Review Article

Patterns of Apoptosis – A Review

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Received: 14-11-2017; **Revised:** 22-03-2018; **Accepted:** 18-04-2018.

ABSTRACT

Apoptosis the programmed cell death is a pathway in which the cells activate enzymes that degrade the cell's own nuclear DNA and cytoplasmic proteins. It is a mechanism used by the multicellular organism to remove the unwanted cells. Apoptosis can occur in different patterns known as patterns of apoptosis. They are Anoikis, Necroptosis, Apoptolysis and Necrobiosis. Anoikis is a form of apoptosis in which there is inappropriate cell to matrix interactions which has adverse effects on the cell survival. It occurs in physiological process of tissue renewal and cell homeostasis. Necrosis has been viewed as an unregulated, uncoordinated cellular event. But now evidences show that necrosis can also occur in a programmed manner called Necroptosis. Apoptolysis is a process in which the autoantibodies react with desmosomal proteins to activate various kinases within keratinocytes, causing their shrinkage, detachment from neighbouring cells and eventually causing death. Necrobiosis which was used before is the death of tissues within the living body. This review article highlights the molecular patterns of apoptosis and hence assists in the understanding of the underlying mechanisms of apoptosis in various diseases.

Keywords: Apoptosis, Necroptosis, Apoptolysis, Anoikis, Necrobiosis

INTRODUCTION

Apoptosis or programmed cell death is a mechanism by which the cells die under normal conditions[1]. It plays a critical role in several physiological and pathological processes[2]. It permits the removal of unwanted or damaged cells from the body through an intrinsic cell-suicide program[3]. Apoptosis was first observed in amphibian metamorphosis, soon it was found to occur in many developing tissues and both vertebrates and invertebrates undergo apoptosis[4]. Apoptosis can be initiated by stress signals from within the cells or by environmental stressors or any toxins. This death signal involves proteolysis by caspases, nucleosomal fragmentation by endonucleases[5]. Caspases are activated from different entry points- at the plasma membrane upon the ligation of death receptors (receptor pathway) or at the mitochondria (mitochondrial pathway) [6]. All the cells undergoing apoptosis show similar characteristics. The morphological features of apoptosis are cell shrinkage with membrane 'blebbing', loss of cell adhesion, nuclear pyknosis, and organelle degradation. These features occur in an orderly manner and do not trigger any inflammatory response[7]. During apoptosis, the nuclear shows striking changes. The chromatin condenses and marginates to the nuclear membrane, forming a ring shaped structure. The chromatin then collapses into two or more particles. Again the reminder of the cell will be devoid of chromatin and will be transparent in appearance. The shape of the cell also shows characteristic changes. The cell becomes smooth surfaced or spherical in some cases[8]. The mechanisms of apoptosis are highly complex and energy dependent cascade of molecular mechanisms. Apoptosis occurs by various mechanisms. The two main mechanisms are extrinsic or death receptor pathway and intrinsic or mitochondrial pathway. There is an additional pathway that involves T-cell mediated cytotoxicity and perforin granzyme dependent killing of the cell. These three pathways end in an execution pathway which is initiated by cleavage of caspase-3. This results in DNA fragmentation, degradation of cytoskeletal proteins, formation of apoptotic bodies, expression of ligands for phagocytic cell receptors and finally uptake phagocytic cells[9]. Caspases are expressed in an inactive form in mot cells and once activated they can activate other procaspases, which initiates the protease cascade. This is a proteolytic cascade, in which one caspase can activate the other caspase, amplifies the apoptotic signalling pathway resulting in rapid cell death[10].

Extrinsic Pathway

The extrinsic signalling pathways involve the death receptors that are members of tumour necrosis factor (TNF) receptor gene superfamily[11]. Members of TNF receptor family share 'death domains'[12] which plays a critical role in transmitting the death signal from the cell surface to the intracellular signalling pathways. The extrinsic phase is characterised with receptors such as FasL/FasR and TNF-alpha. These receptors bind with the homologous trimetric ligand. After ligand binding, cytoplasmic adapter proteins are recruited which exhibit corresponding death domains that bind with receptors[10]. These results in the autocatalytic activation of procaspase-8[13]. Once caspase-8 is initiated, the execution of apoptosis is triggered[14].

