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Original Article

Correlation between ADAM17 protein expression level and prognosis of neuroglioma

Xu Zhai[1], Haitao Wang[2], Jiangjie Wang[3], Peng Xu[2], Yexia Zhao[4], Jianwu Qiu[1]

[1]Department of Neurological Surgery Unit 1, The First Affiliated Hospital of Liaoning Medical University, Jinzhou 121000, Liaoning, China; [2]Neurosurgery Ward 1 Cerebral Section, [3]Neurosurgery Ward 2 Cerebral Section, [4]Department of NICU, Linyi City Yishui Central Hospital, Linyi 276400, Shandong, China

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Abstract: Tumor metastasis is a complicated process involving multiple genes and signals. A disintegrin and metalloproteinase domain 17 (ADAM17) plays an important role in metastasis of glioma. This study thus investigated the expression level of ADAM17 in glioma tissues at different pathological grades, in order to investigate the correlation between ADAM17 expression and pathological feature and prognosis of glioma. Both glioma tissues and normal brain tissues were collected to determine both mRNA and protein levels of ADAM17 by immunohistochemistry (IHC) and PCR. Spearman method was used to analyze the correlation between ADAM17 and malignancy of glioma. Kaplan-Meier method was employed for survival analysis. Cox ratio risk model was adapted for multi-factor survival analysis. ADAM17 protein level was significantly higher in glioma tissues compared to normal brain tissues (χ^2 = 10.5448, P = 0.001). Positive rates of ADAM in advanced grade glioma was 60.92%, which was significantly higher than that in lower grade tumor (25.5%, χ^2 = 8.3576, P = 0.003). mRNA level of ADAM17 was positively correlated with pathological grades (r = 0.712, P<0.05). K-M curve showed significantly shorter survival time of patients with ADAM17 high expression (P<0.05). Cox analysis revealed ADAM17 as one independent risk factor for glioma prognosis. ADAM17 is up-regulated in neuroglioma tissues. The assay of ADAM17 expression can benefit the evaluation of biological behavior of glioma, and may helping the prognostic prediction.

Keywords: A disintegrin and metalloproteinase, neuroglioma cell, prognostic prediction

Introduction

As one common intracranial malignant tumor, glioma has the tendency of over-proliferation, high invasiveness and unfavorable prognosis [1, 2]. Recent advances in treatment for glioma including precise localization radio-therapy, minimal wound neurosurgery and targeted chemotherapy have been developed. The prognosis of glioma, however, did not improve significantly. With further investigation of molecular biology and pathogenesis of glioma, targeted therapy against key modulatory molecule, critical gene and cellular receptor have become the research focus. Due to higher invasiveness of tumor cells, metastasis is one important biological event in the course of glioma and severely affects prognosis [3, 4]. The invasion of tumor is correlated with various genes and signals. Two important members of extracellular matrix degradation system, a disintegrin metalloproteinase (ADAMs) and matrix metalloproteinase (MMPs) may be involved in the metastasis of glioma [5, 6]. As one important member of ADAMs family, ADAM17 participates in the degradation process of various receptors and ligands. It can play a crucial role in glioma metastasis via activating epithelial growth factor receptor (EGFR) related signaling transduction pathway, and hydrolyzing membrane tumor necrosis factor (TNF) [7, 8]. This study investigated the expression level of ADAM17 in glioma tissues with different pathological grades, in an attempt to elucidate the correlation between ADAM17 expression and pathological features and patient prognosis of glioma.

Materials and methods

Clinical information

A total of 111 cases of glioma patients (67 males and 44 females; aging between 21 and 76 years, average age = 61.5 ± 4.3 years) who

received neurosurgery in the First Affiliated Hospital of Liaoning Medical University from January 2010 to December 2014 were enrolled as the disease group. All patients did not receive any chemo-/radio-therapy before surgery. Confirmed diagnosis of glioma was made by two independent pathologists. Based on classification system of central nervous tumor (WHO, 2000), there were 24 cases of low grade glioma (LGG, including 5 cases of grade I astrocytoma, 13 cases of grade II astrocytoma and 6 cases of oligodendroglioma) and 87 cases of high grade glioma (HGG, including 42 patients with anaplastic astrocytoma and 45 patients with glioblastoma multiform). Another 20 samples of normal brain tissues were selected as the control group.

