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Original Article

Gli1 protein expression in nasopharyngeal carcinoma and its clinical significance

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Abstract: Objective: To investigate Gli1 protein expression in nasopharyngeal carcinoma (NPC) tissue and its clinical significance. Methods: Gli1 protein expression in 77 cases of NPC and 20 cases of chronic nasopharyngeal mucositis were detected by immunohistochemistry and its relationship with NPC clinicopathologic features was analyzed. Results: The expression of Gli1 protein in NPC tissues (57.1%) was significantly higher than that in chronic nasopharyngeal mucositis (30.0%, $P < 0.05$); Meanwhile, Gli1 protein expression was related to tumor node metastasis ($P < 0.05$). Conclusion: The upregulated expression of Gli1 protein in NPC tissue may be related to tumor metastasis.

Keywords: Nasopharyngeal carcinoma, hedgehog signal pathway, gli1, immunohistochemistry

Introduction

Nasopharyngeal carcinoma (NPC) is one of the most common tumors in head and neck in southern region of china, and cervical node metastasis may occur in the early stage. Hedgehog (Hh) signal pathway is essential in regulating embryonic development. Recent studies have confirmed that Hh signal pathway is involved in the development of various tumors. Glioma-associated oncogene homologue I (Gli1) is an important nuclear transcription factor of the signal pathway, which is responsible for regulation of downstream effectors. Its expression level directly reflects the activation state of Hh signal pathway. The previous studies have confirmed that Gli1 protein expression is associated with the development of esophageal cancer, lung cancer, breast cancer and other tumors [1-3]. It was also reported that Gli1 gene and protein expression were also increased in NPC tissues [4]. However, there are still no reports about the relationship between Gli1 protein expression and NPC clinicopathologic features. We detected Gli1 expression in NPC tissue by immunohistochemistry streptavidin-perosidase (SP) method, analyzed its relationship with NPC clinicopathologic features, and discussed its function in NPC and clinical significance.

Materials and methods

Clinical data

From April 2013 to April 2014, 77 patients with nasopharyngeal carcinoma in the Department of Oncology in Nanxishan Hospital of Guangxi Zhuang Autonomous Region were collected. Samples were all nasopharyngeal masses collected by the oncologist using fiber nasopharyngoscopy biopsy. The pathological type was all undifferentiated non-keratinizing carcinoma. There were intact related clinical and pathological data, and the patients who received previous chemotherapy, radiotherapy or any other anti-tumor therapies were excluded. Among them, there were 61 males and 16 females; their median age was 52 years. The TNM (tumor, node, metastasis TNM) stage was determined according to the Chinese 2008 staging system for NPC: there were 9 cases of stage II, 27 cases of stage III, 35 cases of stage IVa and 6 cases of stage IVb; regarding to tumor statues of carcinoma, there were 5 cases of T1, 22 cases of T2, 24 cases of T3 and 26 cases of T4;

Table 1. Demographic information and clinical features for both groups

Groups	Age (Year)					Sex				
	< 52	≥ 52	Total	χ^2	*P*	Male	Female	Total	χ^2	*P*
NPC	38	39	77	1.559	0.212	61	16	77	1.076	0.30
Nasopharyngeal mucositis	13	7	20			13	7	20		

Table 2. Expression of Gli1 in NPC and nasopharyngeal mucositis

Group	Gli1 Protein		Total	χ^2	*P*
	Negative	Positive			
NPC	33	44	77	4.683	0.030
Nasopharyngeal mucositis	14	6	20		
Total	47	50	97		

regarding to lymph node status, there were 12 cases of N0, 25 cases of N1, 26 cases of N2, and 14 cases of N3; concerning distant metastasis, there were 71 cases of M0 and 6 cases of M1. Meanwhile, 20 patients with mild chronic nasopharyngeal mucositis during the same period were set as controls. The protocol was approved by Ethics Committee of Nanxishan Hospital of Guangxi Zhuang Autonomous Region, and written informed consent was obtained from all patients.

