Expression of pSTAT3 in human colorectal carcinoma: correlation with clinico-pathological parameters

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Summary

Purpose: Signal transducers and activators of transcription (STATs) are tyrosine phosphorylated transcription factors activated by the Jak family kinases. Various ligands, including interferons and growth factors induce activation of STATs. STATs are key signaling molecules in malignant transformation and tumor progression. Constitutive activation of the STAT3 has been observed in a wide variety of human malignancies. The purpose of this study was to evaluate the clinical significance of phosphorylated (p) STAT3 expression in human colorectal adenocarcinomas (CRC).

Methods: 135 primary human CRC were immunohistochemically studied, from which 11 were intramucosal and 124 invasive carcinomas. The observed pattern of pSTAT3 immunostaining was nuclear and cytoplasmic. Nuclear pSTAT3 staining was calculated as the number of pSTAT3 positive nuclei divided by the total number of nuclei in at least 10 fields, and then expressed as a percentage. Cytoplasmic positivity of pSTAT3 was measured, depending on the intensity of immunoreactivity and scored as mild, moderate and intense.

Results: Positive staining for pSTAT3 immunoreactivity was significantly correlated with the depth of tumor invasion (p<0.001), venous invasion (p<0.05), lymph node metastasis (p<0.05) and advanced Dukes stage (p<0.001). There was no significant correlation between pSTAT3 immunoreactivity and poor differentiation of CRC.

Conclusion: The expression of pSTAT3 is an important factor related to tumor and vascular invasion, nodal involvement and advanced CRC stage.

Key words: colorectal cancer, immunohistochemistry, invasion, prognosis, pSTAT3, tumorigenesis

Introduction

CRC is one of the most frequent neoplastic diseases in humans [1]. Risk factors for developing CRC include inflammatory bowel disease, family history of CRC or polyps and hereditary syndromes. Overall survival has not changed despite the advances in surgical and cytotoxic treatments.

Recent studies have indicated that STATs may be critical mediators in malignant transformation in a number of human malignancies [2,3].

STATs are transcription factors activated in response to cytokines and growth factors. They comprise a family of 7 proteins (STAT1, STAT2, STAT3, STAT4, STAT5$_a$, STAT5$_b$ and STAT6) which transduce extracellular signals and regulate transcription directly. STATs are activated through tyrosine phosphorylation at Tyr, which is regulated by growth factor receptor tyrosine kinases or cytokine receptor associated Jak kinases [4]. The phosphorylated tyrosines provide docking sites for STATs (pSTATs). They can dimerise and translocate to the nucleus, where they control the transcription of target genes [5].

Expression of pSTAT3 has been observed in breast cancer, multiple myeloma, head and neck tumors, ovarian cancer, prostate cancer and most recently in renal tumors [6-8].

The purpose of this study was to investigate the expression of pSTAT3 in CRC and see for any association with clinicopathological features.

Methods

A series of 135 patients underwent surgical resection for primary colorectal adenocarcinoma at the 1st Department of Surgery,

Tzaneio Hospital of Piraeus between 2005 and 2009. Cases of hereditary nonpolyposis CRC syndrome (Lynch syndrome), familial adenomatous polyposis syndrome (FAP) and ulcerative colitis and patients who died in the postoperative period were excluded from the study. None of the patients had received pre- or postoperative radiotherapy or chemotherapy. Clinicopathological stage was based on Dukes classification [9]. Tumors were histologically classified as well, moderate or poorly differentiated adenocarcinoma using the WHO criteria [10].

The observed pattern of pSTAT3 immunostaining was nuclear and cytoplasmic. Nuclear pSTAT staining was calculated as the number of pSTAT3 positive nuclei divided by the total number of nuclei in at least 10 fields, and then expressed as a percentage. Cytoplasmic positivity of pSTAT3 was measured, depending on the intensity of immunoreactivity and scored as mild, moderate and intense.

In addition to stage and grade of differentiation, lymphatic and venous invasion were assessed.

Twenty-five specimens of normal mucosal tissue taken from patients without CRC or other colorectal diseases (e.g. ulcerative colitis) were used as normal controls.

Immunohistochemistry

Formalin-fixed, paraffin-embedded primary CRC tissue sections 4 μm thick were stained with immunoperoxidase, performed in 3 steps using EnVision Dako kit, Denmark.

pSTAT3 polyclonal rabbit antibody (Tyr 705, Santa Cruz, California, USA) was used for recognizing only the phosphorylated form of STAT3. Binding of the primary antibody was assessed using the Dako LSAB2 system detection kit, Denmark. Diaminobenzidine (DAB) was used as chromogen, followed by slight hematoxylin counterstaining. Omission of the primary antibody served as negative control in adjacent normal colorectal mucosa.

