REVIEW PAPER

The Hemopoietic Stem Cell Niche Versus the Microenvironment of the Multiple Myeloma-Tumor Initiating Cell

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Abstract Multiple myeloma cells are reminiscent of hemopoietic stem cells in their strict dependence upon the bone marrow microenvironment. However, from all other points of view, multiple myeloma cells differ markedly from stem cells. The cells possess a mature phenotype and secrete antibodies, and have thus made the whole journey to maturity, while maintaining a tumor phenotype. Not much credence was given to the possibility that the bulk of plasma-like multiple myeloma tumor cells is generated from tumor-initiating cells. Although interleukin-6 is a major contributor to the formation of the tumor's microenvironment in multiple myeloma, it is not a major factor within hemopoietic stem cell niches. The bone marrow niche for myeloma cells includes the activity of inflammatory cytokines released through osteoclastogenesis. These permit maintenance of myeloma cells within the bone marrow. In contrast, osteoclastogenesis constitutes a signal that drives hemopoietic stem cells away from their bone marrow niches. The properties of the bone marrow microenvironment, which supports myeloma cell maintenance and proliferation, is therefore markedly different from the characteristics of the hemopoietic stem cell niche. Thus, multiple myeloma presents an example of a hemopoietic tumor microenvironment that does not resemble the corresponding stem cell renewal niche.

Keywords Multiple myeloma · Tumor-initiating cells · Hemopoietic stem cells · Stem cell niches · Bone marrow microenvironment

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Introduction

Multiple myeloma (MM) is a human malignancy first reported approximately 160 years ago. It apparently initiates by over proliferation of plasma cells, a status called monoclonal gammopathy of undetermined significance (MGUS), that may progress to overt MM in which tumor plasma cells accumulate within the bone marrow microenvironment, causing a bone destructive disease (reviewed by [1–8]). The bone marrow localization of these tumor cells is a most characteristic feature of MM, and it is only at the very late stages of the disease, that the tumor cells may disseminate to other locales. Several drugs, and various other therapeutic modes, such as antibodies to cell surface markers of the tumor cells, are used in the clinic to reduce the disease symptoms (reviewed by [9]. An example is the use of a single-chain Fv against leukocyte antigen-A that causes myeloma cell death [10]. An effective cure for MM is still lacking thereby calling for further investigations into the nature of this disease.

The specific bone marrow localization of MM is reminiscent of the strict dependence of the precursors of plasma cells, i.e., the hemopoietic stem cells (HSCs), upon specific bone marrow niches. This goes along with the cancer stem cell (CSC) theory dealing with heterogeneity among the tumor cell populations (reviewed in [11–36]). It is argued that most cells in the tumor are comparable to mature normal cell counterparts; these "mature" tumor cells are incapable of initiating new tumor formation upon isolation and transplantation into susceptible recipients. By the same token, mature blood cells would be incapable of engraftment and of the establishment of a new bone marrow structure. In contrast, a minor cell population within the tumor, consists of highly proliferating cells capable of tumor formation upon transplantation. These are

said to be the CSCs based on their rareness, possession of cell surface markers similar to their normal counterpart stem cells and by their superior ability to form tumors. This latter function is regarded as the tumor corollary of the ability of the normal HSCs to create a new hemopoietic tissue in animals or humans deficient in this tissue. The analogy made by many investigators, between tumor formation on the one hand, and normal tissue regeneration on the other, seems somewhat exaggerated: normal tissue generation is accurately programmed and is restricted to embryonic life, while tumor formation is highly chaotic, and may occur throughout the entire life span. These processes, therefore, have little in common. However, in this review, the CSC theory will be taken as is, and the question dealt with below is whether CSCs occur in MM or whether they are absent from this disease.

Several reports show that cellular heterogeneity exists in MM. Only rare cells, within mouse plasmacytoma cell lines, formed tumors upon transplantation [37–41]. Similarly, in vitro analysis of primary MM cells indicated a low incidence of clone forming cells [42]. Thus, the heterogeneous MM population contains some rare clonogenic cells that, at least in vitro, show high proliferation potential. More importantly these cells were capable of recreating the disease in recipient immunodeficient animals [38, 40]. Although the human-to-mouse transplantation model is widely used [43] it is unclear how much it represents a true corollary of the human disease (commented on by [44]). Indeed, human MM has an immune component which is of importance [45], and many cytokines that may have relevance to MM, do not operate effectively across species barriers. From this viewpoint, a 3 dimensional reconstruction of the MM microenvironment ex vivo might prove as informative [46]. The data derived from in vitro and in vivo studies on clonogenic and transplantable MM cells show nevertheless that MM contains a tumor-initiating cell (TIC) component (Box-1). Are these TICs, the MM CSCs? One way to define CSCs is to purify them on the basis of surface marker expression. Such an approach suggested that the putative MM CSCs are $CD138^-/CD20^+$ [39]. However, cells capable of transferring the disease were found also in the $CD138^+$ population as well as in the side population (SP) (Commented on by [47]). Whereas the MM TICs were reported to reside within the $CD34^+$ population of hemopoietic progenitors [40], other claims relate these cells to the memory B cell population [48, 49]. It is therefore clear, at this point, that marker expression is not a reliable means of identifying CSCs or TICs with any degree of certainty. This is in fact true in the case of all stem cell type (reviewed by [50, 51]). The findings that most plasma tumor cells are capable of forming tumors in immunodeficient mice, when the appropriate conditions are provided [52, 53] are more dramatic. According to these findings, the rareness of TICs, which is often compared to the low incidence of normal HSCs found in the bone marrow, is just apparent. A high incidence of tumor-forming cells in the cancerous population has been reported [54]. It is probable that TICs seem rare in some assays because of the harsh conditions used that allow only some of the TICs to express their tumor forming potential.

Box-1

Human tumor-initiating cells are often defined as the cells, within an established tumor or a cultured tumor cell line, endowed with a clonogenic capacity in vitro. Herein, a more critical definition is preferred and relates to the capacity of cells, to transfer the disease to immunodeficient animals upon transplantation. The result of this transplantation must be the formation of a tumor, in the mouse, which is of human origin and the disease must share some features with the corresponding human cancer.

