

KAI1 gene expression in colonic carcinoma and its clinical significances

De-Hua Wu, Li Liu, Long-Hua Chen, Yan-Qing Ding

De-Hua Wu, Long-Hua Chen, Department of Radiation Oncology, Nanfang Hospital, First Military Medical University, Guangzhou 510515, Guangdong Province, China

Li Liu, Yan-Qing Ding, Department of Pathology, First Military Medical University, Guangzhou 510515, Guangdong Province, China
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Correspondence to: Dr. Yang-Qing Ding, Department of Pathology, First Military Medical University, Guangzhou 510515, Guangdong Province, China. dyq@fimmu.com

Telephone: +86-20-61642148 **Fax:** +86-20-61642148

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Abstract

AIM: To investigate *KAI1* gene expression in the progression of human colonic carcinoma and its clinical significances.

METHODS: *KAI1* expression was detected by *in situ* hybridization and immunohistochemistry in the 4 established cell lines of colorectal carcinoma with different metastatic potentials, and in 80 specimens of colonic carcinoma, 21 colonic carcinoma specimens with lymphatic metastasis and 20 controls of normal colonic mucosa.

RESULTS: The expressions of *KAI1* in HT29 and SW480 cell lines were higher than those in LoVo and SW620. The expression of *KAI1* gene was significantly higher in colorectal carcinoma compared with normal colonic mucosa and lymphatic metastasis ($\chi^2=46.838$, $P<0.01$). The expression of *KAI1* gene had no relationship with histological grade. The *KAI1* expressions in Dukes A and B carcinoma were higher at both mRNA and protein levels compared to Dukes C carcinoma ($\chi^2=16.061$, $P<0.05$). The expression of *KAI1* in colonic carcinoma specimens with lymphatic metastasis was almost lost. The results of *in situ* hybridization were in concordance with immunohistochemistry.

CONCLUSION: *KAI1* is highly related to the metastasis of colonic carcinoma and may be a useful indicator of metastasis in colonic carcinoma.

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INTRODUCTION

Colorectal carcinoma is one of the most common forms of malignancy, and metastasis is the major cause of mortality in human population. Timely and precise identification of the occurrence of metastasis and its contributive factors are very important in the enhancement of prognosis and effects of treatment. *KAI1* gene was first identified in human prostate carcinoma and mapped to human chromosome 11p11.2^[1]. It encodes 267 amino acids belonging to plasma membrane glycoprotein, which consists of 4 transmembrane domains and

1 large and 1 small extracellular domains. The extracellular domains have 3 potential N-glycosylation sites. According to its special structure, it may be predicted that the function of *KAI1* gene may come down to cell-cell adherence and cell-matrix connection^[2]. The role of *KAI1* in tumor progression may not be limited to prostatic cancer. It was reported to be important in preventing the development of metastases in a wide variety of human tumor types, including cervical^[3], breast^[4], pancreatic^[5], esophageal^[6], bladder^[7], and ovarian cancers^[8]. In the present study, the expression of *KAI1* was detected by *in situ* hybridization in 4 cell lines of colorectal carcinoma, and in paraffin-embedded normal colonic epithelium, carcinoma, and lymphatic metastasis. The purpose was to explore the relationship between *KAI1* expression and colonic carcinoma metastasis and its clinical significances.

MATERIALS AND METHODS

Cell lines and culture conditions

LoVo and HT29 cell lines were cultured in RPMI 1640 supplemented with 100 mL/L heat-inactivated fetal bovine serum (FBS) and 100 U/mL penicillin/streptomycin. SW480 and SW620 were cultured in DMEM medium supplemented with the 100 mL/L FBS. All cells were grown in 50 mL/L CO₂ humidified atmosphere at 37 °C.

Tissue specimens

A total of 80 colonic carcinoma specimens, 20 normal colonic mucosa samples and 20 lymphatic metastasis samples were obtained from Nanfang Hospital. All the samples were routinely fixed in 40 g/L formaldehyde solution, embedded in paraffin, and cut into 4 μm thick sections. Samples were selected according to the pathological diagnosis and reviewed by a pathologist to confirm the diagnosis.

