

Original Article

Quantitative expression analysis of metastasis-related ELAM-1 in nasopharyngeal carcinoma

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Abstract: Objective: To investigate the expression of metastasis-related endothelial leukocyte adhesion molecules-1 (ELAM-1) in patients with nasopharyngeal carcinoma (NPC). Methods: Cancer tissue specimens were obtained nasopharyngeal biopsy of 46 patients with NPC. The quantitative expression of metastasis-related ELAM-1 in all patients was performed using immunohistochemical SP method. These patients were divided into metastasis group and non-metastasis group according to medical imaging examination. The quantitative expression of metastasis related ELAM-1 was analyzed in different groups and clinic-pathologic features. Results: 93.1% (27/29) positive expression of ELAM-1 in 29 metastatic patients was higher than that of 17 non-metastasis (23.5%, 4/17), there was significant difference in the positive expression of ELAM-1 between two groups ($\chi^2=23.607$, $P=0.000$), and there was a positive correlation between ELAM-1 expression and metastasis ($r=0.571$, $P=0.000$), but there was no relationship between ELAM-1 expression and clinical staging, T staging, age and gender ($P>0.05$). Conclusion: Over-expression of ELAM-1 may contribute to metastatic spread, and will serve as a potential prognostic biomarker in patients with NPC.

Keywords: Nasopharyngeal carcinoma, ELAM-1, metastasis

Introduction

Nasopharyngeal carcinoma (NPC) is one of the most common head and neck malignant cancers in south China [1]. The literature have been reported that the 5-year survival rates was about 34 to 52% [2]. Patients with early-stage (I-IIa) NPC are minority, but patients with locally advanced stage (III-IVa) NPC are majority. With the development of medical equipment and technologies, the local control rate of nasopharyngeal carcinoma has made a big progress, especially, concomitant chemoradiotherapy in patients with local advanced NPC made a big progress in overall survival and disease free survival for all histological types and achieved 5-year overall survival rates of about 70% [1, 3]. But distant metastasis is still the main reason for the failed treatment of nasopharyngeal carcinoma, so metastasis is considered as an adverse prognostic factor in patients with NPC, which is essential for the clinical staging and playing a role of determining the

best therapy project. Now, predicting metastasis in patients with NPC remains challenging.

Recently, over expression of adhesion molecules in malignant tumors is an important indicator of distant metastases. Endothelial leukocyte adhesion molecules-1 (ELAM-1), also known as E-selectin, is a member of the selectin family [4, 5]. Normally, ELAM-1 is shed from vascular endothelial cells activated by inflammatory cells [6]. ELAM-1 participates in the initial adhesion of leukocytes, and has a character of type I transmembrane protein. ELAM-1 consists of 589 amino acids, including lectin area, epidermal growth factor area, 4 to 9 short repetitive sequences, a single transmembrane domain and a cytoplasmic, 115 kD after its glycosylation. ELAM-1 gene contains 14 exons, the span is 1.3 kD. When endothelial cells are stimulated by IL1, TNF- α , LPS or the others, the second messenger Cer activates nuclear factor K B (NF- κ B). Under the regulation of NF- κ B, ELAM-1 gene is activated after a series of intra-

Metastasis related ELAM-1 in nasopharyngeal carcinoma

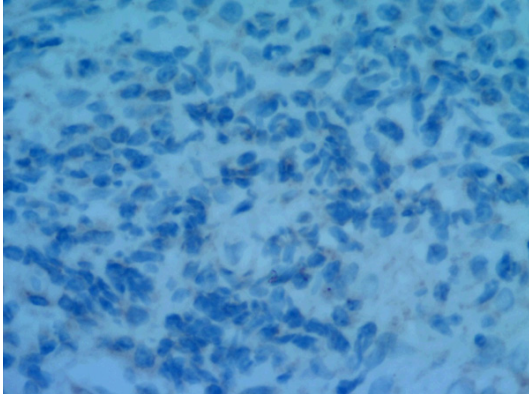


Figure 1. A 58-year male patient with non-metastatic nasopharyngeal carcinoma, ELMA-1 expression of immunohistochemical staining ($\times 400$) had showed negative immunoreactivity (-).

