Array expression meta-analysis of cancer stem cell genes identifies upregulation of *PODXL* especially in *DCC* low expression meningiomas

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Abstract

**Background**

Meningiomas are the most common intracranial tumors, with a subset of cases bearing a progressive phenotype. The DCC netrin 1 receptor (*DCC*) is a candidate gene for early meningioma progression. Cancer stem cell (CSC) genes are emerging as cancer therapeutic targets, as their expression is frequently associated with aggressive tumor phenotypes. The main objective of the study was to identify deregulated CSC genes in meningiomas.

**Materials and methods**

Interrogating two expression data repositories, significantly differentially expressed genes (DEGs) were determined using *DCC* low vs. *DCC* high expression groups and WHO grade I (GI) vs. grade II + grade III (GII + GIII) comparison groups. Human stem cell (SC) genes were compiled from two published data sets and were extracted from the DEG lists. Biofunctional analysis was performed to assess associations between genes or molecules.

**Results**

In the *DCC* low vs. *DCC* high expression groups, we assessed seven studies representing each between seven and 58 samples. The type I transmembrane protein podocalyxin like (*PODXL*) was markedly upregulated in *DCC* low expression meningiomas in six studies. Other CSC genes repeatedly deregulated included, e.g., BMP/retinoic acid inducible neural specific 1 (*BRINP1*), prominin 1 (*PROM1*), solute carrier family 24 member 3 (*SLC24A3*), Rho GTPase activating protein 28 (*ARHGAP28*), Kruppel like factor 5 (*KLF5*), and leucine rich repeat containing G protein-coupled receptor 4 (*LGR4*). In the GI vs. GII + GIII comparison groups, we assessed six studies representing each between nine and 68 samples. DNA topoisomerase 2-alpha (*TOP2A*) was markedly upregulated in GII + GIII meningiomas in
four studies. Other CSC genes repeatedly deregulated included, e.g., ARHGAP28 and PODXL. Network analysis revealed associations of molecules with, e.g., cellular development and movement; nervous system development and function; and cancer.

Conclusions

This meta-analysis on meningiomas identified a comprehensive list of deregulated CSC genes across different array expression studies. Especially, PODXL is of interest for functional assessment in progressive meningiomas.

Introduction

Based on ultrastructural and histologic similarities, meningiomas are described to originate from arachnoidal cells. Meningiomas account for approximately 30% of all primary intracranial brain tumors [1]. The majority of meningiomas are considered benign and are commonly treated by surgery; however, a minority of cases progress further or recur. The clinical behavior of meningiomas is assessed by using the grading system where benign meningiomas are classified as WHO grade I (GI) tumors and more aggressive meningiomas as atypical, grade II (GII) tumors, or as anaplastic, grade III (GIII) tumors. Molecular markers predicting aggressiveness of meningiomas are not well-known [2].

Cancer stem cells (CSCs) represent a population of cells that are implicated in cancer progression as well as chemo- and radioresistance [3–6]. Therefore, CSCs are emerging as therapeutic targets for improving treatment of aggressive types of cancer [7, 8]. The involvement of CSCs in different types of cancer is seemingly complex. Thus far, only a limited number of CSC genes and mechanisms have been identified and characterized in meningiomas [9–13]. Meningioma stromal mesenchymal stem-like cells, similar to bone marrow mesenchymal stem cells (SC), have been detected in meningiomas [3]. A recent immunohistochemical survey in GI meningiomas identified a number of SC markers, including OCT4, NANOG, SOX2, KLF4, and c-MYC, on microvessels that led to the suggestion that these vessels may be associated with meningioma initiation [14]. An immunohistochemical study detected higher expression of nestin (NES), SOX2, and prominin 1 (PROM1), also known as CD133, in more progressive meningiomas [9]. In meningioma cultures, cells with pleomorphic characteristics show markedly increased numbers of CSCs scoring positive for PROM1 and SOX2, or AGR2 and BMI1 [12]. Similarly, meningioma cells expressing PROM1 revealed a higher proliferation rate and the formation of tumorspheres [13].

In the present study, we focused on state-of-the art genome-wide array technology to identify CSC genes, which are deregulated in meningiomas. We performed a meta-analysis using publicly accessible array studies to investigate CSC expression profiles in meningiomas grouped according to their DCC netrin 1 receptor (DCC) expression levels or by tumor grade. DCC functions as a tumor suppressor in various types including malignant astrocytomas where its reduced expression correlates with unfavorable prognosis [15]. In meningiomas, an array expression study has identified DCC as a candidate gene for early meningioma progression [16]. This finding was supported by the fact that 14 of 416 differentially expressed genes (DEGs) that were identified between DCC low and DCC high expression meningiomas were shared with 49 DEGs that were determined using a meta-analysis data set generated from a comparison of less vs. more progressive meningiomas [17]. In contrast, only four of 249 DEGs from the comparison GI vs. GII meningiomas were shared with the 49 DEGs of the meta-
analysis data set. Array meta-analyses using post-statistical assessment of DEG data sets have been successfully conducted in cancer research [17, 18].

Material and methods

Interrogation of data base repositories

We interrogated the Gene Expression Omnibus (GEO) [19] and the ArrayExpress [20] repositories in March 2018 to retrieve published array expression data sets on meningiomas. The search query included meningioma AND expression AND array AND human.

Transcriptome analysis

From the selected array studies, fulfilling the search criteria [21], binary CEL files containing the feature-level extraction output data were imported into the Transcriptome Analysis Console (TAC) version 4.0.1 (ThermoFisher Scientific Inc., Waltham, MA USA; previously branded Affymetrix microarray solutions) that includes the LIMMA (linear modeling for microarrays) package from Bioconductor [22]. In TAC, binary CEL files of each study were normalized utilizing robust multiarray analysis (RMA) algorithm. Array QC metrics analyses were performed using principal component analyses (PCA), 5' and 3' hybridization and labeling control graphs, and signal (log2) intensity bars. Lists of differentially expressed probe sets were generated based on the chosen parameters, including samples (N ≥ 2 per comparison arm) grouped according to the DCC expression values or tumor grade [16]. Samples that ranked in the lowest 30% or in the highest 50% of the DCC log2 intensity values were considered as DCC low or DCC high expression samples, respectively, with the exception of samples from submission GEO88720. This study employed a different array technology resulting in disproportionate log2 intensity values in comparison with other data sets and DCC high expression samples were considered to rank in the highest 30% of the intensity values. In all selected studies, the unitless DCC log2 intensity values ranged between 3.55 and 4.73. Samples with DCC medium expression levels (30% - 50%) were not considered for further DEG analysis. For U133 Plus 2.0 arrays, the 238914_at probe set was utilized to assess DCC expression levels of the samples. Files used for annotation were HuGene-1_0-st-v1.na36hg19.transcript.csv, HuGene-2_1-st-v1.na36hg19.transcript.csv, and HG-U133_Plus_2_na36.annot.csv. Threshold of significance for differentially expressed probe sets and for the subsequently generated DEGs was a p-value < 0.05 and fold change (FC) > 2. Where indicated, a false discovery rate (FDR)-adjusted p-value < 0.05 was employed. In general, the meta-analysis adhered to recommendations outlined in a practical guidance for meta-analysis of gene expression array data sets [21].

