The functional significance of the bone marrow microenvironment in multiple myeloma development and progression

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Abstract

Introduction

Multiple myeloma is one of the most common haematological malignancies, occurring mainly in men over 60 years of age. Despite significant therapeutic progress and a twofold increase in overall survival, multiple myeloma is still an incurable disease. The reason for the relatively poor prognosis for multiple myeloma patients lies in the biology of this tumour, the progressive development of which is closely dependent on the bone marrow microenvironment. The conditions in the bone marrow, in particular, the presence of growth factors for multiple myeloma cells (including interleukin 6, insulin-like growth factor-1, and vascular endothelial growth factor) secreted by different cells, promote their survival and proliferation in the bone marrow niches. Migration and expansion of malignant plasma cells and their mobilisation to/from the peripheral blood, characteristic of myeloma progression, are mainly due to disruption of the stromal cell-derived factor-1/CXCR4 axis caused by numerous molecular extracellular factors. It has been shown that the formation of premetastatic niches in the bone marrow, which are indicative of progression, occurs even before the first metastatic cells, home to the bone marrow, and is affected by cellular and extracellular components of the bone marrow. This close interaction between malignant plasma cells and the bone marrow microenvironment should determine appropriate therapeutic management focused on all elements of this complex biological system for a real improvement in prognosis. This paper reviews the current literature describing the participation of myeloma cells and the bone marrow microenvironment in disease development and progression.

Conclusion

Novel therapeutic approaches should target not only the malignant plasma cell, but also its interaction with the bone marrow microenvironment to sufficiently prevent disease progression. Despite administration of several immunomodulators and proteasome inhibitors, other therapies are still under active investigation.

Introduction

Multiple myeloma is a B-cell malignancy caused by uncontrolled clonal proliferation of plasma cells in the bone marrow or outside it (affecting the liver and spleen). Myeloma plasma cells are similar to long-lived normal plasma cells and, similarly, they show a strong dependence on the bone marrow microenvironment. Probably in the process of malignant transformation, not fully known genetic processes are activated and they alter the surface expression of certain antigens, such as CD19, CD45 and CD561,2, owing to which it is possible to distinguish normal from malignant plasma cells. The phenotype of normal plasma cells is defined as CD38+CD138+CD19+CD45+CD56. In contrast, cancer cells in multiple myeloma exhibit positive expression of CD38 and CD138, but as many as 90% of them are CD19-, 99% CD45- or CD45 low, and 70% CD56+1,2.

The clinical course of multiple myeloma is characterised by frequent occurrence of numerous complications including the presence of osteolytic lesions in bones, anaemia, and immune disorders with reduced levels of serum immunoglobulins. The presence of monoclonal protein produced by malignant plasma cells in serum and/or urine of patients, which often leads to renal dysfunction, is also very characteristic1. Myeloma accounts for approximately 1% of all malignant tumours and approximately 14% of haematological malignancies. The annual incidence in Europe is approximately 4.5/100 thousand. It has been shown that more men than women suffer from the disease, with a ratio of 3:2. The incidence of MM increases with age, with peak incidence in the seventh decade of life (median age, 65 years). Despite the significant progress in MM treatment and longer median lifespan of MM patients (from 3 to 6 years), it is still an incurable disease1. The aim of this review was to discuss the functional significance of the bone marrow microenvironment in multiple myeloma development and progression.

Discussion

The role of the cellular compartment in multiple myeloma development and progression

The bone marrow microenvironment plays an essential role in multiple...
myeloma development and progression, and in the development of cytostatic drug resistance. Conditions of the bone marrow determine both maturation and maintenance of stem cells and blood precursor cells under physiological conditions and in malignancies such as multiple myeloma. Studies on the biology of MM clearly indicate that its induction probably results from engraftment of long-lived normal plasma cells, produced in the germline centres of peripheral lymphoid tissues in the bone marrow. It has been shown that the bone marrow environment per se may promote carcinogenesis, inducing malignant transformation of normal plasma cells. In addition, conditions in the bone marrow, in particular, the presence of a number of myeloma cell growth factors, promote their survival and proliferation in the bone marrow niches.