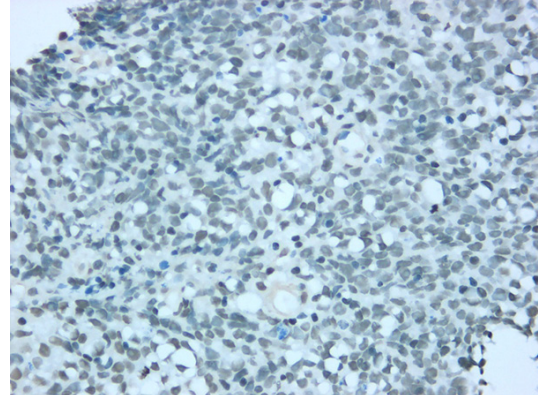


Figure 3. A 45-year male patient with metastatic nasopharyngeal carcinoma, ELMA-1 expression of immunohistochemical staining ($\times 400$) had showed moderate immunoreactivity (++)

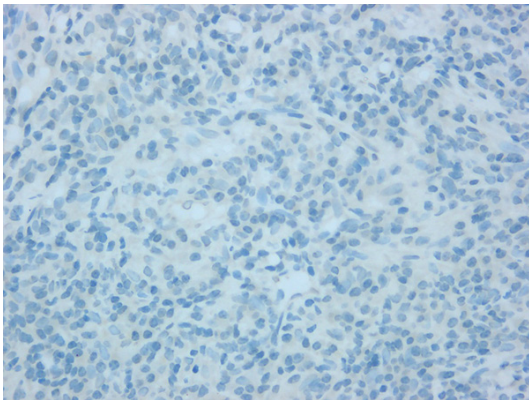


Figure 2. A 65-year female patient with non-metastatic nasopharyngeal carcinoma, ELMA-1 expression of immunohistochemical staining ($\times 400$) had showed weak immunoreactivity (+).

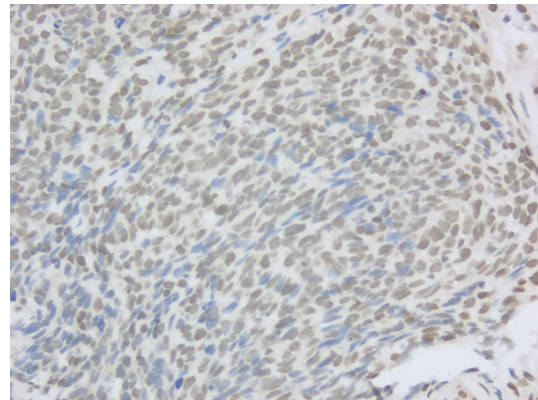


Figure 4. A 53-year male patient with metastatic nasopharyngeal carcinoma, ELMA-1 expression of immunohistochemical staining ($\times 400$) had showed strong immunoreactivity (+++).

cellular information transfer, and the surface of activated endothelial cells expresses ELAM-1 by transcription and translation. Studying the role of expression ELAM-1, patients with growing tumors can provide a greater understanding of NPC development.

Therefore, much is known about ELAM-1 expression within endothelial cells. however, the role of ELAM-1 in patients with NPC and how it affects on metastasis and clinic pathologic features are still unknown. In the paper, the aim was to analyze ELAM-1 expression in 46 patients with NPC by immunohistochemical SP method, and to evaluate its clinical significance and application as a novel tissue biomarker for NPC.

Materials and methods

Patients

46 patients with nasopharyngeal carcinoma were retrospectively analyzed from January 2012 to January 2013 in the Affiliated Tumor Hospital to Guangxi Medical University, including 32 men and 14 women, aged ranging from 31 to 70 years, mean 46 ± 4.3 years. All patients were pathologically proved as poorly differentiated squamous cell carcinoma after nasopharyngeal biopsy. The patients with NPC were regarded as metastasis including following criteria, a. clinical follow-up, b. confirmed by pathology, c. imaging examinations, d. radiological assessment of cervical lymph node

Metastasis related ELAM-1 in nasopharyngeal carcinoma

Table 1. ELAM-1 expression in 46 patients of nasopharyngeal carcinoma

Group	ELAM-1				χ^2	P
	-	+	++	+++		
Metastasis (n=29)	2	4	13	10	23.607	0.000
Non-metastasis (n=17)	13	1	2	1		

Table 2. Comparison between ELAM-1 and clinical features in 46 patients with nasopharyngeal carcinoma

Clinical features	N	ELAM-1				r_s	P
		-	+	++	+++		
Clinical TNM staging							
I	1	0	0	1	0	0.235	0.116
II	6	4	1	1	0		
III	11	4	1	2	4		
IV	28	7	3	11	7		
T staging							
T1	2	0	1	1	0	0.207	0.168
T2	11	5	2	2	2		
T3	11	4	0	3	4		
T4	22	6	2	9	5		
Age							
≤50	21	8	1	8	4	-0.092	0.541
>50	25	7	4	7	7		
Gender							
Male	32	11	3	10	8	-0.007	0.961
Female	14	4	2	5	3		

metastasis was based on UICC 2002. The imaging examination including ultrasound, CT and MRI could provide image evidence to metastasis of parenchymal organs (such as liver, lung, etc). And the radiological evaluation of bone metastasis was based on ECT, PECT bone scan or X-ray, CT or MRI.

