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Signalling and survival pathways in multiple myeloma

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ABSTRACT

The main factors that govern the pathophysiology and malignant growth of multiple myeloma (MM) are genetic defects within the tumour and the interaction between myeloma cells and the bone marrow microenvironment (BMM). This interaction leads to the activation of signalling pathways that promote the expansion of the malignant clone and stimulate neoangiogenesis and osteoclastogenesis. For many years, the cytokine interleukin-6 (IL-6) was considered a central growth factor and was thus believed to play a pivitol role in the pathogenesis of MM. However, increasing numbers of cytokines, chemokines and cell-to-cell contacts provided by the BMM have since been found to support MM cells. It has consistently been demonstrated that oncogenic mutations as well as the BMM stimulate IL-6-independent signalling pathways that protect MM cells from apoptosis. Consequently, multiple targeting of a complex signalling network rather than inhibition of a single pathway or growth factor is required to effectively induce myeloma cell death. Because the tumour suppressor p53 is rarely mutated in MM, non-genotoxic activation of the p53-dependent death pathway could be another attractive therapeutic strategy for this disease. Even though a number of promising new drugs are currently being tested in MM, a comprehensive knowledge of the signalling and survival pathways should pinpoint additional molecular targets and lead to the development of novel and hopefully more effective treatment strategies.

1. Introduction

Multiple myeloma is a malignant disorder of differentiated B-cells (plasma cells). Clonal expansion of the tumour results in the excessive production of monoclonal immunoglobulin (Ig) which is a diagnostic feature of this disease. Deposition of monoclonal Ig light chains in the kidneys can lead to renal failure which is a clinical hallmark of MM. Another characteristic is localisation of the malignant cells to the bone marrow where they lead to osteolytic bone destruction and impaired haematopoiesis. As a consequence, bone pain, hypercalcaemia, anaemia and recurrent infections frequently occur.[1] Despite the development of novel drugs, such as proteasome inhibitors and derivatives of thalidomide, MM remains incurable and the majority of patients eventually succumb to their cancer.

Induction of plasma cell tumours is believed to involve genetic lesions such as translocations between immunoglobulin enhancers and oncogenes, which are then complemented by secondary events that lead to activation of growth and survival pathways and to disruption of apoptotic signalling.[2,3] In addition to genetic alterations, the BMM is involved in the pathophysiology and malignant growth of MM. One consequence of myeloma-microenvironment interaction is enhanced expression of factors that either indirectly promote tumour growth via stimulation of angiogenesis or that directly act as growth factors for the malignant cell.[4] Specifically, the interplay between MM cells and bone marrow

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stromal cells (BMSCs) provides adhesion-mediated drug resistance and leads to activation of signal transduction pathways that promote cell cycle progression and protection from apoptosis.[3,5] Additionally, osteoblasts and osteoclasts are important for the manifestation of the disease. Signals from MM cells block osteoblast differentiation and activity but induce genesis and activation of osteoclasts, resulting in the uncoupling of the bone remodelling process. Because osteoclasts support MM cell growth and survival too, a vicious circle linking osteoclast-mediated bone destruction and myeloma expansion develops.[6,7] In recent years our knowledge about the different factors and components that influence tumour progression has greatly increased, and a number of targets for therapeutic intervention have been proposed and investigated.[8] This analysis, however, is far from complete, and a more comprehensive knowledge about the network of signals and mechanisms that promote tumour growth and prevent tumour death in the complex environment of the bone marrow should help to develop novel therapeutic approaches against myeloma.

