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Expression, correlation and prognostic significance of CD133, P57 and HSF1 in meningioma

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Abstract. – OBJECTIVE: The present study investigated the expressions of CD133, P57 and HSF1 in meningioma so as to explore their protagonists in the prognosis of meningioma.

PATIENTS AND METHODS: The subjects in the present study included 57 cases of pathologically diagnosed patients with meningioma and 38 cases of non-meningioma patients. The immunofluorescence method was used to detect CD133, p57, and HSF1 expressions in the meningioma tissue. The reverse transcription-polymerase chain reaction (RT-PCR) and Western blotting were used for expression studies in both the normal as well as meningioma tissues.

RESULTS: Immunofluorescence results showed high levels of expression of CD133, P57 and HSF1 in meningioma tissues. Further, the results of variance analyses confirmed that the expressions of CD133, P57, and HSF1 in meningioma tissues and normal brains tissue were significantly different ($p < 0.01$).

CONCLUSIONS: Expressions of CD133, p57, and HSF1 in meningioma are exceptionally high. The above observation could be exploited for the development of a novel treatment for a brain tumor and better clinical diagnosis as well.

Key Words:
Meningioma, CD133, P57, HSF1, Prognostic significance

Introduction

Meningioma is one of the common encephalic tumors observed mainly with slow growth and a long course of the disease[1]. However, in few cases, meningioma occurs with invasive growth too[2]. In meningioma treatment, excision or surgical method is given priority but still patients have shown relapse even after surgery[3,4]. At present, the occurrence, transfer and relapse mechanisms of meningioma are still not well understood. Moreover, with rapid development in molecular biology and gene research, a variety of genes including CD133, P57, HSF1 related to the occurrence and development of meningioma have been acknowledged. Moreover, these genes could act as a potential molecular marker with strong specificity and high sensitivity.

CD133 is a novel marker of the broad spectrum in tumor stem cell and is also expressed in multiple tumor stem cells[5]. As a surface molecular marker, now it has been utilized to separate and identify the existence of tumor stem cell in meningioma[6]. P57 is a gene associated with cell cycle control, and is responsible for preventing cells from transferring through G1/S phase. A study in the recent past has suggested its role as a tumor suppressor gene, belonging to negative regulatory factor during the growth regulation of tumor cell[7]. Furthermore, HSF1 is a kind of transcription factor responsible for the activation of heat shock protein gene transcription[8]. The present study detected the expression of CD133, P57, and HSF1 in both the meningioma tissue as well as normal brain tissues by utilizing immunofluorescence, RT-PCR and Western blotting methods, to provide reference basis for the diagnosis/ treatment of meningioma.

Patients and Methods

Patients

Tumor Tissue Collection

57 surgical patients with meningioma, 38 patients with brain tissue excision and normal meninges from neurosurgery in our Hospital were selected. Paraffin specimens of these patients were collected to detect CD133, P57, and HSF1

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by immunofluorescence. Inclusion criteria: confirmed cases; operative specimen for the first time; without chemical treatment and radiotherapy before the operation; Level I and Level II of Simpson grade for resection scope; the dead within 14 days after treatment excluded. There were 23 males with meningioma, with age from 23 to 72 years old, average age was 48.7 years; 33 females with age from 8 to 75 years old, average age was 46.36 years

Primary Reagents

Trizol reagent (Tiangen, Beijing, China); Reverse transcription kit (Tiangen, Beijing, China); monoclonal antibody anti-actin (β-Actin), anti-CD133, anti-P57, anti- HSF1 and immunofluorescence second antibody (CST, Boston, MA, USA); bicinchoninic acid assay (BCA) protein quantification kit (Beyotime, Shanghai, China).

Experimental Methods

Immunofluorescence

Meningioma tissue was conducted with paraformaldehyde fixation of 4% for 48 hours, with conventional paraffin embedding to turn out section with a thickness of 5 μm. Paraffin sections were processed with antigen retrieval after deparaffination and dehydration with graded ethanol. Sections were rinsed with 0.01M phosphate buffered saline (PBS) (pH 7.4) and the process was repeated three times. It was then put into the wet box of 10% bovine serum albumin (BSA) at 37°C for 30 min. Fluorescence mark was diluted properly (dilution with the proportion of 1:50) and was added to the specimen, followed by incubation at 4°C for over night. It was rinsed for three times (5 min/time) with PBS (pH 7.4), followed by addition of second fluorescence antibody and incubation at 37°C for 2 hours. Glycerin sealing sheet was buffered and observation was performed under a fluorescence microscope.

