CHAPTER EIGHT

Proteins of Wnt signaling pathway in cancer stem cells of human glioblastoma

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Abstract

Rationale: Glioblastoma multiforme (GBM) is the most aggressive primary glial brain tumor. The prognosis for GBM patients is not favorable, with the median survival time being 15 months. Its treatment resistance is associated with GBM cell population having cancer stem cells (CSCs). Wnt/β-catenin signaling pathway is a strategically important

International Review of Neurobiology, Volume 151
ISSN 0074-7742
https://doi.org/10.1016/bs.irn.2020.03.006

molecular mechanism, providing proliferation of stem cells of all types. This study compares the expression levels of signaling pathway proteins in CD133(+) CSCs and CD133 (−) differentiated glioblastoma cells (DGCs).

Materials and methods: the present study used U-87MG cells of human glioblastoma, the material was tested for mycoplasma contamination. High-performance liquid chromatography (HPLC) mass spectrometry was used for proteome analysis. Biological and molecular functions, signaling pathways and protein-protein interactions were analyzed using free-access databases: PubMed, PANTHER, Gene Ontology, Swiss-Prot and KEGG. Protein-protein interactions (PPIs) were analyzed using the STRING database (version 10).

Results: There were identified 589 proteins with significantly changed expression in CD133+ CSCs, as compared with CD133-DGCs ($P < 0.05$). Bioinformatics analysis allowed to attribute 134 differentially expressed proteins to 16 signaling pathways. A significant increase in expression of eight Wnt signaling pathway proteins (APC, CSNK1E, CSNK1A, CSNK2A2, CSNK2B, CTNNB1, DVL1, RUVBL) was detected, as well as four proteins of the non-canonical Wnt pathway—RHOA, ROCK2, RAC2, DAAM1. Special attention should be paid to β-catenin (CTNNB1) with more than 13.98-fold increase of expression in CSCs and Disheveled-associated activator of morphogenesis 1 (DAAM1) with 6.15-fold higher upregulation level.

Conclusion: proteins of Wnt/β-catenin signaling cascade are a prospective target for regulating CSCs activity.

1. Introduction

Glioblastoma multiforme (GBM) is one of the most aggressive human brain tumors. It accounts for about a half of primary CNS tumors, up to 80% of gliomas, and about a quarter if all intracranial tumors (Louis, Perry, Reifenberger, et al., 2016). Modern protocol of complex treatment (Stupp, Brada, van den Bent, et al., 2014; Stupp, Mason, van den Bent, et al., 2005; Stupp & Ram, 2018) involves a surgery, radiation and chemotherapy. Treatment results are not very promising (Lukas, Lesniak, & Stupp, 2018), median survival is 15 months, and despite all the efforts of medical staff only 27% of patients manage to live for 2 years after being diagnosed. Treatment resistance (Chang, Graham, Hao, et al., 2016; Perrin et al., 2019) is associated with GBM having cancer stem cells (CSCs) in its cell population.

CSCs play a crucial part in GBM hierarchy (Gimple, Bhargava, Dixit, & Rich, 2019), exhibiting such properties as pluripotency and high proliferative capacity. Only 100 cells, immunopositive to the main CSCs marker—CD133 antigen (Yelle, Bakhshinyan, Venugopal, & Singh, 2019), are

enough to fast-track invasive tumor development in experimental animals. CSCs are highly resistant to radiation (Rycaj & Tang, 2014), modern anti-tumor drugs are unable to eliminate these cells from a patient's body, and the majority of targeted chemotherapeutic agents (Touat, Idbaih, Sanson, & Ligon, 2017) proved to be mainly inefficient in large-scale studies of GBM patients. That is why new molecular targets for regulation of CSCs key properties should be discovered.

