Expression of annexin A5 in serum and tumor tissue of patients with colon cancer and its clinical significance

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Abstract

AIM
To investigate the expression of annexin A5 in serum and tumor tissue of patients with colon cancer and to analyze its clinical significance.

METHODS
Ninety-three patients with colon cancer treated at our hospital between February 2013 and March 2016 were included in an observation group, and 40 healthy individuals were included in a control group. Enzyme-linked immunosorbent assay was performed to determine the serum level of annexin A5, while immunohistochemistry was performed to determine the expression of annexin A5 in cancer tissues.

RESULTS
The serum level of annexin A5 was 0.184 ± 0.043 ng/mL in the observation group, which was significantly higher than that in the control group (P < 0.05). Annexin A5 expression was detected in 79.31% of the patients with lymph node metastasis, which was significantly higher than that in patients without lymph node metastasis (P < 0.05). Moreover, annexin A5 expression was detected in 86.96% of the patients with stage III to IV disease, which was significantly higher than that in patients with stage I to II disease (P < 0.05). The serum level of annexin A5 was 0.215 ± 0.044 ng/mL in patients whose tumors were positive for annexin A5 expression, which was significantly higher than that in patients whose tumors were negative for annexin A5 expression (P < 0.05). The serum level of annexin A5 was correlated with annexin A5 expression in colon cancer tissues.
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CONCLUSION
For patients with colon cancer, annexin A5 expression in cancer tissues is related to lymph node metastasis and tumor grade. Serum level of annexin A5 is related to annexin A5 expression in cancer tissues and is of diagnostic relevance.

Key words: Immunohistochemistry; Annexin A5; Colon cancer; Serum

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Core tip: For patients with colon cancer, annexin A5 expression in cancer tissues is related to lymph node metastasis and tumor grade. Serum level of annexin A5 is related to annexin A5 expression in cancer tissues and is of diagnostic relevance.

INTRODUCTION
Colon cancer is a common malignancy of the digestive tract. Studies have shown that the incidence of colon cancer is ≥ 0.005%[1] and that the incidence has continued to trend upwards in recent years because of risk factors such as diet and smoking[2,3]. Basic cancer research has shown that changes in the levels of certain molecules affect tumor cell proliferation and differentiation, which in turn affect the development and progression of malignant tumors[4]. Annexin A5, first isolated from human placenta, was found to bind to phosphatidylserine in a calcium-dependent manner[5-8]. In the present study, 93 patients with colon cancer who were treated at our hospital between February 2013 and March 2016 were included to investigate the expression of annexin A5 in serum and in cancer tissues, with an aim to investigate its clinical significance.

MATERIALS AND METHODS

General information
Ninety-three patients with colon cancer (observation group) who were treated at our hospital between February 2013 and March 2016 were included in this study. The inclusion criteria were as follows: (1) pathologically confirmed colon cancer; (2) complete clinical and pathological data; and (3) being willing to provide informed consent. The exclusion criterion was incomplete clinical or pathological data. Forty healthy individuals who underwent a routine health checkup at our hospital were included as controls. No significant difference was observed with respect to age or gender between the two groups (Table 1).

Detection of serum level of annexin A5
A fasting venous blood sample was collected from each subject in the morning and centrifuged at 10000 r/min to separate the serum, which was then stored at -20℃ and tested within one week to determine the annexin A5 level. The Roche automated biochemical analyzer E170 module was used for testing, and the assay kit was purchased from Shanghai Taikang Biotechnology Co., Ltd. The assay was performed according to the instructions given in the package insert. Control serum or standard was included with the kit.

Immunohistochemistry
Paraffin sections were deparaffinized, rehydrated, and cut into 3 mm sections. The sections were incubated in 3% H2O2 at room temperature for 5 min, rinsed with deionized water (3 min × 3 times), blocked with 10% milk protein (1 g protein in 100 mL of purified water), and incubated at room temperature for 5 min. Next, the sections were incubated with a mouse anti-annexin A5 antibody (Nanjing Biyuntian Biotechnology Co., Ltd.) for 2 h at 37℃, followed by a PBS wash (5 min × 3 times). Then, the slides were incubated with a horseradish peroxidase-labeled rabbit secondary antibody (Roche) for 30 min at 37℃, followed by a PBS wash (5 min × 3 times). After that, the slides were incubated with NBT/BCIP reagent, which was used to develop the reaction, for 5 min. Finally, the sections were counterstained, dehydrated, cleared, mounted, and observed under an OLIPICS microscope (Shanghai Precision Instrument Co., Ltd). All the required reagents were purchased from Nanjing Taikang Biotechnology Co., Ltd.

Evaluation criteria for immunohistochemical staining
Immunohistochemical staining was considered positive if yellow granules were present in the cytoplasm of tumor cells or stromal cells. The staining intensity was graded as follows: 0, no staining; 1, light yellow; 2, yellow; and 3, brown. The percentage of positive cells was scored as follows: 0, < 5%; 1, 5% to 24%; 2, 25% to 50%; 3, 51% to 74%; and 4, ≥ 75%. The product of the staining intensity and the percentage of positive cells was either < 2 (negative) or ≥ 2 (positive).
Statistical analysis
SPSS v19.0 was used for statistical analyses. Measurement data are expressed as mean ± SD and were analyzed by the t-test. Count data were analyzed by the χ² test. Spearman rank correlation analysis was performed to analyze potential correlations between variables. A receiver operating characteristic curve was used to analyze the diagnostic value of serum annexin A5 level. P < 0.05 was considered statistically significant.

