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The prognostic role of tumor associated glycoprotein 72 (TAG-72) in stage II and III colorectal adenocarcinoma

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ABSTRACT

Tumor associated glycoprotein 72 (TAG-72) is a membrane-bound glycoprotein complex that is overexpressed in many adenocarcinomas. Recently, monoclonal antibody targeting TAG72, minretumomab, have been introduced as a potential therapeutic target in colorectal cancers (CRC) as well as breast and lung cancers. However, the detailed expression profile of TAG72 and its prognostic effect in CRC are not clear yet. We investigated the relationship between tumor associated glycoprotein 72 (TAG-72) expression and clinicopathologic characteristics in CRC using 3E8 antibody, a fully humanized antibody with the highest affinity to TAG-72. Immunohistochemical staining for TAG-72 was performed in 578 CRC patients, and the results were analyzed using a modified Remmele scoring system (score: 0–12). Of the 578 patients, 144 (24.9%) composed the TAG-72 overexpression (TAG-72^{high}) group. TAG-72^{high} was significantly associated with microsatellite stable tumor (P = .002), lymphatic invasion (P = .001), venous invasion (P = .005), and high pN status (P < .001). In survival analyses, TAG-72^{high} group showed shorter disease-free survival in univariate analysis (P = .001), and TAG-72^{high} was found to be an independent prognostic factor in multivariate analysis (P = .028), in addition to TNM stage. In conclusion, TAG-72 is thought to be the factors involved in the progression of CRC and may be considered as one of the potential therapeutic target.

1. Introduction

Colorectal cancer (CRC) is the third most common cancer in men and the second most common in women worldwide [13]. About 20–30% of initially diagnosed CRC patients present with unresectable metastatic disease. The remaining 70–80% newly diagnosed with CRC have localized disease that is amenable to curative surgical resection [28]. However, a substantial proportion of patients (40–50%) experience disease recurrence or development of metastasis after curative resection [14]. In light of this, following curative resection, adjuvant chemotherapy with cytotoxic agents is recommended as standard clinical practice in advanced CRC patients [3]. Recently, the number of targeted agents used in various malignancies has increased dramatically. Currently, there are seven United States Food and Drug Administration (FDA)-approved targeted agents (bevacizumab, cetuximab, panitumumab, ramucirumab, aflibercept, regorafenib, and trifluridine/tipiracil) in metastatic CRC, with many more under development and/or in clinical trials [7,33]. However, CRC remains the fourth leading cause of cancer death in the world [13], and uncovering of new therapeutic target is needed.

Tumor associated glycoprotein 72 (TAG-72) is a membrane-bound glycoprotein complex with the properties of a mucin [24] that is overexpressed in many adenocarcinomas occurring in the colon, stomach, esophagus, ovary, pancreas, breast, and lung, but is not expressed in most normal tissues, except for the endometrium during the secretory phase and fetal tissue [4,6,23,25,31,32,36,41]. TAG-72 is expressed in 80% of CRC, with relatively little expression in the normal mucosa [43]. In analysis of ulcerative colitis patients, the expression of

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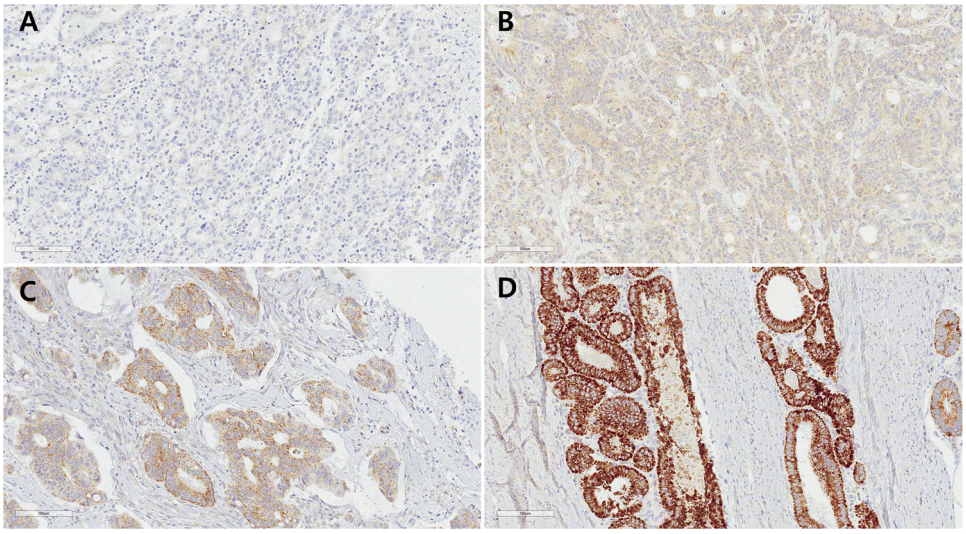

Fig. 1. Representative photographs of TAG-72 immunohistochemistry according to intensity score: (A) Score 0 (B) Score 1 (C) Score 2 (D) Score 3.

