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RESEARCH ARTICLE

Implication of Gastric Cancer Molecular Genetic Markers in Surgical Practice

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Abstract: ***Introduction*:** We have investigated aberrant methylation of genes *CDH1, RASSF1A, MLH1, N33, DAPK*, expression of genes *hTERT*, *MMP7, MMP9*, *BIRC5 (survivin)*, *PTGS2*, and activity of telomerase of 106 gastric tumor samples obtained intra-operatively and 53 gastric tumor samples from the same group of patients obtained endoscopically before surgery. Biopsy specimens obtained from 50 patients with chronic calculous cholecystitis were used as a control group. Together with tissue samples obtained from different sites remote to tumors, a total of 727 samples have been studied. The selected parameters comprise a system of molecular markers that can be used in both diagnostics of gastric cancer and in dynamic monitoring of patients after surgery. Special attention was paid to the use of molecular markers for the diagnostics of malignant process in the material obtained endoscopically since the efficacy of morphological diagnostics in biopsies is compromised by intratumoral heterogeneity, which may prevent reliable identification of tumor cells in the sampling. Our data indicated that certain molecular genetic events provided more sensitive yet specific markers of the tumor. ***Conclusion*:** We demonstrated that molecular profiles detected in preoperative biopsies were confirmed by the material obtained intra-operatively. The use of endoscopic material facilitates gastric tumors pre-operative diagnostics, improving early detection of gastric cancer and potential effective treatment strategies.

Keywords: Gastric cancer, Genetic markers, Molecular profiles, Biopsies, Treatment, Heterogeneous.

1. INTRODUCTION

One of the most important practical challenges in the diagnostics of malignant neoplasms is the development of systems of genetic and epigenetic markers and the design of cost effective diagnostic protocols. The use of cost effective diagnostic protocols will allow in-time diagnostics of oncologic disease and enhance the chance for successful treatments, subsequently.

Oncogenesis involves genetic alterations in the tumor and its microenvironment. It is dependent on the genetic peculiarities of the tumor-hosting organism. The paramount features of a tumor are molecular changes that lead to the deregulation of normal cellular growth. Despite similarities in the genesis of the tumors of a specific organ or tissue, each tumor is unique in disease course, progression and terminal stages. The tumor may be clearly distinguished and molecular evolution of the neoplastic process may be monitored by molecular genetic abnormalities, changes of gene expression and imbalances in protein spectrum [1].

Gastric cancer is a heterogeneous group of diseases among which two major histological types based on Lauren

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classification, diffuse and intestinal gastric cancer types, may be distinguished [2, 3]. These two types possess clinical, morphological and epidemiological differences. Intestinal type is more often observed in elderly patients suffering multifocal atrophic gastritis that progresses to intestinal metaplasia and dysplasia. Diffuse type is commonly found in young patients, and its association with gastritis and metaplasia is not evident. Different mechanisms of tumor development and progression are manifested in clinical differences between these two types [4]. Determination of the key molecular events characterizing the stages of gastric oncogenesis will allow designing systems of molecular markers that would provide valuable clinical information for gastric cancer diagnostics in each individual patient.

2. MATERIALS AND METHODS

2.1. Surgical and Endoscopic Material

Molecular genetic markers were studied using samples from 156 patients, including 106 patients with gastric cancer (case sampling) and a group of 50 patients with cholelithiasis diagnosis (control sampling). A total of 106 gastric tumor samples were obtained intraoperatively, and 53 endoscopically before the surgery. The control group includes tissues from 50 patients with chronic calculous cholecystitis, which were obtained from the intact region of the *corpus ventricle* mucosa during the presurgical endoscopic examination procedure. For each patient we investigated tumor samples and fragments of morphologically normal gastric mucosa located 5 cm above and below the tumor, and a fragment located in the vicinity of the resection edge zone. Altogether, the study involved 727 tissue samples, and all tissue fragments were histologically evaluated for the presence and/or the absence of tumor cells.

2.2. Molecular Genetic Analysis

Aberrant methylation of the CpG islands in the promoter regions of *CDH1, RASSF1A, MLH1, N33, DAPK* genes was probed by multiplex methylation-sensitive PCR as described previously [5]. Genes chosen for methylation analysis exert tumor suppressor properties, and directly influence the restraint of tumor growth. Their inactivation through aberrant methylation is characteristic of various tumor types and dysplastic processes [4-6].

