

Original Article

Expression of RANTES in Tissues of Primary Hepatocarcinoma and Its Relationship to Microvascular Density

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Object: To study on the expressions of regulated-upon activation normal T expressed and secreted (RANTES) and the microvascular density (MVD) in tissues of hepatocarcinoma (PHC), and investigate their relations to the clinicopathological features of PHC and the relationship between the expression of RANTES and the MVD. **Methods** The stains of microvessel and RANTES were completed by the immunohistochemical method of avidin-biotin complex in routine paraffin-embedded sections from 47 cases with PHC. **Results** The positive rate and the scoring mean of RANTES' expressions in the cancer tissues were significantly higher than that in the peri-cancerous tissues (55.32% vs. 19.15%, $P < 0.01$, 1.89 ± 1.77 vs. 0.77 ± 1.29 , $P < 0.01$); The MVD in the cancer tissues were significantly higher than that in the peri-cancerous tissues (67.30 ± 13.68 vs. 37.20 ± 10.58 , $P < 0.01$); Except the MVD in the patients with metastasis was significantly higher than that of non-metastasis (73.50 ± 13.77 vs. 64.10 ± 12.68 , $P < 0.05$). There were no correlations be found among the MVD, the RANTES' expressions and the other main clinicopathological features of PHC (including pathological type, differentiated degree, the value of AFP, cirrhosis, cancer emboli, gross shapes, and maximal diameter of mass, etc); The MVD was significantly correlated with the positive scoring mean of RANTES' expressions in the cancer tissues of PHC ($r = 0.386$, $P < 0.05$). **Conclusion:** The expression of RANTES and the MVD might be closely related to the carcinogenesis and the progress of PHC; RANTES might be closely related to the tumor angiogenesis in PHC.

Key Words:

Primary hepatocarcinoma; Microvascular density; Chemokines; Immunohistochemistry

Tumor angiogenesis have played a crucial role in the occurrence and progress of tumor, which is one of the premises of growth, infiltration and metastasis of tumor. MVD was confirmed by many studies [1] to be the important index of tumor angiogenesis and have close relationship of malignant tumor growth, metastasis and prognosis. As a kind of C-C chemotactic

factor, RANTES play an important role in the regulation of inflammation, anaphylaxis and progression of tumor [2]. In our study, we detected the MVD and the expression of RANTES in PHC to study the relationship of above two with major pathological characteristics of PHC.

1 Material and Methods

1.1 Specimens and general data: 47 cases of cancer tissue and adjacent tissues were obtained from operated patients (had no chemotherapy or radiotherapy before operation) in Hepato-cholo-pancreatic Surgery of Xiangya Second Hospital of Central South University since May 2003 to May 2005. There were 45 males and 2 females with age range of 25~73 ages (47.5 ± 14.0). 42 cases were hepatocellular liver cancer (HCC), and 5 cases were mixed carcinoma of liver (MHC). 29 cases were high and middle

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differentiation and 18 low differentiations, and 42 AFP positive, 5 AFP negative cases. According to the classification standard of liver cirrhosis [3], there were 21 cases of mild cirrhosis and 26 cases of moderate and severe cirrhosis. There were 16 cases accompanied with intrahepatic and (or) extrahepatic metastasis, 39cases were massive type, 8 cases were diffuse type and nodular type; the maximum diameter of neoplasma of 6 cases were < 5 cm, 41 cases were ≥ 5cm. all tumor of cases were successfully cut down and the specimens were diagnosed definitely by pathological examination. The specimens were made continuous 4μmthick sections after 10% formalin fixed and paraffin-embedded.

1.2 Methods and reagents: the test methods of RANTES and MVD were both ABC immunohistochemistry technic. The rabbit anti human RANTES polyclonal antibody and rat anti human CD34 monoclonal antibody were purchased form Wuhan Beshide Company.

1.3 Results: the CD34 positive result was decided by the brownish yellow tubular capsular and vacuoles staining formatted by

endothelial cells or immature endothelial cells the MVCs were assessed according to the method of Weidner et al. The positive expression result of RANTES is detemined by the brown granules in the cytolymph or nucleus which assisted with high power light microscope to observe by two experienced pathologist. To score according to the staining intensity and positive rate and confirm the positive and negative cases.

