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A novel screening test for colon cancer: Talin-1

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Abstract. – OBJECTIVE: Cell adhesion and angiogenesis within the extracellular matrix involve special signaling molecules, such as integrins and the actin binding protein Talin-1. The aim of this study was to investigate and describe the expression of Talin-1 for the early detection of colon cancer.

PATIENTS AND METHODS: Blood serum samples were collected from 50 healthy volunteers and from 90 patients with colon cancer. Using an enzyme-linked immunosorbent assay (ELISA), all 140 samples were analyzed.

RESULTS: Preoperative levels of Talin-1 in the serum were significantly higher in patients with colon cancer ($p < 0.001$). No significant correlation was found between preoperative levels of Talin-1 in the serum and the age and gender of the patients ($p < 0.05$). However, a significant correlation was found between Talin-1 levels and the tumor grade, TNM stage, and lymph node metastasis ($p < 0.001$).

CONCLUSIONS: Talin-1 may play a role in the reinforcement of cell proliferation, cell adhesion, and angiogenesis in colon cancer. Thus, the Talin-1 protein activity may be a novel biomarker to detect colon cancer in humans.

Key words:
Talin-1, Adhesion, Angiogenesis, Colon cancer.

Introduction

Colon cancer was the third most widespread type of malignancy and the third most common cause of cancer-related deaths (for both genders) worldwide in 2011[1]. Although recent progress has been made in the determination of the pathogenesis of colon cancer, early diagnosis remains the most important factor to promote the long-term survival of patients with this disease[2,3]. On the one hand, numerous molecular markers, such as carcinoembryonic antigen (CEA), have been used as diagnostic and or prognostic indicators for colon cancer[4]. On the other hand, many of these markers do not provide uniform sensitivity and reliability in the establishment of new markers that may be used as diagnostic and prognostic markers for this malignancy.

Talin is a high-molecular-weight and ubiquitous cytoskeletal protein that is localized at contact points of the cell[5] as well as at cell-cell contacts in lymphocytes[6,7]. Talin is an inherent cytosolic protein that is found in high concentrations at focal adhesions, where it is responsible for linking integrins to the actin cytoskeleton[8].

Integrins are a family of trans-membrane glycoprotein receptors[9] that affect gene expression, and the regulation of cell survival, differentiation and proliferation. In addition to their physiological roles, integrins are also abnormally expressed in many pathological states, such as inflammation and tumor progression.

In our first study, we investigated the role of Talin-1 in the process of angiogenesis in colon cancer, and have thus incorporated this protein into our current study of patients who were diagnosed with colon cancer and matched controls.

Patients and Methods

Patients

This analysis was designed as a multicenter and prospective study. Patients with familial adenomatous polyposis, hereditary non-polyposis colorectal cancer, and inflammatory bowel disease were excluded from the study. All patients who had undergone a surgical resection for colon cancer. All cancer patients and healthy control subjects underwent colonoscopy and the diagnosed. In the patients who were pathologically confirmed with colon cancer after colonoscopy, blood samples were collected one day before surgery. Additionally, patients with a second primary tumor were also excluded. None of the participants were treated with neoadjuvant chemoradiotherapy. The clinical and pathologic factors collected for each patient

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were as follows: age, gender, tumor size, tumor grade and Tumor-Node-Metastasis (TNM) stage for colon cancer (Table I).

This study was performed in accordance with institutional ethical guidelines and was approved by the Medical Ethics Committee of Sisli Hamidiye Etfal Research and Training Hospital in Istanbul, Turkey.

A total of 50 healthy age-matched volunteers were included in the study as the control group. Immediately after collection, the blood samples were centrifuged at 1000 x g for 15 minutes at 2-8° C. The serum samples were either assayed immediately or were divided into single-use aliquots and stored at –80° C.

ELISA of Talin-1

An ELISA for Talin-1 was performed using a commercially available kit and according to the manufacturer's instructions (Ray Biotech, Inc., Norcross, GA, USA http://www.raybiotech.com). Serum was transferred to the wells of plates precoated with primary antibody. After the recommended incubation and wash times, the substrate solution was added to the compound. The color-reagent and stop solution were then added to the wells. The optical density was determined at a wavelength of a 450 nm using automated optical densitometry. Each sample was run in duplicate, and the mean value was used for the analysis.

