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Original Article

Decreased expression of villin has adverse effect on the prognosis of patients with colorectal cancer

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Abstract

Aim: The expression of villin protein is widely used to determine to the gastrointestinal origin of metastatic tumors. However, little is known about the prognostic value of villin expression in colorectal cancer (CRC). The aim of this study is to investigate the relation between villin expression and basic histopathological features and survival in CRC. Secondarily, investigation of the prognostic significance of the CD4+/CD8+ TILs ratio was adopted.

Materials and Methods: Villin expression and CD4+/CD8+ TILs ratio were evaluated in paraffin-embedded tumor tissues of 147 CRC patients, immunohistochemically.

Results: Decreased villin expression was clearly detected in the tumoral areas compared to non-tumoral colonic mucosa. Loss of villin expression was closely related to high depth of tumor invasion (P = 0.001, ANOVA), increased lymph node metastasis (P <0.001, ANOVA) and high distant metastasis rate (P <0.001, t-test). These findings exposed that decreased villin expression is effective in the progression and aggressiveness of CRC. In addition, high villin expression was closely associated with low overall (p <0.001, Log Rank) and disease-free survival times (p <0.001, Log Rank). Furthermore, multivariate analyses revealed decreased villin expression was independently related with short overall (HR 5.82, 95% CI 3.18-10.65, p <0.001) and disease-free survival (HR 4.08, 95% CI 2.67-6.25, p <0.001). The mean CD4+/CD8+ TILs ratio of all patients was 1.73±1.74 (range 0.14-8.0). High CD4+/CD8+ TILs ratio was positively correlated with high pT and advanced TNM stage (p <0.001, ANOVA). In the univariate Cox regression analyses the high CD4+/CD8+ TILs ratio was an importantly correlated with poor overall outcome (HR 2.22, 95% CI 1.46-3.38, p <0.001).

Conclusion: Decreased villin expression and high CD4+/CD8+ TILs ratio are associated with aggressive pathobiological behavior in CRC. Moreover, decreased villin expression is an independent poor prognostic indicator.

Keywords: Colorectal cancer; CD4+/CD8+ TILs ratio; prognosis; villin

INTRODUCTION

Colorectal cancer (CRC), the third most common cancer in around the world, is one of the most important causes of cancer-related deaths at the same time (1,2). The incidence of CRC tends to increase gradually. So indeed, the number of new cases reported as 1.4 million in 2012 increased to 1.8 million in 2018 worldwide (3). Nowadays, thanks to advanced combined treatments including surgical resection, radiotherapy and chemotherapy, the 5-year survival of CRC has been slightly increased. However, death and relapse rates are still high. Therefore, new molecular biomarkers are needed for new prognostic predictors and more effective targeted therapies (4).

Villin is a member of the gelsolin protein superfamily, which is consists of actin-binding proteins regulated with calcium. The villin protein has firstly isolated and characterized in the microvilli of the intestinal epithelial cells. It has later shown to be found in many absorptive epithelium (5,6). Villin is a unique protein expressed especially in epithelial cells of the gastrointestinal tract, hepatic bile duct and renal proximal tubules (6,7). Villin protein expression specifically shown in CRC, is widely used to differentiate these tumors from cancers without gastrointestinal origin (8,9). Villin has also been found to be expressed in endometrial adenocarcinomas and gastrointestinal neuroendocrine tumors (6,7). However, there are limited studies investigating the effect of villin expression on prognosis in CRC.

The investigation of the effect of tumor-infiltrating lymphocytes (TILs) and host defense on CRC prognosis is one of the other interesting research topics in recent years (10,11). TILs are significant constituent of tumor immune response, and each subgroup of TILs hold a unique antitumor role (12). It has been revealed that CD4+ T helper cells and CD8+ cytotoxic T cells assume

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important tasks in host defense against the tumor (13). Additionally, the CD4+/CD8+ TILs ratio has been shown to correlate significantly with overall survival in patients with CRC (14).

This present study was aimed primarily to investigate the effect of decreased villin expression on the prognosis in CRC. Secondarily, the relation of CD4+/CD8+ TILs ratio with basic prognostic parameters and prognosis in CRC was also analyzed, retrospectively.

