

Expression of CD44 and CD133 in Glioma Stem Cells

Sima Fakhari^{1,*}, Ali Zare Mehrjardi¹, Mitra Noori²

¹Department of Pathology, Iran University of Medical Sciences, Tehran, Iran

²Department of Biology, Faculty of Science, Arak University, Arak, Iran

Abstract Gliomas are the most frequent primary central nervous system neoplasms. Cancer stem cells have been implicated in tumor initiation, progression, metastatic potential and resistance to therapy. Aim of this study is determination of CD44 and CD133 expression level as cancer stem cell markers in different gliomas comparing to normal brain tissue and its correlation with tumor grade. Study carried out on 31 low grades (I & II), 36 high grade (III & IV) glioma patients and 12 normal brain tissue samples. CD44 and CD133 markers expression levels examined using immune-histochemical staining method. Correlation between two these markers with glioma grade and their expression level were studied in different gliomas and human normal brain tissue. Data were analyzed using SPSS in Bivariate correlation and Simple liner regression tests. The positive expression rates of CD44 (44/67) and CD133 (9/67) in patients with gliomas were higher than those in normal brain tissues (both 0/12). These markers were expressed in cell membrane and cytoplasm. Significant correlation was seen between stained cells percent and glioma grade in CD44 marker ($C_s=0.28$). Non liner regression of CD44 and CD133 markers expression was observed in the studied gliomas ($R=0.28$, $P=0.23$).

Keywords Glioma, CD133, CD44

1. Introduction

Glioma is the most common primary neoplasm in the central nervous system that graded as I–IV according to the World Health Organization (WHO) system based on histological features [1]. In recent years the diagnosis and treatment of gliomas have made great progress; however, the prognosis of patients with high grade glioma remains poor [2]. Recent studies have shown that gliomas may be caused by tumor stem cells, along with the expression of some characteristics of neural stem cells [3]. The studies have suggested that tumor biology and treatment resistance are closely related to cancerous stem cells [4, 5]. Cancer stem cells (CSC) are found in a variety of cancers, including glioblastoma. A variety of markers such as CD133, CD15, A2B5, Nestin, Musashi-1, BMI1, SOX2, IB1, Id1, and OCT4 have been studied as glioblastoma stem cells markers [6]. CD133 is known as a marker of stem cells in normal tissue and cancers. Several studies have shown the use of CD133 in the nutrition of cells with fundamental properties. But there is also a great deal of evidence limiting the use of stem cell markers [7-10]. Recent evidence suggests that the correlation between CD133 expression, GSCs and molecular subtypes are more complex than initially proposed [11].

CD44 is a major receptor of stem cell surface that is created as multiple isoforms. These isoforms are produced by intermittent connections of at least 10 exons and encoding portions of the extracellular domain [12].

Determining approved markers for the accurate diagnosis of gliomas in this decade is still controversial [9]. The results of poor recovery of glioblastoma patients suggest that knowledge of markers for diagnosis and progression of disease are vital to effective treatment and the study of CD44 and CD133 markers in patients can be a valuable method in the diagnosis of glioblastoma multiforme in comparison with control. These tumor markers were determined by micro-assay analysis of glioblastoma specimens. The results showed a significant correlation between expressions of five markers, including CD44, with malignant glioma [13]. Anido et al. (2010) showed that the high level of expression of both CD44 and ID1 markers has an inverse correlation with the rate of survival and prognosis in patients with glioblastoma multiforme [14]. Zhang et al. (2008) studied on the association between the expression of Nestin and CD133 markers with glioma grade and their prognostic value using SABC immunohistochemical analysis. Their results showed a dense and dotted stainability, with a significant increase in expression of these two markers by increasing the grade of gliomas. There was a positive correlation between expressions of these two markers in different types of glioma tissues [15]. The cells that express CD133 marker exhibit more proliferation and cells that express the CD44 marker exhibit more invasions. CD133/OLIG2 or CD44 expression can predict response to radiotherapy [16]. The ratio of CD133+ cells as an unrelated risk factor for tumor growth

* Corresponding author:

sima_fakhari1366@yahoo.com (Sima Fakhari)

Published online at <http://journal.sapub.org/ijtt>

Copyright © 2018 The Author(s). Published by Scientific & Academic Publishing

This work is licensed under the Creative Commons Attribution International

License (CC BY). <http://creativecommons.org/licenses/by/4.0/>

and progression in WHO Grade II and III tumors was described [17]. A limited collection of recent reports illustrates the correlation between CD133 expression and poor prognosis in brain tumors. CD133+ brain tumor cells resistant to chemotherapy and radiotherapy can be evaluated with precise and preclinical studies. Different results have been obtained regarding the role of CD133 and other stem cell markers in their classification [18].