Reagents and methods

The rabbit polyclonal primary antibody Gli1 (Lot: ab134906; Abcam, UK), HRP goat anti-rabbit/mouse universal secondary antibody and DAB chromogenic reagent kit (Fuzhou Maixin Biotech. Co., Ltd., Fuzhou, China).

Methods

NPC biopsy specimens were fixed by 10% neutral formalin, embedded in paraffin and serially cut into 5 μm thick sections. Paraffin sections were heated under 73°C for 15 min and under 62°C for another 2 h. Then sections were dew axed, repaired for 20 min, boiled in citrate antigen retrieval buffers (pH=6.0) and incubated with 3% H_2O_2 for 10 min; sections were rinsed with PBS for 3 times, 5 min each. After that, dry the sections and add Gli1 antibody (working concentration: 1:400). Incubate in 37°C for 2 h, and wash with PBS for 3 times, 5 min each. Dry sections, add secondary body and incubate in 37°C for 30 min. Wash for 3 times with 5 min each and dry sections. DAB chromogenic reaction, hematoxylin staining, dehydration with gradient alcohol and dryness were carried out before mounted with neutral gum. For the negative controls, add PBS instead of primary antibody.

Result determination

Gli1 protein was judged as positive expression when granules color changed from light yellow to dark brown in cytoplasm and (or) nucleus [5]. All sections were read by 2 pathologists and discussion was required to reach agreement for the inconsistent results. 5 high power fields were randomly selected and 200 cells were counted in each field. It was scored according to the proportion of positive cells: the positive cells 0-5%, 0; 6-25%, 1; 26-50%, 2; 51-75%, 3; > 75%, 4. It was also scored according to staining degree of tumor cells: non-stained case, 0; light yellow, 1; brown, 2; dark brown, 3. The intensity score and proportion score were multiplied in order to generate an immunoreactive score (IRS), and IRS ≥ 4 was considered positive [5].

Statistical analysis

SPSS18.0 software was used for statistical analysis. The differences among groups were analyzed with χ^2 test or Fisher Exact test, and $P < 0.05$ indicated significant difference.

Results

Characteristics of studied groups

As shown in **Table 1**, there was no significant difference in age and gender between the two groups.

Gil1 expression in NPC and nasopharyngeal mucositis tissues

Gli1 protein which had color change from light yellow to dark brown in cytoplasm or nucleus was considered as positive expression. The expression of Gli1 protein in NPC tissues (57.1%) was significantly higher than that in chronic nasopharyngeal mucositis (30.0%, $P < 0.05$, **Table 2**); in nasopharyngeal mucositis tis-