All slides were read independently by two experienced pathologists. pSTAT3 protein was detected in both the cytoplasm and nucleus.

Immunoreactivity for pSTAT3 was evaluated semi-quantitatively by two observers and, according to the percentage of positive tumor nuclei, was scored as follows:

"negative" for tumors showing <15% immunostained nuclei.
"positive" for tumors showing ≥16% immunostained nuclei.
Cytoplasmic positivity of pSTAT3 was measured depending on the intensity of immunoreactivity and scored as mild (+), moderate (++), and intense (+++).

For positive controls of pSTAT3 expression of normal prostatic tissue was used.

Statistical analysis

Continuous variables were expressed as mean and standard deviation and were analysed with Student's t-test; categorical variables were analysed with x^2 test. The statistical software Minitab (version 14, UK) was used for the analyses and p value<0.05 was considered as the level of statistical significance.

Results

No correlation was noticed between pSTAT3 immunoreactivity and sex, age and site of the primary tumor (Table 1).

Table 1. Epidemiologic, clinical and pathological data for patients with colorectal cancer and the expression of pSTAT3. Continuous variables are expressed as mean ± standard deviation and analysed with the Student's t-test and categorical variables as percentages and analysed with x^2 test

| | pSTAT3 expression | | |
	No (N=31)	Yes (N=104)	p-value
Sex (M/F)	21/10	64/40	NS
Age, years±SD	62 ± 3.45	65±2.5	NS
Location			
Colon/Rectum	19/12	60/44	NS
Tumor depth (T)			
Tis	6	5	NS
T1	5	4	NS
T2	2	5	NS
T3	18	82	p<0.001
T4	0	8	p<0.001
Nodal metastasis	56	79	p<0.05
Differentiation			
Well	6	28	p<0.05
Moderate	9	63	p<0.05
Poor	10	8	NS
Venous invasion	40	95	p<0.05
Lymphatic invasion	110	25	NS
Dukes stage			
A	5	4	NS
B	2	5	NS
C	18	90	p<0.001

SD: standard deviation, NS: non significant, M: male, F: female

Depth of invasion (Figure 1)

Eleven of 135 patients (8.15%) had *in situ* carcinoma (Tis) and 124 (98.15%) had invasive disease: 9 infiltrated the submucosal layer (T1), 7 invaded the muscularis propria (T2), 100 were reaching the subserosa (T3) and 8 infiltrated the serosa or invaded adja-
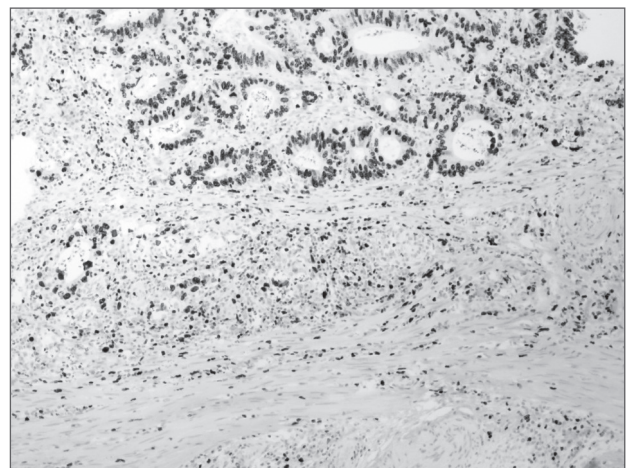

Figure 1. Immunohistochemistry for pSTAT3 in colorectal adenocarcinoma. pSTAT3 shows strong nuclear expression in the deeper parts of invasion (×200).

cent organs (T4). pSTAT3 expression was found in 5 of 11 Tis tumors, 4 of 9 T1 tumors, 5 of 7 T2, 82 of 100 T3 ($p<0.001$) and in all 8 T4 tumors ($p<0.001$).

Lymph node metastasis

Seventy-nine (58.5%) patients had lymph node metastases and positive pSTAT3 expression ($p<0.05$).

Grade of differentiation

Of 124 invasive carcinomas 34 (27.4%) were well differentiated, 72 (58%) were moderately and 18 (14.5%) poorly differentiated.

pSTAT3 expression was found in 28 of 34 (82.35%) well-differentiated, 63 of 72 (87.5%) moderately differentiated and 7 of 18 (44.4%) poorly differentiated CRC. Statistically, there was significant correlation between pSTAT3 immunoreactivity and poor CRC differentiation (Figure 2).

