

Published in final edited form as:

Int J Hematol. 2004 October ; 80(3): 224–231.

Apoptosis of Multiple Myeloma

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Abstract

Multiple myeloma (MM) is a malignancy of terminally differentiated plasma cells. MM cells localize to the bone marrow, where cell adhesion-mediated autocrine or paracrine activation of various cytokines, such as interleukin 6, insulin-like growth factor 1, and interferon α , results in their accumulation mainly because of loss of critical apoptotic controls. Resistance to apoptosis, a genetically regulated cell death process, may play a critical role in both pathogenesis and resistance to treatment of MM. Abnormalities in regulation and execution of apoptosis can contribute to tumor initiation, progression, as well as to tumor resistance to various therapeutic agents. Apoptosis is executed via 2 main pathways that lead to activation of caspases: the death receptor (extrinsic) pathway and the mitochondrial (intrinsic) pathway. Ionizing radiation and chemotherapeutic agents act primarily through the intrinsic pathway, in which mitochondria play the central role. Various therapeutic modalities that are effective in MM modulate levels of the proapoptotic and antiapoptotic Bcl-2 family of proteins and of inhibitors of apoptosis, expression of which is primarily regulated by p53, nuclear factor κ B, and STAT (signal transducers and activators of transcription) factors. This review focuses on the key concepts and some of the most recent studies of signaling pathways regulated in MM and summarizes what is known about the clinical role of these pathways.

Keywords

Multiple myeloma; Apoptosis; Bcl-2 family; TNF ligand and receptor; Ionizing radiation

1. Introduction

Multiple myeloma (MM) is a malignancy of terminally differentiated B-lymphocytes, also known as plasma cells. MM is characterized by accumulation of a monotypic plasma cell population in the bone marrow (BM), serum and/or urine monoclonal immunoglobulin, and osteolytic lesions. Because MM cells are postgerminal, further mutation does not occur [1]. MM constitutes 10% of the hematopoietic malignancies and ranks just behind non-Hodgkin's lymphoma as the second most common of these diseases in the United States [2]. MM is a malignancy characterized by very slow proliferation of malignant plasma cells, which leads to accumulation of these cells within the bone marrow. The existence of this phenomenon suggests that resistance to apoptosis may play a critical role in both pathogenesis and treatment resistance of MM. Moreover, inducers of apoptosis not only may have a lethal effect but also may support their immortalization in BM.

Apoptosis is a morphologically and biochemically distinct form of eukaryotic cell death that occurs under a variety of physiological and pathological conditions [3]. Apoptosis is executed

via 2 main pathways that lead to the activation of caspases: the death receptor (DR) pathway and the mitochondrial pathway [4]. Caspases are members of a cysteine protease family that are synthesized as inactive zymogens. They are responsible for activation of various cellular proteases and endonucleases. The result of activation is cleavage of structural and regulatory cellular proteins and of nuclear DNA. This process leads to morphologic and biochemical cellular changes that are characteristic of apoptosis. The DR pathway is activated by ligation of members of the tumor necrosis factor (TNF) family, such as Fas ligand (FasL; also called Apo1), TNF- α , and Apo2 ligand (Apo2L; also called TNF-related apoptosis-inducing factor [TRAIL]), to DR on the plasma membrane. The receptors of these ligands—Fas, TNFR1 (TNF receptor 1), and DR4/DR5—are members of the TNF receptor superfamily, which is characterized by similar, cysteine-rich extracellular domains and homologous cytoplasmic death domains [5]. Binding of these DRs by their respective ligands recruits the adaptor proteins Fas-associated death domain (FADD) and/or TNFR1-associated death domain (TRADD) and caspase 8 to the death-inducing signaling complex, triggering the proteolytic activation of caspase 8. Caspase 8 in turn activates the effector caspases 3 and 7 [6] (Figure 1). The result is proteolytic targeting of key apoptotic or cell cycle regulatory proteins, such as Bcl-2 [7] and cyclin E [8].

