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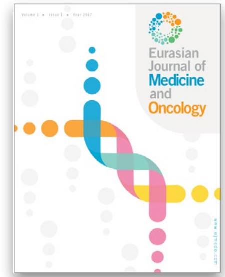

Review

Role of Endothelial Cells in Hematological Malignancies

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Abstract

Endothelial cells (EC) are key elements of vascularized tissues that form a single-cell layer that connects the vessels to the surrounding tissues. EC participate in the regulation of blood hemostasis, leukocyte homing, acute inflammation, wound healing, and antigen presentation. EC subpopulations are characterized by diverse structures, functions, and molecular profiles. In bone marrow, EC are part of the hematopoietic stem cell vascular and endosteal niche, where they play well-defined roles in hematopoietic stem cell functioning and maintenance, and reside surrounding sinusoids and blood vessels. In the past years, the clinical and pathophysiological roles of EC have been explored due to their contribution to neoangiogenesis and alterations in the vascular endothelium functions in hematological diseases. The present review discusses the EC contribution to pathogenesis of hematological malignancies and their potential use as therapeutic target in these diseases.

Keywords: Endothelial cells, hematological malignancies, bone marrow niche, pathogenesis, angiogenesis.

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Nowadays, EC have gained renewed attention due to their association with disease pathogenesis, especially in neoplastic processes.[1] In solid tumor microenvironment, EC are crucial for neoangiogenesis and interaction with tumor cells, as well as provide support, nutrition and anchorage to tumor cells during metastasis.[2] It is known that tumor EC are functionally and phenotypically distinct from normal EC. Tumor EC are resistant to apoptosis, present an activated cyclooxygenase (COX)-2 pathway,[3] downregulate T-cell activation [4] express a variety of chemokine receptors, secrete inflammatory mediators and interleukin-10, and weakly express adhesion molecules.[5] In solid tumors, tumor EC favor immune evasion through induction of tolerance to tumor antigens.

Although the role that tumor EC plays in the pathogenesis and progression of solid tumors is well-known, the role that they play in hematological diseases remains unclear. EC are important components of pathogenesis of hematological malignancies, since they modify bone marrow microvascular density, increase EPC counts, release inflammatory mediators (cytokines and chemokines), and contribute to disease progression.[6,7]

Endothelial Cells: Definition, Morphology, Location and Immunophenotype

EC are key elements of vascularized tissues, including the bone marrow niche, and form a single-cell layer that connects vessels to surrounding tissues and controls the ex-

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change of substances in and out these compartments.[8] EC play essential roles in wound healing, angiogenesis, inflammation, as well as in the pathogenesis of diabetes, cardiovascular diseases, and cancer.[9]

The use of electron microscopy provided the first insights on the EC structural heterogeneity and enabled identification of distinct intercellular junctions, which led to the classification of endothelium as continuous, fenestrated, and discontinuous.[9] Discontinuous endothelium lines blood vessels of the spleen, liver and bone marrow, does not have diaphragm, has considerably larger pores than those of fenestrated endothelium, and is more permeable than continuous and fenestrated endothelium.[9–11]

Pluripotent stem cells from the bone marrow niche give rise to hemangioblasts that are capable of differentiating into hematopoietic progenitor cells or endothelial progenitor cells (EPC). EPC differentiate into circulating endothelial precursors and circulating endothelial cells (CEC). Hematopoietic progenitor cells differentiate into myeloid cells such as monocytes, which can transdifferentiate into myeloid EC. Mature EC that shed from the vessel wall can enter the circulation.[12] EC attached to the endothelium actively participate in the regulation of vasoconstriction, vasodilatation, extravasation of fluids and solutes such as hormones and macromolecules, blood hemostasis, leukocyte homing, acute inflammation, wound healing, atherogenesis, antigen presentation, and catabolism of lipoproteins.[13,14]

EC morphology is typically flat but their shape changes across the vascular tree: they are plump or cuboidal in high endothelial venules; and thin, slightly elongated, and aligned in straight segments but not at branch points of arteries.[10,15] EC dimensions are 30–50 µm length, 10–30 µm wide and thickness, varying from less than 0.1 µm in capillaries and veins to 1 µm in the aorta artery.[16,17]