RT-PCR Detection

About 50 mg meningioma tissues and normal brain tissues were collected respectively, and the RT-PCR detection was performed according to the instructions provided by the kit. According to Table I, primer sequence, convention amplification was applied, and the reaction products were conducted with electrophoretic analysis.

Western Blotting Detection

About 50 mg meningioma tissues and normal brain tissues were collected respectively. Samples with 60 μg protein were proceeded with electrophoretic separation by 10% SDS-PAGE, and the separated protein bands were transferred to polyvinylidene difluoride (PVDF) membrane by the semi-dry process. Skim milk powder of 10% was sealed at room temperature for 1 hour and the primary antibody (1:1000) was incubated overnight at 4°C. After complete membrane cleaning with tris-buffered saline and Tween 20 (TTBS), the second antibody (1:2000) was added and incubated at room temperature for one hour. Blots were then developed and were digitized.

Statistical Analysis

SPSS 17.0 (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. In the analysis of survival, Kaplan-Meier method was taken for single factor analysis and Log-rank method was used for the comparison of the significance of the difference. Cox proportional hazard model was applied to study the influence of various clinical and pathological factors on the recurrence time, $p < 0.05$ standing for the difference with statistical significance.

Results

H&E Staining Results

The hematoxylin and eosin (HE) staining section of meningioma tissue in comparison to normal brain tissue revealed (Figure 1) cells in meningioma tissue. It presented syncytium form arranged in a swirl, a confirmatory pathological feature.

Expression Results of CD133, P57, and HSF1 in Meningioma Tissue and Normal by Immunofluorescence Test

As shown in Figure 2, CD133, P57, and HSF1 in meningioma tissues had fluorescent expres-

Table I. RT-PCR primer sequence of CD44 and GP73 mRNA.

Gene name	Primer sequence
CD133	5'-3' TTCTATGCTGTGTCCTGGGGC 3'-5' TTGTTGGTGCAAGCTCTTCAAGGT
P57	5'-3' CTGATCTCCGATTTCTTCGC 3'-5' TCTTTGGGCTCTAAATTGG
HSF1	5'-3' TCTCCTGTCCTGTGTGCCTACC 3'-5' CAGGTCAACTGCCTACACAGAGC

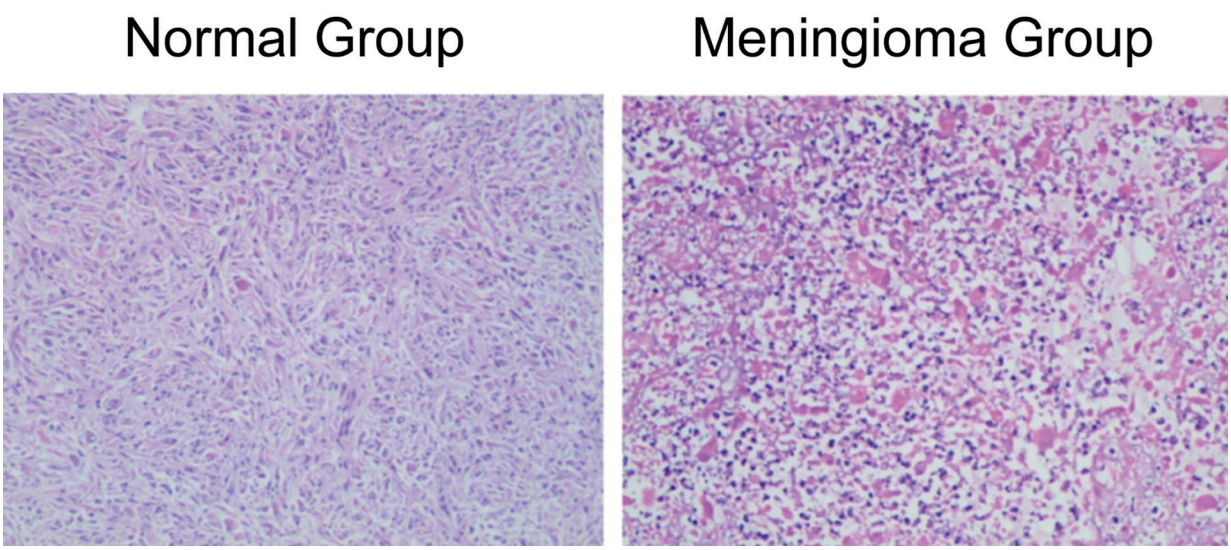

Figure 1. H&E staining result of meningioma tissue and normal brain tissue.

