Multiple myeloma: the bone marrow microenvironment and its relation to treatment

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ABSTRACT

Multiple myeloma is the most common haematological malignancy yet currently it remains incurable. For decades the mainstay in therapy has been non-targeted approaches including genotoxic agents and immunosuppressants. With myeloma predominantly affecting an elderly population, who are vulnerable to aggressive therapy, these non-specific approaches have resulted in poor survival. However, in recent years an explosion of collaborative research into myeloma has identified molecular interactions between myeloma cells and the bone marrow microenvironment as promoting myeloma development and associated complications such as bone lesions due to osteolysis. At the same time, a better understanding of the adhesion molecules, cytokines and signalling pathways involved in myeloma has led to the development of new targeted therapies, which are improving the quality of life for patients and significantly extending median patient survival. This review explores the current understanding of molecular pathways that promote myeloma progression and lead to bone destruction, with particular reference to the influence of interactions with the bone marrow microenvironment. It describes molecular targets for therapy with reference to the new therapeutics and their improved efficacy. While the outlook for myeloma patients has improved in recent years as a result of these new approaches, drug resistance remains a problem and future therapies will also need to address the molecular mechanisms of resistance in order to improve further the outcome for patients with this disease.

KEY WORDS: Antineoplastic agents.
Cellular microenvironment.
Molecular targeted therapy.
Multiple myeloma.

Introduction

According to the most recent survey by Cancer Research UK in 2009, multiple myeloma (MM) is the 17th most common cancer in the UK.[1] In 2010, 4672 people in the UK were diagnosed with myeloma.[2] Multiple myeloma is a disease that predominantly affects the elderly, with 71% of cases diagnosed in people aged 65 years and over. Very few cases are diagnosed in people younger than 40 and most cases are diagnosed in people aged 75–79. Incidence rates increase steadily with age and peak in those aged 85 and over, with the disease twice as common in black people as in Caucasian and Asian people.[1]

Multiple myeloma is a malignant disease of terminally differentiated B cells (plasma cells), characterised by their clonal expansion within the bone marrow (BM), an overproduction of monoclonal immunoglobulin (Ig) in the blood or urine, and destructive bone lesions.[3] Patients typically present with recurrent infection and anaemia due to bone marrow infiltration, as well as renal failure, severe bone pain, multiple fractures and hypercalcaemia. Diagnosis is made by BM aspiration or biopsy. The morphology of the plasma cells of MM patients can vary in appearance, from small, mature differentiated cells resembling typical plasma cells, to large, immature undifferentiated cells of 20–30 µm in diameter.[4]

These malignant plasma cells are believed to rely heavily on their interactions with the surrounding microenvironment (i.e., osteoblasts, osteoclasts, endothelial cells and bone marrow stromal cells) in order to proliferate, and this interaction plays a role in the development of resistance to drugs.[5] In return, however, these interactions can also be disruptive to the environment that supports them.[6] By mechanisms that will be discussed here, bone resorption is enhanced in MM as a result of the increased activation of osteoclasts and the inhibition of osteoblasts. The uncoupling of this fine balance between bone formation by osteoblasts and bone resorption by osteoclasts gives rise to the widespread bone destruction – one of the most detrimental complications of MM. Direct cell-cell contact or the release of soluble factors from nearby BM stromal cells (BMSC) maintains the vicious circle of bone resorption and tumour cell survival.[7] Ultimately, this close interplay between various cells in the BM and the MM cells is critical in the progression and development of the disease.

Multiple myeloma remains incurable, notwithstanding developments in treatment regimens. Research into MM has not only helped in improving treatment and understanding of the disease itself, but has also provided significant knowledge about the BM microenvironment in haematopoietic malignancy. This article will review progress in the study of the interactions of MM cells with their local environment and how treatment strategies aim to influence these interactions.

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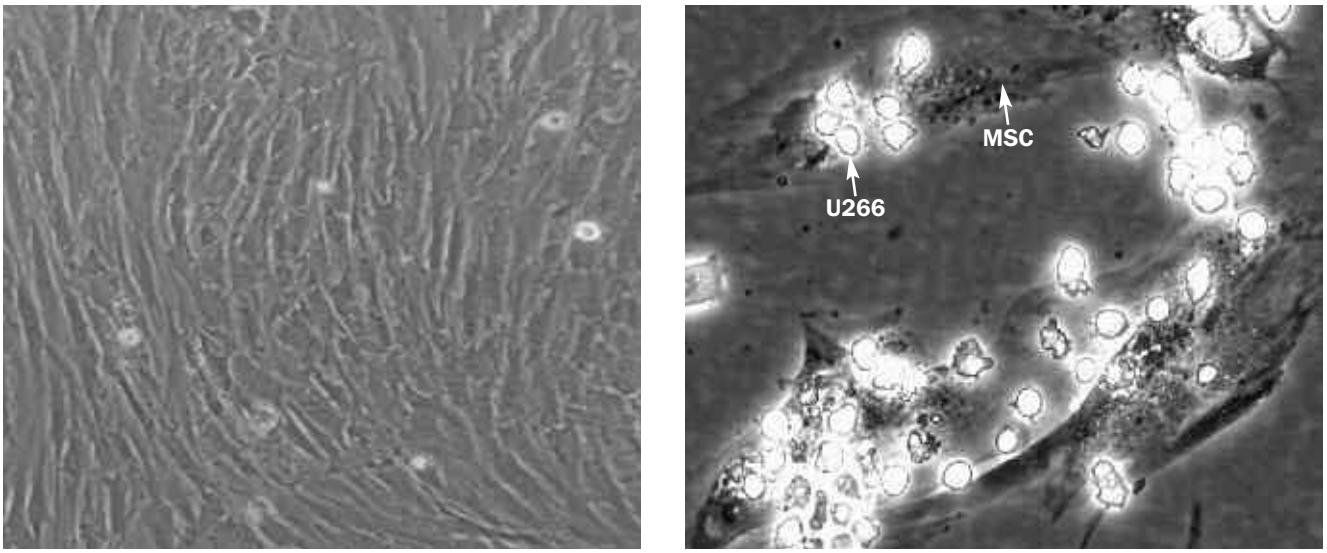