The aim of this study was to determine the expression of CD44 and CD133 markers in different types of gliomas in comparison with normal brain tissue and its correlation with tumor grade in patients referred to Firoozgar Hospital, Tehran, during the years 2014 to 2016.

2. Materials and Methods

2.1. Cases

This study was performed on 67 patients including 25 female patients aged 4 to 71 years old with mean age of 33.83 and 42 males aged 4 to 67 years with a mean age of 41.48 in Neurosurgery Department of Firoozgar Hospital, Tehran, Iran, that had surgically resected gliomas during 2014 to 2016. They had no history of chemotherapy or radiotherapy before surgery. The World Health Organization (WHO) has defined four grades of gliomas, along with increased histological changes in the tumor [7]. Tumors in grades II through IV are diffuse with invasion to normal brain. Grade II tumor are also called low grade gliomas, Grade III tumors are called anaplastic and grade IV tumor are known as glioblastoma multiforme (GBM). In this study, 31 patients were low grade (WHO grade I & II) and 36 patients with high grade (WHO grade III & IV). Twelve normal brain tissues were used as a control sample for immunohistochemical studies.

2.2. Immunohistochemical Staining Method

The expression of CD44 and CD133 markers in glioma tissues was studied by immunohistochemical staining. Samples were fixed in 10% buffered formalin and embedded in paraffin. Anti-CD44 Antibody (Phagocytic Glycoprotein-1, HCAM) (DF1485) prepared from BiogenexCo., and Human Anti-CD133 Monoclonal Antibody (SB-019772) from Sina Biotech Inc. were used. Immunohistochemical staining was performed using Avidin-biotin and Dako kit (DAKO ABC kit, UK). Paraffin blocks were selected for each patient and four micrometers sections were prepared. Deparaffinized sections were then treated with methanol and 3% hydrogen peroxide for 10 minutes. Antigen retrieval method was using a microwave oven at 95°C for 20 minutes and then cooling at 25°C for 2 hours. After washing with PBS, blank serum was added for 10 minutes. The sections were incubated with monoclonal Anti-CD133 (1:30) and Anti-CD44 antibodies (1: 100 ready to use) and negative control samples were incubated with PBS instead of primary antibody for 2 hours at 4°C. The

negative control sections were incubated with PBS instead of the primary antibody. After washing in PBS, a secondary biotin-marked antibody was applied for 10 min followed by peroxidase-marked streptavidin for 10 min. The reaction was visualized using (3,3'-diaminobenzidine tetrahydrochloride and the nuclei were colored by hematoxylin. Negative and positive controls were routinely used. A number of samples were randomly selected and staining repeated.

2.3. Quantification of the Immunostaining in Human Gliomas

Immunohistochemical staining for CD44 and CD133 markers in tumoral cells was scored. The number of positive staining cells indicating immunoreactivity on cell membrane and cytoplasm was counted in 10 microscopic fields and the percentage of positive cells was calculated. Immunoreactivity scores of CD44 and CD133 in tissue sections were negative (0) in no positive cell in the tumor, weak (+1) when less than 30% of the tumor cells were positive, moderate (+2) when about 30%-60% of the tumor cells are positive and strong (+3) when more than 60% of the tumor cells were positive [19].

2.4. Statistical Analysis of Data

After microscopic examination of H & E slides, prepared immunohistochemistry slides and the qualitative attribute of the expression of CD44 and CD133 markers in each patient, the data were analyzed using SPSS 16.0 software. These analyzes were performed using Fisher's, Chi-square, Spearman correlation coefficient and linear regression ($P < 0.05$).

