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CLINICAL EXPERIENCE OF APPLICATION OF MOLECULAR-GENETIC MARKERS IN GASTRIC CANCER SURGERY

Khorobryh T.V.[2], Nemtsova M.V.[3], Shulutko A.M.[2], Agadzhanov V.G.[2], Andriyanov A.S.[1]*

[1] Department of Faculty Surgery, Sechenov University, Moscow, Russia
[2] Department of Faculty Surgery No 2, Sechenov University, Moscow, Russia
[3] Laboratory of Medical Genetics of the Institute of Molecular Medicine, Sechenov University, Moscow, Russia

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Abstract

Present study aimed at improving the outcomes in patients with gastric cancer through the application of molecular genetic methods for examination of the gastric mucosa at the preoperative stage.

The study included 156 patients: 106 patients with gastric cancer (main group) and 50 with cholelithiasis, as a comparison group with unchanged gastric mucosa. All 106 patients with gastric cancer underwent scheduled surgical intervention: in the form of gastrectomy (n=42) or gastric resection (n=64). Genetic tests of the resected tumor material and distant gastric mucosa in the main group and the comparison group were performed.

Locally advanced gastric cancer was verified in 80 (76%) patients of the main group; 58 (72.5%) of them underwent adjuvant chemotherapy with 5-fluorouracil. To assess the efficacy of 5-fluorouracil-based chemotherapy, polymorphisms of the TYMS and TP53 genes, which are detected in locally advanced gastric cancer, were studied.

Genetic tests in patients with gastric cancer reliably confirmed ($p<0.01$) an increase in expression of hTERT, MMP7, BIRC5 markers, as well as telomerase activity, at the same time statistically significant reduction in the surrounding mucosa located 5 cm below and above the tumor node.

When assessing the relationship of TYMS gene polymorphisms with the long-term results of combined treatment of patients with locally advanced gastric cancer: the mean relapse-free survival in patients with the 3R/2R genotype was 34.6±2.2 months, the mean relapse-free survival in patients with the 3R/3R genotype was 25.8±2.5 months. For TP 53 gene polymorphisms: mean relapse-free survival in Arg/Arg genotype is 29.1±2.2 months. Mean relapse-free survival in Arg/Pro genotype is 29.5±3.3 months. Mean relapse-free survival in Pro/Pro genotype is 20.0±3.5 months.

In patients with gastric cancer, analysis of molecular genetic markers is recommended, since the data obtained allow to assess the genetic potential of the mucosa without gross changes and reduce the extent of surgical intervention, as well as to predict the effect of 5-fluorouracil-based adjuvant chemotherapy in patients with locally advanced gastric cancer.

Keywords: *gastric cancer, tumors with microsatellite instability, Epstein-Barr virus.*

Introduction

Gastric cancer is one of the most aggressive malignancies with limited treatment options, resulting in a poor prognosis and low survival [*Ratti M et al., 2018*].

Although the incidence of gastric cancer has declined over the past decades, five-year survival remains low, approximately 10% in patients with advanced gastric cancer. In developed countries such as Japan, where early diagnosis of gastric cancer reaches 50%, five-year survival rate reaches 90% [*Necula L et al., 2019*].

Gastric cancer is a complex disease with significant variability of tumor cell behavior and response to chemotherapy [*Lazar D et al., 2016*].

Gastric tumors are characterized by high molecular heterogeneity, which is responsible for the process of carcinogenesis and metastasis. By identifying the molecular subtype of the tumor, it is possible

Address for Correspondence:

Andrey S. Andriyanov

Department of Faculty Surgery Sechenov University
Dovator Street, 1 building, apt 15, Moscow 119048, Russia
Tel.: +79175903833
E-mail: acandriyanov@rambler.ru

to predict the progression of the tumor process, the outcome for the patient and the approach to treatment in accordance with the genetic and epigenetic profile of the tumor [*Lazar D et al., 2016*].

Many different genetic mutations, epigenetic changes, and unregulated signaling pathways are involved in the pathogenesis of gastric cancer; each of these molecular abnormalities acts at different stages of the disease [*Lazar D et al., 2016*].

Currently, new therapies targeted at some molecular signaling pathways are already included into standard treatment of gastric cancer, while efficacy of others is still to be confirmed in clinical trials [*Lazar D et al., 2016*].