The role of stromal cells
In the process of carcinogenesis within bone marrow stromal elements, structural and functional changes occur and they cause an imbalance between factors that stimulate and inhibit the growth and differentiation of progenitor blood cells. In MM patients, there are changes in interactions between malignant plasma cells derived from multiple myeloma stem cells and the microenvironment. These interactions play a significant role in the proliferation, migration, and survival time of myeloma cells and the development of cytostatic drug resistance. Interactions between myeloma cells, stromal cells, and elements of the extracellular matrix (ECM) occur directly via cell receptors and adhesion molecules, such as integrins, proteoglycans, immunoglobulins, cadherins, selectins, and syndecans, which are present on the surface of myeloma cells. Plasma cell adhesion to extracellular matrix proteins (e.g., collagen, mainly type I and VI), fibronectin, laminin, vitronectin) activates signalling pathways in plasma cells, which affects their proliferation and survival, development of drug resistance, as well as synthesis and secretion of urokinase-type plasminogen activator and metalloproteinases. Some of the adhesion molecules, such as cadherins, facilitate intercellular binding and participate in the formation of functional multicellular structures in the bone marrow by anchoring to the actin cytoskeleton of cell through catenins. Free catenins which accumulate in plasma cells in high concentrations are involved in Wnt signal transduction pathways, activating certain oncogenes (e.g., c-Myc) or cyclin D1, and thereby contributing to the development of cancer.

In the process of adhesion of myeloma cells to stromal cells, an important role is also played by extracellular factors, such as cytokines, chemokines, and growth factors, which have the ability to activate multiple signalling pathways (such as NF-kB and Notch), and increase the expression of cell cycle regulatory proteins (D-type cyclins) and Bcl-2 family anti-apoptotic proteins in both stromal and myeloma cells. The activation of these cells leads to secretion of factors that are of particular importance for the proliferation and survival of myeloma cells, especially IL-6, VEGF, and IGF-1, to the environment and intensification of chemotherapy resistance. It has also been confirmed that factors such as basic fibroblast growth factor (bFGF), angiopoietin-1 (Ang-1), transforming growth factor beta (TGF-beta), platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), and interleukin 1 (IL-1), secreted by stromal cells of patients with MM, affect the growth of myeloma cells. Recently, it has been reported that there is a novel mechanism of intercellular transfer of genetic information which involves stromal cell-derived exosomes, which, after entering myeloma cells, modulate their growth mediated by specific miRNAs.

Stromal cells are also a source of chemokines - CCL2 (MCP-1), CCL8 (MCP-2), and CCL7 (MCP-3) - involved in the migration and homing of myeloma cells to the bone marrow due to the presence of chemokine receptor CCR2 on their surface. However, a special role in this process is attributed to the SDF-1 (CXCL12)/CXCR4 axis. Stromal cells also have the ability to secrete CCL3 chemokine (MIP-1α), affecting the severity of adhesion and interaction between the integrins of myeloma plasma cells and vascular cell adhesion protein 1 (VCAM-1) molecules on the surface of endothelial cells, which in turn promotes neovascularisation in the bone marrow of MM patients. Further expansion of plasma cells in the bone marrowstroma is promoted by cytokines, growth factors, and metalloproteinases (especially MMP-9, MMP-2, IL-6, VEGF, TNFα, SDF-1, MCP-1α, IGF-1, IL-1, IL-3, IL-10 and IL-15), secreted by a variety of bone marrow cells.

The role of endothelial cells and angiogenesis
Clear intensification of angiogenesis in samples from patients with MM, measured by microvessel density (MVD), is observed in patients with active disease as compared to those with inactive MM or monoclonal gammopathy of undetermined significance (MGUS). Bone marrow angiogenesis in multiple myeloma is a determinant of tumour cell growth and disease progression, and a process partially regulated by pro-angiogenic factors released by plasma cells, stromal cells, and osteoclasts. Among these factors, the most important role is attributed to VEGF, bFGF, MMP, IL-6, TNFα, HGF, and chemokines IGF-1, MCP-1, MIP-1, MCP-1, and SDF-1, the secretion of which is a consequence of interaction between stromal cells and myeloma plasma cells. Constitutive secretion of VEGF and bFGF by myeloma cells may also result from the activation of oncogenes and/or genetic mutations.
In order to identify the vascular mechanism underlying metastasis and disease progression, expression profiling of genes involved in the regulation of ECM formation and bone marrow remodelling, angiogenesis, cell cycle regulation, chemotaxis, cell adhesion, and resistance to apoptosis, or processes which promote disease progression, was performed in the endothelial cells of MM patients, showing aberrant expression of 22 genes assayed. These studies have highlighted the role of the microenvironment in the induction of bone marrow neovascularisation, encouraging development of tumour cells and MM progression. This study clearly shows that the use of antiangiogenic factors in multiple myeloma therapy can significantly improve the prognosis.