TAG-72 was associated with the duration of disease and the degree of dysplasia [42]. The employment of anti-TAG-72 monoclonal antibodies has been studied in preclinical animal models as well as in humans for cancer detection based on their high specificity against cancer antigens in various solid cancers. Monoclonal antibodies targeting TAG-72 have been used in surgery for detection of occult tumor [15,21,29,45]. In particular, the combination with CEA has increased the usefulness of TAG-72 [19,20,34,38]. Another monoclonal antibody targeting TAG-72, minretumomab, have been introduced as a potential therapeutic target in CRC as well as breast and lung cancers [37]. Despite these recent advances, however, the detailed expression profile of TAG-72 and its prognostic effect in CRC are not clear yet.

In this study, we investigated TAG-72 expression in CRC using newly developed 3E8 antibody, a fully humanized antibody with the highest affinity to TAG-72, and analyzed the correlations with clinicopathological characteristics. Furthermore, we analyzed the relation between TAG-72 expression and prognosis of CRC patients.

2. Materials and methods

2.1. Patient selection

Patients who underwent surgery for primary CRC from June 2008 to May 2009 at Samsung Medical Center, Seoul, Korea, were eligible for this study. Among them, we excluded patients with American Joint Committee on Cancer (AJCC) stage I or IV, and patients who had received preoperative chemotherapy and/or radiation therapy, and those who had any other uncontrolled cancer at the time of diagnosis of CRC. Following exclusion, 578 consecutive patients with AJCC stage II or III CRC were selected. Four hundred fifty-two patients had received postoperative adjuvant chemotherapy and 73 of them received concurrent radiotherapy [3]. Forty-five patients refused adjuvant therapy for several reasons, no records of adjuvant therapy were available in 81 patients. The mean follow-up period was 56.6 ± 14.3 months. Clinical data such as demographic features, tumor characteristics, and treatment outcomes were obtained by reviewing patient medical records using the intranet resources of Samsung Medical Center. Information on deaths was provided by the national statistical office. Tumor stage was defined according to TNM classification described in the seventh edition of the AJCC cancer staging manual [1]. The institutional review board of Samsung Medical Center approved this study (IRB Number: 2015-08-035), and waived the informed consents.

2.2. Tissue microarray construction

Tissue microarrays (TMA) were constructed using a manual tissue arrayer (Beecher Instruments, Sun Prairie, WI, USA). Two tissue cores with a diameter of 2 mm each were obtained from the most representative tumor areas of formalin-fixed, paraffin-embedded tissue blocks and were arranged in TMA blocks. Hematoxylin and eosin staining of the TMA sections was performed for tissue confirmation.

2.3. Immunohistochemical staining and evaluation

The 3E8 antibody is a fully humanized antibody with the highest affinity to TAG-72, produced by random mutagenesis of HCDR3 (heavy chain complementarity-determining regions residues 3) followed by affinity selection [44]. For the purposes of the current study, this antibody was generously provided by ViroMed Co., Ltd. (Seoul, Korea). The production procedure used is briefly described as follows: CHO cell line stably expressing 3E8 antibody was cultured in serum-free media (CD FortiCHO medium, Thermo Fisher Scientific). The culture supernatant of this cell line was harvested and subjected to affinity chromatography on a HiTrap MabSelect SuRe (GE Healthcare Life Sciences). The integrity and purity of the purified antibody were determined by SDS-PAGE. For quantification of the purified antibody, an optical density of 1.56 at 280 nm was used for a protein concentration of 1 mg/ml.

Immunohistochemistry(IHC) was performed on formalin-fixed, paraffin-embedded, 4-μm-thick tissue sections. After antigen retrieval with pH 8.0 buffer in a 97 °C waterbath (K8004; Dako Denmark A/S, Glostrup, Denmark) and endogenous peroxidase blocking for five minutes, the sections were incubated with an anti-TAG-72 antibody (3E8, diluted 1:1000) for 60 min at room temperature. Antigen-antibody chromogenic reactions were detected using a catalyzed signal amplification system using Code K1500 15 mL (Dako Denmark A/S, Glostrup, Denmark).

