Review

Special Issue: Translational Cell Biology

Evading apoptosis in cancer

Kaleigh Fernald[1] and Manabu Kurokawa[1,2]

[1] Department of Pharmacology and Toxicology, Geisel School of Medicine at Dartmouth, Hanover, NH 03755, USA
[2] Norris Cotton Cancer Center, Dartmouth-Hitchcock Medical Center, Lebanon, NH 03756, USA

Carcinogenesis is a mechanistically complex and variable process with a plethora of underlying genetic causes. Cancer development comprises a multitude of steps that occur progressively starting with initial driver mutations leading to tumorigenesis and, ultimately, metastasis. During these transitions, cancer cells accumulate a series of genetic alterations that confer on the cells an unwarranted survival and proliferative advantage. During the course of development, however, cancer cells also encounter a physiologically ubiquitous cellular program that aims to eliminate damaged or abnormal cells: apoptosis. Thus, it is essential that cancer cells acquire instruments to circumvent programmed cell death. Here we discuss emerging evidence indicating how cancer cells adopt various strategies to override apoptosis, including amplifying the antiapoptotic machinery, downregulating the proapoptotic program, or both.

Regulation of apoptosis in normal and cancer cells

Apoptosis is a cellular suicide program that organisms have evolved to eliminate unnecessary or unhealthy cells from the body in the course of development or following cellular stress. It involves a series of cellular events that ultimately leads to activation of a family of cysteine proteases called caspases. In response to various apoptotic stimuli, 'initiator' caspases (caspase-2, -8, -9, or -10) are activated. Initiator caspases, in turn, cleave and activate the zymogenic forms of 'executioner' caspases (e.g., caspase-3 or -7), resulting in the proteolytic cleavage of specific cellular substrates and, consequently, cell death (Figure 1). In this regard, the cleavage (activation) of executioner caspases is a hallmark of apoptosis. There are two routes to apoptosis: extrinsic and intrinsic. In the extrinsic pathway, initiator caspase-8 and -10 are activated through the formation of a death-inducing signal complex (DISC) in response to the engagement of extracellular ligands [e.g., Fas, tumor necrosis factor (TNF)] by cell surface receptors (Figure 1). In the intrinsic pathway of apoptosis, mitochondrial outer membrane permeabilization (MOMP) is involved; MOMP triggers the release of a group of proapoptotic proteins, including cytochrome *c* and second mitochondria-derived activator of caspases (SMAC), from the mitochondrial intermembrane space to the cytoplasm (Figure 1). In the cytoplasm, cytochrome *c* binds to the adaptor protein apoptotic protease-activating factor 1 (APAF-1) to form a caspase-9-activating complex called the apoptosome (Figure 1). SMAC augments cytochrome *c*-induced caspase activation by binding and neutralizing X-linked inhibitor of apoptosis protein (XIAP), an inhibitor of caspase-3, -7, and -9 (Figure 1).

The balance between pro- and antiapoptotic B cell lymphoma 2 (BCL-2) family proteins is a fundamental determinant for the initiation of MOMP. The BCL-2 family proteins are classified based on the presence of shared blocks of sequence homology, termed BCL-2 homology (BH). The proapoptotic BCL-2 family members, which promote MOMP, include BH3-only proteins [e.g., Bcl2-interacting mediator of cell death (BIM), BH3-interacting domain death agonist (BID), and Bcl2-associated agonist of cell death (BAD), which share only a single block, the BH3 domain, of BCL-2 homology] and multi-BH domain proteins [e.g., Bcl2-associated protein X (BAX) and Bcl2 antagonist/killer (BAK), which share BH1–BH3 domains]. Following cytotoxic or genotoxic stress, BH3-only proteins are activated and promote oligomerization of BAX (or BAK), resulting in MOMP, whereas prosurvival members [e.g., BCL-2, B cell lymphoma extra large (BCL-XL), and induced myeloid leukemia cell differentiation protein (MCL-1), which contain all four BH domains] counteract this process by sequestering proapoptotic family members (Figure 1). Importantly, the interactions between pro- and antiapoptotic BCL-2 family proteins are determined with specific binding specificities [1,2]; for instance, BIM interacts with all six antiapoptotic BCL-2 family proteins as well as BAX and BAK [3], whereas phorbol-12-myristate-13-acetate-induced protein 1 (NOXA) binds to MCL-1 and BFL-1/A1 only.

