Association analyses of the JAK/STAT signaling pathway with the progression and prognosis of colon cancer

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Abstract. The present study investigated the association between the Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway with tumor progression and prognosis of colon cancer. A total of 62 patients with colon cancer were selected as the colon cancer group, and 40 patients with colon lesions were selected as the benign colon lesion group. Immunohistochemistry was used to detect the expression levels of JAK-1 and STAT-3 proteins in colon tissues. The association of JAK-1 and STAT-3 proteins with the pathological parameters and prognosis of colon cancer were analyzed. The total positive rates of JAK-1 and STAT-3 proteins in lesions of patients in the colon cancer group were significantly higher compared with those in the benign colon lesion group (P<0.05). The positive expression of JAK-1 and STAT-3 proteins in patients with colon cancer were not significantly associated with sex, age, tumor differentiation degree and neurovascular invasion (P>0.05), but significantly associated with the clinical stage of colon cancer, tumor infiltration depth and lymph node metastasis (P<0.05). The survival time of patients with colon cancer with positively-expressed JAK-1 and STAT-3 proteins was significantly shorter compared with that of patients with negatively-expressed JAK-1 and STAT-3 proteins (P<0.05). tumor-node-metastasis (TNM) stage, lymph node metastasis and the expression of JAK-1 and STAT-3 proteins in the tumor were associated with the prognosis of patients with colon cancer (P<0.05). TNM stage and the expression levels of JAK-1 and STAT-3 proteins were independent risk factors influencing the prognosis of colon cancer (P<0.05). The JAK/STAT signal may be used as a novel tumor marker and prognostic factor for the diagnosis, assessment and prognosis of colon cancer.

Introduction

Colon cancer is a common malignant tumor of the digestive system in clinical practice. In recent years, its incidence rate has been increasing year by year in China. It is characterized by high malignancy and poor prognosis, which seriously threaten human life and health (1). The pathogenesis of colon cancer is complex; colon cancer cells are prone to invasion and migration, and tumor lesions are prone to metastasis (2). The earlier the diagnosis of colon cancer is, the better the prognosis will be. At present, the clinical diagnosis of colon cancer is mainly based on the results of pathological sections, which is a gold standard, but it is traumatic. In recent years, the application of tumor biomarkers in the diagnosis and prognosis of tumors has gradually become a research hotspot (3). Determining a colon cancer tumor marker in order to accurately assess the progression and prognosis of colon cancer is of great significance. Janus kinase/signal transducer and activator of transcription (JAK/STAT) signal transduction pathway is a common signaling pathway, through which many kinds of growth factors and cytokines transmit signals in cells. Under normal circumstances, this signal transduction pathway plays an important role in the growth and development of the body. However, under pathological conditions, the activation of this signal transduction pathway mediates the proliferation, differentiation and migration of malignant tumor cells and promotes the occurrence and development of many malignant tumors (4). A recent study showed that the JAK/STAT signal transduction pathway is closely related to tumor progression and prognosis of primary liver cancer (5). Wang *et al* revealed that the JAK/STAT signal transduction pathway is activated

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in colon cells (6). Therefore, the association of the JAK/STAT signal transduction pathway with tumor progression and prognosis of colon cancer were explored through the examination of the expression levels of JAK and STAT proteins in the diseased colon tissues of patients with colon cancer, and association analyses of tumor progress and prognosis.

Patients and methods

Study subjects. A total of 62 patients with colon cancer who were treated in the First Affiliated Hospital of Nanchang University (Nanchang, China) from March 2014 to February 2017 were enrolled in the study as the colon cancer group, which included 38 males and 24 females at the age of 22-80 years, with an average age of 56.23±9.03 years. The patient group included 9 cases of cecum cancer, 6 cases of ascending colon cancer, 14 cases of colonic hepatic cancer, 6 cases of horizontal colon cancer, 14 cases of descending colon cancer, 13 cases of sigmoid colon cancer; 38 cases of adenocarcinoma, 16 cases of mucinous carcinoma and 8 cases of undifferentiated carcinoma. Inclusion criteria: i) patients who had complete clinical data and follow-up results; ii) patients receiving no chemotherapy or radiotherapy prior to the pathological examination; iii) patients who would receive radical resection of colon cancer; and iv) patients who were informed (or their family members) of the study and signed the informed consent. A total of 40 patients with benign colon lesions treated in our hospital during the same period were selected as the benign colon lesion group, including 23 males and 17 females at the age of 25-76 years, with an average age of 54.39±10.11 years. This study was approved by the Ethics Committee of the First Affiliated Hospital of Nanchang University and Jiangxi Provincial People's Hospital (Nanchang, China). Signed informed consents were obtained from the patients.

