CHAPTER SIX

Molecular determinants of the interaction between glioblastoma CD133^{+} cancer stem cells and the extracellular matrix

Valeriy Shevchenko[a,b], Natalia Arnotskaya[b], Oleg Pak[c], Aruna Sharma[d], Hari Shanker Sharma[d], Yuri Khotimchenko[a,e], Andrey Bryukhovetskiy[f], Igor Bryukhovetskiy[a,c,e,*]

[a]Department of Fundamental Medicine, School of Biomedicine, Far Eastern Federal University, Vladivostok, Russia
[b]Laboratory of Oncoproteomics, Institute of Carcinogenesis, N.N. Blokhin National Medical Research Center of Oncology, Ministry of Health of Russia, Moscow, Russia
[c]Medical Center, Far Eastern Federal University, Vladivostok, Russia
[d]International Experimental Central Nervous System Injury & Repair (IECNSIR), Department of Surgical Sciences, Anesthesiology & Intensive Care Medicine, University Hospital, Uppsala University, S-75185 Uppsala, Sweden
[e]Laboratory of Pharmacology, National Scientific Center of Marine Biology, Far East Branch of the Russian Academy of Sciences, Vladivostok, Russia
[f]NeuroVita Clinic of Interventional and Restorative Neurology and Therapy, Moscow, Russia
[*]Corresponding author: e-mail address: igbryukhovetskiy@gmail.com

Contents

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Abstract

Glioblastoma multiforme (GBM) is the most common primary tumor of the human brain. It is characterized by invasive growth and strong resistance to treatment, and the median survival time of patients is 15 months. The invasive growth of this tumor type is associated with tumor cells with an aggressive phenotype, while its treatment resistance is attributed to cancer stem cells (CSCs). It remains unclear if CSCs have a more invasive nature than differentiated glioblastoma cells (DGCs), and what contribution CSCs make to the aggressive phenotype of GBM. Interaction with the extracellular matrix (ECM) is a key factor in the development of invasion. The aim of the present study was to compare the expression levels of signaling pathway proteins involved in interaction of receptors with the ECM in CSCs and DGCs. The U-87MG GBM cell line was used in the present study CSCs were extracted from gliomaspheres through magnetic-activated cell sorting based on the expression of cluster of differentiation 133 (CD133); CD133-negative DCGs were used as a control. HPLC and mass spectrometry were also used, and biological and molecular functions, signaling pathways and protein-protein interactions were analyzed using publicly available databases. Increased expression levels of the following 10 proteins involved in interaction with the ECM were identified in CSCs, compared with expression levels in DGCs: COL6A1, COL6A3, FN1, ITGA2, ITGA5, ITGAV, ITGB1, ITGB3, LAMB1 and LAMC1. The proteome of CSCs was observed to have >2-fold higher expression of these key proteins, when compared with the DGC proteome. Increased expression levels of four proteins (FERMT2, LOXL2, HDAC2 and FBN1) involved in activating signaling in response to receptor interaction with the ECM was also observed, indicating that CSCs may have highly invasive nature. LOXL2 expression level was >9-fold higher in CSCs compared to DGCs, suggesting that this protein may have potential as an marker for CSCs and as a target for this cell type in GBM.

1. Introduction

Glioblastoma multiforme (GBM) is one of the most aggressive types of human brain cancer. It is characterized by rapid invasive growth, significant brain infiltration and strong resistance to treatment. Despite advances in surgery, radical elimination of the tumor without causing severe and irreversible neurological damage to the patient remains a challenge (Costa, Lawson, Lelotte, et al., 2019). Therefore, the primary treatment type is high-dose radiation with multiple rounds of chemotherapy (Lukas, Wainwright, Ladomersky, et al., 2019). Prognosis for patients with this type of tumor is unfavorable, as the survival rate at 2 years following diagnosis is 27–43%, and the median survival time of patients with GBM is 15 months (Stupp & Ram, 2018). Due to their invasive ability, cancer cells that have

deeply penetrated the brain tissue from the original lesion inevitably cause relapses, requiring new approaches and treatment protocols for patients with this disease.

The ability to penetrate the brain matter (Perrin, Samuel, Koszyca, et al., 2019) is due to the mesenchymal-like aggressive phenotype of cancer cells, and is influenced by coordinated intercellular interactions with the local microenvironment (Roos, Ding, Loftus, et al., 2017). The key properties of this phenotype include switching from adhesive E-cadherins to migratory N-cadherins, extending the range of integrin receptors on the cellular surface, developing the ability to produce extracellular matrix (ECM) components, synthesizing matrix metalloproteases (Bryukhovetskiy & Shevchenko, 2016).

Focal adhesion proteins are critical to underpinning the invasive potential of cancer cells with an aggressive phenotype (Bolteus, Berens, & Pilkington, 2001). GBM cells penetrate the brain tissue, adhere to ECM proteins and create cellular–matrix connections with structural and signaling functions that interact with the microenvironment (Huttenlocher & Horwitz, 2011). These connections between the ECM and the actin cytoskeleton of the cells also generate tension that is required for migration beyond the primary lesion. Inhibition of the interaction between cancer cells and the ECM is one of the aims of GBM treatment.

