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The role of the bone microenvironment in the pathophysiology and therapeutic management of multiple myeloma: Interplay of growth factors, their receptors and stromal interactions

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ABSTRACT

The close relationship between the biological behaviour of malignant cells and the local microenvironment where they reside is a feature of diverse neoplasias. Multiple myeloma (MM) is considered a main disease model for the study of such interactions and the mechanisms that can lead to bone-related clinical complications, as well as the role of these interactions in attenuating the activity of conventional anti-MM therapeutics, such as dexamethasone and cytotoxic chemotherapeutics. This review focuses on recent progress in the study of interactions of MM cells with their local microenvironment. Major emphasis is placed on how bone marrow stromal cells (BMSCs) and other normal constituents of the bone marrow milieu promote, through cell adhesion- and cytokine-mediated mechanisms, the ability of MM cells to resist conventional anti-MM therapies. The review also addresses ongoing research into these mechanisms, which has already provided several new molecular targets and corresponding therapeutic strategies, such as the proteasome inhibitor bortezomib and thalidomide derivatives (e.g. lenalidomide), for the management of myeloma.

1. Introduction

The close interplay between the biological behaviour of neoplastic cells and the local microenvironment(s) where they reside is not a feature restricted only to multiple myeloma (MM). Instead it is shared by a broad spectrum of solid tumours and haematologic neoplasias[1–4], probably reflecting an underlying biological principle which is pertinent (with tumour type-specific variations) to many, if not most, human malignancies. For example, Stephen Paget proposed already in 1889 the "seed and soild" hypothesis, i.e. that the establishment of tumour metastatic sites is influenced by cross-interaction between selected cancer cells ("seed") and specific organ microenvironments ("soil").[5] Today the concept of tumour-microenvironmental interactions is closely linked in the literature with MM, reflecting the fact that MM has been a prototypical disease model for the study of these interactions.[6] This focus on microenvironmental studies in MM could in turn be traced, at least in part, to the fact that the clinical presentation of the disease, particularly the strong

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predilection of MM cells for establishing lytic bone lesions, makes it more plausible (compared to other neoplasias) to hypothesize a link between the behaviour of tumour lesions and their location. In addition, the substantial morbidity and mortality associated with the skeletal lesions of MM provides a powerful clinically-driven incentive to understand how MM cells perturb bone homeostasis. In the process, it has become apparent that the MM-bone interaction is bi-directional, and that the bone microenvironment is not only radically perturbed by the presence of MM cells, but that it also in turn, changes the behaviour of the tumour cells and renders them more likely to resist the pro-apoptotic effects of conventional therapies, such as dexamethasone and cytotoxic chemotherapeutics.[6–8] The realization that certain novel therapeutics (thalidomide and its derivatives, or the proteasome inhibitor bortezomib) that can counteract or overcome these bone microenvironment-derived protective effects on MM cells[9,10] can also be highly active in patients refractory to these aforementioned conventional drugs[11–13] provides indirect, but powerful, support to the importance of tumour-stromal interactions and further stimulated the already heightened research interest in their comprehensive characterization.

In the present article, we aim to review the recent progress in the study of the interactions of MM cells with their local microenvironment. Particular emphasis is placed on how bone marrow stromal cells (BMSCs) and other normal constituents of the bone marrow (including osteoblasts, osteoclasts and endothelial cells) function to promote, through cell adhesion- and cytokine-mediated mechanisms, the ability of MM cells to resist conventional anti-MM therapies. Ongoing research into these mechanisms has already provided several new molecular targets and corresponding therapeutic strategies for the management of this presently incurable disease.

