MINI-REVIEW

Apoptosis in Cancer - An Update

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Abstract

Apoptosis is programmed cell death which is essential for development and survival of living organisms. It is a sequentially regulated suicidal programme where cells activate certain enzymes which dissolute their own nuclear component and various protein component of nucleus and cytoplasm. Disturbance of this regulatory pathway may lead to various diseases like autoimmune diseases, neurodegenerative diseases and cancers. The potential mechanisms of apoptosis and its role in cancer are discussed. The ability of apoptosis to modulate the life or death of a cell is also recognized for its immense therapeutic potential. Understanding the mechanisms from this review will give us better insight to the pathogenesis of various diseases including cancer and will open new horizons to therapeutic approaches.

Keywords: Apoptosis - caspases - cancer - detection

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Introduction

Homeostatic balance between proliferation of cells and its death is essential for development and maintenance of biological system of a living being. Apoptosis is programmed cell death. It is a widespread phenomenon that plays a vital role in a myriad of physiological and pathological processes. The word apoptosis came from Greek origin; meaning "falling off or dropping off". (Gewies, 2003) It resembles leaves falling off trees or petals dropping off flowers. This analogy emphasizes that the death of living matter is an integral and necessary part of the life cycle of organisms. The mechanism involved in the process of apoptosis in mammalian cells was first studied in a nematode *Caenorhabditis elegans* during its development phase (Horvitz, 1999). In this organism 1090 somatic cells are generated in the formation of the adult worm, of which 131 of these cells undergo apoptosis or "programmed cell death". These 131 cells die at particular points during the development process, demonstrating the remarkable accuracy and control in this system. Apoptosis has since then been recognized and accepted as a distinctive and important mode of "programmed" cell death (Horvitz, 1999; Elmore, 2007).

Apoptosis is the most frequent form of programmed cell death which carries high biological significance. (Cheung et al., 2012). In physiology, it plays a crucial role particularly during embryogenesis and metamorphosis. During growth and development many cells are formed in excess which undergo termination to contribute to sculpturing of many organs and tissue. In limb formation programmed demise of interdigital mesenchymal tissue separate the digits. Death of cells are essential to sculpture hollow structure and formation of reproductive organ as deletion of Mullerian duct is essential for male and Wolffian duct deletion is mandatory for female (Gewies, 2003). In brain development half of the neurons that are initially created die in later stages when the adult brain is formed by the process of apoptosis. Apoptosis is also essential for homeostasis as well as dissolution of auto reactive, damaged or dangerous cells.

Historical Perspective

In the year of 1842, Carl Vogt first hypothesized natural cell death. Later in 1858, Rudolph Virchow describe a natural form of cell death which was distinct from necrosis, he termed it as necrobiosis. Walther Flemming in 1885 named this natural cell death as chromatolysis and clearly describes its morphological alteration (Curtin and Cotter, 2003). Next about 30 year's chromatolysis was reviewed in various literatures. Again in 1960s scientists regained an interest in research of cell death and the term programmed cell death was introduced in 1964 Gewies., 2003). It was proposed that the cell death that occur during development are not an accident; it is a sequence of controlled steps. The term apoptosis was first coined in a publication by Kerr et al in the year 1972 (Gewies, 2003). Later in 1980s it was proved that apoptosis plays a major role in development and disease. At the same time Bcl2 was also identified as an antiapoptotic molecule which started drawing intense interest in the research of the signalling and mechanism of apoptosis. In the year 2002 these research was recognised by the world when three

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pioneers of apoptosis research were awarded Nobel Prize in medicine and physiology (Curtin and Cotter, 2003).

Morphological Changes in Cell Death

Cell death occurs in two ways, apoptosis or necrosis. Apoptosis is physiological whereas necrosis is pathological. In necrosis changes occur in mitochondria first whereas changes in nucleus are very less. These followed by organelles dissolution and loss of selective permeability of cell membrane. As a result oedema formation happens and intracellular contents are released. As lysosome disrupts, hydrolases are released which causes cell degradation. This result in damage to surrounding cells and a strong inflammatory response is evidenced in the corresponding tissue (Searle et al., 1982; Ramírez et al., 1999).