CSC gene selection

We compiled a list of 366 human SC genes (S1 Table), including embryonic (E) SC and induced pluripotent (iP) SC genes, derived from two SC studies with intersecting gene lists [23, 24]. One study used in first instance amniocytes and the other study an N-glycoproteome as SC resource. The database for annotation, visualization and integrated discovery (DAVID) was employed to convert gene IDs to official gene symbols [25].

Biofunctional analysis

Biological significance of identified CSC genes was interpreted using the Ingenuity Pathway Analysis software (IPA; build version 485516M; Ingenuity Systems, Redwood City, CA) that curates a comprehensive pathway knowledge base. Analysis settings comprised direct and
indirect molecular associations. Significant associations between analyzed data set molecules and frameworks prebuilt or generated de novo by IPA were indicated by Fisher’s exact test p-values. The Molecule Activity Predictor was employed to predict expression effects of a molecule on further pathway/network molecules. Network analysis was performed to explore significance of fit, expressed as a score, between molecules of the uploaded data set and networks related to specific diseases and functions. The upstream analysis module was employed to evaluate in how far differences in target gene expression are effected by upstream regulators [26]. The gene ontology (GO) term finder LAGO (https://go.princeton.edu/LAGO/help.html) was employed to assess overrepresentation of GO terms [27].

Results

Using the search query in GEO and ArrayExpress, identified 37 and 27 data sets, respectively (Fig 1). Exclusion criteria from the repositories included data sets that used custom-made, SNP or miRNA arrays; used discontinued array types or brands; contained < 6 meningioma samples or only in vitro/in vivo samples; comprised not the binary CEL files; or were reanalyzed data sets. Two data sets using HuGene 1.0 ST arrays were performed in the same laboratory and samples were pooled for subsequent analysis. In sum, eight studies from both repositories were selected for further analysis (Table 1). Two of eight studies used HuGene 1.0 ST arrays, one study used HuGene 2.1 ST arrays strips, and five studies employed U133 Plus 2.0 arrays. HuGene 1.0 ST arrays and HuGene 2.1 ST arrays strips represent whole transcript chips that interrogate gene expression levels on average with one probe per exon. In contrast, the U133 Plus 2.0 arrays interrogate expression levels primarily at the 3’-region of the genes. Seven selected studies met the criteria to establish sets of differentially expressed CSC genes from the DCC low vs. DCC high expression groups (Fig 2 and S1 Fig) whereas six studies met the criteria to establish sets of differentially expressed CSC genes from the GI vs. GII + GIII comparison groups [16, 28–35].

DCC low vs. DCC high expression groups

In the DCC low vs. DCC high expression groups, podocalyxin like (PODXL) was significantly upregulated in DCC low expression meningiomas in six of seven studies (Table 2). The solute carrier family 24 member 3 (SLC24A3) was significantly upregulated in four studies. CSC genes that were upregulated in three studies include Rho GTPase activating protein 28 (ARHGAP28), Kruppel like factor 5 (KLF5), and leucine rich repeat containing G protein-coupled receptor 4 (LGR4). BMP/retinoic acid inducible neural specific 1 (BRINP1), and PROM1 were significantly downregulated in DCC low expression meningiomas in five studies. Further CSC genes were downregulated in three or four studies, including ADAM metallopeptidase domain 22 (ADAM22), leucine rich repeats, calponin homology domain containing 4 (LRRN1), olfactomedin-like protein 3 (OLFML3), plexin domain containing 2 (PLXDC2), FRY microtubule binding protein (FRY), neural cell adhesion molecule 1 (NCAM1), and Toll-like receptor 2 (TLR2). Fig 3 displays a merged network of the category diseases and functions (Table 3) based on a number of genes including DCC and genes identified in at least three different data sets from the DCC low vs. DCC high expression groups (Table 2). Integrative network molecules presented in Fig 3 were derived from the knowledge base of the software application. Fig 4 illustrates a merged network of three upstream regulators that were significantly associated with a number of regulated genes (p ≤ 6.27E-04; Table 2).
Fig 1. Flow chart for selecting CSC genes from meningioma array studies. Two publicly accessible array expression repositories were assessed to retrieve up-to-date array expression data sets on meningiomas.

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GI vs. GII + GIII comparison groups

DNA topoisomerase 2-alpha (TOP2A) was significantly upregulated in GII + GIII meningiomas in four studies compared with GI (Table 4). Furthermore, adhesion G protein-coupled receptor G2 (ADGRG2), ARHGAP28, laminin subunit alpha 1 (LAMA1), glucosaminyl (N-acetyl) transferase 2 (1 blood group) (GCNT2), PODXL, semaphorin 6A (SEMA6A), and secreted phosphoprotein 1 (SPP1) were upregulated and ADAM22, FRY microtubule binding protein (FRY), hephaestin (HEPH), and LRRN1 were downregulated in two studies. Fig 5 displays a merged network of the category diseases and functions (Table 3) based on genes identified in at least two different data sets of the GI vs. GII + GIII comparison groups and on interconnecting molecules derived from the knowledge base of the software application.

Discussion

In our biostatistical meta-analysis, we identified CSC genes that, according to their expression profiles, are seemingly associated with DCC low expression meningiomas and, in their content, have not been reported before. We combined two resources to compile a list of SC genes that were used to identify differentially expressed CSC genes, which may exert a function in development and/or progression of meningiomas. One resource generated a reference list containing 250 human SC genes that were detected by transcriptome sequencing (RNA-seq) in cultured human amniocytes, or ESC and iPSC and were reported to have a functional relevance in SC maintenance. The authors commented that amniocytes inhere a unique SC identity and exist in a developmentally intermediate, hence uncommitted state [23]. The other resource employed cell surface capture technology and expression array assays to compile a list of 120 human PSC surface N-glycoproteins that were separated from those proteins that were also abundantly expressed in human fibroblasts or other non-diseased tissues [24]. The authors stated that the development of the cell surface capture technology enabled identification of proteins that otherwise are rarely detectable at the transcriptional level.

Evaluation of DCC low vs. DCC high expression groups generally identified more CSC genes than the GI vs. GII + GIII comparison groups. This can be partially attributed to the fact that a subset of benign meningiomas bear the capacity to evolve into more aggressive meningiomas and DCC expression levels are an appropriate molecular discriminator reflecting this characteristics [16]. In addition, it should be stated that the grading system for meningiomas remains suboptimal [36]. Array expression studies on meningiomas are preferentially conducted on a specific array platform [37], which is one of the reasons why this meta-analysis
contained only studies from this brand; however, the selected array studies were performed with three different array types limiting the accumulation of array type specific DEGs. Furthermore, the selected studies were conducted in different geographical regions representing a heterogeneous population group.