Indicator detection. Rabbit anti-human JAK-1 monoclonal antibody (1:600) and rabbit anti-human STAT-3 monoclonal antibody (1:600) were purchased form Cell Signaling Technology, Inc., Danvers, MA, USA (cat. nos. 3344 and 8768), and streptomycin antibiotic protein-peroxidase staining kit was purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). Lesion tissues were taken from all patients in the colon cancer and benign colon lesion groups. The lesion tissues were fixed with 4% paraformaldehyde, and then embedded into paraffin and cut into 3 μm-thick sections. After being baked at 70℃ for 10 min and gradient elution with ethanol was performed, the sections were put into 3.0% H_2O_2 to inactivate endogenous enzymes. PBS was used for rinsing 3 times, each time for 5 min, before incubation in sheep serum (Thermo Fisher Scientific, Inc., Waltham, MA, USA) for 15 min at room temperature. A total of 20-30 μl mouse anti-human JAK-1 monoclonal antibody and mouse anti-human STAT-3 monoclonal antibody were added, respectively, before standing at 4℃ overnight. PBS was used for rinsing 3 times, each time for 5 min, and two antibodies were added. After incubation for 60 min at room temperature, PBS was used for rinsing 3 times, each time for 5 min. DAB was used for coloration for 5 min, and distilled water was used for rinsing. After hematoxylin staining for 5 min, the dilute HCl was used to desalinate for

30 sec. Before dehydration, distilled water was used to wash for 5 min. After transparency and encapsulating, the microscope was used for examination. After being dyed with DAB for 5 min, distilled water was used for rinsing. Then the samples were re-dyed with hematoxylin for 5 min, followed by HCl dilution and desalting for 30 sec. Then distillation followed for 5 min, before dehydration, transparency, film sealing, and microscopy. JAK-1 protein positive signals were indicated by brown-yellow particles in cytoplasm, and STAT-3 protein positive signals were indicated by brown-yellow particles in the cytoplasm or the nucleus. Five fields of view were randomly selected from the stained sections under an optical microscope (x200; Leica Microsystems GmbH, Wetzlar, Germany), and 100 cells were counted in each field of view. The number of positive cells with pale brown particles was counted, and the average number in 5 fields of view was taken. The twice scoring method (staining intensity score and positive cell percentage score) was used to score the staining intensity: 0 point for no yellow or pale brown, 1 point for light yellow, 2 points for pale brown and 3 points for brown. Score of positive cell percentage: 0 point for positive cells <25%, 1 point for ≥25% positive cells but ≤50% cells, 2 points for ≥51% positive cells but ≤75% cells, and 3 points for positive cells >76%. The staining intensity score and positive cell percentage score were added and equally divided into 0 point for negative, 1-2 points for weakly positive, 3-4 points for positive, and 5-6 points for strongly positive. The percentages of positive and strongly positive reactions were recorded as the total positive rates of JAK-1 and STAT-3. Pathological examination of colon samples was performed by two pathologists with extensive experience in the Department of Pathology of the First Affiliated Hospital of Nanchang University. Cancer was diagnosed only when consistent diagnosis was reached.

Treatment and follow-up. Radical colon cancer resection was performed on all patients with colon cancer. Colon cancer was removed and lymph nodes were dissected. Range of lymph node dissection included lymph nodes at middle and blood supply to the root of the blood vessels. Patients underwent radical resection of colon cancer and were followed up to record their disease-free survival (DFS).

Statistical analysis. The collected data in this study were statistically processed by the Statistical Product and Service Solutions (SPSS) 19.0 software (IBM Corp., Armonk, NY, USA). Quantitative data were expressed as mean ± standard deviation (SD), and intergroup differences in the measurement data were statistically analyzed via t-test. Intergroup differences in qualitative data were statistically analyzed by χ^2 test. P<0.05 was considered to indicate a statistically significant difference.

Results

Expression levels of JAK-1 and STAT-3 proteins in lesion tissues of patients of the colon cancer and benign lesion groups. The expression levels of JAK-1 and STAT-3 proteins in the lesion tissues of patients of the colon cancer and benign colon lesion groups were compared. The results showed that JAK-1 and STAT-3 proteins were positively expressed in the lesion tissues of patients in both the colon cancer and

Table I. Expression levels of JAK-1 and STAT-3 proteins in foci of patients of the colon cancer and benign lesion groups.