The structural and functional diversity of EC results from the molecular differences among EC populations.[13] Each EC type and stage of differentiation is identified by a specific expression pattern of gene markers.[11]

The majority of circulating EPC resides in the bone marrow niche in association with hematopoietic stem cells (HSC). EPC are capable of proliferating, migrating, and differentiating into EC-like cells, but they do not acquire phenotype of mature EC.[18] The first EPC from human peripheral blood were isolated in 1997 based on expression of Cluster of Differentiation (CD) 34 and other endothelial markers, such as CD31, Vascular endothelial growth factor receptor 1 (VEGFR1), Vascular endothelial growth factor receptor 2 (VEGFR2) and tie-2.[19]

EPC are classified according to their surface markers into CD45dim/–, CD34+, and CD133+ cells, while CEC are classified into CD45dim/–, CD34+, and CD133– cells.[12,20–22] Numerous blood cell populations express the CD133 and CD34 antigens, which are not specific for EC.[23] Other authors have also used CD146, CD144 and/or VEGFR2 markers for EPC and CEC identification and isolation.[24–26] The table 1 reports the location and the surface markers applied to EC immunophenotyping by flow cytometry.

EC in Hematopoietic Stem Cell Niche

Bone marrow EC are part of the vascular and endosteal HSC niche that play well-defined roles in HSC function and maintenance, and reside surrounding sinusoids and blood vessels.[27] The bone marrow niche is densely vascularized and composed of two major endothelial niches: (1) arteriolar niche, with EC phenotype VE cadherin+ CD31+ endomucin+/– Stem cells antigen 1 (SCA1)high Vascular endothelial growth factor receptor 3 (VEGFR3)-; and (2) sinusoidal niche, with EC phenotype CD144+ CD31+ endomucin+ SCA1, VEGFR3+low[28–30] (Fig. 1).

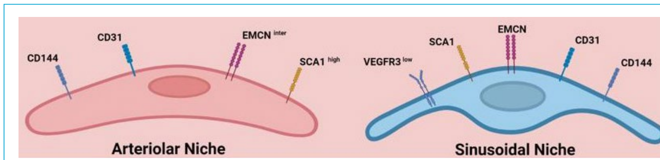

Figure 1. Arteriolar and sinusoidal endothelial cells (EC) immunophenotype in bone marrow niche.

In arteriolar niche, EC are negative for VEGFR3 and positive for CD144 and CD31 markers, present high expression of SCA1 and intermediate expression of endomucin (EMCN). In sinusoidal niche, EC are positive for SCA1, EMCN, CD31 and CD144 markers and present low expression of VEGFR3 marker.

Table 1. Location and surface markers of endothelial cells at different stages of differentiation.

Cell type	Surface markers	Location
Endothelial progenitor cell	CD45low, CD133+, CD34+, KDR+	Bone marrow
Endothelial cell	CD34low, CD31+, CD144+, KDR+	Peripheral blood
Myeloid endothelial cell	CD14+, CD45+, CD34low, CD31+, KDR+	Peripheral blood
Circulating endothelial precursors	CD133+, CD34+, KDR+, CD31+, CD144+	Peripheral blood
Circulating endothelial cells	CD144+, CD31+, CD34+, KDR+	Peripheral blood

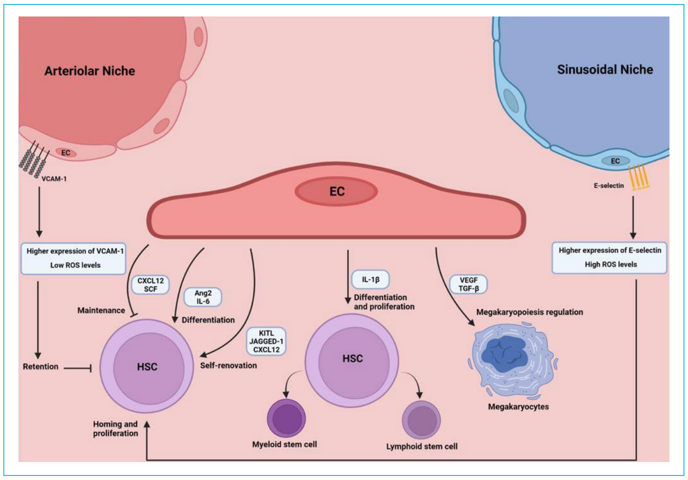

Figure 2. Endothelial cells´ (EC) secretory pattern in healthy bone marrow microenvironment.