Modern classifications of molecular genetic markers

In 2014, based on key DNA defects and molecular abnormalities, the cancer genome atlas analysis identified four distinct genotypes of gastric cancer: tumors were categorized as follows: Epstein-Barr virus-associated (EBV+) gastric cancers (9%), according to the microsatellite instability status (MSI; 22%), and the remaining tumors were classified by the degree of aneuploidy into genomically stable cancers (20%) and those showing chromosomal instability (50%) [*Lazar D et al., 2016*]. The role of genetic subtypes in the treatment of gastric cancer has not been determined, but elements of clinically significant differences are already noted [*Jácome A et al., 2016*].

1. Epstein-Barr virus (EBV+) containing tumors, with widely expressed DNA hypermethylation, JAK2 amplification and known suppressors of the immune response - programmed death ligands 1 (PD-L1) and 2 (PD-L2) (in case of expression of PD-L1 / PD-L2 by tumor cells, these ligands are involved in the mechanisms of tumor escape from immune control). This group accounts for approximately 10% of cases of gastric cancer. EBV-associated subtype is characterized by a younger age group of patients, mainly males [*Charalampakis N et al., 2018; Rosa S et al., 2018*].

2. Tumors with MSI, where a high frequency of mutations, including mutations in genes encoding targeted oncogenic signaling proteins, occurs due to the disruptions in DNA repair mechanisms. They account for 20% of gastric cancers. Disease occurs mostly in women, more common in elderly patients. The intestinal subtype is histologically characteristic. These tumors are resistant to standard chemotherapy [*Smyth E et al., 2017*].

3. Most tumors are classified as "chromosomally instable". These tumors are characterized by multiple chromosomal rearrangements, deletions, translocations, activation of tyrosine kinase receptors, high expression and amplification of HER2; TP53 mutation occurs in 70% of cases. This group is found in about 50% of patients with gastric cancer [*Charalampakis N et al., 2018*].

4. The last group is classified as "genomically stable", and has no molecular characteristics of the other three subtypes. A diffuse growth is characteristic for "genomically stable" tumors. They account for 20% of gastric cancers, characterized by the absence of a high level of aneuploidy and high metastatic potential [*Charalampakis N et al., 2018*].

Thus, most tumors of the diffuse histological subtype belong to the genomically stable group. Chromosomally unstable tumors were mainly at the cardioesophageal junction/cardia, while most EBV+ tumors were located in the fundus or body of the stomach. Genomically stable tumors were diagnosed at an earlier age compared to tumors with microsatellite instability; in most cases, EBV+ subtype tumors were found in men (81%) [*Lazar D et al., 2016*].

This classification can be used in addition to the histopathology to stratify patients as a guideline for targeting agents. Genotypes classified by the cancer genome atlas are now confirmed as prognostic [*Charalampakis N et al., 2018*]. In addition, the Asian Cancer Research Group classified gastric cancer into four subtypes based on gene expression data and assessed correlation with postoperative relapse patterns and survival outcomes [*Charalampakis N et al., 2018*]. The worst prognosis is for mesenchymal tumors, followed by TP53-inactive ones (TP53 is a tumor suppressor gene, located on the short arm of chromosome 17. It encodes p53 protein. Biological role of p53 protein is to ensure genome stability and the ge-

To overcome it is possible, due to the uniting the knowledge and will of all doctors in the world

netic homogeneity of cells in the whole organism [*Shiao Y et al., 1994; Fenoglio-Preiser C et al., 2003*]), TP53-active and the best for tumors with microsatellite instability. The cancer genome atlas and the Asian Cancer Research Group classification systems have similarities but also differences [*Charalampakis N et al., 2018*].

Thus, the molecular classification of gastric cancer can influence clinical decisions: whether to use targeted therapy or immunotherapy, and helps in the development of new drugs or new combination therapies [*Chia N, Tan P, 2016*].

THE USE OF MOLECULAR GENETIC MARKERS IN THE TREATMENT OF GASTRIC CANCER

Genetically stable tumor subtype: In genetically stable subtype, there are no promising markers of targeted chemotherapy that can be used in clinical practice; therefore, for this subtype of tumors, surgery remains the main method of treatment [*Alessandrini L et al., 2018*].

Epstein-Barr virus-associated subtype (EBV+): This subtype is characterized by high immunogenicity (high expression of PD-L1 and high level of CD8+ tumor-infiltrating T-killers), therefore subtype with this characteristic responds well to immunotherapy. Studies have showed that all patients with this subtype (according FISH) had a response to pembrolizumab therapy [*Shinozaki-Ushiku A et al., 2015*].

