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Apoptosis in malignant diseases

Goran R. Brajušković

ABSTRACT

Apoptosis is a special type of cell death essentially different from necrosis in nature and biological significance. It is an active process of genetically regulated cell autodestruction and in most cases has a homeostatic function. Apoptotic cells may be characterized by specific morphological and biochemical changes. A great number of genes are known today, whose protein products take part in regulation of the apoptotic process. Apoptosis or programmed cell death has been implicated in a wide range of pathological conditions. Studies of the correlation of programmed cell death with proliferation and the multistage carcinogenesis process are in the focus of modern research. Mutations and deletions of apoptotic genes play important roles in carcinogenesis, tumor growth, and tumor regression. This article reviews the current knowledge on mutations of apoptosis genes involved in pathogenesis of human cancers. Finally, we have recently summarized achievements in cancer therapy with a focus on the apoptotic genes.

KEY WORDS: *Apoptosis; Neoplasms; Gene Therapy*

Institute of Pathology, Military Medical Academy, Belgrade, Serbia & Montenegro, Address correspondence to: Assist. Prof. Goran R. Brajušković, Ph.D, Institute of Pathology and Forensic Medicine, Military Medical Academy, 11002 Belgrade, Crnotravska 17, Serbia & Montenegro, E-mail: brajuskovic@hotmail.com; The manuscript was received: 04.03.2005, Provisionally accepted: 17.03.2005, Accepted for publication: 04.04.2005

APOPTOSIS IN MALIGNANT DISEASES

What is apoptosis - from the Greek: falling off

Death, along with growth and differentiation, is a critical part of the life cycle of a cell (1). *Apoptosis* was the name given by Kerr, Wyllie and Currie in 1972, to morphologic change culminating in cell death by process clearly distinct from necrosis (2,3). Morphologically, apoptosis is characterized by nuclear and cytoplasmic condensation of single cells (shrinkage) followed by loss of the nuclear membrane; fragmentation of nuclear chromatin, and subsequent formation of multiple fragments of the nuclear material and cytoplasm - apoptotic bodies (4,5). Recent data suggest that apoptosis or "cellular suicide" is a set of ordered events that enables the selective removal of cells from tissue and is essential for homeostasis and proper function of multicellular organisms (6). Cells may also be eliminated by a number of other mechanisms including: autophagy, mitotic catastrophe, dark cell death, paraptosis, chondroptosis, autoschisis, etc. It is important to recognize that these types of cell death differ not only morphologically, but biochemically as well (7). Biochemical features associated with apoptosis include internucleosomal cleavage of DNA, leading to an oligonucleosomal "ladder", phosphatidylserine externalization and proteolytic cleavage of a number of intracellular substrates (8). At the molecular level, the apoptotic cell death machinery forms a complex cascade of ordered events, controlled by the regulated expression of apoptosis - associated genes and proteins. The key components of this self - destruction machinery are members of the caspase family. More than 10 caspases have been identified. Some of them (e.g., caspase 2,8,9,10, and, probably, -11) are involved in the initiation of apoptosis others (e.g., caspase 6 and 7) execute the death order by destroying essential proteins in the cell. The caspase 3 is speculated to have a crucial role in apoptosis and is responsible for the cleavage of many critical cellular substrates, leading to characteristic morphological changes in apoptosis such as chromatin condensation, nucleosomal DNA fragmentation and formation of apoptotic bodies (7,9,10).

Two major apoptosis pathways have been identified (11). One major apoptotic pathway involves cell surface death receptors that transmit an apoptotic signal on binding of a specific death ligand. The largest known family of death receptors is represented by tumor necrosis factor receptors. Upon ligand binding an intracellular "death domain" (DD) of the receptor interacts with a homolog domain in an adaptor protein which recruits caspases. Fas (CD 95, Apo-1) is a transmembrane receptor protein. Binding of its natural ligand, Fas ligand (FasL) triggers apoptosis. The apoptosis signal is transmitted via intracellular DD of Fas. That domain interacts with homologous motif in the adaptor protein FADD (Fas-associated DD protein). FADD protein also contains a DED domain (death effector domain), and this, in turn, recruits pro - caspase 8, which binds via its DED. Via aggregation of two or more procaspase 8 molecules, procaspase 8 becomes activated. Active caspase 8 can then go on to trigger the apoptotic caspase cascade (8,12).

