Introduction

Apoptosis is a type of physiological cell death that occurs during development, normal tissue homeostasis or as a result of different cellular insults.

Apoptosis in development is often referred to as “programmed cell death”.

Apoptosis continues throughout the life in order to maintain tissue homeostasis, that is, a balance between cell proliferation and cell death (15).

Apoptosis is a major barrier to oncogenesis (15) and this concept is now widely accepted (2).

The two best known tumor suppressor gene products in cancer are p53 protein and pRb (retinoblastoma protein) (16). The p53 tumor-suppressor protein has the ability to prevent cells from becoming malignant by inducing growth arrest (18, 19). It is major key regulator of apoptosis and cancerogenesis (3, 10, 13, 15, 20).

The term “apoptosis” was introduced by J.F. Kerr et al. in 1971 to describe the morphological changes of this characteristic form of cell death. These changes involve chromatin condensation, cytoplasmatic and nuclear blebbing, eventual cellular demise without loss of membrane integrity and digestion of the resulting small vesicles by macrophages (15).

The blebbing cells reminded the researchers of leaves that are scattered around a tree in autumn. Nowadays, the Greek word “apoptosis” not only describes the falling of leaves in the autumn, but also the process of a novel form of cell death. Apoptosis has been called a “disassembly” of the cell because everything comes apart. This type of death is therefore different from classical necrosis, which leads to disruption of the cell membrane, necrotic cell bursts and releases of its contents info the surrounding tissue but organelles, such as the mitochondria or the nucleus, remain intact throughout this process (15).

Apoptosis is triggered by a number of factors which act through two major apoptotic pathways: extrinsic and intrinsic pathway (6, 7). These factors include some particular receptors on the cell surface (referred to as “death” receptors) and the components of extrinsic and intrinsic pathways, regulated by the members of a family of proteins called Bcl-2 (promoting mitochondria changes with release of cytochrom-c) (5, 6, 7, 8). Whichever pathway is induced, both lead to activation of the members of highly selective proteases referred to as “caspases” (15). That is why the extrinsic pathway is otherwise known as the “death receptor pathway” and the intrinsic pathway - as the “mitochondrial pathway”. Both extrinsic and intrinsic pathways are activated by tumor-suppressor p53 protein (described later).

The extrinsic apoptotic pathway (Fig. 1)

The extrinsic apoptotic pathway is initiated by the members of tumor necrosis factor (TNF) family including TNFα, FAS/CD95 ligand (FASL) and APO2 ligand (APO2L). They activate their corresponding “death” receptors TNF/NGF receptor family such as TNFR1, FAS/CD95 and APO2 (15). The cell-surface receptor FAS is a key component of the extrinsic pathway. FAS links with a protein FADD (Fas associated death domain) to form a complex called DISC (Death-inducing signaling complex), then DISC activates the pro-caspase 8 and caspase 8 which in turn induces activation of caspase 3 and caspase7 causing apoptosis.

Induced TNFR1 and APO2 also promote cell death through caspase 8.
Caspase 8 can activate the proapoptotic protein BID which is a link between the extrinsic and intrinsic apoptotic pathway (15, 16).

The intrinsic/mitochondrial apoptotic pathway (Fig. 1)
The intrinsic apoptotic pathway is also called “mitochondrial pathway” because it is associated with the release of cytochrome-c protein and other proteins from the mitochondrial intermembrane space into the cytoplasm as a result of activation of proapoptotic members of Bcl-2 family proteins (BID, BAX, BAK and others – described later) which are regulators of mitochondrial outer membrane permeability (5, 8, 15). Activated proapoptotic members of Bcl2 family neutralize the antiapoptotic members of the same family which otherwise inhibits cell death by preventing the release of cytochrome-c from mitochondria (8, 17). Once released into the cytoplasm cytochrome-c binds to an adaptor protein Apaf 1 (Apoptotic protease-activating factor 1) to create “apoptosome”, a complex that activates procaspase 9 (5, 8, 15).

In the presence of nucleotide dATP/ATP caspase 9 is activated, which in turn activate caspase 3 and caspase 7, leading to widespread activation of other caspases (“caspase cascade”) and cell death (8). In vertebrates the majority of apoptosis proceeds through the intrinsic pathway (15).

Some proteins, inhibitors of IAPs (proteins-inhibitors of caspases) such as DIABLO /Smac and Omi/ HtrA2 are also released, as well as other important proteins such as AIF (apoptosis-inducing factor) and endonuclease G (Endo G). They can contribute to apoptosis, even in the absence of caspase activation, creating caspase-independent pathways of cell death (15).

Bcl-2 family of proteins – regulator of mitochondrial outer membrane permeability
Mitochondrial outer membrane permeability is a key process involved in intrinsic apoptotic pathway.

