CHAPTER TEN

Transforming growth factor-β mimics the key proteome properties of CD133^{-} differentiated and CD133^{+} cancer stem cells in glioblastoma

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Abstract

Glioblastoma multiforme is the most aggressive type of primary brain tumor in humans. Its invasive growth is associated with cluster of differentiation (CD)133 cancer stem cells (CSCs) and CD133$^-$ differentiated glioblastoma cells (DGCs) with aggressive phenotype, which are developed under the influence of transforming growth factor (TGF)-β. The present study aimed to compare the proteomes of CD133 CSCs and CD133$^-$ DGCs stimulated by TGF-β, as well as the expression levels of the main proteins responsible for activating the signaling pathway of receptor interactions with the extracellular matrix (ECM). The U87MG GBM cell line was used in this study. CSCs were extracted from gliomaspheres through magnetic-activated cell sorting based on the expression of CD133 (CD133); CD133$^-$ DCGs served as a control. CD133$^-$ DGCs of the U87-MG cell line were treated with 10 ng/mL TGF-β1, and cell proliferation and migration were analyzed via real-time quantitative microscopy. High-performance liquid chromatography mass spectrometry was used for proteome analysis. The results revealed 589 proteins with significantly changes in expression among CD133 CSCs compared with those in CD133$^-$ DGCs ($P < 0.05$). Bioinformatics analysis allowed to attribute 134 differentially expressed proteins to 15 signaling pathways; among these proteins, 14 were involved in signaling cascades associated with the interaction between CSCs and the ECM, and were upregulated >twofold, while four proteins activated this signaling cascade. TGF-β-stimulation increased the mobility, suppressed the proliferation and transformed the proteome profile of CD133$^-$ DGCs. Were identified 13 key proteins that activate the signaling pathway of receptor interaction with the ECM and three proteins activating this signaling pathway in CD133$^-$ DGCs which had the same values as those of CD133 CSCs. In conclusion, TGF-β increased the expression of proteins that activate the signaling pathway of receptor interaction with the ECM in CD133$^-$ DGCs to the level of those in CD133 CSCs.

1. Introduction

Glioblastoma multiforme (GBM) is the most aggressive type of primary brain tumor affecting glial cells in humans. Its characteristics include invasive growth, brain infiltration with cancer cells and unfavorable

prognosis (Costa, Lawson, Lelotte, et al., 2019). Following the modern *Stupp* protocol of complex treatment (Stupp, Brada, van den Bent, Tonn, & Pentheroudakis, 2014) provides a median survival time of 15 months, and only 27% of patients survive 2 years after being diagnosed (Lukas, Wainwright, Ladomersky, et al., 2019), unfortunately only about 5–10% of patients with GBM survive for 5 years (Perrin, Samuel, Koszyca, et al., 2019), while the 10-year survival rates are almost nonexistent. Treatment resistance (Roos, Ding, Loftus, et al., 2017) is associated with the presence of cancer stem cells (CSCs) among the GBM cell population.

CSCs dominate the hierarchy of cancer cells (Singh, Clarke, Terasaki, et al., 2003), and are characterized by their multipotency, high proliferative activity (Gimple, Bhargava, Dixit, et al., 2019) and markedly invasive nature. Only 100 GBM cells immunopositive for the main CSCs marker, that is the CD133 antigen (Singh, Hawkins, Clarke, et al., 2004), are sufficient for a rapid growth of invasive tumors in experimental animals. Nevertheless, multiple data (Colwell, Larion, Giles, et al., 2017; Touat, Idbaih, Sanson, et al., 2017) indicate that cancer cells are able to penetrate the tissue barriers only with enough differentiated glioblastoma cells (DGCs) of a specific phenotype that is developed as a result of complex intercellular interactions with certain cytokines, among which, transforming growth factor (TGF)-β plays a key role (Syed, 2016).

Under physiological conditions, this cytokine suppresses the proliferation and triggers the apoptosis of pathologically modified cells (Xie, Ling, van Dam, et al., 2018). Mutations of components of the TGF-β signaling pathway make tumor growth processes unmanageable (Frei, Gramatzki, Tritschler, et al., 2015), leading to unlimited proliferation of tumor cells and increased speed of synthesis of this cytokine due to "autocrine induction" (Batlle & Massague, 2019). Accumulating in the tumor, TGF-β inhibits immune cells, reduces local inflammation, promotes remodeling of the extracellular matrix (ECM) and induces the aggressive phenotype of GBM cells (Katsuno, Lamouille, & Derynck, 2013).

Intensification of the interaction between cancer cells and the ECM, and destruction of its components are crucial factors for invasive growth (Ramamoorthi & Sivalingam, 2014). Tumor cells adhere to proteins of the ECM, thus creating cellular-matrix connections, and the actin cytoskeleton of the cells also generates the tension that is required for migration beyond the primary lesion (Nalluri, O'Connor, & Gomez, 2015). Upregulation of the intracellular mechanisms involved in the interaction

with the ECM is a key parameter of the proteome of $CD133^+$ CSCs (Bryukhovetskiy, Shevchenko, Kovalev, et al., 2014). However, the contribution of CSCs to the development of the aggressive phenotype of DGCs in GBM remains unclear, and the function of TGF-β in the interaction between DGCs and CSCs has not been defined, which impedes the development of novel approaches for GBM treatment.

The present study aimed to compare the proteomes of $CD133^+$ CSCs and $CD133^-$ DGCs pre-treated with TGF-β in terms of the expression of the main proteins involved in the activation of the signaling pathway of receptor interaction with the ECM.

2. Materials and methods

2.1 CSCs of GBM

The U-87MG GBM cell line was obtained from the American Type Culture Collection (ATCC; cat no. HTB-14™). This cell line is not the original U-87 line established at the University of Uppsala, but derives from a human GBM of unknown origin (Allen, Bjerke, Edlund, et al., 2016). This fact significantly increases the value of the experiment, since the wild type of glioblastoma accounts for more than 90% of cases of this tumor. As demonstrated in our previous study, the stimulation of GBM U-87MG cells with 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 the U-87 GBM cell line (Bryukhovetskiy 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 was studied (Shevchenko, Arnotskaya, Korneyko, et al., 2019). The interactions between U-87 GBM cells and normal stem cells were also assessed in previous studies (Milkina, Ponomarenko, Korneyko, et al., 2018). Thus, U-87MG GBM cells were used as the primary cellular model in the present study. The cell line was tested for mycoplasma contamination with the Universal Mycoplasma Detection Kit (ATCC® 30-1012K™).

