European Review for Medical and Pharmacological Sciences 2016; 20: 3378-3384

Exploring the regulatory role of isocitrate dehydrogenase mutant protein on glioma stem cell proliferation

H.-C. LU[1], J. MA[2], Z. ZHUANG[1], F. QIU[2], H.-L. CHENG[1], J.-X. SHI[1]

[1]Department of Neurosurgery, Jingling Hospital, Medical School of Nanjing University, Nanjing, China
[2]Department of Neurosurgery, Nanjing Brain Hospital, Affiliated to Nanjing Medical University, Nanjing, China

Abstract. – OBJECTIVE: Glioma is the most lethal form of cancer that originates mostly from the brain and less frequently from the spine. Glioma is characterized by abnormal regulation of glial cell differentiation. The severity of the glioma was found to be relaxed in isocitrate dehydrogenase 1 (IDH1) mutant. The present study focused on histological discrimination and regulation of cancer stem cell between IDH1 mutant and in non-IDH1 mutant glioma tissue.

PATIENTS AND METHODS: Histology, immunohistochemistry and Western blotting techniques are used to analyze the glioma nature and variation in glioma stem cells that differ between IDH1 mutant and in non-IDH1 mutant glioma tissue.

RESULTS: The aggressive form of non-IDH1 mutant glioma shows abnormal cellular histological variation with prominent larger nucleus along with abnormal clustering of cells. The longer survival form of IDH1 mutant glioma has a control over glioma stem cell proliferation. Immunohistochemistry with stem cell markers, CD133 and EGFRvIII are used to demonstrate that the IDH1 mutant glioma shows limited dependence on cancer stem cells and it shows marked apoptotic signals in TUNEL assay to regulate abnormal cells. The non-IDH1 mutant glioma failed to regulate misbehaving cells and it promotes cancer stem cell proliferation.

CONCLUSIONS: Our finding supports that the IDH1 mutant glioma has a regulatory role in glioma stem cells and their survival.

Key Words:
Glioma, IDH1 mutant, CD133, EGFRvIII, TUNEL.

Introduction

Glioma a primary form of tumor that occurs in the glial cells of the brain. The glial cells are non-neuronal tissues that provide physical support and supplies nutrient to the neuronal cells. Since the neuronal cells are unable to follow mitosis after certain limits, most of the brain tumor (80%) are associated with dividing glial cells[1]. The mean survival time of a patient with glioma are only minimum and death occurs within 15 months[2]. Most of the brain tumor research are focusing on the comparative analysis of normal and tumor tissue and so the knowledge about the cancer stem cell are limited. Cancer stem cells are the root that helps to understand the cellular regulation of their proliferation and growth. Recent studies show a significant identification of regulatory proteins such as HIF2a[3], L1CAM[4] and Bmi-1[5] that are linked to glioma stem cell. The proteins with similar basis are helped to know their correlation that it has with survival, self-renewal and growth of glioma stem cell. Other than this there are several molecular mechanisms, governing glioma stem cells is to be revealed by the evidence.

Mutational forms of isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) enzymes are identified that increase the survival of glioma patient[6]. The most common mutation occurs in IDH1 are at codon R132 ($IDH1^{R132MUT}$) which are located at the active site of the enzyme[7]. Subsequently, less common IDH2 mutation occurs at the site of R172[8]. The IDH1 mutation is found mostly in lower-grade and anaplastic diffuse gliomas[8,9]. The patient with IDH1 mutant shows higher longevity than patient with non-IDH1 mutant[10,11]. It was previously hypothesized that the mutation in IDH1 results into loss of function of the enzyme that promotes tumorigenesis[11]. However, further studies confirm the IDH1 mutation function as a novel gain of function[12,13]. Several investigations revealed the functional role, which the mutated IDH1 produce oncometabolite named 2-Hydroxyglutarate[14]. The

Corresponding Author: Ji-Xin Shi, MD; e-mail: jixinshi62@gmail.com

2-hydroxyglutarate inhibit many cellular processes[13,15] that disturb cellular differentiation[16] and enhance the transformation of human normal astrocytes[17]. The phenomena in which isocitrate dehydrogenase mutant protein, driving the glioma stem cells are not well known. Additional researches are needed to demonstrate the role of IDH1 mutant in regulating the glioma stem cells.