One important point of note is that high proliferation potential is not a HSC property. The latter renew very slowly and their division is a rare event [55]. This constitutes one major difference between HSCs and clonogenic MM cells. Colony formation in culture is mostly a committed hemopoietic progenitor property. Progenitors do proliferate extensively, but transiently. This is in sharp contrast to HSCs that do not proliferate extensively and perform only rare self-renewal divisions. Therefore the in vitro clonogenic cells differ markedly from HSCs and whether they are identical to in vivo TICs or CSCs is questionable. Although high in vitro proliferation capacity is often regarded as a cancer stem cell property (reviewed by [48, 49, 56]), the link between this property

and the capacity to form tumors is not apparent. The CSC theory is not further analyzed within this review, whereas the similarity/difference between MM TICs versus normal HSCs is examined in detail. The normal HSC in its niche is described first, followed by an analysis of the MM TIC in its bone marrow microenvironment. It is argued that these two cell types and their corresponding microenvironments have little in common.

It is of great importance that a distinction between stem cell niches in particular, and microenvironments in general (Box-2) be made. The microenvironment of an organ contains a wide range of different domains. Some of these are designed to support differentiation, others promote release from the bone marrow compartment and only the highly specialized and rare ones are designed to support stem cell renewal. The latter are designated as the "stem cell niches" (Fig. 1). The function of the stem cell niche is to maintain the stem cell phenotype. The release of the stem cell from the niche will result in differentiation. The terms "niche" and "microenvironment" are often interchangeable in the literature related to stemness. One example is the definition of the niche as an entity essential for survival, growth and differentiation [57]. Clearly, stem cell niches are engaged in antagonism with differentiation rather than with promotion of this process [58–60]. Differentiation inducing domains should therefore not be confused with stem cell maintenance niches. Thus, HSC niches are defined herein as the specific locals within the bone marrow microenvironment, which specialize in maintenance of stem cells in their undifferentiated state.

The Hemopoietic Stem Cell in its Bone Marrow Niche

Hemopoiesis occurs in the bone marrow, within the bone cavity, wherein the hemopoietic cells and their descendents are engulfed and compartmentalized by a microenvironmental tissue composed of various cell types. This is referred to collectively as the bone marrow stroma. Cells that belong in this category are mesenchymal cells (such as adipocytes and adventitial cells) endothelial cells and some derivatives of the HSC, such as macrophages. The bone marrow stroma is apparently vital for the normal functioning of HSCs. The latter cells depend on the stroma for their renewal and differentiation. It is noteworthy, that most experimental data that demonstrate the functions of stromal cells, have been obtained from in vitro studies. Nonetheless, in vivo observations demonstrated close proximity between hemopoietic cells of immature phenotype and stromal elements, strongly supporting the importance of the stroma in the maintenance and regulation of hemopoiesis.

Although not as solid as epithelial organs, the bone marrow is a well-organized tissue. In the former, cell
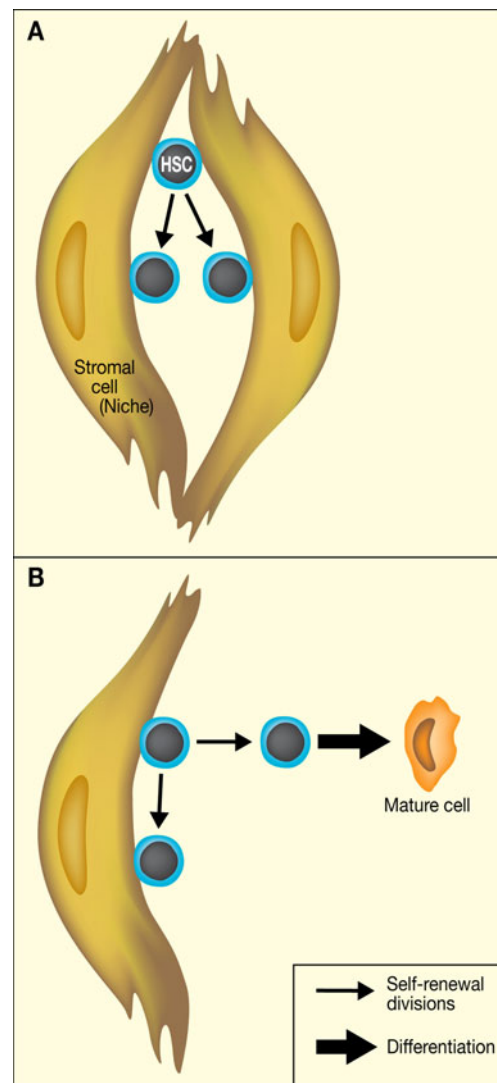
Fig. 1 The general structure of the HSC niche: Self-renewal divisions occurs as long as the stem cell remains within the niche **a**. Detachment from the niche and departure into other microenvironmental domains result in differentiation **b**

contacts are enforced by junctional complexes that tightly bind one cell to another. In contrast, within the bone marrow, cells interact through adhesion molecules but are not tightly bound. Hemopoietic cells are capable of intensive motion and migration, within the bone marrow proper, and also migrate back into it from the blood. The bone marrow is nevertheless well organized into distinct compartments, due to the meshwork of stromal cells. The stroma, including the vasculature forms a sponge-like structure attached to the trabecular bone projections, serves both a structural as well as a regulatory function. The localization of stem cells within the bone cavity is not random. It has been shown, by use of histological examination of the bone marrow following depletion, that foci of hemopoiesis first appear at endosteal and periarteolar sites [61]. These

morphological studies implied that stem cells reside in the specific regions mentioned above, and following injury they start proliferating to supply new mature cells. It was later found that HSCs are specifically localized to the region close to the endosteal bone that was termed the stem cell niche [62, 63].