The mitochondrial pathway is regulated primarily by members of the Bcl-2 family, which comprises both proapoptotic and antiapoptotic proteins. The ratio between these subsets determines the susceptibility of cells to various death signals [4]. All contain at least 1 of the 4 conserved Bcl-2 homology (BH) domains [9]. The antiapoptotic members include Bcl-2, Bcl-xL, Bcl-w, Mcl-1, and Bfl-1/A1, which are characterized by the presence of all 4 BH domains (BH1-BH4). Most members also contain a C-terminal hydrophobic tail, which can target these proteins predominantly to the mitochondria and/or endoplasmic reticulum. These proteins prevent cell death by binding and sequestering proapoptotic proteins. The death promoter members are subdivided according to their function and biochemical structure into multidomain proteins, such as Bax and Bak, that closely resemble Bcl-2 but lack the N-terminal BH4 domain. The other subgroup is represented by BH3-only members that possess the BH3 domain required for binding to other members and for death-promoting activity. The BH3-only proteins include Bik, Bid, Puma/BBC3, and Noxa/APR, each thought to be responsible for transducing a specific cell death signal [10].

After their activation, Bax and Bak undergo conformational changes that contribute to increased permeability of the outer mitochondrial membrane through formation of pores. Bax and Bak also facilitate the release of cytochrome c (cyto c) and sequential activation of the caspase cascade [11]. Cytosolic cyto c binds the adaptor molecule APAF-1 (apoptotic protease activating factor 1) and caspase 9, forming a macromolecular complex, the apoptosome. This process leads to activation of caspase 9 [12]. Activated caspase 9 cleaves caspase 3, which cleaves and activates the other effector caspases (caspases 6 and 7). Additional molecules released from the mitochondria include apoptosis-inducing factor [13] and endonuclease G [14], which exert their apoptotic activity on nuclei. Smac/Diablo [15–17] and Omi/HtrA2 [18,19] facilitate caspase activation by antagonizing molecules belonging to the inhibitor of apoptosis (IAP) family.

A number of recent reviews describe the biology of apoptosis and the clinical applications in MM [1,20–23]. We focus on the key concepts and some of the most recent studies that summarize the field of MM apoptosis.

2. Role of the Mitochondrial Pathway in Apoptosis

Ionizing radiation (IR) and chemotherapeutic agents act primarily through the intrinsic pathway, in which mitochondria play the central role. DNA-damaging agents signal cell death

by altering the mitochondrial transmembrane potential ($\Delta\Psi_m$), activating Bcl-2 family members with subsequent cyto c release, and activating the caspase family of proteins [7,21]. Bcl-2, frequently expressed in follicular lymphomas bearing the t(14;18) chromosomal translocation, is also widely expressed in many other B- and T-cell lymphomas without involvement of a bcl-2 rearrangement [9,21].

The effect of genotoxic agents, such as IR and chemotherapeutic drugs, is directed through the mitochondrial pathway in a p53-dependent manner. BH3-only members of the Bcl-2 family—Bik, Puma, and Noxa—are believed to play a central role in p53-activated cell death [24, 25]. We observed that after IR the BH3-only genes *puma*, *noxa*, and *bik* were up-regulated in MM and lymphoma tumor cells. Eight and 16 hours after IR, ribonuclease protection assays indicated dramatic transcriptional induction of Bik, and there were similar changes in protein levels. In contrast, an increase in Noxa messenger RNA (mRNA) levels was observed as early as 0.5 hours after IR, and Puma levels had increased by 4 hours after IR. The differences in kinetics of induction of these BH3-only proteins indicated their distinct role in apoptosis activation in MM cells (M.O., A.A., unpublished data). Because Bid is not activated by IR [7], the identity of the Bax- and/or Bak-activating BH3-only protein is of great interest.

3. Bcl-2 Proteins Are Key Targets of Therapeutics

Imbalances in expression levels of the Bcl-2 family members result in defects in programmed cell death associated with chemoresistance, malignancy, and aggressiveness of tumors. The expression pattern of the Bcl-2 family of proapoptotic and antiapoptotic genes in MM have been the subject of multiple studies in which the investigators found increased levels of expression of Bcl-2, Bcl-xL, and Mcl-1 are linked to MM cell survival and resistance to chemotherapeutic agents [21,26-28]. The expression pattern of the Bcl-2 family separates the malignant phenotype of MM from normal plasma cells. In MM there is higher expression of the antiapoptotic Bcl-2 and Mcl-1 but not of Bcl-xL, and there is a lower level of expression of Bax [29]. On the other hand, targeted overexpression of Bcl-xL and c-Myc in B-lymphoid cells in mice resulted in lymphoproliferative disease and plasma cell malignancies. These findings were evidence that Bcl-xL can contribute to plasmacytomagenesis [30]. Bcl-xL expression is also associated with drug resistance in MM patients [31].

