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Original Article

Expression of Ten-Eleven Translocation 2 and Glutathione-S-Transferase PI in Colorectal Cancer Patients with and without Type 2 Diabetes Mellitus

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Abstract

Aims: To highlight possible correlations of type 2 diabetes mellitus (T2DM) with microscopic / macroscopic characteristics of colorectal cancer tissues, along with the expression of Ten-Eleven Translocation 2 (TET2) and glutathione-S-transferase pi (GST-pi) proteins.

Materials and methods: Tumors from 46 patients were embedded in paraffin blocks, stained with hematoxylin-eosin and studied microscopically. Immunohistochemical study of TET2 and GST-pi expression was performed. The results were analyzed and correlated with T2DM as comorbidity.

Results: All tumors expressed GST-pi at three levels (weak, moderate, and strong); two out of three tumors showed either weak or moderate TET2 expression. Patients without T2DM tended to have tumors with weak or no expression of TET2 (p=0.038) whereas diabetic patients' tumors showed a significantly higher percentage of strong or moderate GST-pi expression (p=0.034). On binomial logistic regression, tumors excised from T2DM patients were 6.9 times more likely to show moderate (rather than weak and none) TET2 expression compared to tumors from non-diabetic patients (95% CI [1.33, 35.75]), and a 2.7-fold higher relative likelihood of showing strong (rather than moderate and weak) GST-pi expression (95% CI [0.63, 12.09]), taking into account sex, age, and tumor size. The association between T2DM and TET2 expression remains statistically significant in additional binomial analysis that was performed taking into account certain histological tumor characteristics.

Conclusions: TET2 and GST-pi are expressed in malignant colon tumors. T2DM in CRC patients was associated with the highest observed GST-pi expression; absence of T2DM was associated with the lowest observed TET2 expression. T2DM increases the probability of observing GST-pi and TET2 expression at maximum levels, independent of specific tumor microscopic features and certain patient characteristics.

Keywords

colorectal cancer, diabetes mellitus, epigenetics, glutathione S-transferase pi, TET2 protein, type 2

INTRODUCTION

Epigenetics refers to heritable changes in phenotype which result from molecular changes that do not involve gene mutations, yet plays an important role in gene expression or silencing, DNA replication and repair, cell differentiation and stem cell development.[1,2] Epigenetic mechanisms include DNA methylation, histone modifications and non-coding RNA sequences; those mechanisms may lead to reversible epigenetic modifications triggered by several lifestyle and environmental factors, including diet, age, physical activity, smoking and stress.[3]

Colorectal cancer (CRC) remains a major health burden as it is the third most common malignancy and the second most common cancer-related cause of death worldwide.[4] Genetic as well as epigenetic factors seem to play an important role in CRC pathogenesis. Multiple different genes participating in molecular pathways and mutations – namely *APC, BRAF, TP53, FBXW7, TCF7L2*, and *PTEN* – are thought to be involved in the process of malignant transformation[5]; advanced age, a family history of CRC, metabolic comorbidities, inflammatory bowel diseases, immunosuppression as well as alcohol misuse, smoking and processed meat overconsumption are also well-established risk factors for the disease.[6]

Type 2 diabetes mellitus (T2DM) is a major metabolic disorder, which itself has been identified as a CRC-independent risk factor.[7] It is known that T2DM has a strong genetic basis but the crosstalk between genetics and epigenetics is crucial for the establishment of the disease and its complications.[8]

CRC and T2DM share common epidemiological as well as pathogenetic characteristics; they both thrive in Western societies; they are linked to certain dietary and lifestyle habits and they are causally associated.[9] Insulin resistance, obesity advanced glycation end-products (AGEs), low grade inflammation and reactive oxygen species (ROS) are common pathways of colon tumorigenesis as well as compilations in diabetes.[10,11] In respect of genetics, genome wide association studies have shown that there are certain T2DM susceptibility genes, such as *TCF7L2* and *KLF14* which play an important role in CRC development.[12] As far as epigenetics is concerned, T2DM and CRC share some epigenetic alterations, to name but a few: *Sept9* methylation, *PPARGC1A* promoter methylation, *CDKN1A* methylation and miR-21, miR-143, miR-206 and many more.[13-16]

Ten-Eleven Translocation (TET) enzymes play a crucial role in genomic stability, as they are involved in the DNA demethylation process.[17] TET2 hypoexpression has been linked to hematopoietic and solid malignancies and TET2 mutations may have an important role in patients' prognosis and response to treatment.[18] Apart from malignancies, there is accumulated evidence that TET2 dysfunction results in activation of proinflammatory pathways, leading to endothelial dysfunction, atherosclerosis and cardiovascular diseases.[19]