Box-2

A strict distinction is made herein between the terms "niche" and, "microenvironment"; these terms are often used interchangeably in the literature related to stem cell biology. The "stem cell niche" in the sense used herein, means the physical-chemical entity that provides minimal requirements for the survival, and often self-renewal of stem cells. This term does not include other tissue domains in which stem cells may reside transiently while undergoing a process of differentiation. Such sites are referred to as "differentiation domains". The term "microenvironment", used herein, includes all the variety of domains existing within a specific tissue. Thus, when the term microenvironment is mentioned with regard to MM cells, it refers to the fact that MM cells reside within the bone marrow at large, and may be found in a rather widespread pattern crossing different specific bone marrow domains, as tumor cells often do.

One major constituent of the bone marrow stroma is the mesenchymal tissue. These cells are mostly defined through elimination, in lacking properties of well-defined cells such as hemopoietic, epithelial, neuronal, and endothelial cells. The mesenchyme does not exhibit a specific gene set that may be used practically to identify the cells belonging to this category in vivo. Their identification in culture relies on their adhesion, fibroblast-like morphology and migratory properties. In vitro propagation of mesenchymal cells from bone marrow origin and their re-introduction in vivo demonstrated the capacity of these cells to create a local ectopic stromal structure that induces the homing of HSCs and the subsequent initiation of active hemopoiesis; in vitro seeding of bone marrow cells from guinea pigs resulted in death of the hemopoietic cells and growth of adherent fibroblastoid cells. These cells were suggested to be representatives of the bone marrow microenvironment stromata [64]. The transplantation of these cells, under the kidney capsule of recipient guinea pigs, resulted in the formation of bone structures containing hemopoietic cells. Whereas the bone was found to be of donor fibroblast origin, the hemopoietic cells within the bone structure were of recipient origin [65]. Single cells isolated from fibroblastoid colonies were recently shown to form bone structures in vivo in which hemopoiesis occurred [66]. Co-culture of bone marrow fibroblasts, with freshly isolated hemopoietic cells, results under specific in vitro conditions, in long-term hemopoiesis [67]. Overall, the in vitro culture experiments, taken together with the in vivo findings and morphological observations on the bone marrow structure, suggest that the mesenchymal component of the bone marrow, significantly contributes to the formation of the hemopoietic microenvironment and particularly to the maintenance of the HSC pool. These cells may therefore contribute to the formation of the HSC niche.

The implications of the above studies found support in recent experiments in which the bone marrow niche of HSCs was investigated in vivo. It was found that HSCs reside in the endosteal region in the proximity of osteoblastic cells [68]. HSCs were also found in vascular niches [69]. The niche of normal HSCs seems therefore to be made either of osteoblasts or alternatively endothelial cells, reticular adventitial cells or all of these cells. It is unclear whether the above cell types constitute different entities, or whether they are all derivatives of mesenchymal cells.

A long list of molecules is supposed to contribute, in varying degrees, to the stem cell maintaining capacity of the HSC niche. These include adhesion molecules such as N-candherin [70], very late antigen-4 (VLA-4) [71]) and vascular cell adhesion molecule-1 (VCAM-1) [72]. Hormones and growth factors have been similarly implicated including parathyroid hormone acting through its bone

marrow receptors [68], osteopontin [73], bone morphogenic protein (BMP) [74], stromal derived factor-1 (SDF-1) and its receptor CXCR4 [75], angiopoietin through its receptor Tie-2 [76], Wnt pathway activation [77], thrombopoietin and its receptor Mpl [78], stem cell factor and its receptor cKit [79], insulin-like growth fctor2 [80], and other factors (reviewed by [81, 82]). The relative contribution of each of the above to niche formation is not completely understood. It is however obvious that stem cell maintenance requires a plethora of signals, rather then a single putative "stem cell renewal factor", which has been pursued for decades.

The Restrictive Nature of Stem Cell Niches

Notably, several of the factors that emanate from the stem cell niche, listed above as regulators of HSCs, operate in a restrictive manner and do not promote growth. They are often referred to as negative regulators or inhibitors. Earlier attempts to induce in vitro HSC growth, involved a long list of conditions that failed to allow survival of HSCs and their subsequent long-term proliferation. The long-term bone marrow cultures, in which fresh bone marrow is seeded onto pre-formed confluent stromal cell layers, demonstrated the importance and requirement of the stroma for continued hemopoiesis. The combination of stromal cell layers, with hemopoietic colony assays in semisolid medium, allowed the investigation of the events that occur at a single colony forming cell level. The fate of the hemopoietic progenitors in such co-cultures was determined by the stromal cells; hemopoietic colony formation was suppressed by the stroma, and instead of large colonies containing differenti-ated cells, micro clusters of progenitors emerged. The presence of stroma overrides the effect of cytokines that are needed for colony formation. Separation of the hemopoietic cells from the stroma, by an agar layer, leads to the effect of the stroma being abolished [58, 59]. Thus, the proximity of the hemopoietic progenitor cell, to the stroma, represses the capacity of cytokines to induce differentiation and, thereby allowing by default, the maintenance of the undifferentiated cells [51]). These experiments suggested, for the first time, that the stroma protects HSCs from differentiation [83] by antagonizing with the function of differentiation-inducing cytokines. The differentiation restraining activity was ascribed to members of the transforming growth factor (TGF) β family, which are differentiation antagonists [84, 85] (Fig. 2). The latter family in addition to other molecules, were all proposed to take part in the formation of bone marrow stem cell niches.

