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RESEARCH

PHOSPHO-STAT5 Expression is Associated with Poor Prognosis of Human Colonic Adenocarcinoma

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Abstract The signal transducer and activator of transcription-5 (STAT5) protein has been shown to play an important role in tumor progression through stimulating cell proliferation and preventing apoptosis. STAT5 activation has been observed in a variety of human tumors and cancer cell lines. However, it is not clear how activated STAT5 is expressed in colon cancer. In this study, we aimed to investigate phospho-STAT5 (activated form of STAT5) expression and its relationship with the clinicopathological factors and overall survival of patients with colonic adenocarcinoma. A total of 121 histological samples were selected for this study. Immunohistochemistry was used to detect the expression of phospho-STAT5. Analysis of the immunohistochemical staining was based on the proportion of stained cells in the field: positive, >15% stained cells, and negative, <15% stained cells. Survival times were analyzed using the Kaplan-Meier method, and the differences between groups were assessed with the log-rank test. A multivariate Cox regression model was used for prognostic power analysis. Expression of phospho-STAT5 was observed in the cytoplasms of colonic adenocarcinoma cells. Univariate analysis showed that phospho-STAT5 immunoreactivity was correlated with the depth of tumor invasion (P-value=0.009), tumor-node-metastasis (TNM) stage (P-value=0.048) and shorter overall survival times (P-value=0.026). Lymph node metastasis, distant metastasis and TNM stage were associated with shorter overall survival times (P-value range from 0.003-< 0.001). Multivariate analysis showed that only distant metastasis was an independent predictor of overall survival time (P-value=0.016). Our findings first demonstrate that phospho-STAT5 is frequently present and active in colonic adenocarcinoma and related to poor prognosis.

Keywords Colon carcinoma · Immunohistochemistry · Prognosis · Signal transducer and activator of transcription-5 (STAT5)

Abbreviations

STAT	Signal transducer and activator of transcription
p-STAT5	Phospho-STAT5
TNM	Tumor-node-metastasis
CRC	Colorectal cancer
WHO	World health organization
AJCC	American joint committee on cancer
H&E	Hematoxylin and eosin
SP	Streptavidin peroxidase

Introduction

Colorectal cancer (CRC) is one of the most common fatal malignancies in the Western world [1]. Every year, there are more than 150,000 new cases and 55,000 deaths in the United States and approximately 125,000 deaths in Europe [2]. The incidence of this cancer has had an upward trend over recent years in Asia [3]. The prognosis of CRC patients depends on the depth of tumor cell invasion and the presence of lymph node metastasis [4]. At first

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presentation, approximately 30% of patients already have metastases [5]. However, histopathological examination of CRC specimens cannot always assess a prognosis [6]. Recently, signal transducer and activator of transcription (STAT) proteins have been shown to play an important role in cytokine signaling pathways. Constitutive activation of STATs, especially signal transducer and activator of transcription-3 (STAT3) and signal transducer and activator of transcription-5 (STAT5), has been linked to tumor progression by stimulating cell proliferation and preventing apoptosis [7]. However, it is not fully clear how activated STATs (p-STATs) are expressed in cancer.

STATs comprise a family of cytoplasmic transcription factors that are critical for normal cellular processes, such as differentiation, proliferation, cell survival, apoptosis, angiogenesis, and hormone signaling [8]. A total of seven different STAT family members (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6) have been identified [9]. Some reports have demonstrated that STAT proteins, especially STAT3, play an essential role in a variety of cancer types [10–13].

Activation of STAT5, which is dependent on Bcr/Abl kinase activity, has been shown primarily in leukemia [14]. Subsequently, studies have demonstrated that p-STAT5 is linked to the aggressiveness of solid tumors, such as prostate cancer, breast cancer, and hepatocellular carcinoma [15–18]. Later, Xiong et al. [19] reported that STAT5 participated in CRC cell growth, cell cycle progression, invasion and migration through the regulation of target gene expression. Although expression of p-STAT5 has been detected in several cancer types, there have been no studies evaluating the expression of p-STAT5 in colonic adenocarcinoma. In this study, we sought to investigate the expression of p-STAT5 by immunohistochemical staining to identify the relationship between p-STAT5 expression and the clinicopathological features and prognosis of human colonic adenocarcinoma.

