REVIEW

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Microenvironment for myeloma growth and drug resistance

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Bone provides a unique microenvironment for myeloma (MM) cell growth and survival, including niches to foster clonogenic MM cells. MM cells stimulate bone resorption by enhancing osteoclastogenesis, while suppressing bone formation by inhibiting osteoblastic differentiation from bone marrow stromal cells, leading to extensive bone destruction with rapid loss of bone. MM cells alter the microenvironment through bone destruction in bone where they colonize, which in turn favors tumor growth and survival, thereby forming a vicious cycle between tumor progression and bone destruction. MM is still difficult to be cured despite the recent implementation of new agents, and its bone disease also remains a significant clinical problem. Further elucidation of the molecular mechanisms of tumor-bone interactions and tumor growth in the bone microenvironment will provide us with new approaches that have a real impact on both bone disease and tumor progression.

Key words: myeloma niche, Pim-2, Glycolysis, clonogenic myeloma cells, side population

1. Introduction

Multiple myeloma (MM) has a unique propensity to expand almost exclusively in bone, and develop devastating bone destruction. MM cells stimulate bone resorption by enhancing osteoclastogenesis, and suppress bone formation by inhibiting osteoblastic differentiation from bone marrow stromal cells, leading to extensive bone destruction [1,2]. Cytokines aberrantly over-produced by MM cells, including macrophage inflammatory protein (MIP)-1α and MIP-1β as well as receptor activator of nuclear factor-κB (RANK) ligand up-regulated in bone marrow stromal cells play a major role in the enhancement of osteoclastogenesis and bone resorption in MM [3–5]. In contrast to enhanced osteoclastogenesis in MM, osteoblastic differentiation from bone marrow stromal cells is suppressed to cause extensive bone destruction with rapid loss of bone. Although a canonical Wingless-type (Wnt) signaling pathway plays a critical role in osteoblast differentiation, MM cells have been demonstrated to secrete soluble inhibitors of the canonical Wnt signaling pathway, including Dickkopf-1 (DKK-1) [6] and secreted Frizzled-related protein (sFRP)-2 [7]. DKK-1 is also secreted by stromal cells and osteoblasts in the presence of MM cells [8]. The levels of DKK-1 in sera and bone marrow plasma are increased in MM patients and correlated with the presence of osteolytic lesions [9]. Besides these Wnt inhibitors, several factors derived from MM cells and/or their surrounding microenvironment in bone such as IL-3, IL-7, TNFα, and TGF-β appear to suppress osteoblastic differentiation [1,2,10]. More recently, activin A and sclerostin, potent inhibitors of osteoblastogensis, have been demonstrated to be over-produced in bone lesions in MM [11–13]. Therefore, multiple factors act together to eventually develop extensive bone destruction in MM.

Bone provides a unique microenvironment for normal hematopoietic and cancer cell growth, including niches to foster cancer as well as hematopoietic stem cells. The fact that MM cells grow and expand almost exclusively in the bone marrow suggests the importance of the bone marrow microenvironment in supporting MM cell growth and survival. While MM cells perturb a normal bone metabolism to develop bone destruction, a crosstalk between MM cells and the microenvironment in bone lesions leads to the progressive vicious cycle phase of tumor growth and bone destruction [2,14]. In addition to the growth advantage that MM cells have acquired through genetic alterations and clonal selection, the cellular microenvironment skewed in MM endows MM cells with an additional growth and survival potential. In this review, current understanding of the mechanisms of MM progression and drug resistance in bone lesions and therapeutic approaches based on these mechanisms will be discussed.

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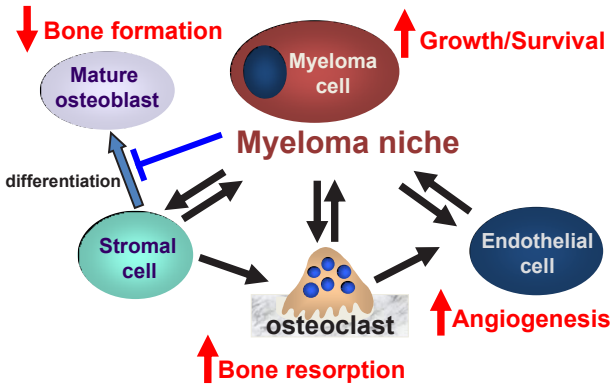

Figure 1. MM niche in bone. MM cells stimulate bone resorption by enhancing osteoclastogenesis, while suppressing bone formation by inhibiting osteoblastic differentiation from bone marrow stromal cells, leading to extensive bone destruction with rapid loss of bone. MM-induced cell types in MM bone lesions, namely osteoclasts, vascular endothelial cells, and bone marrow stromal cells with defective osteoblastic differentiation create suitable for MM growth and survival and confer a drug resistance to MM cells, which can be called an "MM niche".

2. MM niche

By analogy with the formation of a cancer pre-metastatic niche by the migration and seeding of hemopoietic cells to metastatic sites, MM cell-induced cell types in MM bone lesions, namely osteoclasts (OCs), vascular endothelial cells, and bone marrow stromal cells with defective osteoblastic differentiation, appear to play an important role in creating a beneficial cellular environment for MM cell growth and survival as a feeder for MM cells. Such a microenvironment can be construed as the "MM niche" (Figure 1).

2.1. Bone marrow stromal cells

Among cell components in the bone marrow, the roles of bone marrow stromal cells in MM cell growth and survival have been most extensively studied. The interaction between MM cells and stromal cells confers MM cell homing, growth, survival, and resistance to chemotherapy [15]. MM cells stimulate stromal cells to produce various growth and anti-apoptotic factors for MM cells, including IL-6, IGF-1, SDF-1α, IL-21, B-cell-activating factor (BAFF), and VEGF, while inducing RANK ligand to enhance osteoclastogenesis. Importantly, the adhesion of MM cells to stromal cells and their extracellular matrices (ECM) via VLA-4 or VLA-5 confers cell adhesion-mediated drug resistance (CAM-DR) on MM cells [16]. Autocrine activation of VLA-4 on MM cells by MM cell-derived MIP-1 and up-regulation of MIP-1 production by MM cells through VLA-4-VCAM-1 interactions appear to form a positive feedback loop between the adhesion of MM cells and their MIP-1 production [17]. In addition to osteoclastogenesis, MIP-1 has been suggested to promote MM cell homing or colonization in the bone marrow, which up-regulates the production by stromal/pre-osteoblastic cells of growth and anti-apoptotic factors and CAM-DR for MM cells. In contrast to the above factors over-produced in the bone marrow in MM, a murine model permissive for MM growth revealed decreased levels of host-derived adiponectin [18]. Interstingly, adiponectin appears to be decreased in the serum of patients with MGUS that subsequently progressed to MM. Adiponectin was found to increase MM cell apoptosis through activation of the AMP kinase pathway, and tumor burden and bone disease were enhanced in MM models using adiponectin-deficient mice.