GBM relapse and progression is also attributed to cancer stem cells (CSCs) that have unique signaling and morphological properties (Singh, Clarke, Terasaki, et al., 2003), including an ability to initiate and support rapid tumor growth. CSCs occupy the dominant position in the GBM cellular hierarchy, and are detected at the anterior border of the tumor (Gimple, Bhargava, Dixit, et al., 2019), indicating that they are involved in invasive processes. However, it remains unclear if CSCs are more invasive than differentiated tumor cells, and if they contribute to the development of GBM with an aggressive phenotype.

The CD133 antigen is the most reliable marker for CSCs (Singh, Hawkins, Clarke, et al., 2004). This marker is introduced into GBM tissue by the symmetrical division of CSCs and by reprogramming of differentiated CD133-negative (CD133−) tumor cells resulting in phenotypic plasticity (Li, Zhou, Xu, & Xiao, 2013) in the tumor cell population. The ability of to recruit normal neural CD133-positive (CD133+) stem cells further complicates our understanding of its heterogeneity (Okawa, Gagrica, & Blin, 2017). The aim of the present study was, therefore, to compare the expression profiles of proteins involved in the interaction with the ECM,

and signaling pathways in CD133+ CSCs and differentiated glioblastoma cells (DGCs). The present study may lead to the discovery of new molecular targets for regulating the invasive activity of CSCs.

2. Materials and methods

2.1 Human GBM cells

For the present study, the U-87MG GBM cell line was obtained from the American Type Culture Collection (cat no. HTB-14™). This cell line is not the original U-87 line established at the University of Uppsala, but derived from a human glioblastoma of unknown origin (Allen, Bjerke, Edlund, et al., 2016). However, as demonstrated in our previous study, the stimulation of GBM U-87MG cells with transforming growth factor (TGF)-β1 led to a significant increase in the expression levels of proteins associated with the epithelial-mesenchymal transition (Bryukhovetskiy & Shevchenko, 2016), which greatly increased the invasiveness of these cells. Comparative proteome mapping of CD133+ CSCs of U-87 GBM cell line (Bryukhovetskiy, Shevchenko, Kovalev, et al., 2014) and normal CD133+ neural and mesenchymal stem cells of human bone marrow was performed, and the expression of Wnt-signaling pathway proteins in CD133+ CSCs of this GBM cell line were investigated (Shevchenko, Arnotskaya, Korneyko, et al., 2019). The interactions between U-87 GBM cells and normal stem cells were also assessed (Milkina, Ponomarenko, Korneyko, et al., 2018). Due to these previous studies using this cell line, U-87MG GBM cells were used as the primary model for the present study.

The U-87MG differentiated glioblastoma cells (DGCs) were cultured in low glucose Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc.) with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) at 37 °C (5% CO_2). Adhesive cells were cultured until 80% confluent and passaged at a 1:3 ratio.

To obtain gliomaspheres, U-87MG cells were resuspended in serum-free DMEM with L-glutamine (2 mM), B-27 (0.5 mM/mL), basic fibroblast growth factor (20 ng/mL), epidermal growth factor (20 ng/mL), penicillin/streptomycin (100 units/mL) and heparin (5 μg/mL), and then cultured in six-well nonadhesive plastic plates. All chemicals were obtained from Gibco (Thermo Fisher Scientific, Inc.).

U-87MG CSCs were isolated using a CD133 MicroBead kit (cat no. 130-100-857; Miltenyi Biotec, Inc.) according to the manufacturer's protocol. Magnetic beads coated with antibodies against CD133 were used to

isolate CD133+ cells. The purity of CD133+ CSCs was evaluated via flow cytometry after staining with CD133/1-VioBright FITC antibody (cat no. 130-105-226; Miltenyi Biotec, Inc.), and the purity was found to be >90%.

2.2 Preparation of samples for mass spectrometry

Two samples of gliomasphere cells (CD133+ and CD133−) were lysed using the Mammalian Cell Lysis kit (Sigma-Aldrich; Merck KGaA) according to the manufacturer's protocol. Tumor cell lysates were ultra-filtered to eliminate low-molecular compounds. After performing tryptic cleavage of lysate samples, 4 μL peptide solution was analyzed with nano High-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS).

Tryptic peptides were divided into 24 fractions using a Dionex UltiMate 3000 HPLC system (Dionex; Thermo Fisher Scientific, Inc.) equipped with a fraction collector and cation-exchange column MIC-10-CP (Poros 10S; 1 mm × 10 cm; Thermo Fisher Scientific, Inc.). The obtained fractions were then concentrated at 30 °C using a centrifugal concentrator and diluted again with 100 μL formic acid (0.1%).

2.3 Mass spectrometry

Analysis of tryptic peptides was performed with a nano-HPLC Dionex Ultimate 3000. To process the mass spectrometry data, MaxQuant software (version 1.6.1.0; Max Planck Institute of Biochemistry) was used. The table of obtained proteins was processed using Perseus software (version 1.5.1.6; Max Planck Institute of Biochemistry).

