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Immunohistochemical Study of The Expression of TACC3 in Colorectal Carcinoma and its Correlation with Other Pathological Prognostic Factors

Mohamed Elmahdi[1]*, Ahmed Hosain[1], Reham S. E. Esmail[1], Hany Khattab[2]

[1]Department of Pathology, Faculty of Medicine, Fayuom University, Fayoum, Egypt; [2]Department of Pathology, Faculty of Medicine, Cairo University, Cairo, Egypt

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***Correspondence:** Mohamed Elmahdi, Department of Pathology, Faculty of Medicine, Fayuom University, Fayoum, Egypt. E-mail: mhm01@fayoum.edu.eg
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Abstract

BACKGROUND: Colorectal cancer (CRC) is the fourth most common cancer comprising nearly 10% of all cancer cases worldwide. Many tumor markers have been used to expect the prognosis of CRC. Transforming acidic coiled-coil-containing protein 3 (TACC3) is one of the TACC family proteins**.** Physiologically, TACC3 is an important protein in the process of cellular division as it plays a key role in the formation of the mitotic spindle. Pathologically, TACC3 expression was studied in CRC, being found to be a poor prognostic factor.

AIM: The aim of the study was to study the expression of TACC3 and its relationship with other clinical and histopathological prognostic factors in patients with CRC.

METHODS: This is an observational and immunohistochemical study on 45 resection specimens from 45 CRC cases. This study was conducted at the pathology departments of the Faculty of Medicine, Cairo University, and Faculty of Medicine, Fayoum University from July 2019 to February 2020 Tumor tissues were prepared as formalin-fixed and paraffin-embedded specimens. The paraffin blocks were sectioned at the 5 microns thickness. Then, the collected sections were stained with hematoxylin & eosin for histopathological revision and immune-histochemical staining for TACC3 proteins.

RESULTS: The mean immunoreactivity score (IRS) for the TACC3 expression in our sample was 70 ± 89.91**.** TACC3 IRS score was significantly higher in those tumors with N2 stage (IRS = 175 ± 107.1; p = 0.02), and with Stage III tumors (IRS = 136.4 ± 93.5; p = 0.04). The other parameters showed no statistically significant relationship with IRS scores.

CONCLUSION: Immunohistochemical expression of TACC3 would be valuable as a prognostic marker in cases of colorectal adenocarcinoma, where the expression was found to show stronger and more widespread expression in cases with higher stages. Furthermore, TACC3 should therefore be considered as a potential candidate for targeted therapy, where its blockade may hinder the tumor's ability to proliferate and progress.

Introduction

Colorectal cancer (CRC) is the fourth commonest cancer comprising nearly 10% of all cancer cases worldwide with males' predominance over females [1], [2]. Although CRC occurs predominantly in old aged population, rates have been increasing among young adults in different countries [1], [2]. Developed countries have the highest rates globally, with an incidence as much as 8 times higher than some developing countries [3]. The figures in Egypt go parallel to the international ones, even though it is relatively less common compared to the rest of the world. CRC ranks ninth among the commonest malignancies in Egyptian people [4].

Many tumor markers have been used to expect the prognosis of CRC. Many of these markers were related to BRAF and KRAS pathways which are affected in only less than half of CRC. However, up to now, no markers can predict the prognosis accurately enough. We still need to find new biomarkers for a better understanding of the behavior and management of this type of cancer [5], [6]. Transforming acidic coiled-coil-containing protein 3 (TACC3) is one of the TACC family proteins; its gene is located on the short arm of chromosome no. 4 [7]. Physiologically, TACC3 is a target of the Aurora A kinase [8]. It is essential for the growth and stability of microtubules while localizing to the centrosome during mitotic division. This process is important for the formation of the mitotic spindle which is responsible for the final segregation of the chromosomes. Abnormalities of microtubules and centrosomes would result in the defective formation of the mitotic spindle and correlate with tumorigenesis and tumor progression [7]. This explains the involvement of TACC3 reported in various cancers. Expression of TACC3 was found to be a marker of tumor progression in the ovaries [9], lungs [10] CNS [11], esophagus [12], stomach [13], liver [14], and sarcomas as well [15]. TACC3 can therefore be considered as a potential candidate for targeted

therapy, where its blockade may hinder the tumor's ability to proliferate and progress.

Hence, in this study, we aim to study the expression of TACC3 and its relationship with other clinical and histopathological prognostic factors in patients with CRC.