Microsatellite instability subtype: Patients with MSI subtype had a favorable prognosis after surgical treatment only; chemotherapy worsened the prognosis in this group of patients. These findings came from the MAGIC study, which assessed the effect of perioperative therapy, and the CLASSIC study, which assessed adjuvant chemotherapy, as chemotherapy in gastric cancer with a high level of MSI-H destroys the "immune shield" and provokes the development of metastases [*Ratti M et al., 2018*]. Thus, if the tumor shows characteristics of MSI-H subtype, only surgical treatment is indicated, and systemic treatment worsens prognosis. In metastatic cancer, MSI subtype has a predictive role in the planning of immunotherapy (objective response rate in patients with MSI subtype is 50-60%) [*Sato Y et al., 2020*].

Adjuvant chemotherapy in diffuse and intestinal subtypes of gastric cancer

In 2018, a meta-analysis was published in which the authors tried to evaluate and compare the effect of adjuvant chemotherapy on the overall survival of patients with diffuse or intestinal gastric cancer subtypes. In the intestinal subtype, the addition of adjuvant chemotherapy resulted in notable improvement in overall and relapse-free survival. In diffuse subtype, overall survival was exactly the same as with surgical treatment. Thus, adjuvant chemotherapy has been identified as an independent prognostic factor only in intestinal gastric cancer [*Wang K et al., 2018*].

MATERIALS AND METHODS

Since 2008, studies on the use of molecular genetic markers in surgical treatment of gastric cancer are ongoing at the departmental surgery clinics of the Sechenov University.

Original preoperative molecular genetic test panel was used.

The first part of the molecular genetic panel included identification of abnormal methylation of the CDH1, MLH1, N33, RASSF1A, DAPK suppressor genes in tumor tissue.

CDH1 gene, a tumor suppressor gene, encodes the E-cadherin protein, which is responsible for cell adhesion. Reduction in gene activity leads to the acquisition of the ability of the tumor cell to invade and metastasize [*Wang H et al., 2004; Maghraby H et al., 2006; Wang L et al., 2006*].

MLH1 gene is a suppressor gene that encodes a factor involved in the repair of unpaired bases that appear as a result of DNA replication errors [*Kang Y et al., 2002*]. Mutations in this gene lead to the development of gastric cancer.

N33 gene is a suppressor gene. Dysfunction of this gene demonstrates a high level of methylation in gastric cancer.

RASSF1A gene is a suppressor gene, involved in the regulation of cell proliferation and apoptosis, encodes a factor that controls the cell cycle, and its dysfunction contributes to the development of gastric cancer [*Mazoshita T et al., 2003*].

DAPK gene encodes a protein kinase that mediates apoptosis and prevents tumor progression by inhibiting cell polarization. Inactivation of the DAP kinase gene leads to increased migration and invasion of tumor cells, and prevents apoptosis [*Wang W et al., 2002*].

The second part of the molecular markers panel included identification of hTERT, MMP7, BIRC5, TYMS, TP53 genes expression.

Matrix metalloproteinases – proteases that can lyse all structural components of the extracellular matrix; they are produced by stromal cells surrounding the tumor; their high production is associated with metastasis, neoangiogenesis, and poor prognosis.

MMP7 (matrilisin) is a metalloproteinase secreted by the tumor epithelium, its expression correlates with the histologically aggressive behavior of the tumor.

Survivin (Sur) is an endogenous protein that belongs to the family of proteins that inhibit apoptosis. Normally, it participates in the processes of mitosis and embryogenesis; in tumors, survivin gene (BIRC5) is expressed in large quantities and suppresses apoptosis.

TP53 gene encodes a transcription factor that, in case of genome impairment, causes a delay in the cell cycle in the G1 phase and promotes apoptosis. In tumor cells, gene function is disrupted: the apoptosis program switches to the unlimited proliferation pattern.

TYMS gene encodes thymidylate synthase, which is involved in the de novo synthesis of thymine and uracil nucleotides necessary for DNA replication, and therefore serves as a target for drugs, in particular for 5-fluorouracil.

Third part of the panel is aimed at determination of **telomerase activity.** Telomerase is an enzyme encoded by the hTERT gene that is necessary to maintain chromosome stability during cell division and is normally present only in a small pool of actively dividing cells. In a malignant tumor, telomerase plays a key role in maintaining the proliferative potential of cells and further tumor growth [*Yokozaki H et al., 2001*].

The study included 156 patients. All patients were divided into two groups: main group - 106 patients with gastric cancer and the comparison group with unchanged gastric mucosa - 50 patients with cholelithiasis (gallstone disease).