Biological and molecular functions and protein signaling pathways were annotated using the following databases: PubMed (http://www.ncbi.nlm.nih.gov/pubmed/), PANTHER (http://www.pantherdb.org/), Gene Ontology (http://www.geneontology.org/), Swiss-Prot (www.uniprot.org/uniprot) and KEGG (http://www.genome.jp/kegg/). Protein-protein interactions (PPIs) were analyzed using the STRING database (version 10; https://string-db.org).

2.4 Statistical analysis

Analysis was performed using Statistica software (version 12; StatSoft). Significance was identified using Student's *t*-test. $P < 0.05$ was considered to indicate a statistically significant difference.

3. Results

3.1 CSCs have altered proteomes compared to DGCs

Proteome analysis identified 1990 unique proteins. A total of 1891 proteins were identified in the CSC sample, and 1748 proteins were found in the sample of differentiated GBM cells (DGCs). Identified proteins showed a high percentage of overlap between the two cell populations: 1649 proteins were present in all cell lysates; 242 proteins were found only in CSCs; and 99 proteins were observed only in DGCs. Among the discovered proteins, 589 had significantly different expression levels in CSCs ($P < 0.05$) compared to DGCs; the expression levels of 358 proteins were higher, and 231 were lower. The majority of these proteins were localized intracellularly (Fig. 1A), associated with metabolic and cellular processes (Fig. 1B),

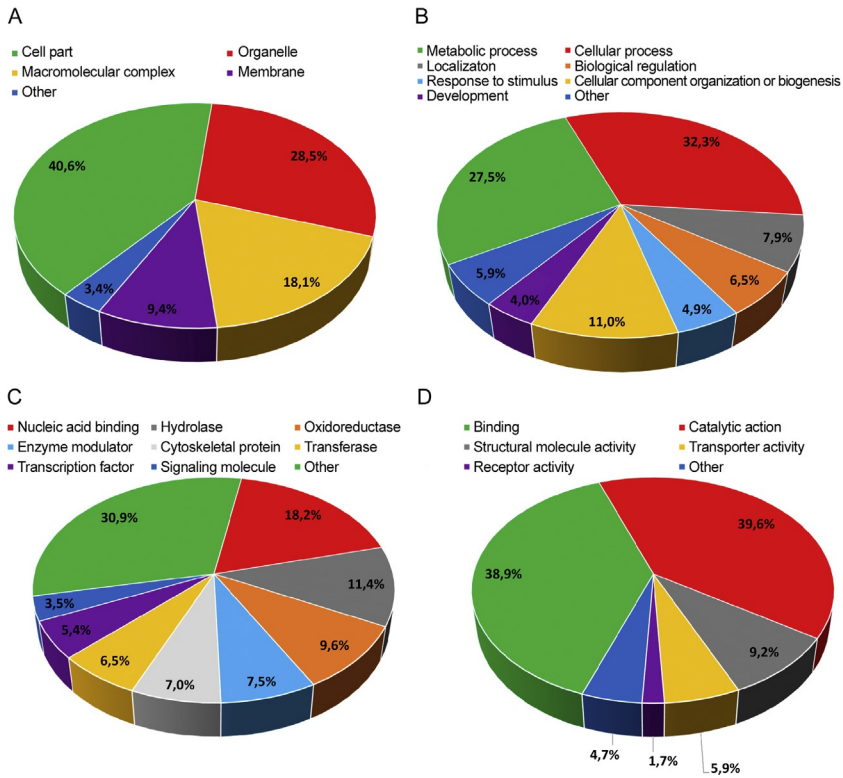

Fig. 1 (A–D) Molecular-biological description of proteins in cancer glioblastoma CD133 + stem cells.

functionally heterogeneous (Fig. 1C) and associated with a class of compounds that exhibit active fermentation properties (Fig. 1D).

3.2 Expression of signaling proteins is increased in CSCs

Bioinformatics analysis identified 14 proteins involved in signaling casades associated with the interaction between CSCs and the ECM (Table 1). Of these, 10 proteins possessed expression levels twofold higher in CSCs compared with that in DGCs. These proteins were collagen VI α1 chain (COL6A1), collagen VI α3 chain (COL6A3), fibronectin (FN1), integrin α2 (ITGA2), integrin α5 (ITGA5), integrin αV (ITGAV), integrin β1 (ITGB1), integrin β3 (ITGB3), laminin β1 (LAMB1) and laminin γ1 (LAMC1). Significantly increased expression levels of fermitin family homolog 2 (FERMT2), lysyl oxidase homolog 2 (LOXL2), histone deacetylase 2 (HDAC2) and fibrillin 1 (FBN1) proteins (Table 2) were also observed in CSCs compared to DGCs. These proteins directly activate signaling following interaction between cells and the ECM (Fig. 2).

Table 1 Determinants of focal adhesion with significantly different expressioin in CSCs and DGCs.

