

# Hypomethylation of the p53 and p16 suppressor genes in cellular aging

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**Abstract.** Protein p53, a product of p53 gene expression, protects the integrity of the genome throughout life and promotes the cell repair in the process of cell aging. In this review, the fundamental significance of the p53 gene was analyzed in the cell aging. The review showed that hypomethylation of the promoter region of the p53 and p16 suppressor genes occurs during aging; the DNA hypomethylation is explained by the possible age-related decrease in the content of CH<sub>3</sub>-groups donor (S-adenosyl monophosphate) in the cell, and by the activity of DNA methyltransferases.

## 1 Introduction

Cellular aging is a genetic program of irreversible cell cycle arrest that blocks the cell's response to proliferative stimuli and growth factors in the presence of unrepairable DNA damage. During the evolution, cellular aging arose to prevent a genetically damaged cell from regenerating into a tumor cell. The tumor suppressor gene p53 plays a decisive role in this process [1]. Its 53 kDa product, consisting of 392 amino acid residues, is expressed ubiquitously in all cell types as an inactive latent transcription factor and is activated only when the cell is exposed to various stresses, such as telomere loss, DNA damage, oncogene activation and oxidative stress [2]. Low levels of p53 contribute to optimal balance of metabolic processes, reduce the risk of mutations and increase the speed of repair processes [3]. Identification of the manifestation of two p53 opposite properties can be helpful in cancer prevention and in slowing down the aging process [4].

## 2 Materials and methods

**Control of cellular aging by the p53 gene.** Cellular aging is a multi-causal process, caused by many factors, the action of which repeats and accumulates throughout life. Among them are the stresses, diseases, activation of free radical oxidation and accumulation of peroxide metabolites, changes in the concentration of hydrogen ions, temperature damage, inadequate removal of protein decay products, hypoxia, etc. [5]. There are a number of hypotheses about the nature of aging. Telomeric aging theory is the most well-known and widely spread one.

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Olovnikov et al. are of opinion, that in the somatic cells, at each replication, due to the specific features of the replication enzyme (DNA-polymerase), the ends of the chromosomes, telomeres, are underreplicated [6]. It is known that telomeric DNA consists of recurrent G-rich hexanucleotides (TTAGGG in vertebrates). Telomeres play a key role in the stabilization of chromosomes during replication, protecting their ends from exonucleases and ligases, and in prevention of degradation and undesirable recombination, e.g. fusion of the terminal sites of broken chromosomes. Due to constant shortening of the chromosomes, typical of every single indirect division, the underreplication gains possession of the genome domains essential for the cell survival, resulting in cell death and aging of organisms. Vaziri et al. set forth current modifications of this hypothesis, stating that the shortening of telomeres activates the synthesis of the p53 protein, the amount of which actually increases with aging of the cultured cells, leading to a block of cell mitosis in the G1 stage, which in turn leads to aging [7]. The most important gene that regulates the functional orientation of cells, the p53 gene, is a suppressor gene or an anti-oncogene; it plays the leading role in regulatory control of normal cell proliferation, protecting somatic cells from accumulation of genomic mutations, including aging [8]. In case of DNA damage under various factors, in particular, under radiation, the level of p53 increases, shutting off the cells in the phase of the G0-cell cycle. This makes possible DNA recovery by blocking the mutant gene's transfer to the daughter cells. Cells that have lost normally functioning p53 can be transformed into tumor cells [9]. By structure, it forms a tetrameric complex capable of regulating the transcription of a number of genes that have specific DNA sequences in their composition. The molecule of p53 maps several functionally significant domains that play an important role in the implementation or regulation of its activity. The N-terminal region is the domain responsible for the transcriptional activation of the target genes [10]. This domain is involved in protein-protein interactions regulating the stability of the p53 molecule. In this domain, there are several residues of serine and threonine, the phosphorylation of which regulates the activity of p53 [11]. The p53 gene encodes a nuclear phosphoprotein. The gene is located on the chromosome 17p13 and has 11 exons. When the normal functioning of p53 changes, the cell that was supposed to die begins to divide uncontrollably and, thus, a tumor arises. If the p53 gene is normal, the programmed cell death system dramatically reduces the incidence of cancer [12]. Dysregulation of p53 gene expression also leads to cell aging; therefore, mutations in the p53 protein, which disturb its functioning, are often found in cells of human malignant neoplasms and in aging cells. Thus, the gene p53 is the center for monitoring the correctness of the genetic programs' performance, and is inevitably related to the aging processes of the organism.