Fig. 1. Representative images of confluent MSC in culture (left) and a U266 multiple myeloma cell line and MSC in co-culture (original magnification x40).

Bone marrow microenvironment and multiple myeloma

The BM microenvironment (stroma) is a complex network of extracellular matrix (mainly collagen) which includes mesenchymal stem/stromal cells (MSC), osteoclasts, osteoblasts, lymphoid cells, fibroblasts and vascular endothelial cells. In 1974, Freidenstein and colleagues first identified and isolated human MSC when they placed whole BM in plastic culture dishes and then after four hours poured off the non-adherent (haematopoietic) cells, leaving a layer of adherent spindle-shaped cells capable of dividing rapidly in culture.[8] These cells are pluripotent and capable of differentiating into a number of mesenchymal cell lineages, including adipocytes, chondrocytes and osteoblasts.[9] Mesenchymal stem cells are relatively easy to isolate from BM and can be expanded *in vitro* using routine cell culture techniques.[10] Under normal conditions, these cells adhere to tissue culture plastic in 24–48 hours. In an undifferentiated state, their morphology resembles that of a fibroblast showing a small cell body with long, thin projections emanating from its centre (Fig. 1).

As there is no single specific marker for MSCs it is their immunophenotypic profile and characteristic morphology that identifies them, along with their extensive capacity for self-renewal while retaining the ability to differentiate along a number of mesenchymal lineages.[11]

As part of the minimal criteria proposed by the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy to define human MSCs,[12] cells must be positive for CD105, CD73 and CD90, and negative for haematopoietic markers such as CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR. Mesenchymal stem cells have been shown to express a number of cell adhesion molecules; for example, activated leucocyte adhesion molecule (ALCAM, currently referred to as CD166), vascular adhesion molecule 1 (VCAM-1/CD106), intercellular adhesion molecule 1 (ICAM-1/CD54), integrins and other adhesion molecules,[13,14] along with the cytokine receptors interleukin (IL)–1R, IL-3R, IL-6R, and tumour necrosis factor (TNF)–R.[15]

Although this review will focus on bone marrow MSC (BMMSC), it is important to note that MSCs are not exclusive to the BM and they can be isolated from virtually all post-natal and extra-embryonic tissues, including amniotic membrane, placenta, umbilical cord and umbilical cord blood.[16] However, MSCs are a rare population in these tissues, and even in the BM, where they are most abundant, their frequency may be as low as 0.001– 0.1% of the total population of marrow nucleated cells.[17] Mesenchymal stem cells are essential in forming the stroma of the bone marrow, which provides support, and the ability to grow and differentiate to primitive haematopoietic cells within the bone marrow.[18] This support is achieved both by direct cell-cell interactions and/or by release or production of cytokines such as IL-6 and granulocyte colony stimulating factor (G-CSF),[19,20] and may also play an important role in the pathogenesis of MM as these support mechanisms are harnessed by MM cells.[21]

In MM, the interaction with cells of the microenvironment determine the survival, migration and proliferation of malignant plasma cells as well as their response to therapy; thus, this stromal environment is essential in supporting tumour progression.[22] Actively growing neoplastic cells recruit MSCs through the release of various chemical signals, thus supporting and enabling them to differentiate into a growing cancer.[21] Over the past decade, molecular biological analysis of MM has improved the understanding of how MM develops and has started to reveal the processes that underpin disease progression. The key factors identified as having crucial roles in MM progression are the adhesion molecules expressed by MM cells and BMMSCs and the effects of cytokines produced by BMMSC and/or MM cells.

Adhesion molecules

The pathogenesis of MM is complex and involves various cytokines and adhesion molecules that provide positive and negative interactions between MM cells and BMMSCs, as well as other cells of the microenvironment (Fig. 2). Following such interactions, proliferative anti-apoptotic signalling pathways are activated in the MM cell,[23] which stimulate osteoclastogenesis[7] and angiogenesis.[24]

Multiple myeloma cells express the adhesion molecules lymphocyte function-associated antigen-1 (LFA-1/CD18),[25] very late antigen 4 (VLA-4/CD49d)[26] and neural cell adhesion molecule (NCAM/ CD56).[27] These molecules bind to their cognate receptor/adhesion molecule on the surface of the MSCs as CD54 is a ligand for CD18 and CD106 is a ligand for CD49d, and thus play an important role in the MM cell and marrow stromal cell interactions *in vivo* and *in vitro*.[28] Adhesion of the MM cells to MSCs activates many pathways, resulting in up-regulation of cell cycle regulating proteins and anti-apoptotic proteins in the MM cell.[5] These pathways, which include the PI-3K/Akt/mTOR/p70S6K cascade, the IKK-α/NF-κB pathway, Ras/Raf/MAPK and JAK/STAT3 pathways, can also be activated by numerous cytokines secreted both by MM cells and MSCs.[29–31]

Using a murine model, Michigami *et al*.[7] found that cell-cell interactions between MM cells and marrow stromal cells that are mediated through VCAM-1 increased the production of osteoclastogenic activity by the MM cells.