Group	n	JAK-1 protein					STAT-3 protein				
		Negative	Weakly positive	Positive	Strongly positive	Total positive rate	Negative	Weakly positive	Positive	Strongly positive	Total positive rate
Benign colon lesion group (n/%)	40	35/87.50	5/12.50	0/0.00	0/0.00	5/12.50	36/90.00	4/10.00	0/0.00	0/0.00	4/10.00
Colon cancer group (n/%)	62	5/8.06	10/16.13	24/38.71	23/37.10	47/75.81[a]	4/6.45	9/14.52	24/38.71	25/40.32	49/79.03[a]

[a]$P<0.05$ vs. the benign colon lesion group. JAK-1, Janus kinase-1; STAT-3, signal transducer and activator of transcription-3.
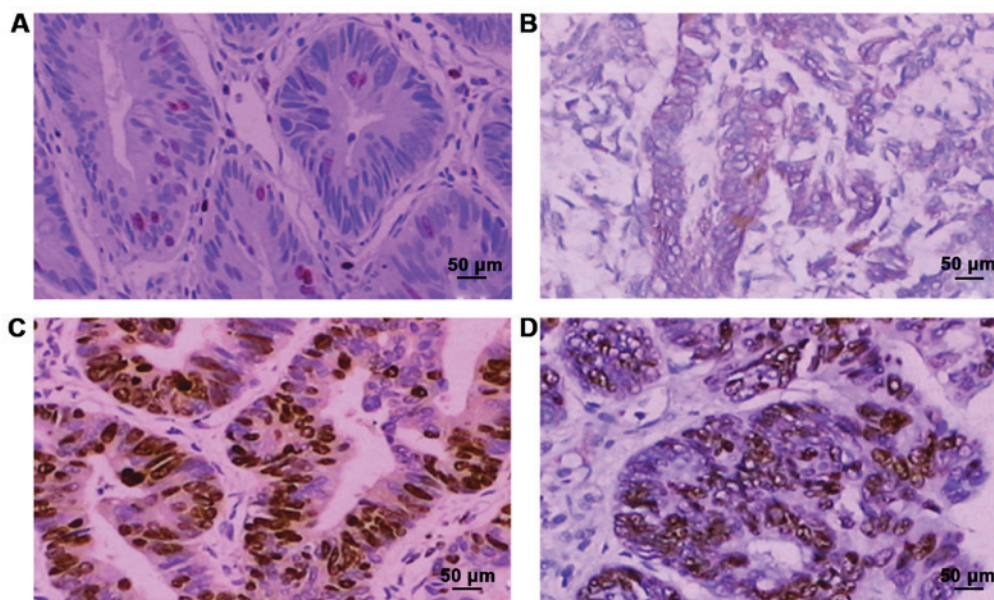

Figure 1. Expression levels of JAK-1 and STAT-3 proteins in lesion tissues of patients of the colon cancer and benign lesion groups. Weak positive (A) JAK-1 and (B) STAT-3 expression in benign lesion tissue. Strong positive (C) JAK-1 and (D) STAT-3 expression in cancer tissue. Magnification, x200. JAK-1, Janus kinase-1; STAT-3, signal transducer and activator of transcription-3.

the benign colon lesion group, but the total positive rates of JAK-1 and STAT-3 proteins in the colon cancer group were significantly higher than those in the benign colon lesion group ($P<0.05$) (Table I, Fig. 1).

Association of JAK-1 and STAT-3 proteins with clinicopathological features of patients with colon cancer. Comparative analyses were conducted for the associations of the expression levels of JAK-1 and STAT-3 proteins in colon cancer tissue with sex, age, tumor differentiation degree, neurovascular invasion, the clinical stage of colon cancer, tumor infiltration depth and lymph node metastasis of patients with colon cancer. The results revealed that the positive expression levels of JAK-1 and STAT-3 proteins were not obviously associated with patients' sex, age, tumor differentiation degree, and neurovascular invasion, but were overtly associated with the clinical stage, tumor infiltration depth and lymph node metastasis of colon cancer. The positive expression rates of JAK-1 and STAT-3 proteins in stage III or IV were significantly higher than those in stage I or II ($P<0.05$). The positive expression rates of JAK-1

and STAT-3 proteins with the infiltration in stage T3/T4 were remarkably higher than those with the infiltration in stage T1/T2 ($P<0.05$). The positive expression rates of JAK-1 and STAT-3 proteins in colon cancer with lymph node metastasis were significantly higher than those in colon cancer without lymph node metastasis ($P<0.05$) (Table II).