3. Results

3.1. Expression of CD44 and CD133 in Human Glioma Tissue

Anti-CD44 and Anti-CD133 expression in 67 patients with primary glioma were analyzed using immunohistochemical analysis. The positive expression rates of CD44 (44/67, 65.67%) and CD133 (9/67, 13.43%) in patients with gliomas were higher than those in normal brain tissues (0/12, 0%) significantly ($P < 0.01$) (Table 1). These two markers were expressed in the cell membrane and cytoplasm that was confirmed by previous studies [8] (Figure 1 A-B). Figures of immunohistochemical staining of CD44 and CD133 markers are shown in Figure 2A-F.

Normal brain tissue sections and paraffin-embedded gliomas, stained with Anti-CD44 and Anti-CD133 markers, have been shown in Figure 2. The Figure 2A show low grade glioma with no staining for CD44.

Figure 2B shows anaplastic oligodendroglioma which are weak positive for CD44. Figure 2C shows glioblastomas that is strong and diffuse positive for CD44 (membranous and cytoplasmic). Normal brain tissue has been shown in Figure 2D with negative staining for CD133. Figure 2E shows low

grade ependymoma that has weak reactivity for CD133. Figure 2F shows GBM that expresses the CD133 marker positively (dense, spotty and membranous).

Table 1. CD44 and CD133 markers expression in glioma and human normal brain tissue

Groups	Cases (n)	0(n) (%)	1+~2+ (%)	3+ (%)	<i>P</i>
CD44					
Gliomas	67	23 (34.3)	15 (22.4)	29 (43.3)	<0.001
Normal	12	12 (100)	0 (0)	0 (0)	
CD133					
Gliomas	67	58 (86.6)	3 (4.5)	6 (9)	<0.001
Normal	12	12 (100)	0 (0)	0 (0)	

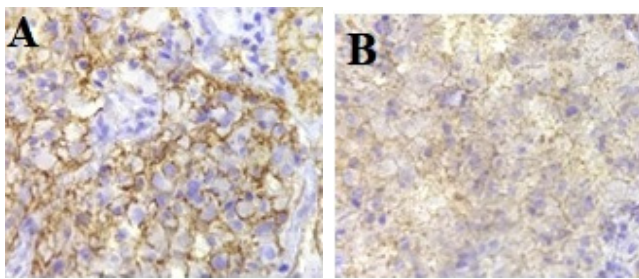


Figure 1. A. Anti-CD44 expression in stem cells membrane and cytoplasm in GBM, B. Anti-CD133 expression in stem cells membrane in GBM ($\times 100$)

3.2. Correlation between Expression of CD44 and CD133 with Clinical Grading of Human Gliomas

The expression of CD44 and CD133 markers in human glioma with different clinical grading is shown in Table 2.

Bivariate correlation (Spearman correlation coefficient) was used to evaluate the relationship between expression of CD44, CD133, and pathologic grading of glioma tumors. There was a significant relationship between the percentage of stained cells with immunohistochemistry and pathological grade of glioma tumors for CD44 marker ($C_s = 0.273$, $P < 0.05$), which indicated a high correlation between higher grades of glioma tumor and high expression of this marker. However, the CD133 marker ($C_s = 0.067$) did not show a significant relationship. Using a simple linear regression showed the relationship between expression of CD44 and CD133 markers $R = 0.277$ ($P = 0.23$), indicating a nonlinear relationship between expression of these two markers in the gliomas of the study (Tables 3 and 4). Figure 3 shows the correlation between expression of CD44 and CD133 markers in glial tumors by P-P plot method.