Many of the adhesion molecules expressed by the MM cell activate the nuclear factor-κB (NF-κB) signalling pathway, which plays a key role in the survival and proliferation of the MM cell.[32] NF-κB signalling protects the cell from apoptosis by activating anti-apoptotic genes of the Bcl-2 family such as *Bcl-XL* and *A1*.[33] In addition, NF-κB can also promote cell growth and differentiation by activating cyclin-D1 expression.[34] NF-κB activation also induces drug resistance in MM cells and up-regulates the expression of adhesion molecules involved in the resistance of MM cells to drugs. The expression of CD49d has been found to be elevated in the melphalan-resistant MM cells that were selected through chronic exposure to the drug.[26] The NF-κB pathway has also been shown to stimulate angiogenesis by inducing vascular endothelial growth factor (VEGF) expression.[35]

Cytokines and signalling pathways

Cytokines and growth factors produced either by MM cells or by stromal cells as a result of intercellular interactions have been implicated in the increase in osteoclast formation and activity. The expanding list of these growth factors includes IL-1β,[36] IL-3,[37] IL-6,[38] TNFα and TNFβ,[39,40] VEGF[41] and macrophage inflammatory protein-1α (MIP1-α).[42] The role of the cytokine IL-6 has been well documented in MM as it has an essential role in MM progression by regulating the growth and survival of tumour cells.[43,44] It is produced by osteoblasts, monocytes, macrophages and MSCs and binds to its cognate receptor IL-6R. IL6 is a pleiotropic cytokine released as a result of pro- and anti–inflammatory stimuli.[45]

Following binding to the gp130-associated receptor IL-6R, the intracellular tyrosine kinase Janus Kinase (JAK) is activated, which phosphorylates and activates the signal transducers and activators of the transcription 3 (STAT3) pathway.[46] Once STAT3 is activated, it translocates to the nucleus where it initiates transcription of IL-6 responsive genes. One such gene is *BCL2L1* that encodes the protein Bcl–XL, which suppresses apoptotic death of haematopoietic cells.[47] This protein works by inhibiting the release of pro-apoptotic molecules from mitochondria.[48]

Catlett–Falcone and colleagues[47] demonstrated that activated STAT3 contributes to the progression of MM by experimentally preventing apoptosis in MM cells by blocking the JAK/STAT pathway and thus inhibiting Bcl-XL expression. The phosphoinositol 3 kinase (PI3K)-protein kinase B (PkB/Akt) pathway is also activated by IL-6 and when activated provides an anti-apoptotic mechanism as well as increasing cell proliferation.[49] This pathway regulates the apoptotic machinery of the plasma cell by phosphorylating and inactivating pro-apoptotic proteins that control the release of cytochrome C from mitochondria.[49]

The release of cytochrome C is crucial for the cell to undergo apoptosis as it is required for the activation of caspases in the cytosol.[50] Cell proliferation is increased following activation of this pathway, by inhibiting the anti-proliferative effects of pro-apoptotic proteins as well as enhancing protein synthesis within the cell.[51] Similarly, IL-6 activates Ras and promotes its translocation to the plasma membrane where it activates Raf, mitogen-activated protein kinase kinase (MEKK) and MAPK, leading to increased proliferation of MM cells.[52]

IL-6 also promotes osteolysis (bone resorption) as it induces the production of the receptor activator of nuclear factor kappa-B ligand (RANKL), found on the surface of BMMSCs and osteoblasts.[29] RANKL interacts with its cognate receptor RANK on the surface of mature osteoclasts, causing their activation, and inhibits differentiation of osteoclast progenitors. In health, this is a tightly regulated mechanism whereby osteoprotegerin (OPG), a decoy receptor, is secreted by osteoblasts and competes with RANK for binding to RANKL, thereby reducing osteoclastogenesis.[53] Osteoprotegerin is a secreted factor that inhibits osteoclast development both *in vitro* and *in vivo*.[53]

Experiments using transgenic mice have highlighted the importance of the OPG/RANKL/RANK system in normal bone remodelling. Mice with a disrupted *RANKL* gene and those that over-expressed OPG had decreased osteoclast formation and developed an excessive accumulation of bone; a condition known as osteopetrosis.[54] Mice deficient in OPG were shown to develop osteoporosis caused by enhanced osteoclast formation and function.[55,56]

In MM, malignant plasma cells stimulate osteoclastogenesis by increasing RANKL and reducing the levels of OPG.[57] The mechanisms through which OPG levels are decreased have yet to be clearly defined, but a study by Standal *et al*.[58] has shown that OPG is bound, internalised and degraded by the MM cells through CD138 (Syndecan-1, a transmembrane protein that controls cell growth and differentiation). Further to this, MM cells may also exhibit an anti-apoptotic effect on osteoclasts by secreting large amounts of M-CSF.[59] As a result, when RANKL binds to RANK in patients with MM there is a dramatic increase in bone resorption. Abe and colleagues[60] identified that MM growth and survival is augmented by the cell-cell contact of MM cells and osteoclasts, and that this mechanism is partially dependent on IL-6 and osteopontin (protein found in osteoblasts). Furthermore, IL-3 has been reported to play a role in bone destruction in MM both by stimulating osteoclasts and indirectly inhibiting osteoblast formation *in vitro*.[61]

