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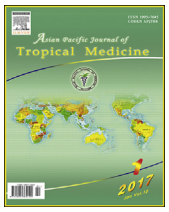

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Correlation of RUNX3 expression with microvessel density in colorectal adenocarcinoma tissues and clinical significance

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ABSTRACT

Objective: To study the expression of RUNX3 in colorectal adenocarcinoma tissues and its correlation with microvessel density (MVD), and investigate the clinical pathological prognostic significance of RUNX3 and MVD in patients with colorectal cancer.
Methods: The expression value of RUNX3 and MVD in 70 specimens' colorectal adenocarcinoma tissues were detected by immunohistochemistry staining technique. The correlation between their expression and the clinicopathologic features was also investigated.
Results: The expression value of RUNX3 and the positive rates of RUNX3 in colorectal adenocarcinoma tissues were 3.25 ± 1.14 and 25.71% (18/70). The expression value of MVD in colorectal adenocarcinoma tissues was 13.14 ± 3.23. Expression of RUNX3 and MVD value were correlated with CEA, serosal invasion, liver metastasis, lymph node metastasis, and TNM stage ($P < 0.01$). The expression value of RUNX3 had negative correlations with that of MVD.
Conclusions: The high expression of RUNX3 could inhibit tumor microvascular generation in order to have negative control response on invasion and distant metastasis.

1. Introduction

Invasion and distant metastasis of tumor are the important factors influencing the prognosis and hematogenous metastasis is one of the important ways. Microvessel density (MVD), as the important index of tumor angiogenic blood vessels, could reflect the progress of malignant tumor [1,2]. Recently, more and more attention has been paid to the antitumor role of RUNX3. RUNX3, as the key transcription factor of transforming growth factor β (TGF-β) downstream, plays negative regulatory role in epithelial cell proliferation differentiation, embryonic development, cell apoptosis, and immunoregulation [3].

This study detected the expression value of RUNX3 and MVD in colorectal adenocarcinoma tissues by immunohistochemistry staining technique and investigated the correlation with clinical pathological characteristics.

2. Materials and methods

2.1. Clinical data

A total of 70 surgical specimens of colorectal adenocarcinoma tissues were collected from August 2013 to June 2015. Cancer tissues were excised from the center. All cases had no other primary tumors, and received no neoadjuvant chemoradiotherapy, biological and immunotherapy. In colorectal adenocarcinoma tissues, 42 cases were male, and 28 cases were female, aged 35–69 years old, with average age as (50.0 ± 4.8) years old. Six cases were ascending colon carcinoma, 4 cases were horizontal colon cancer, 14 cases were descent colon carcinoma, 12 cases were sigmoid colon cancer, and 34 cases were rectal cancer. Thirty nine cases were CEA (+), and 31 cases were CEA (−).

2.2. Methods and reagents

Rabbit Anti-human monoclonal concentrated RUNX3 antibody (cloning ab40371 R2-5G6) was from Abcam Company in Hong Kong; CD34 antibody was from Abnova Company in the United States.

Sections (4 μm) of formalin-fixed paraffin-embedded tissue samples were cut with a microtome and dried overnight at 37 °C on a silanised-slide. Dyeing slides were independently observed

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and diagnosed by pathologist with high qualification 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. The RUNX3 antibody was 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 haematoxylin, 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. Decision criteria of positive result

The positive expression of RUNX3 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: (–) represented 0 or less than 5% tumor cells; (+) represented 6%–25% tumor cells; (++) represented 26%–50% tumor cells; and (+++) represented the strongest staining with more than 50% tumor cells present. (+) ~ (++ +) were considered to be positive.

2.4. MVD detection

CD34 antibody was marked in the membrane and cytoplasm of tumor lymphatic endothelial cell and granular brown substance was considered to be targeting cellular. Firstly, 4 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 MVD value.

2.5. Statistic analysis

The results were analyzed statistically with SPSS 17.0. All enumeration data was expressed as percentage and measurement data was expressed as mean ± SD. The differences were considered to be significant at $P < 0.05$.

