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BRAF PROTEIN EXPRESSION AND MORPHOLOGICAL INDICATORS IN COLORECTAL EPITHELIAL NEOPLASMS

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Abstract
Introduction: Colorectal cancer (CRC) occurs as a result of accumulation of different types of genome damage. BRAF mutations appear at an early stage of CRC carcinogenesis
Purpose: The purpose is to examine and compare BRAF expression in tumor tissue in synchronous/metachronous adenomas and CRC in connection with clinical and morphological indicators and evaluate its role as predictive marker.
Materials and methods: Materials from 18 synchronous/metachronous colorectal adenomas and 21 CRCs were observed. We used a BRAF mouse monoclonal antibody for immunohistochemical analysis.
Results: There are no statistically significant difference between anti-BRAF antibody expression percentage and tested indicators. There was no statistically significant difference between the protein expression and clinico-pathological indicators. Based on logistic regression analysis, a direct correlation was established between BRAF protein expression area and occurrence of lymph node metastases.
Conclusion: Accumulation of BRAF in CRC can be used as a prognostic marker for the risk of lymph node metastases. Despite its low prevalence (5%–8%), BRAF V600 CRC has been widely studied due to the poor prognosis. Some data suggest a residual benefit from monoclonal antibodies such as cetuximab or panitumumab when given in monotherapy or with combination with chemotherapy. Due to the low prevalence of this mutation, it is necessary large-scale studies for further investigations in phase II/III trials in order to improve prognosis, treatment and survival [14].
Keywords: BRAF, synchronous/metachronous adenomas, CRC, lymph node , metastases

1.Introduction

CRC is one of the leading causes of morbidity and mortality in the world [7]. About 1,400,000 new CRC cases and about 700,000 deaths are reported annually worldwide [2]. In terms of geographical distribution, this cancer has increased in industrialized countries with moderate and high living standards [6]. According to the Health Report from 2017 of the Bulgarian National Statistical Institute, the registered cases of colon cancer in 2016 were 18 776, not including carcinomas located in the rectum, anal canal and anus, which were 1 343. As a common complication of colorectal carcinoma in elderly patients is colonic obstruction [16].

CRC onset, like other neoplasms, begins with cell transformation followed by uncontrolled cell proliferation, followed by unlimited autonomic growth even in absence of growth factors [3]. Most often, genetic disorders affect proto-oncogenes, which are normal genes in cells. In mutations, they become oncogenes and, due to overexpression, ensure unlimited growth and

immortality of malignant cells. Mutations that activate proto-oncogenes and convert them to oncogenes are point mutations, chromosomal translocations (changes in chromosome number and arrangement) and gene duplication. Proto-oncogenes are involved in cell growth normal regulation and differentiation, signal transduction and mutagenic signal elimination.
Tumor suppressor genes are normal genes that slow down cell division, protect cells from malignant transformation, correct DNA defects, and, if defects cannot be removed, undergo apoptosis. The BRAF gene is located on chromosome 7q34 and encodes a protein of the same name that is involved in transduction of mitogenic signals from the cell membrane to the nucleus. BRAF mutations occur at an early stage of CRC carcinogenesis and play an important role in the colorectal carcinogenesis pathway. This mutation is characteristic of almost all sporadic CRCs with microsatellite instability (MSI). ‘’The presence or absence of BRAF alterations should be performed at the time of diagnosis as this represents unique CRC subtype with poor prognosis and limited response to standard-of-care therapies’’[20].
In majority of cases, CRC develops against the background of previous benign dysplastic changes.

2.Purpose

The AIM of the present study was to investigate and compare BRAF expression in tumor tissue in synchronous/metachronous adenomas and CRC in connection with clinical and morphological indicators and evaluate BRAF as prognostic marker.

3.Materials and methods

We studied 18 patients with synchronous/metachronous colorectal adenomas and 21 CRC in the University Hospital "St. Marina ‘’- Varna.

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 [15].

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

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

The materials were fixed in 10% neutral formalin and were included in paraffin with a

melting point between 52-54 ° C. 5μ thick cuts were stained by hemalaun-eosin for histological indicators evaluation. We used a BRAF mouse monoclonal antibody for immunohistochemical analysis and mini KIT high Ph DAKO K8024. A five-stage scale was used for evaluation of percentage of positive nuclei: missing expression - 0 (0-5%), 1 (6-25%), 2 (26-50%) and 3 (51-75%) and 4 (>75%). Data were processed statistically and $p<0.05$ was accepted for correlation dependence.

4.Results

13 adenomas (72.22 %) had protein expression, of which in 7 (53.85%) expression percentage was over 75%, in one (7.69%) it was between 51-75%, in 3 (23.08%) adenomas it was between 26-50% and in two cases (15.38%) it was between 6-25%. In the remaining five cases (27.78%), there was no antibody expression. In 17 CRC (80.95%) there was BRAF expression, of which in 16 CRC (94.12 %%) expression percentage was over 75% and in one case (5.88 %) it was between 6%-25%. In 4 cases (19.05 %), antibody expression was absent (Figure 1).