When cell death occurs through apoptosis it can be divided into two stages. In first stage biochemical mediators take an attempt to repair a damaged cell. If they fail then the cell goes into second stage or execution phase. In this phase, structural changes happens which takes the cell to death. Asper described in the publication of Kerr these changes occur in three level; nuclear changes, changes in cell membrane and changes in intra cytoplasmic organelles (Kerr et al., 1972; Chamond et al., 1999).

In the nucleus, changes in both chromatin as well as nuclear membrane happen. Chromatin become dense clumps which shifts towards the nuclear membrane. Though the nuclear membrane remain intact, redistribution of nuclear pores occur. Changes in nuclear protein are also seen (Chamond et al., 1999). In mitochondria there is degradation of DNA. Endoplasmic reticulum loss its structure and there is loss of the mitochondrial transmembrane potential (Chamond et al., 1999). The cytoplasmic membrane of apoptotic cell become deformed and it develop blebbing. In endoplasmic reticulum the cisterns become wide and they fuse. The phospholipids of cell membrane change their orientation and get exposed to external environment. The fragment of cell membrane form apoptotic bodies which is actually cytoplasmic remains surrounded by cell membrane. When the apoptotic bodies are released in external environment they are engulfed by phagocytes. As a result there is no inflammatory reaction. At molecular level there is the activation of proteolytic enzymes which propogate the cleavage of DNA into oligonucleosomal fragments and cleavage of a multitude of specific protein substrates (Ramírez et al., 1999).

Mechanisms of Apoptosis

Apoptosis is a tightly regulated and efficient cell death program which involves multiple factors. Every cell contains an intrinsic mechanism which signals death or survival. Any imbalance in these signals can result in apoptosis.

Caspases take major and a central role in apoptotic mechanism. The term caspases is derived from cysteine-dependent aspartate-specific proteases. There are three pathways to activate caspases. These are intrinsic or mitochondrial pathway, extrinsic pathway (Hassen et al., 2012) or the pathway dependent on the death receptors and recently known less understood intrinsic endoplasmic reticulum pathway. Intrinsic and extrinsic pathway ultimately leads to common pathway or the execution pathway of apoptosis (Rebecca, 2011).

Intrinsic pathway

Factors like hypoxia, genetic damage, high concentration of cytosolic calcium ions, extreme oxidative stress trigger the initiation of the mitochondrial pathway resulting in increased mitochondrial permeability. Apoptotic stimuli trigger the release of apoptogenic factors from the mitochondrial intermembrane space to the cytosol, such as cytochrome C. This pathway is initiated within the cell and regulated by group of proteins which belongs to Bcl-2 family. There are two group of Bcl-2 family proteins (pro apoptotic and anti apoptotic protein) which regulates the pathway. Pro apoptotic proteins are Bax, Bak, Bad, Bcl-Xs, Bid, Bik, Bim and Hrk and anti apoptotic proteins are Bcl-2, Bcl-XL, Bcl-W, Bfl-1 and Mcl-1.Pro apoptotic protein promote release of cytochrome-C from mitochondria whereas antiapoptotic proteins causes its blockage. The initiation of apoptosis depends upon balance between pro and antiapoptotic proteins (Rebecca, 2011; Rahman et al., 2012).

As soon as cytochrome protein is released in cytoplasm it forms apoptosome (made up of cytochrome-C, apoptotic protease activating factor-1 and caspase-9) and activates caspase -3. Other apoptotic factors are apoptosis inducing factor (AIF), second mitochondria-derived activator of caspase (Smac),direct IAP Binding protein with Low pI (DIABLO) and Omi/high temperature requirement protein A (HtrA2) (Rebecca, 2011; Rahman et al., 2012). These are released from the mitochondrial inter membrane space into the cytoplasm. Smac/DIABLO or Omi/HtrA2 bind to