Chemotherapeutic agents, such as doxorubicin (Dox) induce apoptosis by causing cyto c release from mitochondria and subsequent activation of caspases, which are blocked by overexpression of Bcl-2. Treatment of U266 cells with Dox increased activation of Bax and Bak as well as of the BH3-only proteins Bid and Bik [32]. Arsenic trioxide (ATO) has been shown to induce apoptosis in MM cells [33] by directly inducing cyto c release from mitochondria via the mitochondrial permeability transition pore. The voltage-dependent anion channel was identified as a biological target of ATO [34]. Recent studies showed 2 distinct pathways for ATO-induced death in MM, depending on their p53 status. ATO treatment of cells with mutated p53 resulted in G₂/M cell-cycle phase block. In contrast, cells with wild-type p53 were blocked in G₁. Moreover, apoptosis may be activated differentially by ATO, with cells having mutated p53 engaging the extrinsic pathway and those having functional p53 engaging the intrinsic pathway. Finally, ATO treatment led to up-regulation of Apo2L (TRAIL) receptors and down-regulation of decoy receptors, observations that help explain the synergistic effect of ATO with Apo2L [35]. Recent published data from a phase 2 study showed that ATO as monotherapy has therapeutic efficacy in relapsed or refractory MM and that this agent was well tolerated with manageable adverse effects [36].

Overexpression of the antiapoptotic members has been linked to resistance to various chemotherapeutic agents. Increased expression of these proteins after exposure to chemotherapeutic agents of MM cell lines suggested that these agents might contribute to

acquired chemoresistance. Thus regulation of antiapoptotic proteins may represent an important strategy for sensitizing MM cells to various therapeutic agents. Using an antisense strategy, investigators found that Mcl-1, rather than Bcl-2 or Bcl-xL, is an essential survival factor, because Mcl-1 down-regulation induced rapid apoptosis of MM. Although it had no effect, Bcl-2 antisense treatment alone sensitized myeloma cell lines to dexamethasone (Dex), whereas Bcl-xL antisense in combination with Dex had no effect [37]. Pretreatment with Bcl-2-antisense (oblimersen [Genasense]) at clinically relevant doses potentiated Dex-, paclitaxel (Taxol)-, and Ad-p53-induced apoptosis in drug-resistant and freshly isolated myeloma cells [38]. The drug also was effective in patients treated with vincristine and adriamycin and those with Dex-refractory MM [39,40].

Histone deacetylase (HDAC) inhibitors such as depsipeptide induce apoptosis in MM cell lines and primary MM cells by down-regulating Bcl-2, Bcl-xL, and Mcl-1 and increasing Bax levels [41]. Suberoylanilide hydroxamic acid (SAHA) treatment promoted cleavage of Bid in a calpain-dependent but caspase-independent manner. The cleavage was abrogated by overexpression of Bcl-2. In addition, SAHA sensitized MM cells to DR-induced apoptosis, this effect being mediated by down-regulation of the antiapoptotic proteins FLIP (Fas-associated death domain-like interleukin 1 β -converting enzyme [FLICE]-like inhibitory protein), cIAP2, and XIAP [42].

Resistance of tumor cells to standard therapeutic agents has led to the continuous search for identification and characterization of new agents that augment the effects of older ones. Resveratrol treatment induces down-regulation of Bcl-xL and Mcl-1 and significant up-regulation of Bax and Apaf-1 and thus sensitizes MM cell lines to paclitaxel-induced apoptosis [43]. The selective mTOR (mammalian target of rapamycin) inhibitor rapamycin sensitized MM cell lines as well as primary myeloma cells to Dex-induced apoptosis. This effect is associated with concomitant down-regulation of cyclin D2 and the antiapoptotic protein survivin [44].

