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TP53 GENE MUTATIONS – FROM GUARDIAN OF THE GENOME TO ONCOGENE

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TP53 tumor suppressor gene mutations are the most frequent genetic alterations in human cancer affecting a specific gene. The occurrence of *TP53* mutations is considerably influenced by cancer-initiating events, such as DNA damage, the aftermath of which is the promotion of cancer development through the loss of anti-proliferative activities, including apoptosis and cellular senescence.

Over 27.000 *TP53* gene mutations have been discovered and found in more than 50% of human cancers. The most frequent alterations are the point mutations with a single base substitution in gene segment encoding for DNA-binding domaine of p53 molecule, leading to the production of mutant protein that differs from the wild-type protein by one amino acid (missense mutations) usually causing the change in tertiary structure of gene product, thus preventing p53 to bind to DNA and activate transcription of target genes. The result of the mutations may also be the proteins with new, abnormal functions, and the ability to modulate expression of genes responsible for neoangiogenesis, resistance to chemotherapeutics and prevention of tumor initiation and promotion. In such circumstances, not only the mutant *TP53* loses its tumor suppressive function, but acquires oncogenic potential and becomes an active participant in the neoplastic transformation of the cell.

Vast heterogeneity of mutations and methodological approaches in p53 status assessment represent the main difficulties in rapid and effective integration of basic p53 research into clinical practice. *Acta Medica Medianae 2009;48(4):59-63.*

Key words: *TP53 gene, p53 protein, mutations, cancer*

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Introduction

TP53 tumor suppressor gene mutations are the most frequent genetic alterations in human cancer affecting a specific gene (1,2). *TP53* gene encodes p53 protein, involved in numerous anti-proliferative functions through the transcription control of different target genes and through protein-protein interactions. Since *TP53* plays an important role in cell cycle regulation and in maintenance of genome stability by preventing mutations, it is often referred to as "the guardian of the genome" (3).

In a normal cell, p53 protein levels are low because of the active degradation in proteasome, mediated by ubiqitin ligase MDM2. Different types of stress, especially genotoxic damages, stabilize p53 through post-transcriptional modifi-cations and cease p53 degradation. By direct or indirect activation or repression of transcription, stabilized p53 regulates the expression of numerous target genes involved in cell cycle control, apoptosis and DNA reparation.

The loss of p53 function in the cell leads to uncontrolled proliferation and increases the risk of cancer development (4). *TP53* gene mutations are found in almost every type of cancer with various frequencies and are very heterogeneous.

TP53 structure and function

P53 was identified in 1979 (5), but full attention of researchers and scientific community was drawn to p53 in 1989, when the alterations of this gene in human cancers were described for the first time (6). In humans, *TP53* is located on the short arm of the chromosome 17 (17p13.1) (7). The gene encompasses 19,18 kb DNA and is organized in 11 exonic sequences encoding human p53 protein, which consists of 393 amino acids.

Activation of p53 occurs in response to DNA damage, presence of single-stranded DNA, oxidative stress, osmotic shock, ribonucleotide depletion, unregulated oncogene expression and other various forms of distress. In the endangered cells, p53 half-life dramatically increases, followed by its rapid accumulation. Because of the conformation change, p53 undertakes an active role of a transcriptional factor in these cells.

It is generally accepted that *TP53* tumor suppressive function is exerted by multiple mechanisms where p53: 1) activates DNA reparation

proteins after DNA damage occurrence; 2) causes cell cycle arrest in regulatory check-point G1/S, providing time for DNA reparation; 3) initiates apoptosis, programed cell death, in case of irreparable DNA damage.

The question of what directs the p53-induced choice between cell cycle arrest and cell death has not been precisely answered so far. Recent studies suggest that this choice may be influenced by the nature of target genes and timing of their regulation by p53. P53 target genes that mediate cell cycle arrest (WAF1, TIGAR) are rapidly induced by genotoxic stress, while the p53 target genes that mediate apoptosis (PUMA) are induced later when stress is maintained (1). Activated p53 binds to DNA and activates the expression of several genes, including WAF1, encoding for p21 protein (cyclin-dependant kinase inhibitor, CDK1). P21 inhibits G1-S/CDK complex and prevents the cell to enter the synthetic phase of cell cycle before the reparation of damaged DNA. Induction of PUMA gene leads to apoptosis by BAX and BAK protein activation, causing mitochondrial autophagy followed by cytochrome C release and subsequent apoptosis. Induction of PUMA simultaneously causes downregulation of WAF1 and TIGAR genes (8).

