CLINICAL SIGNIFICANCE OF HISTOCHEMICAL EXPRESSION OF MUCINS IN COLORECTAL ADENOCARCINOMA

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Colorectal carcinoma is the most common malignant tumor of the gastrointestinal tract. In the course of colorectal carcinogenesis, in addition to uncontrolled cell proliferation and accelerated angiogenesis, alterations occur in the structure and/or quantity of epithelial mucins, so the aim of our study was to investigate the histochemical expression of mucins in relation to the clinical characteristics of colorectal carcinoma.

The biopsy material of 75 patients operated from colorectal carcinoma, which was routinely processed and molded into paraffin, was used for the examination. On 3-4 μm thick cuts, routine H&E and histochemical AB-PAS pH 2.5 and HID-AB methods were applied. For the statistical analysis, the statistical software package SPSS (version 13) was used.

Mucin alterations occur in colorectal carcinoma and manifest themselves as a trace to moderate secretions of neutral or fucomucins, moderate to hypersecretions of sialomucins and trace to complete secretions of sulfomucins. The fucomucin and sialomucin secretion is associated with a strong, significant and positive coefficient of correlation with the Astler-Coller classification of the tumor stages, with metastases in the lymph nodes and with distant metastases. Unlike fucomucins, sialomucins are associated with a strong, positive, significant coefficient of correlation with the pathological stage of the tumor. Sulfomucins are associated with the significant, but negative coefficients of correlation with the tumor pathological stage, tumor stages according to the Astler-Coller classification, and metastases in the lymph nodes. The secretions of fucomucins and sialomucins are in a good and significant mutual relationship, only the secretion of sulfomucins is in a negative correlation in comparison to the other mucins.

Histochemical expression of mucins may be a useful prognostic indicator of the progression of colorectal carcinoma.

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Introduction

The International Agency for Research on Carcinoma (IARC) has classified colorectal carcinoma among the three most frequent malignant neoplasms in human oncology (1). Alarming is the fact that the incidence and mortality in the last three decades have been rising steadily with an average annual growth rate of around 3% or with more than 400,000 newly diseased during a year. A striking increase in the incidence has been recorded in transition countries and in Australia/New Zealand, Europe and North America (2). In Serbia, colorectal carcinoma is the second leading cause of death with a mortality rate of 16.6/100.000, on the basis of which Serbia is ranked among the countries with high mortality (3).

Colorectal carcinoma is a multifactorial disease resulting from the interaction of hereditary factors and environmental factors (4, 5, 6). The risk for the development of colorectal carcinoma rises significantly after 40 years of age, and about 85% of colorectal carcinoma occurs in people older than 50 years of age (7). The risk of colorectal carcinoma has been estimated to be 15 times higher in people over 50 years of age than in people aged 20-49 years (8).

Based on molecular and cytogenetic studies, it has been established that colorectal carcinoma develops in one of two genetic pathways. The first is the pathway of chromosomal instability or the APC/β-catenin pathway, known as the "adenoma-carcinoma" sequence, and the second is the pathway of microsatellite instability or correction of the wrongly paired DNA (9). During colorectal carcinogenesis, in addition to the uncontrolled cell proliferation and accelerated angiogenesis, alterations in the structure and/or quantity of epithelial mucins with subsequent changes in the mucus protective function and cell signal transduction disorders occur (10, 11). These changes in the expression of mucins or glycosylation affect cell growth, differentiation, transformation, adhesion, invasiveness, and immune control of the tumor (12).

Aim of the study

The aim of our study was to examine the histochemical expression of epithelial mucins in relation to the clinical characteristics of colorectal carcinoma.

Materials and methods

Patients and samples

The study included 75 patients with colorectal adenocarcinoma, who underwent the surgical resection of the tumor in the Center for Abdominal Surgery of the Clinical Center of Montenegro (KCCG). In the Institute of Pathology of CCCG, from each operative resection, depending on the size of the tumor, 5-15 biopsies were taken, including 2-3 biopsies of the surrounding healthy tissue of the colon. After the fixation in a 4% buffered formaldehyde solution, the biopsy material was routinely processed, molded into paraffin blocks and archived.

Tumors tissue samples of colorectal carcinoma made up the study (experimental) group (n = 75). The control group (n = 75) consisted of operative biopsies of adjacent non-tumor tissue of the colon, which were taken from the operative preparation according to the protocol.

This research was carried out in conjunction with the Helsinki Declaration and the World Health Organization's Recommendations for experiments on human material. The consent of the supervisory Ethics Committee exists for the research.

Histopathology and mucin histochemistry

Serial cuts, 3-4 μm thick, have been made from paraffin blocks of all resected tumors and regional lymph nodes, on which routine Hematoxylin-Eosin method, for histopathological verification of lesions, histochemical AB-PAS pH 2.5 (Alcian Blue-Periodic Acid Schiff) method, for the differentiation of neutral from acidic mucins, and histochemical HID-AB pH 2.5 (High Iron Diamine-Alcian Blue) technique, for the differentiation of weak acidic, unsulphated sialomucins from strong acidic, sulphated-sulfomucins, was applied.

