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ORIGINAL ARTICLE

AKAP12/Gravin gene expression in colorectal cancer: clinical importance and review of the literature

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Summary

Purpose: *: Colorectal cancer (CRC) is a common and potentially lethal disease. A number of genetic aberrations is known to take place in colorectal carcinogenesis, which leads to progressive alteration of normal mechanisms controlling cell growth. A-kinase-anchoring protein 12 (AKAP12) plays a role in cell proliferation, angiogenesis and cytoskeletal remodeling. The purpose of this study was to demonstrate the role of the AKAP12 gene expression in CRC patients and to determine its relationship (if any) with prognosis.*

Methods: *AKAP12 gene expression was investigated by immunohistochemistry.*

Results: *A total of 55 patients (63.6% males, 36.4% females) with histologically confirmed CRC were studied. Normal intestinal epithelium showed weak basal staining, dysplastic areas were stained mildly, whereas all of the cancer cells were stained completely with AKAP12.*

Conclusion: *AKAP12 gene seems to play a role in colorectal carcinogenesis.*

Key words: *AKAP12/Gravin, anchoring proteins, carcinogenesis, colon cancer, immunohistochemistry, tumor suppressor gene*

Introduction

CRC is a common and potentially lethal disease. It is the second most common cancer in females and the fifth most common cancer in males in Turkey [1]. Prognostic factors are important in planning treatment for CRC patients.

Carcinogenesis is a multistep process with many factors being involved. Prognosis may be established on the basis of certain clinical and laboratory parameters obtained during diagnosis. Determining the mechanisms that play a part in carcinogenesis may suggest the administration of targeted therapies. Laboratory findings may be helpful in deciding on the treatment intensity for each patient.

Gravin/ AKAP12, a member of A-kinase-anchoring proteins (AKAPs) family, is a tumor suppressor gene. AKAPs family constitute a group of scaffolds that play an essential role in catalyzing the spatial-temporal, dynamic interactions of protein kinase A, protein kinase C, tyrosine kinases, G-protein-coupled receptors and ion channels. Gravin has been studied in a limited number of tumors, such as lung and gastric cancer, and a decrease in the level of its expression has been demonstrated in these malignancies [2-4]. Yildirim et al. found that AKAP12 expression was decreased in cases of acute myeloid and lymphocytic leukaemia and was associated with decreased overall survival [5].

The purpose of this study was to demonstrate the role of the AKAP12 gene expression in CRC patients and to determine its relationship (if any) with prognosis.

Methods

Patients

The study encompassed patients with CRC confirmed histopathologically in the Department of Medical On-

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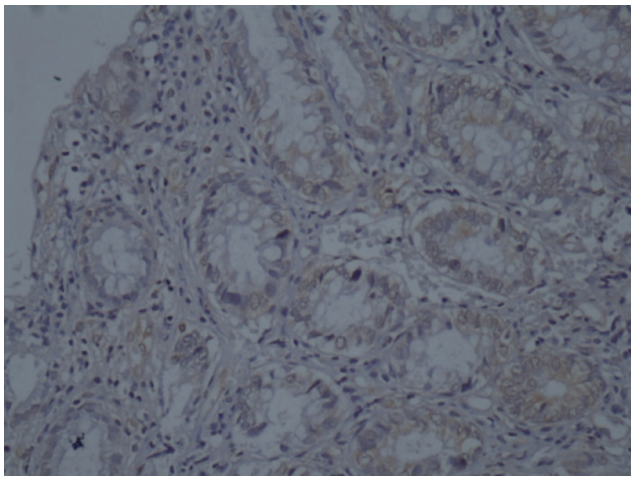

Figure 1. Basal weak staining in normal intestinal epithelium (AKAP12 x200).

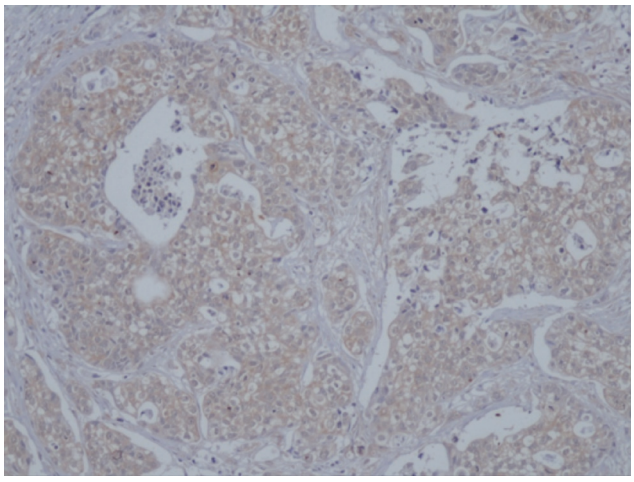

Figure 2. Mild (1+) cytoplasmic staining in tumor cells (AKAP12 x100).