Multiple adhesion molecules, growth factors and stem cell related antigens are expressed in the surface membrane of EC. The EC morphology and immunophenotype is microenvironment-dependent. The, increased expression of VCAM-1 in arteriolar niche is related to downregulation of ROS production, that leads to hematopoietic stem cell (HSC) retention in bone marrow; CXCL12 and SCF signalization dictates the maintenance of the HSC in bone marrow, as indicated by the inhibition arrows. Differently, E-selectin higher expression, in sinusoidal niche, is associated with higher ROS production, that is a key regulator of HSC homing and proliferation. Although there are immunophenotypic, secretory and morphologic differences between sinusoidal and arteriolar EC, the functional activities developed by these cells in both microenvironments are similar. The cytokines and stimulating factors secreted by EC regulates hematopoietic stem cells (HSC) maintenance, differentiation, homing, and self-renewing, and may also control the other cell lineages differentiation and production, such as megakaryocytes. The arrows coming from the EC and pointing to other cells in the niche represent: angiotensin 2 (Ang2) and IL-6 signalization stimulating HSC differentiation; KITL, JAGGED-1 (receptors) and CXCL12 (chemokine) stimulating HSC self-renovation; IL-1β stimulating HSC differentiation in myeloid and/or lymphoid lineages and its proliferation; VEGF and TGF-β stimulating and regulating megakaryopoiesis.

Some authors have explored the contribution of EC to hematopoiesis and maintenance of the HSC niche.[28,31,32] The interaction between EC and HSC occurs during all phases of human development and is important to hematopoiesis.[28] The vascular cells provide specific structures to supporting hematopoiesis in the human bone marrow, since early embryo stages.[31] In adults, the HSC maintenance during homeostasis and regeneration depends on EC support[33] (Fig. 2).

EC and stromal cells produce key growth factors and chemokines for HSC maintenance, such as stem cell factor and C-X-C motif Chemokine (CXCL) 12.[34,35] Bone marrow EC support hematopoietic progenitor cell differentiation and proliferation through IL-3 secretion and cell adhesion contact.[32] HSC and hematopoietic progenitor cell adhesion, homing, and migration also depend on EC[36,37] (Fig. 2).

There is a reciprocal influence among EC, hematopoietic cells, and hematopoiesis. For instance, megakaryocyte production of vascular endothelial growth factor A (VEGF-A) sustains EC survival,[38] while EC regulate megakaryopoiesis by secreting chemokine-mediated interaction and adhesion proteins.[39] Furthermore, the close interactions between HSC and EC are vital for HSC maturation,[27] homing, and proliferation[37] (Fig. 2).

The signaling promoted by endothelial niches[40] in the bone marrow guides the HSC fate. Activation of the mitogen activated protein kinases (MAPK) pathway in EC leads to HSC differentiation into hematopoietic progenitor cells and other terminally differentiated hematopoietic cell types.[40] Activation of the AKT pathway in EC elicits the expression of paracrine factors, including KIT-ligand (KITL), CXCL12, and JAGGED-1, which are related to promotion of HSC self-renovation.[40,41] Endothelial inhibition of the canonical nuclear factor kappa B (NF-κB) pathway improves the self-renovation and regenerative potential of HSC.[41] Vascular permeability also influences the HSC fate; more specifically, the increased endothelium permeability promotes mobilization and proliferation of HSC (Fig. 3).[42]