MATERIALS and METHODS

Patients and pathological investigation

This study was approved by Firat University Faculty of Medicine Ethics Committee on September 17, 2019 with the approval number 13-08. The study included 147 patients with CRC treated by surgical resection between 2013 and 2015 at a single institution. The patients treated with adjuvant or neo-adjuvant chemotherapy were excluded. The clinical and pathological data were obtained from hospital medical archives. Pathological materials were re-evaluated, retrospectively. Histological tumor type and grade were specified according to the World Health Organization (WHO) classification system criteria. Tumor-node-metastasis (TNM) stages of cases were determined according to the American Joint Cancer Committee (AJCC), 7th edition. The survival information of the patients was obtained from the medical data processing records of the hospital. Overall survival (OS) and disease-free survival (DFS) times was determined as the interval between the dates of surgery and death or recurrence.

Immunohistochemistry (IHC)

Immunohistochemical staining was carried out using the indirect immuno-peroxidase technique on formalin-fixed, paraffin-embedded tissues. In the staining process, below antibodies were used: anti-Villin (clone CWWB1, Thermo scientific, Fremont, CA 94538, USA), anti-CD4 (SP35, Ventana, Arizona, USA) and anti-CD8 (SP57, Ventana, Arizona, USA). The prepared paraffinized sections were stained by the Ventana Bench Mark Ultra coater (Ventana, Tucson, AZ-85755, USA) and the ultraView Universal DAB kit (Ventana, Tucson, AZ-85755, USA) considering the manufacturer's instructions. The density and distribution of villin in tumor cells were evaluated immunohistochemically. In addition, the TILs density was assessed by CD4 and CD8 staining.

Scoring of villin expression in tumoral tissues

Villin expression in CRC was evaluated using the IHC scoring method validated in previous studies (7,15). While evaluating the preparations, cytoplasmic and membranous staining in tumor cells was taken into consideration. IHC-score was obtained multiplying the staining intensity [0: no staining, 1: weak staining (light yellow), 2: moderate staining (yellow brown), and 3: strong staining (brown)] by the percentage of positive stained cells (0 = 0%; 1 = 1% –9%; 2 = 10% –50%; and 3 = >50%.). IHC-score ranged from 0 to 9. The mean value of the IHC-score 2.78±1.98 was accepted as the cutoff criterion. The cases with IHC-score below this value were considered as a low-villin expression group and those above were characterized as a high-villin expression group.

Assessment of CD4+/CD8+ TILs ratio

CD4+ and CD8+ TILs density was assessed considering previous studies (16,17). Briefly, five fields with the intense infiltration of CRC were selected from each staining slide and the percentages of CD4+ and CD8+ TILs were evaluated. The average of this five fields was used as the density of TILs. First, a semi-quantitative staining score was determined according to the following scale: 1 (<1% cells); 2 (1-10% cells); 3 (11-33% cells); 4 (34-66% cells); and 5 (67-100% cells). The cells with positive immunereactivity were described as those showing partial or complete positivity in the cytoplasm and/or plasma membrane. Second, staining intensity was scored as follows: 0 (absent), 1+ (slight), 2+ (moderate), and 3+ (intense). Finally, scores were calculated by adding the percentage staining scores and the density scores for each slide (ranging from 1 to 8). The patients were divided into two groups as a low and high density of CD4+ and CD8+ TILs using median value of IHC-score (3.38±1.98, 3.67±1.97, respectively). In addition, CD4+/CD8+ TILs ratio was determined for each case. The mean value for this ratio was accepted as the cut-off criterion (1.73±1.74). The cases with below this value were considered as a low-CD4+/CD8+ TILs ratio group and those above were characterized as a high CD4+/CD8+ TILs ratio group.

Survival and Statistical analysis

In this study, SPSS version 26 software (IBM Corporation, Armonk, NY, USA) was used for statistical analysis of data. Obtained outcomes were expressed as percentages, means, and standard deviations. Normality was checked by the Shapiro-Wilk test. Also, the kurtosis and skewness values between −0.5 and +1.5 were accepted to demonstrate normality. Independent samples t test and ANOVA were used to determine the differences between groups with normal distribution. On the other hand, chi-square, Mann-Whitney U and Kruskal Wallis tests were preferred to determine the differences between groups without normal distribution. Kaplan-Meier method (log-rank test) was used to achieve and compare the survival curves. Univariate and multivariate Cox proportional hazards regression analyses was applied to evaluate independent prognostic indicators associated with survival. For all statistical data, a P value less than 0.05 were considered statistically significant.