2. The interaction of MM cells with bone marrow stromal cells

In the field of MM, the interaction of MM cells with bone marrow stromal cells (BMSCs) is now viewed as a critical component of the overall plexus of biological relationships forged between the tumour and the bone milieu. BMSCs in the MM literature are generally identified descriptively, as a heterogeneous assembly of mesenchymal cells with fibroblast-like morphology. Functionally, BMSCs are able to support normal haematopoiesis *in vitro*. In that respect, the property of BMSCs to support the proliferation, survival and drug resistance of MM could be viewed as an abnormal and pathophysiologically unfavourable recapitulation of their natural role to support haematopoietic cells. MM cells capitalize on the adhesive interaction with BMSCs and the ensuing production of various cytokines to increase their proliferative capacity, and fortify their resistance to various pro-apoptotic stimuli, including various conventional therapeutics.[6] In particular, MM cell adhesion to BMSCs, mediated by various adhesion molecules (e.g. VLA-4, ICAM-1)[14,15] contributes to MM cell proliferation and enhanced viability through several distinct, but mutually complementing, mechanisms, including: a) direct, cell adhesion-mediated, activation of intracellular signalling cascades in MM cells; and b) stimulation of production of cytokines/growth factors in the BM milieu in a paracrine (from the BMSC compartment) and/or autocrine (from the MM cell compartment) manner.[6,14–16] In the *in vitro* mechanistic studies of MM-stromal interactions, the direct, adhesion-mediated, component of anti-apoptotic signalling precedes the secretion of cytokines, but eventually the direct adhesive effects cooperate with cytokine-induced signalling *in vitro* and conceivably both aspects are operational when MM cells interact with their stromal cells *in vivo*. It is not clear if there are specific subpopulations of BMSCs which are more responsible than the rest for the stimulation of MM cell proliferation/survival. A more comprehensive characterization of the biological and phenotypic features of BMSCs is thus warranted.

3. Cytokines, growth factors and their receptors in the context of tumour stromal interactions: An integrative approach to the impact of tumour-microenvironmental interactions in MM

The behaviour of MM cells, at a biological and clinical level, appears not to be solely determined by their underlying genetic features (chromosomal rearrangements, deletions, amplifications or mutational events in individual genes). Instead, the pathophysiology of this disease appears to be significantly affected by a complex network of interactions which converge to a bi-directional relationship of MM cells with their local bone microenvironment.[6] On one hand MM cells perturb the normal process of bone remodelling, leading to the establishment of lytic skeletal lesions, which can be the cause of substantial morbidity and mortality.[17,18] However, in MM, it is not only the neoplastic cells which affect their local milieu, but it is also the bone microenvironment which influences the behaviour of MM cells, by providing them with a multi-faceted web of protective effects against pro-apoptotic stimuli[16], which can include conventional therapeutics, such as steroids, DNA-damaging agents or irradiation.

The complex web of pathophysiolgical mechanisms underlying the development of MM bone lesions are characterized in detail in other articles of this special edition.[113,114] Briefly, under physiological conditions, the bones are subject to a continuous process of structural remodelling in which osteoclasts mediate resorption of old bone tissue, followed by activation of osteoblasts, which are responsible for formation of new bone.[19] However, in MM patients, these two processes are not properly coupled.[20,21] Instead there is a concomitant upregulation of a broad spectrum of factors which stimulate osteoclast formation and function, as well as suppression of negative regulators of osteoclastogenesis and/or positive regulators of bone formation.[18] For instance, it has been proposed that MM cells can contribute to bone resorption by stimulating RANKL expression in BMSCs[22]; causing decrease in the levels of osteoprotegerin (OPG), which functions as decoy against RANKL [23]; and by stimulating the production in the BM milieu of multiple pro-osteoclastogenic cytokines, including IL-6, IL-1α, IL-1β, IL-11, MIP-1α, M-CSF, TNF-α, TNF-β (lymphotoxin-α), PTHrP, or

VEGF.[15,18,24–32] These cytokines, which directly or indirectly stimulate osteoclast maturation and increased resorptive activity, can either be produced by MM cells themselves and/or by host BM cells (e.g. BMSCs) within the context of paracrine/juxtacrine stimulation by MM cells. Furthermore, it is has been proposed that, at least in a subset of MM patients with extensive bone lesions, MM cells express increased levels of transcript for DKK-1, an inhibitor of Wnt signalling, and that this increased *DKK-1* gene expression is associated, in a sizeable sub-population of MM patients, with increased DKK-1 protein levels.[33] The role of DKK-1 in inhibiting differentiation of osteoblast precursor cells suggests that this cascade may contribute to uncoupling of bone formation from excessive resorption in MM[33] and additional research efforts will be important in identifying other proteins which might complement the activity of DKK-1 or substitute for it.

