Expression and clinical significance of survivin and matrix metalloproteinase-7 in colon cancer

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[Abstract] Background and Objective: Responses and prognosis vary in patients with colon cancer of the same stage using the same therapeutic strategy. Finding a good marker to predict the prognosis is necessary. This study was to explore the correlations of Survivin and matrix metalloproteinase-7 (MMP-7) expression to the prognosis and clinicopathologic features of colon cancer. Methods: Clinical data of 620 colon cancer patients, treated in Sun Yat-sen University Cancer Center from January 1995 to May 2003, were analyzed. The expression of Survivin and MMP-7 in the 620 specimens of colon cancer was detected by tissue microarray and immunohistochemistry. Correlations of Survivin and MMP-7 expression to the prognosis and clinicopathologic features were analyzed. Results: The positive rates of survivin and MMP-7 were significantly higher in colon cancer than in normal colon mucosa (41.0% vs. 0, P<0.001; 88.8% vs. 40.9%, P<0.05). There was no relationship between Survivin expression and patients' age, sex, tumor location, gross and histological type, grade, size of colon cancer. The positive rate of Survivin was significantly higher in advanced colon cancer than in early stage colon cancer (P<0.05). There was no relationship between MMP-7 expression and all clinicopathologic factors of colon cancer. Cox univariate and multivariate regression analyses showed that survivin and MMP-7 expression were independent factors for prognosis of colon cancer. Conclusion: MMP-7 and survivin are related to the generation of colon cancer, and are independent factors for prognosis of colon cancer. Key words: colon neoplasm, survivin, matrix metalloproteinase-7, prognosis

At present, individual treatment of colon cancer is mainly based on clinical staging, and Dukes staging and TNM staging are commonly used. These two systems are helpful for predicting the prognosis of patients with colorectal cancer and guiding treatment, but they are based on the depth of tumor invasion, lymph node metastasis, distant metastasis, while do not take the heterogeneity of patients into consideration. Clinically, therapeutic efficacy and prognosis varied for the patients at the same stage and received the same treatments. Researchers have been working on finding out parameters, alone or in combination with clinical staging, to
evaluate the prognosis and guide treatment more accurately. Recently, the idea of molecular pathologic staging is proposed,\textsuperscript{1,2} though no clear concept is available yet. It may be interpreted as re-gard tumors at molecular level using the knowledge of molecular biology, which reflect disease severity and prognosis more thoroughly and accurately as compared with conventional clinicopathologic staging, therefore, guide the prevention and treatment on molecular level.

In this study, we detected the expression of controversial metastasis-associated proteins, matrix metalloproteinase-7 (MMP-7) and Survivin, in 620 specimens of colon cancer using tissue chip technology and immunohistochemical staining, and analyzed clinical and follow-up data of corresponding patients to investigate the relationships of the two proteins to clinicopathologic parameters and prognosis of colon cancer patients, as well as their prognostic significance to provide theoretical basis for molecular staging of colon cancer.

Materials and Methods

Clinical data and samples. All samples were obtained from 620 primary colon cancer patients underwent surgery in Sun Yat-sen University Cancer Center between January 1995 and May 2003. These patients, including 364 men and 256 women with a sex ratio of 1.43:1, aged of 15-86 years with a median of 57 years. All diagnoses were confirmed by pathologic examination. Surgical patterns included right hemicolecotomy, transverse colectomy, left hemicolecotomy and sigmoidectomy. Clinicopathological staging was performed using 2002 TNM staging system. Intraoperative chemotherapy patterns included mesocaval chemotherapy, omental intravenous perfusion, intraperitoneal chemotherapy. Postoperative chemotherapy regimens included 5-fluorouracil/leucovorin (5-FU/CF), 5-FU/levamisole (5-FU/Lev), UFT, FT207 or xeloda, oxaliplatin plus 5-FU/CF (FOLFOX), and so on.

