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Original article

Chemokine C-C motif ligand 18 expression correlates with tumor malignancy in breast cancer

Chimiokine C-C de ligand motif 18 d'expression en corrélation avec la malignité de la tumeur dans le cancer du sein

J. Gao [a,1], Z.-h. Li [b,1], W. Tang [a], Q.-n. Wu [c], G.-h. Liu [c], W.-b. Zheng [a,*]

[a] *Department of Breast Surgery, the First Affiliated Hospital of Guangzhou Medical University, No.151, Yangjiangxi Road, Yuexiu District, Guangzhou 510120, China*
[b] *Department of Oncology, the First Affiliated Hospital of Nanchang University, Prevention and Cure Center of Breast Disease, the Third Hospital of Nanchang City, JiangXi Breast Specialist Hospital, Nanchang 330009, China*
[c] *Department of Pathology, the First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, China*

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ABSTRACT

Purpose of the study. – To investigate whether CCL18 is involved in breast cancer, and the relationship between CCL18 and MVD (MVD was recognized by CD34) which is a well-accepted angiogenic maker of multiple cancers including breast cancer.
Patients and methods. – Immunohistochemistry staining for CCL18 and CD34 was performed on 179 cases, including 29 normal cases as control, 47 cases with benign breast diseases, and 103 cases with breast cancer.
Results. – We found that CCL18 was significantly up-regulated in breast cancer samples as compared with benign tumors or normal breast tissues. Moreover, the expression level of CCL18 increased with the size of tumors, the number of lymph node metastasis, and advancing tumor stage, suggesting that CCL18 expression correlates with tumor malignancy scales. At the same time, we found that MVD was also significantly over-expressed in cancer tissues as compared with normal control group and benign tumor group, but it was not significantly differentially expressed among tumors with different malignancy scale like CCL18, while the expression of MVD in CCL18 positive breast cancer cases was higher than in the CCL18 negative breast cancer cases ($P = 0.016$, $P < 0.05$).
Conclusion. – CCL18 is involved in the development of breast cancer. CCL18 is a better biomarker than MVD in determining whether the tumor is malignant and the severity of malignancy of breast cancer.

Mots clés :
Cancer du sein
CCL18
MVD
Malignité
Tumeur

RÉSUMÉ

Objet de l'étude. – Étudier l'implication de CCL18 et MVD, facteur pro-angiogénique identifié dans plusieurs cancers, dans le développement du cancer du sein.
Patients et méthodes. – Évaluation de l'expression de CCL18 et CD34 par immunohistochimie sur 179 biopsies, dont 29 cas normaux, 47 tumeurs bénignes du sein, et 103 un cancer du sein.
Résultats. – L'expression de CCL18 est significativement augmentée dans les échantillons de cancer du sein par rapport à ceux issus de tumeurs bénignes ou de tissus mammaires normaux. De plus, le niveau d'expression de CCL18 dépend de la taille des tumeurs, du nombre de métastases ganglionnaires et du stade de la tumeur, ce qui suggère que l'expression de CCL18 est corrélée avec la malignité de la tumeur. Dans le même temps, nous avons montré que MVD est significativement surexprimé dans les tissus cancéreux par rapport au groupe témoin et bénin. Cependant, il n'y a pas de corrélation avec la malignité de la tumeur.
Conclusion. – CCL18 est impliqué dans le développement du cancer du sein. CCL18 est un meilleur biomarqueur que MVD pour déterminer si la tumeur est maligne et la gravité de la malignité du cancer du sein.

* Corresponding author.
E-mail address: wenbo_zheng@yeah.net (W.-b. Zheng).
[1] These authors are the co-first authors.

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1. Introduction

Breast cancer is the most frequently diagnosed type of cancer among women worldwide [1]. Despite improvements in screening and treatment, breast cancer remains one of the leading causes of the death in women, mainly because of the invasiveness and metastasis of the tumors. The molecular mechanism of breast cancer progression is very complicated, involving the coordinated efforts of numerous processes within the cancer cell as well as its surroundings. Accumulating evidences showed that tumor microenvironment may release multiple chemotactic factors, such as cytokines, which played a pivotal role in tumor progression and metastasis [2–4].

Chemokine (C-C motif) ligand 18 (CCL18) is a chemokine predominantly produced by the tumor-associated macrophages (TAMs). It was reported to be involved in the immunosuppression of a host antitumor response by attracting tumor-initiating lymphocytes toward TAMs. Such effect is of considerable importance in the context of malignant tumors, since a concomitant reduction in immune function is associated with tumor growth and progression. Currently, enhanced expression of CCL18 production has been demonstrated in various malignancies, including ovarian cancer, gastric cancer, glioma and breast cancer [5–7].

In this study, we analyzed the expression pattern of CCL18 in large cohorts of breast cancer samples. We compared the differential expression of CCL18 between tumors and normal breast tissues and compared the expression of CCL18 in breast cancers with different malignancy scale. Moreover, we compared the differentiating power of CCL18 with another well-accepted malignancy MVD (recognized by CD34).