To obtain gliomaspheres, U-87MG cells were resuspended in serum-free DMEM (Gibco; Thermo Fisher Scientific, Inc.) with L-glutamine (2 mM), B-27 (0.5 mM), basic fibroblast growth factor (20 ng/mL), epidermal growth factor (20 ng/mL), penicillin/streptomycin (100 U/mL) and heparin (5 μg/mL), and then cultured in 6-well non-adhesive 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.).

2.2 DGCs

U-87MG GBM cell were cultured in low-glucose DMEM (Gibco; Thermo Fisher Scientific, Inc.) with 10% fetal bovine serum (FBS; 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. Cells were used in experiments after the third passage since the moment of being obtained from the manufacturer.

2.3 Stimulation of cancer cells with TGF-β1

A total of 1×10^6 U-87MG GBM cell were cultured for 76 h in 6-well plates with DMEM (containing 10% FBS), Antibiotic-Antimitotic 100 × (cat. no. 15240062, Gibco, Thermo Fisher Scientific, US) with 10 ng/mL TGF-1β (cat. no. T7039; Sigma-Aldrich; Merck KGaA) at 37 °C (5% CO_2).

2.4 Scratch wound assay

After U-87MG cells adhered to the surface of the plate the initial density were 8×10^4 cm^2, a scratch was made with a micropipette tip. All cells were cultured in standard conditions in DMEM (Gibco; Thermo Fisher Scientific, Inc.) with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) at 37 °C (5% CO_2). Cell migration toward the scratch was monitored in real time with a high-performance quantitative microscopy system (Cell-IQ; CM Technologies) for 48 h Adobe Photoshop CC 2018 (19.0) (Adobe Systems, Inc.) was used to measure the scratch. The scratch healing rate represented the cell migration.

2.5 Preparation of samples for mass spectrometry (MS)

U-87MG cells pre-treated with TGF-β1, CSCs and control DGCs of the U-87MG cell line were lysed using the Mammalian Cell Lysis Kit (Sigma-Aldrich; Merck KGaA) according to the manufacturer's protocol. Tumor cell lysates were ultrafiltered 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 MS (HPLC-MS/MS). Tryptic peptides were divided into 24 fractions using a Dionex UltiMate 3000 HPLC System (Thermo Fisher Scientific, Inc.), equipped with a fraction collector and a 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 with 100 μL formic acid (0.1%).

2.6 MS data analysis

To process the MS 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 Kyoto Encyclopedia of Genes and Genomes (http://www.genome.jp/kegg/). Protein-protein interactions were analyzed using the STRING database (version 10; https://string-db.org).

2.7 Statistical analysis

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

3. Results

3.1 CD133$^+$ CSCs have altered proteomes compared with those of CD133$^-$ DGCs

Proteome analysis identified 1990 unique proteins. A total of 1891 proteins were identified in CSCs, while 1748 proteins were detected in DGCs. The 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 identified proteins, 589 had significantly different expression levels in CSCs ($P < 0.05$) compared with those in DGCs, and the expression

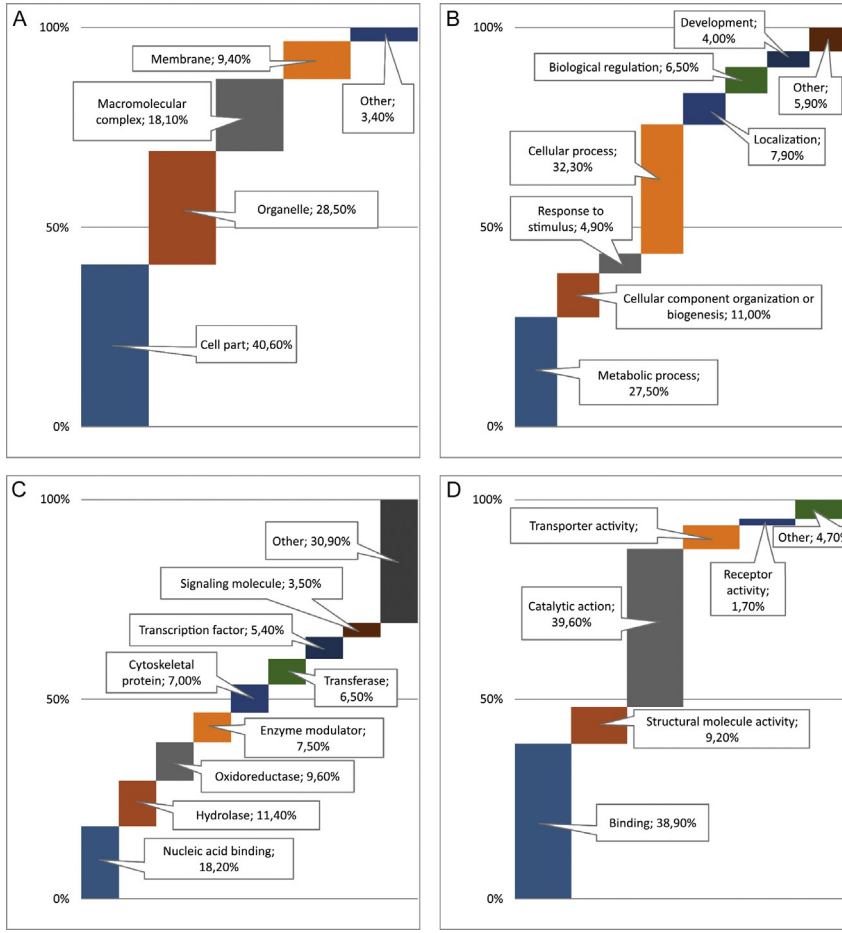

Fig. 1 (A–D) Molecular biological characteristics of the proteins of cancer CD133 + stem cell line U-87MG of human glioblastoma.

levels of 358 proteins were higher while those of 231 were lower. The majority of these proteins were localized intracellularly (Fig. 1A), associated with metabolic and cellular processes (Fig. 1B), functionally heterogeneous (Fig. 1C) and associated with a class of compounds that exhibit active fermentation properties (Fig. 1D).