RESULTS

Various basic clinicopathological parameters were significantly correlate to survival

Of the patients incorporated into the study population, 61 (41.5%) were female and 86 (58.5%) were male. Mean age was 59.7±13.6 years (range 24-83). Most of the tumors were located on the left side (n = 91, 61.9%). The cases mainly consisted of stage III (n = 40, 27.2%) and stage IV (n = 50, 34.0%) patients. In addition, CD8+ TILs density was low (Figure 1A) in 80 (54.4%) of the cases in this series, high (Figure 1B) in 67 (45.6%). Again,

CD4+ TILs density was determined low (Figure 1C) in 75 (51.0%) cases, high (Figure 1D) in 72 (49.0%). The basic clinicopathological features are detailed in Table 1. The follow-up period for survival analysis was determined as 5 years. The mean OS was 45.82±13.67 months and the 5-year survival rate was 38.8%. There was recurrence in 100 (68.0%) of the patients, and the mean DFS was determined to be 40.53±16.31 months. It was revealed that clinicopathological parameters such as gender, age, tumor size and histopathological tumor type had no effect on survival (Table 1). Conversely, according to the data obtained by univariate Cox regression analysis, right tumor site, high histological grade, low CD8+ TILs density, high CD4+ TILs density, high CD4+/CD8+ TILs ratio, advanced depth of tumor invasion (pT), high lymph node metastasis (pN) and advanced TNM stage were found to be significantly associated with poor prognosis (Table 1). While mean survival was 57.39±4.92 months in TNM stage II patients, it was decreased to 31.84±8.1 months in TNM stage IV patients. Moreover, it has been shown that the TNM stage can be used as an independent prognostic parameter according to results of multivariate analyses (HR 3.94, 95% CI 2.46-6.32, p <0.001).

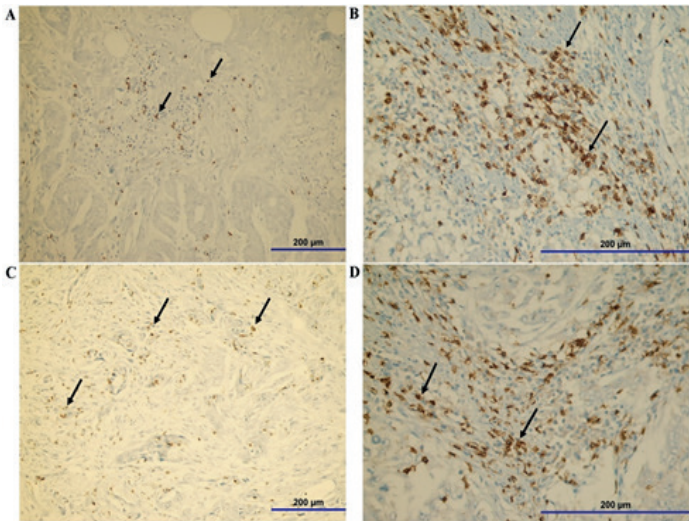

Figure 1. Representative TILs density in colorectal cancer tissues (A) low CD8+ TILs density (arrows) (x200) (B) high CD8+ TILs density (arrows) (x400) (C) low staining intensity for CD4+ T lymphocytes (arrows) (x200) (D) high density of CD4+ TILs (arrows) (x400)

Table 1. Clinical and pathological features of study cases and their relationship with overall survival (N=147)

Parameters	N (%)	Overall survival HR (95% CI)	P value
Gender			
Female	61 (41.5)	1.21 (0.79-1.84)	0.366
Male	86 (58.5)		
Age			
19-44	18 (12.2)	1.02 (0.75-1.37)	0.889
45-54	48 (32.7)		
55 ≥	81 (55.1)		
Tumor size (cm)			
< 5	67 (45.6)	1.45 (0.95-2.20)	0.081
≥ 5	80 (54.4)		
Tumor site			
Left	91 (61.9)	2.58 (1.70-3.82)	< 0.001
Right	56 (38.1)		
Histopathologic type			
Adenocarcinoma	72 (49.0)	0.98 (0.72-1.34)	0.938
Mucinous	55 (37.4)		
Signet-ring	20 (13.6)		
Histologic grade			
Well	29 (19.7)	1.72 (1.22-2.42)	0.002
Moderate	82 (55.8)		
Poor	36 (24.5)		
CD8+ TILs density			
Low	84 (57.1)	0.51 (0.32-0.79)	0.003
High	63 (42.9)		
CD4+ TILs density			
Low	75 (51.0)	1.93 (1.27-2.95)	0.002
High	72 (49.0)		
CD4+/CD8+ TILs ratio			
Low	94 (63.9)	2.22 (1.46-3.38)	< 0.001
High	53 (36.1)		