Myeloma cells also produce large amounts of MIP-1α, which is produced by freshly isolated cells from patients who have extensive bone disease, and this induces osteoclast formation independently of RANKL.[62] MIP-1α also enhances the osteoclast-inducing activity of RANKL and IL-6.[63] Terpos and colleagues[64] noted that the serum levels of

MIP1-α in patients with MM correlated with the extent of bone disease, bone resorption markers and RANKL level. They also noted that the three-year probability of survival with MM decreased with increasing levels of MIP1-α.[64] It has also been shown that MIP1-α induces the activation of the AKT/PKB and MAPK pathway and thus may also directly affect cell signalling pathways that affect growth, survival and migration of MM cells.[42]

Restoring the balance between RANKL and OPG not only stops myeloma-induced bone resorption, but also inhibits growth and survival of MM cells (see section on treatment). Each of these cytokines, as well as the interactions resulting from the adhesion of MM cells to BMMSCs, results in a vicious cycle of increased bone resorption and increased tumour growth.

Functionality of bone marrow in multiple myeloma

Bone remodelling

In addition to increased osteoclast activity and bone resorption, there is reduced bone formation due to the inhibition of osteoblasts.[65] Gilbert and colleagues[66] demonstrated an inhibition of osteoblast differentiation by TNFα *in vitro*. Osteoblasts are derived from MSC progenitors and are stimulated to differentiate during periods of active bone formation. The pathway by which osteoblast progenitors differentiate into mature osteoblasts is known as the canonical Wnt pathway. Briefly, Wnts are cysteine-rich secreted glycoproteins that bind to the Frizzled receptor and low-density lipoprotein receptor-related protein (LRP-5/6) and induce the canonical Wnt pathway. The canonical pathway affects cellular functions by regulating β-catenin levels and thus its nuclear transport and regulation of target genes elicit various effects including induction of differentiation and proliferation of osteoblasts.[67]

In the absence of Wnt signals, a dedicated complex of proteins, including the tumour suppressor gene product adenomatous polyposis coli (APC), axin and glycogen synthase kinase-3β (GSK-3β) controls phosphorylation of specific serine and threonine residues in the *N*-terminal region of β-catenin. This GSK3β-mediated phosphorylation marks β-catenin for ubiquitination and degradation by the proteasome. Signalling by Wnt factors blocks GSK-3β activity, resulting in the accumulation of non-phosphorylated β-catenin, which will translocate to the nucleus and is responsible for the regulation of target genes such as *CCND1*.[68]

The *CCND1* gene is a member of the cyclin-D family involved in a complex pathway that closely regulates physiological cell cycle progression from the G1 to S phase.[69] Over-expression of this gene has been documented in MM patients.[70]

Edwards and colleagues,[71] using an *in vivo* murine model, demonstrated that increasing Wnt signalling in the bone marrow microenvironment can prevent the development of osteolytic bone lesions by increasing osteoblast number and decreasing osteoclast number. Mice treated with lithium chloride (LiCl), an inhibitor of the enzyme GSK-3β, showed increased β-catenin expression in osteoblasts, suggesting that LiCl prevents the development of osteolytic lesions by increasing Wnt signalling in osteoblasts. Extracellular Wnt antagonists that inhibit the Wnt/β-catenin signalling pathway and consequently inhibit osteoblastogenesis have been implicated in MM.

Tian *et al*.[72] analysed the bone marrow of patients with newly diagnosed MM and identified an increase in Dickkopf-1 (Dkk1) in the serum of these patients, and suggested that Dkk1 may inhibit differentiation of BMSC into osteoblasts. It was also noted that the severity of the bone lesion correlated with increased Dkk1 levels in these patients. Finding that a soluble factor produced by MM cells suppresses osteoblast differentiation is significant, although it does not entirely explain why myeloma bone lesions do not heal, even in patients in complete remission. It may be that a long-lasting change in the marrow microenvironment inhibits the ability of osteoblast precursors to differentiate, even in the absence of MM cells.

Further to the findings of Tian and colleagues, Kaiser *et al*.[73] reported a correlation between Dkk1 serum concentrations and the amount of lytic bone disease. As the Dkk1/Wnt pathway is involved in cancer and bone pathophysiology, and the interaction of the cancer and bone marrow microenvironment is crucial to the progression of MM, Dkk1 may represent a potential target for treatment.

In relation to factors inhibiting the differentiation of osteoblasts, Silvestris *et al*.[74] showed that osteoblasts from myeloma patients are functionally exhausted and undergo apoptosis promptly in the presence of MM cells from patients with severe bone disease.

A transcription factor called Runx2 has been shown to be key in driving MSCs to differentiate into osteoblasts, and inhibition of Runx2 has been shown to be a major contributor to osteoblast suppression in MM. Giuliani *et al*.[75] observed decreased osteoblast differentiation when MM cells and osteoblasts were in cell-cell contact, compared with no cellular contact, which suggests that the cellular contact and the release of soluble factors contribute to the block of osteoblastogenesis *in vitro*. They found that in the presence of a blocking anti-CD49d antibody in the co-culture of osteoblast progenitors and MM cells, there was a reduced inhibitory effect on Runx2, suggesting that the CD49d/CD106 interaction could be responsible for blocking osteoblastogenesis by myeloma cells.