sion. On the other hand, no expression was noticed in normal brain tissue. Also, the expressions of CD133 and HSF1 in meningioma were quite high.

RT-PCR Results

Total RNA extracted from meningioma tissue and normal brain tissue samples were detected by RT-PCR respectively. It showed that the expressions of CD133, P57 and HSF1 were higher than that of normal brain tissue, suggesting that the transcription of meningioma cells to CD133, P57, and HSF1 mRNA (Figure 3).

Western Blotting Results

The results of Western blotting showed high expressions of CD133, P57, and HSF1 in meningioma tissues. As shown in Figure 4, the quantitative expressions of CD133 and HSF1 were very high in meningioma.

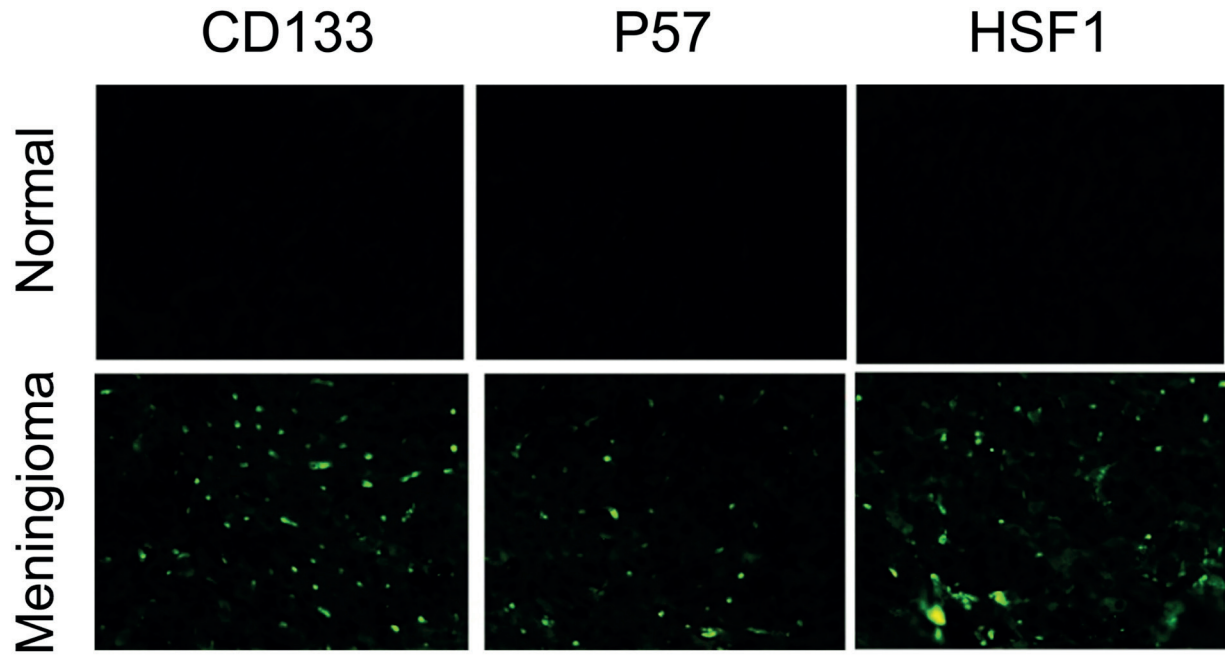

Figure 2. Expression of CD133, P57 and HSF1 in meningioma tissue and normal brain tissue (×400).

