

The Relationship Among Integrin Alpha 7, CD133 and Nestin as Well as Their Correlation With Clinicopathological Features and Prognosis in Astrocytoma Patients

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Abstract

Objective: Integrin alpha 7 (ITGA7), a potential glioma stem cell marker, regulates several other stem cell markers including CD133 and Nestin in several cancers, meanwhile its high expression is related to poor prognosis in multiple solid tumor patients. However, few studies report correlation of ITGA7 with prognosis in astrocytoma patients. Hence, this study aimed to determine the astrocytoma-tissue ITGA7, CD133 and Nestin expressions to explore their relationship and clinical value for astrocytoma management.

Methods: Totally, 124 patients with primary astrocytoma were included. Their tumor tissue ITGA7, CD133 and Nestin expressions were determined by immunohistochemical (IHC) assay and scored by intensity and density ranging from 0-12 points. Besides, their clinical features (such as world health organization (WHO) grade, isocitrate dehydrogenase (IDH) mutation, and adjuvant therapy etc.) were collected, also their overall survival (OS) were analyzed by follow-up data.

Results: The mean IHC scores for ITGA7, CD133 and Nestin were 4.9 ± 2.5 , 2.1 ± 2.6 and 5.8 ± 2.6 , respectively. Moreover, ITGA7 high expression correlated with absence of IDH mutation ($P=0.004$), advanced WHO grade ($P=0.001$) and shorter OS ($P=0.005$). Besides, ITGA7 positively correlated with CD133 ($P=0.001$) and Nestin ($P=0.001$) expressions. Regarding CD133 and Nestin, their high expression also correlated with increased WHO grade and shorter OS. Furthermore, multivariate Cox's regression analysis displayed that only CD133 high expression ($P=0.021$) could independently predict reduced OS, while ITGA7 or Nestin high expression could not.

Conclusion: ITGA7, CD133 and Nestin are intercorrelated, also their high expressions associate with deteriorating disease conditions and poor prognosis in astrocytoma patients.

1 Introduction

Astrocytoma, as the most frequent central nervous system (CNS) tumor, has a high incidence in males compared to females with a ratio of 1.4:1 [1–3]. Regarding its management, different options are applied according to the world health organization (WHO) classification of astrocytoma [1]. In detail, surgery following radiotherapy is commonly applied in low grade astrocytoma patients (WHO grade I and grade II), while surgery in combination with adjuvant chemotherapy and/or radiotherapy is used to treat high grade astrocytoma patients (WHO grade III and WHO grade IV) since most astrocytomas in this classification are in diffuse form and may cause intracranial metastasis [4]. However, even after appropriate management, some astrocytoma patients (such as anaplastic astrocytoma patients) experience multiple, rapid recurrence which further decreases their long-term survival profile [2,5,6]. Therefore, it is necessary and urgent to discover novel biomarkers to improve prognosis in astrocytoma patients.

Integrins are large, membrane-spanning, heterodimeric cell-surface proteins that contribute to cell proliferation, differentiation, adhesion and migration by interacting with extracellular matrix (ECM) and

cytoskeleton [7,8]. Among which, integrin alpha-7 (ITGA7) is a key mediator in rectal cancer, breast cancer and astrocytoma pathogenesis [9–12]. In addition, in the clinical field, ITGA7 high expression correlates with poor prognosis in clear renal cell carcinoma patients, rectal cancer patients and breast cancer patients [10,13,14]. Furthermore, ITGA7 not only acts as a potential cancer stem cell (CSC) marker, but also regulates multiple other CSC marker expressions (including CD44, CD90, CD133, SOX2, OCT3/4 and NANOG), meanwhile, these CSC markers (such as CD133 and Nestin) correlate with shorter overall survival (OS) in astrocytoma patients [9,11,15–17]. Given the fact that ITGA7 regulates CSC marker expressions in several cancers, besides CD133 and Nestin are well-characterised CSC markers in astrocytoma, we hypothesized that ITGA7 assessment might have a potentially clinical value in astrocytoma patients [18–21]. However, no relevant research has been conducted yet. Herein, we performed this study and aimed to explore the relationship among ITGA7, CD133 and Nestin, as well as to explore their correlation with clinicopathological features and prognosis in astrocytoma patients.

2 Materials And Methods

2.1 Patients and specimens

The retrospective study was carried out with the approval from the Institutional Review Board of Hospital of Chengdu Office of People's Government of Tibetan Autonomous Region (Hospital. C.T). Totally, 124 patients with primary astrocytoma who underwent surgical resection in the hospital between January 2016 and June 2020 were included in this study. By reviewing their clinical data, only patients who met the following criteria were screened out for study analysis: (i) histologically-confirmed astrocytoma in accord with World Health Organization (WHO) classification criteria [22]; (ii) age \geq 18 years; (iii) directly treated by surgical resection without neoadjuvant therapy; (iv) formalin fixed and paraffin embedded tissue (FFPE) of astrocytoma was well preserved and available for immunohistochemical (IHC) assessment; (v) major clinical features, treatment information, and survival data were integral and available; (vi) no history of other malignant diseases; (vii) without history of exposure to radiotherapy or chemotherapy before diagnosis of astrocytoma. The written informed consents were obtained from the patients or their relatives. A total of 124 astrocytoma FFPE specimens were acquired from the hospital's sample library, which were used for determination of ITGA7, CD133 and Nestin expression by IHC assay.