Immunohistochemistry

8 μm paraffin sections were stained with HE or subjected to IHC with antibody against ELAM-1 (1:100 dilution) (Boste, Wuhan, China) and visualized with the LSAB-Kit (DakoCytomation, Carpinteria, CA, USA) following the manufacturer's instruction.

The IHC stained sections (400 fold) were evaluated by two experienced pathologists blinded to the patient outcomes. According to IHC-stained sections, five high-power fields were randomly selected to evaluate the expression of ELAM-1. Base on the staining percentage and intensity of positive cells counted in each

core, the final staining score were defined as: -, +, ++, and +++, which stands for a negative, weak or wild, moderate, and strong immunoreactivity, respectively.

Statistical analysis

Statistical analyses were performed statistical software SPSS 18.0. The chi square test was used to compare the positive rate of ELAM-1 expression between the metastasis group and non-metastasis group. Spearman's rank correlation was analyzed the relationship between the grade of ELAM-1 expression and clinic-pathological features, including age, gender, clinical stage, T stage. Pearson correlation coefficients $r_s > 0.7$ was considered as good correlation, $r_s = 0.4-0.7$ as moderate correlation, and $r_s < 0.4$ as poor correlation. $P < 0.05$ was considered statistical significant.

Results

ELAM-1 expression in nasopharyngeal carcinoma

In 46 patients of nasopharyngeal carcinoma, 67.4% (31/46) cases expressed ELAM-1, while the other 15 cases was negatively expressed ELAM-1. Brown-yellow staining of ELAM-1 was mainly located in the cytoplasm of tumor cells. In the 46 cases of nasopharyngeal carcinoma, 63% (29/46) cases occurred metastasis, 17 cases had no detection of metastasis. The expression of ELAM-1 was mainly positive in metastasis group and mainly negative in non-metastasis group (**Figures 1-4**). The positive rate (93.1%, 27/29) of metastasis group was higher than that of non-metastasis group (23.5%, **Table 1**). Difference between the two groups was statistically significant ($P < 0.05$). There was a positive correlation between metastasis and expression level of ELAM-1 ($r_s = 0.571$, $P = 0.000$).

Comparison between ELAM-1 expression and clinical features

ELAM-1 expression and clinical features of 46 patients with nasopharyngeal carcinoma were listed in **Table 2**. Pearson correlation coefficients between the grade of ELAM-1 expression and clinical TNM staging, and T staging

Metastasis related ELAM-1 in nasopharyngeal carcinoma

were 0.235 and 0.207, respectively, and Spearson correlation coefficients between the grade of ELAM-1 expression and age, gender was -0.092 and -0.007, respectively. There were no correlation between *ELAM-1 expression and clinical features* ($P>0.05$).

Discussion

NPC represents a noticeable threat to a large group of population in China, for the clinical symptoms of nasopharyngeal are frequently occult [7]. About 70% patients were diagnosed as mid or late stage when NPC were proved by pathology. Although, the local control and recurrence incidence of patients with NPC are lower in the former than in the latter, its consequences should not be disregarded or underestimated [8]. Although extensive research efforts have been devoted to treatment of NPC, Even with radiotherapy or treatment combined with radiotherapy and chemotherapy, its complexity makes it seem as a difficult puzzle. Distant metastasis is still one of the main reasons for treatment failure of the nasopharyngeal carcinoma, and mortality in patients with distant metastasis of nasopharyngeal carcinoma is up to 57% [9-11]. Thus, distant metastasis is one of the decisive factors in the long-term survival of nasopharyngeal carcinoma patients.