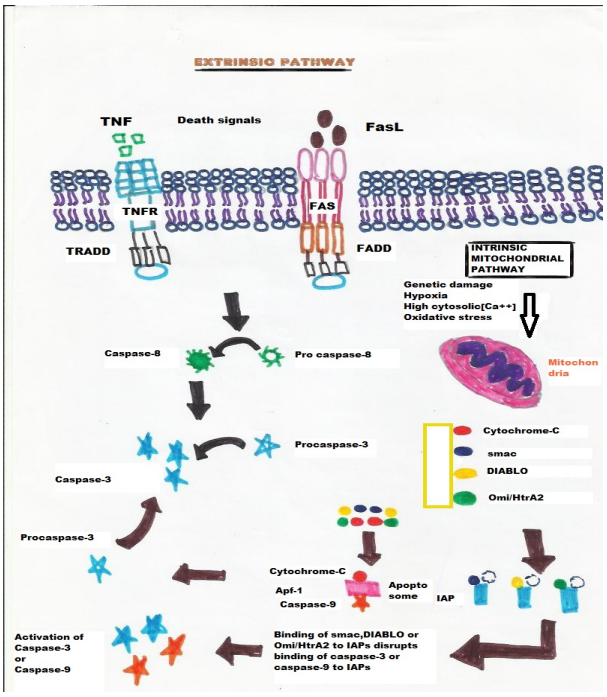

Figure 1. Diagramatic Representation of Extrinsic and Intrinsic Pathway

inhibitor of apoptosis proteins (IAPs) and causes caspases activation which subsequently lead to disruption in the interaction of IAPs with caspase-3 or -9.

Extrinsic pathway

This pathway is mediated by the activation of so called "death receptors" which are cell surface receptors that transmit apoptotic signals after ligation with specific ligands. The best known death receptors are TNFR1 (Tumor Necrosis Factor Receptor 1) related protein called Fas and their ligands, TNF and Fas ligand (FasL) respectively. These death receptors have an intracellular death domain which recruits adaptor proteins. These are TNF receptor associated death domain (TRADD), Fas-Associated Death Domain (FADD) and cysteine proteases like caspase 8. When the death ligand binds to its death receptor it forms binding site for an adaptor protein. The complete ligand-receptor-adaptor protein complex is known as the Death-Inducing Signalling Complex (DISC). The DISC initiates the assembly and activates pro caspase -8. Active caspase-8 then processes downstream effector caspases which subsequently cleave specific substrates resulting in cell death (Rebecca, 2011).

Common pathway

This involves the activation of a series of caspases. The upstream caspases for the intrinsic pathway is caspase-9 and for extrinsic pathway it is caspases -8. The intrinsic and extrinsic pathway converge to caspase-3.Then the caspase-3 cleaves the inhibitor of the caspase-activated Deoxy Ribo Nuclease, that is responsible for nuclear apoptosis. Along with this downstream caspases induce cleavage of protein kinases, cytoskeletal proteins, DNA repair proteins and inhibitory subunits of endonucleases family. All these together produce morphological changes in apoptosis (Rebecca, 2011).

Endoplasmic Reticulum Stress Induced Pathway for Apoptosis

Endoplasmic reticulum (ER) is known for synthesis and folding of different types of protein. Healthy and functioning ER is very essential for survival of cells and maintains its activity. If function of ER is impaired, aggregations of unfolded proteins take place. Whenever ER is subjected to stress its protein folding capacity interrupts. To restore its normal functions transmembrane receptors detect the onset of stress and try to bring the normal ER function back by initiating various protecting mechanisms, collectively called as Unfold Protein Response (UPR) (Szegezdi et al., 2006). If the adaptive response fails or stress is prolonged, apoptosis take place.

The complex cellular process which leads to apoptosis is mediated through three ER transmembrane receptors, namely pancreatic ER kinase (PKR) like ER kinase (PERK), activating transcription factor-6 (ATF-6) and Inositol Requiring Enzyme-1(IRE-1) (Szegezdi et al., 2006). When the cells are in rest, all three ER stress receptors are maintained in an inactive state through their association with the Glucose Regulated Protein 78 (GRP78) and ER chaperone. Due to prolong stress when unfolded protein accumulates, dissociation of GRP78 occurs which activate the UPR, a prosurvival response to restore normal function of ER by reducing the accumulation of unfolded proteins (Schroder and Kaufman, 2005; Szegezdi et al., 2006). After UPR activation, if stress does not restore or protein aggregation persist, prosurvival signalling switches to pro apoptotic signalling. The UPR-mediated signals might elicit apoptosis by three distinct phases namely initiation, commitment and execution. When cells are in rest, the pro-apoptotic Bax and Bak (Bax/Bak) remain inactive by interaction with BCL2 both on the mitochondrial as well as endoplasmic reticulum (ER) membranes, whereas Bim (BH3) is inhibited by binding to cytoskeletal dynein (Szegezdi et al., 2006).

