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Original Article

High expression of ADAM10 predicts a poor prognosis for patients with glioblastoma

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Abstract: A disintegrin and metalloprotease 10 (ADAM10) is a member of the ADAM family of zinc proteases, which are involved in the shedding of extracellular domains of various cell surface molecule. Several ADAM family members, including ADAM10, have been implicated in cancer growth and progression, however the clinical impact of ADAM10 expression in glioblastoma remains unclear. ADAM10 has an essential role in Notch1 signaling, therefore, we examined the expressions of ADAM10 and Notch1 by immunohistochemistry in 50 patients with IDH1 wild-type glioblastoma. ADAM10 was highly expressed in the tumor specimen of 11 patients but ADAM10 was lowly expressed in 39 patients. No ADAM10 expression was found in normal brain tissue. ADAM10 expression was positively correlated with Notch1 expression (P = 0.002). Low expression of ADAM10 had a tendency to be longer survival in our study. In conclusion, the expression of ADAM10 had a correlation with that of Notch1 in glioblastoma and ADAM10 expression might correlate with tumor growth and be a prognostic factor for patients with glioblastoma.

Keywords: ADAM10, Notch1, glioblastoma, immunohistochemistry, prognosis

Introduction

Glioblastoma is the most common and most aggressive malignant primary brain tumor. Despite advances in surgical techniques and concurrent radiochemotherapy regimens, the prognosis remains poor, with a median survival time of less than 2 years from diagnosis [1, 2]. For this reason, a great deal of research has been devoted to identifying the molecular mechanisms driving malignant progression and invasive growth of glioblastoma.

Invasion and migration of tumor cells are characteristics of aggressive malignancies. Disintegrin and metalloproteases (ADAMs) are a family of trans-membrane multi-domain metalloproteases that are important regulators of cell motility and migration, cell adhesion, angiogenesis, and cell signaling in both normal organ development and tumorigenesis [3-5]. ADAM10 is a member of the ADAM family that has been implicated in the shedding of a number of substrates that drive cancer progression [6]. Indeed, ADAM10 expression is associated with the pathogenesis and prognosis of several types of malignancies [7-11]. In *in vitro* glioma experiments, ADAM10 was necessary for the shedding of Cadherin 2 to promote glioblastoma cell migration [12], and L1, an important molecule for cell migration of neural and tumor cells, to increase human glioma cell migration and invasion [13]. Bulstrode et al demonstrated that inhibition of ADAM10/17 function impairs the growth of glioma tumor spheres [14]. Little is known about the clinical significance of ADAM10 expression in glioblastoma beyond a single report correlating ADAM10 expression with the grade of malignancy in human glioma [15].

Recently, Groot AJ et al reported that ADAM10 has an essential role in ligand dependent Notch1 signaling [16]. Notch1 is a multi-functional receptor with a preeminent role in controlling cell fate specification and self-renewal

Table 1. Clinical characteristics of glioblastoma patients

Characteristics	No. of patients (%)
Age (years)	
Median (range)	59.5 (9-77)
≥50 years	40 (80.0%)
<50 years	10 (20.0%)
Gender	
Male	32 (64.0%)
Female	18 (36.0%)
KPS	
Median (range)	80 (20-100)
≥70	37 (74.0%)
<70	13 (26.0%)
Tumor size	
≥5 cm	26 (52.0%)
<5 cm	24 (48.0%)
Surgical extent	
Biopsy	4 (8.0%)
Partial resection	20 (40.0%)
Subtotal resection	12 (24.0%)
Gross total resection	14 (28.0%)
ADAM10	
High	11 (22.0%)
Low	39 (78.0%)
Notch1	
High	12 (24.0%)
Low	38 (76.0%)

processes in stem cell biology [17]. Importantly, inhibition of the Notch1 pathway overcomes apoptosis resistance and sensitizes glioblastoma cells to apoptosis induced by ionizing radiation [18]. The expression of Notch1 is positively correlated with glioma progression, and high Notch1 protein expression is an independent predictor of poor survival in glioma [19]. Notch1 also plays an important oncogenic role in the development and progression of astrocytic gliomas [20].