2. Multiple myeloma: A multistep transformation process

Current models assume that MM evolves through a multistep transformation process. Accompanied by increasing numbers of oncogenic mutations the disease springs from a plasma cell and proceeds via monoclonal gammopathy of undetermined significance (MGUS) to clinically overt myeloma.[2] Translocations involving the Ig heavy chain locus on chromosome 14 (14q32) are commonly observed in MM and MGUS and are therefore thought to be early pathogenetic events. These translocations juxtapose the Ig heavy chain enhancer to a proto-oncogene leading to illegitimate activation of the latter. Examples of recurrent chromosomal partners (and their affected genes) for Ig translocations are 11q13 (*Cyclin D1*), 4p16 (*Fibroblast growth factor receptor 3* (*FGFR3*)), and 16q23 (*C-maf*). Taken together, the combined prevalence of IgH translocations in medullary MM exceeds 50% of cases.[2] Other frequent genetic events in myelomagenesis are activating mutations of the *N-* and *K-ras* genes. *Ras* mutations have been reported in 35–50% of MM patients, but appear to be rare in MGUS, suggesting that they are secondary genetic events that characterise or even cause the transition from MGUS to MM.[9] In contrast to the frequent activation of oncogenic pathways, mutation of tumour suppressors, such as p53, is relatively rare in MM and predominantly associated with advanced disease and extramedullary manifestations.[10–12]

The main functional consequences of these common genetic alterations in MM are cell cycle deregulation and apoptosis resistance. The t(4;14) translocation, for example, leads to overexpression of FGFR3 which stimulates the Ras/mitogen-associated protein kinase (Ras/MAPK) pathway. Alternatively, the same pathway can be activated by *Ras* mutations. Because activating mutations of *FGFR3* and *Ras* have not been found together, these mutations are likely to impart similar effects.[13] Activation of the Ras/MAPK pathway might contribute to cell cycle progression and cellular survival.[14] The t(11;14) translocation leads to overexpression of *Cyclin D1* which contributes to deregulated cell cycle progression, although myeloma cells have a very low proliferation index.[15]

3. The IL-6R/STAT3-pathway

In addition to genetic aberrations the bone marrow microenvironment is critical for the pathophysiology and pathogenesis of MM. The BMM is thought to provide essential support for the propagation and expansion of the malignant clone. The interaction between MM cells and BMSCs leads to enhanced expression and secretion of chemokines and cytokines that stimulate proliferation and protect MM cells from apoptosis. The best characterised myeloma growth factor is the cytokine IL-6 which is presumed to play an important role in the pathogenesis and malignant growth of MM.[16,17] Stimulation of cells by IL-6 leads to signalling through the IL-6 receptor (IL-6R) and triggers phosphorylation of signal transducer and activator of transcription 3 (STAT3) via Janus kinase 1. STAT3 was found to be constitutively activated in primary myeloma cells and inhibition of the IL-6R/STAT3 pathway induces apoptosis in certain human myeloma cell lines *in vitro*.[5,18] Furthermore, IL-6 knock-out mice fail to develop plasma cell tumours.[19] These observations highlighted the potential importance of this pathway for antiapoptotic signalling and made it a prime target for therapeutic intervention. However, the results of early clinical trials with IL-6-blocking antibodies were disappointing and failed to demonstrate substantial clinical responses.[20] Consistent with these observations, MM cells were found to survive IL-6R blockade if they were co-cultured with BMSCs.[5] These results called the essential role of IL-6 into question and suggested that the BMM additionally stimulates IL-6-independent pathways that exert a pro-survival effect. Supporting such a conclusion, a number of additional growth factors, such as stem cell factor (SCF), stromal cell-derived factor 1 (SDF-1), macrophage inflammatory protein 1α (MIP-1α), IL-21, insulin-like growth factor (IGF), and vascular endothelial growth factor (VEGF) have recently been identified that stimulate either the proliferation of MM cell lines or protect primary MM cells from apoptosis.[21–23] Interestingly, these factors might be expressed by bone marrow cells, indicating that the BMM provides multiple factors, rather than just one cytokine, that contribute to the malignant growth of MM.

