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Original Article

Expression profile of ACTL8, CTCFL, OIP5 and XAGE3 in glioma and their prognostic significance: a retrospective clinical study

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Abstract: Background: Cancer/testis antigens (CTAs) are attractive therapeutic targets for tumor immunotherapy due to their restrictive expression in normal testis but excessive in majority of tumor types. ACTL8, CTCFL, OIP5 and XAGE3 are members of the CTAs family. Currently, the data of ACTL8, CTCFL, OIP5 and XAGE3 expression in glioma is limited. *Methods:* ACTL8, CTCFL, OIP5 and XAGE3 mRAN and protein expressions were detected in 108 glioma samples by Reverse Transcriptase-PCR (RT-PCR) and immunohistochemistry and the correlations between their expressions and clinical indexes were analyzed. Furthermore, their clinical significance on glioma prognosis was determined by follow-up data. *Results:* The mRNA positive rate of ACTL8, CTCFL, OIP5 and XAGE3 was 15.74% (17/108), 22.22% (24/108), 13.89% (15/108) and 37.96% (41/108), respectively. At least one CTA mRNA was expressed by 61.11% of glioma tissues, while 2 or more by 29.63%. For protein expression, the positive rate of them was 21.30% (23/108), 34.26% (37/108), 19.44% (21/108) and 23.15% (25/108), respectively. At least one CTA protein was expressed by 58.33% of glioma tissues and 2 or more by 29.63%. Although there were no correlations between their mRNA expressions and clinicopathological parameters, the protein expression of ACTL8, OIP5 and XAGE3 was positively correlated with KPS; while the ACTL8 protein was correlated with gender, and OIP5 protein with gender and WHO grade. Kaplan-Meier analysis revealed a significant negative correlation between the CTCFL protein expression, combined ACTL8 and/or CTCFL protein expression and survival. *Conclusions*: The results suggest that the cohort of glioma does express ACTL8, CTCFL, OIP5 and XAGE3 at both mRNA and protein levels indicating glioma is CTAs-rich tumors. CTCFL protein and the combined ACTL8 and/or CTCFL protein might act as poor prognostic markers for glioma and as potential ideal combined antigens for glioma immunotherapy.

Keywords: Cancer/testis antigen, glioma, ACTL8, CTCFL, OIP5, XAGE3

Introduction

Glioma, the most frequent human primary central nervous tumor, originates from neural mesenchymal cells [1], and is divided into four grades according to WHO classification [2]. Despite continuous advances in surgical treatment and improved systemic chemotherapy and radiotherapy, the survival rate of glioma patients remains poor over the past decades. Especially for glioblastoma multiforme (GBM) which is the most aggressive form in all of human glioma, the mean survival was at approximate 12 months [3, 4]. The high mortality of glioma patients generally results from the lack of effective therapeutic approaches. Therefore, it is extremely crucial to develop new therapeutic approaches for improving the survival of glioma patients.

Currently, the rising cancer immunotherapy is being considered as an attractive strategy for

glioma patient, because it is reported to improve the prognosis of patients with glioma [5]. However, the prerequisite step of effective immunotherapy for glioma is to identify the appropriate neuro-oncology specific target antigen. Cancer/Testis antigens (CTAs) are an immunogenic family of proteins with restricted expression in adult testicular germ cells, and yet are expressed in a wide variety of malignant tumors including glioma [6-11]. And they have been demonstrated to elicit an immune response in glioma patients [11]. These features make CTAs regarded as the potential ideal targets for glioma immunotherapy.

ACTL8, CTCFL, OIP5 and XAGE3 fall into the category of CTAs, and they are also known as CT57, CT27, CT86 and CT12 based on their number in CTA database (http://www.CTA.lncc.br/), respectively. At present, many studies of ACTL8, CTCFL, OIP5 and XAGE3 have been performed on various tumors but very few in brain tumors and thus their information in glioma is very limited. Only one study was reported by Freitas et al [8] in glioma, in which limited cases of glioma was detected at mRNA level and only one of them, CTCFL, at protein level but no others. The expression of their mRNAs in corresponding with their proteins in glioma and their clinical and prognostic significance in a larger cohort have not yet been fully investigated.

In this study, the expression of ACTL8, CTCFL, OIP5 and XAGE3 in large-scale glioma tissues was detected by both Reverse Transcriptase-PCR (RT-PCR) and immunohistochemistry (IHC). Then the correlation with their expression, the clinicopathological parameters and the overall survival of the glioma patients were analyzed. Moreover, we evaluate whether ACTL8, CTCFL, OIP5 and XAGE3 could be used as a prognostic and therapeutic marker for glioma patients.

Material and methods

Patients and specimens

This study protocol was approved by the Medical Ethics Committee of the First Hospital of Guangxi Medical University, and the written informed consent before enrolment was obtained from all patients included in this study. All of the patients were followed up by interview in clinic or phone call. The total period of follow-up was 60-72 months. The overall survival was calculated from the date of surgery until the date of death or last follow-up appointment. All tumor specimens were classified according to World Health Organization (WHO) criteria [2]. One normal brain tissue was collected from cerebral trauma patient who underwent intracranial decompression and one normal testicular tissue was taken from prostate cancer patient undergone surgery by castration.

Collection of clinical parameters

The clinical data of 108 patients with glioma, including patient age, gender, Karnofsky performance status (KPS) [12], tumor size, WHO grade, P53 and Ki-67 expression were collected from the hospital medical records of the patients.

Reverse transcriptase-PCR (RT-PCR)

Total RNA was extracted with RNA Extraction Kit (Takara, Dalian, China) according to the manufacturer's instruction. Total RNA (2 μg) was primed with an oligo $(dT)_{18}$ oligonucleotide and reverse-transcribed with PrimeScript® First Strand cDNA Synthesis Kit (Takara, Dalian, China). Then, cDNA obtained was tested for integrity by amplification of p53 gene as previous report [10]. RT-PCR was performed with ACTL8, CTCFL, OIP5 and XAGE3 specific primers as described by Freitas et al [8]. Human testis cDNA was used as a positive control in PCR reaction. The expression of ACTL8, CTCFL, OIP5 and XAGE3 was considered as positive only if the RT-PCR reaction repeated at least twice with same result.