The following parameters were included in analysis: gender (male, 364 cases; female, 256 cases); age (≤ 40, 84 cases; 41-64, 312 cases; ≥ 65, 224 cases); perioperative blood transfusion (0, 513 cases; ≤ 400 mL, 62 cases; 400-800 mL, 14 cases; ≥ 800 mL, 31 cases); tumor location (ileocecal junction, 47 cases; ascending colon, 107 cases; hepatic flexure, 70 cases; transverse colon, 38 cases; splenic flexure, 21 cases; descending colon, 48 cases; sigmoid colon, 251 cases; multi-focal primary lesions, 38 cases); tumor largest diameter (< 5 cm, 185 cases; ≥ 5 cm, 435 cases); tumor gross type (proliferative type, 180 cases; ulcer type, 322 cases; infiltrating type, 100 cases; unknown, 18 cases); histological type (tubular adenocarcinoma, 445 cases; mucinous adenocarcinoma/signet-ring cell carcinoma, 79 cases; adenoma with canceration, 20 cases; papillary adenocarcinoma, 32 cases; poorly differentiated adenocarcinoma, 32 cases; others, 12 cases); pathologic grade (grade I, 38 cases; grade II, 438 cases; grade III, 130 cases; unknown, 14 cases); intraoperative chemotherapy (no, 309 cases; yes, 311 cases); postoperative chemotherapy (no, 87 cases; yes, 533 cases); TNM stage (stage I, 86 cases; stage II a, 146 cases; stage II b, 110 cases; stage III a, 10 cases; stage III b, 106 cases; stage III c, 24 cases; stage IV, 138 cases); T stage (stage T1, 18 cases; stage T2, 84 cases; stage T3, 266 cases; stage T4, 247 cases; unknown, 5 cases); N stage (stage N0, 392 cases; stage N1, 153 cases; stage N2, 54 cases; unknown, 21 cases); M stage (stage M0, 482 cases; stage M1, 138 cases).

Colon cancer tissue microarray preparation. The sections with HE staining were observed under light microscope to identify and mark 2 spots of typical tumor tissue and 1 spot of normal intestinal mucosa in each specimen. Tissue microarray paraffin blocks with different numbers of samples were prepared using personal tissue arrayer (Beecher Company, USA) and stored at 4°C. The tissue microarray were then cut into 4-μm serial sections.

Immunohistochemistry. The SP immunohistochemistry was applied according to users manuals. Antibody dilutions are as follows: 1:100 for rabbit anti-Survivin (71G4, Cell Signaling Technology), 1: 250 for anti-MMP-7 (clone 141-7B2, Chemicon).
Survivin localized in nuclei and MMP-7 localized in cytoplasm. Five random high-power microscope (× 400) fields of vision in each specimen were observed, and scored according to positive cell proportion and intensity of staining: a specimen with positive cell proportion of <5% was scored 0, 5%-24% scored 1, 25%-49% scored 2, 50%-74% scored 3, ≥ 75% scored 4; no staining was scored 0, weak staining in light yellow scored 1, moderate staining in yellow scored 2, strong staining in yellowish brown scored 3. Multiplying the two scores, the cases with a product of <3 were defined as negative and those with a product of ≥ 3 as positive. PBS, instead of first antibody, was used for blank control; positive sections were used as positive controls. All sections were examined by two pathologists independently.

Follow-up and statistical analysis. The patients were re-examined every 3 months in the first 2 years, every 6 months in the following 3 years, and then once per year. They were followed-up till May 31, 2006, with a median follow-up period of 52 months (1-130 months). All patients were followed up by outpatient review, telephone or mail.

The clinical and laboratory data of all patients were collected as database, processed and analyzed with statistical package SPSS13.0. Survival time was defined as the interval between operation day and the last visit (follow-up). Operation-related and non-tumor-related deaths were defined as trimmed data. \( \chi^2 \) test was used to compare the differences in the expression of tumor markers, life-table method to calculate survival rate, and Kaplan-Meier method to investigate survival. Cox proportional hazards model was used to analyze the prognostic values of the factors with P values of <0.10. A P value of ≤ 0.05 was considered significant.

Results

Expression of Survivin, MMP-7 in normal colon mucosa and cancer tissues. Survivin was expressed in the nuclei of colon cancer cells, as yellow-brown granules (Fig. 1). The positive rate of Survivin was significantly lower in normal colon mucosa than in cancer tissues (0 vs. 41.0%, \( Z = 10.9, P<0.001 \)).

MMP-7 was expressed in cytoplasm of colon cancer cells, as brown granules (Fig. 2). The positive rate of MMP-7 was also significantly lower in normal colon mucosa than in cancer tissues (40.9% vs. 88.8%, \( Z = 6.9, P<0.001 \)).

Relationships of Survivin and MMP-7 expression to clinicopathologic features of colon cancer. The expression of Survivin had no relationships to patients age, sex, tumor location, gross type, maximal diameter, differentiation and

Figure 1  Expression of Survivin in colon cancer tissue (SP ×200)
Survivin is expressed in nuclei of colon cancer cells.