4. The bone marrow microenvironment activates multiple pathways that protect myeloma cells from apoptosis

Detailed pathway analyses revealed that the majority of the myeloma stimulating growth factors activate the Ras/MAPK signal transduction pathway, whereas the STAT3 pathway is primarily stimulated by IL-6 and IL-6-related cytokines, such as leukaemia inhibitory factor (LIF).[5,21] Furthermore, co-culture of MM cells and BMSCs induces the Ras/MAPK pathway as well. Since IL-6R inhibition in this setting blocked STAT3 phosphorylation but did not affect MAPK activation, these experiments indicated that the BMM induces STAT3 activation via IL-6 and the MAPK pathway via IL-6-independent mechanisms.[5] *Ex vivo* experiments showed that *Ras* mutations might lead to constitutive MAPK activity in MM cells

which can be strongly enhanced by BMSCs. Thus, the Ras/MAPK pathway can be activated in MM cells via two different IL-6-independent mechanisms: by the environment and by oncogenic mutations (see above). These observations identified the Ras/MAPK pathway as an interesting target for therapeutic intervention. Surprisingly, blockade of the MAPK pathway alone has no or only minor effects on myeloma growth or survival. However, combined inhibition of both the IL-6R/STAT3 and the Ras/MAPK pathways strongly induces apoptosis of MM cells, even when they are growing in the presence of BMSCs.[5] This clearly indicates that the IL-6R/STAT3 and the Ras/MAPK pathways independently contribute to the survival of MM cells and that both are activated by the BMM. Therefore, in the presence of cells from the BMM, combined targeting of different and independently activated pathways may be required to efficiently induce apoptosis of MM cells. This has direct implications for the design of future therapeutic strategies for MM.

Other pathways/central mediators that have recently been identified to contribute to the proliferation and survival of MM cells involve phosphatidyl/inositol3-kinase/Akt (PI3K/Akt), notch, wingless (WNT), and nuclear factor-kappa B (NF-κB). Like the Ras/MAPK pathway, the PI3K/Akt pathway might be activated by various bone marrow-derived cytokines and chemokines.[24,25] Deletion of *PTEN*, which encodes a phosphatase that negatively regulates the Akt pathway, contributes to sustained activation of Akt in some MM cell lines.[26] Thus, similar to the Ras/MAPK pathway, the PI3K/Akt pathway might be activated by the microenvironment as well as by oncogenic aberrations. However, because the frequency of *PTEN* deletions and mutations in primary MM cells is low, the pathogenetic role of this tumour suppressor remains unclear.[27] Blockade of the PI3K/Akt pathway inhibits proliferation of MM cell lines and enhances apoptosis of primary MM cells.[28]

The notch receptors 1 and 2, which play an important role during lymphoid differentiation, were found to be aberrantly expressed in MM cells. Activation of notch-signalling through jagged-1 enhances MM cell proliferation whereas pharmacological blockade of notch-signalling suppresses proliferation of MM cell lines.[29,30] In contrast, it has been recently published that expression of constitutively active truncated forms of the four notch receptors (ICN1-4) inhibit growth and induces apoptosis in human B-cell lines. These conflicting results need to be analysed in more detail especially regarding the role of notch and the notch-triggered pathways on growth and survival pathways in primary MM.[31]

It has been reported that MM cells have hallmarks of activated WNT signalling.[32] Mutation of WNT pathway components (β-catenin) or of repressors of WNT signalling (adenomatous polyposis coli [APC]) is frequently found in cancers. Accordingly, overexpression of *WNT* genes can lead to cancer. In contrast to normal B-cell populations, MM cells express high levels of β-catenin, including the stabilised unphosphorylated form which acts in the nucleus by activating T-cell factor/lymphoid enhancer factor (TCF/LEF)-mediated transcription of target genes. Stimulation of WNT signalling enhances proliferation of MM cells, whereas its inhibition attenuates growth of MM cell lines *in vitro*. However, the mechanism that leads to activated WNT signalling in MM remains unknown, as no mutations in the genes for adenomatous polyposis coli (APC) and β-catenin have been detected.[32] Recently, it was found that β-catenin stability is functionally linked to the MAPK pathway.[33] MAPK activity leads via inactivation of glycogen synthase kinase (GSK)-3β to a decrease in β-catenin phosphorylation preventing its degradation by the ubiquitin mediated pathway.[33] Thus, MAPK, which is activated by the environment and by oncogenic mutations, might lead to active WNT signalling in MM cells. Interestingly, in addition to its role in MM cells, the WNT pathway plays an important role in osteoblast differentiation and bone formation (see below).

