European Journal of Obstetrics & Gynecology and Reproductive Biology 214 (2017) 178–183

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

Full length article

Association of CXCR4, CCR7, VEGF-C and VEGF-D expression with lymph node metastasis in patients with cervical cancer

Yifei Dai, Rui Tong, Hui Guo, Tingting Yu, Chunyan Wang*

Department of Gynaecologic Oncology, Cancer Hospital of China Medical University & Liaoning Cancer Hospital and Institute, China

ARTICLE INFO

Article history:
Received 14 March 2017
Received in revised form 17 April 2017
Accepted 26 April 2017
Available online xxx

Keywords:
Cervical cancer
Lymph node metastasis
CXCR4
CCR7
VEGF-C
VEGF-D

ABSTRACT

Objective: We attempted to investigate the expression of CXCR4, CCR7, VEGF-C and VEGF-D in cervical cancer specimens, and the association between CXCR4, CCR7, VEGF-C and VEGF-D expression with the clinicopathological parameters of patients with cervical cancer.

Study design: 57 tissue microarrays including 9 normal cervical tissues and 48 cervical cancer tissues were purchased from Biomax. The association between CXCR4, CCR7, VEGF-C and VEGF-D expression with the clinicopathological parameters were evaluated. Then immunohistochemistry was used to assess the expression of CXCR4, CCR7, VEGF-C and VEGF-D in cervical cancer specimens. Finally, Spearman correlations were used for the correlation analyses between CXCR4, CCR7, VEGF-C and VEGF-D.

Results: We revealed that CXCR4 expression was significantly higher in patients with squamous cell carcinomas ($P=0.002$) and lymph node metastasis ($P=0.038$), while CCR7 expression was significantly elevated in patients with lymph node metastasis ($P=0.037$). VEGF-C expression was markedly up-regulated in patients exhibiting FIGO stage II–III tumors ($P=0.015$) and lymph node metastasis ($P=0.038$), while VEGF-D expression was obviously increased in patients displaying FIGO stage II–III tumors ($P=0.025$), squamous carcinomas ($P=0.017$) and lymph node metastasis ($P=0.037$). The correlation analysis indicated that CXCR4, CCR7, VEGF-C and VEGF-D expression have a significant correlation to each other.

Conclusion: These results suggested that CXCR4, CCR7, VEGF-C and VEGF-D expression might have synergistic effects on the lymph node metastasis in patients with cervical cancer.

Introduction

As a malignant disease, cervical cancer is estimated to be one of the leading causes of female cancer-related death [1]. Tumor cell invasion and metastasis through the bloodstream and lymph vessels are critical steps in the progression of cervical cancer. Lymph node state is proved as a pivotal prognostic factor in early-stage cervical cancer [2,3]. However, the mechanisms of lymph node metastasis are not fully elucidated.

Chemokines are well known for their ability to stimulate leucocytes during inflammation, and play important roles in homeostasis, cell proliferation, hematopoiesis, angiogenesis, tumor progression and cancer metastasis [4–6]. The chemokine receptor CXCR4 is thought to regulate the metastasis of solid tumors with the evidence that high expression levels of CXCR4 and positive staining for its ligand CXCL12 might correlate with the presence of metastatic disease in patients with prostate cancer and lung cancers [7,8]. CCR7, the receptor for the chemokine CCL21 and CCL19 is considered to mediate lymphocyte cell trafficking and dissemination to the lymph nodes. Vascular endothelial growth factors (VEGFs) are important angiogenic factors that stimulate the formation of new blood vessels and tumor growth. In a previous study, VEGF-C has been demonstrated to be involved in the promotion of lymph node metastasis in cervical cancer [9]. VEGF-D is proved to promote tumor metastasis by regulating prostaglandins derived from the collecting lymphatic endothelium [10].

Published in vitro studies also showed all these 4 protein factors could cross-regulate each other. Silencing of VEGF-C mediated by RNAi inhibited non-small cell lung cancer progression via down-regulating the expression of CXCR4, CCR7, VEGFR-2 and VEGFR-3, which were involved in ERK, p38 and AKT signaling pathways [11]. Zhuo [12] then found that CXCR4 expression was specifically upregulated by VEGF-C on lymphangiogenic endothelial cells in vitro. Additionally, CCL21/CCR7 was proved to up-regulate VEGF-D

* Corresponding author at: Department of Gynaecologic Oncology, Cancer Hospital of China Medical University & Liaoning Cancer Hospital and Institute, No.44, Xiaoheyan Road, Dadong District, Shenyang 110042, China.
E-mail address: wangchunyancancer@163.com (C. Wang).

http://dx.doi.org/10.1016/j.ejogrb.2017.04.043
0301-2115/

and promote lymphangiogenesis by mediate ERK/Akt pathway in lung cancer [13]. Taking into account the features above, we knew that they were tied up in a single signaling mechanism. Therefore, we selected CXCR4, CCR7, VEGF-C and VEGF-D for further investigation in cervical cancer specimens. In the present study, we aimed to investigate the association between CXCR4, CCR7, VEGF-C and VEGF-D expression and the clinicopathological parameters of cervical cancer was determined. Finally, the correlation between CXCR4, CCR7, VEGF-C and VEGF-D in cervical cancers was assessed.

