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IMMUNOHISTOCHEMICAL EVALUATION OF MUCIN EXPRESSION IN PRECANCEROUS TISSUE OF STOMACH

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Aim: **To assess the profile of mucins (MUC1, MUC2, MUC5AC) in gastric mucosa through immunohistochemical method.** ***Methods:*** **To identify metaplastic areas in gastric mucosa there was used chromoendoscopy with 0.5% solution of methylene blue, the expression of the mucins profile was determined using immunohistochemistry with MUC1, MUC5AC, MUC2 and MUC6 antibodies (clone Ma695, clone CLH2, Ccp58 and CLH5, "Novocastra" Great Britain).** ***Results:*** **In case of the complete intestinal metaplasia (IM) there was maximum MUC2 expression in goblet cells. MUC5AC, MUC1 and MUC6 expression were absent in columnar epitheliocytes with brush border. In case of incomplete IM along with positive MUC2 marking of goblet cells, in 25% of such patients with chronic atrophic gastritis with incomplete IM MUC5AC expression has been observed; but in columnar epitheliocytes MUC5AC expression was observed in 100% of patients, and MUC2 expression was detected in 15% of patients only. Weak expression of MUC2 and MUC5AC in the areas adjacent to gastric adenocarcinoma was registered.** ***Conclusion:*** **MUC5AC expression in columnar epithelial cells and goblet exocrinocytes marks the formation of gastrointestinal phenotype — incomplete IM, along with the simultaneous production of MUC2 by goblet cells. Decrease of MUC 5AC expression in columnar epithelial cells and goblet exocrinocytes was found in areas of severe dysplasia and IM that may serve as an additional criterion of early malignization of gastric mucosa cells.**

Key Words: **mucins, precancerous changes, gastric mucosa.**

Modern understanding of gastrointestinal barrier is associated with the ability of surface and glandular epithelial cells to synthesize mucin [1, 2]. At the same time epithelial mucins are a large group of secreted and embedded in plasmolemma glycoproteins produced by epithelial cells. In epithelial tissues 13 types of mucin are distinguished, forming two classes of compounds: transmembrane and secretory mucins. MUC1 is one of the transmembrane mucins of the digestive tract cells. It is distributed on the cell surface and acts as a signaling molecule. MUC3 and MUC4 are membrane-bound mucins that play important roles in the protection of the epithelial cells and have been implicated in epithelial renewal and differentiation. As for the gel-forming mucins of the digestive tract, they include: MUC2, MUC5AC, MUC4B, MUC6. They are actively expressed on epithelial cells and represent a variable number of peptides rich in serine, threonine and proline, coupled with a large number of oligosaccharide chains. According to the information available [3], the expression of membrane glycoproteins increases nearly tenfold in transformed cells, and carbohydrate chains are becoming shorter. Mechanisms that are responsible for orientation of protein molecules in the bilayer of cytomembrans, stabilization of the spatial structure of proteins, transmembrane and intracellular transport of substances (including transfer of hydrolytic enzymes from the Golgi complex to lysosomes), and molecular mechanisms of intercellular recognition are associated with the specific structure of the carbohydrate determinants of glycoproteins. It is known that these mechanisms play an important role in the maturation and differentiation of cells, histogenesis and organ morphogenesis, contact inhibition, cell proliferation that may be considered as the manifestations of malignant growth [4–7].

Today it is proven that chronic atrophic gastritis (CAG) is one of the main elements of gastric tumorigenesis. According to the recommendations of the International Atrophy Group (2002) two main types of atrophy are distinguished: metaplastic and non-metaplasic [8]. Non-metaplasic atrophy is characterized by loss of glands, accompanied by fibrosis or fibromuscular proliferation in lamina propria of gastric mucosa, as for metaplastic atrophy — there is a replacement of gastric glands by metaplastic ones (including enteric), which can happen in the setting of other signs of atrophy. Disturbance of the synthesis and secrete releasing by glandular cells, its chemical composition change under the influence of exogenous and endogenous factors in CAG with intestinal metaplasia (IM) have not been studied well enough.