Treatment strategies for multiple myeloma

The earliest recorded case of MM is likely to be that of Sarah Newbury in 1844. She suffered from severe back pain and fatigue four years before her death.[76] She went on to develop fractures of both femurs, clavicles, right humerus, right radius and right ulna. At the time, the best available treatment was infusions of orange peel, rhubarb pills and opiates, but these failed to save her. She died on 20 April 1844, five days after being admitted to hospital.[76]

Over a century and a half has passed since then, and cancer research and treatment has improved significantly, with patients living 10–15 years and having a normally quality of life. However, despite recent advances in treatment, MM remains an incurable disease, largely due to the emergence of drug resistance. As mentioned above, the BM microenvironment promotes the survival and growth of malignant plasma cells, leading to the development of treatment strategies that inhibit certain interactions of the

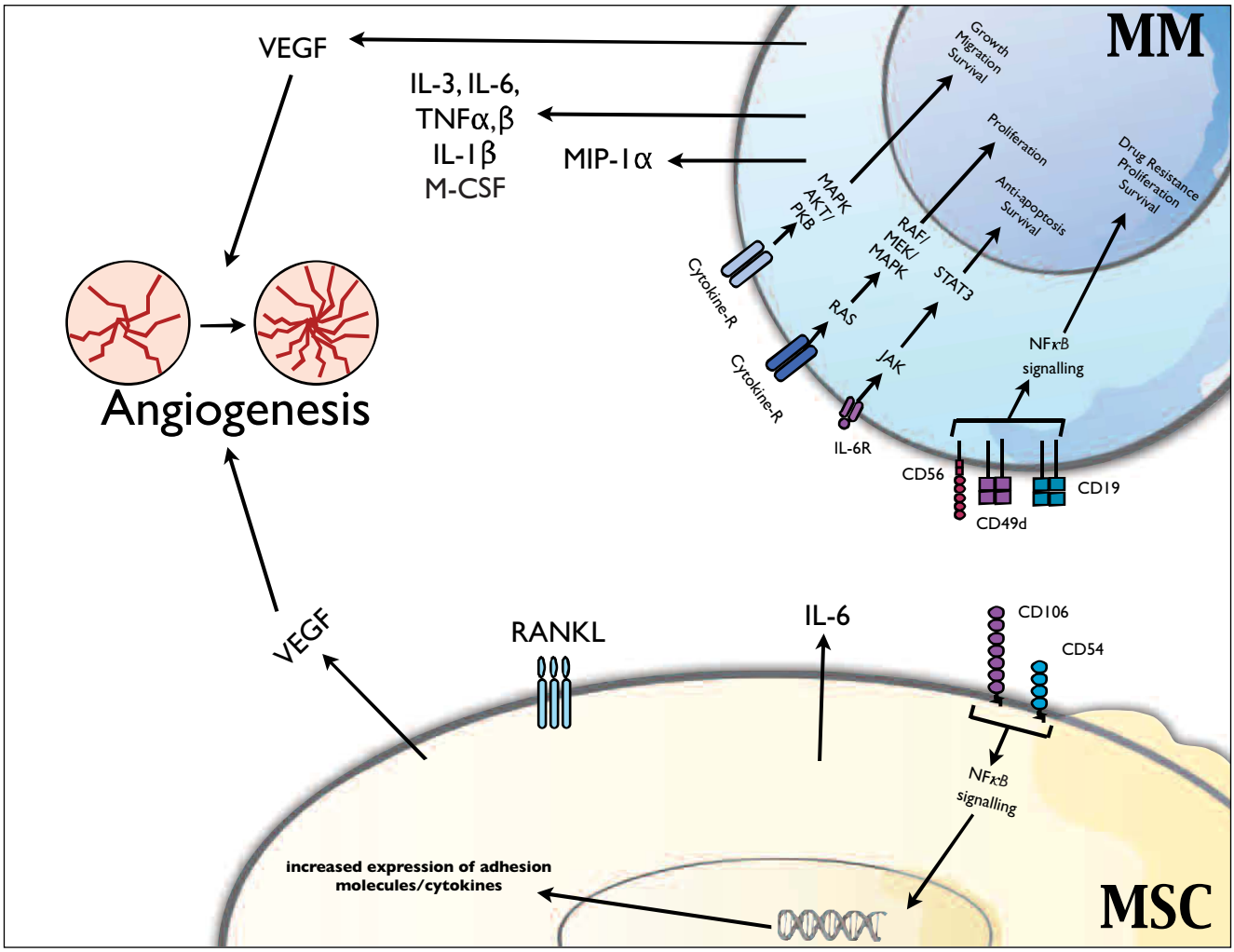

Fig. 2. Cell-cell interactions in MM. The diagram shows the MM cell and BMMSC in the bone marrow, and the pathways and signalling molecules involved in the pathophysiology of MM.
VEGF: vascular endothelial growth factor; IL1β: interleukin-1β; IL-3: interleukin 3; IL-6: interleukin 6; IL-6R: interleukin 6 receptor; JAK: Janus kinase; STAT: signal transduction and activators of transcription; Ras: rat sarcoma; RAF/MEK/MAPK: mitogen-activated protein kinases; MIP1-α: macrophage inflammatory protein 1α; TNFα/β: tumour necrosis factor α/β; OPG: osteoprotegerin; RANKL: receptor activator of nuclear factor-κB ligand; NF-κB: nuclear factor κ-light-chain-enhancer of activated B cells.