The role of differentiation antagonists, in the creation of stem cell niches, has now been well established through studies of *Drosophila Melanogaster* [60]. Both the gonadal stem cells and the niche cells can be morphologically
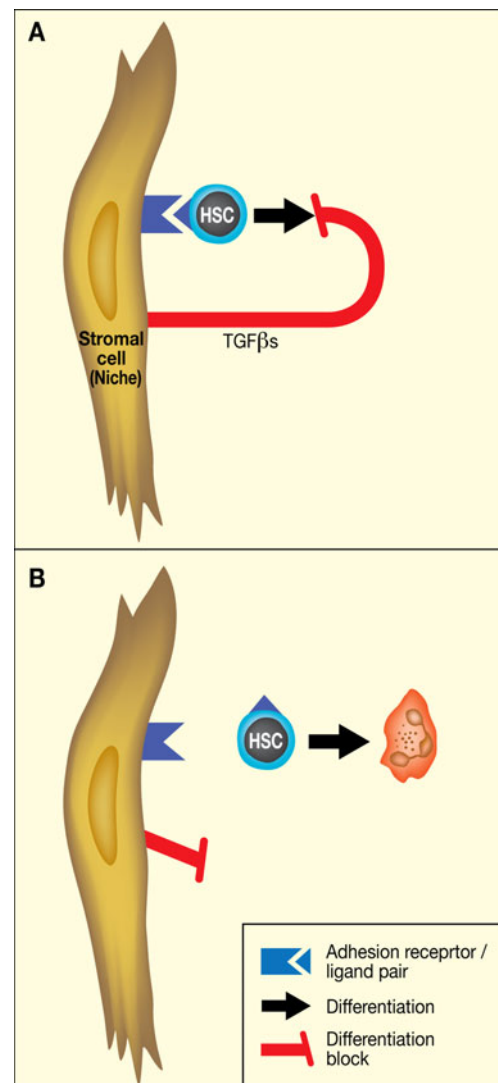
Fig. 2 The mechanism of HSC maintenance within its niche, in an undifferentiated form: Differentiation antagonists such as TGFβs, block the capacity of the HSC to respond to differentiation inducing cytokines **a**. Detachment from the niche releases the stem cell from the effect of the antagonist **b**

identified in situ. This is completely different from mammalian HSCs, and in fact differs from all other mammalian stem cell systems, which are hard to detect and do not have specific markers that identify them unequivocally. The outstanding advantage of *Drosophila* stem cells, in being morphologically conspicuous assisted in identifying factors that maintain stem cells in an undifferentiated state within their niche. First and foremost, the direct contact of the stem cell with the niche cell is obligatory. The second requirement is the activity of decapentaplegic (Dpp), the *Drosophila* homologue of the TGFβ molecule bone morophogenic protein (BMP). Dpp antagonizes the activity of bag of marbles (BOM), which is a differentiation factor, enabling the maintenance of stem

cells in an undifferentiated state. The earlier studies on hemopoietic cells and the later on gonadal cells of the fruit fly, taken together, strongly suggest that the stem cell niche provides antagonism with differentiation. This is mediated through molecules such as TGFβs, thereby protecting stem cells from overdifferentiation, and directing self-renewal by default.

The Stem Cell Niche and the Stem State

It thus appears that a niche cell forms a platform for the adhesion of the stem cell. Physical separation from the niche allows the stem cells to react to differentiation factors. In *Drosophila*, divisions of stem cells that occur while the spindle is perpendicular to the niche, leave one stem cell adherent to the niche. This cell maintains its stemness. The other cell is detached from the niche and starts a differentiation process. However, when such a cell is forced back into the niche, it reassumes a stem cell fate [86] (Fig. 3). Thus, at least in *Drosophila*, de-differentiation occurs and stem cells may be born out of differentiating cells and not only through self-renewal, as hypothesized for HSCs. Does de-differentiation occur in mammalians? Several publications suggest this option [87–89]. In view of the great similarities between stem cell niches in organisms including plants, *Drosophila* and mammalians, de-differentiation in mammalians is a realistic possibility which leads to the suggestion that stemness in mammalians, represents a reversible state in the life cycle of the cell [50, 51]. This view is compatible with the ease by which mature cells may be reprogrammed. The reprogramming of skin fibroblasts, liver and gut cells and B lymphocytes, requires the forced expression of 3-4 genes [90–93] whereas the de-differentiation of neuronal stem cell into induced pluripotent cells requires the overexpression of oct-4 and Klf4 only [94]. More dramatic is the reprogramming of spermatogonia that occurs spontaneously upon culture without the need for forced expression of exogenously introduced genes [95].

The Bone Marrow Microenvironment of MM Tumor-Initiating Cells

The bone marrow microenvironment is supportive of cells that apparently do not belong there, such as, metastasizing cells of small lung cell carcinoma, breast carcinoma and prostate tumors. In addition to these remote tumors, the bone marrow microenvironment is a site for the development and maintenance of MM. This bone marrow localization is a fundamental characteristic of MM. Most stages of the disease occur within the bone marrow while
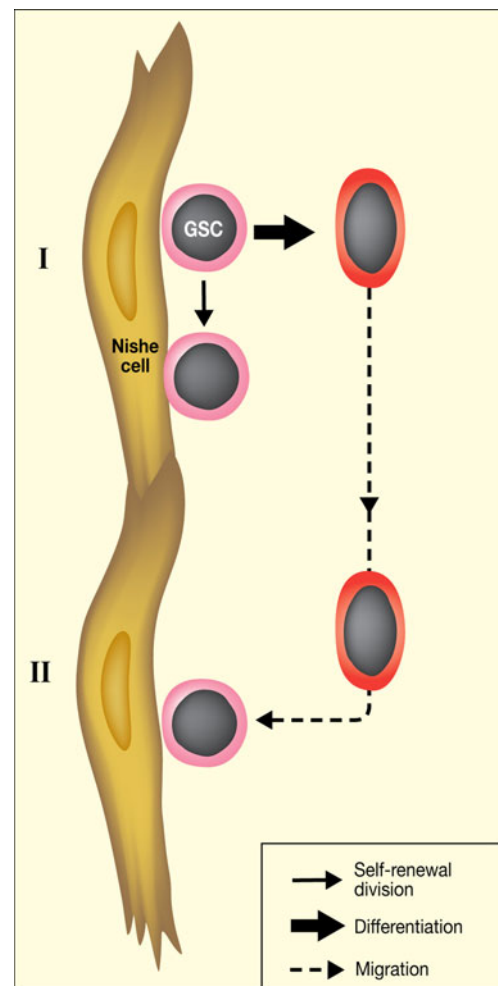
Fig. 3 De-differentiation in Drosophila gonads: The gonadal stem cell (GSC) that remain attached to their niche following division, remain undifferentiated (I) whereas they differentiate upon release from the niche (I). GSC de-differentiate upon return to the niche (II)

dissemination to other tissues and organs is a late occurrence in this disease. It is inferred that the bone marrow microenvironment is required for the development of MM, and that the MM tumor cells, are dependent upon stromal elements of the bone marrow compartment. It has further been suggested that MM cells utilize for their survival and proliferation, molecular cues provided by the bone marrow environment, that are similar to those supporting normal stem cell growth and differentiation. This statement is however hypothetical; it is still not entirely clear what are the molecular cues that the stroma elaborate, which affect hemopoiesis in vivo, both in the case of normal stem cells and in the case of MM cells.