Among the patients included in the study, 55.8% (87) were men, 44.2% (69) were women. The study predominantly included patients over 60 years of age.

All 106 patients from the main group (patients with gastric cancer) underwent elective surgery: gastrectomy in 42 patients (40%) or gastric resection in 64 patients (60%) with lymphadenectomy (D1) – 9 patients (8%), D2 – 84 patients (80%), D3 – 13 patients (12%).

The histological variants of tumors were as follows: adenocarcinoma of various grades (highly differentiated adenocarcinoma - 16 patients (15%), moderately differentiated adenocarcinoma - 34 patients (32%), poorly differentiated adenocarcinoma - 32 patients (30.2%)), signet ring cell carcinoma - 22 patients (20.8%), undifferentiated carcinoma - 2 patients (2%) (Fig. 1).

The stages of the disease in patients with gastric cancer were as follows: stage IA - 16 (15%), stage I B - 20 (18.9%), stage II - 26 (24.6%), stage IIIA - 20 (18.9%), stage IIIB - 10 (9.4%), and stage IV - 14 (13.2%).

Most patients in the main group had locally advanced gastric cancer (T2-T4N0-N3M0) - 80 pa-

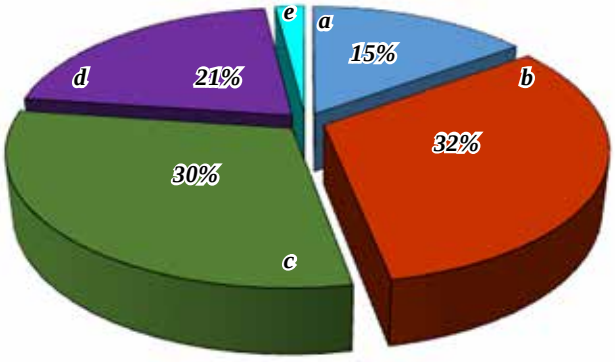

Figure 1. *Histological variants of gastric cancer (106 patients from the main group*
*(**a**)-highly differentiated adenocarcinoma, (**b**)-moderately differentiated adenocarcinoma, (**c**)-poorly differentiated adenocarcinoma, (**d**)-signet ring cell carcinoma, (**e**)-undifferentiated carcinoma (2%)*

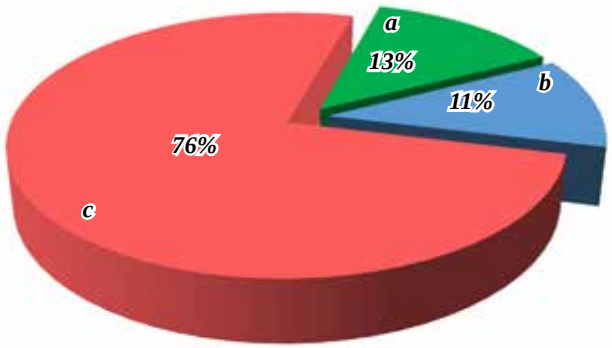

Figure 2. *Main study group (n=106 patients with gastric cancer)*
*(**a**)- early gastric cancer, (**b**)-locally advanced gastric cancer, (**c**)-generalized cancer*

tients (76%); generalized cancer (TanyNanyM1) developed in 14 patients (13%); early cancer (early gastric cancer - invasive carcinoma limited to the mucous membrane or mucous membrane and submucosa, regardless of the involvement of regional lymph nodes (Tis-T1N0-3M0)) developed in 12 patients (11%) (Fig. 2).

After resection or gastrectomy and identification of tumor localization, tumor segment tissue was excised in all 106 patients. Sampling for marker analysis was conducted as follows. The first tissue sample (point 1) was taken from the upper resection margin 5 *cm* above the visible proximal edge of the tumor; the second tissue sample (point 2) is from a visually defined area of the tumor (Fig. 3). Material from the 2e point was obtained endoscopically. The third tissue sample (point 3) was taken 5 *cm* below the visible distal edge of the tumor; the fourth tissue sample (point 4) - at a distance of 0.5 *cm* from the visible margin of the tumor, based on the assumption that the real border of the tumor can be located at a distance of up to 2-3 *cm* in case of exophytic growth, 5-6 *cm* in case of infiltrative tumor growth, and in case of dysplasia and metaplasia - up to 7 *cm* from the visible margin of the tumor. The total volume of tissue obtained from one sampling point was more than 30 μL [29] For each sample (points 1-4), a histopathological examination was performed to identify tumor cells, and when the tumor was identified, its histotyping was carried out.