1.4 The statistical treatment: the data had made t- test, χ² test and correlation analysis using by SPSS10.0 statistical package. P <0.05 was statistical significance difference.

2 Results

2.1 The expression of RANTES and MVD: the immunoreactant of RANTES was mainly localized in cytoplasm(Fig.1), and the CD34 positive staining was mainly localized in the tumor interstitial of vascular endothelial cell(Fig.2). The positive rate and expressive scores of RANTES in tumor tissues was significantly higher than in adjacent tissues. The differences were statistical significance. (Table 1)

Table 1 The expression of RANTES in tumor tissues and adjacent tissues and MVD

	Case	Positive case	RANTES		MVD ($\bar{x} \pm s$)
			Positive rate(%)	Positive score ($\bar{x} \pm s$)	
Tumor tissues	47	26	55.32	1.89±1.77	67.30±13.68
Adjacent tissues	47	9	19.15*	0.77±1.29*	37.20±10.58*

Note: compare to tumor tissues. *P<0.05

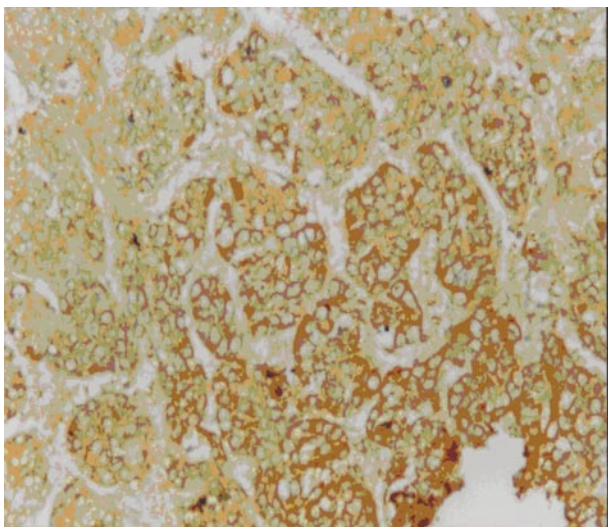


Fig. 1: the positive expression of RANTES in PHC tissues. (ABC immunohistochemistry technic, x200)

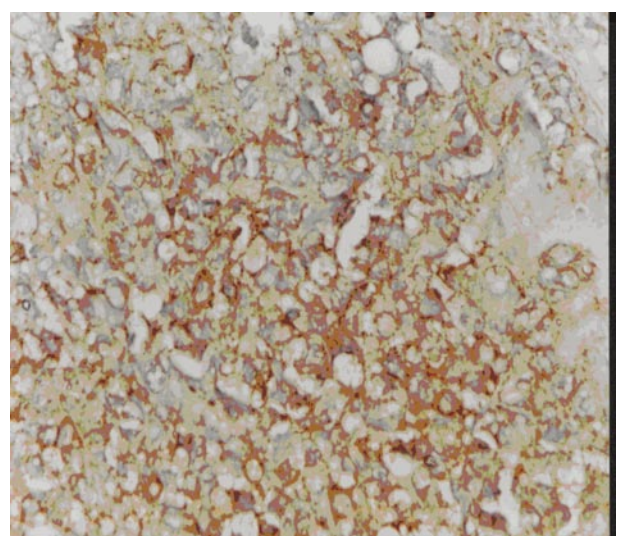


Fig. 2: CD34 staining, PHC, (ABC immunohistochemistry technic, x200 MV counting 130/HP)

2.2 The relationship between the expression of RANTES in tumor tissues and pathological characteristic of MVD: the expression of

RANTES and MVD has no obviously pathological characteristic with PHC except for in the tumor tissues. (Table2)

Table 2 The relationship between the expression of RANTES and MVD and the PHC clinical pathological characteristic