**Study of the p53 gene functional activity during cell aging.** Despite the fact that in the senescent cells the level of the p53 protein or its mRNA does not increase, the degree of its phosphorylation increases and, consequently, the DNA-binding activity is up-regulated. As a result, the level of the main p53target, the p21 protein in the senescent cells is significantly increased, growing with the number of cell divisions [13]. It is p21 that is responsible for the p53-dependent arrest of cell divisions. The p16 protein, like p21, acts as an inhibitor of cyclin-dependent kinases. Expression of p16 significantly increases with age in almost all tissues. This is the result of activation of the p38 mitogen-activated protein kinase in response to oxidative or genotoxic stress, which indirectly increases the expression level of the p16 gene [14]. With this relation, we studied the functional activities of p53 and p16 gene. Synthesis of complementary DNA for p53 and p16 genes was carried out by polymerase chain reaction (PCR) by means of primers specific for these genes, as shown in Table 1.

To synthesize cDNA from the cells of the nodular euthyroid goiter, the total RNA for PCR was prepared by SDS phenol-chloroform method. Table 2 shows the results for the yield of the total RNA.

**Table 1.** Primers for the p53 and p16 genes

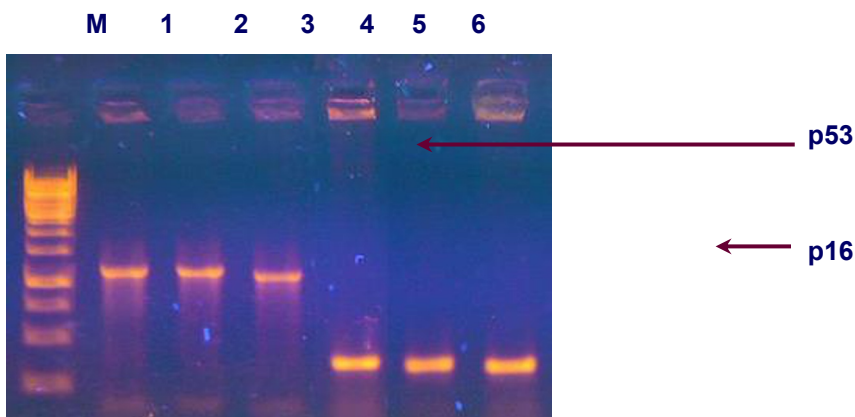
p53 gene	rev: 5 <sup>1</sup> - TGTCACCCAGATTCTCT-3 <sup>1</sup> for: GTGACAAACTCGGAGAG-3 <sup>1</sup>
p16 gene:	rev: 5 <sup>1</sup> AGCCAGCCCCTCCTCTTTCTTC-3 <sup>1</sup> for: 5 <sup>1</sup> -GAACGCACTCAAACACGC- 3 <sup>1</sup>

**Table 2.** Total RNA yield

Object	Total RNA yield (mg/g of crude tissue)
Thyroid gland (of a 40 year-old patient)	35.0±0.48
Thyroid gland (of a 75- year old patient)	22.0±0.64

### 3 Results and discussion

The resulting total RNA was used as a template for cDNA synthesis. To synthesize cDNA, both DNA polymerase I and RNA-dependent DNA polymerase (revertase) were used for p53 and p16 genes. In the PCR, after 55 cycles of amplification, the samples were incubated at 72 ° C for 10 minutes to complete the elongation, and then the degree of amplification by electrophoresis of an aliquot of the reaction mixture (10 ml) in 1.5% agarose with ethidium bromide was checked out (Fig. 1). The p53 protein is also able to function as a transcriptional activator. Multiple DNA damage has been shown to cause the expression of the p53 gene, which in turn activates the transcription of the p16 and p21 genes encoding the inhibitors of cyclin-dependent kinases [15]. The p16 gene consists of three exons and has a rather extended CpG-rich promoter region. Dysregulation of p16 gene expression leads to an uncontrolled cell growth, the onset of a tumor, and cell aging. The functional activity of the p53 and p16 genes was evaluated by methyl-sensitive PCR, which is based on the methylation of the promoter region of these genes using methyl-sensitive restriction enzymes, Hpa II (CCGG) and Hha I (GCGC).