Patients and Methods

Sample Collection

Paraffin-embedded and fresh tissue samples of normal brain, IDH1 mutant glioma and non-IDH1 mutant glioma with earlier stage were obtained from Jingling Hospital, China. Initially, the patients are subjected to pathological as well as molecular diagnosis and get approval from patients and Ethics committees of the hospital for use of their samples for research purposes. The age group of the patient ranges from 16 to 54 years, and samples were collected from the confirmed cases, diagnosed as per the criteria followed by the World Health Organization (WHO).

Immunohistochemistry

For performing Immunohistochemistry, the paraffin embedded tissues are subjected to microtome sectioning of size 7 μm. The consecutive sections on clean glass slides are then de-paraffinized and immersing in freshly prepared 10% H_2O_2 and 10% Methanol in 1X Phosphate Buffered Saline (PBS) for 20 min to block the endogenous peroxidase activity. Trypsin treatment (0.1% Trypsin in 0.1% $CaCl_2$) for 10 min was followed to assess the target protein in the sections. Nonspecific antigens were blocked using serum followed by incubating with either anti-CD133 antibody (Biorbyt, orb18124, labscoop co., Little Rock, AR, USA) or anti-EGFRvIII antibody (Biorbyt, orb47907, labscoop co., Little Rock, AR, USA) for overnight at 4°C. After washing the sections thoroughly with 1X PBS, they are incubated with suitable secondary antibody for 1 hour at room temperature. The sections are then washed to remove the non-specific binding of secondary antibody and stained with DAB (Diaminobenzidine) Kit. Secondary staining was carried out using Ehrlich hematoxylin that facilitates to visualize the individual cells in the section.

Western Blot Analysis

The protein samples were prepared from normal brain, IDH1 mutant and non-IDH1 mutant tissues, and they were resolved in 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel. The Western blotting of samples was carried out as already described by Qi et al[16]. After transferring the gel to a polyvinyl membrane, the membrane are incubated with either anti-EGFRvIII antibody (Biorbyt, orb47907, labscoop co., Little Rock, AR, USA) or anti-CD133 antibody (Biorbyt, orb18124, labscoop co., Little Rock, AR, USA) and steps are followed according to the manufacturer's instructions. After washing the primary antibody, the membrane is incubated with secondary antibody and developed later.

TUNEL Assay

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay aids, to identify cells that are in the last phase of apoptosis with characteristic DNA fragments[18]. Apoptotic cells with DNA fragments are end labelled by incorporating Brdu (bromodeoxyuridine) a thymidine analogue to the 3′ ends of DNA strands. The incorporated Brdu are then detected in the apoptotic cell using anti-Brdu antibody (EMD Millipore, 05-633; Millipore, Billerica, MA, USA). The staining was performed using Diaminobenzidine (DAB Kit, Abcam, Cambridge, UK) and the protocol was followed according to the manufacturer's instructions.

Results

Histological Observation of Normal Brain, IDH1 Mutant and Non-IDH1 Mutant Brain Tissue

Histology was performed to find out the arrangement and appearance of cellular and tissue pattern of the normal brain, IDH1 mutant and non-IDH1 mutant brain tissue. We observed certain morphological criteria that help to differentiate IDH1 mutant and non-IDH1 mutant brain tissue. The normal brain tissue has a uniform arrangement of cells that well adher to the supporting tissues as shown in the Figure 1A. The histology of IDH1 mutant and non-IDH1 mutant brain tissue was viewed with caution and we observed that the IDH1 mutant (Figure 1B) and non-IDH1 mutant (Figure 1C) brain tissue have the infinite replication capability. We also observed that non-IDH1