2.2 Data compilation

The major clinical feature data and treatment information, including patients' age, gender, WHO grade (2016 WHO Classification [22]), isocitrate dehydrogenase (IDH) mutation, preoperative Karnofsky Performance Scale (KPS) score and adjuvant therapy, were collected from the medical documents. In addition, follow-up records of patients were reviewed to abstract the survival information for estimation of overall survival (OS).

2.3 IHC assay

The FFEPT specimens were sliced into sections at a thickness of 4 μm , which were then deparaffinized with xylene, rehydrated with ethanol, and treated with hydrogen peroxide, followed by blocking with normal goat serum. Afterwards, the sections were respectively incubated overnight with primary antibodies (Invitrogen, Carlsbad, California, USA), including ITGA7 Polyclonal Antibody (1:25 dilutions), CD133 Polyclonal Antibody (1:500 dilutions) and Nestin Polyclonal Antibody (1:1000 dilutions). Following that, the sections were incubated with Goat anti-Rabbit IgG (H + L) Secondary Antibody (1:5000 dilutions, Invitrogen, Carlsbad, California, USA). Chromogenic reaction was realized using diaminobenzidine, followed by counterstaining with hematoxylin. At the end of IHC assay, the staining results of sections were viewed and photographed using a microscope. The intensity and density of the IHC staining were scored by two experts, and the detailed scoring procedures were performed in terms of the method described in the published literature [23,9,24]. The IHC score was the product of intensity score and density score, ranging from 0 to 12. The average IHC score of the two experts was used as the final IHC score. In the analysis, the cut-off score of 3 was used to classify the high and low expression, that was: IHC score ≤ 3 , low expression; IHC score > 3 , high expression.

2.4 Statistical analysis

SPSS 26.0 (IBM Corp., Armonk, New York, USA) was applied for data analysis. GraphPad Prism 7.02 (GraphPad Software Inc., San Diego, California, USA) was used for graph construction. Correlation among three markers was checked using McNemar's test. Correlation of three markers with clinical features was analyzed by Chi-square test or Mantel-Haenszel Chi-square test. Correlation of three markers with OS was examined by Kaplan-Meier method and Log-rank test. Prognostic value of three markers was estimated using Cox's proportional hazard regression model analysis with forward stepwise method. A *P* value < 0.05 indicated a statistical significance.

3 Results

3.1 Patients 'clinical characteristics

The mean age of astrocytoma patients was 47.9 ± 14.6 years (**Table 1**). Besides, there were 77 (62.1%) males and 47 (37.9%) females in astrocytoma patients. Regarding their WHO grade, 5 (4.0%), 53 (42.8%), 45 (36.3%) and 21 (16.9%) patients were diagnosed as WHO grade I, WHO grade II, WHO grade III and WHO grade IV astrocytomas, respectively. Furthermore, 64 (51.6%) cases presented with IDH mutation. The detailed clinical features were listed in **Table 1**.

3.2 ITGA7, CD133 and Nestin expressions

In astrocytoma patients, the mean IHC scores for ITGA7, CD133 and Nestin were 4.9 ± 2.5 , 2.1 ± 2.6 and 5.8 ± 2.6 , respectively (**Figure 1A-D**). For further analysis, ITGA7, CD133 and Nestin expressions were categorised into high or low expression based on a cut-off value of 3 on IHC score. Besides, there were 79 (63.7%) cases presented with ITGA7 high expression, while 45 (36.3%) cases presented with ITGA7 low expression. Meanwhile, there were 32 (25.8%) cases presented with CD133 high expression, while 92

(72.4%) cases presented with CD133 low expression. Also, there were 91 (73.4%) cases presented with Nestin high expression, while 33 (26.6%) cases presented with Nestin low expression.

Regarding their relationship, ITGA7 expression positively correlated with CD133 expression and Nestin expression (both $P=0.001$) in astrocytoma patients (**Table 2**). Moreover, CD133 expression positively associated with Nestin expression in astrocytoma patients ($P=0.002$).