Histochemical expression of epithelial mucins has been quantified according to the following scale: - (minus), mucins asecretion; +/- (plus minus), trace secretion of mucins; + (1 plus), moderate secre-tion of mucins; ++ (2 pluses), strongly expressed secretion (hypersecretion) of mucins.

Statistical analysis

For the statistical analysis of the obtained results, the statistical software package SPSS (version 13.0) has been used. The χ^2-test (chi-squared test), Mann-Whitney U test, Kruskal-Wallis test and Student's t-test have been used to analyze the significance of the differences between parametric and non-parametric features between and within the groups. Then, the Kolmogorov-Smirnov test for the normality of distribution, the univariate statistical analysis, the correlation analysis (Spearman's coefficient of rank correlation, Pearson's correlation coefficient for parametric features) have been used. Significance testing has been performed at $p < 0.05$.

Results

In the examined sample of patients, in whom the colorectal carcinoma was removed, there were 45 men (60%) and 30 women (40%). There is no statistically significant difference in the presence of colorectal carcinoma in relation to the gender of the patients, at the adopted level of reliability $p < 0.05$ (χ^2-test = 3.000; p = 0.083 > 0.05).

There was also no statistically significant difference between the groups, by the age of the patients (Mann-Whitney U test = 569.500; p = 0.232). Male patients had an average age of 65.9 ± 10.8 years and women 60.8 ± 14.5 years, which was not a significant difference (Student's t-test = 1.622; p = 0.111).

In the examined sample of patients, the most frequent localisation of the carcinoma was in the rectum. The frequency of this localisation with 65.3% of the cases was highly statistically significant (χ^2-test = 7.053; p = 0.008), compared to all other localisations in the colon.

There was no statistically significant difference in the distribution of colorectal carcinomas with respect to the macroscopic type of the tumor (from 26.7% to 37.3%), but it was noticed that the most commonly occurring type was the ulceroinfil-trative type (37.2% of the cases).

The greatest number of the examined cases (74.7%) of colorectal adenocarcinoma were diagnosed in the third stage according to the pathological classification (pT3), which significantly differed from the number of cases diagnosed as stage pT2 (16%). Colorectal carcinoma rarely occurs in the lowest and highest pT stage (2.7% and 6.7% of the cases).

The distribution of the examined colorectal carcinoma in relation to the Astler-Coller classification showed the lowest frequency of tumors in the C1 stage (one case, 1.3%) and the D stage (7 patients, 9.3%). This frequency was followed by the

B1 stage (11 cases, 14.7%). The highest frequency of patients was in the B2 stage (25 patients, 33.3%) and the C2 stage (31 cases, 41.3%).

Metastases in lymph nodes were found in 39 patients (52%), while in 36 patients (48%) metastases were not found in the lymph nodes. Metastatic deposits in 1-3 lymph nodes were found in 22 patients (29.3%), deposits in 4-6 lymph nodes were found in 8 patients (10.7%), and in 9 patients (12%) deposits were present in more than 7 lymph nodes (Graph 2). Distant metastases were found in 10.7% of the patients.

By examining the histochemical expression of neutral mucins (fucomucins) in the non-tumor tissue, in 97.3% of the cases, their complete asecretion was noticed, which was an obvious significance that did not need to be statistically proven. In the colorectal adenocarcinoma tissue of 48% of the cases, the fucomucine asecretion was verified, in

45.3% of the cases there was a trace secretion present, and in 6.7% of the cases, a moderate secretion of neutral mucins was found (Graph 1).

In the control group (non-tumor tissue), in a significant number of cases, the most common finding was the trace secretion of sialomucins (68%; χ^2-test = 9.720; p = 0.002). The frequency of asecretion (18.7%) or moderate secretion (12%) of sialomucins in this group, was significantly less common and without significant statistical difference. The occurrence of sialomucin hypersecretion in non-tumor surrounding tissue was extremely rare (1.3% of the cases). In contrast to this distribution, in colorectal adenocarcinoma of 42.7% of the cases hypersecretion of sialomucins was found (Figure 1), moderate secretion in 45.3%, trace secretion in 12% of the cases, and in no case the asecretion of sialomucins was found (Graph 2).

