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Correlation of Claudins6 (CLDN6) gene expression in meningioma tissue with the expression of matrix metalloproteinases (MMPs)/tissue inhibitors of matrix metalloproteinase (TIMPs) and epithelial−mesenchymal transition (EMT) genes

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ABSTRACT

Objective: To study the correlation of Claudins6 (CLDN6) gene expression in meningioma tissue with the expression of matrix metalloproteinases (MMPs)/tissue inhibitors of matrix metalloproteinase (TIMPs) and epithelial-mesenchymal transition (EMT) genes. **Methods:** Meningioma tissue samples that were surgically removed in Yibin First People's Hospital between April 2014 and May 2017 were selected, normal arachnoid tissue samples that were collected from decompressive craniectomy in Yibin First People's Hospital during the same period were selected, and the expression of CLDN6, MMPs/TIMPs and EMT genes in tissues were determined. **Results:** CLDN6 protein expression in meningioma tissue was significantly lower than that in normal arachnoid tissue; EMMPRIN, MMP2, MMP9, Vimentin and N-cadherin protein expression in meningioma tissue were significantly higher than those in normal arachnoid tissue while TIMP1, TIMP2, E-cadherin and α-catenin protein expression were significantly lower than those in normal arachnoid tissue; EMMPRIN, MMP2, MMP9, Vimentin and N-cadherin protein expression in meningioma tissue with higher CLDN6 expression were significantly lower than those in meningioma tissue with lower CLDN6 expression while TIMP1, TIMP2, E-cadherin and α-catenin protein expression were significantly higher than those in meningioma tissue with lower CLDN6 expression. **Conclusion:** Lowly expressed CLDN6 gene in meningioma tissue can increase the hydrolysis activity of MMPs, induce epithelial-mesenchymal transition and thus promote the invasive growth of meningioma.

1. Introduction

Meningioma is a common mesenchymal tumor of the central nervous system. It has the characteristics of infiltrative growth and may invade the cerebral dura mater and adhere to it[1,2]. The enhanced hydrolysis activity of matrix metalloproteinases (MMPs) and the abnormal activation of epithelial-mesenchymal transition (EMT) are closely correlated with the invasive growth of meningiomas[3]. Claudins (CLDNs) are a class of cytoskeleton proteins that are involved in the composition of intercellular tight junction, and can form tight junction and adhesion between cells, and inhibit the cell migration and invasion to the distant tissue. In the occurrence and development of many malignant tumors, the expression changes of multiple members in CLDNs family are related to the invasive growth of cancer cells. CLDN6 is a member of CLDNs family with the activity of tumor suppressor genes, and its expression deletion is confirmed to be related to the occurrence of malignant tumors such as gastric cancer and ovarian cancer. However, it is not yet clear about the relationship of CLDN6 with the occurrence and development of meningioma. In the following studies, we analyzed the correlation of CLDN6 gene expression in meningioma tissue with the expression of MMPs/TIMPs and EMT genes.

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2. Clinical sample information and experimental methods

2.1 General information of clinical samples

Meningioma tissue samples that were surgically removed in Yibin First People's Hospital between April 2014 and May 2017 were selected, all patients were with meningioma confirmed by postoperative pathology examination, and there were a total of 52 cases of patients, including 32 men and 20 women that were 42-64 years old; normal arachnoid tissue samples that were collected from decompressive craniectomy in Yibin First People′s Hospital during the same period were selected, all patients had a clear history of craniocerebral trauma, were with intracranial hematoma confirmed by skull CT, and were without previous medical history of intracranial tumor, and there were a total of 36 patients, including 20 men and 16 women that were 39-58 years old. There was no significant difference in the general data between patients with meningiomas and patients with craniocerebral trauma (P>0.05).

2.2 Experimental methods

2.2.1 Clinical sample collection

Moderate amount of meningioma tissue was collected after surgical resection of meningioma, moderate amount of normal arachnoid tissue was collected during decompressive craniectomy, and the tissue was cleaned with saline to remove the residual blood, frozen quickly with liquid nitrogen and then placed in -70 ℃ refrigerator.

2.2.2 Gene protein expression detection

Moderate amount of meningioma tissue and normal arachnoid tissue were taken, added in protein lysis buffer RIPA and fully ground, the obtained tissue grinding liquid was centrifuged for 10 min at a speed of 12 000 r/min, the supernatant was taken to detect total protein content with BCA kit, enzyme-linked immunosorbent assay kit was used to determine CLDN6, EMMPRIN, MMP2, MMP9, TIMP1 and TIMP2 contents, and the CLDN6, EMMPRIN, MMP2, MMP9, TIMP1 and TIMP2 protein expression per mg total protein were calculated.

2.3 Statistical methods

SPSS 19.0 software was used to input data, the median of CLDN6 protein expression in meningioma tissue was calculated, the meningioma tissue with CLDN6 expression ＞ the median was judged as that with higher CLDN6 expression, and the meningioma tissue with CLDN6 expression ＜ the median were judged as that with lower CLDN6 expression. Differences in gene expression between two groups were analyzed by t test and P＜0.05 indicated statistical significance in differences.