In the initiation phase, saevere ER stress leads to activation of c-Jun N-terminal kinase (JNK) and induction of C/EBP homologous protein (CHOP) (Zinszner et al., 1998; Szegezdi et al., 2006). Both JNK and CHOP eliminate the anti-apoptotic effect of BCL2; along with CHOP blocks expression of BCL2, whereas JNK phosphorylates it. JNK also phosphorylates Bim. As JNK phosphorylates Bim, it get release from the cytoskeleton and become activated. It happens in commitment phase.

Together, all these changes allow activation of Bax and Bak, transmission of the signal from the ER to the mitochondria as well as death. It has been proposed that Caspase 12 is a key mediator of ER stress-induced apoptosis. (Szegezdi et al., 2003; 2006). Due to these changes this phase is called execution phase.

Suppressors of Apoptosis

Suppressors of apoptosis are the Inhibitors of Apoptosis (IAP) family proteins. IAP family protein first discover in baculoviruses and are characterised by a novel domain of ~70 amino acids called baculoviral IAP repeat (BIR) (Quinn et al., 1999; Imawati Budihardjo et al., 1999). Till date two IAPs have been discovered in baculoviruses, (cp-IAP and op-IAP), two in drosophilia (DIAP-1 and DIAP-2) and 6 in humans (NAIP, c-IAP1/HIAP-2, c-IAP2/HIAP-1, XIAP/hILP, Survivin, and BRUCE) (Budihardjo et al., 1999; Quinn et al., 1999).

The exact target of inhibition by IAPs is currently unknown two model of action became very popular. The first model propose that IAPs inhibit apoptosis by interfering directly with the catalytic activity of certain caspases (Deveraux et al., 1997; Roy et al., 1997; Budihardjo et al., 1999) and as per second model IAPs may inhibit the procaspases or other proteins which are necessary to activate procaspases (Seshagiri and Miller, 1997; Budihardjo et al., 1999). From studies on mammalian IAPs it is clear that IAPs function in both major pathways of apoptosis, the cell structure death receptors as well as the cytochrome C-dependant (Apafs) pathway.

In the cell surface death receptors pathway IAPs block Caspase-3 and Caspase-7;as a result arrest of caspases-8 initiated apoptosis take place. In cytochrome C dependant pathway which is also called Apafs pathway, IAPs work on three distinct steps; by direct interaction with procaspase-9

as a result by interfering with its processing, through competing for Apaf-1 binding and by direct inhibition of active caspases (Budihardjo et al., 1999).

Aac-11 as an Antiapoptotic Factor

Among the antiapoptotic protein AAC-11 (Antiapoptotic clone-11) prevents apoptosis after growth factor deprivation. AAc-11 is a nuclear protein which is also called as Api (Apoptosis inhibitor-5) or FIF (Fibroblast growth factor-2 interacting factor) (Tewari et al., 1997; Van den Berghe et al., 2000; Morris et al., 2006; Rigou et al., 2009). Though recent studies shows that an antiapoptotic action of AAC-11 is mediated by the suppression of the transcription factor E2F1(E2 promoting binding factor1) induced apoptosis yet exact mechanism of action is till unknown (Morris et al., 2006; Rigou et al., 2009).

It is reported that AAC-11 expression gives a poor outcome in non small cell lung carcinoma patient, whereas its depletion seems to be tumour cell lethal under low serum stress condition (Sasaki et al., 2001; Krejci et al., 2007; Rigou et al., 2009). It was also studied that AAC-11 over expression promotes cervical cancer growth as well as invasiveness. On the other hand study tells AAC-11 gene has been highly expressed in multiple cancer cell lines and some metastatic lymphnode tissue. All these together suggest AAC-11 as a putative metastatic oncogene and suggest it as a therapeutic target in neoplasia (Searle et al., 1982).

Cancer and Apoptosis

Cancer is one of the major threats to public health at present world. The major types which causes death worldwide are lung cancer, stomach cancer,colorectal cancer, livercancer and breast cancer (World Health Organization- NMH Fact sheet, 2010) cancer of head and neck region,specially in oral cavity is mainly squamous cell carcinoma. In numerical term squamous cell carcinoma may be viewed as a small problems but it causes lot of death worldwide (Masthan et al., 2012). In this disease, a normal cell transformed into a malignant one due to succession of genetic changes. On the other hand apoptosis is known to eliminate potentially malignant cells, hence reduction of apoptosis can be considered to play a key role in carcinogenesis. Commonly there are three mechanisms by which apoptosis acquire resistance or reduction. These are disruption in balance of proapoptotic and antiapoptotic proteins, reduction in function in caspases and impaired death receptors signalling.

