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APC PROTEIN EXPRESSION AND CLINICAL AND MORPHOLOGICAL INDICATORS IN COLORECTAL CARCINOMA

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Abstract
Introduction: Colorectal carcinoma (CRC) occurs as a result of accumulation of different types of genome damage. The purpose of the present research was to study and compare APC expression in tumor tissue of synchronous/metachronous adenomas and CRC in connection with clinical and morphological indicators.
Materials and methods: Materials from 18 synchronous/metachronous colorectal adenomas and 21 CRCs were observed. We used an APC mouse monoclonal antibody for immunohistochemical analysis.
Results: There was no statistically significant difference between the percentage of protein expression in synchronous/metachronous adenomas and clinico-morphological indicators expect their differentiated. There was no statistically significant difference between APC expression percentage in CRC and clinico-pathological parameters. Based on logistic regression analysis, we found a statistically significant difference between protein expression area and lymph node metastases.
Conclusion: APC accumulation in CRC can be used as a prognostic sign for the risk of lymph node metastases.
Keywords: APC, CRC, synchronous/metachronous adenomas, prognostic sign, metastases

1.Introduction

CRC is the third most common malignancy and fourth leading cause of death [1]. It is characteristic of him that in the strike and old age he presents himself with obstructive syndrome [2]. In Bulgaria, there is a lasting trend towards increasing CRC morbidity and mortality from CRC. Annual morbidity and mortality increases by 4.1% for men and 3.5% for women. Compared to the European average, CRC incidence and mortality rates in Bulgaria are higher. Forecast data on annual morbidity in Bulgaria is 58.7 for men and for Europe is 55.7 per 100 000, and mortality is 32.3 for Bulgaria and 25.2 for Europe per 100 000. For women, the incidence is 36.4 and mortality rate is 18.2 for Bulgaria, compared to the European average of 34.7 and 15.4 per 100 000, respectively. CRC incidence increases with patients' age. In young people (under 30 years of age) the incidence is 4 per 100 000, while in older individuals (over 75 years), it is 120. CRC reaches its peak in the age group of 75-79 years [3]. Genetic disorders and environmental factors play an important role in the development of CRC. CRC is divided into three forms, depending on etiological factors: sporadic, familial and hereditary form. Adenomas are a common and long-known risk factor for CRC. The risk of neoplastic transformation depends on their size and histological type. At sizes larger than 1 cm, the risk of developing CRC increases by 8% over a period of 10 years. Malignancy is higher in villous

than in tubular adenomas [4]. From environmental risk factors, diet is of the greatest importance for the development of CRC. Risk factors include Western lifestyle, which is characterized by low-fiber, high-saturated fat consumption, red meat consumption, increased alcohol consumption and obesity [5]. There is evidence that smoking is associated with development of larger adenomas and increases the risk of developing CRC [6]. It is believed that there is a direct link between smoking and colorectal adenomas with an induction period for CRC of 30-40 years. Eating high-fiber foods, more fruits and vegetables, fish, calcium, physical activity, aspirin and other NSAIDs are considered factors with a likely protective effect against cancer [7] The APC gene is located on chromosome 21q3. Inactivation of the APC gene by hypermethylation or point mutation is an important early event in the adenoma-carcinoma sequence, leading to accumulation of β-catenin in the cytoplasm and nucleus of the cell with subsequent activation of the WNT signaling pathway.

2.Purpose

The purpose of the present study is to examine and compare APC expression in tumor tissue on synchronous/metachronous adenomas and CRC in connection with clinical and morphological indicators and evaluate its role as predictive marker.

3.Materials and methods

We studied 18 synchronous/metachronous colorectal adenomas and 21 CRC.

The following indicators of adenomas were reported: size, location, histological appearance and signs of dysplasia. The following CRC indicators were analyzed: location, tumor size, depth of invasion, degree of differentiation, presence of lymph node metastases and distant organ metastases. Degree of tumor differentiation and stage were determined according to the seventh revised edition of the 2010 TNM Classification of Malignant Tumors based on histological and imaging studies [8].

We had 18 patients with metachronous/synchronous colorectal adenomas. Their mean age was 69.28±13.70 years. Colorectal adenomas were more common in males: 13 cases (72.22 %), compared to women: 5 cases (27.78%). There were 7 adenomas with sizes between 1.1 cm and 2 cm (38.89%) and 5 were between 0.1 cm and 0.5 cm (27.78%). Two adenomas were over 2 cm in size (11.11%) and 4 adenomas were between 0.6 cm and 1 cm (22.22%). 14 of the synchronous/metachronous adenomas were located in the left half of the column (77.78 %), and 4 in the right half (22.22%). From the synchronous/metachronous adenomas, 9 were tubular (50%), 8 were tubulovillous (44.44%) and one was villous (5.56%). 13 were highly differentiated (72.22%), and the remaining 5 adenomas were poorly differentiated (27.78%).