RESULTS
Annexin A5 expression in cancer tissue
No significant difference was observed in the positive expression rates of annexin A5 among patients of different ages or genders, or those with different tumor diameters. Moreover, 79.31% of the patients with lymph node metastasis expressed annexin A5, which was significantly higher than the percentage of patients without lymph node metastasis (P < 0.05); 86.96% of the patients with stage III to IV disease expressed annexin A5, which was significantly higher than the percentage of patients with stage I to II disease (P < 0.05) (Table 2).

Serum levels of annexin A5 in the two groups
The serum level of annexin A5 was significantly higher in the observation group than in the control group (P < 0.05) (Table 3).

Correlation between serum level of annexin A5 and expression of annexin A5 in tumor tissue
The serum level of annexin A5 was 0.215 ± 0.044 ng/mL in patients whose colon tumors were positive for annexin A5 expression, which was significantly higher than the percentage of patients with stage I to II disease (P < 0.05) (Table 2).

Diagnostic value of serum level of annexin A5
The ROC curve for the serum level of annexin A5 in the diagnosis of colon cancer showed an area under the curve of 0.732 (P < 0.05). At a cutoff value of 0.148 ng/mL, the sensitivity was 83.90%, and the specificity was 57.50% (Figure 1).

DISCUSSION
Changes in diet, excessive alcohol consumption, and genetic susceptibility factors promote the development and progression of colon cancer. In particular, among elderly male smokers aged 45 or older, the incidence of colon cancer is 0.005% or higher and has continued to trend upwards in recent years[7,8]. For colon cancer, the incidence of early metastasis is high, which results in poor patient outcomes: the five years survival rate is < 35%, and the median survival time is < 32 mo[9-11]. Studies on the genetic and biological mechanisms of the development and progression of colon cancer may provide new targets for immune therapy or strategies of comprehensive biological therapy for colon cancer[12,13]. Molecular changes play an important regulatory role in the development of malignant tumors. Cell surface Connexins or membrane proteins can induce the transcription initiation activity of downstream oncogenes, which promotes aberrant activation of the cell cycle in colonic epithelial cells and leads to excessive proliferation of cancer cells[14]. Accumulating experimental data indicate that phosphatidyserine exposition is associated with apoptosis and other cell death programs[15-17], which renders it an attractive target in imaging overall cell death. Annexin A5 is identified in blood vessels as a blood anticoagulation factor and it builds voltage-dependent calcium channel in phosphatidyserine bilayers[18,19]. Corsten et al.[20] showed that through binding with strong affinity to phosphatidyserine, annexin A5 offers an interesting opportunity for visualization of aggregate
cell death\cite{21,22}, thus providing a fit benchmark for in vivo monitoring of anticancer treatment\cite{23-26}. Recently, annexin A5 has been reported as a new mediator of cisplatin-induced apoptosis by inducing voltage-dependent anion channel oligomerization in human kidney epithelial cells\cite{27,28}. Annexin A5 forms N6-acetyllysine at specific positions of the amino-terminal region of the membrane protein, and as a result, it affects the formation of a transcriptional co-inhibitory complex and participates in transcriptional repression and silencing of tumor suppressor genes via H1 phosphorylation\cite{29,30}. Previous studies have investigated the relationship between annexin A5 and liver cancer and its clinical significance. In this study, the authors investigated the expression of annexin A5 in serum and tumor tissues of patients with colon cancer and its clinical significance. In conclusion, annexin A5 is highly expressed in serum and tumor tissues of patients with colon cancer, and its expression is closely related to the clinical stage and presence of lymph node metastasis in patients with colon cancer. Nevertheless, this study has certain limitations. For instance, we did not investigate the relationship between the expression of annexin A5 and the long-term survival of patients with colon cancer.

**COMMENTS**

**Background**

Colon cancer is a common malignancy of the digestive tract. Studies have shown that the incidence of colon cancer is \( \geq 0.005% \) and that the incidence has continued to trend upwards in recent years because of risk factors such as diet and smoking.

**Research frontiers**

Basic cancer research has shown that changes in the levels of certain molecules affect tumor cell proliferation and differentiation, which in turn affect the development and progression of malignant tumors. Annexin A5 is a glycoprotein that contains a multiplex carboxyl terminus binding domain, which influences the differentiation of surface antigens on cancer cells and promotes tumor proliferation and invasion.

**Innovations and breakthroughs**

The objective was to investigate the clinical significance of annexin A5 expression in colon cancer.

**Applications**

For patients with colon cancer, annexin A5 expression in cancer tissues is related to lymph node metastasis and tumor grade. Serum level of annexin A5 is related to annexin A5 expression in cancer tissues and is of diagnostic relevance.

**Peer-review**

In this study, the authors investigated the expression of annexin A5 in serum and tumor tissues of patients with colon cancer and its clinical significance.

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