Int J Biol Med Res. 2012; 3(3): 2306-2308

BioMedSciDirect Publications

Contents lists available at BioMedSciDirect Publications

International Journal of Biological & Medical Research

Journal homepage: www.biomedscidirect.com

Review article

Apoptosis : Understanding of the signaling pathways

Sharma M*, Kumar R, Dhingra R,

**Department of Anatomy, All India Institute of Medical Sciences, New Delhi-110029.*

ARTICLE INFO

Keywords:
Apoptosis
DNA fragmentation

ABSTRACT

Apoptosis (programmed cell death) or cell annihilation imparts important role during various normal physiological systems of human body. Moreover, the inappropriate rate of apoptosis has been linked with pathophysiology of various diseases like preeclampsia, alzheimer's disease, huntington's, disease autoimmune diseases and various cancers. Therefore, understanding the mechanisms of apoptosis becomes the utmost priority. This review focuses on the morphological changes occuring during the process of apoptosis and the major apoptotic signaling pathways involved during this physiological phenomenon.

1.Introduction:

During evolution of cellular life, the mechanisms of cell death has been found equally important as that of cell proliferation and maturation. Throughout the eukaryotes, the cell renewal is brought about by the production of reactive oxygen species. "Apoptosis" (in Greek meaning "falling off") is now considered to be an important and unique process occuring in most of human systems like normal cell turnover, fetal development, immune systems etc.

Apoptosis is a highly organised and well orchestrated process having three phases of morphological changes [1].

In phase 1, chromatin gets condensed at the periphery of nuclei which are reducing in size along with nucleolar disintegration. There is shrinkage of total cell volume, dilation of endoplasmic reticulum and rest all cell organelles gets compactly arranged.

In phase 2, there is formation of membrane bound apoptotic bodies having cytoplasmic and nuclear contents.

In phase 3, the remaining nuclei and cytoplasmic contents get further degenerated. The other mode of cell death is necrosis but apoptosis differs from necrosis by the following [2]: (Fig:I)

a). Apoptosis is the programmed cell death dependent on the internal machinery of the cell. Whereas, necrosis is an unplanned mode of cell death caused by factors external to the cell.

* Corresponding Author : Sharma M
Email: dr.mona18sharma@gmail.com
Phone no: 9968147821

b). Apoptosis has a characteristic morphologic pattern of chromatin condensation and formation of membrane bound apoptotic bodies whereas in necrosis, cell organelles undergo swelling and there is mottled chromatin condensation. The cell membrane ruptures keeping all the cellular contents together.

c). Apoptotic cells undergo rapid recognition and degradation by phagocytes without release of inflammatory contents. Whereas necrosis results in inflammatory tissue injury.

Recent advancements has been made in understanding the pathophysiology of various diseases in which the role of altered apoptosis has been proposed. So, the understanding of apoptosis pathway becomes the initial step which can provide us the various ways to design the future preventive and therapeutic measures for the diseases associated with altered apoptotic phenomenon.

To unlock the mechanism of apoptosis, it is important to understand the key factors taking part in this phenomenon. Caspases (cysteine dependent asparate specific proteases) play the most important role in processing, propagation and amplification of apoptotic signals that result in destruction of the cells. Caspases are present in the cytoplasm, mitochondrial intermembranous space and nuclear matrix of all the cells. Apoptosis can be initiated by the factors which can be the intrinsic stimuli like mitochondrial factors and extrinsic stimuli like cytokines and other death receptors. The caspases bring about the cleavage of various vital cellular proteins after an aspartic acid molecule that ultimately leads to cellular death [3,4]. There are at least 14 members of this family which have been identified in mammals [5] and can be divided into 3 subtypes depending on the presumed role in apoptosis [6]. These include initiators caspases (caspase 2, 8, 9,

executioner caspases (caspase 3, 6, 7) and participants in cytokine activation (caspase 1, 4, 5, 11, 12, 13, 14). Once the intrinsic or extrinsic stimulus is given to the cell, the initiator caspases gets activated which then activate the executioner caspases. The activated executioners bring about the cleavage of cellular key proteins required for the maintenance of homeostasis leading to the collapse and demise of the cell.

Various studies have indicated that caspases cleave a set of key proteins like membrane associated microfilaments that play a role in the accomplishment of apoptotic morphology. But the exact mechanism of how the degradation of these proteins results in apoptotic morphology remains unknown.

