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Expression of LRIG1 in pituitary tumor and its clinical significance

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Abstract. – OBJECTIVE: To analyze the expression of leucine-rich and immunoglobulin-like domain gene1 (LRIG1) in pituitary tumor and its clinical significance.

PATIENTS AND METHODS: Patients were divided into two groups: hypophysoma group (n = 80) and normal group (normal brain tissue, n = 30). The immune tissue chemical streptavidin avidin-peroxidase was applied to detect the expression of LRIG1 of both groups and to analyze its relationship with the patients' prognosis.

RESULTS: The positive expression rate of LRIG1 in normal brain tissues was significantly higher than that in pituitary adenomas (100% *vs*. 53.8%) ($p < 0.05$). The positive expression rate of LRIG1 in pituitary tumors was not significantly related to age and gender, the difference was not statistically significant ($p > 0.05$). The positive expression rate of LRIGl in non-invasive pituitary adenomas was higher than that in invasive pituitary tumors (68.4% *vs*. 21.7%), the difference was statistically significant ($p < 0.05$). Cox multivariate survival analysis showed that LRIG1 can be used as an independent factor for prognosis evaluation. Meier survival analysis showed that the LRIG1 and pituitary tumor types were significantly associated with the prognosis of patients ($p < 0.05$).

CONCLUSIONS: LRIG1 was involved in the occurrence and development of pituitary tumor, the expression of LRIG1 can be used as an indicator for prognosis evaluation, and low expression indicated a poor prognosis.

Key Words:
LRIG1, Pituitary tumor, Prognosis.

Introduction

Leucine-rich and immunoglobulin-like gene1 (LRIGl) is a newly discovered member of the human gene (Gene pool number: AF381545). Studies have indicated that LRIG1 was associated with the occurrence and development of tumor in that it could suppress cancer[1-4]. The role of LRIG1 and the mechanism of tumor suppression are still unknown. LRIG1 is located in chromosome 3p14.3 locus, which is the predilection site for the loss of heterozygosity of different human tumor genes[5]. Studies have found that it might be the mechanism of LRIG1 inhibition of EGFR signaling that inhibited tumor proliferation and induced apoptosis of tumor cells. Because EGFR (epidermal growth factor receptor) itself has tyrosine kinase activity, which once combining with epidermal growth factor (EGF) could initiate related genes in the nucleus, thus, promoting cell division and proliferation. EGFR expressions in gastric cancer, breast cancer, bladder cancer, and head and neck squamous cell carcinoma were higher, but the relationship between LRIG1 and the pituitary tumor was rarely reported. In this study, we have adopted an immunohistochemical method to detect the expression of LRIG1 in pituitary tumors and normal brain tissues, and analyze the expression and clinical significance of LRIG1 in pituitary tumors.

Patients and Methods

Patients

80 patients with pituitary tumor, hospitalized in our hospital from January 2010 to January 2015 were included in pituitary tumor group. All of them accepted pituitary tumor resection in neurosurgery of our hospital. The 80 patients consisted of 33 females and 47 males, ranging from 22-67 years, on average (44.3 ± 15.2) years. 35 patients had prolactin adenomas, 22 patients had growth hormone adenomas, 11 patients had

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sex hormone adenomas, 6 cases had non-functional adenomas, and 6 patients had multiple hormone adenomas. 23 cases were invasive pituitary tumors and 57 cases were non-invasive pituitary tumors. The normal group consists of 30 patients, 18 males and 12 females, ranging from 23-68 years, on average (45.5 ± 14.8) years. There was no difference between the two groups in age and gender ratio ($p > 0.05$) (Table I).

Methods

Immunohistochemical Staining

After formalin fixation, ethanol gradient dehydration, wax dip, and paraffin embedding, the tissue samples were cut into slices of thickness 2 mm. High-pressure thermal remediation was conducted after conventional xylene and ethanol gradient dewaxing and dehydration. Subsequently, 1% hydrogen peroxide was used to inactivate endogenous peroxidase, washed by phosphate buffer for three times (3 minutes). Next, LRIG1 monoclonal antibody (Santa Cruz Co., Santa Cruz, CA, USA) (1:80) was added dropwise, incubated at 37°C for two hours and washed with phosphate buffer for three times (3 minutes). Consequently, the second antibody was added dropwise and incubated for thirty minutes. Finally, the color was developed by diaminobenzidine (DAB) (Dako Company, Glostrup, Denmark) and counter stained with hematoxylin.

Observation Indexes

LRIG1 expresses in nucleus and cytoplasm, and appear as brown or brownish yellow. 10 high power field under ×400 was selected and 500 tumor cells were counted, to calculate the percentage of positive cells. Percentage of positive cells less than 10% was negative (-); 10%-50% was positive (+), > 50% was strongly positive (*).

