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Survival and Proliferation Factors of Normal and Malignant Plasma Cells

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Abstract

Since the first identification of interleukin (IL)-6 as a myeloma cell growth factor by Dr. Kawano's and Dr. Klein's groups 14 years ago, numerous studies have emphasized its major roles in the emergence of malignant plasma cells in vivo and in the generation of normal plasma cells. Four transcription factors control B-cell differentiation into plasma cells. The B-cell transcription factor pax-5 is mainly responsible for a B-cell phenotype, and *bcl-6* represses the plasma cell transcription factor blimp-1 and plasma cell differentiation. *bcl-6* expression is triggered by CD40 and IL-4 activation. A lack of CD40 and IL-4 activation yields a down-regulation of *bcl-6* expression, and IL-6 stimulation yields an up-regulation of blimp-1, mainly through STAT3 activation. Blimp-1 further down-regulates *bcl-6* and *pax-5* expression and makes plasma cell differentiation possible. IL-6 as well as IL-10 up-regulate XBP-1. XBP-1 is another transcription factor that is involved in plasma cell differentiation and whose gene expression is shut down by pax-5. The plasma cell transcription factors blimp-1 and XBP-1 are up-regulated, and the B-cell transcription factors bcl-6 and pax-5 are down-regulated, in malignant cells compared to B-cells. Apart from the recent identification of these 4 transcription factors, the factors involved in normal plasma cell generation are mostly unknown. Regarding malignant plasma cells, 3 categories of growth factors have been identified: (1) the IL-6 family cytokines, IL-10, and interferon α that activate the Janus kinase–signal transducer and activator of transcription (JAK/STAT) and mitogen-activated protein (MAP) kinase pathways; (2) growth factors activating the phosphatidylinositol (PI)-3 kinase/AKT and MAP kinase pathways, unlike the JAK/STAT pathway (insulin-like growth factor 1, hepatocyte growth factor, and members of the epidermal growth factor family able to bind syndecan-1 proteoglycan); and (3) B-cell–activating factor (BAFF) or proliferation-inducing ligand (APRIL) that activate the nuclear factor κB and PI-3 kinase/AKT pathways. BAFF and APRIL bind to BAFF receptor and TACI and are major B-cell survival factors. Recent data indicate that these various growth factors may cooperate to provide optimum signaling because they are localized together and with cytoplasmic transduction elements in caveolin-linked membrane caveolae. The identification of these myeloma cell growth factors and of the associated transduction pathways should provide novel therapeutic targets in multiple myeloma. *Int J Hematol.* 2003;78:106-113.

Key words: Myeloma; IL-6; IGF-1; HGF; EGF; BAFF

1. Introduction

Over the past 15 years, numerous studies have been devoted to the study of myeloma growth factors. These myeloma growth factors may be specific to the myeloma clone or involved in the generation of normal plasma cells. In this article, we briefly review the recent knowledge of the biology of normal plasma cells and then discuss the major growth factors involved in multiple myeloma (MM).

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2. Biology of Normal Plasma Cells

Plasma cells are usually located in the bone marrow, where they represent 0.25% of bone marrow mononuclear cells. Because of the rarity of plasma cells, little is known about the process of generation of normal plasma cells or their biology. Plasma cells are generated in the lymph nodes. B-cells with a high affinity antigen receptor (Ig) are selected by the antigen through mutations of the Ig variable genes in