Upregulation of genes in DCC low expression and/or GII + GIII meningiomas

Upregulation of *PODXL* in DCC low expression and GII + GIII meningiomas. *PODXL* was upregulated in the current study, especially in DCC low expression groups. *PODXL* is a sialomucin and a type I transmembrane protein related to the hematopoietic SC factor CD34 and PODXL2. It exerts functions in regulating cell polarity through actin-dependent microvilli
Table 2. CSC genes significantly deregulated in DCC low vs. DCC high expression groups.

| GEO/ArrayExpress accession number | CSC genes upregulated in DCC low vs. DCC high | CSC genes downregulated in DCC low vs. DCC high |
|-----------------------------------|-----------------------------------------------|------------------------------------------------|
| GSE54934                          | LPAR3, PODXL, SLC24A3                        | ADAM22, CNTFR, LRRN1, OLFML3, PODXL2, PROM1   |
| GSE77259, GSE100534               | LAMA1                                         | BRINP1, CNTFR, FRY, GRID2, NCAM1, OLFML3, PODXL2, PROM1 |
| GSEA88720                         | GAL, KLF2, KLF4, KLF5, LGR4, PODXL, RBX1, SLC24A3 | PLXDC2, TLR2                                   |
| GSE16581                          | ADGRG2, ANOS1, ARHGAP28, CCND1, CRABP1, EPHA7, GCNT2, KLF5, LGR4, LPAR3, PODXL, RBX1, SLC24A3 | ADAM22, BRINP1, CNTFR, FRY, GRID2, NCAM1, OLFML3, PODXL2, PROM1 |
| GSE68015                          | ARHGAP28, JARID2, PODXL                       | ADAM22, BRINP1, GABRB3, LRRN1, NCAM1, NTN1, THY1 |
| E-MTAB-1852                       | PODXL                                         | ATP13A3, BRINP1, IL27RA, KCNE3, MTF2, PLXDC2, PROM1, SLC2A12, SP1, TLR2, ZIC3 |
| E-GEOD-9438                       | APL2, ARHGAP26, CCND1, CD44, FLT1, GCNT2, ID1, KITLG, KLF5, LGR4, PODXL, SALL4, SLC24A3 | ADAM22, AXIN2, BRINP1, CDH3, EED, FRY, LRRN1, NANOS1, OLFML3, PLXDC2, PROM1, SLC15A2, TLR2 |

1CSC genes significantly deregulated in at least three studies. GO annotations of these genes and of DCC are presented in S2 Table.

Expression meta-analysis: Upregulation of PODXL in DCC low expression meningiomas

formation and was revealed to have an anti-adhesin capacity, which increases the adherence of cells to immobilized ligands and accelerates the rate of migration and cell-cell contacts [38, 39]. A meta-analysis on 12 studies revealed that high PODXL expression is significantly associated with worse overall survival in different cancer types [40]. Specifically, PODXL overexpression in MCF-7 breast cancer cells resulted in cell delamination from monolayers by perturbing cell junctions [41]. Furthermore, high PODXL expression was identified to be an independent factor for unfavorable prognosis in breast cancer patients. In mice, induced overexpression of PODXL led MCF-7 cell clusters to bud off from the primary tumor and invade mouse mammary gland stroma [42]. Moreover, in lung adenocarcinoma, PODXL overexpression induced epithelial-to-mesenchymal transition (EMT) [43].

Notably, expression of PODXL exerted a positive correlation with stem-like and EMT core signatures, and contributed to unfavorable prognosis in patients with colon cancer [44]. In addition, PODXL serves a critical role in cancer stemness, invasiveness and conferred chemotherapy resistance in HT29 and HCT15 colon cancer cells that expressed high PODXL levels. Compared with PODXL negative cells, PODXL positive cells express increased levels of progenitor/SC markers Musashi1, SOX2, and BMI1 [45]. The involvement of PODXL in glioblastoma multiforme (GBM) stem-like cell proliferation was demonstrated with PODXL positive cell populations in two GBM oncosphere lines that exhibited significantly elevated growth compared with PODXL negative cells [45]. In astrocytomas, high expression of PODXL is associated with unfavorable prognosis [46]. A newly generated monoclonal antibody directed against an extracellular epitope of PODXL was used in immunohistochemistry to specifically detect PODXL expressing normal renal cells, as well as colorectal, and breast cancer cells [47, 48]. Of notice, in a xenograft mouse model of colorectal adenocarcinoma, a human-mouse chimeric anti-PODXL antibody was shown to inhibit tumor growth of PODXL expressing cancer cells [49]. Preliminary findings of a current project in our labs, wherein we are studying the immunofluorescence staining patterns of PODXL and other stem cell markers in cell cultures derived from progressive meningiomas, let us suggest that PODXL is expressed in these entities. Therefore, based on its association with tumor progression in a number of cancer types, assessment of PODXL for its functional implications in more aggressive meningiomas is envisaged.
Upregulation of TOP2A in GII + GIII meningiomas. TOP2A was determined to be significantly upregulated in four GII + GIII groups but not in any DCC low expression group. Expression level of the cell cycle-dependent DNA topoisomerase peaks at the G2/M cell cycle phase. One of its major functions is decatenation of chromosomes during mitosis. Posttranslational modification of TOP2A, supporting its functions, include phosphorylation, ubiquitination, SUMOylation, and acetylation [50]. Interactions of TOP2A with cell cycle checkpoint protein MDC1 are associated with checkpoint activation and maintenance of genome stability. Another critical interactor of TOP2A is the tumor suppressor and DNA repair gene BRCA1. Abnormal activities of TOP2A and related molecules attribute to the instability of tumor genomes that is linked to tumor progression. TOP2A and HER2 gene amplifications frequently
coincide with a number of malignancies including breast, ovarian, pancreatic, and esophageal/gastroesophageal cancer [51]. An array meta-analysis revealed approximately 10-fold higher expression of TOP2A in GIII compared with GI meningiomas [52]. Due to its central role in decatenation, a number of anticancer compounds have been approved that are either TOP2A poisoning, such as doxorubicin, mitoxantrone, etoposide, and teniposide or TOP2A catalytic inhibitors, such as epirubicin, and idarubicin [50].