Lymphatic and venous invasion

Positive pSTAT3 expression was found in 95 (70.37%) of tumors with venous invasion ($p<0.05$) and only in 25 (18.5%) of tumors with lymphatic invasion.

Dukes stage

The expression of pSTAT3 correlated significantly with advanced Dukes stage ($p<0.001$; Figure 3).

Discussion

CRC is one of the most common human malignant neoplasms and a leading cause of cancer mortality worldwide. The prognosis of patients with CRC is based on the depth of tumor invasion and the presence of lymph node metastasis [11]. These parameters are determined by microscopic examination of tissue sections from the primary tumor and lymph nodes. However, it is difficult to determine the prognosis, based only on the histological examination of primary colorectal specimens. Also, up to 24% of patients with locally advanced, lymph node-positive CRC (Dukes C) experience disease recurrence following surgical treatment [9]. Although a number of genetic markers, such as p53 or k-RAS mutations have been investigated, it is currently not possible to accurately predict the probability of recurrence in Dukes C patients following surgery [12,13].

Recent studies have indicated that STAT3 is a major mediator of tumorigenesis and has been proved to be vital for tumor cell growth, proliferation and apoptosis. STAT3 is activated by phosphorylation at Tyr705 which induces dimerisation, nuclear translocation and DNA binding [14-16]. In the present study we used an antibody to pSTAT3 (Tyr705) which detects STAT3 only when the latter is phosphorylated at Tyr705. Small amounts of pSTAT3 can be detected in the cytoplasm without stimulation [17], although some cytokines upregulate the phosphorylation of STAT3, which then moves from the cytoplasm into the nucleus [18].

In this study the tumor cells expressed pSTAT3 intensely in both the cytoplasm and nucleus, while it was expressed faintly in the cytoplasm of normal mucosa cells. We also found a correlation between the expression of pSTAT3 and the depth of tumor invasion, venous invasion, lymph node metastasis and advanced Dukes stages but not with other parameters (age, sex, tumor location, poor differentiation).
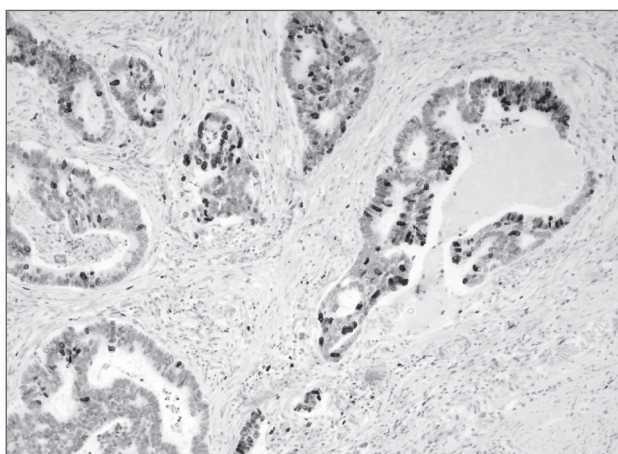

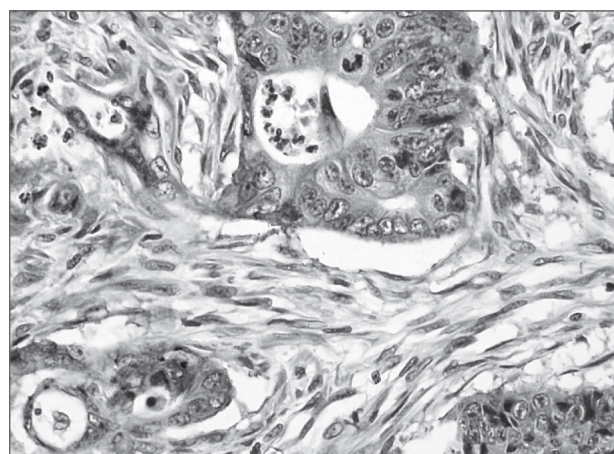**Figure 2.** Colorectal adenocarcinoma showing nuclear expression for pSTAT3 (×200).

Figure 3. Colorectal adenocarcinoma diffusely positive for pSTAT3-cytoplasmic stain (×200).

