Expression and Clinical Significance of Computer-aided HIC-1 in Colon Cancer

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Abstract. Objective: To investigate the expression and clinical significance of HIC-1 in colon cancer. Methods: RT-PCR, immunohistochemistry, and computer image analysis were used to detect the expression levels of HIC-1 gene and protein in 200 patients with colon cancer and the corresponding paracancerous tissues to analyze the relationship between the expressions and clinicopathological characteristics as well as their correlation. Results: the expression of HIC-1 mRNA was up-regulated in the colon cancerous tissues of 141 patients (70.5%) and in the paracancerous tissues of 21 patients (10.5%) with colon cancer, and the difference was statistically significant (P = 0.001). The expression of HIC-1 mRNA was correlated with Dukes stage, lymph node metastasis, and differentiation degree. The up-regulated expression of HIC-1 mRNA in colon cancer and the corresponding paracancerous tissues were 104 (52.0%) and 121 (60.5%), respectively. The expression of HIC-1 mRNA was not correlated with any clinicopathological characteristics of colon cancer. The immunohistochemical staining intensity of HIC-1 in cancerous tissues was 1.79 ± 0.11, higher than that in paracancerous tissues (P = 0.002). The expression level of HIC-1 was correlated with Dukes stage and lymph node metastasis. The expression of HIC-1 in colon cancerous and paracancerous tissues was weaker than that of HIC-1, which was not correlated with clinicopathological characteristics. There was a negative correlation between the expression of computer-aided and HIC-1 in colon cancerous tissues (r = -0.63, P < 0.01). Conclusions: The expression of HIC-1 can promote the progression of colon cancer, and it may have an antagonistic effect.

Keywords: Matrix Metalloproteinase, Tissue Inhibitor, Colon Cancer, Computer-aided

1. Introduction
Colon cancer is a common malignant tumor. In recent years, its morbidity and mortality are increasing over time [1-2]. Invasion and metastasis are the main biological characteristics of the tumor and the leading cause of death of tumor patients. They are also the main factors affecting the therapeutic effect.
and prognosis\textsuperscript{[3]}, Multiple enzymes are involved in this process. Matrix metalloproteinases family (MMPs) is a group of enzymes that have attracted attention in the study of extracellular matrix synthesis and degradation balance in recent years. It can degrade almost all components of the extracellular matrix, which is closely correlated with tumor invasion and metastasis and has become a research hotspot at present\textsuperscript{[4-5]}.

Tissue inhibitors (TIMPs) are a group of low molecular weight glycoproteins, which are extensively distributed in tissues and body fluids. They can be produced and secreted by fibroblasts, epithelial cells, and endothelial cells\textsuperscript{[6]}. Five TIMPs have been identified, which are the main endogenous inhibitors of MMPs in tissues. They can specifically inhibit matrix-degrading enzymes of the MMPs family. By inhibiting the MMPs, they play an important role in normal extracellular matrix remodeling and various pathological processes, such as tumor invasion, diffusion, metastasis, and tissue fibrosis. This study aims to explore the expression of HIC-1 in colon cancer and the correlation and analyze the relationship between HIC-1 and clinicopathological characteristics of colon cancer, providing a theoretical basis for the diagnosis, treatment, and prognosis of colon cancer.

2. Data and methods

2.1. General information

From August 2008 to August 2011, surgical samples from 200 colon cancer patients at Zhongshan People's hospital were collected. The paracancerous tissues were removed from the same patient (about 10-15 cm away from the tumor tissue). 50 mg of each sample was stored in a low temperature refrigerator at -80 °C, 35-40 mg was used to extract total RNA for later use. The rest part was used for histopathological and immunohistochemical detection. No patients received chemotherapy or radiotherapy before the operation. The average age of the patients was (49 ± 7) years, ranging from 21 to 75 years old. The CEA of 157 patients was detected before the operation. The main types of tumor pathology were ulcerative type (123 cases), mass type (42 cases), infiltrative type (35 cases); histological classification: papillary adenocarcinoma (55 cases), tubular adenocarcinoma (52 cases), mucinous adenocarcinoma (81 cases), signet ring cell carcinoma (10 cases), adenosquamous carcinoma (2 cases); high differentiation 87 cases, medium differentiation (55 cases), and low differentiation (58 cases). There were 94 cases of lymph node metastasis and 10 cases of liver metastasis. Dukes stage: 60 cases in a stage, 46 cases in B stage, 75 cases in C stage and 19 cases in the D stage.