1.Pathway of apoptosis

Two pathways of caspase activation have been characterized [7,8]

One ***(Extrinsic or ligand receptor pathway)*** is initiated outside the cell by the stimulation of proapoptotic signals or receptor ligands on the cell surface when the extracellular environment is not appropriate. These ligands include CD95L/FasL and binds to the corresponding receptors like CD95/Fas [7-10]. Once the ligand and receptor binding occurs, there is clustering and recruitment of adaptor protein Fas associated death domain (FADD) and the initiator caspases 8 or 10, thereby forming death inducing signaling complex (DISC)[11-14]. Formation of DISC leads to caspase 8 activation which then activates the executioner caspases [15]. DISC action is inhibited by the interaction of FADD with c- FLIP(Flice inhibitory protein) and therfore caspase activation is inhibited [16]. (Fig:II)

The second ***(intrinsic or mitochondrial pathway),*** gets started from within the cell in response to signals as a result of DNA damage, p53, hypoxia, any defect in cell cycle etc [17-20]. This pathway is also called as mitochondrial pathway as the main regulators of this pathway are the proteins of BCL-2 family which regulate the mitochondrial membrane permeability. This family includes two types of proteins- proapoptotic proteins (BAK,BAX,BAD,BIK) and antiapoptotic proteins(BCL-2,BCL-XL,MCL-1) [21]. The balance between proapoptotic and antiapoptotic proteins is an important checkpoint of apoptosis pathway. Any intrinsic injury within the cell leads to the apoptotic signals to the cell components and stimulation of proapoptotic proteins like BAK/BAX. This leads to the mitochondrial membrane permeabilisation and translocation of cytochrome c and SMAC/DIABLO proteins from the intermembrane space into the cytosol [22]. The released cytochrome c binds to the apoptotic protease activating factor (Apaf-1) and forms apoptosome. Then there occurs recruitment of procaspase 9 into the apoptosome leading to the activation of caspase9 [23]. This ultimately activates the executioner caspases which further cleaves cellular proteins. SMAC/DIABLO protein released from the mitochondria promotes apoptosis by directly interacting with inhibitors of apoptotic proteins(IAPs) and disrupting their ability to inactivate caspases. The IAPs are the important regulators of the apoptosis pathway and the IAP gene family constitutes around 7 members. These IAPs directly bind caspases particularly caspase 3,7 and 9 and inhibit them [24-26]. The balance between IAPs and their negative regulators (SMAC/DIABLO) is another checkpoint of apoptosis pathway. The imbalance caused due to overexpression of IAPs or downregulation of their negative regulator proteins may suppress apoptosis. This is a very common event seen in a variety of carcinoma cell lines and primary tumor samples [27] and is the common baseline for the active investigations regarding the role of IAPs and other regulators in preventive and therapeutic measures. (Fig:III)

Fig I: PROGRAMMED CELL DEATH PATHWAYS

Apoptosis	Necrosis
Caspase activation Inhibition of mRNA translation	Pro-inflammatory signaling and cytokine production
↓	↓
Condensation of cell and organelles	Swelling of the cell and organelles
↓	↓
Chromatin condensation DNA fragmentation	Mottled chromatin condensation
↓	↓
Loss of membrane asymmetry	Loss of membrane asymmetry
↓	↓
Membrane remains impermeable	Rapid loss of membrane permeability
↓	↓
Cells falls apart into apoptotic bodies	Cell membrane explodes Remains stay together

Differences between two modes of cell death: Apoptosis and Necrosis

Fig II: Extrinsic pathway (Death ligand pathway)

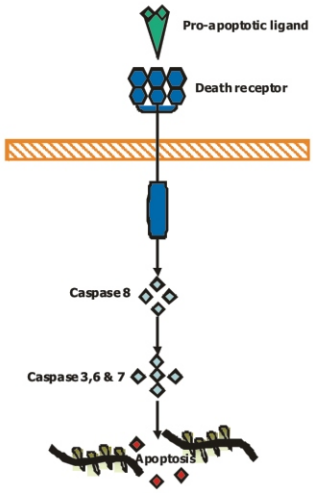
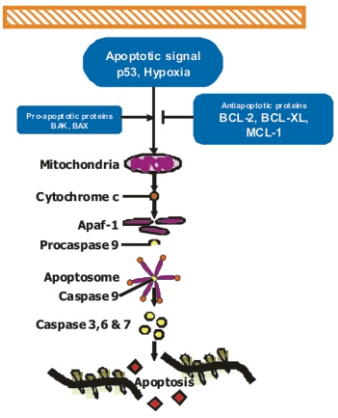


Fig III: Intrinsic pathway (Mitochondrial pathway)



2.Conclusion:

Apoptosis has been found to be a ubiquitous way of cell death in both physiological and pathological conditions. It has also been proposed that diseases can arise from dysregulation of cellular suicide mechanism such as apoptosis. Both intrinsic and extrinsic pathways of apoptosis leads to the cellular death. This process is intricately and precisely regulated inside the cell by various factors. Once the understanding of the apoptotic pathway comes to the edge of our knowledge, it becomes the next step to identify the methods to localise the apoptotic cells in the tissues and this identification can be based on the events occurring during apoptosis pathway like the cleavage of cellular proteins, activation of certain caspases and antibodies directed against the degraded proteins. If we achieve the goal of localizing the apoptotic cells in a tissue, it can lead us to correlate the pathophysiology of various proposed diseases with altered apoptosis and then various antiapoptotic manipulations can be applied to bring about protective and preventive measures against these diseases.