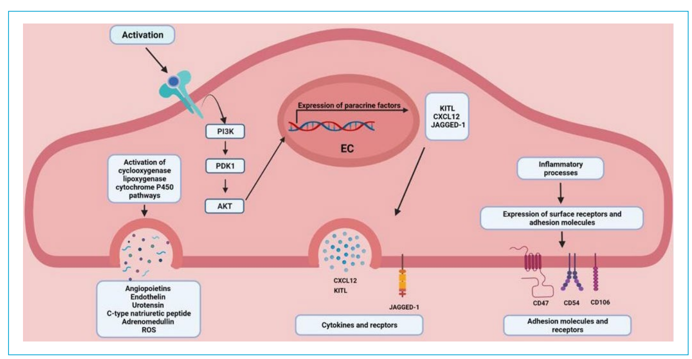

Figure 3. Signaling pathways and biological processes in endothelial cells (EC).

EC are sensitive to numerous molecules signaling, that are involved in the activation of intracellular pathways that regulates the paracrine stimulation, the production of inflammatory mediators and influence in metabolic regulation. For example, mitogen-activated protein kinase (MAPK) receptor activation in EC membrane leads to PI3K/PDK1/AKT pathway activation and stimulates the expression of transcription factors in the nucleus that enhance the synthesis of cytokines like CXCL12 and proteins like JAGGED-1, that interacts with the NOTCH pathway and are related to HSC differentiation. KITL, which interacts with the c-KIT receptor, also stimulates HSC differentiation. The activation of intracellular cascades also promotes the activity of enzymes like cicloxigenases, lypoxigenases and cytochrome P450, that mediates the synthesis of numerous mediators like angiopoietins, endothelin, urotensin, C-type natriuretic peptide, adrenomedullin and ROS, that regulates processes linked to inflammation and coagulation. Inflammatory processes may lead to the remodeling of the surface markers and adhesion molecules expression in the membrane of the EC that directly influences in the course of various diseases, like myeloproliferative neoplasms (MPN). Arrows indicate stimulation and activation.

EC synthesize and release arachidonic acid metabolites, various peptides – such as angiopoietins, endothelin, urotensin, C-type natriuretic peptide and adrenomedullin and reactive oxygen species through the activation of cyclooxygenase (COX), lipoxygenase, and cytochrome P450 pathways capable of modulating coagulation and inflammatory processes (Fig. 3).[14]

It is worth to note that the EC immunophenotype is altered during inflammatory processes and favors transmigration of small molecules and leukocytes, increases expression of surface receptors and adhesion molecules, such as ICAM-1 (CD54), VCAM-1 (CD106), and CD47. Such molecules mediate chemotaxis, interaction with leukocytes and platelets [43] and enhance cytokine expression (Fig. 3).

Taken all these observations together, we hypothesize that EC contribute actively for maintenance of the hematopoietic niche and proliferation, differentiation, and trafficking of HSC.

EC in Hematological Malignancies

CEC are more frequent in patients with chronic myeloid leukemia at blast phase than in patients at chronic and accelerated phases. The high CEC counts in patients with chronic myeloid leukemia (CML) at blast phase is associated with high levels of vascular endothelial growth factor (VEGF) gene expression, indicating that angiogenesis is exacerbated in patients with CML at advanced phase of the disease. In this sense, the increased EC counts may be used as biomarker of CML progression.[44]

Patients with chronic lymphoid leukemia also exhibit increased CEC counts and microvascular density, which are associated with more aggressive disease. In chronic lymphoid leukemia, CEC originate from neoplastic clones and are able to enhance angiogenesis in the bone marrow microenvironment.[45–47] In vitro studies have reported that enhancement of the angiogenic process is associated with the ability of chronic lymphoid leukemia (CLL) cells to secrete VEGF and angiopoietin 2 (ANG2)[48] in bone marrow niche.

Angiogenesis is vital for the establishment and maintenance of acute myeloid leukemia (AML) and acute lymphoid leukemia (ALL). The angiogenesis markers VEGF and ANG2 are associated with worse prognosis in AML and ALL.[49] The increased angiogenesis in bone marrow[50] and CEC is correlated with disease status and treatment response [51] in AML.