Depth of invasion			
Submucosa	20 (13.6)	3.88 (2.40-6.29)	< 0.001
Muscularis propria	33 (22.4)		
Pericolorectal tissues	94 (63.9)		
Lymph node metastasis			
Absent	56 (38.1)	3.17 (2.41-4.16)	< 0.001
1-3	59 (40.1)		
≥ 4	32 (21.8)		
Distant Metastasis			
Absent	97 (66.0)	3.46 (2.66-4.42)	< 0.001
Present	50 (34.0)		
TNM staging			
Stage I	34 (23.1)	5.68 (4.01-8.03)	< 0.001
Stage II	23 (15.6)		
Stage III	40 (27.2)		
Stage IV	50 (34.0)		

HR: hazard ratio, CI: confidence interval

Decreased villin expression was related with aggressive histopathological features

Totally 147 tumor tissues and 50 adjacent non-neoplastic colorectal tissues were stained with anti-villin antibody, immunohistochemically. There was high villin expression in the normal colonic mucosa, prominently (Figure 2A). Distinctly from normal mucosa, villin expression was low intensity in tumoral areas. The main villin IHC staining score was determined as 2.78±1.98. While low villin expression (Figure 2B) was observed in 87 (59.2%) of 147 cases, high expression (Figure 2C) was observed in 60 (40.8%) of the cases. The interrelation between villin expression and clinicopathological features is shown in Table 2. There was no significant relationship between villin expression and CD4+ and CD8+ TILs density according to Mann-Whitney U test (p = 0.103, p = 0.129, respectively). In addition, it was determined that there was no correlation between villin expression and clinicopathological parameters such as gender, age, tumor size and histopathological tumor type.

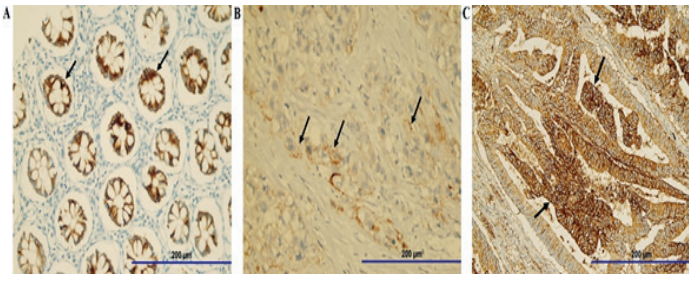

Figure 2. Representative figures of villin expression in colorectal cancer specimens (A) Strong cytoplasmic and luminal villin expression in the normal colonic mucosa adjacent to the tumoral areas (arrows) (x400) (B) decreased cytoplasmic villin expression in poorly differentiated tumoral areas (arrows) (x400) (C) high expression of villin in well differentiated (low grade) tumoral areas (arrows) (x400)

On the other hand, the results of this study proved low villin expression was closely related to high histological grade (P = 0.015, ANOVA), high pT category (P = 0.001, ANOVA), increased pN (P <0.001, ANOVA), high distant metastasis (DM) rate (P <0.001, t-test) and advanced TNM stage (P <0.001, Kruskal-Wallis test). There was also a significant correlation between tumor site and villin expression (p = 0.004, t-test). A significant loss of villin expression was detected in right-settled tumors (2.17±1.64) compared to those with left-settled (3.15±2.09). These data implicated that decreased villin expression is effective in the progression and aggressiveness of CRC.