Glutathione S-transferases (GST) comprise an enzyme superfamily which participates in phase II detoxification, playing a major role in xenobiotic metabolism and in cellular protection against oxidative stress.[20] GST-pi is a member of GST family and contributes to the cellular protection against free radicals; in this way, GST-pi is responsible for tumor resistance to chemotherapy drugs.[21] Additional studies have showed that GST-pi can promote the survival of malignant cells by inhibiting MAPK pathway either through JNK[22] or TRAF2 interplay[23]. As the hyperglycemia-induced oxidative stress is the cornerstone of diabetes vascular complications, GST-pi polymorphisms may aggravate endothelial dysfunction and promote atherogenesis.[24]

AIM

The aim of the study was to examine the microscopic and macroscopic characteristics of colorectal cancer tissues, along with the expression of TET2 and GST3 proteins and highlight the possible correlation of these findings with T2DM as comorbidity.

MATERIALS AND METHODS

Patients

Forty-six patients who were diagnosed with colorectal adenocarcinoma stage I-IV and underwent partial colectomy, hemicolectomy or proctocolectomy were included in the study. None of them was diagnosed with Familial Adenomatous Polyposis or Lynch Syndrome. The patients' characteristics and medical history were retrieved from their medical records. Patients with comorbidities other than T2DM were not included.

Methods

The macroscopic evaluation was followed by microscopic examination of the tissues which were embedded in paraffin, processed and stained with hematoxylin and eosin (H&E). Two independent experienced pathologists assessed the macroscopic characteristics of the tumors, namely size, type, location as well as the microscopic ones, namely T stage, differentiation, modes of invasive growth pattern, vascular invasion, perineural invasion, lymph node infiltration.

For immunohistochemical evaluation, serial 4-μm sections were used. The sections were mounted on saline-coated glass slides. The antibodies used included Anti-GST3/pi antibody [EPR8263] (abcam, ab138491) and Anti-Tet2 antibody [CL6873] (abcam, ab243323).

Protein expression was analyzed by two independent researchers, who were blinded to clinical data of the patients.

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The staining intensity was classified based on the percentage of positive cells, as follows: 0, absent; <10%, weak; 10%-50%, moderate; >50% strong. The results were then quantified using the numerical scale from 0 to 3.

Statistical analysis

Statistical analyses were performed using the IBM® SPSS® Statistics 23 software. The Kolmogorov-Smirnov test was used to estimate the normal distribution of the variables. The mean of continuous data was expressed as $\bar{x}\pm s$. The χ^2 or Fisher's exact test were used to analyze the associations between categorical variables, whereas independent sample *t*-test was used for continuous variables. Non-parametric data were compared using Mann-Whitney U and Kruskal-Wallis tests. Phi and Cramer's V were used as measures of association between nominal variables and Pearson's *r* for continuous variables. Further analyses were performed using binominal logistic regressions. For all analyses, the significance level was set to 0.05.

Ethical considerations

This study was conducted according to the Ethical Guidelines for Medical and Health Research Involving Human Subjects. The present study was approved by the University Ethics Review Committee. No additional permissions were required to review the patient records, including the hospitals from which the records were obtained.

RESULTS

Clinicopathological data

The patients' clinicopathological data are summarized in **Table 1**. 57.8% of the participants were men. The mean age of the patients was 74.11±10.07 years. 56.52% had lymph node metastases and 15.22% presented with distant metastases. One-third of patients had T2DM as comorbidity. Regarding macroscopic examination, the mean tumor size was 4.42±1.75 cm, ranging from 1.5 cm to 8 cm, while 60.87% of the tumors were developed in the left colon. 36.96% of the adenocarcinomas were characterized as annular and 34.78% as ulcerative. Concerning microscopic examination, the majority of tumors (89.13%) were recognized as the widespread streaming form of adenocarcinoma and, therefore, were characterized as infiltrating CRC. Only 2.7% of the tumors were well-differentiated. Vascular and perineural infiltration was noticed in 58.7% and 67.39% of the tumors, respectively.

Immunohistochemistry results

GST-pi was expressed in all examined specimens. More precisely, 36.96% of tumors showed weak GST3 expression, 32.61% moderate and 30.43% strong. After quantification of the above data, GST3 expression was calculated to be 1.93±0.827. In contrast, TET2 expression was negative in almost one-third of the examined tumors, while strong expression was not observed. Quantitatively, TET2 expression was estimated at 0.93±0.772 **(Fig. 1, Table 1)**.

