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Apoptosis in Leukemias: Regulation and Therapeutic Targeting

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Introduction

Nearly 25 years after the seminal publication of John Foxton Kerr that first described apoptosis, the process of regulated cell death, our understanding of this basic physiological phenomenon is far from complete [1]. From cardiovascular disease to cancer, apoptosis has assumed a central role with broad ranging therapeutic implications that depend on a complete understanding of the molecular events involved in orchestrating cellular demise. More than 145,301 (as of April 2008) published works on this subject have increased our understanding of this process, yet have also identified an incredibly complex regulatory system that is critical for development and is at the core of many diseases, challenging scientists and clinicians to step into its molecular realm and modulate its circuitry for therapeutic purposes. This chapter will review our understanding of the molecular circuitry that controls apoptosis in leukemia, and the pharmacological manipulations of this pathway that may yield therapeutic benefit.

Apoptosis pathways

Apoptosis can be orchestrated from within the cell (intrinsic), or from the plasma membrane (extrinsic). Phenotypically, the end results of activating the intrinsic or extrinsic pathways of cell death (plasma membrane blebbing, mitochondrial dysfunction, and chromatin lysis) are indistinguishable; however, these endpoints are achieved via distinct mechanisms. The extrinsic pathway (Figure 1) is activated by ligation of plasma membrane receptors such as FAS, tumor necrosis factor receptor, and TRAIL receptors [2]. The ligands that activate these receptors comprise a superfamily of secreted peptides that may act in an autocrine, or paracrine fashion, and upon binding their cognate receptor, induce a conformational change that recruits various components of the death inducing signaling complex (DISC) resulting in the activation and processing of the initiator caspases, caspase 8 and/or caspase 10 [3–5]. Within the DISC, initiator caspases are activated via the “induced proximity” model [6,7]. In this model, catalytic activation of caspase 8 or caspase 10 occurs by dimerization of the inactive enzymes facilitated by small-scale rearrangements induced by the DISC. The initiator caspases once activated can autocatalytically process themselves, as well as cleave and activate executioner caspases such as caspase 3, caspase 6, or caspase 7. Executioner caspases in turn proteolyze over 100 different proteins involved in various structural and metabolic processes resulting in the characteristic morphological changes associated with apoptosis.

In contrast to the extrinsic pathway, the intrinsic pathway is activated by mitochondrial dysfunction, independent of alterations in the plasma membrane (Figure 2). Initiation of the intrinsic pathway involves an increase in the permeability of the outer mitochondrial membrane (MOMP), which facilitates the release of death promoting proteins such as cytochrome C, AIF, EndoG, Omi, and DIABLO that reside in the intermembrane space of

this organelle [8]. MOMP appears to be mediated by the formation of a supramolecular pore composed of several mitochondrial proteins like VDAC, Cyclophilin D, and ANT [9,10]. Of the death promoting proteins released into the cytosol via MOMP, cytochrome C is essential for the activation of the initiator caspase 9, whereas Omi and DIABLO serve to facilitate the activation of the executioner caspases by caspase 9 [11,12]. Activation of caspase 9 requires the formation of a multiprotein complex called the apoptosome. The apoptosome consists of cytochrome C, Apaf-1, caspase 9, and ATP, and therefore depends on the intracellular energy stores of the cell [13]. Similar to the DISC induced activation of caspase 8, caspase 9 is also activated by “induced proximity” within the apoptosome [14]. Interestingly AIF (Apoptosis Inducing Factor) and EndoG can induce caspase-independent apoptotic cell death by directly mediating chromatinolysis [15,16], an effect that allows mitochondrial dysfunction to orchestrate cell death even in the absence of caspase activation.