Table 2. The correlation between villin expression and histopathological features

Parameters	Villin expression score (Mean±SD)	P value
Histopathologic type		
Adenocarcinoma	2.77±2.15	0.679††
Mucinous	2.90±1.92	
Signet-ring	2.45±1.53	
Histologic grade		
Well	3.44±2.16	0.015††
Moderate	2.86±1.98	
Poor	2.05±1.63	
CD8+ TILs density		
Low	2.66±2.10	0.129‡
High	2.93±1.83	
CD4+ TILs density		
Low	3.00±1.94	0.103‡
High	2.55±2.02	
CD4+/CD8+ TILs ratio		
Low	2.87±1.83	0.427†
High	2.60±2.25	
Depth of invasion		
Submucosa	4.30±2.07	0.001††
Muscularis propria	2.81±1.70	
Pericolorectal tissues	2.44±1.92	

Lymph node metastasis		
Absent	4.03±1.77	<0.001††
1-3	2.16±1.91	
≥ 4	1.71±1.19	
Distant Metastasis		
Absent	3.42±2.09	<0.001†
Present	1.54±0.88	
TNM staging		
Stage I	3.94±1.70	<0.001†††
Stage II	4.65±1.84	
Stage III	2.27±1.98	
Stage IV	1.54±0.88	

†: t-test, ‡: Mann-Whitney U test, ††: ANOVA, †††: Kruskal-Wallis test

High CD4+/CD8+ ratio of TILs negatively affected the prognosis of patients with CRC

The mean CD4+/CD8+ TILs ratio of all patients was 1.73±1.74 (range 0.14-8.0). There was no significant relationship between CD4+/CD8+ TILs ratio and histological type (p = 0.782, Mann-Whitney U), histological grade (p = 0.208, ANOVA) and tumor size (p = 0.331, Mann-Whitney U). On the contrary, high CD4+/CD8+ TILs ratio was positively correlated with high pT (p = 0.004, ANOVA) (Figure 3A), high pN (p = 0.002, ANOVA) (Figure 3B) high DM rate (p <0.001, t-test) (Figure 3C), and advanced TNM stage (p <0.001, ANOVA) (Figure 3D). The effect of CD4+/CD8+ TILs ratio on OS and DFS was also investigated in the study. In the univariate Cox regression analyses the high CD4+/CD8+ TILs ratio was an importantly correlated with poor overall outcome (HR 2.22, 95% CI 1.46-3.38, p <0.001) (Figure 4A) and DFS (HR 2.17, 95% CI 1.45-3.24, p <0.001) (Figure 4B). However, there was no significant relationship between CD4+/CD8+ TILs ratio and OS and DFS in multivariate analyses. These findings shown that the ratio of CD4+/CD8+ TILs in CRC can be used as a reliable prognostic indicator.

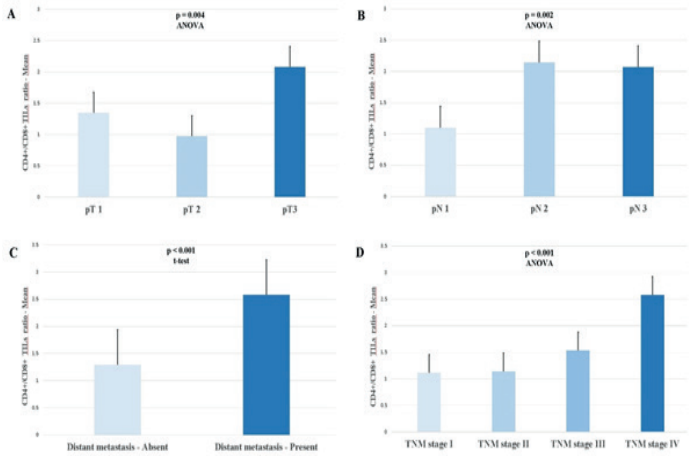

Figure 3. Representative images showing the interrelationship between CD4+/CD8+ TILs ratio and prognostic parameters (A) The ratio of CD4+/CD8+ TILs was higher in patients with advanced depth of tumor invasion (1.34±1.57) compared to those with superficial (2.07± 1.91). (B) There was a positive correlation between high CD4+/CD8+ TILs ratio and lymph node metastasis (p = 0.002, ANOVA) (C) The CD4+/CD8+ TILs ratio was significantly higher in patients with distant metastases (2.58±2.03) than those without metastasis (1.29±1.39) (D) CD4+/CD8+ TILs ratio of patients with TNM stage IV was higher (2.58±2.03) than those with TNM stage I (1.11±1.33)