Expression of *MMP7, MMP9*, *BIRC5* and *PTGS2* genes (Fig. **1**) was analyzed by using RT-PCR expression profiling as described by Dünne *et al.* [7] and Yang *et al*. [8]. RT-PCR products were separated by electrophoresis on 1.8% agarose gels and stained with SYBRGreen I. Bands were visualized under UV light, documented with a CCD camera and analyzed with Image J v.1.35I and Quanty One v.4.4.0 software. The relative mRNA expression was evaluated semi-quantitatively by calculating the ratio of specific gene cDNA amplification signals to the signal of *GAPDH* that arbitrarily was set to 1 (0=not detectable, <0.15=signal weakly detectable, 0.15-0.29=low signal, 0.3-0.54=intermediate, 0.55-0.99=high, >1=very high signal). The choice of genes for expression analysis was based on their involvement in the emergence and progression of gastric and other cancers. Products of *BIRC5 (survivin)* and *PTGS2 (COX-2)* genes are capable of blocking apoptosis as well as of stimulating tumor angiogenesis [9]. Similarly, *MMP7* and *MMP9* support neoangiogenesis, thus promoting tumor growth and invasion [10-14].

2.3. Telomerase Activity

Telomerase activity was measured by modified TRAP method (Fig. **2**) as described earlier [15, 16]. Telomerase is

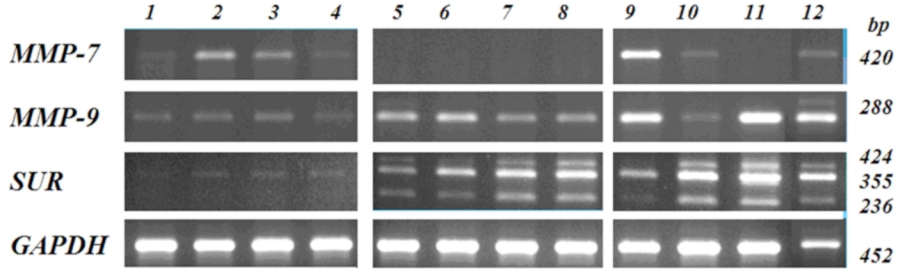

Fig. (1). Analysis of the expression levels of *survivin (BIRC5)*, *MMP7* and *MMP9* by RT- PCR. Lanes 1-4: gastric mucosa samples from patients with cholelithiasis. Lanes 5-8: samples of morphologically normal mucosa adjacent to gastric tumors. Lanes 9-12 correspond to gastric carcinoma samples.

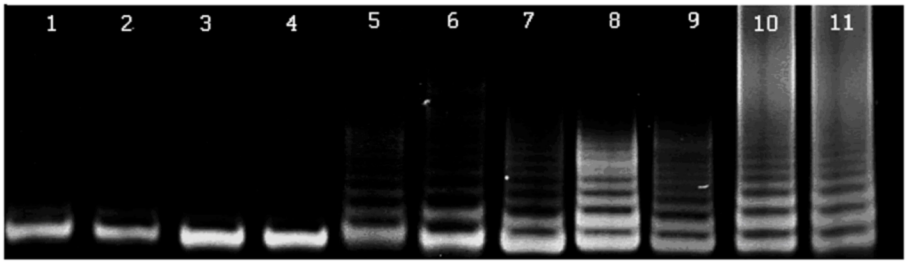

Fig. (2). Analysis of telomerase activity by modified TRAP method. Lanes 2-4: gastric mucosa samples from patients with cholelithiasis. Lanes 5-6: samples of morphologically normal mucosa adjacent to gastric tumors. Lanes 7-10: gastric carcinoma samples. Lane 1: negative control. Lane 11: positive control.

an enzyme necessary for the maintenance of chromosomes stability in the course of cell division that most likely plays a key role in maintaining cellular proliferative potential and tumor growth [17].

In order to estimate diagnostic and prognostic value of gastric cancer molecular markers we evaluated associations between genes methylation, genes expression, telomerase activity and patients' age, tumor type (diffuse or intestinal gastric cancer), tumor process propagation and size of the tumors occupation. In order to provide evidence that epigenetic alterations and gene expression disturbances are associated with tumor formation, we compared marker parameters in the material obtained from gastric cancer patients and from patients with chronic calculous cholecystitis.

