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Original Article

Decreased expression of *ARID1A* is related to the poor prognosis of glioma patients

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Abstract: Purpose: This study intended to determine the expression of AT rich interactive domain 1A (SWI-like) (*ARID1A*) in serum of glioma patients and explore the relevance between *ARID1A* expression and the prognosis of glioma patients. Methods: The expression of *ARID1A* in serum of glioma patients and healthy controls were measured by high performance liquid chromatography (HPLC). Chi-square test was applied to evaluate the statistical difference between *ARID1A* expression and the clinicopathologic characteristics. Kaplan-Meier analysis combing with log-rank test was used to compare the overall survival of glioma patients with different *ARID1A* expression. Cox regression analysis was performed to evaluate the relationship between *ARID1A* expression and the prognosis of glioma patients. Results: The *ARID1A* expression was significantly lower in serum of glioma patients than in the healthy controls ($P < 0.001$). Moreover, the *ARID1A* expression was closely related to pathological grading, age and KPS score ($P < 0.05$), while no relationship was found between *ARID1A* expression and gender, preoperative epilepsy, or tumor range ($P > 0.05$). Besides, the overall survival time of patients with high *ARID1A* expression was significantly longer than those with low *ARID1A* expression according to Kaplan-Meier analysis ($P = 0.005$). Cox regression analysis illustrated that *ARID1A* expression was a potential factor for prognosis of glioma patients and it might be an independent biomarker ($P = 0.002$, HR = 4.992, 95% CI = 1.831-13.611). Conclusion: In a word, our study indicated that down-regulation of *ARID1A* was a promising biomarker for the prognosis of glioma patients.

Keywords: *ARID1A,* glioma, prognosis

Introduction

Tumor is one of the most severe threats for human beings among which glioma is distinct because of the unique micro-environment [1]. Glioma, which arises from glial cells, is one of the most frequent and most aggressive primary brain tumors in clinic [2, 3]. It accounts for 50%-60% of intracranial tumors and has the five-year survival rate only 20%-30% [4-6]. It has been confirmed that the risk of glioma was influenced by many factors such as hereditary disorders and exposure to high doses of ionizing radiation [7, 8]. Currently, despite the therapies for glioma including surgery, radiotherapy and chemotherapy have progressed a lot, the prognosis of this disease is still poor [9]. Therefore, it has been considered a promising approach to find a novel prognostic biomarker for the therapy of glioma patients.

AT-rich interactive domain 1A (*ARID1A*) gene, which is also called *BAF250a*, *p270*, *hOSA1* and *SMARCF1*, is located at the 1p36.11 region of chromosomes [10]. *ARID1A* had been proved to participate in the regulation of various cellular processes, such as development, differentiation, proliferation and chromatin remodeling [11-13]. Evidence has confirmed that mutations and deficient of *ARID1A* were frequently observed in a variety of tumors or cancers, including breast cancer, endometrioid ovarian carcinoma, gastric cancer and Barrett's esophagus carcinoma [14-17]. These observations indicate that *ARID1A* is a potential candidate tumor suppressor. In addition, previous reports have investigated the prognostic significance of *ARID1A* mutation in gastric cancer and found that decreased expression of the *ARID1A* gene was associated with poor prognosis in patients [18], and the same results were also found in

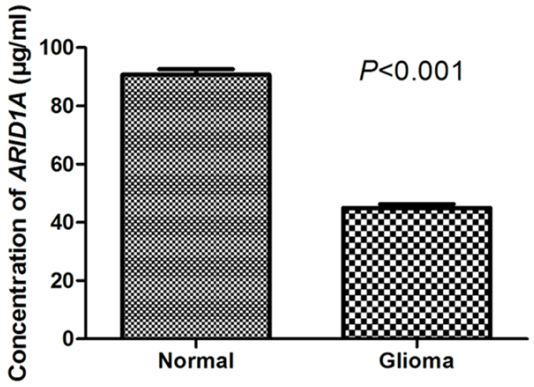

Figure 1. The expression of *ARID1A* in serum of glioma patients and normal controls was assayed by HPLC. *ARID1A* was significantly decreased in glioma patients in contrast to the normal controls ($P < 0.001$).

clear cell renal cell carcinoma [19]. Although the relationship between expression of *ARID1A* and clinicopathological variables and prognostic significance in some cancers have been reported, the role of *ARID1A* in glioma is still unclear.