Regulator of this process is the Bcl-2 (B-cell lymphoma 2/ family of proteins) (5, 15, 16).

The family comprises both antiapoptotic or prosurvival members (with inhibitory effects on apoptosis) and proapoptotic members (which block the effects of inhibitors) (8, 12).

The balance between them determines whether or not a cell commits apoptosis (15). The Bcl-2 family members can be subdivided according to their structure and function.

Antiapoptotic members may be divided into two subclasses: Bcl-2, Bcl-XL and Bcl-w and another comprising Mcl-1 and A1 (15).

Proapoptotic members are subdivided to BAX subfamily (which includes BAX, BAK and BOK) and BH3-only subfamily (which includes BID, BIM, BAD, BIK, BMF, PUMA, NOXA and HRK) (15). BH3-only proteins kept inactive in healthy cells.

During apoptosis proapoptotic BH3-only proteins neutralize the activity of Bcl-2 (16). Bcl-2 is an integral membrane protein
activate each other through multiple feedback loops. In extrinsic apoptotic pathway caspase 8 activate the executioner caspases 3 and 7 (6). Caspase 3, also referred to as “apopain” is the major effector caspase. Caspase 8, with sense activation of “death” receptors, cleaves the proapoptotic protein BID which is a link between the extrinsic and intrinsic apoptotic pathway. Caspase 9 also activates caspase 3 to promote apoptosis. The activity of caspases is essential for both extrinsic and intrinsic pathways (15, 16). Inhibitors of caspases are the members of antiapoptotic IAP (Inhibitors of apoptosis proteins) family. To date 8 human IAPs have been identified /15/. These proteins are able to inhibit apoptosis by direct binding and inactivation of specific caspases (e.g. caspase 9, caspases 3 and 7 and others).

**Tumor suppressor p53 protein – what is it like?**

Tumor suppressor p53 protein, also known as “TP 53” (tumor protein 53), “phosphoprotein p53” and “antigen NY-CD-13” is a product of the tumor suppressor gene p53 located at the short arm of the human chromosome 17 (17p13.1) (10). The location on mouse is chromosome 11, in rat-chromosome 10 and in dog-chromosome 5. The name is due to its molecular mass: it runs as a 53 kDa (kilo Dalton) protein (10). p53 is a nuclear phosphoprotein consisting of 393 aminoacids. In normal cells, p53 is inactive bound to the protein Mdm2 (Hdm2 in humans) which prevents its apoptotic action (10). p53 is the founding of a family of three proteins that also includes p63 (localized at 3q27) and p73 (localized at 1p36), but p53 is the sole member of the family with tumor suppressor activity /15/. p53 protein can arrest cell proliferation at the G1/S cycle through the activation of another protein p21 (also called WAF1), G2/M cycle through activation of 14-3-3 protein, reprimo and b99 and apoptosis (3, 9, 10). Whereas p53 alone is sufficient for induction of p21, the induction of some apoptotic proteins, involved in extrinsic and intrinsic apoptotic pathways, requires p53 together with p63 and p73 (1). Loss of p53 function abolishes growth arrest or apoptosis, which prevents cells properly responding to stress or damage (14, 16, 20). It is believed that the most important function of p53 in protecting us from cancers is its ability to induce cell death (16). Numerous mouse models have shown a critical role for p53 (and regulators of p53) in tumor suppression (15). Due to its tumor suppressor effect p53 has been called “the guardian of genome” (9). Its loss leads to genomic instability and increased mutagenesis (14).

The p53 gene is one of the most frequent targets for mutation in human tumors (14, 15, 20). The resultant protein p53 has an abnormal structure and cell growth can proceed unchecked.

p53 is mutated in about 50% of human cancer (16). Worse, mutant p53 itself can inhibit normal p53. Mutation in p53 can be inherited via the germ line, as in the case with Li-Fraumeni syndrome, where families are predisposed to different types of cancer, including breast cancer, bone, soft tissue, brain, adrenal and colorectal carcinoma, or less frequently melanoma (16). Interestingly, in families affected by this syndrome, the p53 mutations are transmitted from one generation to the next and the cancers generally occur at an increasingly earlier age (16).

How does the p53 protein induce apoptosis? (Fig. 1)

The status of p53 is drastically altered when cells are exposed to stress. Various types of stress lead to p53 stabilization and activation. The signaling pathways involved in p53 activation...
are complex and most certainly still incompletely understood. Activation of p53 is regulated by a number of mediators (ARF, ATM, ATR, Chk1, Chk2 and others). Two of the signaling pathways include ARF-Mdm2-p53 pathway and ATM-ATR-p53 signaling pathway: the first in response to oncogenic stress and the second in response to DNA damage (genotoxic stress) (15).