3.2 The expression of signaling proteins is increased in CSCs

Bioinformatics analysis attributed 134 differentially expressed proteins of $CD133^{+}$ CSCs to 15 signaling pathways (Table 1). Among the proteins with

Table 1 Participation of differentially expressed proteins in $CD133^+$ cancer stem cells of the U87 MG cell line isolated from gliomaspheres in intracellular signaling pathways.

Signaling pathway	Total identified proteins (n)	Upregulated proteins (n)
Adherens junction	4	4
Apoptosis	7	4
Cell adhesion molecules	6	6
Cell cycle	4	4
Chemokine signaling pathway	5	3
Extracellular matrix-receptor interaction	14	14
Focal adhesion	26	16
Gap junction	5	4
Glycolysis/gluconeogenesis	13	12
Insulin signaling pathway	6	1
Integrin signaling pathway	5	2
MAPK signaling pathway	9	3
Regulation of actin cytoskeleton	11	7
Tight junction	9	8
Wnt-signaling pathway	14	12

>twofold upregulation there were 14 proteins (Table 2) involved in the signaling cascade associated with the interaction between $CD133^+$ CSCs and the ECM and four proteins activating this signaling pathway, namely fermitin family homolog 2 (FERMT2), lysyl oxidase homolog 2 (LOXL2), histone deacetylase 2 (HDAC2) and fibrillin 1 (FBN1). Moreover, such signaling proteins of receptor interaction with the ECM as collagen VI α1 chain (COL6A1) and laminin β1 (LAMB1), as well as LOXL2 (the protein activating this signaling pathway) exhibited increased expression by eight- and ninefold, respectively, in CSCs compared with that in DGCs.

3.3 TGF-β influence on DGCs

TGF-β stimulation increased the mobility of $CD133^-$ DGCs and significantly shortened the time of the scratch healing in the cell monolayer.

Table 2 Signaling proteins of extracellular matrix-receptor interaction upregulated in CD133$^+$ CSCs of the U87 MG cell line isolated from gliomaspheres.

ID	Gene name	Ratio CD133$^+$ CSCs/CD133$^-$ DGCs
CD44	CD44 antigen	2.5
HMMR	Hyaluronan-mediated motility receptor	2.2
COL1A1	Collagen type 1 α1	2.8
COL1A2	Collagen type 1	3.2
COL6A1	Collagen type VI α1 chain	8.4
COL6A3	Collagen type VI α3 chain	4.3
FN1	Fibronectin 1	4.1
LAMB1	Laminin subunit β1	8.8
LAMC1	Laminin subunit γ1	3.8
ITGA2	Integrin subunit α2	3.9
ITGA5	Integrin subunit α5	3.6
ITGAV	Integrin αV	5.0
ITGB1	Integrin subunit β1	4.5
ITGB3	Integrin subunit β3	3.2

CSCs, cancer stem cells; DGCs, differentiated glioblastoma cells.

Increased mobility was accompanied by lower levels of cancer cell proliferation, which was reflected in a smaller number of cells separated from the substrate and exhibiting spherical contours, clear cytoplasm and other signs of proliferation (Fig. 2A–D).

Proteome analysis of CD133$^-$ DGCs stimulated by TGF-β identified 2400 proteins, of which 2197 were detected in all cell lysates, 40 were detected only in control DGCs and 163 only in TGF-β1-pre-treated cells. Only 610 identified proteins out of those stimulated with TGF-β1 showed significant changes ($P < 0.05$) in their synthesis level: 488 showed an increase while 122 showed a decrease.

3.4 The expression of signaling proteins is increased in GBM cells stimulated by TGF-β

Proteins with significantly changed expression levels in TGF-β-treated CD133$^-$ DGCs included the following markers of epithelial-mesenchymal

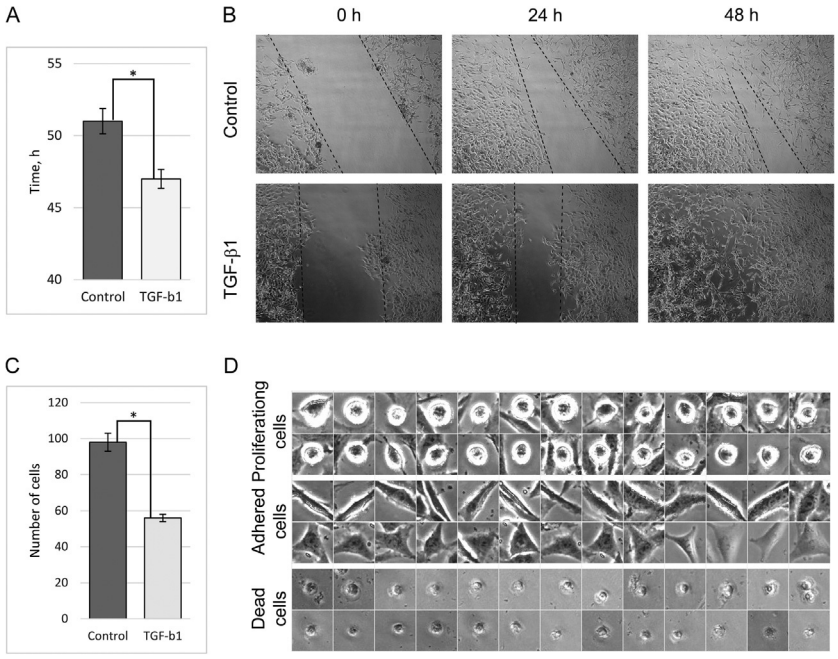

Fig. 2 Characterization of cell culture of the U-87MG line of human glioblastoma when stimulated with transforming growth factor β1 in vitro. Real time, highly efficient robotic quantitative microscopy. (A) The results of the analysis of the migratory activity of tumor cells of the control culture and cells stimulated by TGF-β1. (B) The visual characteristic of the scratch wound assay test at the critical points of the experiment is 0, 24 and 48 h. (C) Results from the analysis of proliferation of tumor cells of the control culture and cells stimulated with TGF-β1. (D) Library of cell images used to "educate" the robotic system. * Results are presented as M ± SEM. * Differences between groups are significant at $P < 0.05$.