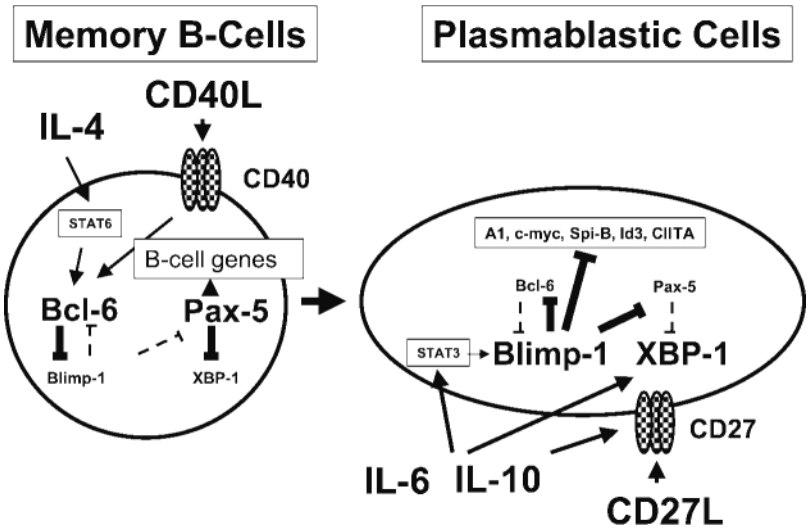

Figure 1. Transcription factors involved in plasma cell differentiation. IL indicates interleukin.

the lymph node. Selected B-cells are then induced to become either memory B-cells or plasmablastic cells. The plasmablastic cells migrate rapidly to the bone marrow where they can find additional survival and differentiation factors, making possible their long-term survival and differentiation into plasma cells. Hallmarks of mature plasma cells are their large amounts of Ig secretion, high expression of the syndecan-1 proteoglycan that is not expressed on B-cells, and a lack of most B-cell markers except CD19. These plasma cells also show high CD38 expression.

The intercellular communication signals that are critical to induce B-cell differentiation into plasmablastic cell are poorly understood. Plasmablastic cells are sometimes greatly expanded in vivo in some patients with acute inflammation. These plasmablastic cells are highly proliferating and short living. They comprise syndecan-1^- immature plasmablastic cells that can yield syndecan-1^+ plasmablastic cells [1]. We recently developed an in vitro model of generation of polyclonal plasmablastic cells (PPC) from peripheral blood B-cells obtained from healthy donors or MM patients [2]. For this generation model we cultured memory B-cells with a CD40 ligand transfectant, interleukin (IL)-4, IL-2, IL-10, and IL-12 for 4 days; plasma cell differentiation was induced by the removal of CD40 stimulation and a change in cytokine combination: removal of IL-4 and addition of IL-2, IL-6, IL-10, and IL-12. These plasmablastic cells were highly proliferating but apoptosed at days 7 to 8 of culture. This model should be critical to better understanding of the mechanisms controlling the survival of plasmablastic cells in the bone marrow, according to the identification of the major transcription factors involved in plasma cell differentiation [3].

As illustrated in Figure 1, we found that memory B-cells express Bcl-6 and Pax-5, whereas these transcription factors are down-regulated in plasmablastic cells. On the contrary, plasmablastic cells expressed the plasma cell transcription factors Blimp1 and XBP-1. Actually, according to data reported in the literature [3], one can hypothesize that IL-4 up-regulates bcl-6 transcription through STAT6 phosphorylation and that CD40 stimulation blocks Bcl-6 degradation. Bcl-6 in turn blocks *Blimp-1* gene expression.

Removal of IL-4 and CD40 signals makes possible down-regulation of bcl-6 and expression of Blimp-1 that is triggered by IL-6 and IL-10 activation of the Janus kinase–signal transducer and activator of transcription (JAK/STAT) pathway, mainly STAT3. Blimp-1 is a transcription repressor that represses *bcl-6* and *Pax5* B-cell genes as well as numerous other B-cell genes. Pax-5 is critical for B-cell maintenance, and its overexpression may block plasma cell phenotype in plasma cell lines. Pax-5 directly represses the *XBP-1* gene that encodes for a second major plasma cell transcription factor whose gene targets are poorly identified. In our model of PPC generation, we found that activated B-cells coexpress CD70 and CD27, suggesting that activation of CD27 together with IL-10 is part of the process of plasmablastic cell generation [2]. Indeed, CD27 is expressed on memory B-cells and highly expressed on plasma cells [4], and triggering CD27 with CD70, the CD27 ligand, together with IL-10, induces plasma cell differentiation in vitro [5]. IL-6 also plays a major role, in part by inducing STAT3 phosphorylation that will trigger *Blimp-1* expression. Jego et al, using plasmablastic cells from patients with reactive plasmacytosis, showed a major role of IL-6 in plasma cell differentiation [1]. In this model, the differentiation of syndecan-1^- plasmablastic cells into syndecan-1^+ early plasma cells was blocked with antibodies to IL-6. This property of IL-6 is not surprising since the IL-6 gene was initially cloned in 1988 as a B-cell differentiation factor [6]. In addition, transgenic mice expressing an IL-6 gene driven by an Eμ promoter develop massive polyclonal plasmacytosis [7], whereas IL-6 knock-out mice have a defect in the production of high-affinity antibodies [8]. This plasma cell differentiation role of IL-6 may be explained by its induction of XBP-1 gene expression [9], a transcription factor critical for plasma cell differentiation [10].

