Expression and Significance of CXCR4 and VEGF in Nephroblastoma*

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ABSTRACT Objective: To detect the expression and significance of CXCR4, VEGF and their correlations in Nephroblastoma, so as to investigate their effect on metastasis and development of the tumor. Methods: SABC immunohistochemistry was used to detect the expression of CXCR4 and VEGF, in 30 samples of Nephroblastoma tissue selected from paraffin wax-embodied specimens with complete clinicopathological data, and other 10 cases of normal kidney tissue. Results: The expression of CXCR4 in normal kidney tissue was lower (30.0%) than that in Nephroblastoma (66.67%), and the difference was significant (X^2=4.126, P<0.05). The expression of VEGF in Nephroblastoma was higher (73.33%) than that in normal kidney tissue (20.0%) and the difference was also significant(X^2=8.889, P<0.05). The expression of CXCR4 was significantly positively correlated with the expression of VEGF in Nephroblastoma (r =0.392, P < 0.05). Conclusions: CXCR4 and VEGF had highly expression in Nephroblastoma. CXCR4 and VEGF may be served as the markers in the estimation of metastasis and dissemination of Nephroblastoma.

Key words: Nephroblastoma; CXCR4; VEGF; Immunohistochemistry

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Introduction

Nephroblastoma or Wilms′ tumor is the commonest malignant tumor of children occurring between 2 and 5 years of age, arising from embryonic kidney tissue. Wilms' tumour is histologically characterised by a triphasic pattern of blastemal, epithelial and stromal components, which have different proliferating potential. Although, with effective treatment, patients with Wilms' tumour have nearly 90% long-term survival for localised disease and >70% for metastatic disease, there is still controversy regarding the best therapeutic strategy and potential molecular targets for antitumour drugs, which can further improve prognosis, reduce treatment-related toxicity and prevent late complications.It is one of the common malignant tumor in children with high malignancy and bad prognosis. The course of its carcinogesis is not clear. The chemokine receptor CXCR4 and its ligand stromal-derived factor-1 (CXCL12) initially have been shown to play a critical role inchemotaxis and homing, especially organ-specific metastasis between the primary and the metastatic site .This CXCR4/ CXCL12 pathway has been shown to be correlated with tumor progression and poor prognosis in several kinds of cancer, but the relationship with Nephroblastoma has been unknown. Immunohistochemistry method was used to detect the expression of CXCR4 and VEGF so as to investigate the relationship between them and Nephroblastoma.

1 Materials and methods

1.1 Tissue sample

Thirty tissue samples of Nephroblastoma were collected from the patients who were in hospital from May 2001 to Dec 2011 including 20 male and 10 female with the average age of 4.8. Ten cases of normal kidney tissue including 8 male and 2 female with the average age of 6.3 years were selected as the control group to Nephroblastoma. All the specimen were fixed by formalin and the paraffin-embedded sections were made with the slip of 4 um. All cases were verified by three experienced pathologists.

1.2 Method

Rabbit-anti-human polyclonal antibody VEGF were purchased from WuHan Boster Bio-engineering Limited Company. Rabbit-anti-human polyclonal antibody CXCR4 was the products of WuHan Boster Bio-engineering Limited Company respectively. SABC immunohistochemistry was used to measure the expression of CXCR4 and VEGF.

1.3 Statistic analysis

The CXCR4 and VEGF positive signals were observed to originate from a brown granular substance, located mainly in the cytoplasm. The cells were detected by a high-power microscope, and 5 to 10 visual fields containing not less than 200 cells in each field were randomly selected. The results were determined on the basis of the percentage of positive cells and the density of staining as follows: (1) Cells in sections were scored according to the density of staining: score 0 = no color; score 1 = light yellow; score 2 = yellow-brown; score 3 = brown (2) according to the percentage of positive cells among the same cells, score 1 for positive cells at ≤ 30%; score 2, 30%-70%; and score 3, >70%. The product obtained by multiplying the score of (1) and (2) was the total score,

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where a total score of 0-1 was considered as a negative score (-); 2-3, a weak positive score (+), and ≥ 4, a positive score (++). The (+) and (+ +) values were combined to form the positive group in order to facilitate the analysis.

The Statistical Package for the Social Sciences (SPSS) 15.0 software was used in the statistical process. The exact fourfold table X^2 test was performed in order to establish the difference among the indicators. The results of the inter-group correlation analysis were confirmed by the Spearman correlation analysis used for intergroup comparison. $P < 0.05$ was considered to represent significant differences.

2 Results

The expression of CXCR4 and VEGF were both located in the cytoplasm. Table 1 indicate that the expression of CXCR4 in normal kidney tissue was lower (30.0%) than that in Nephroblastoma (66.67%), and the difference was significant (X^2=4.126, P<0.05). Table 2 indicate that the expression of VEGF in Nephroblastoma was higher (73.33%) than that in normal kidney tissue (20.0%) and the difference was also significant (X^2=8.889, P<0.05). Table 3 indicate that the expression of CXCR4 was significantly positively correlated with the expression of VEGF in Nephroblastoma (r =0.392, $P < 0.05$).

Table 1 Expression of CXCR4 in Nephroblastoma

Group	CXCR4			
	n	+	-	%
Nephroblastoma	30	20	10	66.67
Normal kidney	10	3	7	30.00

Note:P<0.05, X^2=4.126.