3. Reference:

[1] Wyllie AH, Kerr JFR, Currie AR. Cell death: the significance of apoptosis. Int Rev Cytol. 1980 ; 68: 251-306.

[2] Nanji AA, Sturmhofel HS. Apoptosis and Necrosis. Alchohol health a research world. 1997; 21(4) : 325 - 330

[3] Alnemri ES, Livingston DJ, Nicholson DW, Salvesen G, Thornberry NA, Wong WW, Yuan J. Human ICE/CED-3 protease nomenclature. Cell . 1996; 87:171.

[4] Zhang A, Yan W, Helen WLL, David TY. Apoptosis- A Brief Review. Neuroembryology. 2004; 3:47-59.

[5] Cohen GM: Caspases. The executioners of apoptosis. Biochem J. 1997; 326:1-16.

[6] Thornberry NA. The caspase family of cysteine proteases. Br Med Bull. 1997; 53:478-490.

[7] Ashkenazi A. Targeting death and decoy receptors of the tumor-necrosis factor superfamily. NatRevCancer.2002; 2:420-430.

[8] Fulda S, Debatin KM. Extrinsic versus intrinsic apoptosis pathways in anticancer chemotherapy. Oncogene. 2006;25:4798-4811.

[9] Debatin KM, Krammer PH. Death receptors in chemotherapy and cancer. Oncogene. 2004;23: 2950-2966.

[10] Rowinsky EK. Targeted induction of apoptosis in cancer management: the emerging role of tumor necrosis factor-related apoptosis-inducing ligand receptor activating agents. J Clin Oncol. 2005; 23 :9394-9407.

[11] Boldin MP, Varfolomeev EE, Pancer Z, Mett IL, Camonis JH, Wallach D. A nove protein that interacts with the death domain of Fas/APO1 contains a sequence motif related to the death domain. J Biol Chem. 1995;270 :7795-7798.

[12] Chinnaiyan AM, O'Rourke K, Tewari M, Dixit VM. FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis. Cell. 1995;81 :505-512.

[13] Kischkel FC, Hellbardt S, Behrmann I, et al. Cytotoxicity-dependent APO-1 (Fas/CD95)-associated proteins form a death-inducing signaling complex (DISC) with the receptor. EMBO J. 1995;14: 5579-5588.

[14] Wang J, Chun HJ, Wong W, Spencer DM, Lenardo MJ. Caspase-10 is an initiator caspase in death receptor signaling. Proc Natl Acad Sci USA.2001;98: 13884-13888.

[15] Bao Q, Shi Y. Apoptosome: a platform for the activation of initiator caspases. Cell Death Diff.2007;14:56-65.

[16] Ashkenazi A. Directing cancer cells to self-destruct with pro-apoptotic receptor agonists. Nat Rev Drug Discov. 2008 ; 7: 1001-1012.

[17] Fulda S, Debatin KM. Extrinsic versus intrinsic apoptosis pathways in anticancer chemotherapy. Oncogene. 2006; 25:4798-4811

[18] Coultas L, Strasser A. The role of the Bcl-2 protein family in cancer. Semin Cancer Biol. 2003;13: 115-123.

[19] Thornberry NA, Lazebnik Y. Caspases: enemies within. Science. 1998; 281: 1312-1316.

[20] Letai A. Pharmacological manipulation of Bcl-2 family members to control cell death. J Clin Invest. 2005;115: 2648-2655.

[21] Coultas L, Strasser A. The role of the Bcl-2 protein family in cancer. Semin Cancer Biol. 2003;13: 115-123.

[22] Ashkenazi A. Directing cancer cells to self-destruct with pro-apoptotic receptor agonists. Nat Rev Drug Discov. 2008;7:1001-1012

[23] Bao Q, Shi Y. Apoptosome: a platform for the activation of initiator caspases. Cell Death Diff. 2007; 14:56-65.

[24] Deveraux QL, Takahashi R, Salvesen GS, Reed JC. X linked IAP is a direct inhibitor of cell death proteases. Nature. 1997;388 :300-304

[25] Roy N, Deveraux QL, Takahashi R, Salvesen GS, Reed JC. The c-IAP-1 and c-IAP-2 proteins are direct inhibitors of specific caspases. EMBOJ. 1997; 16:6914-6925.

[26] Tamm I, Wang Y, Sausville E, Scudiero DA, Vigna N, Oltersdorf T, Reed JC. IAP-family protein surviving inhibits caspase activity and apoptosis induced by Fas, Bax, caspases and anticancer drugs. Cancer Res. 1998; 58:5315-5320.

[27] Yang L, Cao Z, Yan H, Wood WC. Coexistence of high levels of apoptosis signaling and inhibitor of apoptosis proteins in human tumor cells. Implication for cancer specific therapy. Cancer Res. 2003; 63: 6815-6824.