THE ROLE OF p53 AND CASPASE IN REGULATION APOPTOSIS ACTIVITY

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ABSTRACT

Apoptosis, or programmed cell death, is a common property of all multicellular organism. Apoptosis occurs normally during development and aging and as a homeostatic mechanism to maintain cell populations in tissue. Apoptosis also accurs as a defense mechanism such as in immune reactions or when cells are damaged by disease or noxious agents. Dysregulation of apoptosis leads to a variety of human pathologies including cancer, autoimmune disease, and neurodegenerative disorders.

Introduction

Exposure to various chemical agents can result in cell damage or death. Whether a cell survives or dies the presence of a chemical insults is often determined by proliverative status, repair enzime capacity, and the ability to induce proteins that either promote or inhibit the cell death process. Much of our understanding about cell death and its involvement in disease relates to its role in regulating tissue turnover or homeostasis. Tissue homeostasis occurs when a balance is achieved between cell renewal and cell death so that no net change in cell number is present. Normal homeostatic cell deletion is controlled, at least in part, by apoptosis.

Apoptosis, a biochemically and morphologically distinct forn of cell death, is an active process associated with cell shrinkage, nuclear and cytoplasmic condensation, release of cytochrome c from mitochondria, caspase activation, plasma membrane blebbing, phosphatidylserine extemalization on the plasma membrane, DNA fragmentation, and the formation of apoptotic bodies that can be taken up and degraded by neighboring cell (11). Apoptosis associated nuclear condensation is usually accompanied by the activation of nucleases that first degrade chromosomal DNA into large 50 to 300 kb subunits and then into smaller units of ~180 pairs (11).

p53 is a master regulator

The ability of p53 to control passage through the cell cycle (in G1 and in G2) and to control apoptosis in response to abnormal proliverative signals and stress including DNA damage is considered to be important for its tumor suppression function (1).

p53 is a transcription factor that establishes programmes for apoptosis, senescence, and repair in response to a variety of cellular stresses, including DNA damage, hypoxia or oncogenic avtivation, and nutrient deprivation, that contribute to tumor suppression either by preventing or repairing genomic damage or through the elimination of potentially oncogenic cells from the proliferating population. Known transcriptional targets for p53 in promoting apoptosis include various pro-apoptotic Bcl2 members, including *puma*, *noxa*, *bid*, and *bax*, as well as component of death-receptor signalling (for example, DR5, Fas/CD95), the apoptotic-effector machinery (for example, caspase-6, Apaf-1, PIDD) and others with less well-defined roles (for example, PERP, PML, p53AIP). Additionally, p53 might directly facilitate cytochrome *c* release (6), (2).

p53 protein becomes stabilized upon its phosporilation by ATM/Chk2 or ATR/Chk1/Casein-kinase 1 and accumulates in the nucleus. There, p53 transcribes a network of genes that initiate DNA repair, growth arrest, senescence, and/or transcribing its negative regulator, the E3 ligase, Mdm2 which ubiquitinates p53 and targets it to the proteasome for degradation (12).

Two main forms of cellular stress lead to activation of p53 for it to function as a transcriptional activator. The first of these, and probably the best studied, is the one initiated by DNA damage which may be caused either by ionizing radiation or by chemotherapeutic drugs, ultraviolet light or protein kinase inhibitor. There are slight differences in which pathway is activated dependent on the mechanism of DNA damage but, in general, the check point proteins which signal to p53 that damage has occured and that cell cycling should be halted until DNA is repaired, are kinase. These include ATM (ataxia telangiectasia mutated), ChK1 and 2, DNA-PK (DNA-dependent protein kinase), JNK (JUN N-terminal kinase) and casein kinase 1 and 2. These kinase all phosphorylate p53 at amino terminal sites in the Mdm2-binding region of the protein, the effect being to block the interaction of Mdm2 and p53 allowing p53 stabilization. Mutations in ATM are associated with the inherited condition, ataxia telangiectasia, and mutation in CHK2 have been found in a few families with Fi-Fraumeni. Mutations in these genes therefore mean that they are no longer able to activate the p53 pathway to allow repair to DNA damage

which consequently leads to the development of cancers (8).

