascular niche, where HPC differentiation occurs. Myeloid progenitor cells (MPC) originate basophils, neutrophils, eosino-phils, monocytes, megakaryocytes (MKs - which give rise to platelets), and erythrocytes; lympho-cyte progenitor cells (LPC) originate lymphocytes B and T, and natural killer cell (NK) cell. BM microenvironment is composed by ECM proteins like collagen (Col), glycoproteins as fibronectin (FN), laminin (LN) and tenascin (TN), glycosaminoglycans (GAGs), and proteoglycans (PG); it also contains soluble factors like growth factors (GF) and matrix metalloproteases (MMPs), and stroma cells (endothelial cells, osteoblasts, fibroblasts, macrophages, adipocytes). Hematopoietic cells associate with ECM molecules throughout cell adhesion receptors (CAR), like integrins.
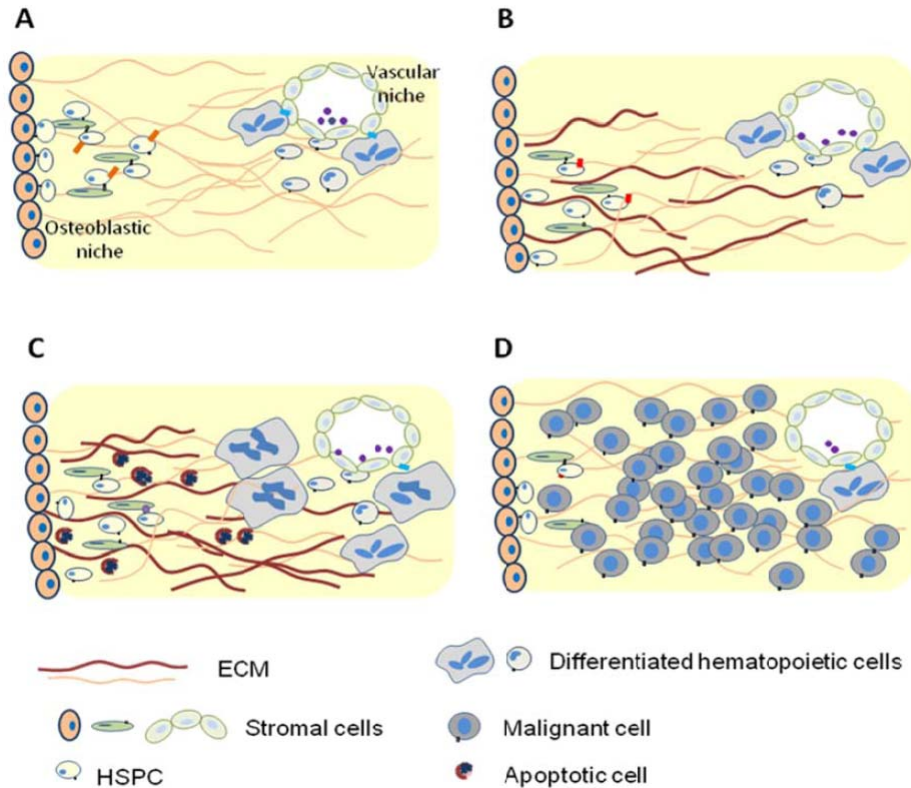
Figure 2. Hematopoietic malignancies are paradigms of dysfunctional cell adhesion mechanisms within bone marrow (BM) microenvironment. A. In a steady-state BM, hematopoietic cells are located in their niches and established appropriated cell-cell and cell-extracellular matrix (ECM) connections with BM microenvironment. B. Alterations in ECM turnover and qualitative and/or quantitative changes in cell adhesion molecules contribute to an unstable BM microenvironment where incorrect cell adhesion pathways/mechanisms occur. C. This situation may create a premalignant condition within BM, characterized by aberrant haematopoiesis, including increased apoptosis. D. BM dysfunction favours clonal selection of malignant hematopoietic cells and leads to the onset and progression of fatal haematological diseases.

4 Final remarks

According to Dührsen and Hossfeld [125], MDS most likely result from a primary lesion in BM stroma, which results in the creation of an unstable microenvironment. Lack of adequate adhesion signals (among others), generates a "suicidal" environment for most hematopoietic cells in the BM; increased sensitivity to apoptosis is a hallmark of MDS. Remarkably, increased cell death is followed by increased proliferation, likely of selected and well adapted cell clones. The generation of such an unstable microenvironment may in fact result in the establishment of a pre-malignant condition. In fact, MDS can progress to AML while PMF can be associated with advanced stages of ET, PV and CLL, representing poor prognosis.

Fibroblasts have been regarded as major producers of ECM in the BM, although an early publication showed the distribution of fibronectin, type I, type III and type V collagens is similar in cultured BM fibroblasts from normal and from MPS patients with and without myelofibrosis [159] Interestingly, MKs are important producers of MMPs [160], fibronectin [161], (our unpublished results), and thrombospondin-1 [162], and they are dysfunctional both in MDS and PMF [153]. This fact raises the possibility that MKs may play a crucial role in regulating ECM turnover, in normal, pre-malignant and in malignant BM.

Two important reports using mice as models have demonstrated that events extrinsic to HSPCs modify BM microenvironment and are the sole cause of an MPS phenotype. One is the loss of the retinoic acid receptor-γ [126] and the other is due to a retinoblastoma protein-dependent interaction between myeloid-derived cells and the micro-environment [127]. Recently, our own studies showed that increased TNF-α levels occurring in BM microenvironment after radiation contribute towards the onset and progression of secondary MDS in mice [163]. The latter report indicated that ECM turnover is altered in irradiated (pre-malignant) BM, and that this contributes towards the onset of hematological dysfunction.

In this review, we have provided examples where hematological malignancies may be seen as paradigms of dysfunctional cell and tissue microenvironments, involving cell-ECM and cell-cell interations, including those mediated by adhesion molecules. Taken together, we propose a tight regulation of the mechanisms that govern cell adhesion to ECM and to other components of the BM microenvironment is essential to assure adequate BM function; conversely, changes in adhesion signaling pathways/mechanisms may contribute towards the generation of a pre-malignant microenvironment and to the onset and progression of fatal hematological diseases (figure 2).

Acknowledgements

Conflict of interest

The authors have declared that no competing interests exist.

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