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REVIEW ARTICLE

What's new in p53

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Abstract

p53 is the main intrinsic factor inducing apoptosis by recognizing the external stimuli and activating the p53 responsive genes to an irreversible series of events. P53 activates the transcription of specific proapoptotic genes called p53 target genes.

A growing number of p53 responsive genes have been identified and numerous studies have demonstrated that p53 proapoptotic factors such as Noxa, Puma and Perp play cell type specific roles in p53's mediated response to certain stimuli. Perp (p53 apoptosis effector related to PMP-22) is a direct proapoptotic target gene encoding a tetraspan protein. Perp is highly expressed in cells undergoing apoptosis compared to cells under G1 arrest and its overexpression is sufficient to cause cell death in fibroblasts. Noxa is another member of the preapoptotic p53 genes family. When expressed Noxa acts in a BH3 motif-dependent localization to mitochondria, causing structural changes, activation of caspase 9 and release of cytochrome c from mitochondria to cytosol. Puma (p53 mutant of apoptosis) is another critical mediator of p53-dependent apoptosis. P53 binds to Puma-promoter gene sites, leading to puma production. The mtCLIC, a member of intracellular chloride channels, is a cytoplasmic and mitochondrial protein positively regulated by p53. Caspase 10 is induced in p53-dependent manner leading to cellular apoptosis. Other newly announced factors are also involved in p53-regulated apoptosis such as brain-specific angiogenesis inhibitor - 1 (BSAI-1), MSOD and GPX genes. A global discussion on this topic is attempted in the present review article. *Hippokratia 2006; 10 (3): 116-119*

Key words: *p53, cancer, apoptosis, cell cycle, tumor suppressor genes*

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Cell cycle is driven by a number of positive and negative signals for phosphorylation and dephosphorylation processes regulated by transcriptional factors. Mutations in genes vital for normal cell proliferation and tissue homeostasis lead to the formation of hyperproliferation and carcinogenesis. Tumor suppressor genes physiologically function as a barrier against mutation formation and accumulation in the genome. DNA damage in tumor suppressors is the key element in oncogenesis. P53, a vital component of tumor prevention, is the gene most frequently mutated in human cancer thus viewed as the "guardian of the genome".

Exposure to cellular stress may trigger the p53 tumor suppressor a sequence specific transcription factor to induce cell growth arrest or apoptosis. A multitude of mechanisms are employed by p53 to ensure efficient induction of cell cycle arrest in a stage and stress signed specific manner.

Apoptosis is a physiological process of programmed cell death mediated by proteins encoded in the cell' s genome. Apoptosis is responsible for tissue development and renewal throughout the whole life span of multicellular organisms. Various factors, both intrinsic and extrinsic, can initiate the cell's decision to "commit suicide". P53 is the main intrinsic factor inducing apoptosis by recognizing the extemal stimuli and activating the p53 responsive genes to an irreversible series of events including morphological series and structural changes, condensation of chromatin, cytoskeleton distraction and fragmentation of the cell into apoptotic bodies. Disrupted apoptotic process participates in the pathogenesis of various diseases such as neoplasms, chronic inflammation, and autoimmune diseases. Understanding the molecular basis of the proapoptotic functions of p53 may assist in our effort to reintroduce p53 in human tumors. The induction of apoptosis is a fundamental mechanism through which p53 suppresses tumor development. P53 activates the transcription of specific proapoptotic genes called p53 target genes.

A growing number of p53 responsive genes have been identified and numerous studies have demonstrated that p53 proapoptotic factors such as Noxa, Puma and Perp play cell type specific roles in p53's mediated response to certain stimuli.

Perp (p53 apoptosis effector related to PMP-22) is a direct proapoptotic target gene encoding a tetraspan protein, localized in the plasma membrane able to stimulate apoptosis via a totally different pathway than Puma and Noxa. Perp is highly expressed in cells undergoing apoptosis compared to cells under G1 arrest and its

overexpression is sufficient to cause cell death in fibroblasts[1].

Noxa is another distinguished member of the preapoptotic p53 genes family. Noxa encodes a Bcl-2 homologue-3 (BH3)-only member of the Bcl-2 family of proteins, functioning as a mediator of p53-dependent apoptosis through mitochondrial dysfunction. When expressed Noxa acts in a BH3 motif-dependent localization to mitochondria, causing structural changes, activation of caspase 9 and release of cytochrome c from mitochondria to cytosol. On the other hand, blockage of Noxa impairs apoptosis[2].