Although, the exact mechanisms of NPC metastasis remain unknown, there are three distinct steps in the course of cancer cell metastasis, including cancer cells detaching from *in situ*, penetrating through the tissue basilar membrane, invading of vasculature and migrating to the specific organs [12, 13]. Here, the adhesion between endothelial cells and cancer cells is crucial for NPC metastasis, which is mediated by intercellular adhesion molecules. These adhesion molecules may play a role in metastatic tumor cell adhesion, such as selectin family. Selectin family are adhesion molecules that mediate the initial bond of leukocytes to microvascular endothelium by lectin-type interactions [14, 15]. One of selectin family is ELAM-1 which is detected on the surface of endothelial cells upon activation by cytokines and binds to target cell surfaces by oligosaccharide recognition. Several studies [4, 5, 16-19] have shown that over expression of ELAM-1 on activated endothelial cells had risk factor of metastasis formation and subsequent colonization, and increased circulating ELAM-1 levels have been associated with metastasis of some can-

cers, such as breast cancer, colon carcinoma, and liver cancer, but some researchers have reported that a negative relationship between ELAM-1 expression in cancer cells and tissues and cancer progression or metastatic potential which has a better prognostic effect [20, 21]. The binding of cancer cells to endothelial cells by ELAM-1 is few reportedly related to NPC metastatic potential.

Results in our study about 46 patients with poorly differentiated nasopharyngeal carcinoma have showed that the ELAM-1 expression was detected in 31 (67.4%) cases. Over expression of ELAM-1 was mainly found in metastasis cases, which was up to 93.1% (27/29) of positive rate. Mainly negative in non-metastasis group was high, which was 76.5% (13/17) of negative rate. The positive percentage of ELAM-1 expression in the metastasis group was significant higher than that of non-metastasis group ($P<0.05$). And at the same time, there was a positive correlation between the grade of ELAM-1 expression and metastasis ($r_s=0.571$, $P=0.000$). As metastasis has a complex multi-step process with a series of continuous interaction between the cancer cells and host cells, in order to form metastases of NPC, tumor cells must success in finishing a sequence of key, inter-related steps initiated by extensive proliferation of the primary tumor cells and their invasion of the surrounding extracellular matrix [22, 23]. First, these malignant cells break away from the primary lesion, intravasate the circulation and form tumor microemboli, which eventually attach to the microvasculature of a target organ [24, 25]. Then, tumor cells attach to the endothelium, extravasate and ultimately colonize to form secondary metastatic lesions. So the abnormal expression of ELAM-1 plays a critical role in hematogenous metastasis of nasopharyngeal carcinoma.

The study shows that there was no relationship between altered expression of ELAM-1 and clinical staging, T staging, age and gender ($P>0.05$). The prognostic factors for nasopharyngeal carcinoma included some clinic pathologic features, such as clinical staging, T staging, age and gender. Most important of all, Perez *et al.* have reported that the prognostic indicators in nasopharyngeal carcinoma were age, stage of the primary tumor, presence of cervical lymphadenopathy and certain technical factors of irradiation [26]. Similarly, Sanguineti *et al.* have indicated that T-stage, N-stage and the radia-

Metastasis related ELAM-1 in nasopharyngeal carcinoma

tion dose to the primary site of NPC were most significant prognostic factors for local and regional control [27]. On the other hand, great majority of patients with nasopharyngeal carcinoma had undifferentiated tumors (WHO type 3) in Chinese population, no significant difference in survival and locoregional control were associated with histological tumor type or degree of differentiation. Furthermore, Cheng's study of 149 patients with nasopharyngeal carcinoma have reported that World Health Organization type II histology, T4 classification and parapharyngeal extension indicated poor prognostic factors for locoregional control, T4, N3 classifications, serum LDH level, parapharyngeal extension and infiltration of the virus predicted poor prognosis for distant metastasis [28, 29]. Therefore, it is difficult to analyze and judge the prognosis of nasopharyngeal carcinoma together with the ELAM-1 expression and clinical features. ELAM-1 may be a new biomarker which will contribute to NPC metastasis, and prediction of survival of patients with NPC.

In a summary, ELAM-1 expression constitutes an unfavorable and independent prognostic marker in NPC, and present an important predictor in advanced-stage NPC, for which modulates metastasis of NPC cells. Not surprisingly, pre-treatment ELAM-1 expression predicts an increased risk of treatment failure in patients with NPC, may be a biomarker of distant metastasis after concurrent chemoradiation. Although, our results suggest that ELAM-1 expression might serve as a potential prognostic biomarker in patients with NPC, which principally constitute the great majority of NPC cases in China. Undoubtedly, further studies have indicated ELAM-1 in large numbers to the tumor site contributed to metastasis, which were needed to confirm the present findings and to examine in depth the prognostic value of ELAM-1 expression in NPC.

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Disclosure of conflict of interest

None.

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Metastasis related ELAM-1 in nasopharyngeal carcinoma

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