The second pathway for activation of p53 occurs in the absence of DNA damage and results from deregulated oncogen expression. This provides a file-safe mechanism to eliminate cell with proliverative abnormalities. The oncogene protein, e.g. RAS or MYC stimulate expression of the ARF gene, the protein product of which interacts with Mdm2 and inhibits its activity, again allowing levels of p53 to increase. The ARF protein also sequesters Mdm2 into the nucleolus so that it cannot interact with p53 in thenucleus (8).

In light of the fundamental restraint yielded by p53, it not surprising then, that it is commonly inactivated in human cancer. Approximately 50% of sporadic human cancers cases, the integrity of the p53 signalling pathways is compromised indirect by various mechanisms, such as upregulation of key negative regulation of p53. Mdm2 or Mdm4 (Mdmx). Likewise downregulation of the p53 activator. ARF leads to mouse tumorigenesis, reminiscent of p53 deficiency. In Li-Fraumeni syndrome patients, germline mutations in p53 results in early onset of multiple cancer types. A knockin of these mutation in mouse models further confirms the oncogenic ability of mutant p53 (8).

Although p53 mutations are fairly ubiquitous and can be found in more than 50% of human tumors, it is now well known that most types of cancer harbor specific genetic defects such as mutations in *APC* in colon cancer, *BRCA1* and *BRCA2* in breast cancer, and *B-RAF* in melanoma. As the p53 network is closely linked to many other cellular pathways, it is likely that defects in any of these pathway, either inherited or acquired somatically, could influence p53 function qualitatively or quantitatively (8). Another example is the relationship betwewn the *BRCA1* and p53 pathways. *BRCA1* acts both as a checkpoint and a DNA damage repair gene that ensures genome integrity. *BRCA1* germ-line mutation are associated with an increased risk of developing breast and ovarian carcinoma (14).

Molecular basis of apoptosis pathway

The mechanisms of apoptosis are highly complex and sophisticated, involving an energy-dependent cascade of molecular events. To date, research indicates that there are two main apoptotic pathways; the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway. However, there is now evidence that the two pathways are linked and that molecules in one pathway can influence the other (4).

Both of these pathways lead to caspase activation and cleavage of spesific cellular substrates. The receptors,

such as Fas, TNF, TRAIL, and downstream molecules (i.e., caspases and Bcl-2 family members). The mitochondria-apoptosome-mediated pathway includes the apoptotic stimuli induced by radiation therapy and chemotherapy, mitochondria, apoptosome, and key effector caspase. Caspase are activated in a cascade-like fashion. Initiator or upstream caspases (caspase 8, 9, 10) can activate affector or downstream caspases, including caspase 3, 6, and 7, with leads to induction of apoptosis (13).

Intrinsic pathway

The intrinsic pathway is activated by various stimuli, such as DNA damage and cytotoxic insult, and acts through the mitochondria, which are controlled by the Bcl-2 family of proteins. In homeostatic conditions, the anti-apoptotic Bcl-2 family members maintain mitochondrial integrity by preventing the pro-apoptotic multidominan Bcl-2 family members Bax and Bak from causing mitochondrial damage. During cellular stress, Bcl-2-homology 3 (BH3)-only proteins are activated and antagonize the anti-apoptotic Bcl-2 family members. Consequently, the inhibition of Bax and/or Bak is relieved, leading to their oligomerization and formation of a channel through which cytochrome c (cyt-c) is released into the cytosol. Then, cyt c associates with Apaf-1 and ATP, forming a platform for recruitment and activation of procaspase-9, also known as the apoptosome. Active caspase-9 cleaves and activates the downstream executioner caspase-3, -6, and -7, which are crucial for the execution of apoptotic cell death. In addition, other pro-apoptotic proteins released from the mitochondria contribute to the cellular suicide mechanism (3).

