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Original Article

Clinical significance of chromosome 1p/19q loss of heterozygosity and Sox17 expression in oligodendrogliomas

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Abstract: Objective: To study chromosome 1p/19q loss of heterozygosity (LOH) and Sox17 protein expression in oligodendrogliomas and correlate this loss with clinicopathological features. Methods: This study included 100 cases of oligodendrogliomas at the First Affiliated Hospital of Xinjiang Medical University from 2003 to 2014. The cases included paraffin-embedded tissues from 50 low-grade oligodendrogliomas and 50 anaplastic oligodendrogliomas. Chromosome 1p/19q LOH was detected by fluorescence *in situ* hybridization (FISH) and Sox17 protein expression was analyzed by immunohistochemistry. Clinicopathological characteristics of the oligodendrogliomas were compared and prognosis analyzed using Cox regression and Kaplan-Meier analyses. Results: The LOH positivity rate of 1p/19q was 52% in 50 cases of low-grade oligodendrogliomas and 68% in 50 cases of anaplastic oligodendrogliomas ($P = 0.102$). The rates of Sox17 expression were significantly different in oligodendrogliomas (82%) and anaplastic oligodendrogliomas (62%, $P = 0.026$). Single factor analysis determined that 1p/19q LOH ($P = 0.000$), Sox17 protein expression ($P = 0.000$), location ($P = 0.001$), chemotherapy ($P = 0.000$), and radiation therapy ($P = 0.001$) were associated with oligodendroglioma patient prognosis. Cox multiple factors regression analysis determined that 1p/19q LOH and Sox17 expression were independent prognostic factors of oligodendrogliomas. Conclusion: In this study, oligodendroglioma patients with 1p/19q LOH and Sox17 protein expression had a better prognosis. Thus, analysis of 1p/19q LOH and Sox17 protein expression could significantly enhance diagnostic accuracy, guide treatment, and improve the prognosis.

Keywords: Oligodendroglioma, 1p/19q LOH, Sox17, prognosis, clinical features

Introduction

Gliomas account for ~60% of intracranial primary tumors and oligodendrogliomas are the most common malignant neoplasms of the central nervous system (CNS), comprising 4-5% of all intracranial tumors [1]. Approximately 50-80% of tumors identified histologically as oligodendrogliomas have concurrent loss of chromosomal regions 1p and 19q [2]. Co-deletion of chromosomes 1p and 19q was associated with improved prognosis and responsiveness to therapy in patients with anaplastic oligodendrogliomas [3]. Sox17 is a recently discovered tumor suppressor gene associated with the occurrence, development, and progression of these tumors [4]. This study focused on the relationship between 1p/19q LOH and Sox17 protein expression and clinical pathological features of oligodendrogliomas.

Materials and methods

Tissue specimens

We enrolled 100 patients with histological diagnosis of the oligodendroglioma at First Affiliated Hospital of Xinjiang Medical University from May 2003 through September 2014. The histological diagnoses were oligodendroglioma Whole Health Organization (WHO) grade II (OII, n = 50) and anaplastic oligodendroglioma WHO grade III (AOIII, n = 50). There were 60 male and 40 female patients with a median age of 42 years (range 6-77). Among the 100 tumors, 89