Figure 2  Expression of matrix metalloproteinase-7 (MMP-7) in colon cancer tissue (SP ×200)
MMP-7 is expressed in cytoplasm of colon cancer cells.
histological type (P>0.05), while only correlated to TNM stage (P<0.05), the more advanced the stage, the higher the positive rate. The expression of MMP-7 had no relationships to the above parameters (P> 0.05).

**Relationships of Survivin and MMP-7 expression to colon cancer-related death.** Cox univariate and multivariate (Enter method) analyses showed that poor differentiation, late TNM stage, MMP-7-positive, and Survivin-positive were independent risk factors for colon cancer-related death, while intraoperative chemotherapy and postoperative chemotherapy were protective factors (Table 1). The 5-year survival rates was 51% for Survivin-positive patients, and 65% for Survivin-negative patients (P<0.05) (Fig. 3), and were 62% for MMP-7-positive patients, and 85% for MMP-7-negative patients (P <0.05) (Fig. 4).

**Discussion**

Studies have proved the occurrence and development of colon cancer is a multi-stage, multi-gene process, which involves a wide range of gene expression changes. In this study, we selected Survivin and MMP-7, which are controversial proliferation and metastasis-related proteins in Wnt/β-catenin signaling pathway, to explore their correlations to clinicopathologic features and prognosis of colon cancer patients. It may provide a theoretical basis for molecular staging of colon cancer.

The concept of tissue microarray (also known as tissue chip) was first proposed by Kononen et al. in the U.S. National Human Genome Research Laboratory in 1998.4 Compared with traditional tissue sections, tissue microarray has the following advantages: (1) high throughput; (2) less experimental errors; (3) time-, labor-, and cost-saving; (4) minor damage to original paraffin blocks. In this study, we produced nine tissue microarrays for immunohistochemical study, saved a lot of time and obtained good results.

Survivin is a new star in inhibitor of apoptosis protein (IAP) family. It is detected in

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MMP-7, matrix metalloproteinase-7.

![Survival curves of colon cancer patients with or without Survivin expression](image1)

![Survival curves of colon cancer patients with or without MMP-7 expression](image2)
most tumors such as gastric cancer, breast cancer, lung cancer, liver cancer, esophageal cancer, bladder cancer, lymphoma tumor and soft tissue sarcoma, but not in normal adult tissues. Our results confirmed this point--the positive rates were 41.0% in colon cancer tissues and 0% in normal tissues, suggesting its significant correlation to the occurrence of colon cancer. Survivin expression in colorectal cancer is not correlated to gender, age, and tumor location, while its correlations to differentiation and tumor stage are controversial.\textsuperscript{5,6} Our results showed that Survivin expression did not relate to patient’s age, sex, tumor location, gross type, tumor maximal diameter, histological type, and differentiation, but relate to TNM stage--the more advanced the stage, the higher the positive rate. Univariate and multivariate analyses showed that Survivin expression was related to tumor-related death, and Survivin-positive patients had shorter survival time, suggesting that Survivin expression is correlated to the occurrence, development, invasion and metastasis of colon cancer, and can affect the prognosis.

Tumor growth, invasion and metastasis depend on angiogenesis, and extracellular matrix degradation which is mainly catalyzed by tumor cell-secreted matrix metalloproteinases (MMPs). MMP-7 is an important member of MMPs family and is the only one specifically expressed by epithelial tumor cells. It mainly functions to degrade elastic fibers, proteoglycans, fibronecrtins, type IV collagen fibers, and a variety of transmembrane proteins inhibiting tumor metastasis. Its expression correlates to the occurrence and development of some human malignant tumors, and plays an important role in the invasion and metastasis of colorectal cancer.\textsuperscript{7,8} The positive rate of MMP-7 varies from 35% to 97% in colorectal cancer, and its relationships to pathologic parameters and prognosis of patients vary among different reports\textsuperscript{9,10}, which may due to using different antibodies and standards. In our study, the positive rate of MMP-7 was significantly higher in colon cancer than in normal mucosa (88.8% vs. 40.9%), suggesting its relationship to the occurrence of colon cancer. Besides, MMP-7 expression did not relate to patient’s age, sex, tumor location, tumor maximal diameter, gross type, histological type, tumor differentiation, TNM stage. Cox univariate and multivariate analyses revealed that it was a risk factor that affected colon cancer-related death. It can be used as a predictor of prognosis; positive patients have poorer prognosis, which may be related to easier metastasis of MMP-7-positive tumors.

Our results suggest that both MMP-7 and Survivin are associated with the occurrence of colon cancer, and are risk factors impacting tumor-related death, thus provides a good basis for the prognosis prediction for colon cancer and lays a theoretical foundation for the molecular staging of colon cancer.

References