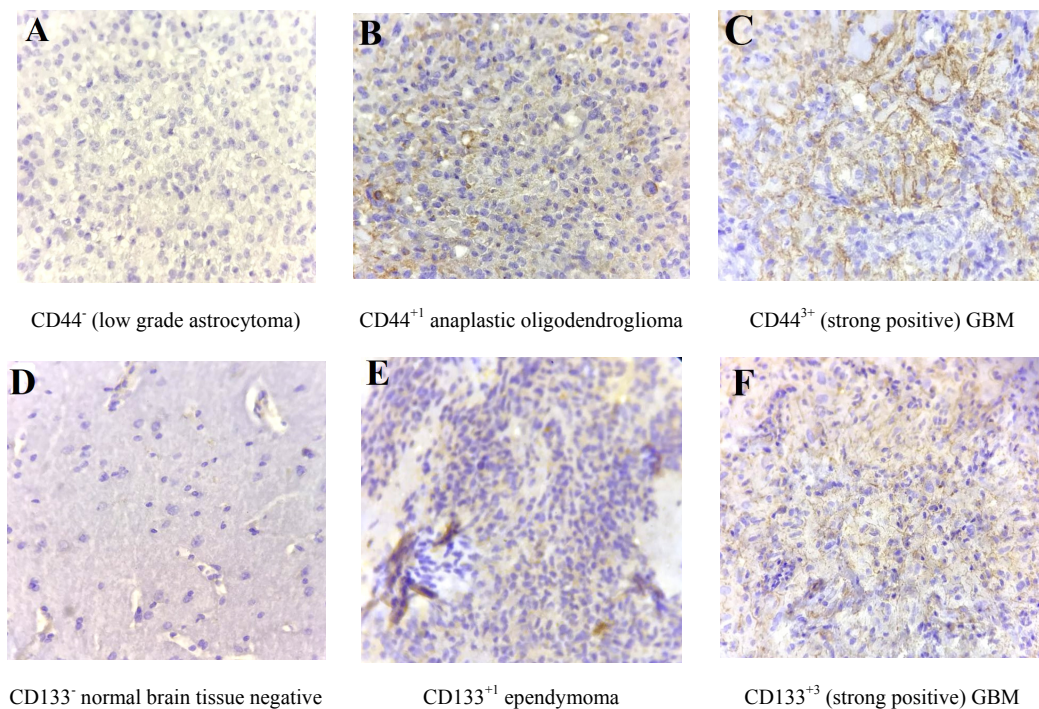


Figure 2. Anti-CD44 and Anti-CD133 immunohistochemical stains ($\times 100$). Paraffin-embedded sections of representative gliomas and normal brain tissues that stained with Anti-CD44 and Anti-CD133 antibodies. A. Negative or weak staining for CD44 in low grade glioma tumors; B. Showing CD44⁺ anaplastic oligodendroglioma; C & F. High expression levels of CD44 and CD133 in glioblastoma respectively; D. showing normal brain tissue, negative staining for CD133; E. Low grade ependymoma with weak staining for cd133

Normal P-P Plot of Regression Standardized Residual

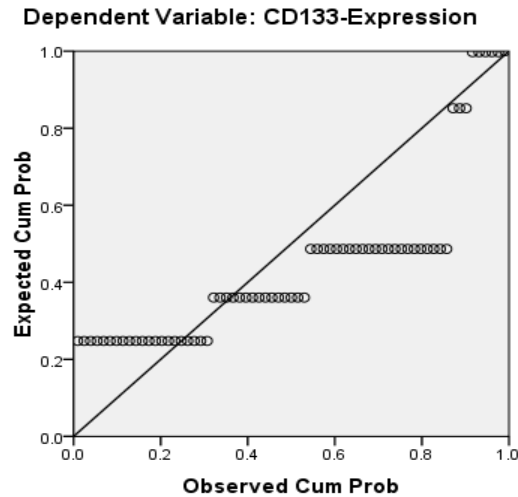


Figure 3. Correlation between CD44 and CD133 markers expression in studied glial tumors using P-P plot test

Table 2. CD44 and CD133 markers expression level in human glioma tissue with different clinical grading

