REGULATORS OF APOPTOSIS

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Abstract

Apoptosis involves a complex network of biochemical pathways that normally ensure a homeostatic balance between cellular proliferation and turnover in nearly all tissues. The key component in apoptosis is the caspase-mediated proteolytic cascade. Apoptosis initiation is tightly regulated by a variety of regulators or factors. Among them, Bcl-2 family proteins, tumor necrosis factor (TNF) and p53 play pivotal roles in the regulation of caspase activation and in the regulation of apoptosis. This brief review summarizes the current knowledge of critical regulators in apoptosis.

Introduction

Apoptosis is a highly regulated process of selective removal of cells involved in development, normal cell turnover, hormone induced tissue atrophy, cell-mediated immunity, tumor regression and a growing number of pathological disorders. Apoptosis is characterized by typical morphological and biochemical hallmarks including cell shrinkage, nuclear DNA fragmentation, and membrane blebbing. Proteolytic enzymes such as caspases are important effector molecules in apoptosis. To avoid inappropriate activation of apoptosis programs, their pathways are tightly controlled. A better understanding of these regulations is important in health and disease. In this review, we provide a summary of critical regulators in apoptosis.

Apoptosis signaling pathways

There are two distinct pathways operated and triggered by cell death stimuli from intra- or extra-cellular environments (Figure 1). The intracellular stimuli trigger the mitochondria-mediated signaling (intrinsic) pathway which is generated by signals arising within the cell mainly by leakage of cytochrome *c* from the mitochondria, while the extracellular (extrinsic) death stimuli induce the receptor-mediated pathway, which is triggered by the binding of death molecules to the cell surface receptors (death receptor-mediated events) (see review, Chowdhury et al., 2006).

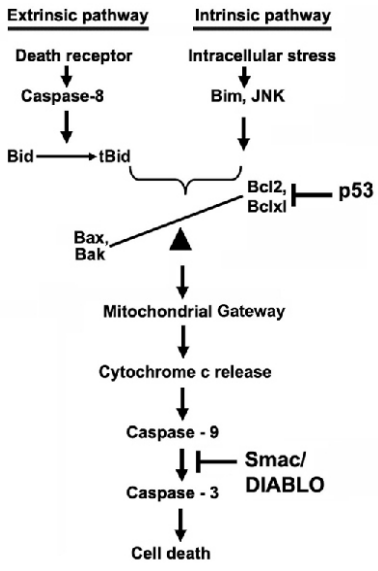

Figure 1: Apoptotic pathways

The mitochondria-mediated apoptotic pathway (the intrinsic pathway)

Cytochrome *c* which is a major component of the mitochondrial electron transport chain is a key

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variable for the activation of caspases; along with a protein known as apoptosis protease-activating factor-1 (Apaf-1) that initiates the intrinsic apoptotic pathway. The death signal (such as DNA damage) induces the pro-apoptotic BH3-only domain proteins (Bid, Bad, Noxa and p53-up-regulated modulator of apoptosis (PUMA) to transfer the signals to the mitochondria. The BH3-only domain proteins facilitate the assembly of other proapoptotic proteins such as Bax and Bak into the pores in the outer mitochondrial membrane, and change the mitochondrial permeability to release or leak out various apoptosis-inducing factors, including cytochrome *c*, through the mitochondrial permeability transition pore (PTP) or voltage-dependent anion channels (VDAC). The released cytochrome *c* and Apaf-1 bind to inactive caspase 9 in the presence of dATP or ATP and form a Apaf-1 complex (7 Apaf-1 + 7 cyt C + 7(d) ATP + 7-Procaspase 9) called the apoptosome or caspase 9-holoenzyme, or "wheel of death", which ultimately activates the effector caspase cascade (caspase 3 and 7), leading to cell death. The activation of the mitochondrial pathway is tightly regulated by the anti-apoptotic Bcl-2 family members via their inhibition of cytochrome *c* release. Mitochondrial permeability is determined by the ratio of proapoptotic and anti-apoptotic members of the Bcl-2 family (see reviews, Chowdhury et al., 2006, 2008).

The death receptor-mediated apoptotic pathway (the extrinsic pathway)