Furthermore, VEGF secreted by EC promotes growth of leukemia cells by paracrine effects.[52] Likewise, recent studies in acute myeloid leukemia have suggested that leukemia cells contribute to EC proliferation, indicating the potential interaction between these cells.[53]

Selectin-mediated homing and rolling seem to be an important crosstalk between leukemic cells and EC in AML.[54] EC and stromal cells prevent spontaneous and therapy-induced blast apoptosis in AML.[55] Thus, the relationship between leukemia cells and bone marrow EC is believed to play a fundamental role in chemotherapeutic drug resistance.

Patients with AML have higher CEC counts at the time of diagnosis than patients who respond to chemotherapy treatment,[56] indicating that EC count is a promising candidate biomarker of patients' response to treatment.[57–59] A study with 40 patients with AML has detected increased CEC and EPC counts at the time of diagnosis when compared with patients responsive to chemotherapy. The patients who achieve complete response to treatment have lower initial CEC and EPC counts than patients who do not respond to treatment, suggesting that CEC and EPC counts correlate with disease status and treatment response.[56]

AML cells interact with, modulate the behavior, and activate resting EC.[60] The leukemia cell adhesion to EC via E-selectin remains in a quiescent state and is unaffected by chemotherapy. Leukemia cells seem to support EC activation and contribute to resistance to chemotherapy.[60]

Altered angiogenesis is another component of the pathophysiology of multiple myeloma (MM). It is assumed that a pre-malignant disease known as monoclonal gammopathy of undetermined significance (MGUS) precedes neovascularization.[61] This early stage of the disease is considered avascular due to the lack of development of blood vessels; angiogenesis occurs only in MM. In addition to oncogenic events, bone marrow angiogenesis and MM-related cytokines play a role in the progression of MGUS to multiple myeloma.[62] Patients with multiple myeloma have increased EPC counts and microvascular density, as compared with healthy controls,[6,7,63–65] which correlate with the multiple myeloma diagnosis parameters, such as protein M and microglobulin-β2 levels. The CEC count can be a useful biomarker of MM progression.[66] Similarly, patients with myelodysplatic syndromes (MDS) and abnormal angiogenesis have increased CEC and EPC counts.[67,68]

Patients with high-risk MDS exhibit an elevated number of activated CEC, bone marrow with high microvascular density, and high levels of basic fibroblast growth factor (bFGF) and soluble VEGFR2.[67] The increased number of functional EPC in MDS strengthens the rationale for therapeutic interventions to restore the normal interaction between hematopoietic progenitor cells and bone marrow microenvironment.[68]

Angiogenesis is exacerbated in three BCR-ABL- myeloproliferative neoplasms (MPN): essential thrombocythemia (ET), polycythemia vera (PV), and primary myelofibrosis (PMF).[69]

Patients with ET and PV display higher CEC counts than normal subjects. In patients with PV, the CEC counts are not associated with the mutation status but correlate with leukocyte counts, and the plasma levels of VEGF and sVEGFR-1 are lower than those detected in patients with ET.[69]

Patients with PMF present high microvascular density associated with elevated levels of proinflammatory cytokines and megakaryocyte counts in the bone marrow.[70] Mice with EC JAK2V617F+ are at higher risk for developing venous thrombosis[71] than their normal counterparts. JAK2V617F-expressing EC contribute to thrombogenesis due to their pro-adhesive phenotype associated with the increased P-selectin and von Willebrand factor expression.[71]

It is worthy to emphasize that MPN are an oncoinflammatory disease characterized by high levels of proinflammatory cytokines and chemokines in bone marrow and peripheral blood.[72,73] The bone marrow and peripheral blood inflammatory microenvironment influence the EC phenotype and function. In this context, the EC activated by cytokines in the hematopoietic niche and peripheral blood of patients with myeloproliferative neoplasms may support the oncoinflammation process by secreting Interleukin (IL) -1, IL-6, IL-8 and granulocyte colony-stimulating-factor (G-CSF) and recruiting inflammatory cells, including neutrophils. Oncoinflammation also leads to cell genetic instability and clonal evolution[74] (Fig. 4). EC also downregulate cell adhesion molecules, reduce immune cell trafficking, and tolerize T-cells.[75]

It seems that "neoplastic bone marrow environment" has immune inhibitory EC, like in solid tumors. Neoplastic cells are capable of directing EC to impair immune response and secrete cytokines that favor disease progression.

