HUMAN PATHOLOGIES RELATED TO DEFECTIVE APOPTOTIC PROTEINS-MOLECULAR BASIS

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ABSTRACT: Research in histology, genetics, and molecular biology during the past many years has revealed that virtually all animal cells are armed with the genetic machinery to commit suicide. Under normal physiological circumstances, damaged cells are removed through a genetically programmed type of cell death. Aberrations in the regulation of genetically programmed cell death have been found to cause disease and deformity. Cell death, the ultimate result of cell injury, is one of the most crucial events in pathology, affecting every cell type and being the major consequence of ischemia (lack of blood flow), infection, toxins and immune reactions. In addition, cell death is critical during normal embryogenosis, lymphoid tissue development and normally induced involution and is the aim of cancer radiotherapy and chemotherapy. This article discusses role of apoptosis in pathologies of malignant and potentially malignant lesions, immune disorders, blood disorders and some neural disorders.

Key words: Apoptotic bodies, BCL-2, Caspases, MDM2 gene, Immunologic diseases

1. APOPTOSIS- INTRODUCTION

The term programmed cell death was introduced in 1964, proposing that cell death during development is not of accidental nature but follows a sequence of controlled steps leading to locally and temporally defined self-destruction. [1] During necrosis, the cellular contents are released uncontrolled into the cell's environment which results in damage of surrounding cells and a strong inflammatory response in the corresponding tissue.[2], [3] Apoptosis, by contrast, is a process in which cells play an active role in their own death. Light microscopic identification of apoptosis usually involves single cells or occasionally small groups of cells. The apoptotic cell shrinks and separates from its neighbors and
is surrounded by a halo-like clear space. The nuclear chromatin breaks up into irregular crescentic, beaded or nodular masses; small basophilic apoptotic bodies may be identified. The complete absence of normal cellular detail is due to protein denaturation and nuclear dissolution. Ultrastructurally necrotic cell initially swells because cell membrane volume control is lost; the cell then lyse; organelles disintegrate, and with nuclear dissolution, there is random DNA degradation. The cytoplasmic contents of lysed necrotic cells excite an inflammatory reaction. Ultrastructurally, apoptosis has a characteristic morphology: initially there is loss of cell junctions and specialized membrane structures, such as microvilli, with the formation of contorted surface protuberances (or) blebs. Condensation beneath the nuclear membrane is irregular, crescentic or bead-like, highly osmiophilic chromatin, which corresponds to cleavage of DNA into large fragments. The cell then breaks up into resistant, membrane bound fragments called apoptotic bodies.

2. APOPTOSIS AT MOLECULAR LEVEL

Important genes involved in cell cycle regulation are: c-myc, c-fos, c-jun, p53, many kinases and phosphatases. The major determinants of the "apoptotic phenotype" in lymphocytes are the levels of expression of Bcl-2, Bcl-xl, Fas and Fas ligand (FasL). Both apoptotic signaling pathways converge at the level of the specific proteases—the caspases (Figs. 1 and 2). There are 14 mammalian caspases which are synthesized as pro-enzymes, which usually undergo proteolysis and activation by other caspases in a cascade. Peptide caspase inhibitors can inhibit downstream caspase activation and subsequently apoptosis. In intrinsic or mitochondrial pathway, internal damage to the cell (e.g., from reactive oxygen species) causes protein, Bax, to migrate to the surface of the mitochondrion where it inhibits the protective effect of Bcl-2 and inserts itself into the outer mitochondrial membrane punching holes in it and causing cytochrome c to leak out. This binds to the protein Apaf-1 ("apoptotic protease activating factor-1") utilising the energy provided by ATP, thus forming apoptosomes. Caspases are activated, thus causing phagocytosis of the cell. In AIF pathway, apoptosis-inducing factor (AIF) is released from intermembranous spaces of mitochondria, binds to DNA, and then triggers the destruction of the DNA and cell death.