The induction of this pathway is initiated by the binding of the death receptors Fas (Apo-1 or CD95), tumor necrosis factor receptor-1 (TNFR-1/p55/ CD120a), interferon (IFN) and TRAIL (TNF-related apoptosis-inducing ligand or Apo2-L) receptors to their ligands in the plasma membrane of the cell. Based on receptor types, there are two major signaling sub-types: the Fas-mediated signaling path and the TRAIL receptor-mediated signaling path. Fas are glycosylated Type-I transmembrane receptors that either activate mitochondria-dependent or mitochondria-independent signaling paths in response to ligands. The mitochondria-independent path is activated by the death-inducing signaling complex (DISC) prior to the loss of mitochondrial transmembrane potential. The mitochondria-dependent path is triggered by activated caspase 8 through the cleavage of the c-terminal fragment of the BH3-only member of the Bcl-2 family from protein Bid to truncated Bid (tBid), which translocates to the outer membrane of the mitochondria, allowing the loss of mitochondrial transmembrane potential and inducing cytochrome *c* release. Thus, Bid mediates the cross-talk from the extrinsic to intrinsic form of cell death. A similar mitochondria- independent event appears to be triggered when TRAIL binds to a different family of death-inducing receptors (DR-3, 4, 5 or 6). Both Fas and TRAIL bindings initiate ligation of the receptors and transmission of the apoptotic signals through the intracellular death domains (DD), death effector domains (DED) and caspase recruitment domains (CARD). The CARD mediates the activation of adaptor proteins and procaspases (procaspase 8 or 10) as DISC, leading to the activation of a cascade of caspases including caspase 3, resulting in cell death (see review, Chowdhury et al., 2006, 2008).

Regulators of apoptosis

Apoptosis requires the concerted effort of many proteins involving regulation at different stages, giving a tight control. These proteins are categorized as the inhibitor of apoptosis proteins (IAP), the Bcl-2 family, the TNF family and p53, which ultimately controls the caspases (see review McConkey and Orrenius, 1996).

Caspases

Caspases are a family of highly conserved aspartate-specific cysteine proteases present in multicellular organisms and are members of the interleukin-1b-converting enzyme family. The caspase gene family consists of 15 mammalian members that are grouped into major sub-families, namely inflammatory caspases and apoptotic caspases. The apoptotic caspases are further subdivided into two sub-groups, initiator caspases and executioner caspases that mediate the regulation and execution of apoptotic cell suicide. All caspases exist within the cell as inactive latent pro-forms as precursor zymogen. These inactive caspases become active in response to specific signals through selective proteolytic processing (two cleavages) at specific aspartic acid residues to produce subunits that form the active heterotetrameric proteases and initiate apoptosis. The signaling of caspases starts with the induction of the apoptotic signal via death receptors resulting in the activation of an initiator caspase such as caspase 8 or caspase 10, whereas the mitochondrial signaling pathway initially involves procaspase 9. These caspases can then activate other caspases in a cascade. This cascade eventually leads to the activation of the effector caspases, principally caspase 3, 6 or 7, which in turn cleave a variety of substrates including the nuclease inhibitor, cytoskeleton and the key cellular proteins, leading to internucleosomal DNA degradation and the cellular dismantling (Chowdhury et al., 2008).

Inhibitor of apoptosis proteins (IAP)

In normal living cells, caspase activation and activity is carefully regulated on several levels by an endogenous family of cellular proteins called the inhibitor of apoptosis proteins (IAP). The IAP includes 8 mammalian family members with highly conserved and differential expression patterns in various tissues. In humans, six IAP relatives have been identified: NAIP, c-IAP1 (HIAP-2), c-IAP2 (HIAP-1), XIAP (hILP), survivin, and BRUCE. The IAP do not bind or inhibit caspase 8, but they do bind to and inhibit its substrate caspase 3, thus arresting the cascade of proteolysis and providing protection from Fas/caspase 8-induced apoptosis. In the mitochondrial pathway, caspase inactivation is done by XIAP, c-IAP1, and C-IAP2, which bind directly to the principal caspase, procaspase 9, thereby preventing its processing and activation induced by cytochrome *c*, both in intact cells and in cell extracts (Chowdhury et al., 2006).

Bcl-2 family proteins

The B-cell/Lymphoma-2 family (Bcl-2) of proteins is a group of evolutionarily conserved regulators of cell death, comprising both anti- and pro-apoptotic members, which operate at the mitochondrial membrane to control caspase activation. The Bcl-2 gene was first discovered in human B-cell lymphomas. At present, more than two dozen Bcl-2 family members have been discovered. The inhibitors or antiapoptotic Bcl-2 family members include Bcl-2, Mcl-1 and Bcl-XL members, while the promoters or apoptotic members include Bax, Bak, Bcl-2-associated X protein members. An important feature of the members of the Bcl-2 family is their ability to form homo- and hetero dimer formation to neurtralize their respective effects; they function either independently or together in the regulation of apoptosis. In the absence of death signals, Bcl-2 proteins are localized to distinct intracellular compartments. Upon receiving death stimuli, the pro-apoptotic members can change their location within cells and undergo various pre- and post-translational modifications. In response to death signals, the cytosolic pro-apoptotic proteins

change conformations and integrate into the outer membrane of the mitochondria, and the anti-apoptotic family members are neutralized. The anti-apoptotic members are initially integral membrane proteins localized to the mitochondria, endoplasmic reticulum and nuclear membranes. In the mitochondria, anti-apoptotic proteins form ion-channels and help to maintain mitochondrial integrity by allowing the export of H+ ions from the inner mitochondrial space. The associations between various Bcl-2 family regulators are not static, but phosphorylation-dependent changes cause them to interact amongst themselves (Hetz and Glimcher, 2007; Chipuk and Green, 2008).

