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Apoptosis and Oncogenesis: Give and Take in the BCL-2 Family

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Abstract

The mitochondrial pathway of apoptosis constitutes one of the main safeguards against tumorigenesis. The BCL-2 family includes the central players of this pathway that regulate cell fate through the control of mitochondrial outer membrane permeabilization (MOMP), and important progress has been made in understanding the dynamic interactions between pro- and anti-apoptotic BCL-2 proteins. In particular, recent studies have delineated a stepwise model for the induction of MOMP. BCL-2 proteins are often dysregulated in cancer, leading to increased survival of abnormal cells; however, recent studies have paradoxically shown that apoptosis induction can under some circumstances drive tumor formation, perhaps by inducing compensatory proliferation under conditions of cellular stress. These observations underline the complexity of the BCL-2 proteins function in oncogenesis.

Introduction

For more than half a century, we have understood that cancer is a consequence of progressive mutations in the genome, resulting in the unregulated expansion of a clone of cells [1]. Several of these steps were functionally grouped into “hallmarks” of cancer [2] and these were subsequently elaborated upon [3]. However, a contrasting viewpoint (or at least, a complementary one) holds that the core changes that convert a cell to a cancer are relatively basic: an increase in proliferation coupled to a decrease in cell death [4]. Many of the hallmarks, in this view, may be regarded as features of any growing tissue, although additional mutations can clearly contribute to the aggressiveness of the tumor. Here, we overview recent progress in the delineation of the mechanisms of cell death as they relate to cancer, and conversely, how features of cancer and its therapeutic manipulation relate to these mechanisms. In particular, we focus on one particular form of cell death, apoptosis, and the major way in which this occurs: the mitochondrial pathway of apoptosis. This avenue to cell death is controlled by proteins of the BCL-2 family, and our discussion is specifically geared to our emerging understanding of how these proteins work, and how this informs our thinking about oncogenesis.

Mitochondria as stepping-stones on the road to ruin

Most cell death in vertebrates occurs via the mitochondrial pathway of apoptosis, in which proteins of the BCL-2 family function to control the integrity of the outer membranes of

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mitochondria in the cell. When the interactions among these proteins results in apoptosis, the two pro-apoptotic BCL-2 effector proteins, BAX and BAK, disrupt this membrane in a process called mitochondrial outer membrane permeabilization (MOMP). If MOMP occurs, proteins present in the mitochondrial intermembrane space gain access to the cytosol and cause the activation of caspases, cysteine proteases that orchestrate the dismantling of the cell (reviewed in detail elsewhere [5]). If such caspase proteases are blocked or their activation is impeded, death can nevertheless occur by a loss of mitochondrial function (mitochondrial catastrophe) [6]••. However, some cells that undergo MOMP can resuscitate, provided sufficient glycolysis is maintained [7] and a small number of mitochondria persist to repopulate the cell [8]••. Such observations may help to explain why tumor cells often display defects in the mitochondrial pathway downstream of MOMP (reviewed in [9] and [10]).

The process of MOMP is antagonized by the anti-apoptotic BCL-2 proteins, such as BCL-2, BCL-W, BCL-xL, A1/Bfl1 and MCL-1, which inhibit the permeabilization function of BAX and BAK. The importance of this effect in cancer is underscored by the observation that oncogenes, such as Myc, which promote proliferation, also promote cell death that is blocked by the anti-apoptotic BCL-2 proteins [11–13]. As a consequence of this interplay, enforced expression of Myc synergizes with any of the anti-apoptotic BCL-2 proteins to transform primary B lymphocytes in vivo [14] (This model, which employs a Myc transgene driven by the immunoglobulin μ (E μ myc) enhancer, has been reviewed elsewhere [15]). This synergy between Myc and the anti-apoptotic BCL-2 proteins is not restricted to B lymphocytes, and has been observed in numerous systems (indeed, too many to cite herein).

Both the anti-apoptotic BCL-2 proteins and the pro-apoptotic effectors are regulated by a third subfamily of BCL-2 proteins, the BH3-only proteins (so named because of the four BCL-2 homology (BH) domains, they contain only BH3). These bind and inhibit the anti-apoptotic BCL-2 proteins with differing efficiency [16,17] and some of these also function to activate the pro-apoptotic effectors [18,19]. Several of these act to inhibit Myc-induced lymphomagenesis such that when ablated, tumor incidence is accelerated. We return to this point below.

