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SIGNIFICANCE OF P53 AND BCL-2 GENES IN CERVICAL CANCER.

Farrukh Sh. Fayziev Associate Professor, Department of Oncology Tashkent Medical Academy. Tashkent, Uzbekistan, ffsh2607@gmail.com;
Saidrasul K. Nasirov Assistant of the Department of Oncology Tashkent Medical Academy. Tashkent, Uzbekistan, nasirov.89@bk.ru.

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Abstract:
Literature data on the effect of the amount of apoptosis and the apoptosis index on the prognosis of malignant tumors are contradictory. Theoretically, active apoptosis in a tumor means its slow growth and low aggressiveness. In addition, the action of many antitumor agents is mediated by the induction of apoptosis.

Keywords: Cervical cancer, endometrial cancer, P53 and BCL-2 genes.

INTRODUCTION:

The literature data on the effect of the amount of apoptosis on the prognosis of VTS are contradictory. The authors note that other factors, including the mitotic index, invasive growth, and other factors, determine the course and prognosis of malignant tumors to a greater extent than the apoptotic index. The number of mitoses or mitotic index is a poorly understood predictor of SITS, however, according to some authors, a mitotic index above 5 indicates a high risk of death, has a correlation with a lower level of differentiation and deep myometrial invasion. Currently, tumor diseases have a steady growth trend. Therefore, scientific research is being carried out to determine the role of oncogenes in the origin of cancer. The p53 protein gene and its mutant form are the most studied. p53 is a transcription factor that controls the cell cycle and acts as a tumor suppressor. This protein gene is normally included in anti-oncogenes and is located in position p13 of chromosome 17. The p53 protein was discovered in 1979. Opened by Levin, D. Lane and W. Old and named by molecular weight (53 kDa). This protein is more abundant in virus-transformed (i.e., potentially oncogenic) cells than in normal cells, so p53 has long been considered an oncogene. Further studies have shown that p53 is normally a tumor suppressor.

Further studies have shown that p53 is normally a tumor suppressor. The p53 protein activates the transcription of some genes and inhibits the transcription of other genes, thereby affecting intracellular processes. One of the functions of p53 is to control the status of cellular DNA. In the presence of damage to the genetic equipment or abnormal processes, activation of p53 occurs, which leads to an acceleration of repair processes or cell cycle arrest and apoptosis under severe stress. Thus, this protein prevents the division and reproduction of potentially oncogenic cells. p53 maintains the genetic identity (stability) of the cells of a multicellular organism, which is why it is called the "guardian of the genome". Damage to the p53 gene causes 50% of oncological diseases, which means that the lack of activity of this protein ensures cell division even with DNA damage. As a result, genetic instability increases and the frequency of mutations increases, which leads to the accumulation of defective tumor suppressors and oncogenes.

Gain-of-function mutant p53 mutations may have additional oncogenic properties and inactivate normal p53. The value of p53 is not limited to protecting the genetic apparatus from negative influences and damage, but in everyday activities this protein regulates metabolism, increases the activity of antioxidant defense and detoxification processes, affects the rate of protein synthesis, controls the repair process of non-dividing cells, stimulates the reproductive function of the body, protects embryos from malformations. It is used to prevent atherosclerosis, metabolic disorders, neurodegenerative pathology and premature aging of the body. Recently, the notion that mutant p53 has a negative dominant inhibitory effect on normal p53 has been developed. Mice expressing mutant p53 have been found to have more aggressive and metastatic tumors than mice with normal p53 or complete loss of the p53 gene without p53 protein expression.

In the studies of D. Driak et al. p53 abnormalities were 4 times more common in sero-papillary carcinoma than in endometrioid adenocarcinoma. p53 is a nuclear protein that identifies a genetic defect and activates mechanisms aimed at cell repair. When damaged DNA cannot be repaired, this protein triggers apoptosis (programmed cell death) instead of activating repair mechanisms and prevents the damaged DNA from replicating. When the p53 gene is mutated, this protein can lose its properties. Mutated p53 has a long half-life compared to other proteins, so it accumulates in the nucleus of the H.E. cell. According to Langlois et al., p53 overexpression correlates with patient survival. In studies, overexpression of p53 was observed in diffuse tumors and was practically absent in localized forms of tumors. X. Matias - Guiu et al., that a significant increase in p53 and p27 is characteristic of all endometrial tumors, even for serous-papillary endometrial cancer; in which they emphasized the characteristic increase in these indicators in low-differentiated and moderately