Immunohistochemistry and evaluation

Immunohistochemistry (IHC) was performed as previous report with minor modification [13]. In brief, formalin-fixed and paraffin-embedded tissue sections were deparaffinized and rehydrated under the routine condition. Subsequently, the sections were treated in Citrate Antigen Retrieval Solution (pH 6.0) for antigen retrieval. After the inactivation of endogenous peroxidase, pre-immune serum from goat was added on the sections for blocking. Then the sections were incubated overnight at 4°C with a series of polyclonal antibodies, respectively. Antibodies purchased from Abcam were ACTL8 (dilution, 1:100), CTCFL (dilution, 1:500), OIP5 (dilution, 1:200) and XAGE3 (dilution, 1:80). Pre-

Table 1. Association between CTAs mRNA and histopathological parameters of glioma

Variables	Cases	ACTL8 positive		*P**	CTCFL positive		*P**	OIP5 positive		*P**	XAGE3 positive		*P**
		n	%		n	%		n	%		n	%	
Age (years)													
<39	53	10	18.9	0.381	13	24.5	0.571	10	18.9	0.142	22	40.0	0.456
≥39	55	7	12.7		11	20.0		5	9.1		19	34.5	
Gender													
Male	71	13	18.3	0.310	15	21.1	0.704	9	12.7	0.614	27	38.0	0.985
Female	37	4	10.8		9	24.3		6	66.7		14	37.8	
WHO grade													
I-II	44	9	20.5	0.265	11	25.0	0.565	9	20.5	0.102	18	40.9	0.601
III-IV	64	8	12.5		13	20.3		6	9.38		23	35.9	
Tumor size													
<5 cm	50	8	16.0	0.945	13	26.0	0.381	8	16.0	0.556	20	40.0	0.685
≥5 cm	58	9	15.5		11	19.0		7	12.1		21	36.2	
KPS													
<70	44	6	13.6	0.300	10	22.7	0.917	6	13.6	0.950	18	40.9	0.601
≥70	64	11	17.2		14	21.9		9	14.1		23	35.9	
Sum	108	17	15.7		24	22.2		15	13.9		41	38.0	

*: Statistically significant (P<0.05); WHO: World Health Organization; KPS: Karnofsky Performance Scale.

immune serum from rabbit was use as negative control. Sections then were incubated with 1:500 diluted secondary antibody (goat anti-rabbit IgG labelled with HR, Long Island Biotec, Shanghai, China). Immunoreactivity was visualized with 3, 3'-diaminobenzidine (DAB) (Maixin Biote, Fuzhou, China) followed by hematoxylin counterstain. Positive immunoreactivity was scored in terms of the percentage of staining intensity and positive cells by two independent pathologists who did not know patients' clinical information. The staining intensity was quantified using the following scores: 0 = no staining, 1 = weak, 2 = moderate and 3 = strong. The percentage of positive tumor cells was defined as follows: 0 = 0-5%, 1 = 6-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%. According to the sum of both points, each section was assigned as no CTAs expression when the sum score was 0, as low CTAs expression when the sum score between 1 and 4, and as high CTAs expression when the sum score more than 4 [13, 14].

Statistical analysis

The software of SPSS 22.0 (IBM, Armonk, NY) and GraphPad Prism 6.0 (San Diego, CA, USA) was used for statistical analysis and graphical representation. The statistical significance was defined as $P < 0.05$. Relationship between the tested CTAs expression and clinicopathological parameters was tested by χ^2 test. Overall survival rate was calculated using the Kaplan-Meier method. The difference in survival curves was compared using the log-rank test. The multivariable Cox regression analysis was performed to detect prognostic factors, and variables with a two-sided *P* value of < 0.05 were entered into the multiple-response analysis.

Results

Clinicopathological parameters of patients

The clinicopathological parameters of the 108 glioma patients are summarized in **Tables 1** and **2**. Of the 108 glioma samples tested, different histological types are as followed: 55 (50.93%) astrocytomas, 14 (12.96%) anaplastic astrocytomas, 32 (29.63%) glioblastomas, 4 (3.70%) oligodendrogliomas and 3 (2.78%) pilocytic astrocytomas. At the last follow-up, 79 patients were followed up successfully. The median overall survival time was 14 months. Further prognostic characteristics was listed in **Table 3**.

mRNA expression of ACTL8, CTCFL, OIP5 and XAGE3 in glioma tissues

The expression of ACTL8, CTCFL, OIP5 and XAGE3 mRNA was evaluated by RT-PCR in 108 glioma tissues, 1 normal brain tissue and 1 testis tissue. As shown in **Figure 1**, the testis tis-

Table 2. Association between CTAs protein tested and histopathological parameters of glioma

Variables	Cases	ACTL8 positive		*P**	CTCFL positive		*P**	OIP5 positive		*P**	XAGE3 positive		*P**
		n	%		n	%		n	%		n	%	
Age (years)													
<39	53	12	22.6	0.737	18	34.0	0.949	13	24.5	0.190	12	22.6	0.902
≥39	55	11	20.0		19	34.5		8	14.5		13	23.6	
Gender													
Male	71	10	14.1	0.011*	25	35.2	0.773	13	18.3	0.680	18	25.3	0.452
Female	37	13	35.1		12	32.4		8	21.6		7	18.9	
WHO grade													
I-II	44	11	25.0	0.593	13	29.5	0.392	13	29.5	0.028*	10	22.7	0.931
III-IV	64	19	29.7		24	37.5		8	12.5		15	23.4	
Tumor size													
<5 cm	50	12	24.0	0.524	18	36.0	0.723	6	12.0	0.070	10	20.0	0.471
≥5 cm	58	11	19.0		19	32.8		15	25.9		15	25.9	
Ki-67													
<10%	62	13	21.0	0.923	21	33.9	0.921	11	17.7	0.604	14	22.6	0.871
≥10%	46	10	21.7		16	34.8		10	21.7		11	23.9	
P53													
<10%	56	9	16.1	0.169	16	28.6	0.196	12	21.4	0.777	11	19.6	0.370
≥10%	52	14	26.9		21	40.4		10	19.2		14	26.9	
KPS													
<70	44	4	9.1	0.010*	11	25.0	0.062	13	29.5	0.038*	5	11.4	0.017*
≥70	64	19	21.9		26	40.6		8	12.5		20	31.3	
Sum	108	23	21.3		37	27.8		21	19.4		25	23.2	

*: Statistically significant (P<0.05); WHO: World Health Organization; KPS: Karnofsky Performance Scale.

sue highly expressed in all of CTAs tested and used as positive control. No mRNA of CTAs tested was found in normal brain tissue. As shown in **Table 1**, XAGE3 was mostly frequently expressed CTA, being present in 37.96% (41/108), followed by CTCFL (22.22%, 24/108), ACTL8 (15.74%, 17/108) and OIP5 (13.89%, 15/108). Notably, 61.11% of the glioma tissues expressed at least one of CTAs mRNA. Among them, 37.96% expressed one of these CTAs mRNA, 17.59% and 5.56% expressed two and three CTAs mRNA, respectively (**Figure 6A**, **6B**). Co-expression of at least two CTAs mRNA occurred in 23.15% of the samples tested.