Comparison of the survival time of colon cancer patients with positively- and negatively-expressed JAK-1 and STAT-3 proteins. The survival time of colon cancer patients with positively- and negatively-expressed JAK-1 and STAT-3 proteins was compared. The results manifested that the survival time of colon cancer patients with positively-expressed JAK-1 and STAT-3 proteins was significantly shorter than that of patients with negatively-expressed JAK-1 and STAT-3 proteins ($P<0.05$) (Table III).

Expression levels of JAK-1 and STAT-3 proteins in the tumor of colon cancer and Cox regression model analyses of prognostic factors. Univariate Cox model analyses showed that

Table II. Association of JAK-1 and STAT-3 proteins with clinicopathological features of patients with colon cancer.

Clinical features	JAK-1 protein positive rate (n/%)	P-value	STAT-3 protein positive rate (n/%)	P-value
Sex		>0.05		>0.05
Male (n=38)	29/76.32		29/76.32	
Female (n=24)	18/75.00		18/75.00	
Age		>0.05		>0.05
≥60 years (n=34)	26/76.47		25/73.53	
<60 years (n=28)	21/75.00		22/78.7	
Tumor differentiation degree		>0.05		>0.05
High differentiation (n=33)	25/75.76		24/72.73	
Low differentiation (n=29)	22/75.86		23/79.31	
TNM stage		<0.05		<0.05
Stage III or IV (n=34)	34/100.00		34/100.00	
Stage I or III (n=28)	13/46.43		13/46.43	
Tumor infiltration depth		<0.05		<0.05
T3/T4 (n=39)	39/100.00		39/100.00	
T1/T2 (n=23)	18/78.26		18/78.26	
Lymph node metastasis		<0.05		<0.05
Yes (n=38)	38/100.00		37/97.37	
No (n=24)	19/79.17		20/83.33	
Neurovascular invasion		>0.05		>0.05
Yes (n=24)	18/75.00		19/79.17	
No (n=38)	29/76.32		28/73.68	

JAK-1, Janus kinase-1; STAT-3, signal transducer and activator of transcription-3; TNM, tumor-node-metastasis.

tumor-node-metastasis (TNM) stage, lymph node metastasis, and the expression levels of JAK-1 and STAT-3 proteins in the tumor were related to the prognosis of patients with colon cancer (P<0.05) (Table IV). Multivariate analyses of the above factors revealed that TNM stage and the expression levels of JAK-1 and STAT-3 proteins in the tumor were independent risk factors for the prognosis of colon cancer (P<0.05) (Table V).

Discussion

The malignancy degree and invasiveness of colon cancer seriously affect the prognosis of the disease. In addition, the pathogenesis of colon cancer is very complicated and not yet fully understood. However, the occurrence and development of colon cancer are multi-step processes involving multiple genes, and the molecular biological bases of the occurrence and development of colon cancer activate oncogenes and inactivate tumor suppressor genes in colon cancer (7). JAK/STAT signal transduction pathway can rapidly transmit extracellular signals to the nucleus, which plays an important role in the processes of the activation of oncogenes and the inactivation of tumor suppressor genes in colon cancer. The signal transduction process of the JAK/STAT signal transduction pathway includes the following steps (8): i) cytokines bind to the corresponding ligands on the cell surface; ii) JAK proteins aggregate with receptors and the JAK proteins nearby are activated by mutual phosphorylation,; iii) the phosphorylation of

Table III. Comparison of the survival time of patients with colon cancer with positively- and negatively-expressed JAK-1 and STAT-3 proteins.

Group	JAK-1 protein	STAT-3 protein
Negative	50.31±4.03	49.20±8.14
Positive	38.52±5.81[a]	38.10±5.39[a]

[a]P<0.05 vs. negatively expressed JAK-1 and STAT-3 proteins. JAK-1, Janus kinase-1; STAT-3, signal transducer and activator of transcription-3.

the structural domains of JAK proteins and the corresponding tyrosine residues of STAT proteins, and the disability of phosphorylated tyrosine on the functional domains and receptors of STAT proteins activate STAT proteins; and iv) STAT proteins enter the nucleus and interact with other nuclear transcription factors to complete gene regulation. JAK/STAT signal transduction pathway can regulate cell apoptosis. JAK/STAT signal transduction pathway can activate gastric motive protein 2, and the activation of gastric motive protein 2 can promote gastric cancer cell apoptosis, and reduce cell viability and cell proliferation (9). The target blocking of the JAK/STAT signal transduction pathway can promote apoptosis through inhibiting the proliferation and migration of hepatic stellate

Table IV. Univariate Cox model analyses of clinical prognostic factors of colon cancer.

