Correlation of *AIB1* overexpression with advanced clinical stage of human colorectal carcinoma ☆

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Keywords:
Colorectal carcinoma;
AIB1;
p53;
Immunohistochemistry;
Amplification;
Chromosome instability;
Tissue microarray

Summary *AIB1*, a member of the steroid receptor coactivator 1 family, has been cloned on 20q12 and is a candidate oncogene in human breast cancer. It is commonly amplified and overexpressed in several types of human cancers. In this study, we examined the expression of *AIB1*, as related to clinicopathologic features, in 85 human colorectal cancers (CRCs). The status of the number of *AIB1* copies, *p53* expression, and DNA ploidy was also analyzed. The overexpression of *AIB1* was detected in 35% of CRCs. Amplification of *AIB1* was observed in 10% of CRCs. In addition, the overexpression of *AIB1* was observed more frequently in CRCs in later clinical stages (T3 N1 M0/T3 N0 2M1), compared with that in T3 N0 M0 stage ($P < .05$). These results suggest that overexpression of *AIB1* might provide a selective advantage for the developmental growth and/or progression of subsets of CRCs. In addition, a significant correlation ($P < .05$) of overexpression of *AIB1* with *p53* overexpression as well as with aneuploid DNA content was observed in these CRCs. The overexpression of *p53* was also correlated significantly with CRC DNA ploidy ($P < .05$). Furthermore, there was a substantial population of CRCs showing overexpression of both AIB1 and p53 protein and all had aneuploid DNA content; most of these were in the later clinical stage. These findings suggest a possible convergence of *AIB1* with a pathway involving *p53*, which might induce chromosomal instability and affect the clinical phenotype of a subset of CRCs.
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1. Introduction

Colorectal carcinoma (CRC) is one of the most common human cancers and a major cause of cancer-related death in developed countries [1]. The incidence of CRC in China, including urban Shanghai, increases rapidly during the last 2 decades of life [2]. Although CRC has been widely

studied, the genetic changes underlying the development and progression of this cancer are not thoroughly understood. In CRC, chromosomal aberrations have been extensively analyzed by comparative genomic hybridization, and several frequently amplified regions including 20q have been identified [3-5]. Amplification of 20q has been also detected frequently in many other cancers, including breast, gastric, bladder, and hepatocellular cancers [6-9].

Amplification and overexpression of an oncogene have been shown to play an important role in the pathogenesis of various cancers, probably because overexpression of the amplified oncogene confers a growth advantage. Several candidate oncogenes have been isolated from 20q, including *AIB1* at 20q12 [10] and *ZNF217*, *BCAS1*, and *CYP24* at 20q13 [11,12]. The *AIB1* gene, also known as *SRC-3*, *p/CIP*, *RAC3*, *ACTR*, and *TRAM-1*, has been found to be involved in many biological processes such as cell proliferation, cell migration, and cell differentiation [13]. The oncogenic role of *AIB1* has been demonstrated in breast, prostate, liver, and gastric cancers [10,14-16].

To investigate the role of *AIB1* in pathogenesis of CRC, the amplification and overexpression of *AIB1* as well as its relationship to clinicopathologic features in 85 CRCs were studied. Because up-regulation of *AIB1* has been correlated with *p53* stabilization in some solid tumors [17] and loss of *p53* function could induce chromosomal instability in cancer cells [18], the status of *p53* expression and DNA ploidy was also studied in these CRC cohorts.

2. Materials and methods

2.1. Patients and tissue specimens

In the present study, 85 patients with CRC were selected from the First Affiliated Hospital of Sun Yat-Sen University (Guangzhou, China) between January 1995 and March 2000. The age of these patients ranged from 36 to 85 years (mean, 59.6 years) at the time of surgery, and the male/female ratio was 1.2:1. All cases selected were moderately differentiated adenocarcinomas.

The specimens were recruited from paraffin blocks of 85 primary carcinomas and available matched paraffin blocks of 22 adenomas, 21 metastatic lymph node lesions, and 21 distant metastatic lesions from the same patients. All primary carcinomas selected were advanced CRCs that had invaded the deep layers of the bowel wall through the muscularis and into the serosa. According to the TNM staging system AJCC [19], 43 CRCs were regarded as T3 N0 M0 tumors with no lymph node and distant metastasis. Among these 43 CRCs, paired adenomatous polyps (tubular, n = 12; tubulovillous, n = 3; villous, n = 7) were recruited from 22 cases. Twenty-one CRCs were in T3 N1 M0 stage with regional lymph node metastasis (pN1, n = 19; pN2, n = 2). Another 21 cases were in T3 N0 2M1 stage with distant metastasis at the time of operation. The sites of

distant metastasis included liver (n = 17), stomach (n = 2), bladder (n = 1), and inguinal region (n = 1).

2.2. Tissue microarray

In this study, a previous constructed tissue microarray (TMA) block [20] containing all 85 CRC cases was used for immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) studies. In this CRC TMA, 6 samples from different pathological and progressive points were selected from each advanced CRC case for the TMA construction. Tissue samples from adjacent nonneoplastic mucosa, primary carcinomas in inner layers (corresponding to layer of mucosa and/or submucosa), muscularis layer, and serosa layer of the bowel wall were selected for all CRC cases. Available matched tissue specimens from 22 adenomas, 21 paired lymph nodes, or distant metastases were also recruited. Hence, the TMA includes a total of 510 (85 × 6) samples. For those cases with paired adenomas, lymph nodes, or distant metastases, TMA tissue specimens were composed of 2 samples from adenoma, lymph node, or distant metastases and 1 sample from normal mucosa, primary carcinoma in inner layer (corresponding to layer of mucosa and/or submucosa), muscularis layer, and serosa layer of the bowel wall. For those cases without paired adenomas, lymph nodes, or distant metastases, TMA tissue specimens for each case were composed of 1 sample from normal mucosa and primary carcinoma in serosa layer of the bowel wall and 2 samples from inner layer and muscularis layer of the bowel wall. Multiple sections (5 μm thick) were cut from the TMA block and mounted on microscope slides.

2.3. Immunohistochemistry

Immunohistochemistry was performed on 5-μm TMA sections rehydrated through graded alcohols. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide for 20 minutes. For antigen retrieval, TMA slides were boiled in 10 mmol/L citrate buffer (pH 6.0) in a pressure cooker for 15 minutes (*AIB1*) or microwave-treated for 10 minutes (*p53*). Nonspecific binding was blocked with 10% normal rabbit serum for 20 minutes. The TMA slides were incubated with either anti-*AIB1* (a monoclonal antibody directed at amino acids 376-389 of *AIB1* (1:50 dilution, Transduction Laboratories, San Jose, CA) or anti-*p53* (Do7, 1:100 dilution, Dako, Glostrup, Denmark) for 60 minutes at 37°C in a moist chamber. The slide was then sequentially incubated with a biotinylated rabbit antimouse immunoglobulin at a concentration of 1:100 for 30 minutes at 37°C and subsequently reacted with a streptavidin-peroxidase conjugate for 30 minutes at 37°C and 3′,3′-diaminobenzidine as a chromogen substrate. The nucleus was counterstained using Meyer hematoxylin. Negative control was performed by replacing the primary antibody with a normal murine immunoglobulin G. Known immunostaining-positive slides were used as positive controls. The malignant and nonmalignant tissues were scored for