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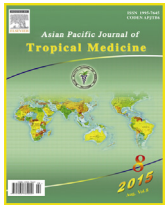

Original research http://dx.doi.org/10.1016/j.apjtm.2015.07.004

Correlation of caveolin-1 expression with microlymphatic vessel density in colorectal adenocarcinoma tissues and its correlation with prognosis

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ARTICLE INFO

Article history:
Received 15 May 2015
Received in revised form 20 Jun 2015
Accepted 15 Jul 2015
Available online 23 July 2015

Keywords:
Colorectal neoplasms
Caveolin-1
Microlymphatic density
Immunohistochemistry

ABSTRACT

Objective: To study the expression of caveolin-1 in colorectal adenocarcinoma tissues and its correlation with microlymphatic vessel density (LMVD), and to investigate the clinical pathological prognostic significance of caveolin-1 and LMVD in patients with colorectal cancer.

Methods: The expression of caveolin-1 and LMVD in 45 specimens of normal colorectal tissues, and 90 specimens of colorectal adenocarcinoma tissues were detected by immunohistochemistry technique. The correlation between their expression and the clinicopathologic features was analyzed. Multivariable Cox regression was used to analyze the association between the laboratory indices and overall survival time.

Results: The positive rates of caveolin-1 in colorectal adenocarcinoma tissues were significantly higher than those in normal colorectal tissues ($P < 0.01$). LMVD in colorectal adenocarcinoma tissues were significantly higher than those in normal colorectal tissues ($P < 0.01$). Mean LMVD in group with caveolin-1 positive was significantly higher than in that with caveolin-1 negative. The median survival time was 26.7 months. Cox regression analysis showed that the caveolin-1 expression, invation depth, lymph node metastasis, TNM stage, liver metastasis and LMVD were independent risk factors of overall survival time of patients with colorectal carcinoma.

Conclusions: Caveolin-1 may contribute to the lymphangiogenesis in the tumor. During the occurrence and development of colorectal adenocarcinoma, there is a close relationship between the expression of caveolin-1 and lymphatic microvessel of tumor. Caveolin-1 expression and microlymphatic vessel density are significant prognostic value of colorectal carcinoma.

1. Introduction

Recently, more and more attention has been paid to caveolin-1. Caveolin-1 is an important carcinogenic factor, and plays a key role in the process of regulating cell signal transduction and induction of proliferation, differentiation, migration and apoptosis [1]. Lymphatic microvessel density (LMVD) can reflect the progression of malignant tumor as important index of micro lymphatics neonatal ability [2,3]. This study detected the expression of caveolin-1 protein, and the average value of LMVD in colorectal adenocarcinoma in order to analyze the relationship between caveolin-1, LMVD and prognosis of colorectal adenocarcinoma.

2. Materials and methods

2.1. Clinical data

A total of 90 surgical specimens of colorectal adenocarcinoma tissues and 45 specimens of normal colorectal tissues were collected from July 2011 to July 2013. Cancer tissues were excised from the center, and normal tissue were excised from stump of the cut edge of the specimen at. All cases had no other primary tumors, and received no neoadjuvant chemoradiotherapy, biological and immunotherapy. In colorectal adenocarcinoma tissues, 57 cases were male, and 33 cases were female, aged 36–74 years old, with average age as (55 ± 2.9) years old; while in normal colorectal tissues, 28 cases were male,

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Peer review under responsibility of Hainan Medical University.
Foundation Project: It is supported by 2013 Mandatory Planning Project of Scientific and Technological Bureau of Zhangjiakou City (1311055D).

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and 17 cases were female, aged 39–71 years old, with average age as (56 ± 2.1) years old. There was no statistical difference in age, gender, *etc* between two groups ($P > 0.05$).

2.2. Methods and reagents

Mouse-anti-human monoclonal concentrated caveolin-1 antibody (cloning ab40278 R3-5G4) was from Abcam Company in Hong Kong; D2-40 was from Abnova Company in the United States.

