Molecular genetic changes in urothelial cells in bladder cancer

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ABSTRACT

The classification of transitional cell forms according to the TNM system, proposed by the International Anti-Cancer Union, does not allow to objectively predict the course of the disease

Keywords:

The classification of transitional cell forms according to the TNM system, proposed by the International Anti-Cancer Union, does not allow to objectively predict the course of the disease. It is proved that the leading role in determining the survival of patients is played by the depth of invasion of neoplasms, the degree of differentiation of tumor cells, the lesion of regional lymph nodes, the presence of insitu carcinoma (CIS)urothelia, the size and number of primary neoplasms, as well as the duration of the relapse-free period.The long-term results of treatment of patients with neoplasms that make up one classification subgroup differ significantly, which is especially noticeable in patients with superficial RMP, therefore, in recent years, attempts have been made to identify additional prognostic factors that would allow distinguishing tumors with a high risk of recurrence and progression from aggressive neoplasms and to apply differentiated treatment

Highly differentiated pTa tumors have less potential for progression than pT1, the probability of muscle invasion is 9 and 18%, respectively. Tumors of G1 progress in 6% of cases, while in G2 the potential for progression is 5 times greater and is 30%[8]. According to foreign and domestic researchers, the recurrence rate of superficial RMP after TUR is 60-90% [9]. In addition to the high frequency of relapses, there is a risk of possible progression of the disease in the form of invasive tumor growth (T) or a decrease in the degree of its differentiation (G), which is an unfavorable prognostic sign of the development of RMP.In general, the rate of progression within 3-5 years after various types of treatment for the Ta stage is 2-4%, T1–29–30%, for CIS about 54% [10]. In Western European countries, cytological examination of urine is the most commonly used method in conjunction with cystoscopy for the diagnosis of new bladder tumors and relapses.The combination of these methods is the "gold standard

The data of molecular biology convincingly prove the role of genetic factors in the initiation of the malignant process and the development of a tumor.However, the regularities of the functioning of the genome of cancer cells are far from being understood[12]. The appearance of a tumor cell occurs as a result of the accumulation in the hereditary apparatus of a normal cell of a certain number of molecular events leading to the activation or inactivation of genes that control the cell cycle. Each such

event in itself does not lead to irreversible damage to the cell, but their accumulation can disrupt the regulation of the cell cycle and, as a consequence, lead to the formation of a stable pathological cell clone with properties such as uncontrolled growth, independence from the regulatory influences of the cellular environment, the ability to invasive growth, metastasis.

At each stage of tumor development, various molecular disorders occur in the cell genome, which may indicate the stage of the disease, determine the speed of the malignant process, the invasiveness and metastatic potential of the tumor, the possibility of recurrence and the effectiveness of treatment.Such disorders can be called "markers of malignant transformation" and used to characterize the tumor process.Among them, there are several groups that have different diagnostic value.Markers of carcinogenesis can be chromosomal anomalies: deletions of chromosomal regions containing suppressor genes, up to the loss of an entire chromosome (monosomy) or, conversely, an increase in the copyicity of chromosomal regions, i.e. hyperamplification of proto–oncogenes.These are major damages to the genetic apparatus of the cell, which are associated with a generalized, irreversible tumor process.Such changes are usually manifested in the last stages of tumor development or indicate

The second group of structural markers characteristic of malignant tumors are mutations.Mutations can be divided into two groups – activating and inactivating.Activating ones take place in oncogenes or their receptors and lead to an increase in gene expression.Inactivating ones are found in genes that negatively regulate the cell cycle, in suppressors of tumor growth, in genes responsible for the repair system.Such mutations lead to a change or absence of a protein product. For most cell types in general, and urothelial in particular, the most powerful stimulators of reproduction are cytokines belonging to the group of growth factors (fibroblast growth factor – FGF, epithelial – EGF, insulin–like – IGF, vascular-endothelial- VEGF, etc.). Their binding to specific receptors induces cascade mechanisms, increases the activity of cyclin-dependent kinases, as a result DNA synthesis and cell division are initiated.Tumor cells themselves acquire the ability to generate

Activating mutations in exons 7 and 10 of the FGFR3 gene encoding the FGF receptor are shown for RMP, and such mutations are rare for low-grade and invasive forms of RMP [19]. B.W.Vanrhijn et al. [19] studied the FGFR3 gene in 72 patients.Mutations were found in 34 out of 53 patients with pTa stage.The frequency of mutations for noninvasive papillary carcinomas was 64%, the remaining 19 patients with a higher stage of the disease called differentiated tumors did not have this mutation ($p<0.0001$).With further follow-up for 12 months, it was found that relapses occur more often in patients with normal FGFR3. The authors suggested that the status of FGFR3 was a more significant predictor of relapse than clinical and morphological characteristics.Another group of scientists investigated FGFR3 mutations in 132 bladder tumors of different stages of malignancy, the mutation frequency was highest in non-invasive papillary tumors of the CT (74%), and no CIS-like mutations were found at all.As a result