The extrinsic pathway in leukemia

The death inducing ligand TRAIL has received considerable attention as a therapeutic strategy for the treatment of human cancer because of its limited toxicity to normal tissues [17]. Interestingly, albeit the majority of leukemia cells express TRAIL receptors, these samples are notoriously resistant to apoptosis induction by TRAIL [18–21]. The reasons for this inherent resistance to TRAIL induced cell death are not completely understood, but may depend on the presence of decoy receptors that bind TRAIL but are not able to induce DISC formation, the expression of FLIP (an inhibitor of caspase 8 activation), or overactivation of NF κ B – a near universal consequence of DR ligation [22–24]. Nevertheless, recent work has demonstrated that a variety of agents can sensitize leukemia cells to apoptosis induction by TRAIL, either by directly affecting the recruitment and assembly of DRs on the cell surface, increasing the expression of DRs, or decreasing the levels of FLIP [25,26]. One such agent is the novel triterpenoid CDDO. CDDO has been shown to sensitize AML cell lines and primary samples to the cytotoxic effects of TRAIL by decreasing the levels of FLIP [27]. On the other hand, several HDAC inhibitors in clinical use have been shown to sensitize leukemia cells by facilitating the formation of an active DISC and increasing expression of the DRs [28]. Finally, since DR5 is a p53-responsive gene, many forms of genotoxic chemotherapy sensitize leukemia cells with an intact p53 pathway to TRAIL induced apoptosis via increased expression of DR5 [29–31]. Currently, two pharmaceutical companies have therapeutic activators of the extrinsic pathway, Genentech with TRAIL itself, and Human Genome Sciences with agonistic antibodies to the DRs that mimic the action of TRAIL. First results with agonistic antibodies are encouraging.

The intrinsic pathway in leukemia

The intrinsic apoptotic pathway is critically regulated by pro- and anti-apoptotic members of the Bcl-2 family. Anti-apoptotic members of the Bcl-2 family contain four conserved Bcl-2-homology (BH) domains, designed BH1–BH4, whereas pro-apoptotic members of this family only contain BH1–BH3 [32]. In addition, there is a subset of pro-apoptotic Bcl-2 family members that contain only the BH3 domain, and are thus termed “BH3-only” proteins. Of these BH3-only proteins, activators, like Bid and Bim are required to directly activate the pro-apoptotic function of the BH1–BH3 proteins Bax and Bak, whereas sensitizers, like Bad, Noxa, or Puma displace Bim or Bid from antiapoptotic Bcl-2 proteins facilitating the subsequent activation of Bax and Bak making BH3 interactions a finely tuned rheostat for apoptotic control [33]. During apoptosis, Bax and Bak oligomerize and form supramolecular pores in the outer mitochondrial membrane leading to the release of pro-apoptotic proteins like cytochrome c and AIF [34]. Interestingly, the expression, intracellular localization and function of pro- and anti-apoptotic Bcl-2 family members can vary widely depending on cell context, and may be temporally regulated throughout

development. For instance, the antiapoptotic Bcl-2 protein Mcl-1 has been shown to be a critical regulator of early hematopoietic development [35,36], and down-regulation of Mcl-1 levels has been shown to be an effective strategy to decrease the viability of multiple myeloma cells [37]. Similarly, the interactions between pro- and anti-apoptotic Bcl-2 proteins display particular selectivity such that the BH3-only proteins Bim, Bmf, and Puma can antagonize Bcl-X_L and Mcl-1, whereas Bid, Bik, and Bad can oppose the anti-apoptotic function of Bcl-X_L, but not of Mcl-1 [38]. This is exemplified by the differences in the subcellular location of Bim, which is sequestered to the dynein motor complex and translocated to the mitochondria upon apoptotic stimuli in some experimental systems [39]. However, Bim is already localized at the mitochondria, complexed with anti-apoptotic Bcl-2 family proteins in hematopoietic cells [40], including AML lines [41]. These observations clearly suggest that the mitochondrial levels of expression of various pro- and anti-apoptotic Bcl-2 family members are important determinants of sensitivity of AML cells to apoptotic insults.

Most genotoxic chemotherapeutics activate the intrinsic apoptotic pathway via activation of p53 signaling resulting in the expression of proapoptotic target genes such as Noxa, Puma, and Bax, as well as the accumulation of cytoplasmic p53 which functions outside the nucleus as a pro-apoptotic protein by antagonizing the function of Bcl-X_L and directly activating bax [42], or by binding to manganese superoxide dismutase and promoting the formation of reactive oxygen species (ROS) [43]. Additionally, alkylating agents can directly promote MOMP by oxidation of critical cysteine residues of components of the supramolecular pore, in particular ANT [44,45]. On the other hand, the novel triterpenoid CDDO and its derivatives have been shown to directly induce MOMP, independently of the formation of a supramolecular pore (Figure 3), possibly by disrupting the impermeability of the inner mitochondrial membrane which leads to matrix swelling with subsequent rupture of the outer membrane [46] [47]. A role of ceramide has also been suggested [48].