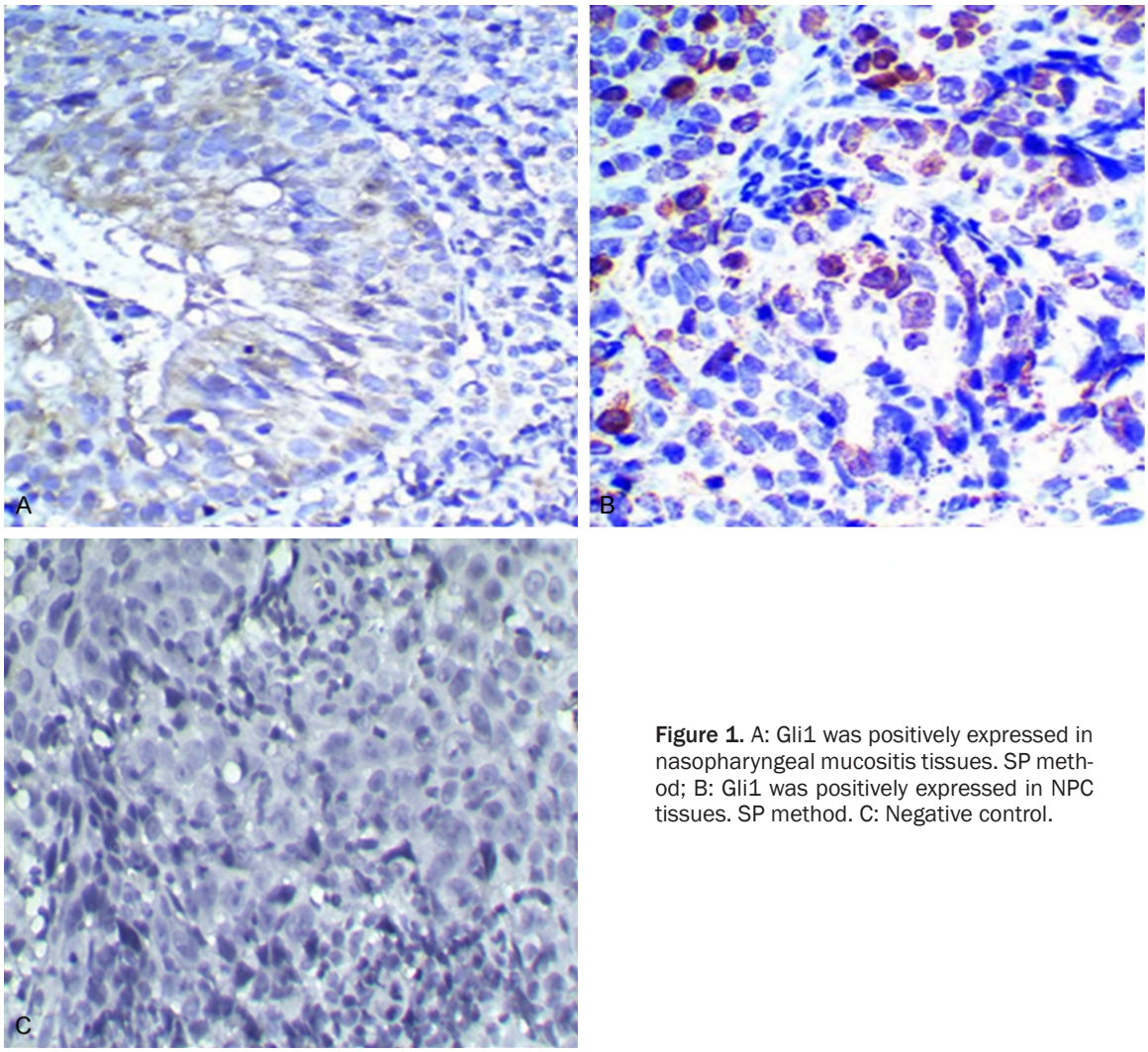

Figure 1. A: Gli1 was positively expressed in nasopharyngeal mucositis tissues. SP method; B: Gli1 was positively expressed in NPC tissues. SP method. C: Negative control.

sues, Gli1 was mainly positively expressed in cytoplasm of mucosal epithelial cells, which had light yellow or brown granules (**Figure 1A**). Meanwhile, in NPC tissue Gli1 was mainly positively expressed in nucleus and cytoplasm of cancer nests cells, which always had dark brown nucleus and light yellow or brown cytoplasm (**Figure 1B**).

The relationship between Gli1 protein expression and NPC clinicopathologic features

In NPC tissues, Gli1 expression was correlated with lymph node metastasis ($P < 0.05$), but it was independent of age, gender, depth of tumor invasion, clinical stages and tumor distant metastasis ($P > 0.05$, **Table 3**).

Logistic regression results

After adjustment of other confounders, Gli1 expression was independent risk factor of NPC (**Table 4**).

Discussion

Hh signal pathway is involved in morphogenesis and growth modulation of most organs during the development of embryo. Meanwhile, it is essential in the development and regeneration of tissues like lung, prostate and intestine. Hh signal pathway is mainly composed of Hh ligand, membrane receptors (Ptch and Smo), nuclear transcription factors Gli and target genes. It was reported that abnormal activation of Hh signal pathway induced generation of tumors, and promoted tumor invasion and metastasis [1-5]. Gli has attracted widespread attention in cancer researches as a key factor of Hh signal pathway. There are 3 kinds of Gli transcription factors in vertebrate, and Gli1 only shows activating ability in Hh signal pathway. Therefore, the expression level of Gli1 directly reflects the activation state of Hh signal pathway.

