

Adenine-and-Uridine-rich element RNA-binding factor 1 (AUF1) as an additional marker in human gliomas

Wataru HARAGUCHI, Takayuki MATSUO, Koichi YOSHIDA, Izumi NAGATA

Department of Neurosurgery, Nagasaki University Graduate School of Biomedical Sciences

AUF1 is one of Adenine-and-Uridine-rich elements binding protein which regulates the mRNA stability of many genes related to growth regulation, cytokines, and cell cycle-regulatory genes. There was no report about the expression of AUF1 in gliomas. Sections of formalin-fixed, paraffin-embedded tissues from 71 gliomas were stained immunohistochemically using a polyclonal antibody against the adenine-and-uridine-rich element RNA-Binding factor 1 (AUF1) oncoprotein. Positive staining, which is known to correlate with gene amplification, was not associated with patients' sex, age, Karnofsky performance status scores (KPS), tumor size, Bcl-2 expression, or longer overall survival. However, positive staining was negatively correlated with the MIB-1 labeling index, while it was positively correlated with the lower grade group of WHO classification. Expression of the AUF1 oncoprotein appears to be an important additional indicator in human gliomas.

ACTA MEDICA NAGASAKIENSIA 59: 7–11, 2014

Key words: glioma, AUF1, Adenine-and-Uridine rich element

Introduction

Glioma originate in the brain or spine and make up approximately 30% of all brain and central nervous system tumors and 80% of all malignant brain tumors [1]. They are fast growing and have a high recurrence rate [2]. The five-year survival rate for glioma patients is low even with surgery, radiotherapy, chemotherapy, and other forms of treatment[3]. Because of the infiltrative growth of gliomas, it is difficult to resect the whole tumor without causing serious damage to brain function [4]. The use of molecular biology methods to explore the pathogenic basis of gliomas may yield further insight into the treatment of this disease.

Adenine-and-uridine rich elements (AREs) represent a class of cis-acting elements that modulate mRNA stability [5]. They are present in a variety of mRNAs of genes that require rapid and fine modulation under particular conditions, such as response to growth factors, serum starvation,

and apoptosis [6.7.8.9]. The adenine-and-uridine-rich element RNA-binding factor 1 (AUF1) protein target mRNAs that also encode mitogenic, immune response, cancer-associated stress response, and cell cycle regulatory proteins[10.11.12]. AUF1 undergoes bidirectional nuclear- cytoplasmic shuttling, with mRNA stabilization or destabilizing activity presumed to occur in the cytoplasm, possibly in association with translational machinery [13]. The mechanism of action of ARE-mediated mRNA decay is under investigation by many laboratories.

At present, the expression status of AUF1 in gliomas and the mechanism of AUF1 action are unclear, and there have been a few studies investigating the behavior of gliomas. Here, we studied the relationship between AUF1 expression and the biological behavior of gliomas in order to provide a theoretical basis for the treatment of gliomas.

Address correspondence: Takayuki Matsuo, 1-7-1 Sakamoto, Nagasaki City, Nagasaki Prefecture, Japan zip:852-8501
Tel: 095-819-7375, Fax: 095-819-7378, E-mail: takayuki@nagasaki-u.ac.jp

Received January 19, 2014; Accepted February 4, 2014

Materials and methods

Clinical specimens and experimental materials

Paraffin-embedded specimens of brain gliomas from 71 patients were collected at Nagasaki University Hospital from January 1990 to December 2004. The average age of enrolled patients was 55.05 ± 21.36 years (range from 0.5 to 85 years). Gliomas can be divided into low-grade (World Health Organization (WHO) Grade I-II) and high-grade gliomas (WHO grade III-IV) depending on their growth rate [14]. Based on histopathological examination, patients were divided into two groups: eighteen patients had low-grade gliomas, and 53 had high-grade gliomas. All patients were assessed by the Karnofsky Performance (KPS) scale: (1) minor disability (80 to 100 points); (2) moderate disability (60 to 70 points); and (3) severe disability (10 to 50 points) [15].

The inclusion criteria were as follows: (a) resected specimens had undergone pathological examination, (b) a complete medical record was available and (c) enrolled cases were successfully followed up.

