FRONTLINE MEDICAL SCIENCES AND PHARMACEUTICAL JOURNAL
(ISSN – 2752-6712)
VOLUME 03 ISSUE 01 Pages: 01-11
SJIF IMPACT FACTOR (2021: 5.14) (2022: 5.605)
OCLC – 1272874727
Publisher: Frontline Journals

Journal Website: https://frontlinejournals.org/journals/index.php/fmspj

Research Article

THE ROLE OF P53 AND BCL-2 GENE EXPRESSION IN BREAST CANCER THERAPY

Submission Date: January 01, 2023, **Accepted Date:** January 05, 2023,
Published Date: January 10, 2023
Crossref doi: https://doi.org/10.37547/medical-fmspj-03-01-01

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ABSTRACT

Breast cancer is one of the most common cancers in women. Statistical data of recent years indicate a steady, intensive increase in the incidence and mortality from breast cancer (BC) No specific genetic or epigenetic changes in tumor cells are known that are responsible for their aggressive behavior the process of metastasis, which is the main cause of death in cancer patients. An important role in the occurrence and development of breast cancer of the p53 gene, which protects the body from mutant cells, has been noted. Mutations in the p53 gene allow cells with damaged DNA to maintain mitotic activity. The incompleteness

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of apoptosis explains the different tumor sizes, non-invasive and slow tumor growth. The bcl-2 gene, in turn, suppresses cell apoptosis. The bcl-2 protein plays a key role in the regulation of apoptosis. Based on the foregoing, the analysis of the expression of the p53 gene, the bcl-2 proto-oncogene and the dependence of their expression on various factors during tumor formation are an important pathophysiological characteristic.

KEYWORDS

Breast cancer, gene expression, p53 and bcl-2 genes, DNA, electrophoresis.

INTRODUCTION

Breast cancer (BC) is one of the most dangerous diseases in the world among women. According to the World Health Organization, 2.3 million women worldwide were diagnosed with breast cancer in 2020, of which 685,000 were fatal [1,2]. BC is the number one cancer in women, which means that one in four (24.2%) women in the world will get BC [3]. In Uzbekistan, 24.6% of women were diagnosed with breast cancer in one year [4,5]. The issue of studying the mechanisms of formation and progression of the disease is an actual problem of modern medicine. Damage of oncosuppressor genes, in particular TP53 gene, which is a key regulator of cellular processes, occupies a special place in the pattern of disorders in breast cancer [6].

Large number of studies are currently underway to study candidate genes that cause breast cancer and many somatic mutations in humans. One of them is the TP53 gene, which is the guardian of the genome. This TP53 gene is a tumor suppressor located at locus 17p13.1 (OMIM, 191170) and contains 11 exons of 20 kb each [6,7]. It consists of 2.8 bp. nucleotides that contain 393 amino acid residues, weighing 53 kDa, to encode the nuclear phosphoprotein p53 The TP53 protein is located in the cell nucleus and is directly linked to Deoxyribonucleic acid (DNA). TP53 is involved in cell cycle control, DNA repair, and regulation of apoptosis [7,8]. The incompleteness of apoptosis explains the different tumor sizes, non-invasive and slow

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tumor growth. The bcl-2 gene, in turn, suppresses cell apoptosis. The bcl-2 protein plays a key role in the regulation of apoptosis [9,10]. Based on the foregoing, the analysis of the expression of the p53 gene, the bcl-2 proto-oncogene and the dependence of their expression on various factors during tumor formation are an important pathophysiological characteristic.

RESEARCH METHODS

When performing the research, DNA samples obtained from peripheral blood leukocytes of patients suffering from breast cancer (180 patients) were used. The blood of oncological patients was obtained at the Department of Oncomammology of the Republican Scientific and Practical Center of Oncology and Radiology of the Ministry of Health of the Republic of Uzbekistan. As a control, peripheral blood leukocyte DNA from healthy donors (20 donors) was used. Blood in a volume of 1 ml was taken using a catheter from the cubital vein into vacuum tubes with 0,5 M EDTA.

Isolation of nuclear DNA from blood leukocytes. DNA isolation from whole blood was performed using the AmpliPrime Ribo Prep and Ribo Sorb AM reagent kit (InterLabService, Russia) according to the standard protocol. The method has been modified. Lysis of biological samples was carried out for 30-40 minutes at a temperature of 65 0 C. Centrifuged at 11 thousand revolution/minute DNA extraction was carried out at a speed of 13 thousand revolution/minute.