In situ hybridization (ISH) assays

The digoxigenin-labeled *KAI1*-cDNA probe for *in situ* hybridization was prepared as described in one of our previous papers^[9]. The ISH was performed according to the instruction of the enhanced sensitive ISH detection kit (purchased from Boster Biotechnology Company). Briefly, the sections were deparaffinized with xylene, dehydrated with graded ethanol and incubated in 30 mL/L H₂O₂ to block endogenous peroxidases for 30 min at room temperature. After being treated for 10 min with Triton X-100 and for 40 s with 3% pepsin, the sections were prehybridized for 3 h and hybridized overnight at 37 °C. The final concentrations of the labeled probes were 0.15 ng/μL. After hybridization, excess probes were removed through rinsing in 2×SSC (1×SSC is 0.15 mol/L NaCl plus 0.015 mol/L sodium citrate pH 7.0), 0.5×SSC, 0.2×SSC, respectively. The sections were incubated with an antidigoxigenin antibody conjugated biotin for 120 min at room temperature and then added strept-avidin biotin complex for 30 min. For color reaction, diaminobenzidine (DAB) was used. If the ISH signals were present, the cytoplasm would be full of brown granules. For ISH of the four colorectal carcinoma cell lines, the cells were incubated and grown on cover slip for 24 h, fixed with 950 mL/L

ethanol and washed with PBS (phosphate-buffered saline pH 7.2) 3 times, and then carried out ISH as described above. For negative controls, the digoxigenin-labeled KAI1-cDNA probe was replaced by prehybridized solution.

Immunohistochemical assays

To confirm the results of *KAI1* gene expression on *in situ* hybridization, immunohistochemical studies were performed as described previously^[9,10]. Briefly, the fixed cells and sections were subjected to immunostaining by using an ultrasensitive streptavidin-peroxidase technique (Maixin Biotechnology Company). Endogenous peroxidases were blocked by incubating with 5 mL/L H₂O₂ for 30 min at room temperature. The cells and sections were subsequently treated for 10 min with Triton X-100 and for 40 s with 30 g/L pepsin. The cells and sections were incubated for 30 min at 37 °C with normal nonimmune serum before overnight incubation at 4 °C with specific monoclonal KAI1-antibody (BD pharmingen technical company) at a dilution of 1:100. The cells and sections were then treated with biotin-conjugated second antibody before adding streptavidin-peroxidase. For color reaction, diaminobenzidine was used. If the positive signals were present, the cytoplasm and membrane were stained brown. For negative controls, the monoclonal KAI1 antibody was replaced by PBS.

Review and scoring of the section

The stained sections were reviewed and scored independently by two pathologists under Olympus microscope. The different degrees of intensity of staining were graded on a scale of 0 to 3 as follows: 0 indicated that the number of positive cells was less than 10%; 1 indicated weak positive and the number of positive cells more than 10%, but less than 30%; 2 indicated positive and the number of positive cells more than 30% but less than 50%; 3 indicated strong positive and the number of positive cells more than 50%.

Statistical analysis

Fisher's exact probability test was adopted to examine the relationship between the variables. A *P* value <0.05 was considered statistically significant.

RESULTS

KAI1 gene expression in colorectal carcinoma cell lines

HT29 and SW480 cell lines were derived from primary colorectal adenocarcinoma. SW620 and LoVo cell lines were established from metastatic colorectal carcinoma. Especially, SW620 cell line was isolated from the same case as was SW480 cell line. The expressions of KAI1 mRNA and protein in HT29 and SW480 cells were positive, whereas those in SW620 and LoVo cells were negative (Figures 1, 2).