In summary, EC play pro-tumor roles in hematological malignancies, which are related to alterations in bone marrow microenvironment, activation of neoangiogenesis, resistance to treatment, and secretion of growth factors important to tumor cells.

Endothelial Cells as Therapeutic Target in Hematological Malignancies

The role of the EC, as a tool, in the regeneration process is widely discussed in the literature. Recent studies have suggested the existence of a subset of tissue-resident EC with regenerative capacity in response to injury, that culminate in changes in its transcriptomic profile during the regenerative process, including upregulation of a number of stress response genes. These EC present stem cell properties and may represent a novel cell-based therapy for various vasculopathies, in addition to representing a new therapeutic approach for vascular regeneration or disruption therapy in states of vascular recruitment to promote tumor growth.[76–80]

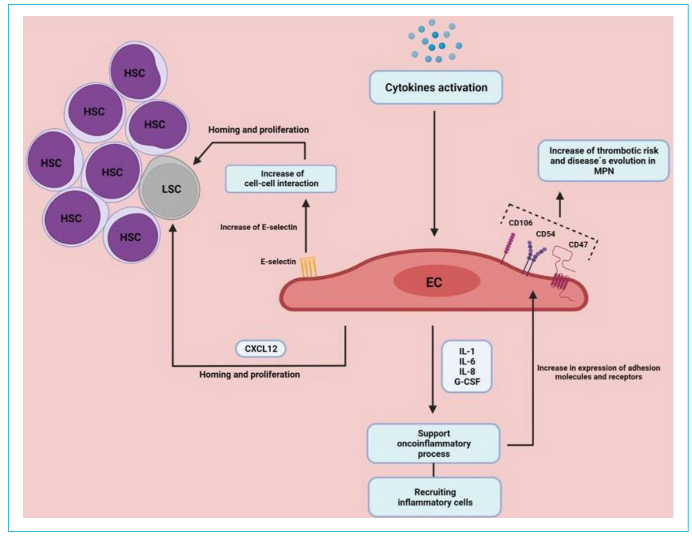

Figure 4. Endothelial cells (EC) secretory pattern in oncoinflammatory neoplastic bone marrow microenvironment.

EC may be activated by the various cytokines that are released in neoplastic bone marrow microenvironment. Once activated, EC has been related to secretion of pro-inflammatory cytokines such as IL-1, IL-6, IL-8 and G-CSF in neoplastic bone marrow niche. In myeloproliferative neoplasms (MPN), the pro-inflammatory cytokines provide support to oncoinflammatory process, increasing adhesion molecules expression like VCAM-1 (CD106), ICAM-1 (CD54) and CD47, that acts recruiting inflammatory immune cells and contributing to high risk to thrombotic events and leukemic transformation. CXCL12 production by EC and E-selectin overexpression in sinusoidal niche are associated with leukemic stem cells (LSC) homing and proliferation. The arrows indicate stimulation and activation.

In cancer scenario, the literature suggests that the EC is a new potential therapeutic target. For example, in AML, the combined use of bevacizumab and OXi4503 affect the function of EC, blocking its neoangiogenic action, leading to leukemia regression by the production of reactive oxygen species and resulting in apoptosis.[81]

Furthermore, AML leukemic cells are able to modulate the behavior and activate resting EC. The leukemia cell interacts with EC by E-selectin molecule inducing activation. The activated EC contribute to AML progression and neoangiogenesis (Fig. 4). These observations suggest that leukemia cells support EC activation contributing to chemotherapy resistance and tumor evasion to immune response.[60]

In a mouse model of AML, inflammatory mediators, released by blasts cells, upregulate E-selectin expression in endothelial niche. These AML-blasts with high E-selectin binding potential are 12-fold more likely to survive to chemotherapy, contributing to disease relapse. Thus, therapeutic blockage of E-selectin inhibits the niche- prosurvival signaling, dampens AML-blast regeneration, and strongly synergizes with chemotherapy, increasing mouse survival over chemotherapy.[82]

