

**UNIVERSITY OF MEDICINE AND PHARMACY
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**PHD THESIS
ABSTRACT**

Colorectal carcinomas with excessive production of mucin.
A clinico-pathological and immunohistochemical study.

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THESIS CONTENTS

INTRODUCTION	4
GENERALITIES – FIELD KNOWLEDGE LEVEL	
CHAPTER I: Etiology and epidemiology of colorectal carcinomas	7
I.A. Etiology of colorectal carcinomas	7
I.B. Epidemiology of colorectal carcinomas	21
CHAPTER II: Colic carcinogenesis	26
II.A. Genetic basis of colorectal carcinogenesis	26
II.A.1 Genetic mechanisms of colorectal carcinogenesis	27
II.A.2. Morphological aspects of colorectal carcinogenesis	34
CHAPTER III : Systems of classification of colorectal carcinomas	43
OBJECTIVES OF THE STUDY	51
SPECIAL SECTION - PERSONAL CONTRIBUTIONS	
CHAPTER IV. Material and Methods	53
IV.A. The studied material	53
IV.B. Methods	55
CHAPTER V: Results	62
V.A.Histopathological study of colorectal carcinomas with excessive mucin	62
V.A.1. Clinical parameters of colorectal carcinomas with excess of mucin	64
V.A.2. Histopathological parameters	66
V.A.2.1. Associated precancerous lesions	71
VA2.2. Colonic mucosal dysplasia	74
V.A.2.3. Multiple primary colorectal malignancies	77
V.A.3. Histopathological parameters with prognostic value	77
V.A.3.1. Vascular invasion	78
V.A.3.2. Perineural invasion	80

V.A.3.3. Tumor implants	80
V.A.3.4. Longitudinal and circumferential resection margins	81
V.A.4. Clinical and pathologic staging parameters	81
V.A.4.1. Tumor progression and peri-colorectal dissemination (pT stage)	81
V.A.4.2. Locoregional lymph nodes status (pN stage)	86
V.A.4.3. Metastases in remote anatomical sites (pM stage)	89
V.A.5. Clinico- pathological staging	90
V.B. Immunohistochemical study	93
V.B.1. Epithelial differentiation markers	93
V.B.2. Cell proliferation markers	112
V.B.3. Cell apoptosis markers	116
V.B.4. Tumor angiogenesis markers	119
V.B.5. Extracellular matrix degradation markers	122
V.B.6. Markers involved in tumor's immunological surveillance	125
V.B.7. Markers to highlight tumor budding	128
CHAPTER VI : Discussion	133
VI.A. Histopathological study of colorectal carcinomas with excess mucus	133
VI.B. Immunohistochemical study of colorectal carcinomas with excess mucus	148
Chapter VII : Conclusions	171
REFERENCES	179

KEYWORDS : colorectal carcinoma, mucinous carcinoma, " signet ring " colorectal carcinoma, adenoma - adenocarcinoma sequence, adenoma, staging, prognostic factors, metastasis, immunohistochemistry, budding .

INTRODUCTION

Colorectal developed carcinomas are the most common epithelial localization for the malignancies of digestive area, while providing the fourth cause of cancer death worldwide. Mucin production is a relatively frequent histologic appearance of colorectal carcinomas, and when it becomes abundant, it forms distinct histopathological entities, classified according to the AJCC as the mucinous (colloid) subtype of adenocarcinomas, and " signet ring " cell subtype. Colorectal carcinomas with excessive production of mucin have a complex pathogenesis, multistage, debuting in normal colorectal mucosa, through the classic adenoma - adenocarcinoma sequence. This evolutionary process involves a number of factors, some related to the patient's particularities: adenomas, familial polyposis, idiopathic inflammatory disease (IBD), colonic mucosal dysplasia, other external factors being related to exposure to pollutants, lack of physical activity, diet (with a role in changing the intestinal flora and potential carcinogenicity).

Although heredity plays an important role, in the genesis of colorectal cancer occur multiple somatic genetic alterations, some of tumor suppressor genes and genes involved in cell stability. These genetic alterations find their counterpart in changes in the specific antigens revealed by immunohistochemistry: mucins, growth factors (eg growth factors of tumor vessels: VEGF), tumor suppressor genes (eg p53), in the "dedifferentiation" at the tumor invasion front (budding phenomenon), in the expression of the immune mechanism of tumor surveillance (CD8).

The aim of this paper is the thoroughgoing study, in terms of etiopathogenesis of colorectal carcinomas with excessive production of mucin, histopathological aspects individualizing them from other colorectal carcinomas, with emphasis on the prognostic value and predictive value of immunohistochemical features .