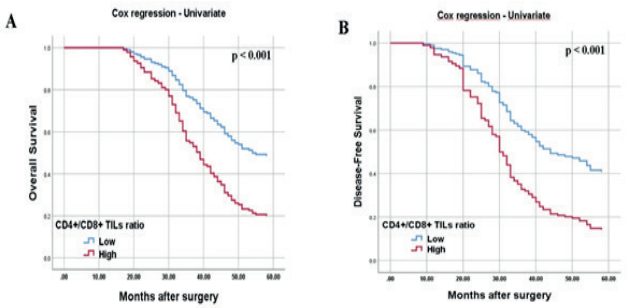

Figure 4. Representative survival curves and Cox proportional regression analyses according to CD4+/CD8+ TILs ratio (A) Patients with high CD4+/CD8+ TILs ratio had a poor overall survival (B) the cases with high CD4+/CD8+ TILs ratio had shorter disease-free survival rates than those with low CD4+/CD8+ TILs ratio

Table 3. The effect of decreased villin expression on overall and disease-free survival (univariate and multivariate Cox regression analyses)

Parameters	Overall survival			
	Univariate		Multivariate	
	HR (95% CI)	P value	HR (95% CI)	P value
Size (<5cm/≥5cm)	1.45 (0.95-2.20)	0.081	1.19 (0.75-1.91)	0.449
Site (left/right)	2.58 (1.70-3.82)	<0.001	1.11 (0.71-1.74)	0.633
Type (adeno/mucinous/signet ring)	0.98 (0.72-1.34)	0.938	1.11 (0.82-1.50)	0.496
Grade (well/moderate/poor)	1.72 (1.22-2.42)	0.002	0.87 (0.61-1.25)	0.470
CD8+ TILs density (low/high)	0.51 (0.32-0.79)	0.003	1.27 (0.61-2.63)	0.517
CD4+ TILs density (low/high)	1.93 (1.27-2.95)	0.002	0.79 (0.44-1.41)	0.428
CD4+/CD8+ TILs ratio (low/high)	2.22 (1.46-3.38)	<0.001	1.62 (0.77-3.40)	0.202
pT (pT1/pT2/pT3)	3.88 (2.40-6.29)	<0.001	1.06 (0.58-1.95)	0.834
pN (absent/1-3/≥ 4)	3.17 (2.41-4.16)	<0.001	1.23 (0.80-1.89)	0.343
TNM stage (I/II/III/IV)	5.68 (4.01-8.03)	<0.001	3.94 (2.46-6.32)	<0.001
Villin expression (low/high)	0.07 (0.04-0.15)	<0.001	3.86 (1.70-8.76)	0.001

Parameters	Disease-free survival			
	Univariate		Multivariate	
	HR (95% CI)	P value	HR (95% CI)	P value
Size (<5cm/≥5cm)	1.37 (0.92-2.04)	0.120	1.00 (0.65-1.55)	0.977
Site (left/right)	2.70 (1.80-4.04)	<0.001	1.24 (0.81-1.90)	0.305
Type (adeno/mucinous/signet ring)	1.02 (0.76-1.37)	0.895	1.18 (0.88-1.59)	0.249
Grade (well/moderate/poor)	1.81 (1.30-2.51)	<0.001	1.01 (0.72-1.42)	0.934
CD8+ TILs density (low/high)	0.51 (0.34-0.78)	0.002	0.76 (0.39-1.49)	0.437
CD4+ TILs density (low/high)	1.95 (1.31-2.91)	0.001	0.98 (0.54-1.76)	0.947
CD4+/CD8+ TILs ratio (low/high)	2.17 (1.45-3.24)	<0.001	1.32 (0.62-2.78)	0.461
pT (pT1/pT2/pT3)	2.58 (1.81-3.68)	<0.001	1.06 (0.66-1.71)	0.788
pN (absent/1-3/≥ 4)	2.76 (2.14-3.56)	<0.001	1.05 (0.72-1.53)	0.793
TNM stage (I/II/III/IV)	3.99 (2.99-5.31)	<0.001	2.61 (1.74-3.93)	<0.001
Villin expression (low/high)	0.08 (0.04-0.14)	<0.001	0.14 (0.07-0.29)	<0.001