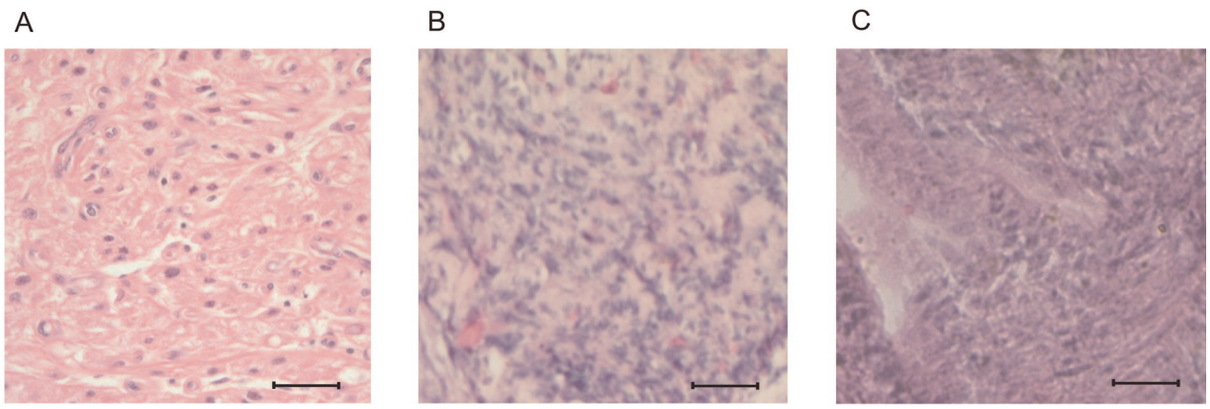

Figure 1. Histological variation between normal brain, IDH1 mutant and non-IDH1 mutant glioma tissue. ***A,*** Histology of normal brain tissue with well organized cellular arrangement. ***B,*** Histology of IDH1 mutant glioma tissue with a proliferative mass of cells. ***C,*** Non IDH1 mutant glioma tissue exhibit abnormal nucleus to cytoplasmic ratio along with cellular clustering. Scale Bar: 100 μm size.

mutant (Figure 1C) brain tissue has a prominent large nucleus with nuclear to a cytoplasmic ratio higher than that of IDH1 mutant brain tissue (Figure 1B). Other than this, the cells of non-IDH1 mutant brain tissue show abnormal aggregations and clustering (Figure 1C).

Validation of CD133 as a Stem Cell Marker

CD133 was used as a neuronal stem cell marker and it was first used to identify glioma stem cell[19]. Recent studies show that there are glioma stem cells without CD133 expression. Also, further studies suggest, there are CD133 positive cells in glioma, which cannot behave like cancer stem cells[20]. Here, we elucidated the presence of stem cells of the brain that are localized in isolated pockets of a normal brain (Figure 2A), but we observed increased expression of CD133 in IDH1 mutant brain tissue (Figure 2B) and also in non-IDH1 mutant brain tissue (Figure 2C).

Identification of Glioma Stem cell Based on EGFRvIII Marker

EGFRvIII was a newly identified glioma stem cell marker based on cancer-specific genetic alteration[21]. The new finding gives a strong correlation to specify cancer stem cells of glioma and it is an interesting aspect to evaluate their expression in IDH1 mutant and in non-IDH1 mutant brain tissue. In the control brain tissue, we observed negligible expression of EGFRvIII protein as shown in the Figure 3A. Interestingly, we examined different regulatory effects of EGFRvIII expression in IDH1 mutant