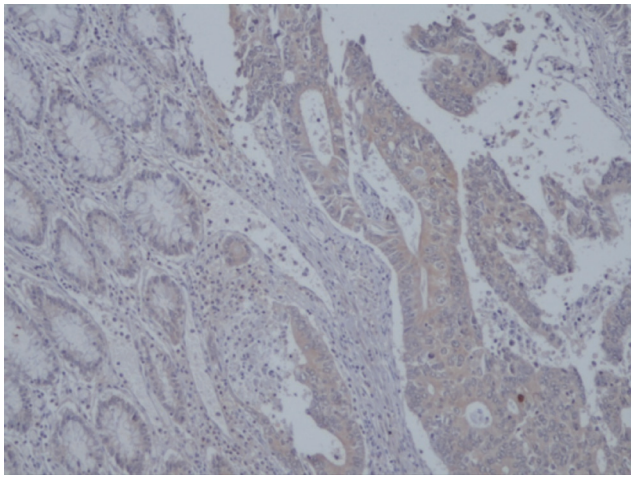

Figure 3. Basal staining in normal intestinal epithelium and moderate (2+) cytoplasmic staining in tumor cells (AKAP12 x100).

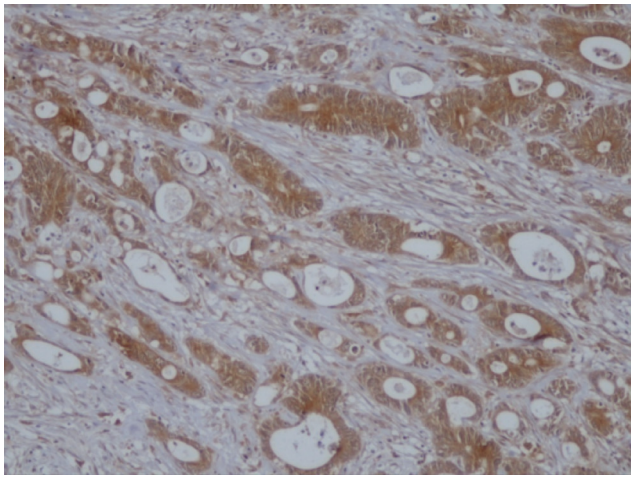

Figure 4. Strong (3+) cytoplasmic staining in tumor cells (AKAP12 x100).

cology of Antalya Education and Research Hospital between 2008-2010. Patients with unconfirmed histopathological diagnosis, and patients who were under treatment in our clinic following a period of treatment that started in other institutions were excluded. The patient files were reviewed retrospectively and data about disease stage and therapeutic modalities applied were registered. All of the patients were staged using the 7th edition of the International Union Against Cancer (VICC) TNM staging system. CRC patients were grouped according to depth of invasion, pathological stage, histological grade, presence or absence of lymph node metastasis and lymphovascular invasion.

Immunohistochemical studies

All specimens were obtained from tissues fixed by formalin and embedded in paraffin. Paraffin blocks were cut into 4 μm sections and were first stained with hematoxylin and eosin for preliminary evaluation. After deparaffinization of the embedded tissues with xylene, gradual rehydration was done with ethanol and the procedure for AKAP12 was performed. In staining the specimens for AKAP12 oncoprotein expression, lyophilized mouse monoclonal antibody (clone100/D5, 1:50, Abcam, USA) was used. Following staining, the slides were evaluated with a Nikon Eclipse 80i microscope.

Immunohistochemical scoring

Expression rates for the positive tumor cells in the specimens were evaluated by two pathologists who were unaware of the patients' clinical features. AKAP12 expression was evaluated according to cytoplasmic staining in the cells. Immunohistochemical staining determined in more than 10% of the tumor cells was considered as positive, whereas if less than 10% of the tumor cells were stained, it was considered as negative. Positive cases were classified according to the intensity of staining in comparison with adjacent non-neoplastic epithelium (Figures 1-4). If the intensity of the staining was similar to adjacent non-neoplastic epithelium, it was scored as mild (1 +), while cases with strong staining were scored as severe (3 +), and the remaining cases were scored as moderate (2 +).

