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Decreased preoperative serum fibulin-3 levels in colon cancer patients

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Abstract. – OBJECTIVE: Fibulin-3 is known to play a role in tumor cell malignancy, invasion and metastasis, as well as in the clinical progression of tumors. This study aimed to assess serum fibulin-3 levels in patients with colon cancer compared with healthy controls and its relationship to demographics and tumor pathology.

PATIENTS AND METHODS: A total of 80 patients (mean age, 58.99 years; 42% males) with colon cancer and 50 controls (mean age, 57.75; 55% males) were included. Serum levels of fibulin-3 were determined using a commercially available sandwich ELISA (Enzyme-Linked ImmunoSorbent Assay).

RESULTS: Preoperative serum fibulin-3 levels were significantly lower in the group of patients with colon cancer (mean, 35.91 ng/mL; range, 10-73 ng/mL) compared with the control group (mean, 96.68 ng/mL; range, 57-168 ng/mL).

CONCLUSIONS: It was concluded that fibulin-3 is expressed at a lower level in colon cancer, and it can serve as a marker for advanced colon cancer.

Key Words:
Fibulin-3, Colon, Cancer.

Introduction

In countries with a westernised lifestyle about half of all deaths are caused by circulatory disease and a quarter by cancer. Worldwide, colorectal cancer represents 9.4% of all incident cancer in men and 10.1% in women. Colorectal cancer, however, is not equally common throughout the world. About 10 million new cases are now diagnosed each year. Potential prognostic and predictive biomarkers of colon cancer are still being investigated. Establishment of these factors, especially those obtained from patient blood samples, may play a key role in prevention, screening, and treatment of colon cancer[1].

It has been reported that carcinoembryonic antigen (CEA) is a key biomarker of colon cancer, which may be used in clinical practice[2]. However, earlier studies showed that serum CEA has limited sensitivity for early diagnosis of colon cancer. Thus, CEA is not suggested as a screening factor for colon cancer, but it may be a marker for monitoring surgical treatment delivery and systemic therapy[1,3,4].

The fibulin-3 gene, also recognized as epidermal growth factor-containing fibulin-like extracellular matrix protein 1 (EFEMP1) on chromosome 2p16[5], is a member of the fibulin gene family that consists of 7 extracellular matrix proteins[6]. Fibulins are widely expressed and localized at basal membranes, stroma and extracellular matrix fibers mediating cell-to-cell and cell-to-matrix conduction[7]. While various functions of the fibulin gene family members have been demonstrated in detail[8-10], the role of fibulin-3 during improvement of tumors has not yet been determined. Albig et al[11] showed that fibulin-3 antagonized tumor angiogenesis and dissolved the blood vessel growth and consistency in the tumor. Sadr-Nabavi et al[12] showed that the level fibulin-3 expression decreased in spaced breast cancers due to anomalous encourager methylation and was related to tenuous survival. Similar results were found in lung cancer[13] and hepatocellular cancer[14]. However, some reports showed that fibulin-3 overexpression caused the proceeds of VEGF and promoted tumor growth and invasion in pancreatic adenocarcinoma[15] and malignant glioma cells[16]. Additionally, fibulin-3 expression was shown to be associated with lymph node metastasis, vascular invasion and poor survival in cervical cancer[17]. These effects of fibulin-3 in colon cancer have never been evaluated.

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In this study, we determined the expression level of fibulin-3 in 80 patients with colon cancer, and the clinico-pathological severity of their disease was assessed.

Patients and Methods

Patients and Controls

From October 2013 to June 2015 80 consecutive patients with histologically confirmed colon cancer and no concomitant diseases that could possibly lead to an increase or decrease of fibulin-3 serum levels (i.e., chronic inflammatory disorders, diabetes mellitus, and ischemic heart disease) were enrolled in the study. Rectal carcinomas were excluded because their biology, surgical treatment options, and clinical behavior can differ greatly from other large bowel carcinomas, thus possibly causing misinterpretation of the results. Informed consent was obtained from all patients. Cancers were staged according to the TNM classification system based on evaluation of findings of physical examination, routine laboratory tests, and diagnostic imaging (chest X-ray; brain, chest, and abdominal computed tomography scans; scintigraphic bone scan; and endoscopy). All patients underwent surgical exploration. Potentially curative surgery was defined. The study was approved by the Institutional Review Board, and informed consent was obtained either from the patient or the patient's family.

Patients with prior radio- or chemotherapy were excluded from the study. The study group consisted of 40 males and 40 females, median age 68 years (interquartile range: 48-72). The control group included 50 healthy blood donors (27 male and 23 female) with a median age of 60 years (interquartile range: 46-69).

The following parameters were recorded in all patients: age, gender, site of tumor (right colon *vs*. left colon), degree of histologic differentiation (well differentiated, moderately differentiated, or poorly differentiated), and TNM stages.