transition: E-cadherin (CDH1), N-cadherin (CDH2), occludin (OCLN) and claudin1 (CLDN1). Upregulated proteins included CDH2, vimentin (VIM), vitronectin (VTN), components of the actin-myosin complex, and the matrix metalloproteinases (MMP)2, MMP9, MMP14 and ADAM metallopeptidase with thrombospondin type 1 motif 1 (ADAMTS1) (Table 3).

Bioinformatics analysis revealed 19 signaling proteins of receptor interaction with the ECM, including the following 13 proteins: CD44 antigen (CD44), hyaluronan-mediated motility receptor (HMMR), collagen I (COL1A2), COL6A1, collagen VI α3 (COL6A3), fibronectin (FN1),

Table 3 Change in the expression of proteins involved in epithelial-mesenchymal transition in the differentiated glioblastoma cells of the U87 MG cell line with TGF-β1 stimulation.

ID	Gene name	Ratio TGF-β1/ control
CDH1	E-cadherin	↓
OCLN	Occludin	↓
CLDN1	Claudin1	0,6
CDH2	N-cadherin	↑
VIM	Vimentin	2.8
VTN	Vitronectin	↑
Actl6a	Actin-like 6A	↑↑
Actn1	Actinin α1	2.0
ARPC3	Like actin related protein 2/3 complex subunit 3	2.3
MYBPC3	Myosin binding protein C, cardiac	5.7
Myo1C	Myosin IC	2.8
MYO5A	Myosin VA (heavy chain 12, myoxin)	↑
Myo7a	Myosin VIIA	↑
MMP2	Matrix metallopeptidase 2	2.8
MMP9	Matrix metallopeptidase 9	3.1
MMP14	Matrix metallopeptidase 14	2.2
ADAMTS1	ADAM metallopeptidase with thrombospondin type 1 motif, 1	↑

↓, proteins that completely disappear after TGF-β1 stimulation; ↑, proteins that appear for the first time TGF-β1 stimulation. TGF-β1, transforming growth factor-β1.

LAMB1, laminin γ1 (LAMC1), integrin α2 (ITGA2), integrin α5 (ITGA5), integrin αV (ITGAV), integrin β1 (ITGB1) and integrin β3 (ITGB3), as well as three proteins activating this signaling cascade fermitin family homolog 2 (FERMT2), lysyl oxidase homolog 2 (LOXL2) and histone deacetylase 2 (HDAC2). Fibrillin 1 (FBN1) in TGF-β-stimulated $CD133^-$ DGCs had increased synthesis, at the same level as that in $CD133^+$ CSCs (Tables 4 and 5, Fig. 3).

Table 4 Signaling proteins of extracellular matrix-receptor interaction upregulated in differentiated $CD133^-$ cells of glioblastoma after TGF-β1 stimulation and in untreated $CD133^+$ CSCs of the U87 MG cell line isolated from gliomaspheres.

ID	Gene name	Ratio $CD133^+$ CSCs/$CD133^-$ DGCs	Ratio $CD133^-$ DGCs TGF-β 1/DGCs $CD133^-$ control
CD44	CD44 antigen	2.5	2.3
HMMR	Hyaluronan-mediated motility receptor	2.2	2.3
Col15a1	Collagen type XV α1	–	2.5
COL1A1	Collagen type 1 α1	2.8	–
COL1A2	Collagen type 1	3.2	3.6
COL6A1	Collagen type VI α1 chain	8.4	8.6
COL6A3	Collagen type VI α3 chain	4.3	4.5
COL7A1	Collagen type VII α1	–	5.7
FN1	Fibronectin 1	4.1	4.2
FNDC3B	Fibronectin type III domain containing 3B	–	2.3
LAMB1	Laminin subunit β1	8.8	8.2
LAMC1	Laminin subunit γ1	3.8	3.7
ITGA2	Integrin subunit α2	3.9	4.1
ITGA5	Integrin subunit α5	3.6	3.5
ITGA8	Integrin subunit α8	–	2.2
ITGAV	Integrin αV	5.0	4.5
ITGAX	Integrin subunit αX	–	2.2
ITGB1	Integrin subunit β1	4.5	4.5
ITGB3	Integrin subunit β3	3.2	3.4

CSCs, cancer stem cells; DGCs, differentiated glioblastoma cells; TGF-β1, transforming growth factor-β 1—this protein was absent in the analyzed sample.

Table 5 Signaling proteins of extracellular matrix-receptor interaction in differentiated $CD133^-$ cells of glioblastoma after TGF-β1 stimulation and in untreated $CD133^+$ CSCs of the U87 MG cell line isolated from gliomaspheres.

ID	Gene name	Ratio $CD133^+$ CSCs/$CD133^-$ DGCs	Ratio $CD133^-$ DGCs TGF-β 1/DGCs $CD133^-$ control
FERMT2	Fermitin family homolog 2	3.5	3.2
LOXL2	Lysyl oxidase homolog 2	9.0	1.3
HDAC2	Histone deacetylase 2	1.7	1.5
FBN1	Fibrillin 1	6.2	5.1

CSCs, cancer stem cells; DGCs, differentiated glioblastoma cells; TGF-β1, transforming growth factor-β1.

