Apoptosis and Necrosis

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Apoptosis is a highly regulated cellular process leading to elimination of excessive, damaged, or unwanted cells. Apoptosis is a physiological mechanism that occurs in multicellular organisms during their ontogenesis, it has also important role in the maturity and it is considered as a type of programmed cell death (PCD) termed PCD-I. The term “apoptosis” is derived from the Greek word describing the falling off of leaves from tree.

Apoptosis vs. necrosis

Apoptosis is an active (requires energy from ATP hydrolysis) and genetically programmed process leading to cell death, that is carried out by proteolytic enzymes called caspases. It can also be characterized by distinct morphological changes. These include chromatin condensation followed by its clustering in cell nucleus periphery, chromosomes loosing from nucleus membrane and activation of endonucleases. Activated endonucleases cleave DNA that takes place between nucleosomes resulting in formation of 180 bp-long DNA fragments and other multiplies of this length. These DNA fragments characteristically organize in electrophoretic gel according to a certain molecular weight to form a typical DNA-ladder (see picture). Cell nucleus, other membrane organelles, and cytoskeleton are degraded and enclosed by cell membranes. The cell connections are disrupted, apoptotic cell is separated from neighboring cells and shrinks. Eventually, the plasmatic membrane forms blebs and the cell breaks up into small membrane-bound fragments termed apoptotic bodies. Apoptotic bodies contain structurally intact cell-like organelles as well as portions of the nucleus and other membrane organelles. Subsequently, the apoptotic bodies are quickly recognized and removed by the process of phagocytosis by neighboring cells without causing an inflammatory reaction.

Compared to apoptosis, the process called necrosis is pathological and accidental mode of cell death. It is a passive (does not need energy from ATP hydrolysis), more chaotic, unplanned and non-programmed type of cell death. It typically mediates cell demise in response to sudden and irreversible cell damage (hyperthermia, hypoxia, detergents, toxic substances or direct cell trauma) and it does not occur during normal development. Mechanistically, necrosis is typically not regulated by Bcl-2 family proteins and its progression is independent on caspases activity. During necrosis, the cells lose the integrity of plasmatic membrane and absorb extracellular fluid, which causes irreversible swelling of the cytoplasm and organelles. Finally, plasmatic membrane ruptures and noxious cell content pours out into extracellular matrix. The rest of necrotic cells is not targeted by phagocytes and engulfed, so the cell content can spread quickly throughout the body and cause immediate reactions in surrounding tissues, leading to local inflammation response accompanied with edemas or even to systemic response. The main differences in the process of apoptotic and necrotic cell death are summarized in Table 1. Moreover, under some pathological conditions both types of cell death may be found and both pathways can switch between each other (e. g. the cell death begins as the apoptosis but due to the lack of energy or caspase mutation cannot be finished, so the necrotic pathway turns on and accomplishes the process of cell death. The decision of the cell to die by necrosis or apoptosis depends largely on the severity of the insult.

The role of apoptosis in health and disease

Apoptosis is a physiological, genetically programmed process that takes part especially in ontogenesis but also in postnatal development of multicellular organisms. It participates in elimination of cells that could be potentially harmful to organisms. It also has an important role in morphogenesis and in maintaining a natural balance between cell proliferation and cell death - tissues homeostasis. Dysregulations of the apoptotic pathway, can cause developmental disorders and many health complications.

Apoptosis in ontogenesis

A general principle of development in human embryogenesis is emerging: excess numbers of cells are made, and then surplus or unwanted cells are removed by apoptosis during the formation of functional organs development and gamete formation. Apoptosis is one of key mechanisms responsible for proper gamete (spermatozoid and oocyte) maturation and embryonic development. As for morphogenesis, massive apoptosis can be found e.g. at bodies’ cavity forming during human ontogenesis. Another example represents development of fingers. First, there is form like a spade and individual fingers are created when cells between them die by apoptotic cell death. Ineffective apoptosis induction results in syndactyly, which is a disease when fingers are not properly separated. It can be either genetically determined (90% of cases) or caused by teratogens.