Methods

Study design and settings

This is an observational and immunohistochemical study on 45 resection specimens from 45 CRC cases. This study was conducted at the pathology departments of the Faculty of Medicine, Cairo University, and Faculty of Medicine, Fayoum University from July 2019 to February 2020.

Inclusion and exclusion criteria

Patients with CRC at the previously mentioned pathology departments department(s) during our study period were included in the study. Patients who received pre-operative chemotherapy and/or radiotherapy with complete or near-complete response were excluded from the study. This is due to the loss of major components of the tumor, which would negatively affect the immunohistochemical analysis. A total of five cases that met our exclusion criteria were excluded. There were only 45 cases were eligible for our study.

Clinical data of included patients

Demographic and clinical data of the included patients were collected from the pathology reports. This included sex, age, anatomical site of the tumor, and pre-operative therapy.

Histopathological data of the specimens

The pathology reports were reviewed for histological data and these data were confirmed by hematoxylin & eosin (H&E) slides. Data included histological variant and grade, the presence of mucinous activity perineural invasion, lymphovascular invasion, T and N staging, and the respective stage group (tumor regression score according to the 8th edition of AJCC staging system for those who received pre-operative therapy.

Specimens' preparation

Tumor tissues were prepared as formalin-fixed and paraffin-embedded specimens. The paraffin blocks were sectioned at the 5 microns thickness. Then, the collected sections were stained with H&E for histopathological revision and immune-histochemical staining for TACC3 proteins.

Immunohistochemical staining technique and analysis

The sections were baked at 60°, deparaffinized in xylene, then rehydrated through a series of decreasingly graded concentrations of alcohol (95–70%) ended with distilled water. After 20 min of cooling, the endogenous peroxidase activity was inhibited by incubation in 3% hydrogen peroxide (H_2O_2) for 5 min. The sections were then boiled for 5 min in citrate solution, with a pH of 6, for antigen retrieval. The sections were incubated with the respective primary antibody for 1 h at room temperature and a dilution of 1:50. The primary antibody used for TACC3 detection was a rabbit polyclonal anti-TACC3 antibody manufactured by Chongqing Biopsies, China. Subsequently, the sections were stained for protein detection in 3, 3-diaminobenzidine for 2 min. Afterward, the sections were rinsed well in tap water and then counterstained with hematoxylin to stain the nuclei. Finally, the slides were cleared in xylene and the coverslips were applied. The positive control for TACC3 was human testicular tissue where it shows cytoplasmic staining in the germ cells of seminiferous tubules.

For immunohistochemical analysis of TACC3 expression, we adopted the methodology described and used by Huang and his colleagues [12]. you should mention the paper's author The expression was evaluated considering both the staining intensity and the extent of staining. The staining intensity was scored as (0: negative staining; 1: weak staining; 2: moderate staining; and 3: strong staining). The extent of staining was scored as the percentage of positive cells. The final immunoreactivity score (IRS) is the product of the intensity score and the extent score.

Statistical analysis

The Microsoft Excel 2010 software and SPSS software (Statistical Package for the Social Science) version 25 were used for the data entry and statistical analysis, respectively. Simple descriptive statistics were used to describe the distribution of various demographic, clinical, and histologic variables as well as the immunohistochemical findings. Inferential statistics were used to determine the existence of a statistically significant relationship between immunohistochemical expression of TACC3 and other variables in the study. The one-way ANOVA test was used for the analysis of TACC3 expression. The level of statistical significance was set at a probability $p < 0.05$.

Ethical consideration

This study was reviewed and approved by the ethical committee, Faculty of Medicine, Fayoum University, Fayoum, Egypt.

Results

General characteristics of the included patients

In this study, we included 45 specimens of colorectal adenocarcinoma from 45 CRC patients. The mean age of our study population was 57.02 ± 12.25. Most of them were females (%) with a male to female ratio of 2:3. Most of our specimens (60%) were obtained from the rectosigmoid anatomical region. Among the 45 included patients, only 7 patients (16%) received pre-operative therapy; Table 1.