ID	Gene name	*P*	CD133+/CD133−
CD44	CD44 antigen	0.046	2.54
HMMR	Hyaluronan-mediated motility receptor	0.006	2.28
COL1A1	Collagen type 1 α1	0.043	2.80
COL1A2	Collagen type 1	0.001	2.27
COL6A1	Collagen type VI α 1 chain	0.001	8.46
COL6A3	Collagen type VI α 3 chain	0.050	2.31
FN1	Fibronectin 1	0.005	2.26
LAMB1	Laminin subunit β 1	0.001	8.84
LAMC1	Laminin subunit γ 1	0.017	2.77
ITGA2	Integrin subunit α 2	0.042	2.90
ITGA5	Integrin subunit α 5	0.008	3.62
ITGB1	Integrin subunit β 1	0.001	4.50
ITGB3	Integrin subunit β3	0.020	2.24
ITGAV	Integrin α-V	0.006	5.07

Table 2 Proteins, activating signaling of preceptor interaction between cells and extracellular matrix.

ID	Gene name	*P*	CD133+/CD133−
FERMT2	Fermitin family homolog 2	0.042	3.56
LOXL2	Lysyl oxidase homolog 2	0.008	9.02
HDAC2	Histone deacetylase 2	0.001	1.7
FBN1	Fibrillin 1	0.020	6.21

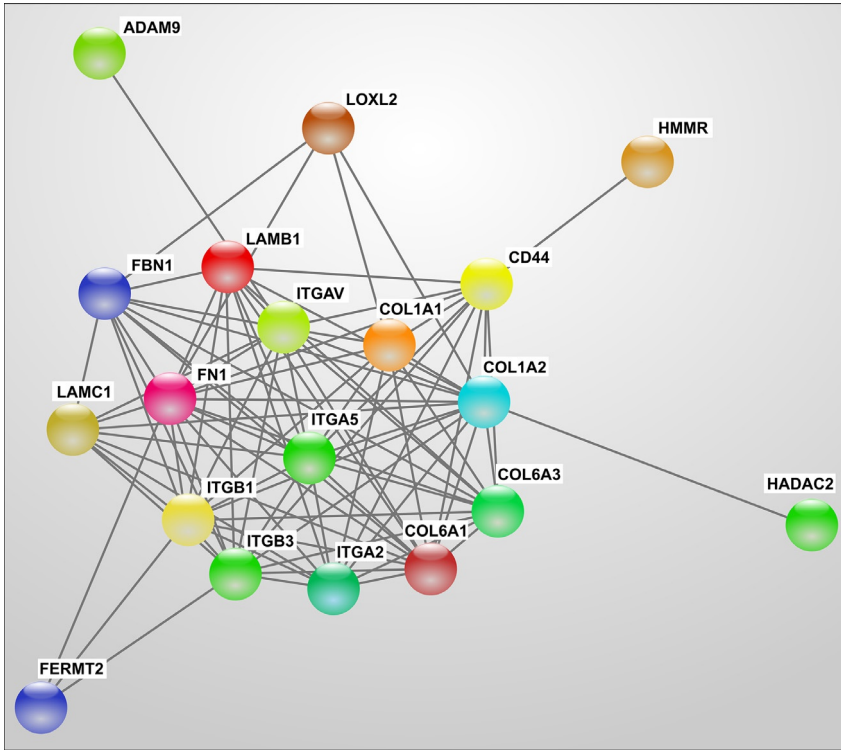

Fig. 2 The interaction between the identified proteins according to the international database of protein-protein interactions STRING v10.

4. Discussion

The present study investigated proteins that are upregulated in CSCs, and that serve a crucial role in their interaction with the ECM. Activation of hyaluronic acid receptors, CD44 (Table 1) and hyaluronan-mediated

motility receptor (HMMR) in CSCs has been found to be a key factor for invasion (Mooney, Choy, Sidhu, et al., 2016). CD44 glycoprotein is described as a CSC marker in various cancer types, including GBM, and it is frequently used as an indicator of tumor cells with aggressive phenotype (Wang, Zheng, Guan, et al., 2018). Despite changes in the expression levels of these proteins, it is unclear whether these $CD133^{+}$ cells can be considered to be CSCs.

After being implanted into experimental animals, CD44+ GBM cells quickly initiate invasive processes in the brain, but due to a low proliferation rate, tumors grow slowly (Brown, Daniel, D'Abaco, et al., 2015). CD44 is a typical marker for DGCs. However, CD133+ CSCs that also express CD44 demonstrate a very high level of invasive activity *in vitro* and *in vivo* (Brown, Filiz, Daniel, et al., 2017). When implanted into the brain, CD44-expressing CD133+ CSCs create large infiltrating tumors with edema, which result in the dislocation of cerebral structures. The findings of the present study revealed that CD44 protein content in CD133+ CSCs was 2.54-fold higher compared with that in DGCs, indicating that these CD133+ cells may have extremely high oncogenic potential.

HMMR is an oncogene that serves an important role in solid tumor progression (Hartheimer, Park, Rao, & Kim, 2019). It is highly upregulated in human glioma tissues, particularly in GBM. HMMR protein creates a complex with CD44, and after binding with hyaluronic acid, activates intracellular signaling pathways (Tilghman, Wu, Sang, et al., 2014) regulating the proliferation and invasion of CSCs. Inhibiting HMMR increases the survival rates of experimental animals (Tilghman et al., 2014). The results of the present study revealed that HMMR expression in CSCs was 2.28-fold higher compared with that in DGCs, providing evidence that these cells have a highly invasive nature.