Next, apoptosis also eliminates other structures that are no longer needed, such as tale in tadpole during its transformation to frog. Similarly, differentiation of male and female genital is also strictly dependent on apoptosis. In early embryo, both genitals basis Müllerian and Wolffian ducts are firstly formed. In the men, the production of anti-Müllerian hormone by Sertoli cells in the testes leads to atrophy of the Müllerian ducts, while in women the absence of this hormone causes apoptotic degradation of Wolffian ducts.

Apoptosis has also a very important role in T-lymphocytes maturation in thymus. Newly derived T-lymphocytes are exposed to a wide variety of self-antigens and undergo two types of selection - positive and negative selection. During the negative selection auto-reactive T-lymphocyte cells, having receptors interacting with own molecules (that are potentially dangerous to organism), are removed by apoptosis. T-lymphocytes that binds too weakly to self-HLA antigens undergo apoptosis too; this process is called positive selection. Both selections finally result in remaining only of those cells which carry adequate affinity to HLA molecules. The positive and negative selections lead to death of almost 95% of T-lymphocytes.

Neural apoptotic cell death has a pivotal role in both the development and pathophysiology (see chapter) of the nervous system. In the developing nervous system apoptosis is observed as early as neural tube formation and persists throughout terminal diferentiation of the neural network. More than 50% of neurons are lost during development as a result of limiting trophic support from the target tissue they are destined to innervate.

Besides described situations, apoptosis is also necessary in development of kidneys, heart, lungs and teeth thus we can even consider this to be as important for proper embryo development as cell proliferation and cell differentiation.

Apoptosis in adult organisms

In humans is the process of apoptosis in balance with cell proliferation to maintain the constant number of cells in various tissues and thus prevent growing and shrinking of organs (see picture). In mature organisms keeps apoptosis its function in tissues responding cyclically to hormones changes (endometrium, prostate, breast cells). It is also necessary in elimination of senescent or damaged cells in renewing tissues and cell systems as intestinal epithelium, skin epithelium, bone marrow, red blood cells etc. Very important role of apoptosis is in elimination of damaged, infected, and mutated cell, which can potentially lead to pathology, e. g. to tumor development.

Apoptosis also has an important role in immune response as it is essential for the T-lymphocyte activity. First, T-lymphocyte is activated and it begins to overexpress Fas ligand (FasL) on its plasma membrane. Next, the lymphocyte kills target cells mentioned above by the activation of death receptor pathway. It starts by binding of Fas receptor on the target cell with FasL on the T-lymphocyte, which enables the forming of the death-induced signaling complex (DISC), recruitment of procaspases 8 and 10 and their activation (see chapter). Besides, apoptosis is also observed in mature peripheral T-lymphocyte, to downregulate the number of reactive cells and to terminate the immune response. Moreover, apoptosis often occurs in B-lymphocytes during their activation. The activated B-lymphocyte requires the signal from helper T-lymphocyte to initiate the formation of immunoglobulin. In case B-lymphocyte does not receive this signal it can undergo apoptosis. This process ensures that there will not be produced any needful antigens in case of stimulation of B-lymphocyte by some accidental antigen which was not detected by T-lymphocyte.

Apoptosis associated with diseases

Too little or too much apoptosis, or apoptosis occurring in the wrong place and/or at the wrong time can result in pathology including autoimmune diseases, viral and bacterial infections, neurodegenerative, and cardiovascular disorders, or cancer. Furthermore, dysregulated apoptosis signaling may impinge on other age-related disorders such as osteoporosis, atherosclerosis and perhaps on the process of aging itself.

Recently, these findings have led to the development of therapeutic approaches based on regulation of apoptosis, some of which are in clinical trials or have entered medical practice.

Cancer

Apoptosis prevents malignant transformation whereas decreased apoptosis can predispose human to cancer. Based on the role of apoptosis in maintaining tissue homeostasis, it is not surprising that alterations of apoptosis play an important role in cancer development, including hyperplasia, neoplastic transformation, tumor expansion, neovascularization, and metastasis. Moreover, defects in the apoptotic pathways are responsible for resistance of cancer cells to cancer therapy. New therapeutic approaches are very often attempting to re-activate these pathways bypassing the apoptotic block. Deregulations of apoptotic pathway leading to its decrease and thus cancer cell survival can result from modulation of the activity of various proteins (inhibiting the activity of tumor suppressor genes, such as Rb and p53 or increasing the activity of proto-oncogenes, such as Bcl-2, Fos and Myc). For example, mutations in r p53 protein are the most common chromosomal aberrations in human cancer. On the other hand, decreased activation of proapoptotic Bcl-2 members, such as Bax or Bak can be found in cancers cells too. In addition, there are often mutations in genes encoding caspases or proteins that regulate caspases activity such as IAPs, Apaf-1, or Smac (see below).