The proliferation speed of GBM cells is inversely proportional to their differentiation level (Friedmann-Morvinski, 2014; Lathia, Mack, Mulkearns-Hubert, Valentim, & Rich, 2015; Mei, Chen, Chen, Xi, & Chen, 2017), therefore, a special attention should be paid to the Wnt signaling pathway—one of the key intracellular mechanisms, regulating differentiation (Tan & Barker, 2018) and proliferation (Kahn, 2018) of normal stem cells. In different types of cancer (Kretzschmar & Clevers, 2017) excessively active Wnt signaling moderates proliferation processes and is directly responsible for GBM cells developing treatment resistance (Kim, Kim, Lee, et al., 2012). The present study aimed at comparing the expression levels of Wnt signaling pathway proteins in CD133+ CSCs and CD133− differentiated cells of human GBM, determining the potential markers and therapy targets.

2. Materials and methods

2.1 Human glioblastoma cells

The present study used U-87MG cells of glioblastoma (cat. no HTB-14™). This cell line is not the original U-87 line established at University of Uppsala but is "likely" to be a glioblastoma of an unknown origin (Allen, Bjerke, Edlund, et al., 2016). Our research team has been using this cell line for CSCs proteome mapping since 2014 (Bryukhovetskiy, Shevchenko, Kovalev, et al., 2014). We demonstrated the multiple similarities between CSCs and normal stem cells proteomes, studied the main differences of invasive cells and CSCs, attempted at identifying Wnt signaling pathway proteins in GBM cells (Shevchenko et al., 2019). A fundamental distinctive feature of this study is using the cell culture, obtained directly from the manufacturer without previously going through multiple passages and having high purity level in the separated fraction of the CSCs, isomorphic to the CD133 marker.

2.1.1 Differentiated glioblastoma cells (DGCs)

GBM cells were cultured in low glucose Dulbecco's modified Eagle medium (DMEM; Gibco, USA) with 10% fetal bovine serum (FBS) in standard conditions (5% CO_2, 37 °C). The cells were cultured until 80% confluence and subcultured at a 1:3 ratio. The cells were used in the experiment after the third passage since the moment of being obtained from the manufacturer. Before the start of the study, the cells were tested for mycoplasma contamination with Universal Mycoplasma Detection Kit (ATCC® 30-1012K™).

2.1.2 Cancer stem cells of GBM (PCK)

To obtain gliomaspheres, the 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 non-adhesive plastic plates. All chemicals were obtained from Gibco (Thermo Fisher Scientific, Inc., USA). The cells were cultured in standard T75 flasks at 37 °C in 5% CO_2 atmosphere. Every 3 days fresh growth factors were added. The extraction of CD133 + cells was performed via immunosorting, using magnetic beads with immobilized antibodies against CD133 (No. 130-100-857, Miltenyi Biotec, USA), the population purity was assessed via flow cytometry with correspondent antibodies (No. 130-105-226, Miltenyi Biotec, USA). The purity of CSCs, that were isolated based on CD133 antigen, was 98.72%.

2.2 Used chemicals

Acetonitrile (ACN) HPLC gradient grade was purchased from Prolabo (VWR BDH Prolabo® USA). Formic acid (FA) from Merck (Germany). Urea (BioUltra for molecular biology, 51456), ammonium bicarbonate (BioUltra, 09830), dithiothreitol (DTT) (BioUltra for molecular biology, 43815), iodoacetamide (IAA, I1149), 2.2.2-trifluoroethanol (TFE, T63002, Reagent Plus), acetic acid (HAc, A6283 Reagent Plus), protease inhibitor cocktail (P8340), mammalian cell lysis kit (MCL1), phosphate buffered saline (PBS, P5493), trypsin (modified, proteomics grade, T7575) ammonium bicarbonate NH_4HCO_3 (BioUltra, 09830), trichloroethylphosphate (TCEP, 07296), trifluoroacetic acids for protein sequence analysis (TFA, 299537), hydrochloric acid (BioReagent, for molecular biology, H1758) were obtained from Sigma-Aldrich (USA). Ultrapure water was prepared by Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.3 Mass spectrometry of samples