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Although studies on the expression of pSTAT3 in gastrointestinal cancers are very few, our results agree with previous reports. Ma et al. reported that there was a significant correlation between the expression of pSTAT3 and the presence of lymph node metastases and invasion in human CRC [19]. In addition, Masuda et al. reported that the expression of pSTAT3 was significantly correlated with the stage in human squamous cell carcinoma of the head and neck [20]. More recently, David et al. indicated in their work on human sarcomas that pSTAT is an important factor related to carcinogenesis of these tumors [21].

In conclusion, pSTAT3 is an important factor related to carcinogenesis of CRC and is significantly associated with some clinicopathological parameters, such as depth of tumor invasion, advanced Dukes stages, and lymph node metastasis. Moreover, it may potentially have diagnostic and prognostic implications in the future.

References

1. Crawford JM, Mand J, Kumar V. The Oral Cavity and the Gastrointestinal Tract. In: Kumar V, Rubins SL (Eds): Basic Pathology (7th Edn). Saunders, Philadelphia and London, 2003, pp 543-590.
2. Bromberg J. Stat proteins and oncogenesis. J Clin Invest 2002; 109: 1139-1142.
3. Darnell JE Jr. STATs and gene regulation. Science 1997; 277: 1630-1635.
4. Sehgal PB. Paradigm shifts in the cell biology of STAT signaling. Semin Cell Dev Biol 2008; 19: 329-340.
5. Zhong Z, Wen Z, Darnell JE Jr. Stat3: a STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. Science 1994; 264: 95-98.
6. Dhir R, Ni Z, Lou W, DeMiguel F, Grandis JR, Gao AC. Stat3 activation in prostatic carcinomas. Prostate 2002; 51: 241-246.
7. Hambek M, Baghi M, Strebhardt K et al. STAT 3 activation in head and neck squamous cell carcinomas is controlled by the EGFR. Anticancer Res 2004; 24: 3881-3886.
8. Huang M, Page C, Reynolds RK, Lin J. Constitutive activation of stat 3 oncogene product in human ovarian carcinoma cells. Gynecol Oncol 2000; 79: 67-73.
9. Edge S, Byrd O, Compton C, Fritz A, Greene F, Tronti A (Eds): Cancer Staging Manual (7th Edn). Springer, 2010, pp 143-159.
10. Hamilton R, Borman F, Boffetta P et al. Tumors of the colon and rectum. In: Borman F, Carneiro F, Hruban R, Theise N (Eds): World Health Organization Classification of the Tumors of the Digestive System (4th Edn). IARC Press, Lyon, 2010, pp 132-182.
11. Talbot IC, Ritchie S, Leighton M et al. Invasion of veins by carcinoma of rectum: method of detection, histological features and significance. Histopathology 1981; 5: 141-163.
12. Newland RC, Dent OF, Lyttle MN et al. Pathologic determinants of survival associated with colorectal cancer with lymph node metastases. A multivariate analysis of 579 patients. Cancer 1994; 73: 2076-2082.
13. Dogan L, Karaman N, Yilmaz KB, Ozaslan C, Atalay C, Altinok M. Characteristics and risk factors for colorectal recurrence. J BUON 2010; 15: 61-67.
14. Bell SM, Scott N, Cross D et al. Prognostic value of p53 overexpression and c-Ki-ras gene mutations in colorectal cancer. Gastroenterology 1993; 104: 57-64.
15. Darnell JE Jr Kerr IM, Stark GR. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science 1994; 264: 1415-1421.
16. Konjevic G, Radenkovic S, Srdic T, Jurisic V, Stamatovic Lj, Milovic M. Association of decreased NK cell activity and IFNγ expression with pSTAT dysregulation in breast cancer patients. J BUON 2011; 16: 219-226.
17. Ihle JN. Cytokine receptor signalling. Nature 1995; 377: 591-594.
18. Pranada AL, Metz S, Herrmann A et al. Real time analysis of STAT3 nucleocytoplasmic shuttling. J Biol Chem 2004; 279: 15114-15123.
19. Ma XT, Wang S, Ye YJ et al. Constitutive activation of Stat3 signaling pathway in human colorectal carcinoma. World J Gastroenterol 2004; 10: 1569-1573.
20. Masuda M, Suzui M, Yasumatu R et al. Constitutive activation of signal transducers and activators of transcription 3 correlates with cyclin D1 overexpression and may provide a novel prognostic marker in head and neck squamous cell carcinoma. Cancer Res 2002; 62: 3351-3355.
21. David D, Rajappan LM, Balachandran K et al. Prognostic significance of STAT3 and phosphorylated STAT3 in human soft tissue tumors - a clinicopathological analysis. J Exp Clin Cancer Res 2011; 30: 56-64.