As an example of inactivating mutations, we can consider mutations of the p53 gene. The p53 protein plays an important role as a keeper of the genome, in response to DNA damage, it triggers mechanisms that block the cell cycle and mechanisms of apoptosis to remove the damaged cell.A high level of expression of this protein keeps the cell in the G0/G1 phase of the cell cycle until DNA damage is corrected using repair systems. Due to this, the probability of accumulation of potentially dangerous cells in the body that can form a malignant tumor is significantly reduced. Mutations of this gene are present in about 50% of all human tumors. D. Esrig et al. [21] evaluated the status of the p53 gene in tumor samples of 243 patients with invasive RMP obtained as a result of radical cystectomy. Patients with a damaged p53 gene had a significantly higher risk of disease recurrence and were characterized by a decrease in overall survival. This relationship was most pronounced in patients whose tumor limited

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The high frequency of mutations of the FGFR3 gene in subsurface papillary cancer, and the p53 gene in invasive RMP allowed A. Vaccagi et al. [25] to study these mutations in patients with newly diagnosed RMP of all stages and degrees of differentiation in order to use the data obtained for molecular classification of tumors. It was determined that the combination of FGFR3 and p53 mutations in one patient is extremely rare. FGFR3 mutations were characteristic of pTa surface tumors (71%) and were significantly less common in pT2-T4 (5%; $p<0.0001$). Mutations of p53, on the contrary, were significantly more often detected at PT2-T4 (47%; $p<0.003$).The results obtained made it possible to divide tumors into two groups with different malignant potential [25]

The conducted studies allowed us to use data on molecular genetic damage in urothelial cells as a system of molecular markers. Investigating deletions of chromosome 9 and mutations and deletions of the p53 gene in bladder tumors, A. Hartmann et al. [26] proposed a scheme for the development of bladder tumors taking into account molecular genetic damage. The authors suggested that the development of papillary cancer with low malignant potential occurs when 9p/9q deletions appear and accumulate in cells.If, during the development of a tumor, at the stage of hyperplasia or dysplasia, damage to the p53 gene occurs as a result of deletion of 17p or mutation, then in this case the tumor will transform into CIS or acquire a higher degree of malignancy[26]

The epigenetic markers of malignant growth today include abnormal methylation of regulatory regions of suppressor genes. It is believed that epigenetic disorders are the earliest and can occur long before the clinical manifestation of the disease [27].Inactivation of genes regulating the cell cycle through abnormal methylation is widely represented in RMP. Abnormal methylation in bladder tumors has been shown for many suppressor genes, but with varying intensity. Interestingly, however, abnormal methylation of the p16 gene occurs quite rarely in RMP, its inactivation in this type of tumor is more often the result of the loss of the 9p21 fragment [28].

Urothelial cells, like other cells of the body, are located in a cellular layer, each element of which occupies a certain position. Cells contact each other and the intercellular matrix with the help of adhesive molecules-cadherins. E-cadherin, a representative of this family, provides the formation of adhesive intercellular contacts in epithelial cells. Malignant tumors are characterized by a loss of expression of E-cadherin, which is associated with the epithelial-mesenchymal transition, providing invasive tumor growth. The progression of RMP can be partly explained by the ability of tumor cells to lose intercellular adhesion and penetrate into surrounding tissues. A decrease in the level of E-cadherin leads to the transformation of superficial transitional cell carcinomas of the bladder into invasive cancer, which confirms the hypothesis of the significance of the CDH1 gene encoding this protein in the carcinogenesis of RMP [29].S.F. Shariat et al. [30] found a relationship between the expression level of E-cadherin determined by immunohistochemistry

The change in the amount of protein is directly related to the inactivation of the CDH1 gene as a result of its abnormal methylation. However, other tumor suppressor genes also exhibit hypermethylation in bladder tumors.W.Y. Michael et al. [31] analyzed 7 genes (RARß,DAPK,CDH1,p16,p15,GSTP1,MGMT) involved in carcinogenesis in 98 bladder tumors. It turned out that the frequency of methylation of the genes RARß,DAPK, CDH1 in tumors is very high – 87, 60 and 63%, respectively. This made it possible to use the methylation of these genes as a marker system for the diagnosis of tumor cells in urine. Using a set of 4 genes (DAPK,RARß,CDH1,p16), the authors found that methylation of at least one of them can be detected in the urine of patients in 90.9% of cases, while urine cytology was informative only in 45.5%. Thus, the combined use of the two methods increases the accuracy of diagnosis, especially for highly differentiated tumors [31].

From the presented literature review, it becomes clear that molecular genetic studies play a central role in the study of the mechanisms of carcinogenesis in RMP.There are three main areas of clinical use of the data obtained. 1. The use of genetic markers as prognostic factors. 2. Development and application of systems for early noninvasive diagnosis of RMP, monitoring of the effectiveness of antitumor therapy. A striking example is the test systems "UroVysion", "ImmunoCyt+", etc

3. The most promising and clinically important direction is the development of "targeted" drugs, which are characterized by an impact on a certain mechanism of carcinogenesis.For the treatment of metastatic kidney cancer, sorafenib, sunitinib, bevacizumab are currently used, which have proven their effectiveness in patients previously considered incurable.These drugs block the cytoplasmic part of the receptors for growth factors, reduce the proliferative activity of tumor cells. Bevacizumab is a human recombinant antibody against VEGF, suppresses the formation of a network of capillaries that supply secondary tumor foci. RMP is a common disease. The life expectancy of patients with distant metastases is about 12 months. Achievements in the field of molecular biology are not only fundamental, but also of practical importance for the creation of antitumor drugs effective in disseminated malignant processes,

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