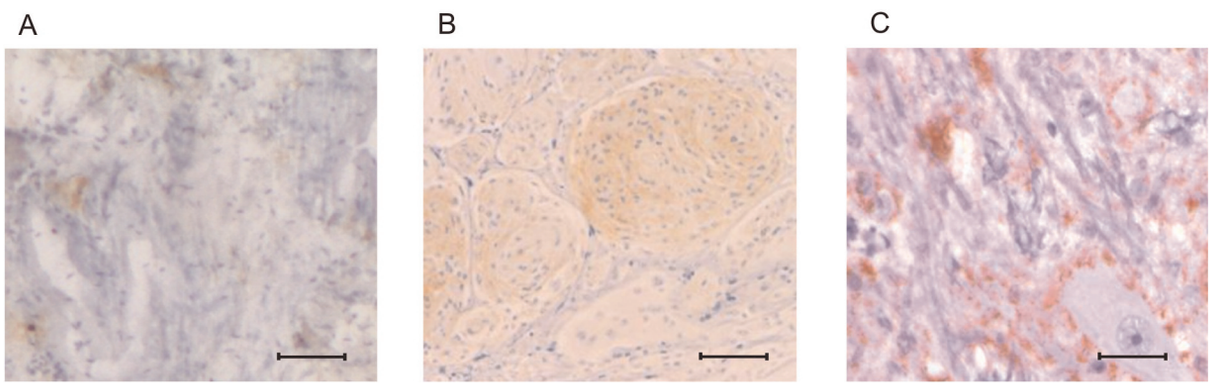

Figure 2. Identification of neuronal and glioma stem cells using CD133 marker. ***A,*** Normal brain tissue immunostain with CD133 protein shows the isolated clustering of neuronal stem cell. ***B,*** IDH1 mutant glioma tissue with CD133 positive signals, which represent the presence of neuronal stem cells as well as with glioma stem cells which are indistinguishable. ***C,*** Aggresive grade, non IDH1 mutant glioma tissue shows positive cells for CD133 marker. Scale Bar: 100 μm size.

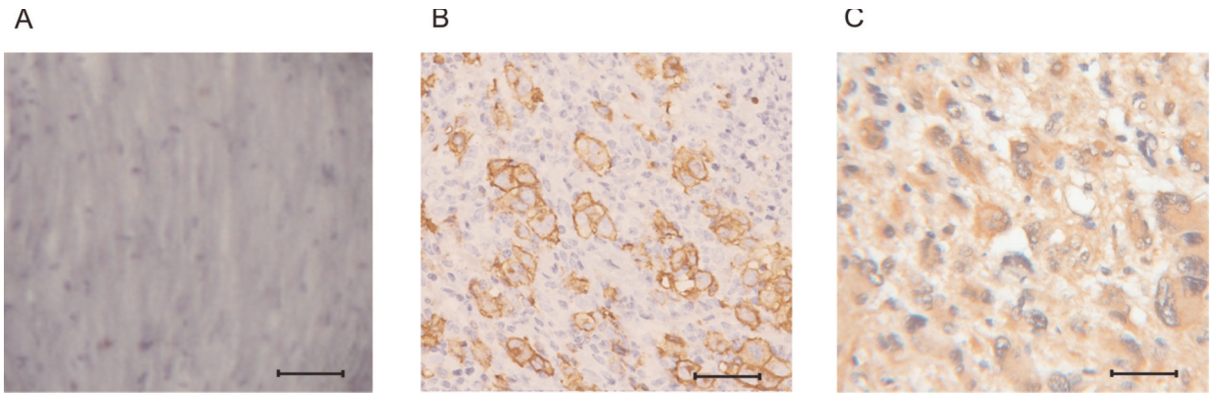

Figure 3. Identification of glioma based stem cells, using exclusive cancer stem cell marker EGFRvIII. ***A,*** normal brain tissue immunostain with EGFRvIII shows no signal. ***B,*** Section of IDH1 mutant glioma tissue with EGFRvIII protein expression. ***C,*** non IDH1 mutant glioma tissue with elevated expression of EGFRvIII protein. Scale Bar: 100 µm size.

and non-IDH1 mutant brain tissue as visualized in Figure 3B and C. The better survival form of glioma, IDH1 mutant glioma tissue shows minimal expression of EGFRvIII (Figure 3B) where as a more aggressive form of non-IDH1 mutant glioma has an elevated expression of EGFRvIII expression (Figure 3C).

Elevated Level of Apoptosis Cells in IDH1 Mutant Glioma

Tunnel assay was performed to characterize the suppression phenomena of abnormal cells through apoptosis. Normal brain tissue shows no signal for Tunnel staining (Figure 4A). But we observed strong signal over IDH1 mutant glioma tissue as shown in the Figure 4B. Perhaps, the more aggressive non-IDH1 mutant glioma shows only mild positive signals (Figure 4C).