p53

p53 is a stress-response 53-kDa nuclear protein which exists as a tetramer, accumulates in the cytoplasm during the G1 (GAP1) phase and migrates to the nucleus at the start of the S (synthesis) phase. p53 is well described as a transcription factor that can induce the expression of multiple different proapoptotic gene products, including inhibitors of cell cycle advancement, regulators that control p53 activity in negative feedback loops, mediators of oxidative stress and endoplasmic reticulum (ER) stress, components of the death receptor signaling pathway, and caspase activators and pro-apoptotic proteins of the Bcl-2 family by catalyzing mitochondrial outer membrane permeabilization (MOMP). However, p53 has another, transcription-independent pro-apoptotic effect, which involves direct interactions between p53 and MOMP inducers at the mitochondrial level. DNA damage results in dramatic changes in p53. The activation of p53 as a transcription factor arrests the cell cycle, acting as the emergency brake of a cell. Once p53 is accumulated, it binds to DNA and mediates two major effects: a) allowing time for cells to repair damaged DNA, or b) activation of apoptosis inducing genes, especially Bax, by up-regulating its transcription and down regulating Bcl-2, thus favoring mitochondria-dependent apoptosis. In addition, p53 up-regulates the transcription of Fas to support Fas-mediated apoptosis (see review Zamzami and Kroemer, 2005; Harris and Levine, 2005).

Tumor necrosis factor (TNF)

TNF is a soluble pleiotropic cytokine that mediates apoptosis, cell proliferation, immunomodulation, inflammation, allergy and autoimmune disease, among others. The members of the TNF ligand family exert their biological functions via interaction with their cognate membrane receptors. There are two distinct TNF-receptors: type I (TNF-R1; CD120a; p55/60) expressed in all cell types; and type II (TNF-R2; CD120b; p75/80) expressed only on the cells of the immune system and endothelial cells. They bind membrane-integrated TNF (mem-TNF) and soluble TNF (sTNF), but also the secreted homotrimeric molecule lymphotoxin-alpha (LT-alpha). The signal transduction of cell death from TNF-R1 is via its cytoplasmic death domain (DD) by the activation of caspase 8 alone to activate caspase 3 or by the activation of the mitochondria-dependent amplification loop. TNF-R2 directly recruits TNF receptor-associated factors (TRAF), induces gene expression and intensively cross-talks with TNF-R1 (see review Chowdhury and Bhat, 2009).

FLIP (FADD-like-ICE-inhibitory protein/FLICE inhibitory protein)

The signaling from death receptors through their adaptors to procaspase 8 is a well-regulated path, controlled by a polypeptide named FLIP (FADD-like-ICE-inhibitory protein/FLICE

inhibitory protein) that contains a pro-domain similar to that of procaspase 8, but lacks a caspase active site. FLIP (cFLIP) proteins are well-known inhibitors of death receptor-induced apoptosis (Kataoka et al., 1998). There are three known c-FLIP isoforms: c-FLIPL, c-FLIPs and c-FLIPR. FLIP binds to FADD and competitively inhibits recruitment of procaspase 8 and procaspase 10, thereby interrupting signaling initiated by various death receptors.

Other regulators of apoptosis

Other regulators of the apoptosis include cytosolic Ca^{2+}, protein kinase C (PKC), protein tyrosine kinase, cyclin dependent kinase, cAMP, ceramide, intracellular acidification, oxygen radicals etc. The cytosolic Ca^{2+} concentration and/or intracellular Ca^{2+} compartmentalization are involved in the regulation of apoptosis. The process of Ca^{2+} compartmentalization is altered by antiapoptotic Bcl-2 members. The PKC activation inhibits apoptosis by inhibiting endonucleases, whereas cAMP activation promotes apoptosis. Protein tyrosine kinases (PTKs) play important roles in suppressing apoptosis. Ceramide and oxygen free radicals are well known apoptotic agents produced naturally in the cells. Ceramide is a sphingomyelin hydrolytic product that activates both protein kinases and phosphatases.

Therapeutic application of apoptotic regulators

Modulating of apoptosis is a novel therapeutic strategy in treatment of different diseases. These include situations with unwanted cell accumulation (cancer) and failure to eradicate aberrant cells (autoimmune diseases) or disorders with an inappropriate loss of cells (heart failure, stroke, AIDS, neurodegenerative diseases, and liver injury). Many approaches including gene therapy, antisense strategies and numerous apoptotic drugs to target specific apoptotic regulators are currently being developed (see review Fischer and Schulze-Osthoff, 2005; Mousavi et al., 2008).

Conclusions

Apoptosis plays a central role in physiological growth control and regulation of tissue homeostasis. Tipping the balance between cell death and proliferation in favor of cell survival may result in tumor formation or other cellular growth. Emerging evidence indicates that apoptosis is regulated by multiple signal transduction pathways. A better understanding of these diverse modes of regulators in apoptosis may provide a molecular basis for new strategies targeting death pathways in resistant forms of cells.

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