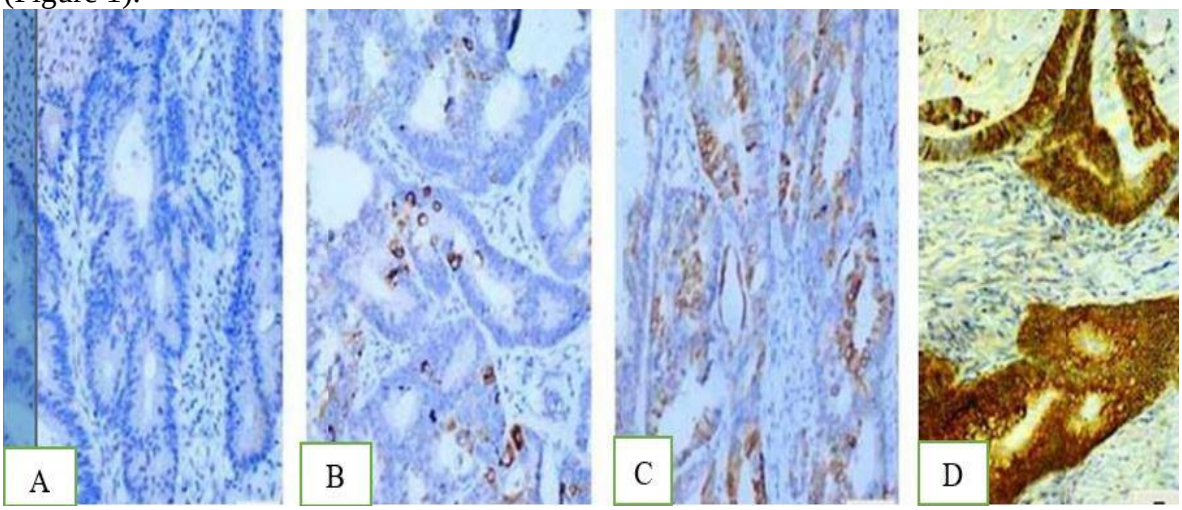

Fig.1. Expression of antiBRAF antibody in colorectal carcinoma: A.missing expression; Б. expression (6-25%); B expression >75%).(*x100*)

Of the 6 CRCs in T4 stage, 4 had protein expression >75%, of the 11 CRCs in T3 stage, 8 had expression above >75% . The mean percentage of protein expression was 47.78±40.81. There was no statistically significant difference between the percentage of antiBRAF antibody expression in synchronous/metachronous adenomas and studied morphological parameters ($p>0.05$). There was no statistically significant difference between the protein expression percentage and clinico-pathological indicators: sex, age, size, location and T- and M-stage ($p>0.05$). Based on logistic regression analysis, a direct correlation was found between the intensity and area of BRAF protein expression and occurrence of lymph node metastases ($p< 0.05$).

5.Discussion

Analysis of BRAF tissue expression in CRC tumor tissue showed expression in 80.95 % of cases. The results we obtained do not differ significantly from data in literature, according to which, protein expression varies between 45% and 66% [1,10,12]. Other authors report low antibody expression [5]. There are few and contradictory data from literature on BRAF protein expression in relation to clinical and morphological parameters in CRC [10]. We did not find a statistically significant difference between the intensity, area and overall rate of protein expression and sex of CRC patients ($p> 0.05$), which was consistent with the results of some authors [8]. According to other authors [4,5], BRAF protein expression was higher in women

than in men. Similar to Jang MH et al. (2017) [9], we found no relationship between BRAF protein expression and age of CRC patients ($p>0.05$). According to Capper D et al. (2013) [4] protein expression increases in proportion to age. There was no statistically significant relationship between BRAF protein expression and tumor size ($p> 0.05$). Our results differ from Yujie Li and Weier Li (2017) [18], who found a correlation between protein expression and tumor size based on their meta-analysis, which included two studies: the first one with 399 patients and second with 4 154 patients. In our opinion, these differences were most likely due to the small number of patients included in the current study. We did not establish a relationship between CRC localization and BRAF protein expression. These results are consistent with the results of Capper D et al. (2013) [4] who also found no relationship between tumor localization and BRAF protein. The results obtained by us and those of Capper D et al. (2013) [4] differed from data from a study of 5 307 patients, according to which high protein expression was associated with CRC localization in the proximal part of the column. According to other studies, BRAF mutations are more common in carcinomas of the right half of the colon, in poorly differentiated adenocarcinomas, and in the mucinous histological type of carcinoma. Regardless of CRC location and morphological characteristics, according to Zlobec I et al. (2010) [19] high BRAF expression selected patients in need of a more aggressive course of chemotherapy. In analyzing the relationship between area of BRAF protein expression, we, similar to Kanik P et al. (2018)[10], found a higher protein epression percentage in 104 patients with advanced CRC stage (T3 and T4) as compared to stage T2, although the difference was not statistically significant ($p>0.05$). According to Selingmann JF et al. (2017) the occurrence of a mutation in the BRAF gene determines an unfavorable prognosis in patients, regardless of clinical and pathological characteristics. Evidence from literature shows that the BRAF mutation is not only a prognostic marker but also an important biomarker for determining target therapy [11]. Protein expression is crucial for CRC resistance to conventional therapy [10]. Patients with BRAF V600E mutations showed a weaker response to targeted therapy with a monoclonal body directed against EGFR. Detection of BRAF mutation may play a predictive role in the choice of treatment for patients with metastatic CRC, suitable for anti-ERGF therapy.

Conclusion: Accumulation of BRAF in CRC can be used as a prognostic marker for the risk of lymph node metastases Despite its low prevalence (5%–8%), BRAF V600 CRC has been widely studied due to the poor prognosis. Some data suggest a residual benefit from monoclonal antibodies such as cetuximab or panitumumab when given in monotherapy or with combination with chemotherapy. Due to the low prevalence of this mutation, it is necessary large-scale studies for further investigations in phase II/III trials in order to improve prognosis, treatment and survival [14].

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