APOPTOSIS - NEW CONCEPTS IN MOLECULAR MEDICINE

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Molecular medicine encompasses molecular components that determine cellular division, homeostasis, cellular behaviour, gene expression and interaction, and cell death. Conceptual as well as practical advances in molecular medicine are changing our understanding of disease pathogenesis and opening new avenues for therapeutic intervention.

Apoptosis/programmed cell death is a well conserved fundamental physiological mechanism of active self destruction/cell death, that is genetically governed and occurs throughout life span. It is an essential feature in both development and maintenance of multicellular animals. It provides an efficient mechanism for eliminating unwanted cells. The morphological changes that typify apoptosis from the pathological cell death i.e. necrosis are: cell shrinkage, collapse of nucleus, cell membrane blebbing, formation of apoptotic bodies and chromatin condensation. The cells are disposed off by phagocytosis by macrophages or neighbouring cells and there is no inflammation. In most cases cleavage of DNA at linker regions between nucleosomes resulting in an oligonucleosomal ladder is observed. Activation of a cascade of specific apoptotic genes, in turn, leads to the induction of a signalling cascade involving a Ca^{2+}/Mg^{2+} dependent endonuclease which is responsible for cleaving nuclear DNA. The DNA fragmentation in several cases can be inhibited by protein kinase C. Signalling can be modulated by intracellular Ca^{2+} level, protein phosphorylation and dephosphorylation, deprivation of growth factors, hormones and the expression of various growth related genes (1,2). In many instances protein and RNA synthesis inhibitors have been shown to block apoptosis.

Various factors both intrinsic as well as extrinsic such as radiation, virus, certain chemicals, hormones (dexamethasone), cytokines (Tumor necrosis factor alpha) can stimulate apoptosis. Removal of growth factor from factor dependent cells triggers apoptosis. Ceramide, a sphingolipid breakdown product is found to play a role in induction of apoptosis (3). Apoptosis occurs in diverse cell types, ranging from thymocytes to hormonally sensitive prostate and breast cells, immune T and NK cells, neurons etc (4). The reason for cell death to occur is different from one cell type to another as is the signalling mechanism. Apoptosis represses tumor formation whereas abnormal apoptosis can promote cancer. Diverse chemotherapeutic drugs kill sensitive cells by inducing apoptosis such as topoisomerase inhibitors, antimetabolites and alkylating agents that complex with DNA (5). Drugs that induce apoptosis can amplify the effects, of chemotherapeutic agents on resistant cells. Thus activation of apoptosis pathway serves as a common final pathway for most cytotoxic agents, irrespective of the primary mechanism of action.

Several genes have been found to modulate apoptosis, some genes induce and others inhibit apoptosis. Some of the genes found to modulate apoptosis are those previously shown to be involved in controlling mitosis, such as c-myc, c-jun, c-fos. It appears that cell proliferation and apoptosis may be subjected to coordinated but inverse regulation. TRPM-2 also acts as a mediator of apoptosis. P53 under various conditions may regulate the metabolic activity of the apoptotic pathway (6,7). Mutated P53 blocks apoptosis in growth activated cells. However bcl-2 acts as suppressor of apoptosis. The genetic basis for apoptosis and its antidote has come from embryological studies in the nematode C elegans. The evolutionary conservation between the nematode apoptosis suppressor gene i.e. ced-9 and mammalian bcl-2 underscores the importance of apoptosis as a basic cellular control mechanism (8). The bcl-2 encoded protein is overexpressed when bcl-2 juxtaposed to the Ig heavy chain gene in the t (14: 18), a gene arrangement associated with follicular lymphomas and in Hodgkins disease. Like the C elegans ced-9 gene product, the bcl-2 encoded protein blocks apoptosis

and enhances cell longevity. Abrogation of apoptosis may be a pivotal lesson in the emergence and or progression of certain malignancies. The molecular dissection of bcl-2 related pathway may allow design of specific therapies aimed at inactivating or circumventing this natural inhibitor of apoptosis. Bax heterodimerizes in vivo with bcl-2. A pre-set ratio of Bcl-2/ Bax appears to determine the survival or death of cells following an apoptotic stimulus. BCR-ABL expression inappropriately prolongs the growth factor-independent survival of CML myeloid progenitor and granulocytes by inhibiting apoptosis. Inhibition of BCR-ABL expression by antisense oligonucleotide reversed the suppression of apoptosis as well as the enhancement of survival. The decreased rate of apoptosis appears to be the primary mechanism by which BCR-ABL affects expansion of the leukemic clones in chronic myelogenous leukemia. Reopening the apoptotic cell death pathway by antisense approaches to suppress bcl-2 activity is a novel approach for follicular B cell lymphomas and can be used as an adjunct to existing therapies. Targeting these antisense molecules regionally would minimize the potential of solemic toxicity.

Poly ADP-ribosylation is a post translational modification of chromatin proteins. Poly ADPR Transferase enzyme, catalyzes the polymerization of NAD^+ onto nuclear proteins, in particular histones. This reconfigurates the chromatin structure in a way that transiently inhibits DNA metabolism (9). Extensive DNA damage can lead to lowering of intracellular pools of NAD^+ and eventually cell death, or poly ADP ribosylation may alter chromatin structure and might expose sites for endonuclease action or else directly activate the Ca^{2+}, Mg^{2+} endonuclease responsible for apoptosis. Thus poly ADP ribosylation may play a critical role in apoptosis.

Modulation of gene expression by gene therapy is currently undergoing rapid clinical development for prevention and therapeutic purposes. The development of clinically testable oligonucelotide constructs that focus their activation on tumor specific gene or their transcribed product and spare the normal counterpart is an exceedingly important direction for continued development. Use of antisense oligonucelotides which can selectively target and inhibit the net expression of the targeted tumor cell gene, tumor cell proliferation and survival, without affecting normal cells, are under preclinical development for brain tumors. But one must not rule out nonspecific effects. Many human cancers have mutation in p53. Its overproduction in some myeloid leukemias induces apoptosis. Gene therapy/transfecting p53 to stem cells with mutant p53 allele appears promising. c-myc antisense oligonucleotide is reported to block apoptosis.

The understanding of the molecular mechanisms will bring unprecedented opportunities to uncover the broad principles that govern the interplay between intrinsic and extrinsic factors that in turn modulate net gene expression and cellular functions of many cell types, for advances in detection, therapy and ultimately prevention of disease based on knowledge of specific molecules or interaction at the molecular level. New avenues for antitumor therapy besides cytotoxic therapy, now include agents that induce differentiation or apoptosis. The phenomenon of apoptosis may be fundamental to the understanding of malignancy and may underlie the effectiveness of chemotherapy as well as interferons. New therapies may arise from exploiting apoptosis using noncytotoxic pathways. Differentiation is an alternative to the induction of apoptosis. Malignant cells may be induced to differentiate into mature, non replicative cells through exposure to retinoids, interferons, low doses of cytotoxic drugs. Use of all-trans-retinoic acid showed induction of complete remission in patients with acute promyelocytic leukemia, even in drug-resistant patients and prevented the occurence of second primary carcinomas in patients with head and neck cancer, and with alpha interferon, produced response in cervical carcinomas. The important implication of apoptosis concept for medicine is clearly the possibility that cell deletion by apoptosis might be selectively and predictably controlled in disease states.

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