These compounds have shown promise in numerous preclinical investigations [49] [50] and in clinical trial in leukemias [51]

Endogenous inhibitors of caspase activity

The inhibitors of apoptosis proteins (IAPs) are family of proteins that suppress both mitochondrial and death receptor mediated apoptosis primarily by inhibiting caspases. Currently, eight IAP members have been identified in humans. Among them, XIAP is the most potent cellular caspase inhibitor. XIAP directly binds to caspase-9 by BIR3 domain and caspases-3 and -7 by BIR1 and BIR2 domains (Deveraux QL and Reed JC 1999 EMBO J 18:5242–5252) and inhibits their activities. The antiapoptotic function of XIAP is supported by multiple *in vitro* and *in vivo* studies demonstrating that overexpression of XIAP confers resistance to both mitochondrial and death receptor pathway activation (Wilkinson JC and Duckett CS 2004 Mol Cell Biol 24:7003–7014; Berezovskaya O and Schimmer AD et al. 2005 Cancer Res 65:2378–2386; Tong QS and Zheng LD 2005 Cancer Gene Ther 12:509–514). Decreasing XIAP levels with siRNA or antisense oligonucleotide restores chemosensitivity to malignant cells (Sasaki H and Sheng Y et al 2000 Cancer Res 60:5659–5666; Holcik M and Yeh C et al. 2000 Oncogene 19:4174–4177; Chawla-Sarkar M and Bae SI et al., 2004 Cell Death Differ 11:915–923; McManus DC and Lefebvre CA et al. Oncogene 23:8105–8117) [52]. We have demonstrated in HL-60 cells that knockdown XIAP with antisense oligonucleotide induces caspase activation, apoptosis, and enhances Ara-C induced cell death (Carter B et al., 2003 Leukemia). Phenylurea-based small-molecule XIAP inhibitors, which bind to BIR2 domain of XIAP, induce apoptosis in various AML cell lines and patient blasts in a caspase-3 dependent manner (Carter B et al., Blood 2005). Triptolide, a diterpenoid isolated from a Chinese herb, inhibits XIAP and potently

induces AML cell death (Carter B et al., Blood 2006). Interestingly, XIAP knockout mice looked normal and showed no apparent defect in apoptosis (Harlin H and Reffey SB et al., 2001 Mol Cell Biol 1:3604–3608).

Overexpression of XIAP has been reported by several groups [53–58]. In addition, we have found that XIAP expression is higher in both AML stem (CD34+/CD38–) and progenitor (CD34+/CD38+) cells than in their normal counterparts by Taq-Man RT-PCR (Andreeff M et al., unpublished results). Clinically, increased XIAP expression is correlated with decreased survival in AML [59,60]. We have demonstrated that the expression of XIAP is induced by cytokines through the MAPK and the PI3K pathways in AML [61,62] suggesting that hematopoietic growth factors promote AML cell growth and survival at least in part through upregulating XIAP protein expression. Nuclear factor- κ B (NF- κ B) also participates in the upregulation of XIAP levels in various malignancies [63]. In AML, NF- κ B is constitutively activated [64–66] which undoubtedly contributes to XIAP overexpression.

Survivin, another member of the IAP family, has an interesting dual function as caspase inhibitor, and as a component of the apoptotic spindle. Inhibition of survivin by survivin AS ODN in leukemia cells results in G₂M block, followed by apoptosis. In CML, survivin levels are entirely dependent on bcr-abl signaling: bcr-abl inhibition with imatinib abrogates survivin expression, while in imatinib-resistant CML there is no effect [67]. In this setting, survivin AS ODN induces pronounced apoptosis [67]. Clinical trials with survivin AS ODN are in progress.

Caspase-independent cell death: the role of apoptosis inducing factor (AIF)