Protein expression of ACTL8, CTCFL, OIP5 and XAGE3 in glioma tissues

The protein of ACTL8, CTCFL, OIP5 and XAGE3 was mainly observed in cytoplasm and nucleus of spermatogenic cells in seminiferous tubules of testis tissue which was considered as the positive control (**Figures 2E**, **5E**). There was no protein expression of ACTL8, CTCFL, OIP5 and XAGE3 detected in normal brain tissues (data not shown). While in glioma tissues, the protein of ACTL8, CTCFL, OIP5 and XAGE3 was found in 21.30% (23/108), 34.26% (37/108), 19.44% (21/108) and 23.15% (25/108), respectively (**Table 2**). The highest incidence of protein positivity was CTCFL, followed by XAGE3, ACTL8 and OIP5. As shown in **Figure 6C**, **6D**, glioma tissues expressed at least one CTA protein occupied 58.33%, among which 28.70% expressed only one of these CTAs proteins, 21.30% and 8.33% expressed any two and three of these CTAs proteins, respectively.

As regard to the protein localization and intensity, ACTL8 protein was discovered in both of cytoplasm and nucleus of glioma cells. Weak staining of ACTL8 was found in 14 of 23 specimens (60.87%), moderate staining in 6 of 23 (26.09%), and strong intensity in 3 of 23 (13.04%) (**Figure 2A-D**). Similarly, CTCFL protein was also localized in cytoplasm and nucleus of tumor cells (**Figure 3A-D**). Of specimens with CTCFL protein positive staining, 17 of 37 (45.95%) showed weak staining, 13 of 37 (35.14%) moderate staining and 7 of 37 (18.92%), respectively. As shown in **Figure**

Table 3. Cox proportional hazard model to analyze the clinicopathological parameters and the overall survival of glioma patients

Parameter	Subgroup	n	Univariate		Multivariable	
			HR (95% CI)	*P* value	HR (95% CI)	*P* value
Gender	Male	55	1.280 (0.689-2.385)	0.437		
	Female	24				
Age (years)	≥39	42	2.777 (1.455-5.299)	0.002*	2.219 (1.052-4.681)	0.036*
	<39	37				
Tumor size (cm)	≥5 cm	40	1.136 (0.632-2.040)	0.671		
	<5 cm	39				
KPS score	≥70	48	0.669 (0.362-1.236)	0.199		
	<70	31				
WHO grade	I-II	33	0.385 (0.200-0.739)	0.040*	0.479 (0.197-1.163)	0.104
	III-IV	46				
Ki-67 expression	≥10%	31	2.104 (1.167-3.790)	0.013*	1.228 (0.570-2.645)	0.600
	<10%	48				
p53 expression	≥10%	39	0.469 (0.257-0.857)	0.014*	0.539 (0.280-1.039)	0.065
	<10%	40				
ACTL8 expression	positive	17	1.508 (0.673-3.381)	0.318		
	negative	62				
CTCFL expression	positive	30	1.831 (1.015-3.305)	0.045*	1.530 (0.517-4.526)	0.442
	negative	49				
OIP5 expression	positive	15	1.052 (0.506-2.187)	0.891		
	negative	64				
XAGE3 expression	positive	19	1.250 (0.645-2.422)	0.509		
	negative	60				
ACTL8 and/or CTCFL expression	positive	39	1.848 (1.019-3.352)	0.043*	2.570 (0.866-7.625)	0.089
	negative	40				
ACTL8 and/or OIP5 expression	positive	29	1.349 (0.717-2.538)	0.353		
	negative	50				
ACTL8 and/or XAGE3 expression	positive	31	1.087 (0.593-1.990)	0.788		
	negative	48				
CTCFL and/or OIP5 expression	positive	38	1.619 (0.898-2.922)	0.109		
	negative	41				
CTCFL and/or XAGE3 expression	positive	36	1.6849 (0.934-3.305)	0.083		
	negative	43				
OIP5 and/or XAGE3 expression	positive	31	1.381 (0.767-2.489)	0.282		
	negative	48				
ACTL8, CTCFL and/or OIP5 expression	positive	44	1.602 (0.875-2.933)	0.127		
	negative	35				
ACTL8, CTCFL And/or XAGE3 expression	positive	45	1.769 (0.957-3.270)	0.069		
	negative	34				
ACTL8, OIP5 and/or XAGE3 expression	positive	40	1.184 (0.659-2.127)	0.573		
	negative	39				
CTCFL, OIP5 and/or XAGE3 expression	positive	43	1.704 (0.929-3.125)	0.085		
	negative	36				
ACTL8, CTCFL, OIP5 and/or XAGE3 expression	positive	49	1.719 (0.911-3.244)	0.094		
	negative	30				

*: Statistically significant (P<0.05); n: No. of patients; WHO: World Health Organization; KPS: Karnofsky Performance Scale.

4A-D, OIP5 protein was mostly distributed in cytoplasm of tumor cells, showing 14 of 21 (66.67%) weak staining, 5 of 21 (23.81%) moderate staining and 2 of 21 (9.52%) strong staining. Both cytoplasm and nuclear staining was demonstrated in XAGE3 (**Figure 5A-D**). Intensity of XAGE3 protein was different as followed: 15 of 25 (60.00%) weak staining, 7 of 25 (28.00%) moderate staining and 3 of 25 (12.00%) strong staining.