For evaluation of IHC of TAG-72, we used the 12-point Remmele scoring system (score: 0–12) [35]. To calculate the score, one of the numbers 0, 1, 2, or 3 is chosen according to the intensity of the stain, and one of the numbers 0, 1, 2, 3, or 4 is chosen according to the percentage of positive tumor cells (Fig. 1). The final score is calculated by multiplying the number reflecting the stain intensity by the number reflecting the percentage of positive tumor cells, with a maximum score of 12 (3 × 4). When there is heterogeneity in one TMA core, each intensity score is multiplied by its corresponding proportion score, and the values are summed. For example, if one core has 25% intensity

score 3, 50% intensity score 2, and 25% intensity score 0, the final score is 7 [(3 × 1) + (2 × 2) + (0 × 1)]. We evaluated two TMA cores for every patient and calculated the mean value. The IHC slides were interpreted independently by two pathologists (JC and SYH). In cases of disagreement, the final interpretation was determined by consensus using a multi-head microscope.

2.4. Statistical analysis and cutoff value setting

Statistical analyses were performed using the SPSS statistical software program (IBM Corporation, Armonk, NY, USA). Chi-square tests (Pearson's chi-square test or chi-square test using linear by linear association) were used to analyze correlations between IHC results and clinicopathologic parameters. P values < 0.05 were considered to be statistically significant. Survival curves were plotted using the Kaplan–Meier method, and the significance of differences between survival curves was determined using the log-rank test. Univariate survival analyses were performed using the log-rank test, and multivariate survival analyses were performed using the Cox proportional hazards regression model.

Survival analysis by Kaplan–Meier method was performed for all values at intervals of 0.5 points from score 3 to score 9 to find out the most appropriate cutoff value for separating the TAG-72-overexpression (TAG-72^{high}) and TAG-72-underexpression (TAG-72^{low}) groups. Based on this preliminary analysis, patients with a score of 6 or more were assigned to the TAG-72^{high} group.

3. Results

The clinicopathological characteristics of the 578 CRC patients are summarized in Table 1. The mean age was 59.2 (range, 28–90). The ratio of male to female was 1.6:1. The distribution of TAG-72 score of all cases is depicted in Fig. 2. Most cases were distributed in the range of 2–7 points. Among the 578 CRC patients, TAG-72^{high} was 144 (24.9%) and TAG-72^{low} was 434 (75.1%). The overexpression of TAG-72 was associated with tumor located in the left colon (P = .027), non-mucinous histology (P = .005), microsatellite stable tumor (P = .002), lymphatic invasion (P = .001), venous invasion (P = .005), high pN status (P < .001), and high TNM stage (P < .001). Age, sex, perineural invasion, and pT status did not show significant correlation with TAG-72 expression in CRC (Table 1).

Five-year disease-free and overall survival rates were 83.5% and 92.5%, respectively. In univariate survival analysis, TAG-72^{high} was associated with shorter disease-free survival (DFS) (P = .001) (Fig. 3). In multivariate analysis, TAG-72^{high} remained an independent prognostic factor for DFS (hazard ratio (HR) = 1.781, P = .028) with adjuvant therapy (HR = 0.426, P = .020) and TNM stage III (HR = 1.802, P = .029). In both univariate and multivariate survival analyses, TAG-72 expression was not significantly related with overall survival (OS) (univariate, P = .271; multivariate, P = .691) (Table 2).

4. Discussion

In recent years, the progress in precision medicine has brought about a new era of cancer treatment [2]. The exploration of novel biomarkers is one of the most important parts of precision medicine, and numerous diagnostic, predictive, and prognostic biomarkers have been proposed and investigated with tremendous interest. Various biomarkers are currently used in CRC [5], *BRAF* [10], microsatellite instability (MSI) [12], *KRAS* [9,11], *NRAS* [9,11], *PIK3CA* [9], *EGFR*

Table 1
Correlation between TAG-72 expression and clinicopathological features of colorectal cancer patients.

