UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA

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EPITHELIAL-MESENCHIMAL TRANSITION IN COLONIC ADENOCARCINOMAS

SUMMARY

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CONTENTS

INTRODUCTION

KNOWLEDGE STAGE

CHAPTER I - Risk actors of colonic adenocarcinomas

CHAPTER II - Carcinogenic mechanisms in colonic adenocarcinomas

CHAPTER III - Epithelial-mezenchimal transition in colonic adenocarcinomas

PERSONAL RESEARCH

PURPOSE AND OBJECTIVES OF THE STUDY

CHAPTER IV - Material and methods

IV. 1. Studied material

IV. 2. Methods used in research

CHAPTER V – Results

V. 1. Histopathological Study of Colonic Adenocarcinomas

V. 2. Statistical correlation between histopathological parameters

V. 3. Immunohistochemical Study of Colonic Adenocarcinomas

V. 4. Statistical correlation between histopathological markers and immunohistochemical

CHAPTER VI – Discussions

VI. 1. Discussions on histopathological study of colonic adenocarcinomas

VI.2. Discussions on immunohistochemical study of colonic adenocarcinomas

CHAPTER VII – Conclusions

SELECTIVE BIBLIOGRAPHY

Introduction

Epitelial-mezenchimal tranzition (EMT) is involved both during embryonic development and in the physiological response to various aggressions [249]. The completion of TEM is signaled by the degradation of the basal membrane and the acquisition of a mezenchimal phenotype, characterized by migratory capacities, invasivness and increased resistance to apoptosis [86]. Thus, an alteration of intercellular junctions occurs with progressive loss of cellular polarity and reorganization of the cytoskeleton. Moreover, it decreases E-caderine expression, which maintains intercellular adhessions and the organization of the cytoskeleton, and the expression of N-caderine will increase under the action of the Twist, Snail, SLUG and ZEB1 factors [48].

The association of EMT with progression and prognosis of patients with CRC is controversial and further investigations are needed to identify and establish its role. The present study aims to assess the expression of markers involved in EMT(cellular adhesion markers, transcription factors, epithelial markers as well as mezenchimal markers), but also their association with investigated histopathological parameters. Taking into account the role of this process in cancer progression, the proteins involved in the conduct of EMT could provide a targeted therapeutic strategy with the aim of preventing invasion, metastasis and tumor recurrences.

Key words: adenocarcinomas of the colon,histhological type, grade of differentiation, E-caderine, N-Caderine, P-Caderine, Snail, Slug, Twist, ZEB1, AE1/AE3, Fibronectin, Vimentine.

KNOWLEDGE STAGE

CHAPTER I. Risk factors of colonic adenocarcinomas

In this chapter data from published literature was described in regards to age and sex group distribution. Also, protecting and predisposing factors for colonic adenocarcinoma were described.

CHAPTER II. Carcinogenic mechanisms in colonic adenocarcinomas

In this section, pathogenic pathways in colorectal carcinogenesis were presented, as well as genetic predisposition and non-hereditary predisposing factors.

CHAPTER III. Epithelial-mesenchimal transition in colonic adenocarcinomas

This section includes recent data regarding the aspects of epithelial-mesenchimal transition, as well as the primary mechanisms that induce this process.

PERSONAL RESEARCH

PURPOSE AND OBJECTIVES OF THE STUDY

The study carried out proposed a complete and detailed evaluation of colorectal carcinogenesis, especially colonic adenocarcinomas, as well as identification factor implicated in the unfavorable evolution of them, with the purpose of identifying possible prognostic and therapeutic targets.

CHAPTER IV. Materials and methods

The study included a number of 276 cases of colonic adenocarcinomas , diagnosed in the period 2017-2018.

The **clinico-epidemiological** study took into account the following parameters: sex, age and age distribution.

The **histopathological** study of the colonic adenocarcinomas investigated, pursued the identification of the main histopathological parameters in relation to prognosis: tumour growth pattern, grade of tumour differentiation , inflammatory infiltratate, necrosis, vascular invasion, perineural invasion, depth of invasion, metastatic adenopathy, remote metastases .

For the **immunohistochemical** study, a number of 60 cases were selected, with the diagnosis of colon adenocarcinoma, for which the expression of some markers implicated in EMT, such as E-caderine, N-caderine, Snail, Slug, Twist, ZEB1, AE1/AE3, Fibronectin and Vimentin, was evaluated.

Obtained results were the subject of statistical analysis, using statistic tests for the evaluation of differences between the immunoreactivity scores obtained for each tumor, considering a significant difference ff $p<0.05$.