MATERIALS AND METHODS

Patients and methods. During 6 years there were examined 98 patients who were sent to endoscopy departments of Vinnytsya hospitals to clarify the clinical diagnosis. There were 52 male (53%) and 46 female patients (47%). Among these 98 patients we selected 68 with chronic atrophic gastritis (CAG) with IM that were taken as a main group for further investigation because IM was closely associated with this disease. In this group 42 patients were diagnosed as *H. pylori*-infected and 26 patients as *H. pylori*-noninfected. The comparison group included 30 people with CAG without IM (16 pateints *H. pylori*-infected and 14 — -noninfected). The average age of patients examined in dynamics was 53 ± 1.1, average duration of disease at the time of IM di-

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Abbreviations used: CAG – chronic atrophic gastritis; IM – intestinal metaplasia.

agnosis — 2.6 ± 0.6 years. As the first-line therapy for eradication of *H. pylori*, all patients were treated by 10-day triple therapy with omeprazole at a dose of 20 mg (twice daily), amoxicillin at a dose of 1 gm/day, and either metronidazole at a dose of 500 mg (twice daily).

Moreover, surgical specimens of gastric adenocarcinoma as well as adjacent mucosa were investigated (54 patients were enrolled into study: 28 adenocarcinoma, 12 signet-ring cell carcinoma and 14 undifferentiated tumor, a distribution of patients according to TNM staging is given in table 1).

Table 1. Distribution of patients with gastric cancer according to TNM staging

Stage	Number of patients	
	n	%
IA (T1N0M0)	4	7.4
IB (T2N0M0)	21	38.9
II (T3N0M0)	12	22.2
IIIA (T3N1M0)	4	7.4
IIIB (T3N2M0)	4	7.4
(T4N0M0)	2	3.7
IV (T4N1M0)	2	3.7
(T4N2M0)	3	5.5
(T3N2M1)	1	1.9
(T4N2M1)	1	1.9
Total	54	100

Multiple biopsies were performed during endoscopy and chromoendoscopy with 0.5% solution of methylene blue (two biopsies from the body and antrum and one — from an area of the angle of the stomach) taking into account the requirements of the modified Sydney system of chronic gastritis diagnosis and stained areas of gastric mucosa with the following histological study of biopsies. Biopsy material was processed by conventional histologiocal methods. To define metaplastic changes of gastric mucosa the following methods were used: staining with hematoxylin and eosin and van Gisone, combined high iron diamine and alcian blue technique, orsein combined with alcian blue, Gomory aldehyde fuchsin, alcian blue with pH 1.0 and 2.5 together with the PAS reaction. *H. pylori* persistence has been conducted with a rapid urease test and with a Pappenheim stain as well as with Romanovsky — Giemsa and toluidine blue staining.

Immunohistochemistry. Immunohistochemical studies were performed on paraffin sections using streptavidin-biotin method ("DAKO", Denmark, LSAB2 Systems, HRP. Expression of the mucins profile was determined using antibodies MUC1, MUC5AC, MUC2 and MUC6 (clone Ma695, clone CLH2, Ccp58 and CLH5, "Novocastra", Great Britain). In preparations at 400-fold magnification we determined the rate of intestinal differentiation (nuclear label CDX2) in 5 randomly selected fields of view (⩾ 500 cells) as a percentage share of positively stained nuclei of epithelial cells of gastric mucosa in three compartments (I — surface and foveolar epithelium; II — neck region, III — base of the glands, middle and lower third of the glands to the basal sections). To evaluate the expression of mucins (MUC5AC, MUC2, MUC6) in gastric mucosa in similar areas there was used semi-quantitative color intensity rating scale : 0 (none) — no positive reaction in the cells, 1 (weak) — up to 30% of the cells reacted positively, 2 (moderate) — 31–60%, 3 (strong) — 60% or more stained cells [9].

All sections were examined by three independent investigators. The mucins expression was evaluated by cytoplasmic staining. The results were expressed semiquantitatively for each histological group as the number of sections positively labeled, the predominant cell type labeled, and the average score of the positively labeled cells. Positive Control Sections: control tissues taken from colon and stomach, with previously identified MUC gene expression patterns were included with each batch of sections for immunohistochemistry. Negative Control Sections: the primary antibody was omitted as a negative control to test the specificity of the antibodies utilized for each section.