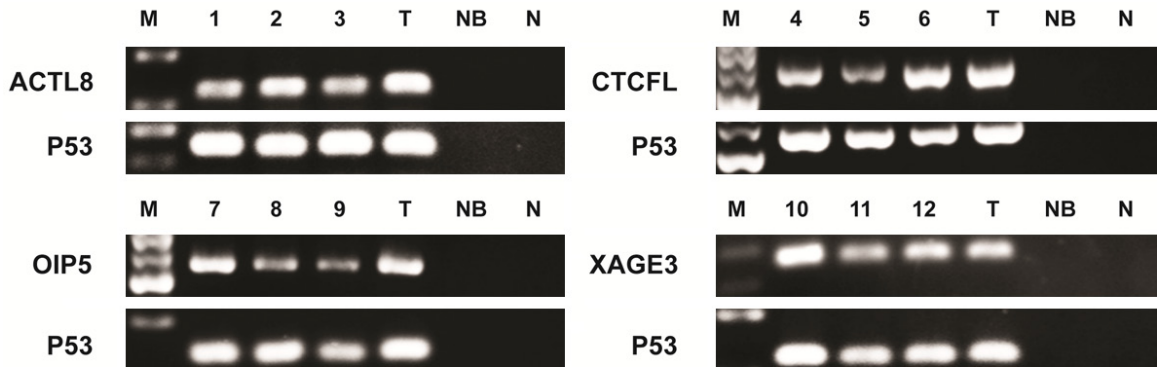


Figure 1. Representative RT-PCR analysis of four CTAs mRNA expression in glioma, Testis and normal brain tissues. M: Marker; 1-12: glioma tissue; T: Testis tissue (positive control); NB: Normal Brain tissue; N: Negative control (no cDNA template). P53 was used as internal control for the parallel PCR analysis of the same sample.

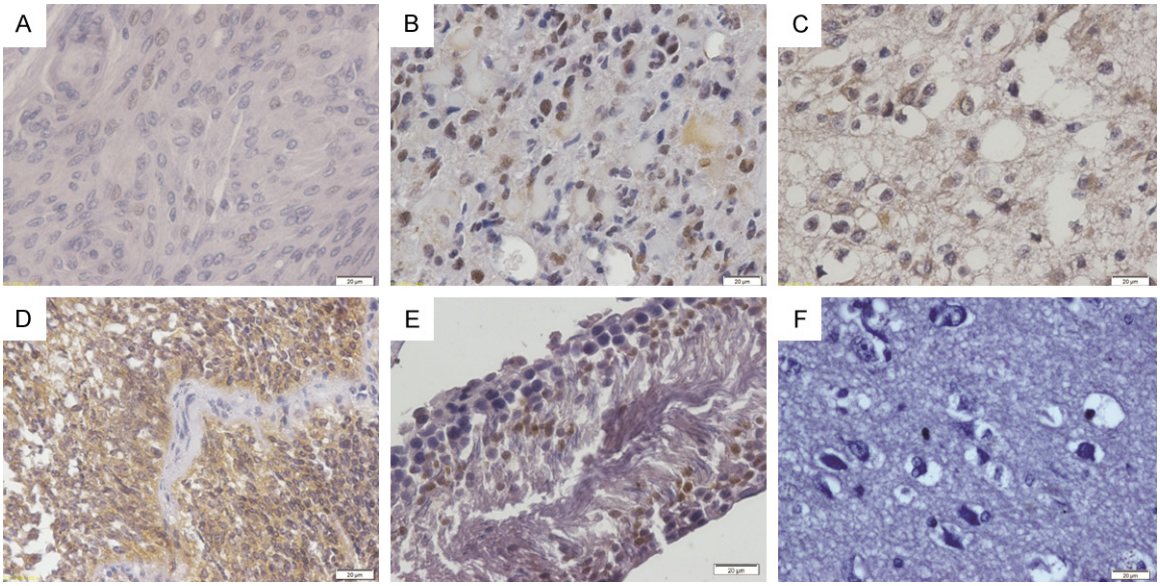


Figure 2. Representative immunohistochemical staining of ACTL8 protein in glioma and testis tissues. Typical immunohistochemical staining of ACTL8 protein were shown in glioma tissues (A-D). Testis germinal epithelium and sperm cell were demonstrated with strong staining in normal testis (E). Glioma tissue probed with pre-immune serum as negative control (F). Bar, 20 μm.

Correlation between ACTL8, CTCFL, OIP5 and XAGE3 and the clinicopathological parameters of glioma patients

The correlation between the expression of CTAs tested and the clinicopathological parameters was statistically assessed. Although there were no correlations between the mRNA expression of CTAs and clinicopathological parameters (**Table 1**), the protein expression of ACTL8, OIP5 and XAGE3 was positively correlated with KPS; while the ACTL8 protein was correlated with gender, and OIP5 protein was correlated with gender and WHO grade (**Table 2**).

Correlation between ACTL8, CTCFL, OIP5 and XAGE3 protein and the overall survival of glioma patients

The association of ACTL8, CTCFL, OIP5 and XAGE3 protein expressions with the overall survival of glioma patients was analyzed. Of 108 patients enrolled in this study, 79 patients were successfully followed up. The range of follow-up time was 60-72 months. Firstly, each of CTAs protein tested was analyzed with correlation of overall survival by Kaplan-Meier. Only CTCFL protein was associated with overall survival of glioma patients (**Figure 7**). Then, the any com-

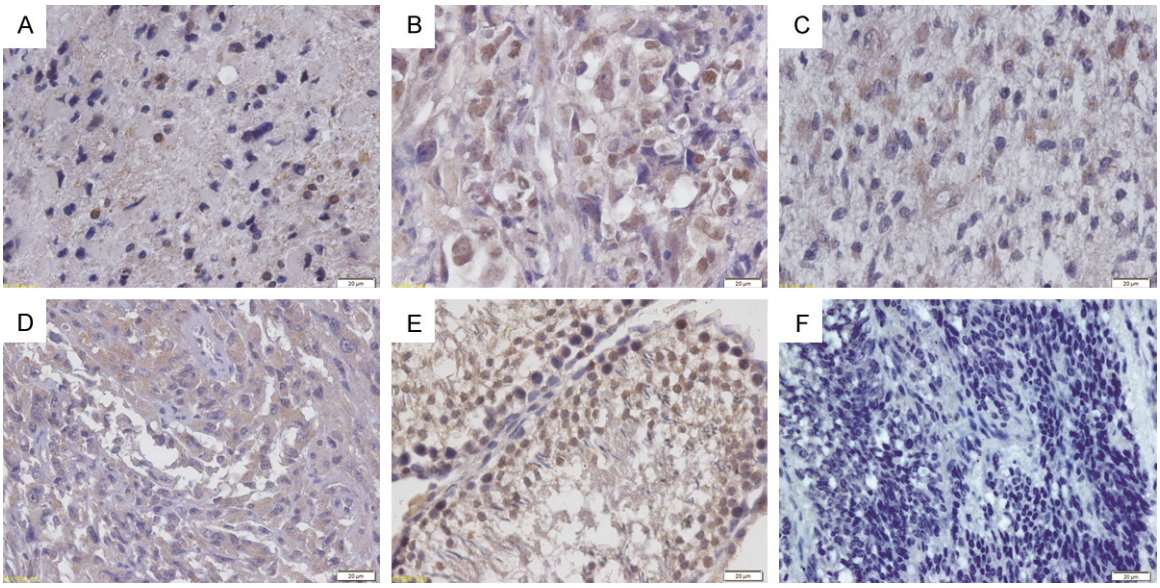


Figure 3. Representative immunohistochemical staining of CTCFL protein in glioma and testis tissues. Typical immunohistochemical staining of CTCFL protein was shown in glioma tissues (A-D). Testis germinal epithelium and sperm cell were demonstrated with strong staining in normal testis (E). Glioma tissue probed with pre-immune serum as negative control (F). Bar, 20 μm.