Puma (p53 mutant of apoptosis) is another critical mediator of p53-dependent apoptosis. P53 binds to Puma-promoter gene sites, leading to puma production. Puma also belongs to BH-3 only proteins, interacting with Bcl-X(L) protein and causing mitochondrial translocation and mu1ti-merization of Bax, which in turn allows mitochondrial permeabilization[3]. Genetic disruption of Bax turns cells resistant to apoptosis mediated by puma. Bax also causes cytochrome c release that associates with Apaf-1 and caspase 9 to form the apoptosome. Active caspase 9 activates caspases 7 and 3, which execute the death programmed[4]. In addition, p53 localized in the cytosol can directly activate Bax in the absence of p53 transcriptional mediators and permeabilizes mitochondria engaging the apoptotic effect[5].

On the other hand, mtCLIC (member of intracellular chloride channels) is a cytoplasmic and mitochondrial protein positively regulated by p53 through an independent proapoptotic gene-involved pathway. Following p53 activation mtCLIC releases cytochrome c activates caspases without association with Bax protein[6].

Caspase 10 is also induced in p53-dependent manner, with an increase in caspase cell concentration in both mRNA and protein level leading to cellular apoptosis. P53 binds to multiple p53-binding sites located within the caspase 10-gene locus[7].

Finally, other newly announced factors are also involved in p53-regulated apoptosis. Brain-specific angiogenesis inhibitor - 1 is reduced in tumor tissues. Experiments conducted in vitro have demonstrated that in the presence of p53 BSAI -1 levels were up regulated thus allowing it to suppress tumor angiogenesis and delay tumor growth[8].

GPX promoter regions, MSOD and GPX genes code for magnesium superoxide dismutase and glutathione peroxidase respectively, and the concentrations of these two enzymes were increased leading to oxidative cellular stress and apoptosis[9].

Determination of cell fate is controlled by p53-dependent p53RDL1 (p53 regulated receptor for death and life) a newly recognized gene regulating cell survival.

The DNA damage response includes checkpoint, direct activation of DNA repair mechanisms and apoptosis. Functional defects of DNA repair have shown to be associated with genomic instability and cancer. The complexity of the p53 pathway depends upon a variety of genes regulated by p53. It has long been known that in response to stressful stimuli p53 proceeds to a cell-cycle arrest in the G1 phase by direct activation of transcription of p21 (WAF1/CIP1), which in turn inhibits cyclin dependent kinases, proteins that control entry into the cell cycle. The challenge nowadays was to provide p53' s implication in controlling entry to mitosis when cell enter G2 phase or are arrested in the S phase. Chk1 and Chk2 (checkpoint kinases 1 and 2) are essential in inhibiting the promotion of cell cycle to the G2 phase. Chk2 is activated in response to ionizing radiation causing cell cycle arrest in a p53 dependent fashion and acts as an indirect factor contributing to up regulation of p53's target genes. P53 is a tetrameric protein phosphorylated in its BOX-1 transactivation domain by Chk2 at specific Chk2 docking sites (trh-18, ser-20) in response to DNA damage[10].

Following activation, p53 blocks cell cycle in the G2 phase via multiple pathways. Cdc2, the cyclin-dependent kinase needed to enter mitosis, is inhibited by p53 through repression of the cdc2 gene and through production of three proteins (Gadd45, p21, 14-3-3sigma) that inhibit cdc2. Atm and Atr also activate Chk2 upon genotoxic stress thus contributing to p53-regulated G2 phase arrest. Finally, p53 enhances gene transcription of proteins (B99, reprimo, mcg10) that reinforce its result in G2 arrest[11].

P53R2 is a newly recognized gene, which encodes a 351-amino acid peptide, considered as a new member of ribonucleotide reductase family. Ribonucleotide reductase(RR) is responsible for the de novo conversion of the ribonucleoside diphosphates to deoxyribonucleoside diphosphates, which are essential for DNA synthesis and repair. It is a directly regulated p53 target gene carrying a p53-binding site in intron1. P53R2 plays a major role in DNA repair and determination of cell fate. Induction of p53R2 causes G2/M arrest and at that point p53R2 becomes a nucleotide supplier for DNA repair by maintaining RR activity (acting as coenzyme of ribonucleotide reductase), while dysfunction of this pathway may lead to p53 dependent apoptosis. In the future, presence of p53R2 expression may be associated with carcinogenesis and may act as a predictor factor of tumor development[12].

Recent experiments have shown that p53 may lead to p53-mediated G1 arrest via inactivation of MAPKs. MKPI mRNA and protein levels are up regulated upon p53 binding to the second intron of the MPKI gene. MKPI in turn inactivates MAPKs thus causing cell cycle control in the absence of DNA damage[13].

