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The correlation between the expression of ADAM17, EGFR and Ki-67 in malignant gliomas

J. SUN, D.-M. LI, J. HUANG, J. LIU, B. SUN, D.-L. FU, G.-S. MAO

Departments of Neurosurgery, Chinese People's Armed Police Force General Hospital, Beijing, China

Abstract. – OBJECTIVE: The aim of this study was to analyze the depolymerization in metalloproteinase (ADAM17), epidermal growth factor receptor (EGFR), the expression of Ki-67 of glioma patients and the correlations with malignancy.

PATIENTS AND METHODS: 53 brain glioma samples resected from patients who had surgery from April 2015 to May 2016 at Chinese People's Armed Police Force General Hospital were selected. According to the degree of malignancy: 22 patients were divided into a deterioration group (stage I to II); 31 patients in highly deteriorated group (stage III to IV); 14 brain tissue samples of traumatic decompression from the hospital as control group. The immunohistochemical method was used to detect the expression of ADAM17, EGFR, and Ki-67 in three groups, and the correlation between the expression of ADAM17, EGFR, and Ki-67. Thus, the stages of malignancy were analyzed.

RESULTS: ADAM17, EGFR, and Ki-67 had no expression or weak expression in the control group, and increased in the low stage of deterioration group; the differences were statistically significant ($p < 0.05$). The positive expression rates of ADAM17, EGFR, and KI-67 were significantly higher in the high deterioration group than that in the control group ($p < 0.05$). Moreover, the analysis showed that the expression of ADAM17, EGFR, and Ki-67 were positively correlated with the stage of malignancy (R = 0.823, $p = 0.000$; R = 0.804, $p = 0.000$; R = 0.811, $p = 0.000$).

CONCLUSIONS: The results suggested that there was a significant positive correlation between ADAM17, EGFR, and Ki-67 with the stage of malignancy.

Key Words:
Brain glioma, Deterioration, EGFR, ADAM17, Ki-67.

Introduction

Glioma is a type of intracranial tumor, which occurs in 40%-50% of all brain tumors worldwide[1]. Since there are high growth rate and high stage of malignancy of gliomas, it is difficult to complete the radical resection[2]. Therefore, there is great clinical need to learning the factors which correlate to the malignant degree of gliomas. A disintegrin and metalloproteinase 17 (ADAM17) is an important member of the ADAM family[3]. It has been found that ADAM17 works as protein cleavage enzyme and plays an important role in tumor proliferation and metastasis[4]. At present, it has been confirmed that ADAM17 is highly expressed in cervical cancer, breast cancer, esophageal cancer and other malignant tumors[5,6]. Epidermal growth factor receptor (EGFR) is a receptor with tyrosine kinase activity, which further activates the signaling pathway by binding and phosphorylating with the ligand and then promotes the invasion and development of tumor. Ki-67 is a cell proliferation associated nuclear protein, and its expression level is related to the proliferation of tumor cells[7]. In recent years, it has been reported that ADAM17 may be involved in the activation of EGFR and take part in tumor development. The aim of this study was to analyze the expression of ADAM17, EGFR, and Ki-67 in patients with glioma and study correlations between the proteins and the degrees of malignancy.

Patients and Methods

Patients

From April 2015 to May 2016, 53 patients with glioma were selected in our hospital (Chinese People's Armed Police Force General Hospital, Beijing, China). There were 32 male and 21 female patients with age ranged 28-71 years old (43.3 ± 10.4).

Inclusion criteria: (1) All patients were examined by CT or MRI before surgery and were

Corresponding Author: Gengsheng Mao, MD; e-mail: tvbop194@sina.com

diagnosed with glioma. (2) The patients had no radiotherapy and chemotherapy treatment before surgery. (3) Informed consent was signed by all patients.

Exclusion criteria: (1) Secondary brain tumor. (2) Patients with other malignancies. (3) Patients with severe disorder of consciousness.

According to the WHO classification of tumors of the nervous system[8], 22 cases were divided into low deteriorate group (I-II stage), 31 into high deteriorate group (III-IV stage). And 14 brain tissue samples of traumatic decompression from our hospital were selected as control group.

This study was approved by medical Ethical Committee of our hospital (Chinese People's Armed Police Force General Hospital, Beijing, China).