A common misconception arises from these considerations: because resistance to apoptosis is necessary for oncogenesis, tumors are necessarily resistant to apoptosis. But this is not quite the case (and indeed, if it were, the majority of therapeutic agents would invariably fail, as these act via engagement of MOMP and the mitochondrial pathway). Instead, only those apoptotic signals that are promoted by the relevant oncogenes and tumor suppressors need be damped to the point that proliferation exceeds cell death. As a result, many tumors are observed to be “primed to die.” By administering specific BH3 only proteins (or only the peptides corresponding to their BH3 regions) directly to the mitochondria of tumor cells, a profile of susceptibility upon derepression of the anti-apoptotic BCL-2 proteins can be obtained [17]. Such BH3 profiling predicts responses to drugs that mimic BH3-only proteins, such as ABT-737 (which targets only a subset of anti-apoptotic proteins such as BCL-2 and BCL-xL [20]) and further, demonstrate that many, but not all primary tissues are not so primed [21].

Exceptions include platelets [22,23], immature T lymphocytes [24]• and mast cells [25]. Therefore, to gain a deeper understanding of how tumor cells resist some apoptotic signals and how they can be triggered to undergo apoptosis, we turn to the mechanisms by which BAX and BAK are activated to effect MOMP.

Stalking the killers

BAX and BAK are superficially redundant proteins required for MOMP. That is, in the absence of both, MOMP does not occur, and each is capable of permeabilizing lipid membranes. Such permeabilization appears to be in the form of large, round holes of 25–100 nm [26]• that may represent lipidic pores [27–29], and allow the diffusion of proteins through the membrane. However, it is not fully understood how BAX and BAK cause such permeabilization: While structural data exists for each in their inactive forms, the structures of the active forms remain elusive. Upon activation, BAX and BAK form homo-oligomers, and these are assumed to be the agents of MOMP [30–32]. Despite the absence of structural details of MOMP, there has been progress towards this goal.

The activation of BAX or BAK to cause MOMP can be triggered by the BH3-only proteins BID, BIM, and perhaps PUMA (or by peptides corresponding to the BH3 regions of BID and BIM) [18,19,33,34], and by some proteins that lack BH3 domains, such as cytosolic p53 [35,36]. BAX and BAK activation is a necessary function for direct activator BH3-only proteins to fully exert their pro-apoptotic potential. This phenomenon has recently been demonstrated for BIM *in vivo* using a series of knock-in mice in which the BIM BH3 domain was replaced by that of BAD, NOXA, or PUMA [37]••. BIM^{PUMA-BH3} or a combination of BIM^{BAD-BH3} and BIM^{NOXA-BH3} mutants, which display the same binding pattern as wild-type BIM for pro-survival BCL-2 proteins, did not fully compensate for the loss of BIM in mice. This observation suggests that inhibition of anti-apoptotic BCL-2 family members and activation of the effectors BAX and BAK are two necessary features of BIM pro-apoptotic activity. Recently, two other direct activators of BAX and BAK have been proposed: IRF3, a transcription factor that induces interferon expression that appears to activate BAX and/or BAK in a transcription-independent manner [38], and endophilin B, involved in mitochondrial dynamics [39]. The latter appears to induce BAX-BAK-dependent MOMP independently of its role in mitochondrial fission.

In the case of BID-induced BAX activation, physico-chemical studies have delineated an ordered series of events [40]••. First, active BID (i.e. cleaved BID) rapidly associates with the mitochondrial membrane, where it then transiently binds and activates BAX. Activated BAX subsequently associates with the membrane, where it then binds other BAX molecules. This is followed by membrane permeabilization.

The transient interaction of the BH3 region of BIM with BAX, leading to at least the first steps of BAX activation has been analyzed structurally [41]••. Although BAX possesses a hydrophobic groove homologous to that in anti-apoptotic proteins that binds to BH3 regions, BIM BH3 does not appear to associate with this groove. Instead, BIM BH3 interacts with a hydrophobic groove situated at the “back” of the protein, rotating the first alpha helix ($\alpha 1$) to produce further conformation changes in BAX. These include the exposure of an epitope (6A7) associated with the early stages of BAX activation.

The next step in the process is suggested by biochemical studies on the activation of BAK [42]••. By placing cysteines at strategic sites in BAK and subjecting them to cross-linking, it was found that activation of BAK by activated BID results in two BAK-BAK interfaces. One involves exposure of the BAK BH3 region and reciprocal insertion of the BH3 regions from two BAK molecules into the hydrophobic groove formed upon such exposure (“nose to nose”) [43]••. The second involves another face of the protein, resulting in an $\alpha 6$ - $\alpha 6$ association (“back to back”) [42]••. These interactions may explain the formation of BAK tetramers, but higher order oligomers, predicted by cross-linking and electrophysiological studies [44,45] would presumably require additional interactions. Interestingly, BAX appears to oligomerize with the same dynamics in detergents that promote oligomerization

by forming first a homodimer through the BH3-domain/hydrophobic groove “nose to nose” interface and then larger oligomers through the second “back to back” interface [46]•.