4. Reactive Oxygen Species as Apoptosis Mediators

An increase in generation of reactive oxygen species (ROS) within cells has been shown to induce loss of $\Delta\Psi_m$, release of cyto c and other apoptotic factors, and activation of caspases. IR treatment of MM cells results in ROS production associated with an increase in permeability transition pore opening and a decrease in $\Delta\Psi_m$ and glutathione, effects associated with caspase activation and cyto c release [45]. IR, exogenous ROS, and caspase 3 all induce a decrease in $\Delta\Psi_m$ and release of cyto c from mitochondria, effects that may be prevented by molecular (dominant-negative caspase 9) and pharmacologic (zVAD-fmk) caspase inhibitors and overexpression of Bcl-2. Exogenous ROS also induces mitochondrial permeability transition pore opening and cyto c release in isolated mitochondria that may be blocked by inhibition of the permeability transition pore with cyclosporin A. Results of studies conducted on MM cell lines sensitive or resistant to Dex indicated that treatment with 2-methoxyestradiol (2ME2) but not Dex induced generation of superoxide (O_2^-). 2ME2-induced apoptosis was associated with a decrease in $\Delta\Psi_m$, an increase in O_2^- , and release of cyto c and Smac [46]. Dex-triggered apoptosis involved a decrease in $\Delta\Psi_m$ without a concurrent increase in O_2^- , a process associated with Smac release. The results of that study suggested that 2ME2 triggers O_2^- generation, which occurs upstream of caspase activation [46]. The synthetic oleanane triterpenoid 2-cyano-3,12-dioxoolean-1,9-dien-28-oic acid (CDDO) and its derivative CDDO imidazolide ester (CDDO-Im) have been shown to induce apoptosis in MM and primary cells by decreasing the intracellular amount of reduced glutathione and increasing ROS levels, this effect being associated with FLIP down-regulation and caspase 8 activation [47]. Amplifying the levels of ROS within the tumor cells with inhibitors such as 2ME2 or CDDO may help increase the efficacy of standard treatments. It is interesting that 2ME2 and PS-341 activate c-

jun NH2-terminal kinase (JNK), which translocates to mitochondria [48]. JNK recently was shown to activate a novel apoptotic pathway by releasing Smac rather than cyto c during TNF-induced apoptosis [49], a process similar to Dex-induced apoptosis in MM [23].

5. Role of the DR Pathway in Apoptosis

Activation of cell surface DRs, such as DR4 and DR5, by the Apo2L ligand induces a signaling cascade culminating in activation of caspases [5] (Figure 2). In addition to being tumor specific, Apo2L-based therapy is attractive because it is not dependent on p53 and therefore should be effective in p53-deficient tumors. Apo2L potently induces apoptosis of MM cells from patients and the majority of MM cell lines, including cells sensitive or resistant to Dex, Dox, melphalan, and mitoxantrone. Apo2L also has overcome the survival effect of interleukin 6 (IL-6). A recent report indicated that Apo2L is expressed in plasma cells and may be responsible for apoptosis of plasma cells in a caspase-independent manner after antibody secretion [50].

Interferons (IFNs) have been used as effective pharmacological agents in treating a variety of cancers and viral diseases in the last 30 years. Our studies have shown that type I but not type II IFNs induce apoptosis through activation of the Apo2L pathway and modulation of the Bcl-2 family of proteins in MM cell lines and patient-derived primary cells [6,21]. IFNs play an important role in the immune system and may influence expression of a number of genes associated with both apoptosis and cell cycle progression [51]. One mechanism by which IFNs may achieve their role in immune surveillance may be through regulation of other cytokines. Our data provided evidence that IFNs exert their profound effect by inducing apoptosis in MM. The data also suggested that Apo2L may be an important mediator of these effects. We have identified and isolated the promoter region of Apo2L and found that it contains IFN-stimulated regulatory elements that can be regulated in MM [52]. Cytokines suppress apoptosis by preventing Apo2L expression through modulation of the forkhead FOXO3a transcription factor [53]. These findings support a rationale for exploring Apo2L as well as the therapies that modulate its expression in the management of MM, especially in instances in which IFN- α and IFN- β may be effective, such as in advanced MM. Given the lack of cytotoxicity of Apo2L toward most other types of blood cells, as well as other cell types in mice and nonhuman primates, it would be of interest to examine whether Apo2L, which is expected to enter clinical trials, could be used for treating MM patients.

Apo2L induction was at least partially necessary for proteolytic cleavage-dependent activation of apoptotic activity of Bcl-2 and Bid (Figure 2). In addition, we observed that IFN- α induces down-regulation of Bcl-xL, which is expressed at high levels in these cells (A.A., Q. Chen, unpublished data, 2002). Other reports have indicated that the effect of several therapeutic agents could be mediated by down-regulation of Bcl-2 family protein expression [21]. Expression of these proteins also may be regulated by the myeloma survival factors IL-6, IFNs, and insulin-like growth factor 1 (IGF-1) [54].

Recent studies have further demonstrated the role of the mitochondrial pathway in IFN- α -induced apoptosis in MM by showing that IFNs activate sequentially the proapoptotic Bcl-2 family members Bak and Bax [32]. IFN- α -induced apoptosis can be blocked through inhibition of the phosphatidylinositol 3-kinase (PI3K)/mTOR pathway. Because many tumor types are resistant to IFN treatment, these findings are an aid to understanding the variability of cellular response to IFN treatment and emphasize the importance of knowledge of the signaling pathways responsible for apoptosis induction [6,55].