3.3 Correlation of ITGA7 expression with clinical features

In astrocytoma patients, ITGA7 high expression was correlated with elevated WHO grade ($P=0.001$) (**Figure 2C**) and absence of IDH mutation ($P=0.004$) (**Figure 2D**). While there was no correlation of ITGA7 expression with age ($P=0.265$) (**Figure 2A**), gender ($P=0.257$) (**Figure 2B**), KPS score ($P=0.696$) (**Figure 2E**), adjuvant radiotherapy ($P=0.079$) (**Figure 2F**) or adjuvant chemotherapy ($P=0.545$) (**Figure 2G**).

3.4 Correlation of CD133 and Nestin expressions with clinical features

CD133 high expression positively correlated with WHO grade in astrocytoma patients ($P=0.012$) (**Figure 3C**). However, there was no correlation of CD133 expression with age ($P=0.728$) (**Figure 3A**), gender ($P=0.186$) (**Figure 3B**), IDH mutation ($P=0.149$) (**Figure 3D**), KPS score ($P=0.622$) (**Figure 3E**), adjuvant radiotherapy ($P=0.121$) (**Figure 3F**) or adjuvant chemotherapy ($P=0.308$) (**Figure 3G**).

Regarding Nestin, its high expression correlated with advanced WHO stage ($P<0.001$) (**Figure 3J**), absence of IDH mutation ($P=0.043$) (**Figure 3K**) and presence of adjuvant radiotherapy ($P=0.043$) (**Figure 3M**) in astrocytoma patients. While no correlation of Nestin expression with age ($P=0.481$) (**Figure 3H**), gender ($P=0.528$) (**Figure 3I**), KPS score ($P=0.334$) (**Figure 3L**), or adjuvant chemotherapy ($P=0.877$) (**Figure 3N**) was observed.

3.5 Correlation of ITGA7, CD133 and Nestin expressions with OS

ITGA7 high expression ($P=0.005$) (**Figure 4A**), CD133 high expression ($P=0.006$) (**Figure 4B**) and Nestin high expression ($P=0.003$) (**Figure 4C**) all correlated with reduced OS in astrocytoma patients.

Univariate Cox's regression analysis revealed that ITGA7 high expression ($P=0.007$), CD133 high expression ($P=0.007$) and Nestin high expression ($P=0.006$) all correlated with reduced OS in astrocytoma patients (**Figure 5A**). Further multivariate Cox's regression analysis displayed that only CD133 high expression ($P=0.021$) (**Figure 5B**) could independently predict reduced OS in astrocytoma patients, while ITGA7 high expression or Nestin high expression could not.

4 Discussion

ITGA7 is a cell adhesion molecule which is critical for cell migration, homeostasis and translocation [7,8]. Apart from its critical role in normal cell biology, ITGA7 is also able to induce cell malignant behaviours in several cancers [8,7,9–11]. One study displays that ITGA7 regulates phosphatidylinositol 3 kinase

(PI3K)/protein kinase B (Akt) pathway to further promote glioma CSC proliferation and clonogenic survival [12]. Recently, ITGA7 has been identified as a novel biomarker in several cancer patients. For example, ITGA7 expression positively correlates with pathological grade and TNM stage in clear cell renal cell carcinoma patients, breast cancer patients and rectal cancer patients [10,14,13]. Furthermore, ITGA7 high expression is related to worse OS in rectal cancer patients and breast cancer patients [10,14]. However, limited study can be found in terms of the correlation of ITGA7 with prognosis in astrocytoma patients. Therefore, we performed this study and discovered that ITGA7 high expression correlated with advanced WHO grade and poor prognosis in astrocytoma patients. The possible reasons to explain these findings were: (a) ITGA7 might promote astrocytoma cell proliferation, invasion and metastasis through the regulation of PI3K/Akt pathway, therefore led to deteriorating clinical features in astrocytoma patients [12,17]. (b) ITGA7 might regulate ERK signalling pathway to mediate cell differentiation, thereby led to increased WHO grade in astrocytoma patients [25]. (c) Regarding their survival profile, ITGA7 high expression might lead to increased chance of developing chemotherapy resistance due to the clonogenic survival of CSC, therefore resulted in poor prognosis in astrocytoma patients [26].