A combination of high-performance liquid chromatography and mass spectrometry was applied. The label-free method was used to evaluate the changes in proteins expression level. Two cell samples—CD133+ CSCs and CD133− DGSs—were lysed with mammalian cell lysis kit, low-molecular compounds were removed, then enzymatic cleavage was performed, and each 4 μL of solution was analyzed, using mass spectrometry for controlled trypsinolysis. The samples were incubated at 30 °C in a Labconco CentriVap centrifugal concentrator to remove ammonium bicarbonate. Tryptic peptides were diluted during the mobile stage (30% acetonitrile, 70% water and 0.1% formic acid, pH 2.7) and divided into 24 fractions, using a Dionex UltiMate 3000 chromatograph (Dionex, Sunnyvale, CA, USA), equipped with a fraction collector and cation exchange column MIC-10-CP (Puros 10S, 1 mm × 10 cm, Thermo, USA). The obtained fractions were concentrated at 30 °C in the centrifugal concentrator and diluted again with 100 μL of 0.1% formic acid.

Tryptic peptides were analyzed with Dionex Ultimate 3000 (Dionex, Netherlands) and LTQ Orbitrap XL mass-spectrometer (Thermo) with NSI ionization. Peptide division was performed with Acclaim C18 PepMap100 column (75 μm × 150 mm, grit size—3 μm, Dionex), equipped with a precolumn. Mass-spectrometry data were processed with (23) MaxQuant (version 1.6.1.0) and Perseus software (version 1.6.1), Max Planck Institute of Biochemistry (Germany). Biological processes, molecular functions, cell location and protein signaling pathways were annotated, using such databases as PubMed (http://www.ncbi.nlm.nih.gov/pubmed), PANTHER (http://www.pantherdb.org), Gene Ontology (http://www.geneontology.org), KEGG (http://www.genome.jp/kegg/) and STRING v10 (https://string-db.org/).

2.4 Statistical analysis

Statistical significance was calculated, using Student's *t*-test. $P < 0.05$ was considered to indicate a statistically significant difference.

3. Results

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

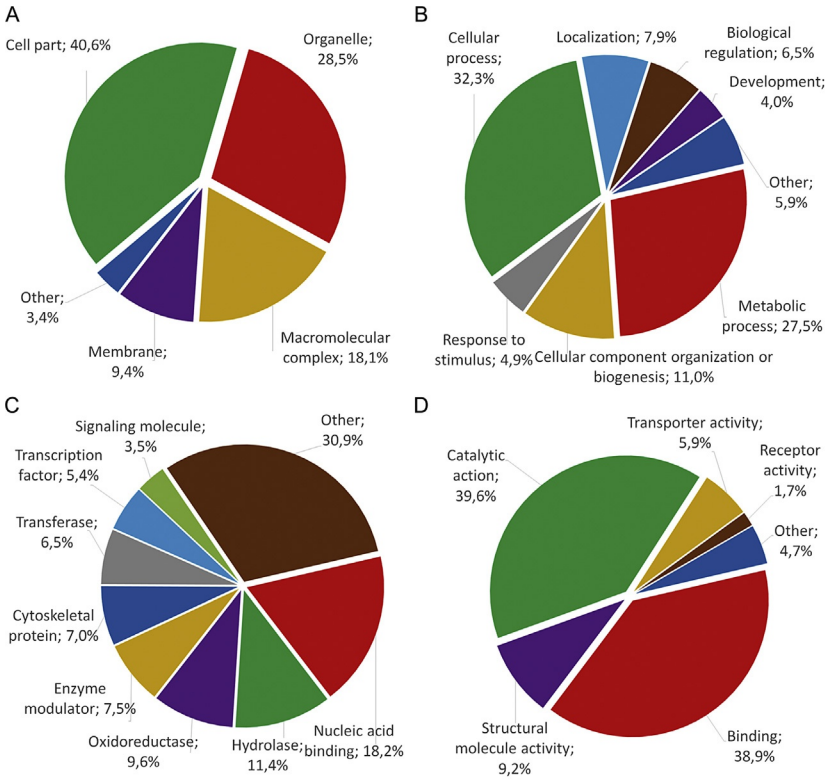

Fig. 1 (A–D) Molecular-biological description of proteins in cancer glioblastoma CD133 stem cells: (A) claster of localization, (B) claster of intracellular processes, (C) claster of functional activity, and (D) claster of enzymatic activity.

showed a high percentage of overlap between 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), they were associated with metabolic and cellular processes (Fig. 1B), were functionally heterogeneous (Fig. 1C) and associated with a class of compounds that exhibit active fermentation properties (Fig. 1D).

