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Clinical and pathological importance of TIGD3 gene expression in colorectal cancer patients

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ABSTRACT

Background: Advanced human cancers exhibit significant levels of TIGD3 expression. The purpose of this study was to look at possible associations between positive TIGD3 expression and clinical, pathological aspect in colorectal cancer patients.

Methods: In this cross-sectional investigation, TIGD3 expression levels were measured in 100 colorectal cancer tissues using western blot and immunohistochemical labeling. Following that, immunohistochemical-staining outcomes were contrasted with clinicopathological characteristics.

Results: In 82 out of 100 colorectal cancer samples, TIGD3 expression was shown to be present. TIGD3 expression was substantially correlated with higher stages of cancer TNM III-IV (p=000117), lymph vascular invasion (p=000045) and angiovascular invasion (p=00001), in contrast to other clinicopathological criteria.

Conclusion: The results of this investigation imply that elevated or positive TIGD3 expression may be linked to an advanced stage and a bad prognosis.

Keywords: cancer, clinical, colorectal, expression, pathological, TIGD3

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INTRODUCTION

The second leading cause of cancer-related death universally is colorectal cancer, which will account for 10% of all cancer cases in 2020 [1]. Similar to other cancers, colorectal adenocarcinoma develops through a number of pathways by which healthy endothelium cells become cancerous via genetic and epigenetic mechanisms [2]. The genetic instability of humans may be significantly influenced by neogenes, which are created through the molecular process of molecular domestication on DNA transposons. One of these Neogenes is the protein TIGD3, which belongs to the Tigger-derived (TIGD) family and has different functions in the human genome [3-7]. TIGD3 is one of the genes that have been linked to lung squamous cell carcinoma and may serve as a predictive biomarker [8]. TIGD3 was highly expressed in colorectal cancer cell lines derived from advanced stage of cancer [9].

Western blot and immunohistochemistry were used in this work to look at the tissues of colorectal cancer patients that exhibit the TIGD3 protein. The link between elevated expression of this protein and the clinicopathological features of the patients was then investigated.

MATERIAL AND METHODS

In this cross-sectional investigation, 100 colorectal adenocarcinoma samples of patients with colorectal cancer who underwent surgery between 2008 and 2010 were gathered from histopathology department, Trousseau hospital, Tours/France. These samples were formalin-fixed, paraffin-embedded, and attributed to colorectal cancer patients. A straightforward technique was used for sampling. All samples were examined using a checklist that includes demographic data, clinical observations, and histopathological results. The inclusion criteria were the presence of colorectal cancer and adequate pathology information. Insufficient pathology data is one of the exclusion criteria. It was assessed how each patient's clinical and pathological characteristics. Among the parameters for stratification were age, gender, the location and size of the tumor, the stage of tumor pathology, differentiation of the tumor, TNM stage, lymph node metastases, and vascular and lymphatic invasion. Two pathologists examined the slides of hematoxylin and eosin.

Obtaining proteins for the western blot method

Protein extractions from tissue samples: slices of tissue samples were cut using a cryostat and stored at -80°C. Similar to the method

originally developed by Arnaoty et al., protein lysates were made from these tissue slices. The Bradford technique was used to measure the protein extracts and kept at a freezing temperature [10].

Analysing proteins with acrylamide gel electrophoresis

Analyzing proteins with acrylamide gel electrophoresis SDS-PAGE, Arnaoty et al., had previously described this technique [10]. 50 micrograms of tissue-specific protein extracts were added for every well of a polyacrylamide gel.

Western Blot

The methodology was already thoroughly explained by Arnaoty et al. using a dilution of 1:250 of the anti-TIGD3 primary antibody (In Cell Art, Nantes, France) [10]. Then, for an additional hour at room temperature, a secondary antibody (Amersham, GE Healthcare) coupled to goat anti-mouse IgG-HRP was incubated. The membranes were then put through a chemiluminescence procedure for examination after that images were acquired using the FUGI LAS4000 imager (Amersham ECL Advance Western Blotting Detection Kit, GE Healthcare).

Immunohistochemical technique

Two FFPE blocks from each patient with colorectal cancer were chosen, and 10 μm thick slices from each block were put on poly l-lysine slides to study how well the TIGD3 gene is expressed. Slides were first deparaffinised, to do this, the slides were heated to 60°C for 15 minutes, followed by three 5-minute deparaffination cycles in xylene. After 5 minutes in distilled water and alcohols 70%, 90%, and 100%, the tissues were rehydrated. With the aid of sodium citrate buffer pH of 6, antigen retrieval was carried out for 20 min at 97°C. Using peroxidase 0.3% solution, endogenous peroxidase activity was blocked for 15 minutes after chilling. Blocking solution was applied for 15 minutes at room temperature after being washed twice in PBS to avoid background staining. The tissues were incubated with TIGD3 antibody (In Cell Art, Nantes, France) for 1 hour at room temperature following three washings in PBS solution for 5 min each. Goat anti Mouse IgG-HRP (Amersham, GE Healthcare) secondary antibody incubation and DAB staining were carried out after a PBS wash. Tissue dehydration was carried out in accordance with usual method after counterstaining with hematoxylin dye. Negative control samples were those in which primary antibody incubation had been removed.