Statistical Analysis

SPSS19.0 statistical software (SPSS Inc., Chicago, IL, USA) was applied for data analysis. Chi-square test was used to compare the sample rate, Kaplan-Meier method was used to make single factor survival analysis, Log-rank test was applied to compare the significant difference, and Cox risk ratio model analysis was applied to make the multivariate survival analysis. $p < 0.05$ was considered statistically significant.

Results

Expression of LRIG1 in Different Tissues

LRIG1 positively expressed in the nucleus and cytoplasm, and its color was brown or brown yellow. The results showed that the positive expression rate of LRIG1 in normal brain tissues was 100.0% (30/30), in brownish yellow (Figure 1A). The positive expression rate of LRIG1 in pituitary tumor rate was 53.8% (43/80), in black blue (Figure 1B, 1C). The positive expression rate of the former was higher than that of the latter, and the difference was statistically significant ($p < 0.01$) (Table I).

Relationship of LRIG1 Expression with age, Gender and Type of Pituitary Tumor

The differences on LRIG1 high expression rate (54.8% *vs.* 55.1%) and low expression rate (45.1% *vs.* 44.9%) between the pituitary tumor patients £45 years and > 45 years were not statistically significant ($p > 0.05$). Differences on LRIG1 high expression rate (51.1% *vs.* 54.5%) and low expression rate (48.9% *vs.* 45.5%) between the male patients and female patients were not statistically significant ($p > 0.05$). LRIG1 high expression rate of non-invasive patients (68.4% *vs.* 21.7%) was significantly higher than that of invasive patients (68.4% *vs.* 21.7%), and the difference was statistically significant ($p < 0.05$). LRIG1 low expression rate of invasive patients was significantly higher than that of non-invasive patients (78.3% *vs.* 31.6%), and the difference was statistically significant ($p < 0.05$) (Table II).

Table I. General information of the two groups, and the comparison of the expression of LRIG1 in normal brain tissues and pituitary tumors.

Groups	Pituitary tumor group (n = 80)	Normal group (n = 30)	p
Male/female	47/33	17/13	> 0.05
Age range	22-67	23-68	> 0.05
Average age	44.3 ± 15.2	45.5 ± 14.8	> 0.05
LRIG1 positive expression rate	53.8% (43/80)	100% (30/30)	< 0.001

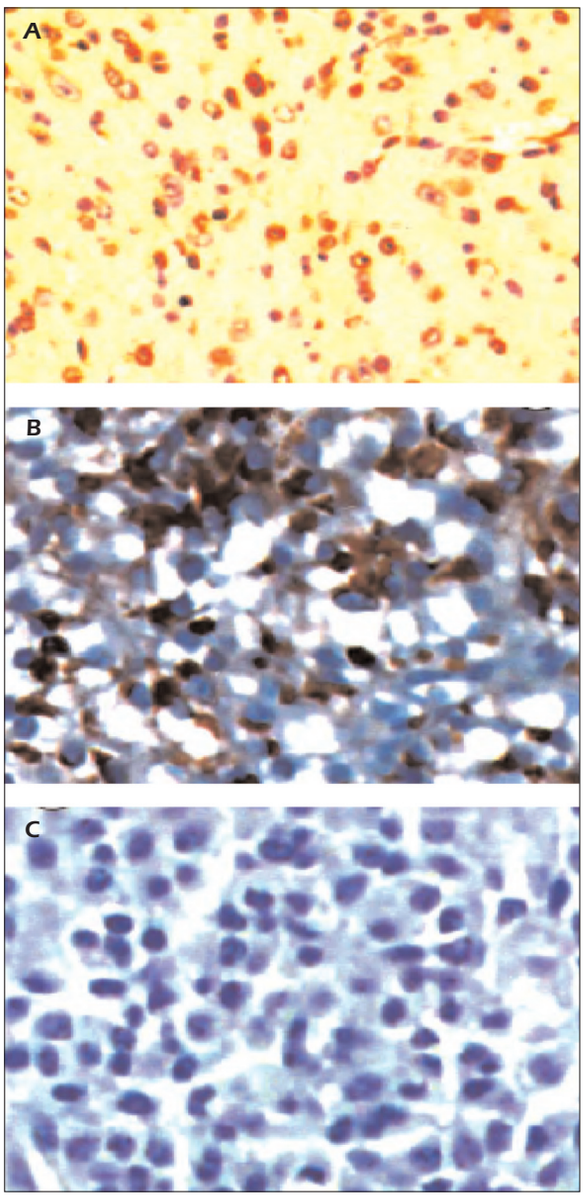

Figure 1. Expression of LRIGl in normal brain tissue and pituitary tumor (immunohistochemical staining, ×400). ***A,*** Normal brain tissue. ***B,*** Noninvasive pituitary tumor. ***C,*** Invasive pituitary tumor.