Materials and Methods

Patients

A total of 121 histological samples of human colonic adenocarcinoma (excluding rectal adenocarcinoma) were selected for this study. The samples were obtained from surgically treated patients at the Third Affiliated Hospital of Harbin Medical University in Heilongjiang Province between June 2003 and May 2004. These patients had not received chemotherapy or radiation therapy before surgery. Tumors were staged using the TNM staging system of the American Joint Committee on Cancer (AJCC sixth edition) [20]. There were 12 cases of stage I, 62 cases of stage II, 34 cases of stage III, and 13 cases of stage IV. Histological type was classified according to the World Health Organization (WHO) classification system [21]. Tumors were

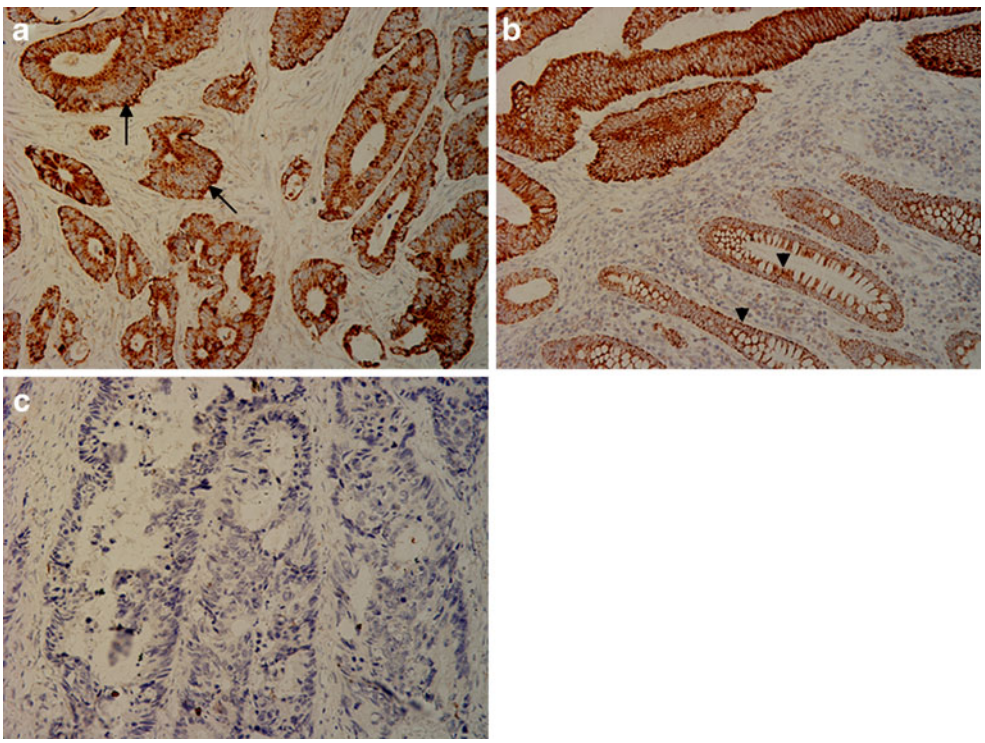

Fig. 1 Immunohistochemical staining of p-STAT5 in human colonic adenocarcinoma. **a** P-STAT5 expression was confined to the cytoplasms of tumor cells within the glandular structure (*arrows*; magnification, 200×). **b** P-STAT5 expression was observed in the cytoplasms of the normal epithelium adjacent to tumor cells (*arrowheads*; magnification, 200×). **c** There was no immunoreactivity when non-immune serum was substituted for the anti-p-STAT5 antibody in the immunohistochemical process (*magnification*, 200×)

stratified into well, moderately, poorly differentiated and mucinous adenocarcinomas. Seventy-five patients were male, and 46 were female. The mean age of the patients was 59.1 years (SD, 11.3 years; range, 25–87 years). Sixty-seven tumors were located in the left-sided colon, and 54 were in the right-sided colon. All patients were followed up for overall survival after surgery for at least 5 years.

The protocol for our study was approved by the Ethics Committee of the Third Affiliated Hospital of Harbin Medical University, and the research project was performed in accordance with the provisions of the Helsinki Declaration of 2004.