An additional characteristic feature of MM is the increased osteolytic activity, leading to the high incidence of bone fractures. Bone homeostasis is maintained normally by two opposing activities. Osteoblasts build bone by depositing extracellular-matrix that undergoes mineralization.

Osteoclasts degrade bone structures by creating a sealing zone into which they release protons and proteases that dissolve the mineral and proteins. The balance between bone formation by osteoblasts and degradation by osteoclasts, results in normal bone remodeling and maintenance of an intact bone structure. It is believed that MM cells cause bone damage by inducing osteoclastogenesis and by diminishing osteogenic functions.

Evidence for the dependence of MM cells upon stromal cells from the bone marrow is mostly indirect. A common way to demonstrate the contribution of a particular cell to a specific function would be cell ablation. Thus, knockout of the T cell receptor (TCR) would result in a lack of mature T cells and is a clear-cut demonstration of the subsequent lack of cellular immunity. A technology for bone marrow stromal cell ablation is unavailable. There are only circumstantial indications for the dependence of MM cells on the bone marrow stroma. Moreover, as will emerge from the following description of experimental systems used to study this issue, most information is based on cell cultures. Several animal models have been constructed for the study of MM. In the mouse, subcutaneous tumors are often used to study the disease. These, however, are rather remote from the human situation. In contrast, the 5T22 tumor model allows for the growth of MM cells within the bone marrow compartment, and therefore this model is the closest to human MM [96]. Immunodeficient mouse models are employed in order to enable the study of human MM cells. One example is the transplantation of human bone into these mice, followed by intra-bone implantation of MM cells [43]. While this model allows the study of human MM cells in vivo, it lacks compatibility in hormonal and cytokine signaling. Furthermore, the model does not include human immune cells, such as T regulatory cells, which are thought to contribute to the pathogenesis of MM.

The general view of MM is that the tumor cells adhere to the bone marrow stroma, and are dependent for their survival and proliferation on growth factors elaborated in the bone marrow microenvironment. Subsequent to their adhesion to the stroma, MM cells induce the secretion of various cytokines, thereby enhancing osteoclastogenesis and damaging osteobalstogenesis. In MM the predominance of the osteoclastic activity brings about bone lesions and fractures, further enhancing the elaboration of cytokines and enhancing MM cell proliferation. These general features of MM are further elaborated on below.

Adhesion of MM Cells to Bone Marrow Stromal Cells

The adhesion of MM cells to the mesenchymal stroma of the bone marrow has been suggested as being crucial for the proliferation of MM cells. Interference with adhesion

processes reduced MM growth, secretion of interleukin (IL)-6 and osteolysis. MM cell lines, co-cultured with a stromal cell line, are often used to study the interactions between MM cells and the stroma. The 5TGM1 mouse myeloma was shown to adhere to the stromal cell line ST2 in one such study. This adherence is mediated by $\alpha 4\beta 1$ integrin expressed by the myeloma cell line and VCAM-1 found on the surface of the stromal cell [97]. The interaction between the myeloma and stromal cell caused release of bone resorbing activity. The role of $\alpha 4\beta 1$ integrin was further substantiated in an in vivo study, in which the 5TGM1 myeloma was transplanted into mice, thereby causing osteolysis. This bone damage could be reduced by treatment with antibodies to $\alpha 4$ [98]. An additional integrin, VLA-4, expressed by human primary MM cells or MM cell lines, made a major contribution to the in vitro interaction of these MM cells with fibronectin and VCAM-1 [99] (Fig. 4). The adhesion of MM cell lines, from both humans and mice, was shown to be down regulated by agonists of the peroxisome proliferators-activated receptor (PPAR)γ [100]. Adhesion interactions, described in this section, are common to many cellular systems. It is therefore not surprising that some of them are also shared by HSCs.

Drug Resistance Conferred on MM Cells Interacting with Stromal Cells

Although alkylating drugs and authologous bone marrow transplantation, as well as newly developed drugs such as bortezomib, thalidomide and lenalidomide, improve the health status of MM patients, there is thus far no treatment regimen that results in a complete cure. MM patients often exhibit resistance to drugs and this may in part be due to the adhesive interactions of MM cells with the stroma, which render these cells resistant to the effect of chemotherapeutic agents. The direct adherence of human MM cell lines to primary bone marrow stroma increased their resistance to a
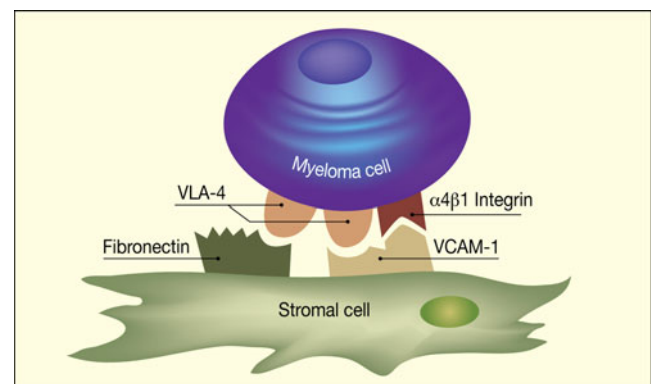

Fig. 4 MM cells adhere to bone marrow stromal cells

topoisomerase II inhibitor, mitoxantrone. Such resistance could also be conferred by soluble factors released during the interactions between the MM cell line and the stroma [101]. Similar stromal effects mediated by direct interactions and soluble factors conferred resistance of MM cell lines to the apoptosis induced by the Apo2 ligand/TRAIL [102] (Fig. 5) (see below, under "inhibitory cytokines"). The protective effect of the stroma is not general; whereas stromal cells protect a MM cell line from dexamethasone, the same stroma caused increased sensitivity to doxorubicin and melphalan [103]. The resistance granted is due in part to direct cell-to-cell interactions through adhesive interactions but is also conferred by the soluble factors IGF-I and IGF-II [104].