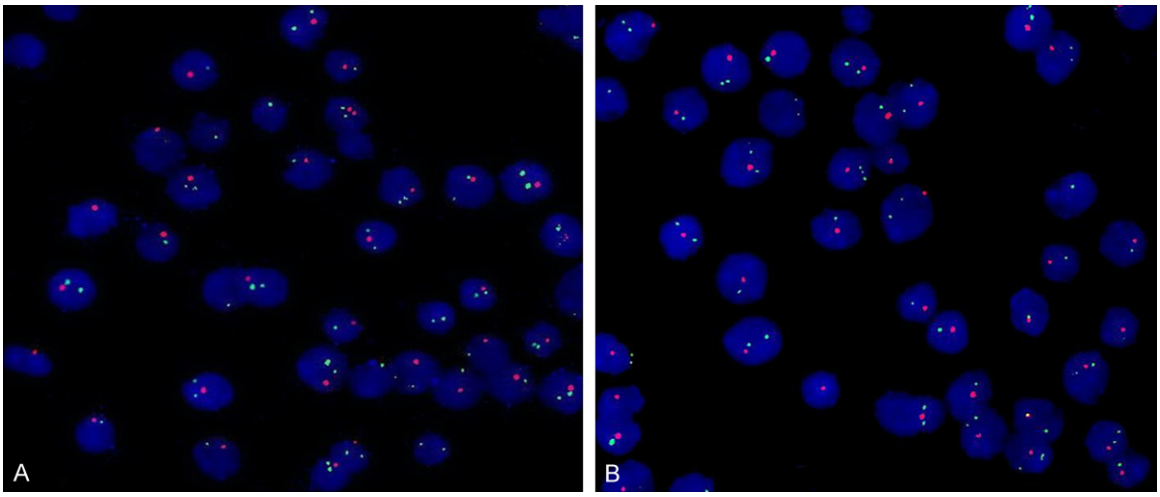

Figure 1. Deletions in chromosome 1p/19q in oligodendrogliomas. A: 1p deletion with two green (1q) and one red (1p) signal in most nuclei. B: 19q deletion with one red (19q) and two green (19p) signals.

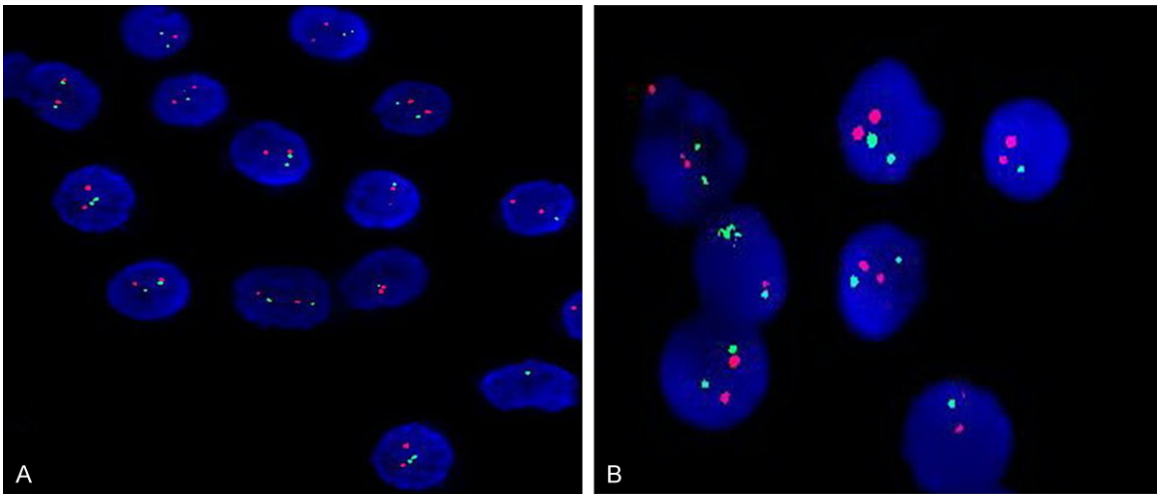

Figure 2. A: Normal chromosome 1 with two green and two red signals. B: Normal chromosome 19 with two green and two red signals.

were located in the cerebral hemisphere, 6 in the cerebellum, 3 in the thalamus, and 1 in the spinal cord. There were 45 cases treated with radiation and 48 cases treated with chemotherapy. All specimens were independently re-evaluated by two experienced neuropathologists who were blinded to the clinical outcome of the patients, according to the 2007 WHO classification of CNS tumors.

From this patient cohort, clinical histories were if available researched for the following clinical data: patient age, gender, tumor location, adjuvant therapy details including chemotherapy and radiation therapy, and duration of survival.