CHAPTER V. Results

The clinico-epidemiologic study was composed of 276 cases of colon adenocarcinoma (CAC) selected in the time interval of 2017-2018, resulting in the incidence being higher in 2017

(144 cases – 52,2%), 178 (64,5%) of patients being males and 100 (36,2%) pertaining to the 71-80 year bracket.

Of the analysed cases, most were of the mucinous type (91- 33%), the majority being G2 in 178 cases (64,5%). Imflammatory infiltrate was present in 140-50,7% of cases, necrosis in 130-47,1% of cases, vascular invasion in 130 of cases (47,1%) and perineural invasion in 71 of cases (25,5%). In regards to the depth of tumoral invasion, we observed that the majority of diagnosed tumours were T3 (135- 48,9%), and many of the alaysed cases (133- 48,2%) were N0, although in 30 cases (10,9%), metastasis of the lung and liver were found. Thus a great part of the studied cases fell within Tumour Stage T3 (113-40,9%). We obtained statisticaly significant associations between tumour stage and tumour grading, tumour stage and vascular invasion, also with perineural invasion, but also between tumour grade and both vascular and perineural invasion ($p<0.05$, chi-square test).

Immunohistochemical analysis was composed of 60 cases of CAD, obtained results being afterwards stasticaly interpreted in comparison with histhopathological parameters. E-caderine immunoreaction was identified in 77% of the 60 analysed CAD cases, and in comparison with the advance in tumoral stage a decrease of immunomarking was observed. Furthermore, we highlighted statisticaly significant aspects of E-caderine expression in comparison with tumour grading, tumoral stage and perineural invasion ($p<0.05$). Immunoreaction for P-caderine was identified in 74,6% of cases, and for N-caderine in 56% of cases, in both situations an increase of immunomarking in comparisson with tumour stage was observed, as well as a statiscaly significant association between them. The study of immunoexpression of transcription factors showed a positive reaction for Twist, Snail, Slug, ZEB1 in 75,5%, 74,66%, 75,83% and 72,58% of cases, showing an increase of markings of tumour cells with the advancement in tumoral stage, theese being statisticaly significant with tumour stage and perineural invasion ($p<0,05$). AE1/AE3 immunoexpression was identified in 85,08% of cases and Fibronectin in 78,5% of cases, both increasing with tumoral stage advancement and a statisticaly significant aspect in comparison with tumoral grading ($p<0,05$), Vimentine was negative in all cases of CAD, on an epithelial level. Statistic analysis of caderine levels revealed, linear negative correlation between E-caderine and N-caderine (Pearson test). E-caderine also presented linear negative correlation with AE1/AE3 (Pearson test), N-caderine with AE1/AE3, as well as with Fibronectin, these presenting a linear

positive aspect (Pearson test). Last but not least a positive linear correlation between P-caderine and AE/1/AE3 was discovered.

CHAPTER VI. Discussions

CRC is the third most diagnosed type of cancer and one of the main causes of death, and DAC represents a subtype of CRC which develops from the colonic mucosa, being an epithelial tumour [83]. The majority of CRC (70%) are diagnosed as being moderately differentiated [19,110], and tumour staging este by far the most important prognostic predictor of clincal evolution for patient with CRC.

Cadherins are a transmembranary component of adherence junctions. These mediate intercelullar adhesion and cannect to actine cytoskeleton by associating with cathenins [108].
E-cadherine is a calcium dependent transmembranary glycoprotein, expressed in the majority of epithelial tissues, who form a firm junction which connects adjacent cells. One of the main discoveries of EMT is the loss of cell adhesion with the decrease of E-cadherine expression, which leads to tumour progression, metastasis and unfavorable prognosis [72]. P-cadherine is a calcium dependant adhesion glycoprotein, which holds a crucial role in preserving structural integrity of epithelial tissue. It regulates many homeostatic cellular processes, of which maintaining tissular arhitecture and is important in cell differentiation, form, polarity, cell growth but also migration of the cell [291]. The EMT process, is also known as the Cadherine Switch, which leads to loss of E-cadherine and gaining n-cadherine, which is a mesenchimal amrker which can promote motility, but also tumour cell invasion [249].

