PROGNOSTIC VALUE OF THE MUCIN IMMUNOHISTOCHEMICAL PROFILE OF EARLY GASTRIC CANCER

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The mucin immunohistochemical profile of early gastric cancer (EGC) has certain clinicopathological features that are independent of the histological type of the tumour. In this study, we identified the prevalence and prognostic value of the mucin profile of EGC.

Aims

The aim of the study was to compare the mucin profile variants based on clinicopathological characteristics and histological type of early gastric cancer (EGC).

Materials and Methods

Immunhistochemistry analysis was performed on the surgical material of 227 cases of EGC, with testing the expression of marker mucins MUC 5 AC, MUC6, MUC2, and CD10. The mucin immunophenotype variants were classified as gastric, intestinal, mixed, and null immunophenotype according to the immunopositivity of the above markers.

Results

EGC with the gastric immunophenotype was characterised by more aggressive morphological features – it occurred significantly more often (compared to the intestinal phenotype) in the 0 III macroscopic type, ulcerated type, K. Nakamura undifferentiated histological type, in P. Lauren diffuse type, and JGCA signet ring cell histological type of EGC. Also, EGC with the gastric immunophenotype showed a higher rate of metastasis compared to the intestinal one (20.6% vs. 11.7%), and the metastasis rate in differentiated EGC with the gastric immunophenotype were notably higher than in the intestinal one (9/2; 22.3% vs. 40/3; 7.0%), but the data did not reach significance due to the small sample size. EGC with the intestinal immunophenotype had more favourable morphological features and was significantly more common with intramucosal localisation, 0 I macroscopic type, K. Nakamura differentiated type, P. Lauren intestinal type, and JGCA highly differentiated adenocarcinoma, and had the lowest incidence of ulceration.

Conclusions

The results suggest that the mucin profile of EGC is associated with tumour progression. The gastric immunophenotype of EGC was associated with more aggressive morphological characteristics than the intestinal one.

Keywords: early gastric cancer, mucin immunophenotype

INTRODUCTION

Early gastric cancer (EGC) is characterized by high heterogeneity. However, histological classifications such as Lauren P. [1] and Japanese Gastric Cancer Association (JGCA) (2014) [2] classifications and the classification of Nakamura K. [3] that provides grounds for the indication of endoscopic JGCA resection [4], do not reflect all the clinical and prognostic peculiarities of EGC. Taking into account the presence of minimal risk for lymphogenic metastasizing in cases that meet the extended indications for endoscopic JGCA resection, there is a necessity for additional methods for evaluating the malignant potential of EGC in order to verify the risk of lymphogenic metastasizing and help to choose the treatment tactics. The mucin profile of EGC is an important immune histochemical marker of the disease prognosis. Mucins are glycosylated glycoproteins that look like a cohesive gel secreted by the epithelium of the gastrointestinal tract. The identification of the mucin profile (immune phenotype) of EGC is based on the immune histochemical establishment of the expression of markers to mucins of normal epithelial cells of the

gastrointestinal tract in tumor cells. According to a traditional classification [5, 6], the mucin profile of EGC is divided into 4 variants: intestinal (expression of the minimum one of the intestinal markers MUC 2, CD 10), gastric (expression of the minimum one of the gastric markers MUC 5 AC, MUC 6), mixed (a combination of expression of gastric and intestinal markers), and null (non-classified – no expression). It is known that variants of the mucin immune phenotype of EGC are spread significantly and associated with malignant potential. However, the data on the association between the mucin profile of EGC and tumor behavior are controversial [7, 8].

The study was aimed to perform a comparative evaluation of the variants of the mucin profile depending on the clinical-morphological characteristics and histological type of EGC. The authors believe that the identification of the mucin immune histochemical profile of EGC as a prognostic factor can be an integral part of the preoperational stage of patients' examination for the choice of treatment tactics.

MATERIALS AND METHODS

General characteristics. The authors conducted a retrospective study that included operational material obtained from 277 patients with EGC (113 (49.8%) males and 114 (50.2%) females) that underwent radical surgery with a lymph node dissection at the department of abdominal surgery of the N.N. Blokhin National Medical Research Center of Oncology. The volume of surgery and the level of lymph node dissection were estimated according to the JGCA recommendations [4]. The mean age of patients was 58.5±11.4 years old (from 26.4 to 90 years old, median 59 years).

Histological study. The operational material (hematoxylin-eosin stained microslides) was retrospectively studied by two pathologists and reclassified according to the histological classification of tumors using Lauren P. [1], Nakamura K. [3], and JGCA 2014 classifications [2].