2.4. Statistical Analysis

Data processing was carried out using the methods of variation statistics. Student's t-test was used to assess the significance of differences. The probability level of more than 95% was adopted as the basis for the assessment of statistical confidence. The minimum value of the t criterion, where the difference is considered reliable, is ≥ 2 ($p < 0,05$).

3. RESULTS

Each gastric tumor from the collection has demonstrated abnormal methylation of at least one gene under study. The highest frequencies of methylation in tumor tissues were revealed for *N33, CDH1* and *DAPK* genes (Fig. **3**). Methylation was observed in both tumors and in morphologically intact gastric mucosa located 5 cm from the tumor nodules. Abnormal methylation of the *RASSF1A* and *MLH1* genes was registered at a lower frequency (less then 20%), and almost solely in tumor samples. For *RASSF1A* and *MLH1* differences in methylation frequencies observed in tumors *versus* other sites of gastric mucosa are statistically significant (Fig. **3**).

In order to exclude possible association of the *CDH1, RASSF1A, MLH1, N33* and *DAPK* genes abnormal methylation with age (type A methylation, age-specific [18]), we calculated methylation frequencies in the age subgroups (Table **1**). *N33* and *MLH1* genes appeared to be significantly more frequently methylated in tumors of the patients in the early age subgroup (< 50 years old), and for the remaining genes we found no significant association between aberrant methylation and the age of patients, which excludes type A (age-specific) methylation of all the five genes included in our study.

To evaluate the possibility of using methylation of the *CDH1, RASSF1A, MLH1, N33* and *DAPK* genes as clinical markers, we determined the associations between methylation frequencies and clinical features of gastric tumors. Table **2** presents methylation frequencies in intestinal and diffuse type tumors subgroups. The classification of the subgroups was divided according to the classification of P. Lauren [2]. We observed no significant differences in gene methylation frequencies except for *CDH1*, whose methylation level was significantly higher in diffuse type tumors.

Table **3** summarizes gene methylation frequencies in the subgroups of early gastric carcinoma (T_{is}-$T_1N_0M_0$), locally advanced ($T_{2-4}N_{0-3}M_0$) and generalized ($T_{any}N_{any}M_1$) gastric carcinomas cases. It reveals significant differences in gene methylation between subgroups for N33 methylation frequency gradually growing with tumor progression, and *CDH1* methylation that is more frequent in locally advanced tumors. The latter is in agreement with *CDH1* inactivation co-occurring with the time point when the tumor begins to metastasize to lymph nodes. Thus, abnormal methylation of the *CDH1* and *N33* genes is a potential marker of poor prognosis associated with tumor progression and metastases. Abnormal methylation of *DAPK* gene, in contrast, is a marker of favorable prognosis [19], being more frequently methylated in non-metastatic gastric carcinomas ($p<0,05$), as shown in Table **4**.

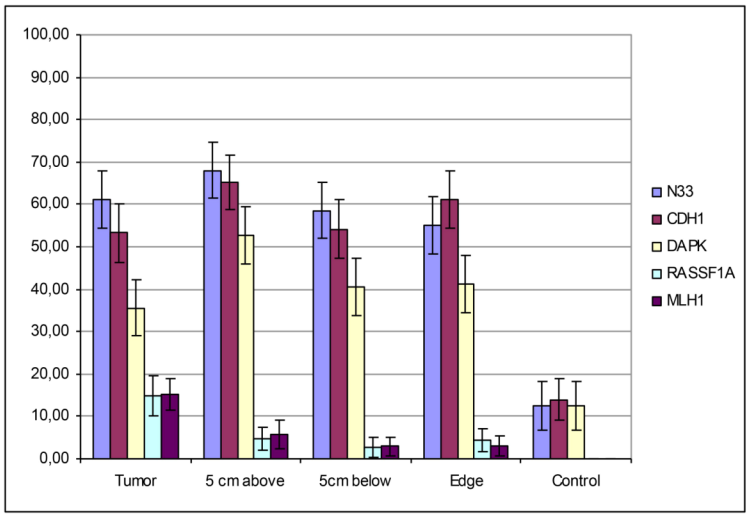

Fig. (3). Frequencies of DNA methylation in promoter regions of tumor suppressor genes in gastric tumor samples, fragments of morphologically normal gastric mucosa located 5 cm above and below the tumor, fragments located in the vicinity of the resection edge and in control samples (gastric mucosa from patients with chronic calculous cholecystitis).