A second major apoptotic pathway involves mitochondria. A great number of mitochondrial proteins take part in the regulation of apoptosis (13). Cytochrome c, a component of the mitochondrial electron transfer chain, initiates caspase activation when released from mitochondria during apoptosis (14). Cytosolic cytochrome c forms an essential part of the apoptosome, which is composed of cytochrome c, Apaf-1 and procaspase 9. Only the caspase 9 bound to the apoptosome is able to efficiently cleave and activate downstream executioner caspases such as caspase 3 (Figure 1) (13,14).

The mitochondrial pathway is regulated by members of Bcl-2 protein family. The principal mechanism by which Bcl-2 family proteins regulate apoptosis is probably by controlling cytochrome c release. Some of these proteins (such as Bcl-2, Bcl X_l) are anti-apoptotic while others (such as Bax, Bad and Bak) are proapoptotic. While the anti-apoptotic proteins work to prevent cytochrome c release from mitochondria, the proapoptotic members play a major role in initiating cytochrome c release (8).

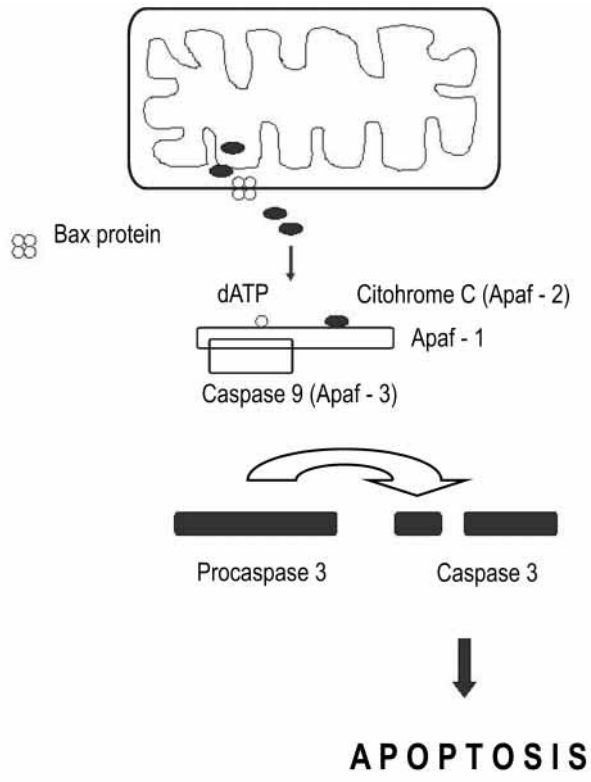

Figure 1. Induction of apoptosis via the mitochondrial pathway

It is known that the Bcl-2 protein plays a role in the promotion of cell survival and in the inhibition of apoptosis. The Bcl-2 protein prevents cytochrome c release (15,16). Bcl-2 - associated protein X (Bax) was the first proapoptotic protein isolated by co-immunoprecipitation with Bcl-2 which is primarily localized to the cytosol. Death signals direct Bax protein from the cytosol to the mitochondrial outer membrane. In the same time, Bak protein, which resides in the mitochondrial membrane in an inactive form, will be activated. Together they allow release of cytochrome c from the intermembrane space of the mitochondria (11,17,18). Early experiments showed that over expression of anti-apoptotic proteins Bcl-2 or Bcl X_l could inhibit the proapoptotic activity of Bax. The mechanism by which Bcl-2 blocks apoptosis is by preventing Bax relocation to the mitochondria (11,17). Korsmayer and his associates showed that Bcl-2 and Bax proteins could make homo- and heterodimers. The antiapoptotic effects of Bcl-2 protein is based on its possibility to bind Bax protein in the heterodimer form, and in that way blocks making Bax/Bax proapoptotic homodimers. The ratio of Bcl-2/Bax represents a cell autonomous rheostat that predetermines a cells life or death response to an apoptotic stimulus (19).