Immunohistochemistry

Tumor tissue blocks were freshly cut into 4- μ m thick sections. Sections were fixed on slides and dried for 12 to 24 h at 37°C. Sections were subsequently deparaffinized in xylene and rehydrated through a graded series of ethanol to distilled water. After antigen retrieval, sections were incubated for 60 min with primary antibody to AUF1 (07-260; Upstate, Hamilton, U.S.A), bcl-2(M0887; DAKO, Glostrup, Denmark) or Ki-67(M7240; DAKO). Following washing with PBS, sections were incubated for 30 min with the biotinylated secondary antibody (multi-link swine anti-goat/mouse rabbit immunoglobulin; Dako). After washing, an avidin/Biotin Complex (1:1000; Vector Laboratories, Burlingame, CA, USA) was applied to the sections for 30 to 60 min at room temperature. The sections were washed and the products were visualized by oxidation of 3,3'-diaminobenzidine (DAB) by horseradise peroxidase (HRP) for 20s in the presence of H₂O₂. Sections were then counterstained in Gill's hematoxylin and dehydrated in ascending grades of methanol before clearing in xylene and mounting under a coverslip.

For negative controls, the primary antibody was replaced with 0.01M PBS. Cells positive for AUF1 and bcl-2 were defined as those with brown granules in the nucleus. Two hundred cells from two representative fields of each section were counted by two independent observers to determine

the immunostaining intensity. The staining intensity was recorded on a 4-point scale: 0, no staining; 1, light brown (weak immunostaining); 2, brown (moderate immunostaining); 3, dark brown (strong immunostaining). In addition, the extent of staining was assessed on a 4-point scale: 1, <25% positive cells; 2, 26-50% positive cells; 3, 51-75% positive cells; 4, >75% positive cells. Scores from the two scales were combined and each section was classified as low/no AUF1 expression (1-4 points) or high AUF1 expression (5-7 points). Ki-67 expression was classified semi-quantitatively classified based on positive staining for Ki-67 in the nucleus.

Statistical analysis

All data were analyzed with SPSS statistics software (Version 13.0; SPSS, Inc., Chicago, IL, USA). The relationships between AUF1 and other parameters were studied using the chi-square test, Fisher's exact test, or independent t tests. Disease-specific survival was analyzed using the Kaplan-Meier method. The log-rank test was used to analyze differences in survival. A p value of less than 0.05 was considered statistically significant.

Results

Expression of AUF 1 in gliomas

Immunohistochemical analyses showed that AUF1 localized in the nucleus of the glioma cells (Figure 1). Based on the 4-point scales used to grade AUF-1 staining, 9 (12.7%) glioma section scored 2 points, 18(25.4%) scored 3 points, 14(19.7%) scored 4 points, 9(12.7%) scored 5 points, 9(12.7%) scored 6 points, and 12(16.9%) scored 7 points.

The relationship between AUF1 expression and clinico-pathological characteristics

Chi-square analysis showed that AUF1 expression was closely related to the Ki-67 expression and the WHO grade ($P=0.0309$ and 0.001 , respectively), but it was not related to patient sex, age, KPS Score, tumor diameter or Bcl-2 expression (Table1).

Prognostic analysis

Survival analysis showed that patients with high AUF1 expression tended to have better postoperative disease-specific survival than those with low AUF1 expression, although

the difference was not statistically significant (Figure 2). Kaplan-meier & Log-rank analysis identified the WHO

grade and Ki-67 expression as independent prognostic factors ($P=0.001$ and 0.011 , respectively).