Expression of wnt signaling proteins is increased in CSCs. Bioinformatics analysis identified 16 signaling pathways in GBM CSCs (Table 1). Fourteen

Table 1 Participation of differentially expressed proteins of glioblastoma CD133+ stem cells in the 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
ECM-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

proteins of Wnt signaling cascade were discovered, while 12 of them had a more than twofold higher synthesis level, as compared with that of DGCs (Table 2). Eight of upregulated proteins belonged to the canonical Wnt cascade: adenomatous polyposis coli (APC), casein kinase 1, epsilon (CSNK1E), casein kinase 1α (CSNK1A), casein kinase 2α2 (CSNK2A2), casein kinase 2β (CSNK2B), catenin β1(CTNNB1), Disheveled 1 (DVL1), and Pontin 52 (RUVBL1), while four proteins (Table 3) were components of the non-canonical Wnt signaling pathway: Ras homolog gene family, member A (RHOA), Rho-associated, coiled-coil containing protein kinase 2 (ROCK2), Ras-related C3 botulinum toxin substrate 2 (RAC2), Disheveled-associated activator of morphogenesis 1 (DAAM1).

Table 2 Changes in expression of canonical Wnt signaling proteins in CSCs relative to DGCs.

ID	Protein name	Ratio ($P < 0.05$) CSCs/DGCs
APC	Adenomatous polyposis coli	2.01
CSNK1E	Casein kinase 1, epsilon	2.21
CSNK1A	Casein kinase 1α	2.23
CSNK2A2	Casein kinase 2α2	3.01
CSNK2B	Casein kinase 2β	3.04
CTNNB1	Catenin β1	13.98
DVL1	Disheveled 1	3.21
FRAT1	FRAT regulator of WNT signaling pathway 1	1.08
GSK3B	Glycogen synthase kinase 3 beta	1.01
RUVBL1	RuvB like AAA ATPase 1, Pontin 52	3.22

Table 3 Changes in expression of non-canonical Wnt signaling pathway proteins in CSCs relative to DGCs.

ID	Protein name	Ratio ($P < 0.05$) CSCs/DGCs
RhoA	Ras homolog gene family, member A	2,88
ROCK2	Rho-associated, coiled-coil containing protein kinase 2	3,18
RAC2	Ras-related C3 botulinum toxin substrate 2 (rho family, small GTP binding protein Rac2)	2.07
DAAM1	Disheveled-associated activator of morphogenesis 1	6.54

4. Discussion

Based on the fundamental notion (Bradshaw, Wickremesekera, Brasch, et al., 2016) of CSCs having a dominant position in the GBM cell hierarchy, the data of bioinformatics analysis shed some light on a series of crucial aspects in the biology of these cells. Firstly, CD133+ cells are characterized by upregulation of all proteins of glycolysis/gluconeogenesis signaling pathways (Table 1), indicating their ability to survive in the tough

conditions of hypoxia that is inevitable in GBM growth process. Hypoxia is very likely to be the major factor in development of tumor cells hierarchy. Thus, on the one hand, GBM therapy should be aimed at elimination of local hypoxia, while, on the other hand, it should involve antiangiogenic drugs, since CSCs differentiation into the cells of angiogenic endothelium is the main way (Hambardzumyan & Bergers, 2015) for them to fulfill their differentiation potential.

Secondly, total protein upregulation of adherens junction, cell adhesion molecules, tight junction and gap junction signaling pathway indicates a special role that intercellular interactions play in regulating basic properties of CSCs. Intensified interactions in gliomaspheres is accompanied by the increased synthesis level for eight key proteins of the canonical Wnt signaling pathway and four proteins of the non-canonical one.