HR: hazard ratio, CI: confidence interval

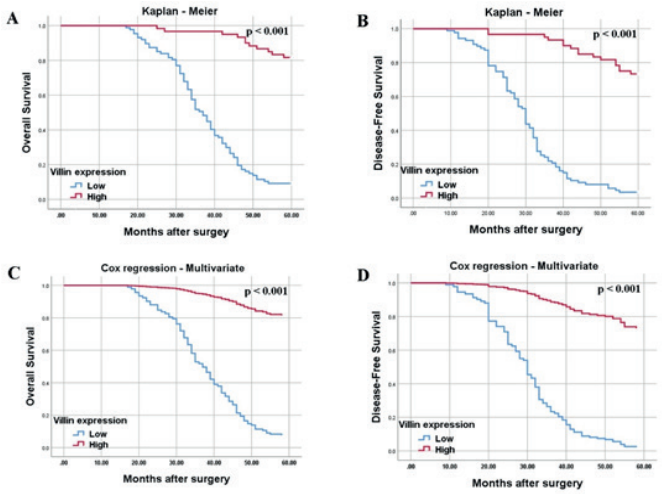

Figure 5. Representative Kaplan-Meier survival curves and Cox proportional regression analyses according to villin expression (A) Patients with high villin expression had a better overall survival (p <0.001, Log Rank) (B) The cases with decreased villin expression had shorter disease-free survival rates than those with high expression (p <0.001, Log Rank) (C) and (D) In multivariate Cox regression analyzes, decreased villin expression was significantly correlated with both poor overall (p <0.001) and disease-free survival (p <0.001), independently

Decreased villin expression in CRC arose as an independent poor prognostic indicator

Whether villin expression could be used as a prognostic biomarker was evaluated using Kaplan-Meier analysis and Cox regression models. The survival curves displayed that loss of villin expression was importantly associated with poor OS (p <0.001, Log Rank) (Figure 5A) and short DFS time (p <0.001, Log Rank) (Figure 5B) of CRC patients. The cases with low villin expression exhibited worse biological behavior than those with high expression. According to univariate analysis low villin expression was substantially related with poor OS (HR 0.07, 95% CI 0.04-0.15, p <0.001) and DFS (HR 0.08, 95% CI 0.04-0.14, p <0.001) in CRC (Table 3). The patients with low villin expression had significantly shorter mean OS and DFS time than those with high expression. Furthermore, multivariate analyses revealed that decreased villin expression was independently correlated with short OS (HR 3.86, 95% CI 1.70-8.76, p = 0.001) (Figure 5C) and DFS (HR 0.14, 95% CI 0.07-0.29, p <0.001) (Figure 5D) in patients with CRC. In short, univariate and multivariate analyses brought out that decreased villin expression was an independent biomarker indicating poor prognosis for CRC patients.

DISCUSSION

Despite the slight increase in survival rates in recent years, CRC still maintains its importance as a devastating health problem. While there are dazzling new developments every day in targeted therapies used in the treatment of many malignant neoplasms, new prognostic biomarkers are also needed for CRC. Therefore, the effect of villin protein expression on tumor progression and prognosis of CRC was investigated in this study. In addition, the prognostic significance of TILs density and CD4+/CD8+ TILs ratio was also examined.

Villin is a 95 kDa protein belonging to the Gelsolin family of actin binding proteins regulated by calcium (18,19). The villin protein is an essential component of the brush border cytoskeleton and functions in capping, cutting, and bundling actin filaments (18). Thus, it has an important role in the regulation of cell morphology and cell-specific epithelial anti-apoptotic mechanisms (20). The pattern of villin expression is mainly limited to the intestinal epithelium and proximal tubules of kidney (6,7,19). Villin expression has been demonstrated in intestinal metaplasia areas found in Barrett's esophagus and chronic atrophic gastritis. The immunexpression of villin has also been specified in several different malignant tumors including CRC (6,7,20). Moreover, villin immunoexpression is widely used both in the primary diagnosis of CRC and in determining the colonic origin of metastatic carcinomas of unknown origin (8,19). However, there are a limited number of studies examining the effect of villin expression on prognosis in CRC in large clinical