FUCOMUCINS

■ no secretion   ■ +/-   ■ (+)
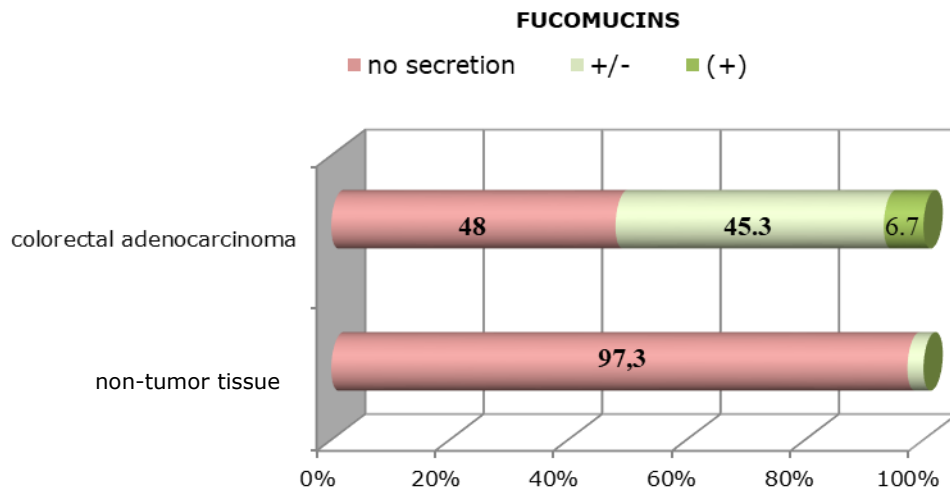
Graph 1. Distribution of neutral – fucomucins in colorectal carcinoma and adjacentnon-tumoral tissue

SIALOMUCINS

■ no secretion   ■ +/-   ■ (+)   ■ (++)
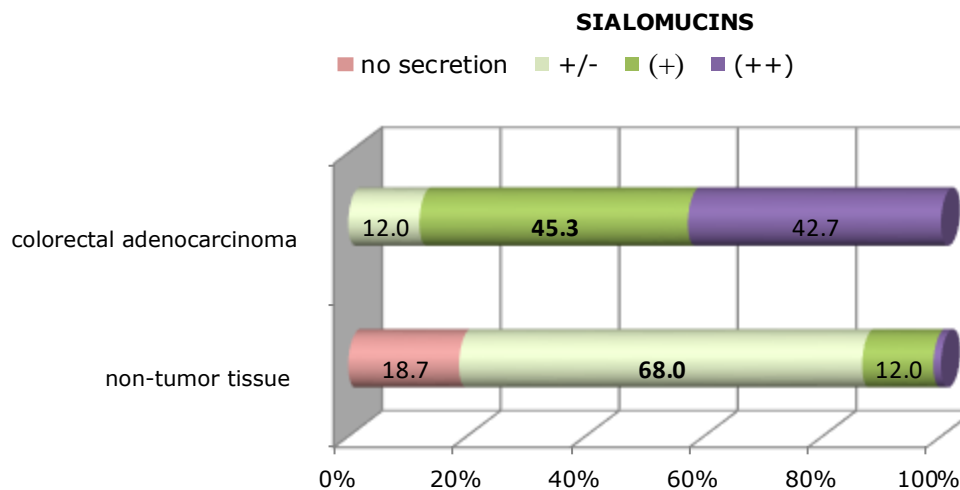
Graph 2. Distribution of poorly acidic – sialomucins in colorectal carcinoma and adjacent non-tumor tissue

SULPHOMUCINS

■ no secretion　■ +/-　■ (+)　■ (++)
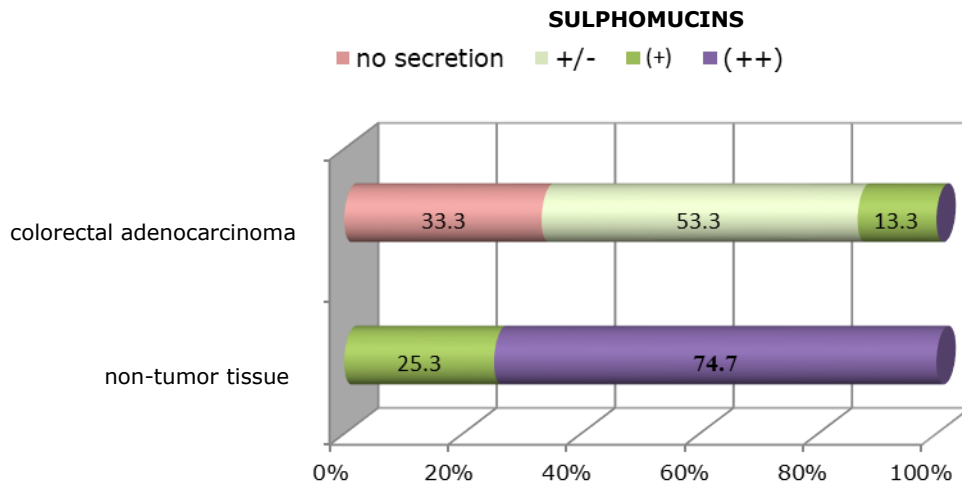
Graph 3. Distribution of highly acidic – sulfomucins in colorectal carcinoma and adjacent non-tumor tissue
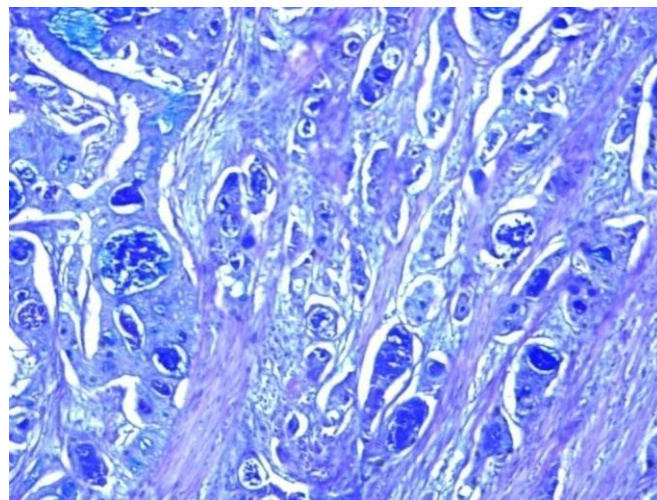