Cellular senescence and tissue ageing are important biological consequences of p53 activation by genotoxic stress and significantly contribute to its tumor suppressive activity. In *TP53* +/m mice, the expression of a mutant allele in which the first six exons of *TP53* are deleted giving rise to a truncated p53 protein resulted in a decreased number of spontaneous tumors, but induced an accelerated aging compared with wild-type carriers (9). This phenotype was shown to be caused by permanent p53 activation resulting from the interaction of the mutant and normal p53. In the investigation of cellular response to inflammation induced by nitric oxide donors, it was found that p53 initiates senescence by downstream effector microRNA miR-34a. Overexpression of miR-34a causes growth and replication arrest, leading to tissue ageing (10).

Tumor suppressive activity of p53, which is still poorly understood, is its ability to modulate cell migration. It has been shown that p53 inhibits CDC42-induced filapodia formation. Loss of p53 function increases cell motility and may thus contribute to tumor invasiveness (11).

TP53 mutations

Somatic mutations of *TP53* gene are found in majority of sporadic human cancers and their frequency varies from 5% to 70%, depending on tumor type and tumor stage (12). Over 27.000 different *TP53* mutations have been described in scientific literature. Their presence may carry prognostic information in tumor response to therapy and survival.

Major significance of p53 in cancerogenesis is supported by the fact that even if the gene itself has not been hit by a mutation, p53 physiological pathways are usually inactivated by indirect mechanisms like MDM2/MDMX amplification, leading to p53 destabilization (13). In cervical cancer, human papilloma virus (HPV) produces E6 viral protein that specifically bindes p53 inducing its degradation (14), which explains rare p53 mutations in this type of cancer.

TP53 gene mutations occur early during the cancerogenesis and are often discovered in pre-malignant lesions, especially when there is exposure to environmental cancerogenes. Investigation of liver and esophageal cancers has shown that *TP53* mutations might occur at the very beginning in the natural history of these malignancies (15). *TP53* mutations have no crucial significance for the initiation of cancerogenesis, but they allow the cells to overcome division constraints in state of distress and ignore the signals that lead altered cells into apoptosis or cell cycle arrest. In this way, cells with damaged DNA are given a short-term proliferative advantage, which ultimately enhances the risk of progression to cancer.

Somatic mutations of *TP53* gene in cancer

The most frequent alterations of the *TP53* gene in human neoplasms are the point mutations with a single nucleotide substitution, leading to synthesis of a mutant protein that differs from the wild-type protein by one amino acid (missense mutations). This usually causes the change in tertiary structure of the molecule, or disables DNA-binding because of arginine residues substitution within the DNA-binding core domen of p53, which directly interacts with DNA (16), thus causing the failure of transcriptional activation of the target genes.

P53 molecules, products of *TP53* mutated in oligomerization domen (OD), dimerize with wild-type gene products, preventing them to activate transcription. OD mutations express dominant negative effect on p53 function, because altered genetic product antagonizes wild-type p53.

Numerous studies have proven the existence of p53 proteins with undoubted oncogenic potential, which actively participate in neoplastic transformation (17,18,19). The products of mutant genes modulate expression of target genes in a specific manner, different from normal p53. It has been found that R175H, R273H and D281G mutations of *TP53* encode proteins capable to induce NF-κB2 gene expression, increasing the resistence of tumor cells to chemotherapeutics (20). Gene product with R175H mutation may enhance ID4 gene expression and thus induce ID4-dependant neo-angiogenesis in vivo, favorizing tumor growth (1). It has been recently implied that in vitro, in mammalian cells, mutant p53 in R175H and R248W mutations inhibits the expression of transforming growth factor beta (TGFβR2), which plays a key role in prevention of cancer initiation and promotion (21). Under these circumstances, the mutant p53 acts like oncogenic transcriptional factor.

Molecular analysis of *TP53* mutations

Direct sequencing of the *TP53* gene after PCR amplification remains the golden standard in molecular analysis of *TP53* alterations. This approach is facilitated by the fact that 10 coding exons are smaller than 350 bp and can therefore be easily amplified individually.

Considering the mutational effect, 75% of mutational events represent missense mutations, leading to synthesis of abnormal protein that accumulates in the nuclei of tumor cells. 20-25% of mutations do not result in protein synthesis. Usually, these are nonsense mutations with stop-codon formation or either small deletions or insertions, leading to frameshift mutations. Larger deletions are extremely rare. Functionally, missense mutations found in human cancers are usually joined with the loss of transactivational activity. In over 80% of these mutations, the protein product has no ability to act as a transcriptional factor. Function of the mutant protein is completely or partially preserved in 8.8% and 11.1% respectively (12,22).