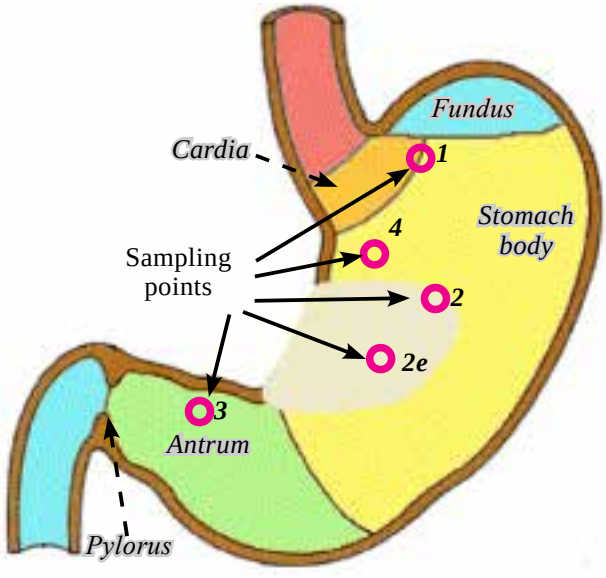

***FIGURE 3**. Areas of tissue sampling in the study group of patients*

Abnormal methylation of CDH1, RASSF1A, MLH1, N33 DAPK genes was investigated in all 106 patients in each of the samples (points 1-4) obtained intraoperatively, and additionally in 53 (50%) patients - in the samples obtained endoscopically (point 2e) using methyl-sensitive polymerase chain reaction using the restriction endonuclease Hpall. Also, the expression of the hTERT, MMP7, BIRC5, TP53 genes was assessed. Analysis of the expression of the studied genes of metalloproteinases MMP7, BIRC5, TP53, and hTERT was performed using reverse transcription polymerase chain reaction. In addition, in each of the surgical samples of all patients, telomerase activity was investigated using modified TRAP method.

In the comparison group (patients with cholelithiasis), to analyze molecular markers, endoscopic material was used, which was taken at the stage of preoperative examination for subsequent cytological, histological and molecular genetic studies.

To assess the role of molecular genetic changes in the combined treatment of gastric cancer, study was carried out with 80 patients with locally advanced gastric cancer (stages IB - IIIB) after radical surgery: subtotal distal gastric resection in 16 patients (20%), marginally subtotal distal gastrectomy in 22 patients (27.5%), and gastrectomy in 42 patients (52.5%).

Intraoperative pathomorphological examination of the material was conducted in all cases.

Adjuvant chemotherapy with the inclusion of 5-fluorouracil (5-FU) for locally advanced gastric cancer was performed in 58 patients.

Out of 80 patients with locally advanced gastric cancer, 50 (62%) were men and 30 (38%) were women.

To develop a system of individual factors for predicting the clinical course of locally advanced gastric cancer, as well as the efficacy of chemotherapy with 5-FU derivatives, genotyping for TYMS gene (R2/R3, G>C, Del 6bp) and TP53 gene (72 Arg>Pro, Insl6bp) polymorphisms was carried out in all patients.

Results

Genetic study of tumor material and distant gastric mucosa showed that the surgical material tes results completely coincided with the preoperative data obtained by endoscopic biopsy, which proves the possibility of use of the genetic material after endoscopic biopsy at the preoperative stage (Tables 1, 2, 3).

To assess the possible correlation between CDH1, RASSF1A, MLH1, N33, DAPK genes methylation and the development of gastric cancer, the methylation of these genes in the tumor and non-tumor (borderline) epithelium of the gastric mucosa was studied. N33, CDH1, and DAPK genes had the highest frequency of abnormal methylation; methylation of these genes was identified in most samples obtained from each patient, including grossly unchanged tissue in the proximity of the upper and lower resection margins. On the contrary, RASSF1A and MLH1 genes did not show methylation in the mucosa surrounding the tumor (points 1, 3, 4), and their methylation occurred mainly in tumor samples (point 2). Statistically significant differences were noted in the levels of methylation of these genes in the tumor and in other parts of the mucosa.