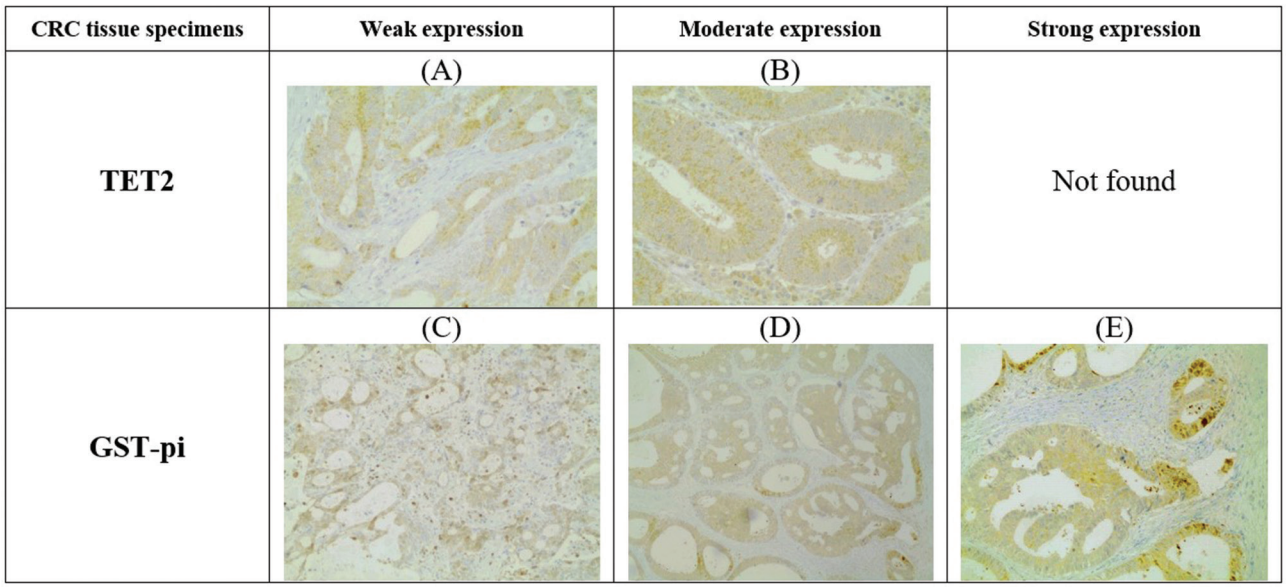

Figure 1. Immunohistochemical staining of the expression levels of GST-pi and TET2 in CRC tissue specimens. Representative staining patterns of CRC with weak (**A**) and moderate (**B**) staining intensity of TET2 as well as weak (**C**), moderate (**D**), and strong (**E**) staining intensity of GST-pi. Magnification, ×200. CRC: colorectal cancer; TET2: Ten-Eleven Translocation 2; GST-pi: glutathione-S-transferase pi.

Table 1. Clinicopathological characteristics of patients with colorectal cancer included in the study

Characteristics	Frequency, N (%) / Mean ± SD
Total number of patients	46
Age (years)	74.11±10.07
Sex	
Male	27 (58.7)
Female	19 (41.3)
T2DM	
Yes	15 (32.6)
No	31 (67.4)
pT stage	
T1	0 (0)
T2	3 (6.5)
T3	36 (78.3)
T4	7 (15.2)
pN stage	
N0	20 (43.5)
N1	19 (41.3)
N2	7 (15.2)
pM stage	
M0	39 (84.8)
M1	7 (15.2)
Tumor location	
Right colon	13 (28.3)
Left colon	28 (60.9)
Transverse colon	5 (10.9)
Tumor size	4.42±1.75
Vascular invasion	
Yes	27 (58.7)
No	19 (41.3)
Lymphatic invasion	
Yes	26 (56.5)
No	20 (43.5)
Perineural invasion	
Yes	31 (67.4)
No	15 (32.6)
Tumor differentiation	
Well differentiated	1 (2.2)
Moderately differentiated	41 (89.1)
Poorly differentiated	4 (8.7)
Tumor growth pattern	
Exophytic	11 (23.9)
Pedunculated	2 (4.35)
Ulcerating	16 (34.8)
Annular	17 (36.9)
Invasion type through the mucosa to the subserosa	
Infiltrating	41 (89.1)
Expanding	5 (10.9)
GST-pi expression	
Strong	14 (30.4)
Moderate	15 (32.6)
Weak	17 (37)
Negative	0 (0)
TET2 expression	
Strong	0 (0)
Moderate	12 (26.1)
Weak	19 (41.3)
Negative	15 (32.6)