Figure 1. Pronounced histochemical expression of sialomucins in colorectal carcinoma
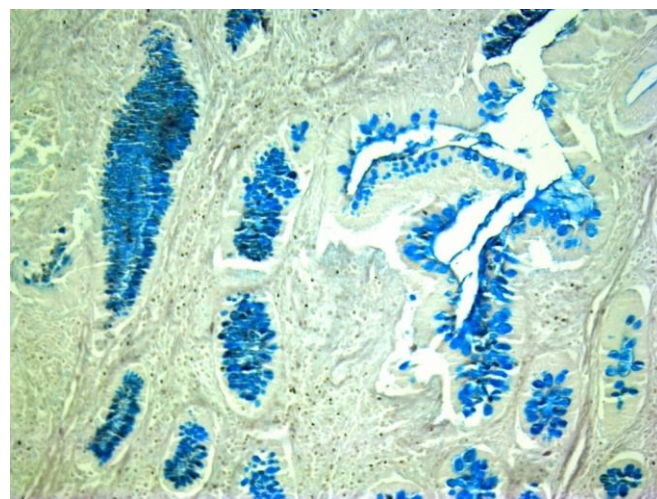

Figure2. Excessive sulfomucins reduction (black) and hypersecretion of sialomucins in colorectal carcinoma (HID-AB x200).

By analyzing the non-tumor tissue, significant hypersecretion (74.7%) of strong acidic sulphomucins was observed, which is a statistical significance that should not be specifically proven. Only in about 25.3% of the cases a moderate secretion was found, while trace secretion and asecretion of sulphomucins were not detected in this group of subjects. Contrary to this finding, in the colorectal adenocarcinoma group, hypersecretion of sulphomu-cins was not identified in any case, and moderate secretion was a significantly rare occurrence (13.3%) compared to the remaining cases. In most of the remaining cases, the trace secretion of sulphomucins (53.3% of the cases) (Figure 2), or the complete asecretion of mucins (33.3% of the cases) was found (Graph 3).

The connection of the mucin secretion with the clinical parameters of colorectal carcinoma

By examining the mucin secretion in relation to the gender and age of patients and by testing the significance (Mann-Whitney U test), no statistically significant differences were found in either parameter (fuco-, sialo-, and sulphomucins) between the comparable gender and age groups.

No statistically significant difference in the expression of mucins (fuco-, sial- and sulphomucins) (Kruskal-Wallis test; p = 0.212 to p = 0.833) was found compared to the macroscopic type of tumor.

Mucin expression in relation to colorectal carcinoma localisation also did not show statistically significant differences (Kruskal-Wallis test; p = 0.212 to p = 0.833).

The distribution of all the analysed mucins in relation to the pathological stage of the tumor (pT) showed a change in the secretion from the second (pT2) to the third (pT3) pathological stage in the following manner: the secretion of neutral-fucomucins and weak acidic sialomucins increased significantly (p = 0.008 and p = 0.001) and of strong acidic-sulphomucins decreased significantly (p = 0.003). The described mucin secretion trend was also observed in the highest pathological stage of the carcinoma, but due to the low number of data (5 cases), was not the subject of statistical comparison (Table 1).

Mucin expression in relation to the Astler-Coller tumor stage is shown in Table 2. It was noticed that mucin secretion, picturesquely divided the stages according to the Astler Coller classification into two parts, wherein the mucine secretion parameters were arranged in a similar manner in stages B1 and B2 versus stages C1 to D. It was also observed that the expressions of mucins within the B1 and B2 groups, but also within the C1 to D groups, were statistically indifferent (Mann-Whitney U test for B1-B2; p = 0.074 to p = 0.839 and C1 to D; p = 0.053 to p = 0.658). However, when the whole group B1-B2 was compared with the C1-D group, a highly significant difference in the expression of mucins (p < 0.0001) was obtained.