While direct activator BH3-only proteins seem to be required to recruit and activate pro-apoptotic BCL-2 effector proteins, additional proteins in the mitochondrial outer membrane may potentiate BAX/BAK induced MOMP [26]. One of these proteins has been identified as MTCH2/MIMP (mitochondrial carrier homologue 2/Mit-induced mitochondrial protein), an outer mitochondrial membrane protein that facilitates the recruitment of cleaved BID to mitochondria, increasing BAX/BAK activation, MOMP, and apoptosis [47]•. Additionally, DRP-1 (Dynamin-related protein-1) a GTPase that controls mitochondrial fission may facilitate BAX oligomerization by promoting membrane remodeling [48]•. The putative steps in the activation of BAX and BAK are illustrated in Figure 1.

Further complexity in the regulation of the BCL-2 proteins

BAX and BAK, as well as the anti-apoptotic proteins, are controlled at several steps in addition to their interactions with BH3-only proteins. Recent studies have highlighted some novel ways in which such regulation comes about. BAX can be inhibited by the function of the peptidyl-proline isomerase PIN1 [49], presumably affecting its conformation (although other possibilities certainly exist). BAX is also targeted by the E3 ligase IBRDC2 for ubiquitylation and degradation [50]. Intriguingly, an alternatively spliced variant of BAX, BAX β , appears to be constitutively active, and although it is expressed in some tissues, such as brain, the protein is rapidly degraded by the proteasome [51]•. Whether this is an effect of PIN1, IBRDC2, or another mechanism is currently unknown. Although the expression of BAK generally suffices to permit MOMP in the absence of BAX [52], the regulation of BAX may be particularly important in at least some cancers. In the *E μ -myc* model, mentioned above, lack of BAX profoundly accelerates lymphomagenesis [53], while effects of ablation of BAK alone has not been described (possibly because no effect has been observed). In another model system, ablation of BAX promoted outgrowth of pancreatic islets in which Myc was expressed, while ablation of BAK had no effect [54]. The reasons for this remain unclear, but suggest that mechanisms that specifically control BAX or its activation may be important in cancer.

Of the anti-apoptotic BCL-2 proteins, MCL-1 is the most labile, possessing a short half-life mediated by ubiquitin-dependent [55] and -independent [56] proteasomal degradation (Figure 2). Its expression is of practical importance, as the BH3-mimetics ABT-737 and its soluble/orally-available derivative ABT-263 target only BCL-2 and BCL-xL; MCL-1 levels appear to be the major determinant of resistance to these drugs [57–60]. Phosphorylation of MCL-1 by GSK3 links its ubiquitylation by β -TrCP and half-life to growth factor signaling [61], while translational control of MCL-1 is similarly linked by the TORC1 complex [62]•, resulting in decreased MCL-1 upon growth factor deprivation. Alternatively, phosphorylation by CDK1 under conditions of stalled mitosis promotes its degradation via APC/C-mediated ubiquitylation [63]. Finally, MCL-1 can be ubiquitylated following DNA damage through direct binding of MCL-1 Ubiquitin Ligase E3 (MULE, also known as ARF-BP1, HectH9, LASU1, and HUWE1), a HECT domain containing ubiquitin ligase that regulates multiple signaling pathways through ubiquitylation various targets including p53 [64], myc [65,66], Cdc6 [67] and DNA polymerase β [68]. MULE displays a BH3 domain that specifically recognizes and binds MCL-1 hydrophobic pocket [69,70]. Conversely, a deubiquitinase (DUB), USP9X, acts to stabilize MCL-1 [71]••, and this DUB is frequently expressed in tumors and tumor lines. Knockdown of USP9X in such lines destabilizes MCL-1, making such cells sensitive to apoptosis induction by ABT-737.

Intriguingly, the BH3 domain of MCL-1 has been found to bind exclusively to MCL-1 itself, inactivating it [72]•. While increased expression of conventional MCL-1 (MCL-1L) produces only anti-apoptotic effects, alternatively spliced variants, such as MCL-1S and MCL-1ES, may cause apoptosis in some cells. These shorter forms of MCL-1 oligomerize with MCL-1L [73–75], and thus may do so by exposing their BH3-domain to MCL-1L. Interestingly, the regulation of alternative splicing of MCL-1 and BCL-xL mRNA may be coupled to the cell cycle. Induction of mitotic arrest appears to promote pro-apoptotic splicing of MCL-1 and BCL-xL [76].