Associations between patients' characteristics and histopathological findings

No statistically significant differences were observed in terms of location, degree of differentiation, vascular and perineural infiltration between male and female patients. On the contrary, statistically significant differences emerged regarding the T staging of the primary tumor (p=0.028), the identification of lymph node infiltration (p=0.049), and the existence of distant metastases (p=0.015). More specifically, it appeared that more female patients were diagnosed at stage T4 instead of T3 while more male patients at stage T3 instead of T4 and more women presented with lymph node as well as distant metastases compared to the men. Exophytic tumors, compared to all other morphological subtypes, were prominent in female patients (p=0.036) and annular tumors in male patients (p=0.002). Finally, it was shown that sex is related to the pattern of tumor invasion in the deep tissues as male patients exhibited higher frequency of infiltrating rather than expanding pattern (p=0.008) **(Table 2)**. Patients' age did not appear to be related with tumor localization, tumor macroscopic appearance, pattern of penetration into the deep tissues, degree of differentiation, T stage of the tumor, frequency of vascular and perineural infiltration as well as the incidence of distant metastases, to a statistically significant degree. On the contrary, it was found that the mean age of the patients who presented with lymph node metastases was lower compared to those who did not show lymph node involvement (p=0.01).

Associations between patients/tumors characteristics and immunohistochemistry findings

No statistically significant difference was found when comparing the different degrees of TET2 and GST-pi expression between male and female patients. Additionally, there was no

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Table 2. Significant differences in histopathological findings between male and female patients

		Male N (%)	Female N (%)	*p*
T staging	T3	24	12	0.015
	T4	1	6	
Lymphatic invasion	Yes	12 (26.1)	14 (30.4)	0.049
	No	15 (32.6)	5 (10.9)	
Distant metastases	Yes	1 (2.2)	6 (28.3)	0.015
	No	26 (56.5)	13 (13)	
Invasion type	Infiltrating	27 (58.7)	14 (30.43)	0.008
	Expanding	0 (0)	5 (10.87)	
Tumor growth pattern	Annular	15 (32.61)	2 (4.35)	0.002
	All other subtypes	12 (26.09)	17 (36.96)	
	Exophytic	2 (4.35)	9 (19.57)	0.004
	All other subtypes	25 (54.35)	10 (21.74)	

statistically significant differences between the mean age of the patients categorized in the different levels of expression of the above proteins. No association was noticed between the different levels of TET2 / GST-pi expression and the histopathological findings of the examined tumors **(Table 3)**.

Association between the expression of TET2 and GST-pi

When grouping the levels of expression of TET2 and GST-pi in two categories, namely a group that includes the tumors with the maximum observed protein expression and a group with all the other levels of protein expression and comparing them, it was found that high expression levels of TET2 were more frequently observed in tumors with high GST-pi levels (p=0.003).

Association between T2DM and clinicopathological characteristics of the patients.

No statistically significant differences were found between patients with and without T2DM in terms of the degree of differentiation, the presence of distant metastases and lymph node infiltration, the tumor size and macroscopic appearance. A statistically significant difference was observed between the patients who had T2DM and those who did not, concerning the location of tumors along the colon; more specifically, it was shown that, compared to the rest of the locations, T2DM patients presented with tumors in the right colon more frequently (p=0.002), while non-diabetic patients had left colon tumors more frequently (p=0.008). The correlation between T2DM and tumor location appeared to be very strong (Cramer's V=0.49). Additionally, it was found that patients with T2DM were mainly diagnosed with T4 tumors and tumors with expanding growth pattern compared to patients without T2DM who mainly presented with T3 tumors (p=0.028) and tumors with infiltrating growth pattern (p=0.033). Finally, only 18.5% of the tumors with vascular infiltration were excised from patients with T2DM (p=0.015).

Association between T2DM and immunohistochemistry results

The comparison between patients with and without T2DM in terms of positive (any grade) and negative expression of TET2 did not reveal any statistically significant difference. Furthermore, after quantifying the levels of expression of TET2 and GST-pi and comparing their means between patients with and without T2DM, no statistically significant difference was found **(Fig. 2)**. On the contrary, patients without T2DM show a significantly higher percentage of weak or no expression of TET2 compared to T2DM patients (p=0.038). In fact, a strong correlation between the absence of T2DM and low/no TET2 expression was documented (Cramer's V=0.326, p=0.027). Additionally, patients with T2DM show a significantly higher percentage of moderate and strong GST-pi expression compared to patients without T2DM (p=0.034) **(Fig. 3)**.