Clinical Grading	Number	CD44 (n, %)			CD133 (n, %)		
		0	1+~2+	3+	0	1+~2+	3+
Low-grade tumors	31	16 (51.61)	5 (16.12)	10 (32.25)	28 (90.32)	2 (6.45)	1 (3.22)
Astrocytoma	15	9 (60)	3 (20)	3 (20)	14 (93.33)	0 (0)	1 (6.66)
Oligodendroglioma	6	3 (50)	1 (16.66)	2 (33.33)	6 (100)	0 (0)	0 (0)
Ependymoma	4	2 (50)	0 (0)	2 (50)	3 (75)	1 (25)	0 (0)
Pilocytic astrocytoma	6	2 (33.33)	1 (16.66)	3 (50)	5 (83.33)	1 (16.66)	0 (0)
High-grade tumors	36	7 (19.45)	10 (27.78)	19 (52.77)	30 (83.33)	1 (2.78)	5 (13.89)
GBM	27	4 (14.82)	8 (29.63)	15 (55.55)	23 (85.18)	1 (3.70)	3 (11.11)
Anaplastic astrocytoma	4	2 (50)	1 (25)	1 (25)	4 (100)	0 (0)	0 (0)
Malignant oligodendroglioma	4	1 (25)	1 (25)	2 (50)	3 (75)	0 (0)	1 (25)
Pleomorphic xanthoastrocytoma	1	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)

Table 3. Spearman correlation for determination of relationship between CD44 and CD133 markers expression levels and tumor grade

		CD133-Expression	CD44-Expression	Tumor Grade
CD133-Expression	Correlation Coefficient	1.000	.311*	.067
	Sig. (2-tailed)	.	.010	.588
	N	67	67	67
CD44-Expression	Correlation Coefficient	.311*	1.000	.273*
	Sig. (2-tailed)	.010	.	.026
	N	67	67	67

*. Correlation is significant at the 0.05 level (2 tailed).

**. Correlation is significant at the 0.01 level (2-tailed)

Table 4. Simple linear regression with two variants, CD44 and CD133 expression level in human glial tumors

Model Summary ^b				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.277 ^a	.077	.062	8.55015E-1

a. Predictors: (Constant), CD133-Expression

b. Dependent Variable: CD44-Expression

4. Discussion and Conclusions

Malignant gliomas are highly recurrent tumors even after surgery, chemotherapy, radiotherapy and immunotherapy. Gliomas treatment strategy has not been tangibly changed by the reason their limited biology finding [20]. In central nervous system tumors, cancer cells differ in their ability to form tumors. While the majority of the cancer cells have a limited ability to divide, a population of cancer stem cells that has the exclusive ability to extensively proliferate and form new tumors can be identified based on marker expression [21]. However, there is little information about the expression of these markers in brain tumors, especially with association to the malignant grades of these tumors [15]. In order to identify novel prognostic markers in gliomas, several CSC markers have been investigated [6]. Singh et al. (2003) showed that the identification of a brain tumor stem cell (BTSC) provides a powerful tool to investigate the tumorigenic process in the central nervous system and to develop therapies targeted to the BTSC [22]. This study was undertaken to explore the expression of stem cell marker CD44 and CD133 in human gliomas. In our study, CD44 expression was significantly correlated with the histological grade of the tumor, but there was no significant correlation between the expression of the CD133 marker and the histological grading of the tumor (Table 3). The expression of the CD133 stem cell marker may be related to the proliferation of glioma tumor cells and mesenchymal invasion (23). The expression of CD133 marker in grade IV glial tumors was higher than that of grade II tumors [17]. However, in other studies, there was no association between CD133 expression and tumor grade and its outcome, which may be due to differences in the methods of conducting studies [17, 24]. In Table 1 and Figure 2, the expression of the CD133 marker is 9% strong and 4.5% moderate and weak. This marker does not express in normal brain tissue. As Table 2 shows, the expression level of CD133 is only 11.11% of the glioblastomas. In Table 3, there is no significant correlation between histologic grade of tumor and CD133 expression. Wu et al 2016 studies showed no correlation between CD133 protein expression and patient's survival. Also no correlation between CD133 protein expression and CD133 promoter methylation status was found [25]. CD44 is a glycoprotein marker that is expressed in a variety of malignancies [26]. Xu et al (2010) found that in GBM xenograft models, knockdown of CD44 inhibited cell growth and improved response to chemotherapy [27]. The CD44 marker is strongly expressed in mesenchymal stem cells [28]. As shown in Table 1 and Figure 2, the expression of the CD44 marker was 43.3% strong and 22.4% weak and moderate in gliomas. This marker is not expressed in the normal tissue of the brain. In table 2, the expression of the CD44 marker is shown in a variety of gliomas, the highest of which is 55.55%, in the glioblastoma multiforme. Table 3 shows a significant correlation between the

expression of CD44 marker and tumor grade. As shown in Table 4-4 and Figure 3, there is a consistent and non-linear correlation between the expression of CD44 and CD133 markers in glial tumors of the study.