Table 1

Gene methylation frequency

Types of genes	II-o point (surgical (resected) material)	II-e point (material obtained endoscopically)
N33	61.15±6.7	63.0±6.6
CDH1	53.25±6.	63.0±6.6
RASSF1A	14.75±4.7	13.1±4.3
MLH1	15.15±3.7	14.8±4.7
DAPK	35.55±6.6	50.0±6.8

Table 2

Gene expression level analysis

Parameter	II-o sampling point	II-e sampling point	p
BIRC5	0.97±0.06	0.89±0.05	>0.05
MMP7	0.8±0.07	0.77±0.06	>0.05
hTERT	0.42±0.043	0.49±0.05	>0.05

Table 3

Telomerase activity analysis

Parameter	II-o point	II-e point	p
Telomerase activity	66.1±5.8	62.0±4.9	>0.05

In the main group, methylation of the RASSF1A and MLH1 genes was determined in the tumor tissue; in the comparison group, there was no methylation of RASSF1A and MLH1 genes. However, according to the study data, frequency of methylation of the RASSF1A and MLH1 genes in the tumor is low and does not exceed 20% (Table 4).

The comparison group showed low methylation levels of N33, CDH1, DAPK genes and the absence of methylation of RASSF1A, MLH1 genes, low expression of hTERT, MMP7, BIRC5, and low telomerase activity.

Thus, these markers can potentially be used as markers of changes in the mucosa characteristic of carcinogenesis (Tables 4, 5).

Analysis in this group of patients with gastric cancer confirmed ($p<0.01$) an increase in expression of hTERT, MMP7, BIRC5 markers, as well as telomerase activity, at the same time statistically significant reduction in the surrounding mucosa located 5 *cm* below and above the tumor node (Table 6).

In a tissue with normal morphology surrounding a tumor, high methylation of the CDH1, N33, and DAPK genes may indirectly indicate the involvement of this tissue in the tumor process. The study of this issue has not yet been fully completed; it requires a deeper investigation of the tumor material and the surrounding non-tumor tissue.

Thus, molecular genetic markers analysis allows to assess the genetic potential of the mucosa without gross changes and to reduce the extent of resection.

Among 64 study participants with gastric resection: 62 patients underwent R0 gastric resection; 2 patients with an extreme degree of anesthetic risk for life indications underwent cytoreductive surgery - R1 gastric resection. In 62 patients who underwent R0 gastric resection, control test of the unchanged mucosa of the stump did not show an increase in the expression of markers hTERT, MMP7, BIRC5, as well as telomerase activity, i.e. there were no genetic alterations in the mucosa, which confirms the curative nature of the surgeries; in 2 patients who underwent cytoreductive surgery - R1 gastric resection, an increase in the expression of hTERT, BIRC5 markers, as well as in-

TABLE 4

Gene methylation analysis

Types of genes	Main group, patients with gastric cancer (II point, tumor)	Comparison group, patients with cholelithiasis (IIe - point)
N33	61.15±6.7	12.5±5.6
CDH1	53.25±6.8	25.0±6.5
RASSF1A	14.75±4.7	0.0
MLH1	15.15±3.7	0.0
DAPK	35.55±6.6	12.5±5.6

TABLE 5

Telomerase activity analysis

	Telomerase activity
Point 1	31.0±4.8
Point 2 (TUMOR)	66.1±5.8
Point 3	32.6±4.9
Point 4	27.6±5.1
Interval for II	77.7–54.5

TABLE 6

Level of expression of genes BIRC5, MMP7, hTERT in the main group (patients with gastric cancer) and in the comparison group

	BIRC5	MMP7	hTERT
Point 1	0.57±0.06	0.3±0.06	0.15±0.02
Point 2 (TUMOR)	0.97±0.06*	0.8±0.07*	0.42±0.04*
Point 3	0.78±0.078	0.3±0.04	0.26±0.038
Point 4	0.61±0.06	0.4±0.067	0.25±0.28
Patients with cholelithiasis	0.45±0.17	0.34±0.06	0.15±0.07

Note: ** Indicators of gene expression at sampling point 2 statistically significantly differ from ($p<0.01$) from the indicators in the comparison group*

crease in telomerase were noted in the unchanged mucosa of the stump, which requires additional monitoring in this group of patients in the postoperative period.

In order to study the use of TYMS and TP53 gene polymorphisms as criteria for individual selection of treatment, we investigated the relationship between specific genotypes and long-term results of combined treatment in 58 patients with locally advanced gastric cancer who, after radical surgery, underwent chemotherapy according to the fluoropyrimidine-based regimens (median follow-up was 33 months).

In patients with locally advanced gastric cancer, molecular genetic changes in tumor tissue (surgical material) were determined by genotyping for polymorphisms of the TYMS (R2/R3, G>C, Del6bp) and TP53 (72 Arg>Pro, Ins16bp) genes.