3. Results

3.1 CLDN6 expression in meningioma tissue

CLDN6 protein expression in meningioma tissue and normal arachnoid tissue were (3.79±0.52) ng/mg protein and (8.15±1.06) ng/mg protein respectively. t test showed that CLDN6 protein expression in meningioma tissue was significantly lower than that in normal arachnoid tissue. Differences were statistically significant in CLDN6 protein expression in meningioma tissue and normal arachnoid tissue (P<0.05).

3.2 MMPs/TIMPs expression in meningioma tissue

Analysis of EMMPRIN (ng/mg protein), MMP2 (ng/mg protein), MMP9 (ng/mg protein), TIMP1 (pg/mg protein) and TIMP2 (pg/mg protein) protein expression in meningioma tissue and normal arachnoid tissue was as follows: EMMPRIN, MMP2 and MMP9 protein expression in meningioma tissue were significantly higher than those in normal arachnoid tissue while TIMP1 and TIMP2 protein expression were significantly lower than those in normal arachnoid tissue. Differences were statistically significant in EMMPRIN, MMP2, MMP9, TIMP1 and TIMP2 protein expression in meningioma tissue and normal arachnoid tissue (P<0.05).

Analysis of EMMPRIN, MMP2, MMP9, TIMP1 and TIMP2 protein expression in meningioma tissue with different CLDN6

Table 1.
MMPs/TIMPs expression in meningioma tissue and normal arachnoid tissue.

Tissue origin	n	EMMPRIN	MMP2	MMP9	TIMP1	TIMP2
Meningioma	52	3.28±0.62	7.48±0.93	6.61±0.82	186.53±22.35	126.26±16.28
Normal arachnoid	36	1.17±0.20	2.94±0.41	3.02±0.46	459.79±61.27	294.52±42.48
T		17.398	16.463	11.236	13.587	12.526
P		＜0.05	＜0.05	＜0.05	＜0.05	＜0.05

Table 2.
MMPs/TIMPs expression in meningioma tissue with different CLDN6 protein expression.

CLDN6	n	EMMPRIN	MMP2	MMP9	TIMP1	TIMP2
Low expression	26	4.41±0.76	9.93±1.18	9.16±1.09	117.65±15.28	82.31±11.29
High expression	26	2.14±0.34	5.13±0.72	4.28±0.59	264.27±29.47	174.42±22.31
T		10.398	9.038	11.237	14.245	10.917
P		＜0.05	＜0.05	＜0.05	＜0.05	＜0.05

Table 3.

EMT gene expression in meningioma tissue and normal arachnoid tissue.

Tissue origin	*n*	E-cadherin	-catenin	Vimentin	N-cadherin
Meningioma	52	1.57±0.22	79.42±9.51	2.38±0.41	1.28±0.17
Normal arachnoid	36	4.52±0.59	198.48±25.57	0.98±0.12	0.52±0.07
T		17.049	12.352	14.527	13.418
P		<0.05	<0.05	<0.05	<0.05

Table 4.

EMT gene expression in meningioma tissue with different CLDN6 protein expression.

CLDN6	n	E-cadherin	-catenin	Vimentin	N-cadherin
Low expression	26	0.92±0.11	52.34±7.52	3.31±0.56	1.83±0.22
High expression	26	2.24±0.38	116.58±15.522	1.42±0.22	0.71±0.09
T		13.478	11.917	12.038	12.846
P		<0.05	<0.05	<0.05	<0.05

protein expression was as follows: EMMPRIN, MMP2 and MMP9 protein expression in meningioma tissue with higher CLDN6 expression were significantly lower than those in meningioma tissue with lower CLDN6 expression while TIMP1 and TIMP2 protein expression were significantly higher than those in meningioma tissue with lower CLDN6 expression. Differences were statistically significant in EMMPRIN, MMP2, MMP9, TIMP1 and TIMP2 protein expression in meningioma tissue with different CLDN6 protein expression (P<0.05).

3.3 EMT gene expression in meningioma tissue

Analysis of EMT genes E-cadherin, α-catenin, Vimentin and N-cadherin protein expression in meningioma tissue and normal arachnoid tissue was as follows: E-cadherin and α-catenin protein expression in meningioma tissue were significantly lower than those in normal arachnoid tissue while Vimentin and N-cadherin protein expression were significantly higher than those in normal arachnoid tissue. Differences were statistically significant in E-cadherin, α-catenin, Vimentin and N-cadherin protein expression in meningioma tissue and normal arachnoid tissue (P<0.05).

Analysis of EMT genes E-cadherin, α-catenin, Vimentin and N-cadherin protein expression in meningioma tissue with different CLDN6 protein expression was as follows: E-cadherin and α-catenin protein expression in meningioma tissue with higher CLDN6 expression were significantly higher than those in meningioma tissue with lower CLDN6 expression while Vimentin and N-cadherin protein expression were significantly lower than those in meningioma tissue with lower CLDN6 expression. Differences were statistically significant in E-cadherin, α-catenin, Vimentin and N-cadherin protein expression in meningioma tissue with different CLDN6 protein expression (P<0.05).

