Apoptosis’ activation associated to BH3 only domain and BCL-2 homology domains proteins: new way to design anti-cancer drugs

Abstract

Cancer is one of the main health problems we face today. A possible way to develop specific treatments is through the identification and understanding of proteins responsible for the regulation of apoptosis. Apoptosis is a cell suicide that is critically important for organ development and tissue turnover.1 The BCL-2 homology domain (BHD) and BH3-only domain (BOD) are pro-apoptotic proteins that mediate mitochondrial damage, inducing intrinsic pathway cell death. In order to develop treatments that can promote apoptosis in cancer cells, this study want to clarify how BOD and BHD proteins trigger mitochondrial events associated to apoptosis.

Keywords: apoptosis, BH3-only domain, BIM, BID, BAX, BAK, BCL-2 homology domain

Introduction

Apoptosis or programmed cell death is a genetically controlled mechanism that plays a crucial role in regulation and maintaining tissue homeostasis leading the elimination of old or damaged cells. Cancer is a disease that appears when a group of malignant or abnormal cells growth out of control dividing too quickly invading and destroying adjacent tissues forgetting how to die in the body. For these reasons, control the pathway of apoptosis could help to find or improve treatments for a lot of diseases, especially cancer. Decrease or increase in apoptosis, plays a key role in a great many diseases because unhealthy cells become immortal or on the contrary, healthy cells are indiscriminate killed, respectively.

The cell regulates apoptosis via intrinsic and extrinsic pathways. Pro-apoptotic ligands (Apo2L/TRAIL and CD95L/FasL) interact with pro-apoptotic receptors (DR4/DR5 and CD95/Fas, respectively) on the cell surface, activating the extrinsic pathway apoptosis. To induce intrinsic pathway cell death, BH3-only domain (BOD) proteins interacts with BCL homology domain (BHD) proteins triggers mitochondrial permeabilization and finally, apoptosis (Figure 1).

BCL proteins have essential roles on determining if a cell lives or dies because either promote or block apoptosis pathway. We found on the literature two groups of BCL family: the antiapoptotic members including BCL-XL, Bcl-2, Bcl-w, MCL-1, Boo, NR-13, BHRF1, LMW5-HL, ORF16, KS-Bcl-2, E1B-19K, and Ced-9; and the pro-apoptotic members BCL-2 homology domains (BHD) including BAX, BAK, NOXA, PUMA, BIK and BAD. Both pro-apoptotic and anti-apoptotic share conserved up to fourth similar structural domains and a C-terminal membrane anchor. Other pro-apoptotic proteins are the BH3-only domain (BOD) that forms amphipatic α-helices and as their name says, only one conserved domain. One of these α-helices interacts with BHD’s active site promoting mitochondrial membrane damage.

A convergence of genetic and biochemical studies has established that in the intrinsic apoptotic pathway, BOD proteins display selective binding to specific BHD family members integrating diverse apoptotic stimuli acting at an upstream point in an apoptotic signal-transduction cascade that leads ultimately to cytochrome C release from mitochondria and activation of caspases-9 and caspases-3.2 Elucidation of the signaling circuitry governing the apoptotic program has revealed how apoptosis is triggered in response to various physiologic stresses that cancer cells experience during the course of tumorigenesis or as a result of anticancer therapy.3 The cells induce apoptosis when they are exposing to oxidative stress, or when increase levels of oncogenes (P53) and/or DNA mutations occur but when tumors are growing the cell are very resistant to apoptotic stimulus and insensitive to proteins that control cell division. In this review, we are going to focusing on intrinsic pathway, antiapoptotic BCL-XL, proapoptotic BHD BAX/BAK and proapoptotic BOD BIM/ BID to explain the apoptosis’ initiation as a viable manner to stop the uncontrolled growth of cancer cells.

Intrinsic and extrinsic pathways of apoptosis

Apoptosis is a tightly regulated and at the same time highly efficient cell death program which requires the interplay of a multitude of factors. Apoptotic cells can be recognized by stereotypical morphological changes: the cell shrinks, shows deformation and loses contact to its neighboring cells. Its chromatin condenses and margiates at the nuclear membrane, the plasma membrane is blebbing or budding, and finally the cell is fragmented into compact membrane-enclosed structures, called ‘apoptotic bodies’ which contain cytosol, the condensed chromatin, and organelles. At the same time, internally biochemical features of apoptosis result from cellular degradation precipitated by intracellular cysteine proteases (caspases), which cleave substrates after specific aspartate residues. These proteases are present in healthy cells aszymogens with low, yet significant, enzymatic activity. In response to apoptotic signals, they become fully activated through adaptor protein-mediated aggregation, which facilitates autocatalytic processing, or through proteolysis by other caspases already activated.4