The minimum detectable level of human Talin-1 was typically less than 5.86 pg/mL. This assay shows high sensitivity and specificity for the detection of human talin-1. No significant cross-reactivity or interference between human Talin-1 and any known homologues were observed. The detection range was 23.44-1500 pg/mL.

Table I. The clinicopathologic features of patients and controls.

	Controls (n=50)	Patients (n=90)	p
Age (y)	59.4 ± 7.58	59.87 ± 6.12	< 0.05
Gender (M/F)	25/25	45/45	
Tumor size			
≤ 4 cm	13		
> 4 cm	77		
TNM stage			
TI	20		
TII	14		
TIII	23		
TIV	33		
Invasion			
T1	20		
T2	14		
T3	22		
T4	34		
Lymph node metastasis			
N0	13		
N1	17		
N2	60		
Metastasis			
Present	71		
Not present	19		

Determination of CEA

Preoperative serum CEA levels were determined an enzyme immunoassay test kit DPC (Diagnostic Product Co., Los Angeles, CA, USA) with the upper limit of 5 ng/ml^{-1} defined as normal ac- cording to the manufacturers of the kits used.

Statistical Analysis

One-sample Kolmogorov-Smirnov and Shapiro Wilk normality test control charts were drawn in addition to a histogram. Data are presented as the mean and the standard deviation; the median, minimum, and maximum are also shown and are presented as frequencies and percentages. Normally distributed variables were compared by *t*-test for independent samples. To determine the normal distribution variables, Mann-Whitney U test, Kruskal-Wallis one-way analysis of variance and Bonferroni-corrected Mann-Whitney U tests were performed with binary comparisons. Yates-corrected chi-square and chi-square tests were used for nominal variables; a p value <0.05 was considered to be statistically significant. The statistical software used for the present study was the SPSS 21.0 package program (SPSS Inc., Chicago, IL, USA).

Results

Table I provides the demographics and clinicopathologic data for all the patients. The levels of Talin-1 in the serum were measured in 50 healthy participants (25 men and 25 women) with a median age of 59.4 (range: 33-71 years). The study group was composed of 90 patients with colon cancer (45 men and 45 women) with a median age of 59.87 (range: 40-71 years) ($p > 0.05$). A total of 13 patients (14.4%) presented with a tumor circumference of less than 4 cm, and 71 (78.8 %) patients had distant metastasis. The postoperative T stages of the patients were T1, T2, T3, and T4 in 19, 14, 23, and 44 patients, re-

Table II. Preoperative serum Talin-1 levels and CEA in all patients and controls (Mean ± SD) (Min-Max).

	Controls	Patients	p
Talin-1 (pg/mL)	11.7 ± 2.09 (5.6-17.4)	264.21 ± 204.38 (21.3-724.3)	< 0.001
CEA (ng/mL)	3.5 ± 1.2 (1-6)	13.4 ± 1.8 (10-17)	< 0.001

spectively. The postoperative TNM stages were TI, TII, TIII, and TIV in 20, 14, 24, and 43 patients, respectively.

Table II shows the serum levels of Talin-1 in all of the cancer patients and of those in the control group. Neither group demonstrated any significant age-related differences ($p > 0.05$) (Figures 1 and 2). In the matched controls, the mean ± SD serum level of Talin-1 was 11.7 ± 2.09 pg/mL. The mean ± SD serum levels of Talin-1 in the 90 patients with colon cancer was 264.21 ± 204.38 pg/mL. The serum levels of Talin-1 were significantly higher in the patients with colon cancer than in the patients in the control group ($p < 0.001$).

Table III illustrates the serum levels of Talin-1 in the patients with colon cancer according to the clinicopathologic variables.