The role of osteoclasts and osteoblasts

The observed severe bone destruction in the immediate vicinity of myeloma cells indicates their participation in the process of osteoclastogenesis. In particular, it has been shown that the interaction of myeloma cells with stromal cells in the bone marrow leads to increased expression of RANKL (receptor activator of NF-κB ligand) on myeloma cells, thus generating a signal for the activation of osteoclast precursors that express the cell-surface receptor RANK. The RANKL and OPG (osteoprotegerin) molecules, which are their competitive receptors, are assigned the most important role in the regulation of bone resorption. In physiological conditions, a dynamic balance between RANKL and OPG is maintained by their competitive receptors, which are assigned the most important role in the regulation of bone resorption.

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The impact of the extracellular environment on multiple myeloma development

Complex positive and negative interactions mediated by various adhesion molecules, cytokines, and their receptors occur between individual bone marrow cells and myeloma cells. Some of the growth and survival factors for myeloma cells, such as IL-6, are produced by many types of bone cells (osteoblasts, osteoclasts, stromal cells) and the MM cells themselves. Additional external factors such as hypoxia or internal signals generated by a deregulated c-Myc oncogene in myeloma cells lead to hypoxia-inducible factor 1α (HIF-1α) activation and VEGF secretion by myeloma cells. VEGF, in turn, stimulates endothelial cells to secrete IGF-1, which induces proliferation of myeloma cells. Thus, interleukin-6, VEGF, and IGF constitute a network of factors essential for MM development and progression.

Interleukin-6

Interleukin-6 (IL-6) is a key growth and survival factor for multiple myeloma cells, originally produced by stromal cells, osteoclasts, and malignant plasma cells, and affecting tumour growth by autocrine and paracrine mechanisms. IL-6 secretion by medullary stromal cells is regulated directly by adhesion to myeloma cells, and indirectly by soluble factors secreted by these cells (TNFα, VEGF, IL-1β, TGFβ), which lead to activation of the transcription factor NF-κB in plasma cells. Thus, a cross-regulation network develops between the tumour cells and the microenvironment, which promotes myeloma progression. After binding to its receptor on myeloma cells, IL-6 induces a signalling pathway that leads to activation and proliferation of plasma cells, inhibits the activity of p27 and p21 inhibitors, and activates anti-apoptotic proteins (Mcl-1 and Bcl-x) and c-Myc. It has been observed that a high level of IL-6 in the serum of MM patients is correlated with poor prognosis and an increased percentage of proliferating myeloma cells in the peripheral blood.
Insulin-like growth factor 1

Insulin-like growth factor 1 (IGF-1) is a factor promoting the carcinogenesis of many cancers. IGF-1 is secreted by stromal cells, endothelial cells, and bone marrow osteoblasts, and in the paracrine mechanism, it is conducive to MM development by binding to its receptor on tumour cells. This interaction leads to a shift in the balance of factors associated with apoptosis towards cell death inhibitors in myeloma cells.

Vascular endothelial growth factor

It has been shown that increased bone marrow vascularisation is positively correlated with a poor prognosis of cancer patients; the growth of new blood vessels significantly improves the conditions for nutrient and oxygen transport to cells, facilitating further growth of the tumour. Vascular endothelial growth factor (VEGF), as a known pro-angiogenic factor in a number of haematological malignancies including multiple myeloma, is clearly associated with disease progression. Secreted by stromal and myeloma cells under the influence of cytokines and other growth factors, such as IL-6, bFGF, TGFβ, and TNFα, present in the bone marrow of MM patients, it enhances the development of a favourable microenvironment conducive to gradual progression of the disease.