The p53 protein is a nuclear protein that functions as a transcription factor capable of regulating a range of downstream genes. The p53 tumor suppressor limits cellular proliferation by inducing cell cycle arrest and apoptosis in response to cellular stresses such as DNA damage, hypoxia, and oncogene activation. Many apoptosis-related genes that are transcriptionally regulated by p53 have been identified (12,20).

Apoptosis and tumorigenesis

Apoptosis has been implicated in a wide range of pathological conditions. Increased apoptosis has been associated with acute ischemic diseases, neurodegenerative diseases such as Alzheimer's and Parkinson's diseases, AIDS, diabetes and hepatitis. Decreased apoptosis is involved in cancer and autoimmune disorders (21).

Malignant transformation is a process where genetic occurrences are accumulated. In the recent past, molecular oncologists focused their studies primarily on the cellular pathways controlling proliferation. Neoplastic disease was typically envisaged as resulting from defects in these pathways leading to excess cell division (22). During the last 12 years, however, overwhelming evidence has accumulated which suggests that the other side of the balance - the rate of cell death - is just as important. Studies of the correlation of programmed cell death with proliferation and the multistage carcinogenesis process are in the focus of modern research. Mutations and deletions of apoptotic genes play important roles in carcinogenesis, tumor growth, and tumor regression (12). Alterations of genes along the Fas-mediated apoptosis pathway have been reported in many human cancers. Mutations of Fas gene are rare in primary gastric MALT lymphomas (5.6%) but occur in a subset of primary gastric diffuse large B-cell lymphomas (14.3%) and suggest that these mutations contribute to the pathogenesis of gastric lymphomas by rendering lymphocytes resistant to apoptosis (23). Fas gene mutations were also detected in Mycosis fungoides (24); Hodgkin's disease (12); bladder cancer (12); plasmocytomas (25), etc.

Genetic alternations of the Bcl-2 gene have been identified in hematologic malignancies (12). The pathogenesis of follicular lymphoma and chronic lymphocytic leukemia involves the dysregulation of the Bcl-2 protein (26). The Bcl-2 was first identified at the chromosomal breakpoint of t(14;18) in a human leukemia line and was later shown to be a common event in follicular lymphoma (5). The clinical significance of this feature is still controversial. Current analysis of Bcl-2 expression by multicolor flow cytometric assists in the diagnosis of follicular lymphoma in lymph node and bone marrow (27). Inhibition of apoptosis and upregulation of the Bcl-2 protein are key elements of the pathophysiology of chronic lymphocytic leukemia (28). Aberrant expression of Bcl-2 protein in CLL is associated with poor response to chemotherapy and decreased overall survival (29).

Bax mutations were detected in about 50% of human colon carcinomas with a microsatellite mutator phenotype (12). In one third of breast adenocarcinomas reduced Bax expression has been detected. Decreased Bax expression associated with increased expression of the anti-apoptotic family members has been shown in tumors in colon cancer patients (11). The p53 tumor suppressor proapoptotic protein as transcription factor prevents tumor developments through induction cell cycle arrest and apoptosis. Around half of all human tumors carry p53 mutation. From a clinical standpoint, mutations in p53 are usually a poor prognostic indication in a variety of tumor types including gastrointestinal, hematopoietic, breast, and genitourinary cancers (22). Other tumors that frequently contain p53 mutations (melanoma, lung cancers, bladder and prostate cancer) often respond poorly to radiation or chemotherapy (12). In general, p53 mutations are more frequent in aggressive disease and are associated with poor survival (30).

Apoptosis and cancer therapy

Experimental studies, both *in vitro* and *in vivo*, have shown the capability of antineoplastic agents to induce the process of apoptosis. Studies conducted up to now on various models systems (acute lymphoblastic leukemia, lymphomas, mycosis fungoides, breast cancer, lung cancer, etc.) have shown that the ability of antineoplastic agents to induce apoptosis of neoplastic transformed cells represent a positive prognostic parameter in the treatment of malignity (21,31). Also, cytotoxic therapy and immunotherapy of leukemia and lymphoma predominantly mediates cell death through induction of apoptosis (32). Last ten years, resistance to antitumor treatment is also considered from the viewpoint of the loss of the capability of a certain antineoplastic agent to induce apoptosis of malignant cell (33,34). Many investigators are starting to exploit the recent discovers about apoptosis to develop new treatments. Gene therapy is still experimental. It is being studies in clinical trials (research studies with humans) for many different types of cancer and for other diseases. Cancer gene therapy (based on genetic differences between normal and transformed cells) has interest in developing approaches to inactive oncogenes or replace nonfunctioning tumor suppressor genes. Transfer of various tumor suppressor genes directly to cancer cells has been demonstrated to suppress tumor growth via induction of apoptosis and, in some cases, with evidence for bystander effects. Various studies also have shown that combination of tumor suppressor gene therapy with conventional anticancer