Zhang et al (2008) studies using immunohistochemistry method showed that expression level of anti-Nestin and CD133 markers increased significantly with increased glioma tumor grade ($P < 0.05$). There was a positive correlation between expression of these two markers in different glioma tissues ($r = 0.89$). Therefore, the expression of CD133 and Nestin can be suggested as an important feature of human glial tumors, and simultaneous finding of Nestin and CD133 in predicting the invasive nature of this tumor is useful [15]. In Ma et al. (2007) study, the expression of CD133, Nestin, SOX-2, Musashi-1, CXCR4, Flt4 and Endoglin markers on the protein and mRNA levels were used to determine the association between malignancy degree of different types of astrocytoma and the expression of these genes in proliferating glial cells. CD133⁺ cells were seen with higher levels (15.6%) in astrocytomas with WHO grade IV and 2.3% in normal brain tissue that associated with malignant grades in these tumors. Results in Real Time RT-PCR test were reported as 12.5% in grade IV astrocytoma and 8.5% in normal brain tissue [3]. Also Dalhort et al (2014) using fluorescence staining method reported that CD133 expression was not associated with WHO grade and overall survival rate of patients. Positive expression of the Nestin marker was associated with the glioma WHO grade. Patients expressing high levels of CD133 and Nestin showed low survival [29]. The use of different CD133 antibodyclones possibly recognizing different CD133 splice variants with frail epitopes of different glycosylation status may explain the different findings. It is therefore most likely the distribution of certain CD133 antigens and not the distribution of the CD133 protein itself that is of prognostic value [6].

An attempt has been made to find alternative methods for the isolation of cancer stem cells. Stem cell therapies and isolation and nutritional methods for cancer stem cells continue to be a major challenge [9]. The expressions of CD133, Olig2 and CD44 are correlated with the proliferative or invasive states of a GBM cell. Proliferation in turn correlates with sensitivity to therapy. By monitoring the changes in cellular phenotype using CD133/Olig2 and CD44 markers in the clinic, an improved therapeutic regimen could be designed to minimize acquisition of non-genetic tumor resistance [11].

In this study, the relationship between CD44 and glioma tumor grade was significant. This marker looks helpful in predicting tumor grade when tissue specimen is small and all the criteria of grade III or IV tumor cannot be found. Using alternate methods for the isolation of cancerous and non cancerous stem cells and determining of CD44 and CD133 expression levels in other stem cell markers can be suggested in gliomas comparing with normal brain tissues.

ACKNOWLEDGEMENTS

Authors would like to thank the supervisor and technicians of Department of Pathology in Tehran Firoozgar Hospital, Iran for their helps.