2.2. OCs

Other than stromal cells, OCs induced by MM cells are also among the major cellular components of the bone marrow microenvironment. Intriguingly, OCs enhance MM cell growth and survival via an interaction between MM cells and OCs [19,20]. The effects of OCs on MM cell growth seem more potent than those of primary bone marrow stromal cells, while MM cells more firmly adhere to stromal cells and confer drug resistance [20]. Similar to bone marrow stromal cells, OCs protect MM cells from apoptosis induced by anti-cancer drugs, including doxorubicin. BAFF and a proliferation-inducing ligand (APRIL), members of the tumor necrosis factor (TNF) family, have been implicated as survival factors for myeloma cells [21,22]. OCs are a predominant cell type producing these factors in the bone marrow in MM [23]. Treatment with TACI-Fc, a decoy receptor for both BAFF and APRIL, markedly enhanced apoptosis of MM cells in the presence of OCs, indicating a supporting role for BAFF and APRIL in OC-mediated MM cell growth and survival [24,25]. Ge et al. reported that the serine protease fibroblast activation protein (FAP) is up-regulated in OCs after coculture with MM cells [26]. Knockdown of FAP expression reduced MM cell survival in cocultures. In addition, FAP is also expressed in osteogenic cells, bone marrow stromal cells, adipocytes, and vascular endothelial cells in the bone marrow.

Hexokinase II (HKII), a key enzyme of glycolysis, is widely over-expressed in cancer cells [27] (Figure 2). HKII has been demonstrated to be over-expressed in most MM cells [28]. The inhibitor of HKII 3-bromopyruvate (3BrPA) promptly and substantially suppresses ATP production and induces cell death in MM cells [29]. Interestingly, cocultures with OCs but not bone marrow stromal cells enhanced the phosphorylation of Akt along with an increase in HKII levels and lactate production in MM cells. The PI3K-Akt signaling has been demonstrated to phosphorylate HKII to facilitate its association with a mitochondrial membrane, and thereby stabilize HKII to post-translationally increase intracellular HKII levels [30]. Consistently, the enhancement of HKII levels and lactate production in MM cells by OCs

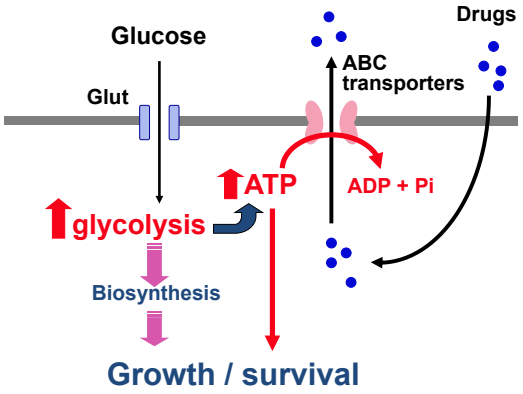

Figure 2. Aerobic glycolysis (the Warburg effect). Malignant cells increase their expression of glycolytic enzymes and glucose uptake to markedly enhance glycolysis (aerobic glycolysis; the Warburg effect), which leads to the production of a large amount of ATP and biomass such as nucleic acids and lipids essential for cell survival and division. ABC transporters are aberrantly over-expressed in drug-resistant cancer cells and side population (SP) cells. ABC transporter function in these cells appears to be mediated by ATP abundantly produced through enhanced glycolysis. The inhibition of glycolysis can abolish ATP production as well as biomass synthesis in cancer cells without compromising ATP production and cell metabolism in normal quiescent cells; and thus enhanced glycolysis may become a novel cancer-specific target for anti-cancer treatment.

were mostly abrogated by the PI3K inhibitor LY294002, suggesting activation of glycolysis in MM cells by OCs via the PI3K-Akt-HKII pathway. Treatment with 3BrPA was able to markedly induce cell death in MM cells even in cocultures with OCs.

These observations collectively suggest the contribution of OCs to the aggressiveness or drug resistance of MM cells. Thus, MM cells and OCs directly interact to augment their growth and activity, thereby forming a vicious cycle that leads to extensive bone destruction and MM expansion (Figure 1). These observations provide a rationale for therapeutic strategies targeting the OC-MM interaction.

2.3. Angiogenesis

Similar to osteoclastogenesis, angiogenesis is enhanced in the bone marrow in MM in parallel with tumor progression [31,32]. Bone marrow stromal cells as well as MM cells secrete angiogenic factors including vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) [33,34]. In vascular endothelial cells, the VEGF receptor VEGF-R2 mutually interacts with $\alpha V\beta_3$ integrin after the binding of their respective ligands to efficiently transduce their downstream signaling and induce angiogeneisis. Of note, OCs constitutively secrete a large amount of the proangiogenic factor osteopontin, a ligand of $\alpha V\beta_3$ integrin, which cooperates with VEGF secreted from MM cells to enhance angiogenesis and induce the production of osteoclastogenic activity by vascular endothelial cells [35]. Osteopontin is subject to enzymatic cleavage, and its fragments become functionally active [36]. OCs also produce matrix metalloproteinase (MMP)-9, which has been demonstrated to be responsible for angiogenesis induced by OCs [37]. MMP-9 may affect the activity of other factors elaborated in bone lesions in MM including osteopontin. Therefore, a close link between MM cells, OCs, and vascular endothelial cells can be established in MM bone lesions, thereby forming a vicious cycle involving bone destruction, angiogenesis, and MM expansion (Figure 1).

2.4. Niches for MM cancer stem cell (CSC)-like cells

The presence of CSCs or cancer-initiating cells have been demonstrated in various types of cancers, and regarded as a predominant cause of drug resistance. Likewise, MM CSCs have been postulated, and are considered to contribute to disease relapse through their drug-resistant nature [38,39]. Thus, MM CSCs become among the most important targets in the treatment for MM. However, MM CSCs are still conceptual in many senses, because of the lack of sensitive and reliable methods to identify them. Besides clonotypic B cells, phenotypically distinct MM plasma cell fractions have been demonstrated to possess a clonogenic capacity [40–42], leading to long-lasting controversies regarding the cells of origin in MM or MM-initiating cells. It is hard to explain the clonogenic capacity of differentiated MM cells only with the notion of a one-way hierarchical model from MM CSCs to differentiated MM cells. MM CSCs may not be a static population but survive as phenotypically and functionally different cell types via the transition between stem-like and non-stem-like states in local microenvironments, as observed in other types of cancers.