Role of Stimulatory Cytokines in MM

IL-6, which is one product of bone marrow stromal cells but is expressed by many other cell types, is thought to be a major contributor to MM cell survival and growth in the bone marrow microenvironment. In vitro data show that mouse plasmacytomas are often IL-6 dependent. However, cell lines that are IL-6 independent, in that they do not require for in vitro growth the addition of the cytokine, are rather prevalent. Some of the latter strive on autocrine secretion of IL-6, while others have a modified, constitutively active, IL-6 signaling cascade. Human MM cells that are otherwise IL-6 dependent, seem to lose this dependence when in contact with bone marrow stromal cells [105]. Toll-like receptor ligands mediate survival of MM cells by inducing autocrine secretion of IL-6 [106]. Human MM cells isolated from the bone marrow are growth stimulated in vitro by IL-6. Their survival is however limited in time. The lack of plasmacytoma development in mice, that are IL-6 deficient, strongly supports the critical role of this molecule in the emergence of this disease [107]. Yet, therapeutic attempts using antibodies to this cytokine have
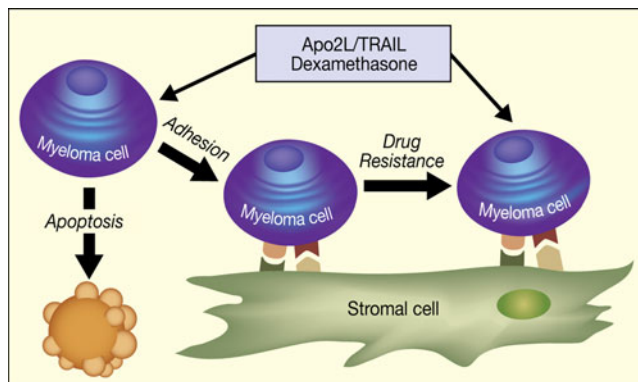
not yielded promising results probably due to the multitude of growth factors that could compensate for the absence of IL-6. It is noteworthy that IL-6 has a direct differentiation inducing effect on HSCs and it effectively synergizes with other cytokines to induce massive differentiation. In contrast, it is not known to promote HSC renewal and is not listed amongst the molecules that form the self-renewal niche for HSCs.

Several other cytokines have been implicated as growth factors for MM cells (Fig. 6). These include IL-11, insulin like growth factor (IGF)-1, IL-3 [108], leptin [109], and hedgehog (hh) [110, 111]. It remains to be determined how each of these molecules contributes to the survival of myeloma cells within the bone marrow compartment. Analysis of intracellular signaling events activated in MM cells, or evoked in them by the growth factors on which they are dependent, indicated a role for several intracellular signaling cascades. These include PI3K, JAK/STAT, Raf and MEK/ERK and NFkB pathways (Fig. 6) (reviewed by [7]). Newly added signaling cascades are the mTORC1 and MTORC2 pathways, which are known to regulate normal cell survival and growth and are activated in many cancers. The molecule DEPTOR suppresses mTORC kinase activity and is therefore expressed at a low level in tumors. By contrast, in a subset of MM, DEPTOR is highly expressed [112]. Similarly to HSCs, the migration of MM cells is influenced by the SDF-1/CXCR4 axis [113].

Role of Inhibitory Cytokines in MM

Apoptosis is a mode by which cells are killed physiologically in a manner that maintains the intracellular constituents within membranal particles removed by phagocytosis. This ensures that cells are removed from the organism without spilling intracellular molecules that could cause inflammation. Agents that cause apoptosis of MM cells should therefore be considered as possible drugs. Tumor necrosis factors (TNFs) are such death inducing molecules.

Fig. 5 Adhesion of MM cells to stroma protects the tumor cells from apoptosis
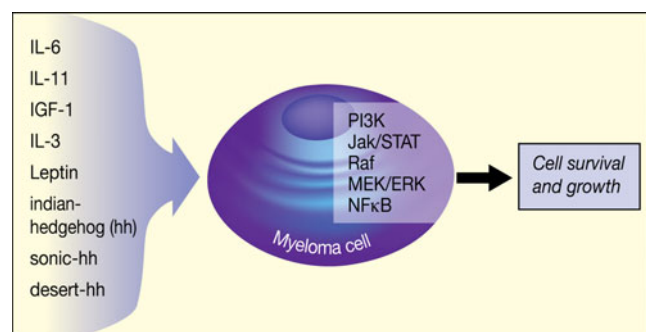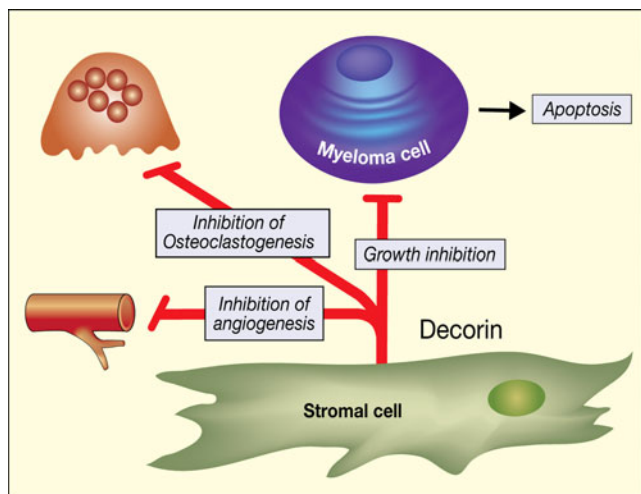