Table 1. Patient characteristics

Characteristics	*Mean± SD*	*Median*	*Normal range*
Age (years)	60.8±12	62	
Height (cm)	163.1±8.6	164	
BUN (mg/dl)	14±4.5	13	7-25
Cre (mg/dl)	0.81±0.23	0.8	0.5-1.3
AST (U/L)	22.3±15	18	10-41
ALT (U/L)	20.1±17.4	15	9-37
LDH (U/L)	200±68.5	182.5	240-480
WBC (10^3/mm^3)	7.61±5.18	6.76	4-11
PTL (10^3/mm^3)	264.6±99.6	246.5	150-440
Hb (g/dl)	12.4±1.5	12.1	12-16

BUN: blood urea nitrogen, Cre: creatinine, AST: aspartate amino transferase, ALT: alanine amino transferase, LDH: lactic dehydrogenase, WBC: white blood cells, PLT: platelets, Hb: hemoglobin, SD: standard deviation

Statistics

Statistical analyses were performed using the SPSS software version 15. The association between AKAP12 and other prognostic factors (tumor stage, grade, lymphovascular invasion and lymphocytic reaction) was evaluated by chi-square test. A p-value of less than 0.05 was accepted as statistically significant.

Results

A total of 55 patients (N=35; 63.3% males and N=20; 36.4% females), were studied. The mean patient age was 60.8±12 years (range 26-82). Table 1 displays some basic lab examinations. All of the cases were of the usual gland-forming adenocarcinoma.

Patients were evaluated according to the depth of invasion, one of whom (1.9%) had T1, 3 (5.4%) T2, 41 (78.2%) T3, 6 (10.9%) T4 and 4 (7.3%) Tx disease. In relation to TNM stage one patient (1.8%) had stage 1, 19 (34.5%) stage 2, 30 (54.5%) stage 3 and 5 (9.1%) stage 4. Tumor grades were as follows: 1,2 and 3 in 7 (12.7%), 43 (78.2%) and 5 (9.1%) of the patients, respectively. Twenty-five (45.5%) cases had no lymph node metastasis, 14 (25.5%) had ≤3 metastatic and 11 (19.9%) had >3 metastatic lymph nodes (Table 2).

Lymphovascular invasion was detected in 33 (60%) patients, perineural invasion in 17 (30.9%) and lymphocytic reaction in 14 (25.5%) patients. The amount of mucin component in the tumor cells was <5% in 42 (76.4%), 5-10% in 7 (12.7%), 10-50% in 5 (9.1%) and >50% in 1 (1.8%)) of the patients (Table 2).

Table 2. AKAP 12 and other prognostic factors

Prognostic factors	*AKAP12 (+) N=41 N (%)*	*AKAP12 (-) N=14 N (%)*	*All patients N=55 N (%)*	*p-value*
T stage				0.324
T1	0	1 (7.1)	1 (1.9)	
T2	3 (7.3)	0	3 (5.4)	
T3	31 (75.6)	10 (71.4)	41 (74.5)	
T4	3 (7.3)	3 (21.5)	6 (10.9)	
Tx	4 (9.8)	3 (21.5)	4 (7.3)	
Grade				0.312
1	5 (12.2)	2 (14.3)	7 (12.7)	
2	34 (82.9)	9 (64.3)	43 (78.2)	
3	2 (4.9)	3 (21.4)	5 (9.1)	
Lymphovascular invasion				0.398
Negative	15 (36.6)	6 (42.9)	21 (38.1)	
Positive	25 (61)	8 (57.1)	33 (60)	
Missing	1 (2.4)	0	1 (1.9)	
Perineural invasion				0.995
Negative	27 (65.9)	10 (71.4)	37 (67.2)	
Positive	13 (31.7)	4 (28.6)	17 (30.9)	
Missing	1 (2.4)	0	1 (1.9)	
Lymphocytic reaction				0.438
Negative	28 (68.3)	12 (85.7)	40 (72.6)	
Positive	12 (29.3)	2 (14.3)	14 (25.5)	
Missing	1 (2.4)	0	1 (1.9)	
Mucin content (%)				0.029
<5	33 (80.6)	9 (64.3)	42 (76.4)	
5-10	6 (14.6)	1 (7.1)	7 (12.7)	
10-50	1 (2.4)	4 (28.6)	5 (9.1)	
>50	1 (2.4)	0	1 (1.8)	

Normal intestinal epithelium and apical surfaces showed nonspecific immunostaining, dysplastic areas were stained mildly, whereas all of the cancer cells were stained completely with AKAP12.

According to the intensity of immunostaining, AKAP12 gene expression in patients with CRC was as follows: 1+ in 14 (25.5%) patients, 2+ in 24 (43.6%) and 3+ in 17 (30.9%). As it cases were considered negative, 41 (74.5%) patients were considered as AKAP12 positive and 14 (52.5%) as negative. There was no statistically meaningful relationship determined between AKAP12 gene expression and stage, histological grade, lymphovascular invasion, perineural invasion and lymphocytic reaction (p=0.324, p=0.312, p=0.398, p=0.995, and p=0.438, respectively). There was statistically meaningful relationship determined between AKAP12 gene expression and mucin content in tumor cells (p=0.029). A decrease in AKAP12 gene expression level was detected in tumor cells with increased mucin content (Table 2).