The trend of the distribution of neutral-fucomucins was such that at the B1 and B2 stage the frequency of the cases with increased secretion increased, but it was still similar. However, it was noticed that there was no significant difference be-

tween the high frequency of the cases without the secretion of neutral mucins in stage B1 (81.8%) and 64% of the cases in stage B2. A significant increase in trace fucomucins occured in C2 stage (67.7%) and continued in stage D with the occurrence of cases with moderate secretion of this mucin (42.9%). The trend of the increase of the secretion of neutral mucins followed an increase in the Astler Coller stage (χ^2-test = 5.444; p = 0.020).

By examining the expression of weak acidic-sialomucins in relation to the Astler Coller Stages, it was observed that the trace presence of this mucin in the B1 and B2 stage (36.4% and 12%) linearly increasesed by the intensity of the secretion up to the hypersecretion that prevailed in the C2 stage and especially in the D stage (61.3% and 71.4% of the cases, respectively), while C2 and D stage trace secretion was extremely rare (6.5% and 0% respectively).

Asecretion of the strong acidic-sulphomucins was a rare occurrence in stages B1 and B2 (0% and 16%), as opposed to C2 and D stages, where there was no secretion of this mucins in 58.1% and 42.9% of the cases. The trace secretion of sulphomucins did not differ significantly relative to the tumor stage (χ^2-test = 0.900; p = 0.343). Moderate secretion of strong acidic mucins was a more frequent occurrence in stage B1 (45.5%), while it was significantly rarer in higher stages (16% in B2, 0% in C2 and 14.3% in D). (χ^2-test = 11.560; p = 0.001).

Examination of mucin secretion in relation to the occurrence of metastases in the lymph nodes and in relation to the number of affected lymph nodes showed that groups, classified by metastases in the lymph nodes, were significantly different only in relation to the cases without metastases (Table 3).

A group of the cases without metastatic deposits was significantly different in the distribution of mucins in relation to the each individual group with a presence of deposits in the lymph nodes. (Kruskal-Wallis test; p < 0.001 to p < 0.007). When the distribution of mucin secretion was considered within the groups without metastases, it was noticed that the absence of deposits in the lymph nodes was approaching the distribution of mucins according to the characteristics of normal colorectal tissue because it did not deviate much from the normal tissue.

The occurrence of metastases in the lymph nodes significantly changed the secretion of the mucins, increasing the intensity of fucomucins and sialomucins secretion with the increase in the number of affected nodes, while the secretion of sulphomucins was decreasing. However, these changes in the secretion of mucins did not show a significant difference between the groups with deposits. That is, the groups of lymph nodes with metastatic deposites did not significantly differ between themselves in relation to mucin expression (Kruskal-Wallis test; p = 0.119 to p = 0.755).

Table 1. Distribution of mucin secretion in relation to the pathological stage of the tumor

Parameters	Pathological stage of the tumor							
	Stage I		Stage II		Stage III		Stage IV	
FUCOMUCINS	n	%	n	%	n	%	n	%
No secretion	1	50.0	10	**83.3**	23	41.1	2	40.0
+/-	0	0.0	2	16.7	29	51.8	3	60.0
(+)	1	50.0	0	0.0	4	7.1	0	0.0
SIALOMUCINS	n	%	n	%	n	%	n	%
+/-	0	0.0	4	33.3	5	8.9	0	0.0
(+)	1	50.0	8	**66.7**	23	41.1	2	40.0
(++)	1	50.0	0	0.0	28	50.0	3	60.0
SULFOMUCINS	n	%	n	%	n	%	n	%
No secretion	0	0.0	0	0.0	21	37.5	4	80.0
+/-	1	50.0	8	**66.7**	30	53.6	1	20.0
(+)	1	50.0	4	33.3	5	8.9	0	0.0

Table 2. Distribution of mucin secretion according to Astler-Coller tumor classification

Parameters	Astler-Collerstage of the tumor									
	B1		B2		C1		C2		D	
FUCOMUCINS	n	%	n	%	n	%	n	%	n	%
No secretion	9	**81.8**	16	**64.0**	0	0.0	10	32.3	1	14.3
+/-	1	9.1	8	32.0	1	100.0	21	**67.7**	3	**42.9**
(+)	1	9.1	1	4.0	0	0.0	0	0.0	3	**42.9**
SIALOMUCINS	n	%	n	%	n	%	n	%	n	%
+/-	4	**36.4**	3	**12.0**	0	0.0	2	6.5	0	0.0
(+)	6	**54.5**	16	**64.0**	0	0.0	10	**32.3**	2	**28.6**
(++)	1	9.1	6	24.0	1	100.0	19	**61.3**	5	**71.4**
SULFOMUCINS	n	%	n	%	n	%	n	%	n	%
No secretion	0	0.0	4	16.0	0	0.0	18	**58.1**	3	**42.9**
+/-	6	**54.5**	17	**68.0**	1	100.0	13	**41.9**	3	**42.9**
(+)	5	**45.5**	4	**16.0**	0	0.0	0	0.0	1	14.3