In binomial logistic regression, tumors excised from T2DM patients were approximately 7 times more likely to have moderate (rather than weak and none) TET2 expression compared to tumors from non-diabetic patients (OR 6.9, CI [1.33, 35.75]), and a 2.7-fold higher relative likelihood of showing strong (rather than moderate and weak) GST3 expression than tumors derived from non-diabetic patients (OR 2.7, CI [0.63, 12.09]), taking into consideration sex, age and tumor size. The association between T2DM and the group of maximum observed TET2 expression remains statistically significant in an additional binomial analysis that was performed examining the histological characteristics of the tumors, namely vascular infiltration, perineural infiltration and lymph node invasion (OR 4.3, CI [1.09, 17.4]) **(Tables 4A, 4B)**. In a similar analysis for GST3, the results were close to statistical significance **(Tables 5A, 5B)**.

Table 3. Associations between GST-pi and TET2 levels and the clinicopathological factors of patients with colorectal cancer. Each subscript letter denotes a subset of each variable categories whose column proportions do not differ significantly from each other at the 0.05 level

Characteristics	N (%)	GST-pi expression			*P*-value	TET2 expression			*P*-value
		Weak	Moderate	Strong		Negative	Weak	Moderate	
Sex					0.38				0.78
Male	27 (58.7)	8_a (17.4)	9_a (19.6)	10_a (21.7)		8_a (17.4)	11_a (23.9)	8_a (17.4)	
Female	19 (41.3)	9_a (19.6)	6_a (13)	4_a (8.7)		7_a (15.2)	8_a (17.4)	4_a (8.7)	
Tumor location					0.89				0.57
Right colon	13 (28.3)	5_a (10.9)	5_a (10.9)	3_a (6.5)		3_a (6.5)	5_a (10.9)	5_a (10.9)	
Left colon	28 (60.9)	10_a (21.7)	8_a (17.4)	10_a (21.7)		11_a (23.9)	12_a (26.1)	5_a (10.9)	
Transverse colon	5 (10.9)	2_a (4.3)	2_a (4.3)	1_a (2.2)		1_a (2.2)	2_a (4.3)	2_a (4.3)	
Tumor growth pattern					0.31				0.81
Annular	17 (37)	5_a (10.9)	5_a (10.9)	7_a (15.2)		5_a (10.9)	6_a (13)	6_a (13)	
Ulcerating	16 (34.8)	6_a (13)	6_a (13)	4_a (8.7)		4_a (8.7)	8_a (17.4)	4_a (8.7)	
Exophytic	11 (29.3)	6_a (13)	2_a (4.3)	3_a (6.5)		5_a (10.9)	4_a (8.7)	2_a (4.3)	
Pedunculated	2 (4.3)	0_a (0)	2_a (4.3)	0_a (0)		1_a (2.2)	1_a (2.2)	0_a (0)	
Invasion type					0.52				0.91
Infiltrating	41 (89.1)	14_a (30.4)	14_a (30.4)	13_a (28.3)		13_a (28.3)	17_a (37)	11_a (23.9)	
Expanding	5 (10.9)	3_a (6.5)	1_a (2.2)	1_a (2.2)		2_a (4.3)	2_a (4.3)	1_a (2.2)	
Tumor differentiation					0.24				0.57
Well differentiated	1 (2.2)	0_a (0)	0_a (0)	1_a (2.2)		1_a (2.2)	0_a (0)	0_a (0)	
Moderately differentiated	41 (89.1)	14_a (30.4)	15_a (32.6)	12_a (26.1)		12_a (26.1)	18_a (39.1)	11_a (23.9)	
Poorly differentiated	4 (8.7)	3_a (6.5)	0_a (0)	1_a (2.2)		2_a	1_a (22)	1_a (2.2)	
Vascular Invasion					0.18				0.06
Yes	27 (58.7)	12_a (26.1)	6_a (13)	9_a (19.6)		7_a	15_a	5_a	
No	19 (41.3)	5_a (10.9)	9_a (19.6)	5_a (10.9)		8_a	4_a	7_a	
Perineural invasion					0.2				0.37
Yes	31 (67.4)	14_a (30.4)	8_a (17.4)	9_a (19.6)		9_a (19.6)	15_a (32.6)	7_a (15.2)	
No	15 (32.6)	3_a (6.5)	7_a (15.2)	5_a (10.9)		6_a (13)	4_a (8.7)	5 (10.9)	
Lymphatic invasion					0.59				0.9
Yes	26 (56.5)	8_a (17.4)	9_a (19.6)	9_a (19.6)		9_a (19.6)	10_a (21.7)	7_a (15.2)	
No	20 (43.5)	9_a (19.6)	6_a (13)	5_a (10.9)		6_a (13)	9_a (19.6)	5_a (10.9)	
Distant metastases					0.8				0.61
Yes	7 (15.2)	2_a (4.3)	3_a (6.5)	2_a (4.3)		2_a (4.3)	4_a (8.7)	1_a (2.2)	
No	39 (84.8)	15_a (32.6)	12_a (26.1)	12_a (26.1)		13_a (28.3)	15_a (32.6)	11_a (23.9)	