Multiple myeloma progression

In most cases, myeloma cells develop primarily in the bone marrow, although in aggressive forms of the disease, malignant plasma cells can also be home to extramedullary sites, such as spleen, liver, and body fluids. Symptomatic MM progression is associated with further expansion of myeloma cells within the bone marrow and spreads to secondary sites in bone marrow by the bloodstream. Myeloma cell migration from the blood to the bone marrow (called homing) is a multi-stage process actively managed by several interactions in the bone marrow, primarily initiated by the activation of selectins. The next stages of homing, such as adhesion and extravasation, when myeloma cells exit the capillaries, occur by the activation of integrins LFA-1 and VLA-4 on their surface. However, substantial mobilisation of malignant plasma cells from the peripheral blood to the bone marrow occurs due to activity of the SDF-1/CXCR4 axis. SDF-1 chemokine, which induces homing of myeloma cells into the bone marrow, is secreted by stromal cells (e.g. endothelial cells, mesenchymal stem cells), and binds specifically to the cell-surface receptor CXCR4 expressed on plasma cells. Worth mentioning is the large diversity of CXCR4 expression on myeloma cells, confirmed on the surface of 10% to 100% of the cells. It has been found that blocking CXCR4 inhibits migration of myeloma cells to SDF-1 chemokines in the bone marrow. In turn, migration and expansion of myeloma cells in the bone marrow and their mobilisation or egress into the peripheral blood occur due to disruption of the SDF-1/CXCR4 axis, which involves weakening of SDF-1 expression under the influence of bone marrow environment proteases and intensification of CXCR4 expression following hypoxic myeloid niches (1%-2% O₂).

Nevertheless, the bone marrow microenvironment is not sufficiently prepared for metastatic myeloma cell engraftment. The requirement for MM dissemination within the bone marrow is the adaptation of myeloid niches to conditions that enable further development of MM, which is mediated by ECM components, stroma, endothelial cells, cytokines, chemokines, and growth factors, in particular IL-6, IGF-1, and APRIL. It has been demonstrated that myeloma cells can invade large areas of the bone marrow with particular ease by entering into abnormal interactions with both ECM proteins and bone marrow stromal cells, on which they are closely dependent. The formation of premetastatic niches in the bone marrow, which are an expression of a pathological response of the bone marrow environment to the presence of myeloma cells, occurs even before the first arrival of metastatic cells and substantially facilitates their distribution, thus promoting the creation of new malignant foci. This results in characteristic premetastatic disorders in the said components of the bone marrow microenvironment, which together facilitate the growth and survival of the metastasizing myeloma cells. This specific bone marrow microenvironment remodelling is also affected by increased osteoclast activity mediated by RANKL/RANK and MIP-1alpha, osteoblastogenesis attenuated by DKK1 and IL-3, as well as the previously mentioned intensive neangiogenesis. It is believed that myeloma cells circulating in the peripheral blood and molecular factors secreted by them, including metalloproteinases, are also of particular importance in the phenomenon of MM bone marrow metastases. Metalloproteinases (MMPs) have the ability to degrade ECM, and at the same time, they stimulate angiogenesis, which promotes the spread of malignant cells. Among the various MMPs, considerable importance in MM progression is attributed mainly to gelatinases MMP-2 and MMP-9, which degrade type IV collagen, the major component of the basal membrane of cells, and thus affect their ability to spread myeloma. Among other factors involved in premetastatic bone marrow remodelling, there are also adhesion molecules, characterised by increased VLA-4 expression on the surface of myeloma cells, and exosomes which induce neoangiogenesis in metastatic lesion foci. Additionally, cells of haematopoietic origin that exhibit the expression of fibronectin (VLA-4) and VEGF (VEGFR1) receptors on their surface allow implantation.
of new myeloma cells into the bone marrow.

Conclusion
The recent studies clearly emphasize a strong interaction between myeloma cells and elements of the bone marrow microenvironment both in MM development and progression. It even seems that the role of each component of the tumour-host interactions is equivalent in MM pathogenesis. Novel therapeutic approaches should target not only the malignant plasma cell, but also its interaction with the bone marrow microenvironment, to sufficiently prevent disease progression. Despite administration of several immunomodulators and proteasome inhibitors, other therapies are still under active investigation.

Abbreviations list
Ang-1, angiopoietin-1; bFGF, basic fibroblast growth factor; ECM, extracellular matrix; HGF, hepatocyte growth factor; HIF-α, hypoxia-inducible factor 1-α; KIF-1, insulin-like growth factor 1; IL-1, interleukin 1; IL-6, interleukin 6; MM, multiple myeloma; MGUS, monoclonal gammopathy of undetermined significance; MVD, microvessel density; PDGF, platelet-derived growth factor; RANKL, receptor activator of NF-κB ligand; TGF-beta, transforming growth factor beta; VCAM-1, vascular cell adhesion protein 1.

References
Critical review

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Competing interests: none declared. Conflict of interests: none declared.

All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript.

All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.