3. APOPTOSIS IN HUMAN PATHOLOGIES

Apoptosis is useful for normal physiological conditions, as in formation of the fingers and toes of the fetus, sloughing off of the inner lining of the endometrium at the start of menstruation, formation of the proper connections (synapses) between neurons in the brain. The majority of the developing lymphocytes die either during genetic rearrangement events in the formation of the antigen receptor, during negative selection or in the periphery, thereby tightly controlling the pool of highly efficient and functional cells and at the same time keeping lymphocyte numbers relatively constant. Programmed cell death is needed to destroy cells that represent a threat to the integrity of the organism. The process of apoptosis is controlled by a diverse range of cell signals, which may originate either extracellularly (extrinsic inducers) or intracellularly (intrinsic inducers). Extracellular signals may include toxins, hormones, growth factors, nitric oxide or cytokines, and therefore must either cross the plasma membrane or transduce to effect a response. These signals may
positively or negatively induce apoptosis. The binding and subsequent initiation of apoptosis by a molecule is termed positive, whereas the active repression of apoptosis by a molecule is termed negative. In multicellular organisms, homeostasis is maintained through a balance between cell proliferation and cell death. Abnormal regulation of apoptosis has been implicated in the onset and progression of many diseases. Many disorders can be classified based on whether they are associated with too much or too little apoptosis. During their pathogenesis, however, most apoptotic disorders feature too much apoptosis of one type of cell and too little elimination of another kind of cell.

3.1 Leukoplakia & Lichen planus

The leukoplakia lesions showed positive keratinocyte staining for P53, P21, MDM2 and BCL-2, and these positive cells were distributed in the basal and prickle cell layers. In contrast oral lichen planus lesions shows keratinocytes positive for P53, P21, MDM2 and BCL-2, that were distributed predominantly in the basal strata of the mucosa. [19] There was a prominent expression of FaSR/ FaSL in oral lichen planus, with a rather uniform distribution throughout the inflammatory cell infiltrate. This expression of Fas proteins was more abundant in the basal cell area compared to the suprabasal cell layer. Quantitative assessment of apoptosis in oral lichen planus done by Balvinder K. et al, given conclusion that, approximately apoptotic cell was detected per millimeter of basal layer, cell death increasing with lymphocytic infiltration. Epithelial cell proliferation did not correlate with apoptosis, BCL-2 expression was weak (or) absent in basal cells, and Bax was localized to upper prickle cells. The topographical distribution of keratinocytes expressing proteins involved in apoptotic signal transduction pathways reflects some aspects of pathogenesis of each lesion. The percentage of supra basal keratinocytes expressing BCL-2 and MDM2 in leukoplakia lesions were higher than those in Oral lichen planus. Topographical distribution of two oncoproteins, BCL-2 and MDM2 in leukoplakia suggest that leukoplakia has more potential for malignant transformation than oral lichen planus.

3.2 Oncology

Experimental studies, known that tumor cell apoptosis is regulated by numerous oncogenes and tumor suppressor genes. Apoptotic index (AI) is defined as the percentage of apoptotic cells bodies in the total number of tumor cells. According to Arends et al studies, tumors can have low AIs intermediate AIs, or high AIs. Tumors of a particular type had an AI that was restricted within narrow range. There is an increase in apoptosis from prostatic intra epithelial neoplasia to prosthetic carcinoma in which the AI correlates with Gleason grade. Increased apoptosis occurs as an anti hormonal effect during androgen ablation. Expression of the apoptosis inhibitor bcl-2 is associated with the emergence of androgen – independent prostatic carcinoma. Normal human melanocytes and melanocytes in benign nevi express BCL-2 and this correlates with minimal apoptosis. (Klein-Parker et al. 1994) malignant melanomas also have a low AI. BCL-2 expression is reduced or absent as these tumor acquire a more malignant phenotype and in metastases, correlating with the melanoma cells acquiring independence of Bcl-2 for survival. [6] The carcinogenic transformation is postulated to result from alterations of apoptotic signal transduction proteins in epithelial cells A recent hypothesis implicates malfunction in apoptotic signaling events as the cause of malignant changes in a variety of cell types. (Thompson C.B. 1995). This hypothesis is supported by the findings that several proteins identified in apoptotic signaling pathways regulate cell growth and cell division. Such apoptotic signaling proteins include tumor suppressive protein P31, and cyclin dependent kinase (CDK) inhibitor P21 and onco proteins MDM2 and BCL-2.[6] MDM2 onco-protein, originally cloned from a spontaneously transformed BALB/c mouse cell line, is a known inhibitor of P21 mediated transcriptional activation. The MDM2 genes possess P35 response elements, and inhibit P33 dependent cell cycle arrest and apoptosis. Similarly increased expression of BCL-2 has been reported in nasopharyngeal and prostatic carcinomas.
3.3 Auto-immune diseases