Recently, Chaidos et al. elegantly addressed a phenotypic and functional interconvertible state between $CD138^+$ and $CD138^-$ cells [43]. They identified a distinct subpopulation with $CD19^-CD38^{++}CD319^+CD138^-$ immunophenotype in clonotypic cells in both the bone marrow and circulation in patients with MM, and termed as pre-plasma cell. They identified a $CD138^+$ plasma cell to pre-plasma cell transition within clonotypic subpopulations in the bone marrow samples of 30 patients with MM by dynamic mathematical models. Together with the observations with the forward transition of clonotypic $CD19^+$ cells and/or $CD138^{low}$ cells to differentiated $CD138^+$ MM plasma cells [38,39,44], bidirectional transitions between differentiated and undifferentiated MM clonotypic cells are suggested in patients with MM (Figure 3). Because the inoculation of phenotypically differentiated MM cells alone could form MM in xenograft models, which is transplantable to secondary and tertiary recipients [40,41], MM-initiating cells seem to arise from MM cell population *in vivo*. Collectively, these findings

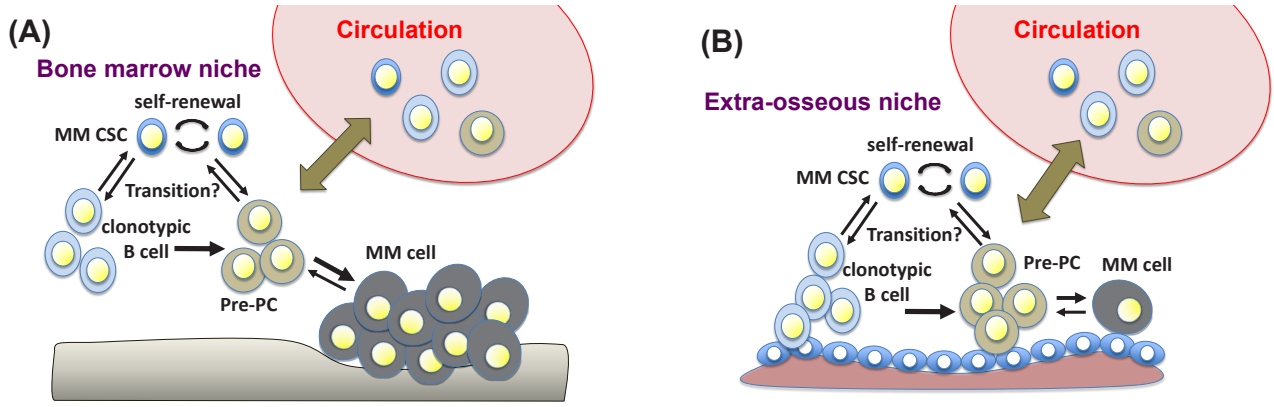

Figure 3. Localization of clonotypic cells in MM and transitions between MM progenitors and differentiated cells. A. Bone marrow (BM) niche for MM cells and their progenitors. MM cells are phenotypically and functionally heterogeneous populations. Clonotypic B cells have been demonstrated to be able to give rise to monoclonal immunoglobulin-secreting plasma cells. Besides clonotypic B cells, phenotypically distinct MM plasma cell fractions have been demonstrated to possess a clonogenic capacity. A distinct subpopulation termed as a pre-plasma cell (pre-PC) with $CD19^{-}CD38^{++}CD319^{+}CD138^{-}$ immunophenotype in clonotypic cells is identified in both the bone marrow and circulation of patients with MM. Bidirectional transitions between pre-PC and differentiated MM cells have been demonstrated. These clonotypic cells egress from the bone marrow into circulation and home back to the bone marrow. The existence of phenotypic as well as functional plasticity between MM cancer stem cells (CSCs) and non-CSC populations is also suggested. B. Extra-osseous niches for MM progenitors. When human $CD138^{+}$ mature MM cells were injected via tail veins in human MM-permissive mice, the spleen and liver were found to contain predominantly immature clonotypic plasma cells, including pre-PCs, rather than $CD138^{+}$ mature MM cells, although $CD138^{+}$ mature MM cells composed the majority of clonotypic cells in the bone marrow. Thus, extra-osseous tissues are thought to harbor rather immature clonotypic cells egressed from the bone marrow, and provide niches for clonogenic MM cells to foster them. Immature clonotypic cells or clonogenic MM cells colonized in extra-osseous may egress again and home to the bone marrow.

imply the existence of phenotypic as well as functional plasticity between CSCs and non-CSC populations in MM cells.

As reported for SCID-hu or SCID-rab models in which human MM cells were injected into bone marrow cavities of subcutaneously implanted human or rabbit bones, MM growth was restricted to the implanted bones, suggesting that human or rabbit bones serve niches for MM CSCs in these experimental models [40,45]. Furthermore, Yaccoby demonstrated that in cocultures with osteoclasts, differentiated MM cells became plasmablastic along with reduced expression of CD38 and CD138, and developed resistance to dexamethasone-induced apoptosis [46]. Interestingly, Chaidos et al. found that when human $CD138^{+}$ mature MM cells were injected into immunodeficiency mice via tail veins, the spleen and liver contained predominantly immature clonotypic cells, namely $CD19^{-}CD38^{++}CD319^{+}CD138^{-}$ pre-plasma cells and $CD138^{low}$ plasma cells, rather than $CD138^{+}$ MM cells, although $CD138^{+}$ mature MM cells composed the majority of clonotypic cells in the bone marrow [43] (Figure 3). Furthermore, clonotypic B cells appear to play a role in disease progression or recurrence in MM. The frequency of circulating clonotypic B cells have been demonstrated to increase in parallel with disease progression [43]. Clonotypic B cells are detected not only at diagnosis or at relapse but also persist even in patients with MM achieving a molecular remission in bone marrow samples after treatments [43]. Therefore, clonotypic B cells appear to be a reservoir for MM-initiating cells to cause relapse. Taken together, these results suggest dedifferentiation of mature MM cells *in vivo* and preferential localization of immature MM clonotypic cells or MM progenitors in certain extra-osseous microenvironments as well as the bone marrow. Further studies are needed to explore the localization of MM CSC-like cells and the mechanisms of their tumorigenicity and plasticity upon interaction with their preferential microenvironment.

3. Targeting MM cell-MM niche interaction

MM remains incurable even with the implementation of novel anti-MM agents, high-dose chemotherapies, and immunotherapies, and thus there is a need to target and disrupt the MM niche to improve the efficacy of the present therapeutic modalities against MM progression as well as MM bone disease.

3.1. Inhibition of osteoclastogenesis

The administration of inhibitors of OC activity, including bisphosphonates, RANK-Fc, and osteoprotegerin, not only prevents MM-induced bone destruction but also interferes with tumor progression in animal models of MM in some reports [47–49]. The repeated administration of bisphosphonates has also been reported to reduce the tumor burden without chemotherapy in a portion of MM patients [50,51]. These observa-

tions are consistent with the notion that an interaction between OCs and MM cells may play an important role in MM expansion in the bone marrow. In addition, the MM-OC interactions create an acidic milieu by protons produced by OCs and lactate by proliferating MM cells (the Warburg effect), which blunts cytotoxic effects of various chemotherapeutic agents as well as the activity of cytotoxic immune cells. Therefore, potent anti-resorptive therapy together with tumor reduction is needed to effectively suppress osteoclastogenesis, thereby disrupting the vicious cycle between osteoclastogenesis and MM expansion.