Approximately 280 of 393 *TP53* codons are affected by mutations, but mutations are mainly clustered on DNA-binding domen, comprising exons 5-8. Alterations of codons 175, 248 and 273 constitute 19% of all described *TP53* mutations. Therefore, these codons are considered the mutational "hot spots". Recent studies add codons 245, 249 and 282 to this most frequently affected group (15).

The analysis of all-point mutation has shown that 51% of mutations represent GC>AT transitions, 59% of them affecting CpG dinucleotides, DNA regions where cytosine nucleotide is followed by a guanine nucleotide along the linear DNA sequence (23). In mammalian cells, cytosine is often methylated within these dinucleotides and it has been shown that there are 42 CpG spots in *TP53* gene where cytosine is normally methylated (24). It has been suggested that higher rate of 5-methylcytosine deamination leads to T/G mismatch, which is not repaired efficiently, causing the higher frequency of *TP53* transitions. Cytosine deamination leads to U/G mismatch, which is repaired more efficiently. Exogenous carcinogens, such as UV radiation and benzopyrene, express stronger affinity toward the methylated CpG dinucleotides (25). It is plausible that endogenous carcinogens, arising from the altered cellular metabolism, may also target methylated CpG dinucleotides and cause higher transition rate.

The pattern of mutational events inactivating *TP53* is cancer specific. There is a high frequency of transitions in colorectal cancer and brain tumors, while in lung cancer the leading mutation is GC>AT transversion (22).

TP53 mutations as a carcinogen fingerprint

The studies of molecular epidemiology have shown the correlation between the exposure to specific carcinogens and characteristic *TP53* mutational scheme in development of certain neoplasms (12,26). The marked example is frequent occurrence of tandem mutations in basal-cell and squamous-cell skin carcinoma, which are quite rare in other malignancies (27). In these tumors, high frequency of C>T transitions have been found in CC foci; thus it is considered that this mutation is caused by UV radiation exposure.

High frequency of G>T transversion is often found in lung cancer, esophageal cancer and head and neck tumors. G>T transversion of codons 157, 158, 248 and 273 occurs in 30% of lung cancer cases, and in less than 10% of other tumors and is considered the consequence of tobacco smoke exposure, i.e. of mutagenic effect of benzopyrene (25).

Exposition to aflatoxin B1 correlates to G>T transversion of codon 249, since this mutation is found in more than 50% of liver cancer, while the frequency in other tumors is lower than 2% (28). The specificity of G>T transversion of codon 249 for hepatocellular carcinoma allows the development of sensitive screening methods for mutation detection in serum DNA in inhabitants of regions where food is possibly contaminated with the fungus producing aflatoxin.

Strong correlation between p53 mutational spectrum and exposure to exogenous carcinogens is influenced by upstream genetic filters that modulate carcinogen activation, detoxification, and/or DNA repair. However, tissue specificity, genetic polymorphisms associated with the p53 pathway or p53 mutant heterogeneity can also act as a second set of downstream filters that have a profound impact on the spectrum of p53 mutations (23).

TP53 germline mutations and LI-fraumeni syndrome

Distinctive from somatic mutations, occurring sporadically in somatic cells, germline mutations are present in reproductive cells and are transmitted to offspring. Clinical repercussions of *TP53* germline mutations are Li-Fraumeni syndrome (LFS) and LFS-related syndromes. To date, 535 germline mutations have been reported.

Li-Fraumeni syndrome was first described in 1988. as a clustering of early onset tumors (in childhood and adolescence), with a predominance of sarcomas, in members of certain families (29). Two years later, it was discovered that the syndrome is caused by germline mutations of *TP53* gene with autosomal dominant transmission (30). It is clear today that *TP53* gene alterations are the main cause of LFS and that different types of mutations are associated with different penetrance and phenotypes. *TP53* gene deletions are present only in a small number of patients. Missense mutations represent the predominant mechanism of gene function loss and are associated with earlier onset of more aggressive neoplasms (2). *TP53* mutation carriers develop sarcomas (osteosarcomas, soft tissue sarcomas), breast carcinomas (bilateral tumors in young girls), brain tumors, leukemia/lymphomas, adreno-cortical cancers in childhood, gastric cancer, Wilms's tumor and other malignancies with far

earlier onset and higher frequency of occurence. It is estimated that there are about 400 families with LFS worldwide today.