Table 1. Frequencies of DNA methylation in the promoter regions of tumor suppressor genes in gastric tumors subdivided by the age of patients.

—	< 50 Years Old (Subgroup 1) Methylation Frequency, %	50-60 Years Old (Subgroup 2) Methylation Frequency, %	> 60 Years Old (Subgroup 3) Methylation Frequency, %	Confidence of Methylation Frequency Differences Between Age Subgroups 1, 2 and 3 (p)		
				1 *vs.* 2	2 *vs.* 3	1 *vs.* 3
N33	83,5 ± 5,0	64,7 ± 6,5	56,0 ± 6,7	**<0,05**	>0,05	**<0,01**
CDH1	66,7 ± 6,4	55,9 ± 6,7	54,0 ± 6,7	>0,05	>0,05	>0,05
RASSF1A	-	20,55 ± 5,5	12,5 ± 4,4	-	>0,05	-
MLH1	33,3 ± 6,4	3,1 ± 2,7	20,8 ± 5,5	**<0,01**	**<0,01**	>0,05
DAPK	33,3 ± 6,4	29,45 ± 6,3	40,0 ± 6,7	>0,05	>0,05	>0,05

Table 2. Frequencies of DNA methylation in promoter regions of tumor suppressor genes in the two major histological subgroups based on P. Lauren classification.

—	Intestinal Type Subgroup, Methylation Frequency, %	Diffuse Type Subgroup, Methylation Frequency, %	p
N33	57,5 ± 6,8	64,0 ± 6,7	>0,05
CDH1	40,0 ± 6,7	64,0 ± 6,7	**<0,05**
RASSF1A	12,5 ± 4,5	10,4 ± 4,1	>0,05
MLH1	78,9 ± 5,6	89,6 ± 4,1	>0,05
DAPK	37,5 ± 6,7	34,0 ± 6,5	>0,05

Table 3. Frequencies of DNA methylation in promoter regions of tumor suppressor genes in the subgroups of early (T_{is}-$T_1N_0M_0$), locally advanced ($T_{2-4}N_{0-3}M_0$) and generalized ($T_{any}N_{any}M_1$) gastric carcinomas.

—	Early (Subgroup 1)	Locally Advanced (Subgroup 2)	Generalized (Subgroup 3)	Confidence of Differences Between Subgroups 1, 2 and 3 (p)		
				1 *vs.* 2	2 *vs.* 3	1 *vs.* 3
N33	50,0 ± 6,8	61,0 ± 6,7	71,4 ± 6,2	>0,05	>0,05	**< 0,05**
CDH1	33,3 ± 6,5	61,0 ± 6,7	35,7 ± 6,6	**< 0,05**	**< 0,05**	>0,05
RASSF1A	-	16,1 ± 5,0	21,4 ± 5,6	-	>0,05	-
MLH1	-	18,9 ± 5,4	14,3 ± 4,8	-	>0,05	-
DAPK	33,3 ± 6,5	37,3 ± 6,6	28,6 ± 6,2	>0,05	>0,05	>0,05

Levels of mRNA expression measured for *hTERT*, *MMP7, MMP9*, *BIRC5* and *PTGS2* genes in gastric tumor samples, fragments of morphologically normal gastric mucosa located 5 cm above and below the tumor, fragments located in the vicinity of the resection edge and in control samples are shown in Fig. (**2**). We have detected significant increase in *BIRC5*, *MMP7* and *hTERT* genes expression in the tumors as compared (Fig. **4**) to the borderline morphologically intact tissue ($p<0.01$). No differences have been observed between gene expression in the gastric tissues adjacent to tumors and the control group mucosa samples.

Gene expression levels in the subgroups of early gastric carcinoma, locally advanced and generalized gastric carcinomas Table **5** reveal statistically confident elevation of *BIRC5, MMP7* and *PTGS2* expression across the subgroups from early to generalized cancer. Telomerase activity in the tumors was significantly higher than that in the remote sites near the borders of resection and in the biopsies of gastric mucosa from non-tumor patients.