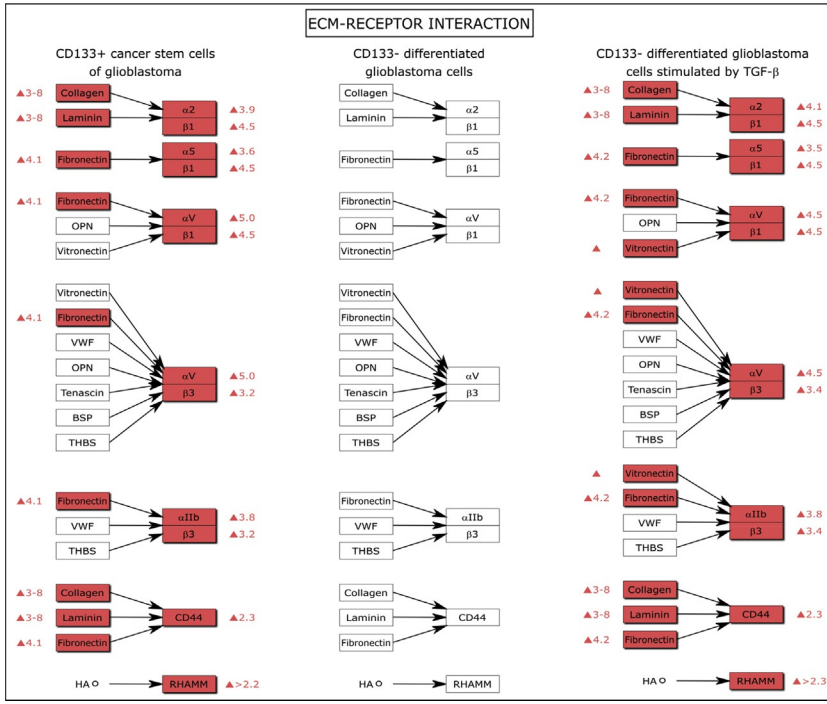

Fig. 3 Key proteins (marked with a red light) of the ECM-receptor interaction signaling pathway which were upregulated in the $CD133^+$ CSCs of U87MG line of GBM and significantly ($P < 0.05$) enhanced expression in $CD133^-$ DGCs after stimulation by TGF-β1.

4. Discussion

CSCs is the key problem of GBM therapy (Roos et al., 2017) and the main reason for treatment failure. All modern protocols of GBM therapy are based on the idea of eliminating CSCs or regulating their reproductive potential by using targeted drugs (Roos et al., 2017). However, the lack of remarkable progress in this area (Touat et al., 2017) indicates certain gaps in our understanding of the role of CSCs in GBM, and requires the development of new approaches to treat this disease.

Bioinformatics analysis suggests that $CD133^+$ CSCs are a unique living system that is well adjusted to surviving in tough conditions of intratumoral hypoxia, which is confirmed by a significant upregulation of 12 out of 13 signaling proteins involved in glycolysis/gluconeogenesis (Table 1). Hypoxic metabolism is one of the most important characteristics of malignant tumor cells, especially for U87 cells of wild-type GBM (Allen et al., 2016). Thus, significant differences in the proteome profile of $CD133^+$ CSCs and $CD133^-$ DGCs indicate that the selection process in serum-free medium with low-glucose content creates conditions that are identical to those inside the tumor lesion, promoting the selection of cells with unique morphogenetic and signaling properties.

This hypothesis is supported by upregulation of 8 out of 10 proteins belonging to the Wnt-signaling pathway, which is a key embryonic mechanism regulating reproduction and pluripotency of cells, accompanied by increased synthesis of cell cycle, adherens junction, cell adhesion molecules and tight junction proteins in $CD133^+$ CSCs. A close association between the upregulation of adherens junction proteins and the synthesis level of β-catenin, a key component of the Wnt-signaling pathway, in $CD133^+$ CSCs, was presented in our previous study (Shevchenko et al., 2019). This connection is likely to be due to the specific nature of $CD133^+$ CSC proliferation in gliomaspheres, where the increase in interaction changes the proliferation program and induces cells to exit from the gliomaspheres to differentiate and create a monolayer.

$CD133^+$ CSCs (Singh et al., 2003) have a high proliferation rate. However, invasive growth is a complex morphogenetic program where the proliferation process is integrated with other independent phenomena (Nalluri et al., 2015), including the crucial increased receptor interaction between cancer cells and the ECM. The invasive potential of such cells is demonstrated by the upregulation of 14 signaling proteins of receptor

interaction with the ECM (Table 2). This molecular arsenal allows $CD133^+$ CSCs to fast-track the invasive process that supports the experimental data (Singh et al., 2004) on the extreme oncogenetic nature of these cells.

The number of CSCs in a tumor is relatively small (Singh et al., 2003, 2004); this is why GBM progression and thus neoplastic cell penetration into the surrounding tissues outside the primary tumor lesion is possible only when reaching a certain qualitative level that could be achieved only via a set of biochemical modifications that $CD133^-$ DGCs undergo after interacting with CSCs.

The present study indicates TGF-β to be an important moderator of such modifications in GBM lesions and able to significantly change the proteome profile of DGCs by inhibiting adhesive E-cadherin synthesis, intensifying the production of migratory N-cadherin, and upregulating actin-myosin complex components, marker proteins of mesenchymal phenotype and matrix metalloproteinases in DGCs. Increased mobility of GBM cells and inhibition of their proliferation speed indicate these changes, thus, suggesting significant alterations in their interaction with the ECM.

However, these changes are relatively well-studied parameters, indicating transformation of the molecular profile of cancer cells due to TGF-β exposure. Our study revealed that TGF-β-stimulated $CD133^-$ DGCs had upregulation of 19 signaling proteins of receptor interaction with the ECM, and 13 of those proteins increased their synthesis level by >twofold, achieving values comparable to those of $CD133^+$ CSCs, which suggests that CSCs can be considered as a biological matrix, determining the most important properties of DGCs' aggressive phenotype.

Activation of hyaluronic acid receptors, CD44 and 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 (Jun, Hong, Liu, et al., 2017), including GBM, and it is frequently used as an indicator of tumor cells with aggressive phenotype (Wang, Zheng, Guan, et al., 2018). After being implanted into experimental animals, $CD44^+$ GBM cells quickly initiate invasive processes in the brain (Brown, Daniel, D'Abaco, et al., 2015), but due to a low proliferation rate, tumors grow slowly.