OBJECTIVES

This study, performed retrospectively on a group of 82 patients with the diagnosis of colorectal carcinoma with excessive production of mucin, involved the following objectives:

- Increasing the range of knowledge of histopathological and immunohistochemical features of colorectal carcinoma with excessive mucin, relative to tumor phenotype, to expand the knowing of carcinogenic mechanisms.
- Identification of morphological parameters and precursor lesions in order to detect as early as possible and to identify possible molecular targets.
- Completion of morphological parameters of diagnostic assessment.
- Identifying mechanisms and markers involved in colorectal excess mucin secreting cancer invasion and aggression.
- Identifying the specific markers of prognosis and metastatic potential of these cancers.
- Identifying significant statistical correlation with the goal of revealing the prognostic value factors.

MATERIAL AND METHODS

The material studied was collected from a total of 82 patients with the diagnosis of colorectal carcinoma with excessive mucin, established following surgical treatment in the clinics of the Emergency County Hospital Craiova, in a period from January 2008 to December 2011. The number of cases with this diagnosis showed an upward trend, the lowest number of cases was in 2008 (16 surgical resections) and the highest in 2010 (26 cases).

The methods, described in detail in this paper, followed the analysis of the cases studied, for the evaluation of clinical and epidemiological parameters, morphological preneoplastic lesions and possible correlations between these histopathological and molecular parameters investigated. Through the immunohistochemical study of excessive mucin secreting colorectal carcinomas, we followed tumor aggressiveness assessment, evaluating the expression of: 1) markers for highlighting epithelial differentiation (MUC1, MUC2,

MUC5AC, CDX2) and the immune profile MUC1/MUC2 and CK7/CK20, 2) markers of proliferation (Ki -67) and apoptosis (p53); 3) tumor angiogenesis markers (VEGF); 4) markers to highlight the immunological mechanism with the role of surveillance of tumor proliferation (CD8); 5) markers to highlight the tumor stroma (MMP9); 6) markers for assessing tumor budding (AE1/AE3). We used morphometric analysis to quantify the density of outbreaks of "tumor budding" to guide the local invasive capacity and prognosis of colorectal carcinoma that composed the study group. Statistical analysis of data intended to evaluate correlations between clinico-pathological and immunohistochemical parameters by calculating correlation coefficients (p) .

RESULTS

The histopathological and immunohistochemical study of our cases was performed in the Laboratory of Pathology of the Emergency County Hospital Craiova. The immunohistochemical study included the Department of Pathology of the University of Medicine and Pharmacy Craiova.

Cases with diagnosis of colorectal adenocarcinoma with excessive secretion of mucin were from patients whose age ranged within limits between the third and eighth decades of life, most cases belonging to the 7th decade (34 cases). The cases represented by males were more noticeable (58.53 % of mucinous adenocarcinomas). Compared to the three main anatomical sites arbitrarily set, the location of colorectal cancers with excess production of mucin showed preponderant number of cases (40 cases -48 , 78 % of the total) located in the right colon compared to the other two anatomical sites groups . The colorectal presence of risk lesions could be identified by studying the associated adenomas (25 cases, the percentage 30,48 %) and adenomas included in the periphery of the tumor – waste adenomas (20 cases, 24.39 % percentage).

The low-grade and high-grade dysplasia was present in most of adenomas associated with mucinous adenocarcinoma - 19 cases (76 % of the adenomas associated with this type of cancer), and all residual adenomas revealed, 20 patients (100 % in this group study) . Villous growth pattern and the tubulo - villous growth pattern provided the highest percentage of low grade and high-grade dysplasi, both in associated adenomas (8 cases representing 32% of adenomas associated with mucinous carcinoma) and in the residual adenoma type (all 20

cases in this category). The size of adenomas was also an important risk factor in the development of dysplasia, with numeric and percentage higher risk of dysplasia in adenomas greater than 1 cm in diameter. Vascular invasion was a prognostic parameter more common in non-mucinous adenocarcinomas (18.07%) rather than mucinous (10.97%) and also for tumors with excessive production of mucin, more frequent in tumors with the " signet ring " histology (14.28 %) than in mucinous tumors with extracellular secretion (10,66 %).

Perineural invasion was present both in 8% (6 cases) of colloid adenocarcinomas and in 28% (2 cases) of " signet ring " carcinomas that composed the study group.

Most (65 adenocarcinomas with excess mucin secretion) of the study group were cases that at the time of surgery, involved the whole intestinal wall and were classified as stage pT3 (79.26 % of total casuistry).

The absence of locoregional lymph node carcinoma metastasis (stage pN0) was observed in 37 cases of the 75 mucinous carcinomas with extracellular secretion (49.33 %), forming the largest category of this type of adenocarcinoma . pN1a and pN1b stages were established only for colloid adenocarcinomas of the study group: 6 cases pN1a (8% of colloid adenocarcinomas) and 9 cases of colloid adenocarcinomas pN1b (12%). pN2a stage was met both for colloid adenocarcinomas (6 cases , 8%) as well as for the "signet ring" secretion type (1 case, 14.28 %). Extensive involvement of lymph nodes (pN2b) was present in both histological types of mucinous secretion carcinoma.