From the 21 studied CRC patients 7 (33.33%) were women and 14 (66.67%) were men. Seven of them (33.33%) were aged between 61 and 70 years, and there were 4 (19.05%) cases between 71-80, 81-90 and 51-60 years. Higher percentage of CRC were localized in left half of the column: 15 (71.43%) cases, and the remaining 6 (28.57%) were found in his right half. Tumors were predominantly between 2 and 3 cm in size: 13 (61.91%), only only one (4.76%) was 4.5 cm, and the remaining 7 (33.33%) tumors were between 3.1 and 4 cm. All carcinomas had moderate differentiation (G2). The majority of CRC patients were in advanced T3 stage: 11(52.38%), 6 patients (28.57%) were in T4 stage and 4 (19.05%) in T2 stage. A little more than half of the patients had no metastases in lymphatic nodes: 13 (61.90%) cases and 15 (71.43%) had no organ metastases

4.Results

Protein expression was present in 14 (77.78%) adenomas: in 4 (28.57%) cases it was between 6% and 25%, in one (7.14%) between 26% and 50 %, in one (7.14%) between 51% and 75% and in 8 (57.14%) cases expression was over 75%. In 4 (22.22 %) cases, there was no antibody

expression

In the tumor tissue of 10 (47.62%) CRC there was no APC protein expression. In 11 (52.38%) CRC there was expression of the protein in the tumor tissue: in 4 (36.36 %) cases the expression was between 6% and 25%, in 1 (9.09%) case between 50% and 75% and in 6 (54.55%) cases over 75% (figure 1).

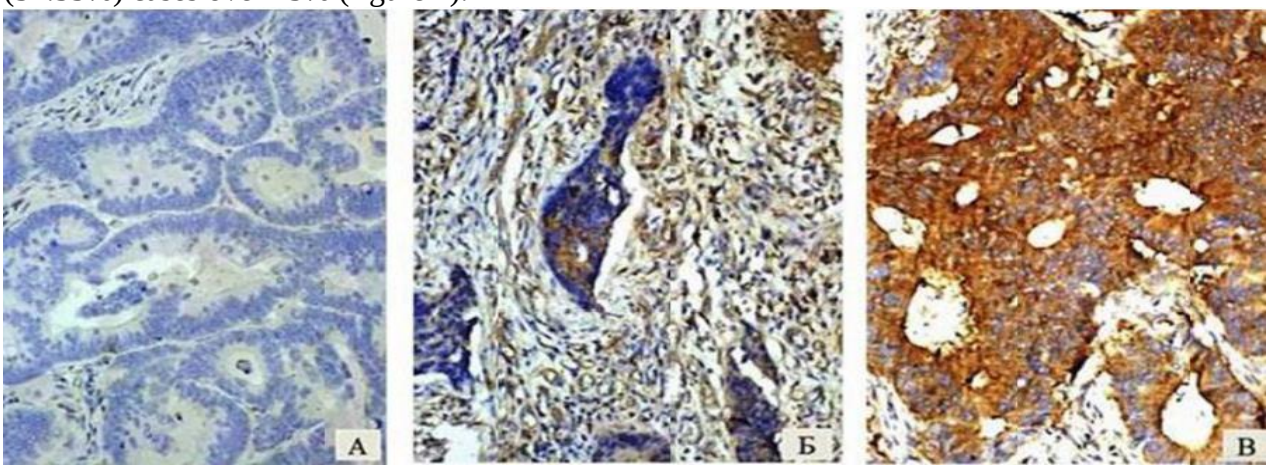

Fig. 1. Expression of antiAPC antibody in colorectal carcinoma. A. missing expression B. expression 6%-25% C. expression 51%-75% D expression >75% (A,B,C x 200, D x 400)

There was no statistically significant difference between protein expression percentage in synchronous/metachronous adenomas and the clinico-morphological parameters: size, location and histological type of the adenoma ($p>0.05$). We found such a relationship between expression area and degree of differentiation ($p<0.05$). Antibody expression was higher in highly differentiated than poorly differentiated adenomas. There was no statistically significant difference between APC expression percentage and clinical and pathological indicators: sex, age, size, location and TNM stage ($p>0.05$). Based on logistic regression analysis, we found a statistically significant difference between protein expression area and lymph node metastases.

5.Discussion

We, similar to Zsuzsanna P et al. (2012) [9], found that there was no statistically significant difference between the intensity and area of protein expression and localization of adenomas in the left and right halves of the column ($p>0.05$), but there was a higher percentage of expression in highly differentiated than poorly differentiated adenomas, indicating that the APC protein is likely to block the transition of cells from G1 to S phase of the cell cycle (Powell SM et al., 1992)[10]. According to the clinical and morphological profile, the malignant potential of adenomas is determined by the size: >1 cm, low degree of differentiation and presence of villous component [11]. Similar to other studies [12], we did not find a statistically significant difference between these parameters and protein expression. Some authors report a higher incidence of APC gene mutation in tubulovillous and villous adenomas and its involvement in tumor progression [13].

We did not find a statistically significant difference between APC expression in CRC and following clinical and morphological parameters: sex, age, size, location and TNM-stage ($p> 0.05$). The data obtained are consistent with the results of Lugli A et al. (2007)[14], who also found no relationship between sex, age, size, CRC localization and APC expression. Birnbaum DJ et al. (2012)[15], apart from the indicators listed above, did not establish a relationship between T- and M-stages of CRC and APC protein expression ($p>0.05$). Based on an extensive meta-analysis involving 24 studies and 2 025 patients, no statistically significant difference was found between the hypermethylation of the APC protein responsible for its absence and clinical morphological indicators [16]. According to the same

authors, APC mutation is an early event in carcinogenesis and may be an early diagnostic CRC marker. In addition, they noted that APC methylation was not associated with overall patient survival. In our opinion, hypermethylation of APC, which leads to loss of APC expression, is also an early event that occurs in synchronous/metachronous adenomas. We found an inverse correlation between protein expression and lymph node metastases. Logistic regression analysis showed that low APC protein expression increased the risk of metastasis in 91 regional lymph nodes. These results are consistent with those of Li BQ et al. (2017)[17]. The results obtained should be taken into account when determining prognosis and therapeutic approach in CRC patients.

In conclusion, we can claim that APC protein is involved not only in the early stages of colorectal carcinogenesis, but is also a risk factor for metastasis of CRC.

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