Biochemical Analyses

Samples were allowed to clot for two hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000x g. Samples were assayed immediately or stored in aliquots at -20°C or -80°C. Serum levels of fibulin-3 were determined using a commercially available sandwich ELISA (Biocompare, Catalog No: ABIN421041 Aachen, Germany) (Detection range: 1.563-100 ng/mL; minimum detection limit: 0.65 ng/mL; Intra-assay: CV less than 10%; Inter-assay: CV less than 12%; High sensitivity and excellent specificity; no significant cross-reactivity or interference between fibulin-3 and analogues was observed).

Statistical Analysis

Shapiro-Wilk normality control and one-sample Kolmogorov-Smirnov tests were performed, and histogram charts were drawn. Average, medians, including min. and max., were presented in the form of frequency and percentage. Groups of two independent samples *t*-test (for variables with normal distribution) and Mann-Whitney U test (for normally distributed variables) were evaluated. Fib3 variable was achieved by taking the natural logarithm of normality because it deviated from normality. Logarithmic values were used for analysis of covariance. Age groups in fibulin-3 variable age for the patients and controls were evaluated by analysis of covariance for different variables on the covariance. Three or more groups were compared by Kruskal-Wallis one-way analysis of variance. Afterwards, the binary comparisons (post hoc) were performed by Dunn's test. Gender variables between the 2 groups were analyzed by chi-square test and Yates correction. The significance limit was taken as $p < 0.05$ and duplex. Analyses were performed using SPSS 21v and NCSS10 programs.

Results

Patient characteristics are summarized in Table I. Preoperative fibulin-3 serum levels were significantly lower in patients with cancer (mean, 35.91 ng/mL; range, 10-73 ng/mL) compared with the levels in the control group (mean, 96.61 ng/mL, range, 57-168 ng/mL) (Table II) (Figure 1).

Table III presents the serum fibulin-3 levels of patients with colon cancer according to clinicopathologic variables.

The serum fibulin-3 levels were significantly lower in patients with colon cancer with reducing tumor burden, lymph vascular involvement, distant metastasis, lymph node metastasis, and TNM stages ($p < 0.001$). The serum fibulin-3 levels decreased from T1 tumors to T4 tumors, and this difference was statistically significant ($p < 0.001$) (Table III) (Figure 2).

We showed a significant correlation between serum fibulin-3 levels and tumor size with lower

Table I. The clinico-pathologic features of patients and controls.

	Controls (n = 50)	Patients (n = 80)	p
Age (y)	57.75 ± 6.01	58.99 ± 5.47	< 0.05
Gender (M/F)	27/23	42/38	
	55%/45%	53%/47%	
Tumor size			
≤ 4 cm			
> 4 cm		39	
		41	
TNM stage			
TI		20	
TII		18	
TIII		18	
TIV		24	
Invasion			
T1		18	
T2		18	
T3		19	
T4		25	
Lymph node metastasis			
N0		28	
N1		22	
N2		30	
Metastasis			
Present		59	
Not present		21	

Table II. . Preoperative serum Fibulin-3 levels of patients and controls (mean) (min-max).

	Controls	Patients	p
Fibulin-3 (ng/mL)	96.61 (57-168)	35.91 (10-73)	< 0.001

fibulin-3 levels detected at the > 4 cm tumor size ($p = 0.002$), and between serum fibulin-3 levels and T stages ($p = 0.003$). The serum fibulin-3 levels were correlated significantly with distant metastasis, lymph node metastasis, and lymph vascular involvement ($p = 0.004$, $p = 0.005$, $p = 0.003$, respectively).

Discussion

The results of this study have demonstrated that the levels of fibulin-3, which was detected in the blood directly derived from the tumor, were related to tumor stage, and the contribution of fibulin-3 expression was more substantial than any other clinical parameters.

Tumor angiogenesis, which is the ability of tumors form new blood vessels, describes a major step in tumor development through which the tumor is invigorated by a substantive procurement of blood procuring, leading to maintenance of tu-

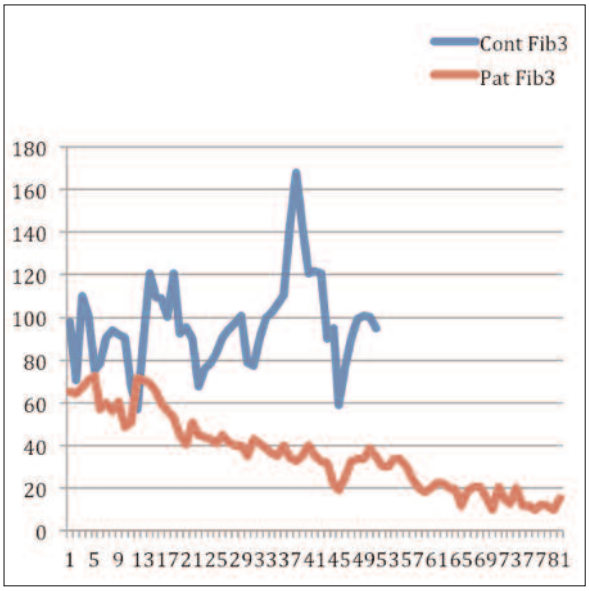

Figure 1. The histogram of the levels of fibulin-3 in controls and in all patients groups. Cont Fib3: The levels of fibulin-3 in control groups. Pat Fib3: the levels of fibulin-3 in patients groups.

