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REVIEW ARTICLE

Apoptosis: Dual Role in Aetiology and Cure

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ABSTRACT

Apoptosis is a programmed cell death which occurs following a variety of stimuli. Physiologically the process is important for morphogenesis of organs and homeostasis of different types of cells. Apoptotic cell death is responsible for a variety of pathologic states such as elimination of cell death in mutated cells, infected cells, tumour cells and transplant rejection well as the pathological atrophy. In this review, there is discussion about the control of apoptosis, detection methods of apoptosis, its association with infectious and non-communicable diseases. Intracellular microorganisms survive through inhibition of host cell apoptosis as well as they destroy the parenchymal cells causing impaired functions. It plays important role in tumourigenesis. There are possible therapeutic roles of drugs that modify apoptosis in human diseases.

INTRODUCTION

Apoptosis is a process of programmed cell death and the word was derived from Greek word meaning "falling off" which is an analogy to leaves falling off trees[1]. Apoptosis follows a specific stimulus and stimulated by rapid activation of endonucleases[2 – 4]. It is a physiological process occurring during organogenesis in embryonic life, tissue homeostasis such as atrophy of cells in senility, shrinkage of breast and reproductive organs after reproductive period. It has a role in some pathological conditions such as removal of DNA damaged cells and immune cells on removal of cytokines and growth factors[5, 6].

Recent studies found that some DNA lesions such as base-alkylations, DNA cross-links and DNA-double-strand breaks (DSBS) can trigger apoptosis[7]. Programmed cell death is initiated by activations of endonucleases which causes proteolysis of DNA repair proteins, cytoskeletal proteins, and the inhibitor of caspase-activated deoxyribonuclease[8, 9].

Detection of Apoptosis

Histologically apoptotic cells are characterized under light microscope by cell shrinkage, blebbing of plasma membrane, small fragmentation of cell cytoplasm containing pyknotic remnants of nuclei. It can also be detected by terminal deoxynucleotidyltransferase (TdT) mediated dUTP-biotin nick end labelling (TUNEL) method to see the nuclear fragmentation[10] which is based on specific binding of TdT to 3'-OH ends of DNA of nuclei undergoing apoptosis on the histological sections or cell preparation in flow or laser scanning cytometry[10, 11]. Degradation of genomic DNA results in formation of DNA fragments which on agar rose electrophoresis show laddered DNA[12]. Ligation-mediated polymerase chain reaction (LMPCR) can amplify DNA fragmentation specific to apoptotic cell death[13, 14]. Ultrastructurally, nuclear chromatin condensation, cells break up into membrane-bound bodies, apoptototic bodies, which are phagocytosed and degraded by cells such as histiocytes, endothelial cells, epithelial cells or neoplastic epithelial cells[1, 13]. Oligonucleotide and protein microarray methods detect the genes and proteins that regulate apoptosis through the Fas system[15, 16].

Control of Apoptosis

Apoptosis is initiated by the extrinsic pathway, which can be triggered by ligation of death receptors which activates caspase-8 and the intrinsic pathway initiated by cellular stress by activation of caspase-9 following the cellular stress or the granzyme B pathway, which uses granzyme B to kill the target cells[17]. Double-strand specific caspase is activated and cleaves the chromosomal DNA into large fragments of 50 – 300 kb[18]. Some studies describe about the caspase independent processes which requires apoptosis inducing factor (AIF)[17]. There are many genes identified in recent years which are responsible for apoptosis[20]. *Bcl2* oncogene promotes cell survival by blocking programmed cell death that prevents the permeability of mitochondrial outer membrane[21]. Mutation of transmembrane receptor protein, CD95/Fas, that interact with Fas associated death domain (FADD) to recruit pro-caspase 8[22]. Bax which is a bcl2 homologous protein when stimulated inserts into the mitochondrial outer membrane to release cytochrome C in apoptosis. P53 has a major role in inducing apoptosis: it regulates cell cycles by allowing damaged DNA to repair, inducing Bax expression[23], through activation of Noxa[24], increasing the level of reactive oxygen radicals (ROS), all of which can trigger release of cytochrome C. Inhibitors of apoptosis proteins (IAPs) are composed of 70 amino acids termed BIR (baculoviral IAP repeat) discovered in genome of baculoviruses[25]. Cyclin D1 promotes cell proliferation and inhibits drug-induced apoptosis[26]. Apoptotic peptidase activating factor-1 (Apaf-1) is responsible for initiating apoptosis downstream mitochondrial damages. Loss of expression of Apaf-1 is associated with recurrences of cancer[27].

Apoptosis and Non-communicable Diseases

Apoptosis is a very important process in development of mammals. It deletes some organs during organogenesis, controls the number of cells, elimination of potentially dangerous cells. Mutation of FANCC, which has effects on the failure of apoptosis of haemopoietic stem cells (HSC) on exposure to growth factor deprivation causing DNA damage, is described in Fanconi anaemia (FA)[28]. Defective Fas-induced apoptosis can lead to autoimmune lymphoproliferative syndrome in human[29] which present with