Experimentation protocols were submitted to and approved by the ethics committee of the First Affiliated Hospital of Liaoning Medical University.

Research methods

A retrospective study was performed to analyze clinical information including sex, age, KPS before surgery. To minimize the effect of treatment plan and pathological grade on survival time, we defined the inclusive criteria: (1) HGG cases with no history of chemo-/radio-therapy; (2) No acute infection, other malignant tumor or diabetes; (3) Morality caused by other disease. After radical surgery to remove the whole or major parts of tumors, post-operative chemotherapy (temozolomide or fluticasone + semustine) or radiotherapy (60 Gy dosage) was applied. Excluding those patients lost during postoperative follow-ups, a total of 79 HGG patients were recruited in this study. Using Kaplan-Meier survival analysis, follow-ups were performed until the endpoint (June 2015) or patient death.

Immunohistochemistry (IHC)

Samples of both glioma and normal brain tissues were fixed in 4% neutral buffered formalin, embedded in paraffin block, and were sectioned into consecutive slices (4 μm thickness). IHC staining was performed using SP method. In brief, tissue slices were de-waxed and dehydrated, followed by heated antigen retrieval. Endogenous peroxidase activity was blocked by 3% hydrogen peroxide for 15 min. Anti-ADAM17 primary antibody (1:100, Boster Biotech, China) was added for overnight incubation at 4°C. On the next day, slices were rinsed in PBS, followed by secondary antibody (Boster Biotech, China) incubation. 10 fields were randomly selected under light field microscope. The positive result of IHC staining was deduced by dual standards [9]. The staining intensity was classified as 0, 1 2, or 3 points for no positive cell, light yellow, yellow-brown and dark brown color, respectively. The positive rate was given scores from 0 to 3 for those positive percentages at 0%, less than 10%, between 10% and 50%, and more than 50%. The overall staining index was calculated as intensity score times positive score. Those samples with index larger than 4 were identified as high expression, otherwise samples had low expression for ADAM17.

PCR

Total RNA was extracted by TRIzol reagent (Beyozol, US) following tissue homogenization. UV spectrometer was used to determine the purity of RNA. In PCR amplification, specific primers for ADAM17 and beta-actin (Sangon, China) were used under the following conditions: 95°C denature for 5 min, followed by 40 cycles each containing 94°C denature for 50 sec, 52°C annealing for 1 min and 72°C elongation for 1 min. Eagle eye type II imaging analysis system was used to scan the DNA bands. Relative level of ADAM18 mRNA was determined as the ratio of absorbance value between ADAM17 and beta-actin. Primers used were: ADAM17-forward, 5'-GCACA GGTAA TAGCA GTGAG TGC-3'; ADAM17-reverse, 5'-CACAC AATGG ACAAG AATGC TG-3'; beta-actin-forward, 5'-TTCCA GCCTT CCTTC CTGG-3'; beta-actin-reverse, 5'-TTGCG CTCAG GAGGA GCAAT-3'.

Statistical analysis

SPSS 20.0 software was used to process all collected data, of which enumeration data were tested by chi-square test. Those data fitted normal distribution were presented as mean ± standard deviation (SD) and were analyzed by analysis of variance (ANOVA) or student t-test. Spearman test analyzed the correlation between ADAM17 level and glioma malignancy. Kaplan-Meier method was employed for survival analysis. Multi-variate survival analysis was performed using Cox ratio risk model. A statistical significance was defined when $P<0.05$.