Adenomatous polyposis coli (APC) is a protein of the canonical Wnt signaling cascade. Mutation of APC gene that codifies this protein is a key component of Turcot syndrome, also known as the Mismatch repair cancer syndrome. This syndrome is associated with biallelic DNA mismatch repair mutations, accompanied by development of bowel polyps, colon cancer and malignant brain tumors (Dipro, Al-Otaibi, Alzahrani, et al., 2012). This study showed 2.01-fold higher upregulation of APC in CSCs, as compared with DGCs. Upregulation of this protein is typical for CSCs (Shevchenko et al., 2019) and indicates the scope of systemic molecular-genetic disorders, leading to GBM development.

Casein kinase 1ε (CSNK1E) is a protein of the canonical Wnt signaling cascade, but it stayed off the neurooncologists' radar until recently. This protein ensures the survival of GBM cells (Varghese et al., 2018) and is an important biomarker for glial tumors (Varghese et al., 2016). Our research showed the content of CSNK1E in CSCs to be 2.21-fold higher than in DGCs.

Casein kinase CK1α (CSNK1A) is a main component of the canonical Wnt/β-catenin signaling pathway (Cruciat, 2014). This protein phosphorylates regulatory molecules, involved in the cell cycle, transcription, translation and intercellular adhesion (Schittek & Sinnberg, 2014). It has been suggested (Mishra, 2014) treating CK1α as a possible antagonist of Zinc finger protein GLI2—one of the key mediators of Sonic hedgehog (Shh) pathway, involved in GBM cells proliferation. Our study showed the amount of CSNK1A in CSCs to be 2.23-fold larger than in DGCs.

Casein kinase CK2 (casein kinase 2) is a component of the canonical Wnt/β-catenin signaling pathway and a multifaceted regulator (Rowse et al., 2017) of the cell cycle, transcription and apoptosis (Nitta et al., 2015).

CK2α expression increases in cells of many tumors (Ferrer-Font, Villamañan, Arias-Ramos, et al., 2017), including CSCs of GBM (Rowse et al., 2017). We identified two catalytic subunits (CSNK2A2 and CSNK2B) with higher expression levels (3.01 и 3.04 ± fold) in CSCs as compared with DGCs.

β-Catenin is a key component of Wnt signaling pathway. When there is no Wnt signal, this proteins is phosphorylated by β-catenin destruction complex, which includes the following proteins: Axin, APC, GSK3 and CK1α. Wnt signal on the surface of the cell membrane inhibits this destruction complex, leading to the increase of β-catenin in the cell (Krishnamurthy & Kurzrock, 2018) that allows it to activate T cell-specific factor (TCF)/lymphoid enhancer-binding factor (LEF) and co-activators, like Pygopus and Bcl-9, to turn on the Wnt target genes such as c-Myc, cyclin D1 and Cdkn1a. β-Catenin accumulation in GBM cells induces the synthesis of telomerase catalytic subunits (TERT), resulting in immortalization of tumor cells with high reproductive potential (Suh et al., 2017).

Our study demonstrated (Table 2) the increased synthesis (two- to three-fold) of some destruction complex components in CSCs, as compared with the same in DGCs. Despite that, the larger amount of β-catenin (CTNNB1) in CSCs—13.98 fold more than in DGCs—indicates the high activity of this pathway in gliomasphere, proving the earlier stated notion (Náger et al., 2015) about the sharp increase in β-catenin synthesis in glioma cells and its importance for CSCs survival, invasion and proliferation.

β-Catenin is one of the most well-known markers of CSCs in stomach cancer (Tanabe, Aoyagi, Yokozaki, & Sasaki, 2016) and colon cancer (Farhana et al., 2016), our research data allow considering CTNNB1 to be a crucial marker of glioblastoma CSCs. β-Catenin suppression (Zhang et al., 2017) reduces the amount of CSCs, produces cytostatic effect (Kierulf-Vieira et al., 2016) and inhibits the ability of GBM cells to create gliomaspheres in vitro.

