Tropical Journal of Pharmaceutical Research October 2024; 23 (10): 1599-1604
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v23i10.2

Original Research Article

TAG-72 expression has no clinical significance in colorectal cancer

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Sent for review: 14 May 2023 *Revised accepted: 7 October 2024*

Abstract

Purpose: *To determine the expression patterns of TAG-72 and its association with factors as an index of its clinical significance and function in the etiology of colorectal cancer (CRC).*
Methods: *This retrospective study involved histopathological reports, archival blocks, and slides of patients with CRC at King Abdulaziz University Hospital. Immunohistochemistry procedures were carried out utilizing TAG-72 monoclonal antibody and tissue microarray (TMA) slides of 155 CRC cases and 33 specimens of normal colon tissue.*
Results: *TAG-72 was found in 55 (35.5 %) of CRC cases, out of which 13 (8.1 %) cases showed weak immunostaining, while 8 (24.2 %) of the 33 normal colon mucosal specimens showed diffuse-to-moderate TAG-72 levels. Cytoplasmic staining was seen in each positive case in CRC and healthy colon mucosa. However, no correlation was noted between TAG-72 and any of the parameters. The Kaplan-Meier survival curves and Cox proportional hazards model did not show differences in survival behavior.*
Conclusion: *TAG-72 has no association with clinicopathological parameters of CRC, and has no diagnostic and prognostic values in CRC.*

Keywords: *TAG-72, Colorectal cancer, Immunohistochemistry*

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INTRODUCTION

Colorectal tumors are one of the top three cancers all over the world and are major causes of cancer-related death [1]. One million, four hundred thousand (1,400,000) cases of CRC are diagnosed each year, with 50 % mortality rate [2]. Amongst the Saudis, approximately 1,465 CRC cases were recorded in 2015, accounting for 12.2 % of freshly diagnosed cancers [3]. Adenocarcinoma was the most reported histologic type of colorectal cancer, followed by mucinous cancer, signet ring cell cancer, and others [3].

The management protocol of colorectal cancer is mainly established through data of patients, including TNM stage, grade, infiltration, and lymph node metastasis [4]. Most cases of colorectal cancer are probably curable through tumor resection [5]. However, about thirty percent of stage I to III CRC cases and almost

sixty-five percent of stage IV cases may recur, and most advanced CRC patients have poor prognosis [6]. Nevertheless, this procedure does not exactly influence patient survival, perhaps due to heterogeneity of the disease and heterogeneity of patients. This makes CRC a main medicinal challenge. Therefore, the discovery of new predictive biomarkers for classifying CRC cases into low and high-risk groups is essential for evolving strategies for efficient and personalized CRC therapy [4].

Tumor-associated glycoprotein 72 (TAG-72) is a 1000 kDa mucin-like oncofetal antigen [7]. This antigen has been found on the surface of malignant cells of several human tumors, including breast, ovary, uterus, lung, pancreas, and stomach cancers [8-13]. Nonetheless, it is sometimes but not frequently found in non-cancerous and normal tissues [14,15]. This glycoprotein is found in more than eighty percent of CRC cases, with comparatively much less presence in normal mucosa expression [16]. Earlier reports revealed about 40 % increase in serum TAG-72 levels in gastric cancer and CRC patients. The TAG-72 level has been significantly correlated with tumors at higher stages [17]. Furthermore, it was noticed that almost 50 % of the colorectal cancer cases with untraceable levels of CEA showed noticeable levels of TAG-72 [18]. This study aimed to examine the expression patterns of TAG-72 and its association with clinical indices to determine if TAG-72 has relevance and function in the etiology of CRC.

METHODS

This retrospective study which was based on paraffinized tissue blocks retrieved from pathology department archives, received ethical approval from the Unit of Biomedical Ethics at King Abdulaziz University (approval no. 1127-13), and it was conducted in accordance with the guidelines of the Helsinki Declaration [19]. This manuscript was prepared following the standards for reporting diagnostic accuracy studies (STARD) guidelines.

Samples collected comprised paraffinized tissue blocks from 155 CRC cases and 33 healthy colon mucosa specimens from patients screened for non-CRC conditions. Care was taken to eliminate blocks from patients treated with chemotherapy**.** The blocks were sliced into 4 µm sections and subjected to routine staining with hematoxylin and eosin (H&E), followed by reassessment. Biodata and detailed medical records on CRC patients were obtained from the King Faisal Specialist Hospital & Research Center medical records. The CRC staging was consistent with the WHO guidelines.