OCs produce the anti-apoptotic factors BAFF and APRIL, as noted above. BAFF is also produced by bone marrow stromal cells [52]. Gene expression of TACI, a receptor for these factors, in MM cells can distinguish tumors with a signature of microenvironment dependence [23]. To target these factors, TACI-Ig (atacicept) and anti-BAFF neutralizing antibody have been developed and shown to exert anti-MM effects in preclinical animal models [25]. Denosumab, a fully human monoclonal antibody against RANK ligand that inhibits the RANK ligand-RANK system, is under worldwide clinical trials with patients with MM.

3.2. Induction of bone formation

A reverse correlation between osteoblastic differentiation and MM tumor growth has been reported in patients with MM treated with the proteasome inhibitor bortezomib [53–55] and carfilzomib [56]. Serum levels of bone-specific alkaline phosphatase were found to be elevated after treatment with bortezomib, which were inversely correlated with a reduction in the tumor burden [57,58]. Such bone anabolic effects of bortezomib and their correlation with tumor regression were further demonstrated in MM animal models [59]. MM growth inhibition associated with osteoblastic differentiation has also been observed in MM animal models treated with other anabolic agents such as anti-DKK1 antibody [60,61], lithium chloride [62], and activin A inhibitor [12,63], as well as with enforced expression of Wnt3a within bone [64]. These observations suggest that anti-MM activity emerges with osteoblastic differentiation, and that MM cells may protect themselves from such osteoblast-mediated growth suppression by inhibiting the terminal differentiation of osteoblasts (Figure 1). The pro-anabolic agent BHQ880, an anti-DKK-1 monoclonal antibody, is currently being evaluated in clinical trials to test the promising preclinical results [65].

Transforming growth factor-β (TGF-β) is a potent inhibitor of terminal osteoblastic differentiation and mineralization [66]. It is produced by osteoblasts and osteocytes, and deposited in bone matrices as its latent form [67]. In MM bone destructive lesions, TGF-β appears to be abundantly released from bone matrices through enhanced bone resorption and activated by acids and MMPs secreted from OCs. We have demonstrated that blockade of TGF-β action releases stromal cells from their differentiation arrest by MM, and that terminally differentiated osteoblasts inhibit MM cell growth and survival and potentiate responsiveness to anti-MM agents [10]. These results support the idea that the suppression of osteoblastic differentiation by MM both accelerates bone loss and creates an MM niche to provide favorable conditions for MM growth and survival (Figure 1). Thus, the induction of osteoblastic differentiation through TGF-β inhibition may provide a novel approach to ameliorating bone destruction and tumor progression in MM.

Activin A is a TGF-β superfamily member associated with a variety of fundamental biological processes, including embryogenesis, organ development, gonadal hormone signaling, and erythropoiesis. It also affects bone remodeling, and plays a crucial role in the skeleton [68]. It has been reported that bone marrow plasma levels of activin A are increased in MM patients with bone lesions [11]. MM cells induce the release of activin A from bone marrow stromal cells in part via activation of their JNK pathway [11] (Figure 1). Furthermore, activin A inhibits osteoblastic differentiation and bone formation by stimulating intracellular signaling protein Smad2 activity and inhibiting distal-less homeobox (DLX)–5 expression. The soluble activin type IIA (ActRIIA)-Fc fusion protein is now under clinical development for patients with MM.

A large amount of ECM proteins is accumulated with osteoblastic differentiation. TGF-β-binding small leucine-rich proteoglycans, biglycan and decorin, were found to be among the most up-regulated proteins produced by terminally differentiated osteoblasts [10]. These proteoglycans bind various growth factors and modulate their actions. Decorin has been identified among osteoblast-derived factors responsible for the suppression of MM cell growth and survival [69]. Modification of the sulfation status of extracellular proteoglycan chains markedly regulated the binding and signal transduction of a number of growth factors and cytokines. Forced expression of extracellular heparan sulfate 6-O-endosulfatases, which are down-regulated in MM cells, was reported to markedly promote the deposition of ECM within the tumor microenvironment, along with a marked reduction in MM tumor growth *in vivo* [70]. These findings suggest that modification of the sulfation status of proteoglycans can have a considerable impact on MM cell growth and survival. A variety of ECM proteins and enzymes affecting the sulfation status, including sulfatases and sulfotransferases, have been found to be targets of Runx-2, an essential osteogenic transcription factor, and up-regulated in mature osteoblasts [71].

3.3. Targeting MM cell signaling pathways up-regulated in bone lesions

A number of intracellular signaling mediators, including NF-κB, Akt, p38MAPK, and the adaptor protein p62, have been well demonstrated to be activated in both MM cells and their surrounding environmental cells and regulate the pathological bone metabolism and tumor growth in MM.

In pursuing factors responsible for MM tumor survival in bone lesions, we have found that the serine/threonine kinase Pim-2 is constitutively over-expressed as a critical pro-survival mediator in MM cells, and its expression is further up-regulated in cocultures with bone marrow stromal cells or OCs [72] (Figure 4). Pim-2 has been identified using cDNA microarrays as one of the 34 most highly over-expressed genes in MM cell lines in comparison with lymphoma and AML cell lines [73]. Although Pim-2 appears to be the most up-regulated member of the Pim family, MM cells also expressed Pim-1, but rarely Pim-3, at various levels. Pim-1 and Pim-2 were first identified as oncogenic factors based on observations of the development of lymphomas in transgenic mice over-expressing these kinases [74,75]. In addition to enhancing oncogenic and survival signals, Pim kinases have also been shown to be involved in the expression and activation of drug efflux transporters [76]. Pim-1 phosphorylates the ATP-binding cassette (ABC) transporter ABCG2, also known as breast cancer resistance protein (BCRP), and thereby promotes its multimerization with its stable membrane expression, leading to drug resistance [77]. Because ABCG2 is preferentially expressed in MM cells, the up-regulation of Pim kinases may involve a drug efflux in MM cells to reduce the efficacy of drug treatment. Sensitization of MM cells to chemotherapeutic agents by Pim inhibition warrants further study on combinatory treatment of anti-MM agents such as bortezomib and melphalan with Pim inhibitors. Inhibition of the IGF-1/PI3K/Akt pathway by Akt inhibitors has drawn considerable attention as a new therapeutic modality against MM [78,79]. However, because the survival pathways elicited by Pim kinases seem independent of the PI3K/Akt pathway [72,80], Pim kinases should be targeted together to improve the anti-MM efficacy of PI3K/Akt pathway inhibitors. As Pim-2 expression is not increased in normal bystander cells, Pim-2 seems to be a specific target for the treatment of MM. Specific inhibitors for Pim-2 are under development at several pharmaceutical companies as anti-cancer agents [76], and may be available in the near future.