Albeit caspases are central players during apoptosis induction, many agents induce apoptosis-like cell death even in the absence of caspase activation or in the presence of pharmacological inhibition of these thiol proteases. In fact, we have reported that in AML cell lines treatment with traditional chemotherapeutic agents like Ara-C, doxorubicin, vincristine, and paclitaxel induce caspase activation and apoptosis, but pharmacological blockade of caspase activity does not prevent cell death induced by any of these agents, and did not inhibit, or only partially inhibited, mitochondrial release of apoptosis-inducing factor (AIF) and loss of mitochondrial membrane potential [68]. Caspase inhibition also did not protect AML blasts from chemotherapy-induced cell death in vitro. These results suggest that cytotoxic insults that promote the release of AIF from mitochondria may not require caspase activation to induce cell death. AIF is a bifunctional apoptogenic flavoprotein with NADH oxidase activity resides in the intermembrane space of the mitochondria in healthy cells and has a vital function in oxidative phosphorylation (OXPHOS), possibly due to indirect modulation of OXPHOS complex I [69,70]. AIF is synthesized in the cytosol as a 67KDa precursor molecule with a predicted mitochondrial localization sequence (MLS) in its N-terminal prodomain. AIF is matured into a 57KDa protein that lacks the first 100 amino acids, and resides in a soluble form in the mitochondrial intermembrane space. In response to certain proapoptotic stimuli that elicit MOMP, AIF is released into the cytoplasm. AIF will then translocate into the nucleus and induce caspase-independent large scale DNA fragmentation (~50 Kbp) and chromatin condensation. AIF contains a DNA binding domain that is necessary and sufficient to induce apoptosis [71]. In contrast, the NADH oxidase domain is not necessary for apoptosis induction, but is required for the vital role of AIF in modulating OXPHOS [72]. Interestingly, it has been reported that the release of AIF may require caspase activation under some conditions, suggesting that this protein may also amplify caspase-dependent apoptosis [73]. Notably, the synthetic triterpenoid CDDO promotes the release of AIF via perturbations of the permeability of the inner mitochondrial membrane that lead to caspase-independent MOMP [74], suggesting that

agents that directly target the mitochondrial membrane could provide therapeutic efficacy even in the context of caspase dysfunction or inhibition.

Targeting the p53 pathway to induce apoptosis in leukemia

The transcription factor and tumor suppressor p53 is the most frequently mutated gene in cancer [75]. Activation of the transcriptional activity of p53 by a variety of stress signals leads to the expression of genes, which may orchestrate growth arrest, apoptosis, or both [76]. In addition, p53 has also been shown to function outside the nucleus as a pro-apoptotic protein by antagonizing the function of Bcl-XL and directly activating bax [77], or by binding to manganese superoxide dismutase and promoting the formation of reactive oxygen species (ROS) [78]. The levels of p53 are controlled by the ubiquitin ligase MDM-2 which targets this tumor suppressor protein for proteosomal degradation [79]. Interestingly, mutations in p53 have been found in only ~5% of patients with AML [80,81], but it has been proposed that overexpression of MDM-2 may abrogate the function of p53 in this malignancy [82,83]. Indeed, MDM-2 overexpression was observed in up to 53% of leukemias, and was associated with unfavorable cytogenetic characteristics and poor prognosis [84–86]. Recently, potent and selective small-molecule antagonists of MDM-2, Nutlins, have been reported to activate the p53 pathway in cancer cells leading to growth arrest and apoptosis *in vivo* and *in vitro* [87]. It has been reported that Nutlins potently induce p53-dependent apoptosis in primary leukemia samples and leukemia cell lines with wild-type p53 [88,89]. Nutlin activity was positively correlated with baseline MDM2 levels suggesting that the inhibition of MDM-2 may offer considerable clinical benefit for the therapy of leukemia.

MDM-2 inhibition activates transcription-dependent and -independent pro-apoptotic effects of p53 in a cell context-dependent manner [89]. For instance, confocal microscopy analysis revealed that MDM-2 inhibition induced predominantly nuclear accumulation of p53 in the AML cell line MOLM13 (Figure 2), and in these cells apoptosis was greatly dependent on *de novo* protein synthesis. Conversely, MDM-2 inhibition induced mostly cytoplasmic accumulation of p53 in OCI-AML3 cells (Figure 4), and in these cells inhibition of *de novo* protein synthesis did not markedly abrogate apoptosis. Nevertheless, in both cell types, induction of the p53 targets Noxa and PUMA, as well as complex formation between p53 and Bcl-xL was observed supporting the notion that p53 modulates the Bcl-2 apoptotic rheostat directly, by behaving as a BH3-only protein, and indirectly by increasing the levels of sensitizer BH3-only proteins. Interestingly, Deng et al have reported that p53 can directly bind to Bcl-2 and induce a conformational change that exposes the BH3 domain of this protein, converting Bcl-2 into a proapoptotic molecule, adding yet another mechanism by which p53 modulates the Bcl-2 apoptotic rheostat [90]. HDM4 inhibits p53 transcriptionally and can either be inhibited specifically [91,92] or non-specifically, as it has been shown to be downregulated by genotoxic stress including chemotherapy [93].