Bcl-2 family proteins play a vital role in regulation of apoptosis via intrinsic pathway predominantly. They are comprised of both pro-apoptotic and anti-apoptotic proteins. Based on their function and bcl-2 homology, domain bcl-2 family members can be divided into three groups. First group is made up of antiapoptotic proteins. On the other hand second and third group comprise of pro apoptotic proteins. Disruption in the balance of proapoptotic and antiapoptotic protein causes disregulated apoptosis. p53 directly interact with members of the Bcl-2 family and influences apoptosis (Hanahan and Weinberg, 2000;, Dewson and Kluc, 2010; Rebecca, 2011; Tiwari, 2012).

Apoptotic Markers

The main aim of therapeutic strategies for malignant tumours is to restore the balance between degeneration and proliferation of cells. Whenever physiological apoptosis occurs most of the products of cell death are effectively removed by macrophages and neighbouring cells whereas in pathological conditions this system is impaired or overloaded and appreciable quantity of cell death products can accumulate in the circulation.

The most frequent markers investigated in cancer patients have been the soluble form of apoptosis receptor, sFas and its ligand Fasl, various cytokeratin and circulating DNA fragments. As markers circulating in the blood are very easily accessible, they are very useful for serial measurement but quantification in blood might lack organ and cell death specificity. These products are very useful in diagnosis, prognosis and monitoring of disease.

Study shows that compared to healthy individual anti apoptotic soluble form of the fas-receptor (sFas) and the proapoptotic fas-ligand (Fas-L) is elevated in patients with various kind of cancers. In some of these tumors sFas and sFasL is also correlate with stage of tumour. Other markers include CYFRA21-1(cytokeratin 19-fragments), Tissue polypeptide antigen (TPA,Cytokeratin 8-,18- and 19- fragments). These all are elevated in variety of benign and malignant disorders (Holdenrieder and Stieber, 2004).

After the diagnosis of cancer has been established, the measurement of the concentration of apoptotic markers is often carried out prior to therapy. Along with clinical markers they are used to estimate overall survival and progression free survival. Organ specific apoptotic markers have considerable potential for estimating prognosis of cancer. In univariate analysis, high sFas levels have been shown to be related to poor overall survival in many tumours. Measurement of parameter is useful for monitoring treatment efficacy also. Rapid decrease in the marker levels indicates therapeutic efficacy, on the other hand increase or a slow decrease is associated with insufficient response to treatment. CYFRA21-1 is very useful for monitoring lung, cervical and head and neck cancer; whereas TPA and TPS are helpful for breast, ovarian, colorectal cancer. In addition, circulating DNA fragments have shown potential for the monitoring of therapy in various solid tumours (Holdenrieder and Stieber, 2004).

An increase of several apoptotic markers in cancer patients has also been described prior to or at the time of tumour recurrence. These include CYFRA21-1, TPS, TPA and circulating DNA fragments in various cancers. As tumour markers are neither organ nor tumour specific measurement of their concentration over time should interpreted very carefully (Holdenrieder and Stieber, 2004).

Detection and study of apoptosis

There are various techniques to detect and study of

apoptosis. Light and electron microscopy are very useful for this process. Due to lack of cellular synchronization in apoptosis and as apoptotic cells are rapidly engulfed through phagocytosis the study method based on morphologic criteria are enough for its demonstration but not particularly useful for its quantification (Chamond et al., 1999).

Alteration in plasma membrane

At the time of apoptosis the lipid phosphatidylserine translocates from the inner to the outer leaflet of the plasma membrane. Annexin V is a calcium-dependent protein that preferentially binds phosphatidylserine with high affinity. If it is conjugated to a fluorescent tag, Annexin V can be used to detect this early cell surface change of apoptosis (Apoptosis detection guide, Nzo life sciences., 2010).