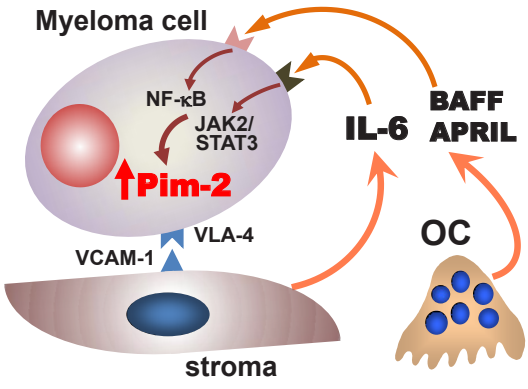

Figure 4. Enhanced expression of Pim-2 in MM cells in bone lesions. Pim-2 is a novel pro-survival mediator for MM cells. MM bone marrow microenvironment up-regulates Pim-2 expression in MM cells through activation of the JAK2/STAT3 pathway for IL-6 and the NF-κB pathway for TNF family cytokines, TNFα, BAFF and APRIL, to promote MM cell survival. Pim-2 over-expressed in MM cells in the MM bone marrow microenvironment appears to be an important therapeutic target.

3.4. Targeting glycolysis in drug-resistant and clonogenic MM cells

The most important property of CSCs is that they are resistant to chemotherapeutic agents. Targeting MM CSCs is clinically relevant, and various approaches have been proposed to target molecular, metabolic and epigenetic signatures and the self-renewal signaling pathway characteristic of MM CSC-like cells.

The side population (SP) phenotype is characteristic of stem cells in various normal tissues [81]. CSCs also exhibit high levels of ABC transporter activity and confer drug resistance besides their clonogenic or tumor-initiating capacity. SP cells are identified by their ability to efflux Hoechst33342 dye, a substrate for ABCG2, suggesting that SP cells have high ABC transporter activity. SP cells have been also demonstrated as a drug resistant fraction in many cancers, and are considered to contain their CSCs [81–85]. In MM, the distinct fraction of SP cells has been detected in both MM cell lines and primary MM cells [86].

Malignant cells increase their expression of glycolytic enzymes and glucose uptake to markedly enhance glycolysis (aerobic glycolysis; the Warburg effect), which leads to the production of a large amount of ATP and biomass such as nucleic acids and lipids essential for cell survival and division [28,87–89] (Figure 2). In parallel with enhanced glycolysis, ATP production by oxidative phospohorylation in the tricarboxylic acid (TCA) cycle in mitochondria is suppressed through oncogenic alterations including the mutation of p53. Glycolysis in MM cells has been demonstrated to be further up-regulated in cocultures with OCs [29]. Because ABC transporter activity is dependent on ATP [90,91], and because ATP production in cancer cells is largely dependent on enhanced glycolysis, inhibition of glycolysis by 3BrPA was found to promptly and effectively suppress ATP production and ABC transporter activity in ABC transporter-expressing malignant cells and restored their

susceptibility to anti-cancer drugs [29]. Although the relationship between ABCG2 expression and SP phenotype has been established, other ABC transporters are also involved in the SP phenotype with high ABC transporter activity and drug resistance. Therefore, the efficacy of strategies targeting a single transporter appears to be limited. In contrast, inhibition of glycolysis is able to simultaneously inactivate all types of ABC transporters in cancer cells including SP cells, because each transporter is dependent on ATP generated largely through enhanced glycolysis. The SP cells exhibited the increased expression of genes involved in the glycolytic pathway including *GLUT1, GLUT3, PDK1* and *PFK2*; and generated a larger amount of ATP and lactate per cell compared to MP cells [29]. Thus, glycolysis appears to be highly accelerated in SP cells. Furthermore, the ability of RPMI8226 cells to form colonies was completely abolished by relatively low concentrations of 3BrPA. From these results, it is plausible to assume that inhibition of glycolysis can disrupt their clonogenic capacity and drug resistant nature in SP cells.

4. Perspectives

MM cells and their surrounding cells in the bone marrow microenvironment closely interplay with each other to create an MM niche in the bone marrow to promote MM cell growth and protect MM cells from spontaneous and drug-induced apoptosis (Figure 1). In addition to cytotoxic agents for MM cells, novel agents targeting bone disease appear to be promising therapeutic strategies for the treatment of MM tumor expansion. However, because MM remains incurable even after effectively debulking the tumor mass, the idea of MM CSCs has been proposed to represent functional properties of small numbers of cells that have innate resistance to chemotherapeutic agents and the ability to live long and regrow in local microenvironments. Given the interconversion between MM CSC-like cells or undifferentiated clonotypic cells and MM cells, we should target all types of clonotypic cells from MM CSCs to mature MM cells. Although MM cells preferentially grow within the bone marrow, we also need to clarify where MM CSCs localize and how they self-renew upon interaction with MM microenvironment. The establishment of sophisticated, refined and humanized methods to recapitulate MM microenvironment, which supports MM CSC-like cell survival and self-renewal, is urgently needed. Further elucidation of the molecular mechanisms that keep MM clonogenesity and drive the transition between stem-like and non-stem-like states in local microenvironments will provide us with new approaches with crucial impact on the therapeutic paradigm in MM.

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References

1) Raje N, Roodman GD. Advances in the biology and treatment of bone disease in multiple myeloma. Clin Cancer Res. 2011; 17: 1278-86.
2) Abe M. Targeting the interplay between myeloma cells and the bone marrow microenvironment in myeloma. Int J Hematol. 2011; 94: 334-43.
3) Choi SJ, Cruz JC, Craig F, Chung H, Devlin RD, Roodman GD, et al. Macrophage inflammatory protein 1-alpha is a potential osteoclast stimulatory factor in multiple myeloma. Blood. 2000; 96: 671-5.
4) Han JH, Choi SJ, Kurihara N, Koide M, Oba Y, Roodman GD. Macrophage inflammatory protein-1alpha is an osteoclastogenic factor in myeloma that is independent of receptor activator of nuclear factor kappaB ligand. Blood. 2001; 97: 3349-53.
5) Abe M, Hiura K, Wilde J, Moriyama K, Hashimoto T, Ozaki S, et al. Role for macrophage inflammatory protein (MIP)-1alpha and MIP-1beta in thedevelopment of osteolytic lesions in multiple myeloma. Blood. 2002; 100: 2195-2.
6) Tian E, Zhan F, Walker R, Rasmussen E, Ma Y, Barlogie B, et al. The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma. N Engl J Med. 2003; 349: 2483-94.
7) Oshima T, Abe M, Asano J, Hara T, Kitazoe K, Sekimoto E, et al. Myeloma cells suppress bone formation by secreting a soluble Wnt inhibitor, sFRP-2. Blood 2005; 106: 3160-65.
8) Gunn WG, Conley A, Deininger L, Olson SD, Prockop DJ, Gregory CA. A crosstalk between myeloma cells and marrow stromal cells stimulates production of DKK1 and interleukin-6: a potential role in the development of lytic bone disease and tumor progression in multiple myeloma. Stem Cells. 2006; 24: 986-91.
9) Kaiser M, Mieth M, Liebisch P, Oberlander R, Rademacher J, Jakob C, et al. Serum concentrations of DKK-1 correlate with the extent of bone disease in patients with multiple myeloma. Eur J Haematol. 2008; 80: 490-4.
10) Takeuchi K, Abe M, Hiasa M, Oda A, Amou H, Kido S, et al. TGF-beta inhibition restores terminal osteoblast differentiation to suppress myeloma growth. PLoS One. 2010; 5: e9870.
11) Vallet S, Mukherjee S, Vaghela N, Hideshima T, Fulciniti M, Pozzi S, et al. Activin A promotes multiple myeloma-induced osteolysis and is a promising target for myeloma bone disease. Proc Natl Acad Sci U S A. 2010; 107: 5124-9.
12) Colucci S, Brunetti G, Oranger A, Mori G, Sardone F, Specchia G, et al. Myeloma cells suppress osteoblasts through sclerostin