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differentiated forms of endometrioid cancer and the absence of their increase in highly differentiated forms that affect the prognosis of the disease. In a comparative analysis of p53 expression in endometrial clear cell carcinoma and endometrioid cancer, T. Yalta et al. observed a consistent increase in p53 in clear cell tumors (64%) compared with the control group (19%). Y. Chen et al consider p53 expression to be an important prognostic factor in sero-papillary endometrial carcinoma, such as clear cell carcinoma, according to which abnormal p53 expression was associated with poor survival in 45% and 73% of cases, respectively. Bcl-2 and similar cytoplasmic proteins are key regulators of apoptosis, a cell suicide program that is critical for tissue development, homeostasis, and pathogenesis. The proteins closest to Bcl-2 inhibit the adapters necessary for the activation of proteases (caspases) involved in cell decay and ensures cell viability at the expense of ash. Their more distant relatives, in contrast, induce apoptosis by triggering mechanisms that remove adapters from pro-viable proteins. Thus, for many signals of apoptosis, the balance between competing processes determines cell fate. Proteins of the Bcl-2 family are necessary to maintain order in many organ systems, and mutations that damage them play an important role in carcinogenesis. Proteins of the Bcl-2 family control apoptosis at the mitochondrial level. The anti-apoptotic proteins of this large family are Bcl-2 and Bcl-xL, Bcl-w, Mcl-l, Al, Boo; proapoptotic proteins include Bax, Bad, Bok, Bcl-xS, Bak, Bid, Bik, Krk, and Mtd. Bcl-2 overexpression blocks mammalian cell apoptosis triggered by various stimuli such as growth factor depletion, radiation, s-myc, or antitumor drugs. Bcl-2 derivatives belong to a new class of proto-oncogenes that inhibit apoptosis and are induced by cisplastin and etoposide. Thus, this protein increases tumor resistance to chemotherapeutic agents. Many androgen-dependent tumors express Bcl-2.

Various members of the CED-9/Bcl-2 family may form dimers with each other, where one may enhance or inhibit the function of the other. In this case, the ratio of inhibitors or activators can determine the cell's susceptibility to apoptosis. The absence of functionally active Bcl-x or Bcl-2 in mice leads to embryonic or postnatal lethality of animals, which, in turn, leads to death in various organs due to increased programmed cell death. Conversely, if Bax is damaged, normal cell death does not occur. Another mechanism for controlling the activity of members of the Bcl-2 family is their phosphorylation. For example, Bad, a pro-apoptotic member of the Bcl-2 family, is phosphorylated by a growth factor-activated kinase. How does Bcl-2 inhibit apoptosis? A study of ced-9, ced-4 and ced-3 mutants showed that ced-9 inhibited cell death independently of ced-4 and ced-3. Biochemical mechanisms that stop apoptosis using ced-9/Bcl-2 and members of their family are poorly understood. Bcl-2 and Bcl-x are located on the outer mitochondrial membrane, endoplasmic reticulum and nucleus. It consists of two hydrophobic alpha chains similar to pore-forming bacterial toxins such as diphtheria toxin and colicins.

According to M. Kudela et al., in Bcl-2-positive tumors, a well-developed network of vessels is detected (in more than 73% of cases), and in Bcl-2-negative endometrial tumors, the density of vessels is generally low. Unlike Bcl-2-negative endometrial tumors, Bcl-2-expressing tumors usually retain PR receptors. The obtained results indicate that endometrial malignant tumors have a certain differentiation depending on the level of Bcl-2 expression. Analyzing data on neoangiogenesis and activity of estrogen metabolism and enzyme synthesis in tumors, Bcl-2 positive tumors are more aggressive than their biological potential, high levels of neoangiogenesis were found in these tumors along with high levels of estrogen synthesis, and in most cases these tumors retained PR.