Upregulation of SLC24A3, ARHGAP28, KLF5, and LGR4 in DCC low expression and/or GII + GIII meningiomas. SLC24A3, also known as NCKX3, is a member of the sodium/potassium/calcium exchangers. Its expression is most abundant in brain and smooth muscle [53, 54]. In tumorspheres of EBV positive nasopharyngeal carcinoma cells, SLC24A3 was one of several CSC markers that were upregulated compared to corresponding monolayer cells [55]. Furthermore, tumorspheres were enriched in CD44 positive cells and these cells exhibited higher chemotherapeutic resistance. Knockdown of expression of transcription factor TFAP2C in hormone responsive breast carcinoma cells resulted in deregulation of a number of target genes including SLC24A3, which was downregulated [56]. ARHGAP28 encodes a Rho GTPase activating protein (RhoGAP) that downregulates RhoA activity resulting in inhibition of actin stress fiber formation [57]. In mouse embryos, Arhgap28 exhibits a spatial and temporal expression pattern in tissues where a stiff extracellular matrix is assembled. ARHGAP28 has been previously reported in array expression studies as a DEG and, using a platform not included in our meta-analysis, it was found to be upregulated in GII and, with statistical significance, in GIII compared with GI meningiomas [58]. In GBM-derived radioresistant tumor-initiating and PROM1-positive cell populations, ARHGAP28 was upregulated upon treatment with the anticancer compound resveratrol that resulted in induction of apoptosis and elevated radiosensitivity through repression of STAT3 pathway signaling [59]. KLF5 is an evolutionary conserved zinc finger transcription factor that is known to participate in a number of key pathways including the Wnt, Ras, TGFβ, and Notch signaling pathways [60]. Knockdown experiments in murine embryonic SCs demonstrated that Klf5 exerts critical functions in inhibiting mesoderm differentiation [61]. Based on its implications in various signaling pathways and cancers, KLF5 has become a therapeutic target for cancer therapy development [60]. LGR4 is a G-protein-coupled transmembrane receptor for R-spondins and a regulator of the Wnt/β-catenin signaling pathway [62, 63]. Higher LGR4 expression correlates with unfavorable prognosis in breast and prostate cancer [62, 64]. In a xenograft mouse model, LGR4 silencing in prostate cancer cells led to a delay of metastases and reduced expression of EMT markers [64].
Downregulation of genes in DCC low expression and/or GII + GIII meningiomas

Downregulation of BRINP1 and PROM1 in DCC low expression meningiomas.

BRINP1, alias DBC1, is a putative tumor suppressor gene that is a negative regulator of G1/S transition [65]. Reduced expression of BRINP1 caused by different mechanisms, such as promoter hypermethylation, has been revealed in a number of tumor types including lymphoproliferative malignancies, non-small cell lung carcinomas, and astrocytomas [66–68]. Furthermore, in non-muscle-invasive bladder cancer, lower expression of BRINP1 is associated with unfavorable prognosis [69]. PROM1 is a pentaspan transmembrane glycoprotein that has been described by different research groups as a CSC factor in meningiomas [9, 10, 12, 13]. In our survey, PROM1 was markedly downregulated in DCC low expression meningiomas.

Table 4. CSC genes significantly deregulated in GI vs. GII + GIII comparison groups.

| GEO/ArrayExpress accession number | CSC genes upregulated in GII + GIII vs. GI | CSC genes downregulated in GII + GIII vs. GI |
|----------------------------------|-------------------------------------------|-------------------------------------------|
| GSE54934                         | ARHGAP28<sup>1</sup>, PODXL<sup>1</sup>, SEMA6A<sup>1</sup>, TOP2A<sup>1</sup> | GJA1, LRRN1<sup>1</sup> |
| GSE77259, GSE100534              | ANOS1, CD24, GPM6A, PODXL<sup>1</sup>, PTRZ1, TOP2A<sup>1</sup>, ZIC3 | CNTFR |
| GSE88720                         | CLUL1, GABRA3, GCNT2<sup>1</sup> | HEPH<sup>1</sup> |
| GSE16581                         | ADGRG2<sup>2</sup>, ARHGAP28<sup>1</sup>, CRABP1, EPHA7<sup>9</sup>, LAMA1<sup>1</sup>, OLFM4<sup>9</sup>, SPP1<sup>1,4</sup>, TOP2A<sup>1,4</sup> | ADAM22<sup>1</sup>, FRY<sup>1</sup>, HEPH<sup>1</sup>, LRRN1<sup>1</sup>, NPY1R, TLE1 |
| GSE68015                         | CBX5, CCND2, CD44, DPP6, EZH2, KLF12, LAMA1<sup>1</sup>, LRIG1, MED13L, RBP4, SALL1, SEMA6A<sup>1</sup>, SPP1<sup>1</sup>, TNFRSF21, TOP2A<sup>1</sup> | ADAM22<sup>1</sup>, CDH3, FRY<sup>1</sup> |
| E-MEXP-3586                      | ADGRG2<sup>2</sup>, GCNT2<sup>1</sup>, IL27RA, KITLG, NANOS1, SLC15A2 | |
malignant meningiomas in five array expression studies. Several controversies remain connected with the expression of the gene, its role as CSC marker, and its impact on tumor progression [70]. Multiple transcript variants of PROM1 encoding different isoforms have been described [71]. Immunostaining in consecutive tissues using different PROM1 antibodies showed different protein expressions and characteristics [72]. GBM tumors, initiated directly from biopsies and engrafted intracerebrally into nude rats, expressed little or no PROM1 [73]. During serial passaging in vivo, the tumors gradually displayed increased PROM1 expression. In mice, GBM xenografts from PROM1-negative cells showed more proliferative and angiogenic features compared to that from PROM1-positive cells [74]. Previous work in meningiomas revealed different co-expression patterns of PROM1 and SOX2 in tissues compared to corresponding cell lines [12]. Whereas the average number of cells positive for both SOX2 and PROM1

Fig 5. The top two merged networks of genes that were present in at least two different data sets from the GI vs. GII + GIII comparison groups. Genes/molecules include ADAM22, ADGRG2, ARHGA28, FRY, GCNT2, HEPH, LAMA1, LRRN1, PODXL, SEMA6A, SPP1, and TOP2A. Integrative network molecules comprise, adenosine A3 receptor (ADORA3), alkB homolog 5, RNA demethylase (ALKBHS), adaptor related protein complex 4 subunit beta 1 (AP4B1), adaptor related protein complex 4 subunit sigma 1 (AP4S1), amyloid beta precursor protein (APP), beta-estradiol, caveolin 1 (CAV1), cyclin dependent kinase like 2 (CDKL2), chondroitin sulfate B, CKLF like MARVEL transmembrane domain containing 3 (CMTM3), catenin beta 1 (CTNNB1), dynactin subunit 6 (DCTN6), erb-b2 receptor tyrosine kinase 2 (ERBB2), ERK1/2, Gpcr, glutathione S-transferase zeta 1 (GSTZ1), heterogeneous nuclear ribonucleoprotein A2/B1 (HNRNPA2B1), interferon gamma (IFNG), IL12 (complex), Irp, ISL LIM homeobox 1 (ISL1), lysine methyltransferase 2D (KMT2D), laminin subunit alpha 3 (LAMA3), long intergenic non-protein coding RNA 461 (LINC00461), Mek, NFkB (complex), O-GlcNAcase (OGA), PI3K (complex), POU class homeobox 1 (POU4F1), pyrophosphate, regulating synaptic membrane exocytosis 4 (RIMS4), semaphorin 6C (SEMA6C), solute carrier family 39 member 6 (SLC39A6), SPARC like 1 (SPARCL1), TCF, TEP5IN adaptor related protein complex 4 accessory protein (TEPSIN), and transforming growth factor beta 1 (TGFB1). S3 Table contains the relationships of molecules.