Table 3. Distribution of mucin secretion in relation to metastases in the lymph nodes

Parameters	Metastases in the lymph nodes							
	Without deposits in lymph nodes		Deposits in 1-3 lymph nodes		Deposits in 4-6 lymph nodes		Deposits in more than 7 lymph nodes	
FUCOMUCINS	n	%	n	%	n	%	n	%
No secretion	25	69.4	8	36.4	1	12.5	2	22.2
+/-	9	25.0	12	54.5	6	75.0	7	77.8
(+)	2	5.6	2	9.1	1	12.5	0	0.0
SIALOMUCINS	n	%	n	%	n	%	n	%
+/-	7	19.4	2	9.1	0	0.0	0	0.0
(+)	22	61.1	5	22.7	4	50.0	3	33.3
(++)	7	19.4	15	68.2	4	50.0	6	66.7
SULFOMUCINS	n	%	n	%	n	%	n	%
No secretion	4	11.1	11	50.0	3	37.5	7	77.8
+/-	23	63.9	11	50.0	4	50.0	2	22.2
(+)	9	25.0	0	0.0	1	12.5	0	0.0

Table 4. Correlation analysis - interdependence of parameters - significance and degree of dependence

		Fucomucins	Sialomucins	Sulfomucins	pT stage	AC stage	Metastases in the lymph nodes	Distant metastases
Fucomucins	cc	1.00	**0.37***	**-0.34***	0.22	**0.43***	**0.39***	**0.33***
	p	.	0.00	0.00	0.06	0.00	0.00	0.00
Sialomucins	cc	**0.37***	1.00	**-0.44***	0.35	**0.47***	**0.42***	0.23
	p	0.00	.	0.00	0.00	0.00	0.00	0.04
Sulfomucins	cc	**-0.34***	**-0.44***	1.00	**-0.45***	**-0.50***	**-0.50***	-0.10
	p	0.00	0.00	.	0.00	0.00	0.00	0.39
pT stage	cc	0.22	**0.35***	**-0.45***	1.00	**0.60***	**0.44***	0.17
	p	0.06	0.00	0.00	.	0.00	0.00	0.13
AC stage	cc	**0.43***	**0.47***	**-0.50***	**0.60***	1.00	**0.84***	**0.54***
	p	0.00	0.00	0.00	0.00	.	0.00	0.00
Metastases in the lymph nodes	cc	**0.39***	**0.42***	**-0.50***	**0.44***	**0.84***	1.00	0.23
	p	0.00	0.00	0.00	0.00	0.00	.	0.05
Distant metastases	cc	**0.33***	**0.23***	-0.10	0.17	**0.54***	0.23	1.00
	p	0.00	0.04	0.39	0.13	0.00	0.05	.

* Significant $p < 0.05$, cc - Spearman's correlation coefficient

Mucin secretion significantly differed only in relation to the metastases in the lymph nodes compared to the cases without the metastases, and a significant difference in the secretion of mucins between the cases with metastatic deposits and the cases without deposits was observed.

In a preliminary analysis, the cross-examination of the tested parameters indicated a high significance relationships in certain interrelated relationships. The correct measure of their connection was shown by a correlation analysis where the significance of that relationship was proved by the significance of the correlation coefficient, and the strength of the connection by its size. In Table 4, all the parameters that were the subject of the analysis in this study are shown through the correlation coefficient (cc) with its statistical significance (p).

A group of the cases without metastatic deposits was significantly different in the distribution of mucins in relation to the each individual group with a presence of deposits in the lymph nodes. (Kruskal-Wallis test; $p < 0.001$ to $p < 0.007$). When the distribution of mucin secretion was considered within the groups without metastases, it was noticed that the absence of deposits in the lymph nodes was approaching the distribution of mucins according to the characteristics of normal colorectal tissue because it did not deviate much from the normal tissue.

The occurrence of metastases in the lymph nodes significantly changed the secretion of the mucins, increasing the intensity of fucomucins and sialomucins secretion with the increase in the number of affected nodes, while the secretion of sulphomucins was decreasing. However, these changes in the secretion of mucins did not show a significant difference between the groups with deposits. That is, the

groups of lymph nodes with metastatic deposites did not significantly differ between themselves in relation to mucin expression (Kruskal-Wallis test; $p = 0.119$ to $p = 0.755$).

