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***Observational Study***

**Expression of Annexin A5 in serum and tumor tissues from patients with colon cancer and its clinical significance**

 Sun CH *et al.* Expression of Annexin A5 in serum and tumor tissue

Chong-Bing Sun, Ai-Yan Zhao, Shuai Ji, Xiao-Qing Han, Zuo-Cheng Sun, Meng-Chun Wang, Fu-Chang Zheng

**Chong-Bing Sun,** **Ai-Yan Zhao, Xiao-Qing Han**, **Zuo-Cheng Sun, Meng-Chun Wang,** Department of General Surgery, Weifang People's Hospital, Weifang 261000, Shandong Province, China

**Shuai Ji,** Department of Anus and Intestine Surgery, Linqu People's Hospital, Weifang 261000, Shandong Province, China

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**Correspondence to:** **Fu-Chang Zheng**, **Associate Chief Physician,** Department of General Surgery, Weifang People's Hospital, 151 Guangwen Road, Weifang 261000, Shandong Province, China. zhengfc@yeah.net

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**Abstract**

***AIM***

To investigate the expression of Annexin A5 in serum and tumor tissues from patients with colon cancer and its clinical significance.

***METHODS***

In all, 93 patients with colon cancer who were treated at our hospital between February 2013 and March 2016 were included in the observation group, and 40 healthy individuals were included in the 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 (*r* = 0.312, *P <* 0.05); when the cutoff of > 0.148 ng/mL for the serum level of Annexin A5 was used in the diagnosis of colon cancer, the sensitivity was 83.90%, and the specificity was 57.50%.

***CONCLUSION***

For patients with colon cancer, Annexin A5 expression in cancer tissues was related to clinicopathological indicators; specifically, the serum level of Annexin A5 was related to Annexin A5 expression in cancer tissues and presented a certain diagnostic value.

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

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**Core tip:** For patients with colon cancer, Annexin A5 expression in cancer tissues was related to clinicopathological indicators; specifically, the serum level of Annexin A5 was related to Annexin A5 expression in cancer tissues and presented a certain diagnostic value.

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**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 proliferation and differentiation, which then affect the development and progression of malignant tumors[4]. Annexin A5 was discovered from human placenta for the first time and was found binding to phosphatidylserine in a calcium-dependent manner[5,6]. In this 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 the serum and in cancer tissues. The objective was to investigate the relationship between Annexin A5 and colon cancer.

**MATERIALS AND METHODS**

***General information***

In all, 93 patients with colon cancer (the 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) patients with pathologically confirmed colon cancer; (2) complete clinical and pathological data; and (3) informed consent and signed consent. The exclusion criteria were as follows: incomplete clinical or pathological data. Moreover, 40 healthy individuals who underwent a routine health check at our hospital were included as the control group. No significant difference was observed with respect to age or gender between the two groups (Table 1).

***Detection of the serum level of Annexin A5***

A fasting venous blood sample was collected in the morning and was centrifuged at 10000 r/min to separate the serum, which was then stored at -20 °C 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 Company Limited (Co. Ltd). The assay was performed according to the instructions given in the package insert. Control serum or standard was included with the kit. All procedures were performed in strict accordance with the instructions for use.

***Immunohistochemistry***

Paraffin sections were deparaffinized and rehydrated; 3 mm 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 (1g 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 °C, followed by a PBS wash (5 min × 3 times). Then, the slides were incubated with an HRP-labeled rabbit secondary antibody (Roche) for 30 min at 37 °C, followed by a PBS wash (5 min × 3 times). Finally, the slides were incubated with NBT/BCIP reagent, which was used to develop the reaction, for 5 min; the sections were counterstained, dehydrated, rendered transparent, mounted, and then observed under an OLIPICS electron microscope (Shanghai Precision Instrument Co. Ltd). All required reagents were purchased from Nanjing Taikang Biotechnology Co. Ltd.

***Criteria for test results***

 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 determined as follows: 0, < 5%; 1, 5% to 24%; 2, 25% to 50%; 3, 51% to 74%; and 4, and ≥ 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 analysis. The measurement data were expressed as mean ± SD were analyzed by *t*-test. Count data were analyzed by the *χ*2 test. Spearman rank correlation analysis was performed to analyze potential correlations. A receiver operating characteristic curve was used to analyze the diagnostic value *P <* 0.05 was considered statistically significant.

**RESULTS**

***Annexin A5 expression in cancer tissue***

No significant difference was observed in the positive expression rate of Annexin A5 among patients of different ages and 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).

***The serum level of Annexin A5 in the observation group and the control group***

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 the serum level of Annexin A5 and the 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 corresponding value in patients whose colon tumors were negative for Annexin A5 (0.180 ± 0.021 ng/mL) (*t* = 4.599, *P <* 0.05). A Spearman rank correlation analysis showed that the serum level of Annexin A5 was related to the expression of Annexin A5 in tumor tissues (*r* = 0.312, *P <* 0.05).