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significantly increased in GII + GIII meningioma cell lines, they significantly decreased in GII + GIII meningioma tissues compared to GI entities [10]. Taken together, these observations are compatible with a cyclic and microenvironmental-influenced expression of PROM1 [75, 76].

**Downregulation of ADAM22, LRRN1, OLFML3, PLXDC2, FRY, NCAM1, and TLR2 in DCC low expression and/or GII + GIII meningiomas.** ADAM22 is a member of the ADAM family of disintegrins. Downregulation of ADAM22 by siRNA in endocrine resistant cell populations resulted in impaired cell migration and reconstituted differentiation [77]. Similarly, treatment with recombinant LGI1, that serves as a ligand for ADAM22, impaired cell migration. In breast cancer, ADAM22 has been identified as an estrogen receptor independent predictor of disease-free survival and has been assessed as a target for endocrine resistant breast cancer therapy [78]. LRRN1 is associated with neuroepithelial boundary formations and its temporal expression changes during mammalian neural progenitor cell development [79, 80]. In embryonic SCs, LRRN1, alias NLRR1, was one of four genes markedly higher expressed than in fibroblasts [81] and in a xenograft mouse model, LRRN1 expression in neuroblastoma cells resulted in enhanced tumor growth [82]. OLFML3 has been identified as a proangiogenic factor [83]. It binds to BMP4 and in xenograft mice models of Lewis lung carcinoma cells, Olfrm3 was expressed in tumor endothelial cells and pericytes. OLFML3 may constitute a target for antiangiogenic therapy as further in vivo experiments demonstrated that anti-Olfml3 antibodies impaired tumor growth and angiogenesis. Comparably higher expression of Olfrm3 has been detected in the stroma transcriptome of osteoblastic bone metastases of prostate cancer [84]. PLXDC2 is a type I transmembrane protein that has been identified as component of network molecules implicated in modulating proliferation and differentiation of the developing nervous system. In embryonic neuroepithelial cells, PLXDC2 functions as a mitogen [85]. The evolutionarily conserved FRY protein is a microtubule binding factor that exerts critical functions in maintaining structural integrity of mitotic chromosomes in spindle bipolarity [86]. Especially, FRY silencing in in vitro experiments resulted in chromosome misalignment and multipolar spindle formation. NCAM1 is a cell adhesion molecule expressed preferentially in neurons, glia, skeletal muscles, and T cells. In vitro and in vivo experiments indicated that NCAM1 is necessary for EMT induction and maintenance and furthermore, high NCAM1 expression level was found to be associated with tumor invasion [87]. In a mouse model of intracerebral hemorrhage, increased expression of Tlr2 was observed, resulting in a proinflammatory gene profile with activation of Mmp9 [88]. Furthermore, blood-brain-barrier permeability was decreased in Tlr2 knockout mice compared with wild type mice. In vitro microglial experiments demonstrated that Tlr2 induces random-like migration involving the Akt pathway [89].

In conclusion, there is compiling evidence that CSC are present in meningiomas and in this regard, a number of crucial SC markers are used to characterize CSC population; yet, the functional relevance of CSC in meningiomas requires further detailed studies [90]. Our meta-analysis identified a number of CSC genes that were repeatedly deregulated in the analyzed data sets in either or both of the comparison groups. Some of the identified CSC genes already represent valuable targets for specific inhibitors while others may represent new candidate genes. In particular, POXDL, which encodes a sialomucin and type I transmembrane protein and known to be involved in cell migration processes, is of interest for assessment of its functional implications in progressive meningiomas.

**Supporting information**

S1 Fig. Categorization of GEO data sets according to their DCC expression values. Array expression studies on meningiomas were used to extract expression of CSC genes from human

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SC and iPSC gene compilations. (A) GSE54934, (B) GSE88720, (C) GSE16581, (D) GSE68015, (E) E-MTAB-1852, and (F) E-GEOD-9438. Blue bars, DCC high expression samples; red bars, DCC medium expression samples; and purple bars, DCC low expression samples. Dots indicate expression values of individual samples. Except for GSE68015, significance between DCC low and DCC high expression groups is based on an FDR-adjusted p-value < 0.05. Of notice, using normalized, unlogged expression values and, with exception of GSE88720 samples, utilizing adapted, but unified, threshold values, the same gene lists for DCC low and DCC high expression samples were generated as specified in Table 2.

**S1 Table. Compiled list of SC genes.** List is combined from two studies on SC genes [23, 24] and served to extract CSC genes from array expression studies on meningiomas.

**S2 Table. Gene ontology annotations for the data set genes used to generate Figs 3 and 5.** GO term finder LAGO was used to assess overrepresentation of GO terms.

**S3 Table. Relationships of molecules of Figs 3 and 5.**

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**References**

1. Shibuya M. Pathology and Molecular Genetics of Meningioma: Recent Advances. Neurologia medico-chirurgica. 2015; 55(1):14–27. https://doi.org/10.2176/nmc.ra.2014-0233 PMID: 25744347; PubMed Central PMCID: PMC4533397.
2. Pećina-Slaus N, Kafka A, Lechpamar M. Molecular Genetics of Intracranial Meningiomas with Emphasis on Canonical Wnt Signalling. Cancers. 2016; 8(7):67. https://doi.org/10.3390/cancers8070067 PMID: 27429002

3. Lim HY, Kim KM, Kim BK, Shim JK, Lee JH, Huh YM, et al. Isolation of mesenchymal stem-like cells in meningioma specimens. International journal of oncology. 2013; 43(4):1260–8. Epub 2013/08/08. https://doi.org/10.3892/ijo.2013.2053 PMID: 23921459.

4. Krause M, Dubrovskas A, Linge A, Baumann M. Cancer stem cells: Radioresistance, prediction of radiotherapy outcome and specific targets for combined treatments. Advanced drug delivery reviews. 2017; 109:63–73. Epub 2016/02/16. https://doi.org/10.1016/j.addr.2016.02.002 PMID: 26877102.