Determination of the concentration and purity of nucleic acids. The concentration of the resulting nucleic acid preparation in the samples was determined by the spectrophotometric method on a BioSpec-nano spectrophotometer (Shimadzu Biotech, Japan). The optical density (E) of the DNA preparation was measured at three wavelengths: 230 nm, 260 nm and 280 nm, against TE buffer. The purity of the sample was determined based on the ratio of E260/E280. The value of E260/E280 ≥1.8 showed that the DNA preparation was pure and did not contain impurities. mRNA expression of the studied p53 and bcl-2 genes was determined ¬by a semiquantitative reverse transcription polymerase chain reaction method. The amplification reaction was carried out according to the following scheme: denaturation - 94 °C, 10 s; annealing - 10 s; synthesis - 72 °C, 20 s. GAPDH expression was determined as an internal control to assess the amount of RNA taken into the

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reaction. The reverse transcription polymerase chain reaction products ¬were analyzed by electrophoresis in 2% agarose gel with the addition of 0.5 μg/ml ethidium bromide. The E260/E230 value was 1.8.

Reverse transcription (RT-PCR) Total RNA was obtained using the EZ1 RNA Tissue Mini Kit for RNA extraction from tissues (for 48 samples). Producer "InterlabService", Moscow, Russia. To obtain cDNA on an RNA matrix, a REVERTA- L kit kit , Russia, was used. The reverse transcription reaction was performed according to the manufacturer's protocol. We used primers for p53 genes and bcl -2. Primers of the GAPDH reference gene were used as a control . Polymerase Chain Reaction (PCR) was carried out on a US BioRad amplifier . PCR products were stored at -20 0 C.

Electrophoresis of PCR products was carried out in 2% agarose in TAE buffer, 100 V, 1 h. The results of electrophoretic analysis in the gel were visually observed through transmitted UV rays on a Bio - Rad transilluminator. The agarose gel after electrophoresis was scanned on a densitometer, which analyzes the luminescence intensity of PCR products . The resulting image of the bands was processed using a computerized densitometer (Gel - Pro - Analizer 4.0).

RESULTS AND DISCUSSION

The key function of p53 is to arrest the cell cycle and increase the duration of the premitotic phase for repair synthesis. The activated p53 protein is able to influence the regulation of the cell cycle through a number of mechanisms [11]. Mutations in the p53 gene allow cells with damaged DNA to maintain mitotic activity. The incompleteness of apoptosis explains the different sizes and different degrees of maturity of the nodes, the benign nature of the tumor, non-invasive and slow tumor growth. During tumor formation, a higher content of the bcl-2 protooncogene , which is one of the main inhibitors of apoptosis, is noted [12]. An increase in the expression of bcl-2 in a cell leads to a change in the normal course of apoptosis and an increase in the life span of this cell. The bcl-2 gene is of great importance in tumor growth, being one of the regulators of the balance between cell proliferation and death. High expression of this gene is associated with a low level of apoptosis; there is an inverse correlation between the p53 and Bcl -2 genes [13].

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Table 1 shows the primer sequence of the p53 and GAPDH genes. Figure 1 shows a quantitative assessment of p53 mRNA expression in peripheral blood lymphocytes of patients with breast cancer. The human reference gene GARDH was used as a control for the analysis of the expression level of the p53 gene. The luminescence intensity in UV PCR of the GARDH gene product is taken as 100%.

Table 1.

Primer sequence used in studies

Genes	Primer sequence	Size bp
p53 gene	For: 5'-GCCCCCACGGGGAGCACT-3' Rev: 5'-GGAGAGGAGCTGGTGTTG-3	321
GAPDH Referent gene (control).	For: 5' -CCATCACCATCTTCCAGGAG -3' Rev: 5' -CCTGCTTCACCACCTTCTTG -3'	576

The levels of mRNA of the p53 gene in lymphocytes were determined by RT-PCR using GARDH mRNA as an internal standard. To determine the level of mRNA expression, the integral optical density of the bands of the studied samples corresponding to gene-specific PCR products was normalized by % optical density by luminescence in UV PCR of the GARDH gene product . The expression of the p53 and bcl-2 genes in breast cancer was studied by RT PCR. Figure 2 shows data on the expression of p53 and bcl -2 genes in breast cancer. This figure shows that the expression of the p53 gene is higher than the expression of the bcl- 2 gene . An important role in the induction of apoptosis belongs to the p53 protein, which contributes to cell retention in the G1 phase of the cell cycle and thereby protects the body from mutant cells capable of malignant or benign transformation.