Great attention has been paid in discovering the properties of CSC since they are responsible for cancer metastasis, recurrence and drug resistance [27,28]. CD133 and Nestin are two heavily investigated CSC markers, meanwhile they are commonly co-expressed in neural stem cells [29]. In the clinical field, CD133 and Nestin correlated with advanced WHO grade, also their high expressions correlate with shorter OS in astrocytoma patients [15]. However, no relevant research reports their relationship with ITGA7 in astrocytoma patients. In the present study, we discovered that ITGA7 expression positively correlated with CD133 and Nestin expressions in astrocytoma patients. Besides, both CD133 and Nestin expressions correlated with advanced WHO stage and unfavourable survival profile in astrocytoma patients. Furthermore, CD133 high expression could independently predict shorter OS in astrocytoma patients, which could be explained as follows: (a) Regarding the correlation of ITGA7 expression with CD133 and Nestin expressions, high ITGA7 expression suggested increased CSC cell proportion and density as discussed earlier, meanwhile CD133 and Nestin were glioma CSC markers, therefore, ITGA7 positively correlated with CD133 and Nestin in astrocytoma patients [29]. (b) In terms of the correlation of CD133 and Nestin expression with WHO grade, elevated CSC marker expressions (CD133 and Nestin in this case) suggested inhibition of glioma CSC apoptosis while promotion of CSC angiogenesis through Wnt/ β -catenin and PI3K/Akt pathways, therefore led to clonogenic survival of glioma CSC and further led to differentiation of glioma CSC into astrocytoma cells, which resulted in elevated astrocytoma cell density and irregular nuclear morphology, therefore led to increased WHO grade in astrocytoma patients [27,28]. (c) In terms of the correlation of CD133 and Nestin with prognosis, elevated CSC marker expressions (CD133 and Nestin) indicated a higher proportion of CSC, which led to increased chance of developing drug resistance and distal metastasis, therefore resulted unfavourable survival profile in astrocytoma patients [28,27].

There were several limitations in the current study. Firstly, the interacting mechanisms among ITGA7, CD133 and Nestin was not discovered in the present study, and further study was needed. Secondly, the sample size in our study was relatively small, therefore further study with larger sample size to validate

the prognostic value of these biomarkers was needed. Thirdly, we detected ITGA7, CD133 and Nestin expressions from astrocytoma tissue samples harvested from patients, therefore these marker expressions from blood samples were not detected and further study could investigate this aspect. Finally, further experiments were warranted to determine the effect of ITGA7 on chemotherapy resistance in astrocytoma patients.

In conclusion, ITGA7, CD133 and Nestin are intercorrelated, also their high expressions correlate with deteriorating disease condition and poor prognosis in astrocytoma patients, suggesting their potentially clinical value for astrocytoma management.

Declarations

Ethics approval and consent to participate

The retrospective study was carried out with the approval from the Institutional Review Board of Hospital of Chengdu Office of People's Government of Tibetan Autonomous Region (Hospital. C.T). The written informed consents were obtained from the patients or their relatives.

Consent for publication

Not applicable.

Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors have no conflicts of interest to declare.

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None.

Authors' contributions

CL and JL conceived, designed, performed all experiments and wrote the manuscript. WY and CC confirm the authenticity of all the raw data. BW was responsible for the collection and follow-up of clinical cases. WY and BW were responsible for data statistics. All authors have read and approved the manuscript.

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Tables

Table 1. Clinical characteristics of astrocytoma patients

Items	Astrocytoma patients (N = 124)
Age (years), mean±SD	47.9±14.6
<60 years	92 (74.2)
≥60 years	32 (25.8)
Gender, No. (%)	
Male	77 (62.1)
Female	47 (37.9)
WHO grade, No. (%)	
I	5 (4.0)
II	53 (42.8)
III	45 (36.3)
IV	21 (16.9)
IDH mutation, No. (%)	64 (51.6)
KPS score, median (IQR)	60.0 (60.0-70.0)
<70	69 (55.6)
≥70	55 (44.4)
Adjuvant treatment, No. (%)	
Radiotherapy	101 (81.5)
Chemotherapy	84 (67.7)

SD, standard deviation; WHO, World Health Organization; IDH, isocitrate dehydrogenase; KPS, Karnofsky performance status; IQR, interquartile range.

Table 2. Correlation among ITGA7, CD133 and Nestin expressions

Items	ITGA7		<i>P</i> value	Nestin		<i>P</i> value	
	Low (n = 45)	High (n = 79)		Low (n = 33)	High (n = 91)		
CD133	Low (n = 92)	41 (44.6)	51 (55.4)	0.001	31 (33.7)	61 (66.3)	0.002
	High (n = 32)	4 (12.5)	28 (87.5)		2 (6.2)	30 (93.8)	
Nestin	Low (n = 33)	20 (60.6)	13 (39.4)	0.001	-	-	-
	High (n = 91)	25 (27.5)	66 (72.5)		-	-	

ITGA7, integrin alpha 7.