secretion. Blood Cancer J. 2011; 1: e27.
13) Terpos E, Christoulas D, Katodritou E, Bratengeier C, Gkotzamanidou M, Michalis E, et al. Elevated circulating sclerostin correlates with advanced disease features and abnormal bone remodeling in symptomatic myeloma: reduction post-bortezomib monotherapy. Int J Cancer. 2012; 131: 1466-71.
14) Yaccoby S. Advances in the understanding of myeloma bone disease and tumour growth. Br J Haematol. 2010; 149: 311-21.
15) Anderson KC, Carrasco RD. Pathogenesis of myeloma. Annu Rev Pathol. 2011; 6: 249-74.
16) Landowski TH, Olashaw NE, Agrawal D, Dalton WS. Cell adhesion-mediated drug resistance (CAM-DR) is associated with activation of NF-kappa B (RelB/p50) in myeloma cells. Oncogene. 2003; 22: 2417-21.
17) Abe M, Hiura K, Ozaki S, Kido S, Matsumoto T. Vicious cycle between myeloma cell binding to bone marrow stromal cells via VLA-4-VCAM-1 adhesion and macrophage inflammatory protein-1alpha and MIP-1beta production. J Bone Miner Metab. 2009; 27: 16-23.
18) Fowler JA, Lwin ST, Drake MT, Edwards JR, Kyle RA, Mundy GR, et al. Host-derived adiponectin is tumor-suppressive and a novel therapeutic target for multiple myeloma and the associated bone disease. Blood. 2011; 118: 5872-82.
19) Yaccoby S, Wezeman MJ, Henderson A, Cottler-Fox M, Yi Q, Barlogie B, et al. Cancer and the microenvironment: myeloma-osteoclast interactions as a model. Cancer Res. 2004; 64: 2016-23.
20) Abe M, Hiura K, Wilde J, Shioyasono A, Moriyama K, Hashimoto T, et al. Osteoclasts enhance myeloma cell growth and survival via cell-cell contact: a vicious cycle between bone destruction and myeloma expansion. Blood. 2004; 104: 2484-91.
21) Novak AJ, Darce JR, Arendt BK, Harder B, Henderson K, Kindsvogel W, et al. Expression of BCMA, TACI, and BAFF-R in multiple myeloma: a mechanism for growth and survival. Blood. 2004; 103: 689-94.
22) Moreaux J, Legouffe E, Jourdan E, Quittet P, Reme T, Lugagne C, et al. BAFF and APRIL protect myeloma cells from apoptosis induced by interleukin 6 deprivation and dexamethasone. Blood. 2004; 103: 3148-57.
23) Moreaux J, Cremer FW, Reme T, Raab M, Mahtouk K, Kaukel P, et al. The level of TACI gene expression in myeloma cells is associated with a signature of microenvironment dependence versus a plasmablastic signature. Blood. 2005; 106: 1021-30.
24) Abe M, Kido S, Hiasa M, Nakano A, Oda A, Amou H, et al. BAFF and APRIL as osteoclast-derived survival factors for myeloma cells: a rationale for TACI-Fc treatment in patients with multiple myeloma. Leukemia. 2006; 20: 1313-5.
25) Yaccoby S, Pennisi A, Li X, Dillon SR, Zhan F, Barlogie B, et al. Atacicept (TACI-Ig) inhibits growth of TACI(high) primary myeloma cells in SCID-hu mice and in coculture with osteoclasts. Leukemia. 2008; 22: 406-13.
26) Ge Y, Zhan F, Barlogie B, Epstein J, Shaughnessy J, Jr., Yaccoby S. Fibroblast activation protein (FAP) is upregulated in myelomatous bone and supports myeloma cell survival. Br J Haematol. 2006; 133: 83-92.
27) Mathupala SP, Rempel A, Pedersen PL. Glucose catabolism in cancer cells. Isolation, sequence, and activity of the promoter for type II hexokinase. J Biol Chem. 1995; 270: 16918-25.
28) Nakano A, Miki H, Nakamura S, Harada T, Oda A, Amou H, et al. Up-regulation of hexokinaseII in myeloma cells: targeting myeloma cells with 3-bromopyruvate. J Bioenerg Biomembr. 2012; 44: 31-8.
29) Nakano A, Tsuji D, Miki H, Cui Q, El Sayed SM, Ikegame A, et al. Glycolysis inhibition inactivates ABC transporters to restore drug sensitivity in malignant cells. PLoS One. 2011; 6: e27222.
30) Pastorino JG, Shulga N, Hoek JB. Mitochondrial binding of hexokinase II inhibits Bax-induced cytochrome c release and apoptosis. J Biol Chem. 2002; 277: 7610-8.
31) Kumar S, Witzig TE, Timm M, Haug J, Wellik L, Kimlinger TK, et al. Bone marrow angiogenic ability and expression of angiogenic cytokines in myeloma: evidence favoring loss of marrow angiogenesis inhibitory activitywith disease progression. Blood. 2004; 104: 1159-65.
32) Bhatti SS, Kumar L, Dinda AK, Dawar R. Prognostic value of bone marrow angiogenesis in multiple myeloma: use of light microscopy as well as computerized image analyzer in the assessment of microvessel density and total vascular area in multiple myeloma and its correlation with various clinical, histological, and laboratory parameters. Am J Hematol. 2006; 81: 649-56.
33) Jakob C, Sterz J, Zavrski I, Heider U, Kleeberg L, Fleissner C, et al. Angiogenesis in multiple myeloma. Eur J Cancer. 2006; 42: 1581-90.
34) Corre J, Mahtouk K, Attal M, Gadelorge M, Huynh A, Fleury-Cappellesso S, et al. Bone marrow mesenchymal stem cells are abnormal in multiple myeloma. Leukemia. 2007; 21: 1079-88.
35) Tanaka Y, Abe M, Hiasa M, Oda A, Amou H, Nakano A, et al. Myeloma cell-osteoclast interaction enhances angiogenesis together with bone resorption: a role for vascular endothelial cell growth factor and osteopontin. Clin Cancer Res. 2007; 13: 816-23.
36) Takafuji V, Forgues M, Unsworth E, Goldsmith P, Wang XW. An osteopontin fragment is essential for tumor cell invasion in hepatocellular carcinoma. Oncogene. 2007; 26: 6361-71.
37) Cackowski FC, Anderson JL, Patrene KD, Choksi RJ, Shapiro SD, Windle JJ, et al. Osteoclasts are important for bone angiogenesis. Blood. 2009; 115: 140-9.
38) Matsui W, Huff CA, Wang Q, Malehorn MT, Barber J, Tanhehco Y, et al. Characterization of clonogenic multiple myeloma cells. Blood. 2004; 103: 2332-6.
39) Matsui W, Wang Q, Barber JP, Brennan S, Smith BD, Borrello I, et al. Clonogenic multiple myeloma progenitors, stem cell properties, and drug resistance. Cancer Res. 2008; 68: 190-7.
40) Yaccoby S, Epstein J. The proliferative potential of myeloma plasma cells manifest in the SCID-hu host. Blood. 1999; 94: 3576-82.
41) Hosen N, Matsuoka Y, Kishida S, Nakata J, Mizutani Y, Hasegawa K, et al. CD138-negative clonogenic cells are plasma cells but not B cells in some multiple myeloma patients. Leukemia. 2012; 26: 2135-41.
42) Kim D, Park CY, Medeiros BC, Weissman IL. CD19-CD45 low/- CD38 high/CD138+ plasma cells enrich for human tumorigenic myeloma cells. Leukemia. 2012; 26: 2530-7.
43) Chaidos A, Barnes CP, Cowan G, May PC, Melo V, Hatjiharissi E, et al. Clinical drug resistance linked to interconvertible phenotypic and functional states of tumor-propagating cells in multiple myeloma. Blood. 2013; 121: 318-28.
44) Boucher K, Parquet N, Widen R, Shain K, Baz R, Alsina M, et al. Stemness of B-cell progenitors in multiple myeloma bone marrow. Clin Cancer Res. 2012; 18: 6155-68.