Factor	Hazard ratio value	95% confidence interval	P-value
Sex (male vs. female)	1.185	0.617-2.276	0.611
Age (≥60 years vs. <60 years)	2.539	0.898-6.180	0.078
Tumor differentiation degree	1.716	0.777-3.362	0.063
TNM stage (stage III or IV vs. stage I or II)	0.885	0.442-1.783	0.025
Tumor infiltration degree (T3/T4 vs. T2/T1)	1.685	0.863-3.134	0.122
Lymph node metastasis	1.147	0.602-2.174	0.035
Neurovascular invasion	0.952	0.489-1.818	0.883
JAK-1 protein expression	1.278	0.884-3.353	0.045
STAT-3 protein expression	1.245	0.810-3.104	0.032

TNM, tumor-node-metastasis; JAK-1, Janus kinase-1; STAT-3, signal transducer and activator of transcription-3.

Table V. Multivariate Cox model analyses of clinical prognostic factors of colon cancer.

Factor	Hazard ratio value	95% confidence interval	P-value
TNM stage (stage III or IV vs. stage I or II)	0.588	0.228-1.512	0.037
Lymph node metastasis	0.549	0.122-3.315	0.096
JAK-1 protein expression	0.631	0.341-1.889	0.041
STAT-3 protein expression	0.579	0.231-1.568	0.045

TNM, tumor-node-metastasis; JAK-1, janus kinase 1; STAT-3, signal transducer and activator of transcription 3.

cells (8). JAK/STAT signal transduction pathway can promote the expression of many downstream growth factors [such as vascular endothelial growth factor A (VEGFA), insulin-like growth factor-1 and matrix metalloproteinase], and can activate, as well as promote, angiogenesis at the tumor site, thus promoting cell proliferation and survival and inhibiting apoptosis (10-12). In addition, JAK/STAT signal transduction pathway also plays regulatory roles in glycolysis, inflammatory response and epithelial mesenchymal transformation (13,14). However, the regulation processes of apoptosis, glycolysis, inflammatory response, epithelial mesenchymal transformation and angiogenesis, by the JAK/STAT signal transduction pathway are accompanied by tumor cell metastasis. Besides, studies have shown that using JAK protein inhibitor AG2490 *in vitro* can effectively promote the cell apoptosis of acute lymphoblastic leukemia (15).

JAK protein family includes JAK-1, JAK-2, JAK-3, and Tyk-2 members and STAT protein family includes STAT-1, STAT-2, STAT-3, STAT-4, STAT-5, and STAT-6 members. JAK1 in the JAK protein family and STAT-3 in STAT family are closely associated with cancer development. Therefore, JAK-1 in JAK protein family and STAT-3 in STAT protein family were selected to study the involvement of JAK/STAT signal transduction pathway in the disease. In this study, JAK-1 and STAT-3 proteins, the important members of the JAK/STAT signal transduction pathway in the colon, were examined via immunohistochemistry. The results manifested that the expression intensities and positive rates of JAK-1 and STAT-3 proteins in colon cancer tissues were higher than those

in benign lesions, suggesting that the activation of JAK-1 and STAT-3 protein expression levels may be related to the occurrence of colon cancer. Further analyses of the association of JAK-1 and STAT-3 proteins with clinicopathological features of patients with colon cancer revealed that the expression levels of these two proteins were associated with the clinical stage, tumor infiltration depth and lymph node metastasis in colon cancer, indicating that the JAK/STAT signal transduction pathway further strengthens the invasion and metastasis processes of colon cancer and promotes the progress of colon cancer. The survival time of colon cancer patients with positively-expressed JAK-1 and STAT-3 proteins was significantly shorter than that of patients with negatively-expressed JAK-1 and STAT-3 proteins. Moreover, Cox model analyses revealed that the expression levels of JAK-1 and STAT-3 proteins in the tumor and TNM stage were independent risk factors for the prognosis of patients with colon cancer, suggesting that the JAK/STAT signal transduction pathway may have an association with the prognosis of colon cancer, and reflect the patient's prognosis to some extent.

In summary, this study not only explored the association between the JAK/STAT signal transduction pathway and colon cancer, but also found that the JAK/STAT signal transduction pathway can be used as a diagnostic and prognostic marker for colon cancer.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ST, XY, XT and QL contributed to the conception and design of this study. YC and JS were responsible for the collection of the data. ST, XY, YC and JS analyzed and interpreted the data. ST, XY, YC, JS, XT and QL contributed to the writing of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the First Affiliated Hospital of Nanchang University (Nanchang, China) and Jiangxi Provincial People's Hospital (Nanchang, China). Signed informed consents were obtained from the patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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