Neurological diseases

Apoptosis plays a key role in central and peripheral nervous system development and up to 50% of neurons die before embryonic development is complete (see above). It has become apparent that excessive or inadvertent apoptosis also plays role in the pathogenesis of several diseases, including neurodegenerative diseases and acute injury. Unnaturally high rate of apoptosis is typical for the pathogenesis of Alzheimer’s, Parkinson’s and Huntington’s diseases, spinal muscular atrophy or amyotrophic lateral sclerosis.

Autoimmune diseases

A common feature of autoimmune diseases is altered tolerance to self-antigens and generation of autoantibodies. Immune homeostasis and maintenance of immune tolerance are strongly dependent on apoptosis, moreover defective clearance of dying cells results in persistence of auto antigens, therefore autoimmune diseases can arise both from defective clearance of auto reactive cells or by delayed elimination of auto antigens.

The activation of T-lymphocyte is known to lead to the upregulation of Fas receptor and Fas ligand and to the susceptibility of these cells to Fas-mediated killing (so called AICD). AICD is essentially a mechanism for switching off the immune response and serves to limit the intensity of immune responses. Some chronic inflammatory disease, such as asthma, could result from an escape of activated T-lymphocytes from Fas/Fas ligand-mediated cell death. This indicates that accelerated induction of apoptosis in T-lymphocyte can limit auto antigen-driven immune response and could be a novel strategy for the treatment of autoimmune disease.

Infectious diseases

Pathogenic microorganisms (bacteria, protozoa) and viruses, once present inside a host must avoid their detection and destruction. Several pathogens can trigger or inhibit apoptosis in eukaryotic host cells thus escaping the activity of immune system. On the other hand, the host can use apoptosis to defend himself from spreading of pathogens. Thus apoptosis has a fundamental role in intracellular pathogen propagation.

Considering viral infection, it causes induction of apoptosis in the host cell in normal conditions by the recognizing of the virus in the cell by immune system. The elimination of the host cell by the apoptotic process reduces production and spreading of new virions and it represents a key defense mechanism preventing viral propagation. Viruses have therefore developed several strategies to inhibit or delay apoptotic cell death. For example, adenoviruses in the host cell produce a protein E1B 19K, a viral homologue of anti-apoptotic protein Bcl-2. Next, another adenovirus protein termed RID (receptor internalization and degradation) mediates internalization of the cell surface Fas and subsequent destruction inside lysosomes, which allow cells to resist Fas-mediated death and promote survival of the virus.

On the other hand, some pathogens, especially bacteria induce apoptosis to kill cells of the immune system. For example macrophage undergo apoptosis upon infection with Salmonella, Shigella or Yersinia. It has been implicated that the induction of macrophage death is important to initiate infection, promotes bacterial survival and to enable escape from the host immune responses.

The course of apoptosis

The process of apoptosis can by divided in several stages that include:

1. receiving an apoptotic signal that
2. turning on the cascade of intracellular activities leading to cell decomposition and apoptotic bodies formation, which are eventually
3. removed by phagocytosis.

Apoptotic signal

Apoptosis is regulated by three fundamentals different pathways. First, many cells in multicellular organisms require specific signals from other cells to stay alive. This mechanism ensures cell proliferation and survival only in the right time and the right place. In the absence of such survival signals, often referred to as trophic factors, these cells activate apoptotic program. Next way is receiving of specific signal that induce apoptosis in particular cell. Besides these two physiologically regulated initiations of apoptotic pathway, apoptosis can be triggered by many other non-physiological conditions, including DNA damage caused by radiation or chemical substances, oxidative stress, hypoxia or by presence of foreign agents in cytosol (bacteria, viruses, toxic substances). According to extra or intracellular origin of apoptotic signals we can distinguish apoptosis stimuli to two different apoptotic signaling pathways leading to cell death - intrinsic or extrinsic apoptotic pathway.