MM cell and BM microenvironment. Together, adhesion molecules and cytokines mediate MM pathogenesis by stimulating the expansion of MM cell populations that, as a result, contribute to bone destruction. Over recent years, the treatment of MM has undergone significant development and the introduction of new therapies has resulted in improved survival (Fig. 3).

Most treatment now aims to prolong survival. While few patients achieve a complete response (CR) with conventional chemotherapy regimens, rates have improved with the use of high-dose therapy (HDT) followed by autologous stem cell transplantation (auto-SCT) and the introduction of new therapies, such as thalidomide, lenalidomide and bortezomib. High-dose therapy supported by auto-SCT is now recommended for newly diagnosed MM patients under the age of 65 years[77] but is not indicated for older patients. Therefore, determining whether or not they would be candidates for stem cell transplant is one of the first steps in choosing an initial therapy for symptomatic MM patients.

For decades the standard treatment for patients consisted of the oral alkylating agent melphalan in combination with prednisone (MP).[78] With this regimen, the overall response rate is 50–60% and patient median survival is two to three years.[79] Although the introduction of melphalan and its combination with prednisone was important in the management of MM, patient survival remained unsatisfactory. The combination of vincristine, adriamycin and dexamethasone (VAD) later became a common initial therapy in preparing patients for auto-SCT.

Dexamethasone (Dex) was later found to achieve most of the plasma cell reduction with VAD and that survival times with VAD or Dex were similar.[80] Dex induces growth arrest and apoptosis in MM cells via activation of related adhesion focal tyrosine kinase (RAFTK).[81] Increased rates of survival were found in younger patients who received an auto-SCT and as a result it became the standard of care, while MP remained the treatment in older and less-fit patients.

In order to prevent recurrent MM and induce complete remission, combination therapy is usually used alongside auto SCT.[82] Maintenance treatment with active anti-myeloma agents post-transplant may successfully eliminate minimal