Western Blotting Analysis to Validate the Expression of CD133 and EGFRvIII in Glioma Tissue

The protein samples were isolated from normal brain, IDH1 mutant and non-IDH1 mutant glioma tissue are subjected to Western blot analysis. The correlation between stem cell and cancer stem cell of glioma are analyzed using stem cell marker CD133 and EGFRvIII. The results demonstrate that the level of stem cell marker, CD133 expression shows minimal expression in control tissue (Figure 5A, Lane 1), but no significant variation were observed in the samples of IDH1 mutant glioma tissue and non-IDH1 mutant glioma tissue (Figure 5A, Lane 2 and 3). A similar expression of EGFRvIII was studied to trace out the glioma based cancer stem cell. From the Figure 5B it is evident that the

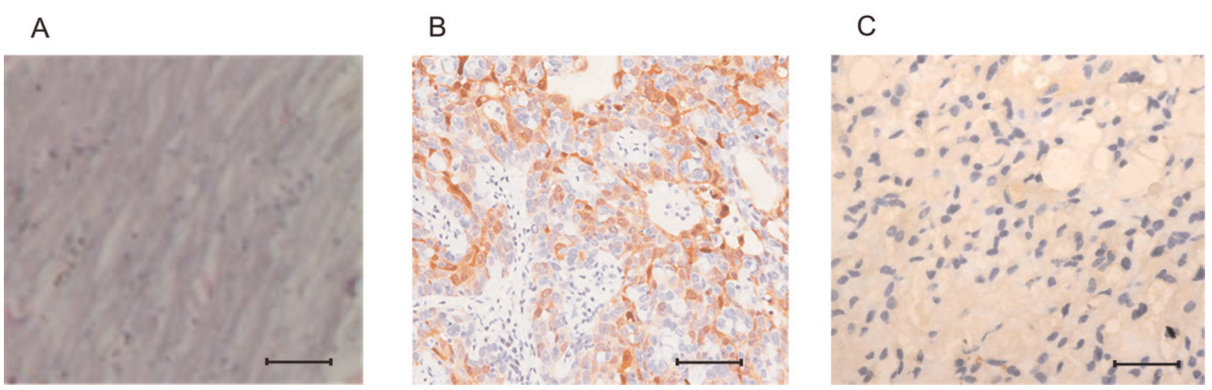

Figure 4. Depletion of apoptotic cells in non IDH1 mutant glioma tissue. ***A,*** Normal brain tissue with no specific apoptotic signal. ***B,*** IDH1 mutant glioma tissue with an elevated apoptotic signal. ***C,*** Section of non IDH1 mutant glioma tissue with a depleted apoptotic signal. Scale Bar: 100 µm size.

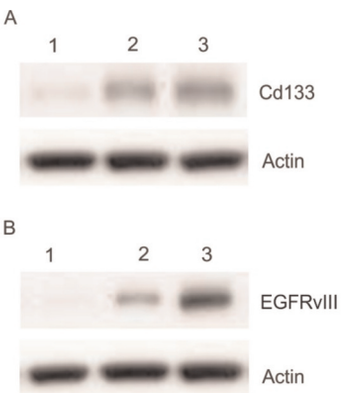

Figure 5. Validation of CD133 and EGFRvIII expression by Western blotting. ***A,*** Cell Lysate of Normal brain, IDH1 mutant and non IDH1 mutant glioma tissue are subjected to immunoblot with the anti-CD133 antibody. ***B,*** Western blot, demonstrating the expression of EGFRvIII protein in the normal brain, IDH1 mutant and non IDH1 mutant glioma tissue. In Figure A and B, Lane 1, Lane 2, Lane 3 represent protein samples from normal brain, IDH1 mutant glioma and from non IDH1 mutant glioma tissue respectively. Actin was used as a control.

normal brain shows little or no expression of EGFRvIII (Figure 5B, Lane 1). The result with IDH1 mutant and from non-IDH1 mutant glioma tissue shows the expression of EGFRvIII was doubled in non-IDH1 mutant glioma when comparing with the IDH1 mutant glioma (Figure 5B, Lane 2 and 3).