Still, the amount of β-catenin in CSCs can vary widely. In our previous research (Shevchenko et al., 2019), based on the U87MG cell line, there was a more than sixfold increase in the amount of this protein in CSCs. The results of the present study prove the idea (Fedele, Cerchia, Pegoraro, Sgarra, & Manfioletti, 2019) that GBM cells, expressing isotopic cell surface antigens, do not always have similar molecular phenotype and possess different properties. However, these differences may be determined by the nature of interactions with microenvironment (Dirkse et al., 2019) in gliomasphere, indicating the high plasticity (Friedmann-Morvinski, 2014) of CSCs as a key property of this cell type.

The high proliferation activity of Wnt signaling pathway in CSCs is proven by more than threefold higher upregulation level of Disheveled 1 (DVL1). When cells receive Wnt protein, it binds the membrane receptor of the Frizzled family that, in its turn, activates the Disheveled, inhibiting the destruction complex, thus, resulting in a lower speed of β-catenin degradation. Our study showed the amount of DVL1 in CSCs to be 3.31-fold larger, as compared with that in DGCs.

FRAT Regulator of WNT signaling pathway 1 (FRAT1) is a component of the canonical Wnt signaling pathway, directly responsible for regulating colon cancer cells proliferation (Zhu, Guo, Wang, & Yu, 2016), it ensures the creation of hepatocellular carcinoma cells with high invasive activity (Fan, Du, Liu, & Chen, 2016) and is associated with the aggressive phenotype of glial brain cancer cells (Guo et al., 2015). Our study had the amount of FRAT1 in CSCs 1.08-fold more than that in DGCs.

GSK3β is an important component of the β-catenin destruction complex, it ensures GBM cells survival and proliferation, inhibits apoptosis (Majewska & Szeliga, 2017), participates in repairing the DNA double-strand breaks (Yang et al., 2017), caused by radiation. Our study demonstrated the slight increase in the amount of this protein in CSCs, extracted from gliomaspheres. GSK3β expression level is likely to depend on microenvironment influence and can vary widely. Low content of FRAT1 and GSK3β in CSCs as compared with DGCs does not prove the popular idea (Kim, Park, Jun, et al., 2017) of total upregulation of Wnt signaling pathway in glioblastoma CSCs.

RUVBL1 is a nuclear protein, necessary for cancer cells growth (Mao & Houry, 2017), it is crucial for mitosis, chromatin remodeling, transcription and DNA repairs. As the experiment suggests, RUVBL1 level is significantly increased in CSCs as compared with DGCs. In our study the amount of RUVBL1 in CSCs was 3.22-fold higher than in DGCs, giving the ground to consider this proteins as a potential target for CSCs regulation. Still, proteins of the non-canonical Wnt signaling that regulates the processes of morphogenesis and cell polarity, are worth to be analyzed as well (Table 3).

Ras homolog gene family, member A (RHOA) is a component of the non-canonical Wnt signaling pathway (Fig. 2), it is a part of Rho small GTPases family that is responsible for modifying actin cytoskeleton, regulating key properties of normal stem cells and CSCs activation (Yoon et al., 2016), ensuring their chemotherapy resistance. In our study RHOA was 2.88-fold more upregulated in CSCs, as compared with DGCs.