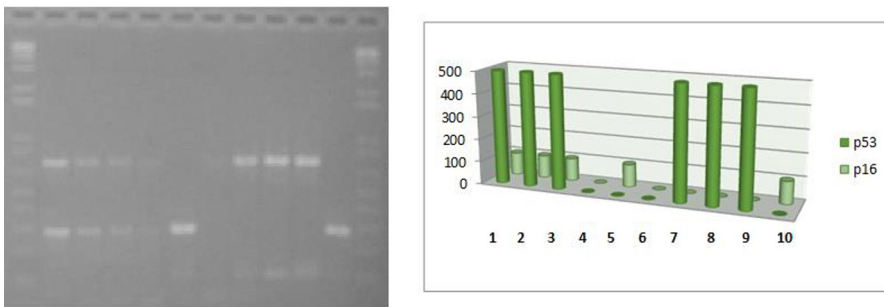
**Fig. 1.** Electrophoresis of PCR products of p53 and p16 genes, modified from Ref. [16].

It is known that the promoter region of genes is represented by CpG-islands, which are untranslated regulatory DNA sequences from 500 to 2000 bp characterized by a high content of guanine and cytosine ( $G + C > 60$  percentage). As a result of methylation, cytosine facing guanine is converted to methylcytosine. As a consequence, the recognition of a particular gene sequence by proteins and transcription factors is damaged which leads to the blocking of transcription. Methylation of CpG-islands can promote the interaction of DNA with

nonspecific DNA-binding proteins, which also repress transcription [16]. In some cases, the disruption of the gene's activity associated with methylation of the promoter region is a cause for of the specified suppressor genes' inactivation. The methyl-sensitive PCR is based on the ability of methylation-sensitive restriction enzymes to cleave an unmethylated DNA molecule and to keep regions containing methylcytosine unhydrolyzed. As a template for polymerase chain reaction, DNA isolated from white blood cells of elderly people (practically healthy, with nodular euthyroid goiter), pre-treated with methyl-sensitive restriction enzymes HpaII (CCGG) was used. Methylation status of the promoter region of the p53 and p16 genes can be seen in Fig.2. It was found that in the case of methylation of the promoter region, no hydrolysis of DNA occurs and the PCR product is detected in the gel. In the absence of methylation, complete hydrolysis of the DNA takes place, and the PCR product is not detected in the gel. It has been established that, during aging, there was no hypomethylation of the promoter region of the p53 and p16 genes, and the PCR product was not detected in the gel.

## 4 Conclusion

Methylation of the promoter region of the p53 and p16 genes is a factor influencing the gene expression. It is shown that in the elderly people DNA a reduction in methyltransferase activity, as compared with the normal condition, is the cause for hypomethylation. The change in the activity of DNA methyltransferases, as well as the age-related changes in the structure of chromatin, which lead to a disruption in the availability of methylated DNA sites for DNA methylases, can be explained by the possible age-related decrease in the content of the CH<sub>3</sub>- donor (S-adenosylmonophosphate) in the cells. Thus, aging is a multi-causal process caused by a number of factors. The change in the expression of certain genes, observed with aging, can be a response to accidental damage or may reflect the side effects and multiple effects of genes controlling growth, development and metabolism. Our results from the study on the functional activity of the p53 gene in cell aging provide key information on the involvement of the gene in the control of cell aging processes and open new possibilities for the study of p53 function.



**Fig. 2.** PCR of the methylation of the promoter region of the p53 and p16 DNA from white blood cell leukocytes in different age groups. M – markers; 1,2,3,7,8,9 – the p53 gene methylated promoter regions; 4, 5, 6, 10 – the p53 gene unmethylated regions; 1, 2, 3, 5, 10 – the p16 gene methylated promoter regions; 4, 6, 7, 8, 9 – the p16 gene unmethylated regions, modified from Ref

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