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Original contribution

Down-regulated *GAS1* expression correlates with recurrence in stage II and III colorectal cancer☆

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Summary Growth arrest–specific gene 1 had been associated with cell-cycle arrest, proliferation, and apoptosis. The aim of this study was to investigate the correlations between clinicopathologic factors and survival time and growth arrest–specific gene 1 expression in patients with stage II and III colorectal cancer (CRC). Quantitative real-time polymerase chain reaction was performed in 64 fresh CRC tissues to examine growth arrest–specific gene 1 mRNA expression. Six metastasis-derived and primary-derived cell lines were subjected to quantitative real-time polymerase chain reaction and Western blotting for further examination of both mRNA and protein concentrations. Growth arrest–specific gene 1 protein was immunostained in 118 paraffin-embedded specimens. Growth arrest–specific gene 1 expression was down-regulated both in tissues with recurrence and in metastasis-derived cell lines. Expression was unrelated to sex, age, tumor grade, or lymphovascular or perineural invasion. However, it was positively related to disease-free survival time ($P < .05$). Furthermore, lower growth arrest–specific gene 1 expression indicated a poorer survival rate ($P < .05$; log-rank test). Multivariate analysis also showed weak growth arrest–specific gene 1 protein expression to be an independent adverse prognosticator ($P < .05$). Taken together, our results support the idea that growth arrest–specific gene 1 contributes to predicting metastasis or recurrence in stage II and III CRC.
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1. Introduction

Colorectal cancer (CRC) is responsible for more than 500 000 deaths worldwide every year [1]. Stage II and III tumors together represent approximately 70% of CRC patients [2]. Multidisciplinary treatments induce clinical remission in about 60% of stage II and III cases. However, 40% to 50% of patients will relapse, and most of them will die from secondary disease [2,3]. Tumor stage, histologic grade, and histologic tumor characteristics are the main prognostic factors used in clinical practice. However, they are not sufficient to evaluate the likelihood of survival. Therefore, biomarkers are urgently needed to predict prognosis and facilitate treatment.

Growth arrest–specific-gene 1 (*GAS1*) encodes a pleiotropic 45-kDa protein inserted into the cell membrane through a glycosyl-phosphatidylinositol anchor [4]. It has been directly related to cell arrest in the G0 to S phase transition [5-7]. *GAS1* also shows a role in growth suppression in different cell systems [5,8-11], and it is involved in apoptosis in different types of cells in context-dependent manners [9,10,12]. Taken together, these findings indicate that *GAS1* could be critical in the regulation of cell growth and apoptosis.

An emerging role for *GAS1* in carcinogenesis has been discovered recently. The *GAS1* gene has been located in a

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fragile chromosome site that is frequently deleted in cancerous cells [13-15]. Overexpression of *GAS1* has been found to inhibit tumor growth and promote apoptosis in glioma cells [10,11], suggesting that it may be critical for cancer cell survival. Previous studies also showed that *GAS1* expression inhibits melanoma [16] and prostate cancer metastasis [17,18]. However, the clinical significance of *GAS1* expression in tumors remains unclear. There is no report concerning the prognostic value of *GAS1* in CRC samples, especially in stage II and III samples. In the present study, we investigated *GAS1* expression using quantitative real-time polymerase chain reaction (qPCR), Western blotting, and immunohistochemistry. The correlations between GAS1 expression and clinicopathologic features and survival time were analyzed.

2. Materials and methods

2.1. Patients and tissue specimens

To detect the differences of *GAS1* expression in cases with and without recurrence, 64 fresh stage II and III CRC tissues, 36 with recurrence and 28 without, were collected from the Department of Colorectal Surgery at the Cancer Hospital of Fudan University (Shanghai, China) between April 2000 and November 2004 for mRNA examination. Meanwhile, 118 paraffin-embedded CRC tissues, 78 with and 40 without recurrence, were collected retrospectively from archival material stored in the Department of Pathology at the Cancer Hospital between February 1993 and March 2004 for protein analysis; these samples were from different patients from those used for mRNA analysis. The characteristics of the samples are shown in Tables 1 and 2.

All specimens were taken from vital cores of histopathologically confirmed cancers at primary curative surgery in patients who did not undergo any chemotherapy or radiotherapy before operation. Tumor samples were reviewed by at least 2 experienced pathologists, and tumor stage was assigned on the basis of the system of the International Union Against Cancer. No stage II but all stage III patients received postoperative chemotherapy, but no patient received radiotherapy.

Disease-free survival was defined as the time elapsed from the date of the initial diagnosis to the appearance of local relapse or distant metastasis. The research protocol was approved by the ethics committee at the Cancer Hospital of Fudan University, and informed consent was obtained from all patients before tissue acquisition.

Table 1 Characteristics of 64 patients with CRC

Characteristic	Without recurrence (n = 28) (%)	With recurrence (n=36) (%)	P [a]
Age (y)			.454
<80	26 (93)	31 (86)	
≥80	2 (7)	5 (14)	
Sex			.450
Male	16 (57)	24 (67)	
Female	12 (42)	12 (33)	
Size (cm)			.615
≤5	14 (50)	21 (58)	
>5	14 (50)	15 (42)	
Grading			.889
Well	6 (21)	9 (25)	
Moderate	18 (64)	21 (58)	
Poor	4 (14)	6 (17)	
Type			.555
Non–mucin-producing	23 (82)	27 (75)	
Mucin-producing	5 (18)	9 (25)	
Location of tumor			.597
Colon	11 (39)	11 (31)	
Rectum	17 (61)	25 (69)	
Disease-free survival (y)			.077
≥5	11 (39)	23 (64)	
<5	17 (61)	13 (36)	
TNM stage			.615
II	13 (46)	14 (39)	
III	15 (54)	22 (61)	
Adjuvant chemotherapy			.615
Yes	15 (54)	22 (61)	
No	13 (46)	14 (39)	

[a] P values are obtained using χ^2 test.

2.2. Cell lines

Six human CRC cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA). Three are primary-tumor-derived lines (SW480, Caco-2, and HCT116), 2 are lymph-node-metastases-derived lines (SW620, LoVo), and 1 is an abdominal dropsy-metastases-derived line (Colo205). Cell lines LoVo and Colo205 were cultured in RPMI-1640 medium, whereas Caco-2 and HCT116 were cultured in Eagle's minimum essential medium and McCoy's 5a medium modified, respectively. Both SW480 and SW620 were cultured in Leibovitz's L-15 medium. All media were supplemented with 10% fetal bovine serum (GIBCO, USA), penicillin 100 IU/mL, and streptomycin 100 μg/mL at 37°C in a 5% CO_2-humidified atmosphere.

2.3. mRNA analysis by qPCR and reverse transcriptase PCR

Total RNA was isolated with an RNeasy Mini Kit (Qiagen GmbH, Hilden, Germany) and treated with DNase. According to the manufacturer's instructions, cDNAs were

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