The extrinsic apoptotic pathway

The extrinsic apoptotic pathway is triggered by extracellular apoptosis stimuli, particularly by specific ligands binding to so called death receptors, or receptors with death domain, (DRs). DRs are members of the tumor necrosis factor (TNF) superfamily and include Fas (see above, also called CD95 and Apo1), TNF receptor-1 (TNFR1), TNF-related apoptosis-inducing ligand receptor 1 (TRAIL-R1, also called DR4) and receptor 2 (TRAIL-R2, also called DR5). These receptors are characterized by the presence of up to six cysteine-rich domains (CRD), that define their ligand specificity and by the presence of death domain (DD) in their C-terminal intracellular tail, which is essential for apoptosis induction. The binding of specific ligands (Fas ligand, TNF, TRAIL) to the death receptor causes its homodimerization and recruitment of adapter proteins, e. g. FADD, to the DD. Adapter proteins cause the recruitment of inactive procaspase-8 or -10 to the intracellular site of DRs and its dimerization and activation. Active initiator caspases-8 and -10 can then cleave and activate executioner caspases-3, -6 and -7.

The intrinsic apoptotic pathway

The intrinsic apoptotic pathway is activated in response to cellular stress induced by many stimuli such as oxidative stress, hypoxia, DNA damage, accumulation of unfolded proteins, cytoskeletal disruption, but also growth factors deprivation, and many others. This pathway is also known as mitochondrial apoptotic pathway because it depends

on pro-apoptotic factors released from mitochondria that subsequently activate caspases. The intrinsic and extrinsic apoptotic pathways are not orchestrated separately, but they rather cooperate and complement with each other to amplify the apoptotic signal.

Bcl-2 family proteins

Initiation of intrinsic apoptotic pathway is regulated by Bcl-2 (B-cell lymphoma) family proteins that count at least fifteen different members in mammalian cells. These proteins can be divided in the two antagonistic groups according to their function in apoptosis induction. Bax, Bak, Bid or Bim are pro-apoptotic Bcl-2 proteins, while Bcl-2, Bcl-xL, or Mcl-2 have anti-apoptotic function. Bcl-2 family members possess up to four conserved α-helical domains, designated BH1, BH2, BH3 and BH4. Pro-apoptotic Bcl-2 family proteins differ in numbers of BH (Bcl-2 homology) domains and can be divided into multi-domain proteins having three domains and BH3-only proteins, anti-apoptotic Bcl-2 family proteins are all multi-domain having four domains. Some of these proteins also contain hydrophobic helical transmembrane domain on their C-terminal for anchoring these proteins to cellular membranes (see picture).

Bcl-2 family proteins activity can be regulated by phosphorylation mediated by cell kinases. Very important way of regulation is also carried out by homodimeric or heterodimeric interactions of individual members belonging to this protein family that are performed by their BH domains. Concurrent inhibition of the activity of anti-apoptotic Bcl-2 proteins and increased activation of pro-apoptotic Bcl-2 proteins is a key mechanism in initiation of intrinsic apoptotic pathway.

The role of mitochondria in the intrinsic apoptotic pathway

The Bcl-2 family proteins are primarily involved in the regulation of mitochondrial membrane integrity. When apoptotic signaling inactivates anti-apoptotic Bcl-2 proteins, it then enables activation of pro-apoptotic Bcl-2 proteins (Bax, Bak). Activated Bax and Bak increase the permeabilization of the outer leaf of membrane leading to decrease of mitochondrial membrane potential by opening of permeability transition pore (PTP) or by pores made directly of pro-apoptotic proteins Bax and Bak. Increased permeability of mitochondrial membrane leads to the translocation of proapoptotic molecules (cytochrome c and SMAC/Diablo) from intermembrane space of mitochondria to cytosol and facilitates progress of the apoptotic cascade. Cytochrome c is probably most important protein released from mitochondria. It can be found in intermembrane space of mitochondria to be essential component of the electron transport chain. During apoptosis, translocated cytochrome c, protein Apaf-1, dATP and procaspase-9 form a heptameric complex called apoptosome and allows auto-cleavage and activation of procaspase-9.