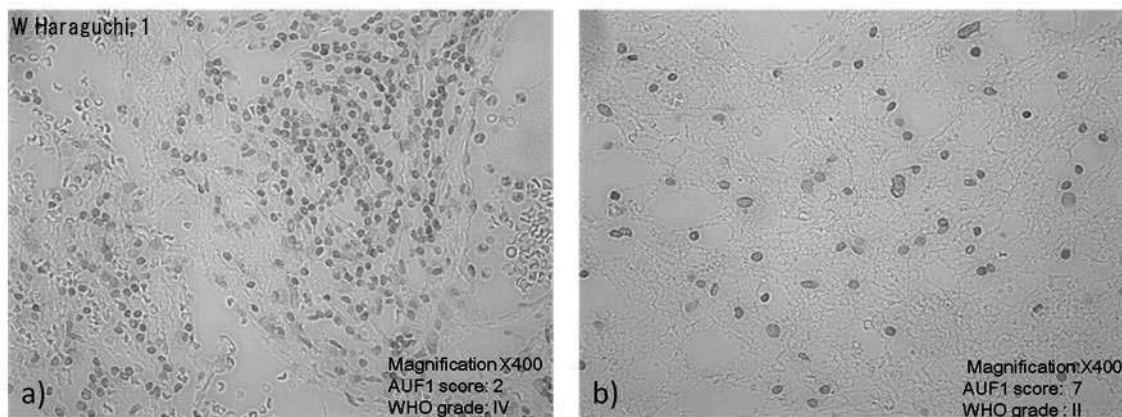


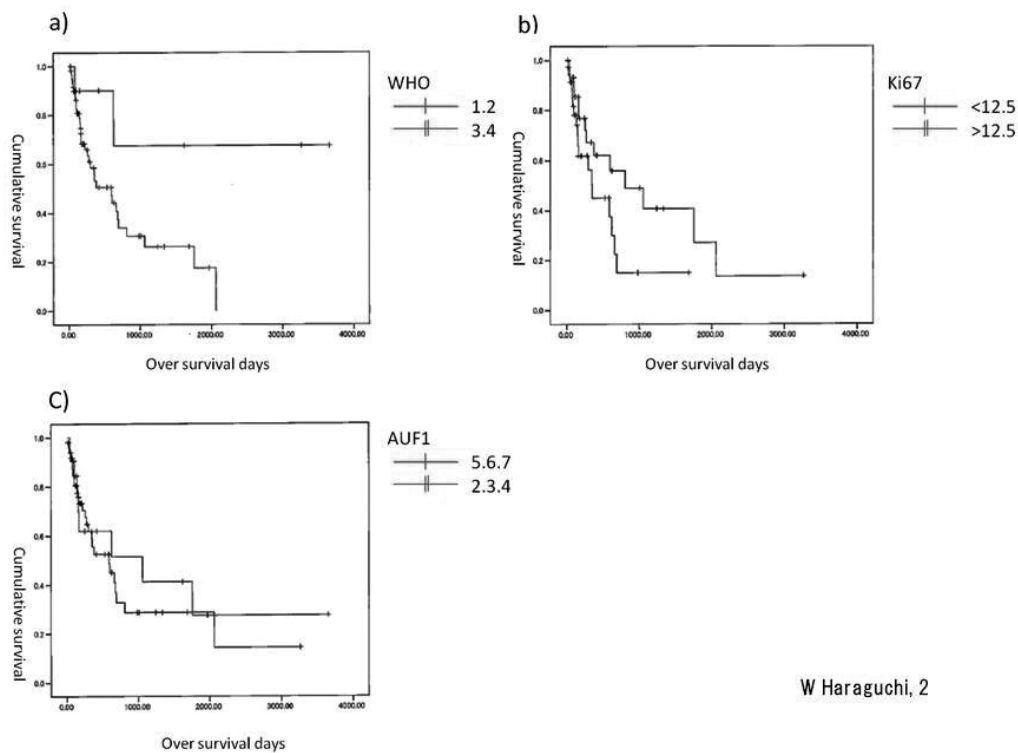
Figure 1. Expression of AUF1 in glioma tissues (X400).

a) High grade glioma stained for AUF1; b) low-grade glioma stained for AUF1.