Fluorescence in situ hybridization (FISH)

Dual-color FISH analysis of chromosomes 1 and 19 was performed as described previously [5]. Briefly, representative unstained sections of 3-μm thickness were cut from archival formalin-fixed, paraffin-embedded blocks and were deparaffinized, dehydrated, washed in citrate buffer at 100°C for 30 min, and air cooled. The sections were then digested in pepsin solution for 10 minutes at 55°C, washed in distilled water at room temperature, and air-dried. Dual-probe hybridization was performed using a digoxigenin-labeled locus-specific 1p or 19q probe and a Spectrum Green-labeled

Table 1. Effects of genetic factors on 1p/19q and Sox17 protein expression in oligodendrogliomas

Variable	N	Sox17		1p/19q LoH	
		+	-	+	-
Histological type	100	72 (74.5.0)	28 (25.5)	60 (35.5)	40 (64.5)
OG	50	41 (82.0)	9 (18.0)	26 (52.0)	24 (48.0)
AO	50	31 (62.0)	19 (38.0)	34 (68.0)	16 (32.0)
P		0.026		0.102	

AO, anaplastic oligodendroglioma; OG, oligodendroglioma.

probe (Vysis, Downers Grove, IL) mapping to 1q and 19p, respectively. Paired probes for 1p32/1q42 and 19p13/19q13 and target DNA were denatured simultaneously in an 80°C oven for 5 min, followed by overnight incubation at 42°C. Slides were then washed in 0.4× SSC/0.3% NP-40 at 67°C for 2 min, 2× SSC/0.1% NP-40 at 37°C for 2 min, and in 2× SSC at 37°C for 2 min. After washing, the slides were air-dried in the dark and counter-stained with 10μl 4',6-diamidino-2-phenylindole (DAPI) applied to the target area.

Green and red fluorescent signals were enumerated under an Olympus BX60 fluorescence microscope with appropriate filters (Olympus, Melville, NY). For each hybridization, a minimum of 200 interphase non-overlapping nuclei were assessed in each case, with the absence of one of two signals interpreted as hemizygosity for the corresponding chromosomal region. At least 50% or more nuclei had to show one signal to be scored as a deletion. The presence of multiple (> 2) signals were indicative of polysomy was also documented for each chromosome if at least 5% of the cells had such alterations.

Immunohistochemistry

Experimental procedures were performed as described previously. A Sox17 antibody was purchased from Thermo (City, Country) and used at a dilution of 1:100. Expression was visualized using 3,3'diaminobenzidine (EnVision, City, Country). The slices were incubated in 0.05 mol/L (pH 6.0) boiling citric acid buffer for 30 min for antigen repair and analyzed semi-quantitatively. Under normal circumstances, Sox17 protein expression is present in the cell membrane. However, in oligodendroglioma, Sox17 expression was observed in the cytoplasm and/or the nucleus. The positive rate was defined as the number of positive cases and ratio of the total. A positive degree of classification was defined as the number of positive cells and the ratio to the total number of cells (-: 0, +: < 25%, ++: 25%~50%; +++: > 50%).

Survival data

Overall survival (OS) was defined as the period from the first operation to death or last follow-up. Patients who were still alive at last follow-up were considered as a censored event in analysis. The average follow-up time in the available78 cases was 2.8 years.

Statistical analysis

All statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS, ver. 17). For group comparisons of proportions, the χ^2 and/or Fisher's exact test were conducted. For survival data, the LIFETEST procedure in SPSS was used to compute and plot Kaplan-Meier survival curves, and the log-rank test was used to test for survival differences among groups. $P < 0.05$ were considered to indicate statistical significance.

Results

Chromosome 1p/19q alterations and Sox17 expression

The majority of oligodendrogliomas had a co-deletion of 1p/19q (**Figures 1** and **2**). The positivity rate of 1p/19q co-deletion was 52% (26/50) in low-grade oligodendrogliomas and 68% (34/50) in anaplastic oligodendrogliomas ($P = 0.102$, **Table 1**).