		TAG-72				Total (%) (N = 578)		*P* value
		Low (%) (N = 434)		High (%) (N = 144)				
Age								.940
	60 <	197	(45.5)	65	(45.1)	262	(45.4)	
Sex								.897
	male	268	(61.9)	90	(62.5)	358	(62.0)	
Location*								.027
	right colon†	83	(19.1)	17	(11.8)	100	(17.3)	
	mid colon†	45	(10.4)	9	(6.3)	54	(9.3)	
	left colon†	306	(70.5)	118	(81.9)	424	(73.4)	
Histology								.005
	mucinous	49	(11.3)	5	(3.5)	54	(9.3)	
MSI†								.002
	high	28	(6.6%)	0	(0.0)	28	(5.0)	
Lymphatic invasion								.001
	present	101	(23.3)	53	(36.8)	154	(26.6)	
Venous invasion								.005
	present	50	(11.5)	30	(20.8)	80	(13.8)	
Perineural invasion								.864
	present	32	(7.4)	10	(6.9)	42	(7.3)	
pT status								.163‡
	1	10	(2.3)	0	(0.0)	10	(1.7)	
	2	20	(4.6)	6	(4.2)	26	(4.5)	
	3	353	(81.3)	119	(82.6)	472	(81.7)	
	4	51	(11.8)	19	(13.2)	70	(12.1)	
pN status								< .001‡
	0	264	(60.8)	43	(29.9)	307	(53.1)	
	1	138	(31.8)	72	(50.0)	210	(36.3)	
	2	32	(7.4)	29	(20.1)	61	(10.6)	
TNM								< .001‡
	II	264	(60.8)	43	(29.9)	307	(53.1)	
	III	170	(39.2)	101	(70.1)	271	(46.9)	

TAG-72, tumor associated glycoprotein 72; MSI, microsatellite instability.
* Right colon, cecum-ascending colon; mid colon, hepatic flexure-splenic flexure; left colon, descending colon-rectum.
† MSI was evaluated by immunohistochemistry, MLH1, MSH2, MSH6 and PMS2. MSI was not evaluated in 13 patients. (N = 565).
‡ linear-by-linear association.

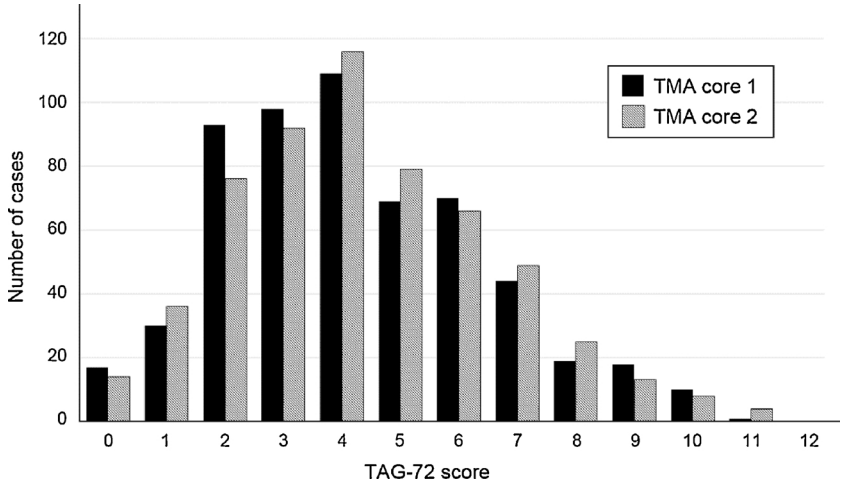

Fig. 2. The distribution of the score of tumor associated glycoprotein 72 expression in each 2 tissue microarray cores.

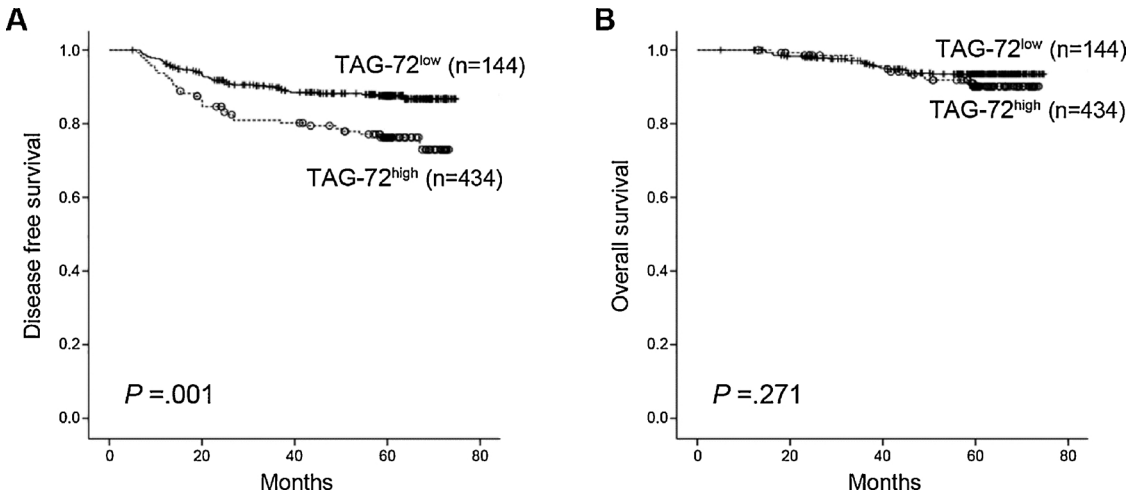

Fig. 3. Kaplan-Meier survival curves for tumor associated glycoprotein 72 expression in 578 colorectal cancer patients; (A) Disease free survival (B) Overall survival.