Change in mitochondria

Dysfunction of mitochondria occurs during apoptosis and it is accompanied by a decreased membrane potential. Membrane potential assays test the mitochondria's ability to concentrate a cationic dye using its proton gradient. The detection of released cytochrome c is specific for all stages of apoptosis (Apoptosis detection guide, Nzo life sciences, 2010).

Cytoplasmic changes

Activation of caspases is characteristic change in the cytoplasm during apoptosis. Caspase assays may detect early to late stages of apoptosis (Apoptosis detection guide, Enzo life sciences, 2010).

DNA changes

Chromatin condensation and DNA fragmentation is one of the apoptotic hallmark. At the late stage of apoptosis, caspase activated endonucleases break the double-stranded DNA. These apoptotic nucleosomal fragments can be resolved by gel electrophoresis as typical DNA ladders. The TUNEL (terminal deoxy-nucleotidyl transferase [TdT] dUTP nick end labelling) assay useses TdT to mark those breakpoints with tagged (e.g. biotinylated) nucleotides, which are then detected by using enzyme-tagged (for IHC) or fluorescent labelled (for FACS) antibodies (Apoptosis detection guide, Enzo life sciences, 2010).

Role of apoptosis in various diseases

Diseases as a consequence of apoptosis can be divided into two groups, disease caused as a result of inhibition of apoptosis resulting in increased cell survival or due to hyperactive apoptosis which leads to increase in cell death. The diseases which occur due to inhibition of apoptosis are various neoplasms, autoimmune diseases mainly *Myastenia gravis* and Systemic lupus erythematosus, inflammatory diseases like Bronchial asthma, Inflammatory intestinal disease and viral infections caused by Adenovirus, Baculovirus (Chamond et al., 1999).

In neoplastic disorders there are defects in gene such as p53, ras or c-myc either by mutation, inactivation or dysregulation resulting in cell accumulation, resistance to therapy and defective tumor surveillance by the immune system. The tumor suppressor gene plays a central role in the defense against malignant transformation. This gene is found to be inactivated in 50% of all human cancers There is also modification of expression of bcl-2gene in tumor cells contributing to cancer cell survival through direct inhibition of apoptosis (Chamond et al., 1999; Rebecca, 2011).

In autoimmune disease there is a mutation of genes which resist auto reactive lymphocytes for apoptosis whereas in a normal immune response there is apoptotic death of clones of auto reactive lymphocytes. Same thing also happen in viral disorders. Certain viruses are able to inhibit apoptosis of infected cells. The diseases which occur due to excess apoptosis are AIDS, various neurodegenerative disorders, haematological diseases and diseases due to tissue damage like myocardial infarction, cerebrovascular accident, ischaemic renal damage and polycystic kidney (Ramírez et al., 1999).

AIDS is due to infection by Human Immunodeficiency Virus (HIV). In AIDS there is loss of balance between number of CD4+ lymphocytes and the ability of bone marrow to generate new mature cells. This virus infects CD4+ T cells by binding to the CD4 receptor. The virus is subsequently internalized into the T cell where the HIV Tat protein is thought to increase the expression of the Fas receptor, resulting in excessive apoptosis of T cells (Chamond et al., 1999; Elmore, 2007). In neurodegenerative disorders the neuronal death appears to be associated with increase susceptibility for apoptosis in this cells.Hematopoiesis is regulated by certain trophic factors such as erythropoietin, colony stimulating factors, cytokines etc. In haematological disorders the presence of abnormal levels of normal trophic factor favours the accumulation of immature cells. In ischemic cell sudden increase in reactive oxygen radicals induce apoptosis in these cells.

Various studies show that ageing is also associated to an increase cell fragility which causes lymphocytes to undergo apoptosis when activated. Apoptosis may also be associated with allergy such as asthma, allergic rhinitis and allergic dermatitis. Eosinophils appear to actively collaborate in the resolution of the inflammation which is characteristic of asthma (Chamond et al., 1999).

Conclusions

In the human body a homeostasis is maintained between cells produced by mitosis and cell death by apoptosis. Understanding apoptotic signalling mechanisms becomes important as its dysregulation contributes to a wide variety of diseases. An insight to apoptosis will also allow us to develop effective and specific therapeutic approaches like targeted activation of proapoptotic tumor suppressors or the blockade of antiapoptotic oncogenes in the cancer and treatment of premature cell death in neurodegeneration (Schroder and Kaufman, 2005).

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