In a cohort of de novo AML patients, it was observed higher number of both CEC and EPC patients at diagnosis, as well as, after induction of chemotherapy in comparison to healthy controls. Patients who achieved complete response presented lower initial CEC and EPC levels compared with patients who did not responded to treatment, suggesting that CEC and EPC levels may correlate with disease status and treatment response.[56]

Higher vascularization was observed in patients with CLL with advanced clinical stage and poor outcome that seems to be associated with increased serum levels of VEGF and ANG2 produced by EC.[45,46,48,83] These data as supported by in vitro studies with CLL cells that showed increase angiogenesis throughout secretion of VEGF and ANG2.[48]

Lin et al. (2016) [84] demonstrated that there was no difference in proliferation between normal and mutant hematopoietic stem/progenitor cells (HSPC) when cultured with normal EC, while JAK2V617F+ HSPCs showed growth advantage over normal HSPCs when cultured with JAK2V617F+ EC, thus suggesting that the JAK2V617F+ vascular niche may preferentially promote the expansion of JAK2V617F+ HSCs.

In patients with MPN, disease recurrence is observed in approximately 40% of cases.[85] Lin et al. (2018) [85] analyzed the apoptosis of normal HSCs transplanted in mice with both normal EC and JAK2V617F+ EC and irradiated a few weeks after transplantation. The authors reported that the apoptotic activation of HSCs was reduced in mice with JAK2V617F+ EC compared to mice with normal EC. These data indicate that the mutant vascular niche may contribute to the radioprotection of HSCs and make the medullary microenvironment conducive to disease recurrence even in patients undergoing curative treatment.

In vitro tests have shown that JAK2V617F+ ECs exhibit significantly increased cell proliferation, cell migration, angiogenesis and decreased apoptosis (after irradiation) compared to normal ECs,[84] in addition to having increased levels of expression of essential factors, CXCL12 and KITL, in mice, for the maintenance of HSC.[34,86] Furthermore, the proportion of HSC expressing C-X-C chemokine receptors (CXCR) 4 and c-KIT was significantly increased in cells carrying the mutation compared to normal cells,[34,87] indicating that the mutation in JAK2 with the consequent increase in the expression of CXCL12 and SCF can act in the expansion of the vascular niche and clonal expansion of JAK2V617F+ HSC. It was also reported that the increased expression levels of CXCL12, epidermal growth factor and pleiotrophin in irradiated EC mutants may suggest that the JAK2V617F+ vascular niche contributes to the radioprotection of JAK2V617F+ HSC due to the expression of cytokines and chemokines responsible for activating HSC.

In MPN JAK2V617F+ patients the thrombosis is one of the main causes of morbidity and mortality and the recent identification of the presence of JAK2V617F mutation in EC of MPN patients, open new perspectives in the pathogenesis of thrombosis in these diseases.[71] Guy and collaborators showed, in a mice model, that endothelial cells JAK2V617F+ present higher risk for venous thrombosis, once JAK2V617F-expressing EC present a pro-adhesive phenotype associated with increased endothelial P-selectin expression. They also demonstrated that P-selectin blockade or hydroxyurea therapy are able of reduce the propensity thrombosis through reduction of endothelial P-selectin expression.[71]

Taking in account all this information, we may conclude that endothelial cells have a relevant role in hematological malignancies pathogenesis and prognosis and seems to be a promising target in the treatment of these neoplasms.

Conclusion

The abovementioned studies emphasize the EC contribution to the pathogenesis and progression of hematological malignancies. We may speculate that EC contribute to a more aggressive course of hematological malignancies. Additional studies are required to better elucidate the cellular and molecular mechanisms involved in the interaction among EC, HSC, and neoplastic cells, and to develop novel treatments for hematological malignancies. Moreover, the oncoinflammatory process and vasculogenesis-mediated CEC are interesting therapeutic targets to stop disease progression.

Disclosures

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