However, $CD133^+$ CSCs that also express CD44 demonstrate a 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 the

CD44 protein content in $CD133^+$ CSCs was 2.5-fold higher compared with that in DGCs, indicating that these $CD133^+$ cells may have a markedly high oncogenic potential. In addition, the level of CD44 protein in cells with aggressive phenotype, which was developed after stimulation with TGF-β, became 2.3-fold higher in comparison with that in untreated cells.

HMMR is an oncogene (Hartheimer, Park, Rao, & Kim, 2019) that serves an important role in solid tumor progression. It is highly upregulated in human glioma tissues, particularly in GBM. HMMR protein creates a complex with CD44 (Tilghman, Wu, Sang, et al., 2014), and after binding with hyaluronic acid, activates intracellular signaling pathways, regulating the proliferation and invasion of CSCs. Inhibition of HMMR suppresses and radiosensitizes cells of GBM (Li, Ji, & Wang, 2018). The results of the present study revealed that HMMR expression in CSCs was 2.2-fold higher compared with that in DGCs, providing evidence that these cells have a highly invasive nature. The synthesis level for this protein increased 2.3-fold in differentiated cancer cells with aggressive phenotype, which was developed after stimulation with TGF-β.

Special attention (He, Lee, & Jiang, 2016) should be paid to the components of the ECM, namely collagen, fibronectin and laminin, which are synthesized as part of a complex invasive growth program. The present study demonstrated >twofold higher expression of collagens types I and IV in CSCs compared with those in DGCs. Also, TGF-β-stimulation of DGCs increased the synthesis of the collagen subunits COL1A2, COL6A1 and COL6A3, reaching the levels found in CSCs.

COL1A2 was upregulated 3.2-fold in CSCs compared with its expression in DGCs; TGF-β-stimulation of differentiated cells increased the synthesis of this protein by 3.6-fold. The present study (Hirose, Nakahara, & Miyakoshi, 2003) used this protein as a molecular focus of GBM cell migration in the magnetic field and as a strategic marker of prognosis in stomach (Rong, Huang, Tian, et al., 2018) and prostate cancer. Suppressing COL1A2 gene inhibits invasive and metastatic processes in tumors of the digestive system (Ao, Guan, Wang, et al., 2018; Penet, Kakkad, Pathak, et al., 2017).

COL6A1 was 8.4-fold upregulated in CSCs compared with its expression in DGCs, but TGF-β-stimulation of tumor cells increased the synthesis level of this protein by >8.4-fold. COL6A1 upregulation in GBM cells is associated with their resistance to treatment (Turtoi, Blomme, Bianchi, et al., 2014), and is considered (Fujita, Sato, Festa, et al., 2008) to be one of the most significant markers of invasive glial tumors.

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 patients with GBM. Upregulated FN1 modulates adhesion, proliferation, differentiation and chemoresistance of CSCs (Yu, Xue, Liu, et al., 2018). The results of the present study revealed that the expression of this protein in CSCs was 4.1-fold higher than that in DGCs. Also, TGF-β-stimulation led to a 4.2-fold increase in FN1 synthesis in tumor cells.

The expression of LAMB1 and LAMC1 was 8.8- and 3.8-fold higher, respectively, in $CD133^+$ CSCs of GBM compared with that in DGCs. GBM cell stimulation intensified the production of these laminin molecules by 8.2- and 3.7-fold, respectively. The dominant role of LAMB1 (Chen, Lu, Cai, et al., 2014) and LAMC1 (Qin, Rodin, Simonson, & Hollande, 2017) in the GBM invasive processes has been previously described.

Adherence to collagen, laminin and fibronectin leads to restructuring in the cytoplasmatic domain of integrin receptors (Huttenlocher & Horwitz, 2011), with the creation of an adhesive patch necessary for amoeboid migration to the surrounding tissues. Thus, the extensive range of integrin receptors on the cell surface is one of the most important changes that occur during the development of an aggressive phenotype.

It is accepted (Arnaout, Goodman, & Xiong, 2007) that the apical-basal polarity of epithelial cells is associated with the expression of integrin receptors, similar to the ECM main proteins, which are located on the basal surface of such cells. The possibility of amoeboid migration to the adjacent tissues arises when integrin receptor expression occurs on the whole surface of the basal membrane as a result of epithelial-mesenchymal transition triggered by TGF-β (Roth, Silginer, Goodman, et al., 2013), which indicates its readiness to interact with the ECM components.

The extensive range of integrin receptors appearing due to TGF-β stimulation is demonstrated based on the cellular models of human lung adenocarcinoma with the A549 cell line (Shevchenko, Bryukhovetskiy, Nikiforova, et al., 2017) and GBM (Allen et al., 2016). The present study revealed a significant increase in the aggressive phenotype of five proteins in DGCs, which is similar to the values observed in CSCs.

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 3.9-fold higher expression of ITGA2 compared with that of DGCs.

TGF-β-stimulation of tumor cells promotes a 4.1-fold increase in the synthesis level of this protein.

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). In addition, ITGA5 positively regulates cell stemness in triple-negative breast cancer (Xiao, Li, Tao, et al., 2018), and supports the formation of gliomaspheres *in vitro* (Blandin, Noulet, Renner, et al., 2016) and the migration of GBM cells. The results of the current study indicated that CSCs had 3.6-fold higher expression of ITGA5 compared with that of DGCs. Furthermore, TGF-β-stimulation of tumor cells led to ~3.5-fold increase in the synthesis level of this protein.

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 TGF-β (Silginer, Burghardt, Gramatzki, et al., 2016) through an aryl hydrocarbon receptor-dependent mechanism and the SMAD signaling pathway, and induces the differentiation of CSCs when interacting with ECM components. These proteins play an important part in the positioning of progenitor-like phenotype cells, and promote the creation of chains of integrins, leading to migration of neuroblasts from the subventricular zone of the brain to the olfactory bulb (Niibori-Nambu, Midorikawa, Mizuguchi, et al., 2013). The current study revealed 5.0-fold higher expression of ITGAV in CSCs compared with that in DGCs. TGF-β-stimulation of tumor cells resulted in a 4.5-fold increase in the synthesis level of this protein. Integrins are the main receptors of VTN, FN1, fibrinogen, laminin and other ECM components (Fig. 3).