A. Activation of p53 by ARF-Mdm2-p53 signaling pathway

Oncogenes (from Greek “oncos”, a tumor) such as MYC, RasV12, E2F-1 and others can mediate apoptosis through its indirect activation of p53 via an important tumor suppressor protein ARF (p19ARF in mouse or p14ARF in humans) (11).

ARF is a nucleolar protein encoded by a transcript that is read from a same exon that the one encoding INK4a (INK4-inhibitory protein of cyclin-dependent kinase 4) but in Alternative Reading Frame (hence ARF) (11). Transcription of ARF is induced by MYC, E2F1 and others. Unlike p53, ARF is not induced by DNA damage (16).

ARF expression is repressed by repressor proteins such as Twist, TBX2, TBX3, Bmi1 and others (for example Twist is overexpressed in rhabdomyosarcoma).

In normal cells, p53 is inactive, bound to the protein Mdm2 (Hdm2 in humans) which prevents p53 protein in response to oncogenic stress.

ARF activates p53 by sequestration of Mdm2 and inhibition of Mdm2 to induce cell cycle arrest or apoptosis. The levels of p53 are kept in check by a balance between ARF and Mdm2 (15).

B. Activation of p53 by ATM-ATR-p53 signaling pathway

Activation of p53 in result of DNA damage, induced by genotoxic stress (e.g. irradiation-X ray, ultraviolet-UV, cytotoxic chemotherapeutic drugs and carcinogens) is regulated via kinases ATM and ATR (14, 19).

This normally results in phosphorylation of 53, which causes disruption of its interaction with Mdm2 and activation of p53 (18).

ATM (Ataxia telangiectasia mutated) and ATR (ATM- and Rad3-related) are members of phosphatidyl – inositol-3-kinases (PI3K) family. ATM activates various targets including another kinase CHK2. ATM can also activate the proapoptotic BH3-only protein BID which can mediate both growth arrest in its full form and apoptosis in its truncated form (tBID) (8).

The mechanism by which activated p53 exerts its apoptotic effects appear to be multifactorial, very complex and incompletely understood. Once activated by the stresses p53 induces or inhibits the expression of more than 150 genes (9, 10).

One of mechanism by which p53 promotes apoptosis includes proapoptotic proteins which are the major p53-transcriptional targets (8).

When overexpressed p53 activates PUMA (called also Bbc3), a member of proapoptotic BH-3 only family, which was found to play a key role in p53-dependent apoptosis. PUMA localizes to mitochondria where its overexpression has been shown to bind to all prosurvival Bcl-2 family proteins (15). Proapoptotic BH-3-only protein NOXA binds only to antiapoptotic Mcl-1 and A1 (15).

Other transcriptional targets of p53 are the proapoptotic proteins BID and BAX. BID in its truncated form tBID neutralize the activity of antiapoptotic Bcl-2 and activate BAX and BAK, which play a key role in the intrinsic apoptotic pathway (8). p53 target is also the proapoptotic protein BAD with activation result in BAX releasing and translocation to mitochondria (8). p53 activates the proapoptotic protein BID and BMF, which binds to antiapoptotic Bcl-2, Bcl-XL and Bcl-w. The p53 protein directly induces Apaf-1 expression (5). The p53 protein has also been shown to antagonize the proapoptotic proteins Bcl-2 and Bcl-XL at the mitochondrial outer membrane by directly binding to them (15). All these facts come to stress the important role of p53 protein in intrinsic apoptotic pathway.

The p53 protein also activates the “death” receptors (belonging to the TNF-R family) and directly caspase 8 – both components of extrinsic apoptotic pathway.

Aside from transcriptional activation, p53 can downregulate the transcription of genes that inhibit apoptosis (for example such as IAPs member Survivin) (15).

Conclusions

The p53 tumor suppressor protein can intervene at every major step in apoptotic pathways: from the extrinsic “death” receptor signaling through the proapoptotic protein BID to the intrinsic mitochondria pathway culminating in direct caspase activation and apoptosis. At present p53 protein has been recognized as a key regulator of apoptosis and cancerogenesis. All factors that neutralize the function of p53 (for example via overexpression of high levels of Mdm2 protein) or activate antiapoptotic components of apoptotic pathways (such as Bcl-2 proteins) or provoke overexpression of inhibitors of apoptosis (such as IAPs or other inhibitors), greatly facilitate tumor development and progression.

Since defects in apoptosis are likely to be universal lesions in tumor progression, restoring or activating apoptosis in tumors is an active area of cancer research (15). Preclinical trials have validated the antitumor efficacy of this approach and clinical trials are currently ongoing for various apoptosis – activating strategies (15).

REFERENCES