Tissue microarray construction

The tissue blocks (155 CRC and 33 normal) were processed into Tissue Microarray (TMA) as described previously [20]. Then, the TMA blocks were sectioned into 4 µm slices and coated with aminosilane.

Immunohistochemistry staining procedure

This protocol was carried out using BenchMark Autostainer, as reported previously [21]. The TAG-72 polyclonal immunoglobulin (Cell Marque, CA, USA; 1:100 dilution) was subjected to immunohistochemistry staining and visualized, with lung adenocarcinomas slide as +ve control. A slide treated with Tris buffer in place of TAG-72 antibody was employed as negative control. A brown coloration in the cytoplasm of more than 5 % of cancer cells was recorded as positive. The results of immunohistochemical staining were analyzed by 2 pathologists.

The population of positively-stained cells was estimated using a quasi-quantitative method at x40 magnification, and the intensity of TAG-72 staining was graded 1, 2 and 3 (positive), as a function of degree of staining. Negative stain was graded 0.

Statistical analysis

Data was processed using IBM-SPSS version 21. Results are presented as ratios and incidences. The association between CRC and TAG-72 levels was assessed with χ^2 and Fisher tests. The relevance of clinical indices for CRC prognosis was determined with Cox Proportional Hazards model. The Kaplan-Meier survival curve was used to analyze the influence of TAG-72 staining intensity on survival patterns. Values of $p < 0.05$ indicated significant differences.

RESULTS

The TAG-72 expression levels in the 155 colorectal cancer cases, and their clinicopathological characteristics, are displayed in Table 1. Twenty-one cases showed lympho-vascular invasion; 63 cases revealed involvement of lymph nodes, while 42 tumors had remote metastases. In this study, TAG-72 was found in 55 (35.5 %) cases of CRC, out of which 13 (8.1 %) cases showed weak immunostaining (Figures 1A and 1B). Sixty percent of positive tumors displayed immunostaining in less than half of tumor cells,

while only 8 out of 33 healthy colon mucosal specimens (24.2 %) showed faint-to-moderate TAG-72 levels. All the positively stained cases of cancerous and healthy mucosa showed staining in the cytoplasm.

Regarding the stage of CRC, 81 % of positive cancer cases were of high stages (3 and 4), but there was no significant correlation between TAG-72 and CRC stage (p = 0.750; Table 1). The TAG-72 phenotype did not significantly correlate with tumor differentiation (p = 0.060) and tumor site (p = 0.695). Furthermore, the results did not reveal any substantial statistical relationship between TAG-72 expression and tumor size, invasion of lymph nodes, invasion of blood vessels, remote metastasis, recurrence, or relapse. Moreover, TAG-72 was not a predictor of survival (Table 2). The Kaplan-Meier curves did not reveal any marked variations in survival (Figure 2).

DISCUSSION

A few studies have described TAG-72 immunohistochemistry in colorectal cancers and reported the association between its expression patterns and parameters of CRC. Nevertheless, the relationship between TAG-72 phenotype and colorectal cancer has not been elucidated, and the results of these studies remain controversial.

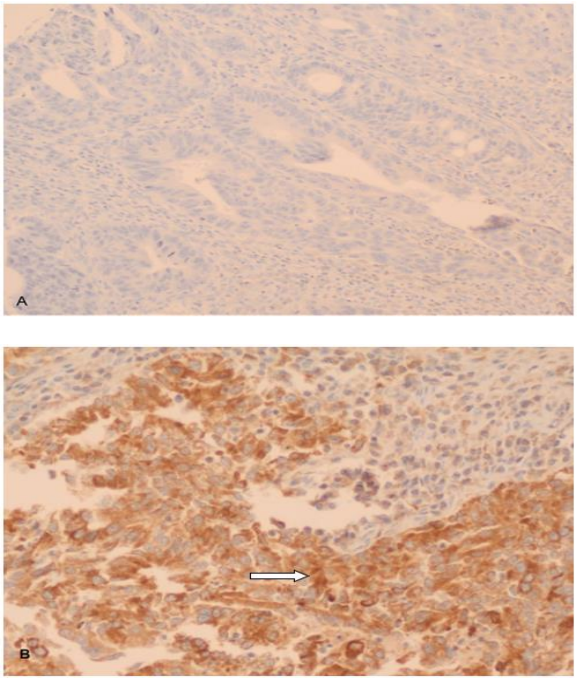

Figure 1: TAG-72 immunostaining pattern in colorectal cancer. (A) negative staining (x20); (B) moderate-to-strong positive staining as the white arrow indicates (x20)