An essential factor in invasion is the adhesion of GBM cells to the basal membrane (Bauer, Ratzinger, Wales, et al., 2011), which is influenced by the production of collagen and other ECM components. The present study demonstrated >2-fold higher expression levels of COL1A1, COL1A2, COL6A1 and COL6A3 collagens in CSCs compared with those in DGCs. The importance of collagens in the development of malignant tumors is well recognized (Bryukhovetskiy & Shevchenko, 2016). A notable finding of the current study was an 8-fold increase in the expression levels of COL6A1, which has previously been associated with treatment resistance (Turtoi, Blomme, Bianchi, et al., 2014) and poor prognosis of GBM. FN1 is a key ECM component and increased expression levels of FN1

(Serres, Debarbieux, Stanchi, et al., 2014) have been identified in the majority of GBM patients. Upregulated FN1 (Yu, Xue, Liu, et al., 2018) modulates adhesion, proliferation, differentiation and chemoresistance of CSCs. The results of the present study revealed that expression of this protein in CSCs was 2.26-fold higher than that in DGCs. Laminins belong to a family of large adhesive glycoproteins that are crucial components of basal membranes (Arnaout, Goodman, & Xiong, 2007). Laminins are localized around blood vessels and serve an important role in tumor invasion, cell differentiation and wound healing (Humphries, Byron, & Humphries, 2006). The current study identified that the expression of LAMB1 and LAMC1 was 8.84 and 2.77-fold higher, respectively, in CD133+ cancer stem cells of GBM compared with that in DGCs.

Integrins are heterodimeric transmembrane molecules involved in cell adhesion that interact with the ECM, regulate the form and mobility of tumor cells, and activate proliferation, migration, invasion, angiogenesis and survival (Arnaout et al., 2007; Humphries et al., 2006). Cancer cells have increased expression of integrin receptors, located all on the cell membrane, and they act as receptors for many ECM components (Danen, 2005), including collagens, laminins, vitronectins, fibronectins and other ligands. The expression of integrin receptors in tumor tissue is correlated with the invasive ability of the cells (Madamanchi, Santoro, & Zutter, 2014). Integrins increase the expression of transforming growth factor β (Roth, Silginer, Goodman, et al., 2013), and are involved in regulating the biological effects of this a key mediator in GBM cells with an aggressive phenotype.

ITGA2 is involved in the invasive progression of tumors, including melanoma (Adorno-Cruz & Liu, 2018). ITGA2 contributes to the survival and invasion of prostate cancer cells (Ojalill, Parikainen, Rappu, et al., 2018) and is a marker of trophoblast progenitor cells (Lee, Turco, Gardner, et al., 2018). The current study revealed that CSCs had 2.9-fold higher expression of ITGA2 compared with that in DGCs. ITGA5 is a marker of unfavorable prognosis in cases of esophageal cancer (Xie, Guo, Wu, et al., 2016) and colorectal adenocarcinoma (Starchenko, Graves-Deal, Yang, et al., 2017), positively regulates cell stemness in triple-negative breast cancer (Xiao et al., 2018), and supports the formation of glio-maspheres *in vitro* (Blandin, Noulet, Renner, et al., 2016) and the migration of GBM cells. The results of the current study indicated that CSCs had 3.62-fold increased expression of ITGA5 compared with DGCs. ITGAV is upregulated in primary brain tumor cells and metastases of melanoma, lung, renal and breast cancer (Vogetseder, Thies, Ingold, et al., 2013). ITGAV

regulates the biological effects of TGFβ (Silginer, Burghardt, Gramatzki, et al., 2016) through an aryl hydrocarbon receptor-dependent mechanism and the SMAD signaling pathway, and induces differentiation of CSCs when interacting with ECM components (Silginer et al., 2016). The current study found 5.07-fold higher expression of ITGAV in CSCs compared with that in DGCs. Expression levels of ITGB1 and ITGB3 were also increased in CSCs, 4.52 and 2.24-fold, respectively. These proteins play an important part in the positioning of progenitor-like phenotype cells, and they promote the creation of chains of integrins, leading to migration of tneuroblasts from the subventricular zone of the brain to the olfactory bulb (Niibori-Nambu, Midorikawa, Mizuguchi, et al., 2013). Due to these alterations in protein expression, CSCs may be well equipped to create cell-matrix adhesion complexes that regulate intracellular processes and interact with the microenvironment (Arnaout et al., 2007). CSCs may bind ECM components to the actin cytoskeleton of the cells, generating tension, which is necessary for the migration and invasion of the cells (Kawamura, Hamilton, Miskiewicz, et al., 2018; Niibori-Nambu et al., 2013; Silginer et al., 2016).

When analyzing the proteomic changes in CSCs, proteins that are involved in the regulation of receptor-matrix interaction were identified (Table 2). FERMT2 is an important regulator of integrin activity in cell-cell and cell-ECM interactions that modulates integrin-mediated (Kawamura et al., 2018) adhesion and invasion of trophoblasts into the uterine wall, and also participates in carcinogenesis and tumor progression. The results of the present study revealed that CSCs had 3.56-fold increased expression of FERMT2 compared with DGCs.