The PUMA paradox

PUMA is a BH3-only protein that binds and inhibits all of the anti-apoptotic BCL-2 proteins [16,17]. It is a direct transcriptional target of p53 and is also induced by FOXO3a under conditions of growth factor deprivation [77–79]. PUMA may also activate BAX and BAK [33,34,80], although it has been shown to promote MOMP predominantly through displacement of other proteins with this function from anti-apoptotic BCL-2 proteins [81]. Loss of PUMA accelerates Myc-induced lymphomagenesis [82,83]. Importantly, in a survey of a large number of human tumors of different types, deletion of PUMA was found to be one of the most common copy-number abnormalities [84]••.

It was therefore very surprising to find that genetic ablation of PUMA (which, unlike p53, does not result in spontaneous oncogenesis [85–87]) actually *delays* tumor formation in one model system. Sub-lethal irradiation of mice promotes the development of thymomas, an effect that is dramatically accelerated in animals lacking p53. Radiation induces massive apoptosis in thymocytes, which is dependent on p53 and its target gene, PUMA [85]. Thus, it was expected that loss of PUMA would accelerate radiation-induced transformation. Remarkably, mice lacking PUMA are instead *resistant* to this effect [88]•• [89]••.

Two sets of observations help to shed some light on this paradox. The first concerns when, in the progression of cancer in this model of radiation-induced thymoma, p53 acts to prevent oncogenesis [90]. Using a novel “switchable p53” (p53 with a germline engrafted estrogen receptor steroid-binding domain, such that p53 is only functional in the presence of tamoxifen), mice were irradiated and p53 was made functional at different times. If p53 were inactive at the time of irradiation and made functional only later, no apoptosis occurred in the thymus (the signal from DNA damage quickly waned), but no cancer manifested. In contrast, if p53 were enabled at the time of irradiation (such that thymic apoptosis occurred) cancer arose with high frequency. Thus, in this system, p53 functions not to eliminate damaged cells that might have oncogenic mutations, but rather when such mutations actually drive oncogenesis. Because, as mentioned above, PUMA is frequently deleted in human cancers [84]••, we can speculate that loss of PUMA only at later times after any mutagenic event leads to oncogene activation (phenocopied by enforced expression of Myc) would similarly promote oncogenesis.

But this does not explain why apoptosis in the tissue functions to promote thymoma. To begin to understand this, we can examine recent studies on progenitor cell competition [91,92]••. Hematopoietic stem cells (HSCs), treated with sub-lethal doses of radiation or other DNA-damaging agents, can effectively reconstitute myelo-ablated animals. If, however, they are mixed with p53-deficient HSCs, the latter effectively outcompete them to dominate in the reconstituted mice [92]••. This competition requires that the stem cells are driven into reconstituting the tissue, and we can readily envision that such repopulation and selection provides the background against which oncogenic mutations subsequently appear. Under these experimental conditions, however, p53 does not trigger apoptosis—enforced BCL-2 expression, for example, does not offer such competitive advantage—and we can

predict that loss of PUMA would similarly not provide a competitive edge. However, in the absence of PUMA, irradiation does not ablate the thymus, and thus no repaid repopulation event occurs. This is consistent with the finding that resistance to radiation-induced thymoma in PUMA-deficient mice was effectively overcome by induction of apoptosis by combining irradiation with administration of glucocorticoids, which induce thymocyte apoptosis in a PUMA-independent manner [89]••.

It is possible that apoptotic cells themselves might help to create favorable conditions for thymomagenesis. Cells undergoing apoptosis induce proliferation of neighboring progenitor or stem cells and promote tissue regeneration through a paracrine mechanism [93]••. This phenomenon is dependent on activation of effector caspases-3 and -7 that trigger the signaling cascade leading to apoptosis-induced compensatory proliferation. Accordingly, mice lacking either of these caspases are deficient in skin wound healing and in liver regeneration [93]••. It is therefore conceivable that PUMA-induced apoptosis and subsequent caspase activation provides the milieu for enforced proliferation of surviving HSCs, and those harboring mutations that provide a competitive advantage may then acquire secondary mutations leading to oncogenesis (Figure 3).

While these considerations hold promise for resolving the PUMA paradox, we are left with another: Loss of p53 eliminates the ablation of the thymus following irradiation; why does irradiation profoundly accelerate thymomagenesis in p53-deficient mice? Thus, the PUMA paradox remains incompletely resolved.