Figures

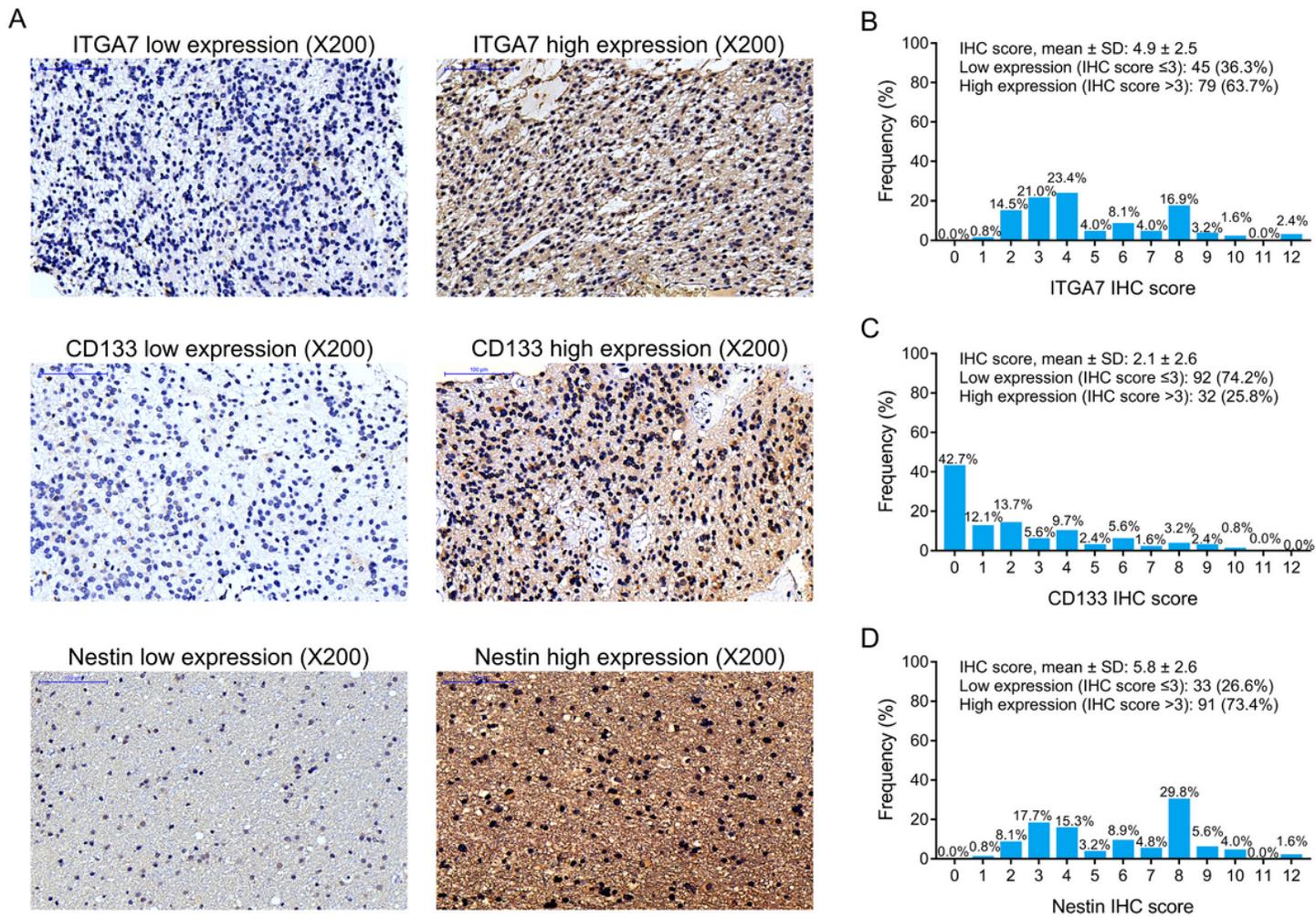


Figure 1

ITGA7, CD133 and Nestin IHC scores. The distribution of tumor tissue ITGA7 (A, B), CD133 (A, C) and Nestin (A, D) IHC scores in astrocytoma patients. ITGA7: Integrin alpha 7. IHC: immunohistochemical.