Immunohistochemical study. An immunohistochemical study was performed by a peroxidase-antiperoxidase method on waxed 5 μm sections according to a standard protocol. The authors used the most representative waxed block of the operational material obtained from 227 patients with EGC (monoclonal antibodies to mucins MUC 5 AC, MUC 6, MUC 2 – Novocastra, CD10 – DAKO). The expression of MUC 5 AC, MUC 6, MUC 2, and CD10 was evaluated depending on the intensity of staining. The criteria for positive expression of markers to mucins were 20% of stained tumor cells and 10–5% of CD 10 positive cells [9]. For internal control, non-tumor gastric mucosa was used. The percentage of positively stained cells was estimated by two independent pathologists. The discrepancy in the interpretation of the estimation was solved based on consensus. Cases of EGC were divided into four mucin immune phenotypes: gastric, intestinal, mixed, and null based on the recommendations [5, 6].

Statistical analysis. The statistical analysis of the obtained data and calculation of the parameters were performed in Microsoft Excel, Statistica for Windows v.10, and SPSS v.21. Continuous variables were compared using Student's t-test or Mann-Whitney U-test. Categorical variables were compared using Chi-square or Fisher's exact test. The differences were considered significant at $p<0.05$.

RESULTS

General characteristics of the mucin profile of EGC (n=227). Patients with EGC with the gastric immune phenotype were significantly younger (the mean age of patients was 55.6±11.4 years old in comparison with the null immune phenotype (p=0.046) and intestinal immune phenotype (p=0.038)). In the group of patients with the gastric immune phenotype, the share of patients older than 60 years old (35.3%) was significantly lower than in the group with the intestinal immune phenotype (50.0%) and null immune phenotype (55.6%) (p=0.038). In patients with the intestinal immune phenotype, tumors with intramucosal localization prevailed (70%) (p=0.023) in comparison with the null immune phenotype (47.2%). The lowest rate of occurrence was observed in tumors with the depth of invasion SM 2–10.0% (p=0.049) in comparison with the null immune phenotype (25.0%). In patients with the gastric immune phenotype, tumors with ulceration were observed significantly more often 48.5% (p=0.025) in

comparison with the intestinal phenotype (30.0%), 0 IIc macroscopic type (38.2%) (p=0.018) in comparison with the null phenotype (16.7%), and 0 III macroscopic type (25.0%) (p=0.043) in comparison with the intestinal phenotype (11.7%). In patients with EGC of gastric immune phenotype, a higher rate of metastasizing was observed than in patients with the intestinal immune phenotype (20.6% versus 11.7%). The highest rate of lymphogenic metastasizing was revealed in patients with the null immune phenotype.

Variants of the mucin immune phenotype of EGC did not have significant differences by the localization and maximal size of the tumor and the presence of lymph-vascular invasion. A detailed characteristic of the variants of the mucin immune phenotype of EGC (n=227) is presented in Table 1.

Table 1

Characteristics of the variants of mucin immune phenotypes in the group of patients with EGC (n=227)

		Mucin immune phenotypes							
		null		gastric		intestinal		mixed	
N		36		68		60		63	
		abs.	%	abs.	%	abs.	%	abs.	%
Sex	male	19	52.8	29	42.6	32	53.3	33	52.4
	female	17	47.2	39	57.4	28	46.7	30	47.6
Age	< 60 years old	16	44.4	44	64.7	30	50.0	37	58.7
	> 60 years old	20	55.6	24	35.3*	30	50.0	26	41.3
Ulceration and fibrosis	no	21	58.3	35	51.5	42	70.0	34	54.0
	yes	15	41.7	33	48.5+	18	30.0	29	46.0+
Lymph-vascular invasion	no	31	86.1	62	91.2	52	86.7	52	82.5
	yes	5	13.9	6	8.8	8	13.3	11	17.5
Depth of invasion	M	17	47.2	41	60.3	42	70.0*	35	55.6
	SM1	10	27.8	18	26.5	12	20.0	15	23.8
	SM2	9	25.0	9	13.2	6	10.0*	13	20.6
Localization	L and LD	14	38.9	34	50.0	31	51.7	31	49.2
	M and ML.	16	44.4	27	39.7	22	36.7	26	41.3
	U all with E -	5	13.9	5	7.4	4	6.7	3	4.8
	U (E+)	1	2.8	2	2.9	3	5.0	3	4.8
Macroscopic type	0 I	19	52.8	7	10.3*+	17	28.3*	12	19.0*
	0 II a	1	2.8	11	16.2*	13	21.7*	7	11.1
	0 II b	4	11.1	7	10.3	3	5.0	9	14.3
	0 II c	6	16.7	26	38.2*	20	33.3	25	39.7*
	0 III	6	16.7	17	25.0+	7	11.7	10	15.9
N+		8	22.2	14	20.6	7	11,7	9	14,3
Maximum size		2.97±1.59		2.98±1.50		2.64±1.46		2.48±1.39	
Mean age		60.4±11.5		55.6±11.4*+		59.6±9.7		59.6±12.3	