There were 72 of 100 oligodendrogliomas positive for Sox17 (**Figures 3** and **4**); 28 of them showed negative expression (**Figure 3**). The positivity rate of Sox17 expression was 82% in low-grade oligodendrogliomas and 62% in anaplastic oligodendrogliomas ($P = 0.026$, **Table 1**).

Correlation analysis of 1p/19q co-deletion and Sox17 expression in oligodendrogliomas by Spearman's rank correlation test showed a significant positive correlation ($r = 0.521$, $P = 0.521$).

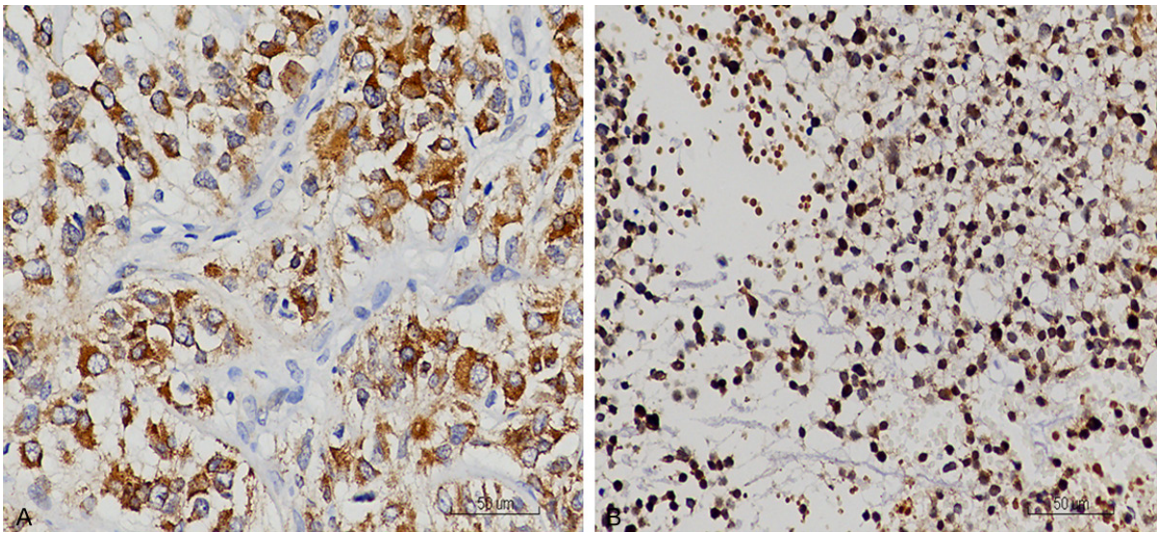

Figure 3. A: Expression of Sox17 protein in the cytoplasm of oligodendrogliomas (EnVision 40 × 10. B: Sox17 protein expression in the nucleus of oligodendrogliomas (EnVision 40 × 10).

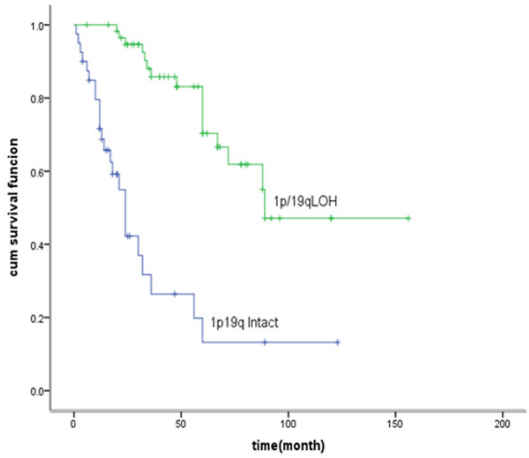

Figure 4. The relationship between survival time after surgery and the presence of 1p/19q in oligodendrogliomas.