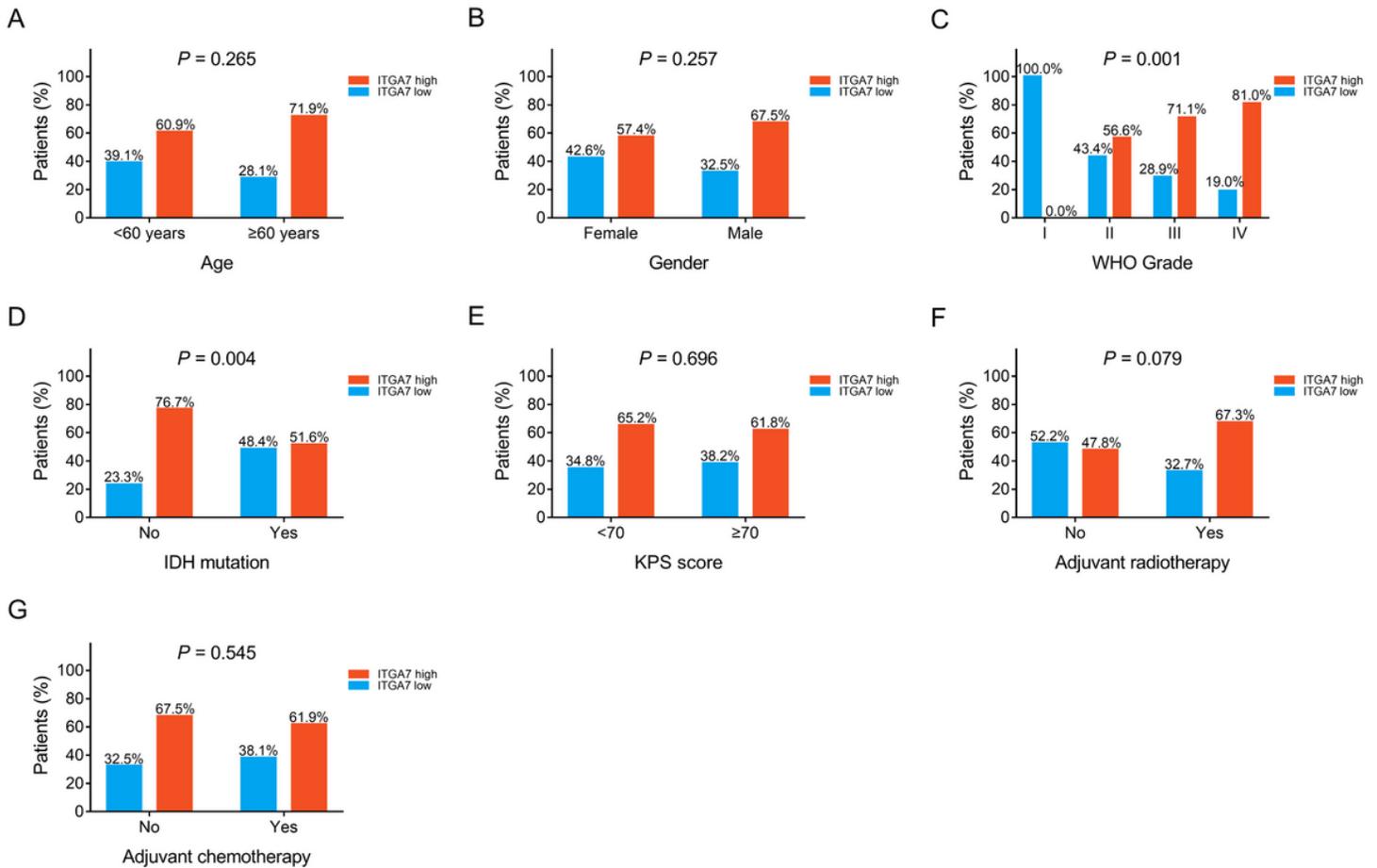


Figure 2

ITGA7 high expression correlated with absence of IDH mutation and elevated WHO grade. Correlation of ITGA7 expression with age (A), gender (B), WHO grade (C), IDH mutation (D), KPS score (E), adjuvant radiotherapy (F) and adjuvant chemotherapy (G) in astrocytoma patients. ITGA7: Integrin alpha 7, WHO: world health organisation, IDH: isocitrate dehydrogenase, KPS: Karnofsky Performance Scale.