Methods

The specimens were placed in 10% formaldehyde solution, and 4 um serial sections were prepared by conventional paraffin embedding. The slices were dehydrated in gradient ethanol solution, PBS (phosphate-buffered saline) solution repair, high-temperature antigen repair, then put in 3% H_2O_2 for 5-10 min at 37°C. Then, antigen blocking was used with specific serum. When the blocking was completed, the I antibodies of ADAM17, EGFR and KI-67 with the dilution ratio of 1:100 were added and were set overnight under constant 4°C. The specimens were washed with PBS, dropped with II antibody, and incubated for 30 min. After that, the specimens were washed with PBS again, the adding of streptavidin-biotin-peroxidase complex were continued and they were incubated for 30 min. Gradient ethanol hydration was performed, as well as DAB (3,3'-diaminobenzidine) staining (TaKaRa, Dalian, Liaoning, China), hematoxylin and eosin (HE) staining (Beyotime, Shanghai, China). Finally, they were observed under the microscope. The main reagent was Rabbit anti-human ADAM17 polyclonal antibody, mouse anti-human EGFR monoclonal antibody, rabbit anti-human monoclonal antibody KI-67, and DAB chromogenic agent and immunohistochemistry kit. They were all purchased by TaKaRa (Dalian, Liaoning, China).

Evaluation Criterion[9]

Put the slices under the microscope, 5 views under the light of 400 times were randomly selected. ADAM17 and EGFR protein were localized in the cytoplasm, and EGFR protein was localized in the nucleus. According to the percentage of positive cells, the 0 score meant no positive cells, 1 meant positive cells < 30%; 2 meant the percentage of positive cells was 30%-70%; 3 meant positive cells > 70%. In addition, according to the intensity of staining, it can be divided into 4 grades, 0 score was no positive cells: light yellow staining had 1 point, yellow staining had 2 points, yellow-brown had 3. The above two scores were added as a comprehensive score: 0 point for the negative (-); 1-2 weakly positive (+); 3-4 positive (+ +); 5-6: strong positive (+ + +).

Positive rate = weak positive rate + positive rate + strong positive rate

Statistical Analysis

SPSS 21.0 statistical software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The percentage data were compared using the χ^2-test. The correlation of ADAM17, EGFR and Ki-67 expression with the degree of malignancy was analyzed by the Spearman correlation analysis, $p < 0.05$ was statistically significant.

Results

Comparison of the Expression of ADAM17 in Three Groups

The positive expression rate of ADAM17 in high deteriorate group was 93.55% (29/31), and was significantly higher than that in the low deteriorate group 59.09% (13/22). The low deteriorate group was significantly higher than that of control group 28.57% (4/14). The difference was statistically significant ($p < 0.05$), as shown in Table I.

Comparison of the Expression of EGFR in Three Groups

The positive expression rate of EGFR in high deteriorate group was 70.97% (22/31), which was significantly higher than that of low deteriorate group 36.36% (8/22). The low deteriorate group was significantly higher than that of control group 0.00% (0/14). The difference was statistically significant ($p < 0.05$), as shown in Table II.

Comparison of the Expression of Ki-67 in Three Groups

The positive expression rate of Ki-67 in high deteriorate group was 80.65% (25/31), which was significantly higher than that of low deteriorate

Table I. Comparison of ADAM17 expression between the three groups (No., %).

Groups	No.	-	+	++	+++	Positive rate (%)
High deteriorated group	31	2	4	11	14	29 (93.55)#,*
Low deterioration group	22	9	7	6	0	13 (59.09)*
Control group	14	10	4	0	0	4 (28.57)
χ^2-value	–	–	–	–	–	26.394
p-value	–	–	–	–	–	0.000

Note: Compared with the low grade deterioration group, #$p < 0.05$. Compared with the control group, *$p < 0.05$.

Table II. Comparison of EGFR expression between the three groups (No., %).

Groups	No.	-	+	++	+++	Positive rate (%)
High deteriorated group	31	10	3	6	12	22 (70.97)#,*
Low deterioration group	22	14	3	5	0	8 (36.36)*
Control group	14	14	0	0	0	0 (0.00)
χ^2-value	–	–	–	–	–	21.492
p-value	–	–	–	–	–	0.000

Note: Compared with the low grade deterioration group, #$p < 0.05$. Compared with the control group, *$p < 0.05$.

group 31.82% (7/22). The low deteriorate group was significantly higher than that of control group 0.00% (0/14). The difference was statistically significant ($p < 0.05$), as shown in Table III.

Expression of ADAM17, EGFR, and Ki-67 in Gliomas and Its Correlation With Malignancy

By correlation analysis, the expression of ADAM17, EGFR, and Ki-67 in glioma patients was significantly positively correlated with the stage of malignancy ($R = 0.823$, $p = 0.000$; $R = 0.804$, $p = 0.000$; $R = 0.811$, $p = 0.000$).