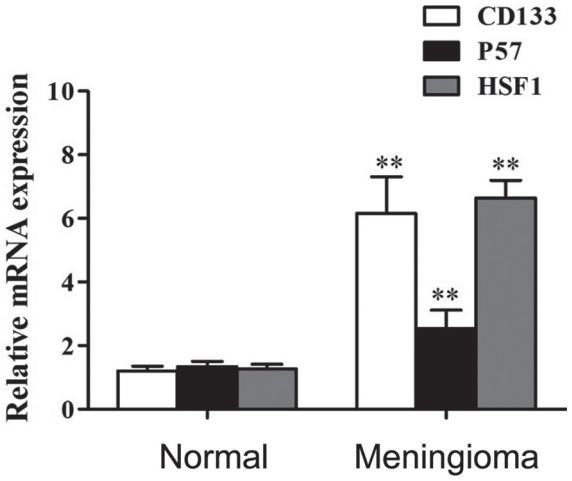

Figure 3. Expression of CD133, P57 and HSF1 in meningioma tissue and normal brain tissue.

Kaplan-Meier Survival Analysis Results

The results of Kaplan-Meier survival analysis illustrated that overall survival of negative expression group of CD133, P57, and HSF1 was superior to that of the obviously positive expression group ($p < 0.05$).

Discussion

Meningioma is a common central nervous system tumor, which originated from arachnoid villus cell[9]. Meningioma is a common intracranial tumor, accounting for 20% of primary intracranial tumors, which could be divided into benign (I Level), atypical (II Level), malignancy meningioma (III Level) according to the Meningioma Classification Standards that was issued by WHO. I Level of meningioma accounts for about 90%, II Level for 4.7%-7.2% and III Level for 1%-3% in the pathological pattern. Meningioma could form space occupying lesion, so headache, epilepsy, and diminution of vision were the most common clinical symptoms[10-12]. Surgery is the main treatment of meningioma at present, and other treatment approaches included radiotherapy, chemotherapy, and biotherapy. However, partial meningioma could manifest invasiveness growth, with easy recurrence after excision and distant metastasis. These are the key factors that influenced patient prognosis. At present, most retrospective studies[13,14] hold that the recurrence rate of II and III Levels is pretty high, and the pathological grading is the key that influences prognosis. The occurrence and development of meningioma are still not clear so far.

The emerging tumor stem cell theory believed that they are theses cell masses are few but have abilities to keep the self-renewability and multi-directional differentiation in tumor tissue. Further, CDl33 is a kind of membrane protein that was observed in recent years with conservativeness among species. The existing research verified that CDl33 antigen could be a surface marker for the brain cancer, prostate cancer, pancreatic cancer, large intestine cancer and other cancers[15,16]. Furthermore, researches[16,17]

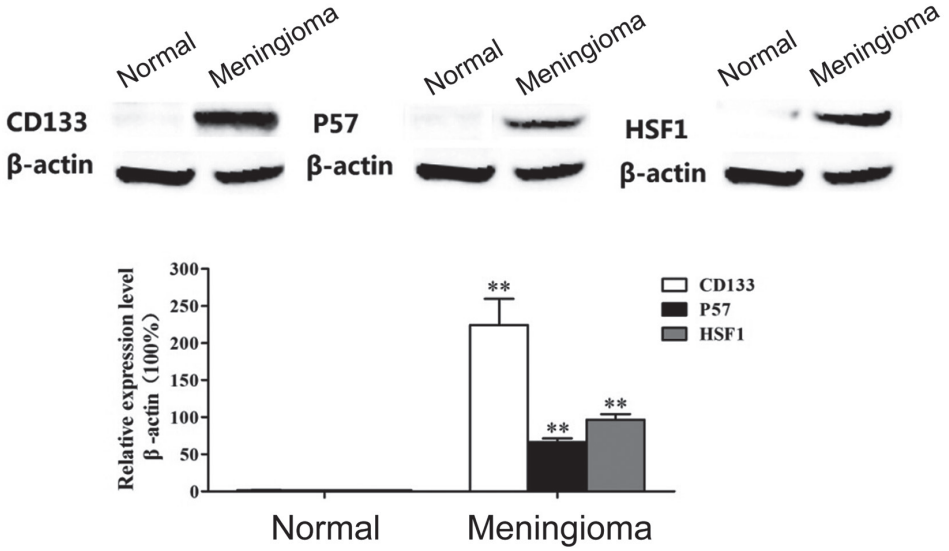

Figure 4. Expression of CD133, P57 and HSF1 in meningioma tissue and normal brain tissue.