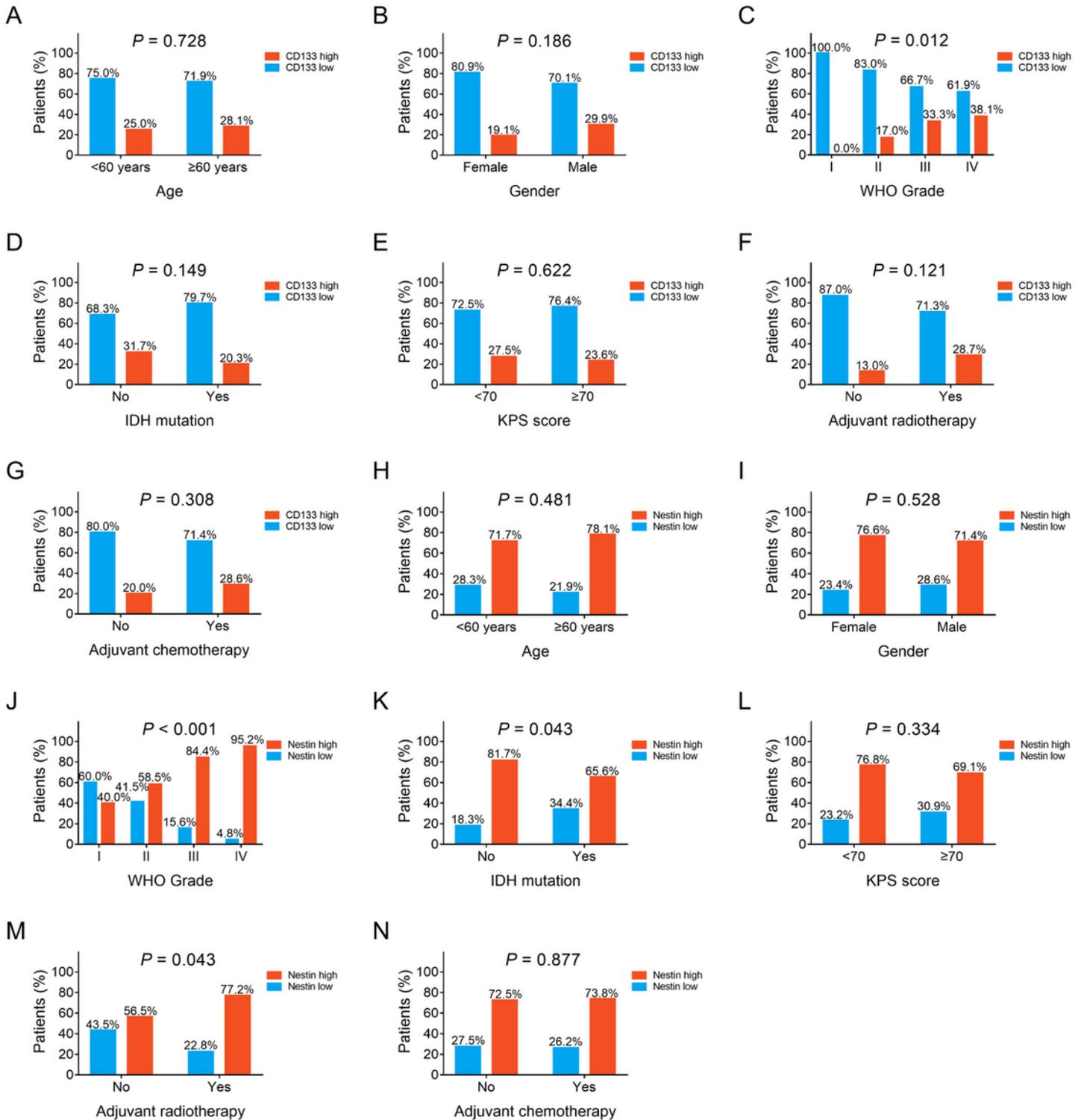


Figure 3

CD133 and Nestin high expressions correlated with increased WHO grade. Correlation of CD133 expression with age (A), gender (B), WHO grade (C), IDH mutation (D), KPS score (E), adjuvant radiotherapy (F) and adjuvant chemotherapy (G) in astrocytoma patients. Correlation of Nestin expression with age (H), gender (I), WHO grade (J), IDH mutation (K), KPS score (L), adjuvant radiotherapy (M) and adjuvant chemotherapy (N) in astrocytoma patients.

(M) and adjuvant chemotherapy (N) in astrocytoma patients. WHO: world health organisation, IDH: isocitrate dehydrogenase, KPS: Karnofsky Performance Scale.

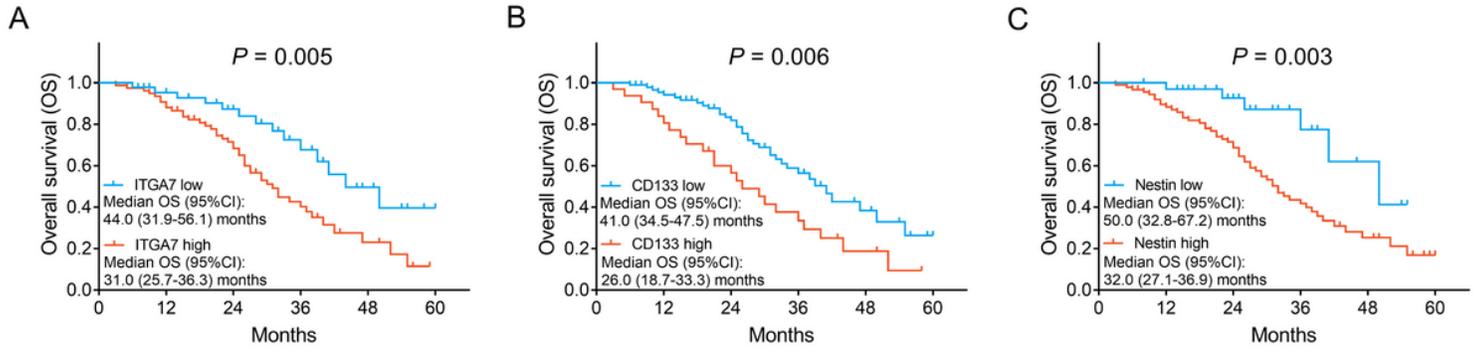
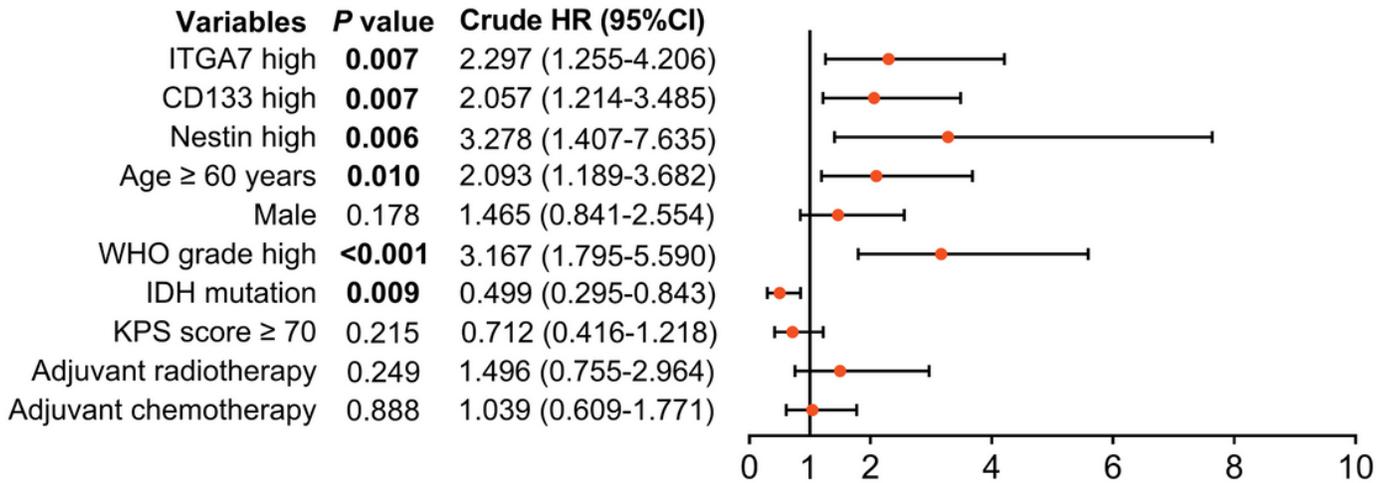