DISCUSSION

The present study described the histopathological characteristics of colorectal adenocarcinomas in a cohort of patients with and without T2DM and investigated the relationship between TET2 and GST-pi expression in these patients. In the present study, patients with T2DM showed higher rates of adenocarcinomas in the right colon, compared to non-diabetic patients in accordance with a recent meta-analysis.[25] With regard to T staging, in the present study a statistically significant difference between T2DM patients and non-T2DM patients in terms of the predominance of stage T4 and T3 tumors, respectively, was found; such a difference did not reach the level of statistical significance in previous studies focusing on colon tumorigenesis and hyperglycemic tumor microenvironment.[26] Despite the fact that hyperglycemic tumor microenvironment promotes the proliferation as well as the hematogenous dissemination of malignant cells through a variety of modifications in endothelial cells' ligands[27], our study failed to demonstrate a statistically significant relationship between T2DM and vascular infiltration in the examined tumors.

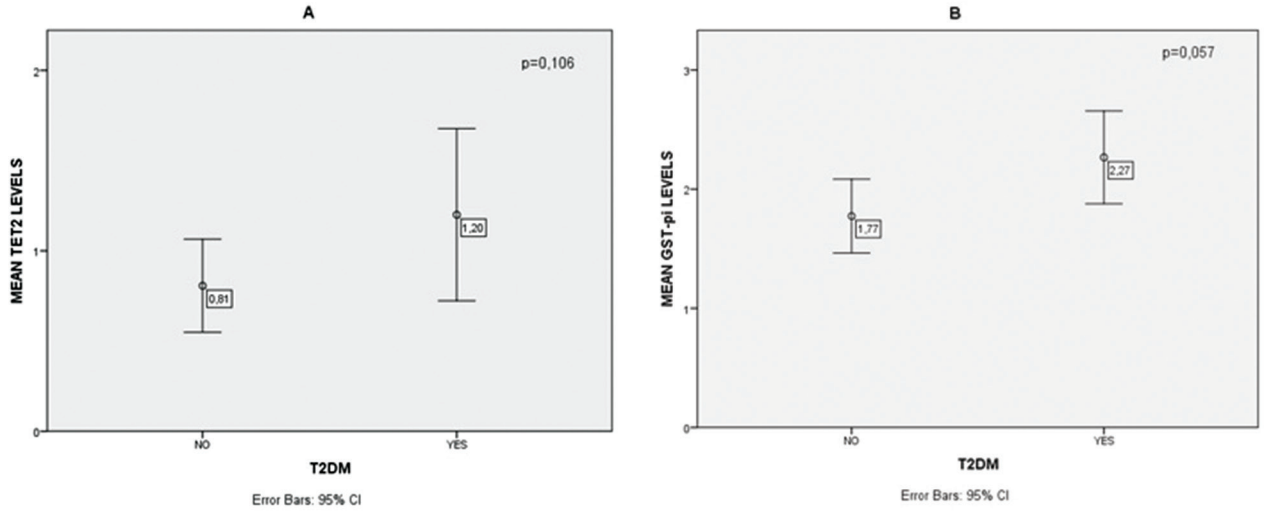

Figure 2. Comparison of mean TET2 (**A**) and GST-pi (**B**) levels in tumors excised from patients with and without T2DM.