Discussion

CRC is the fourth most frequently diagnosed cancer and the second leading cause of cancer deaths in the United States [6]. A number of genetic aberrations is known, which leads to progressive alteration of normal mechanisms controlling cell growth [7]. AKAP12 belongs to the AKAP family and has been detected initially in the serum samples of patients with myasthenia gravis in 1992. Since it was firstly detected in patients with myasthenia gravis, it was named Gravin [8]. AKAP12 plays a role in cell proliferation, angiogenesis and cytoskeletal remodeling [9].

AKAP12 gene is located on chromosome 6q24-25.2. Three distinct isoforms of AKAP12 (named as AKAP12/A, AKAP12/B and AKAP12/C) have been detected: 305, 287 and 250 kDa. AKAP12 can regulate Raf/MEK/ERK pathways through direct scaffolding functioning upstream of ERK, JNK, and PKC signaling. Since AKAP12 suppresses oncogenic proliferation, invasion and neovasculari-

Table 3. AKAP12 gene expression in malignancies

Author	*Tumor type*	*Material*	*Method*	*Result*	*References*
Frankfort	v-src oncogene transformation	NIH3T3 fibroblast cell culture	PCR	Decreased expression	13
Xia	Prostate cancer	Cell culture, normal and hyperplastic prostate tissue samples	FISH NB WB IF	Role of metastasis development	14
Wikman	Lung adenoCa	18 patient and 4 control tissue samples	cDNA-Array RT-PCR	Decreased expression	15
Wasenius	Papillary thyroid cancer	18 patient and 3 control tissue samples	RT-PCR cDNA array IH	Decreased expression	16
Choi	Gastric cancer	Cell culture, 18 patients' normal and tumor tissues	NB WB RT- PCR MSP-PCR	AKAP12 gene, methylation, decreased expression	3
Huang	Breast cancer	MMTV-Wnt-1 transgenic mouse breast cancer model	WB IH	Decreased expression	17
Lahav	Malignant melanoma	Melanoma cell culture	RT-PCR cDNA array	Increased AKAP12 expression with ETRB inhibition	18
Bilban	B-CLL	42 B-CLL samples	RT-PCR cDNA array	Increased AKAP12 expression in B-CLL	11
Boultwood	AML, CML and MDS	41 AML, 36 CML, 10 MDS	RT-PCR	Decreased expression	12
Yildirim	AML, ALL	100 AML, 37 ALL	RT-PCR	Poor prognostic factor in leukemia	5

B-CLL: chronic lymphocytic leukemia, AML: acute myeloid leukemia, CML: chronic myeloid leukemia, MDS: myelodysplastic syndrome, ALL: acute lymphocytic leukemia, PCR: polymerase chain reaction, FISH: fluorescence in situ hybridization, NB: northern blot, WB: western blot, IF: immunofluorescene, CDNA: complementary DNA, RT-PCR: reverse transcription polymerase chain reaction, IH: immunohistochemistry, ETRB: endothelin receptor B

zation, it functions as tumor suppressor gene [10]. Our study revealed that AKAP12 expression was detected in tumor cells with variable intensity.

Although the number of studies about AKAP12 expression in malignant tumors is limited, an association between AKAP12 gene expression and tumor behavior has been reported. AKAP12 levels or expressions have been found to be decreased in prostate, lung, breast, thyroid, and gastric cancers and malignant melanoma. Also, AKAP12 expression in leukemia have been investigated in two studies [11,12]. In these studies mainly FISH, Northern blot, Western blot analysis of the immune fluorescence analysis and immunohistochemical methods were used. The available studies that have evaluated AKAP12 expression in various malignancies have been summarized in Table 3.

A few studies have investigated the relationship between CRC and AKAP12 expression. Liu et al. applied methylation-specific high resolution melting (MS-HRM) technology to detect quantitatively AKAP12 methylation in the peripheral blood from 100 CRC patients and 50 healthy volunteers and in 3 CRC cell lines. They found that AKAP12 methylation was significantly higher in well differentiated CRC samples [19]. In another study it was demonstrated that AKAP12 expression suppressed tumor growth by inducing apoptosis in a human CRC cell line [20].

Our study shows that AKAP12 might play a role in the colorectal carcinogenesis. However, we found no relationship between AKAP12 gene expression and stage, histological grade, lymphovascular invasion, perineural invasion and lymphocytic reaction, but we found that AKAP12 gene expression was detected in all of the cells of colon cancer.

Colorectal carcinogenesis is a multistep process. To investigate in which step of colorectal carcinogenesis AKAP12 plays a role, we suggest that determination of the expression of AKAP12 in colonic adenomas is necessary

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