REFERENCES

- [1] LOUIS DN, OHGAKI H, WIESTLER OD, et al. 2007. The 2007 WHO classification of tumors of the central nervous system. *Acta Neuropathol.*; 114: 97–109.
- [2] STUPP R, TONN JC, BRADA M, PENTHEROUDAKIS G, 2010. High-grade malignant glioma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2010; 21(Suppl 5): v190–3. doi: 10.1093/annonc/mdq187.
- [3] MA Y-H, MENTLEIN R, KNERLICH F, KRUSE M-L, MEHDORNH M, HELD-FEINDT J. 2008. Expression of stem cell markers in human astrocytomas of different WHO grades. *J Neuro oncol.*; 86: 31–45.
- [4] BAO S, WU Q, MCLENDON RE, HAO Y, SHI Q, HJELMELAND AB, DEWHIRST MW, BIGNER DD AND RICH JN. 2006. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature*; 444: 756760.
- [5] KONG BH, MOON JH, HUH Y-M, SHIM J-K, LEE J-H, KIM E-H, CHANG JH, KIM D-S, HONG Y-K, KIM SH, LEE S-J, AND KANG S-G. Prognostic value of glioma cancer stem cell isolation in survival of primary Glioblastoma Patients, *Stem Cells Int.* 2014; 2014: 838950, Published online 2014 Dec 11. doi:10.1155/2014/838950, PMCID: PMC4279114.
- [6] DAHLROT, R. H., HERMANSEN, S. K., S., KRISTENSEN, B. W. 2013. What is the clinical value of cancer stem cell markers in gliomas?, *Int J Clin Exp Pathol.* 6(3): 334–348.
- [7] BEIER D, HAU P, PROESCHOLDT M, LOHMEIER A, WISCHHUSEN J, OEFNER PJ, AIGNER L, BRAWANSKI A, BOGDAHN U, BEIER CP. 2007. CD133(+) and CD133(-) glioblastoma-derived cancer stem cells show differential growth characteristics and molecular profiles. *Cancer Res.* 67(9): 4010–4015. doi: 10.1158/0008-5472.CAN-06-4180.
- [8] WANG J, SAKARIASSEN PØ, TSINKALOVSKY O, IMMERVOLL H, BØE SO, SVENDSEN A, et al. 2008. CD133 negative glioma cells form tumors in nude rats and give rise to CD133 positive cells. *Int J Cancer.* 122: 761–768. doi: 10.1002/ijc.23130.
- [9] BRESCIA, P, RICHICHI, C, PELICCI, G. 2012. Current strategies for identification of glioma stem cell, *Journal of Oncology*, ID: 376894, [Http://dx.doi.org/10.1155/2012/376894](http://dx.doi.org/10.1155/2012/376894).
- [10] BARRANTES-FREER A, RENOVANZ M, EICH M, BRAUKMANN A, SPRANG B, SPIRIN P, et al, 2015. CD133 Expression Is Not Synonymous to Immunoreactivity for AC133 and Fluctuates throughout the Cell Cycle in Glioma Stem-Like Cells. Harrison JK, editor. *PLoS ONE*. 10 e0130519 doi: 10.1371/journal.pone.0130519 (PMC free article).
- [11] BROWN, DV, FILIZ, G, DANIEL, PM, HOLLANDE, F, DWORKIN, S, AMIRIDIS, S., KOUNTOURI, N, NG W, MOROKOFF, A, MANTAMADIOTIS, T. 2017. Expression of CD133 and CD44 in glioblastoma stem cells correlates with cell proliferation, phenotype stability and intra-tumor heterogeneity, *PLOS*, Published: February 27, <https://doi.org/10.1371/journal.pone.0172791>.
- [12] KAAIJK, P, PALS, ST, MORSINK, FHM, TROOST, D. 1997. Differential expression of CD44 splice variants in the normal human central nervous system, *Journal of Neuroimmunology*, 73 (1-2): 70–76.
- [13] WEI KC, HUANG CY, CHEN PY, FENG LY, WU TW, CHEN SM, TSAI HC, LU YJ, TSANG NM, TSENG CK, PAI PC, SHIN JW. 2010. Evaluation of the prognostic value of CD44 in glioblastoma multiforme, *Anticancer Res.* 30 (1): 253–259.
- [14] ANIDO J, SAEZ-BORDERIAS A, GONZALEZ-JUNCA A et al. 2010. “TGF- β receptor inhibitors target the CD44 (high)/Id1(high) glioma-initiating cell population in human glioblastoma,” *Cancer Cell*, 2010; 18, 6: 655–668.
- [15] ZHANG, M, SONG, T, YANG, L et al. 2008. Nestin and CD133: valuable stem cell-specific markers for determining clinical outcome of glioma patients, *Journal of Experimental & Clinical Cancer Research*, 27: 85.
- [16] BROWN DV, DANIEL PM, D’ABACO GM, GOGOS A, NG W, MOROKOFF AP, et al. 2015. Coexpression analysis of CD133 and CD44 identifies Proneural and mesenchymal subtypes of glioblastoma multiforme. *oncotarget*, 6: 6267–6280. doi: 10.18632/oncotarget. 3365 [PMC free article].
- [17] ZEPPERINCK F, AHMADI R, CAMPOS B, DICTUS C, HELMKE BM, BECKER N, LICHTER P, UNTERBERG A, RADLWIMMER B, HEROLD-MENDE CC. 2008. Stem cell marker CD133 affects clinical outcome in glioma patients. *Clin Cancer Res.* 1; 14(1): 123–9. doi: 10.1158/1078-0432.CCR-07-0932. PubMed PMID: 18172261.
- [18] CHENG JX, LIU BL, ZHANG X. 2009. How powerful is CD133 as a cancer stem cell marker in brain tumors? *Cancer Treat Rev.*, 35 (5):403–8. doi: 10.1016/j.ctrv.2009.03.002. Epub 2009 Apr 14. Review. PubMed PMID: 19369008.
- [19] LIANG ML, MA J, HO M, SOLOMON L, BOUFFET E, RUTKA JT, HAWKINS C. 2008. Tyrosine kinase expression in pediatric high grade astrocytoma. *J Neurooncol.* 87: 247–253.
- [20] GALLI R, BINDA E, ORFANELLI U, CIPELLETTI B, GRITTI A, DE VITIS S, FIOCCO R, FORONI C, DIMECO F, VESCOVI A. 2004. Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. *Cancer Res.* 64: 7011–7021.
- [21] AL-HAJJ M, CLARKE M. 2004. Self-renewal and solid tumor stem cells, *Oncogene*. 23 7274–7282, doi: 10.1038/sj.onc.1207947.
- [22] SINGH SK, CLARKE ID, TERASAKI M, BONN VE, HAWKINS C, SQUIRE J, DIRKS PB. 2003. Identification of a cancer stem cell in human brain tumors. *Cancer Res.*; 63(18): 5821–8. PubMed PMID: 14522905.
- [23] JOO KM, KIM SY, JIN X, SONG SY, KONG DS, LEE JI, JEON JW, KIM MH, KANG BG, JUNG Y, JIN J, HONG SC, PARK WY, LEE DS, KIM H, NAM DH. 2008. Clinical and biological implications of CD133-positive and