4. Discussion

Meningiomas have strong invasiveness and can invade the dura mater and cause tissue adhesion[4,5], but the specific mechanism for regulating the invasion of meningioma cells is not yet clear. CLDN6 is a member of the CLDN family and contains four transmembrane domains, and the structure of carboxyl terminal participates in the composition of intercellular tight junction. CLDN6 expression is rich in the cell membrane of the adjacent surface of epithelial cells, and can maintain the polarity and directional arrangement between epithelial cells, and prevent the material dispersion among different functional areas, which makes cells anchor in local tissue and avoids abnormal cell migration and invasion. In the occurrence and development process of malignant tumors such as lung cancer, gastric cancer and breast cancer, CLDN6 shows the characteristics of the tumor suppressor genes and presents the tendency of lower expression in tumor lesions, and lower expression of CLDN6 can promote cancer cell migration and invasion[6-9]. In order to define whether the abnormal CLDN6 expression was related to the occurrence of meningioma, the CLDN6 expression in meningioma tissue was analyzed in the study, and the results showed that CLDN6 protein expression in meningioma tissue was significantly lower than that in normal arachnoid tissue. This indicates that the low expression of CLDN6 is related to the occurrence of meningiomas, and the reduction of CLDN6 expression can result in the loss of intercellular tight junction and the enhanced properties of cell migration and invasion.

The degradation of extracellular matrix and cellular basement membrane is the important pathological link for tumor cells to leave the primary lesion and infiltrate towards the adjacent tissue[10]. MMP2 and MMP9 in MMPs family on the components such as IV collagen, laminin and elastin in the extracellular matrix and cellular basal membrane, and can promote can promote cell migration and invasion. EMMPRIN is an inducer of MMP2 and MMP9 expression, which can significantly increase the expression of MMP2 and MMP9 and enhance their hydrolytic activity [11-12]; TIMP1 and TIMP2 are the inhibitory molecules of various MMPs, which can be combined

with MMP2 and MMP9 to inhibit their hydrolysis activity[13,14]. In the study, analysis of the expression of MMPs/TIMPs in meningioma tissue showed that EMMPRIN, MMP2 and MMP9 protein expression in meningioma tissue were significantly higher than those in normal arachnoid tissue while TIMP1 and TIMP2 protein expression were significantly lower than those in normal arachnoid tissue. This indicates that the high expression of MMP2 and MMP9 as well as the low expression of corresponding inhibitory molecules are related to the occurrence of meningiomas, and the enhancement of MMP hydrolysis activity is beneficial to the invasive growth of meningiomas. Further analysis of the correlation between CLDN6 expression and MMPs/TIMPs expression showed that EMMPRIN, MMP2 and MMP9 protein expression in meningioma tissue with higher CLDN6 expression were significantly lower than those in meningioma tissue with lower CLDN6 expression while TIMP1 and TIMP2 protein expression were significantly higher than those in meningioma tissue with lower CLDN6 expression. It show that low expression of CLDN6 in meningioma tissue can affect the balance of MMPs/TIMPs, increase the expression of MMP2 and MMP9 and reduce the expression of corresponding inhibitory molecules so as to enhance the hydrolytic activity of MMPs and promote the invasive growth of meningioma.

On the basis of degrading extracellular matrix and cellular basement membrane, tumor cells can obtain strong movement performance through epithelial mesenchymal transition, which makes the cells leave the primary lesions and transfer to the distant tissue. E-cadherin is a marker molecule of epithelial phenotype cells, which can mediate the calcium-dependent intercellular adhesion and is beneficial to the formation of intercellular tight junction; -catenin is able to attach E-cadherin to actin and provide strong mechanical junction for intercellular adhesion and cytoskeleton[15,16]. Vimentin and N-cadherin are markers of interstitial phenotype cells that can promote cell movement and migration[17]. In the study, analysis of EMT gene expression in meningioma tissue showed that E-cadherin and α-catenin protein expression in meningioma tissue were significantly lower than those in normal arachnoid tissue while Vimentin and N-cadherin protein expression were significantly higher than those in normal arachnoid tissue. It means that the decreased expression of epithelial phenotype markers and the increased expression of mesenchymal phenotype markers are associated with the occurrence of meningioma, excessive EMT process can weaken the intercellular adhesion and enhance the cell movement ability, and it is conducive to invasive growth of meningioma. Further analysis of the correlation between CLDN6 expression and EMT gene expression indicated that E-cadherin and α-catenin protein expression in meningioma tissue with higher CLDN6 expression were significantly higher than those in meningioma tissue with lower CLDN6 expression while Vimentin and N-cadherin protein expression were significantly lower than those in meningioma tissue with lower CLDN6 expression. It means that the low expression of CLDN6 in meningioma tissue can decrease the expression of epithelial phenotype markers and increase the expression of mesenchymal phenotype markers so as to promote the EMT process and the invasive growth of meningioma.

The CLDN6 gene is lowly expressed in in meningioma tissue; lowly expressed CLDN6 can affect MMPs/TIMPs balance, enhance MMPs hydrolysis activity, and also mediate the epithelial-mesenchymal transition.

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