When studying the correlation between TYMS gene polymorphisms and long-term results of combined treatment of patients with gastric cancer, the following data were obtained: mean relapse-free survival in the 3R/3R subgroup was 25.8±2.5 months (95% confidence interval [95% CI] 20.8-30.7), median survival – 30 months. Mean relapse-free survival in the 3R/2R subgroup was 34.6±2.2 months. (95% CI 30.4-38.9) (Fig. 4).

Mean overall survival in the 3R/3R subgroup was 28.6±2.4 months. (95% CI 24.0-33.2), median survival - 30 months. Mean overall survival in the 3R/2R subgroup was 34.9±2.5 months. (95% CI 29.9-39.8) (Fig. 5).

When studying the correlation between relapse-free survival and TP53 gene polymorphisms, it was found that relapse-free survival in patients with Pro/Pro genotype is significantly lower vs. patients with Arg/Arg and Arg/Pro genotypes ($p=0.013$ and $p=0.015$, respectively; log-rank test) (Fig. 6).

In the study of the correlation between TP 53 gene polymorphisms and long-term results of combined treatment in patients with gastric cancer, the following results were obtained: mean relapse-free survival in patients with Arg/Arg genotype is 29.1±2.2 months (95% CI 24.7-33.5), median survival 31 months. Mean relapse-free survival in

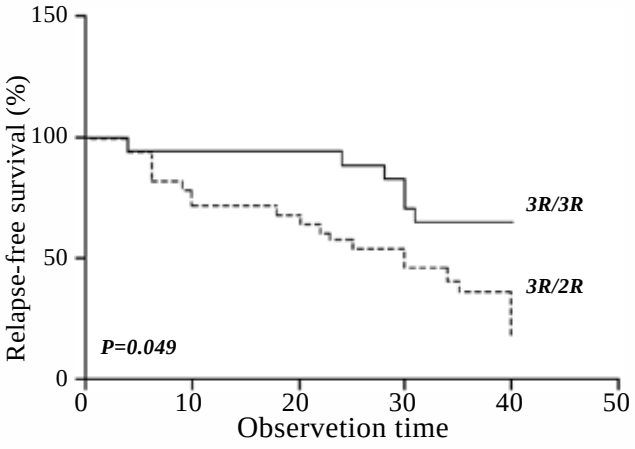

FIGURE 4. *Relapse-free survival in patients with locally advanced gastric cancer with 5`UTR VNTP polymorphisms of the TYMS gene*

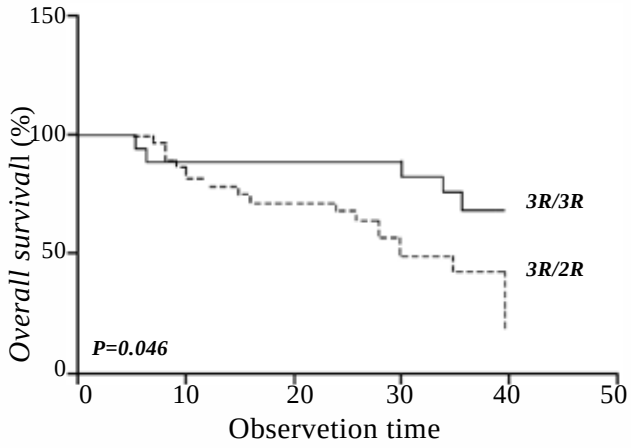

Figure 5 *Overall survival in patients with locally advanced gastric cancer with 5`UTR VNTP polymorphisms of the TYMS gene*

Arg/Pro genotype is 29.5±3.3 months (95% CI 23.1-36.0), median survival has not been achieved. Mean relapse-free survival in Pro/Pro genotype is 20.0±3.5 months (95% CI 13.2-26.8), median survival is 23 months.

According to the genetic study, it was found that A-6/A-6, 2G/3C (TYMS), Arg/Pro (TP53) genotypes are factors indicating favorable prognosis in locally advanced gastric cancer. 2R/2R, 3G/3G (TYMS), Pro/Pro (TP53) genotypes are factors of poor prognosis in locally advanced gastric cancer. 2R/3R, Arg/Pro, and Arg/Arg genotypes are associated with improved efficacy of 5-FU-based chemotherapy regimens; 3R/3R and Pro/Pro genotypes are associated with the worst efficacy of 5-FU-based chemotherapy regimens.

Thus, preoperative genotyping should be applied to assess possible effect of fluoropyrimidines-based adjuvant chemotherapy regimens after curative surgical treatment in patients with locally advanced gastric cancer.