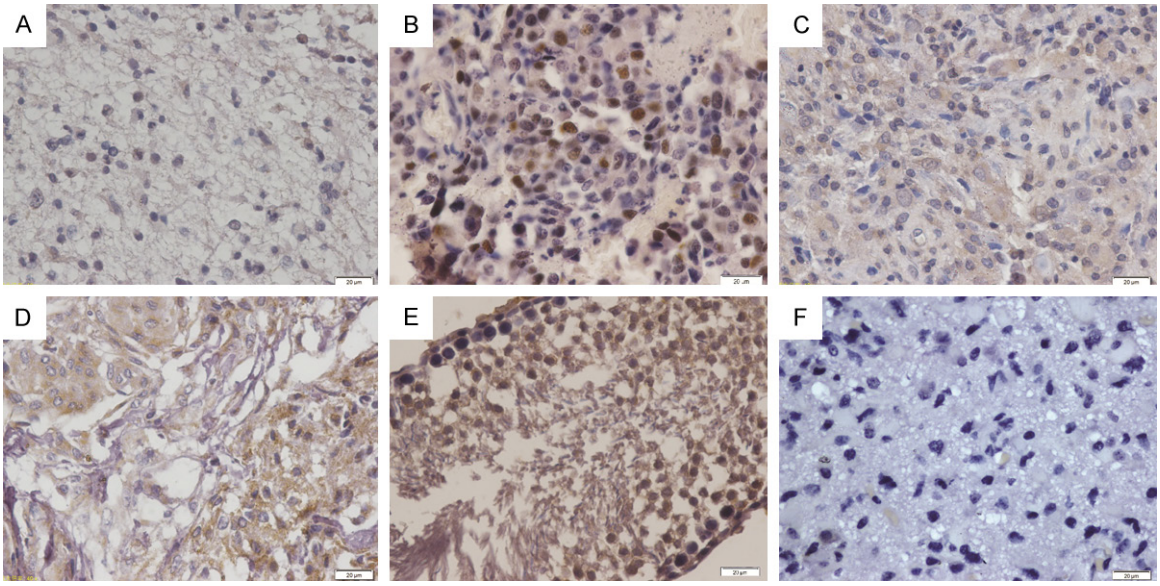


Figure 4. Representative immunohistochemical staining of OIP5 protein in glioma and testis tissues. Typical immunohistochemical staining of OIP5 protein were shown in glioma tissues (A-D). Testis germinal epithelium and sperm cell were demonstrated with strong staining in normal testis (E). Glioma tissue probed with pre-immune serum as negative control (F). Bar, 20 μm.

bined expression of CTAs protein tested was further analyzed for correlation of overall survival. As shown in **Figure 8**, the overall survival of patients only with combination of ACTL8 and/or CTCFL protein was significantly shorter than the patients without them. This suggested that the CTCFL protein and the combination of ACTL8 and/or CTCFL protein were the predicted poor prognostic factors of glioma patients.

Multivariate Cox regression analysis

To further evaluate the prognostic significance of CTCFL protein expression and the combined

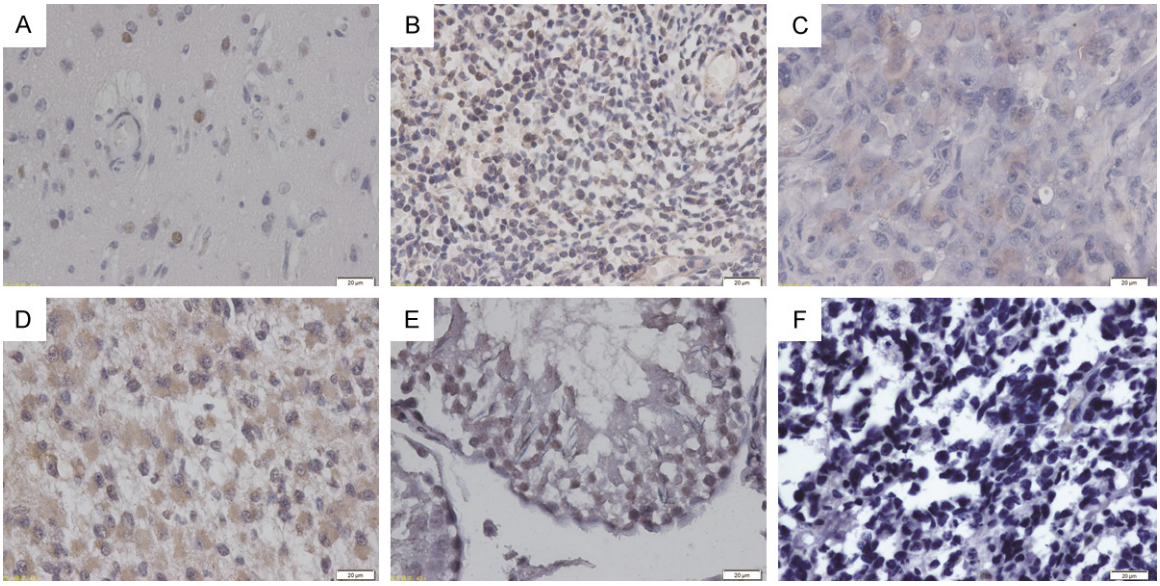


Figure 5. Representative immunohistochemical staining of XAGE3 protein in glioma and testis tissues. Typical immunohistochemical staining of XAGE3 protein were shown in glioma tissues (A-D). Testis germinal epithelium and sperm cell were demonstrated with strong staining in normal testis (E). Glioma tissue probed with pre-immune serum as negative control (F). Bar, 20 μm.