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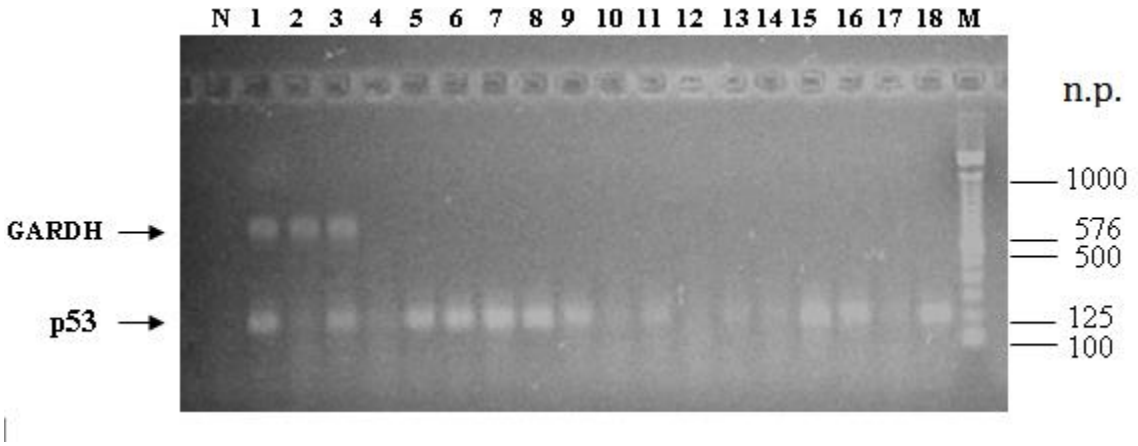

Fig.1. Analysis of p53 gene expression in blood lymphocytes, RT-PCR

Lanes: N , control (primers + water), M, 100 bp marker , GARDH , reference gene (luminescence intensity in the gel, PCR product, this gene is taken as 100%, control), p53, tumor suppressor. Tracks: 1-18 - samples under study; 1-3 – cDNA + GARDH gene primers . Electrophoresis was carried out in 2% agarose gel TAE buffer, 15 min at 20 V, then 1 h at 120 V. The gel was stained in an aqueous solution of ethidium bromide (0.5 μg/ml) for 15 min and then photographed through the transmitted beams of a UV transilluminator (Bio - Rad).

The bcl -2 gene is of great importance in tumor growth, being one of the regulators of the balance between cell proliferation and death. A feature of the control of p53 gene expression is that it is predominantly carried out at the post-translational level. Despite the fact that the p53 gene is constantly transcribed and translated in all cells of the body, the level of its protein product in the cells of most tissues remains extremely low [14].

Against the background of reduced expression of p53, which is manifested by a low rate of recognition and elimination of functionally defective and damaged cells, a clinically manifested formation and growth of a tumor occurs.