Table 1

Variable	n	AUF1 n(%) 2/3/4	AUF1 n(%) 5/6/7	X2	P value
Sex				0.8480	0.357
Male	40	25(62.5)	15(37.5)		
Female	31	16(51.6)	15(48.4)		
Age(Years)				0.003	0.9593
<35	14	8(57.1)	6(42.9)		
>35	57	33(57.9)	24(42.1)		
KPS				0.005	0.9444
60-70	30	17(56.7)	13(43.3)		
80-100	40	23(57.5)	17(42.5)		
Tumor diameter				0.016	0.8992
<30mm	15	9(60.0)	6(40.0)		
>30mm	55	32(58.2)	23(41.8)		
Ki67				4.656	0.0309
<12%	27	11(40.7)	15(59.3)		
>12%	38	27(71.1)	12(28.9)		
Bcl-2				0.128	0.7206
2/3/4	27	14(51.9)	13(48.1)		
5/6/7	11	5(45.5)	6(54.5)		
WHO grade				16.667	<0.001
I-II	18	3(16.7)	15(83.3)		
III-IV	53	38(71.7)	15(28.3)		

Clinical characteristics in patients of glioma with low AUF1 expression group and high AUF1 expression group. KPS, Karnofsky performance status. WHO, world health organization.



W Haraguchi, 2

Figure 2: Kaplan-meier curve for overall survival by a) WHO classification, b) Ki-67 expression, and c) AUF1 expression.

Discussion

We have determined that the mRNA-binding protein AUF1 localized primarily in the nucleus in the low-grade gliomas and in the gliomas with a relatively low Ki-67 positive staining. However, we could not identify a relationship between the AUF1 expression level and prognosis of glioma patients.

AUF1 is an mRNA-binding protein and is known to affect target mRNA destabilization by binding A+U-rich elements [20]. In general, mRNA-binding proteins, including AUF1, regulate cellular processes including differentiation, survival, senescence, and the response to stress and immune signals [17,18]. AUF1 target mRNAs that encode proteins implicated in processes such as cell-cycle progression (e.g., cyclin D1, p21, c-Myc), apoptosis (e.g., Bcl-2), and the stress response (e.g., Gadd45 a, ATF3) [19,20,21]. In addition, overexpression of AUF1 triggers sarcoma development [22], and high AUF1 levels have been detected in numerous malignancies, including cancers of the breast, skin, thyroid, and liver [23].

Our data showed a significant relationship between AUF1 expression and WHO grade, as well as AUF1 expression and Ki-67 expression. However, there was no relationship be-

tween AUF1 expression and survival of the glioma patients. As such, AUF1 expression is not a prognostic factor in patients with gliomas. Previously it had been shown that AUF1 over-expression leads to tumorigenesis in several in vitro models [22]. Several studies have also demonstrated that AUF1 is involved in apoptosis, tumorigenesis, and development by its interactions with ARE-bearing mRNAs. Trojancowicz et al. determined that AUF1 in complexes with ARE-bearing mRNAs was crucial for proliferation and the cell cycle of thyroid carcinomas [24]. AUF1 may control the balance between stabilizing and destabilizing effects, both of which are exerted on the cell cycle machinery in tumors. Although we cannot explain the role of AUF1 in the tumorigenesis of gliomas, AUF1 may be considered as an additional marker for glioma grading.

One limitation of our study is that immunohistochemical analysis is not a strictly quantitative method. Different scores and cut-off values exist and the interpretation of staining intensity remains partially subjective. Another limitation is the small number of study cases.

Despite these limitations, our study suggests that the immunohistochemical staining of AUF1 in gliomas may be important. Further investigations of AUF1 will reveal the potential value of AUF1 as a biomarker of gliomas.