In the present study, we analyzed ADAM10 and Notch1 expression levels in glioblastomas using immunohistochemical staining, and compared these data with the clinicopathological features of the respective patients. We also evaluated the prognostic significance of ADAM10 expression in the tumors of glioblastoma patients and assessed the correlation of ADAM10 and Notch1 expression with clinical outcome. To our knowledge, the current study is the first to investigate the expression of ADAM10 immunohistochemically in relation to survival in patients with glioblastoma.

Materials and methods

Patients

This study was approved by the Ethics Committee of Shinshu University School of Medicine (registration numbers 2604). Formalin-fixed and paraffin-embedded (FFPE) tissue samples from 50 patients with IDH1 wild-type glioblastoma. The negative expression of IDH1 was determined by immunohistochemical evaluation. These data collected from consecutive patients with IDH1 wild-type glioblastoma initially treated at our institute from 1989 to 2012. Furthermore, neurologically normal brain tissues from donor bodies from Shinshu University Hospital were used as normal controls in this study.

Immunohistochemical analysis

FFPE glioblastoma samples were washed free of fixative with Tris-buffered saline (TBS; pH 7.6), and exposed to 0.3% H_2O_2 in TBS for 10 min to inactivate endogenous peroxidase. For Notch1 staining, samples were pretreated with EDTA for 30 min and microwaved. No antigen retrieval was used before ADAM10 immunostaining. For single labeling, FFPE sections were incubated with ADAM10 antibodies (1:200, Merck Millipore, Darmstadt, Germany) or Notch1 (1:100, Santa Cruz Biotechnology, Inc., Dallas, TX, USA) in a humidity chamber at 4°C overnight. As the secondary antibody, polyclonal rabbit IgG antibody for ADAM10 and polyclonal goat IgG antibody for Notch1 were applied to the sections for 30 min. Staining was developed with 0.02% 3,3'diaminobenzidine tetrahydrochloride and 0.006% H_2O_2 in TBS. The slides were washed three times in TBS and counterstained with Mayer's hematoxylin. ADAM10 and Notch1 expression levels were evaluated as both the proportion of stained cells (PS) and the intensity of staining (IS). The PS was stratified into four grades: undetectable, PS grade 0; detectable but ≤33%, PS grade 1; 34-66%, PS grade 2; and ≥67%, PS grade 3. Similarly, IS was also stratified into four grades: undetectable, IS grade 0; weak, IS grade 1; moderate, IS grade 2; and strong, IS grade 3. The final score was obtained