Physiologic regulation of cell death is essential for the removal of potentially auto reactive lymphocytes during development and for removal of excess cells after the completion of immune response. [16], [17] Failure to remove autoimmune cells that develops as a result of somatic mutation during an immune response can result in auto immune disease. Fas, molecule cell surface receptor, is believed to regulate cell death in lymphocytes. Two forms of hereditary autoimmune diseases have been attributed to alterations in Fas – mediated apoptosis. MRL- lpr mice, which develops fatal systemic lupus erythematosus by 6 months of age, have a mutation in Fas receptor. GLD mouse which develops a similar illness, has a mutation in the Fas ligand. Patients with systemic lupus erythematosus have elevated levels of soluble Fas, which may competitively inhibit Fas-FasL interactions. The resultant decreases in Fas mediated apoptosis may contribute to the accumulation of autoimmune cells in this disorder. Role of apoptosis in the development of rheumatoid arthritis, psoriasis, inflammatory bowel disease and autoimmune diabetes mellius is being investigated. [20] Alterations in the susceptibility of lymphocytes by apoptosis in vitro have been reported in several diseases.

3.4 Viral infections

The disruption of cell physiology as a result of viral infection can cause an infected cell to undergo apoptosis (B. Levine et al 1993). Apotosis prevents viral propagation by inducing death of infected cells. Cytotoxic T cells prevent viral spread by recognising and killing cells that present viral peptides in association with cell surface major histocompatibility complex (MHC) class I molecules (P.A. Henkart, 1994). These induce apoptosis either by activation of Fas receptor on the surface of the target cell or by introduction of proteases, such as granzyme B, which activate cell death program within cytoplasm. A number of viruses have developed mechanisms to disrupt the normal regulation of apoptosis within the infected cell. The EIB 10KD protein has been shown to block apoptosis directly in viral infections. The BHRF1 gene of Epstein – Barr virus and LMWS- HL gene of African swine fever virus both have sequence and functional similarity to BCL-2. P35 gene and the inhibitor of apoptosis gene (IAP) found in baculo viruses can inhibit apoptosis in response to a wide variety of stimuli. Pox virus appears to inhibit apoptosis by producing an inhibitor of the death effector molecule, interleukin IB (IL-IB) converting enzyme (ICE). (M. Miura et. al. Cell 75, 653: 1993) establishes a latent infection in B cells. The viral gene LMP-1 in Epstein Barr-virus, produced during latency specifically up-regulates the expression of BCL-2, thus preventing apoptotic pathways. The Cowpox gene cmrA is a member of the serpin family of protease inhibitors and acts as a specific inhibitor of ICE. The cmr A gene can inhibit apoptosis in response to a number of stimuli and has also been shown to be required to inhibit the development of an inflammatory response to virally infected cells.

3.4 A.I.D.S

Stimulation of the CD4 T-cells occurs by binding to the soluble viral product gp 120, results in the enhanced susceptibility of T-cells to undergo apoptosis. In vitro activation of mature T cells from asymptomatic, HIV infected individuals by polyclonal activators such as calcium ionophore induces apoptosis in a fraction of CD4+ T cells and CD8+ T cells. Specific cell death of CD4+ T cells is observed upon activation of T cells by major histocompatibility complex (MHC) class II dependent bacterial superantigens. In the absence of activating agents, high background amount of apoptosis occurs in cultured lymphocytes of HIV infected patients, which is not dependent on protein synthesis. The exl glycoprotein gp 120-gp 41 complex expressed on the surface of HIV infected cells induces a cell death program in surrounding cells by receptor activation. Additional mechanisms may contribute to programmed cell death induction in HIV infection including defects in activation signaling optimal activation of T cells requires T cell receptor stimulation and a second signal delivered from an antigen presenting cell. [21] Recognizing the importance of apoptosis in AIDs
pathogenesis may have dramatic consequences for the conception of new strategies of research and treatment for combating the disease. In order to prevent apoptosis, it is essential to dissect the subtle interaction of molecules involved in process.