The serum Talin-1 levels were significantly higher in patients with T4 colon cancer than in patients with T1 tumors ($p < 0.001$) (Figure 3). This finding shows that serum Talin-1 levels are directly proportional to tumor size. Additionally, serum Talin-1 levels were significantly higher in patients

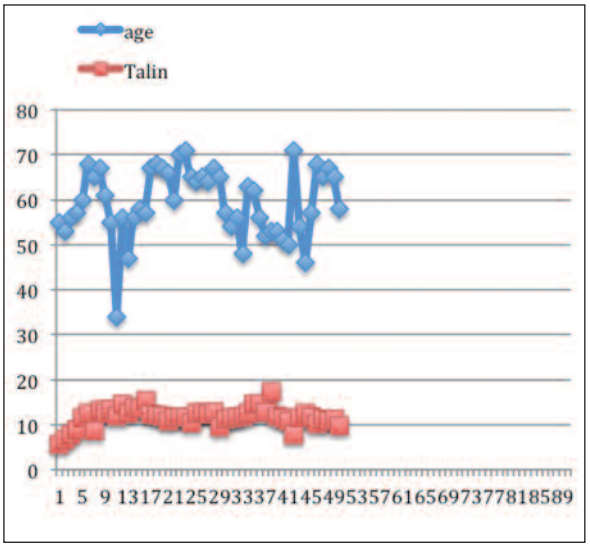

Figure 1. The histogram of the control group.

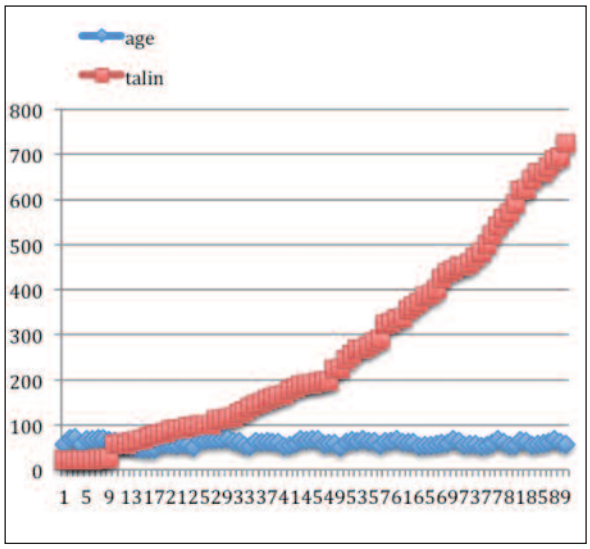

Figure 2. The histogram of all colon cancer patients.

with lymphovascular involvement, distant metastasis, and lymph node metastasis ($p < 0.001$).

Tumor size, lymphovascular involvement and distant metastasis each demonstrated a similar significant correlation with the age variable between the two groups ($p = 0.573$, $p = 0.523$, and $p = 0.412$, respectively). Both lymph node metastases and distant metastases showed a similarly significant relationship to the gender variable between the two groups ($p = 0.997$ and $p = 0.8$, re-

Table III. Preoperative serum Talin-1 levels in relation to the clinicopathologic variables of the patients (Mean ± SD) (Minimum-Maximum).

	Talin-1 (pg/mL)
Tumor size	
≤ 4 cm	49.05 ± 25.49 (21.3-88.9)
> 4 cm	325.68 ± 190.92 (90.5-724.3)
TNM stage	
TI	49.05 ± 25.49 (21.3-88.9)
TII	109.95 ± 15.21 (90.5-139.2)
TIII	208.70 ± 46.34 (145.7-290.1)
TIV	499.11 ± 125.07 (321.5-724.3)
Invasion	
T1	49.0 ± 25.01 (21.3-85.2)
T2	105.4 ± 10.9 (88.9-143.8)
T3	208.7 ± 42.9 (145.7-310.8)
T4	501.5 ± 110.71 (330.6-724.3)
Lymph node metastasis	
N0	49.05 ± 25.49 (21.3-88.9)
N1	142.58 ± 37.98 (90.5-198.0)
N2	447.75 ± 149.11 (222.5-724.3)
Metastasis	
Present	355.04 ± 183.19 (113.5-724.3)
Not present	63.07 ± 31.27 (21.3-110.5)