Extrinsic pathway

The extrinsic pathway of apoptosis is induced upon stimulation of death receptors belonging to the TNFR family, such as TNFR, Fas and TRAIL-R. Signalling by these receptors can induce a variety of cellular responses, including proliferation, differentiation and cell death. Apoptosis is induced by the formation of a death-inducing signaling complex (DISC). In this complex, Fas-associated death domain (FADD) recruits the initiator caspase-8 and/or -10 via homotypic death domain interaction. In contrast to signaling induced by Fas and TRAIL-R, TNFR1 aggregation leads to the sequential formation of two complex. Complex 1 consists of TNFR1, TNFR-associated death domain (TRADD). TRAF2, RIP1, cIAP1 and cIAP2 and is formed at the plasma membrane. These proteins are important mediators of TNF-induced activation of NF-κB and MAPKs. Endocytosis of TNFR1 is followed by the formation of complex II, which is analogous to the receptor-proximal DISC

induced by FasL and TRAIL and includes TRADD, FADD, and caspase-8 and/or -10. Activation of caspase-8 and -10 leads to activation of the downstream executioner caspases (3).

Caspase are the central component of the apoptotic response.

Caspases, being the key effector molecules in apoptosis, are potential targets for pharmacological modulation of cell death (5).

Caspases (which are so-named as they are cysteine proteases that cleave after an aspartate residu in their substrates) are a conserved family of enzymes that irreversibly commit a cell to die. Although the first caspase; interleukin-1β- converting enzyme (ICE; also known as caspase-1), was identified in humans. Caspases and their homologues have been reported in species that range from the nematode to the dipteran *Drosophila melanogaster* and the lepidopteran Spodoptera frungiperda, and even the yeas*t Saccharomyces cerevisiae* (9).

Caspases are zymogens (inactive enzyme precursors, which require a biochemical change to become an active enzyme) that consist of an N-terminal prodomain followed by a large subunit of about 20 kDa, p20, and small subunit of about 10 kDa, p10. In a number of procaspases, the p20 and p10 subunits are separated by a small linker sequence. Depending on the structure of the prodomain and their function, caspases are typically divided into 3 major groups. The caspases with large prodomains are referred to as inflammatory caspases (group I) and initiator of apoptosis caspases (group II), while caspases with a short prodomain of 20-30 amino acids are named effectoe caspases (group III) (9).

Once activated, initiator caspases are responsible for cleaving and activating effector pro-caspases. Effector caspases, in turn, cleave various proteins leading to morphological and biochemical features characteristic of apoptosis (10).

Caspase inhibition

In mammals, inhibition of caspase is assiciated with the proteins belonging to IAPs (inhibitors of apoptosis) family (e.g. cIAP-1, cIAP-2, ILP-2, survivin, XIAP, Livin, BIRC, NAIP) that are differentiated by the presence of three baculoviral IAP repeats (BIR) and in some, a RING-finger motif at the C-terminus. The IAPs family proteins inhibit the function of some initiator (e.g. casp-9) and effector caspases (casp-3, and 7) by different processes, although several several initiator as well as effector caspases are resistant to inhibition by XIAP.c-IAP1, dan cIAP2. The action of caspase-8 has been found to be regulated by a family of proteins known as FADD-like ICE (FLICE)-inhibitory proteins (FLIPs). It has been found that FLICE-inhibitory proteins (FLIPs) derived from a virus (v-FLIPs) are

capable to bind with FADD and casp-8 by homotypic interactions and block the recruitment of casp-8 to the DISC leading to casp-8 inactivation. Regulation of caspase-9 activity can also be achieved by the release of mithocondrial proteins Smac/DIABLO and HtrA2/Omi. Now it has become clear that ubiquitin-mediated degradation is implicated in the regulation of IAPs and proteins with which they interact (13).

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