Correlation of 1p/19q co-deletion and Sox17 expression with clinicopathological parameters

As shown in **Table 2**, the prevalence of 1p/19q co-deletion and Sox17 expression was higher among younger adults 18 to 60 years of age when compared to older adults > 60 years of age in juveniles; this difference was significant for oligodendrogliomas (P = 0.000). A 1p/19q co-deletion in a frontal, temporal location was more frequent than in other locations (P = 0.050). However, there was no significant relationship between 1p/19q co-deletion and gender, ethnic group or between expression of Sox17 and gender, ethnic group, or location.

Correlation of 1p/19q LOH and Sox17 expression with survival time

Seventy-three oligodendroglioma patients were analyzed at the last follow-up visit. Univariate analysis determined that 1p/19q LOH (**Figure 4**, P = 0.000) Sox17 expression (**Figure 5**, P = 0.000), chemotherapy (P = 0.000) and radiation therapy (P = 0.001) were related to patient survival time, while histologic subtype (P = 0.214), patient age (P = 0.297), ethnicity (P = 0.583), and gender (P = 0.783) were not. Cox multiple factors regression analysis further determined that 1p/19q co-deletion and Sox17 expression were independent prognostic factors of oligodendrogliomas.

Discussion

Oligodendrogliomas occur in the cerebral cortex; 50% are reported in the frontal lobe and the remaining 50% in the parietal and temporal lobes [1]. In recent years, great progress has been made in our understanding of the molecular biology of gliomas. Which can be used to improve the diagnosis, prognosis and provide evidence for the selection of targeted therapy. In the present study, the co-deletion of chromosomes 1p/19q was a common feature of oligodendrogliomas.

Ranghavan et al. reported that oligodendrogliomas with a co-deletion of 1p/19q in older children tended to have a greater overlap with their adult counterparts, and patients > 60 years of

Table 2. Correlations of 1p/19q co-deletion and Sox17 expression with clinicopathological parameters in oligodendrogliomas

Variable	Number	Sox17		*P*	1p/19q co-deletion		*P*
		+	-		+	-	
Age							
< 18 y	8	2 (25.0)	6 (75.0)		1 (12.5)	7 (87.5)	
18-60 y	80	68 (85.0)	12 (15.0)	0.000	58 (72.5)	22 (27.5)	0.000
> 60 y	12	3 (25.0)	9 (75.0)		1 (8.3)	11 (91.7)	
Gender							
Male	60	42 (70.0)	18 (30.0)	0.408	35 (58.3)	25 (41.7)	0.677
Female	40	31 (78.0)	9 (22.0)		25 (62.5)	15 (37.5)	
Ethnic group							
Ethnic Han	61	42 (69.0)	19 (31.0)	0.243	38 (62.3)	23 (37.7)	0.558
Ethnic minority	39	31 (79.0)	8 (21.0)		22 (56.4)	17 (43.6)	
Location							
Frontal, Parietal	64	49 (77.0)	15 (23.0)	0.285	43 (67.2)	21 (32.8)	0.050
Other lobes	36	24 (67.0)	12 (33.0)		17 (47.2)	19 (52.8)	

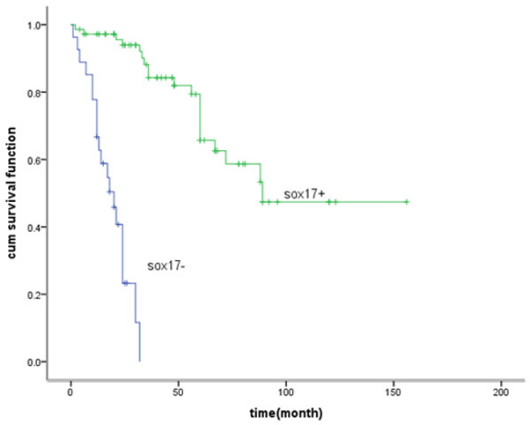

Figure 5. The relationship between survival time after surgery and the expression of Sox17 in oligodendrogliomas.

age had significantly fewer co-deletions of 1p/19q than younger patients [6, 7]. These results are consistent with our observations. This demonstrates that 1p/19q LOH is not a distinct molecular alteration in children and older patients. Age is likely an important factor influencing molecular genetic abnormalities of oligodendrogliomas due to the existence of distinct pathogenetic pathways in the genesis of these oligodendrogliomas or reduced tumor suppressor gene deactivation due to 1p/19q LOH.