Mitochondrion is the central regulator of intrinsic apoptosis pathways, but the extrinsic pathway begins on the bilayer membrane cell. The activation of extrinsic pathway apoptosis in the type II cells coming from the activation of dead receptors (DR) that are on the cell surface. These DR emit apoptotic signals when are bound to their specific ligands. The family of the receptors is composed of TNFR-1, Fas/CD95, and the TRAIL DR-4 and DR-5.5 When the receptor
recognized their ligands starts a trimerization and activation of the receptor. Cytoplasmic part of the DR sends the signals inside the cell. But these cells harbor the capacity to induce direct caspase-dependent apoptosis pathways. For this reason, proapoptotic and antiapoptotic proteins help to initiate mitochondria-dependent apoptotic pathway. BIM is the key to links caspase cascade and mitochondrial damage. Activated form of BIM induces the oligomerization of BAX on the mitochondrion, simultaneously BAD sequesters BCL-XL, Caspase-8 cleavages BID to forms truncated BIM (tBID) that translocates to the mitochondria Bax and Bak to induce the release of cytochrome C and other mitochondrial factors. The released cytochrome c activates, in turn, a cascade of caspases that act via their proteolytic activities to induce the multiple cellular changes associated with the apoptotic program (Figure 2).

### Proapoptotic and prosurvival proteins

There has been quite some debate about how the BCL-2 family controls apoptosis. Commonly, the activation of apoptosis is regarded to occur when a cell encounters a specific death-inducing signal such as the ligation of a death receptor by its ligand. This is the same that occurs in the cell when proapoptotic proteins BOD are activated and the interaction with BHD proteins or with BCL-XL, initiate or block apoptosis, respectively.

BHD and BCL-XL display sequences conservation in all BH domains, all of which include α-helical segments. BAX activation is believed to be a highly regulated by the contact with BOD, multi-step process involving an interaction-triggered conformational change, mitochondrial translocation, and oligomerization of BAX and Bak that ultimately leads to mitochondrial dysfunction, cytochrome C release, caspases activation and apoptosis. Anti-apoptotic BCL-XL binds directly to the pro-apoptotic members BOD, neutralize their activities, and abolish the apoptotic signaling. In healthy cells, BAX and BAK exist as monomers and when receive a variety of dead stimuli, BAX replaces loosely attached into the mitochondrial membrane. When BAK is inactive resides at the mitochondrion and in response to dead stimuli a conformational change occurs to BAK.

BOD proteins have amphipathic BH3 α-helical domain that it’s required for dead activation. BOD couldn’t initiates apoptosis without BH3 sequence because this domain is necessary to binds with BAX (Figure 3). BIM and BID BOD proteins bind to BAX and BAK BHD as well as prosurvival BCL-XL. On the contrary, BAD and NOXA BOD preferred to bind antiapoptotic proteins. BOD family are regulated by phosphorylation and its retention in the cytosol when receive survival signals. Different studies (Figure 4) demonstrate that BIM, BID and BAD couldn’t induce apoptosis in the absence of BAX or BAK. Determine the activation site of BAX when interact with BIM at the α1 and α6 using NMR technique. Other researchers utilized multiple binding assays, including yeast two-hybrid, co-immunoprecipitation from detergent-solubilized cell lysates, and in vitro pull-down experiments, indicate that individual BH3-only molecules display some selectivity for multidomain BCL-2 members. Some studies reveal that BOD like BAD is the most prominently demonstrates the capacity to overcome BCL-XL protection of mitochondria. BAD itself can’t activate BAX or release cytochrome c, probably because BAD sensitize mitochondria to BID or BIM by successfully binding to BAX. Additionally, Bcl-2 inhibitors may directly induce apoptosis in angiogenically active endothelial cells in vitro, in similar fashion to their effect on tumor cells, and endothelial cell-specific apoptosis can disrupt neovascularization in vivo. In vivo studies show that both BIM and BID are the most potent proapoptotic proteins inducing BAX/BAK oligomerization, membrane permeabilization, cytochrome C releasing and cascade caspases’ activation killing human leukemia cells. The cytochrome C release occurs by distinct mechanisms that are either Ca^{2+}-dependent or Ca^{2+}-independent. Previous studies validated that Ca^{2+}-independent cytochrome C release seems to be governed by different members of proapoptotic BHD, especially oligomeric form of BAX permealizing the outer mitochondrial membrane. Another important factor is P1i that induces apoptosis by up-regulating expression of the Noxa and Puma BOD, doing so in response to substantial levels of DNA breaks and other chromosomal abnormalities. In addition, decrease in interleukin-3 or insulin-like growth factor in epithelial cells can induce apoptosis through activation of BIM. All of this concepts show that cancer cells have
a plan to disrupt and stop the activation of apoptosis to preserve the growth, proliferation invasion, and the vascularization of the tumor. We are trying to explain the correlation between cancer and proteins involved in apoptosis pathway; and how we can take advantage of this process to kill cancer cells without affect healthy cells.