Immunohistochemically, out of the 82 cases, MUC1 immunostaining was positive in 70 cases (85.3%), MUC2 in 85.3 % and phenotype MUC2 + / MUC1 + was the best represented numerically (69 carcinomas, 84.14 %). This phenotype was especially noted in the studied tumors with lymph node and distant metastases, highlighting their metastatic potential. Immunostaining of MUC5AC was detected in 23 cases (28% of the study group). Analysis of CDX2 expression in the 82 colorectal carcinomas with excessive mucin secretion revealed the presence of immunostaining in 69 cases of mucinous tumors (84.14 %) and the CK20 + / CK7 immune profile was the most common of the four types of immunofixation. These markers of epithelial differentiation outlined the immune profile of the studied colorectal carcinomas. Colorectal carcinomas (especially the colloid type) were more frequent tumors of moderate proliferative activity (IP ++). In the study group, p53 overexpression was given by 14/82 cases (17.07 %), and VEGF immunostaining was expressed in 51 cases (percentage 62.19 %). MMP- 9 was expressed with variable intensity in 60 cases (73.17 %) of

the cases studied, and in 72 cases (87.80 %) CD8 positive immunostaining was noticed. Analysis of tumor budding by using pan- cytokeratins AE1/AE3 immunostaining revealed the existence of this mechanism in a total of 52 cases (63.41 %).

DISCUSSION

Our study showed that mucinous histology of colorectal carcinoma seems to have no epidemiological implication on the age of the patients compared to different forms of non-mucinous colorectal cancer, so that in the group we now investigated, the maximum age of patients with more than 70 years, was close to the seventh decade, recorded as peak of incidence (for colorectal cancer) in the literature. Is noticed a dominance of the right colon excessive mucin secretion carcinoma cases encountered in women , however, the frequency was in favor of males both in the left colon and the rectum . This change in sex distribution is recorded in most studies. Note the random distribution throughout the colic, sometimes very close to the tumor, sometimes far away from it as well as a variable number of adenomatous polyps diagnosed concurrently with colorectal mucinous adenocarcinoma. The conclusion expressed by our study, as do the majority of the literature, is that adenomas are the most common pre-neoplastic lesions in the colon, while over 95% of colorectal carcinomas develop at the level of an adenoma. I noticed that there is a higher percentage of cases of non-mucinous adenocarcinomas with vascular invasion compared with mucinous colorectal tumors. Perineural invasion was also highlighted in our study, with studies highlighting that the association of perineural invasion with vascular invasion worsens prognosis. Is noticed the increased invasiveness of colorectal carcinomas with excessive production of mucin studied, most cases being diagnosed as pT3 and pT4 tumoral stages.

Development of colorectal carcinoma frequently associates qualitative and quantitative alterations of mucins expression. Such mucins expression pattern was expressed very heterogeneous in mucinous colorectal carcinomas (MUC1 is frequently expressed at a higher level than in normal intestinal mucos; MUC2 - diffusely expressed and MUC5AC with weak and focal immunoreactivity).

The tumor budding, examined in the light of the prognostic factor represented by lymphatic and vascular invasion of investigated colorectal carcinomas, revealed a high

intensity of the phenomenon of budding (≥ 10 areas / microscopic field) in a significant number of cases.

CONCLUSIONS

The histopathological and immunohistochemical study conducted in the period 2008-2011, on a number of 82 colorectal carcinomas with excessive production of mucin, was followed by these findings :

- The group of colorectal carcinomas with mucin secretion excess studied represented a percentage of 13.62 % of the total of 602 cases of colorectal carcinomas, with investigated casuistry more common in males and people over 70 years of age.
- Associated precancerous lesions (remote or tumor periphery included adenomas) were significantly correlated with mucinous histology of studied colorectal tumors.
- Histopathological parameters with prognostic value (vascular, lymphatic and perineural invasion, tumor implants and circumferential resection margin) revealed no statistically significant correlations ($p > 0.05$) with mucinous or non- mucinous types of colorectal carcinoma in the period studied from 2008 to 2011.
- For excess mucin secreting colorectal carcinoma, we believe that the expression of mucins (MUC1, MUC2 and MUC5AC), the expressed MUC2/MUC1 and CK20/CK7 phenotypes, nuclear transcription factor CDX2, and that of the oncoprotein p53, reveals the connection between colorectal carcinogenesis and tumor phenotype, while expression of the proliferation marker Ki -67, vascular growth factor (VEGF), the immune marker CD8 (cytotoxic T lymphocytes involved in the immune mechanism of tumor), in conjunction with the expression of MMP - 9 (important in the degradation of extracellular matrix) and tumor budding phenomenon, provide information about the biological behavior of studied tumors.