NF-κB was originally described as a B-cell transcription factor, which is required for proper regulation of normal B-cell differentiation. Constitutive DNA-binding and transactivation activity of NF-κB has been described in many tumours. In particular, Hodgkin and Reed-Sternberg cells of classical Hodgkin lymphoma show pronounced constitutive NF-κB activity and inhibition of NF-κB in these cells can block tumour growth *in vitro* and *in vivo*.[34] NF-κB was also found constitutively active in primary MM cells, and blockade of this transcription factor leads to apoptosis.[18,35] Although there is evidence that in some cell types Akt activates NF-κB via phosphorylation and thus inactivation of IκB kinase (IKK), the mechanism of NF-κB activation in MM cells is still unclear.[36] Interestingly, activation of NF-κB is not restricted to MM cells but it might also play a role in cells of the BMM (see below).

In summary, it appears that both the bone marrow microenvironment and oncogenic mutations activate a complex signalling network consisting of the IL-6R/STAT3, Ras/MAPK, PI3K/Akt, notch, WNT, and NF-κB pathways, which sustains MM cell survival and promotes tumour expansion. If and how these pathways are functionally linked to each other is largely unknown.

Osteoclasts constitute yet another important cellular component of the BMM that has been reported to support MM propagation and expansion. *In vitro* observations suggest that osteoclasts may be even more effective than BMSCs to promote the survival of MM cells. Primary MM cells appear to survive co-culture with *in vitro* differentiated osteoclasts for up to 13 weeks, whereas MM cells co-cultivated with BMSCs usually die earlier. Experiments using a non-contact co-culture showed a strong reduction in survival and viability of the myeloma cells indicating the need for a direct cell-to-cell contact.[7] In addition, osteoclasts survive longer in co-cultures with MM cells and in inhibition experiments using antibodies against cell adhesion molecules, it has been shown that very late antigen 4 (VLA-4) and $\alpha_v\beta_3$ integrin-mediated cell-to-cell contact is important.

5. The bone marrow microenvironment: direct and indirect growth support and bone destruction

The interplay between MM cell and its bone marrow microenvironment induces signalling pathways in both partners (Fig. 1). Specifically, the attachment of MM cells to BMSCs induces NF-κB activation and leads to up-regulation of IL-6, VEGF and receptor activator of NF-κB ligand (RANKL) in BMSCs. IL-6 and VEGF might act directly as growth factors for MM cells (see above) and might indirectly support tumour

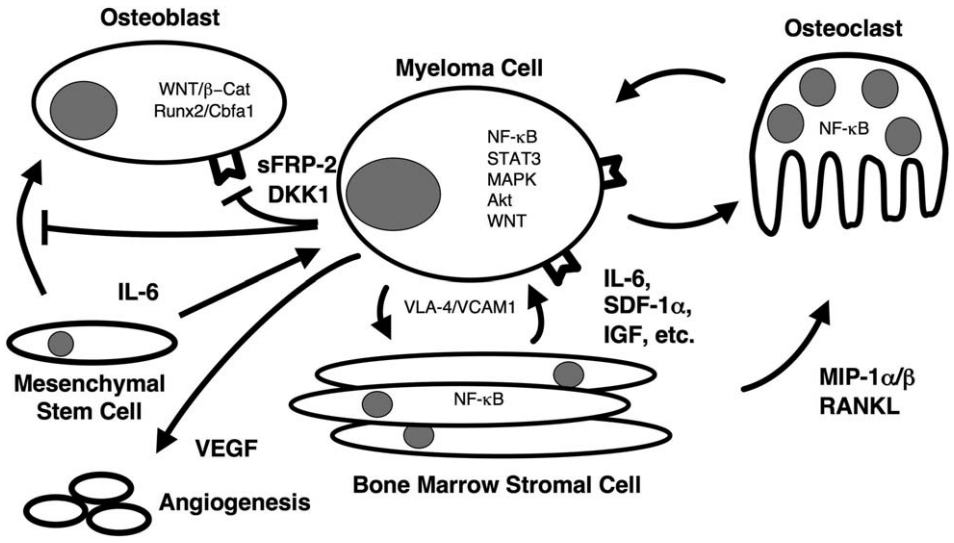

Fig. 1 – Interactions between multiple myeloma cells and cells of the bone marrow mircroenvironment. The diagram shows the cells in the bone marrow and pathways and signalling molecules involved in the pathophysiology of multiple myeloma.