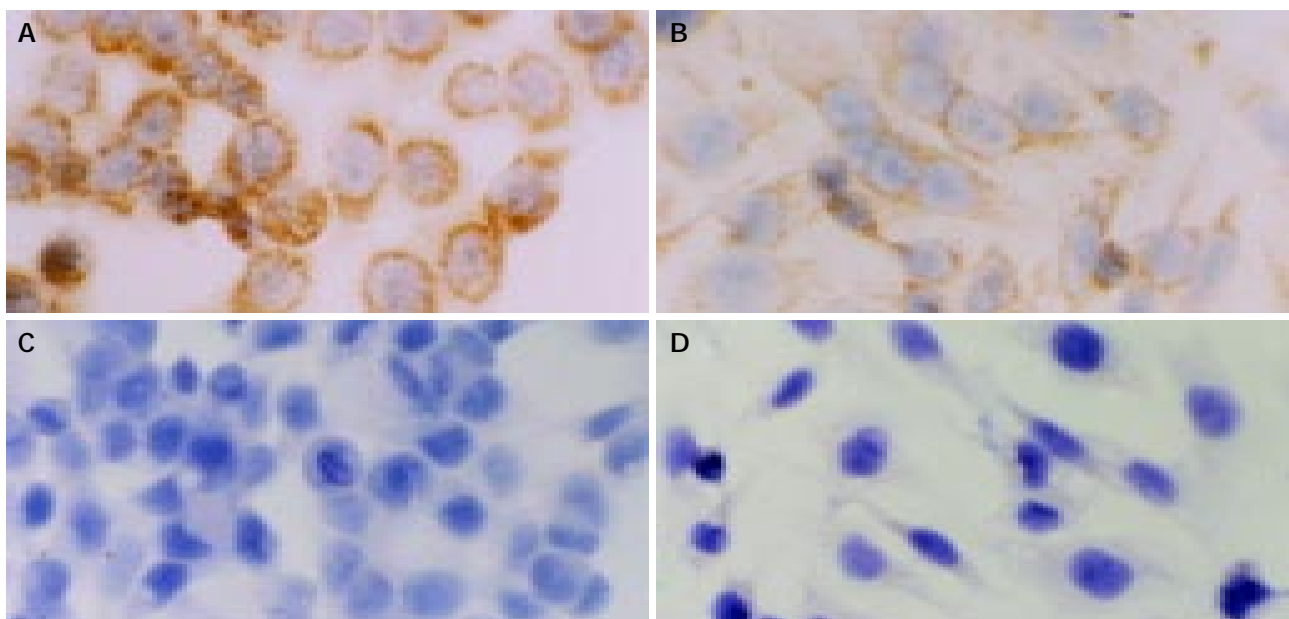


Figure 1 *In situ* hybridization detection of expression of KAI1 mRNA in colorectal carcinoma cell lines (Original magnification: ×400). A: In HT29 cells, the positive expression (brown granule) was located in cytoplasm; B: In SW480 cells, the positive expression was located in cytoplasm; C: In SW620 cells, the expression of KAI1 mRNA was negative; D: In LoVo cells, the expression of KAI1 mRNA was negative.

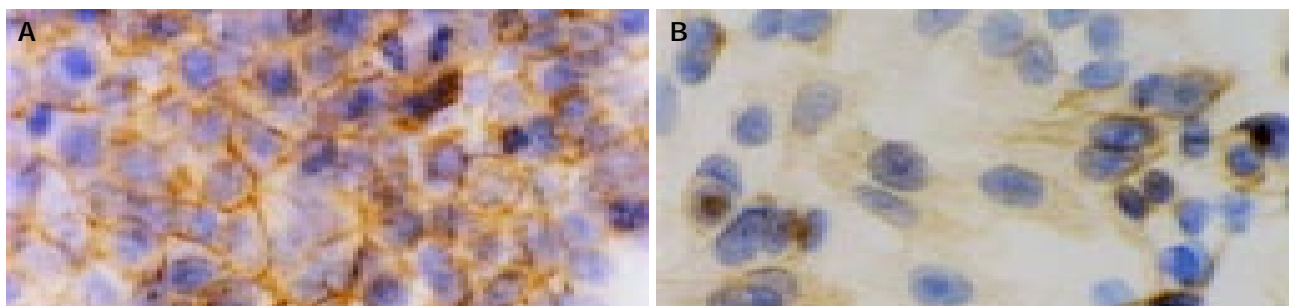


Figure 2 Expression of KAI1 protein detected by immunohistochemistry in colorectal carcinoma cell lines (Original magnification: ×400). A: In HT29 cells, the positive expression located in cytoplasm and membrane; B: In SW480 cells, the positive expression located in cytoplasm and membrane.

Heterogeneous expression of *KAI1* mRNA in colonic carcinoma tissue

Of the 80 colonic carcinoma specimens, 56(70%) were classified as *KAI1* positive (Figure 3). There were 4 cases (20%) with positive *KAI1* mRNA expression in normal colonic mucosa and 2 cases (10%) in lymphatic metastasis. The results are shown in Table 1. The positivity ratio was significantly higher in colonic carcinoma than that in normal colonic mucosa and in lymphatic metastasis ($\chi^2=46.838$, $P<0.01$). The expression in lymphatic metastasis was almost lost.