As LFS patients are prone to develop secondary neoplasms after conventional anticancer treatments; currently gene therapy approach is in intensive development and is expected to overcome occurrence of new and more aggressive tumors. Local intratumoral injection of adenoviral vector containing a normal *TP53* tumor suppressor gene in progressive carcinomas of LFS patients, resulted in complete and durable remission of lesions, what was confirmed by positron emission tomography scan evaluation. Safety and efficacy of these therapy approaches are still in phase of clinical evaluation (31).

TP53 gene mutations: new clinical marker

Large number of studies is focused on p53 status assessment in cancer, as factor important for prognosis and therapeutic response. It is known that the activity of numerous molecules applied in cancer chemotherapy is based on p53-dependant apoptosis induction. However, uniform consensus strategies still do not exist in this field. Direct implementation into clinical routine is interfered by multiple factors: diversity of methodologies for p53 assessment (PCR, immuno-histochemistry, functional essays), vast heterogeneity in mutant p53 behavior and especially limited knowledge on p53 signaling pathways (23). Genomics, which allows simultaneous uncovering of mutations and epigenetic alterations in large number of genes, offers fresh opportunities in the development of biomarkers and accelerates their clinical application (32).

Several meta-analyses have suggested the prognostic significance of p53 status in non-small-cell lung cancer (33) and in breast cancer (34). In breast cancer, different types of mutations are associated with different clinical outcome, with non-missense *TP53* mutations being in correlation with the worst prognosis (34).

Conclusion

Tumor suppressor gene *TP53* is one of the most frequently altered genes in human malignancies. Point mutations with single base alteration in gene segment encoding for DNA-binding domen of p53 represent the biggest part of *TP53* mutations. Their result is the synthesis of mutant p53 protein, which is incapable of transcriptional activation of downstream target genes regulating cell cycle and apoptosis. Functional consequences of mutations may be: 1) the loss of transactivational function of the mutant protein; 2) dominant negative effect – hetero-oligomerization of a more stable mutant p53 with wild-type p53 expressed by the residual normal allele; 3) gain-of-function mutation – mutant p53 acquires new, abnormal function. In such circumstances, not only the mutant *TP53* loses its tumor suppressive function, but acquires oncogenic potential and becomes active participant in the neoplastic transformation of the cell.

Although it has been more than 20 years since the first *TP53* mutation discovery, the assessment of p53 status still does not carry unequivocal clinical significance. Vast heterogeneity of mutations and methodological approaches interferes with the establishment of uniform criteria for p53 status assessment in the purpose of diagnosis and prognosis. Further efforts are encouraged by recent success of therapy approach based on p53 reactivation in p53-deficient tumors, leading to potent apoptosis or cellular senescence. In the era of individualized therapy (35), it is rational to expect that with the establishment of firm scientific justification of patient's p53 tumor genotype determination, the implementation into clinical oncology will become reality.

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MUTACIJE *TP53* GENA – OD ČUVARA GENOMA DO ONKOGENA

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Mutacije tumor supresorskog gena *TP53* najfrekventnije su genetske alteracije u humanom kanceru koje pogađaju specifični gen. Na nastanak *TP53* mutacija u velikoj meri utiču kancer-inicirajući događaji, kao što je oštećenje DNK. Efekat tih mutacija je promocija razvoja kancera kroz gubitak antiproliferativnih aktivnosti, uključujući apoptozu i ćelijsko starenje.

Otkriveno je preko 27.000 mutacija *TP53* gena koje se sreću u više od 50% sporadičnih humanih kancera. Najučestalije su tačkaste mutacije sa izmenom samo jedne baze u segmentu gena koji kodira DNK-vezujući domen p53 molekula, dovodeći do stvaranja mutantnog proteina, koji se od produkta divljeg alela razlikuje u jednoj aminokiselini (pogrešno smislene mutacije). One obično uzrokuju izmenu tercijarne strukture genskog produkta, te izostaje vezivanje p53 za DNK i transkripciona aktivacija ciljnih gena. Rezultat mutacija mogu biti i proteini sa novim, abnormalnim funkcijama, sa sposobnošću da modulišu ekspresiju gena odgovornih za neoangiogenezu, rezistenciju na hemioterapeutike i prevenciju tumorske inicijacije i promocije. U takvim okolnostima, mutantni *TP53* ne samo da gubi funkciju tumor supresorskog gena, već stiče onkogeni potencijal i postaje aktivni učesnik u neoplastičnoj transformaciji ćelije.

Velika heterogenost mutacija i metodoloških pristupa u proceni p53 statusa glavne su prepreke brze i efikasne integracije rezultata bazičnih p53 istraživanja u kliničku praksu. Acta *Medica Medianae 2010;49(1):59-63.*

***Ključne reči**: TP53 gen, p53 protein, mutacije, kancer*