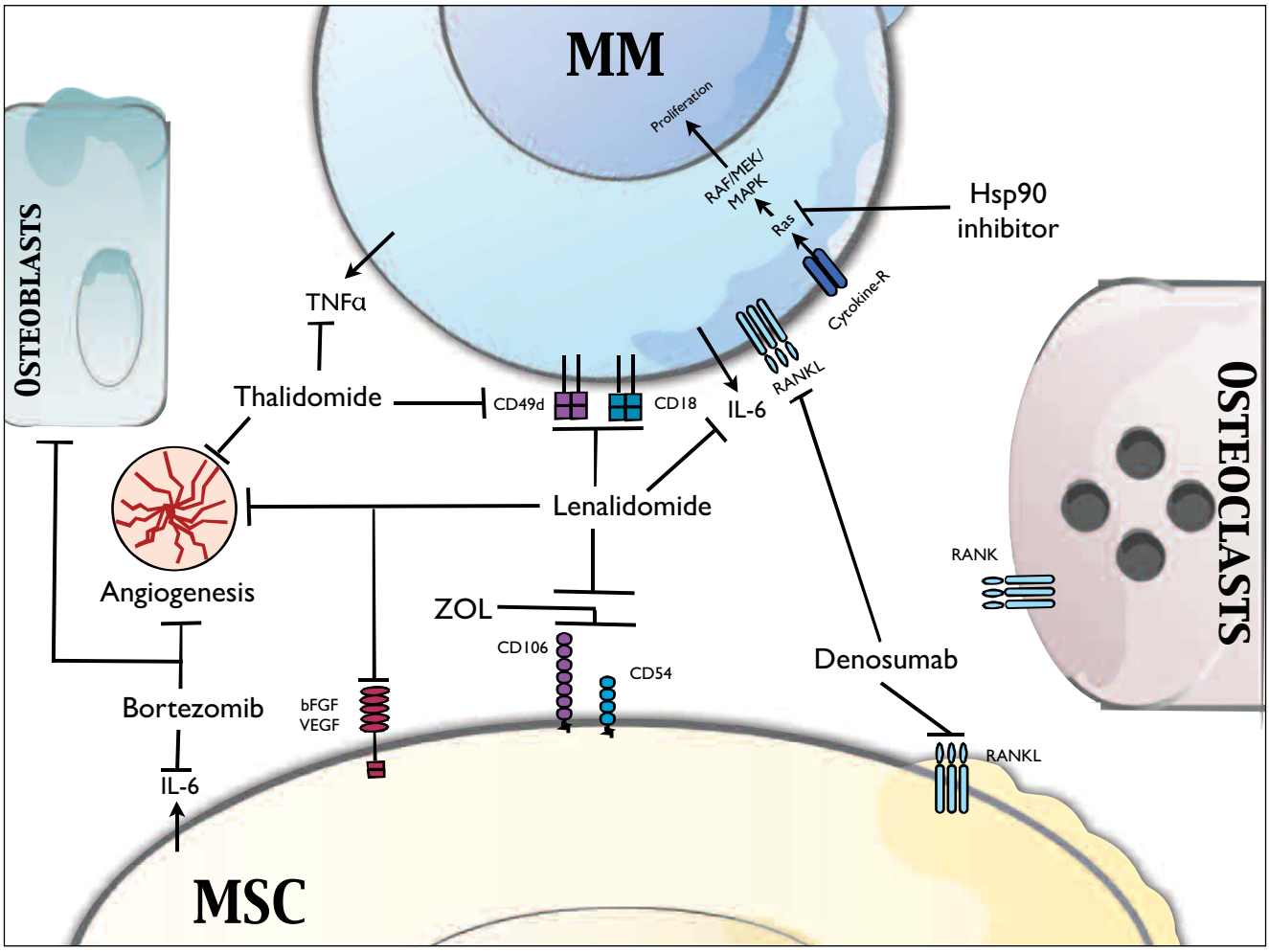

Fig. 3. Demonstrates the novel therapies designed to target the molecular pathways involved in the progression of MM. VEGF: vascular endothelial growth factor; bFGF: basic fibroblast growth factor; IL-6: interleukin 6; IL-6R: interleukin 6 receptor; Ras: rat sarcoma; RAF/MEK/MAPK: mitogen-activated protein kinases; TNFα: tumour necrosis factor-α; RANK/RANKL: receptor activator of nuclear factor-κB/ligand; ZOL: zoledronic acid; Hsp90: heat shock protein 90.

residual disease, delay disease recurrence and potentially extend survival.

Thalidomide, the first in the class of immunomodulatory drugs, has a broad spectrum of activity in MM. Its oral route of administration and minimal myelosuppressive effect makes it an attractive agent for maintenance therapy following autologous transplant. Thalidomide is a synthetic glutamic acid derivative first synthesised in 1953. Initially, it was used as a sleeping aid and an antiemetic in pregnant women. It was later withdrawn from use when it was reported that the drug produced severe, life-threatening birth defects. Thalidomide has since been found to significantly improve the management of MM as it possesses unique immunomodulatory, anti-inflammatory and anti-angiogenic properties.[83]

Angiogenesis is the formation of new blood vessels and is a fundamental process of normal development.[84] In cancer, angiogenesis is essential for tumour growth and metastasis, and increased angiogenesis has been documented in MM.[84] Thalidomide's ability to inhibit angiogenesis was first discovered in 1994, when it was demonstrated that thalidomide inhibited neovascularisation induced by basic fibroblast growth factor (bFGF) in the rabbit cornea micropocket assay.[85] In a mouse model, thalidomide inhibited angiogenesis induced by bFGF and VEGF.[86] Thalidomide may also inhibit adhesion of MM cells to marrow endothelial cells, as it can decrease the density of TNFα-induced CD54, CD106, and CD62E and CD62L on the endothelial cells of human umbilical vein.[87]

In order to improve the therapeutic index of thalidomide it may be combined with other active agents against MM. Thalidomide combined with dexamethasone was shown to induce a high response frequency, rapid onset of remission and low incidence of serious, irreversible toxicity compared to thalidomide alone in patients with previously untreated MM.[88] Thalidomide and dexamethasone also produced higher response rates compared to dexamethasone alone.[89]

Although thalidomide was shown to be useful in MM, modifications to its structure led to the formation of a new, less-toxic immunomodulating drug (IMD) called lenalidomide.[90] Like thalidomide, lenalidomide has anti-angiogenic properties and is a powerful inhibitor of TNFα; it also inhibits the adhesion of BMSCs and the release of growth and survival factors.[91] Palumbo *et al.*[92] conducted a double-blind trial comparing melphalan-prednisone-lenalidomide followed by lenolidomide maintenance (MPR-R), melphalan-prednisone-lenalidomide (MPR) and melphalan prednisone (MP) followed by placebo. Median

progression-free survival was significantly longer with MPR-R (31 months) than with MPR (14 months) or MP (13 months), with the greatest benefit observed in patients in the 65–75 age group.[92]

The introduction of the bisphosphonates has also improved the management of MM bone disease.[93] Bisphosphonates are pyrophosphate analogues that inhibit bone resorption by increasing osteoclast apoptosis.[94] They thereby have a direct effect on the BM microenvironment and are of particular clinical relevance in patients who have symptomatic bone loss. Bisphosphonates such as zoledronic acid (ZOL) has been of particular importance in MM due to its bone-protective effects.[95] In addition, ZOL down-regulates the expression of BMSC adhesion molecules (i.e., CD54, CD106, CD49d and CD40) that are involved in the cell-cell contact with MM cells.[96] As a result, IL-6 production is decreased, thereby reducing MM proliferation.