The expression levels of ITGB1 and ITGB3 were also increased in CSCs by 4.5- and 3.24-fold, respectively. TGF-β-stimulation of tumor cells led to a 4.5- and 3.5-fold increase in the synthesis level of this protein compared with that of untreated DGCs. ITGB1 synthesis level rises in GBM cells that are resistant to bevacizumab, and antiangiogenic therapy becomes more efficient (Carbonell, DeLay, Jahangiri, et al., 2013) after inhibiting this target. Increased ITGB3 production (Liu, Han, Dong, et al., 2016) is typical for GBM cells isolated from hypoxic tumor areas with abundant VTN, and high invasive and metastatic activity. Expression of these proteins in colorectal cancer cells (Dong, Qian, Chen, et al., 2019) is suggested to be a molecular marker of distant metastases spreading to the brain.

TGF-β-stimulation is accompanied by upregulation of various signaling proteins of receptor interaction with the ECM, which makes the molecular

phenotype of DGCs similar to the one of CSCs. Furthermore, bioinformatics analysis revealed that a series of proteins that activate the signaling pathway of receptor interaction with the ECM (Fig. 3) are upregulated in DGCs after TGF-β stimulation and reach values similar to those in CSCs.

FERMT2 is an important regulator of integrin activity in cell-cell and cell-ECM interactions. FERMT2 modulates integrin-mediated (Kawamura, Hamilton, Miskiewicz, et al., 2018) adhesion and invasion of trophoblasts into the uterine wall, and participates in carcinogenesis and tumor progression. The results of the present study revealed that CSCs had 3.5-fold increased expression of FERMT2 compared with that of DGCs. TGF-β-stimulation of tumor cells increased the synthesis level of this protein by 3.2-fold compared with that in untreated 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 (Xie, Yu, Wang, et al., 2019) and other aggressive tumors. The current analysis identified a 9.0-fold increase in LOXL2 expression in CSCs compared with that in DGCs. However, TGF-β-stimulation of tumor cells did not have a significant effect on the synthesis level of this protein in DGCs compared with that in untreated DGCs, indicating a specific function of this enzyme in CSC biology.

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 of LOXL2 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 participate in neoplastic processes in this type of cells. LOXL2 changes histone structure and, thus, modifies the shape of cells, facilitating metastasis development and invasion (Du & Zhu, 2018).

LOXL2 expression level correlates with HDAC2 expression (Cierna, Mego, Jurisica, et al., 2016), which suggests that these proteins may play a synergistic oncogenic role. In the present study, the expression of HDAC2 was 1.7-fold higher in CSCs compared with that in DGCs. TGF-β-stimulation of tumor cells increased the synthesis level of this protein by 1.5-fold compared with that in CSCs.

FBN1 is a protein of the fibrillin family that is involved in supporting the pluripotency of normal embryonal stem cells and CSCs (Lerner-Ellis, Aldubayan, Hernandez, et al., 2014), and enabling the biological effects of TGF-β. The expression level of FBN1 in CSCs was found to be 6.21-fold higher than that in DGCs. TGF-β-stimulation of tumor cells increased the synthesis level of this protein by 5.1-fold compared with that in CSCs.

Therefore, TGF-β-stimulation inhibits the proliferation, increases the mobility and creates the aggressive molecular phenotype of GBM cells, which is characterized by transitioning from adhesive E-cadherins to migratory N-cadherins, producing markers of tissues with mesodermal origin, increasing the synthesis of actin-myosin complex proteins, producing matrix metalloproteases, upregulating 19 proteins and activating the signaling pathway of receptor interaction with the ECM. Of note, the synthesis level of 13 proteins out of those 19 increased >twofold, reaching the values of CSCs. Development of aggressive phenotype in GBM cells is accompanied by increased synthesis of molecules that directly interact with the upregulated proteins. The expression of LOXL2 was >ninefold higher in CSCs compared with that in DGCs, suggesting that CSCs may have a highly invasive nature, and that this protein may be an important CSC marker and a promising target for eliminating these cells in GBM. The production of LOXL2 marker distinguishes DGCs with aggressive phenotype from CSCs.

The present study suggests that the most important way of managing the invasive nature of CSCs could be inhibiting TGF-β transmission in the tumor lesion that mimics the key proteome properties of $CD133^{-}$ differentiated and $CD133^{+}$ cancer stem cells of GBM. Theoretically speaking, it is possible provided the pro-inflammatory activation of the resident tumor microglia.

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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.

Authors' contributions

I.B. wrote the manuscript, proposed the study idea, designed the study, offered support with the experiments, put together the scientific team, cultured the cancer cells, isolated the cancer $CD133^{+}$ stem cells of GBM for the experiments, provided scientific guidance and contributed to the bioinformatics analysis. S.Z. performed mathematical processing of experimental results, studied the processes of cell migration and proliferation using a system of high-performance quantitative microscopy in real time; V.S., N.A., Z.V. and T.K. prepared and analyzed the samples, and performed cell lysis, chromatography and MS experiments, and contributed to the bioinformatics analysis. Y.K., O.P., A.B. and H.S. discussed, analyzed and interpreted the results of the study, and wrote the manuscript. All authors read and approved the manuscript and agreed to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of this work are appropriately investigated and resolved.

Ethics approval and consent to participate

Informed consent was obtained from all individual participants involved in the study. All the procedures conducted in studies involving human participants were in accordance with the ethical standards of the Far Eastern Federal University.

In this work, we did not use material obtained directly from patients.

Patient consent for publication

In this work, material obtained from patients was not used.

Competing interests

The authors declare that they have no competing interests.

References

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

Ao, R., Guan, L., Wang, Y., et al. (2018). Silencing of COL1A2, COL6A3, and THBS2 inhibits gastric cancer cell proliferation, migration, and invasion while promoting apoptosis through the PI3k-Akt signaling pathway. *Journal of Cellular Biochemistry*, *119*(6), 4420–4434.