To further investigate the relationship between the expression of *KAI1* and the clinical pathology, we sorted the colonic carcinomas based on histological grades and Dukes stages. Histological grade I means well differentiated adenocarcinoma, II means moderately differentiated and III means poorly differentiated. The results indicated that *KAI1* expression had no relationship with histological grades ($\chi^2=3.887$, $P>0.05$). The expression in Dukes A and B carcinoma was markedly higher in comparison with Dukes C carcinoma ($\chi^2=16.061$, $P<0.01$).

KAI1 protein expression analyzed by immunohistochemistry

There were 52(65%) cases with positive *KAI1* protein expressions in the colonic carcinomas (Figure 3). The positive ratios of normal colonic mucosa and lymphatic metastasis were 25% and 10%, respectively. The *KAI1* expressions in 3 groups were significantly different ($\chi^2=28.298$, $P<0.01$). Overall, the immunohistochemical results agreed well with those from the *in situ* hybridization assays.

DISCUSSION

Loss of the function of metastasis suppressor genes is an important step in the progression of a tumor type. Several candidate antimetastasis or anti-invasion genes have been studied in colorectal carcinoma, including nm23^[11], E-cadherin^[12], and CD44^[13], but inconsistent findings have been reported. For example, in separate studies, nm23 expression has been found to be directly correlated, not to be correlated, or inversely correlated with metastatic potential in colorectal cancer^[14-16]. *KAI1* has been thought to be one of such metastasis suppressor genes, because it was shown to suppress the ability of human prostatic cancer cells to metastasize when the tumor was transplanted into nude mice^[1] and because *KAI1* mRNA expression was reduced in advanced pancreatic cancer^[17] so that the pancreatic cancer cells spread to lymph nodes and distant organs. Furthermore, the transfer of the *KAI1* gene into mammary cancer cells has been shown to lead to suppression of their metastatic potential, whereas their primary tumor growth has not been affected^[18-20].

KAI1 is a member of the transmembrane-4 superfamily (TM4SF), many of which, including *KAI1*, are CD antigens present on the surface of leukocytes^[21,22]. At least three TM4SF members are implicated in metastasis, including CD9/MRP-1, CD63/ME491, and CD82/*KAI1*^[23]. *KAI1* and other TM4SF members, such as integrins and E-cadherin, have been demonstrated to bind to each other and relay extracellular signals to signal transduction pathways that are important in cellular adhesion, invasive motility, and metastasis^[24-27].

In our previous studies, we transfected the *KAI1* cDNA

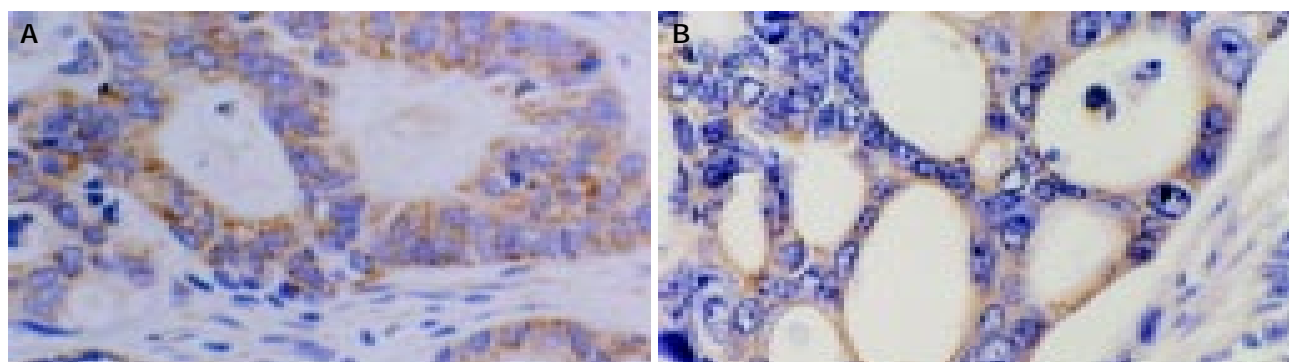


Figure 3 *In situ* hybridization and immunohistochemical detection of *KAI1* mRNA and protein in colonic carcinoma (Original magnification: $\times 400$). A: *In situ* hybridization detection found that strong *KAI1* mRNA staining was located in cytoplasm (brown granule); B: Detection by immunohistochemistry showed strong *KAI1* protein staining was in cytoplasm and membrane.