Characterizing of TIGD3 expression

Two seasoned pathologists blindly examined the slides to look for immune staining patterns and intensities. In over 96% of the instances, the outcomes were related. When the remaining cases were examined again, there was only one possible explanation. Each slide's stromal and inflammatory cells were recognized as positive internal controls and given a +2 score, after which the degree of staining of the cancer cells was contrasted with them. When the staining intensity matched the positive control, it was given a score of +2, when it declined or lesser than that of the internal control, it received a score of +1, and when there was no immunostaining at all, it received a score of negative. A score of +3 was assigned to immunostaining that was more intense than positive control cells. Given the heterogeneity of cancer cells, positive results were determined when more than 12% of cancer cells were stained, regardless of intensity (Figure 1).

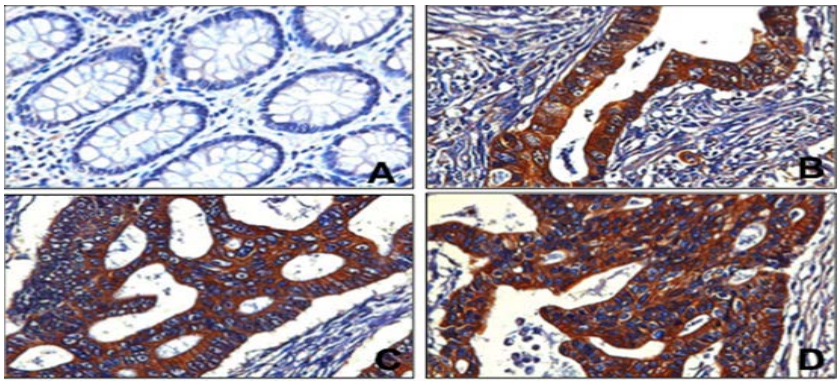

Fig. 1. Immunohistochemistry expression pattern of TIGD3 in normal mucosa and stages I–III colorectal carcinoma (CRC). A) Negative TIGD3 in epithelial normal mucosa; B) Positive TIGD3 in cancer of stage I CRC; C) Positive TIGD3 in cancer of stage II CRC; D) Positive TIGD3 in cancer of stage III CRC

Statistical analysis

All factors that are related to the expression of the TIGD3 gene and clinical plus pathological components of the patients such as gender, tumor location, a subtype of histopathology, pathological tumor stage, differentiation of tumor, lymphatic and vascular invasion, number of lymph nodes metastasis, TNM stage, A p-value of 0.05 or below was regarded statistically significant when doing the statistical analysis using SPSS 20 (SPSS Inc., Chicago, Ill, US).

RESULTS AND DISCUSSION

TIGD3 Expression by western blot

When anti-TIGD3 antibody (In-Cell-Art, Nantes, France) was used, each sample examined (transfected Hela, normal colon tissue, and stage I–III colorectal cancer tissue) exposed a single TIGD3 expression product with a molecular weight of 52 kDa, the same as the TIGD3 transposase [11, 12]. In contrast to colorectal cancer, which exhibits strong expression, particularly as the stage of the disease progressed from stage I to stage III, TIGD3 expression was observed to be very low or absent in normal colon tissue as illustrated in Figure 2.

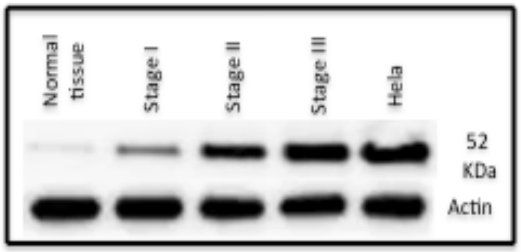

Fig. 2. TIGD3 western blot analysis

Tab. 1. The pathological and clinical features of 100 colorectal adenocarcinomas were associated with the level of TIGD3 expression