5. Abdullah LN, Chow EKH. Mechanisms of chemoresistance in cancer stem cells. Clinical and Translational Medicine. 2013; 2:3. https://doi.org/10.1186/2001-1326-2-3 PMID: 23369605; PubMed Central PMCID: PMCPMC3565873.

6. Schonberg DL, Lubelski D, Miller TE, Rich JN. Brain tumor stem cells: Molecular characteristics and their impact on therapy. Molecular aspects of medicine. 2014; 39:82–101. Epub 2013/07/09. https://doi.org/10.1016/j.mam.2013.06.004 PMID: 23831316; PubMed Central PMCID: PMCPMC3866208.

7. Chiu HY, Lai WK, Huang LC, Huang SM, Chueh SH, Ma HI, et al. Valproic acid promotes radiosensitization in meningioma stem-like cells. Oncotarget. 2015; 6(12):9959–69. Epub 2015/04/22. https://doi.org/10.18632/oncotarget.3692 PMID: 25895030; PubMed Central PMCID: PMCPMC4496410.

8. Codd AS, Kanaseki T, Torigo T, Tabi Z. Cancer stem cells as targets for immunotherapy. Immunology. 2018; 153(3):304–14. Epub 2017/11/19. https://doi.org/10.1111/imm.12866 PMID: 29150846; PubMed Central PMCID: PMCPMC5795182.

9. Xiao ZY, Chen XJ, Pan Q, Yang QZ, Li KZ. Expression of Nestin, CD133 and Sox2 in Meningiomas. Turkish neurosurgery. 2017;10.5137/1019-5149.JTN.21234-17.2. Epub 2018/01/26. https://doi.org/10.1186/s12935-018-0571-6 PMID: 29368320; PubMed Central PMCID: PMCPMC4496410.

10. Alghamdi F, Qashqari H, et al. Pleomorphism and drug resistant cancer stem cells are characteristic of aggressive primary meningioma cell lines. Cancer Cell Int. 2017; 17:72. Epub 2017/07/25. https://doi.org/10.1186/s12935-017-0441-7 PMID: 28736504; PubMed Central PMCID: PMCPMC5521079.

11. Schulten HJ, Hussein D, Al-Adwani F, Karim S, Al-Maghrabi J, Al-Sharif M, et al. Expression meta-analysis: Upregulation of PODXL in DCC-low expression meningiomas. PLOS ONE | https://doi.org/10.1371/journal.pone.0215452 May 13, 2019 15 / 20
Expression meta-analysis: Upregulation of *PODXL* in DCC low expression meningiomas

19. Clough E, Barrett T. The Gene Expression Omnibus Database. Methods in molecular biology (Clifton, NJ). 2016; 1418:93–110. Epub 2016/03/24. https://doi.org/10.1007/978-1-4939-3578-9_5 PMID: 27008011; PubMed Central PMCID: PMCPMC4944384.

20. Rustici G, Kolesnikov N, Brandizi M, Burdett T, Dylag M, Emam I, et al. ArrayExpress update—trends in database growth and links to data analysis tools. Nucleic acids research. 2013; 41(Database issue): D987–90. Epub 2012/11/30. https://doi.org/10.1093/nar/gks1174 PMID: 23193272; PubMed Central PMCID: PMCPMC3531147.

21. Ramasamy A, Mondry A, Holmes CC, Altman DG. Key issues in conducting a meta-analysis of gene expression microarray datasets. PLoS medicine. 2008; 5(9):e184. Epub 2008/09/05. https://doi.org/10.1371/journal.pmed.0050184 PMID: 18767902; PubMed Central PMCID: PMCPMC2528050.

22. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic acids research. 2015; 43(7):e47. Epub 2015/01/22. https://doi.org/10.1093/nar/gkv007 PMID: 25605792; PubMed Central PMCID: PMCPMC4025510.

23. Maguire CT, Demarest BL, Hill JT, Palmer JD, Brothman AR, Yost HJ, et al. Genome-wide analysis reveals the unique stem cell identity of human amniocytes. PloS one. 2013; 8(1):e53372. Epub 2013/01/18. https://doi.org/10.1371/journal.pone.0053372 PMID: 23326421; PubMed Central PMCID: PMCPMC3542377.

24. Boheler KR, Bhattacharya S, Kropp EM, Chuppa S, Riordon DR, Bausch-Fluck D, et al. A human pluripotent stem cell surface N-glycoproteome resource reveals markers, extracellular epitopes, and drug targets. Stem cell reports. 2014; 3(1):185–203. Epub 2014/07/30. https://doi.org/10.1016/j.stemcr.2014.05.002 PMID: 25068131; PubMed Central PMCID: PMCPMC4110789.

25. Jiao X, Sherman BT, Huang DW, Stephens R, Baseler MW, Lane HC, et al. DAVID-WS: a stateful web service to facilitate gene/protein list analysis. Bioinformatics (Oxford, England). 2012; 28(13):1805–6. https://doi.org/10.1093/bioinformatics/bts251 PMID: 22543366; PubMed Central PMCID: PMCPMC3381967.

26. Kramer A, Green J, Pollard J Jr., Tugendreich S. Causal analysis approaches in Ingenuity Pathway Analysis. Bioinformatics (Oxford, England). 2014; 30(4):523–30. Epub 2013/12/18. https://doi.org/10.1093/bioinformatics/btt703 PMID: 24336805; PubMed Central PMCID: PMCPMC3928520.

27. Boyle EI, Weng S, Gollub J, Jin H, Botstein D, Cherry JM, et al. GO::TermFinder—open source software for accessing Gene Ontology information and finding significantly enriched Gene Ontology terms associated with a list of genes. Bioinformatics (Oxford, England). 2004; 20(18):3710–5. Epub 2004/08/07. https://doi.org/10.1093/bioinformatics/bth456 PMID: 15297299; PubMed Central PMCID: PMCPMC3037731.

28. Torres-Martín M, Lasaletta L, Isla A, De Campos JM, Pinto GR, Burbano RR, et al. Global expression profile in low grade meningiomas and schwannomas shows upregulation of PDGFD, CDH1 and SLIT2 compared to their healthy tissue. Oncology reports. 2014; 32(6):2327–34. Epub 2014/10/22. https://doi.org/10.3892/or.2014.3526 PMID: 25333347; PubMed Central PMCID: PMCPMC4240498.

29. Schulten HJ, Bangash M, Karim S, Dallol A, Hussein D, Merdad A, et al. Comprehensive molecular biomarker identification in breast cancer brain metastases. Journal of translational medicine. 2017; 15(1):269. Epub 2017/12/31. https://doi.org/10.1186/s12967-017-1370-x PMID: 29287594; PubMed Central PMCID: PMCPMC5747948.