As pointed out above, the polyclonal plasmablastic cells generated in our in vitro model rapidly apoptose in vitro, on

days 7 to 8 after starting the cultures of B-cells, 3 to 4 days after removal of CD40 stimulation, despite the addition of various cytokines: IL-6, sIL-6R, IL-10, IL-2, IL-12. This apoptosis is associated with a rapid down-regulation of several genes coding for antiapoptotic proteins, the A1 protein of the bcl-2 family member and the c-IAP2 inhibitor of caspase activity. Conversely, we found an up-regulation of the Bik, caspase 3, and caspase 10 genes, coding for proapoptotic proteins. The down-regulation of A1 is likely a direct consequence of Blimp-1 expression that blocks *A1* transcription.

Thus, in conclusion, only 2 intercellular communication pathways have been described for normal plasma cells: IL-6 and activation of CD27 and IL-10. It is not presently known whether the factors known to induce the growth of malignant plasma cells—insulin-like growth factor 1 (IGF-1), epidermal growth factor (EGF) family, hepatocyte growth factor (HGF), and B-cell–activating factor/proliferation-inducing ligand (BAFF/APRIL)—are also involved in the biology of normal plasma cells. Also unknown are the transduction pathways that are activated in normal plasma cells, resulting in their cell survival and proliferation. We can expect that at least some of the growth factors recently identified for malignant plasma cells are also involved in normal plasma cell biology.

3. Myeloma Cell Survival and Proliferation Factors

Numerous studies have been devoted to the identification of myeloma cell growth factors and to the signaling pathways leading to survival and/or proliferation of myeloma cells. A first category of factors activates the JAK/STAT and mitogen-activated protein (MAP) kinase pathways (mainly IL-6). Another category involves the phosphatidylinositol (PI)-3 kinase/AKT as well as MAP kinase and nuclear factor (NF)-κB pathways.

3.1. Factors Activating the JAK/STAT and MAP Kinase Pathways: IL-6, Cytokines of the IL-6 Family, and Interferon α

IL-6 binds to a specific receptor (IL-6R), and the complex IL-6/IL-6R binds and induces the homodimerization of the gp130 IL-6 transducer [11]. A remarkable feature of IL-6R is that its soluble form (sIL-6R) is an agonist molecule. It binds IL-6 with the same affinity as membrane IL-6R, and the complex IL-6/sIL-6R binds and activates gp130 [11]. Evidence for a major role of IL-6 in the survival and proliferation of malignant plasma cells has accumulated since the initial reports by others and us 15 years ago [12,13]. The evidence consists of the following:

(1) Antibodies to IL-6 block myeloma cell proliferation and reduce by 50% the number of myeloma cells in in vitro culture of patient bone marrow cells [12-14].
(2) Injection of anti–IL-6 monoclonal antibody inhibited myeloma cell proliferation in patients with terminal disease [15,16] if the antibody was injected at a sufficient concentration to block the large amount of IL-6 production in vivo [17].
(3) Serum levels of IL-6 and soluble IL-6R are increased in patients with MM in association with a poor prognosis [18,19].
(4) The bone marrow environment of patients with MM, consisting mainly of monocytes, myeloid cells, and stromal cells, overproduces IL-6 [13,20]. This production of IL-6 by the tumor environment is mostly mediated by IL-1 that is produced by monocytes and myeloma cells [20,21]. IL-1 induces PGE2 synthesis that further triggers IL-6 production [21]. Thus inhibitors of IL-1, such as the IL-1 receptor antagonists, or of PGE2 synthesis might block IL-6 production in patients with MM. A similar mechanism was shown in the model of murine plasmacytoma in BALB/C mice. The generation of plasmacytomas was blocked by chronic administration of indomethacin that inhibited PGE2 synthesis and the large IL-6 production by the inflammatory environment [22]. Myeloma cells can also directly trigger IL-6 production by direct contact with the bone marrow stromal cells by unidentified mechanisms [23,24].
(5) Cell lines whose survival is dependent on addition of exogenous IL-6 can be obtained from patients with extramedullary proliferation [25].
(6) Mice transgenic with an IL-6 gene driven by the Eμ promoter develop massive polyclonal plasmacytosis [26]. When crossed with murine BALB/c mice that spontaneously develop plasmacytomas, these crossed mice develop malignant plasma cells [27]. In addition, knock out of the IL-6 gene abrogated the generation of malignant plasmacytomas in BALB/C mice primed with mineral oil [28].