Table 1: General, clinical, and histopathological characteristics of the study population. Remove P value from this table, it is not the tabe of relations (DONE)

Parameters	TACC3 sections, (*n*=45) (%)
Age, median (Range), years	60 (29–83)
Sex, *n* (%)	
Males	18 (40)
Females	27 (60)
Anatomical site, n (%)	
Right colon	12 (27)
Transverse colon	3 (6)
Left colon	30 (67)
Pre-operative therapy	
Received	7 (16)
Not received	38 (84)
Histological variant	
Conventional	38 (84)
Mucinous	5 (11)
Signet ring	2 (4)
Mucinous activity	
Present	30 (67)
Absent	15 (33)
Histologic grade	
Low grade	38 (84)
High grade	7 (16)
Perineural invasion	
Present	17 (38)
Absent	28 (62)
Vascular invasion	
Present	31 (69)
Absent	14 (31)
T Stage	
T2	5 (11)
T3	28 (62)
T4	12 (27)
N Stage	
N0	20 (44)
N1	18 (40)
N2	7 (16)
Clinical stage group	
I	4 (9)
II	16 (36)
III	25 (55)

Histological findings

Most of our cases (84%) showed the morphology of conventional colorectal adenocarcinoma. In addition, most of our cases (84%) were classified as low-grade carcinoma according to the two-tier grading system accepted by the WHO [16] Regarding tumor invasion, perineural and vascular invasion was seen in 38% and 69% of cases. As for the grading of the tumors, 72% of cases were classified as T3 and T4 stages. Moreover, most of the specimens were of Stage III (55%).

Immunohistochemical expression of TACC3

The mean IRS for the TACC3 expression in our sample was 70 ± 89.91. TACC3 IRS score was significantly higher in those tumors with N2 stage (IRS = 175 ± 107.1; p = 0.02), and with Stage III tumors (IRS= 136.4 ± 93.5; p = 0.04). The other parameters showed no statistically significant relationship with IRS scores; Table 2.

Table 2: Relationship between TACC3 Expression general, clinical, and histopathological data of the study population

Parameters	TACC3 expression	p-value
Sex		
Male	112.2 ± 102.4	0.77
Female	103.9 ± 82.5	
Age		
20–29	140	0.75
30–39	138.8 ± 135.5	
40–49	125 ± 94.4	
50–59	118 ± 92.4	
60–69	93 ± 89.7	
70–79	69.3 ± 68.6	
80–89	195	
Anatomical site		
Cecum & Ascending	91.25 ± 100.6	0.53
Transverse	53.33 ± 37.9	
Descending	101.67 ± 106.8	
Sigmoid	107.78 ± 90.6	
Rectum	147.22 ± 81.25	
Preoperative therapy		
No therapy	100 ± 91.3	0.21
Received	146.4 ± 76.3	
Histologic variant		
Conventional	111.7 ± 90.1	0.31
Mucinous	54 ± 48.8	
Signet ring	155 ± 162.6	
Mucinous activity		
Present	82 ± 79.3	0.19
Absent	119 ± 93.5	
Histologic grade		
Low grade	105.1 ± 89	0.72
High grade	118.6 ± 101.4	
Perineural invasion		
Present	135.3 ± 89.6	0.1
Absent	90.2 ± 87.3	
Vascular invasion		
Present	121.8 ± 92.7	0.11
Absent	75 ± 76.8	
T Stage		
T2	126 ± 118.9	0.87
T3	132.5 ± 86.1	
T4	154.17 ± 104.1	
N stage		
N0	70.8 ± 71.9	0.02*
N1	121.4 ± 86.2	
N2	175 ± 107.08	
Clinical stage group		
I	42.5 ± 51.9	0.04*
II	77.81 ± 75.8	
III	136.4 ± 93.5	

TACC3: Transforming acidic coiled-coil-containing protein 3, *Statistically significant difference

Discussion

This study was designed to assess the suitability of using the immunohistochemical expression of TACC3 as a prognostic factor in colorectal carcinoma by comparing it to other clinicopathologic findings. We found that the mean IRS score of 45 CRC specimens was

70 ± 89.91, with significantly higher scores in those tumors with N2 stage and Stage III tumors. On the other hand, IRS showed no statistically significant relationship with the remaining clinical and histopathological parameters.

Physiologically, TACC3 is an important protein in the process of cellular division as it plays a key role in the formation of the mitotic spindle [17]. Pathologically, TACC3 expression was studied in a variety of tumors, being found to be an independent poor prognostic factor [18].