6. Initiation and Amplification Stages in Apoptosis

The link between the extrinsic and intrinsic pathways of apoptosis through engagement of mitochondria by truncated Bid provides amplification of the apoptotic signal and further

supports the critical role of mitochondria in apoptosis. In contrast, genotoxic agents such as IR and IFNs cause MM apoptosis by 2 distinct stages of cyto c release and a positive feedback loop linking caspase activation to cyto c release and mitochondrial dysfunction [6,7]. In addition, we found that in MM and all other hematopoietic tumor cells examined, a caspase 3-generated p18-kd proteolytic fragment of cyclin E produced during genotoxic stress-induced apoptosis [8] will further amplify the process by activating Bax. Bax is sequestered to cytoplasmic proteins, such as the DNA repair protein Ku70, and is released after p18 cyclin E binding to Ku70 (A.A., S. Mazumder, unpublished data, 2004) or acetylation [56]. Radiotherapeutics and chemotherapeutics also induce apoptosis through increased expression of cyclin E [57], perhaps by providing more substrate for genesis of p18 cyclin E.

7. Growth and Survival Factors Prevent Apoptosis

MM cells accumulate in the BM, where they acquire growth and survival properties conferred by the BM microenvironment [22]. BM stromal cells (BMSCs) secrete the chemokine stromal cell-derived factor 1 α (SDF-1 α). This chemokine plays a major role in homing, because MM cells express the SDF-1 α receptor, CXCR4 (Figure 3). Once in the BM, integrin $\alpha_4\beta_1$ (VLA-4) mediates attachment of MM cells to the stroma [58], and this process confers the cells a survival advantage. Adhesion of MM cells to fibronectin (FN) is known to protect the cells from drug-induced apoptosis [23]. Although nearly all myeloma cells respond to growth and survival factors, such as IL-6, only some require them, and only some produce them. Those that do not produce IL-6 and require it rely on its production by BMSCs [1,59]. The pathophysiology of MM has been attributed to dysregulation of autocrine growth loops (including IL-6 and IL-1) and various survival pathways, including NF- κ B/I κ B (nuclear factor κ B/inhibitory unit of NF- κ B), JAK2/STAT3 (Janus kinase 2/signal transducers and activators of transcription 3), PI3K/Akt, and Ras/Raf/MAPK (Ras/Raf/mitogen-activated protein kinase) [23,60], which act through targets such as Bcl-2 or IAPs to prevent their apoptotic demise.

NF- κ B refers to a group of dimeric transcription factors regulating various genes the functions of which include cell growth, angiogenesis, cell adhesion, and protection from apoptosis. NF- κ B exists in the cytoplasm in an inactive form bound to I κ B α , one of its interacting negative regulators, which has to be phosphorylated to mark it for degradation by the 26S proteasome. This process leaves NF- κ B free to translocate to the nucleus [61], where it binds to its targets, such as cyclin D1 [61], Bcl-xL, and IAPs [62]. NF- κ B, because of its striking importance in MM, continues to be a compelling target for drug development. Dex, thalidomide, PS-341, and curcumin all inhibit NF- κ B [23,63]. Combined with the Chk1 abrogator UCN-01, Bay 11-7082, an irreversible inhibitor of I κ B α phosphorylation, and the NF- κ B inhibitor SN50 effectively induce apoptosis [60]. NF- κ B is responsible for up-regulation of IAPs and Bcl-2 [62] to control cell survival and for expression of ICAM1 and VCAM1 (intercellular and vascular adhesion molecules 1) on MM cells and BMSCs [1]. Adhesion molecules such as these and LFA-1 (lymphocyte function-associated antigen 1) facilitate the BMSC-MM cell interaction [20,64].

Posttranslational modifications involve ubiquitination and proteasome degradation. Proteasome inhibitors block accumulation of I κ B in the cytoplasm, inhibiting the NF- κ B cell survival pathway and leading to apoptosis. A transcriptional profile of MM.1S cells treated with the bortezomib/proteasome inhibitor PS-341 (Velcade) revealed distinct patterns of coordinated changes in a range of transcripts. The changes included down-regulation of growth and antiapoptotic transcripts and induction of apoptotic members, such as the Fas ligand and receptor. The effect of the agent was blocked by a dominant-negative Fas [65]. Moreover, coadministration of proteasome and HDAC inhibitors in MM cell lines sensitive or resistant to established cytotoxic agents resulted in a synergistic increase in mitochondrial injury, caspase activation, and apoptosis [66]. Furthermore, IL-6-induced growth was associated with

high levels of CDC34 (ubiquitin-conjugating enzyme [UBC3]), IL-6-mediated protection from Dex-induced apoptosis being dampened by blocking of CDC34. CDC34 is responsible for ubiquitination of various proteins, including I κ B α . MM cells express high levels of CDC34 at both the mRNA and the protein levels. Dex, 2ME2, and PS-341 treatments are associated with decreased CDC34 expression. Blocking CDC34 augments sensitivity to these therapies [67]. Targeting mitochondria may be an effective way to overcome conventional resistance and Velcade resistance in MM [68].