Formalin-fixed paraffin-embedded tissue samples were cut into sections (4 μm) with a microtome and dried overnight at 37 °C on a silanised-slide. Dyeing slides were independently observed and diagnosed under optical microscope. Samples were deparaffinised in xylene at room temperature for 30 min, rehydrated with graded ethanol and washed in phosphate-buffered saline (PBS). The samples were then placed in 10 mm citrate buffer (pH 6.0) and boiled in a microwave for 10 min for epitope retrieval. Endogenous peroxidase activity was quenched by incubating tissue sections in 3% H_2O_2 for 10 min. Caveolin-1 antibody were used overnight at 4 °C at dilutions of 1:150. The slides were washed and secondary antibody was applied for 30 min after rinsing in PBS. The slides were then washed and treated with the chromogen 3,3,-diaminobenzidine for 5 min, then rinsed in PBS, and counterstained with hematoxylin, dehydrated in graded ethanols (80%, 85%, 90%, 95%, 100%), cleared in xylene and transparent for 5 min and sealed by neutrality. PBS instead of first antibody was treated as negative control.

2.3. Criteria of positive result

The positive expression of caveolin was identified as yellow and granular brown substance in cell nucleus. The immunoreactivities were graded as (−), (+), (++), and (+++) according to the percentage of positive tumor cells identified: (−) represents 0 or less than 5% tumor cells; (+) represents 6%–25% tumor cells; (++) represents 26%–50% tumor cells; and (+++) represents the strongest staining with more than 50% tumor cells present. (+)–(+++) are considered to be positive.

D2-40 was marked in the membrane and cytoplasm of tumor lymphatic endothelial cell and granular brown substance was considered to be targeting cellular [4,5]. Firstly, 5 densely area full of vascular (hot area) were selected under optical microscope of 40 times, and then individual and cluster endothelial cells with coloring were identified as the observation area under optical microscope of 400 times, finally, where the average number of targeting cellular was counted as LMVD value.

2.4. Statistic analysis

The results were analyzed with SPSS 14.0. All enumeration data was expressed as percentage, and measurement data was expressed as mean ± SD. Multi-factor analysis of prognosis were use by Cox Risk Model. The differences were considered to be significant at $P < 0.05$.

3. Results

3.1. Immunohistochemical staining of caveolin-1 protein and LMVD

The positive expression rate of caveolin-1 in colorectal adenocarcinoma tissues and normal colorectal tissues were 73.33% (66/90) and 11.11% (5/45), and the difference was statistically significant ($P < 0.05$). LMVD in colorectal adenocarcinoma tissues and in normal colorectal tissues was 18.25 ± 2.36 and 3.14 ± 1.58, and the differences was statistical significant ($t = 32.00$, $P < 0.05$) (Figure 1).

3.2. Relation of caveolin-1 protein and LMVD

Mean LMVD in group with caveolin-1 positive (17.81 ± 2.15) was significantly higher than in that with caveolin-1 negative (6.15 ± 2.21), and the differences was statistical significant ($t = 21.42$, $P < 0.05$).

3.3. Relation between caveolin-1 protein, LMVD and pathological prognosis

The clinical data of 14 cases was lost and follow-up data of 76 cases was complete, including 42 male and 34 female cases,

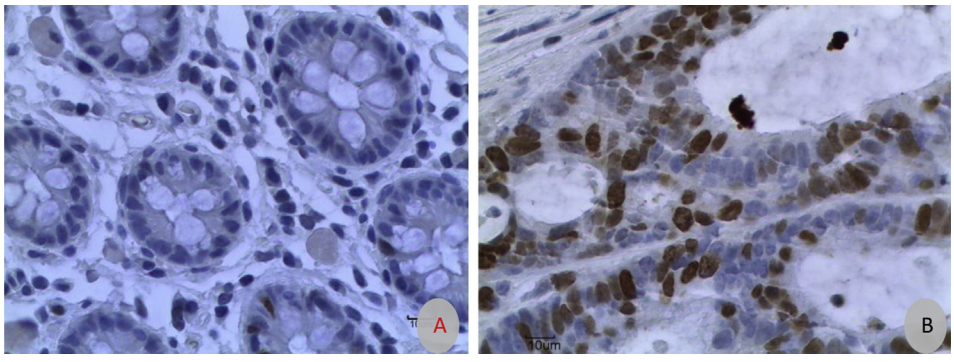

Figure 1. Immunohistochemical staining of caveolin-1 protein and LMVD (IHC × 400).
A: Normal colorectal tissues; B: Colorectal adenocarcinoma tissues.