- CD133-negative cells in glioblastomas, *Lab Invest.* 88 (8): 808-15. doi: 10.1038/labinvest.2008.57.
- [24] CHRISTENSEN K, SCHROEDER HD AND KRISTENSEN BW. 2008. CD133 identifies perivascular niches in grade II-IV astrocytomas. *J Neurooncol*; 90 (2): 157-170.
- [25] WU X, WU F, XU D, ZHANG T.2016. Prognostic significance of stem cell marker CD133 determined by promoter methylation but not by immunohistochemical expression in malignant gliomas. *J Neurooncol*; 27: 221-232.
- [26] LI C, HEIDT DG, DALERBA P, BURANT CF, ZHANG L, ADSAY V, WICHA M, CLARKE MF, SIMEONE DM.2007. Identification of pancreatic cancer stem cells. *Cancer Res.* 67(3):1030-7. PubMed PMID: 17283135.
- [27] XU Y, STAMENKOVIC I, YU Q.2010. CD44 attenuates activation of the hippo signaling pathway and is a prime therapeutic target for glioblastoma. *Cancer Res.* 70 (6):2455-2464. doi: 10.1158/0008-5472.CAN-09-2505. Epub 2010 Mar 2. PubMed PMID: 20197461; PubMed Central PMCID: PMC2840073.
- [28] MAO P, JOSHI K, LI J, KIM SH, LI P, SANTANA-SANTOS L, LUTHRA S, CHANDRAN UR, BENOS PV, SMITH L, WANG M, HU B, CHENG SY, SOBOL RW, NAKANO I.2013. Mesenchymal glioma stem cells are maintained by activated glycolytic metabolism involving aldehyde dehydrogenase 1A3. *Proc Natl AcadSci U S A.* 110 (21): 8644-9. doi: 10.1073/pnas.1221478110. Epub 2013 May 6. PubMed, PMID: 23650391; PubMed Central PMCID: PMC3666732.
- [29] DAHLROT, RH, HANSEN, S, JENSEN, SS, SCHRØDER, HD, HJELMBORG, J, KRISTENSEN, BW. 2014. Clinical value of CD133 and nestin in patients with glioma: a population-based study, *Int J Clin Exp Pathol.* 7(7): 3739–3751. Published online 2014 Jun 15. PMCID: PMC4128985.