The uncoupling of bone formation from bone resorption in the MM bone milieu is not impacting only the formation of bone lesions and resulting in increased risk for spontaneous fractures and hypercalcemia[18,21], but can also have impact on the responsiveness of MM cells to anti-tumour therapeutics. The microenvironment of the bone generally constitutes a favourable niche for the proliferation, survival and drug resistance of neoplastic cells, which can explain, at least, in part the propensity of, not just MM, but several other neoplasias as well (e.g. breast or prostate cancer) for formation of bone metastatic lesions.[34] Some of the growth factors (e.g. IL-6 and IGFs.) released during the process of bone remodelling not only serve to regulate the maturation and function of the local cells participating in these cascades, but can also function to stimulate the proliferation of tumour cells, such as MM cells, and promote their resistance to drug-induced apoptosis.[6] Therefore, the bone microenvironment is a fertile ground for the homing of MM cells, while their ability to further perturb the normal homeostatic regulation of bone remodelling generates a vicious circle in which bone resorption feeds the MM cell compartment with high local levels of growth-promoting/anti-apoptotic cytokines and the MM cells, in turn, further deregulate bone remodelling in favour of increased resorption, thereby further enhancing the production of the cytokines which support the viability of MM cells in the BM milieu.[6]

This concept that the tumour microenvironment may contribute to drug resistance is not restricted to MM[1] and does not exclude other concurrent or independent mechanisms (e.g. "tumour stem cells", genetic mechanisms of resistance) that have been proposed to explain the lack of curative responses of MM to conventional or even some novel treatments.[35,36] While more research may still be needed to further clarify the role of the proposed "MM tumour stem cells" in the pathophysiology of the disease, there is extensive evidence from other fields of study, including normal haematopoietic stem cells[37], that clearly suggest that stem cells, "tumour" or "normal" are not functioning completely independently of their local milieu, but are significantly influenced by it.[38] In addition, the concept of genetically-determined mechanisms potentially guiding the drug responsiveness and prognosis of the respective patients is not mutually exclusive with the concept of microenvironmental regulation. There are several levels at which these mechanisms can intersect and cooperate, e.g. specific cytogenetic abnormalities of MM cells can modulate the pattern of adhesion molecules involved in MM-stromal interaction (e.g. as shown by the effect of c-maf overexpression on integrin expression in MM cells).[39]

4. Signalling cascades triggered in MM cells interacting with their local microenvironment

When MM cells adhere to extracellular matrix proteins, BMSCs and other cells of the BM milieu, the direct contact with these structural and cellular constituents of the microenvironment[40–42], as well as the ensuing stimulation of autocrine/paracrine production of cytokines[15,16,43] activates in MM cells a broad spectrum of proliferative/anti-apoptotic signalling pathways. These pathways, which include the PI-3K/Akt/mTOR/p70S6K cascade[16,44], the IKK-α/NF-κB pathway[16,45], as well as the Ras/Raf/MAPK [16], and JAK/STAT3[46–48] signal transduction pathways, can be activated by upstream binding of cytokines to their respective receptors (e.g. IL-6R, IGF-1R, c-met, IL-1R, IL-21R) or by direct cell adhesion-mediated activation of proliferative/anti-apoptotic signalling through adhesion-triggered kinase pathways e.g. integrin-linked kinase/focal adhesion kinase. Despite a different starting point for these cytokine- vs. adhesion-triggered signalling cascades, their functional sequelae are not dissimilar, since they eventually converge to the same aforementioned downstream pathways (PI-3K/Akt/mTOR/p70S6K cascade, the IKK-α/NF-κB pathway, as well as the Ras/Raf/MAPK, and JAK/STAT3), which in turn trigger further downstream events, including cytoplasmic sequestration of pro-apoptotic Forkhead transcription factors[16], upregulation of D-type cyclins[16], increased intracellular levels of caspase inhibitors (e.g. FLIP, cIAP-2, survivin), and anti-apoptotic Bcl-2 family members (e.g. A1/Bfl-1, Mcl-1).[16] In addition, these adhesion/cytokine-triggered pathways appear to further stimulate the activity of pathways which conceivably contribute to enhanced replicative potential (telomerase activity); degradation of pro-apoptotic mediators and negative regulators of cell cycle (chymotryptic-like activity of 20S proteasome); and ability of tumour cells to recruit new vessels under hypoxic conditions (HIF-1α).[16,44,49] As previously mentioned, these molecular events are triggered either directly, via cell adhesion molecule-mediated interactions of MM cells with BMSCs, osteoblasts, other BM cellular compartments or the extracellular matrix[42]; or indirectly, via cytokines/growth factors released by BMSCs and/or MM cells and engaging their respective receptors on the MM cell surface.