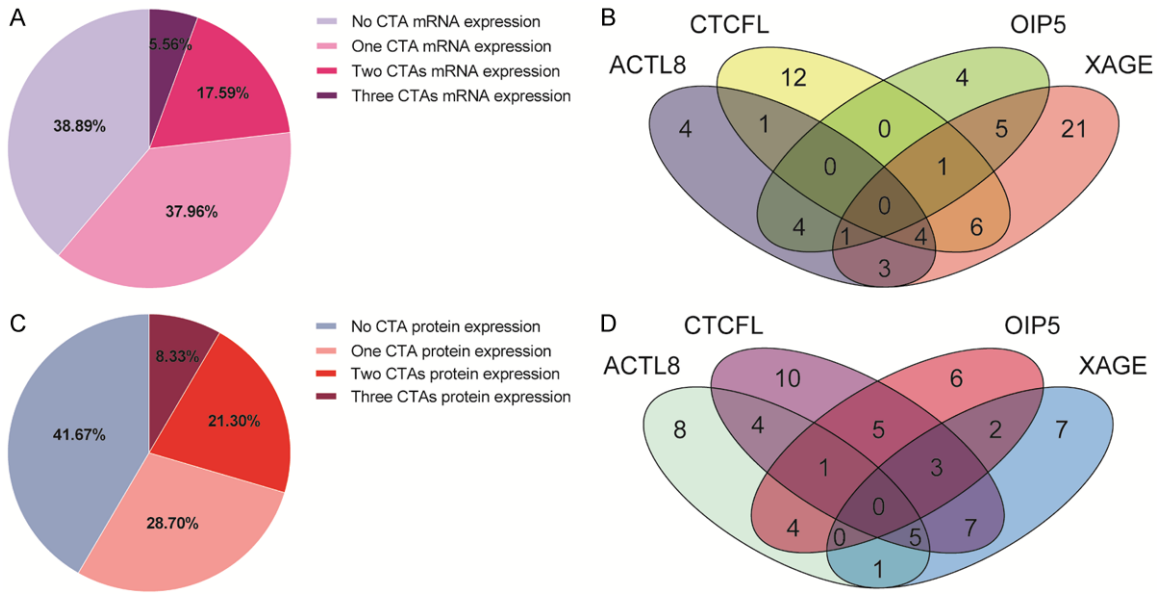


Figure 6. Coexpression of four CTAs in glioma tissues. A: Percent of mRNA coexpression among four CTAs in glioma tissue. B: Venn digram demonstrated the overlap mRNA experession among four CTAs. C: Percent of protein coexpression among four CTAs in glioma tissue. D: Venn digram demonstrated the overlap protein experession among four CTAs.

ACTL8 and/or CTCFL protein expression in multivariate analysis, the univariate analysis for each clinicopathological factor in relation to overall survival of glioma patients was performed. As shown in **Table 3**, age (P = 0.002), WHO grade (P = 0.040), Ki-67 (P = 0.013), P53 (P = 0.014), CTCFL protein expression (P = 0.045) and combined ACTL8 and/or CTCFL protein expression (P = 0.043) were significantly relevant with overall survival in glioma patients. However, the multivariate Cox regression analysis of the above factors disappointingly showed