Immunohistochemistry

Principal tumor blocks were selected by checking their hematoxylin and eosin (H&E) stained slides. Immunohistochemical staining was carried out on 5-μm-thick formalin-fixed and paraffin-embedded sections using standard streptavidin peroxidase (SP) method. Briefly, sections were deparaffinized, rehydrated, immersed in an EDTA buffer (pH 8.9) and boiled for 20 min in a pressure cooker. Sections were then left to cool at room temperature and washed in PBS (pH 7.4). After individual incubations in 3% hydrogen peroxide and normal goat or rabbit serum for 10 min at room temperature, a primary rabbit polyclonal anti-human p-STAT5 antibody (Tyr 694; diluted at 1:100; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) was added to the sections and incubated overnight at 4°C. Sections were washed in PBS (pH 7.4) and individually incubated with a 1:50 dilution of biotinylated goat anti-rabbit immunoglobulin G and a 1:50 dilution of streptavidin-biotin-peroxidase complex for 10 min at room temperature. Finally, enzyme reaction was developed using 3,3′-diaminobenzidine chromogen and the slides counterstained with hematoxylin. Non-immune serum was substituted for primary antibody as a negative control. Breast cancer tissue positive for the p-STAT5

Table 1 Relationship between clinicopathological factors and p-STAT5 expression in colonic adenocarcinoma

Variable	Number	P-STAT5 Negative(%) (n=44)	Expression Positive(%) (n=77)	*P*-value
Age (yr)				0.192
>60	59	18(30.5)	41(69.5)	
≤60	62	26(41.9)	36(58.1)	
Sex				0.915
Male	75	27(36.0)	48(64.0)	
Female	46	17(37.0)	29(63.0)	
Tumor size (cm)				0.630
>5	38	15(39.5)	23(60.5)	
≤5	83	29(34.9)	54(65.1)	
Primary site[a]				0.369
Rt colon	54	22(40.7)	32(59.3)	
Lt colon	67	22(32.8)	45(67.2)	
Histological type[b]				0.173
Well, mod	86	28(32.6)	58(67.4)	
Por, muc	35	16(45.7)	19(54.3)	
T stage				0.009*
T1 and T2	15	10(66.7)	5(33.3)	
T3 and T4	106	34(32.1)	72(67.9)	
Lymph node metastasis				0.154
Negative	81	33(40.7)	48(59.3)	
Positive	40	11(27.5)	29(72.5)	
Distant metastasis				0.174
Negative	108	42(38.9)	66(61.1)	
Positive	13	2(15.4)	11(84.6)	
TNM stage				0.048**
I and II	74	32(43.2)	42(56.8)	
III and IV	47	12(25.5)	35(74.5)	

[a] Rt colon, right colon; Lt colon, left colon

[b] Well, well differentiated adenocarcinoma; mod, moderately differentiated adenocarcinoma; Por, poorly differentiated adenocarcinoma; muc, mucinous adenocarcinoma

* $P<0.01$: T1 and T2 vs. T3 and T4

** $P<0.05$: stage I and stage II vs. stage III and stage IV

antibody was used as a positive control. Analysis of the immunohistochemical staining was carried out independently by two investigators considering the mean percentage of positive tumor cells in at least five areas. Immunoreactivity was classified as follows: (a) positive, more than 15% of carcinoma cells stained; (b) negative, no detectable staining or less than 15% of carcinoma cells stained [6].

Statistical Analysis

The SPSS 10.0 software package was used for all statistical analyses. The relationship between p-STAT5 expression and variables was analyzed by chi-squared test ($\chi 2$ test). Survival time was analyzed using the Kaplan-Meier method, and differences between groups were evaluated with the log-rank test. A multivariate Cox proportional hazards model was used to analyze the prognostic power of p-STAT5 expression and pathological variables. A P-value<0.05 was considered to be statistically significant.

Results

Expression of P-STAT5 in Colonic Adenocarcinomas

Immunoreactivity to p-STAT5 staining was confined to the cytoplasms of tumor cells; no nuclear staining was found in tumor cells (Fig. 1a). Occasionally, cytoplasmic staining was detected in the normal epithelium adjacent to tumor cells (Fig. 1b). Non-immune serum was substituted for primary antibody as a negative control (Fig. 1c). Positive p-STAT5 protein staining was identified in 77 of the 121 cases (63.6%).