Table 3. The relationship between Gli1 protein expression in NPC tissue and clinicopathologic features

Clinicopathological Parameter	n	Gli1		χ^2 value	*P* value
		+	Positive rate (%)		
Gender					
Male	61	36	59.02	0.421	0.517
Female	16	8	50.00		
Age (years)					
≥ 52	39	26	66.67	2.927	0.087
≥ 52	38	18	43.37		
Clinical stages					
II	9	5	55.56		
III	27	14	51.85	0.564	0.755
IVa+IVb	41	25	60.98		
Depth of tumor invasion					
T1+T2	27	14	51.85	0.475	0.491
T3+T4	50	30	60.00		
Lymph node metastasis					
-	12	3	25.00	5.997	0.014*
+	65	41	63.08		
Distant metastasis					
M0	71	39	54.17	0.847	0.357
M1	6	5	83.33		

*$P < 0.05$.

Table 4. Logistic regression results

Variables	B	SE	χ^2	*P*	95% ci
Sex	0.763	0.576	1.758	0.185	2.416 (0.649-6.631)
Age	-0.650	0.542	1.437	0.231	0.522 (0.180-1.511)
Glil expression	0.319	0.142	5.032	0.025	1.376 (1.041-1.820)
Constant	0.063	0.739	0.007	0.932	

It was reported that Gli1 was highly expressed in esophageal cancer, glioma cancer and medulloblastoma cell lines, promoting tumor cell proliferation and differentiation; its expression in breast cancer, gastric cancer and liver cancer tissues was higher than that in the adjacent tissues [3, 7-9]; its positive rate in cervical intraepithelial neoplasia and cervical squamous cancer of stage CIN2 and CIN3 was significantly higher than that in normal cervical squamous epithelium and cervical intraepithelial neoplasia tissues of stage CIN1 [10]. Thus Hh signal pathway is abnormally activated in tumor tissues. Abnormal activation of Hh signal pathway promotes proliferation and differentiation of tumor cells, inhibits tumor cell apoptosis, enhances epithelial-mesenchymal transformation and increases tumor invasiveness. Upregulation of Gli1 promotes the transformation of low metastatic cells into highly metastatic tumor cells, increasing capacity of invasion and metastasis of tumor cells. In addition, Gli1 expression is related to tumor grade, lymph node metastasis and prognosis [1, 8-11], indicating that Gli1 is correlated with invasion and metastasis of tumors.

In this study, Gli1 expression was higher in NPC tissues than chronic nasopharyngeal mucositis tissues, indicating that Hh signal pathway was abnormally activated in NPC. The result was also in accordance with study of Yue and his colleagues [4], and similar to that of other solid tumors [1-3, 5-10]. Further study showed that Gli1 expression was related to NPC lymph node metastasis, which corresponded to studies on esophageal, gastric and liver cancer [1, 8, 9]. It was confirmed that Hh signal pathway promoted tumor lymphangiogenesis and improved ability of tumor lymph node metastasis [12]. In our early experiments, it was found that there was lymphangiogenesis in NPC tissues, which was propitious to development of lymphatic metastasis, and resulted from tumor cells' invasion to lymphangion [13]. It was suggested that Gli1 may lead to lymph node metastasis of cancer by promoting lymphangiogenesis in NPC tissues. Although Gli1 was proved to be one of the important activating transcription factors of Hh signal pathway, it remains to be elucidated how Hh signal pathway is activated in NPC tissues as well as the specific mechanism of promoting tumor metastasis.

In conclusion, the abnormal expression of Gli1 protein is of vital importance in invasion and metastasis of tumor cells, and its specific mechanism still needs further investigation. Gli1-targeted therapies may become a new direction of NPC treatment.

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Disclosure of conflict of interest

None.

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