*Significant differences in comparison with the null phenotype, p<0.05.
+ Significant differences in comparison with the intestinal phenotype, p<0.05.

Mucin profile of EGC in respect to the morphological characteristics of the tumor

The distribution of the variants of the mucin immune phenotype with respect to the tumor size.

The variants of the mucin immune phenotype did not correlate with the maximal size of the tumor (Table 1). However, it should be noted that among minor tumors (1–2 cm), a mixed immune phenotype of EGC prevailed (33.7%). Along with an increase in the tumor size (2.01–3 cm and >3 cm), the number of cases with the mixed immune phenotype decreased (25.0% versus 22.1%) and the number of cases with null immune phenotype insignificantly decreased (M – 13.3%, SM 1 – 15.4%; SM2 – 19.5%).

The characteristic of the variants of the mucin immune phenotype with respect to the depth of invasion of EGC

The intestinal immune phenotype was more often observed in cases with intramucosal EGC (31.1%) and significantly rarer in cases with the depth of invasion SM2 (16.2%; p=0.053). *The gastric immune phenotype* in cases with intramucosal EGC was met rarer (30.4%) than intestinal but the rate of occurrence did not change with an increase in the depth of tumor invasion unlike the intestinal immune phenotype (SM1 – 32.7%; SM 2 – 24.3%). In cases with intramucosal EGC, the *null immune phenotype* (12.6%) was observed significantly rarer than the other phenotypes (gastric p=0.0003, intestinal p=0.0002, mixed p=0.004). In cases with a maximal depth of invasion SM2, the *mixed immune phenotype* was observed two times more often than intestinal (35.1%; p=0.055) (Table 2).

The characteristics of the variants of the mucin immune phenotype of EGC with respect to a macroscopic type of tumor

The gastric immune phenotype was observed significantly more often in cases with 0III macroscopic type (42.5%; p=0.014 in comparison with intestinal 17.5%). The lowest occurrence rate of the gastric immune phenotype was observed in cases with 0I macroscopic type (12.7%) (data were significant in comparison with the null immune phenotype (34.5%; p=0.006) and intestinal immune phenotype (30.9%; p=0.018). *The intestinal immune phenotype* was met significantly more often in cases with 0I and 0II macroscopic type (in comparison with gastric 30.9%; p=0.018 and null 40.6%; p=0.0002). The lowest occurrence rate of this immune phenotype was observed in cases with 0III macroscopic type 17.5% (in comparison with gastric; p=0.014).

The association between the mucin immune phenotype of EGC with ulceration. In cases with EGC with ulceration, the gastric phenotype was met significantly more often (34.7%) in comparison with intestinal 18.9% (p=0.011) and null 15.8% (p=0.002). In cases with non-ulcerous EGC, the rate of occurrence of the intestinal immune phenotype (31.8%) was significantly higher than in cases with ulcerous cancer (18.9%, p=0.021).

Table 2

Characteristics of the variants of the mucin immune phenotype of EGC depending on the depth of the invasion

Mucin phenotype	Depth of invasion							
	M		SM1		SM2		Total	
Number of patients	135		55		37		227	
	Abs	%	Abs	%	Abs	%	Abs	%
null	17	12.6	10	18.2	9	24.3	36	15.9
gastric	41	30.4*	18	32.7	9	24.3	68	30.0
intestinal	42	31.1*	12	21.8	6	16.2	60	26.4
mixed	35	25.9*	15	27.3	13	35.1	63	27.8

*Significant changes in comparison with null phenotype, p<0.05

Table 3

Characteristics of the mucin profile of EGC depending on the Lauren classification

	Intestinal		Diffuse		Mixed		Total	
Number of patients	123		72		32		227	
	Abs.	%	Abs.	%	Abs.	%	Abs.	%
null	25	20.3	10	13.9	1	3.1	36	15.9
gastric	13	10.6	43	59.7*#	12	37.5*#	68	30.0
intestinal	51	41.5*+	4	5.6	5	15.6	60	26.4
mixed	34	27.6	15	20.8	14	43.8*#	63	27.8