Table 2 Expression of VEGF in Nephroblastoma

Group		VEGF		
		+	-	%
Nephroblastoma	30	22	8	73.33
Normal kidney	10	2	8	20.00

Note: P<0.05, X^2=8.889.

Table 3 Relationship between VEGFand CXCR4 expression in Nephroblastoma

CXCR4	n	VEGF	
		-	+
-	8	0	8
+	22	4	18

Note r =0.392, $P < 0.05$.

3 Discussion

Chemokine is a superfamily of small cytokines with the ability to chemoattract cells to the target tissues. CXC chemokine receptor 4 (CXCR4)-CXCL12 interaction plays an important role in the regulation of hematopoiesis, migration of hematopoietic cells and angiogenesis [1]. Accumulating evidence indicated that chemokines and their receptors regulated homing and proliferation of cancer cells at specific secondary sites. It has been shown that human malignant breast tumors and metastases expression increased chemokine receptor CXCR4. By comparison, the CXCR4 ligand CXCL12 is abundantly expressed in the local cancer site and draining lymph node [2]. Ex vivo studies further demonstrated that the metastasis of breast cancer cells with CXCR4 gene deficiency was impaired [3]. Moreover, it was shown that CXCR4 had highly expression in a number of human tumors such as oral squamous cell carcinoma, colorectal cancer and breast cancer and was an important prognostic factor in patients [4-6].

Angiogenesis has been known to play an important role in the development of tumor growth and metastasis[7]. Vascular endothelial growth factor (VEGF) potently increases vascular permeability and promotes the formation of new blood vessels in tumor and it is regarded as the main growth stimulatory factor in the tumor-related angiogenesis [8]. The prognostic value of high expression of VEGF has been demonstrated in various types of human tumors [9,10]. Most recent studies demonstrated that stimulation of CXCR4 enhanced the secretion VEGF, which led to accelerating angiogenesis in tumors and the subsequent tumor metastasis[11-13]. These observations suggested that the expression level of CXCR4 and VEGF might be an appropriate biomarker for predicting the metastatic ability of tumor cells.

Recently, there is growing interest in CXCR4 enhancing tumor growth and angiogenesis through various signaling pathways. CXCL12 has previously been found to be able to induce secretion of VEGF in human arterial endothelial cells [14]. New evidences demonstrated that stimulated CXCR4 expression by CXCL12 or overexpression of CXCR4 by transduction in human tumor cell lines increased aberrant production of VEGF and neovascularization [11,13]. Furthermore, these functional effects of CXCR4 induced by CXCL12 could be prevented by the in vitro administration of AMD3100, a bicyclam noncompetitive antagonist of CXCR4[15]. It is likely that the interaction of CXCR4 and CXCL12 may promote tumor progression and metastasis via the induction of VEGF-mediated angiogenesis. However, no data are currently available on the biological relationship between these two markers in Nephroblastoma. This study showed there was significant relationship between the high expression of CXCR4 and VEGF in human Nephroblastoma samples. Therefore, altered expression of CXCR4 and VEGF may play an important role in Nephroblastoma growth and metastasis.

4 Conclusion

In this study, the expression of CXCR4 in normal kidney tis-

sue was low while the expression rate of CXCR4 in Nephroblastoma was high, and the difference between them was significant. The expression rate of VEGF in Nephroblastoma was high, while the expression rate of VEGF in normal kidney tissue was low, and the difference between them was also significant. The expression of CXCR4 was significantly positively correlated with the expression of VEGF in Nephroblastoma. In summary, CXCR4, and VEGF had highly expression in Nephroblastoma samples as demonstrated using immunohistochemistry. CXCR4 and VEGF may be served as the markers in the estimation of metastasis and dissemination of Nephroblastoma. If further studies confirm the utility of CXCR4 as a novel marker of biological behavior in this disease, it could represent a new therapeutic target.

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CXCR4 及 VEGF 在肾母细胞瘤中的表达及其意义 *

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摘要 目的:检测趋化因子受体 4 (CXCR4) 及血管内皮生长因子 (VEGF) 在肾母细胞瘤中的表达,探讨其在肾母细胞瘤的发生、发展、浸润和转移中的作用。**方法**:选择临床及病理资料齐全的存档肾母细胞瘤标本 30 例,另取正常肾脏标本 10 例作对照。采用免疫组化 (SABC) 方法检测 CXCR4 及 VEGF 的表达。**结果**:CXCR4 在肾母细胞瘤组织中高表达(66.67%),在正常肾脏组织中低表达 (30.0%),两者相比有统计学意义 (P<0.05), VEGF 在肾母细胞瘤组织中高表达(73.33%),在正常肾脏组织中低表达 (20.0%),两者相比同样有统计学意义(P<0.05),肾母细胞瘤组织中 CXCR4 与 VEGF 的表达两者之间呈正相关性 (r = 0.392,P<0.05)。**结论**:肾母细胞瘤组织中 CXCR4 和 VEGF 均高表达。CXCR4 及 VEGF 可能成为预测肾母细胞瘤浸润转移的重要指标。

关键词:肾母细胞瘤;趋化因子受体 4;血管内皮生长因子;免疫组织化学

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