LOXL2 is a secreted copper-dependent amino oxidase of the LOX family; its substrates are collagen and elastin (Zhang, Huang, You, et al., 2019). Increased expression of LOXL2 in cells of different cancer types correlates with reduced survival rates in patients, and is associated with invasive and metastatic activity of breast cancer (Yang, Geng, Wang, et al., 2019), hepatocellular adenocarcinoma (Shao, Zhao, Liu, et al., 2019), prostate cancer cells (Xie, Yu, Wang, et al., 2019) and some other aggressive tumors. The current analysis identified a 9.02-fold increase in LOXL2 expression in CSCs compared with that in DGCs. LOXL2 may therefore be a notable marker of CSCs and a promising therapeutic target. LOXL2 expression level is positively regulated by hypoxia-inducible factors (Schietke, Warnecke, Wacker, et al., 2010) and increased expression is frequently observed in invasive tumors of the breast, head and neck. Therefore, the large increase in LOXL2 expression observed in CSCs in the present study indicates that

this protein may serve a role in neoplastic processes in this cell type. LOXL2 changes histone structure and, thus, modifies the shape of cells, facilitating metastasis development and invasion (Schietke et al., 2010; Shao et al., 2019; Xie et al., 2019). LOXL2 expression level correlates with HDAC2 expression (Du & Zhu, 2018), indicating that these proteins may play a synergistic oncogenic role. In the present study, expression of HDAC2 was 1.7-fold higher in CSCs compared with that in DGCs.

FBN1 is a protein of the fibrillin family that is involved in supporting the pluripotency of normal embryonal stem cells and CSCs (Cierna, Mego, Jurisica, et al., 2016), and enabling the biological effects of TGFβ (Lerner-Ellis, Aldubayan, Hernandez, et al., 2014). The expression level of FBN1 in CSCs was found to be 6.21-fold higher compared with that in DGCs.

The current study presented the comparative mapping and bioinformatics analysis of protein expression in CD133+ CSCs and CD133− negative DGCs, and identified proteins involved in the interaction of cells with the ECM. The upregulation of 10 proteins involved in signaling pathways was revealed in CSCs. CSCs were also found to have increased expression levels of four proteins involved in the activation of signaling due to the interaction of receptors with the ECM. The expression of LOXL2 was >9-fold higher in CSCs compared with DGCs, suggesting that CSCs may have a highly invasive nature, and that this protein may by an important CSC marker and a promising target for eliminating these cells in GBM.

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Availability of data and materials

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

Ethics approval and consent to participate

Ethics approval and consent to participate.

Informed consent was obtained from all individual participants included in the study and all procedures performed in studies involving human participants were in accordance with the ethical standards of the Far Eastern Federal University and University of Uppsala.

Authors' contributions

V.S. and N.A. prepared and analyzed the samples, as well as performed the cell lysis, chromatography and mass spectrometry, and contributed to the bioinformatics analysis. S.A. provided and performed the statistical analysis and was responsible for the mathematical

process of the results. Y.K. and H.S. discussed, analyzed and interpreted the results of the study, and also worked on the manuscript. I.B. wrote the manuscript, proposed the study idea, designed the study, offered support with the experiments, organized the scientific team, cultured the cancer cells and isolated the cancer CD133+ stem cells of glioblastoma for the experiment, provided scientific guidance and contributed to the bioinformatics analysis. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Informed consent for publication was obtained from all individual participants included in the study.

Conflict of interest

The authors declare there are no conflicts of interests.

References

Adorno-Cruz, V., & Liu, H. (2018). Regulation and functions of integrin α2 in cell adhesion and disease. *Genes & Diseases*, *6*(1), 16–24.

Allen, M., Bjerke, M., Edlund, H., et al. (2016). Origin of the U87MG glioma cell line: Good news and bad news. *Science Translational Medicine*, *8*, 354re3.

Arnaout, M. A., Goodman, S. L., & Xiong, J. P. (2007). Structure and mechanics of integrin-based cell adhesion. *Current Opinion in Cell Biology*, *19*, 495–507.

Bauer, R., Ratzinger, S., Wales, L., et al. (2011). Inhibition of collagen XVI expression reduces glioma cell invasiveness. *Cellular Physiology and Biochemistry*, *27*(3–4), 217–226.

Blandin, A. F., Noulet, F., Renner, G., et al. (2016). Glioma cell dispersion is driven by α5 integrin-mediated cell-matrix and cell-cell interactions. *Cancer Letters*, *376*(2), 328–338.

Bolteus, A. J., Berens, M. E., & Pilkington, G. J. (2001). Migration and invasion in brain neoplasms. *Current Neurology and Neuroscience Reports*, *1*(3), 225–232.

Brown, D. V., Daniel, P. M., D'Abaco, G. M., et al. (2015). Coexpression analysis of CD133 and CD44 identifies proneural and mesenchymal subtypes of glioblastoma multiforme. *Oncotarget*, *6*(8), 6267–6280.