Other cytokines of the IL-6 family are also myeloma cell growth factors because of the expression of specific receptors: OSM, CNTF, IL-11, and LIF [29]. But these factors are not likely to be involved in the emergence of the disease in vivo, because they are weakly produced by the tumor or its environment [30]. In our hands, we found that interferon α (IFNα) is also a myeloma cell survival factor that is independent of IL-6 [31,32]. IFNα activated the JAK/STAT and MAP kinase pathways, in particular STAT3 phosphorylation, as did IL-6 [32]. Other groups found that IFNα could block myeloma cell proliferation. This discrepancy might be explained by the ability of IFNα to induce P19 inhibitor in some cell lines yielding to apoptosis [33]. Finally, we found that IL-10, a potent plasma cell differentiation factor, is also a myeloma cell growth factor [34]. IL-10 works through induction of autocrine loops of IL-6 family cytokines [35].

The myeloma cell survival activity of these cytokines is partly mediated by the phosphorylation of STAT3 by JAK kinases activated by the gp130 IL-6 transducer or IFN receptor. Blockade of the JAK/STAT pathway by AG490 inhibits STAT3 phosphorylation and induces myeloma cell apoptosis [36]. STAT3 binding elements are found in the promoters of several antiapoptotic proteins: Mcl-1, Bcl-2, and Bcl-xL. Among 10 antiapoptotic and proapoptotic proteins, we found that only Mcl-1 was regulated by IL-6 or IFNα [37]. Other groups suggested that bcl-xL was the main antiapoptotic protein controlled by IL-6 in myeloma cells [38,39], but a recent study emphasized that only a blockade of Mcl-1, unlike bcl-2 or bcl-xL, could inhibit myeloma cell survival [40]. In addition, we found that induction of the constitutive production of Mcl-1 by retroviral vector is sufficient to promote myeloma cell proliferation independently