MM cell adhesion to BMSCs up-regulates secretion of vascular endothelial growth factor (VEGF) [64] and IL-6 by BMSCs. IL-6 secretion by BMSCs induces MM cells to produce their own IL-6. Secretion by MM cells in turn increases IL-6 secretion by BMSCs. VEGF is produced and secreted by both MM cells and BMSCs [64]. FLT1, a high-affinity VEGF receptor on MM cells, when phosphorylated by VEGF activates p42/p44/MAPK, leading to MM cell proliferation. VEGF is known to stimulate angiogenesis but also induces up-regulation of MCL-1 (myeloid cell leukemia 1), thus protecting MM cells against apoptosis [69]. In a PI3K/Akt/PKC (protein kinase C)-dependent manner, VEGF promotes migration of MM cells from BM [1]. IGF-1 mediates migration through PI3K as well [70]. CD40, a TNF family transmembrane protein expressed in most MM cells, may have a role in MM cell homing and migration by induction of VEGF [71].

IL-6, a major survival factor for MM tumor cells, induces signaling through the STAT proteins. STAT3 is constitutively activated in BM mononuclear cells from patients with MM and in the IL-6-dependent human MM cell line U266 [27]. IL-6-induced Dex resistance occurs in part through JAK/STAT3 signaling [1,31]. MM cells from patients have been shown to express constitutively active forms of NF- κ B and STAT3, whereas cells from healthy subjects have not [31]. Up-regulation of Bcl-xL and Mcl-1 via retroviral insertion of activated STAT3 and STAT5A, targets of both IL-6 and IGF-1, causes cell growth, oncogenesis, and cytokine independence [59]. Fibroblast growth factor receptor 3 (FGFR3), frequently overexpressed owing to a t(4:14) translocation, also confers a growth and survival advantage, possibly by activating STAT3 and resulting in Bcl-xL expression [72]. Bcl-xL expression can be inhibited by blocking IL-6 receptor signaling from JAKs to the STAT3 protein, demonstrating that STAT3 signaling is essential for the survival of MM tumor cells [27]. IL-6, via Shp2/RAFTK (related adhesion focal tyrosine kinase) prevents a Dex-induced increase in Smac, thus preventing apoptosis. Dex, in addition to triggering apoptosis, has the effect of transiently increasing expression of IL-6 receptor and transforming growth factor β receptor II and increasing production and release of IL-6 [22].

IL-6 and IGF-1 afford protection against Dex-induced apoptosis by up-regulation of Bcl-xL, and Mcl-1 (through STATs) and activation of the MAPK and PI3K/Akt pathways, which contribute to inactivation of caspase 9 [61,64]. IGF-1 has been reported to have multiple functions that lead to sustained activation of NF- κ B and Akt, phosphorylation of FKHRL-1 (forkhead homolog of rhabdomyosarcoma receptor ligand 1), up-regulation of FLIP, survivin, cIAP-2, Bfl-1, and XIAP, and a decrease in Apo2L sensitivity [23,73]. More recently it has been shown that, via extracellular signal-regulated kinase (ERK), IGF-1 also induces VEGF secretion [70]. IL-6 and IGF-1 have separate receptors but have substantial downstream overlap of pathways. Simvastatin (a 3-hydroxy-3-methylglutaryl coenzyme A inhibitor) induces apoptosis in vitro despite protective influences of IL-6, IGF-1, and VLA-4 (which allows MM cell adhesion to FN). This finding suggests a common downstream pathway for IL-6, IGF-1, and VLA-4, possibly through PI3K [74].