2.2. Main instruments and reagents

The real-time fluorescence quantitative RT-PCR instrument icycler IQ was purchased from Bio Rad, USA, Olympus dd70 BX51 image acquisition system was purchased from Olympus, Japan was purchased from USA, and ultra-low temperature refrigerator (-80 °C) was purchased from Sanyo, Japan. The 3-phosphoglyceraldehyde dehydrogenase (GAPDH) probe and primer, HIC-1 probe and primer were purchased from Takara (Dalian) Co., Ltd.; mouse anti-human HIC-1 monoclonal antibody, sheep anti-mouse peroxidase labeled streptavidin/peroxidase staining kits were purchased from ZYMED, USA; concentrated DAB kit was purchased from Zsibo, Beijing.
2.3. Histopathological examination
Fixed with 10% formaldehyde for more than 24 hours, paraffin embedded, sectioned and stained with he.

2.4. Detection of hic-1 and hic-1 mrna expressions in colon cancer
Probes and primers were synthesized by Takara (Dalian) Co., Ltd. GAPDH was used as the internal reference gene and HIC-1 and HIC-1 as the target genes. The probe and primer sequences were retrieved from gene bank by NCBI and spanned the adjacent regions of two exons, with a length of about 20bp, (Table 1).

Table 1. primers and probes

| Gene      | Sequence                                      | Length(bp) |
|-----------|-----------------------------------------------|------------|
| HIC-1     |                                               |            |
| Upstream primers | 5'-TTGACACGCGACAAGAAGT-3'                     | 19         |
| Downstream primers | 5'-GGGCGAGGACCATAGA-3'                     | 16         |
| probe     | 5'-CAGCATGTGTTACTCGACTA-3'                    | 20         |
| HIC-1     |                                               |            |
| Upstream primers | 5'-GTTTGTTCGCTGGCGTGATAG-3'                  | 20         |
| Downstream primers | 5'-TGTGGGACCTGTGGAAGTA-3'                  | 19         |
| probe     | 5'-ATCTGCGCGATGGCAGG-3'                       | 21         |
| GAPDH     |                                               |            |
| Upstream primers | 5'-CCTCAAGATCAGCAAT-3'                    | 19         |
| Downstream primers | 5'-CCATCCACAGTCTTCTGGGT-3'                  | 20         |
| probe     | 5'-FAMACCACAGTCCATGCCATCAC-TAMRA-3'          | 20         |

2) Extraction of Total RNA
Take 35 ~ 40mg of each sample, cut it into pieces, extract the total RNA by Trizol method, and detect the RNA level by spectrophotometer. The total RNA was dissolved in RNA free enzyme water treated by DEPC and stored at -80 °C.

3) RT-PCR Detection
The conditions of RT and PCR amplification were optimized according to the instructions, and PCR amplification was performed using the icyclir IQ system. Based on the formula ratio = (e target gene) ^ CT target gene (control sample)/(egapdh) ^ CTgapdh (control sample), ratio is the relative expression ratio of HIC-1 and HIC-1 gene to GAPDH Gene in each sample (> 1 indicated the up-regulated expression), e represents the amplification efficiency, e = 10-1/slope (slope represents the slope of amplification efficiency curve), control represents the control group, and sample represents the colon cancer group.

2.5. Detection of HIC-1 protein expression in colon cancer by immunohistochemistry
Conventional section was performed at about 4 μm thick. Dewaxing and hydration with xylene were carried out. After 3% H2O2 enzyme treatment and washing, mouse anti-human HIC-1 monoclonal antibody was added according to the recommended dilution and incubated in a refrigerator overnight at 4°C. The secondary anti-mouse and horseradish peroxidase - ovalbumin - biotin labeling complex were added in turn, followed by DAB staining, hematoxylin re-staining, and sealing. Olympus dd70 BX51 image acquisition system was used to collect the images. Results: 10 high power visual fields were randomly selected for each section. The background staining intensity was 1. The relative ratio
of absorbance value (a) was read, and the mean value was taken as the staining intensity. The staining intensity was divided into negative (<1).