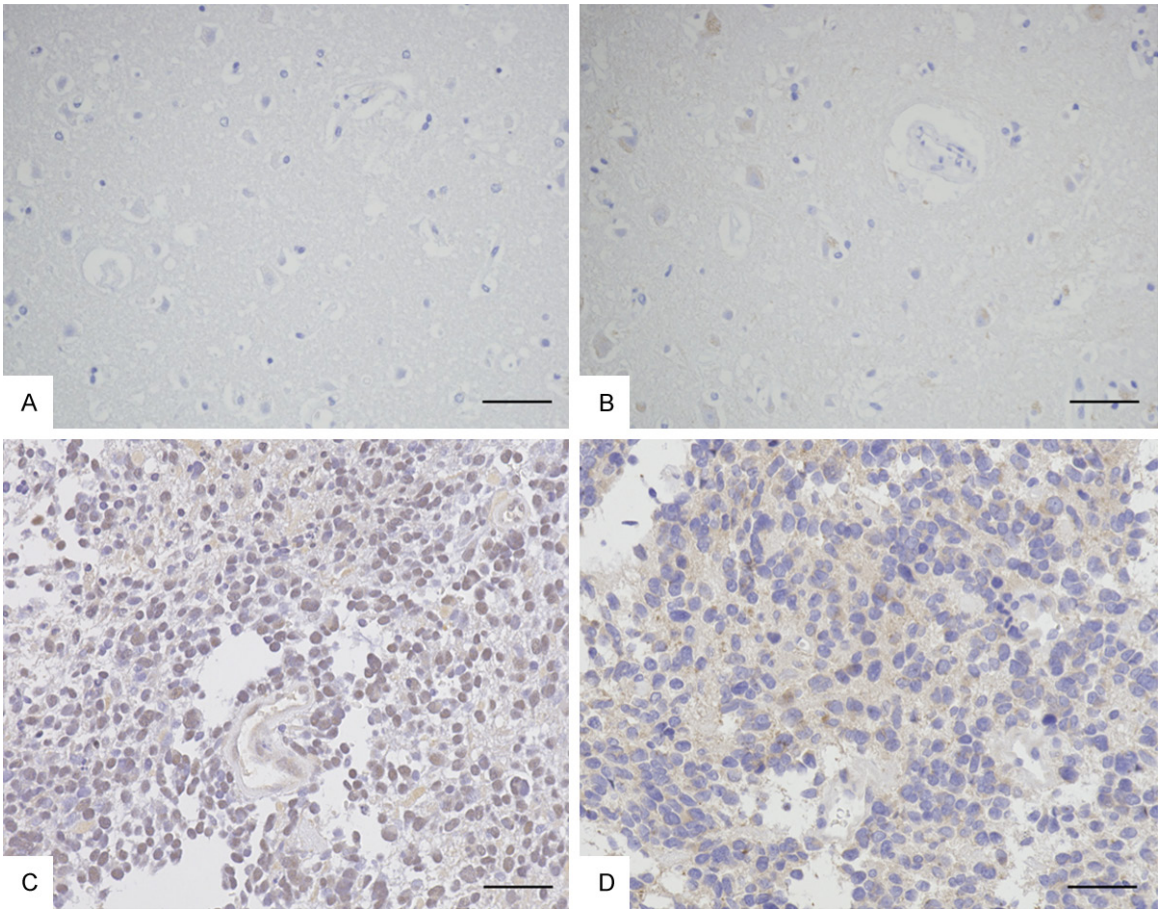

Figure 1. Immunohistochemical staining of ADAM10 and Notch1. In normal brain, (A) ADAM10 was not expressed and (B) Notch1 was slightly expressed in the cytoplasm of neurons. In glioblastoma, (C) ADAM10 was expressed in the cytoplasm and nucleus of tumor cells and endothelial cells. (D) Notch1 was mainly expressed in the cytoplasm of tumor cells. Scale bars 50 μm.

by multiplying the IS and PS grades. Final scores of ≥6 were defined as high, and those of <6 were defined as low.

Statistical analysis

The significance of correlations between ADAM10 expression levels and age, gender, Karnofsky Performance Status (KPS), tumor size and Notch1 expression were assessed using the Fisher's exact test. The Kaplan-Meier method was used to estimate survival with comparison made between groups based on ADAM10 expression levels. Log-rank test was used to test differences in survival between the ADAM10 high and ADAM10 low groups. Univariate and multivariate analyses were performed using the Cox proportional hazards model. Probability values of less than 0.05 ($P<0.05$) were considered significant. Xlstat® software for Mac (Addinsoft SARL, Paris, France) was used for the statistical analysis.

Results

Clinical characteristics

The characteristics of the 50 patients enrolled in the study are presented in **Table 1**. The median age was 59.5 years (range 9-77 years). Forty patients were 50 years or older, and 10 patients were under 50 years of age. There were 32 male patients and 18 female patients. The median KPS was 80 (range 20-100). Twenty-six tumors were ≥5 cm in diameter and 24 tumors were <5 cm. All patients underwent surgery. Fourteen patients were treated with gross total resection, 12 patients with subtotal resection, 20 patients with partial resection, and four patients with biopsy. High expression of ADAM10 was observed in 11 patients and low expression of ADAM10 was observed in 39 patients. High expression of Notch1 was found in 12 patients and low expression of Notch1 was observed in 38 patients.

Table 2. Correlation between ADAM10 expression and clinicopathological factors

Clinicopathological factors	n	ADAM10 expression		*p-value*
		High	Low	
Age, Median (range)	59.5 (9-77)			
≥50 years	40	8	32	0.671
<50 years	10	3	7	
Gender				
Male	32	8	24	0.724
Female	18	3	15	
KPS, Median (range)	80 (20-100)			
≥70	37	7	30	0.508
<70	13	4	9	
Size				
≥5 cm	26	4	22	0.314
<5 cm	24	7	17	
Notch1 expression				
High	12	7	5	0.002*
Low	38	4	34	

*Statisitical significance was determined with Fisher's exact test.