8. Future Directions

Novel therapeutic targets in MM emerge from proteins identified through microarray [1,75] and proteomics strategies [20]. Gene expression profiling provides essential information on expression and regulation of many genes in MM, including cell DRs (eg, DR4/5, BAFFR [B-cell-activating factor receptor], BCMA [B-cell maturation antigen], TACI [transmembrane activator and calcium modulator and cyclophilin ligand interactor]) and their ligands (Apo2L/TRAIL, APRIL [a proliferation-inducing ligand], BAFF, and CD40) [75] (M.O., A.A., unpublished data). Exogenous cytokine-dependent MM cells were reported to overexpress receptors for APRIL and BAFF. All 3 BAFF receptors—TACI, BCMA, and BAFFR—activate NF- κ B. APRIL induces up-regulation of Mcl-1 and Bcl-2, and IL-6 up-regulates Mcl-1 only. Both BAFF and APRIL have been shown to be as effective as IL-6 in preventing Dex-induced apoptosis. IL-6 has induced phosphorylation of STAT3, MAPK, and Akt (activating the JAK/STAT, MAPK, and PI3K/Akt cascades, respectively) [2]. IL-6-producing MM cells, via activated STAT3, are resistant to Fas [59]. IGF-1 has been shown to activate MAPK and PI3K/Akt. BAFF and APRIL have induced phosphorylation of only MAPK and Akt (activating the ERK1/2 and PI3K/Akt cascades, respectively) [76]. Establishing the function of these molecules in MM and their validation as targets in clinical therapy will broaden our understanding of the molecular determinants of the disease and provide additional tools that may help to cure it.

Acknowledgements

Supported in part by research grants CA81504 and CA82858 to A.A. from the National Cancer Institute.

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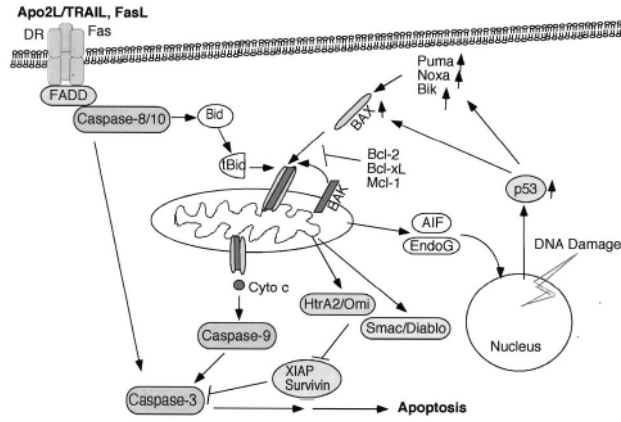


Figure 1. Apoptotic pathways. The death receptor (DR) pathway is activated by ligation of the death ligands (eg, Apo2L/TRAIL [tumor necrosis factor–related apoptosis-inducing factor ligand] and FasL) to their cognate receptors on the cell surface. This process results in sequential binding of Fas-associated death domain (FADD) and pro–caspase 8. Active caspase 8 cleaves and activates caspase 3. In addition, caspase 8 cleaves Bid, and truncated Bid translocates to the mitochondria to promote the release of cytochrome c (Cyto c). The mitochondrial pathway is activated by a number of stimuli, including chemotherapeutic drugs and ionizing radiation (IR). All these stimuli result in activation and oligomerization of Bax and Bak. These changes contribute to pore formation in the outer mitochondrial membrane and the release of cyto c and other apoptogenic factors. Cyto c promotes activation of caspase 9 and of the effector caspases. DNA-damaging agents such as IR induce activation of the p53 that promotes transcription of proapoptotic Bcl-2 family members such as the multidomain Bax and the Bcl-2 homology 3 (BH3)-only proteins Puma, Noxa, and Bik. Activation of the BH3-only molecules either directly or indirectly results in activation of Bax and Bak. AIF indicates apoptosis-inducing factor.

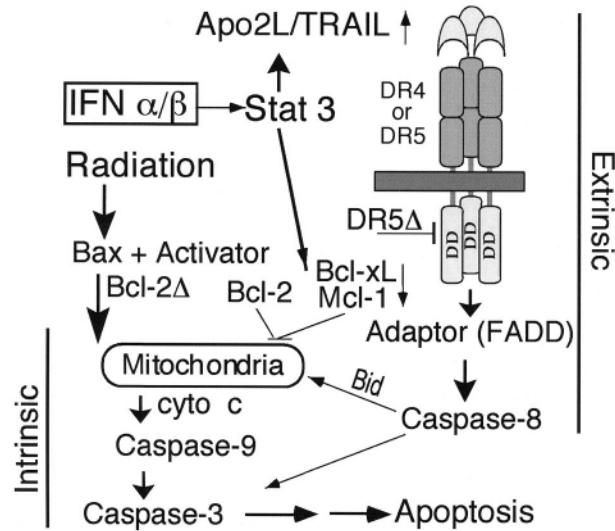


Figure 2.