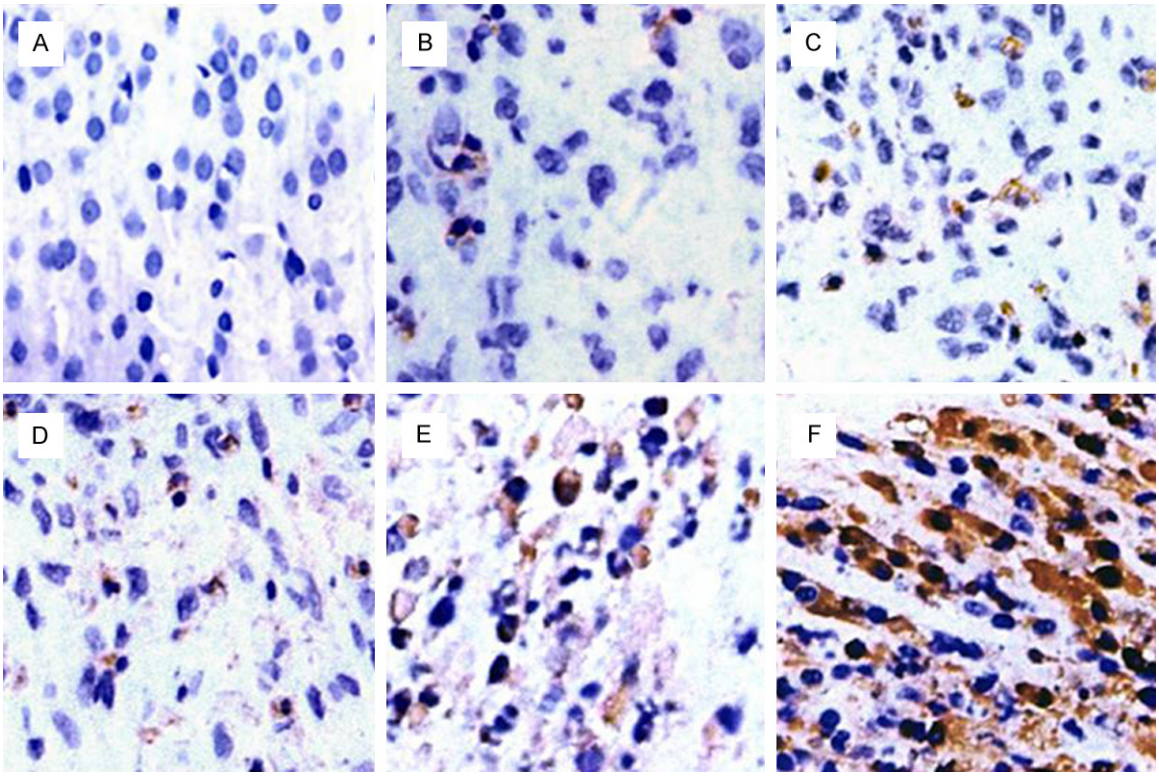

Figure 1. ADAM17 protein expression in glioma tissues. A: Negative control; B: Normal brain tissue; C: Grade I glioma; D: Grade II glioma; E: Grade III glioma; F: Grade IV glioma.

Table 1. Clinical features and ADAM17 expression

Clinical feature	N	ADAM17 positive rate	χ^2 value	*P* value
Age (years)				
≤60	48	25 (52.08)	0.0258	0.872
>60	63	33 (52.38)		
Sex				
Male	67	34 (50.75)	0.1536	0.695
Female	44	24 (54.55)		
Clinical stage				
I + II	24	6 (25.5%)	8.3576	0.003
III + IV	87	53 (60.92%)		
KPS score				
≤80	52	27 (51.92)	0.0042	0.948
>80	59	31 (52.54)		

Results

IHC staining of ADAM17 protein

Positive expression of ADAM17 mainly existed on membrane and cytoplasm. With advanced pathological grade of glioma, positive rate of ADAM177 protein was gradually elevated. In glioma tissues (**Figure 1C-F**), the positive rate was 53.55% (58/111), which was significantly higher than normal brain tissues (**Figure 1B**, 10.0%, 2/20, χ^2 = 10.5448, P = 0.001). HGG (**Figure 1E** and **1F**) had higher positive rate than LGG cases (**Figure 1C** and **1D**, positive rate 60.92% vs. 25.5%, χ^2 = 8.3576, P = 0.003).

ADAM17 expression and clinical features

HGG (grade III + grade IV glioma) patients had 60.92% positive rate of ADAM17 expression, which was significantly higher than LGG (grade I + grade II) patients (25.5%, χ^2 = 8.3576, P = 0.003). No significant correlation has been identified (P>0.05, **Table 1**).

ADAM17 mRNA expression level

PCR results showed significantly elevated mRNA levels of ADAM17 in glioma tissues compared to control group (P<0.05). With higher malignancy of tumors, ADAM17 mRNA level was significantly elevated (**Table 2**). A positive correlation existed between ADAM17 mRNA level and malignancy of glioma (r = 0.712, P<0.05, **Figure 2**).