The list of paracrine/autocrine proliferative/survival factors produced in the local BM microenvironment and affecting the pathophysiology of MM cells is continuously expanding and currently includes interleukin-6 (IL-6), insulin-like growth factors (IGFs), IL-1α, IL-1β, hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), stromal-derived factor (SDF-1, also known as CXCL12), TNF-α or various Notch family members.[8,16,43,49–52] The downstream signalling cascades utlilized by these factors are often overlapping, but the biological effects of these molecules are not identical. For some of these factors, their contribution to

MM pathophysiology extends beyond the stimulation of tumour cell proliferation and survival. For instance, IL-6, IL-1, VEGF can stimulate bone resorption[17,24,28,29,32,53], while TNF-α can modify, in an NF-κB-dependent fashion the profiles of cell adhesion on the surface of MM cells and BMSCs[54], leading to more pronounced MM-BMSC adhesive interaction and ensuing production of cytokines, such as IL-6.[54] Other factors such as the chemokine SDF-1/CXCL12 function to promote MM cell homing to the BM milieu[8], and therefore, even though the actual direct proliferative/anti-apoptotic stimulatory activity of SDF-1 (or its receptor CXCR4) is rather minimal, SDF-1 signalling appears to be an important determinant of MM cell locomotion and, especially, helps MM cells attract themselves to the BM niche where their microenvironmental conditions are more conducive to the proliferation and viability of the tumour cells.

For many years, IL-6 was considered a major, if not the major, cytokine-based driving force for the proliferation and survival of MM cells. Extensive studies from various groups have shown that IL-6 stimulates, via its the gp130 receptor IL-6R, the activation of PI-3K/Akt and JAK/STAT3 signalling, with ensuing sequelae in terms of suppressing induction of apoptosis by dexamethasone and potentially other conventional therapeutics, such as cytotoxic chemotherapeutics and (at least partially) irradiation. Furthermore, the well-documented role of IL-6 in stimulating osteoclastogenesis[55], as well as in promoting differentiation of B-lineage cells to normal plasma cells[56], seemed to compose a comprehensive rationale for IL-6 as a central regulator of the biological behaviour of MM cells, thereby validating the IL-6 pathway, in general, and its individual components, in particular, as prime therapeutic targets for this disease. However, in recent years, it has become apparent that not all aspects of the pathophysiological behaviour of MM cells can be explained exclusively by IL-6 signalling. For instance, although IL-6 is traditionally viewed as the primordial proliferation factor for MM cells, only a subset of MM cell lines respond significantly to IL-6 stimulation *in vitro* (while even fewer are dependent on IL-6 stimulation for the long-term culture) and only at concentrations several logs higher than the levels of IL-6 detected in the peripheral blood plasma (or even bone marrow aspirate plasma) of MM patients.[16] Interestingly, anti-IL-6 neutralizing antibodies were tested in clinical trials of MM patients in the 1990s [57] and showed robust activity in suppressing the circulating levels of C-reactive protein (CRP), as a surrogate marker for IL-6 bioactivity, which unfortunately did not translate into the type of major clinical responses that the myeloma community would have hoped for based on and the pre-clinical data and preliminary clinical experience.[58] It is conceivable that some of these patients had disease with too high a production of IL-6[59]; or that for pharmacokinetic reasons, administration of a single anti-IL-6 antibody cannot achieve sufficient clearance of the cytokine[60]; or that enrolled patients had very aggressive biological features and advanced course, which are less likely to correspond to a biological setting of IL-6-dependence of the MM cells. In addition, it could be argued that MM cell lines are derived mostly from patients with plasma cell leukaemia and extramedullary plasmacytomas, and therefore are representative of the genetic and molecular features of patients with very aggressive late-stage disease (i.e. at a time where MM tumour cells are considered to be largely independent of the BM microenvironment and its cytokines) rather than patients with earlier stage disease (e.g. newly diagnosed MM). However, it is unlikely that all these results can be attributed solely to the disease stage. For instance, if this was the case, then administration of exogenous IL-6 would be expected to be sufficient for long-term culture of MM cells *in vitro*. However, MM tumour cells purified from BM aspirates of early stage patients can be maintained in culture *in vitro* only for short periods of time, despite exposure to high doses of IL-6 and/or co-culture with BMSCs (C. Mitsiades unpublished observations). It is also notable that thalidomide, immunomodulatory thalidomide derivatives (e.g. lenalidomide), or the proteasome inhibitor bortezomib have been shown to have substantial clinical activity not only in the setting of relapsed or refractory MM[11–13,61], but also in earlier stages of the disease, including newly-diagnosed patients[62–65], i.e. those clinical settings where MM cells are more likely to be responsive to inhibition of IL-6 signalling. In fact these agents affect MM cells, at least in part, by inhibiting the production of IL-6 in the BM milieu and/or its signalling on MM cells[66]. In contrast, when MM patients become resistant to some or many of these new therapies, the stage of the disease is generally advanced and MM tumour cells are more likely to already be IL-6-independent. In that respect, it remains to be seen whether any new future more specific inhibitors of IL-6 signalling will be able to offer additional benefit to MM patients or whether the effects of these agents will be overlapping with those of agents recently developed and already extensively applied for clinical management of myeloma.