Perforin/Granzyme Pathway

In T-cell mediated cytotoxicity, a variant form of type IV hypersensitivity, CD8+ cells kill antigen bearing cells. These cytotoxic T lymphocytes kill the target cells via the extrinsic pathway. They also exert their cytotoxic effects on tumour cells and virus infected cells which involves the release of cytoplasmic granules into the target cell[16]. Granzyme A and granzyme B are present within the cytoplasmic granules. Granzyme B will cleave proteins at aspartate residues and will activate procaspase 10[17]. It can utilise the mitochondrial pathway for amplification of the death signal and induction of cytochrome c release[18, 19]. Thus there is direct induction of the execution phase of apoptosis. It is shown that both the mitochondrial pathway and activation of caspase-3 are critical for granzyme-B induced killing[20]. Granzyme A is also important in cytotoxic T cell induced apoptosis and activates the caspase independent pathways[21].

Intrinsic Pathway

The intrinsic signalling pathways are mitochondrial initiated events. They involve a non-receptor mediated stimuli that produce intracellular signals that act directly on targets and initiate apoptosis. The stimuli that initiate the intrinsic pathway could be either in a positive or negative fashion. Negative signals involve the absence of certain growth factors, cytokines which leads to failure of suppression of death programs, leading to apoptosis. Positive stimuli are not limited to radiation, toxins, viral infections or free radicals[10]. These stimuli cause changes in the inner mitochondrial membrane which leads to opening of the mitochondrial permeability transition (MPT) pore and there is release of two main groups of normally sequestered proapoptotic proteins into the cytosol[22]. The first group consists of cytochrome c and the second group consists of serine protease[23]. These proteins activate the mitochondrial pathway. Cytochrome-c binds and activates the procaspase-9 forming an apoptosome[24, 25]. The second groups of proapoptotic proteins are released from the mitochondria during apoptosis, but this is late event that occurs after the cell has committed to die [26]. The tumour suppressor proteins p53 plays a critical role in the regulation of bcl-2 family of proteins[27]. These Bcl-2 families of proteins govern the mitochondrial membrane permeability and can be either prop-apoptotic or anti-apoptotic. It is thought that the main mechanism of action of Bcl-2 family of proteins is the regulation of cytochrome-c release from the mitochondria[10].

Execution Pathway

The extrinsic and intrinsic pathways both end at the point of execution phase, called the final pathway of apoptosis. The activation of the execution caspases begins the phase of apoptosis. Caspase-3, caspase-6 and caspase-7 are executioner caspases cleaving various substrates like cytokeratins, plasma membrane cytoskeletal protein alpha fodrin, and others, ultimately causing the morphological and biochemical changes seen in apoptotic cells[28]. Caspase-3 is the most important of the executioner caspases. In apoptotic cells, activated caspase-3 cleaves ICAD to endonuclease CAD[28]. CAD chromosomal DNA within the nuclei and cause chromatin condensation. It also induces cytoskeletal reorganisation and disintegration of the cell into apoptotic bodies[10].

Phagocytic uptake of apoptotic cells is the last phase component of apoptosis. Phospholipid asymmetry and externalisation of phosphatidylserine on the surface of apoptotic cells is the hallmark of this phase[29]. The appearance of phosphatidylserine o the outer leaflet of apoptotic cells then facilitates non-inflammatory phagocytic recognition, allowing for their early uptake and disposal[30].

Inhibitors of Apoptosis

The anti-apoptotic therapy includes stimulation of the IAP (inhibitors of apoptotic proteins) family of proteins, caspase inhibition, stimulation of the PKB/Akt (protein kinase B) pathway and inhibition of Bl-2 proteins[10]. The IAP family of proteins is the most important regulators of apoptosis as they regulate both the extrinsic and intrinsic pathways[31]. A study shows that eight human IAP proteins have now been identified although XIAP(X-linked mammalian inhibitor of apoptosis protein) and surviving remain better members[32]. ICE (Interleukin-1 beta converting enzyme), also called caspase I, is a cysteine protease that appears to mediate protein degradation during apoptosis[33]. Infusion of insulin-like growth factor (IGF-1), which stimulates PKB/Akt signalling and promotes cell survival, was shown to be beneficial in animal models of myocardial ischaemia[34]. As the molecular and biochemical complexities of apoptosis continue to be elucidated, new therapeutic strategies continue to develop.