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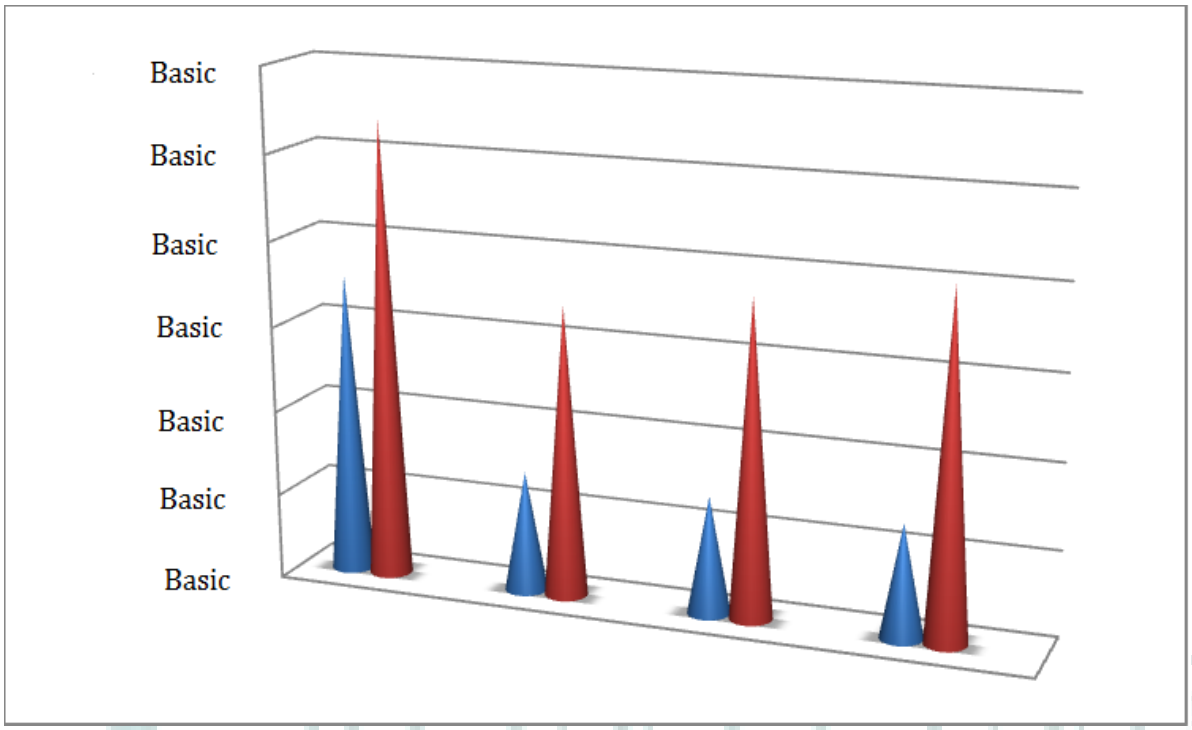

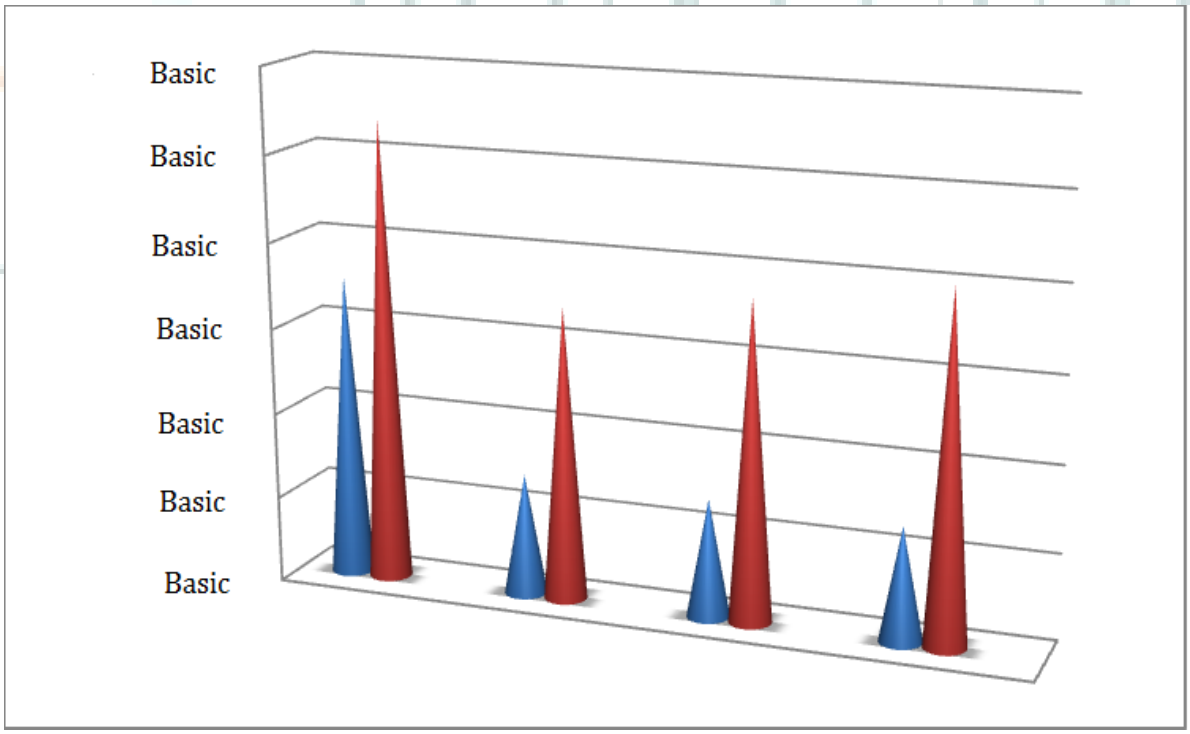

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Fig.2. Expression of the p53 and bcl -2 genes in breast cancer patients (blue columns bcl-2 gene, red columns p53 gene). x 2 = 4.84, p = 0.0185, OR = 6.84; 95% CI (34-26.6).