Methods

Patients and tissue samples

57 tissue microarrays including 9 normal cervical tissues and 48 cervical cancer tissues were purchased from Biomax, Inc. (Rockville, MD, USA). The average age was 43.73 ± 1.50 years (ranging from 25 to 67 years). Tumor issues were obtained at the time of surgery and were immediately fixed by 10% formalin and embedded by paraffin. Clinical staging was assessed based on the International Federation of Gynecology and Obstetrics (FIGO) staging system as follows: 37 were classified as cervical cancer at stage I A and B, and 11 as cervical cancer at stage II–III. Histological cell typing was conducted according to the World Health Organization classifications as follows: 33 were allocated to squamous cell carcinomas, and 15 to adenocarcinomas. Histological differentiation was identified as follows: 8 were identified as well differentiations, 31 as moderate differentiations and 8 as poor differentiations, and 1 case was unable to analyze pathologic stage due to the limitation of sampling. There were 9 patients with lymph node metastasis and 39 patients with no lymph node metastasis. Informed consents were obtained from all patients before sample collections.

Immunohistochemistry

5 μm tissue sections were obtained from several representative areas of each tumor specimens and were mounted on to glass slides for immunostaining according to the UltraVision Quanto kit instructions (Thermo Fisher Scientific, Lafayette, Colorado, USA). Finally, sections were analyzed with a microscope (BH-2 OLYMPUS, Tokyo, Japan).

Staining evaluation

CXCR4 and CCR7 exhibited positive expression with yellow or brown particles in cytoplasm and cell nucleus; VEGF-C and VEGF-D expressions were positively with brown particles appeared in cytoplasm, and sometimes in cell nucleus. For evaluation of chemokine receptors and VEGFs expression, a score corresponding to the product of both (a) staining (0 = negative; 1 = canary; 2 = yellow; 3 = brown) and (b) percentage of positive cells (0 = < 5% positive cells; 1 = 5%–10% positive cells; 2 = 11%-50% positive cells; 3 = 51%-75% positive cells; 4 = > 75% positive cells) was established. The product of (a) * (b) was considered as comprehensive evaluation scores (0–1 = negative; 2–3 = weakly positive; 4–5 = positive; >6 = strongly positive). A score greater than 2 was the value of a positive immunohistochemical assay [14].

Statistical analysis

SPSS16.0 statistical software (SPSS Inc, Chicago, IL) was used for all statistical analysis. The association between the variables was analyzed using the chi-square test. Spearman correlations were used for the correlation analysis. All tests were two-tailed and the level of statistical significance was set at 0.05.

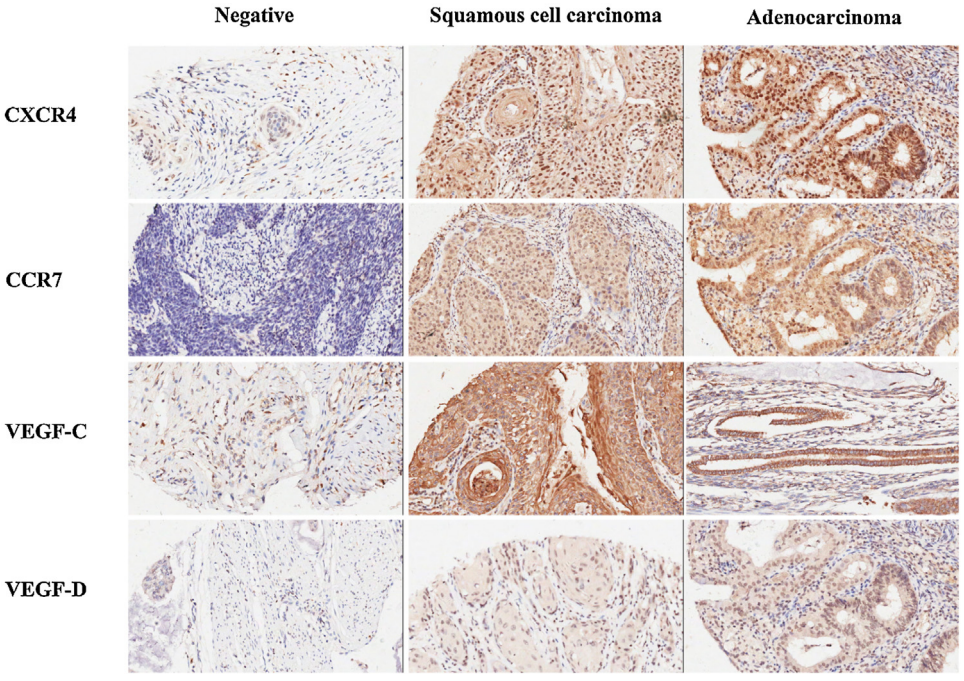

Fig. 1. Immunohistochemical staining for CXCR4, CCR7, VEGF-C and VEGF-D in cervical cancer tissues. Original magnification ×200.