therapy can yield synergistic therapeutic benefits (35). Several therapy strategies have been designed to restore p53 function in human tumors, including p53 gene therapy, reactivation of mutant p53 and activation of wt p53 by inhibition of the p53 antagonist MDM 2 (30). The first work with p53 gene therapy was conducted by Jack Roth and colleagues. They reported the results of the first clinical trials using p53 delivered by a retrovirus vector to treat patients with non-small-cell lung cancer who had failed other treatments. The recombinant adenovirus was administered intratumorally and caused no toxic side effects up to 5 months later. Wild - type (wt) p 53 was detected in lung biopsy by in situ hybridization and PCR amplification, and apoptosis was increased in posttreatment biopsy samples (36). Furthermore, combined treatment of head and neck cancer with p53 gene replacement and chemotherapy achieved a high proportion of complete response. By 6 months, none of the responding tumors had progressed, whereas all tumors treated with chemotherapy alone had progressed (12).

Because many tumor suppressors are currently known to function in the mediation of apoptosis, with p53 being a prime example, another strategy for cancer gene therapy has been designed to introduce directly proapoptotic genes into tumor cells. Two groups of apoptosis inducing genes have been extensively studies: the proapoptotic members of the Bcl-2 gene family and the Fas death receptor members (37). Several laboratories have demonstrated that gene transfer of FasL prevented growth and induced tumor regression *in vivo*. The finding that gene transfer of FasL is able to generate a potent apoptotic response suggests that molecular genetic intervention may be successful and must be further elucidated and pursued (9). Ad-Bax (adenoviruses with Bax gene) infection of human lung cancer cell lines, both *in vitro* and *in vivo* caused apoptosis that was independent of endogenous p53 status. Interestingly, lung cancer cell line resistant to Ad-p53 were susceptible to apoptosis following Ad-Bax (37).

Over expression of anti apoptotic Bcl-2 family members can promote tumorigensis and chemoresistance, suggesting that functional inhibition of these protein might be lethal to cancer cells (5). Bcl-2 has proved a provocative target for therapeutic intervention with the aim of inducing apoptosis of malignant cells. Reduction of Bcl-2 level using antisense treatment or intracellular expression of anti-Bcl-2 antibody may render tumor cells susceptible to drug induced apoptosis (9). Bcl-2 antisense oligonucleotide therapy in patients with non-Hodgkin's lymphoma showed first positive effect. Recent results of a Phase I trial of a 14-day continuous infusion of an 18-mer phosphorothioate oligonucleotides to first six codons of the Bcl-2 open reading frame (G3139) have shown minimal toxicity ands some evidence of antitumor activity. This oligonucleotide has also been used in phase I and II trials in combination with dacarbazine for the treatment of melanoma resulting in 40% reduction in Bcl-2 protein levels and an increase in tumor cell apoptosis as well as a considerable increase in the apoptotic response to dacarbazine (38,39). The reduction in Bcl-2 gene expression used antisense therapy was associated with increase in Bax gene and increased apoptosis in CLL cells (40). Caspases seem to be the key executioners of apoptosis, and thus, represent an attractive target for therapeutic intervention. Modulation of caspase activity is currently the focus of both commercial and academic research. The team of company "Maxim Pharmaceuticals" created "small molecule" caspase activator called MX-2060. As a potential anticancer agent, MX-2060 treated in human cancer xenograft animal models (39).

The near future of cancer therapy will most likely rely on the combined application of apoptosis - sensitizing strategies described above, and conventional radio- and chemotherapy. Unfortunately, the fundamental problem still remains - How do we selectively activate apoptosis in an intrinsically resistant tumor cell?

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