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Expression of GLUT-1 in nasopharyngeal carcinoma and its clinical significance

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Abstract. – OBJECTIVE: To investigate the relationship between the expression of glucose transporter-1 (GLUT-1) and the clinicopathological features and prognosis of patients with nasopharyngeal carcinoma (NPC).

PATIENTS AND METHODS: Sixty-three patients with NPC (the NPC group) and 24 patients with chronic nasopharyngitis (the control group) who were treated between December 2014 and February 2016 were selected for this study. Pathological nasopharyngeal tissues were collected from patients. The expression of GLUT-1 was detected by immunohistochemistry. The expression of GLUT-1 was correlated with clinicopathological features and survival time.

RESULTS: The positive GLUT-1 expression rate in the NPC group was 58.73% (37/63), which was significantly higher than in the control group (29.17%, 7/24) ($p<0.01$). The positive GLUT-1 expression rate was significantly correlated with clinical stage, lymph node metastasis, and Epstein-Barr (EB) virus infection ($p<0.05$). The 3-year survival rate of GLUT-1-positive NPC patients was 75.00% and was significantly lower than that of GLUT-1-negative NPC patients (88.89%) ($p<0.05$).

CONCLUSIONS: GLUT-1 was highly expressed in the nasopharyngeal tissues of patients with NPC, and its expression was associated with clinical stage, lymph node metastasis, and EB virus infection.

Key Words:
Nasopharyngeal carcinoma, Glucose transporter-1, Clinicopathological features, Prognosis.

Introduction

Nasopharyngeal carcinoma (NPC) is a malignant tumor with high incidence in southern China. It is believed to be caused by Epstein-Barr (EB) virus infection and other environmental factors[1,2]. Because the lesions of NPC are deep and closely linked to the skull base, NPC is highly invasive, and can easily migrate to the skull base, parapharyngeal area, carotid sheath, and other surrounding tissues[3]. Early treatment of NPC can achieve good therapeutic results, with a 5-year survival rate of up to 85% or more. However, because the clinical symptoms of early NPC are usually atypical and unobvious, patients often have already reached the advanced stage when they are diagnosed. Therefore, early diagnosis and treatment of NPC are critical for improving the prognosis of patients[4]. Glucose metabolism depends on the uptake of glucose by cells. However, glucose cannot freely pass through the lipid bilayer of cell membranes. Glucose uptake depends on glucose transporters (GLUTs) expressed on the cell membrane[5-7]. GLUTs are expressed in various tissues and cells of the body, and they are divided into two categories: sodium-dependent glucose transporters (SGLTs) that transport glucose against the concentration gradient, and GLUTs that facilitate the diffusion of glucose without consuming energy. GLUTs are synthesized inside the cell and cannot be supplemented exogenously[8]. The expression of GLUTs can reflect the status of tumor tissue metabolism to some extent[9]. The aim of this study was to investigate the expression of glucose transporter-1 (GLUT-1) in patients with NPC, to understand its correlation with clinicopathological features of the disease.

Patients and Methods

Patients

Sixty-three patients with nasopharyngeal squamous cell carcinoma confirmed by pathological examination and treated in the ENT Department of the Second Clinical Medical College of Jinan University between December 2014 and February 2016 were included in the NPC group. The Ethics Com-

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mittee of the Second Clinical Medical College of Jinan University approved the study. Twenty-four patients with chronic nasopharyngitis confirmed by endoscopy and pathological examinations, and treated during the same period were included in the control group. Examination by computed tomography was used to determine lymph node metastasis. Patients in the NPC group were from 26-72 years old, with an average age of 42.7 ± 13.9 years old. There were 41 males and 22 females. According to clinical stage, there were three cases in stage I, 25 cases in stage II, 22 cases in stage III, and 13 cases in stage IV. Among them, 51 patients had cervical lymph node metastasis. The control group was aged from 22-66 years old, with a mean age of 43.1 ± 9.7 years old. There were 14 males and 10 females. There were no significant differences in age or sex between the two groups ($p<0.05$).