Figure 4

ITGA7, CD133 and Nestin high expressions correlated with shorter OS. ITGA7 (A), CD133 (B) and Nestin (C) high expression correlated with shorter OS in astrocytoma patients. ITGA7: Integrin alpha 7, OS: overall survival.

A

Univariable Cox's proportional hazard regression model analysis for OS



B

Multivariable Cox's proportional hazard regression model analysis for OS

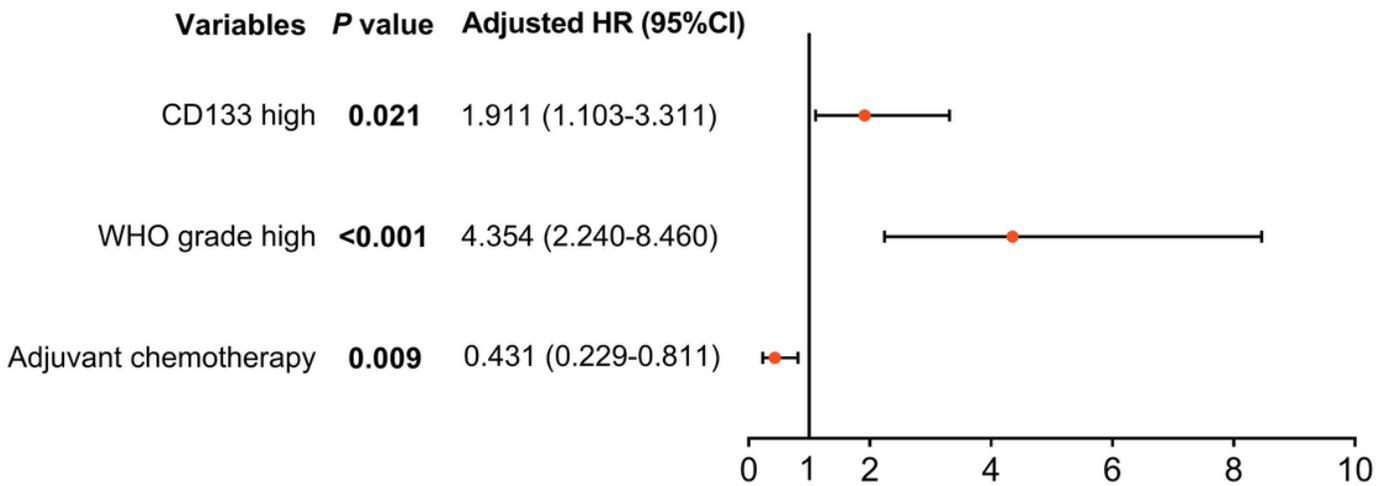


Figure 5

CD133 high expression independently correlated with reduced OS. Univariable (A) and multivariate (B) Cox's regression analyses of factors related to OS in astrocytoma patients. ITGA7: Integrin alpha 7, OS: overall survival, WHO: world health organisation, IDH: isocitrate dehydrogenase, KPS: Karnofsky Performance Scale.