Acknowledgements

no financial and other conflicts of interest for the work

References

- [1] Mamelak AN, Jacoby DB. Targetted delivery antitumoral therapy to glioma and other malignancies with synthetic choleroxin(TM-601). *Expert Opin Drug Deliv* 4:175-186,2007
- [2] Venkataramana NK, Venkatesh PK, Dwarakanath BS, Vani S. Protective effect on normal brain tissue during a combinational therapy of 2-deoxy-glucose and hypo fractionated irradiation in malignant gliomas. *Asian J Neurosurg* 8:9-14,2013
- [3] Zhao S, Wu J, Wang C et al. Intraoperative fluorescence-guided resection of high grade malignant gliomas using 5-aminolevulinic acid induced porphyrins; a systemic review and meta-analysis of prospective studies. *Plos One* 8:e63682,2013
- [4] Hoover JM, Nwojo M, Puffer R, Mandrekar J, Meyer FB, Parney IF. Surgical outcome in recurrent glioma. *J Neurosurg* 118:1224-1231,2013
- [5] Chen CY, Shyu AB. AU-rich elements: characterization and importance in mRNA degradation. *Trends Biochem Sci* 20:465-470,1995
- [6] Scheiavone N, Rosini P, Quattrone A et al. A conserved AU-rich element in the 3' untranslated region of bcl-2 mRNA is endowed with a destabilizing function that is involved in bcl-2 down-regulation during apoptosis. *FASEB J* 14:174-184,2000
- [7] Xu N, Loflin P, Chen Cy, Shyu AB. A broader role for AU-rich element-mediated mRNA turnover revealed by a new transcriptional pulse strategy. *Nucleic acids Res* 26:558-565,1998
- [8] Rodriguez-Pascual F, Hausding M, Ihriq-Biedert I et al. Complex contribution of the 3'-untranslated region to the expressional regulation of the human inducible nitric-oxide synthase gene. Involvement of the RNA-binding protein HuR. *J Biol Chem* 275:26040-26049,2000
- [9] Madireddi MT, Dent P, Fisher PB. Regulation of mda-7 gene expression during human melanoma differentiation. *Oncogene* 19:1362-1368,2000
- [10] Ross J. mRNA stability in mammalian cells. *Microbiol Rev.* 59:423-450,1995
- [11] Bhattacharya S, Giordano T, Brewer G, Malter JS. Identification of AUF1 ligands reveals vast diversity of early response gene mRNAs. *Nucleic Acids Res* 27:1464-1472,1999
- [12] Lin S, Wang W, Wilson GM et al. Downregulation of cyclin D1 expression by prostaglandin A2 is mediated by enhanced cyclin D1 mRNA turnover. *Mol Cell Biol* 20:7903-7913,2000
- [13] Jacobson A, Peltz SW. Interrelationships of the pathways of mRNA decay and translation in eukaryotic cells. *Annu Rev Biochem* 65:693-739,1996
- [14] Louis DN, Ohgaki H, Wiestler OD et al. The WHO classification of tumors of the central nervous system. *Acta Neuropathol* 114:97-109,2007
- [15] Wen PY, Brandes AA. Treatment of recurrent high-grade gliomas. *Curr Opin Neurol* 22:657-664,2009
- [16] Sirenko OI, Lofquist AK, DeMaria CT, Morris JS, Brewer G, Haskill JS. Adhesion-dependent regulation of an ARE-binding activity associated with AUF1. *Mol Cell Biol* 17:3898-3906,1997
- [17] Apponi LH, Corbett AH, Pavlath GK. RNA-binding proteins and gene regulation in myogenesis. *Trends Pharmacol Sci* 32:652-658,2011
- [18] Abdelmohsen K, Yamanaka K, Srikantan S, Yoon JH, Kang MJ, Gorospe M. RNA-binding protein AUF1 represses Dicer expression. *Nucleic Acids Research* 40:11531-11544,2012
- [19] Zucconi BE and Wilson GM. Modulation of neoplastic gene regulatory pathways by the RNA-binding factor AUF1. *Front Biosci* 16:2307-2325,2011
- [20] Gratacos FM and Brewer G. The role of AUF1 in regulated mRNA decay. *Wiley Interdiscip Rev RNA* 1:457-473,2010
- [21] Mameczark MK, Kuwano Y, Zhan M et al. Identification of a signature motif in target mRNAs of RNA-binding protein AUF1. *Nucleic Acids Res* 37:204-214,2009
- [22] Gouble A, Grazide S, Meggetto F, Mercier P, Delsol G, Morello D. A New Player in Oncogenesis: AUF1/hnRNP Overexpression Leads to Tumorigenesis in Transgenic Mice. *Cancer Research* 62:1489-1495,2002
- [23] Zucconi BE, Wilson GM. Modulation of neoplastic gene regulatory pathways by the RNA-binding factor AUF-1. *Front Biosci* 16:2307-2325,2011
- [24] Trojanowicz B, Broudauf L, Sekulla C et al. The role of AUF1 in thyroid carcinoma progression. *Endocr Relat Cancer* 16:857-871,2009