Model for activation of apoptosis in multiple myeloma by interferons (IFNs). After transcriptional induction by IFNs, Apo2 ligand (Apo2L) engages its death receptor 5 (DR5) or DR4 and through an adaptor Fas-associated death domain (FADD) recruits caspase 8 to the cell membrane, which can be blocked by a dominant-negative DR5Δ. After caspase 8 activation by proteolysis, Bid is cleaved and translocates to mitochondria, causing release of low levels of cytochrome c (cyto c) into the cytosol, a process that leads to caspase 9 and 3 activation. This process results in attack of the antiapoptotic protein Bcl-2 on the mitochondrial membranes, producing a truncated Bcl-2Δ protein, which causes release of more cyto c, caspase activation, and apoptosis. Bcl-xL or Mcl-1 transcriptional down-regulation mediated by signal transducers and activators of transcription 3 (Stat 3) is an additional mechanism by which IFNs may decrease levels of antiapoptotic proteins and shift the balance toward a proapoptotic state. (Modified from [6], with permission.) TRAIL indicates tumor necrosis factor-related apoptosis-inducing factor ligand; DD, death domain.

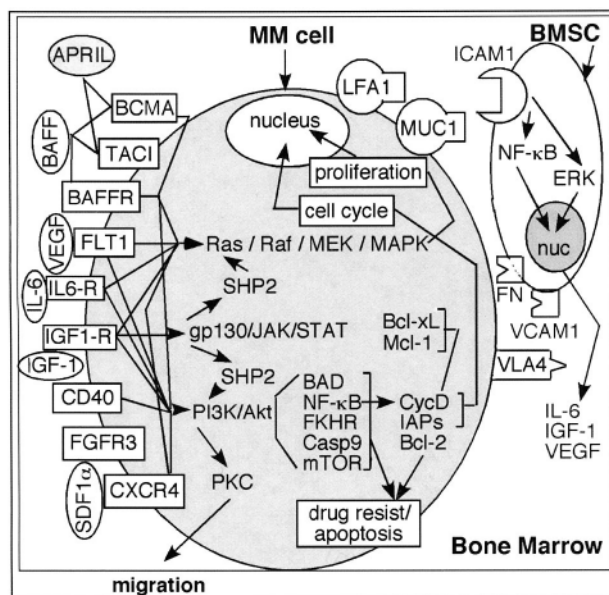


Figure 3.

Growth and survival pathways. Receptors for a proliferation-inducing ligand (APRIL) and B-cell-activating factor (BAFF) include B-cell maturation antigen (BCMA), transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), and BAFF receptor (BAFFR). FLT1 is a high-affinity vascular endothelial growth factor (VEGF) receptor. CXCR4 is a receptor for stromal cell-derived factor (SDF1 α). These receptors, along with interleukin 6 receptor (IL6-R), insulin-like growth factor 1 receptor (IGF1-R), and CD40, mediate downstream activation of the various pathways. These pathways include nuclear factor κ B (NF- κ B), Janus kinase/signal transducers and activators of transcription (JAK/STAT), phosphatidylinositol 3-kinase (PI3K)/Akt, and Ras/Raf/mitogen-activated protein kinase (MAPK). These pathways ultimately lead to cytokine production (IL-6, IGF-1, VEGF), resistance to apoptosis (Bcl-2, inhibitors of apoptosis [IAPs]), cell proliferation, and migration of multiple myeloma (MM) cells. The bone marrow stromal cell (BMSC)/MM cell interaction is mediated by the surface receptors lymphocyte function-associated antigen 1 (LFA1), mucin, (MUC1) integrin $\alpha_4\beta_1$ (VLA4), intracellular adhesion molecule 1 (ICAM1), vascular adhesion molecule 1 (VCAM1), and fibronectin (FN). Activation of ICAM by cell-cell interaction leads to secretion of various cytokines from the BMSCs. FGFR3 indicates fibroblast growth factor receptor 3; MEK, mitogen-activated protein/ERK kinase; SHP2, Src homology region 2 domain-containing phosphatase 2; gp130, glycoprotein 130; PKC, protein kinase C; BAD, Bcl-2 antagonist of cell death; FKHR, forkhead homolog of rhabdomyosarcoma; Casp9, caspase 9; mTOR, mammalian target of rapamycin; CycD, cyclin D; ERK, extracellular signal-regulated kinase; nuc, nucleus.