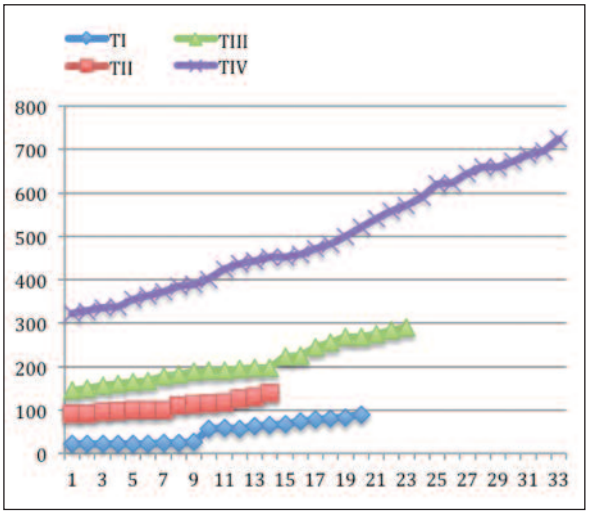

Figure 3. The histogram of TNM stages.

spectively). Gender was distributed homogenously between T stages and lymph node metastasis, but this finding was not statistically significant (p = 0.995 and p = 1, respectively).

We also observed a significant correlation between serum Talin-1 levels and tumor size. Higher Talin-1 levels were detected in tumors that were greater than 4 cm (p = 0.003). In addition, we found a correlation between serum Talin-1 levels and the T stage (p = 0.002). The serum Talin-1 levels were also significantly correlated with distant metastasis, lymph node metastasis and lymphovascular involvement (p = 0.004, p = 0.005, and p = 0.003, respectively).

Discussion

Colon cancer is a common malignancy and is a leading cause of death for the patients[1]. Identifying the factors that affect the behavior of colon cancer is necessary for an accurate diagnosis and prognosis.

Recently, the early diagnosis of colon cancer has become the most important step in the management of colon cancer. A colonoscopy and most imaging techniques can help detect carcinoma of the colon relatively soon after the inception of the initial tumor. In most instances, in addition to imaging, surgeons and oncologists depend on the detection of CEA, which is a widespread and achievable marker, for assessing colon cancer. However, CEA has not been defined as a reliable marker for the detection of early colon cancer, and its role in prevention and therapy is unknown due to its low specificity and sensitivity. Furthermore, colonoscopy is always considered as an invasive procedure.

Previous studies have shown a relationship between serum talin-1 levels and hepatocellular carcinoma[10]. Our current study demonstrates that concentrations of Talin-1 in the serum are also correlated with colon cancer.

Talin-1 is a cytoskeletal protein that is a notable component of focal adhesion complexes of adherent cells[11]. It has been demonstrated that Talin-1 expression increases cell adhesion, migration, and invasion in prostate cancer. Moreover, although the role of Talin-1 overexpression in hepatocellular carcinoma is still contentious, we found significant differences in Talin-1 expression in patients with colon cancer compared to matched controls. Additionally, Talin-1 concentrations were associated with the TNM stage, tumor size, and lymph node metastasis. Talin-1 can modulate growth, cell cycle progression, and metastasis. This effect of Talin-1 on tumor progression has been shown by Zhang et al in 2009[12].

Talin-1 insures cell spreading and adhesion, which involves the activation of SFK-linked signaling pathways and actin polymerization by the ligand-bound integrins. After the initial spreading, the contraction of actomyosin is activated, and in the absence of the full-length talin protein, the actin that is merged in the periphery is condensed towards the central cytoplasm, which holds the microtubules and cytoplasmic vesicles in place. The head of the Talin protein promotes integrin binding to the extracellular matrix. Thus, in the presence of full-length talin, the contractions of actin filaments pull on the ligand-bound integrins, which causes the assembly of focal adhesions and the activation of force-dependent signaling. Thus, Talin confers a mechanical linkage between integrins and actin filaments during the process of cell spreading, which leads to increased cell adhesion and angiogenesis[12,13]. Further changes in the expression pattern of Talin is one of the general features of malignant cells and often results in changes in cell polarization and the disruption of adherens junctions[13,14].

Conclusions

To our knowledge, this is the first study to show the detection of Talin-1 in colon cancer patients, with a validation by ELISA. According to our study, Talin-1 may be a valuable biomarker

for colon cancer, which may be essential for the early detection, especially in combination with other markers in population screenings that are currently used to detect the colon cancer. Moreover, if further studies of Talin-1 in colon tumors are permitted, the inclusion of additional patients for analysis are needed for confirmation and to generalize the present results.

Conflict of interest

The Authors declare no conflict of interests.

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