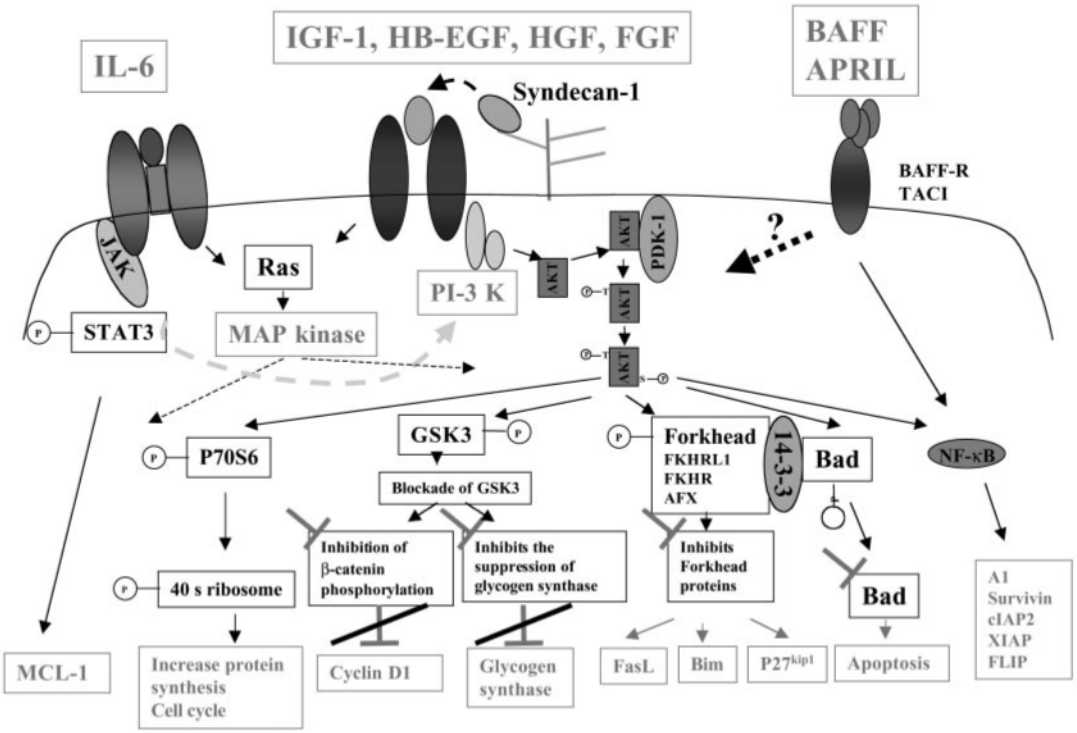

Figure 2. Growth factors and transduction pathways involved in myeloma cell survival and proliferation. IL indicates interleukin; IGF, insulin-like growth factor; HB-EGF, heparin-binding epidermal GF–like GF; HGF, hepatocyte GF; FGF, fibroblast GF; BAFF, B-cell–activating factor; APRIL, apoptosis-inducing ligand; STAT, signal transducer and activator of transcription; MAP, mitogen-activated protein; PI, phosphatidylinositol; GSK, glycogen synthase kinase.

of IL-6 [41]. IL-6 was reported to activate AKT kinase that is able to trigger various signaling pathways in myeloma cells [42]. AKT activation can be mediated by STAT3 phosphorylation and a ras pathway that can trigger PI-3 kinase activation [43]. We found weak AKT phosphorylation in only some IL-6–dependent cell lines (results not shown). Actually, the IL-6–induced AKT phosphorylation in myeloma cells is weak and transient compared to that induced by IGF-1 [44]. PI-3 kinase–mediated AKT phosphorylation appears critical in promoting proliferation of myeloma cell lines, because PI-3 kinase inhibitors, unlike MAP kinase inhibitors, abrogate this process [45,46].

These transduction pathways activated by IL-6 and other myeloma cell growth factors are schematized in Figure 2.

3.2. Factors Activating the PI-3 Kinase/AKT Pathway: IGF-1, Heparin-Binding Growth Factors

3.2.1. Insulin-Like Growth Factor 1

IGF-1 is a survival and proliferation factor for most myeloma cell lines [47-49]. Its effect is independent of activation of the JAK/STAT pathway [49,50]. IGF-1 induced the PI-3 kinase pathway, in particular the phosphorylation of AKT protein [42,50]. IGF-1 also induced MAP kinase phosphorylation [49,50]. The myeloma growth factor activity of IGF-1 is blocked by an inhibitor of the PI-3 kinase pathway unlike a MAP kinase inhibitor [45,46]. One mechanism of action of AKT is phosphorylation of the proapoptotic protein Bad, a process that induces Bad sequestration by the 14-3-3 protein and prevents its migration to the mitochondrial membrane [50,51]. Other proteins are phosphorylated by the PI-3 kinase/AKT pathway in myeloma cells: P70S6-kinase, forkhead proteins, and the glycogen synthase kinase-3 β (GSK3β) [45,46,51]. Phosphorylation of these proteins contributes to blockade of apoptosis and activation of the cell cycle in various models. In particular, IGF-1 induces cyclin D1 and Skp2 expression and down-regulation of P27kip1 in myeloma cells [46]. In addition, it was shown in one myeloma cell line that the PI-3K/AKT pathway may activate the NFκB pathway and expression of several targets of NFκB involved in cell survival: A1/Bfl1, cIAP2, XIAP, survivin, and FLIP [44].