Nutlins potently synergized with doxorubicin, cytarabine and fludarabine to induce apoptosis in primary leukemia samples [94,95], suggesting the possibility that potentiation of p53-mediated apoptosis may occur through an increase in steady-state p53 protein levels, as effected by nutlin 3a, in combination with further activation of p53 signalling induced by traditional genotoxic chemotherapeutics. Combinations of Nutlin with an aurora kinase inhibitor [96], a dual inhibitor of P13 kinase and mTOR [97], and a CDK1 inhibitor [98] all point to potential clinical combination therapies. Alternatively, it has recently been reported that DNA damage directly activates the BH3-only activator protein Bid via ATM-dependent phosphorylation [99,100] and decreases the levels of MDM-4 [101,102], a transcriptional repressor of p53, suggesting that the synergistic nature of nutlin 3a and genotoxic agent combinations may be mediated directly at the level of the MOMP, or by enhancement of

p53-dependent transcription. Notably, nutlins fail to inhibit the binding of p53 to MDM-4 [103], suggesting that neutralizing the function of this MDM-2 homolog would result in increased transcription-dependent proapoptotic activity of the nutlins.

Targeting the Bcl-2 rheostat to induce apoptosis in leukemia

Bcl-2 overexpression or unchanged levels of Bcl-2 after chemotherapy confers drug resistance to both solid tumors and hematological malignancies [104–107]. Since the discovery of Bcl-2 in 1984 several approaches have been investigated to decrease bcl-2 levels and increase the efficacy of chemotherapy including antisense oligonucleotides and small molecule BH3 mimetics. Small molecule BH3 mimetics bind to the hydrophobic pocket of antiapoptotic Bcl-2 proteins releasing proapoptotic Bcl-2 proteins, which in turn induce apoptosis in leukemia cell lines and primary samples via activation of the mitochondrial pathway of cell death [108]. The scientific literature has reported many naturally occurring as well as synthetic compounds that bind to Bcl-2 and antagonize its prosurvival function. Amongst these are antimycin A [109], epigallocatechin gallate (EGCG) [110], HA14-1 [111], vitamin E [112], gossypol [113], thiazolidinediones [114], ABT-737 [115,116], BH3I-1 and -2 [117], and GX015-070 [118,119]. Albeit all these various compounds induce apoptosis, bind to Bcl-2, and dissociate BH3 peptides from Bcl-2, only ABT-737 demonstrates an absolute requirement for both Bax and Bak to induce apoptosis suggesting this agent induces apoptosis solely through perturbations in the Bcl-2 rheostat. Interestingly, ABT-737 was originally postulated to only induce apoptosis in combination with chemotherapy; however, early evidence suggested that this agent could induce apoptosis in a cell context dependent manner, and this depended on the composition of the Bcl-2 rheostat – particularly the levels of BH3-only activator proteins [120,121]. Additionally, our observations suggested that the activity of ABT-737 was minimized by the expression of Mcl-1, a finding that at second glance was not surprising since ABT-737 bound Bcl-2, Bcl-xL, and Bcl-w, but failed to interact with Mcl-1 or A1 [122]. This peculiar property resulted from the NMR-guided, structure based design used to generate ABT-737 where the hydrophobic pocket of Bcl-2 served as the docking target for lead compounds. More interestingly, however, was our observation that ABT-737 also induced apoptosis in primary leukemia samples and cell lines at low nanomolar doses, and this was independent of Bim expression since siRNA ablation of this BH3-only activator failed to rescue leukemia cells from apoptosis induced by this agent [123]. Furthermore, in contrast to the findings in the original report by Oltersdorf et al., we observed that ABT-737 induced cytochrome c release from isolated leukemia cell mitochondria [124]. Taken together, the above observations suggest that leukemia mitochondria, but not mitochondrial from solid tumors, are poised to activate free Bax and Bak, even in the absence of BH3-only activator proteins, to induce apoptosis via MOMP.