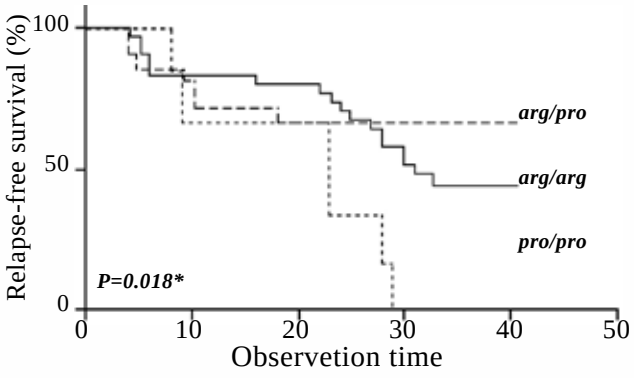

Figure 6. *Relapse-free survival of patients with locally advanced gastric cancer with Arg>Pro TP53 gene polymorphisms in codon 72*

Discussion

Modern molecular genetic study methods can reduce the extent of surgical intervention in gastric cancer, and also allow to predict the effect of adjuvant chemotherapy in patients with locally advanced gastric cancer [*Li Q et al., 2010*].

Indications for organ-sparing surgeries can be expanded through the use of molecular genetic markers proposed in the study. It is advisable to analyze the grossly unchanged mucosa in the area of gastric stump to be spared, for hTERT, MMP7, BIRC5 expression markers, as well as to assess telomerase activity, which allows to reduce the volume of surgical aggression in gastric cancer and improve the patient's quality of life.

The high methylation level of the CDH1, N33, DAPK genes is a prognostic factor for performing surgical intervention in the extent of gastrectomy, since these molecular genetic markers may indicate the involvement of the grossly unchanged mucosa.

Resistance to fluoropyrimidines develops as a result of a series of changes in the biochemical processes in tumor cells. 5-fluorouracil is converted into the anti-tumor 5-fluoro-2'-deoxyuridine monophosphate (FdUMP) in the liver. FdUMP blocks DNA synthesis by binding to the thymidylate synthetase (TS) enzyme and inactivating it. The conversion of 5-FU into FdUMP may occur in different ways, and the resistance of cells to the drug is associated with a reduced activity of the enzymes involved in this conversion [*Ichikawa W, 2006; Moon J et al., 2017*].

Thus, studies of TYMS and TP53 gene polymorphisms, which are detected in tumors in locally advanced gastric cancer, should be used at the preoperative stage to predict the effect of 5-FU-based adjuvant chemotherapy.

The study demonstrated that molecular genetic test should be carried out in locally advanced gastric cancer, since 5-FU-based adjuvant chemotherapy may be ineffective. It was found that genotypes 2R/3R, Arg/Pro, and Arg/Arg are associated with better efficacy of 5-FU-based chemotherapy regimens in locally advanced gastric cancer, 3R/3R and Pro/Pro genotypes are associated with the worst efficacy of 5-FU-based chemotherapy regimens. It indicates the need to change treatment tactics in patients with locally advanced gastric cancer.

CONCLUSION

Genetic test of the unchanged gastric mucosa at the preoperative stage allows to avoid gastrectomy and reduce the surgery volume in favor of gastric resection.

Genetic test of the gastric mucosa predicts the response of patients with MRC to chemotherapy.

High level of methylation of N33, CDH1, DAPK, RASSF1A, MLH1 genes, and high expression rates of hTERT, MMP7, BIRC5, increased telomerase activity in tumor tissue may indicate that these molecular genetic markers can potentially be used as markers of changes in the mucosa. characteristic of carcinogenesis.

In patients with gastric cancer, analysis of molecular genetic markers is recommended, since the data obtained allow to assess the genetic potential of the mucosa without gross changes and reduce the extent of surgical intervention, as well as to predict the effect of 5-fluorouracil-based adjuvant chemotherapy in patients with locally advanced gastric cancer. The long-term results allow to consider the proposed methods very promising.

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Address for correspondence:

Yerevan State Medical University
2 Koryun Street, Yerevan 0025,
Republic of Armenia

Phones:

(+37410) 582532 YSMU
(+37410) 580840 Editor-in-Chief
Fax: (+37410) 582532
E-mail: namj.ysmu@gmail.com, ysmiu@mail.ru
URL: http//www.ysmu.am

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Saryan St., 4 Building, Area 2
Phone: (+374 10) 52 02 17,
E-mail: collageltd@gmail.com

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