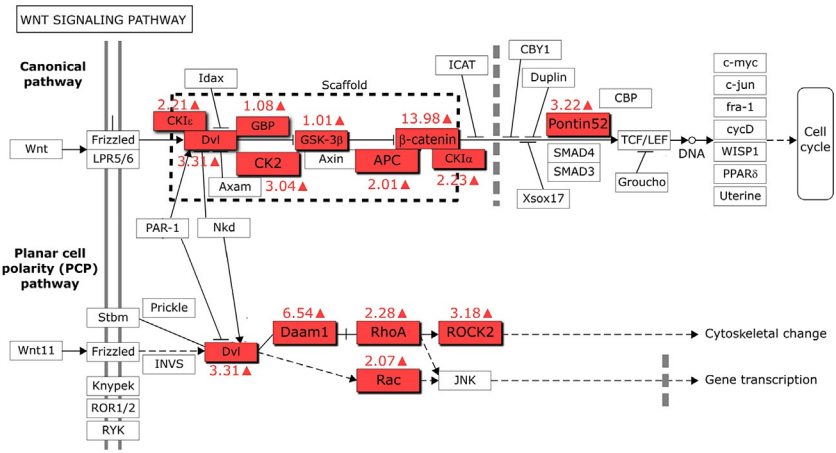

Fig. 2 Wnt signaling pathway according to the Kyoto Encyclopedia of Genes and Genomes database. The red marker indicates upregulated proteins in the cancer stem cells of glioblastoma.

Rho-associated, coiled-coil containing protein kinase 2 (ROCK2) is a component of the non-canonical Wnt signaling cascade (Fig. 2), like RHOA, it belongs to the small GTPase RhoA family. ROCK2 function is related to regulating cell mobility and polarity, it prevents migration of inflammatory cells, acts as a negative regulator of VEGF-induced activation of endothelial cells. In our study ROCK2 was 3.18 more upregulated in CSCs, as compared with DGCs. Higher expression level of RHOA and ROCK2 is typical (Pranatharthi, Ross, & Srivastava, 2016; Wei, Surma, Shi, Lambert-Cheatham, & Shi, 2016) for cells of the most aggressive cancer types.

Ras-related C3 botulinum toxin substrate 2 (RAC2) is a cytosolic protein and a component of the non-canonical Wnt signaling pathway (Fig. 2) from the small GTPase family. RAC2 is a small G-protein and regulates cell movement, proliferation, and survival. In our study ROCK2 was 2.07-fold more upregulated in CSCs than in DGCs. Inhibition of this protein hinders gliomaspheres creation and Cd133+ and Sox 2 expression (Lai et al., 2017).

Disheveled-associated activator of morphogenesis 1 (DAAM1) is an indispensable component of the Wnt signaling cascade (Fig. 1), involved in cell mobility, adhesion and migration. This protein regulates the Wnt5a-induced invasion of GBM cells (Liu, Yan, Li, et al., 2018). Our study showed the 6.15-fold higher upregulation level of DAAM1 in CSCs as compared with DGCs, leading us to an assumption of its potential for becoming a target in CSCs regulation.

In the light of the above mentioned, special attention should be paid to β-catenin, a key protein of the canonical Wnt signaling pathway, that has 13-fold higher upregulation level in CSCs, and DAAM1—a protein of non-canonical Wnt signaling pathway—that is also involved in Wnt/Ca2+ and planar cell polarity pathway (PCP). The first target could be used for regulation of the CSCs reproductive properties, the second one—for suppression of invasive processes. Undoubtedly, GBM growth always results from the complex coordination of those processes that has been demonstrated through studying Wnt signaling pathway in CSCs. Practically speaking, including β-catenin inhibitors into a certain GBM chemotherapy protocol together with using temozolomide and antiangiogenic agent is worth considering and requires further studies.

To sum up, human glioblastoma CSCs have eight upregulated proteins of the canonical Wnt signaling cascade (APC, CSNK1E, CSNK1A, CSNK2A2, CSNK2B, CTNNB1, DVL1, RUVBL) and four proteins of non-canonical Wnt pathway (RHOA, ROCK2, RAC2, DAAM1). β-Catenin (CTNNB1) deserves special attention among the proteins with its more than 13-fold synthesis level increase, since it is the protein that becomes a potential target for managing the key properties of GBM CSCs.

Funding

This study was funded by the Ministry of Science and Higher Education of Russia (Contract no. 14.584.21.0027 ID: RFMEFI58417X0027).

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

VS and NA prepared and analyzed the samples, as well as performed the cell lysis, chromatography and mass spectrometry, and contributed to the bioinformatics analysis. SZ provided and performed the statistical analysis and was responsible for the mathematical process of the results. YH, HS and AS discussed, analyzed and interpreted the results of the study, and also worked on the manuscript. IB 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.

Conflict of interest

The authors declare there are no conflicts of interests.

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