The present study attempted to examine the expression of *ARID1A* in glioma patients and evaluate the prognostic value of *ARID1A*.

Materials and methods

Patients and specimens

A total of 83 patients who were pathologically diagnosed with glioma were selected from the Second Hospital of Hebei Medical University. Among the patients, there were 49 males and 34 females with the age range from 10 to 70 years. None of the patients had received radio- or chemo- therapy before serum collection. In addition, another 46 healthy human serum specimens (the biochemical indicators and immune indexes were in the normal range) were collected and regarded as controls. The study obtained approval of the Ethic Committee of the Second Hospital of Hebei Medical University. All the participants were asked to sign the informed written consents in advance.

The serum of glioma patients and healthy controls were collected and put into EDTA collection tubes, then stored at -80°C for using. The clinicopathologic characteristics including age, gender, preoperative epilepsy, tumor range, KPS score, and pathological grading were recorded in a database. A 5-years follow-up was conducted and the information was gotten via a telephone or questionnaire. The overall survival period was defined from the day of diagnosis to the day of death. Patients who died from unexpected events or other diseases were excluded from our study.

High performance liquid chromatography analysis

The concentration of *ARID1A* in serum was determined by high performance liquid chromatography (HPLC). 5 mL 5% perchloric acid was added into 5 mL serum sample for protein precipitation. The residue was separated and centrifugated at 4000 r/min for 10 min. The supernatant was collected and the precipitation was dropped. Then the supernatant was separated and centrifugated at 4000 r/min for 10 min three times. The precipitum of each sample was respectively affiliated and dried at 40°C under nitrogen stream. Then the sediments were reconstituted with 50 μl methyl cyanide and 20 μl of the solution was used for sample injection. HPLC was carried out with C_{18} column (12 × 4 mm, 5 μm) at room temperature and the column temperature was 30°C. The mobile phase consisted of ultrapure water, methyl cyanide and trifluoroacetic acid (500:400:0.5). The flow rate was set at 1.0 mL/min, and the detection wavelength was 282 nm. 0.01 g BAF250a was weighted and diluted in a 50 mL volumetirc flask for standard curve.

Statistical analysis

All the data were carried out by SPSS 18.0 software (SPSS Inc, IL, USA). The difference of *ARID1A* expression between glioma patients and healthy controls was analyzed through Students't test. The statistical significance of *ARID1A* expression and clinicopathologic characteristics of glioma patients was evaluated by Chi-square test. Kaplan-Meier analysis was used to describe the overall survival rate of glioma patients with different *ARID1A* expression. Cox regression analysis was adopted to evaluate the correlation between *ARID1A* expression and the prognosis of glioma patients. $P < 0.05$ was considered to be statistically significant.

Table 1. Statistical difference in *ARID1A* expression of patients with various clinical features

Characteristics	Case (n)	*ARID1A* Expression		χ^2	*P* value
		Low	High		
Gender				0.042	0.838
Male	49	37	12		
Female	34	25	9		
Preoperative epilepsy				0.669	0.413
Yes	38	30	8		
No	45	32	13		
Tumor range				0.478	0.489
Single lobe of brain	46	33	13		
Multiple lobe of brain	37	29	8		
KPS score				4.371	0.037
< 80	44	37	7		
≥ 80	39	25	14		
Age				5.552	0.018
≤ 50	46	39	7		
> 50	37	23	14		
Pathological grading				4.490	0.034
I, II	35	22	13		
III, IV	48	40	8		

Results

Low expression of ARID1A was found in glioma patients

The *ARID1A* expression was examined in 83 glioma serum specimens and 46 healthy human serum samples by HPLC. The concentration of *ARID1A* in serum of glioma patients was 44.98 ± 11.87 (mean ± SD), while that in the healthy human serum was 90.75 ± 12.89 (mean ± SD). A significant decrease of *ARID1A* expression was found in the glioma samples compared to the controls, indicating that *ARID1A* might be a tumor suppressor in glioma patients (**Figure 1**, $P < 0.001$).

