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Prognostic value of matrix metalloproteinase 9 expression in patients with juvenile nasopharyngeal angiofibroma: Tissue microarray analysis

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A B S T R A C T

Objective: Although JNA is a benign neoplasm histopathologically, it has a propensity for locally destructive growth and remains a higher postoperative recurrence rate. The aim of this study was to analyze the expression and localization of MMP-9 in JNA using tissue microarray to elucidate its correlation with clinicopathological features and recurrence.

Materials and methods: The expression of MMP-9 was assessed by immunohistochemistry in a tissue microarray from 70 patients with JNA and 10 control subjects. Correlation between the levels of MMP-9 expression and clinicopathologic variables, as well as tumor recurrence, were analyzed.

Results: MMP-9 was detected in perivascular and extravascular less differentiated cells and stromal cells of patients with JNA but not in the matured vascular endothelial cells of these patients. The presence of MMP-9 expression in JNA was correlated with patient's age ($p = 0.001$). Spearman correlation analysis suggested that high expression of MMP-9 in JNA had negative correlation with patient's age ($r = -0.412$, $p < 0.001$). The recurrence rate in JNA patients with high MMP-9 expression was significantly higher than those with low MMP-9 expression ($p = 0.002$). In multivariate and ROC curve analysis, MMP-9 was a good prognostic factor for tumor recurrence of JNA.

Conclusion: Higher MMP-9 expression is a poor prognostic factor for patients with JNA who have been surgically treated.

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1. Introduction

Juvenile nasopharyngeal angiofibroma (JNA) is a rare, nonencapsulated, highly vascularized benign tumor of the nasopharynx. It accounts for less than 0.05% of all head and neck tumors, and primarily affects adolescent males [1–3]. Although histologically benign in appearance, JNA has a propensity for locally destructive growth with bone erosion. JNA is considered to originate from the superior margin of the sphenopalatine foramen or pterygoid canal [4]. Skull base invasion is present in 20% and intracranial involvement in 10–20% of all patients with JNA and are more frequent in younger adolescents [5–8].

Surgery is the mainstay of the treatment, but the best approach to JNA has been a dilemma to the otorhinolaryngologist. The recurrence rate after surgical excision of JNA is high and fairly heterogeneous, varying from 20 to over 50% [5,7,9–13]. The factors of recurrence of JNA include age at diagnosis, extension to neighboring structures, tumor size, previous treatment approaches, and Radkowski classification [7,10].

Although its exact nature and pathogenesis is remaining unknown, JNA is thought to have a vascular origin [1,7]. Angiogenesis is an essential component of solid tumor growth and metastasis [14]. Recent studies showed that MMPs had a vital role in tumor invasion and angiogenesis. Among all of the MMPs, MMP-9 is considered to play a critical role in tumor aggressiveness and metastasis. Increased MMP-9 levels were associated with poor prognosis in nasopharyngeal carcinoma, non-small cell lung cancer, breast cancer and gastrointestinal cancer [15–18]. So, the aim of this study was to analyze the expression and localization of MMP-9 in JNA using tissue microarray to elucidate its correlation with clinicopathological features and disease outcome.

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2. Materials and methods

2.1. Patients and tissue samples

This study protocol was approved by the institutional review board of Fudan University. The study included 70 cases with JNA. All clinical data were obtained from patients' medical records. Patients with histologically proven JNA underwent surgery between 2003 and 2007 at the Eye, Ear, Nose and Throat Hospital of Fudan University. All patients with JNA were male and ranged in age from 9 to 41 years old (median age, 17 years) at the time of surgery. The follow-up period ranged from 69 to 127 months, with a mean of 95 months. The clinicopathological characteristics of all patients are shown in Table 1. Preoperative evaluation of paranasal computed tomography (CT) and/or MRI was used to determine the extent of tumor growth. Tumor size was determined by measuring the major diameter of the lesion observed on a CT scan or MRI. Tumor staging was performed according to the classification of Radkowski and colleagues [19]. Completeness of the resection was defined when no macroscopic residual tumor remained and was further confirmed with surgical bed histology, as well as postoperative CT scan or MRI scan. A diagnosis of relapse was based on clinical or imaging study demonstrating tumor presence in the nasopharynx or neighboring structures, with confirmed major symptoms including epistaxis or nasal obstruction after the first surgical treatment. Different hospitals have different therapeutic principles and surgical techniques for patients with JNA. Therefore, the history of previous operations at other hospitals was not considered for the relapse analysis in this study.