To the best of our knowledge, two studies considered TACC3 in CRC. One of them discussed its value as a poor prognostic factor [19]. The other was more specific, relating it to poor therapy response in rectal cancer [20]. The IRS was the accepted method for assessing TACC3 expression in both studies which is the same score used in our study. The method of calculation, however, was different. While the intensity of staining was assessed similarly in all studies, the amount of staining in our study was assessed as a percent of all tumor tissue with 5% increments. In Du's and Ma's studies, the amount of staining was given a score from 0 to 4. Moreover, the percentage of the amount of staining was multiplied by the intensity score. In their study or your study???. This is different from what we did in our study. In our study, we did not use a (0–4) score; we used the *percent of stained cells (0–100).* They added the amount score to the intensity score to give the final IRS. Subsequently, they classified tumors with IRS more than 5 as tumors with "high" expression and those with IRS of 5 or less as tumors with "low" expression. Why you use different method from them? We adopted the methodology, we believed to be more accurate in assessing immunohistochemical staining.

As in most studies, males were the dominant group in Du's and Ma's studies. They represented 56% and 64% out of 161 and 152 studied individuals, respectively. This was different from the sex distribution in our study, in which the females were more than males representing 60% of the study's population. The small size of our sample may be a reasonable explanation of this difference. Regardless of this difference, sex was not significantly related to TACC3 expression in all studies.

As for the age of the patients, our sample was similar to that of Du *et al*. and Ma *et al*. They included patients with a mean age of 53–55 years old, respectively, which is nearly similar to our mean age group (60 years old). This represents a similar age distribution across the different study groups. Moreover, they found no statistically significant correlation between TACC3 expression and age group. The anatomical distribution of CRC s in our study was different from that of Du *et al*. study [19]. Rectal tumors formed 20% of all cases in our study, while they formed nearly 40% in Du's study and in Ma's study [19], [20], which included only patients with rectal tumors. This may be explained by the different epidemiologic factors in each study population. In addition, the small sample size of our study may influence those results. However, the specific site of the tumor was found to be unrelated to TACC3 expression.

In our study, 16% of our included that patients were subjected to chemotherapy and/or radiotherapy preoperatively. On the other hand, all of the patients in Ma *et al*. [20] study received chemoradiotherapy before undergoing surgical excision. This may account for the differences between both studies' results. However, the question of our study regarding this point is completely different from that of Ma *et al*. [20]. We analyzed data to test whether TACC3 expression was different between those who received pre-operative therapy and those who did not, regardless of their response. On the other hand, Ma *et al*. wanted to know if the response to therapy can be reliably predicted using TACC3 expression. They found that those who had a stronger expression of TACC3 showed poorer responses to therapy. Although In Du *et al*. study [19], none of the study population received any form of pre-operative therapy, they recommended giving more aggressive therapy to those who have high TACC3 expression due to its correlation with poorer prognosis. Hence, TACC3 may be used as a prognostic factor to determine the response to therapy.

The adopted grading system differed between our study and the other studies. We adopted the simple and recently recognized two-tier system, where 84% of the cases were low grade and the other 7% were high grade. Du *et al*. [19] adopted the more traditional three-tier system with quite sufficiently similar findings, as 95% of his cases proved to be well or moderately differentiated, that is, low grade, while the rest of the cases were poorly differentiated, that is, high grade. Ma *et al*. [20] followed an even more complex four-tier system adding the undifferentiated category. Yet, the results were again similar; 85% of the cases here were well and moderately differentiated. In the three studies, regardless of the used grading, the differences in TACC3 expression between different tumor grades were not statistically significant.

The T3 stage was the most common stage among the cases in our study (62%)., A lesser number of cases were staged as T4 (27%), while T2 tumors were the least common (11%). None of our cases were excised at the T1 stage. In Du *et al*. study [19], T4 tumors were much more common forming more than half of the study population (55%). The T3 and T2 tumors were found in 25% and 20% of cases. The paucity of T1 and T2 tumors reflect the problem of early detection of CRCs.

Limitations

The sample size of each trial represents the major limitation. It was relatively small; which makes it difficult to give a representative result. Therefore, this should be considered in the upcoming trials. We recommend further studies on a larger number of patients of different ages,

and ethnic groups to confirm the prognostic role of TACC3 in CRC such as recurrence rates and survival rates.

Conclusion

Immunohistochemical expression of TACC3 would be valuable as a prognostic marker in cases of colorectal adenocarcinoma, where the expression was found to show stronger and more widespread expression in cases with higher stages. Furthermore, TACC3 should therefore be considered as a potential candidate for targeted therapy, where its blockade may hinder the tumor's ability to proliferate and progress.

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