All patients were thoroughly informed about the study that was approved by the local ethics committee.

Statistical analysis. Results of immunohistochemical alterations were compared to the clinicopathologic features using chi-square test with two tailed p-value, $p < 0.05$ was considered as significant.

RESULTS

It was shown that a certain cell phenotype corresponds to some certain type of mucin. So, MUC5AC was found in the epithelium pits, MUC1 — in mucous neck cells, MUC6 — in the pyloric exocrinocytes, MUC2 — in goblet cells. The fact that MUC5AC is selectively synthesized by epithelial cells of pits and necks of the gland cells of normal gastric mucosa allowed us to draw a conclusion that the epithelial cells of the necks of the glands may be classified as those producing mucus. Due to the low specificity of MUC1 binding of cytoplasmic mucus, we observed its weak expression in the cytoplasm of mucous neck cells only in 2 patients with CAG, and due to lack of positive binding in IM case we excluded it from further analysis. MUC5AC marking was highly informative in all the investigated cases. Thus, the expression of MUC 5AC has not been defined in columnar epitheliocytes areas of complete IM despite strong and moderate expression in areas with incomplete IM (Fig. 1 *a*, *b*).

In *H. pylori*-infected group associated HAG the MUC5AC labeling was moderate and weak. After eradication of *H. pylori* infection the expression of several of them was amplified. MUC5AC expression prevailed in columnar epithelial cells in 21% of patients with incomplete IM in the cytoplasm and membranes of goblet cells. In patients with a long history of CAG (over 3 years) and over 6 years in the group subjected to dynamic monitoring, in 34% of patients loss of MUC5AC was observed in areas of incomplete IM and mild and severe dysplasia (Fig. 2).

Decrease of the number of mucin-positive cells was visible in areas of incomplete IM adjacent to tumor border and in cancer cells. However, in 37% of patients the MUC5AC expression remained in the IM areas adjacent to tumor and in neoplastic cells, which may predominantly indicate a gastric type of adenocarcinoma (Fig. 3).

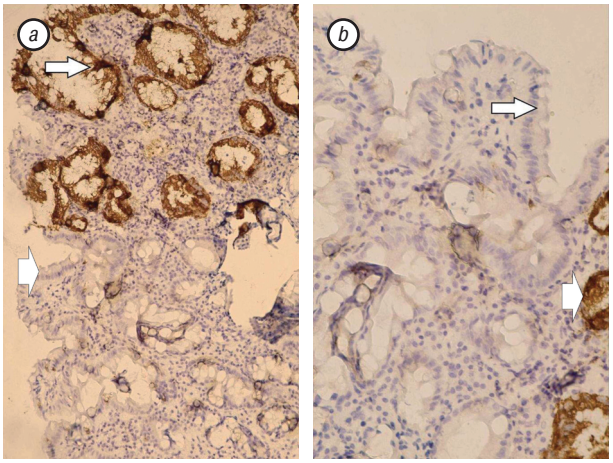

Fig. 1. *a*) Chronic atrophic gastritis with complete and incomplete intestinal metaplasia (InCIM). Strong expression of MUC5AC in areas of incomplete IM (arrow) and lack of mucin expression in the areas of the complete IM (arrowhead). Immunohistochemical staining, x100. *b*) Chronic atrophic gastritis with complete IM (CIM). Lack of MUC5 expression in epitheliocytes with complete IM (arrow) and moderate expression in superficial epitheliocytes (arrowhead) of the adjacent to metaplasia area. Immunohistochemical staining, x200.