30. Dalan AB, Gulluoglu S, Tuysuz EC, Kuskucu A, Yaltirik CK, Ozturk O, et al. Simultaneous analysis of miRNA-mRNA in human meningiomas by integrating transcriptome: A relationship between PTX3 and miR-29c. BMC cancer. 2017; 17(1):207. Epub 2017/03/23. https://doi.org/10.1186/s12953-017-1398-4 PMID: 28327132; PubMed Central PMCID: PMCPMC5361823.

31. Lee Y, Liu J, Patel S, Cloughesy T, Lai A, Farooqi H, et al. Genomic landscape of meningiomas. Brain pathology (Zurich, Switzerland). 2010; 20(4):751–62. Epub 2009/12/18. https://doi.org/10.1111/j.1750-3639.2009.00356.x PMID: 20015288; PubMed Central PMCID: PMCPMC3167483.

32. Gump JM, Donson AM, Birks DK, Amani VM, Rao KK, Griesinger AM, et al. Identification of targets for rational pharmacological therapy in childhood craniopharyngioma. Acta neuropathologica communications. 2015; 3:30. Epub 2015/06/21. https://doi.org/10.1186/s40478-015-0211-5 PMID: 25980246; PubMed Central PMCID: PMCPMC4438576.

33. Claus EB, Park PJ, Carroll R, Chan J, Black PM. Specific genes expressed in association with progesterone receptors in meningioma. Cancer research. 2008; 68(1):314–22. Epub 2008/01/04. https://doi.org/10.1158/0008-5472.CAN-07-1796 PMID: 18172325; PubMed Central PMCID: PMCPMC3256746.

34. Serna E, Morales JM, Mata M, Gonzalez-Darder J, San Miguel T, Gil-Benzo R, et al. Gene expression profiles of metabolic aggressiveness and tumor recurrence in benign meningioma. PLoS one. 2013; 8(6):e67291. Epub 2013/07/11. https://doi.org/10.1371/journal.pone.0067291 PMID: 23940654; PubMed Central PMCID: PMCPMC3696107.
Choy CT, Kim H, Lee JY, Williams DM, Palethorpe D, Fellows G, et al. Anosmin-1 contributes to breast tumor malignancy through integrin signal pathways. Endocrine-related cancer. 2014; 21(1):85–99. Epub 2013/11/06. https://doi.org/10.1530/ERC-13-0181 PMID: 24189182; PubMed Central PMCID: PMCPMC3869950.

Willis J, Smith C, Ironside JW, Erridge S, Whittle IR, Everington D. The accuracy of meningioma grading: a 10-year retrospective audit. Neuropathology and applied neurobiology. 2005; 31(2):141–9. Epub 2005/03/18. https://doi.org/10.1111/j.1365-2900.2004.00621.x PMID: 15771707.

Aarhus M, Lund-Johansen M, Knappskog PM. Gene expression profiling of meningiomas: current status after a decade of microarray-based transcriptomic studies. Acta neurochirurgica. 2011; 153(3):447–56. https://doi.org/10.1007/s00701-010-0906-0 PMID: 21234620; PubMed Central PMCID: PMCPMC3040823.

Nielsen JS, Graves ML, Chelliah S, Vogl AW, Roskelley CD, McNagny KM. The CD34-related molecule podocalyxin is a potent inducer of microvillus formation. PLoS one. 2007; 2(2):e237. Epub 2007/02/22. https://doi.org/10.1371/journal.pone.0000237 PMID: 17311105; PubMed Central PMCID: PMCPMC1798660.

Nielsen JS, McNagny KM. Novel functions of the CD34 family. Journal of cell science. 2008;121(Pt 22):3683–92. Epub 2008/11/07. https://doi.org/10.1242/jcs.037507 PMID: 18987355.

Wang J, Zhao Y, Qi R, Zhu X, Huang C, Cheng S, et al. Prognostic role of podocalyxin-like protein expression in various cancers: A systematic review and meta-analysis. Oncotarget. 2017; 8(32):52457–64. Epub 2017/09/08. https://doi.org/10.18632/oncotarget.14199 PMID: 28681743; PubMed Central PMCID: PMCPMC581042.

Somasiri A, Nielsen JS, Makretsov N, McCoy ML, Prentice L, Gilks CB, et al. Overexpression of the anti-adhesive podocalyxin is an independent predictor of breast cancer progression. Cancer research. 2004; 64(15):5068–73. Epub 2004/08/04. https://doi.org/10.1158/0008-5472.CAN-04-0240 PMID: 15289306.

Graves ML, Cipollone JA, Austin P, Bell EM, Nielsen JS, Gilks CB, et al. The cell surface mucin podocalyxin regulates collective breast tumor budding. Breast cancer research: BCR. 2016; 18(1):11. Epub 2016/01/23. https://doi.org/10.1186/s13058-015-0670-4 PMID: 26796961; PubMed Central PMCID: PMCPMC4722710.

Kusumoto H, Shintani Y, Kanzaki R, Kawamura T, Funaki S, Minami M, et al. Podocalyxin influences malignant potential by controlling epithelial-mesenchymal transition in lung adenocarcinoma. Cancer Science. 2017; 108(3):325–35. Epub 2016/12/23. https://doi.org/10.1111/cas.13142 PMID: 28004467; PubMed Central PMCID: PMCPMC5378270.

Lee WY, Kuo CC, Lin BX, Cheng CH, Chen KC, Lin CW. Podocalyxin-Like Protein 1 Regulates TAZ Signaling and Stemness Properties in Colon Cancer. International journal of molecular sciences. 2017; 18(2). Epub 2017/01/05. https://doi.org/10.3390/ijms18020247 PMID: 28946619; PubMed Central PMCID: PMCPMC5666729.

Binder ZA, Siu IM, Eberhart CG, Ap Rhys C, Bai RY, Staedtke V, et al. Podocalyxin-like protein is expressed in glioblastoma multiforme stem-like cells and is associated with poor outcome. PloS one. 2013; 8(10):e75945. Epub 2013/10/23. https://doi.org/10.1371/journal.pone.0075945 PMID: 24146797; PubMed Central PMCID: PMCPMC3797817.

Huang T, Jin X, He L, Zhang M, Wu J, Wang Y, et al. Role of podocalyxin in astrocyoma: Clinicopathological and in vitro evidence. Oncology letters. 2013; 6:1390–6. Epub 2013/01/02. https://doi.org/10.3892/ol.2013.1556 PMID: 24179530; PubMed Central PMCID: PMCPMC3813577.

Ogasawara S, Kaneko MK, Yamada S, Honma R, Nakamura T, Saidoh N, et al. PcMab-47: Novel Anti-human Podocalyxin Monoclonal Antibody for Immunohistochemistry. Monoclonal Antibodies in Immunodiagnosis and Immunotherapy. 2017; 36(2):50–6. https://doi.org/10.1089/mab.2017.0008 PMID: 28384052; PubMed Central PMCID: PMCPMC404275.