3. Results

3.1. Immunohistochemical staining of RUNX3 protein and MVD

There were obvious differences among the shape, size, and distribution of microvessel in intercellular substance of colorectal adenocarcinoma tissues marked by CD34 antibody and irregular counter of vascular lumen. The MVD value of 70 colorectal adenocarcinoma tissues was 13.14 ± 3.23 (8–45/high power field) (Figure 1A). The positive expression of RUNX3 was 25.71% (18/70) and showed an uneven distribution in colorectal adenocarcinoma tissues, in which 5 cases were strong positive, 5 cases were middle positive, and 8 were weak positive (Figure 1B). The negative expression of RUNX3 was 52 cases and the expression value was 3.25 ± 1.14.

3.2. Expression value of different pathological feature

The expression value of RUNX3 and MVD gradually and significantly decreased along with the deepening of infiltration depth and TNM staging ($P < 0.05$). The expression value of RUNX3 and MVD with liver metastasis, lymphatic metastasis, and CEA (+) were significantly lower than those without liver metastasis, lymphatic metastasis, and CEA(–) ($P < 0.05$) (Table 1).

3.3. Correlation of RUNX3 and MVD with clinical characteristic

The expression of RUNX3 in colorectal adenocarcinoma tissues had negative correlation with CEA, serosal invasion, liver metastasis, lymph node metastasis, and TNM stage, while the MVD value had positive correlation with CEA, serosal invasion, liver metastasis, lymph node metastasis ($P < 0.01$; Table 2).

3.4. Correlation of RUNX3 and MVD

The MVD value with the positive expression of RUNX3 ($n = 18$) in colorectal adenocarcinoma tissues was 9.21 ± 3.42, while the MVD value with the negative expression of RUNX3 ($n = 52$) in colorectal adenocarcinoma tissues was 16.09 ± 4.25, and there was significantly differences ($P < 0.01$, $t = 18.515$).

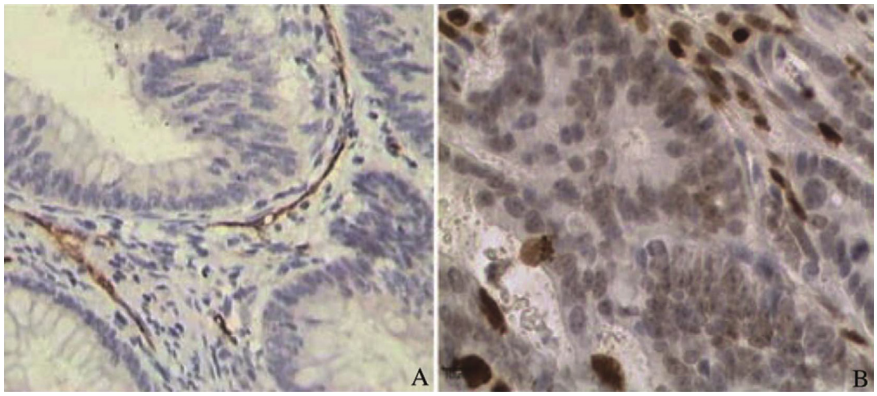

Figure 1. The number of microvessel in colorectal adenocarcinoma tissues (marked by CD34) (A) and the positive expression value of RUNX3 in colorectal adenocarcinoma tissues (IHC × 400) (B).

Table 1
Expression value of RUNX3 and MVD in different pathological feature of colorectal adenocarcinoma tissues.

Pathological feature	*n*	RUNX3			MVD		
		Mean ± SD	*t*	*P*	mean ± SD	*t*	*P*
Serosal invasion							
Yes	44	4.24 ± 1.21	2.251	0.013	16.03 ± 4.21	12.640	0.000
No	26	6.11 ± 1.89			9.53 ± 3.10		
TNM stage							
I + II	27	6.04 ± 1.65	6.013	0.000	9.29 ± 3.13	10.365	0.000
III + IV	43	2.51 ± 1.40			15.24 ± 3.91		
Lymphatic metastasis							
Yes	48	2.12 ± 1.16	8.149	0.000	17.29 ± 3.16	9.591	0.000
No	22	7.20 ± 1.21			10.61 ± 2.58		
Liver metastasis							
Yes	23	3.55 ± 1.31	4.651	0.000	18.55 ± 3.69	21.995	0.000
No	47	7.09 ± 1.17			8.22 ± 4.18		
CEA(+)							
Yes	45	3.60 ± 1.33	4.131	0.000	16.41 ± 3.66	8.153	0.000
No	25	6.81 ± 1.34			10.12 ± 3.50		

Table 2
Correlation of RUNX3 and MVD with clinical characteristic in the colorectal adenocarcinoma tissues.