expansion by promoting angiogenesis. RANKL binds to its receptor which is expressed by osteoclasts and osteoclast precursors and stimulates their differentiation and activation. This effect is mediated by activation of NF-κB in the osteoclasts, and inhibition of NF-κB blocks osteoclastogenesis.[37] Other cytokines secreted by MM cells are MIP-1α and MIP-1β, which belong to the RANTES family (regulated on activation, normal T cell expressed and secreted) of chemokines. It has been shown that MIP-1α can induce differentiation of osteoclast progenitors and osteoclasts in bone marrow culture. In addition, both cytokines can enhance RANKL expression in BMSCs.[38,39] On the other hand, similar to BMSCs, osteoclasts strongly enhance contact-mediated growth and survival of MM cells, entertaining a vicious circle between bone destruction and tumour expansion.[6] Thus, it appears that NF-κB contributes to the pathophysiology and pathogenesis of myeloma in multiple ways: it is activated not only in the malignant cell but also in BMSCs and osteoclasts which results in bone destruction and direct and indirect tumour growth support.

In addition to its role in tumour growth the WNT pathway is important for osteoblast differentiation and bone formation. Expression of the WNT signalling antagonist dickkopf 1 (DKK-1) or the soluble WNT inhibitor secreted frizzled-related protein 2 (sFRP-2) by myeloma cells, prevents WNT-mediated terminal differentiation of mesenchymal stem cells (MSC) and osteoblast progenitors into osteoblasts. Production of DKK-1 and sFRP-2 by myeloma cells has been shown to be associated with the presence of bone lesions in MM patients.[40,41] In addition, it has been shown that MM cells can suppress the activity of the transcription factor Runx2/Cbfa1, a critical transcription factor in pre-osteoblasts, in a cell contact-dependent way.[42] Thus, MM cells promote bone destruction by uncoupling the normal bone remodelling: 1) by inhibition of osteoblast differentiation and activity via inhibition of WNT signalling; and 2) by induction of osteoclast differentiation via activation of the NF-κB pathway.

6. Heat shock proteins contribute to the maintenance of the multiple myeloma signalling network

Heat shock proteins (HSP) are constitutively expressed molecular chaperones which support correct protein folding, intracellular disposition and turnover of key regulators of cell growth and survival. In response to protein-denaturing stresses, such as temperature changes, the intracellular level of HSPs increases in order to restore protein integrity and to facilitate cell survival.[43] As molecular chaperones HSPs might stabilise components of central signalling pathways such as Akt, MAPK, WNT, and NF-κB.[44] Furthermore, HSPs can stabilise protein complexes that keep apoptosis-inducing proteins such as e.g. caspases in their inactive state.[45] HSPs are overexpressed in many cancers and it is presumed that they are required to sustain the aberrant signalling in malignant cells. Thus, HSPs allow mutant and deregulated oncoproteins to gain function while permitting the transformed cell to tolerate the imbalanced signalling that oncoproteins might create. Recently, it was reported that pharmacologic inhibition of HSP90 decreases the activity of some pathways of the myeloma signalling network and induces apoptosis in MM cell lines and of primary MM cells. Furthermore, HSP90 blockade showed anti-myeloma activity in SCID mice transplanted with cells from a human MM cell line.[44] This suggests that HSP90 contributes to the maintenance of the MM signalling and survival network and might therefore be an attractive novel therapeutic target.