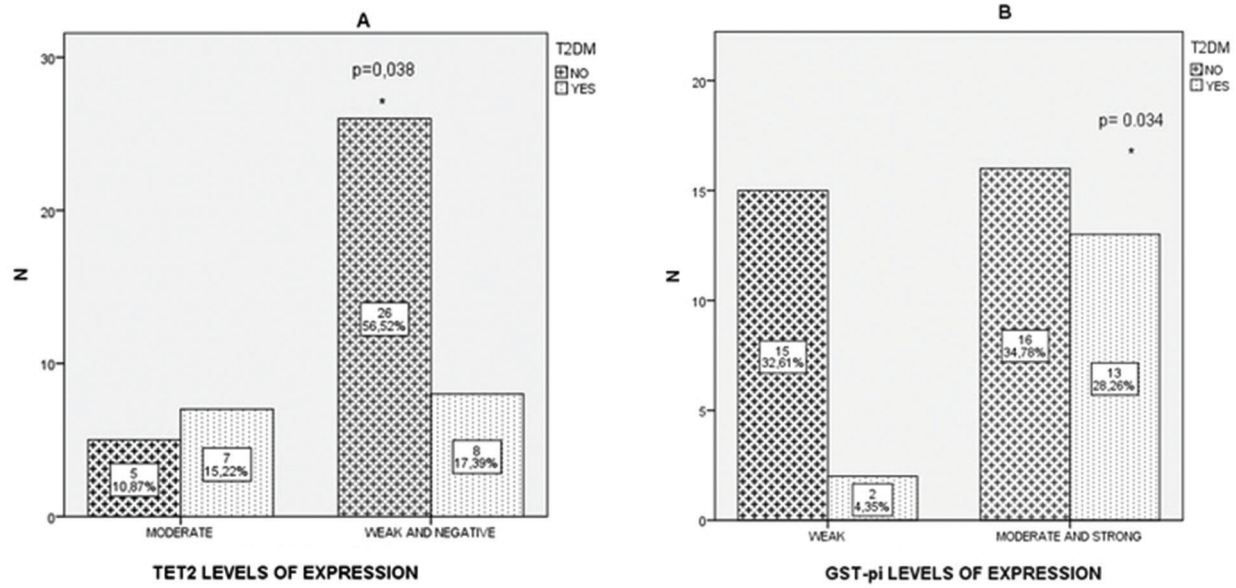

Figure 3. Comparison of the different levels of expression of TET2 (**A**) and GST-pi (**B**) in tumors excised from patients with and without T2DM.

Table 4. Binary logistic regression and relevant probability of maximum level of TET2 expression and T2DM, when examining clinical (**A**) or histopathological (**B**) characteristics of the tumors

		TET2		
		Relative probability	**95% Confidence Intervals**	***P*-value**
A				
	T2DM	6.886	1.326 – 35.748	0.022
Clinical characteristics	Age	0.963	0.897 – 1.033	0.292
	Sex	0.264	0.050 – 1.384	0.115
	Tumor size	1.045	0.697 – 1.567	0.831
B				
	T2DM	4.316	1.089 – 17.39	0.043
Histopathological characteristics	Vascular infiltration	0.454	0.066 – 3.128	0.422
	Perineural infiltration	1.439	0.196 – 10.58	0.721
	Lymphatic infiltration	1.277	0.289 – 5.651	0.747

Table 5. Binary logistic regression and relevant probability of maximum level of GST-pi expression and T2DM, when examining clinical (**A**) or histopathological (**B**) characteristics of the tumors

		GST-pi		
		Relative probability	**95% Confidence Intervals**	***p*-value**
A				
	T2DM	2.75	0.626 – 12.078	0.18
Clinical characteristics	Age	1.009	0.741 – 1.081	0.806
	Sex	0.323	0.071 – 1.474	0.145
	Tumor size	1.139	0.783 – 1.656	0.496
B				
	T2DM	2.331	0.528 – 10.284	0.264
Histopathological characteristics	Vascular infiltration	4.084	0.398 – 41.945	0.236
	Perineural infiltration	0.312	0.029 – 3.381	0.338
	Lymphatic infiltration	1.682	0.426 – 6.635	0.458

To the best of our knowledge, the concurrent immunohistochemical study of TET2 and GST-pi expression in colon adenocarcinoma tissues has not been studied before. Previous studies[28] have examined the expression of TET2 in cytoplasm; yet we observed that GST-pi was predominantly expressed in the cytoplasm and cytoplasmic membrane of malignant cells while TET2 enzyme had predominantly nuclear expression. Data analysis in the present study documented a weak negative correlation between TET2 expression levels and the size of the studied tumors. Although the association was not statistically significant, similar associations between TET2 expression and malignancies have also been reported; in head and neck squamous cell carcinoma, it appeared that low TET2 levels were significantly associated with larger tumor size, advanced clinical stage and worse prognosis.[29] Accordingly, in an experimental model of glioblastoma, it was shown that TET2 overexpression in tumor cells resulted in the development of smaller tumors.[30] Furthermore, no statistically significant difference was observed between GST-pi expression levels and vascular and perineural infiltration. Yet, Tan et al. previously showed that high GST-pi expression levels were associated with adverse histological features and decreased overall survival in patients who had undergone surgery for stage C colon adenocarcinoma.[31]