Unlike normal cells, cancer cells are under constant stress, including oncogenic stress, genomic instability, and cellular hypoxia. In response to such apoptotic stimuli, the intrinsic pathway of apoptosis would normally be activated. Yet cancer cells can often avoid this cellular response by disabling the apoptotic pathways. Notably, mouse genetic models have shown that genetic inactivation of a BH3-only protein or a caspase can not only lead to resistance to certain proapoptotic stimuli but also accelerate tumor formation in mice [4–6]. Moreover, forced expression of antiapoptotic BCL-2 family proteins can significantly augment tumor development induced by an oncogene, such as *MYC*, although overexpression of an antiapoptotic BCL-2 family protein alone may not result in tumor formation similar to that seen with an oncogene alone [7–10]. Thus, it is strongly suggested that inhibition of apoptosis plays a critical role in cancer cell survival and tumor development.

Corresponding author: Kurokawa, M. (manabu.kurokawa@dartmouth.edu).
Keywords: BH3; caspase; MOMP; phosphorylation; ubiquitination.

0962-8924/$ – see front matter
 http://dx.doi.org/10.1016/j.tcb.2013.07.006

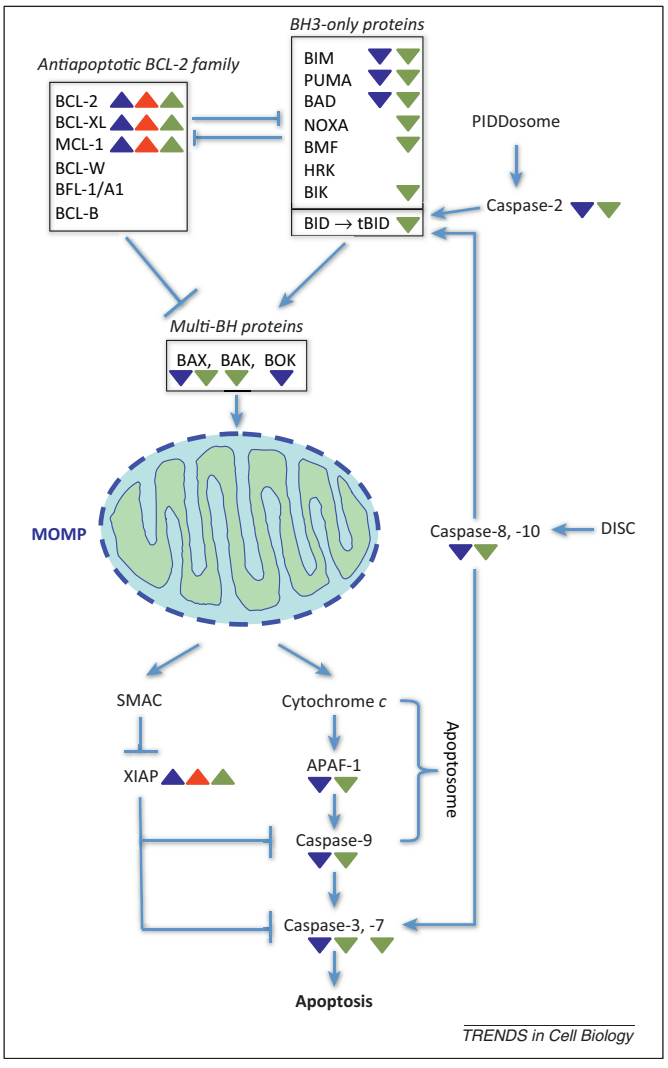

Figure 1. Apoptosis is triggered in response to internal or external stimuli. The intrinsic pathway to apoptosis is initiated by activation of BH3-only protein. Among the BH3-only proteins, expression of which is often transcriptionally induced following an apoptotic stimulus, BID is unique in that it is activated by caspase cleavage (by caspase-2 or -8/10); cleaved BID is termed tBID. BH3 proteins trigger the activation of multi-BH domain proteins by direct binding or by inhibition of antiapoptotic BCL-2 family proteins. Once activated, multi-BH domain proteins form oligomers that induce MOMP. MOMP prompts the proapoptotic proteins SMAC and cytochrome *c* to be released to the cytoplasm, where cytochrome *c* induces formation of the apoptosome. The initiator caspase-9 activated within the apoptosome initiates the activation of executioner caspase-3 and -7, resulting in apoptosis. Of note, caspase-2 is activated via formation of the PIDDosome, a complex comprising the adaptor PIDD and RAIDD. However, despite identification of this complex, the precise mechanism of caspase-2 activation remains elusive. In addition, only a few cellular substrates of caspase-2, including BID, have been identified. Colored triangles denote that the indicated gene or gene product may be transcriptionally (blue), translationally (red), or post-translationally (green) up- or downregulated in cancer cells (see text and Table 1).

Cancer cells can modulate apoptotic pathways transcriptionally, translationally, and post-translationally. In some cases, cancer cells may escape apoptosis by increasing expression of antiapoptotic genes or by decreasing expression of proapoptotic genes. Alternatively, they may inhibit apoptosis by stabilizing or destabilizing anti- or proapoptotic proteins, respectively. Moreover, cancer cells may also prevent apoptosis by changing the functions of anti- or proapoptotic proteins through post-translational modifications such as phosphorylation. Importantly, these mechanisms are not mutually exclusive and cancer cells may employ one or multiple mechanisms to evade apoptosis. In this review, we highlight the core antiapoptotic machinery that cancer cells may employ for survival, focusing our discussion on transcriptional, translational, and post-translational modifications to evade apoptosis (note that recent studies suggest that cancer metabolism can also modulate this machinery to protect cells from apoptosis [11,12]; however, this is outside the scope of this discussion).