P53 protects against tumorigenesis through its ability to cause cell cycle arrest and apoptosis under a large variety of cellular stresses. The mechanism by which so many signals are transmitted by a signal molecule still remains obscure. However, it is obvious that certain factors such as ultraviolet radiation, gamma irradiation,

oxidative stress, the use of certain chemotherapeutic agents and other genotoxic stresses cause a p53-mediated response. The common denominator in all p53-regulated processes is disruption of the nucleolus[14]. Tumor suppressor protein p53 is localized in the cytoplasm and in response to DNA damage is transported to the nucleus via the dynein protein. P53 nuclear accumulation is necessary to induce the production of p53-regulated target genes Puma, Noxa etc[15]. Certain nuclear factors are released upon cellular stress leading to up regulation of p53 levels. Under normal homeostatic circumstances the p53 concentration levels in the cytosol are basal. DNA damage causes an increase in STAT-1 concentration. STAT-1 is a transcription factor that interacts with p53 and inhibits cell growth by promoting apoptosis. It acts as a coactivator for p53, enhances transcription of p53 responsive genes, and negatively regulates Mdm2, which is a p53 inhibitor[16].

Furthermore, increased superoxide generation activates NF kappa B that upregulates p53 levels, increases its stability and affinity to the p53-dependent genes, and induces the expression of p53 target genes such as Puma and p21/WAFl resulting in cellular apoptosis. NF kappa B also negatively binds to cyclin D1 thus assisting in G1 arrest[17]. ASPP group of proteins is related as well to p53 regulation acting in the same way with NF kappa B protein.

On the other hand, there are also some down regulators of p53, which have recently been described. HGTSE-l (human G2 and S phase expressed-l) is a cell cycle regulated protein, found in the cytoplasm, able to decrease p53' s ability to induce apoptosis. HGTSE-l binds to p53 protein's c-terminal and represses its ability to induce apoptosis, enhances p53 cytoplasmic localization (p53 nuclear accumulation renders p53 active) always in the presence of Mdm2. Mdm2 is a protein whose expression is regulated by p53. Mdm2 contains an E3 ligant and a ring finger protein that binds to p53 and allows its degradation via ubiquitation. Accordingly, COPl (Constitutively Photomorphogenic 1) is also a ring finger-containing protein leading to p53 degeneration by the proteasome, increasing p53 turnover rate independently of Mdm2 activity[18].

Additionally, DNA repair protein hHR23 is involved in p53 degradation by the proteasome. Experiments have shown that hHR23 is intrinsically involved in the delivery of p53 molecules to the proteasome by binding to the polyubiquitinated p53 via its carboxyl-terminal ubiqnitin associated domain and then by interacting with the 26S proteasome.

Endoplasmic reticulum alterations lead to accumulation of proteins harmful for cell survival. Cell adaptation to endoplasmic reticulum stress was recently found to inhibit p53 mediated apoptosis thus leading to prolongation of cell life. Certain signals not yet defined increase p53' s localization and destabilization by phosphorylation of the p53 protein at Ser 315 and 376. It is clearly noted however, that the mechanisms used in this case are different compared to the ones used by other p53 negative regulators[19].

Finally, mutant p53 protein expressed in most human cancer cell lines exceeds a dominant effect on wild type p53, reducing its ability to bind to p53 proapoptotic genes, therefore diminishing its role in protection of the genome[20].

Environmental genotoxins such as ultraviolet radiation and chemical carcinogens trigger a p53 dependent pathway of DNA repair by up regulation of Nucleotide Excision Repair (NER) mechanism. Expression of genes such as XPC and XPE, considered essential for Global Genomic Repair (GGR) is markedly elevated upon p53 activation following cellular stress (GGR handles with all repairable lesions of the genome). On the other hand, in Base Excision Repair (BER) p53 is directly coupled to proteins directly involved in BER specific pathways[21].

The p53 tumor suppressor acts to integrate multiple stress stimuli into a series of various antiproliferative responses. The p53 protein adapts multiple cellular functions, such as gene transcription, DNA synthesis and repair, cell cycle arrest, senescence and apoptosis. These processes act in concert in order to execute the programmed cell death. Disruption of these processes can lead to genetic instability, tumor progression and chemoresistance. A1though not yet undoubtedly defined, our current understanding of p53 illustrates how cell cycle arrest, DNA repair and apoptosis can be included into a larger network manipulated by diverse signals, extrinsic and intrinsic factors and cell type. Elaboration of this network will provide insights into cancer and autoimmune diseases and will introduce policies to ameliorate their treatment. Additionally, determination of p53 mutation load in non tumorous tissue may help in identifying individuals at risk of cancer development.

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