Brown, D. V., Filiz, G., Daniel, P. M., et al. (2017). Expression of CD133 and CD44 in glioblastoma stem cells correlates with cell proliferation, phenotype stability and intra-tumor heterogeneity. *PLoS One*, *12*(2), e0172791.

Bryukhovetskiy, I., & Shevchenko, V. (2016). Molecular mechanisms of the effect of TGF-β 1 on U87 human glioblastoma cells. *Oncology Letters*, *12*(2), 1581–1590.

Bryukhovetskiy, A., Shevchenko, V., Kovalev, S., et al. (2014). To the novel paradigm of proteome-based cell therapy of tumors: Through comparative proteome mapping of tumor stem cells and tissue-specific stem cells of humans. *Cell Transplantation*, *23*(Suppl. 1), S151–S170.

Cierna, Z., Mego, M., Jurisica, I., et al. (2016). Fibrillin-1 (FBN-1) a new marker of germ cell neoplasia in situ. *BMC Cancer*, *16*, 597.

Costa, E., Lawson, T. M., Lelotte, J., et al. (2019). Long-term survival after glioblastoma resection: Hope despite poor prognosis factors. *Journal of Neurosurgical Sciences*, *63*(3), 251–257.

Danen, E. H. (2005). Integrins: Regulators of tissue function and cancer progression. *Current Pharmaceutical Design*, *11*, 881–891.

Du, X. G., & Zhu, M. J. (2018). Clinical relevance of lysyl oxidase-like 2 and functional mechanisms in glioma. *Oncotargets and Therapy*, *11*, 2699–2708.

Gimple, R. C., Bhargava, S., Dixit, D., et al. (2019). Glioblastoma stem cells: Lessons from the tumor hierarchy in a lethal cancer. *Genes & Development*, *33*(11 – 12), 591–609.

Hartheimer, J. S., Park, S., Rao, S. S., & Kim, Y. (2019). Targeting hyaluronan interactions for glioblastoma stem cell therapy. *Cancer Microenvironment*, *12*(1), 47–56.

Humphries, J. D., Byron, A., & Humphries, M. J. (2006). Integrin ligands at a glance. *Journal of Cell Science*, *119*, 3901–3903.
Huttenlocher, A., & Horwitz, A. R. (2011). Integrins in cell migration. *Cold Spring Harbor Perspectives in Biology*, *3* a005074.
Kawamura, E., Hamilton, G. B., Miskiewicz, E. I., et al. (2018). Fermitin family homolog-2 (FERMT2) is highly expressed in human placental villi and modulates trophoblast invasion. *BMC Developmental Biology*, *18*(1), 19.
Lee, C. Q. E., Turco, M. Y., Gardner, L., et al. (2018). Integrin α2 marks a niche of trophoblast progenitor cells in first trimester human placenta. *Development*, *145*(16). pii: dev162305.
Lerner-Ellis, J. P., Aldubayan, S. H., Hernandez, A. L., et al. (2014). The spectrum of FBN1, TGFβR1, TGFβR2 and ACTA2 variants in 594 individuals with suspected Marfan syndrome, Loeys-Dietz syndrome or thoracic aortic aneurysms and dissections (TAAD). *Molecular Genetics and Metabolism*, *112*(2), 171–176.
Li, P., Zhou, C., Xu, L., & Xiao, H. (2013). Hypoxia enhances stemness of cancer stem cells in glioblastoma: An in vitro study. *International Journal of Medical Sciences*, *10*, 399–407.
Lukas, R. V., Wainwright, D. A., Ladomersky, E., et al. (2019). Newly diagnosed glioblastoma: A review on clinical management. *Oncology (Williston Park, NY)*, *33*(3), 91–100.
Madamanchi, A., Santoro, S. A., & Zutter, M. M. (2014). Alpha2beta1 integrin. *Advances in Experimental Medicine and Biology*, *819*, 41–60.
Milkina, E., Ponomarenko, A., Korneyko, M., et al. (2018). Interaction of hematopoietic CD34+ CD45+ stem cells and cancer cells stimulated by TGF-β1 in a model of glioblastoma in vitro. *Oncology Reports*, *40*(5), 2595–2607.
Mooney, K. L., Choy, W., Sidhu, S., et al. (2016). The role of CD44 in glioblastoma multiforme. *Journal of Clinical Neuroscience*, *34*, 1–5.
Niibori-Nambu, A., Midorikawa, U., Mizuguchi, S., et al. (2013). Glioma initiating cells form a differentiation niche via the induction of extracellular matrices and integrin αV. *PLoS One*, *8*(5), e59558.
Ojalill, M., Parikainen, M., Rappu, P., et al. (2018). Integrin α2β1 decelerates proliferation, but promotes survival and invasion of prostate cancer cells. *Oncotarget*, *9*(65), 32435–32447.
Okawa, S., Gagrica, S., & Blin, C. (2017). Proteome and secretome characterization of glioblastoma-derived neural stem cells. *Stem Cells*, *35*(4), 967–980.
Perrin, S. L., Samuel, M. S., Koszyca, B., et al. (2019). Glioblastoma heterogeneity and the tumor microenvironment: Implications for preclinical research and development of new treatments. *Biochemical Society Transactions*, *47*(2), 625–638.
Roos, A., Ding, Z., Loftus, J. C., et al. (2017). Molecular and microenvironmental determinants of glioma stem-like cell survival and invasion. *Frontiers in Oncology*, *7*, 120.
Roth, P., Silginer, M., Goodman, S. L., et al. (2013). Integrin control of the transforming growth factor-β pathway in glioblastoma. *Brain*, *136*(Pt. 2), 564–576.
Schietke, R., Warnecke, C., Wacker, I., et al. (2010). The lysyl oxidases LOX and LOXL2 are necessary and sufficient to repress E-cadherin in hypoxia: Insights into cellular transformation processes mediated by HIF-1. *The Journal of Biological Chemistry*, *285*(9), 6658–6669.
Serres, E., Debarbieux, F., Stanchi, F., et al. (2014). Fibronectin expression in glioblastomas promotes cell cohesion, collective invasion of basement membrane in vitro and orthotopic tumor growth in mice. *Oncogene*, *33*(26), 3451–3462.
Shao, B., Zhao, X., Liu, T., et al. (2019). LOXL2 promotes vasculogenic mimicry and tumour aggressiveness in hepatocellular carcinoma. *Journal of Cellular and Molecular Medicine*, *23*(2), 1363–1374.
Shevchenko, V., Arnotskaya, N., Korneyko, M., et al. (2019). Proteins of the Wnt signaling pathway as targets for the regulation of CD133+ cancer stem cells in glioblastoma. *Oncology Reports*, *41*(5), 3080–3088.