Transcriptional supressors of E-cadherine, ZEB1/ZEB2, Twist and Snail/Slug, are associated with EMT. Twist is a transcription factor which belongs to the helix-loop-helix protein family, implicated in EMT and invasive processes [101]. Snail is one of the best promotors of EMT, being considered with a prognostic role in CRC. Similarly to Snail, Slug is a zynk finger transcription factor, having the same properties as Snail, such as E-cadherine supression and anti-apopthotic activity, with a crucial role in organogenesis and neutralisation [86]. ZEB1 supresses the interleukin 2 gene, but also the gene for the E-caderine promotor, aspect which induces EMT [48]. Furthermore, it helps in the process of invasion and metastasis [178]. AE1/AE3 belongs to the cytokeratin class, which are intermediary fillaments of keratin found in intercyoplasmatic cytoskeleton of epithelial tissues. The cytokeratin cocktail, is appropriate to distinguish carcinomas

from non-epithelial malignancies and is used to facilitate tumour classification. Fibronectin is a glycoprotein of the extracellular matrix, which plays a crucial role in cell adhesion, growth, migration and differentiation [160,318]. It was discovered that Fibronectin is strongly expressed in tumoral vascularisation and mediates angiogenesis during tumorgenesis, offering a potential role in tumoral progression [318].

CHAPTER VII. Conclusions

- Clinico-epidemiological analysis for the period of time between 2017-2018, indicated that the highest incidence was recorded in 2017 (52.2%), with the majority of patients being male (64.5%), aged in the range of 71-80 years (36.2%);
- The histopathological study of the analysed cases revealed 33% cases of Mucinos type CAD, 31.2% cases with CAD cribriform type comedo-carcinoma, 14.1% CAD with signet cells, 10.5% serated type, 5.8% micropapilary and 5.4% medullary;
- Study of the degree of tumour differentiation, revealed 9.1% of cases with G1, 64.5% G2 and 26.4% G3, being significantly associated with vascular invasion (P = 0,000), perineural invasion (P = 0,031) and the presence of inflammatory infiltrate (P = 0,047), most of them being G2 ;
- Tumour stage analysis showed that most of the cases studied were classified in stage III (40.9%), being moderately differentiated (30.1%) and were significantly associated with vascular invasion (P = 0,000) and perineural invasion (P = 0,000);
- The analysis of the inflammatory infiltrate revealed its presence in 50.7% of CAD, and its distribution according to the growth pattern showed that it was found in 80% of cases of medullary CAD and 67.4% of the cribriform type comedo-carcinoma;
- Tumour necrosis was discovered in 47.1% of cases, without statistical correlation with other histopathological parameters analysed, and the distribution according to the growth pattern showed that it was present in 79.1% of the CAD cribriform type comedo-carcinoma and in 46.7% of medullary type;
- Vascular invasion was present in 47.1% of the cases analysed and perineural invasion was found in 25.7% of them;

Immunohistochemical study was done on 60 cases of CAD, using markers implicated in EMT:

• Immunorection for E-Caderine was identified in 77% of the CAD, with markings being superior in the case of G1 tumors, as well as in early stages of disease, and statistical analysis revealed significant associations with tumour differentiation grade (P = 0,028) , tumour stage (P = 0,034) and perineural invasion (P = 0,050);

• P-Caderine immunomarking was identified in 74.6% of cases, being superior in the case of G3 tumours and in the advanced stages of disease and statistical analysis revealed significant statistical associations with the degree of tumour differentiation (P = 0,043), tumour stage (P = 0.002) and vascular invasion (P = 0,006), in addition a positive linear correlation between P-Caderine and AE1/AE3 was discovered;

• N-caderine marking was found in 56% of the cases analysed, being better highlighted in G3 and the tumor III/IV stages, having a statistically significant association with tumour grade (P = 0,009);

• Twist immunoexpression was identified in 75.5% of the 60 cases analysed, having a statistically significant association with tumour grade (P = 0,010), tumour stage (P = 0,000), vascular invasion (p = 0.001) and perineural invasion (P = 0,000);

• The Snail's immunomarking was present in 74.66% of cases, with a statistically significant association with tumour stage (P = 0.002), vascular invasion (P = 0,009) and perineural invasion (p = 0.001);

• Slug immunorection was identified in 75.83% of CAD cases analysed, having a statistically significant association with the tumour stage (P = 0,000), vascular invasion (P = 0,024) and perineural invasion (P = 0,006);

• ZEB1 reaction was identified in 72.58% of cases, with a statistically significant association between the ZEB1 marking, the tumour stage (P = 0,021) and the perineural invasion (p = 0,014);

• Immunoexpression AE1/AE3 was identified in 85.08% of the 60 cases, with a statistically significant association with tumour grade (P = 0.004), tumour stage (P = 0.001) and perineural invasion (P = 0,017);

• Immunoreaction of Fibronectin was identified in 78.5% of CAD having a statistically significant association with tumour grade (P = 0,038) and vascular invasion (P = 0.002);

• The expression of Vimentine was negative at the epithelial level in all 60 cases of ADC analysed;

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