	Case	RANTES			MVD
		Positive case	Positive rate (%)	Positive score($\bar{x} \pm s$)	($\bar{x} \pm s$)
Pathological type					
HCC	42	23	54.76	1.88±1.78	66.56±13.88
MHC	5	3	60	2.00±1.87	74.48±11.06
Differentiation					
High and moderate differentiation	29	18	62.07	2.14±1.77	65.48±14.33
Low differentiation	18	8	44.44	1.50±1.76	70.22±12.36
AFP (μg/L)					
Negative	5	1	20	0.80±1.79	74.20±15.51
30-1000	15	9	60	2.07±1.79	62.89±17.24
>1000	27	16	56.26	2.00±1.75	67.91±10.84
Hepatic cirrhosis					
No-light	21	11	53.28	1.81±1.83	66.10±13.52
Moderat-severe	26	15	57.69	1.96±1.75	68.27±13.98
Metastasis					
No	31	15	48.39	1.58±1.69	64.10±12.68
Yes	16	11	68.75	2.50±1.83	73.50±13.77*
General figure					
Massive type	39	21	53.85	1.85±1.79	66.38±13.14
Diffuse type and nodular type	8	4	62.50	2.13±1.81	71.15±16.23
Maxium diameter					
< 5cm	6	3	50	1.83±2.04	71.33±13.88
≥ 5cm	41	23	56.10	1.90±1.76	66.71±14.50

Note: compare to cases had no metastasis. * $P < 0.05$

2.3 The relationship of the expression of RANTES in PHC tumor tissues with MVD: the associativity analysis indicated that the expressive scores of RANTES in PHC tissues had strong positive correlation with MVD ($r=0.386$, $P < 0.05$)

3 Discussion

Chemokine is a kind of cytokine which has chemotaxis to many cells, its mainly affects the migration of cells in

inflammation and anaphylactic reaction places. RANTES is a typical kind of mediators of inflammation created by CD₈T cells, platelets, endothelial cells and fibroblasts. Its main function is to cause the migration of leucocytes, mediate the inflammatory reaction and has strong chemotaxis to monocytes, NK cells, T cells, eosinophil and basophil. In spite of important role in inflammatory and anaphylactic reaction, a lot of reports indicated many chemokine could play important role in growth, infiltration and metastasis of tumor which secreted by tumor cells

or inflammatory cells^[2]. As a kind of chemokine, the expression of RANTES is significantly strengthened in many malignant tumor tissues. The study found that RANTES could promote the development of tumors through paracrine secretion. Otherwise, RANTES could significantly enhance the expression of matrix metalloproteinases (MMP)-9 to strengthen the invasive ability and improve the tumor angiogenesis^[7]. Some study found that RANTES could inhibit the immune response of T cells and promote the growth of tumor^[8]. Our study found that the positive expression rate and scores of RANTES in PHC tissues was higher than in adjacent tissues ($P<0.01$), which explained that RANTES possible had close relationship with the development of PHC. Our research also indicated that inhibitor of RANTES or low expression of RANTES in PHC tissues maybe has latent clinic significance in bioremediation of PHC. Our research had no discovery that the expression of RANTES in PHC tissues had relationship with main clinic pathological characteristic. It indicated that the extensional mechanism of action of RANTES in PHC need deeply research.

Tumor angiogenesis has important role in the occurrence, development and metastasis. Our study found that the MVD was significantly higher in PHC tumor tissues than in adjacent tissues of 47 cases, and there was significant difference between them ($P<0.01$). The tumor angiogenesis is a stepped chain reaction including many regulation of mediator. The mechanism of regulation is complex. Chemokine had been confirmed to be main regulation factor in angiogenesis in pathologic processes, such as the growth and traumatic cicatrization^[9]. Azenshtein^[8] found that RANTES had strong chemotaxis to monocytes and spurred a lot of MMPs, which induced the degradation of basilar membrane and extracellular matrix (ECM), promote the tumor angiogenesis and development of tumor. Adler etc.^[7] also found RANTES could promote the tumor angiogenesis through enhancing the expression of MMP₉. Our research found the expression of RANTES in the PHC tissues had close positive correlation with MVD ($P<0.05$).

It explained that RANTES could promote the occur of hepatic cancer microvascular, and had promoting role in the development of tumor. It could have relationship to the chemotaxis to monocytes by RANTES induced many MMPs releasing and degradation of ECM. Nevertheless, the extensional mechanisms need further research.

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