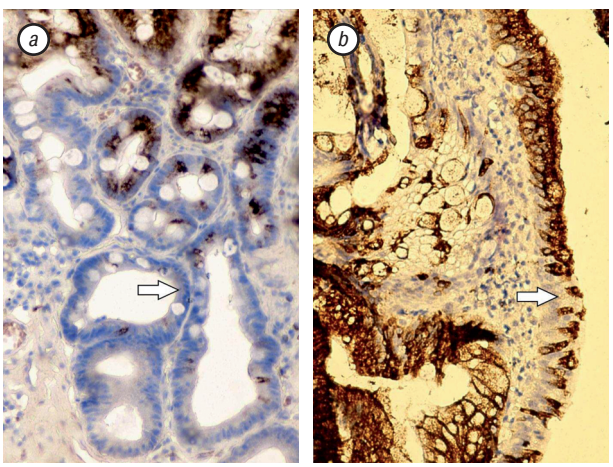

Fig. 2. Loss of MUC5AC expression in the foveolar epithelium (*a*) and surface epithelial cells (*b*) of incomplete IM areas and dysplasia in patients with chronic atrophic gastritis with incomplete intestinal metaplasia (arrow). Immunohistochemical staining, *a*) x400, *b*) x200

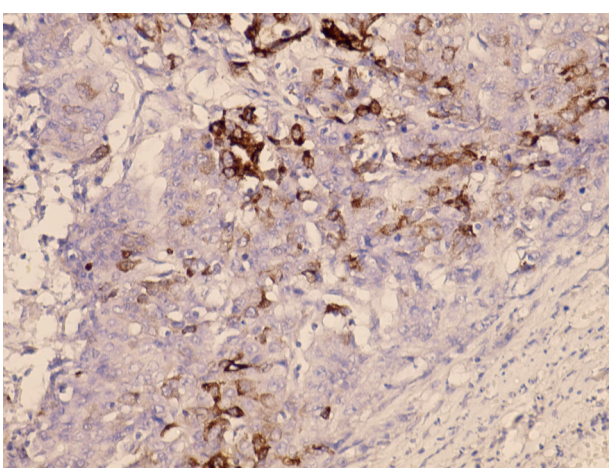

Fig. 3. Moderate MUC5AC expression in poorly differentiated gastric adenocarcinoma. Immunohistochemical staining, x400.

It should be noted that the loss of mucin-producing properties was observed mainly in patients with poorly differentiated adenocarcinoma, but in 44% of patients with cancer signet-ring cells carcinoma retained MUC5AC labeling. MUC2 expression was moderate in the cytoplasm and sometimes heavy in the membranes of goblet cells and remained during the observation in cases of complete and incomplete IM (Fig. 4). Goblet cells in the initial phases of secretion were wedge-shaped with the apex, directed to the basal parts, with the foundation towards the lumen of the stomach.

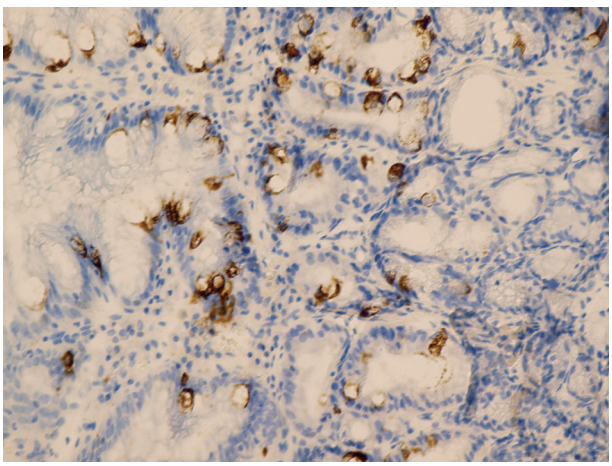

Fig. 4. Moderate expression of MUC2 in goblet cells of stomach glands. Chronic antral atrophic gastritis with areas of complete and incomplete IM. Immunohistochemical staining, x200

In areas adjacent to adenocarcinoma the weak expression of MUC2 was registered. During the observation we found no positive labeling of MUC2 in the group of patients with gastric signet-ring cell adenocarcinoma. MUC6 expression was observed mainly in the pyloric exocrinocytes of deep parts of lamina propria, it was weak in the areas of incomplete IM and it was lacking in complete IM. Thus the more profound was IM spread, the weaker was MUC6 expression, indicating a progressive displacement of pyloric glands with neogenic intestinal epithelium and severe IM (stage III of chronic atrophic metaplastic gastritis). While studying biopsies from the fundus we have noted the positive labeling of exocrinocytes of the oxyntic glands (Fig. 5), that confirms their restructuring and the development of pyloric metaplasia (pylorization).