7. The p53 tumour suppressor pathway

Whereas pro-survival signalling provides an essential contribution to support tumour development, it is often complemented by inactivation of tumour suppressor mechanisms. Tumour suppressor protein p53, known as “guardian of the genome”, is a central transcriptional regulator of apoptosis,

cell-cycle progression and DNA repair under conditions of stress, including oncogenic transformation.[46] However, it has also been found to directly trigger the mitochondrial death programme.[47] p53 is kept at very low levels by its inhibitor murine double minute 2 (MDM2), which targets the protein for rapid degradation and is itself transcriptionally regulated by p53. If this autoregulatory feedback loop is disturbed through stress-induced stabilisation of p53 or destabilisation of MDM2, the level of p53 rises and the p53 pathway becomes activated.[48] Underscoring the central role of p53 for tumour suppression, its gene is believed to be the most frequently mutated in human cancer. However, in MM, p53 is rarely mutated or deleted at diagnosis and in medullary manifestations, although both types of lesion become more frequent in advanced and extramedullary disease.[11,49,50] Therapeutic activation of p53 might therefore offer a particularly suitable approach for the treatment of MM. Recently, Vassilev and colleagues reported on a novel class of competitive small-molecule antagonists of MDM2 (nutlins), that hog the main p53 binding site and specifically block the interaction between p53 and MDM2.[51] Selective pharmacologic inhibition of MDM2 with nutlin-3 activates the p53 pathway and induces apoptosis in about 90% of primary MM tumour samples.[11] Thus, it appears that the p53 tumour suppressor pathway remains functionally intact in the majority of medullary MM cases. Because nutlin-mediated p53 activation avoids the DNA damage inflicted by conventional chemotherapies, it may help to reduce the severe genotoxic side-effects of current treatments. In contrast to cancer cells, non-malignant cell types appear to be more tolerant to nutlin exposure. For example, the viability and the growth-supporting effect of human BMSCs on MM cells are not affected by nutlin treatment.[11] Similar observations have been made with primary human haematopoietic stem cells and treatment of nude mice with nutlins is well tolerated.[51,52] Because cancer cells that retain wild type p53 are often charged with a high burden of apoptotic triggers that needs be kept at bay, they may be exquisitely sensitive to specific p53 induction therapy.[46] Furthermore, the above mentioned observations suggest that this new class of selective MDM2 antagonist might be an attractive treatment strategy for patients with multiple myeloma.

8. Conclusion and perspectives

Multiple myeloma is presumed to evolve as a multipstep transformation process which requires the accumulation of oncogenic mutations. Genetic aberrations and the interaction between MM cells and the bone marrow microenvironment are of central importance for the pathophysiology and malignant growth of MM. Thus, both, the BMM and oncogenic mutations, activate a complex signalling network that sustains survival of the malignant cell and mediates tumour progression and drug resistance. Major signalling pathways involved are the IL-6R/STAT3, Ras/MAPK, PI3K/Akt, notch, WNT-, and NF-κB pathways. Heat shock proteins appear to contribute to the maintenance of the myeloma signalling network. Therefore, pharmacologic inhibition of this signalling network, for example via blockade of heat shock proteins, could be an interesting approach to develop novel therapies for MM. On the other hand, MM cells induce signalling in cells of their microenvironment which results in osteolytic bone destruction, neoangiogenesis and indirect tumour growth support. In contrast to oncogenic pathways, which are regularly affected by mutations, tumour suppressor genes are rarely mutated in MM. Selective activation of the p53-dependent pathway by pharmacologic antagonists of the p53-inhibitor MDM2 induces apoptosis of primary MM cells. Therefore, this non-genotoxic activation of the p53 pathway might be a promising therapeutic approach that could potentially be combined with inhibitors of the signalling network. Thus, combined targeted activation of p53-dependent and p53-independent death pathways, through direct activation of p53 and blockade of pro-survival signalling, might lead to the development of effective and less toxic therapies for MM patients. In addition to successful new drugs, such as bortezomib and the thalidomide derivative lenalidomide, that are already used for treatment, the coming years should witness more successes in translating the rapidly expanding knowledge on the biology of MM into the clinic.

Conflict of interest statement

None declared.

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