Fig. 6 IL-6 is a major survival and growth factor for MM cells but other cytokines also affect MM cell survival by evoking intracellular signaling cascades
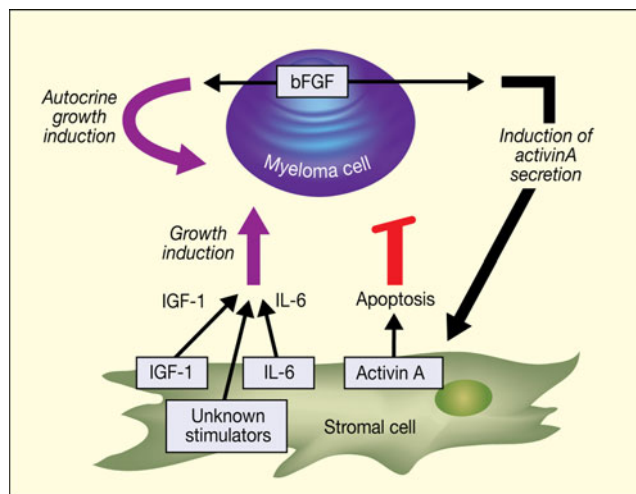
TNF related apoptosis-inducing ligand (TRAIL), also called Apo2 ligand (Apo2L), interacts with surface receptors to generate a proapoptotic signal through interactions of the receptor intracellular death domains with adaptor proteins that activate proteolytic caspases. TRAIL/Apo2L has been reported to overcome drug resistance of MM cell lines, as well as MM tumor cells obtained from patients [114] (Fig. 5). In addition to its direct effect on MM cells, it has been suggested that TRAIL kills osteoblasts; untreated osteoblasts were resistant to TRAIL, while osteoblast first incubated with MM cells were killed by this molecule [115]. The interactions between MM cells and the stroma, are also affected by decorin, which is a small leucine-rich proteoglycan. Its elimination from the stromal osteoblasts increased the growth of MM cells by inhibiting several pathways that otherwise contribute to myeloma progression (Fig. 7) [116]. Activin A, a member of the TGFβ superfamily, is expressed by mouse and human bone marrow stromal cells. This molecule has a dual inhibitory effect on MM cells (Fig. 8). Firstly, activin A causes a block in cell cycle progression [117]. Secondly, this molecule is a competitive antagonist of IL-6 [118, 119]. Thus, the molecule kills MM cells that are IL-6 dependent through both mechanisms, and would also affect MM cells that are IL-6 independent by directly affecting their cell cycle progression.

The stroma is apparently a supportive niche for MM cells. Therefore the finding that a stromal factor, activin A, is capable of mediating MM cell death, is seemingly a contradiction. However, the case may be that in normal bone marrow, the balance between activin A and IL-6 is in favor of the former, while in MM the titer of IL-6 is high enough to overcome the inhibitory function of activin A. Indeed, overdoses of IL-6 overcome the in vitro killing
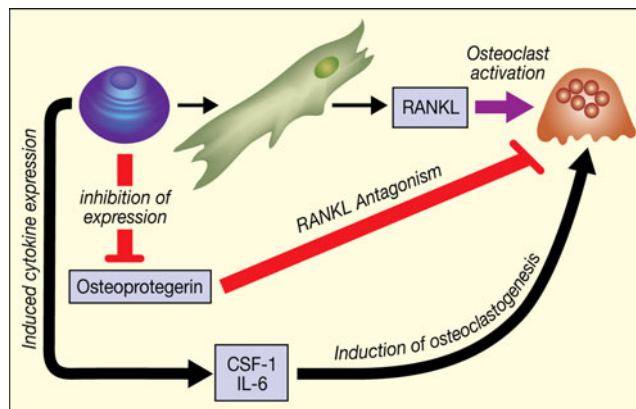

Fig. 8 The stromal cell release the TGFβ cytokine activin A causes apoptosis of myeloma cells. Additional cytokines that take part in the interactions between the MM cell and the stroma are indicated

effect of activin A [118]. The latter was also demonstrated to inhibit plasmacytoma tumor development in vivo [120].

It is noteworthy that neither of the above listed inhibitors have been implicated as a major player in the HSC self-renewal process.

Osteoclastogenesis Mediated by MM Cells

The osteolysis occurring in MM patients is probably due to the presence of MM cells within the bone cavities. MM cells were shown to induce the expression of the receptor activator of NF-kB ligand (RANKL), a major osteoclast activator, in bone marrow stromal cells (Fig. 9) [121, 122]. Moreover, these cells suppress the expression of osteoprotegerin (OPG), a RANKL antagonist. This last finding was recently used in an in vivo disease model. A MM cell line transplanted into β2mNOD/SCID mice developed a bone

Fig. 7 Decorin, a stroma cell surface proteoglycan elicits several functions that negatively regulate MM cell survival within the bone marrow


Fig. 9 Several MM cell induced functions lead to osteoclast activation

marrow disease involving an increase in osteoclast number and bone loss. Upon treatment with multipotent stromal cells, (MSC) transduced with hOPG, bone loss was diminished [123]. MM may thus involve an imbalance between osteoclast stimulation, mediated by RANKL and MIP1α, and inhibitory agents such as OPG and DKK1. In addition, the occupancy of the bone marrow with MM cells causes an accumulation of molecules that potentially augment osteoclastogenesis, including CSF-1, IL-6. Indeed it was found that not only RANKL but also MM cells themselves up-regulate the expression of IL-6 and IL-11 by bone marrow stromal cells [124]. These findings suggest that in fact the progression of osteolysis would enhance MM growth and dissemination. In line with this conclusion, the treatment of diseased mice with inhibitors of osteoclast activity, reduced bone resorption and also inhibited myeloma growth [125]. One component in the mediation of osteolysis are matrix-degrading proteases. Overall, increased osteoclastogenesis causes bone damage, along side with the secretion of cytokines that promote further myeloma growth and spread (Fig. 10).