Table 1: TAG-72 expression levels in the 155 CRC cases and their clinical features

		TAG-72 Immunostaining				
		Negative		***Positive***		
Parameter	**Item**	*n*	*(%)*	*n*	*(%)*	*P-value*
Group	Colorectal cancer	100	(64.5)	55	(35.5)	0.107
	Non-cancerous	25	(75.8)	8	(24.2)	
Differentiation	Well-differentiated	20	(57.1)	15	(42.9)	0.060
	Mod differentiated	72	(70.6)	30	(29.4)	
	Poorly differentiated	8	(44.4)	10	(55.6)	
Tumor site	Right colon	25	(61.0)	16	(39.0)	0.695
	Left colon	65	(67.0)	32	(33.0)	
	Rectum	10	(58.8)	7	(41.2)	
Tumor Size	< 5cm	49	(73.1)	18	(26.9)	0.050
	≥ 5cm	51	(58.0)	37	(42.0)	
Tumor stage	1	2	(100.0)	0	(.0)	0.750
	2	19	(65.5)	10	(34.5)	
	3	73	(64.0)	41	(36.0)	
	4	6	(60.0)	4	(40.0)	
Lymphovascular invasion	Negative	88	(65.7)	46	(34.3)	0.448
	Positive	12	(57.1)	9	(42.9)	
Serosa Resected Margin	Negative	96	(64.9)	52	(35.1)	0.676
	Positive	4	(57.1)	3	(42.9)	
Lymph Node Metastasis	Negative	55	(64.7)	30	(35.3)	0.458
	Positive	39	(61.9)	24	(38.1)	
Distant Metastasis	Negative	74	(65.5)	39	(34.5)	0.679
	Positive	26	(61.9)	16	(38.1)	
Local recurrence	Negative	70	(65.4)	37	(34.6)	0.725
	Positive	30	(62.5)	18	(37.5)	
Relapse	Negative	65	(65.7)	34	(34.3)	0.693
	Positive	35	(62.5)	21	(37.5)	

Table 2: Means and medians for survival time

Tag-72 Immunostaining	Mean[a]				Median			
	Estimate	*Standard Error*	*95% confidence interval*		*Estimate*	*Standard error*	*95% confidence interval*	
			Lower bound	*Upper bound*			*Lower bound*	*Upper bound*
0	116.656	11.071	94.958	138.355	166.000	0.000	0.000	0.000
1+2	70.991	9.063	53.228	88.754	0.000	0.000	0.000	0.000
Overall	110.115	9.824	90.861	129.370	166.000	0.000	0.000	0.000