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Fucomucins were in a good, positive, and significant correlation with the Astler-Coller tumor stage (cc = 0.43), with the metastases in the lymph nodes (cc = 0.39) and with the distant metastases (cc = 0.33).

Sialomucins were in an even stronger, significant relationship with the Astler-Coller tumor stage (cc = 0.47), with metastases in the lymph nodes (cc = 0.42) and with the distant metastases (cc = 0.23). In contrast to neutral mucins, sialomucins were with the good, positive and significant correlation coefficient (cc = 0,35) associated with the pathological stage of the tumor.

Sulphomucins were with the good, significant, but negative correlation coefficients associated with

the pathological stage of the tumor (cc =-0.45), with the Astler-Coller tumor stage classification (cc =-0.50), and with lymph node metastases (cc =-0.50).

The secretions of neutral and weak acidic mucins were in a good and significant mutual relationship (cc = 0.37), only the secretion of sulphomucins was in the negative correlation with the other mucins (cc =-0.34, cc =-0.44).

Discussion

Mucus (phlegm) is a viscous colloidal substance produced by specialised goblet cells, and mucocytes of mucous or mixed seromucous glands. In the form of a thin layer, the water-insoluble gel covers the epithelium of the tubular organs and separates it from the lumen content. The mucus covers the mucous membranes that directly or indirectly come into contact with the outer environment, where it primarily has a protective role (13). Mucus contains water, antiseptic enzymes, immunoglobulins, inorganic salts, proteins and glycoprotein macromolecules-mucins (14). The most present component of mucus are the mucins that are responsible for the biochemical and biophysical properties of the mucus. They are divided into two large groups: membrane and secretory mucins (15).

Mucins represent a selective molecular barrier on the epithelial surface, provide protection of the cell surface and participate in morphogenetic cell transduction (16, 17). Based on the histochemical characteristics, they are divided into neutral fucomucins, weak acidic or sialomucins and strong acidicsulphomucins. Fucomucins contain N-acetyl derivates of hexosamine, D-galactose and L-fructose. Fukomucins do not have acid groups, so they are stained using PAS (Periodic Acid Schiff) method (12). Sialomucins contain hexosamine, glucuronic and sialic acid, and sulfomucins besides hexosamine and uronic acid also contain sulphate groups (11, 18, 19). Specific molecular structure contributes to the specialised role of membrane mucins through which the information about the conditions in the external environment is transferred into the epithelial cells, and so they serve as receptors and sensors on the cell surface. They carry signals to extreme stimuli, thus leading to a coordinated cell response that includes differentiation, apoptosis, and secretion of specific products (11, 17).

The secretion of mucus glycoproteins is a slow but continuous process, which maintains a thin layer on the surface of the mucous membrane. Accelerated secretion occurs under the influence of various stimuli, physical or chemical factors. Mucins that are released from the cytoplasmic vesicles bound themselves to water and in the form of gel coat cover the mucous membrane (20).

In healthy colon mucosa the secretion of strong acidic sulphated mucins dominates, while neutral and weak acidic-sialomucins are mostly present in traces (21). In accordance with this, we have verified the pronounced and moderate secretion of strong acidic sulphomucins and a complete absence or trace secretion of neutral and weak acidicsialo-

mucins in the non-tumor tissue, in the vicinity of the carcinomas of all of our patients.

The first significant aberrations of the mucin secretion have been observed in the second and third pT tumor stages, when the secretion of neutral and weak acidic mucins significantly increases, and the secretion of strong acidic sulphomucins significantly decreases. In relation to the Astler-Coller classification, a slight increase in the secretion of neutral mucins is observed already in B1 to B2 stages, while a significant increase in the secretion of fucomucins occurs in the C2 stage and continues in D stage with the occurence of moderate secretion. A slight increase in the secretion of weak acidic mucins has also been observed in the stages B1-B2, and then the secretion intensity increases linearly all the way to the hypesecretion of sialomucins, which dominates in the C2 stage, and especially in the D stage. In the C2 and D stages, a high significant absence of secretion (asecretion) or a trace secretion of strong acidicsulphomucins is observed. It has been observed in the literature that an increased quantity of sialomucins, besides in the tissue of the colorectal carcinoma, is also found in the transitional (transient) zone that surrounds the primary carcinoma. The same authors have claimed that the occurrence of sialomucins hypersecretion in this zone has been associated with poor prognosis and that the morphological and mucin components of the transitional zone are the prognostic factors for the progression of colorectal carcinoma (22).

In our research, we have observed that with the occurrence of metastases in the lymph nodes and the increase in the number of affected lymph nodes the secretion of neutral fucomucins and weak acidicsialomucins significantly increases, while the secretion of strong acidic-sulphomucins decreases. It is thought that increased sialinisation stimulates the migration of tumor cells through the extracellular matrix (23, 24), and it has been emphasized in numerous studies that the expression of sialomucins affects the processes of invasion and metastasis of the tumor (25).