Expression and Activity of Proteinases in MM

A host of proteases have been reported to be expressed in MM. These include the matrix metalloprotease (MMP)-1, MMP-2, MMP-7, MMP-8, MMP-9 and MMP-13, as well as urokinase plasminogen activator (uPA). Several of the MMPs degrade collagen, a major constituent of the bone matrix. uPA cleaves non-collagenous proteins. Therefore, the collective expression of these molecules may contribute to bone lesions in MM. in vitro experiments using both primary myeloma and MM cell lines cultured with osteoblasts isolated from human bone marrow showed that MM cells induce the expression of MMP-1, MMP-2 and uPA in the osteoblasts [126, 127]. Stromal cells suppress plasma cell IgG production by secreting MMPs that
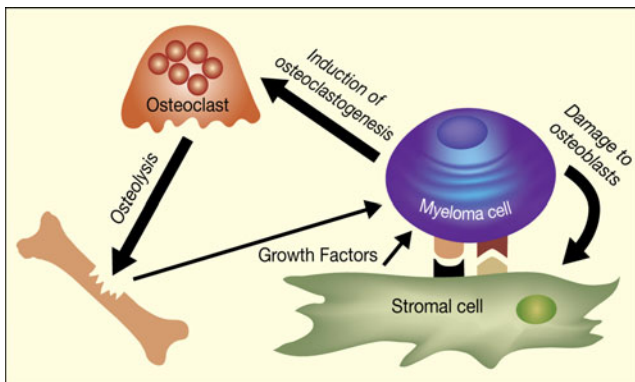
Fig. 10 MM cells induce osteolysis and the release of cytokines that further promote MM cell survival and growth

degrade the chemokine ligands CCL2 and CCL7, such that antagonistic degradation products are produced [128].

The Status of the Bone Marrow Mesenchymal Stroma and Osteoblasts in MM

While osteoclastogenesis is an overt occurrence in MM due to the bone lesions, the possible involvement of osteoblasts in the course of the disease is less pronounced. However, several reports indicate that osteoblasts are targeted in MM. As has been indicated above, cultured MM cells induce the secretion of a variety of cytokines by the mesenchymal stroma and by osteoblastic cells in particular. In addition, protease expression is augmented in the stroma. A number of studies point to a possible damage caused to osteoblasts by MM cells. These experiments imply that the stroma in MM maybe defective. This could be due to the effect of the MM cells themselves. Otherwise, primary defects in the stroma could contribute to the emergence of the disease. The question raised in both cases is whether the bone marrow stroma in MM patients is deficient. Analysis of adhesion molecules expressed by MM stroma, compared to that of normal controls, did not reveal differences. Adhesion of MM cell lines to stroma from patients and normal individuals was shown to be similar [129]. Other studies examined the properties of MSCs. One set of data showed identity in differentiation potential between MM derived and normal MSCs [130, 131] whereas another study detected a deficient capacity to osteoblastic differentiation of MM derived MSCs [132]. The latter function was examined by Pevsner-Fischer et al., and was found to be intact in MM MSCs (unpublished observations). This issue is therefore unresolved and further studies are needed. A study using comparative genomic hybridization, indicated that stromal cells from MM patients, show genomic imbalances that were not observed in stroma from normal controls [133]. It remains to be seen whether these occurrences affect MSC biological functions.

Conclusions

The discussion above highlighted the major features of the HSC niche that maintains the stem cell phenotype. This niche is made of several cellular elements, which are still poorly characterized. Therefore, a clear conclusion as to whether HSCs and MM TIC share the same supportive stromal cell type is premature. It has recently been suggested that macrophages protect MM cells from apoptosis. These cells are an ample source of cytokines and if anything, their presence in the HSC niche would promote differentiation, rather than renewal. More importantly, it

seems that the macrophages that promote MM survival, are tumor specific cells and may differ from normal macrophages [134]. Thus, the nature of the cells that form the MM microenvironment and those that create the HSC niche is still an open question. However, based on current knowledge the microenvironment of MM tumor seems to markedly differ from that of the normal bone marrow.

In contrast to the vagueness in regards to the exact cell types that form the MM environment versus those that make the HSC niche, it is obvious that HSCs and MM TICs differ markedly in their cytokine requirements. The above summary of cytokines, that affect HSCs self-renewal, highlighted the importance of molecules that antagonize differentiation processes. For example, TGFβs reduce differentiation and angiopoietin and thrombopoietin keep HSCs quiescent. Thus, such molecules block the option for differentiation and consequently allow stem cell renewal to occur. The vast majority of MM cells are fully mature plasma-like cells that secrete antibodies. These are end cells in the differentiation cascade and therefore the issue of blockade of differentiation does not apply in MM. It is often argued that most of the MM plasma cells are quiescent and therefore do not contribute to the increase in tumor load. However, TICs are expected to be quiescent and indeed most MM plasma-like cells were found to contribute to tumor formation in a human/mouse model. Certainly, in the mouse, as few as 100 plasmacytoma cells may form a tumor under the skin of Nude mice [120]. The survival of MM cells is dependent on several growth factors, the major one being IL-6. This molecule makes little, if any, contribution to the HSC niche. MM cannot be induced in IL-6 deficient mice while these mice do not lack HSCs. A major contribution to MM cell survival and growth within the bone marrow compartment is ascribed to osteoclastogenesis, the subsequent osteolysis and release of inflammatory cytokines. The latter promote MM cell survival and growth and allow the spread of the disease within the bone marrow. The complete opposite is true for the HSC. Increased osteoclastogenesis generates signals that drive HSCs away from their niches. It is therefore concluded that there is no solid evidence for any true similarity between HSCs and MM TICs, and these cell types represent distinct biological entities.

One question raised is whether the information available, to date, provides an explanation for the residence of MM cells within the bone marrow. The molecules that have been detailed above as major players in MM, like the adhesion pair VLA-4/VCAM-1 and the cytokine IL-6, are clearly not bone marrow specific. It follows that the specificity in bone marrow localization should be sought in the bone itself. Indeed, osteoclasts are clearly affected by MM, but compelling evidence for the absolute necessity of these cells for the bone marrow localization and retention of MM

cells is not available. It would be of importance to examine mice deficient in osteoclasts to clarify this point.

Other candidate cells contributing to the MM TIC microenvironment are the osteoblasts. These cells may be modified in the myelomatous bone compartment, and may therefore serve as a docking site for the malignant cells. However, data available thus far does not support this possibility. It seems that too little effort has been put in the analysis of stromal cells from MM patients. A few studies suggest that MM stroma diverges from normal, while in other studies the MM stroma was found to be normal. Further analysis is needed to resolve this issue.

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