reactive lymphadenopathies and autoimmune manifestations. Apoptosis plays a role in pathogenesis of gout. Delayed spontaneous and TNF-a induced apoptosis is observed in acute gouty arthritis[30] and renal damage is caused by apoptosis of renal tubules induced by uric acid[31]. Mutation of p53, a gene that blocks the damaged cell to enter cell cycle and directs towards apoptosis, leads to genetic instability characteristic of many cancer cells[2]. Apoptosis and the genes that control it have a significant effect on the carcinogenesis in the steps of initiation and progression, tumour biological behaviour such as metastasis and the morphologic types of cancer[22]. Mutation of caspase-8 gene is associated with advanced gastric cancer, hepatocellular carcinoma[32] leukaemia[33] and medulloblastoma[34]. Bcl-2, a protein that blocks apoptosis, is associated with B cell lymphoma and inhibitors of apoptosis proteins (IAPs) is found in MALT lymphoma[35]. Massive and selective apoptosis lead to neurodegenerative diseases and neural tube defects[36]. Mutation of neuronal apoptosis inhibitory protein (NAIP) was shown in spinal muscular atrophy[37]. Apoptosis of cardiomyocytes induced by non-encoding RNAMeg3 is associated with myocardial infarction and biomarkers of cell death such as MAC were studied in post-mortem cases in an attempt to identify the new diagnostic markers[38, 39].

Apoptosis and Infections

Bacteria-induced host cell death by apoptosis to enhance their replication and survival[40]. Intracellular organisms such as *L. pnemophilia*, and *Chlamydia* spp. survive in their host cells by inhibiting the macrophage apoptosis[41]. A mutant gene nuoG in *M. tuberculosis* decreased the ability to inhibit macrophage apoptosis and subsequently reduced the virulence of the organism[42]. Bacterial infection of central nervous system with group B Streptococcus lead to apoptosis of neurons in the presence of microgli[43]. Regeneration of neural tissue occurs after injury, but it is not sufficient to replenish the cells required for normal function. Apoptosis and necroptosis of neurons and glial cells occur in meningitis and it is responsible for neurological complications[43]. HIV 1 virus evades macrophage apoptosis by transcription of prosurvival genes via MAP2K1/ERK2 pathway[44]. Placental malaria usually complicated by poor neonatal outcome due to growth retardation is related to apoptosis-related mechanism[45]. Hepatitis B and C viruses cause chronic liver disease and parenchymal damage by escaping viral clearance by apoptosis[46]. Levels of Caspase-3, which triggers apoptosis, are low in *Mycobacterium tuberculosis* infected pulmonary alveolar macrophages rendering them to escape from apoptosis and create a more favourable environment for intracellular growth of bacteria[47]. Apoptosis is both qualitatively and quantitatively increased in lepra type 1 reaction and proposed to be one of the variables for high detection of the reaction[59] and lepromatous leprosy[48]. High level of nerve growth factor (NGF), a neurotrophin that causes apoptosis of Schwann cells and nerve damage, is correlated with nerve damage[49]. Pathogens like *Leptospira interrogans* escape phagocytosis by rendering apoptosis of macrophages through Fas-FasL/Caspase-8/-3 pathway[49].

Application in Therapeutics

Apoptosis is involved in tumourigenesis and other human diseases, it is also a centre of target for treatment. Inhibitors of apoptosis such as IAP may serve as a molecular target for specific anticancer therapy. IAPs can also be used as an indicator of prognosis after therapy as highly expressed in tumour cells[50]. IAP counter-reacts the high basal caspase activity of tumour cells selectively[51, 52]. Survivin, a member of IAP, is implicated management of cancer in different aspects such as targeting the tumorgenesis, neovascular angiogenesis, and immunotherapy target. Recombinant survivin protein used as a immunotherapy was effective as an adjuvant therapy in human melanoma cells[53, 54]. Some advances have been made to focus on TNF-related

apoptosis-inducing ligand (TRAIL) for cancer treatment[55]. Agonistic antibodies to TRAIL receptors can induce apoptosis of the tumour cells. Antiapoptotic- Bcl-2 family proteins are implicated in indicators of chemotherapy resistance in prostate cancer and other solid cancers and haematological malignancies[56]. Parenchymal cell death in diseases is a major concern in treating many diseases. Caspase-8, after activation by caspase cascade, is responsible for tumour cell motility by acting on focal adhesion complexes[57]. Evidence showed that pan-caspase inhibitors have profound effect on acute TNF- and Fas/FasL mediated apoptosis in hepatocytes and caspase mediated metastasis[57]. Short term uses of inhibitors of apoptosis are implicated in conditions such as ischaemic stroke, spinal cord injury, reperfusion injury and organ transplant. Arthritis, Alzheimer's disease, and Huntington's disease may benefit from their long-term usage[58]. Anti-angiogenic cancer therapy targets the vascular endothelial cells by inducing the Fas-ligand and initiating the apoptosis cascade[59]. Understanding the underlying mechanisms such as MAP2K1/ERK2 pathway and host cell K+ channels inhibition helps to design effective antiviral drugs for HIV and HCV[44, 46].

CONCLUSION

Apoptosis, programmed cell death, is an essential process in development, control of immune system and tissue homeostasis in living. In addition to its physiological functions, it also plays a role in aetiology of tumour formation and in the mechanism to reduce tumour cell burden in cancer caused by apoptosis of tumour cells. However, there are still some controversial theories on the use of apoptosis modifying therapeutics. Attempts should be made to consider several therapeutic aspects of apoptosis to improve the patient's outcome.

CONFLICT OF INTEREST

The authors declare that they have no competing interests in publishing this article.

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