The search for cytokines/growth factors which are implicated even at the late stages of MM pathophysiology has recently underscored the notion that insulin-like growth factors (IGFs) and their receptor IGF-1R (CD221) play a more prominent role in the pathophysiology of MM than previously appreciated.[16] Extensive, but sporadic, research data from various types of neoplasias, have been suggesting for many years now that IGFs and IGF-1R are implicated in the pathogenesis of multiple forms of solid tumours. For instance, prospective epidemiological studies have indicated that higher circulating levels of IGF-1 are associated with higher risk for development of various types of epithelial cancers (reviewed in Ref. [70]). Several pre-clinical models have also documented that expression of IGF-1R is necessary for malignant transformation of normal cells.[67–69] Various reports (reviewed in Ref. [70]), including studies with MM cell lines, have indicated that IGF-1 can stimulate variable increases in the proliferative rates of neoplastic cell lines *in vitro*. However, for many years this pathway was not considered an attractive target for development of novel therapeutics, not only because of concerns about the potential safety of anti-IGF-1R signalling strategies, but also because of the fact that the relative significance of this cascade, compared to other growth factor receptor pathways, had not been clearly appreciated. Indeed, the substantial degree of homology of IGF-1R to its closely related insulin receptor (IR)[71], as well as the widespread expression of IGF-1R in normal cells[71] (often at levels comparable to those present in their neoplastic counterparts), had established a widely accepted notion that selective small molecule inhibitors of IGF-1R kinase domain could not be developed or

that even strategies more conducive to selective IGF-1R blockade (e.g. monoclonal antibodies) could not be administered without catastrophic toxicities due to inhibition of IGF-1R function in normal tissues. While specific anti-human IGF-1R neutralizing monoclonal antibodies have existed for at least two decades[72–75], the data which showed *in vivo* anti-tumour activity of these antibodies against human tumour cell lines xenografted in rodents[76,77] did not manage to alleviate the concerns regarding possible toxicities in human. This was due in part to the legitimate concern that anti-human IGF-1R mouse monoclonal antibodies did not cross-react with the endogenous rodent version of the receptor in normal cells of the host animal, thereby limiting the ability of these models to provide meaningful information on possible toxicities in humans (particularly when the target receptor is so broadly expressed in normal tissues), and therefore overestimate the maximum tolerated dose that might be achievable in human patients.[70]

This major hurdle in the development of anti-IGF-1R therapeutics was overcome partly because of studies, which focused early on MM, as a disease model with substantial responsiveness to anti-IGF-1R inhibition. Indeed, the synthesis and pre-clinical study of selective small molecule inhibitors of the IGF-1R kinase domain[16,78] was an important step forward, not only as an advance in medicinal chemistry of selective inhibition of related kinase targets, but also because of the property of the aminopyrrolopyrimidine IGF-1R kinase inhibitors to block the function of both human and rodent IGF-1R versions. This allowed, for the first time, the study of IGF-1R inhibition in pre-clinical models which were informative, in a more clinically-relevant manner than in previous cases, about potential toxicities of IGF-1R kinase inhibitors. Importantly, the safety and efficacy of IGF-1R kinase inhibitors were tested in SCID/NOD models of diffuse skeletal lesions of MM, i.e. in a model where MM cells were placed in a microenvironmental context relevant to the pathophysiology of bone lesions in human MM.[16]