Relevance of ARID1A expression and the clinicopathologic characteristics of glioma patients

In order to elucidate the clinical significance of *ARID1A* in glioma, we estimated the association between *ARID1A* expression and the clinicopathologic characteristics. The specimens were divided into two groups manually with the median expression of *ARID1A* in glioma patients: the patients with an *ARID1A* expression of no less than 51.01 μg/ml were attributed to the high *ARID1A* expression group while the others were belonged to the low *ARID1A* expression group. The result revealed that *ARID1A* expression level was correlated with age ($P = 0.018$), KPS score ($P = 0.037$), and pathological grading ($P = 0.034$), but shared no significant relationship with gender, preoperative epilepsy and tumor range ($P > 0.05$), as shown in **Table 1**. This might indicate that the *ARID1A* expression was related to the development of glioma.

Correlation between ARID1A expression and the overall survival of glioma patients

During the follow-up, 61.3% (38 out of 62) cases died in the low *ARID1A* expression group, and 23.8% (5 out of 21) patients died in the high *ARID1A* expression group. Kaplan-Meier analysis manifested that patients with low *ARID1A* expression had shorter overall survival time than those with high *ARID1A* expression (**Figure 2**, Log-rank test, $P = 0.005$). Cox regression confirmed that *ARID1A* acted as an independent prognostic factor for glioma patients (**Table 2**, $P = 0.002$, HR = 4.992, 95% CI = 1.831-13.611).

Discussion

Gliomas represent a series of low and high grade brain tumors that belong to the central nervous system [20]. These tumors are classified as grade I-IV by the World Health Organization (WHO) according to the histology and morphological characteristics [21]. In addition, it has been confirmed that sophisticated gene interactions and molecular modulations were involved in the development of glioma. Glioma also has a highly invasive rate which makes the complete resection difficult and lead to a poor prognosis [22]. Therefore, we aimed to find a candidate biomolecular for prognosis of glioma patients and expected to provide a new therapy for this disease.

ARID1A encode a large nuclear protein which can interact with other proteins and form a switch/sucrose nonfermentable (SWI/SNF)