series. According to Al-Maghrabi et al., expression of the villin has downregulated in CRC compared to normal colonic mucosa (7). In their study, while the percentage of positively stained cancer cells decreased significantly in some cases, the intensity of expression was found to be much lower than that of normal mucosa in other ones. It has been stated that these findings are conforming the results of Arango et al., and Werling et al. (19,21). However, other researchers have noted that villin are expressed in almost all of both primary and metastatic CRCs (22,23). Consistent with previous reports, the present study showed decreased expression of villin in tumoral areas compared to normal colon mucosa. Low villin expression was detected in 59.2% of the cases. In the study of Arango et al., It was stated that there is a loss of villin expression in microsatellite instable (MSI) CRCs, which are frequently show right colon involvement. In their data, the percentage of villin-positive tumor cells was prominently lower in MSI CRCs compared to those with microsatellite stable (MSS) (19). Additionally, ıt was demonstrated that the intensity of villin expression in both MSI and MSS groups was significantly lower in tumors with poorly differentiated histology (minimal glandular differentiation or no glandular differentiation) compared to those with well / moderately differentiated histology (existence of glandular differentiation). On the contrary, Altintas et al.'s study claimed that there was no significant relationship between villin expression and tumor differentiation. Moreover, it was reported that no significant difference was found any of the clinicopathological parameters including age, tumor site, pT, pN and TNM stage in terms of villin expression (5). Similarly, in the study of Al-Maghrabi et al., It was suggested that there was no statistically significant relationship between villin expression and classical parameters such as age, tumor location, pT and lymphovascular invasion (7). In this study, contrary to previous findings, it is clearly revealed that low villin expression is closely related to high pT category, high pN category, high DM rate and advanced TNM stage. In present study, an important correlation was also found between the tumor site and the expression of villin. Supporting Arango et al., a significant loss of villin expression was showed in right site CRCs compared to those with left-settled. In a recent study by Azizi et al., It was stated that decreased villin expression in gastric cancer could act as an influential factor causing loss of specific structure of the cell and thereby the epithelial-mesenchymal transition (EMT) and metastasis (24). Similarly, Patnaik et al. stated that a change in villin expression pattern such as accumulation in the nucleus is associated with mouse models of tumorigenesis and nuclear villin may play a role in the regulation of EMT (25). In present study, an important finding was observed that supports the current reports mentioned above. In this series, lymph node metastasis and distant metastasis were more common in cases with decreased villin expression than those with high expression. All these consistent data indicate that decreased of villin expression plays an effective role in the progression and aggressiveness of CRC.

There are very few clinical studies investigating the effect of villin expression on the prognosis and survival of patients with CRC (7,19). Furthermore, the results of these studies are incompatible. According to Al-Maghrabi et al., CRC patients with higher villin expression were related with better survival time (7). On the contrary, Arango et al. didn't find out such a relationship between villin expression and OS in patients with CRC (19). In present study, it has revealed by univariate Cox regression analyzes that decreased villin expression had a negative effect on overall and disease-free survival times in CRC patients. Moreover, according to the data obtained from multivariate analysis, decreased villin expression could be used as independent poor prognostic indicator in CRC.

TILs are one of the important parameters representing immune host defense against tumor (11). There are studies indicating that it can be used as a useful biomarker to predict survival and therapeutic outcomes in patients with various cancers (7,14,26,27). Therefore, a series of reports on TILs continue to be published (11). However, new studies are needed to determine which of the T cell subpopulations are associated with better prognosis and survival. There are studies showing that infiltration of CD8+ T cells within cancer tissues contributes to better survival of CRC patients (10,11,14,28). In addition, it has been reported that CRC patients with low CD4+/CD8+ TILs ratio have longer overall survival than those with high CD4+/CD8+ TILs ratio (14,29). Consistent with previous reports, a positive correlation was observed between high CD8+ TILs density and survival in present study. High CD8+ TILs density had a positive effect on disease-free survival. Further, the effect of CD4+/CD8+ TILs ratio on prognosis in patients with CRC was evaluated. It was found that lymph node metastasis and distant metastasis rates were higher in cases with high CD4+/CD8+ TILs ratio than those with low. Moreover, high CD4+/CD8+ TILs ratio was significantly related with poor OS and DFS. All these findings support that high ratio of CD8+ T lymphocytes in tumoral tissue positively affects the prognosis and CD8+ TILs are an important component of the host defense against CRC.

CONCLUSION

As a summary, the expression of villin in cancerous tissues has downregulated compared to normal colonic mucosa. Decreased villin expression in CRC is closely related to aggressive pathological features and poor survival outcomes. And also, higher CD8+ TILs density in CRC is associated with better survival times. On the contrary, the increasing CD4+/CD8+ TILs ratio negatively affects the prognosis. More in vivo and in vitro studies are needed to elucidate the effects of villin expression and CD8 + TILs density on the prognosis of patients with CRC.

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