Table 2. ADAM17 mRNA level across different glioma tissues

Group	N	Mean	Standard deviation	Standard error	95% CI of means		F value	*P* value
					Lower	Upper		
Control	20	0.2530	0.06003	0.01342	0.2249	0.2811	54.607	0.000
Grade I glioma	5	0.3962	0.07722	0.03453	0.3003	0.4921		
Grade II glioma	19	0.5184	0.10347	0.02374	0.4685	0.5682		
Grade III glioma	42	0.5738	0.12717	0.01962	0.5342	0.6134		
Grade IV glioma	45	0.6626	0.10495	0.01565	0.6311	0.6942		
Total	131	0.5405	0.17357	0.01517	0.5105	0.5705		

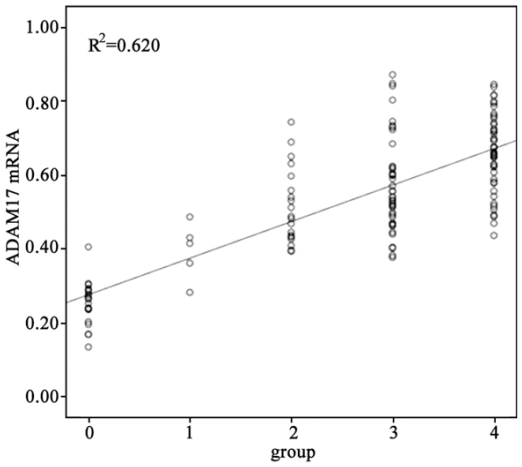

Figure 2. Correlation between ADAM17 mRNA and malignancy of glioma.

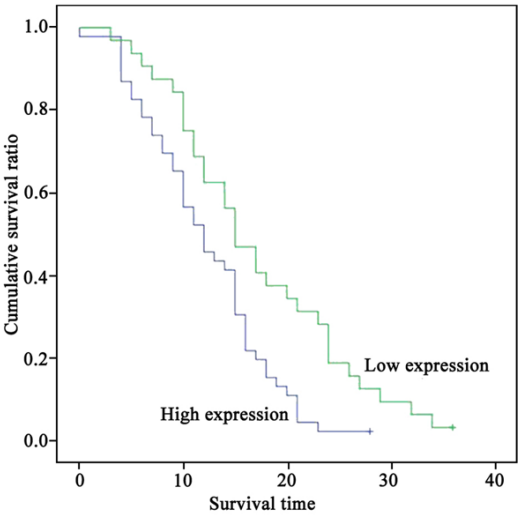

Figure 3. Survival curve of glioma patients with different levels of ADAM17.

ADAM17 and prognosis

Using Kaplan-Meier survival curve, we found the average value of ADAM17 mRNA relative expression was 0.620 ± 0.03. Using 0.65 as the cutting-off value, we divided all patients into high expression (N = 46) and low expression (N = 33) groups based on level of ADAM17 mRNA. In postoperative follow-ups until June 2015, the survival period of high expression was significantly shorter than those in low expression group (**Figure 3**; **Table 3**).

Cox regression analysis

We further replenished multiple risk factors for prognosis including age, sex, clinical stage, ADAM expression level and KPS score into Cox ratio risk model. Results indicated clinical stage III + IV and high ADAM level as risk factors for unfavorable prognosis (**Table 4**).

Discussion

More than 70% of primary intracranial malignant tumors belong to glioma, whose 5-year survival rate was only 25%. Therefore the targeted treatment against critical genes during the transformation of tumor in order to inhibit proliferation/differentiation is of critical importance [10, 11]. ADAM17 has multiple functional domains with bioactivities including secreting active factor, hydrolyzing protein and transducing intracellular signals [12, 13]. It can activate multiple signal pathways such as Ras/Raf/MAPK and PI3K/AKT to participate in tumor invasion, migration, proliferation and differentiation by editing ligands of transforming growth factor (TGF) and amphiregulin receptors [14, 15]. ADAM17 is highly expressed in various tumor tissues including breast cancer, ovarian cancer and renal cell carcinoma. The hyperactivation of EGFR has been related with ADAM17 over-expression during the pathogenesis of renal cancer. In breast cancer tissues, the number of lymph node metastasis is positively correlated with ADAM17 expression level, which is also related with pathological grades.