Clinico-pathologic Feature	TIGD3 Expression	
	Positive %	Negative %
Gender		
Male	39 (36.4) [0.19]	17 (19.6) [0.34]
Female	26 (28.6) [0.24]	18 (15.4) [0.44]
Age		
<60	21 (24.32) [0.45]	17 (13.68) [0.81]
≥ 60	43 (39.68) [0.28]	19 (22.32) [0.49]
Tumor location		
Proximal colon	19 (21.12) [0.21]	14 (11.88) [0.38]
Distal colon	28 (26.24) [0.12]	13 (14.76) [0.21]
Rectum	17 (16.64) [0.01]	9 (9.36) [0.01]
Size of tumor (cm)		
<5	22 (20.35) [0.13]	15 (16.65) [0.16]
≥ 5	33 (34.65) [0.08]	30 (28.35) [0.1]
PN stage		
PN0	32 (36.58) [0.57]	30 (25.42) [0.83]
PN1-2	27 (22.42) [0.94]	11 (15.58) [1.35]
PT stage		
PT1-2	13 (12.16) [0.06]	6 (6.84) [0.1]
PT3-4	51 (51.84) [0.01]	30 (29.16) [0.02]
Differentiation		
Well	38 (36.48) [0.06]	19 (20.52) [0.11]
Moderate	18 (20.48) [0.30]	14 (11.52) [0.53]
Poor	8 (7.04) [0.13]	3 (3.96) [0.23]
Mucinous component		
Absent	48 (46.97) [0.02]	29 (30.03) [0.04]
Present	13 (14.03) [0.08]	10 (8.97) [0.12]
Lymphovascular invasion		
Absent	40 (30) [3.33]	20 (30) [3.33]
Present	10 (20) [5]	30 (20) [5]
Angiovascular invasion		
Absent	20 (31.5) [4.2]	50 (38.5) [3.44]
Present	25 (13.5) [9.8]	5 (16.5) [8.02]
TNM stage		
I-II	48 (39) [2.08]	12 (21) [3.86]
III-IV	17 (26) [3.12]	23 (14) [5.79]

Tab. 2. The pathological and clinical features of 100 colorectal adenocarcinomas associated with the level of TIGD3 expression (statistical relations)

Clinico-pathologic Feature	chi-square statistic	p-value	Significance
Gender	1.2059	0.272138	Not significant p>0.05
Age	2.0306	0.154163	Not significant p>0.05
Tumor location	0.9407	0.624794	Not significant p>0.05
Size of tumor	0.4719	0.492114	Not significant p>0.05
PN stage	3.6806	0.55049	Not significant p>0.05
PT stage	0.199	0.655535	Not significant p>0.05
Differentiation	1.3738	0.503143	Not significant p>0.05
Mucinous component	0.2518	0.615808	Not significant p>0.05
Lymph vascular invasion	16.6667	0.000045	Significant p<0.05
Angio-vascular invasion	25.4449	0.00001	Significant p<0.05
TNM stage	14.8352	0.000117	Significant p<0.05

DISCUSSION

Choosing a treatment plan that will be as successful as feasible requires finding biomarkers that affect patients' clinical results. Identification of the markers that influence illness prognosis or lead to treatment resistance is the goal of research on targeted therapy [12]. The relevance of the TIGD3 gene in the development or progression of cancer is still not fully understood. There are no data available that explain TIGD3 expression in human cancer tissue.

In previous research done on colorectal cancer cell lines by the western blot technique was used to examine TIGD3 expression [9]. Our western blot results revealed that TIGD3 expression increased from normal colon tissue to advanced stage of colorectal cancer. This outcome is consistent with past study on colorectal cancer cell lines, they discovered that colorectal cancer cell lines from advanced stages of the illness showed elevated expression of TIGD3 when compared with healthy colon tissue by western blot approach [9]. In this study, immunohistochemical findings showed that 82% of tumors expressed TIGD3 in a positive manner. No data are available to compare our findings with about the immunohistochemistry-based TIGD3 expression profile in colorectal cancer. Additionally, we did this study for the first time and could not find any prior research that showed a connection between TIGD3 expression and clinicopathological characteristics in colorectal cancer. Our study showed a relationship between elevated TIGD3 expression and lymph vascular, angiovascular invasions of colorectal cancer as well as advanced tumor stage TNM III-IV. Illustratable findings from our study include a link between high TIGD3 expression and tumor progression (higher stage) and metastasis of colorectal cancer.

Because positive changes in TIGD3 expression would promote cell proliferation, delay apoptosis, and increase tumor aggressiveness. Our research revealed that the amount of TIGD3 expression was not substantially influenced by age, size of tumor, gender, tumor location, mucinous component, pathological tumor stage and differentiation.

CONCLUSION

According to our research, increasing or high levels of TIGD3 expression may be related to tumor growth, and positive TIGD3 expression has been associated with a poorer prognosis and a higher tumor stage. To completely corroborate these findings, another study needs be conducted with bigger sample sizes.

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ETHICAL CLEARANCE

The Trousseau hospital in Tours, France, histopathology department provided samples for the subjects who volunteered to take part in this kind of research.

CONFLICT OF INTEREST

There are no known conflicts of interest from the author.

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