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.

Batlle, E., & Massague, J. (2019). Transforming growth factor-β signaling in immunity and cancer. *Immunity*, *50*(4), 924–940.

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.

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, 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.

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.

Carbonell, W. S., DeLay, M., Jahangiri, A., et al. (2013). β1 integrin targeting potentiates antiangiogenic therapy and inhibits the growth of bevacizumab-resistant glioblastoma. *Cancer Research*, *73*(10), 3145–3154.

Chen, Q., Lu, G., Cai, Y., et al. (2014). MiR-124-5p inhibits the growth of high-grade gliomas through posttranscriptional regulation of LAMB1. *Neuro-Oncology*, *16*(5), 637–651.

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.

Colwell, N., Larion, M., Giles, A. J., et al. (2017). Hypoxia in the glioblastoma microenvironment: Shaping the phenotype of cancer stem-like cells. *Neuro-Oncology*, *19*(7), 887–896.

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.

Dong, L., Qian, J., Chen, F., et al. (2019). LINC00461 promotes cell migration and invasion in breast cancer through miR-30a-5p/integrin β3 axis. *Journal of Cellular Biochemistry*, *120*(4), 4851–4862. https://doi.org/10.1002/jcb.27435.

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.

Frei, K., Gramatzki, D., Tritschler, I., et al. (2015). Transforming growth factor-β pathway activity in glioblastoma. *Oncotarget*, *6*(8), 5963–5977.

Fujita, A., Sato, J. R., Festa, F., et al. (2008). Identification of COL6A1 as a differentially expressed gene in human astrocytomas. *Genetics and Molecular Research*, *7*(2), 371–378.

Gimple, R. C., Bhargava, S., Dixit, D., et al. (2019). Glioblastoma stem cells: Lessons from the tumor hierarchy in a lethal cancer. *Genes and 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.

He, X., Lee, B., & Jiang, Y. (2016). Cell-ECM interactions in tumor invasion. *Advances in Experimental Medicine and Biology*, *936*, 73–91.

Hirose, H., Nakahara, T., & Miyakoshi, J. (2003). Orientation of human glioblastoma cells embedded in type I collagen, caused by exposure to a 10 T static magnetic field. *Neuroscience Letters*, *338*(1), 88–90.

Huttenlocher, A., & Horwitz, A. R. (2011). Integrins in cell migration. *Cold Spring Harbor Perspectives in Biology*, *3*, a005074.

Jun, F., Hong, J., Liu, Q., et al. (2017). Epithelial membrane protein 3 regulates TGF-β signaling activation in CD44-high glioblastoma. *Oncotarget*, *8*(9), 14343–14358.

Katsuno, Y., Lamouille, S., & Derynck, R. (2013). TGF-β signaling and epithelial-mesenchymal transition in cancer progression. *Current Opinion in Oncology*, *25*(1), 76–84.

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, J., Ji, X., & Wang, H. (2018). Targeting long noncoding RNA HMMR-AS1 suppresses and radiosensitizes glioblastoma. *Neoplasia*, *20*(5), 456–466.

Liu, Z., Han, L., Dong, Y., et al. (2016). EGFRvIII/integrin β3 interaction in hypoxic and vitronectinenriching microenvironment promote GBM progression and metastasis. *Oncotarget*, *7*(4), 4680–4694.

Lukas, R. V., Wainwright, D. A., Ladomersky, E., et al. (2019). Newly diagnosed glioblastoma: A review on clinical management. *Oncology (Williston Park, N.Y.)*, *33*(3), 91–100.

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.

Nalluri, S. M., O'Connor, J. W., & Gomez, E. W. (2015). Cytoskeletal signaling in TGFβ-induced epithelial-mesenchymal transition. *Cytoskeleton (Hoboken)*, *72*(11), 557–569.

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.

Penet, M. F., Kakkad, S., Pathak, A. P., et al. (2017). Structure and function of a prostate cancer dissemination-permissive extracellular matrix. *Clinical Cancer Research*, *23*(9), 2245–2254.

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.

Qin, Y., Rodin, S., Simonson, O. E., & Hollande, F. (2017). Laminins and cancer stem cells: Partners in crime? *Seminars in Cancer Biology*, *45*, 3–12.

Ramamoorthi, G., & Sivalingam, N. (2014). Molecular mechanism of TGF-β signaling pathway in colon carcinogenesis and status of curcumin as chemopreventive strategy. *Tumour Biology*, *35*(8), 7295–7305.

Rong, L., Huang, W., Tian, S., et al. (2018). COL1A2 is a novel biomarker to improve clinical prediction in human gastric cancer: Integrating bioinformatics and meta-analysis. *Pathology Oncology Research*, *24*(1), 129–134.

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.

Shevchenko, V. E., Bryukhovetskiy, I. S., Nikiforova, Z. N., et al. (2017). The transforming growth factor beta-1 in the oncogenesis of human lung adenocarcinoma. *Advances in Molecular Oncology*, *4*(3), 67–74. in Russia https://doi.org/10.17650/2313-805X-2017-4-3-67-74.

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., Brada, M., van den Bent, M. J., Tonn, J. C., & Pentheroudakis, G. (2014). ESMO guidelines working group. High-grade glioma: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Annals of Oncology*, *25*(Suppl. 3), iii93–101.

Syed, V. (2016). TGF-β signaling in cancer. *Journal of Cellular Biochemistry*, *117*(6), 1279–1287.

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.

Touat, M., Idbaih, A., Sanson, M., et al. (2017). Glioblastoma targeted therapy: Updated approaches from recent biological insights. *Annals of Oncology*, *28*(7), 1457–1472.

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, F., Ling, L., van Dam, H., et al. (2018). TGF-β signaling in cancer metastasis. *Acta Biochimica et Biophysica Sinica Shanghai*, *50*(1), 121–132.

Xie, J. J., Guo, J. C., Wu, Z. Y., et al. (2016). Integrin α5 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 EMT via HIF-2α signaling. *Cancer Science*, *110*, 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*, 21369–21379. https://doi.org/10.1002/jcp.28746.