45) Yata K, Yaccoby S. The SCID-rab model: a novel in vivo system for primary human myeloma demonstrating growth of CD138-expressing malignant cells. Leukemia. 2004; 18: 1891-7.
46) Yaccoby S. The phenotypic plasticity of myeloma plasma cells as expressed by dedifferentiation into an immature, resilient, and apoptosis-resistant phenotype. Clin Cancer Res. 2005; 11: 7599-606.
47) Croucher PI, Shipman CM, Lippitt J, Perry M, Asosingh K, Hijzen A, et al. Osteoprotegerin inhibits the development of osteolytic bone disease in multiple myeloma. Blood. 2001; 98: 3534-40.
48) Yaccoby S, Pearse RN, Johnson CL, Barlogie B, Choi Y, Epstein J. Myeloma interacts with the bone marrow microenvironment to induce osteoclastogenesis and is dependent on osteoclast activity. Br J Haematol. 2002; 116: 278-90.
49) Croucher PI, De Hendrik R, Perry MJ, Hijzen A, Shipman CM, Lippitt J, et al. Zoledronic acid treatment of 5T2MM-bearing mice inhibits the development of myeloma bone disease: evidence for decreased osteolysis, tumor burden and angiogenesis, and increased survival. J Bone Miner Res. 2003; 18: 482-92.
50) Dhodapkar MV, Singh J, Mehta J, Fassas A, Desikan KR, Perlman M, et al. Anti-myeloma activity of pamidronate in vivo. Br J Haematol. 1998; 103: 530-2.
51) Kondo H, Mori A. Anti-tumor activity of pamidronate in human multiple myeloma. Leuk Lymphoma. 2002; 43: 919-21.
52) Tai YT, Li XF, Breitkreutz I, Song W, Neri P, Catley L, et al. Role of B-cell-activating factor in adhesion and growth of human multiple myeloma cells in the bone marrow microenvironment. Cancer Res. 2006; 66: 6675-82.
53) Lund T, Soe K, Abildgaard N, Garnero P, Pedersen PT, Ormstrup T, et al. First-line treatment with bortezomib rapidly stimulates both osteoblast activity and bone matrix deposition in patients with multiple myeloma, and stimulates osteoblast proliferation and differentiation in vitro. Eur J Haematol. 2010; 85: 290-9.
54) Lee SE, Min CK, Yahng SA, Cho BS, Eom KS, Kim YJ, et al. Bone scan images reveal increased osteoblastic function after bortezomib treatment in patients with multiple myeloma. Eur J Haematol. 2011; 86: 83-6.
55) Delforge M, Terpos E, Richardson PG, Shpilberg O, Khuageva NK, Schlag R, et al. Fewer bone disease events, improvement in bone remodeling, and evidence of bone healing with bortezomib plus melphalan-prednisone vs. melphalan-prednisone in the phase III VISTA trial in multiple myeloma. Eur J Haematol. 2011; 86: 372-84.
56) Zangari M, Aujay M, Zhan F, Hetherington KL, Berno T, Vij R, et al. Alkaline phosphatase variation during carfilzomib treatment is associated with best response in multiple myeloma patients. Eur J Haematol. 2011; 86: 484-7.
57) Zangari M, Esseltine D, Lee CK, Barlogie B, Elice F, Burns MJ, et al. Response to bortezomib is associated to osteoblastic activation in patients with multiple myeloma. Br J Haematol. 2005; 131: 71-3.
58) Ozaki S, Tanaka O, Fujii S, Shigekiyo Y, Miki H, Choraku M, et al. Therapy with bortezomib plus dexamethasone induces osteoblast activation in responsive patients with multiple myeloma. Int J Hematol. 2007; 86: 180-5.
59) Yaccoby S, Wezeman MJ, Zangari M, Walker R, Cottler-Fox M, Gaddy D, et al. Inhibitory effects of osteoblasts and increased bone formation on myeloma in novel culture systems and a myelomatous mouse model. Haematologica. 2006; 91: 192-9.
60) Yaccoby S, Ling W, Zhan F, Walker R, Barlogie B, Shaughnessy JD, Jr. Antibody-based inhibition of DKK1 suppresses tumor-induced bone resorption and multiple myeloma growth in vivo. Blood. 2007; 109: 2106-11.
61) Heath DJ, Chantry AD, Buckle CH, Coulton L, Shaughnessy JD Jr., Evans HR, et al. Inhibiting Dickkopf-1 (Dkk1) removes suppression of bone formation and prevents the development of osteolytic bone disease in multiple myeloma. J Bone Miner Res. 2009; 24: 425-36.
62) Edwards CM, Edwards JR, Lwin ST, Esparza J, Oyajobi BO, McCluskey B, et al. Increasing Wnt signaling in the bone marrow microenvironment inhibits the development of myeloma bone disease and reduces tumor burden in bone in vivo. Blood. 2008; 111: 2833-42.
63) Chantry AD, Heath D, Mulivor AW, Pearsall S, Baud'huin M, Coulton L, et al. Inhibiting activin-A signaling stimulates bone formation and prevents cancer-induced bone destruction in vivo. J Bone Miner Res. 2010; 25: 2633-46.
64) Qiang YW, Shaughnessy JD Jr., Yaccoby S. Wnt3a signaling within bone inhibits multiple myeloma bone disease and tumor growth. Blood. 2008; 112: 374-82.
65) Fulciniti M, Tassone P, Hideshima T, Vallet S, Nanjappa P, Ettenberg SA, et al. Anti-DKK1 mAb (BHQ880) as a potential therapeutic agent for multiple myeloma. Blood. 2009; 114: 371-9.
66) Maeda S, Hayashi M, Komiya S, Imamura T, Miyazono K. Endogenous TGF-beta signaling suppresses maturation of osteoblastic mesenchymal cells. EMBO J. 2004; 23: 552-63.
67) Matsumoto T, Abe M. TGF-beta-related mechanisms of bone destruction in multiple myeloma. Bone. 2010; 48: 129-34.
68) Lotinun S, Pearsall RS, Davies MV, Marvell TH, Monnell TE, Ucran J, et al. A soluble activin receptor Type IIA fusion protein (ACE-011) increases bone mass via a dual anabolic-antiresorptive effect in Cynomolgus monkeys. Bone. 2010; 46: 1082-8.
69) Li X, Pennisi A, Yaccoby S. Role of decorin in the antimyeloma effects of osteoblasts. Blood. 2008; 112: 159-68.
70) Dai Y, Yang Y, MacLeod V, Yue X, Rapraeger AC, Shriver Z, et al. HSulf-1 and HSulf-2 are potent inhibitors of myeloma tumor growth in vivo. J Biol Chem. 2005; 280: 40066-73.
71) Teplyuk NM, Haupt LM, Ling L, Dombrowski C, Mun FK, Nathan SS, et al. The osteogenic transcription factor Runx2 regulates components of the fibroblast growth factor/proteoglycan signaling axis in osteoblasts. J Cell Biochem. 2009; 107: 144-54.
72) Asano J, Nakano A, Oda A, Amou H, Hiasa M, Takeuchi K, et al. The serine/threonine kinase Pim-2 is a novel anti-apoptotic mediator in myeloma cells. Leukemia. 2011; 25: 1182-8.
73) Brocke-Heidrich K, Kretzschmar AK, Pfeifer G, Henze C, Loffler D, Koczan D, et al. Interleukin-6-dependent gene expression profiles in multiple myeloma INA-6 cells reveal a Bcl-2 family-independent survival pathway closely associated with Stat3 activation. Blood. 2004; 103: 242-51.
74) van Lohuizen M, Verbeek S, Krimpenfort P, Domen J, Saris C, Radaszkiewicz T, et al. Predisposition to lymphomagenesis in pim-1 transgenic mice: cooperation with c-myc and N-myc in murine leukemia virus-induced tumors. Cell. 1989; 56: 673-82.
75) Allen JD, Verhoeven E, Domen J, van der Valk M, Berns A. Pim-2 transgene induces lymphoid tumors, exhibiting potent synergy with c-myc. Oncogene. 1997; 15: 1133-41.
76) Isaac M, Siu A, Jongstra J. The oncogenic PIM kinase family regulates drug resistance through multiple mechanisms. Drug