4. DISCUSSION

4.1. Abnormal DNA Methylation in Gastric Tumors

The methylation frequencies of the *N33, CDH1, DAPK, RASSF1A* and *MLH1* genes in tumor material detected in our study are in agreement with the previously reported results

[20]. In this study, we demonstrate high levels of the *CDH1, N33, DAPK* genes methylation in the morphologically normal tissue remote to tumors, which we hypothesize to reflect surrounding tissue involvement in tumor development. Additional monitoring of this category of patients in postsurgical period is required to clarify this suggestion. The levels of aberrant methylation in the surgically excised samples of gastric cancer and adjacent morphologically unchanged tissues were significantly higher ($p<0.01$) than in the biopsies from the patients group with cholelithiasis and chronic calculous cholecystitis, further confirming our hypothesis. The changes found in the apparently normal tissues might be potentially used in gastric cancer diagnostics practices as specific markers for the evaluation of tumor progression as well as for the investigation of dynamic changes of gastric stump mucosa after resection, although such possibility requires further detailed investigation.

Table 4. Frequencies of DNA methylation in promoter regions of tumor suppressor genes in gastric carcinomas in the cases subdivided by lymph node status (N0 *vs.* N1-3).

——	N0	N1,2,3	P value
N33	65,2 ± 6,6	55,3 ± 6,8	>0,05
CDH1	51,9 ± 6,9	55,3 ± 6,8	>0,05
RASSF1A	19,2 ± 5,4	8,4 ± 3,9	>0,05
MLH1	18,8 ± 5,4	10,5 ± 4,3	>0,05
DAPK	44,2 ± 6,8	23,6 ± 5,8	**< 0,05**

Aberrant *N33* and *CDH1* genes methylation is a marker of negative prognosis for gastric cancer that is associated with tumor process generalization and metastasizing [21]. The presence of *DAPK* gene methylation [22], on the other hand, is a marker of more favorable prognosis. Methylation of this gene is less frequent in cases where lymph node metastases are present. Assessment of this gene methylation can further find practical application in lymph node metastases diagnostics during pre-surgical period, but the data obtained in this study require additional analysis on larger patient groups.

We have also found that *CDH1* gene methylation level is significantly higher in diffuse than in intestinal type tumors ($p<0.05$), which is in accordance with the reported data [6]. This confirmation indicates that most likely molecular differences between these types of gastric cancer, which is further reflected in different morphogenesis and clinical behavior of the tumors.

4.2. Elevated *BIRC5*, *MMP7* and *PTGS2* Expression in Gastric Tumors

Increased *hTERT, PTGS2*, *MMP7, MMP9* and *BIRC5* genes expression has previously been demonstrated of breast, lung, and others types of the tumors growth and metastasis [7, 8, 10-14, 23, 24]. Here we report significant increase in *BIRC5*, *MMP7* and *hTERT* genes expression in the tumors as compared to the borderline morphologically intact tissue. Taken into the account that we have found no differences in gene expression in the borderline gastric tissue compared to the control group mucosa samples, we suggest a possibility for *BIRC5*, *MMP7* and *hTERT* gene expression assessment can be used in the gastric cancer diagnostics, as well as in differential diagnostics with gastroduodenal zone benign lesions on the background of chronic inflammation. According to our results, *BIRC5, MMP7* and *PTGS2* expression is significantly higher in generalized than in early and locally advanced cancer ($p<0.05$), which may reflect the role for these genes in tumor development, increase of tumor size, and metastasis [10, 11, 25-30]. Therefore, the increased expression of these genes might be useful tools as a clinical marker for the gastric tumors and metastasis.

4.3. Telomerase Activity Assessment

In our study telomerase activity in the tumor was significantly higher than that in the remote sites near the borders of resection and in the biopsies of gastric mucosa from non-tumor patients [31, 32], suggesting a possible diagnostic application.

4.4. Diagnostic Applications of DNA Methylation, Gene Expression and Telomerase Activity Tests

From the above results, it is clear that the aberrant *CDH1, RASSF1A, MLH1, N33, DAPK* tumor suppressor genes methylation, increased expression of *MMP7, BIRC5* and *hTERT* genes, as well as increase in telomerase activity may be hallmarks of the characteristic of gastric cancer, although they tend to be pronounced differently in certain gastric tumor types. The levels of *RASSF1A* and *MLH1* genes methylation*, hTERT, MMP7*, *BIRC5* expression, and telomerase activity in gastric tumors are significantly higher than in morphologically unchanged adjacent gastric mucosa tissues. The values of the parameters studied in the tumors differ significantly from those in the control group (gastric mucosa biopsies from patients with cholelithiasis), and consequently may comprise a system of molecular genetic markers of gastric cancer. In our understanding most likely this system might be useful for the both gastric cancer diagnostics and monitoring of the cancer dynamic in the patients after the surgical procedure.