Discussion

The origin of glioma stem cell are still unresolved and it has two possibilities, whether it emerges from the neuronal stem cell or it may evolve from dedifferentiated cells which behave similarly to stem cell[22]. Patient with an IDH1 mutant form of glioma has longer survival[23,24]. Our data support these phenomena which are confirmed through experimental basis. The comparative study of histology (Figure 1A-C) helps to revive how cells are misbehaving in the IDH1 mutant glioma (Figure 1B) as well as in non-IDH1 mutant glioma. The cell clustering and higher nucleus to cytoplasmic ratio of non-IDH1 mutant glioma itself show the aggressive nature of the glioma of non-IDH1 mutant patients. The data of histological interpretation were further supported by the experiments performed with stem cell markers.

The CD133 is not the absolute cancer stem cell marker; it was initially used to identify tumor repopulation cells in leukemia[25,26]. Later it was detected as a brain tumor stem cell marker in many *in vivo* and *in vitro* studies[19,27]. Still, it cannot able to play a significant role to identify cancer stem cells exclusively because there are many tumor cells without the essential role of CD133[20]. The immunohistochemistry performed using CD133 marker elucidate that their expression is almost equal in IDH1 mutant glioma (Figure 2B) and non-IDH1 mutant glioma (Figure 2C) when compared to the normal brain tissue (Figure 2A). The data reveal that the aggressive form of non-IDH1 mutant glioma has cancer-based stem cells and shows no variation on CD133 expression. The aggressive cancer stem cells are analyzed using EGFRvIII marker which can exclusively detect glioma stem cell (Figure 3B, 3C). As we expected, the low survival as well as an aggressive form of non-IDH1 mutant glioma tissue, overexpress EGFRvIII than IDH1 mutant glioma tissue, which implies the severity of the disease condition. The high survival IDH1 mutant having glioma tissue shows an underdetermined expression of EGFRvIII which reveals that the proliferation of glioma stem cells is under controlled conditions.

Stem cells of its own have the ability to profound anti-tumor effect[28]. Cells with abnormal behaviour should be recognized and subjected to apoptosis, the failure or defect in the apoptosis pathway pays the way for tumor initiation and malignancy[29]. The collective data from Figure 4A-C helps to investigate that the high survival of IDH1 mutant glioma patient is due to the apoptotic regulation over the abnormal behaving cells where it lacks in an aggressive form of non-IDH1 mutant glioma tissue.

Conclusions

There is a significant variance in the histology of IDH1 mutant and non-IDH1 mutant glioma tissue that helps to understand the level of misbehaving cells. Also, the aggressive form of non-IDH1 mutant glioma shows increased expression of the EGFRvIII protein with a poor apoptosis signal. The high survival rate of IDH1 mutant glioma is due to significant regulation of apoptosis over abnormal cells and keeps a limit over glioma stem cells than that of non-IDH1 mutant glioma.