***Diagnostic value of the serum level of Annexin A5***

The ROC curve for the value of 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 the 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 blood and lymph node 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 targeted immune therapy or methods 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 activity of transcription initiation factors of downstream oncogenes, which promotes aberrant activation of the cell cycle in colonic epithelial mucosa cells and leads to excessive proliferation of cancer cells[14]. Accumulating experimental data indicate that phosphatidylserine 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 vessel as a blood anticoagulation factor and it builds voltage-dependent calcium channelin phosphatidylserine bilayers[18,19]. Corsten *et al*[20] showed that through binding with strong affinity to phosphatidylserine, Annexin A5 offers an interesting opportunity for visualization of aggregate cell death[21,22], thus providing a fit benchmark for *in vivo* monitoring of anticancer treatment[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 kidneyepithelial cells[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[29,30]. Previous studies have investigated the relationship between Annexin A5 and liver cancer or esophageal cancer and showed that uH2B-related monotone generalization increased the risk of malignant digestive tumors and promoted clinical progression[31-34]. This study explored not only the expression of uH2B in colon cancer tissue but also the diagnostic value of its serum level in the diagnosis of colon cancer.

In this study, immunohistochemical staining showed significantly high expression of Annexin A5 in colon cancer tissue and demonstrated that the positive expression rate of Annexin A5 was significantly higher in patients with lymph node metastasis than in those without lymph node metastasis. This suggests that Annexin A5 may play a role in the promotion of the invasion of lymph nodes by colon cancer cells. Furthermore, approximately 80% of the patients with late-stage (III and IV) colon cancer expressed Annexin A5, which was significantly higher than the percentage of patients with stage II disease, which suggests that Annexin A5 significantly promotes the clinical progression and worsening of colon cancer. Annexin A5 induces the activation of second messengers in cancer cells, which promotes the production of cancer cell differentiation antigens, the proliferation and differentiation of colon cancer cells, and clinical progression.

In conclusion, Annexin A5 was highly expressed in the serum and tumor tissues of patients with colon cancer, and its expression was 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 ≥ 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***

To introduce briefly the current hotspots or important areas in the research field as related to your study. Basic cancer research has shown that changes in the levels of certain molecules affect tumor proliferation and differentiation, which then 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***

To summarize and emphasize the differences, particularly the advances, achievements, innovations and breakthroughs, as compared to other related or similar studies in the literature, which will allow the readers to assimilate the major points of your article. The objective was to investigate the relationship between Annexin A5 and colon cancer.

***Applications***

To summarize the practical applications of your research findings, so that readers may understand the perspectives by which this study will affect the field and future research.

For patients with colon cancer, Annexin A5 expression in cancer tissues was related to clinicopathological indicators; specifically, the serum level of Annexin A5 was related to Annexin A5 expression in cancer tissues and presented a certain diagnostic value.

***Peer-review***

In this manuscript, the authors investigate the expression of Annexin A5 in serum and tumor tissues from patients with colon cancer and its clinical significance.

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Grade D (Fair): 0

Grade E (Poor): 0

**Table 1 General information (mean ± SD)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Group** | ***n*** | **M/F** | **Age (yr)** |
| Observation group | 93 | 54/39 | 53.29 ± 9.49 |
| Control group  | 40 | 27/13 | 52.17 ± 8.14 |
| *t*/ |   | 1.046 | 0.65 |
| *P* | 　 | > 0.05 | > 0.05 |

**Table 2 Relationship between Annexin A5 and clinicopathological features of patients with colon cancer *n* (%)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Clinicopathological Features** | ***n*** | **Positive** | ***χ*2** | ***P* value** |
| Gender | 　 | 　 | 　 | 　 |
| M | 54 | 31 (57.41) | 0.023 | > 0.05 |
| F | 39 | 23 (58.97) |
| Age, yr |   |   |   |   |
| ≥ 55  | 47 | 29 (61.70) | 0.516 | > 0.05 |
| < 50  | 46 | 25 (54.35) |
| Lymph node metastasis |   |   |   |   |
| Yes | 29 | 23 (79.31) | 7.812 | < 0.05 |
| No | 64 | 31 (48.44) |
| Tumor diameter (cm) |   |   |   |   |
| ≥ 5  | 51 | 29 (56.86) | 0.067 | > 0.05 |
| < 5  | 42 | 25 (59.52) |
| Tumor stage |   |   |   |   |
| I to II | 47 | 14 (29.79) | 31.204 | < 0.05 |
| III to IV | 46 | 40 (86.96) |

**Table 3 Serum level of Annexin A5 in the two groups (****mean ± SD, ng/mL**)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Group** | ***n*** | **Annexin A5** | ***t*** | ***P* value** |
| Observation group | 93 | 0.184 ± 0.043 | 2.904 | < 0.05 |
| Control group | 40 | 0.159 ± 0.051 |



**Sensitivity**

**1-Specificity**

**Figure 1 ROC curve for the value of the serum level of Annexin A5 in the diagnosis of colon cancer.**