Transfection of myeloma cells with activated AKT enhances tumor growth and protects from DEX-induced apoptosis, and expression of an AKT dominant negative results in inhibition of IL-6–induced proliferation of myeloma cells [52]. The importance of the PI-3 kinase/AKT pathway for the survival and proliferation of myeloma cells is emphasized by deletion/mutation of the PTEN gene in some myeloma cells [53]. PTEN is a phosphatase inhibiting the PI-3 kinase/AKT pathway, and its deletion results in a high activation of the PI-3K/AKT pathway.

IGF-1 probably plays a major role in myeloma in vivo. Indeed, IGF-1 serum levels are predictive of poor survival in patients with MM, although they are not increased [54].

IGF-1 is mostly produced by the liver, but it is also produced by osteoblasts in the bone matrix where myeloma cells survive and proliferate in vivo. The biology of IGF-1 is complex, because 4 IGF binding proteins, mostly IGF-BP3, circulate at high concentrations and neutralize IGF-1 [55]. Cells may also express IGF-binding protein that contributes to the biological activity of IGF-1 and disrupts the circulating IGF/IGF-BP complexes. Using Atlas macroarrays, we found that myeloma cells variably express IGF-BP3 or IGF-BP4 genes [56].

3.2.2. Heparin Binding Factors

A hallmark of plasma cell differentiation is the expression of the proteoglycan syndecan-1 [57,58]. This heparan-sulfate protein has many biological activities and, in particular, is able to bind heparin-binding growth factors and to present them to their specific receptors [59]. Thus it is not surprising that several recently reported myeloma cell growth factors are heparin-binding growth factors.

3.2.2.1. Heparin-Binding EGF-Like Growth Factor

Using Atlas macroarrays, we found that myeloma cell lines overexpressed the heparin-binding EGF-like growth factor (HB-EGF) gene compared to Epstein-Barr virus–transformed B-cell lines or normal plasmablastic cells and that inhibitors of HB-EGF can block the IL-6–dependent survival of these myeloma cell lines [56]. Actually, we found that HB-EGF cooperates with IL-6 to trigger optimal survival of myeloma cells, likely through an interaction between the transducer chains, gp130, and EGF receptors [60]. HB-EGF can activate 2 of the 4 members of the EGF receptor family, ErbB1 and ErbB4, which are variably express by myeloma cells. HB-EGF triggers the PI-3K/AKT pathway in myeloma cells, unlike STAT3 phosphorylation (unpublished data). Several coreceptors can enhance the binding of HB-EGF and increase its biological activity: syndecan-1, the tetraspanin CD9, and the integrin $\alpha 3\beta 1$ [61]. Using Affymetrix microarrays and fluorescence-activated cell-sorting analysis, we found that these coreceptors are overexpressed in myeloma cells compared to B-cells or plasmablastic cells [2]. In addition, because there are 11 members of the EGF family able to activate the ErbB receptors [62], it is likely that other EGF members may be involved in myeloma cell biology. Several inhibitors of EGF activity (humanized monoclonal antibodies, inhibitors of ErbB kinase activity) have been investigated clinically in patients with epithelial cancers [63]. Our recent data indicate that ErbB inhibitors can potentiate dexamethasone-induced apoptosis of myeloma cell lines and of primary myeloma cells of most patients. These data also suggest that ErbB inhibitors might improve treatment of patients with MM.

3.2.2.2. Hepatocyte Growth Factor

A recent study has shown that HGF is also a growth factor for myeloma cell lines [64]. HGF activity is blocked by removal of heparan sulfate chains of syndecan-1 with heparitinase. This result indicates that syndecan-1 is critical in the capture of heparin-binding HGF and its presentation to its receptor, cMet. Whether HGF cooperates with IL-6 to trigger myeloma cell survival was not investigated. It is noteworthy that the XG-1 cell line used in this study was initially obtained in our laboratory and produces a low amount of autocrine IL-6 [31] that is sufficient to induce the HB-EGF activity [60]. HGF is likely involved in the biology of myeloma. Indeed, the serum level of HGF is increased and is a prognostic factor in patients with MM [65]. Because HGF increases bone resorption, it may also be involved in the abnormal osteoclast resorption in patients with MM [66].