Another peculiar property of ABT-737 was observed in the context of Bcl-2 phosphorylation. Studies in our laboratory have identified Bcl-2 phosphorylation in most primary AML samples, and this was associated with increased antiapoptotic potential, and resistance to ABT-737 induced apoptosis. Since ERK is a Bcl-2 kinase, we hypothesized that the ERK and MEK1 inhibitor PD98059 would sensitize cells expressing phosphorylated Bcl-2 to the apoptotic effects of ABT-737. As predicted, PD98059 potentially synergized with ABT-737 to induce apoptosis in leukemia cells. Unexpectedly, however, it was observed that PD98059 in addition to decreasing Bcl-2 phosphorylation, also decreased the levels of Mcl-1, suggesting that multiple effects were responsible to the powerful synergy in between ABT-737 and ERK/MEK1 inhibition [125]. In support for the criticality of Mcl-1 in preventing ABT-737 induced apoptosis, it was observed that ABT-737 inhibited clonogenic growth in primary AML samples, and their relative sensitivity to this agent was highly correlated to Mcl-1, but not to Bcl-2 protein expression. Moreover, multicolor flow

cytometric analysis of primary AML samples treated with ABT-737 or cytarabine revealed that ABT-737, but not cytarabine, preferentially induced apoptosis in the leukemia stem cell compartment – a critical requirement for maximal therapeutic efficacy of any drug (Figure 5). In contrast, no inhibition was observed of colony formation by normal bone marrow cells treated with ABT-737, and this was associated with high expression of Mcl-1 in these cells. Clinically, this identifies leukemia as a bona fide tumor target for ABT-737, as long as Mcl-1 is not the dominant antiapoptotic Bcl-2 protein.

We have also demonstrated that the combination of nutlin 3a and ABT-737 synergistically induce mitochondrial apoptosis in leukemia cells, via in part an interplay of cell cycle modulation with the bcl-2 apoptotic rheostat [126]. While nutlin-3a induced p53-mediated apoptosis predominantly in S and G2/M cells, ABT-737 induced apoptosis predominantly in G1, the cell cycle phase with the lowest Bcl-2 protein levels and Bcl-2/Bax ratios. The complementary effects of Nutlin-3a and ABT-737 in different cell cycle phases, as well as the induction of proapoptotic Bcl-2 family proteins in response to Nutlin 3a, account in large part for their synergistic activity. Our data suggest that combined targeting of Mdm2 and Bcl-2 proteins could offer considerable therapeutic promise in AML[127]. ABT-737 lowers the apoptotic threshold and should therefore be synergistic or at least additive with other agents. One potential choice is the combination with Gleevec (imatinib) in CML [128] and with the FLT3-ITD inhibitor Sorafenib [129,130]

Another Bcl-2 “inhibitor” with a detailed preclinical and recently also clinical history [131] is Obatoclax Mesylate (GX-015-070). This agent was shown to target the BH3 region, although probably at a lower affinity than ABT-737. A clinical trial has been reported in AML with inconclusive results [131]. No on-target effects on platelets (which contain large amounts of Bcl- X_L and are highly sensitive to ABT-767 and ABT-263) have been observed, and no dose- dependent anti-leukemia effects. As Obatoclax targets Mcl-1, unlike ABT-737, potential synergistic effects with ABT-737 could be exploited and Mcl-1 dependent malignancies could be targeted. Another group of compounds, Gossypol and Apo-Gossypol target the BH3 hydrophobic groove, but other established mechanisms make these compounds less specific, which is also borne out in the reported toxicities of Gossypol [132–134].

Interestingly, recent observations suggest that the FLT3 inhibitor sorafenib can activate the expression of multiple proapoptotic Bcl-2 proteins such as Bim, Bad, Bax and Bak [135], while simultaneously decreasing the levels of Mcl-1 and XIAP, changes that render AML cells exquisitely sensitive to ABT-737. These observations support the combinatorial use of sorafenib with BH3 mimetics as a novel therapeutic strategy in AML. Promising results of a Phase I study in hematological malignancies utilizing ABT-263, the orally available clinical analog of ABT-737, are evolving and Sorafenib showed high activity as single agent in FLT3-ITD AML [136]. Optimized, mechanism-based combinations of targeted agents should result in “synthetic lethality” overcoming mechanisms of resistance of their combinatorial partner agents.