Discussion

Glioma is a common malignant tumor of the brain. The malignant degree is high, and the cure rate is quite low. However, the pathogenesis of glioma remained unclear. It is of great importance to make a judgment on the malignant stage of glioma by histological diagnosis and specific molecular diagnosis[10]. ADAM17, as one of the ADAM family members, is a gene molecule newly discovered in recent years. ADAM17 has a significant effect on the protein-cutting enzyme, and also activates cell cycle key molecules in order to promote tumor cell proliferation, fusion, invasion, etc[11]. At present, some studies have suggested that the expression level of ADAM17 in patients with glioma is usually at a high level, which is abnormal[12]. EGFR is an important member of the epidermal growth factor receptor family. The level of the expression of EGFR is correlated to the malignant stage of the tumor. Studies have shown that EGFR can bind to tyrosine kinase activity, which is correlated to the occurrence and to the development of different tumors *in vivo*[13]. In recent years, researchers have

Table III. Comparison of Ki-67 expression between the three groups (No., %).

Groups	No.	-	+	++	+++	Positive rate (%)
High deteriorated group	31	7	3	7	14	25 (80.65)#,*
Low deterioration group	22	15	5	2	0	7 (31.82)*
Control group	14	14	0	0	0	0 (0.00)
χ^2-value	–	–	–	–	–	23.373
p-value	–	–	–	–	–	0.000

Note: Compared with the low grade deterioration group, #$p < 0.05$. Compared with the control group, *$p < 0.05$.

found that ADAM17 may be involved in the activation of EGFR, and played an important role in tumor development[14]. Therefore, this paper analyzed the correlation between the expressions of ADAM17, EGFR, and Ki-67 and the stages of malignancy in patients with glioma; furthermore, it enriched the pathogenesis of glioma.

In this study, we compared the different stages of brain glioma and brain injury. Results suggested that the positive expression rate of ADAM17 in high deteriorate group was significantly higher than in the low deteriorate group; and the low deteriorate group was significantly higher than that in control group. This showed similar results to the research by Chen et al[15]. The current studies have shown that the expression of ADAM17 in gastric cancer, liver cancer and lung cancer and other cancer cells increased abnormally. Its major mechanism is to accelerate the proliferation, invasion, and migration of tumor cells through EGFR-PI3K-AKT signaling pathway[16]. The results suggested that the expression of ADAM17 was closely correlated to the malignant stage of glioma patients. The results were similar to the research by Andersson et al[17]. The positive expression rate of EGFR in high deteriorate group was significantly higher than that in the low deteriorate group; and the low deteriorate group was significantly higher than that in control group. Thereafter, the expression of EGFR was closely correlated to the malignant stage of glioma. EGFR, as an epidermal growth factor, is an important expression product of proto-oncogene HER1. EGFR combines with tyrosine kinase active molecules and activates multiple signaling pathways which regulate cellular growth, proliferation, and differentiation. It has been found that EGFR gene was overexpressed in many solid tumors, such as lung cancer, cervical cancer, liver cancer and so on[18]. Results have found that the expression level of EGFR was significantly high in poorly differentiated gliomas. The high expression rate of EGFR was closely correlated with the differentiation of glioma, as well as the malignant stage of glioma[19]. In addition, the results also showed that the positive expression rate of Ki-67 in high deteriorate group was significantly higher than that in the low deteriorate group; and the low deteriorate group was significantly higher than that in the control group, which suggested that the expression of Ki-67 is closely correlated to the malignant stages of gliomas. Ki-67 is one of the proliferating cell nuclear antigens. The expression level of Ki-67 was correlated with the proliferation of tumor cells[20]. With the development of a tumor, the positive expression rate of Ki-67 increased gradually. The results of correlation analysis showed that the expression of ADAM17, EGFR and Ki-67 in patients with glioma was significantly positively correlated with the stage of malignancy[21].

Conclusions

To sum up, ADAM17, EGFR, and Ki-67 had no expression or weak expression in the control group, and increased expression in the low deteriorate group. The malignant stage of gliomas was positively correlated with the expression of ADAM17, EGFR, and Ki-67, which indicated that in clinical work, we can further learn the changes of patients' condition, and provide the reference for the clinical treatment plan by examining the positive expression rate of ADAM17, EGFR, and Ki-67.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Lu Y, Chopp M, Zheng X, Katakowski M, Buller B, Jiang F. MiR-145 reduces ADAM17 expression and inhibits in vitro migration and invasion of glioma cells. Oncol Rep 2013; 29: 67-72.

2) Jain KK, Sahoo P, Tyagi R, Mehta A, Patir R, Vaishya S, Prakash N, Vasudev N, Gupta RK. Prospective glioma grading using single-dose dynamic contrast-enhanced perfusion MRI. Clin Radiol 2015; 70: 1128-1135.

3) Wang Z, Fei X, Dai X, Chen H, Tian H, Wang A, Dong J, Huang Q. Differentiation of glioma stem cells and progenitor cells into local host cell-like cells: a study based on choroidcarcinoma differentiation of choroid plexus of GFP transgenic nude mouse. Cancer Biother Radiopharm 2015; 30: 225-232.