Note: a. Estimation is limited to the largest survival time if it is censored

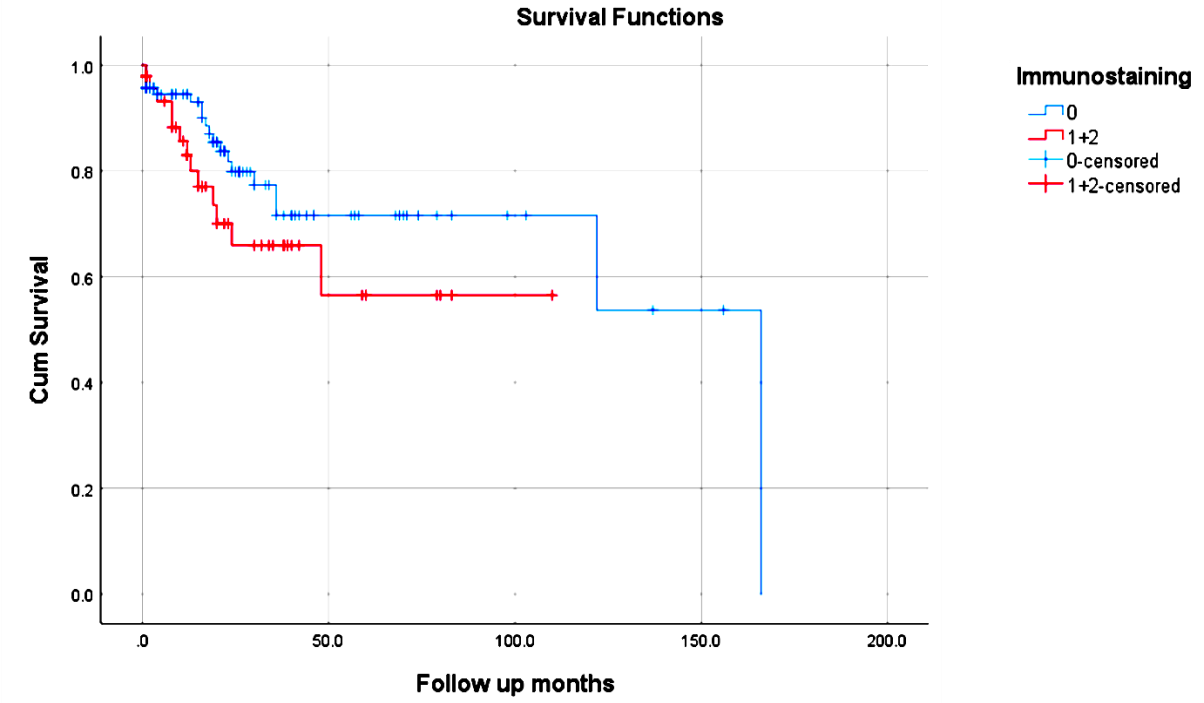

Figure 2: Kaplan-Meier survival curves by various clinicopathological variables with Tag-72 immunostaining in colorectal adenocarcinoma

In a study, Xu *et al* [22] showed that TAG-72 was strongly expressed in all 21 specimens of transitional mucosa and 17 (81 %) colorectal cancer specimens. Staining was strong and homogeneous in transitional mucosa, whereas it was weaker and more heterogeneous in malignant tissues. Based on these data, Xu *et al* proposed that the expression of TAG-72 increases in the early phases of the transformation process in the colon, but becomes down-regulated in later phases [22].

Listrom *et al* [23] reported that although TAG-72 appeared in transformed cells of colorectal tumors and the benign specimens, different TAG-72 phenotype patterns may characterize the tumor development process. The staining pattern in colorectal carcinomas was observed to be located at the luminal surface, and it was also diffusely cytoplasmic [23]. In a recent study that included only samples from patients with stages II and III TNM, Cho *et al* reported that TAG-72 was significantly correlated with microsatellite stable neoplasms, invasion of veins and lymph vessels, and lymph node metastasis. Cases with high TAG-72 expression levels had short survival times, and the expression of TAG-72 appeared to be an independent prognostic factor [23].

On the other hand, the results reported by Guadagni *et al* [20] showed a substantial increase in TAG-72 expression (about 10-fold) in the malignant cells of colorectal cancer, when compared with the phenotype of TAG-72 of normal mucosa from the same CRC cases. The TAG-72 expression of normal mucosa from colorectal cancer patients was five times greater than the corresponding expression in the healthy control group. However, there was no significant relationship between the phenotype of TAG-72 and the tumor clinicopathological factors [24]. The results from this study are consistent with the findings of Guadagni *et al*, which confirms that TAG-72 has no clinical significance in

diagnosis, prognosis, and overall survival of colorectal cancer patients [20].

The discrepancies between the present study and previous ones, with respect to the results on the connection between the expression of TAG-72 and clinicopathologic factors, may be due to variations in histological types of cancers, differences in tumor stages, immunoglobulin and/or methodology sensitivity, and differences among subjects and/or number of specimens. On the other hand, it may be that TAG-72 has no specific role in cancer etiology and prognosis.

Limitations of the study

This study had some limitations such as comparatively low population size and weak quantitative technique. Therefore, there is a requirement for additional and more comprehensive investigations on the relevance of this glycoprotein (TAG-72) in cancer pathogenesis, diagnosis and outcomes.

CONCLUSION

This study has shown that TAG-72 has no association with the parameters of CRC and may not be relevant as a diagnostic and prognostic index in CRC.

DECLARATIONS

Acknowledgment

The authors thank the Deanship of Scientific Research (DSR) at King Abdulaziz for their technical and financial support.

Funding

This project was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University (KAU), Jeddah, under grant number (G-535-140-1441).

Ethical approval

Ethical approval was received from the Unit of Biomedical Ethics at King Abdulaziz University (approval no. 1127-13).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the contents of this article will be borne by the authors. Jaudah Al-Maghrabi: study design, acquisition of data, analysis, and interpretation of data; Mohamad Nidal Khabaz: corresponding author, analysis and interpretation of data, article draft, revised article critically.

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