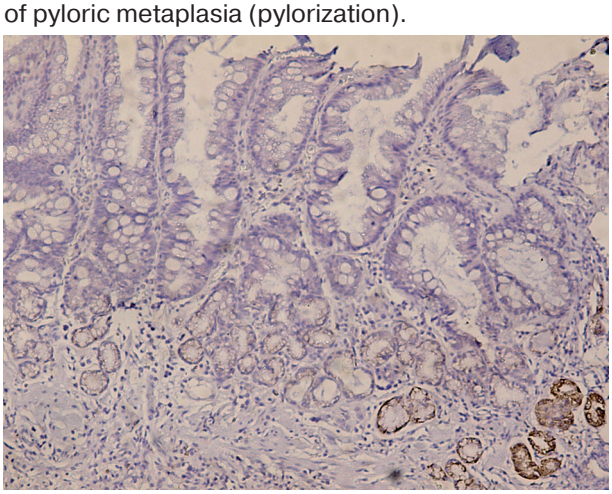

Fig. 5. Moderate expression of MUC6 in the pyloric exocrinocytes and focal expression in the oxyntic glands. Chronic atrophic fundic gastritis with incomplete IM. Immunohistochemical staining, x100

So the findings of immunohistochemical analysis of mucins profile indicate some mucin specificity in different IM types (Table 2, 3).

Table 2. Chronic atrophic gastritis with complete intestinal metaplasia

Mucins	Specificity						
	SFE	MNC	OE	PC	PE	GS	CCBB
MUC1	-	+	-	-	-	-	-
MUC2	+/-	-	-	-	-	+++	-
MUC5AC	-	-	++	-	-	-	-
MUC6	-	-	-	-	+++	-	-

Notes: SFE – superficial and foveolar epithelium; MNC – mucous neck cells; OE – oxyntic exocrinocytes; PC – parietal cells; PE – pyloric exocrinocytes; GS – goblet cells; CCBB – columnar epithelial cells with brush border; MUC – mucin; – no expression; +/– weak or absent; + weak expression; ++ moderate; +++ strong expression.

Table 3. Chronic atrophic gastritis with incomplete intestinal metaplasia

Mucins	Specificity						
	SFE	MNC	OE	PC	PE	GS	CCBB
MUC1	+	+	-	-	-	+++	-
MUC2	-	-	-	-	-	+++	+/-
MUC5AC	+++	++	++	-	-	+/-	+++
MUC6	-	+	+	-	+++	-	-

Notes: SFE – superficial and foveolar epithelium; MNC – mucous neck cells; OE – oxyntic exocrinocytes; PC – parietal cells; PE – pyloric exocrinocytes; GS – goblet cells; CCBB – columnar epithelial cells with brush border; MUC – mucin; – no expression; +/– weak or absent; + weak expression; ++ moderate; +++ strong expression.

As it can be seen from table 3, when the IM was complete, the MUC2 maximum expression was visible in goblet cells, thus MUC5AC, MUC1 and MUC6 marking were absent in columnar epitheliocytes with brush border. When the IM was incomplete and MUC2 marking of goblet cells was positive, in 25% of patients with CAG with incomplete IM, the gastric mucin (MUC5AC) was observed as well as for columnar epitheliocytes, it was characteristic that the gastric mucin (MUC5AC) has been found in 100% of patients, and as for MUC2, it was observed in 15% of patients.

DISCUSSION

IM has been recognized as either a complete or an incomplete type IM, or as a small- (Type I and Type II) or large- (Type III) intestinal-type IM [10, 11]. Though these classifications have been generally accepted, they overemphasize the characteristics common to cells in the small intestine, while neglecting the existing gastric phenotype. But the results of our investigations coincide with other authors [12, 13] that suggested dividing intestinal metaplasia (IM) into two categories, i.e., a mixed gastric and intestinal (GI) type, and a solely intestinal (I) type, based on the residual gastric phenotype cells.

Thus separation of gastrointestinal phenotype of IM unlike previous classification [10, 11], which offered only intestinal types (mainly intestinal and colonic), will let us consider gastric and intestinal phenotype. It will let us deepen our knowledge about the histogenesis of gastric cancer and it confirms the heterogeneity of IM.

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