In spite of the high degree of homology between IR and IGF-1R, in terms of their kinase domain sequences and structures, Garcia-Echeverria synthesized 2 pyrrolo [2,3-d] pyrimidine compounds, NVP-AEW541 and NVP-ADW742,[78], which were tested *in vitro* and *in vivo* models of MM and other neoplasias, exhibiting several favourable properties, including high selectivity against IGF-1R (both in human and rodent cells)[16,78]; oral biovailability; lack of major side effects (including lack of hyperglycemia); and importantly, anti-tumour activity in clinically-relevant *in vivo* models of diffuse distribution of MM bone lesions, which simulated the diffuse distribution and anatomic localization of these lesions in human MM patients.[16] The anti-tumour activity of this class of IGF-1R inhibitors included both decrease in MM tumour burden (quantified by whole-body bioluminescence imaging) and, importantly, increase in the overall survival of IGF-1R inhibitor-treated MM-bearing mice vs. control mice.[16] This *in vivo* anti-MM activity was observed both with oral and with parenteral administration of the inhibitors. These results were important because they showed that kinase inhibitors with sufficient selectivity against IGF-1R vs. IR, can achieve *in vivo* anti-tumour activity without catastrophic toxicities. In more general terms, these results have broader implications, as they validated the feasibility of IGF-1R inhibition as an *in vivo* therapeutic approach, thus providing *in vivo* proof-of-concept for other strategies targeting IGF-1R, e.g. antibodies or siRNA constructs. Importantly, IGF-1R inhibitors caused dose-dependent inhibition of cell proliferation and induction of cell death of cell lines from a very broad spectrum of haematologic malignancies and solid tumours. MM cell lines were in general among the most sensitive lines to IGF-1R kinase inhibition.[16] This suggested that perhaps MM could represent a disease setting where intact IGF-1R function may be more important for the survival of the malignant cells, as opposed to other tumour types, thus identifying MM as a possible "niche" for early clinical applications of IGF-1R inhibitors. Furthermore, these efforts generated a roadmap for potential applications of IGF-1R inhibition in a broader spectrum of other haematologic malignancies and solid tumours, by capitalizing on the pleiotropic drug-sensitizing properties of IGF-1R inhibitors.

Indeed, IGF-1R inhibitors were not only active as single agents, but also exhibited potential for *in vitro* and *in vivo* sensitization of MM cells to other anti-cancer drugs, including cytotoxic chemotherapeutics. These results were consistent with the fact that in MM, as well as in other neoplasias, IGF-1R appears to function as a pleiotropic regulator of diverse anti-apoptotic pathways, implicated in the response of tumour cells to various conventional, as well as novel agents. Indeed, IGF-1R signalling potently activates telomerase and proteasome activities[16]; stimulates the production of anti-apoptotic caspase inhibitors[16], contributing to resistance against various anti-tumour drugs (including dexamethasone, cytotoxic chemotherapy and, in part, proteasome inhibitors). In addition, IGF-1R primes MM cell responsiveness to other cytokines (e.g. IL-6); and stimulates production of angiogenic cytokines[16], which further contribute to an increased ability of the MM cell to survive. Importantly, the *in vivo* chemosensitizing effect of IGF-1R inhibitors was also confirmed in experiments where both the IGF-1R inhibitor NVP-ADW742 and melphalan were administered at doses that were sub-therapeutic for each drug alone, thus highlighting the appealing potential that IGF-1R inhibition can be combined with other anti-MM drugs, allowing for use of the latter at doses lower than in current protocols for single-agent activity, which could potentially contribute to lower frequency of adverse events. Concurrently with the development of pyrrolo [2,3-d] pyrimidine inhibitors for IGF-1R, members of the class of cyclolignans (e.g. picropodophyllin, PPP), emerged as another group of small molecules which can selectively inhibit IGF-1R, but not IR activity. Consistent with the results on pyrrolo [2,3-d] pyrimidines, PPP inhibited malignant cell growth both *in vitro* and *in vivo*[79], including studies in MM.[80,81]