Relationship of LRIG1 Expression Rate and Pituitary Tumor type with Survival and Prognosis

LRIG1 expression and pituitary tumor types were significantly correlated with the prognosis of patients. The results of single factor survival analysis under Kaplan-Meier showed that the median survival time of patients with high expression of LRIG1 was significantly higher than that in patients with low expression ($p < 0.01$). Compared with patients with non-invasive pituitary tumors, the median survival time of patients with invasive pituitary tumors was significantly ($p < 0.05$). Cox multivariate survival analysis showed that LRIG1 can be used as an independent factor for prognosis evaluation of patients with pituitary tumor while pituitary tumor type cannot be used as an independent factor for prognosis evaluation of pituitary tumors.

Discussion

LRIG1 is a newly discovered tumor suppressor gene, which is one of the members of the LRIGs family LRIGs family including LRIG1, LRIG2 and LRIG3. LRIG1 widely exists in mammals. Hedman et al[6] found that LRIG1 had different expressions in various tissues of human body, its expression in spleen was the lowest while in the brain tissues was the highest and the difference was 240 times, and LRIG1 transcripts were largely expressed in heart, brain, kidney, skeletal muscle, stomach and testis. The mRNA transcript by LRIG1 and proteins expressed by LRIG1 in tumor cells, such as lung cancer cells, colon cancer cells, clear cell carcinoma cells, prostate cancer cells, and skin squamous cell carcinoma were significantly lower than those in normal tissues[7-9]. The study of Qi et al[10] showed that over-expression

Table II. Relationship of LRIG1 expression with age, gender and type of pituitary tumor.

Clinical parameters		Case	LRIG1 high expression	LRIG1 low expression
Age	≤ 45	31	17 (54.8%)	14 (45.1%)[1]
	> 45	49	27 (55.1%)	22 (44.9%)
Gender	Male	47	24 (51.1%)	23 (48.9%)[2]
	Female	33	18 (54.5%)	15 (45.5%)
Type	Non-invasive	57	39 (68.4%)	18 (31.6%)[3]
	Invasive	23	5 (21.7%)	18 (78.3%)

[1]Compared with > 45, $p > 0.05$; [2]Compared with female group, $p > 0.05$; [3]Compared with invasive $p < 0.05$.

of LRIG1 could significantly inhibit the expression of EGFR in mRNA and protein in Hep-G2 cells. A study[11] has shown that after chemotherapy on glioma patients with cisplatin, LRIG1 enhanced the sensitivity of P-EGFR cells to cisplatin by down regulating P-EGFR, and promoted the apoptosis of glioma cells. The study of Yang et al[12] and Liu et al[13] showed that inhibiting or interfering the expression of LRIG1 will result in the proliferation of GL15 of human brain glioma cell line. Lindstrom et al[14] found that all of the 128 cervical carcinoma patients in their study had LRIG1 expression, and as clinical stage increased, the expression of LRIG1 decreased. In contrast to most studies, it is shown that 40% (11/28) of the expression of LRIG1 in breast cancer was positive[15], Ingrid et al[16] discovered that LRIG1 expression of one patient was positive. Some other studies[17] also indicated that the expression of LRIG1 of leukemia patients was up-regulated.

The results of our investigation indicated that the positive expression rate of LRIG1 in pituitary tumor was 53.8%, which was significantly higher than that of normal brain tissue (100%), indicating that the occurrence of pituitary tumor was related to the down-regulation of LRIG1, and LRIG1 was likely to be involved in the occurrence of pituitary tumor as a kind of tumor suppressor. Han et al[18] suggested that LRIG gene family and EGFR gene had different expressions in HP75 cell line and normal pituitary tissues. Wang et al[19] indicated that pre-operative EGFR detection can reflect the proliferation of pituitary tumors to a certain extent, and pre-operative EGFR detection may be a good indicator for the diagnosis of pituitary tumor invasiveness and the evaluation of prognosis. And the tumor suppression effect of LRIG1 was realized by participating in the formation of EGFR negative feedback loop, which confirmed the results of our study. Also, the results of our study indicated that the high expression of LRIG1 in non-invasive patients was significantly higher than that in patients with invasive invasion (68.4% *vs.* 21.7%) ($p < 0.05$). The low expression of LRIG1 in invasive patients was higher than that in non-invasive patients (78.3% *vs.* 31.6%), the difference was statistically significant ($p < 0.05$), which confirmed that the expression of LRIG1 was correlated with tumor invasion. By the comparison and the analysis of the expression of LRIG1 in 74 pituitary tumors and 20 normal brain tissues, Zhang et al[21] concluded that LRIG1 expression was associated with tumor invasion. Our study based on the postoperative survival data of the patients, showed that the median survival time of patients with high LRIG1 expression was significantly longer than that of low expression.

Conclusions

LRIG1 was involved in the occurrence and development of pituitary tumor, the expression of LRIG1 can be used as an indicator for prognosis evaluation, and low expression indicated poor prognosis.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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