Targeting XIAP to potentiate apoptosis in leukemia

Giving its role in opposing apoptosis and promoting chemoresistance, as well as its overexpression in malignant cells over normal cells, XIAP has attracted great attention as a potential therapeutic target for cancer. A phase I/II clinic trial with a XIAP antisense oligonucleotide [137] alone or in combination with chemotherapies is ongoing in various solid tumors and AML. Various small molecules that inhibit BIR3 or BIR2 domains of XIAP are under preclinical development [138–143]. Among them, polyphenylurea based small molecular BIR2 inhibitors of XIAP induce apoptosis in various AML cell lines and

patient blasts in a caspase-3 dependent manner [144]. By screening a library of molecules isolated from Chinese herbs, Nikolovska-Coleska et al. identified embelin, a natural product from the Japanese *Ardisia* plant that binds the surface groove in the BIR3 domain of XIAP. Its binding affinity is similar to the SMAC peptide and induces apoptosis of malignant prostate cancer cells at low micromolar concentrations [145]. Our group has found that triptolide inhibits XIAP mRNA and protein and potently induces AML cell death at low nanomolar concentrations [146]. Curiously, triptolide can also activate p53 resulting in increased expression of DR5 and potent synergism with TRAIL to induce apoptosis of AML cell lines and primary samples [147]. A clinical trial with a water-soluble derivative of triptolide in solid tumors is being conducted in France. The potent effect of triptolide in leukemia cells in culture warrants its clinical evaluation in AML.

Conclusions

The findings presented here support hypothesis-driven therapeutic concepts to maximize the induction of apoptosis in leukemia cells. For example, the inactivation of p53 by HDM2 is frequent in leukemias and can be overcome by agents that disrupt the HDM2-p53 interaction. HDM4 which inhibits p53 through a different mechanism will also need consideration. Second, the inactivation of certain pro-apoptotic by anti-apoptotic Bcl-2 family members is very frequent in leukemias and both protein-protein interactions are presently being investigated in targeted clinical trials. The inhibitor-of-apoptosis (IAP) family proteins are likely to contribute to apoptosis resistance, and clinical trials targeting XIAP and survivin are ongoing (Schimmer, Andreeff 2009, In Press). The notion that p53 modulates the Bcl-2 apoptotic rheostat directly, by behaving as a BH3-only protein, and indirectly by increasing the levels of sensitizer BH3-only proteins, provides a rationale to combine HDM2 inhibitors with BH3 mimetics. Ongoing work has indeed revealed that nutlins potently synergize with ABT-737 to induce apoptosis in AML cell lines and primary samples. Second, the finding that inhibition of MAPK signaling decreased the levels of Mcl-1 and abrogated Bcl-2 phosphorylation resulting in increased sensitivity to ABT-737 demonstrates that the true “Achilles heel” of leukemia may be exploited by targeting both signal transduction and apoptotic programs simultaneously (“synthetic lethality”). Third, XIAP and survivin inhibitors have progressed to clinical trials to maximize the contribution of caspase activation to cell death. While still incomplete, our present understanding of the apoptotic circuitry of leukemia cells is illustrated in Figure 6. Activation of the intrinsic apoptosis pathway, particularly the induction of MOMP, is a central event in promoting cell death of leukemia cells, whether by activated p53 signaling, BH3 mimetics, or IAP inhibition alone or in combination strategies with kinase inhibitors and classical apoptosis inducers, such as chemotherapeutic agents. Critical knowledge will be gained in carefully analyzed clinical trials. The expected surprises will extend our knowledge of the apoptotic machinery of leukemia cells, lead to refinements of the basic concepts discussed here, and the development of new agents that will need to exert pro-apoptotic activity against leukemic stem cells in hypoxic microenvironments that takes into account biochemical and molecular realities not yet fully appreciated in our current concepts. [148–150]

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Figure 1.
The extrinsic apoptotic pathway.

Figure 2.
The intrinsic apoptotic pathway.

Figure 3.

The CDDO derivative, CDDO-Me, directly induces MOMP via disruption of inner mitochondrial membrane impermeability. *Top* – U937 cells treated with 0.1% DMSO. *Bottom* – U937 cells treated with 300 nM CDDO-Me for 3 h. Images on left were captured at 10,000X direct magnification (bar = 2 microns); images on right correspond to 50,000X direct magnification of boxed area (bar = 500 nm).

Figure 4. Subcellular distribution of nutlin 3a-induced p53 is cell context regulated. COX-IV is a mitochondrial OXPHOS protein used to delineate cytoplasmic localization of p53.

Figure 5.
ABT-737, but not AraC, induces apoptosis in the CD34+/38-/123+ leukemia stem cell compartment.

Figure 6.
Targeting the apoptotic circuitry in leukemia cells.