PUMA is not the only paradoxical BCL-2 protein in cancer. The survey of human cancers, mentioned above, revealed only three other copy number abnormalities in BCL-2 family genes common across tumor types [84]••. Two of these are amplification of MCL-1 and amplification of BCL-xL, which are not surprising given our discussions in the previous sections. The other, however, is the deletion of BOK, a BCL-2 protein with no known function, although it appears to be pro-apoptotic [94,95]. The role of BOK as a tumor suppressor may be under-appreciated, and its mechanism of action under-explored.

Conclusion

The process of oncogenesis ensures that cancer cells display dysregulation of some apoptotic pathways, that is, those pathways that are engaged by the specific tumor suppressor mechanisms that would serve to limit tumorigenesis in these cells. As a consequence, tumor cells may actually be *more* sensitive to engagement of other pathways of apoptosis than are primary cells, since tumor expansion requires only that cell death occur at a lower frequency than that of cell division. The upregulation of anti-apoptotic BCL-2 proteins is a common feature in cancer, and these serve to inhibit pro-apoptotic BCL-2 proteins that are induced as a tumor suppressor mechanism during oncogenesis. Thus, many malignant cells are “primed for death” and will undergo apoptosis if the function of the anti-apoptotic proteins is therapeutically impaired. In this context, BH3-mimetic agents represent a promising avenue of anti-cancer therapy, either alone or in combination with other modalities. The most specific BH3 mimetics, ABT-737 and ABT-263, target BCL-2 and BCL-xL, but not MCL-1, and thus the control of MCL-1 has become an area of intense investigation. Similarly, the proapoptotic BCL-2 proteins determine whether or not disruption of the anti-apoptotic proteins will drive cell death, and therefore the regulation and function of these proteins remain issues of fundamental importance. The complex interplay among the BCL-2 family proteins and their involvement in oncogenesis, tumor expansion, and resistance to tumor therapy, have only begun to yield their secrets.

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FIGURE 1.

Stepwise model for BAX and BAK activation.

(a) In healthy cells, BAX is a cytosolic protein, its C-terminal α -helice (**CT**) being sequestered in a hydrophobic pocket, while BAK is constitutively anchored into the outer mitochondrial membrane (OMM). Following apoptotic stimulation, BH3-only proteins are produced and/or activated. In the particular case of cleaved BID, targeting to the OMM is facilitated by mitochondrial proteins such as MTCH2/MIMP. (b) Binding of BAX or BAK by BH3-only activator through an interface close to the α -helice 6 (**$\alpha 6$**) triggers a series of extensive conformational changes including exposure of the N-terminal α -helice (**NT**) and transient exposure of the BH3-domain (**BH3**). Additionally, BAX activation induces displacement of the C-terminus which targets it to the OMM and frees the hydrophobic groove (c) Next, BAX and BAK further insert into the membrane and dimerize “nose to nose” though reciprocal BH3-domain-groove interaction. (d) Further oligomerization is then achieved though α -helix 6 interactions in two dimers (“back to back”). Induction of MOMP by formation of higher order oligomers is facilitated by DRP-1 induced membrane curvature.

FIGURE 2.

Regulation of MCL-1 anti-apoptotic activity.

MCL-1 pro-survival function is regulated by two mechanisms: control of protein level and inhibition by BH3-only proteins. The PI3K/AKT signaling pathway increases MCL-1 translation through mTOR signaling complex 1. MCL-1 protein level is also tightly regulated by proteasomal degradation. Three E3 ubiquitin ligase complexes MULE, β -TrCP, APC/C and one deubiquitinase USP9X, have been identified as direct regulators of MCL-1 ubiquitylation. Alternatively, MCL-1 stability is also controlled by ubiquitin-independent proteasomal degradation. The second mode of regulation of MCL-1 pro-survival function is by occlusion of MCL-1 hydrophobic groove by BH3-only proteins. Additionally, shorter versions of MCL-1, generated by alternative splicing, could have a similar function by engaging MCL-1L through their BH3 domains.

FIGURE 3.

Model for apoptosis driven tumor formation.

In wild type mice, γ -irradiation simultaneously induces massive apoptotic cell death in the haematopoietic compartment and oncogenic mutations in the surviving population.

Leukocyte depletion, and possibly surrounding apoptotic cells, induces compensatory proliferation of the hematopoietic stem/progenitor cells (HSCs). Some mutant HSCs clones, such as the one with disrupted p53 function, may display a proliferative advantage and outcompete non-mutated cells. This could provide favorable conditions for accumulation of secondary mutations eventually leading to malignant transformation. In the absence of PUMA, cell loss via apoptosis does not occur, and no compensatory proliferation follows. This model suggests that compensatory proliferation and growth advantage of some mutations are necessary for oncogenesis in this system of radiation-induced tumorigenesis.