3.5 Neurodegenerative disorders

Many neurological diseases are characterized by the gradual loss of specific set of neurons. Such disorders include Alzheimer’s disease, Parkinson’s disease, amyotrophic lateral sclerosis (ALS), retinitis pigmentosa, spinal muscular atrophy, various forms of cerebellar degeneration. The cell loss in these diseases does not induce an inflammatory response and apoptosis appears to be mechanism of cell death. Oxidative stress, calcium toxicity, mitochondrial defects, excitatory toxicity, deficiency of survival factors have all been postulated to contribute to the pathogenesis of these disorders. Over expression of BCL-2 decreases the neurotoxicity of each of these potential inducers of cell death (L.T. Zhong et al 1993). Retinal degeneration associated with retinitis pigmentosa occurs as a result of mutations in any one of three photoreceptor specific genes: rhodopsin, the subunit of cyclic guanosine monophosphate phosphodiesterase and the pheripherin gene. All three mutations lead to photoreceptor apoptosis. Apoptosis may be initiated in response to the accumulation of mutant proteins or as a result of the altered functional properties of the mutant proteins. Alzheimers disease is associated with increased apoptosis by progressive accumulation of β-amyloid peptide.

3.6 Cardio-haematopoietic disorders

Disorders of blood cell production, such as myelodysplastic syndrome and some forms of aplastic anemia are associated with increased apoptotic cell death within the bone marrow. [22] These disorders could result from the activation of genes that promote apoptosis in stromal cells or hematopoietic survival factors or the direct effects of toxins and mediators of immune responses. The regulation of hematopoiesis is influenced by a number of growth factors, including stem cell factor, erythropoietin colony stimulating factor, thrombopoietin. (R.A. Fleischman., 1993) Hematopoietic stem cells in which apoptosis is suppressed by overproduction of BCL-2, can differentiate in the absence of extracellular growth factors or cell division.

Apoptosis participates in the genesis and pathophysiology of cardiomyopathy, paraxysmal arrhythmias, or conduction disturbances, focal fibro-muscular dysplasia of small coronary arteries and arrhythmogenic right ventricular dysplasia. In ischemic cells of some cardiac disorders, cells in the centre appear to die rapidly as a result of necrosis while peripheral cells appear to die by apoptosis. Free radical production and increase in intracellular calcium act as potent inducers of apoptosis. Ultrastruturally, the death of cardiomyocytes that occurs during reperfusion, show all features of apoptosis.

4. TREATMENT PERSPECTIVES

Insight into the mechanisms of apoptosis leads to a surge in research into novel therapies for degenerative, neoplastic, and autoimmune disorders potential therapies fall into 3 broad categories.

1. Gene therapy (eg. Replacement of P53)
2. Injectable molecules targeted at the upstream modulators of apoptosis (eg. Growth factors (or) soluble FaSL).

For apoptosis based therapy to be feasible, therapeutic molecules must be delivered to and active only in specific target cells. Indiscriminate inhibition of apoptosis could lead to wide spread hyperplasia, and in appropriate promotion of
apoptosis might lead to undesirable tissue degeneration. Considering the complexity of apoptosis signaling combinational therapies that target multiple cell death molecules for activation or inactivation may offer the best hope of therapeutically modulating apoptosis.

5. CONCLUSION

Understanding the physiological process of apoptosis at the molecular level not only affords insights into diseases pathogenesis but also opens new avenues for developing diagnostic, prognostic and therapeutic tools. Understanding the molecular apoptotic pathway will allow the development of non-pharmaceutical therapies in future.

REFERENCES