Transcriptional/translational regulation

Genomic and epigenomic abnormalities are a characteristic of cancer cells. This includes gene copy number amplification, gene deletion, gene silencing by DNA methylation, and activation (or inactivation) of transcription factors that have an impact on the expression of apoptotic regulators [13]. In addition, miRNAs negatively control gene expression by targeting the 3′ untranslated region (3′-UTR) of mRNAs, although they could function as tumor suppressors or oncogenes, depending on the target messengers [14]. Thus, transcriptional and translational alterations can be a means for cancer cells to directly raise the threshold for apoptotic induction at gene expression levels. In this section, we discuss how cancer cells may increase or block the expression of anti- or proapoptotic gene products, respectively (see Table 1 for loss or amplification of apoptotic genes identified in cancer tissues).

Inducing antiapoptotic protein expression

To overcome stress signals, cancer cells frequently overexpress antiapoptotic proteins, especially antiapoptotic BCL-2 family proteins. Cancer cells prevent MOMP by amplification of antiapoptotic BCL-2 family proteins. Consequently, inhibition of one or multiple antiapoptotic BCL-2 family proteins causes apoptosis in cancer cells but not in healthy normal cells – it is often said that cancer cells are primed for death or addicted to antiapoptotic BCL-2 family proteins. Overexpression of antiapoptotic BCL-2 family proteins is often associated with poor prognosis, recurrence, and resistance to cancer therapeutics [15–17]. The *BCL-2* gene was originally found in B cell follicular lymphoma, where genetic translocation resulted in constitutive expression of BCL-2 [18–20] (Table 1). Amplification of the *BCL-XL* gene has also been reported in various cancers, including lung and giant-cell tumor of bone [21–23] (Table 1). Likewise, frequent amplification of the *MCL-1* gene has been reported in breast and lung cancer samples [23,24] (Table 1).

In normal cells, MCL-1 transcription is induced upon cytokine and growth factor stimulation [25,26], whereas its synthesis is inhibited in response to various cellular stresses, including DNA damage [27–29]. In particular, it was shown that the transcription factor E2F1, known to control proliferation and apoptosis, could repress *MCL-1* gene expression by binding directly to the *MCL-1* promoter [30], resulting in increased apoptosis. Alternatively, the transcription factors cAMP response element-binding protein (CREB) and signal transducer and activator of transcription 3 (STAT3), which are known to activate genes responding to prosurvival stimuli, can transactivate the *MCL-1* gene [25,27]. Importantly, whereas these signaling pathways operate to maintain cellular homeostasis in normal cells, they are often deregulated in cancer cells.