Pathways Leading to Apoptosis

Genetic regulators

P53

P53 has a central role in the maintenance of genomic stability and preventing the mutation and deletion of functional genes. P53 is involved n G1-cycle arrest and apoptosis induced by DNA damage[35].

C-myc

The requirement of C-myc is increased for the switch from proliferation to apoptosis. Studies show that elevated levels of C-myc has an undetermined role in prostate cancer[36].

Rb gene

The suppression of tumour suppressor gene retinoblastoma has been correlated with cancer progression. Studies show that the loss of heterozygosity of Rb gene occurs in over half of the patients with

prostate cancer, suggesting its loss is closely related to tumour development[37].

Cellular regulators

Fas/TNF signals

The TNF receptor-1 (TNFR1) and Fas initiate death signals when they interact with corresponding antigens TNF-alpha and Fas ligand (FasL) [39-42]. A series of murine mutations in Fas (Ipr) or in Fas ligand (gld) have provided a means of detecting the involvement of this system in various apoptosis activating pathways[43].

Mitochondria

Mitochondria is a central executioner of cell death. Studies have shown that mitochondrial disruption precedes nuclear DNA fragmentation, induced by several apoptosis inducing agents[44]. Permeabilisation of inner and outer membrane of mitochondria has resulted in the release of cytochrome-c and caspase activating factor. Cytochrome-c has been shown to activate caspase cascade[45]. Further evidence has shown that inhibitors of mitochondrial permeability block the induction of apoptosis[46].

Caspase

The central regulating signal to apoptosis is the caspases[1]. Caspases has now been considered as the exclusive common pathway to apoptotic death[47]. Their primary function is to cleave specific protein in the cell, leading to their deactivation and this result in apoptotic cell death[48].

Here in this review we will learn the various patterns of apoptosis.

Necrobiosis

Necrobiosis is death brought on by (altered) life- a spontaneous wearing out of living parts- the destruction and annihilation consequent upon life- natural as opposed to violent death. This term also means to imply slow death or death of tissues within the living body. As necrobiosis was a vague and ambiguous term, it disappeared[49].

Apoptolysis

Pemphigus vulgaris (PV) is an autoimmune disorder where there is keratinocyte detachment leading to blistering[50]. Studies indicate that PVIgG can induce supra basal acantholysis which is a histopathologic hallmark of PV[51]. The supra basal split occurs due to differences between basal and supra basal cells in their responses to PVIgG, as was predicted by the Basal Cell Shrinkage hypothesis[52]. In Steven Johnson syndrome (SJS) there is cell death in all epidermal layers and sloughing of full thickness necrotic epidermis. The difference in PV is that basal cells shrink but do not die, rendering a tombstone appearance to the basal layer. So a new term was required to distinguish cell damage and detachment in PV from other forms of cell death[53]. This pathologic mechanism was designated as apoptolysis. The major steps of apoptolysis in PV are:

Apoptolysis in pemphigus is triggered by binding of autoantibodies to the PV antigens capable of transducing apoptolytic signals from the keratinocyte plasma membrane. The ligated antigen elevates intracellular calcium and launches cell death cascade. Collapse and retraction of tonofilaments cleaved by executioner caspases results in basal cell shrinkage, while most desmosomes remain intact. Supra basal acantholytic cells die, thus allowing the formation of supra basal blister and rendering tombstone appearance to surviving basal cells[50]. Understanding the death pathway of keratinocytes in PV may be a key to development of novel treatments. Appreciation of new concept that cell detachment and death in PV occurs via apoptolysis helps to resolve confusion that acantholysis may develop without apoptosis[54].