Table 1 *KAI1* gene expression in normal colonic mucosa, colonic carcinoma and lymphatic metastasis *n* (%)

Tissue	KAI1/CD82 expression				Total
	0	1	2	3	
Normal colonic mucosa	16(80.0)	3(15.0)	1(5.0)	0	20
Colonic carcinoma					
Histological grade					
I	6(23.1)	2(7.7)	12(46.2)	6(23.1)	26
II	10(26.3)	12(31.6)	14(36.8)	2(5.3)	38
III	8(50.0)	6(37.5)	2(12.5)	0	16
Dukes stage					
Dukes A	1(4.3)	6(26.1)	9(39.1)	7(30.4)	23
Dukes B	3(17.6)	6(35.3)	5(29.4)	3(17.6)	17
Dukes C	20(50.0)	10(25.0)	8(20.0)	2(5.0)	40
Lymphatic metastasis	18(90.0)	2(10.0)	0	0	20

into a colorectal carcinoma cell line, LoVo, and found that the KAI1 transfectant exhibited significantly increased homotypic cell adhesion and suppressed invasion and metastasis^[9]. Our findings were consistent with those in the studies of prostatic cancer^[2] and breast cancer^[18] cells.

In this study, we first investigated KAI1 mRNA expression in 4 colorectal carcinoma cell lines and found *KAI1* gene expression was much higher in HT29 and SW480 cell lines than in SW620 and LoVo cell lines. SW480 was isolated from a high-grade primary colonic tumor, and SW620 was isolated from a metastatic lymph node from the same patient 1 year later at the time of clinical relapse. KAI1 protein expression was high in SW480 but reduced in SW620. The colonic cancer cell line derived from metastatic lesions, LoVo, hardly exhibited any expression. In our observation we found that *KAI1* gene expressions both at mRNA and protein level, were inversely correlated with the metastatic potential of some established colorectal carcinoma cell lines.

We next examined KAI1 expression in normal colonic mucosa, carcinoma and lymphatic metastasis by *in situ* hybridization. The results indicated that KAI1 gene was highly expressed in colonic carcinoma compared with normal colonic epithelium and lymphatic metastasis. We found that although the expression had no relationship with histological grades, it was significantly correlated with Dukes stages. This indicates that the KAI1 mRNA expression may be positively related with the Dukes stages. The data shown in Table 1 demonstrate that KAI1 expression increases at an earlier tumor stage of colonic carcinoma, while decreases at advanced stages, and is possibly lost in metastases. That is to say, the expression of KAI1 has a reverse correlation with metastasis in colorectal carcinoma.

How does reduced TM4SF expression cause changes of invasive ability of tumor cells? It was hypothesized that tetraspanins might be implicated in the assembly of integrin-contained signaling complexes, thus modulating the function of integrin receptors in cell migration^[24]. Some results indicated that integrin-tetraspanin protein complexes played an important role in regulating protrusive activity of the tumor cells and contributed to extracellular matrix-induced production of matrix metalloproteinase 2 (MMP-2), and as a consequence, the invasive ability of cells^[28].

KAI1 has been extensively studied for its involvement in the progression of different human cancers. The mechanism of down-regulation of KAI1 has also been analyzed, however, much debate still exists. Recent study found a putative p53 consensus-binding site within the promoter region of KAI1 and demonstrated that the loss of p53 function, which was commonly observed in many types of cancer, led to the down-regulation of the *KAI1* gene, which might result in the progression of metastasis^[29]. But other data suggested that the down-regulation of KAI1 was not associated with either mutation, allelic loss, methylation of the promoter, or p53 regulation^[30]. Our previous study also demonstrated that mutation of the KAI1 gene, methylation of CpG islands and the abnormality of p53 were not related to low expression of KAI1.

In conclusion, KAI1 expression increases in an earlier tumor stage of colonic carcinoma, decreases in advanced stages, and is possibly lost in lymphatic metastases. The loss of KAI1 expression is correlated with higher stage, a surrogate marker for metastatic potential. The down-regulation of KAI1 in Dukes C and loss in metastasis demonstrate that loss of KAI1 expression occurs in cancer progression. The selection of cells that have the ability to spread from the primary tumor to the metastasis may favor those cells that have lost KAI1 expression. Those cells would be expected to be less adhesive, more invasive, and more motile^[31], and these characteristics are necessary for metastasis.

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