Caspases

Caspases are the executive proteins that play key role in both intrinsic and extrinsic apoptotic pathways. They belong to the family of endoproteases that hydrolyze peptide bonds. They have cysteine residue in their active site and the cleavage occurs only after aspartic acid residues present in their substrate. At the time, it is known at least fifteen different caspases in mammals. Caspases involved in apoptosis can be divided into two groups: initiator caspases, (caspase-9, -8 and -10) and executioner caspases (caspase-3, -6 and -7). The main function of initiator caspases is to cleave and thus activate the executioner caspases. As to executioner caspases, once activated, they can cleave death substrates Death substrates are proteins that ensure cell compactness such as proteins of nuclear lamina, cytoskeletal proteins, or inhibitors of endonucleases cleaving DNA to fragments (described above). Briefly to the caspase-driven cascade, one molecule of activated executioner caspase can activate other molecules of executioner as well as initiator caspases, leading to an accelerated feedback loop of caspase activation.

Caspase-3 is most important executioner caspase responsible for activation of essential enzyme CAD (Caspase-activated DNase). CAD is normally inhibited by death substrate ICAD (Inhibitor of caspase-activated DNase), after its cleavage by caspase-3 CAD is activated. This protein breaks up the DNA during apoptosis at inter-nucleosomal linker sites between nucleosomes. Executioner caspases-3 and -7 have a similar substrate specifity and are partially replaceable. Categorization of caspases-1, -2, -4, -5 and -12 is still somehow unclear, they can for instance participate in forming of pro-inflammatory cytokines during inflammatory response (see picture).

Caspases are initially produced as inactive monomeric zymogenes, termed procaspases. Activation of caspases is strictly regulated and occurs only during apoptosis, because the process of apoptosis is irreversible from the point, when caspases are activated. Dimerization of procaspases followed by auto-cleavage represents basal way of regulation of their activation. Caspase activity can be also regulated by IAP (Inhibitors of apoptosis) family proteins (XIAP, cIAP survivin, livin), that bind and inactivate caspases, which finally blocks progression of apoptosis. Interestingly, regulatory mitochondrial proteins SMAC/Diablo and HTRA2/Omi bind proteins from the IAP family and neutralize their inhibiting function and thus progression of apoptosis.

The role of endoplasmic reticulum in intrinsic apoptotic pathway

Except of mitochondria, endoplasmic reticulum (ER) is another cell organelle which can participate significantly in activation of intrinsic apoptotic pathway. In ER, the apoptotic signal leads to accumulation of unfolded or misfolded proteins, which in turn activate the signaling pathway called UPR (unfolded protein response). During UPR, the expression of proteins that can help proteins to maintain their native structure and thus restore the function of ER is increased in cells. When the ER damage is irreversible, the Ca2+ translocate from the ER lumen to cytosol Releasing Ca2+ from the ER can be regulated by Bcl-2 family proteins too, especially by inactivated protein Bcl-2, that can stimulate opening of channels linked to inositol-1,4,5-triphosphate receptor (IP3R) and release Ca2+ ions. The dimer of pro-apoptotic proteins Bax and Bak can also directly penetrate ER membrane to form pores. Cytosolic

Ca2+ can subsequently stimulate the translocation of proapoptotic molecules to cytosol or activate Ca2+-dependent enzymes (caspase-4, -12, calpain) which can finally activate apoptotic pathway. Cytochrome c or caspase-3 activated in the intrinsic apoptotic pathway can also stimulate the release of Ca2+ from ER to amplify the apoptotic signal in cells.

Elimination of the apoptotic cell

Apoptotic bodies formed in the final stages of apoptosis change plasmatic membrane composition and display phagocytogenic molecules, such as phosphatidylserine, on their cell surface. In physiological condition, phosphatidylserine is strictly localized in the inner leaf of the plasma membrane. During apoptosis, phosphatidylserine is redistributed to the outer leaf of the plasmatic membrane by an enzyme known as scramblase. The cells labeled by phosphatidylserine are then quickly recognized by phosphatidylserine receptor on the phagocyte cells and are engulfed by neighboring cells, macrophages or other phagocyting cells without causing an inflammatory response. The removal of apoptotic cells by neighboring phagocyting cells is sometimes termed efferocytosis.

Links

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