Despite the fact that survival analysis goes beyond the remit of the current study, it is worth mentioning that different studies have related the level of expression of GST-pi with overall survival in patients with colon cancer; Jankova et al. showed that survival did not differ among patients with low GST-pi expression who had been on adjuvant chemotherapy, patients with low GST-pi expression who had not been on chemotherapy and patients with high GST-pi expression who had been on chemotherapy. Only patients with high GST-pi expression who had not received chemotherapy had poorer survival when compared to the other three groups.[32] On the other hand, Kang et al. showed that the 5-year overall survival as well as the 5-year disease-free survival rate of patients with high expression levels of TET2 were significantly higher compared to that of patients with low TET2 levels.[28]

Finally, the present study demonstrated that colon tumors excised from patients with T2DM were more likely to exhibit high GST-pi expression and moderate TET2 expression. It remains controversial whether certain GST-pi genetic polymorphisms have an impact on susceptibility to T2DM; yet there is evidence that GST-pi polymorphism may affect the onset of T2DM and especially in patients below the age of 40 years.[33,34] Furthermore, certain TET2 mutations have been suggested to correlate with T2DM.[35]

Some of the limitations of the present study include its retrospective nature, the relatively small number of the included patients and the lack of some data concerning T2DM, such as disease duration, which is partially known.

CONCLUSION

To conclude, our study supports the idea that GST-pi and, mainly, TET2 could serve as useful immunohistochemical markers of colorectal cancer tumors in patients with T2DM and no other comorbidities. TET2 and GST-pi are highly expressed in malignant colon tumors. History of T2DM in CRC patients was associated with the highest observed GST-pi expression and the absence of T2DM was associated with the lowest observed TET2 expression in the studied tumors. High levels of TET2 expression are significantly more likely to be observed in tumors from patients with T2DM, regardless of the clinical (age, sex, tumor size) and histopathological (vascular, perineural, lymphatic invasion) characteristics. In the era of precision medicine, treatment, prevention, and diagnostic methods are not one-size-fits-all approaches; further studies are needed to fully establish GST-pi and TET2 as a predictive biomarkers or therapeutic targets for CRC among the diabetics.

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Conflict of Interest

None.

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Экспрессия Ten-Eleven Translocation 2 и глутатион-S-трансферазы пи-1 у пациентов с колоректальным раком с сахарным диабетом 2 типа и без него

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Резюме

Цели: Выделить возможные корреляции сахарного диабета 2 типа (СД2) с микроскопическими/макроскопическими характеристиками тканей колоректального рака, а также с экспрессией белков Ten-Eleven Translocation 2 (TET2) и глутатион-S-трансферазы пи-1 (GST-pi).

Материалы и методы: Опухоли 46 пациентов были помещены в парафиновые блоки, окрашены гематоксилином-эозином и исследованы микроскопически. Было проведено иммуногистохимическое исследование экспрессии TET2 и GST-пи-1. Результаты были проанализированы и соотнесены с СД2 как сопутствующим заболеванием.

Результаты: Все опухоли экспрессировали GST-пи-1 на трёх уровнях (слабый, умеренный и сильный); две из трёх опухолей показали либо слабую, либо умеренную экспрессию TET2. Пациенты без СД2, как правило, имели опухоли со слабой или отсутствующей экспрессией TET2 (p=0.038), тогда как опухоли пациентов с диабетом показали значительно более высокий процент сильной или умеренной экспрессии GST-пи-1 (p=0.034). При биномиальной логистической регрессии опухоли, вырезанные у пациентов с СД2, в 6.9 раза чаще демонстрировали умеренную (а не слабую и никакую) экспрессию TET2 по сравнению с опухолями у пациентов без диабета (95% ДИ [1.33, 35.75]), и в 2.7 раза более высокую относительную вероятность демонстрации сильной (а не умеренной и слабой) экспрессии GST-пи-1 (95% ДИ [0.63, 12.09]), принимая во внимание пол, возраст и размер опухоли. Связь между СД2 и экспрессией TET2 остаётся статистически значимой в дополнительном биномиальном анализе, который был проведён с учётом определённых гистологических характеристик опухоли.

Заключение: TET2 и GST-пи-1 экспрессируются в злокачественных опухолях толстой кишки. СД2 у пациентов с КРР был связан с самой высокой наблюдаемой экспрессией GST-пи-1; отсутствие СД2 было связано с самой низкой наблюдаемой экспрессией TET2. СД2 увеличивает вероятность наблюдения экспрессии GST-пи-1 и TET2 на максимальных уровнях, независимо от конкретных микроскопических особенностей опухоли и определённых характеристик пациента.

Ключевые слова

колоректальный рак, сахарный диабет, эпигенетика, глутатион-S-трансфераза пи-1, белок TET2, тип 2