Acknowledgements

This work was supported by Jingling Hospital, Medical School of Nanjing University.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Goodenberger ML, Jenkins RB. Genetics of adult glioma. Cancer Gen 2012; 205: 613-621.
2) Rich JN, Eyler CE. Cancer stem cells in brain tumor biology. In: Cold Spring Harbor symposia on quantitative biology 2008; Cold Spring Harbor Laboratory Press, 2008; pp. 411-420.
3) Lee J, Kotliarova S, Kotliarov Y, Li A, Su Q, Donin NM, Pastorino S, Purow BW, Christopher N, Zhang W, Park JK, Fine HA. Tumor stem cells derived from glioblastomas cultured in bFGF and EGF more closely mirror the phenotype and genotype of primary tumors than do serum-cultured cell lines. Cancer Cell 2006; 9: 391-403.
4) Bao S, Wu Q, Li Z, Sathornsumetee S, Wang H, McLendon RE, Hjelmeland AB, Rich JN. Targeting cancer stem cells through L1CAM suppresses glioma growth. Cancer Res 2008; 68: 6043-6048.
5) Abdouh M, Facchino S, Chatoo W, Balasingam V, Ferreira J, Bernier G. BMI1 sustains human glioblastoma multiforme stem cell renewal. J Neurosci 2009; 29: 8884-8896.
6) Li S, Chou AP, Chen W, Chen R, Deng Y, Phillips HS, Selfridge J, Zurayk M, Lou JJ, Everson RG, Wu KC, Faull KF, Cloughesy T, Liau LM, Lai A. Overexpression of isocitrate dehydrogenase mutant proteins renders glioma cells more sensitive to radiation. Neuro Oncol 2013; 15: 57-68.
7) Xu X, Zhao J, Xu Z, Peng B, Huang Q, Arnold E, Ding J. Structures of human cytosolic NADP-dependent isocitrate dehydrogenase reveal a novel self-regulatory mechanism of activity. J Biol Chem 2004; 279: 33946-33957.
8) Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, Kos I, Batinic-Haberle I, Jones S, Riggins GJ, Friedman H, Friedman A, Reardon D, Herndon J, Kinzler KW, Velculescu VE, Vogelstein B, Bigner DD. IDH1 and IDH2 mutations in gliomas. N Engl J Med 2009; 360: 765-773.
9) Balss J, Meyer J, Mueller W, Korshunov A, Hartmann C, von Deimling A. Analysis of the IDH1 codon 132 mutation in brain tumors. Acta Neuropathol 2008; 116: 597-602.
10) Nobusawa S, Watanabe T, Kleihues P, Ohgaki H. IDH1 mutations as molecular signature and predictive factor of secondary glioblastomas. Clin Cancer Res 2009; 15: 6002-6007.
11) Zhao S, Lin Y, Xu W, Jiang W, Zha Z, Wang P, Yu W, Li Z, Gong L, Peng Y, Ding J, Lei Q, Guan KL, Xiong Y. Glioma-derived mutations in IDH1 dominantly inhibit IDH1 catalytic activity and induce HIF-1 . Science 2009; 324: 261-265.
12) Gross S, Cairns RA, Minden MD, Driggers EM, Bittinger MA, Jang HG, Sasaki M, Jin S, Schenkein DP, Su SM, Dang L, Fantin VR, Mak TW. Cancer-associated metabolite 2-hydroxyglutarate accumulates in acute myelogenous leukemia with isocitrate dehydrogenase 1 and 2 mutations. J Exp Med 2010; 207: 339-344.
13) Figueroa ME, Abdel-Wahab O, Lu C, Ward PS, Patel J, Shih A, Li Y, Bhagwat N, Vasanthakumar A, Fernandez HF, Tallman MS, Sun Z, Wolniak K, Peeters JK, Liu W, Choe SE, Fantin VR, Paietta E, Löwenberg B, Licht JD, Godley LA, Delwel R, Valk PJ, Thompson CB, Levine RL, Melnick A. Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. Cancer Cell 2010; 18: 553-567.
14) Ward PS, Patel J, Wise DR, Abdel-Wahab O, Bennett BD, Coller HA, Cross JR, Fantin VR, Hedvat CV, Perl AE, Rabinowitz JD, Carroll M, Su SM, Sharp KA, Levine RL, Thompson CB. The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting α-ketoglutarate to 2-hydroxyglutarate. Cancer Cell 2010; 17: 225-234.
15) Xu W, Yang H, Liu Y, Yang Y, Wang P, Kim SH, Ito S, Yang C, Wang P, Xiao MT, Liu LX, Jiang WQ, Liu J, Zhang JY, Wang B, Frye S, Zhang Y, Xu YH, Lei QY, Guan KL, Zhao SM, Xiong Y. Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of -ketoglutarate-dependent dioxygenases. Cancer Cell 2011; 19: 17-30.
16) Qi S, Song Y, Peng Y, Wang H, Long H, Yu X, Li Z, Fang L, Wu A, Luo W, Zhen Y, Zhou Y, Chen Y, Mai C, Liu Z, Fang W. ZEB2 mediates multiple pathways regulating cell proliferation, migration, invasion, and apoptosis in glioma. PLoS One 2012; 7: e38842.
17) Koivunen P, Lee S, Duncan CG, Lopez G, Lu G, Ramkissoon S, Losman JA, Joensuu P, Bergmann U, Gross S, Travins J, Weiss S, Looper R, Ligon KL, Verhaak RG, Yan H, Kaelin WG Jr. Transformation by the (R)-enantiomer of 2-hydroxyglutarate linked to EGLN activation. Nature 2012; 483: 484-488.
18) Fulda S, Pervaiz S. Apoptosis signaling in cancer stem cells. Int J Biochem Cell Biol 2010; 42: 31-38.
19) Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J, Dirks PB. Identification of a cancer stem cell in human brain tumors. Cancer Res 2003; 63: 5821-5828.
20) Beier D, Hau P, Proescholdt M, Lohmeier A, Wischhusen J, Oefner PJ, Aigner L, Brawanski A, Bogdahn U, Beier CP. CD133+ and CD133– glioblastoma-derived cancer stem cells show differential growth characteristics and molecular profiles. Cancer Res 2007; 67: 4010-4015.
21) Yiin J-J, Hu B, Schornack PA, Sengar RS, Liu K-w, Feng H, Lieberman FS, Chiou S-H, Sarkaria JN,