3.2.2.3. Fibroblast Growth Factor

A role of fibroblast growth factor (FGF) in myeloma is suggested by the finding of a t(4;14) translocation affecting the FGF receptor type 3 (FGR3) in 15% of patients with MM [67]. Whether FGR3 translocations have prognostic value is controversial [67,68]. In addition, mutations of FGR3 that make possible ligand-independent FGR3 activation are found in some myeloma cell lines [69]. These mutations are involved in thanatophoric dysplasia. Although serum levels of FGF2 are increased in myeloma [70], there is no published direct evidence that FGF or FGFR3 expression plays a role in the survival or proliferation of human myeloma cells. FGFs likely play an important role in myeloma biology, because they bind syndecan-1 as HB-EGF or HGF, and activation of FGR3 may induce the PI-3K/AKT pathway [71] that is critical for myeloma cell survival and proliferation. Indirect evidence obtained using murine B9 hybridoma or 3T3 cells suggests that constitutive FGR3 expression may increase resistance to dexamethasone or tumorogenicity [72,73].

3.3. Factors Activating NFκB: BAFF Family

BAFF and APRIL belong to the tumor necrosis factor (TNF) family and activate at least 3 receptors of the TNF receptor family: BAFF-R, BCMA, and TACI. BAFF proteins are critical for the survival of B-cells and may be involved in systematic lupus erythematosus [74]. Activation of the BAFF receptor family results in triggering the NFκB pathway and likely other unidentified pathways [75]. Using DNA microarrays, others and we observed overexpression of two BAFF receptors, BCMA and TACI [2,76]. This observation prompts us to look for a role of the BAFF family in the survival/proliferation of myeloma cells. We found that BAFF or APRIL is a potent survival and proliferation factor of myeloma cells, depending on expression of BAFF-R or TACI on myeloma cells. In addition, BAFF can protect myeloma cells from dexamethasone-induced apoptosis [77]. These data suggest that BAFF inhibitor could be useful in the treatment of patients with MM in association with dexamethasone.

3.4. Cross-Activation of Growth Factor Receptors and Potential Clinical Applications

Conceptualization of the contribution of these various myeloma growth factors to myeloma disease can be simpli-

fied by considering the transduction pathways that are critical to the promotion of myeloma survival and the cell cycle. As indicated above, at least 4 transduction cascades are activated by these various factors: the JAK/STAT pathway induced by IL-6 cytokines and IFNα; the PI-3 kinase/AKT pathway strongly activated by IGF-1 and heparin binding factors and weakly induced by IL-6; the NFκB pathway activated by IGF-1 and BAFF proteins; and the MAP kinase pathway induced by all factors. We recently pointed out that cooperation between IL-6 and HB-EGF triggers myeloma cell survival and proliferation [60]. The effect of HB-EGF is dependent on weak gp130 activation by IL-6. This cooperation likely reflects a cross-talk of the transduction elements and activation of various antiapoptotic proteins [60].

This effect was recently demonstrated for the gp130 IL-6 transducer and IFN or IGF receptor. Indeed, Jelinek's group showed that IFNα could activate the phosphorylation of endogenous gp130. This cross-activation is not reciprocal, because IL-6 cannot cross-phosphorylate the endogenous IFN receptor [78]. The same group also showed that IFNα induced a cross-phosphorylation of the endogenous ErbB3 receptor in a myeloma cell line [79]. This ability of IFNα, unlike IL-6 or IGF-1, to induce these cross-phosphorylations of other transducer chains, is linked to its ability to trigger a large and long-lasting activation of JAK1 and Tyk2 kinases compared to IL-6 [78]. More recently, it was shown that the gp130 and IGF-1R are both colocalized in caveolin-associated membrane caveolae in human myeloma cells together with PI-3 kinase and src kinase and that abrogation of caveolae by cholesterol inhibitors blocks IL-6 or IGF-1–induced transduction, in particular the PI-3K/AKT pathway [80]. Of major interest is the finding that the caveolin 1 gene is overexpressed on malignant plasma cells compared to normal B-cells or plasma cells. Taken together these data suggest that gp130 IL-6 transducers, IGF-1 receptors, EGF receptors, and eventually coreceptors such as syndecan-1 and CD9 are colocalized within caveolin-associated caveolae. In particular, myeloma cells overexpress the CD9 gene [2] coding for a tetraspanin involved in the formation of membrane multimolecular complexes [81].

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