Table 3. Survival time of glioma patients with different levels of ADAM17

ADAM level	Mean				Median			
	Estimate	Standard error	95% CI		Estimate	Standard error	95% CI	
			Lower	Upper			Lower	Upper
High	12.174	0.895	10.420	13.928	12.000	1.689	8.689	15.311
Low	17.219	1.509	14.261	20.176	15.000	1.694	11.680	18.320
Total	14.304	0.877	12.586	6.022	14.000	1.038	11.966	16.034

Table 4. Cox regression analysis for prognostic risk factors

Factor	β	$S^{\bar{x}}$	Wald χ^2	*P* value	OR value	95% CI
Clinical stage	0.992	0.480	4.276	0.041	2.697	1.053~6. 905
ADAM expression level	1.054	0.557	3.584	0.033	2.869	0.963~8.544

In gastric carcinoma tissues, ADAM17 expression level was elevated, and can facilitate gastric carcinoma cell proliferation via releasing ligands of EGFR. In liver cancer tissues, mRNA of ADAM17 is also higher than peripheral tissues, and is related with differentiation stage of tumors. ADAM17 has been shown to facilitate tumor cell migration of head-neck tumors by inducing the release of EPGR ligands [16, 17]. Study results suggested the possible relation between tumor malignancy and ADAM17 expression level, which may thus affect prognosis. Recent studies have shown elevated expression of ADAM17 in human glioma tissues, indicating its possible involvement in tumor formation. After transfecting by ADAM17-shRNA vector, the expression of ADAM17 was silenced in cultured glioma cells, whose proliferation and invasiveness were inhibited, leading to higher cell apoptosis [10, 13]. Currently there are few correlative studies regarding ADAM17 expression and glioma prognosis. This study thus investigated the expression level across glioma tissues with different pathological grades, in an attempt to elucidate the clinical implication of ADAM17.

ADAM17 function is correlated with certain biological behaviors such as cell-to-cell adhesion, extracellular matrix degradation-reformation. This study showed significantly elevated ADAM17 protein level in glioma tissues compared to normal brain tissues. HGG tissues had even higher protein positive rate than LGG samples. Furthermore, ADAM17 level was not correlated with patient age, sex or KPS score, suggesting the *de novo* gene mutation during pathogenesis of glioma, whose progression is related with ADAM17. Therefore, ADAM17 has certain implications in the differential diagnosis of glioma. ADAM17 mRNA was also shown to be positively correlated with pathological grades, suggesting the reference value of ADAM17 in prognostic evaluation. K-M survival curve showed significantly lower survival time in ADAM17 high expression patients compared to those with low-expression ones. Cox analysis also revealed ADAM17 high expression and clinical stage as independent risk factors for prognosis of glioma. Overall speaking, ADAM17 may predict the survival time of glioma patients and work as one independent affecting factor for prognosis. During the malignant transformation of glioma, ADAM17 exerts critical functions for accelerating tumor invasion and growth. Via binding integrin on cell surface, ADAM17 protein can regulate cell adhesion; alter extracellular matrix structure and cytoskeleton. It can also cut tumor metastasis related adhesion molecule (CD44, E/N-cadherin), regulate interaction between tumor cells and matrix, degrade matrix barrier, affect vascular endothelial growth factor expression, facilitate glioma cell migration/invasion and enhance cell adhesion, proliferation and invasion abilities [18, 19]. ADAM17 can also participate in glioma invasion via hydrolyzing multiple ligands including interleukin 6 receptor, cell adhesion molecule 1, EGFR, and biregulin [20, 21]. Due to the wide spectrum of ADAM17 functions and its correlation with various important membrane molecule hydrolysis, the application of ADAM17 inhibitor may cause various adverse effects. The detailed mechanism of ADAM17 in facilitating tumor progression remains to be elucidated. In summary, the elevation of ADAM17 in glioma tissues suggested the implication in prognostic prediction of glioma.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jianwu Qiu, Department of Neurological Surgery Unit 1, The First Affiliated Hospital of Liaoning Medical University, Jinzhou 121000, Liaoning, China. Tel: +86-416-4197026; Fax: +86-416-4197026; E-mail: Jianwu-0909@126.com

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