Mutations in the p53 gene allow cells with damaged DNA to remain viable in mitosis and thus lead to tumor transformation [15]. Figure 3 shows the results of the regulation of bcl-2 gene expression in the treatment of patients with breast cancer at different stages of the disease. This figure shows an increase in the expression of the bcl -2 gene in the treatment of breast cancer. High expression of this gene is associated with a low level of apoptosis; there is an inverse correlation between the p53 and bcl -2 genes. It is rather difficult to unambiguously say how the p53 protein is involved in the pathogenesis of breast cancer, however, it is already clear that the p53 gene polymorphism, leading to a change in the activity of the protein, can be considered as a genetic risk factor for the development of breast cancer, ineffective therapy for the severe clinical course of this disease.

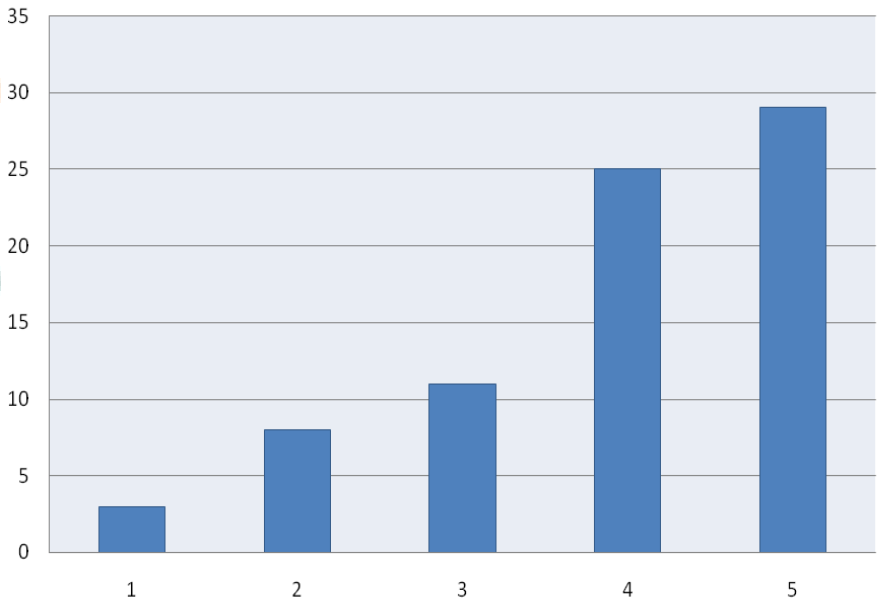

Fig.3. Expression of the bcl -2 gene in the treatment of patients with breast cancer at different stages (x 2 = 5.62, p = 0.018, OR = 7.91; 95% CI (44-36.90). Each column is a group of patients with

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different stages of breast cancer 1 - healthy donors (20 donors), 2 - stage I (65 patients), 3 - stage II (57 patients), 4.5 - stages III and IV of breast cancer (58 patients).

CONCLUSION

Based on the data obtained, it is possible to build a model of the influence of p53 and bcl-2 gene disorders on certain manifestations of tumor progression during the development of breast cancer. In addition, additional criteria can be developed for the formation of groups of increased oncological risk and prognosis of the disease. Identification of risk groups will allow to carry out preventive measures from an early age and carry out dispensary observation in order to identify precancerous conditions of breast cancer. Based on the contribution of genetic aberrations of the p53 gene to specific manifestations of tumor progression, it is possible to develop targeted therapy.

1. The study of the expression of p53 and bcl-2 genes in breast cancer was carried out.
2. It has been shown that tumor cells in patients with stage 4 PMC have a higher expression of the proto-oncogene bcl -2, which is one of the main inhibitors of apoptosis.

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