Expression of ADAM10 and Notch1 in normal brain and glioblastoma

In normal brain, ADAM10 was not expressed, and Notch1 was slightly detected in the cytoplasm of neurons (**Figure 1A**, **1B**). In a case of glioblastoma with high expression of ADAM10 and Notch1, ADAM10 was mainly expressed in the cytoplasm and nucleus of tumor cells, and weak staining was observed in infiltrating endothelial cells (**Figure 1C**). Notch1 was mainly expressed in the cytoplasm of tumor cells (**Figure 1D**).

Correlation between ADAM10 expression and clinicopathological factors

We found a significant positive correlation between the expression of ADAM10 and the expression of Notch1 (P = 0.002). No significant correlations were detected between ADAM10 expression and age, gender, KPS score, or tumor size (**Table 2**).

Kaplan-Meier survival curves and log-rank tests for ADAM10

Kaplan-Meier survival curves were used to compare patients with high or low ADAM10 expression levels. The median overall survival (OS) was 478 and 633 days in ADAM10 high and ADAM10 low patients, respectively. Survival was significantly longer in the patients with ADAM10 low compared to ADAM10 high by log-rank test (P = 0.033, **Figure 2A**).

Kaplan-Meier survival curves and log-rank tests for ADAM10 in patients who underwent subtotal and gross total resection

We evaluated the prognosis for patients who underwent subtotal or gross total resection. The median OS was 319 and 725 days in ADAM10 high and low patients, respectively. Survival was significantly longer in the patients with ADAM10 low compared to ADAM10 high by log-rank test (P = 0.006, **Figure 2B**). However, there was no statistically significant difference in OS between ADAM10 high and ADAM10 low patients who underwent partial resection and biopsy (data not shown).

Univariate and multivariate analyses of prognostic factors for overall survival

Univariate and multivariate analyses were performed for the survival rates of patients by age, KPS, extent of resection, ADAM10 expression and Notch1 expression. Younger age tended to be associated with better survival with a hazard ratio of 0.434 by univariate analysis (P = 0.067). The gross total resection patient group tended to be better survival, although the difference was not statistically significant. The ADAM10 low group was associated with significantly better survival with a hazard ratio of 0.432 (P = 0.038) and 0.298 (P = 0.048) by univariate and multivariate analyses, respectively. However, KPS and Notch1 expression was not associated with survival (**Table 3**).

Discussion

ADAM10 is a multipotent molecule involved in regulating ectodomain shedding, and is upregulated in various types of cancers, contributing to cancer progression and metastasis [21-23]. In a previous study of glioma, Bulstrode et al demonstrated that ADAM10 was overexpressed in human glioma tissue and was required for growth of glioma tumor spheres [14]. In this study, we used immunohistochemical staining to examine ADAM10 expression. ADAM10 was detected not only in the cytoplasm of glioblas-