We correlated 1p/19q LOH with tumor location and found results similar to prior reports [8-11], in terms of a positive association between 1p/19q LOH and tumor location. Frontal and parietal lobe oligodendrogliomas have more frequent 1p/19q co-deletions than their morphologically indistinguishable counterparts in other lobes. Simultaneously, tumor sites in these lobes were more responsive to chemotherapy than those in other lobes, and patients had better outcomes [12]. We suspect that this distinction may be because of the special cells external environment of cerebrum different location supply growth vigor respectively for development of tumor distinct molecular abnormality, just as Micro environment determinate biological behaviour of neural stem cell and neoplastic stem cell.

In our study, all patients underwent radiotherapy or chemotherapy but because of financial difficulties, not all patients completed the treatment. Oligodendroglioma patients showed a trend toward longer overall survival time in patients with 1p/19q co-deletion. Based on the literature [13-16] and our results, 1p/19q LOH is a prognostic factor only in oligodendroglioma patients treated with formal chemotherapy or/and radiotherapy.

The Sox17 gene is a member of the high mobility superfamily of transcription factors. It is highly conserved during evolution and is closely related to embryonic stem cell development and differentiation, and involved in the formation of oligodendrocytes, blood vessels, and the differentiation of the endoderm [17, 18]. Zhang et al. found that epigenetic inactivation of Sox17 by methylation played a role in the increased incidence of colon cancer, as well as liver and breast cancer [19-21]. However, little of the role of Sox17 in oligodendrocyte neoplasia is known.

In our study, we use an immunohistochemical method to detect Sox17 expression in oligodendrogliomas. Sox17 expression in anaplastic oligodendrogliomas was significantly lower than in oligodendrogliomas, and so may play an important role in the occurrence and development of oligodendrogliomas. Moreover, oligodendrogliomas with 1p/19q LOH had similar results, while the expression of Sox17 was considerably lower in elderly patients and children with Sox17 protein expression. Thus, age is likely an important influencing factor for the genetic abnormalities associated with oligodendrogliomas.

Sox17 has been correlated with tumor cell differentiation, metastasis, and prognosis in a variety of human tumors. Yin et al. [22] reported that Sox17 methylation was associated with the degree of tumor differentiation in lung cancer tissue, while another study [20, 23] reported that Sox17 methylation was closely related to the stage and lymph node metastasis of breast cancer. However, in liver cancer, the expression of Sox17 was negatively correlated with mobility and invasion force. Our results also showed that the prognosis of oligodendrogliomas was improved in those expressing Sox17; these findings are consistent with previous reports that Sox17 expression is reduced during cancer cell invasion and metastasis.

There was a positive correlation between 1p/19q LOH and Sox17 protein expression in oligodendrogliomas. However, the mechanisms underlying the effect of 1p/19q LOH in oligodendrogliomas on chemotherapy sensitivity remain unclear Several studies have reported a correlation 1p/19q LOH and Sox17 protein expression, suggesting a causal relationship, as co-deletion of 1p/19q resulted in increased Sox17 expression, leading to reduced alkylating agent resistance that improved both sensitivity to chemotherapy and efficacy, and prolonged survival.

Testing for 1p/19q LOH testing is costly. However, prediction of 1p/19q LOH by determining Sox17 protein expression would be a more economical alternate detection method. Thus determination of 1p/19q LOH and Sox17 protein expression in oligodendrogliomas will improve the accuracy of clinical diagnosis, guide treatment, and improve the prognosis.

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Disclosure of conflict of interest

None.

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