Parameter		Serosal invasion	TNM stage	Lymphatic metastasis	Liver metastasis	CEA (+)
RUNX3	*r*	−0.039	−0.741	−0.422	−0.0007	−0.371
	P	0.008	0.000	0.002	0.0000	0.000
MVD	*r*	0.287	0.314	0.384	0.4920	0.378
	P	0.021	0.014	0.000	0.0000	0.001

4. Discussion

The generation of tumor microvessels plays an important role in supporting and guiding cell infiltration and distant metastasis and impacts hematogenous metastasis and prognosis. The expression of MVD is the most reliable quantitative evaluation index for measuring the generation of tumor microvessels. Detection MVD values can not only predict the progress of tumors, but also make accurate evaluations on the efficacy of adjuvant radio-chemotherapy and biotherapy [4,5]. For example, some drugs with angiogenesis-inhibiting can obviously inhibit the growth rate of tumors, control further development, infiltration and metastasis, and meanwhile provide reliable clinical basis for gene therapy.

RUNX3 is a heterodimer formed jointly by α and β subunits, which has a specific Runt domain (RD) structural domain folded by S-type immune globulin at its amino terminal. α-subunit mediates the target combination of RD and DNA, and strengthens the interaction of proteins; while β-subunit can further enhance the cohesion of RD and target-DNA [6,7]. RUNX3 protein can induce the activated smad protein compound through the conductive process of TGF-β signal to the specific target spot in cell nucleus, and conduct specific combination to activate target gene in order to induce cell differentiation, period regulation and apoptosis acceleration, *etc* [8,9]. The previous studies have proven that RUNX3 could weaken its behavior of lymphatic metastasis through inhibiting the generation of tumor micro-lymphvessels [10], additionally, some literature reported that RUNX3 could inhibit the angiogenesis and hematogenous metastasis in prostate and kidney cancers [11,12].

Currently, MVD as the important detection index of the generative capacity of cancer tissue microvessels has been confirmed in the study of breast cancer, gastric cancer, prostate cancer, liver cancer and urologic neoplasms [13–15], while there are fewer applications in colorectal cancer. In this study, MVD in colorectal cancer had taken on inhomogeneous distribution, morphological difference of pipe diameter and heterogeneity, which was corresponding to the report by Gang-Gang Shi, *et al* [16]. The study by Yan *et al* [17] confirmed that the MVD distribution in normal mucous membrane of colorectum, adenoma and adenocarcinoma took on a gradually rising trend, and the difference was significant. The MVD dense zone in the adenocarcinoma zone was significantly higher than that in the normal intestinal wall zone, and the maximum value in the former was approximately 4 higher than that in the latter. Moreover, from the normal zone to the lesions zone, the morphology of microvessels in mesenchyme tended to disorder, and the number increased gradually until the densest zone was the center of cancer. This experiment also showed MVD expression value decreased gradually along with the deepening of infiltration depth and TNM staging. The MVD value with liver metastasis, lymphatic metastasis, and CEA (+) were significantly higher than that without liver metastasis, lymphatic metastasis, and CEA (−).

In addition, this study confirmed that RUNX3 expression value decreased gradually along with the deepening of infiltration depth and TNM staging. The expression value of RUNX3 with liver metastasis, lymphatic metastasis, and CEA (+) were significantly lower than those without liver metastasis, lymphatic metastasis, and CEA(−). Moreover, this study showed that RUNX3 could obviously inhibit the malignant biological behaviors such as infiltration and metastasis of colorectal cancer etc and according to the correlation analysis, the expression of RUNX3 had significantly negative correlation with infiltration degree, liver metastasis, lymphatic metastasis, and CEA (+), which implied that RUNX3 could obviously inhibit the generation of tumor microvessels. It was considered that RUNX3 might inhibit the tumor angiogenesis through hindering the expression of the positive regulating factor of angiogenesis (VEGF) in order to inhibit the growth and proliferation. However, the specific mechanism still needs to be further studied, which will also be the future research direction of this subject.

Conflict of interest statement

We declare that we have no conflict of interest.

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