Instruments and Reagents

The main instruments used in this study included a Rotary microtome (Microm, Germany; Refrigerator (Electrolux, Changsha, China); and Optical microscope CX21 (Olympus, Tokyo, Japan).

The main reagents used in this study included Eosin staining solution (BASO, Taiwan); GLUT-1 (sc7903) antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA); and an S-P kit (Beijing Zhongsheng Jinqiao Bioengineering, Beijing, China).

Immunohistochemistry

Immunohistochemistry was used to determine the expression of GLUT-1 in nasopharyngeal tissues. H&E staining was conducted first. Paraffin-embedded tissues were sectioned continuously at 4 μm and were placed in a 60°C oven for 40 min. After the sections had been dewaxed, they were incubated for 10 min in 3% hydrogen peroxide solution to block the activity of endogenous peroxidases. Then, the primary antibody was added and the slides were placed in a humid chamber at 4°C overnight. After addition of the enhancer of the S-P kit, slides were incubated at room temperature for 24 min. Next, the enzyme-linked anti-mouse secondary antibody was added, followed by incubation for 30 min at room temperature. Three rinses were performed with phosphate buffered saline (PBS) (pH 7.4) for 3 min each. PBS was removed and freshly prepared diaminobenzidine (DAB) solution was added. The slides were then observed under a microscope.

Evaluation of Immunohistochemical Results

Positive GLUT-1 expression was marked by the presence of yellow or brown granules in the cytoplasm or nucleus of cells. Grading was based on the degree of staining as follows: colorless, 0 points; light yellow, 1 point; brownish yellow, 2 points; brown, 3 points. Grading based on the percentage of positively-stained cells was as follows: <10%, 0 points; 10–25%, 1 point; 26–50%, 2 points; >50%, 3 points. The final scores were taken as the sum of the results of the above two grading methods: > 2 points were determined as positive expression, and ≤ 2 was determined as a negative expression.

Statistical Analysis

Data were analyzed with SPSS21.0 (SPSS Inc., Chicago, IL, USA) software. Quantitative data are presented as mean ± standard deviation ($\overline{X}$ ± s). Categorical data are presented as percentage (%). The comparison of the rates of positive GLUT-1 expression was made by the χ^2-test. The Kaplan-Meier method was used to analyze the survival data, and the log-rank method was used for comparisons between groups. $p<0.05$ indicated a statistically significant difference.

Results

Comparison of GLUT-1 Expression Between the two Groups of Patients

The positive rate of GLUT-1 expression in the NPC group was 58.73% (37/63). The number was significantly higher than in the control group (29.17%, 7/24) ($p<0.01$) (Table I and Figure 1).

Correlation of the Expression of GLUT-1 with Various Clinicopathological Features

Positive GLUT-1 expression in patients with NPC was significantly correlated with clinical stage, lymph node metastasis, and EB virus infection ($p<0.05$), but not with sex or age ($p>0.05$) (Table II).

Expression of GLUT-1 and Survival Status during the 3-year follow-up of Patients with NPC

After the 3-year follow-up, the survival data of patients were analyzed using the Kaplan-Meier test. The 3-year survival rate of the GLUT-1-positive patients was 75.00%, which was significantly lower than that of the GLUT-1-negative patients (88.89%) ($\chi^2 = 2.482$, $p = 0.035$, <0.05). These results suggested that positive GLUT-1 expression was not favorable for the prognosis of patients with NPC.

Table I. Expression of GLUT-1 in the two groups of patients [n (%)].