2.2. Tissue microarray

The preparation of tissue microarray for JNA was reported in our previous report [7]. Briefly, a total of 70 formalin-fixed (buffered neutral aqueous 4% formalin solution), paraffin-embedded tumors were obtained from the Department of Otolaryngology – Head and Neck Surgery, Eye, Ear, Nose, and Throat Hospital, Fudan University, China. A representative paraffin block was selected from each case after histologic review of slides stained with hematoxylin–eosin (H&E). Two representative tumor areas were selected, and the corresponding areas were marked on the surface of the standard paraffin block. Tissue cores were punched from the designated area using a biopsy needle. The normal middle turbinates of 10 male patients with a median age of 21.4 years (range, 13–26 years) were used as normal controls. Two cores were taken from each representative tissue, and all tissue cores (1.5 mm in diameter) were put into a tissue microarray (TMA) block that covered up to 160 cores. Samples were processed in collaboration with Shanghai Biochip Company, Shanghai, China. Serial 4 μm sections were placed on 3-aminopropyltriethoxysilane-coated slides.

Table 1
Clinicopathological characteristics of 70 patients with JNA.

	Number of patients with JNA
Total number	70
Sex	
Male	70
Female	0
Median age, year	17
≤17	39
>17	31
Tumor stage	
Stage Ia	4
Stage Ib	7
Stage IIa	6
Stage IIb	7
Stage IIc	34
Stage IIIa	5
Stage IIIb	7
Operation history	
No	40
Yes	30
Operation approach	
With endoscope	17
Without endoscope	53
Volume of intraoperative hemorrhage (ml)	
≤800	26
>800	44

Abbreviations: JNA, juvenile nasopharyngeal angiofibroma.

2.3. Immunohistochemical study and microscopic analysis

Immunodetections of MMP-9 was performed using monoclonal antibodies (1:100; Abcam, UK) with a microwave antigen retrieval method. 0.01 M phosphate-buffered saline (PBS) (pH 7.4) was used as the dilutions of all the antibodies. Tissue sections were deparaffinized and rehydrated. Endogenous peroxidase was blocked by placing slides in 3% hydrogen peroxide in methanol for 15 min Slides were then washed with deionized water and placed in PBS for 5 min. After preincubation with 10% normal serum in 1% bovine albumin phosphate-buffered saline solution for 30 min to avoid non-specific binding, the primary antibodies were stored overnight at 4 °C. Slides were washed three times with PBS and consecutively incubated with biotinylated secondary antibody for 30 min; again washed three times with PBS and incubated with streptavidin horseradish peroxidase conjugate for 20 min; washed again three times with PBS. Detection system with 0.05% diaminobenzidine (DAB) as chromagen (DAB; DAKO, Denmark) was used. Then, the samples were counterstained with hematoxylin for 2 s, washed, dehydrated, and mounted. Negative controls, which were included in all assays, were treated identically to the samples without the addition of primary antibodies.

The intensity of the immunoreactivity and the percentage of positive cells were initially estimated semi-quantitatively by light microscopy in stromal cells of JNA. The overall immunoreactivity in stromal cells was scored in a 4-tier scale system based on the percentage of positive cells and the intensity of immunoreactivity in a semi-quantitative method as previously described [1,20]. Immunoreactivity was scored as follows: − = negative result; + = weak immunoreactivity regardless of the percentage of the cells being positive; ++ = moderate immunoreactivity in less than 75% or strong immunoreactivity in less than 25% of the cells; and +++ = moderate immunoreactivity in 75% or more or strong immunoreactivity in 25% or more of the cells. Only cytoplasmic reactivity was considered specific for MMP-9. For further analysis, the patients were divided into two groups, low MMP-9 expression (0—+) versus high MMP-9 expression (++—+++).

Microvessel density (MVD) count was carried out using immunostaining with the anti-CD105 antibody, as described in our previous report [7]. The vascular hot spot technique was used according to Weidner et al. [21]. For further survival analysis, MVD was classified into two groups, high (≥10 vessels per 200× visual field) versus low (<10 vessels per 200× visual field).

2.4. Statistics

The association between clinicopathologic variables and expression of MMP-9 in JNA were analyzed by Pearson's chi-square test. Overall survival was calculated by the Kaplan–Meier survival curves. The impact of the expression of MMP-9 on the time to recurrence of JNA patients was investigated by Kaplan–Meier survival curves, and the difference was determined by the log-rank test. The Cox regression model served for multivariate survival analysis. Receiver operating characteristic (ROC) curve analysis was used to determine the predictive value of the parameters. A p values <0.05 were considered statistically significant. All statistical analyses were performed with SPSS 19.0 statistical software (SPSS, Chicago, IL).

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