Wiener EC, Ma HI, Cheng SY. ZD6474, a multitargeted inhibitor for receptor tyrosine kinases, suppresses growth of gliomas expressing an epidermal growth factor receptor mutant, EGFRvIII, in the brain. Mol Cancer Ther 2010; 9: 929-941.

22) Furnari FB, Fenton T, Bachoo RM, Mukasa A, Stommel JM, Stegh A, Hahn WC, Ligon KL, Louis DN, Brennan C, Chin L, DePinho RA, Cavenee WK. Malignant astrocytic glioma: genetics, biology, and paths to treatment. Gene Dev 2007; 21: 2683-2710.

23) Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, Olivi A, McLendon R, Rasheed BA, Keir S, Nikolskaya T, Nikolsky Y, Busam DA, Tekleab H, Diaz LA Jr, Hartigan J, Smith DR, Strausberg RL, Marie SK, Shinjo SM, Yan H, Riggins GJ, Bigner DD, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW. An integrated genomic analysis of human glioblastoma multiforme. Science 2008; 321: 1807-1812.

24) Weller M, Felsberg J, Hartmann C, Berger H, Steinbach JP, Schramm J, Westphal M, Schackert G, Simon M, Tonn JC, Heese O, Krex D, Nikkhah G, Pietsch T, Wiestler O, Reifenberger G, von Deimling A, Loeffler M. Molecular predictors of progression-free and overall survival in patients with newly diagnosed glioblastoma: a prospective translational study of the German Glioma Network. J Clin Oncol 2009; 27: 5743-5750.

25) Bühring HJ, Seiffert M, Marxer A, Weiss B, Faul C, Kanz L, Brugger W. AC133 antigen expression is not restricted to acute myeloid leukemia blasts but is also found on acute lymphoid leukemia blasts and on a subset of CD34+ B-cell precursors. Blood 1999; 94: 832-833.

26) Horn PA, Tesch H, Staib P, Kube D, Diehl V, Voliotis D. Expression of AC133, a novel hematopoietic precursor antigen, on acute myeloid leukemia cells. Blood 1999; 93: 1435-1437.

27) Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB. Identification of human brain tumour initiating cells. Nature 2004; 432: 396-401.

28) Ganta C, Ayuzawa R, Rachakatla R, Pyle M, Andrews G, Weiss M, Tamura M, Troyer D. Rat umbilical cord stem cells completely abolish rat mammary carcinomas with no evidence of metastasis or recurrence 100 days post–tumor cell inoculation. Cancer Res 2009; 69: 1815-1820.

29) Thompson CB. Apoptosis in the pathogenesis and treatment of disease. Science 1995; 267: 1456-1462.