A new and clinically effective therapeutic agent known as denosumab has recently been developed for targeting osteoclasts. Denosumab is a fully human monoclonal antibody to RANKL that mimics the effects of OPG, thereby binding to and neutralising RANKL, leading to inhibition of osteoclast function.[97] A recent study by Henry *et al*.[98] compared denosumab to ZOL in preventing or delaying first on-study skeleton-related events in myeloma patients and patients with advanced cancer metastasising to bone and found that it was comparable to ZOL. Like other monoclonal antibodies, denosumab does not depend on renal clearance and can be administered by subcutaneous injection, providing many potential benefits with reduced side effects compared to those of ZOL. It should be noted, however, that as both agents target osteoclasts, osteonecrosis of the jaw is a potentially serious side effect of both therapies.[99,100]

A widely used agent in the treatment of MM is the proteasome inhibitor; bortezomib (Velcade, PS-341), which targets the 26S proteasome complex.[101] The 26S proteasome complex is the central proteolytic machinery of the highly conserved ubiquitin proteasome system (UPS), which controls basic cellular functions such as cell cycle progression and cell death. In the BM microenvironment, bortezomib inhibits the binding of MM cells to BM stromal cells, which in turn inhibits the production of IL-6 in the stromal cells as well as inhibiting angiogenesis.[102] Bortezomib blocks NFκB activation and thus makes the MM cell more susceptible to apoptosis. Bortezomib increases the susceptibility of MM cells to chemotherapeutic agents by regulating the expression of proteins involved in cell cycle progression (e.g., p21,p27) and apoptosis (BCL2).[103,104]

In 2001, Hideshema and colleagues[105] demonstrated that bortezomib acts directly on MM cells and alters cellular interactions and cytokine secretion in the BM milieu to inhibit tumour cell growth, induce apoptosis and overcome resistance to conventional therapies. Furthermore, bortezomib has also been shown to enhance osteoblast differentiation *in vitro* and *in vivo* in MM patients.[106] Bortezomib-induced osteoblast differentiation via Wnt-independent activation of β-catenin suggests that bortezomib might overcome Dkk1-mediated inhibitory effects on this pathway.[107] Terpos and colleagues[108] showed that bortezomib reduces Dkk1 and RANKL serum levels in patients with MM.

A trial by Mateos *et al*.[109] highlighted the benefits of using bortezomib in combination with melphalan and prednisone (VMP) when compared to the standard MP regimen. Overall response rate was 89% (32% CR) with VMP compared with 42% overall response rate for MP. In addition, the 16-month event-free survival rate was significantly higher with VMP than MP (83% versus 51%, $P<0.001$).[109]

Recently, heat shock protein 90 (Hsp90) has emerged as a potential target for treatment of MM.[110] Hsp90 are ubiquitous and abundant stress-inducible-related proteins that act as molecular chaperones, stabilising many 'client' proteins that are involved in proliferation and apoptosis.[111] Many of the client proteins crucial to the signalling pathways previously mentioned, such as Akt (PI3K/Akt pathway), FAK (integrin pathway), Bcr-Abl (RAS/ERK pathway) and Apaf-1 (apoptosis), are regulated by Hsp90, and hence inhibition of Hsp90 affects all these pathways.[112–114] As a result, the protective qualities provided by BMSC to the MM cells, which normally aids in MM cell survival, are diminished.[115] A combination of the Hsp90 inhibitor KW-2478 and bortezomib greatly reduce tumour burden *in vivo* and *in vitro*.[116]

Recent advances in the understanding of the pathophysiology of MM have allowed the production of new therapies against this disease, many of which target the malignant cell and the bone marrow microenvironment. However, resistance to chemotherapeutic drugs remains a major problem in the treatment of MM. While patients usually respond to initial chemotherapy, drug resistance subsequently appears and patients succumb to refractory myeloma.[117]

In order for the antitumour agent to exert its desired effect, it must reach the plasma cell in sufficient concentration. Reduced cellular drug accumulation may arise due to alterations in the uptake or efflux of the drug and could be responsible for the acquisition of resistance.[118] Transporter proteins called ATP-dependent multidrug transporters associated with resistance are multidrug resistance protein (MDR; P-glycoprotein, P-gp), multidrug resistance-associated protein (MRP1), lung resistance-related protein (LRP) and breast cancer resistance protein (BRCP), as these proteins play a major role in removing the drug from the cells.[119,120] As well as these proteins, intrinsic cell survival mechanisms including the over-expression of anti-apoptotic proteins (Bcl-XL), activation of NF-κB and Akt/MAPK signalling pathways leads to the malignant transformation of the plasma cell and ultimately drug resistance.[121] As new therapies for MM are established that target these pathways, the challenge now is for pharmaceutical companies to develop drugs that either evade efflux or inhibit the function of efflux transporters.

Conclusions

Multiple myeloma is characterised by severe bone destruction with reduced or no new bone formation. Various factors have been discussed related to the microenvironment or plasma cell that lead to this bone destructive process and disease progression. Our understanding of MM has come a long way since the first reported case in 1844. The introduction of melphalan led the way and remains a treatment option for some patients.

Continued investigation into the disease biology and tumour microenvironment has led to the development of new therapies that target specific proteins and pathways, and these have significantly improved the outlook for patients with MM. Moreover, the combination of these therapies with other agents may also lead to improved responses in this patient population. Indeed statistics from Cancer Research UK demonstrate clear improvements in survival rates, with one-year survival almost doubling between the 1970s and 2009 (35% versus 70%), whereas five- and 10-year survival rates have tripled and quadrupled (11% versus 37% and 5% versus 19%), respectively. Furthermore, current Cancer Research UK data may underestimate the survival rates for myeloma patients diagnosed today.[122] However, conventional treatment remains unsatisfactory due to drug resistance.

Further investigation into the complex pathogenesis of myeloma and the bone marrow microenvironment and its interactions is required, in order to combat the development of resistance to chemotherapeutics. Such new targeted therapies are starting to emerge and a continually improving rate of patient survival, quality of life and perhaps even a cure for this debilitating disease may not be too far from our reach. □

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