Group	No.	Positive	Negative
NPC	63	37 (58.73)	26 (41.27)
Control	24	7 (29.17)	17 (70.83)
χ^2		11.633	
p		0.001	

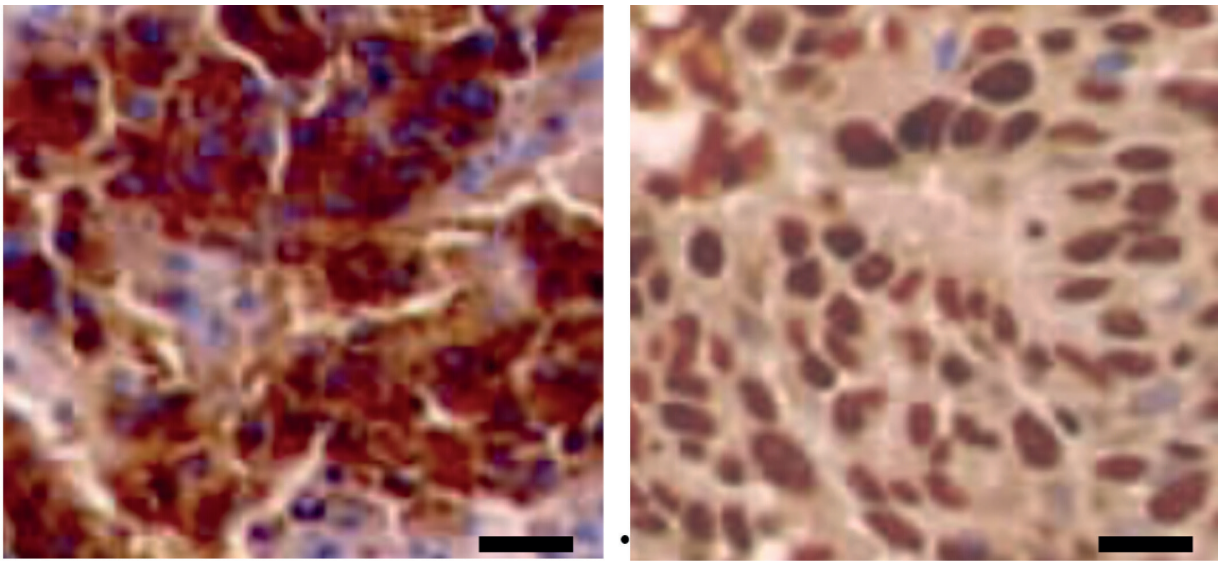

Figure 1. Immunohistochemical staining results (400×). Left: Expression of GLUT-1 in NPC tissue. Right: Expression of GLUT-1 in normal tissue. Compared with NPC tissue, the expression of GLUT-1 in normal tissues was significantly decreased.

Table II. Correlation of GLUT-1 expression with various clinicopathological features.

Item	Positive	Negative	Positive expression rate (%)	χ^2	*p*
Sex				0.389	0.682
Male	25	16	60.98		
Female	12	10	54.55		
Age				0.387	0.541
<50	23	18	56.1		
≥50	14	8	63.64		
Clinical classification				7.382	0.005
I-II	11	17	39.29		
III-IV	26	9	74.29		
Lymph node metastasis			9.389	0.003	
Yes	34	17	66.67		
No	3	9	25		
Histopathological classification				10.293	0.002
High	28	10	73.68		
Moderate	2	9	18.18		
Low	7	7			
Received radiation therapy			1.287	0.192	
Yes	20	10	66.67		
No	17	16	51.51		
EB virus infection			9.482	0.002	
Yes	29	8	78.38		
No	8	18	30.77		