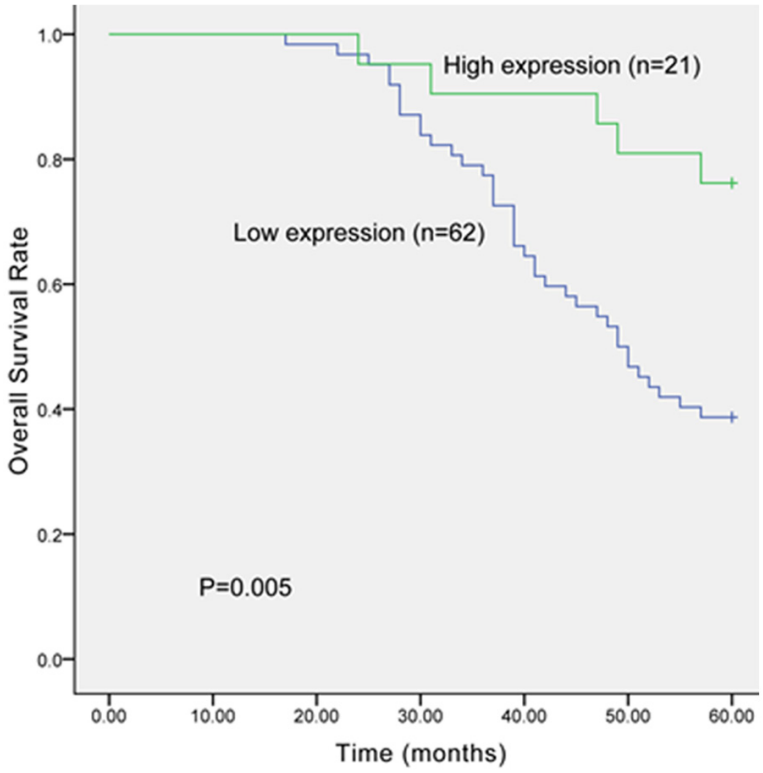

Figure 2. Kaplan-Meier analysis was made to evaluate the overall survival rate of glioma patients. Low *ARID1A* expression appeared to be correlated with unfavorable overall survival rate of glioma patients (P = 0.005). The P value was determined by log-rank test.

Table 2. Multivariate analysis for the prognostic factors in the patients with glioma

Characteristics	*P* value	HR	95% CI
Gender	0.424	0.724	0.327-1.599
Age	0.636	1.184	0.588-2.387
Pathological grading	0.921	1.044	0.446-2.446
ARID1A expression	0.002	4.992	1.831-13.611

chromatin remodeling complex [11, 23]. And it has been repeatedly reported to be mutated in various cancers as chromatin remodeling complexes. Loss or decreased expression of *ARID1A* is often found in a variety of cancers [18, 19], which has been proved to significantly relate to cancer recurrence and tumor aggravation. For instance, Ozawa Y et al. found *ARID1A* was decreased in esophageal squamous cell carcinoma and was a positive factor to the infiltrative growth of this tumor [24]. Itamochi H et al. investigated the effects of *ARID1A* and confirmed its down-regulation contributed to a poor prognosis in the stage I/II clear cell carcinoma of the ovary [25]. In the study of Cho H et al. the decrease of *ARID1A* participated in the tumor progression and predicted the prognosis of cervical cancer [26]. Besides, *ARID1A* also a prognostic factor in gastric cancer, breast cancer, and colorectal cancer because of its decreased or deleted expression in these cancers [18, 27, 28]. According to previous studies, *ARID1A* has often emerged as a novel tumor suppressor. However, its role in glioma had never been reported.

In the present study, we detected the expression of *ARID1A* in serum of glioma patients and found it was significantly decreased in the patients. Our finding was in agreement with a series of researches that *ARID1A* expression was frequently decreased or lost in a variety of cancers and indicated *ARID1A* might be a tumor suppressor in glioma. Then in order to estimate whether *ARID1A* was involved in the development of glioma, the relationship between its expression and clinicopathologic characteristics of glioma patients was analyzed. The result demonstrated that *ARID1A* expression was associated with the KPS score, age and pathological grading. So we inferred that *ARID1A* might participate in the progression of glioma development.

As a variety of studies all confirmed that the *ARID1A* expression was linked with the prognosis of cancers, we speculated that it was also associated with the prognosis of glioma. As shown in this study, Kaplan-Meier analysis verified a significant correlation between the decrease of *ARID1A* expression and overall survival of glioma patients. The results revealed that the overall survival time of patients with high *ARID1A* expression lived longer than those with low *ARID1A* expression. Next, Cox regression analysis further confirmed that the association between the expression level of *ARID1A* and the prognosis of glioma. The outcome proved that *ARID1A* expression could impact

the prognosis and might be an independent prognostic indicator for glioma patients. However, the mechanism of this effect of *ARID1A* on glioma has not been fully understood. Some researchers had considered nonsense or deletion mutation of *ARID1A*, the alterations of PI3K-AKT pathway, and the p53 expression were all relative factors for the function mechanism of *ARID1A* in several cancers [29-32]. Therefore, we conjectured that loss or decreased expression of *ARID1A* in glioma was correlated with PI3K-AKT or p53 pathways, and this deduction needs to be further investigated in the future studies.

In conclusion, the current study confirmed that the *ARID1A* expression was down-regulated in glioma and explained the clinical significance of *ARID1A*. What's more, abnormal expression of *ARID1A* was proved to be an independent prognostic marker and correlated with unfavorable prognosis in glioma patients.

Disclosure of conflict of interest

None.

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