4.4.1. Potential Preoperative Applications of Molecular Genetic Markers

One of the main goals of this study was to assess the applicability of gastric cancer molecular genetic markers in the preoperative period. Molecular assessment of material obtained in the course of endoscopic examination in the presurgical period may considerably improve the efficiency of gastric cancer diagnostics. In ambiguous cases, determination of molecular markers allows to complement histological investigation and to differentiate tumor and non-tumor gastric lesions. Thus, in our cohort of patients the results of presurgical histological analysis of gastric mucosa biopsies obtained endoscopically did not confirm gastric cancer in roughly half of biopsies. During morphologic examination of this material no tumor cells were found. However, the patients were operated after the results of investigations based on the system of molecular genetic markers described above

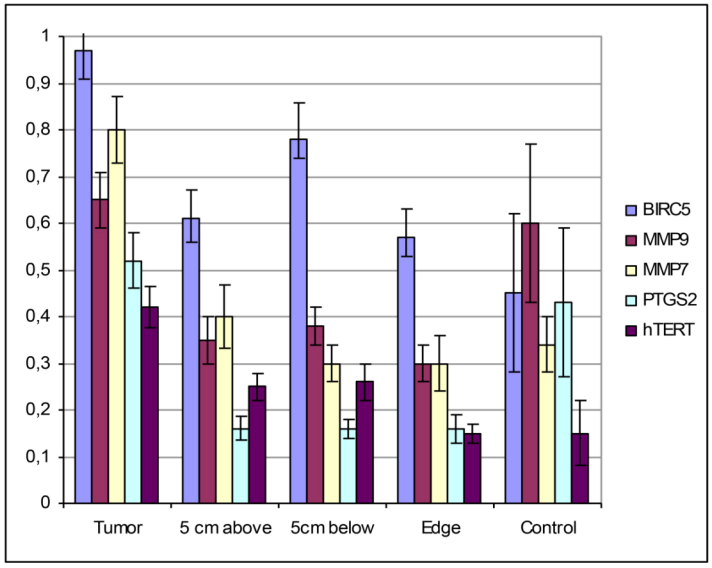

Fig. (4). Levels of *hTERT*, *MMP7, MMP9*, *BIRC5* and *PTGS2* mRNA expression measured in gastric tumor samples, fragments of morphologically normal gastric mucosa located 5 cm above and below the tumor, fragments located in the vicinity of the resection edge and in control samples (gastric mucosa from patients with chronic calculous cholecystitis).

Table 5. Frequencies of DNA methylation in promoter regions of tumor suppressor genes in the subgroups of early (T_{is}-$T_1N_0M_0$), locally advanced ($T_{2-4}N_{0-3}M_0$) and generalized ($T_{any}N_{any}M_1$) gastric carcinomas.

—	Early (1)	Locally advanced (2)	Generalized (3)	Confidence of Differences Between Subgroups 1, 2 and 3 (p)		
—	—	—	—	1 *vs.* 2	2 *vs.* 3	1 *vs.* 3
BIRC5	0,63 ± 0,14	0,98 ± 0,07	1,19 ± 0,1	**<0,01**	**<0,05**	**<0,01**
MMP9	0,73 ± 0,2	0,63 ± 0,06	0,7 ± 0,08	>0,05	>0,05	>0,05
MMP7	0,54 ± 0,13	0,79 ± 0,07	1,0 ± 0,15	**<0,01**	>0,05	**<0,01**
PTGS2	0,12 ± 0,03	0,53 ± 0,07	0,69 ± 0,14	**<0,01**	>0,05	**<0,01**
hTERT	0,32 ± 0,1	0,42 ± 0,05	0,48 ± 0,09	>0,05	>0,05	>0,05

suggested the presence of tumor specific features. Subsequent examination of surgical material unequivocally confirmed cancerous nature of the extirpated gastric lesions.