Hypersecretion of sialomucins with the reduction of sulfomucins has been observed in adenoma-carcinoma sequences in humans (26) and in the "aberrant cryptal focus" (ACF) in rats (27). If ACF is known to be the earliest known precancerous lesion, then it is clear that the occurrence of the hypersecretion of sialomucins is an early event in colorectal carcinogenesis.

Aberrations in the secretion of mucins simultaneously with the disorder of the protective function of mucus in the intestines also cause a disturbance of the signal transduction in which the mucin molecules are involved. This triggers the inflammatory processes in the intestinal mucous membrane and predisposes the development of the carcinoma. Chronic inflammation in the intestines leads to a change in glycosylation and causes deregulation of the mucins, and it is therefore thought that precisely this aberrant mucin expression represents a link between inflammation and carcinoma (28).

The mechanism by which the mucins participate in the pathogenesis of the carcinoma is not fully

clear, but there is a presumption that the tumor growth area is hypoxic, acidic and full of proteases and other biologically active substances, and so the tumor tissue likely uses mucins for the configuration of the microenvironment during invasion, metastasis and growth under unfavorable conditions (18). Increased concentrations of mucin glycoproteins in serum are in correlation with an increased risk for tumor formation and poor prognosis, and therefore it is thought that excessive expression, inadequate expression, or the expression of aberrant mucin forms contributes to the pathogenesis of the carcinoma (14, 29).

In this study it has been noticed that mucin secretion does not depend on the demographic characteristics of the patients, on the localisation and on the macroscopic type of the tumor. The secretion of weak acidic sialomucins is associated with strong, significant and positive correlation coefficients with the pathological stage of the tumor, with the stages of the tumor according to the Astler-Coller classification and with metastases in the lymph nodes.

Sulphomucins are associated with significant, but negative correlation coefficients with the pathological stage of the tumor, with the stages of the tumor according to the Astler-Coller classification and with metastases in the lymph nodes.

Conclusion

In colorectal carcinoma, numerous alterations in the secretion of epithelial mucins occur, which are primarily manifested as a moderate to hypersecretion of sialomucins, trace or a moderate secretion of fucomucins and a trace or a complete asecretion of sulphomucins. Changes in the quality and quantity of mucins depend on the pathological stage of the tumor, on the Astler-Coller classification of the tumor stage, on the metastases in the lymph nodes and distant metastases, which indicates that the histochemical expression of mucins may be a useful prognostic indicator of colorectal carcinoma progression.

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KLINIČKI ZNAČAJ HISTOHEMIJSKE EKSPRESIJE MUCINA U KOLOREKTALNOM ADENOKARCINOMU

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Kolorektalni karcinom je najučestaliji maligni tumor gastrointestinalnog trakta. Tokom kolorektalne karcinogeneze, osim nekontrolisane proliferacije ćelija i ubrzane angiogeneze nastaju i alteracije u strukturi i/ili količini epitelnih mucina, pa je cilj našeg rada ispitivanje histohemijske ekspresije mucina u odnosu na klinčke karakteristike kolorektalnog karcinoma.

Za ispitivanje je korišćen biopsijski material 75 bolesnika operisanih od kolorektalnog karcinoma, koji je rutinski obrađivan i kalupljen u parafin. Na rezovima debljine od 3 µm do 4 µm, primenjene su rutinska H&E i histohemijske AB-PAS pH 2,5 i HID-AB metode. Za statističku analizu korišćen je statistički programski paket SPSS (verzija 13).

U kolorektalnom karcinomu nastaju alteracije mucina koje se manifestuju kao sekrecija u tragu do umerene sekrecije fukomucina, umerene do hipersekrecije sijalomucina i sekrecije u tragu do potpune asekrecije sulfomucina. Sekrecija fukomucina i sijalomucina je jakim, signifikantnim i pozitivnim koeficijentima korelacije povezana sa stadijumima tumora po Astler-Coller klasifikaciji, sa metastazama u limfnim žlezdama i sa udaljenim metastazama. Za razliku od fukomucina, sijalomucini su jakim, pozitivnim, značajnim korelacionim koeficijentom povezani i sa patološkim stadijumom tumora. Sulfomucini su signifikantnim, malim negativnim koeficijentima korelacije povezani sa patološkim stadijumom tumora, sa stadijumima tumora po Astler-Coller klasifikaciji i sa metastazama u limfnim žlezdama. Sekrecije fukomucina i sijalomucina u dobroj su i značanoj uzajamnoj povezanosti, jedino je sekrecija sulfomucina u negativnoj korelaciji prema ostalim mucinima.

Histohemijska ekspresija mucina može biti koristan prognostički pokazatelj progresije kolorektaknog karcinoma.

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Ključne reči: *kolorektalni karcinom, epitelni mucini, histohemija*