Relationship Between P-STAT5 Expression and Clinicopathological Characteristics

Table 1 shows the correlations between p-STAT5 expression and various clinicopathological characteristics of colonic adenocarcinomas. As shown, significant differences were found between p-STAT5 expression and tumor depth

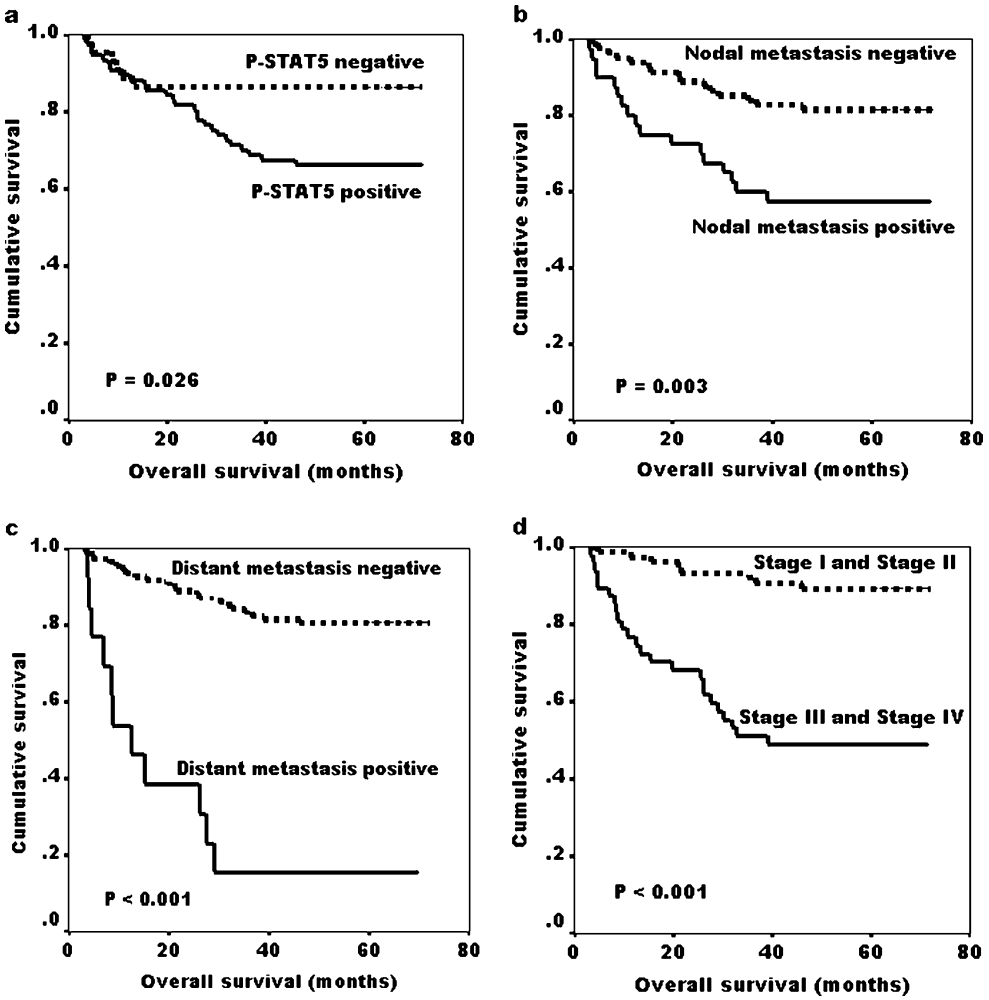

Fig. 2 Kaplan-Meier survival curves for patients with colonic adenocarcinoma. Relationships between overall survival and p-STAT5 expression (**a**), lymph node metastasis (**b**), distant metastasis (**c**), TNM stage (**d**) respectively

(*P*-value=0.009) and TNM stage (*P*-value=0.048). No associations were found between p-STAT5 expression and age, sex, primary site, tumor size, differentiation, lymph node metastasis, or distant metastasis.

Analysis of Prognostic Factors in Colonic Adenocarcinoma Patients

As shown in Fig. 2a, the overall five-year survival rates for patients with p-STAT5-positive colonic adenocarcinomas (66.2%; 51 of 77) were lower than those for patients with p-STAT5-negative tumors (86.4%; 38 of 44). This difference was statistically significant (*P*-value=0.026; log-rank test). Univariate analysis also showed that lymph node metastasis, distant metastasis, and TNM stage were connected with shorter overall survival (Table 2, Fig. 2b–d). In addition, Cox multivariate analysis confirmed that only distant metastasis was an independent predictor of shorter overall survival (*P*-value=0.016; Table 2).

Discussion

Although p-STAT5 has been observed in a variety of human tumors, it is not clear how p-STAT5 is expressed in colon cancer. To assess p-STAT5 expression and its relationship with the clinicopathological factors and overall survival of patients with colonic adenocarcinoma, immunohistochemistry was used to detect the expression of p-STAT5. In the present work, we first showed that p-STAT5 was frequently present and active in colonic adenocarcinoma clinical samples. In addition, we also showed that p-STAT5 expression was associated with colonic adenocarcinoma invasion behavior and poor prognosis. Our findings suggest that p-STAT5 plays a key role in colon cancer progression.