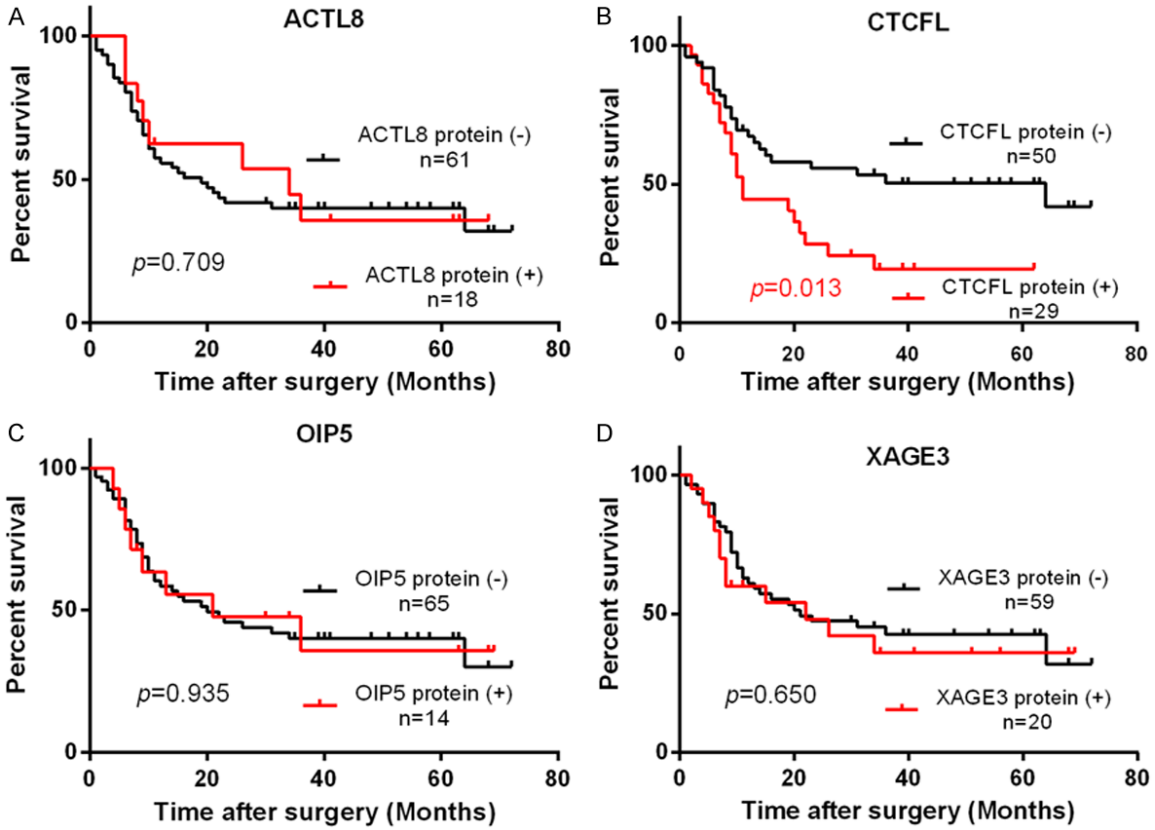


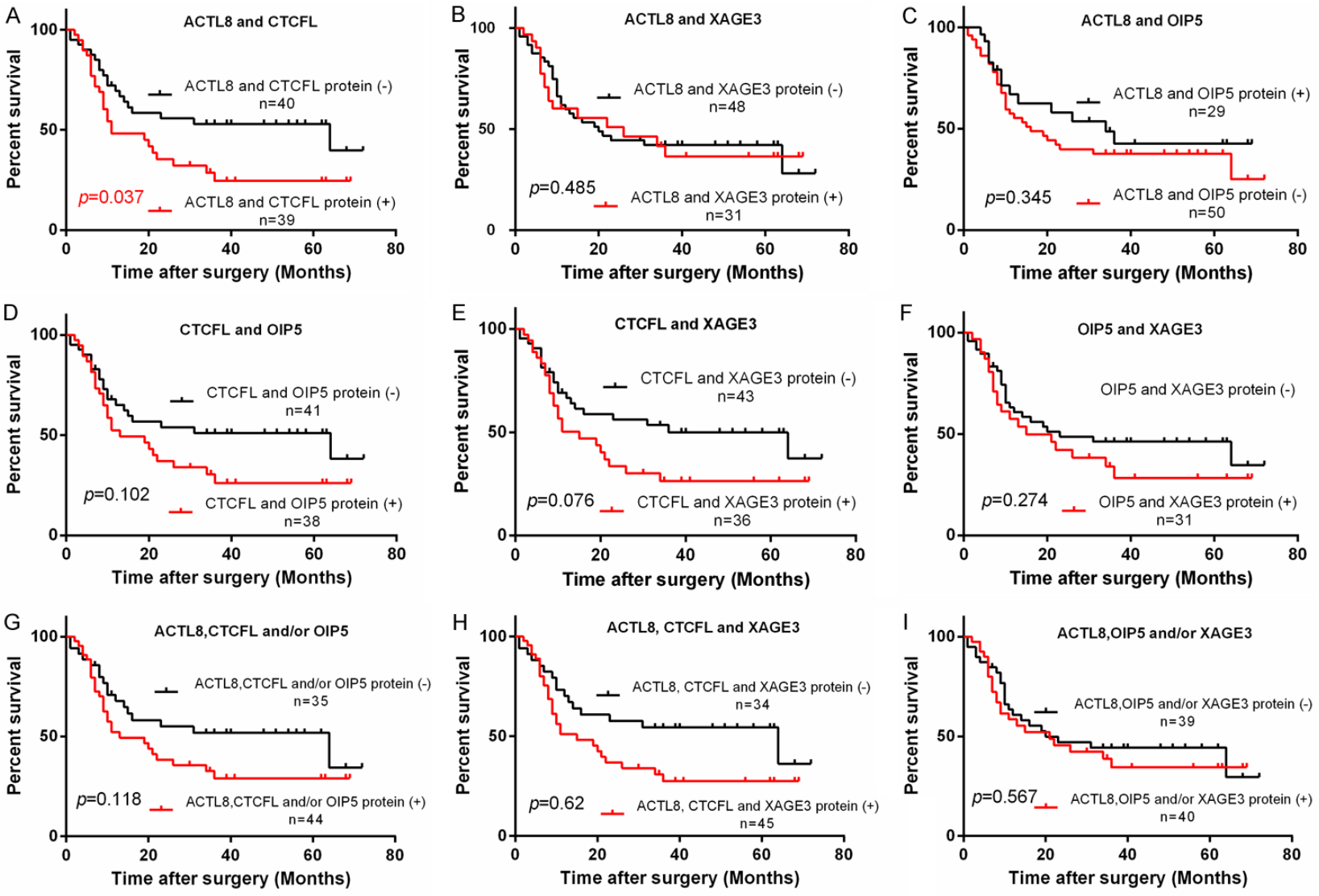
Figure 7. Correlation between CTA protein expression and overall survival of glioma patients. Kaplan-Meier analysis was performed to evaluate four CTAs protein in 79 patients with glioma, categorized as negative and positive (A-D). The results showed only CTCFL protein expression had statistically significant difference with the overall survival (B).

that CTCFL protein cannot serve as an independent prognostic factor for glioma patients as well as combined ACTL8 and/or CTCFL protein.

Discussion

Despite treatment with aggressive surgical resection followed by radiotherapy and chemotherapy, the clinical outcome of patients with glioma still remains poor [15], emphasizing the crucial need for new therapeutic approaches. Immunotherapy is a promising therapeutic approach to improve the survival and clinical outcomes for patients with glioma [5]. Recently, many CTAs have been reported to be strictly tumor specific and frequently expressed in glioma [9, 11, 16, 17]. Therefore, CTAs are considered as ideal candidates of immunotherapy for glioma.

Although many CTAs have been identified in glioma, the number of CTAs suitable as vaccines for gliomas is still little, in large part, due to lack of clear data of their expression either individual or combined. Therefore, in present study, a panel of CTAs (ACTL8, CTCFL, OIP5 and XAGE3) was tested in both mRNA and protein to get complete expression profile in glioma. Our result revealed that the mRNA expression of ACTL8, CTCFL, OIP5 and XAGE3 in glioma was found at various frequencies. The overall frequencies of four CTAs mRNA expression in our result are lower than that reported by Freitas et al [8], although we applied the same primers in PCR detection. For protein expression, CTCFL was mostly frequently expressed CTA, being present in 34.26%, followed by XAGE3, ACTL8 and OIP5. To our knowledge, there has been no study of the protein expression of these CTAs in glioma, except for CTCFL. CTCFL protein expression in glioma has been tested only by Freitas et al [8]. Compared with their result, the higher frequency of CTCFL protein in glioma was shown in our study by using the same antibody. The discrepancy may be due to multiple factors, such as variation (number, type of sample), sampling, staining proto-


A
ACTL8 and CTCFL
Percent survival
ACTL8 and CTCFL protein (-)
n=40
p=0.037
ACTL8 and CTCFL protein (+)
n=39
Time after surgery (Months)
B
ACTL8 and XAGE3
ACTL8 and XAGE3 protein (-)
n=48
p=0.485
ACTL8 and XAGE3 protein (+)
n=31
C
ACTL8 and OIP5
ACTL8 and OIP5 protein (+)
n=29
p=0.345
ACTL8 and OIP5 protein (-)
n=50
D
CTCFL and OIP5
CTCFL and OIP5 protein (-)
n=41
p=0.102
CTCFL and OIP5 protein (+)
n=38
E
CTCFL and XAGE3
CTCFL and XAGE3 protein (-)
n=43
p=0.076
CTCFL and XAGE3 protein (+)
n=36
F
OIP5 and XAGE3
OIP5 and XAGE3 protein (-)
p=0.274
OIP5 and XAGE3 protein (+)
n=31
G
ACTL8,CTCFL and/or OIP5
ACTL8,CTCFL and/or OIP5 protein (-)
n=35
p=0.118
ACTL8,CTCFL and/or OIP5 protein (+)
n=44
H
ACTL8, CTCFL and XAGE3
ACTL8, CTCFL and XAGE3 protein (-)
n=34
p=0.62
ACTL8, CTCFL and XAGE3 protein (+)
n=45
I
ACTL8,OIP5 and/or XAGE3
ACTL8,OIP5 and/or XAGE3 protein (-)
n=39
p=0.567
ACTL8,OIP5 and/or XAGE3 protein (+)
n=40