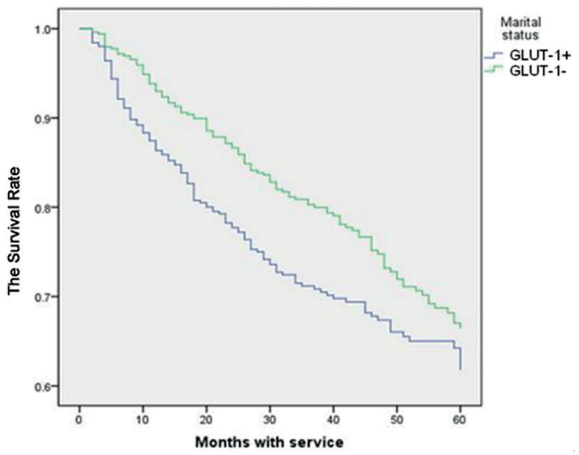

Figure 2. Survival curves of patients with NPC with different GLUT-1 expression. The 3-year survival rate of GLUT-1-positive patients was 75.00%, which was significantly lower than that of patients negative for GLUT-1 expression (88.89%) ($\chi^2 = 2.472$, $p = 0.035$, <0.05).

Discussion

NPC is a malignant epithelial cell carcinoma that originates from the lining of epithelia of the nasopharyngeal mucosa. It is prone to occur in the posterior wall of the nasopharynx and pharyngeal recess, with fewer occurrences in the pharyngeal opening of the eustachian tube and posterior nares[6]. Because NPC is highly invasive, it often expands to the oropharynx, nasal cavity, orbital, and even the skull base and intracranial space, causing corresponding clinical manifestations. In general, upper cervical lymph node metastasis, which gradually affects the lower cervical lymph nodes, appears in the early stage of NPC. Furthermore, it can retrograde spread to the submandibular and submental lymph nodes[7]. As the location is difficult to detect and early symptoms are mild, NPC has become one of the most common head and neck cancers in China. The main treatment for NPC is radiotherapy. Platinum-based chemotherapy is more effective than other methods. However, distant metastasis and local recurrence are major factors leading to treatment failure[9,10].

The occurrence and development of malignant tumors are closely related to the over-proliferation of cells. Because aerobic metabolism during cancer cell proliferation consumes large amounts of oxygen, hypoxia is a common phenomenon in tumor development, and it is one of the characteristics of the tumor microenvironment[11]. Hypoxia can enable cancer cells to change their biological behavior to adapt to the hypoxic environment, thereby increasing their invasiveness and metastatic potential[9-12]. Under such circumstances, the energy required for cancer cell growth mainly comes from anaerobic glycolysis. Therefore, with the progression of cancer cell metastasis, the expressions of GLUTs continue to increase to meet the demands of glucose metabolism in cancer tissue. Currently, nine GLUTs have been identified. GLUT-1 was found to be an important cellular carrier for transmembrane glucose import, and an important regulator of glucose transport[13,14]. Yang et al[12] showed that GLUT-1 expression was not detected in normal tissues or benign tumors, whereas high levels of GLUT-1 were generally found in malignant tumors. The mechanism was that GLUT-1 increased glucose uptake and transport. Thus, GLUT-1 is considered one of the early markers of cell malignancy. At present, numerous studies have shown that GLUT-1 is overexpressed in a variety of tumors and its expression level is closely related to clinical stage and metastasis[15-18]. We showed that the positive rate of GLUT-1 expression in patients with NPC was significantly higher than in the control group. This finding suggested that GLUT-1 was involved in NPC. We also found that positive GLUT-1 expression in patients with NPC was significantly correlated with clinical stage, lymph node metastasis, and EB virus infection. These results indicated that GLUT-1 may play an important role in the occurrence, development, and metastasis of NPC. GLUT-1 can serve as a molecular marker to predict the prognosis of NPC, and possibly as a therapeutic target of NPC. The GLUT-1-positive patients had a lower 3-year survival rate than GLUT-1-negative patients, suggesting that GLUT-1 is related to the long-term prognosis of patients with NPC.

Conclusions

We found that GLUT-1 was expressed at high levels in patients with NPC, and its expression was related to clinical stage and lymph node metastasis. GLUT-1 was also related to the long-term prognosis of patients. GLUT-1 can serve as a prognostic molecular indicator of NPC and may represent a potential therapeutic target of NPC.

Conflict of interest

The authors declare no conflicts of interest.

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