4) Jiang CM, Wang XH, Shu J, Yang WX, Fu P, Zhuang LL, Zhou GP. Analysis of differentially expressed genes based on microarray data of glioma. Int J Clin Exp Med 2015; 8: 17321-17332.

5) Fu XR, Sun ZC, Chang Y. Expression and clinical significance of P53, O6-methylguanine-dna methyltransferase and epidermal growth factor receptor in glioma. J Biol Regul Homeost Agents 2015; 29: 853-858.

6) Chang CY, Li JR, Wu CC, Ou YC, Chen WY, Kuan YH, Wang WY, Chen CJ. Valproic acid sensitizes

human glioma cells to gefitinib-induced autophagy. IUBMB Life 2015; 67: 869-879.

7) Lin T, Wang M, Liang HS, Liu EZ. The expression of p53, mgmt and egfr in brain glioma and clinical significance. J Biol Regul Homeost Agents 2015; 29: 143-149.

8) Pedeutour-Braccini Z, Burel-Vandenbos F, Gozé C, Roger C, Bazin A, Costes-Martineau V, Duffau H, Rigau V. Microfoci of malignant progression in diffuse low-grade gliomas: towards the creation of an intermediate grade in glioma classification? Virchows Arch 2015; 466: 433-444.

9) Nordfors K, Haapasalo J, Mäkelä K, Granberg KJ, Nykter M, Korja M, Paavonen T, Haapasalo H, Soini Y. Twist predicts poor outcome of patients with astrocytic glioma. J Clin Pathol 2015; 68: 905-912.

10) Jing SY, Jing SQ, Liu LL, Xu LF, Zhang F, Gao JL. Down-expression of miR-373 predicts poor prognosis of glioma and could be a potential therapeutic target. Eur Rev Med Pharmacol Sci 2017; 21: 2421-2425

11) Seong MW, Ka SH, Park JH, Yoo HM, Yang SW, Park JM, Park D, Lee ST, Seol JH, Chung CH. Deleterious c-Cbl exon skipping contributes to human glioma. Neoplasia 2015; 17: 518-524.

12) Warram JM, de Boer E, Korb M, Hartman Y, Kovar J, Markert JM, Gillespie GY, Rosenthal EL. Fluorescence-guided resection of experimental malignant glioma using cetuximab-IRDye 800CW. Br J Neurosurg 2015; 29: 850-858.

13) Chen Y, Henson ES, Xiao W, Shome E, Azad MB, Burton TR, Queau M, Sathya A, Eisenstat DD, Gibson SB. Bcl-2 family member Mcl-1 expression is reduced under hypoxia by the E3 ligase FBW7 contributing to BNIP3 induced cell death in glioma cells. Cancer Biol Ther 2016; 17: 604-613.

14) Wu Z, Wang G, Xu S, Li Y, Tian Y, Niu H, Yuan F, Zhou F, Hao Z, Zheng Y, Li Q, Wang J.Effects of tetrandrine on glioma cell malignant phenotype via inhibition of ADAM17. Tumour Biol 2014; 35: 2205-2210.

15) Chen J, Chen X, Wang F, Gao H, Hu W. Dihydroartemisinin suppresses glioma proliferation and invasion via inhibition of the ADAM17 pathway. Neurol Sci 2015; 36: 435-440.

16) Zheng X, Jiang F, Katakowski M, Lu Y, Chopp M. ADAM17 promotes glioma cell malignant phenotype. Mol Carcinog 2012; 51: 150-164.

17) Späth F, Andersson U, Dahlin AM, Langseth H, Hovig E, Johannesen TB, Grankvist K, Björkblom B, Wibom C, Melin B. Pre-diagnostic serum levels of EGFR and ErbB2 and genetic glioma risk variants: a nested case-control study. Tumour Biol 2016; 37: 11065-11072.

18) Ghasimi S, Wibom C, Dahlin AM, Brännström T, Golovleva I, Andersson U, Melin B. Genetic risk variants in the CDKN2A/B, RTEL1 and EGFR genes are associated with somatic biomarkers in glioma. J Neurooncol 2016; 127: 483-492.

19) Nitta Y, Shimizu S, Shishido-Hara Y, Suzuki K, Shiokawa Y, Nagane M. Nimotuzumab enhances temozolomide-induced growth suppression of glioma cells expressing mutant EGFR in vivo. Cancer Med 2016; 5: 486-499.

20) Zeng A, Hu Q, Liu Y, Wang Z, Cui X, Li R, Yan W, You Y. IDH1/2 mutation status combined with Ki-67 labeling index defines distinct prognostic groups in glioma. Oncotarget 2015; 6: 30232-30238.

21) Miao C, Wang Z, Yang J, Li J, Gao X. Expression and mutation analysis of Cyclin A and Ki-67 in glioma and their correlation with tumor progression. Oncol Lett 2015; 10: 1716-1720.