STAT5 activation has been previously reported in hematopoietic malignancies, prostate cancer, breast cancer, hepatocellular carcinoma, and nasopharyngeal carcinoma [14, 15, 17, 18, 22]. For prostate cancer, STAT5 activation was associated with not only a high histological grade [16] but also a shorter progression-free survival time [15, 23]. Other studies also found that poor prognosis was associated with the expression of p-STAT5 in breast cancer, hepatocellular carcinoma, and nasopharyngeal carcinoma [17, 18, 22]. In the present work, our results were consistent with the above-mentioned studies showing that p-STAT5 expression was associated with poor prognosis in patients with colonic adenocarcinoma.

STAT5 is a latent cytoplasmic protein which is composed of two highly homologous isoforms, STAT5a and STAT5b. STAT5 becomes activated by rapid phosphorylation of a specific tyrosine residue at the C-terminal region, tyrosine 694 in STAT5a and tyrosine 699 in STAT5b. P-STAT5 proteins dimerize and translocate to nucleus to regulate target genes transcription [9]. Ren et al. have reported that loss of STAT5a delays mammary cancer progression in mouse model studies [24] and Cotarla et al. have demonstrated that STAT5a is related to human breast cancer progression [25]. These data raised our interest in examining the expression of STAT5 phosphorylation on Y694 in colon cancer. Some studies have indicated that p-STAT5 is generally present in the nuclei of both breast cancer cells and prostate cancer cells [17, 23]. Xiong et al. [19] reported that p-STAT5$^{Tyr694/Tyr699}$ was present in the cytoplasms of colorectal adenocarcinoma cells, but was also present in the nuclei of normal epithelium cells. In contrast to those studies, our results first revealed that p-STAT5^{Tyr694} was present in the cytoplasms of almost all colonic adenocarcinoma cells and some neighboring normal epithelium cells. No nuclear staining for p-STAT5 was found in either of these cell types.

Table 2 Univariate and multivariate analyses of overall survival in 121 patients with colonic adenocarcinoma[a]

Variable	Mean survival (mo)	Univariate analysis[b]		Multivariate analysis[c]		
		Log rank	*P*-value	HR	95% CI	*P*-value
Lymph node metastasis		8.9	0.003	1.10	0.25–4.81	0.900
Negative	63					
Positive	49					
Distant metastasis		49.1	<0.001	6.05	1.40–26.24	0.016
Negative	62					
Positive	22					
TNM stage		27.1	<0.001	3.76	0.65–21.73	0.140
I and II	67					
III and IV	44					
P-STAT5		5.0	0.026	1.30	0.50–3.39	0.597
Negative	63					
Positive	55					

[a] *HR* Hazard ratio; *CI* Confidence interval

[b] Univariate analysis estimated using Kaplan- Meier test

[c] Multivariate analysis estimated using Cox proportional hazards model

A recent study indicated that p-STAT5 was localized in the cytoplasm and formed a complex signaling pathway with PI3K and with consecutive Akt activation in patients with mastocytosis [26]. Harir et al. [27] also found that persistently p-STAT5 had a cytoplasmic localization in primary cells of myeloid leukemia patients. Based on the p-STAT5 protein expression in human colonic adenocarcinoma tissues, we hypothesized that p-STAT5 might be involved in a complex cytoplasmic mechanism and might interact with members of other signal transduction pathways. Our findings indicate that p-STAT5 proteins might play a key role in the cytoplasm both as transcriptional activators and as cytoplasmic signaling effectors. A recent report documented that STATs were downstream of JAKs which could be activated through cytokine receptors or even receptor tyrosin kinases, which were abundant on colonic mucosa [28]. However, at present, little is known about the mechanism of STAT5 activation and regulation in human colon carcinoma. Our data suggest that it is important to examine STAT5 activation in colon carcinoma.

Our study may be affected by the number of histological samples or the use of only immunohistochemical staining for the detection of p-STAT5 protein expression. Further studies on the prognostic significance of p-STAT5 protein expression are required.

In conclusion, our findings suggest that p-STAT5 plays an important role in the malignant progression of colon carcinoma and contributes to a poor prognosis.

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Conflicts of interest No conflicts of interest exist in the submission of this manuscript.

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