Table 2
Univariate and multivariate survival analyses in 578 colorectal cancer patients.

	Disease-free survival				Overall survival			
	Univariate *P* value	Multivariate HR	95% CI	*P* value	Univariate *P* value	Multivariate HR	95% CI	*P* value
Adjuvant therapy	.075			.020	< .001			< .001
None		1				1		
CTx. and/or RTx.		0.426	0.208-0.873			0.156	0.064-0.382	
TAG-72	.001			.028	.271			.691
Low		1				1		
High		1.781	1.065-2.978			0.833	0.339-2.049	
TNM stage	.002			.029	.008			.011
II		1				1		
III		1.802	1.062-3.057			3.186	1.310-7.749	

HR, hazard ratio; CI, confidence interval; CTx, chemotherapy; RTx, radiotherapy.

[39], *MET* [39], *ERBB2* [39], programmed death-ligand 1(PD-L1) [22], and so on. Monoclonal antibodies (cetuximab and panitumumab) targeting *EGFR* have been approved by the FDA in the United States for CRC patients without *RAS* mutations [9,11]. Immune checkpoint protein, programmed death 1 (PD-1) and its ligand PD-L1 are also one of the emerging therapeutic targets in CRC [8]. Pembrolizumab, an anti-PD-1 immune checkpoint inhibitor, has been shown to be beneficial in CRC patients with MSI [27]. However, not all patients are receiving optimal treatment through the above-mentioned biomarkers. Therefore, the development of novel therapeutic targets and corresponding biomarkers to cover more cancer patients is highly required.

In a study by Stramignoni et al. (1983), the anti-TAG-72 monoclonal antibody B72.3 demonstrated reactivity for colon adenocarcinomas (82%), while not in adenomas [40]. Since then, TAG-72 usually has

been utilized as a serum marker for CRC. Approximately 40% of CRC patients had positive TAG-72 serum level at the time of diagnosis, while only 3% of patients with benign disease were positive [17,18]. In addition to B72.3, various monoclonal antibodies have been used for the evaluation of TAG-72 [26]. CC49, a second-generation higher affinity monoclonal antibody, has demonstrated overexpression of TAG-72 in the majority of malignant epithelial cells compared to normal or benign lesions. Due to its high specificity and strong immunoreactivity to the target antigen, the CC49 antibody has entered clinical trials for the imaging and treatment of various carcinomas [16,30]. However, the correlation between TAG-72 expression and CRC was not clear enough. In the current study, we hypothesized that TAG-72 is a sensitive prognostic biomarker in CRC. Thus, we performed TAG-72 immunohistochemistry using the newly developed 3E8 antibody, a fully humanized antibody with the highest affinity to TAG-72, in 578 CRC patients and analyzed clinicopathologic characteristics and prognosis of patients.

In this study, TAG-72 overexpression was significantly associated with well-known poor prognostic features, including lymphatic invasion, venous invasion, and lymph node metastasis. These findings suggest that TAG-72 is associated with the motility and invasiveness of cancer cells. Because our cohort was composed of only TNM stages II and III patients, TAG-72 in tumor with distant metastasis (stage IV) was not assessed. The depth of invasion was not associated with TAG-72, presumably because most cases were concentrated in pT3. MSI-high and associated features, right colon predominance and mucinous histology were associated with TAG-72 under-expression. Especially, there was no MSI-high CRC in the TAG-72^{high} group in our study. In survival analyses, TAG-72^{high} patients showed shorter DFS. In our cohort, there were 45 patients who did not receive adjuvant therapy for personal reasons, despite the need for chemotherapy and/or radiotherapy. These patients may have acted as a confounding factor. In multivariate survival analysis, TAG-72^{high} was an independent prognostic factor with adjuvant therapy and TNM stage in DFS.

5. Conclusion

TAG-72 was more expressed in cases of CRC of higher TNM stage, and TAG-72^{high} was associated with shorter DFS. These findings suggest that the expression of TAG-72 in CRC could be related to tumor progression, and that TAG-72 needs to be considered as a promising therapeutic target in CRC. Therapeutic strategies targeting TAG-72 have the potential to improve the prognosis of patients with CRC. Further studies are required to identify the specific mechanisms by which TAG-72 affects the tumorigenesis and progression of CRC and to examine the clinical utility of anti-TAG-72 agents.

Conflict of interest

The authors declared that they have no conflicts of interest related to this work.

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