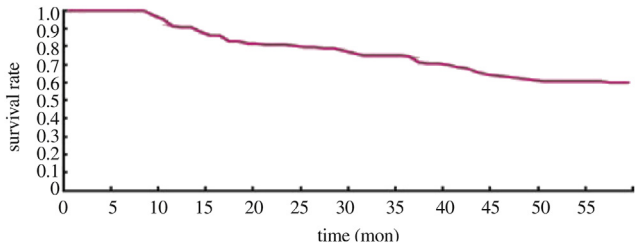

Figure 2. Overall survival curve.

Table 1
Multiple factor analysis of the prognosis of 76 cases with colorectal adenocarcinoma.

Risk factors	RE	SE	SV	P	HR	95%CI
Tumor size	0.170	0.541	0.099	0.754	1.185	0.410–3.422
Histological types	–0.024	0.363	0.004	0.947	0.976	0.479–1.989
Differentiation	0.162	0.334	0.234	0.629	1.175	0.611–2.261
Depth of invasion	2.405	0.810	8.823	0.003	11.083	2.266–54.198
Lymph node metastasis	1.555	0.611	6.482	0.011	4.736	1.430–15.682
TNM stage	2.514	0.810	9.627	0.002	12.353	2.524–60.453
Liver metastasis	2.079	0.811	6.574	0.010	8.000	1.632–39.212
Caveolin-1	3.209	0.675	22.601	<0.001	24.750	6.592–92.921
LMVD	1.239	0.615	4.057	0.044	3.453	1.034–11.532

aged 44–73 years old, with average age as (57.2 ± 6.1) years old. The median survival time was 26.7 months (8–56 months), and 1, 3 year survival rates were 91.60% and 75.15%, respectively (Figure 2). Multi-factor analysis showed that caveolin-1, LMVD value, invation depth, TNM stage, liver metastasis, lymph node metastasis were independent risk factors (Table 1).

4. Discussion

Caveolin-1 gene as a member of caveolin family is located on chromosome 7q31, containing 178 amino acid residues, which is divided into two subtypes of α and β. The structure of caveolin-1 has a special domain, in which *N*-terminal amino acid contains caveolin binding sequence similar to activation center of a variety of signaling molecules [Caveolin-1 scaffolding domain (CSD)]. CSD can specially combine a variety of cellular signaling proteins, such as G-protein-subunits, Src, Fyn, HA2Ras, EGF receptor, insulin receptor, eNOS, PKC, and so on in order to play a role of targeted adjustment [6,7]. Its C-terminal area containing Tyr14 phosphorylation, could specially bind to Ab1, Frn, Src, and other tyrosine kinases, leading to the phosphorylation, and thus induce a series of biochemical reactions [8]. In addition, caveolin-1 can inhibit the system of Ras/ERK, and MAPK/ERK, and induce cell excessive proliferation and apoptosis, so as to promote their growth [9]. Caveolin-1 may promote the permanent withdrawal from the cell cycle and induce apoptosis or necrosis.

In this study, high expression of caveolin-1 protein in colorectal adenocarcinoma may be closely-related to invasion depth, liver metastasis and lymph node metastasis, which was basically consistent with studies of Yang *et al.* [10] and Xu *et al.* [11]. Further research showed that the value of LMVD in group with caveolin-1 positive expression was significantly higher than that in group with caveolin-1 negative expression. Caveolin-1 may induce lymphangiogenesis and promote tumor metastasis through the lymphatic system in the colorectal cancer [12].

Furthermore, multiple factors analysis of prognosis showed that the survival rate of patients with high LMVD value and high caveolin-1 expression is significantly lower than that with low LMVD value and low caveolin-1 expression in the colorectal cancer, which reveals that LMVD value and caveolin-1 have important value in evaluating the prognosis of patients with colorectal cancer. After colorectal cancer radical surgery, monitoring the tumor lymphatic could be used to predict the progress of the tumor and the prognosis in order to provide valuable reference index of treatment and postoperative follow-up.

Caveolin-1 and LMVD are independent prognosis indicators for colorectal cancer, and the detection of caveolin-1 expression and LMVD level helps to assess the malignant degree of tumor, so as to provide further guidelines for clinical diagnosis and treatment. At the same time, the study of mechanism of inducing tumor lymphangiogenesis and its gene targeting therapy may lead to new inspiration and direction in the treatment for colorectal cancer.

Conflict of interest statement

We declare that we have no conflict of interest.

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