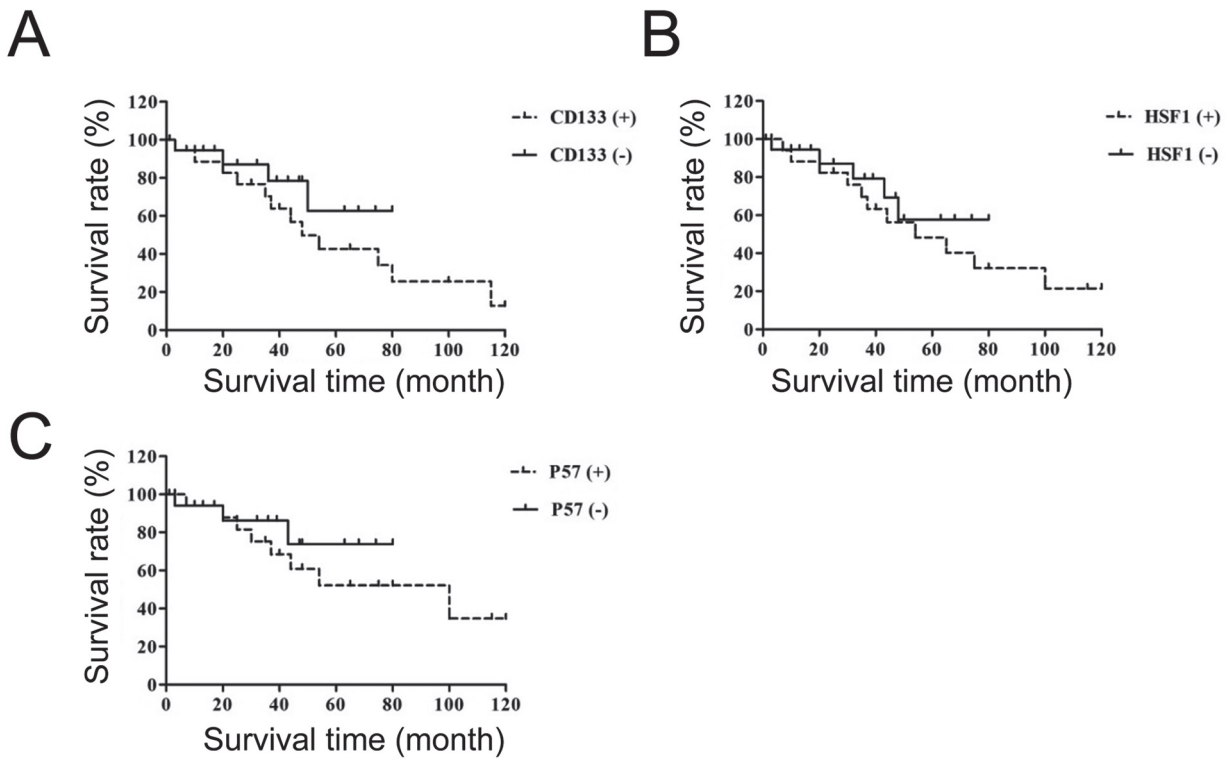

Figure 5. Relationship between CD133, P57 and HSF1 and prognostic of patients with meningioma. ***A,*** CD133; ***B,*** P57; ***C,*** HSF1.

also found that CDl33 could act as a marker to predict tumor biological behavior. Further, P57 protein has the function of restricted regulation of cell cycle[18], which realized its effect mainly by suppressing Cyclin CDK compound to pass G1 stage[19,20]. In eukaryotes, all inducible transcriptions of heat shock protein need a trans-regulator protein-heat shock factor (HSF) for opposite integration, as the necessary special sequence for the genetic transcription, namely, heat shock element (HSE)[21,22]. The study showed that the expression of CD133, P57, and HSF1 in meningioma tissue were pretty high. The results of RT-PCR and Western blotting indicated that the comparison between CD133, P57, and HSF1 in normal brain tissue and those in meningioma tissue were statistically significant ($p < 0.01$).

Conclusions

CD133, P57, and HSF1 played an important role in the growth and development of meningioma, which could act as the potential target for meningioma treatment, providing a new basis for clinical diagnosis.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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