Silginer, M., Burghardt, I., Gramatzki, D., et al. (2016). The aryl hydrocarbon receptor links integrin signaling to the TGF-β pathway. *Oncogene*, *35*(25), 3260–3271.
Singh, S. K., Clarke, I. D., Terasaki, M., et al. (2003). Identification of a cancer stem cell in human brain tumors. *Cancer Research*, *63*(18), 5821–5828.
Singh, S. K., Hawkins, C., Clarke, I. D., et al. (2004). Identification of human brain tumor initiating cells. *Nature*, *432*(7015), 396–401.
Starchenko, A., Graves-Deal, R., Yang, Y. P., et al. (2017). Clustering of integrin α5 at the lateral membrane restores epithelial polarity in invasive colorectal cancer cells. *Molecular Biology of the Cell*, *28*(10), 1288–1300.
Stupp, R., & Ram, Z. (2018). Quality of life in patients with glioblastoma treated with tumor-treating fields-reply. *Journal of the American Medical Association*, *319*(17), 1823.
Tilghman, J., Wu, H., Sang, Y., et al. (2014). HMMR maintains the stemness and tumorigenicity of glioblastoma stem-like cells. *Cancer Research*, *74*(11), 3168–3179.
Turtoi, A., Blomme, A., Bianchi, E., et al. (2014). Accessibilome of human glioblastoma: Collagen-VI-alpha-1 is a new target and a marker of poor outcome. *Journal of Proteome Research*, *13*(12), 5660–5669.
Vogetseder, A., Thies, S., Ingold, B., et al. (2013). αv-integrin isoform expression in primary human tumors and brain metastases. *International Journal of Cancer*, *133*(10), 2362–2371.
Wang, F., Zheng, Z., Guan, J., et al. (2018). Identification of a panel of genes as a prognostic biomarker for glioblastoma. *eBioMedicine*, *37*, 68–77.
Xiao, Y., Li, Y., Tao, H., et al. (2018). Integrin α5 down-regulation by miR-205 suppresses triple negative breast cancer stemness and metastasis by inhibiting the Src/Vav2/Rac1 pathway. *Cancer Letters*, *433*, 199–209.
Xie, J. J., Guo, J. C., Wu, Z. Y., et al. (2016). Integrin alpha5 promotes tumor progression and is an independent unfavorable prognostic factor in esophageal squamous cell carcinoma. *Human Pathology*, *48*, 69–75.
Xie, P., Yu, H., Wang, F., et al. (2019). Inhibition of LOXL2 enhances the radiosensitivity of castration-resistant prostate cancer cells associated with the reversal of the EMT process. *BioMed Research International*, *2019*, 4012590.
Yang, H., Geng, Y. H., Wang, P., et al. (2019). Extracellular ATP promotes breast cancer invasion and epithelial-mesenchymal transition via hypoxia-inducible factor 2α signaling. *Cancer Science*, *110*(8), 2456–2470. https://doi.org/10.1111/cas.14086.
Yu, Q., Xue, Y., Liu, J., et al. (2018). Fibronectin promotes the malignancy of glioma stem-like cells via modulation of cell adhesion, differentiation, proliferation and chemoresistance. *Frontiers in Molecular Neuroscience*, *11*, 130.
Zhang, X., Huang, J., You, F., et al. (2019). Prognostic and clinicopathological significance of LOXL2 in cancers: A systematic review and meta-analysis. *Journal of Cellular Physiology*, *234*(11), 21369–21379. https://doi.org/10.1002/jcp.28746.