2.6. Statistical analysis
The expression of mRNA was analyzed by the software rest and paired randomization. Pearson correlation test was used to analyze the correlation between mRNA and protein expression and clinicopathological characteristics, as well as between computer-aided analysis and HIC-1 expression.

The “diversity” defined in this paper represents the ratio between the saved resources and the output error value under the similar operation.

\[ \text{Xbility} = \frac{E_{\text{CAI}}} {Q_{\text{save}}} \]  

Where the measured value should be determined according to the actual problems. For example, in the process of clinical treatment, the signal-to-noise ratio (PSNR) of colon cancer peak value is generally taken as the measurement standard according to a similar order. The main content of this paper is the time consumed in the classification stage. According to the operating system level and time-consuming factors, the time of the classification stage can be divided into the following:

\[ E = E_{\text{AES}} + E_{\text{ALMA}} + E_{\text{ACP}} \]  

Where it represents the use time of CAI benefit evaluation. In this paper, it mainly refers to the time of saving Cai benefit evaluation to DRAM; it represents the time of CAI benefit evaluation.

3. Results
In colon cancer, the positive cells of HIC-1 immunohistochemical staining were diffusely distributed in the cancer nests, and the cytoplasm showed brownish yellow, weakly expressed in the paracancerous tissues. The difference was statistically significant (P = 0.003). The expression of HIC-1 was not correlated with clinicopathological characteristics. There was a negative correlation between the expression of HIC-1 and computer-aided in colon cancer (r = -0.63, P < 0.01). See Table 2 and figure 1.

Table 2. Relationship between the expression of HIC-1 and clinicopathological characteristics of colon cancer [cases (%)]

| Clinopathological parameters | mRNA | Protein |
|-----------------------------|------|---------|
| Age (year)                  |      |         |
| Less than 50                |      |         |
| >50                         | 23(27.38) | 61(72.62) | 40(47.62) | 44(52.38) | 0.006 | 0.853 |
| Gender                      |      |         |
| male                        | 36(31.03) | 80(68.97) | 50(43.10) | 66(56.90) | 0.004 | 0.970 |
| female                      | 44(32.59) | 91(67.41) | 50(37.04) | 85(62.97) | 0.004 | 0.970 |
| Dukes staging               |      |         |
| A+B                         | 15(23.08) | 50(76.92) | 40(61.54) | 25(38.46) | 0.006 | 0.853 |
| 46(43.40) | 60(56.60) | 43(40.57) | 63(59.43) | 0.004 | 0.970 |
| Lymph node metastasis       |      |         |
| negative                    | 13(13.83) | 81(86.17) | 47(50.00) | 47(50.00) | 0.006 | 0.853 |

0.690 0.023 0.730 0.010
The expression of HIC-1 was negatively correlated with computer-assisted therapy ($r = -0.63, P < 0.01$). The study showed that the balance between MMP-1 and TIMP1 was broken in patients with gastric cancer. The low expression of TIMP1 could promote the occurrence and progression of gastric cancer. In this study, 121 patients (60.5%) showed up-regulated expression of HIC-1 mRNA in paracancerous tissues, higher than that in colon cancerous tissues (104 cases (52.0%)). This suggested that HIC-1 may have an inhibitory effect on tumor development. Reis et al. found that the expression of HIC-1 was high, while that of HIC-1 was low in prostate cancer, and the balance between HIC-1 and HIC-1 was broken, which facilitated the progression of prostate cancer. However, the above balance theory has not been fully elucidated until the present. The exact regulatory mechanism and antagonistic factors of HIC-1 expression in the the up-regulation of colon cancer are still unclear. We believe that the deepening of studies and the clarification of the above issues will open a new chapter for the prevention and treatment of colon cancer.

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