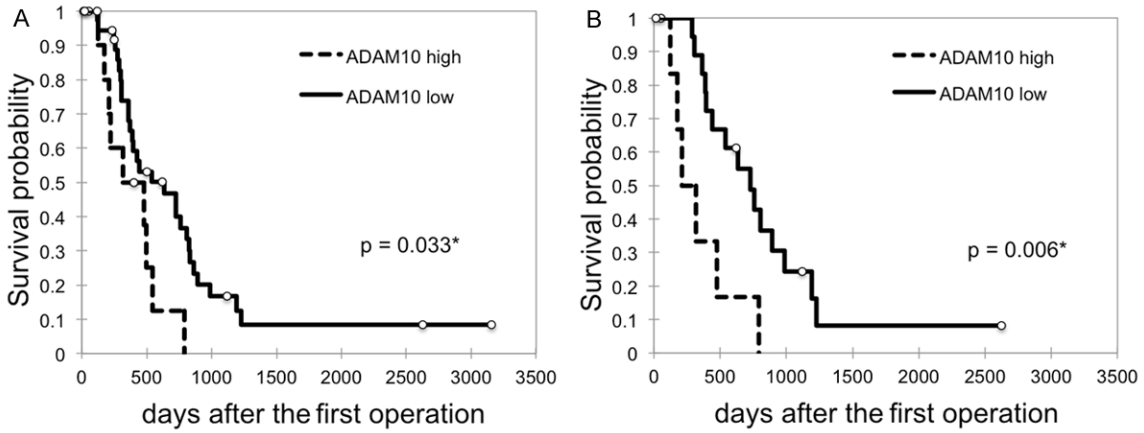

Figure 2. Kaplan-Meier survival analysis for overall survival based on ADAM10 expression in patients with glioblastoma. A. Survival was significantly longer in the patients with low ADAM10 expression compared to high ADAM10 expression in 50 patients with glioblastoma (P = 0.033, log-rank test). B. Survival was significantly longer in the patients with low ADAM10 expression compared to high ADAM10 expression in 26 patients treated with subtotal and gross total resection (P = 0.006, log-rank test).

Table 3. Univariate and multivariate analyses of factors predicting overall survival

	Univariate			Multivariate		
	Hazard ratio	95% CI	*p-value*	Hazard ratio	95% CI	*p-value*
Age <50 years	0.434	0.177-1.061	0.067	0.565	0.202-1.584	0.278
KPS <70	1.212	0.550-2.671	0.633	1.355	0.569-3.231	0.493
Surgical extent[a]	2.055	0.948-4.453	0.068	2.063	0.895-4.753	0.089
ADAM10 low	0.432	0.196-0.955	0.038*	0.298	0.090-0.991	0.048*
Notch1 low	1.120	0.538-2.331	0.763	2.170	0.617-7.633	0.227

[a]: without vs. with gross total resection. *Statisitical significance was determined with Cox proportional hazards model.

toma cells, but also in the nucleus of glioblastoma cells and infiltrating endothelial cells. Arima et al reported that in prostate cancer the ADAM-10 protein showed a nuclear localization [9]. They found a positive correlation between nuclear ADAM-10 staining intensity and progression of prostate cancer. The results suggested that nuclear translocation of ADAM10 was linked to the pathogenesis and progression of human prostate cancer [9]. In addition, ADAM10 regulates endothelial permeability and T-cell transmigration by shedding of vascular endothelial VE-cadherin [24], suggesting that the expression of ADAM10 in infiltrating endothelial cells may be involved in promoting tumor associated angiogenesis through regulation of Vascular Endothelial Growth Factor (VEGF)-induced endothelial cell function [25]. Overall, these data together with confirmation that ADAM10 was expressed in glioblastoma and not in normal brain tissue, suggest that ADAM10 may be a valid therapeutic target for glioblastoma.

Our immunohistochemical study revealed that the expression of ADAM10 was significantly positively correlated with Notch1 expression. This result suggested that ADAM10 may be an endogenous Notch1 activator in glioblastoma, an observation that is consistent with studies of other cancers including non-small cell lung cancer [26], and leukemia, breast cancer, lung cancer and glioma [16]. Notch1 plays a role in glioma cell migration and invasion [27] and activation of Notch is involved in the regulation of vessel sprouting [28]. Li et al reported that Notch1 expression is correlated with glioma progression, and high Notch1 protein expression is an independent predictor of poor survival in patients with glioma [29]. However, in our study Notch1 was not correlated with a prognosis for patients with glioblastoma.

In conclusion, we found that high expression of ADAM10 and Notch1 were observed in the 22% and 24% patients in IDH1 wild-type glioblastoma respectively, furthermore, ADAM10 expression was positively correlated with Notch1 expression. However, there was no ADAM10 expression in normal brain tissue. In our

small study, low ADAM10 expression in the patients with IDH1 wild-type glioblastoma had a tendency to be longer survival compared to high ADAM10 expression, especially in the patients who underwent subtotal or gross total resection of the tumor. However, the patient number of this study was small and the patients' background such as age, treatment was not homogeneous. Although this study was preliminary, our data suggest that therapeutic ADAM10 inhibitors may potentiate anti-cancer therapy for glioblastoma patients, more extensive studies on a larger cohort of patients are required to definitively elucidate the function and prognostic significance of ADAM10 expression in glioblastoma.

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Disclosure of conflict of interest

None.

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