2. Materials and methods

2.1. Patient sample collection

The samples were collected from 103 patients with breast cancer and 47 with benign cases. Adjacent normal tissues were also collected from 29 benign lesions to serve as controls. All of the cases were women who had not received any treatment before surgery. All samples were collected between January 2011 and June 2012 at the First Affiliated Hospital of Guangzhou Medical University. Two pathologists independently examined all archival specimens. Tumors were staged according to the TNM staging system.

2.2. Immunohistochemistry (IHC)

The immunohistochemistry (IHC) staining was performed according to the reported method [7,8]. Briefly, tissues were fixed in 10% neutral buffered formalin and embedded in paraffin. Then, the specimens were cut into 4 μm sections and mounted on slides. The slides were baked for 1 h at 67 °C, deparaffinized in xylene and rehydrated through a series of graded alcohols. Antigen retrieval was achieved through incubation of the slides in sodium citrate (pH 6.0) buffer, which was microwave heated (5 min, twice), and allowed to naturally cool between heating. The slides were then washed with PBS 3 times and incubated for 2 h with primary antibody against either CCL18 (1/200, Abcam, Cambridge, UK) or CD34 (1/100, Zsgb-bio, Beijing, China) at 37 °C. The EnVision™ Detection kit (DAKO, Copenhagen, Denmark) was then used according to the manufacturer's protocol. All slides were counterstained with hematoxylin.

2.3. IHC evaluation

The number of CCL18 positive cells was counted as described by Chen et al. [7]. Slides were identified as positive when brown immunostained cytoplasm was observed. MVD positive staining was determined as previously described by Weidner et al. [9]. All observations were performed using a Nikon microscope (Nikon, Tokyo, Japan). For quantification, five areas depicting the invasive component of the tumor with the highest number of microvessels — the tumor "hot spot" — were identified at low magnification (×40). Then, in each area, the number of individually stained vessels was counted at ×400 magnification (0.307 mm^2) and MVD was calculated per HP (high power, ×400 magnification) field-of-view. A single microvessel was defined as any brown immunostained endothelial cell that was separated from adjacent microvessel tumor cell and other connective tissue elements. MVD was expressed as the mean number of microvessels per HP field-of-view. The average from five "hot spot" counts was calculated. All specimens were examined by two blinded pathologists independently. In cases of disagreement (score discrepancy), the slides were reexamined and a consensus was reached by the observers.

2.4. Statistical analysis

All statistical analyses were performed using SPSS 18.0 statistical software package (SPSS Inc., Chicago, IL, USA). The χ^2 test was used to analyze the expression of CCL18, and the relationship between CCL18 and CD34, while the expression of CD34 was analyzed by t test. In all cases, $P < 0.05$ was considered statistically significant.

3. Results

3.1. Patients' characteristics

The average age of the patients was 44.59 ± 7.59 years old in control group, 43.91 ± 6.9 years old in benign breast disease group, and 47.21 ± 9.92 years old in breast cancer group, respectively. There were no significant differences among the three groups ($P > 0.05$; Fig. 1).

The benign breast disease group included 22 cases with non-proliferative lesions, 20 cases with proliferative lesions and without atypia, and 5 cases with proliferative lesions and with atypia (Fig. 2).

The breast cancer group included 5 cases with carcinoma in situ, 74 cases with infitrating ductal carcinoma, 12 cases with infiltrating lobular carcinoma, 5 cases with mixed carcinoma, 4 cases with tubular and mucinous, and 3 cases with other types breast carcinoma. The construction of all the types of breast carcinoma is showed in Fig. 3.

3.2. The expression of CCL18

In all of the 47 cases of benign breast disease group, we did not detect the expression of CCL18 in the benign lesions and the tissues nearby. Similarly, no CCL18 was observed in the 29 control cases. While CCL18 was expressed in 84 cases of breast cancer. We found CCL18 was scatterly distributed at the tumor margin scattered in the edges of the tumors, but not distributed in the center (Fig. 4). The positive rate of CCL18 in the breast cancer group was 81.55% (84/103). The expression of CCL18 was significantly higher in

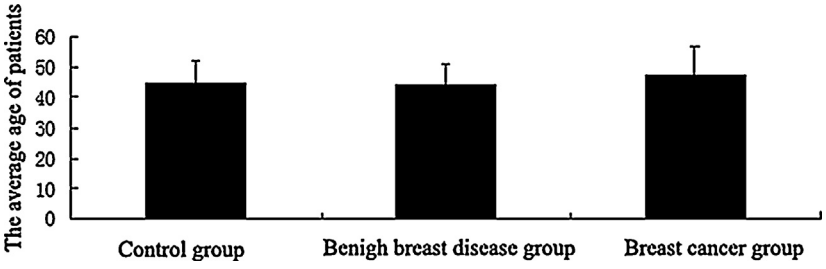

Fig. 1. The average age of the patients in different groups.