IGFs and their IGF-1R receptor are not the sole cytokines capable of stimulating MM proliferation or of opposing the activity of certain anti-MM drugs. Many of the signalling cascades downstream of IGF-1R are also employed by other cytokines involved in MM pathophysiology (e.g. IL-6, HGF can also activate PI-3K/Akt).[44,94] Therefore, it is not entirely clear why IGF-1R inhibition appears to have more impact on MM cell survival than some of the other cytokine pathways currently evaluated in this disease. However it has been observed that,

primary tumour cells from plasma cell leukaemia (PCL) patients can proliferate and survive independently of many BM-derived cytokines (e.g. IL-6), but they maintain significant responsiveness to selective inhibition of IGF-1R signalling.[16] Conceivably, part of the answer may be that, compared to many other cytokines reported to be involved in MM pathophysiology, the levels of IGFs are high in serum, while they are even higher locally in the BM milieu due to paracrine release by osteoblasts and BMSCs.[16] The high local bioavailability of IGFs in the bone milieu could explain a role for IGFs in medullary MM, while the high levels of IGFs in the circulation could perhaps account for the continued responsiveness of malignant plasma cells to IGF-1R inhibition even in cases of extramedullary MM. In terms of the overlap of signalling cascades triggered by IGFs vs. other cytokines, their differential functional outcomes may be related to the fact that IGFs can stimulate more pronounced and sustained activation of proliferative/anti-apoptotic signalling (e.g. PI-3K/Akt, IKK/NF-κB) than certain other BM-derived cytokines.[16] This observation, along with the sustained *in vivo* exposure of MM cells to high levels of IGFs (either in medullary or extramedullary MM) might explain, at least in part, the differences in functional impact on MM cells by IGF-1R vs. certain other cytokine/growth factor receptors. However, further research studies will be required to provide more discrete molecular documentation for the precise mechanisms responsible for these observations.

5. Current and future perspectives on tumour-stromal interactions as targets for therapeutic interventions in MM

The aggregate clinical impact of the bi-directional interactions between MM cells and the BM milieu is unfavourable not just because of the direct repercussions due to the development of osteolytic lesions, but also because of the indirect consequences of the epigenetic resistance that the BM milieu confers to MM cells against conventional chemotherapeutics or glucocorticoids.[16,44] Even in cases where MM cells are still early in the disease course and may not harbor genetic lesions that could confer constitutive drug resistance, the epigenetic protection provided by the stromal microenvironment may allow some MM cells to survive long enough to develop those additional genetic events that may be required for the emergence of clinical resistance and frank relapse of the MM tumour population after an apparent clinical remission. Not surprisingly, some of the more recently developed therapies for MM e.g. thalidomide, its derivatives, and the proteasome inhibitor bortezomib, which can be active even steroid- and/or chemorefractory patients, can also overcome the protective effects of BMSCs on MM cells. This provides indirect support to the notion that the interaction of MM cells with the BM milieu constitutes a legitimate nexus of events and corresponding molecular targets that should be pursued for further therapeutic interventions towards the goal of longer disease control.

There are several potential levels at which this therapeutic targeting of the tumour-microenvironmental interaction can occur. The stromal cells and other accessory cells supporting the MM cell survival in the bone microenvironment constitute bona fide targets for therapeutic interventions, some of which are already applied in clinical practice. For instance, the tumour-associated endothelium is the target of entire classes of anti-angiogenic therapies, while thalidomide/IMiDs are reported to operate *in vivo*, at least in part, by anti-angiogenic effects.[82,83] There are also reports about the effect of this class of agents on the growth of the endothelial compartment associated with the MM cells.[84,85] Bisphosphonates, aside from their potential direct anti-tumour effects[86,87], target the osteoclasts and inhibit the sequelae of their resorptive activity (e.g. cytokine release).[53] Stromal cells themselves are also subject to targeting for therapeutic intervention, since they are viewed as key regulators of *in vivo* MM cell growth in the bone. Perhaps one potential limitation of attempting to directly target stromal cells is their similar role in supporting normal haematopoiesis.[88] Instead, it may be more feasible to target the network of cytokine/growth factors mediating the effects of stromal cells on MM cells, e.g. suppression of the production of these mediators by stromal cells, other cells of the host microenvironment, or even MM cells, or inhibition of the local bioavailability of these factors in the local milieu. For instance, agents such as histone deacetylase (HDAC) inhibitors, apart from their direct pro-apoptotic activity against MM cells[89,90], can suppress the stromal production of IL-6 in co-culture models of MM and BMSCs.[90] Osteoprotegerin (OPG) or RANK-Fc can serve to block the stimulation of osteoclastogenesis.[91,92] Antibodies against or soluble antagonistic forms of receptors for VEGF, HGF, DKK-1 as well as small molecule inhibitors of the respective signalling cascade could serve to counteract the role of these mediators in angiogenesis, tumour cell proliferation and inhibition of osteoblast maturation/function, respectively.[33,93,94]