Resist Updat. 2011; 14: 203-11.
77) Xie Y, Xu K, Linn DE, Yang X, Guo Z, Shimelis H, et al. The 44-kDa Pim-1 kinase phosphorylates BCRP/ABCG2 and thereby promotes its multimerization and drug-resistant activity in human prostate cancer cells. J Biol Chem. 2008; 283: 3349-56.
78) Hideshima T, Catley L, Yasui H, Ishitsuka K, Raje N, Mitsiades C, et al. Perifosine, an oral bioactive novel alkylphospholipid, inhibits Akt and induces in vitro and in vivo cytotoxicity in human multiple myeloma cells. Blood. 2006; 107: 4053-62.
79) McMillin DW, Ooi M, Delmore J, Negri J, Hayden P, Mitsiades N, et al. Antimyeloma activity of the orally bioavailable dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor NVP-BEZ235. Cancer Res. 2009; 69: 5835-42.
80) Hammerman PS, Fox CJ, Birnbaum MJ, Thompson CB. Pim and Akt oncogenes are independent regulators of hematopoietic cell growth and survival. Blood. 2005; 105: 4477-83.
81) Challen GA, Little MH. A side order of stem cells: the SP phenotype. Stem Cells. 2006; 24: 3-12.
82) Ho MM, Ng AV, Lam S, Hung JY. Side population in human lung cancer cell lines and tumors is enriched with stem-like cancer cells. Cancer Res. 2007; 67: 4827-33.
83) Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, et al. Identification of pancreatic cancer stem cells. Cancer Res. 2007; 67: 1030-7.
84) Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C, et al. Identification and expansion of human colon-cancer-initiating cells. Nature. 2007; 445: 111-5.
85) Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med. 1997; 3: 730-7.
86) Loh YS, Mo S, Brown RD, Yamagishi T, Yang S, Joshua DE, et al. Presence of Hoechst low side populations in multiple myeloma. Leuk Lymphoma. 2008; 49: 1813-6.
87) Buchakjian MR, Kornbluth S. The engine driving the ship: metabolic steering of cell proliferation and death. Nat Rev Mol Cell Biol. 2010; 11: 715-27.
88) Kondoh H. Cellular life span and the Warburg effect. Exp Cell Res. 2008; 314: 1923-8.
89) Cairns RA, Harris IS, Mak TW. Regulation of cancer cell metabolism. Nat Rev Cancer. 2011; 11: 85-95.
90) Fletcher JI, Haber M, Henderson MJ, Norris MD. ABC transporters in cancer: more than just drug efflux pumps. Nat Rev Cancer. 2010; 10: 147-56.
91) Ding XW, Wu JH, Jiang CP. ABCG2: a potential marker of stem cells and novel target in stem cell and cancer therapy. Life Sci. 2010; 86: 631-7.