*Significant differences in comparison with the null phenotype, p<0.05
+Significant differences in comparison with the gastric phenotype, p<0.05 #Significant differences in comparison with the intestinal phenotype, p<0.05

Table 4

Characteristic of the mucin profile of the differentiated and non-differentiated types of EGC

	Differentiated type		Non-differentiated type		Total	
Number of patients	91		136		227	
	Abs.	%	Abs.	%	Abs.	%
null	13	14.3#	23	16.9+	36	15.9
gastric	9	9.9#	59	43.4*	68	30.0
intestinal	43	47.3	17	12.5*+	60	26.4
mixed	26	28.6#	37	27.2+	63	27.8

*Significant differences between the types by the Nakamura classification, p<0.05
+Significant differences in comparison with the gastric phenotype, p<0.05
Significant differences in comparison with the intestinal phenotype, p<0.05

The Lauren classification [1]. In the general sampling (n=227), more than half of cases of EGC had intestinal type (intestinal – 54.1%; diffuse – 31.7%; mixed – 14.2%; p=0.00001). The intestinal type of EGC was not completely represented by the intestinal immune phenotype. Still, this mucin profile prevailed (41.5% of cases) in comparison with the gastric phenotype (10.6%; p=0.00001) and null phenotype (20.3%; p=0.0003). In half of the cases, the diffuse type of EGC had the gastric immune phenotype (59.7%) in comparison with intestinal (5.6%; p=0.00001) and null (13.9%; p=0.00001). In cases with mixed histological type, the mixed phenotype prevailed (43.8%) in comparison with the intestinal (p=0.014) and null (p=0.0001) phenotypes and the gastric phenotype (37.5%) prevailed in comparison with the null (3.1%) (p=0.0006) and intestinal (15.6%) (p=0.044). (Table 3).

Histological classification by Nakamura K. [3]. In cases with differentiated type by the classification of Nakamura, the *intestinal phenotype* was observed significantly more often (47.3%; (p=0.00001 in comparison with gastric, p=0.00001 – null, p=0.007 – mixed). In cases with non-differentiated type, the *gastric immune phenotype* prevailed (43.4%; p=0.00001 in comparison with null, p=0.00001 – intestinal, p=0.004 – mixed). The comparison of histological types in cases of a differentiated type showed that the intestinal immune phenotype (p=0.00001) was observed more often than in cases of a non-differentiated type; in cases of a non-differentiated type, gastric phenotypes prevailed (p=0.00001) (Table 4).

The study of regional metastasizing in cases of a differentiated type of EGC with the gastric immune phenotype showed that the rate of metastasizing was significantly higher (9/2; 22.3%) than in cases with the intestinal phenotype (43/3; 7.0%). However, these data did not meet the criterion of significance because of a small sampling. In cases with a non-differentiated type, the rate of lymphogenic metastasizing in patients with EGC with the gastric and intestinal immune phenotypes was similar (20.3% and 23.5%, respectively).

Histological classification JGCA 2014 [2]. *The intestinal immune phenotype* was observed significantly more often in cases with highly differentiated adenocarcinoma (more than in half of the cases – 58.6). Along with a decrease in the differentiation of EGC, the number of cases with the intestinal immune phenotype decreased (non-solid low-differentiated adenocarcinoma (17.1%; p=0.00001), solid low-differentiated adenocarcinoma (20.0%; p=0.027), colloid cancer (4.2%; p=0.00001). On the contrary, the *gastric immune phenotype* was rarely met in cases with highly-differentiated adenocarcinoma (3.4%) but significantly more often in cases with the colloid histological type of EGC (70.4%; p=0.00001 in comparison with highly differentiated adenocarcinoma – 3.4%, p=0.0001 in comparison with moderately differentiated adenocarcinoma – 17.6%, p=0.003 in comparison with low differentiated solid adenocarcinoma – 20.0%, p=0.00001 with low differentiated non-solid adenocarcinoma – 14.6%, p=0.00001 with papillary adenocarcinoma – 18.2%).

The null immune phenotype was observed in cases with such an unfavorable type of cancer as low differentiated solid adenocarcinoma (50%) (in comparison with highly differentiated adenocarcinoma – 8.6% (p=0.004), moderately differentiated adenocarcinoma – 5.9% (p=0.015), colloid cancer – 7.0% (p=0.0019), and papillary adenocarcinoma – 40.9% (in comparison with highly differentiated adenocarcinoma – 8.6% (p=0.0017), moderately differentiated adenocarcinoma – 5.9% (p=0.014), colloid cancer – 7.0% (p=0.0005)).