Cancer

When damaged cells acquire the capability to proliferate with uncontrolled growth, a mass of cancer cells develop. Growth factor ligands are produced, to which cells can respond via the expression of cognate receptors. Alternatively, cancer cells send signals to stimulate normal cells to support tumor formation reciprocating with growth factors. Somatic mutations were revealed after performed DNA sequencing analyses of cancer cell. Additionally, cancer cells can break away from this original mass, travel through the blood and lymph systems, and lodge in other organs where they can again repeat the uncontrolled growth cycle. This process of cancer cells leaving an area and growing in another body area is termed metastatic spread.

Over the last two decades, the researchers have been trying to understand how cancer cells avoid apoptotic machinery. The concept that programmed cell death, especially intrinsic pathway, by apoptosis serves as a natural barrier to cancer development has been recently established after compelling functional studies. The strategies of cancer cell to inhibit apoptosis are the deactivation of tumor suppressor protein of P<sub>53</sub>, increase the production of anti-apoptotic proteins as BCL-2, BCL-XL and diminish or inactivate the pro-apoptotic proteins as BIM, BID, BAD, BAX and BAK. The overexpression of anti-apoptotic proteins such as BCL-2, BCL-XL, and MCL-1, which bind to the BCL-2 a-helical domain of pro-apoptotic proteins such as BAX, BAK, BAD, and BIM, and inhibit their function.20

New therapies in cancer

Recent studies are trying to develop new drugs using proteins that are very important to induce apoptosis. Pro-survival proteins (BCL-2, BCL-XL) contribute to oncogenesis and drug resistance inhibiting pro-apoptotic activity of BAX, BAK, BAD and BIM. BOD proteins such as BIM, BID, BIK, NOXA and PUMA can engage pro-apoptotic and anti-apoptotic proteins in the same way. Some researchers in the last decade have been working on discovering and construct possible molecules or peptides that could promote the activation of apoptosis. A lot of studies are focused on activation of BHD proteins (BAX, BAK).21 found that short peptides representing the α-helical BH3 domains of BID or BIM are capable of inducing oligomerization of BAK and BAX releasing cytochrome c. BIM peptide is the BH3 domain of this protein that binds with BAX protein and it has been demonstrated.3,13,22 that the sequence between residues 146-166 are sufficient to interact with BAX and activate apoptosis. Recent studies.23 revealed that BH3 domain peptides (BIM or BID) with enhanced α-helical structures bound with greater affinity to BAX, activating cell death. The use of peptides as therapeutics is, however, limited by their low bioavailability, their inefficiency in crossing cell membranes (due primarily to their size), and their poor metabolic stability in vivo.24 To improve the poor physicochemical properties of peptides,6 developed a stapling method to stabilized alpha-helix of BOD domains peptides (BIM SAHBSs) that directly initiates BAX-mediated mitochondrial apoptosis. Aileron Therapeutics is a biopharmaceutical company that was founded in 2005 by Dr. Walensky, leading in the development of a completely new therapeutic modality that has an optimized version of the published Stapled BIM peptide into late-stage preclinical studies (Drugs.com). Other researchers are using this pathway to design inhibitors for pro-survival proteins as novel anti-cancer drugs. Most recent discoveries are using non-peptidic molecules (Figure 5) that mimic the BH3 binding groove of pro-survival proteins disrupting BCL-2 or BCL-XL and MCL-1 binding to BAX, BAK, BAD, or BIM, freeing up pro-apoptotic proteins, which leads to the release of cytochrome c, activation of caspases and induction of apoptosis in a BAX- and BIM-dependent manner in human cancer cells.25 Other experiments reveal that the benefits of green and black tea polyphenols, in particular catechins and the aflavins have remarkably broad therapeutic properties and are commonly listed as small-molecule Bcl-2 inhibitors and inhibits epidermal growth factor receptor, and metastasis-associated laminin receptor inducing apoptosis in tumor cells in vitro and in vivo.26 These results show different ways to activate intrinsic apoptosis directly or indirectly in cancer cells giving us multiple alternatives to design new treatments without side effects and low toxicity.

Concluding remarks

All the experimental apoptotic activators discussed above have biochemical mechanistic differences because they are trying to activate apoptosis interacting with BHD such as BAX or inhibiting BCL-2 but the similarity is that all utilize mitochondrial permeabilization pathway. Mitochondrion has a key role in a lot of processes that occurs inside the cell that allows sustainability, growth, or dead; and in tumor growth could be the same history. Cancer development...
could be stop and the cell could be reprogrammed to kill tumor. The experiments performed to prove the effectiveness of these small drugs or peptides have had great advantages above the actual approved clinical treatments such as chemotherapy and radiotherapy. Some of these new discoveries are on preclinical stages giving a hope to find better targeted treatments against cancer. But a lot of different cancers are affecting humans, including each cancer could developing in a different way depends the organ or tissue and these types of possible treatments cannot attack all of them. Finally, these recent studies show that we are on the right way to understand the mechanism of cancer’s development but obviously, a lot of studies have to be done first because internal and external environment of cell is a very complex place.

Figure 5  Chemical structures of various small-molecule inhibitors of survival proteins Bcl-2 and BCL-XL allowing initiation of apoptosis.17

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Conflicts of interest

Author declares that there is no conflicts of interest.

References

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