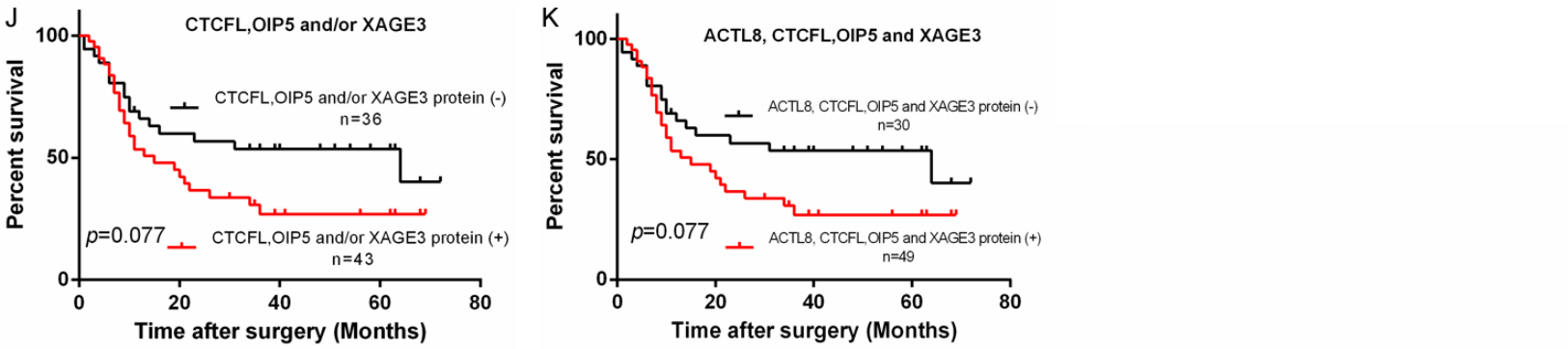


Figure 8. Correlation between any combined CTAs protein expression and overall survival of glioma patients. Any combined expression of four CTAs protein in 79 patients with glioma was tested by Kaplan-Meier analysis, respectively (A-K). But only the combined ACTL8 and/or CTCFL protein expression had statistically significant difference with the overall survival (A).

col, scoring method and ethnics, etc. Previous studies have also demonstrated differential expression of several CTAs in different ethnic groups at the same tumor type. For example, NY-ESO-1 protein was expressed in melanoma at much lower frequency in Australia than in Japanese [18, 19]; whereas MAGE-A1 protein was expressed in melanoma at much higher frequency in American than in Australia [18, 20]. Meanwhile, our study also indicated more than half of samples tested expressed one of these CTAs mRNA and protein, which implicated that many patients with glioma would be eligible for the specific immunotherapy with at least one of these CTAs, while the co-expression of these CTAs either at least two or three was much less. Therefore, additional CTAs must be identified for glioma, especially if the goal is set to develop multivalent vaccine for a majority of patients.

Interestly, although both mRNA and protein expression of ACTL8, CTCFL, OIP5 and XAGE3 were found in various frequencies in our study, they were not well paralleled. This discrepancy might be considered as the following factors. First, the sensitivity of detection is different between the immunohistochemistry and PCR-based method. Second, different areas of the same tumor were sampled for PCR and immunohistochemistry test, respectively. Third, additional processes such as post-transcriptional regulation, translation modification, and proteasomal degradation in these CTAs might also play a role [21, 22]. Accordingly, it emphasizes the need to analyze protein rather than only mRNA as the protein may play biological function.

Some kinds of CTAs, such as MAGE-A1, MAGE-A9, PRAME, NY-ESO-1 and OY-TES-1 were found to be the predictive factors for poor overall survival in many types of tumors including glioma [9, 23, 24]. Currently, the prognostic significance of ACTL8, CTCFL, OIP5 and XAGE3 protein expression in glioma has not been investigated. Therefore, we evaluated the correlation between the protein expression of ACTL8, CTCFL, OIP5 and XAGE and overall survival in glioma patients. A novel finding in our study was the association of CTCFL protein expression and combined ACTL8 and/or CTCFL protein expression with worse clinical outcome in glioma patients. Interestingly, Freitas et al [8] reported that mRNA positivity for OIP5 and combined above 3-4 CTA genes showed a significant association with better overall survival. Several previous studies have found an inverse correlation of many CTA expressions and survival in many types of tumors [6, 23-27]. However, reports of CTA expression being associated with better survival have been rare, such as CT10 [28]. Potential reasons for the discordance between our results and those from Freitas et al [8] may include the races difference, the number of samples tested, heterogeneity in the grades of evaluable tumors, and different immunohistochemical methods across studies [28]. Therefore, further prospective studies with glioma patients are needed to confirm the correlation between the four CTAs expression and clinical outcome.

Although the biological function of these four CTAs remains unclear, previous studies reported that CTCFL, also known as BORIS, can act as a transcriptional regulator directing the epigenetic reprogramming at CTCFL target sites in normal and tumor tissues [29, 30]. It has also been proposed as a mediator of the induction/derepression of other genes and deregulation of X chromosome inactivation in females [30-32]. OIP5 was shown to be involved in chromatin reorganization during the cell cycle. Furthermore, it was demonstrated that OIP5 mRNA was highly expressed in a wide variety of tumors including glioblastoma, promoting the growth of tumor cells [33-40]. ACTL8 has been described to promote the proliferation, migration and invasion in colorectal cancer and head and neck squamous cell [41, 42]. The function of XAGE3 is poorly understood. All were considered as potential biomarkers and immunotherapeutic targets for some cancers [6, 43-45]. At the same time, they may contribute to the genesis and progress of gliomas, and the further investigations of the functions of these CTAs in gliomas are clearly needed.

In summary, the present study provided evidence that the cohort of glioma does express ACTL8, CTCFL, OIP5 and XAGE3 at both mRNA and protein levels. The expression of CTCFL protein and the combined expression of ACTL8 and/or CTCFL protein showed the significant association with overall survival of glioma patients, suggesting they might act as poor prognostic markers for patients with glioma and as potential ideal combined antigens for glioma immunotherapy.

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Disclosure of conflict of interest

None.

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