The morphological characteristics of the tumor that provided grounds for the evaluation of the risk of lymphogenic metastasizing in patients with EGC and indications for endoscopic resection cannot be precisely identified during the preoperative examination of a patient [10-12]. Besides, the expanded indications for endoscopic resection in patients with EGC are not always safe [13]. The authors believe that such an additional factor of prognosis of the progression of EGC as a mucin profile of the tumor can help in the choice between EGC endoscopic resection and surgical operation.

The majority of the studies on the mucin profile of EGC were conducted in Asian countries. The present study was one of the first in Russia. It showed that the rate of occurrence of the variants of mucin immune phenotypes in the general sampling (n=227) was uniform (intestinal – 26.40%, gastric – 30.00%, mixed – 27.80%, null – 15.90%). The intestinal type of carcinoma by the classification of Lauren and the differentiated type by the classification of Nakamura K. were characterized not only by an intestinal immune phenotype but also gastric, mixed, and null mucin phenotypes, which agreed with the available published data [5, 6, 9]. According to the published data, an increase in the size of the tumor and depth of invasion led to a phenotypic shift in the mucin profile of EGC from the gastric to intestinal immune phenotype [14, 15]. On the contrary, some other studies showed that the number of cases with gastric and intestinal immune phenotypes increased and the number of cases with mixed mucin phenotype decreased ($p < 0.05$) [16]. The present study showed that an increase in the size and depth of tumor invasion led to an insignificant increase in the number of cases with the gastric immune phenotype and a decrease in the number of cases with intestinal immune phenotypes.

It is known that the intestinal and gastric immune phenotypes of EGC have different occurrence rates and are associated with different malignization potential. However, these data on the association of mucin immune phenotypes with tumor behavior are controversial [14]. The majority of researchers believe that the gastric immune phenotype of EGC is prognostically more unfavorable than intestinal. The authors report on a significantly deeper invasion, a higher rate of lymphovascular invasion and ulceration, a higher rate of venous invasion, and metastasizing into regional

lymph nodes in cases with the gastric immune phenotypes of EGC in comparison with intestinal [8, 17, 18]. In the present study, EGC with gastric immune phenotype was also characterized by more aggressive morphological features. It correlated with 0 III macroscopic type, ulceration, and low differentiated histological type. Besides, in cases of EGC with the gastric immune phenotype, a higher level of metastasizing was observed in comparison with intestinal (20.6% and 11.7%, respectively). On the contrary, the intestinal phenotype was revealed significantly more often in cases with intramucosal EGC (depth of invasion M – 70%, p=0.023; depth of invasion SM 2–10%; p=0.049) and highly differentiated histological type, which agreed with the available published data [7, 18, 19].

Besides, EGC of a differentiated type by the classification of Nakamura K. [3] with the gastric immune phenotype has unique characteristics that distinguish it from differentiated EGC with the intestinal immune phenotype because of higher malignization potential and tendency to metastasize. It is noted that EGC with the gastric mucin immune phenotype has a larger diameter and deeper invasion of a submucosal layer than the intestinal phenotype [17, 18]. Besides, it is more often observed in cases with colloid cancer and tends to a faster transformation into a non-differentiated type, ulceration, and metastasizing at the initial stage of cancerogenesis [9, 20]. According to the published data, the rate of occurrence of the gastric immune phenotype in cases with differentiated EGC varied from 7.9% to 23.9% [14, 21] and could reach 52.38% [9]. In the present study, in cases with a differentiated type of EGC, the gastric immune phenotype was observed in 9.8% of cases (9/91), intestinal – in 47.2% (43/91). The importance of the study of the mucin profile of EGC is explained by the fact that differentiated EGC (by the classification of Nakamura), especially with a diameter < 2 cm, is a generally accepted criterion of the indication for endoscopic resection of EGC proposed by JGCA [4]. The results of the present study showed that in cases with differentiated EGC with gastric immune phenotype, the rate of metastasizing was significantly higher than with intestinal (22.3% and 7.0%, respectively).

CONCLUSIONS

The authors believe that the gastric immune phenotype of EGC is an unfavorable prognostic feature. For this reason, the identification of the mucin profile of EGC can become an additional prognostic factor for the choice of treatment tactics for EGC. The immunohistochemical identification of the mucin profile of EGC is a simple and cheap method that can help specify and expand the indications for endoscopic resection of EGC.

CONFLICTS OF INTEREST

The authors declare no conflict of interest

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