Material obtained endoscopically may differ from surgical material as a consequence of intratumoral tissue heterogeneity. This may prevent reliable morphologic diagnosis based on the identification of tumor cells in the sampling. Our investigation proves that certain molecular genetic events provide more sensitive yet specific markers of the tumor as far as we have found no significant differences in gene methylation, gene expression, and telomerase activity between tumor samples obtained at presurgical period and those obtained in the course of gastric cancer surgery. We have found that the amount of DNA and RNA obtained from endoscopic sample is quite sufficient for a series of molecular-genetic investigations. The use of endoscopic material facilitates gastric tumors preoperative diagnostics that can be basis for improving early detection of gastric cancer.

4.4.2. Adjustment of Surgical Tactics Based on the Molecular Genetic Markers

Taking into consideration significant differences between tumor material and gastric mucosa biopsy samples from patients with cholelithiasis in terms of gene methylation, gene expression, and telomerase activity, we postulate that the tumors were distinguished by certain molecular changes that did not occur in gastric mucosa in the absence of the malignancy. At the same time finding the border of the area in gastric mucosa where these changes occur most likely suggesting the location of the margin between the tumor and normal mucosa and to refine the edge of resection during surgery. However, the results obtained in our study suggest that morphologically normal mucosa distant from the tumor areas is characterized in some patients by the presence of epigenetic changes attributable to tumor tissues rather than to the normal ones (gastric mucosa from patients with cholelithiasis). High level of the *CDH1, N33, DAPK* genes methylation in morphologically unchanged tumor adjacent mucosa

in gastric cancer patients may indirectly indicate an involvement of this tissue into the malignancy, and requires monitoring in the postsurgical period. Investigation of this aspect is far from being completed and requires more in-depth study of tumor and adjacent non-tumor tissue samples. Thus, the level of *CDH1, N33, DAPK* genes methylation might not be appropriate candidate for including into the marker system for size of the resection of the tumor margins. The latter purpose can be achieved by measuring expression markers of *hTERT, MMP7*, *BIRC5*, as well as telomerase activity that indicates were their levels increase in the tumor solely and are significantly lower in surrounding mucosa located 5 cm above or below the tumor nodule. The sites of morphologically normal mucosa 5 cm above or below the tumor tissues that determined in our study are located closer to the nodule than those in proximal and distal resection margins. The absence of elevated marker of the genes expression allows us to conclude that surgical operations such as subtotal distal resection or proximal stomach resection that are performed in the cases of limited antral or proximal tumor growth are appropriate because they are performed not only in the borders of morphologically unchanged tissues, but also on the background of insignificant molecular and genetic changes. Taking into consideration the results of our present investigation of molecular genetic markers, one may assert that operation volume (subtotal distal resection and proximal resection) is adequate, and the markers themselves can serve as prognostic values of the *"molecular genetic clearance of resection edge"* which are correlating with the histological examination.

Although molecular genetic markers do not allow identification of malignant gastric lesions with 100% accuracy, they may serve as valuable additional diagnostic markers for the assessment of the nature of the lesion in ambiguous cases when contradiction between clinical, instrumental, laboratory data and the results of preoperative cytological and histological investigations occurs.

CONCLUSION

Based on our results we concluded that the adequate assessment of molecular genetic gastric mucosa changes (*MMP7, hTERT*, *BIRC5* genes expression, as well as telomerase activity) in patients with gastric cancer provides early diagnosis of disease progression at various stages of the tumor growth and metastasis. Indeed, the results presented – comparing cancer and control tissues – remain valid although in the same promoter, as in the case of that for transglutaminase gene [33, 34], both methylated and non-methylated CpG domains can be located. Therefore, the analysis of *CDH1, RASSF1A, MLH1, N33, DAPK* genes aberrant methylation may provide useful tools in the assessment of cancer metastases into the mucosa after stomach resection in order to predict tumor recurrence, as well as future prognosis of the cancer growth and metastases in general.

LIST OF ABBREVIATIONS

CDH1	=	Cadherin 1, type 1, E-Cadherin
RASSF1A	=	RAS Association Domain Family 1A
MLH1	=	MutL Homolog 1
N33	=	Tumor Suppressor Candidate 3 Gene
DAPK	=	Death Associated Protein Kinase 1
hTERT	=	Human Telomerase Reverse Transcriptase
MMP7	=	Matrix Metallopeptidase 7
MMP9	=	Matrix Metallopeptidase 9
BIRC5	=	Baculoviral Inhibitor of Apoptosis Repeat-Containing 5
PTGS2	=	Prostaglandin-Endoperoxide Synthase 2

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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