Another broad category of approaches that can target tumour-microenvironmental interactions involves the perturbation of the ability of MM tumour cells to communicate with their microenvironment. This can be potentially achieved either by altering the physical adhesive interaction of MM cells with the extracellular matrix or other cells in the BM milieu, or by abrogating the ability of the cells to receive the soluble signals that the microenvironment offers in the form of cytokines and growth factors. Indeed, monoclonal antibodies against cell adhesion molecules (e.g. integrins) involved in MM cell adhesion in the BM have been evaluated pre-clinically as targets for therapeutic intervention, in MM models, where e.g. preventative administration of anti-α4 integrin antibody decreased tumour burden of 5TGM1 murine myeloma cells and also led to decreased bone destruction with diminished number of osteoclasts, and prolonged survival of 5TGM1-bearing mice.[95] The targeting of the ability of the MM cell to receive its microenvironment cues and respond to them represents a very broad category of therapeutic interventions exemplified by the diverse strategies employed to abrogate growth factor/cytokine signalling either at the level of the respective cell surface receptors (e.g. IGF-1R kinase inhibitors[16]; FGF-R3 kinase inhibitors[96–98]); or at diverse downstream cascades necessary for MM cell responses to microenvironmental stimuli. Examples of this latter approach could include inhibitors of Ras farnesylation[99–102] inhibitors of the IKK/NF-κB axis (e.g. inhibitors of the kinase activity of IKK[45]) or inhibitors of the nuclear translocation of NF-κB[103]); inhibitors of the PI-3K/Akt/mTOR axis (e.g. mTOR

inhibitors[104–108]; telomerase inhibitors[109,110]; or agents which can simultaneously inhibit multiple levels of these aforementioned signalling cascades, including hsp90 inhibitors, which can simultaneously affect the 3-dimensional structure and function of several of the previously mentioned signalling proteins involved in tumour cell proliferation, survival and drug resistance, including members of the PI-3K/Akt/mTOR, Ras/Raf/MAPK and IKK/NF-κB pathways.

Not unlike the complexity of genetic lesions leading to myelomagenesis[111], MM cell-driven bone resorption and BM microenvironment-determined drug resistance also appear to be highly multi-factorial, with extensive groups of mediators and regulators, and with substantial potential for inter-patient heterogeneity. For example, polymorphisms in genes promoter regions for cytokines/growth factors (similar to those described for TNFα[112]) could explain differences between individual patients in regards to the relative contribution of these signalling cascades in the biological and clinical behaviour of the MM cells or perhaps account, at least partly, for the differences in the patterns and aggressiveness of the bone lesions of the disease. Furthermore, the potential for substantial redundancy between the diverse modulators of tumour-stromal interactions may also be an underlying source for eventual development of resistance to treatments targeting only individual mediators of these processes.[6] Therefore, the bench-to-bedside translation of more effective therapies targeting the MM-microenvironmental interactions will require not only further refinements of existing pre-clinical models, so that they simulate as faithfully as feasible the complex network of tumour-BM interactions, but also will have to take into account to the fullest possible extent the highly multi-factorial nature of the tumour microenvironment, in order to allow for rational development of strategies to selectively and comprehensively inhibit all those interactions between MM and their milieu which contribute to the pathophysiologic sequelae and the propensity of this disease for eventual development of resistance to conventional and investigational agents.

Conflict of interest statement

None declared

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