These genes are identified by immunohistochemistry. There are several methods for conducting IGX, one of which is the avidin-biotin method. J.L. After the studies of Gesdon and co-authors, the substances of biotin and avidin began to be used in immunohistochemistry. Biotin (vitamin N) is stored in large quantities in the egg white of poultry, which is associated with a large glycoprotein, avidin. Avidin has a high affinity for biotin, one molecule of which can bind 4 molecules of biotin. Biotin can bind to Fc-immunoglobulins, each biotin molecule has one binding site, but several biotins can bind to one immunoglobulin molecule. Like biotin, avidin can be labeled with a fluorescent target, an enzyme, ferritin, or a gold molecule. The method using avidin-biotin complexes is more sensitive than the PAP method. In this method, the first layer is formed by primary rabbit antibodies, the second layer by biotinylated goat anti-rabbit immunoglobulins; the third layer is formed by the avidin-biotin complex. Avidin reacts with biotinylated horseradish peroxidase in such a proportion that the three binding sites of avidin react with biotinylated peroxidase, leaving one site in each molecule free for biotin to interact with the second antibody layer. Later, a method was proposed to block non-specific interactions with tissue biotin. Unconjugated avidin is added to the tissue to bind biotin, and then large amounts of unconjugated biotin are added to prevent binding of the avidin-biotin complex. New methods for imaging immunohistochemical reactions using conjugated polymers have now been developed by various companies. A large amount of enzyme is attached to a long polymer molecule. The sensitivity of the method is increased by increasing the concentration of the

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enzyme. In this case, primary antibodies and EPOS (Enhanced Polymer One Step Staining) enzyme molecules from Dako can be mixed with the polymer. When secondary antibodies and an enzyme (Dako, En Vision) are conjugated with a polymer molecule, the immunohistochemical reaction proceeds in 2 steps, which achieve high sensitivity compared to other imaging methods.

Immunohistochemical reaction protocol recommended by Dako.

Preparation of paraffin sections on slides coated with poly-L-lysine, deparaffinization and rehydration in TBS (Dako TBS, S196830; add 0.05% Tween 20). 2. Remove excess fluid around cuts and drip 1% hydrogen peroxide for 10 minutes. 3. Wash in TBS. 4. Removal of excess fluid around incisions. 5. Instillation of primary (mouse or rabbit) antibodies. Incubate for 30 minutes at room temperature in a humid chamber. 6. Wash in TBS. 7. Removal of excess fluid around the incisions. 8. Secondary instillation of antibodies (mixture of anti-mouse or anti-rabbit biotinylated antibodies). Incubate for 15-30 minutes at room temperature in a humid chamber. 9. Rinse in TBS. 10. Removal of excess fluid around incisions. 11. Instillations of streptavidin conjugated with peroxidase. Incubate for 15-30 minutes at room temperature in a humid chamber. 12. Wash in TBS. 13. Removal of excess fluid around incisions. 14. Histochemical determination of peroxidase activity with diaminobenzidine solution for 5-10 minutes. 15. Washing in water. 16. Stain nuclei with Mayer's hematoxylin for 1-2 minutes. 17. Rinse the sections under running water. 18. Dehydration of climbing batteries of alcohol. 19. Embedding cuttings in Canadian balsam.

CONCLUSION:

Thus, the expression of the mutant p53 gene in the analysis of postoperative materials after preoperative brachytherapy in patients with the tumor became bcl-2-negative, in 80% of patients the expression of the mutant p53 gene was not detected.

Expression of bcl-2, p53 and tumor invasion into myometrium - T ($t = 0.469$ and $t = 0.700$; $p < 0.05$) and BTS risk level - G ($t = 0.318$ and $t = 0.514$; $p < 0.05$) were found . have a positive correlation. There is a correlation between bcl-2 expression and the mutant p53 gene, but the correlation was not statistically significant ($r=0.131$). Taking into account the main clinical and morphological factors, independent risk factors for death in patients with BPS are age over 50 years, pathogenetic type II, contiguous extragenital pathology CD+GC, obesity grade III, histological type, localization and depth of the tumor. invasions, high expression of mutant p53 and bcl-2 genes, such signs as loss of expression of these genes after preoperative radiation treatment.

When analyzing the dependence of gene expression (p53, bcl-2) on overall and relapse-free survival in patients with SBTS: the risk ratio and confidence interval were 3.67 (2.31-5.82) for the loss of expression of the mutant p53 gene after HT. It was 2.67 (1.98-4.95) when bcl-2 gene expression was lost. In patients with BPS, treatment volume combined with preoperative radiotherapy has been shown to improve subsequent outcomes.

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