Table III. Preoperative serum fibulin-3 levels of the clinicopathologic variables in patients (mean) (minimum-maximum).

	Fibulin-3 (ng/mL)
Tumor size	
≤ 4 cm	49.65 (33-73)
> 4 cm	21.84 (10-39)
TNM stage	
TI	60.09 (40-73)
TII	40.21 (33-51)
TIII	31.71 (19-40)
TIV	16.51 (10-25)
Invasion	
T1	62.04 (49-73)
T2	41.23 (35-51)
T3	31.87 (19-40)
T4	16.51 (10-25)
Lymph node metastasis	
N0	54.19 (35-73)
N1	33.62 (19-43)
N2	18.21 (10-34)
Metastasis	
Present	27.32 (10-45)
Not present	58.97 (40-73)

mor growth, invasion, and metastasis[18]. This process is regulated through a number of pro- and anti-angiogenic factors released by the tumor and microenvironment[19], and tumors are poised to prompt the acquisition of their own blood during progression.

Fibulins, members of the family of extracellular matrix glycoproteins, are comprised of a stretch of calcium-binding epidermal growth factor-like modules followed by a singular C-terminal fibulin-type module. Fibulins behave not only as intermolecular bridges within the extracellular matrix to form super-molecular temperaments but also as mediators of cellular processes and tissue modulation[6,7]. Until now, dysregulation of these molecules has been found in relation to vasculo-genesis and cancer biology[20]. Fibulin-3 may be a probable tumor suppressor gene, which functions as the antagonist of angiogenesis.

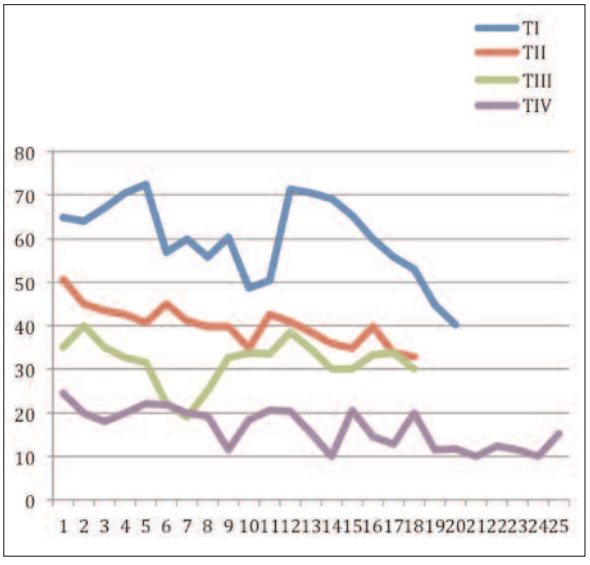

Figure 2. The histogram of the levels of fibulin-3 in TI-IV stages.

Our results also demonstrated that down-regulation of fibulin-3 in colon cancer was correlated with tumor stage, lymph node metastasis and poor disease-free survival. Recent reports from cancer studies have revealed the crucial role of angiogenesis in cancer progression. These findings suggest that down-regulation of fibulin-3 may refer the patient prognosis by promoting the angiogenic activity of colon cancer cells, and fibulin-3 may be an original molecular biomarker for the prognosis of colon cancer.

Various new studies reported that fibulin-3 was up-regulated in glioma[16] and cervical cancer[17]. However, endothelial cell proliferation and migration were not regulated by fibulin-3, indicating an indirect angiogenic impact. This discrepancy with our results may have occurred because fibulin-3 regulates cellular processes in a context-specific manner, the variant histological types of tumors with different microenvironments may have dissimilar expression patterns of fibulin-3, and the tumor microenvironment affects the tumor-associated genes to mediate angiogenesis and metastasis through their protein-protein interactions[21,22]. For instance, tissue inhibitor of metalloproteinases-3 (TIMP-3) is a binding partner of fibulin-3, and their interaction may promote VEGF binding to VEGF receptor-2, resulting in an inhibition of angiogenesis[23]. Furthermore, fibulin-3 competes with epidermal growth factor for binding to the epidermal growth factor receptor, and it activates MAPK and Akt pathways in pancreatic cancer[24]. However, the full mechanism required still entails further study.

Conclusions

The results of the current study support the hypothesis that fibulin-3 is down-regulated in colon cancer and may be a consequential factor in colon cancer. Our results also provide evidence

that serum fibulin-3 levels may be useful for predicting the outcomes of patients who undergo surgery. Elucidation of the role of serum fibulin-3 down-regulation levels in broader patient populations will require further research.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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