Necroptosis

Apoptosis is a programmed and regulated cell death. In contrast to regulated cell death, Necrosis is considered as an unprogrammed, unregulated cell death. Recent in vitro and in vivo studies have emerged that have characterised new form of regulated cell death (other than apoptosis). One of these is the programmed necrosis called Necroptosis[55]. Necrosis is different from apoptosis. This cell death is described as uncontrolled and accidental necrosis characterised by loss of plasma membrane integrity and cellular collapse though nuclei remain intact[56-58]. Degterev and co-workers in their study[59] demonstrated that treatment of cultured cells with TNF-alpha, leads to necrotic or non-apoptotic cell death in the presence of caspase inhibitors or in the absence of Fas associated death domain (FADD). Although necrosis and apoptosis were initiated by same stimulus (TNF-alpha), the morphological changes occurring with necroptosis were characteristic of necrosis, which was assumed to represent uncontrolled cell death occurring as a consequence of overwhelming stress[60]. Necroptosis was prominently found when intracellular apoptotic signalling was inhibited. It was characterised by necrotic cell death morphology, concomitant activation of autophagy ad dependency on the function of R1PK1[61]. During necroptosis, RIPK3 is a downstream target of RIPK1, and forms a complex that may or may not contain RIPK1[62]. The core molecular complex of R1PK1 and RIPK3 is recognised as the necrosome[63].

This regulated necrosis can be inhibited by Necrostatin-1[64]. RIP3K can also form complexes with DNA- dependent activator of interferon regulatory factor (DAI) and the adaptor molecule TRIF (TIF-domain-containing adaptor-inducing interferon-beta; adaptor protein downstream of TLR3), leading to RIP3K-dependent programmed necrosis[65].

Apart from necrosis like morphological changes observed, necroptosis was found to be associated with activation of autophagy. Studies showed that electron microscopic examination of necroptotic jurkat cells revealed the presence of electron-dense double membrane enclosed vesicles characteristic of autophagy i.e. autophagosomes[66-68].

Anoikis

Anoikis is defined as the apoptosis of the cells induced by inadequate or inappropriate cell matrix interactions. Anoikis is a Greek term, which means 'homelessness' or 'loss of home.' It was first defined by Steven M. Frisch[69]. Integrin receptors, mediators of cell-ECM interaction not only provide physical links with the cytoskeleton but also transduce signals from the ECM to the cell, which is mandatory for several cellular processes including migration, proliferation and survival[70-74]. Anoikis plays an essential role in the development and organisation of normal tissues through its inhibitory effect on unfavourable cellular proliferation at inappropriate locations. Thus it contributes to the maintenance of physiologic state. Disturbance of anoikis allows inappropriate cell growth and metastasis of several types of carcinoma cell[75, 76].

The initiation and execution of anoikis is mediated by different pathways, all of which terminally converge into the activation of caspases and downstream molecular pathways, culminating in the activation of endonucleases, DNA fragmentation and cell death[77]. The induction of the anoikis program occurs through two apoptotic pathways, namely the perturbation of mitochondria (intrinsic pathway) or the triggering of cell surface death receptors (the extrinsic pathway) [78, 79].

In the intrinsic pathway, caspase activation occurs as a consequence of mitochondrial permeabilisation[80, 81]. This is regulated by the Bcl-2 family of proteins, which control the formation of pores in the mitochondrial membrane (OMM), releasing proapoptotic factors such as cytochrome c, which activates caspase[82]. The extrinsic pathway is initiated by the ligation of death receptors on the cell surface, such as TNFR or Fas, resulting in the assembly of death inducing signalling complex (DISC). The role of DISC is to recruit and activate caspase 8, via an adaptor molecule FADD[83]. Caspase 8 cannot by itself initiate apoptosis but instead cleaves the BH3-only protein Bid, which then initiates the intrinsic apoptotic pathway.

Anoikis is thus an essential mechanism to maintain the correct position of cells. Induction of anoikis occurs when the cells lose attachment to ECM or adhere to inappropriate type of ECM[78].

CONCLUSION

Programmed cell death not just involve traditional death mechanism apoptosis, but also occurs by various patterns like programmed necrosis, anoikis, apoptolysis etc. The huge increase in studies related to cell death, has contributed to wealth of knowledge in facilitating a better understanding of cancer pathogenesis and therapeutics. Many regulatory genes are common to more than one module; therefore programmed cell death should be regarded as a network of interconnected pathways.

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Source of Support: Nil, **Conflict of Interest:** None.