Itai S, Yamada S, Kaneko MK, Kato Y. Determination of critical epitope of PcMab-47 against human podocalyxin. Biochemistry and biophysics reports. 2018; 14:78–82. Epub 2018/06/07. https://doi.org/10.1016/j.bbrep.2018.04.003 PMID: 29872738; PubMed Central PMCID: PMCPMC5986553.

Kaneko MK, Kunita A, Yamada S, Nakamura T, Yanaka M, Saidoh N, et al. Antipodocalyxin Antibody chPcMab-47 Exerts Antitumor Activity in Mouse Xenograft Models of Colorectal Adenocarcinomas. Monoclonal Antibodies in Immunodiagnosis and Immunotherapy. 2017; 36(4):157–62. Epub 2017/07/01. https://doi.org/10.1089/mab.2017.0020 PMID: 28665782.

Chen T, Sun Y, Ji P, Kopetz S, Zhang W. Topoisomerase IIα in Chromosome Instability and Personalized Cancer Therapy. Oncogene. 2015; 34(31):4019–31. https://doi.org/10.1038/onc.2014.332 PMID: 25328136; PubMed Central PMCID: PMCPMC4404185.

Heestand GM, Schwaederle M, Gatalica Z, Arguello D, Kurzrock R. Topoisomerase expression and amplification in solid tumours: Analysis of 24,262 patients. European journal of cancer (Oxford,
Yu CY, Liang GB, Du P, Liu YH. Lgr4 promotes glioma cell proliferation through activation of Wnt signal-

Gronbaek K, Ralfkiaer U, Dahl C, Hother C, Burns JS, Kassem M, et al. Frequent hypermeth ylation of 

Beetz C, Brodoehl S, Patt S, Kalff R, Deufel T. Low expression but infrequent genomic loss of the puta-

Izumi H, Inoue J, Yokoi S, Hosoda H, Shibata T, Sunamori M, et al. Frequent silencing of DBC1 is by 

Nishiyama H, Gill JH, Pitt E, Kennedy W, Knowles MA. Negative regulation of G1/S transition by the 

Yue Z, Yuan Z, Zeng L, Wang Y, Lai L, Li J, et al. LGR4 modulates breast cancer initiation, metastasis, 

Aksoy I, Giudice V, Delahaye E, Wianny F, Aubry M, Mure M, et al. Klf4 and Klf5 differentially inhibit 

Luo W, Tan P, Rodriguez M, He L, Tan K, Zeng L, et al. Leucine-rich repeat-containing G protein-coupled receptor 4 (Lgr4) is necessary for prostate cancer metastasis via epithelial-mesenchymal transition. The Journal of biological chemistry. 2017; 292(37):15525–37. Epub 2017/08/05. https://doi.org/10.1074/jbc.M116.771931 PMID: 28768769; PubMed Central PMCID: PMC5602409.

Nashiyama H, Gill JH, Pitt E, Kennedy W, Knowles MA. Negative regulation of G1/S transition by the candidate bladder tumour suppressor gene DBCCR1. Oncogene. 2001; 20(23):2956–64. Epub 2001/06/23. https://doi.org/10.1038/sj.onc.1204432 PMID: 11420708.

Izumi H, Inoue J, Yokoi S, Hosoda H, Shibata T, Sunamori M, et al. Frequent silencing of DBC1 is by genetic or epigenetic mechanisms in non-small cell lung cancers. Human molecular genetics. 2005; 14 (8):997–1007. Epub 2005/03/05. https://doi.org/10.1093/hmg/ddi092 PMID: 15746151.

Beetz C, Brodoehl S, Patt S, Kalff R, Deufel T. Low expression but indifferent genomic loss of the putative tumour suppressor DBCCR1 in astrocytoma. Oncology reports. 2005; 13(2):335–40. Epub 2005/01/12. PMID: 15643521.

Gronbaek K, Ralkiaer U, Dahl C, Hother C, Burns JS, Kassem M, et al. Frequent hypermethylation of DBC1 in malignant lymphoproliferative neoplasms. Modern pathology: an official journal of the United States and Canada. 2006; 19(1):20–5. https://doi.org/10.1097/01.ppm.0000191775.63194.8b PMID: 16544798.

Yu CY, Taylor SH, Garvan R, Holmes DF, Zeef LA, Soininen R, et al. Arhgap28 is a RhoGAP that regulates centrosome duplication. The Journal of biological chemistry. 2017; 292(37):15525–37. Epub 2017/08/05. https://doi.org/10.1074/jbc.M116.771931 PMID: 28768769; PubMed Central PMCID: PMC5602409.

Yang YP, Chang YL, Huang PI, Chiou GY, Tseng LM, Chiou SH, et al. Resveratrol suppresses tumorigenicity and enhances radiosensitivity in primary glioblastoma tumor initiating cells by inhibiting the STAT3 axis. Journal of cellular physiology. 2014; 229(2):796–803. Epub 2013/12/10. https://doi.org/10.1002/jcp.22806 PMID: 21503893.

Gao Y, Ding Y, Chen H, Chen H, Zhou J. Targeting Kruppel-like factor 5 (KLF5) for cancer therapy. Current topics in medicinal chemistry. 2015; 15(8):699–713. Epub 2015/03/04. PMID: 25732792.

Aksoy I, Giudice V, Delahaye E, Wianny F, Aubry M, Mure M, et al. Klf4 and Klf5 differentially inhibit mesoderm and endoderm differentiation in embryonic stem cells. Nature communications. 2014; 5:3719. Epub 2014/04/29. https://doi.org/10.1038/ncomms4719 PMID: 24770696.

Yang YP, Chang YL, Huang PI, Chiou GY, Tseng LM, Chiou SH, et al. Resveratrol suppresses tumorigenicity and enhances radiosensitivity in primary glioblastoma tumor initiating cells by inhibiting the STAT3 axis. Journal of cellular physiology. 2012; 227(3):976–93. Epub 2011/04/20. https://doi.org/10.1002/jcp.22806 PMID: 21503893.

Yeung CY, Taylor SH, Garvan R, Holmes DF, Zeef LA, Soininen R, et al. Arhgap28 is a RhoGAP that inactivates RhoA and downregulates stress fibers. PloS one. 2014; 9(9):e107036. Epub 2014/09/12. https://doi.org/10.1371/journal.pone.0107036 PMID: 25211221; PubMed Central PMCID: PMC3528656.

Luo W, Tan P, Rodriguez M, He L, Tan K, Zeng L, et al. Leucine-rich repeat-containing G protein-coupled receptor 4 (Lgr4) is necessary for prostate cancer metastasis via epithelial-mesenchymal transition. The Journal of biological chemistry. 2017; 292(37):15525–37. Epub 2017/08/05. https://doi.org/10.1074/jbc.M116.771931 PMID: 28768769; PubMed Central PMCID: PMC5602409.

Nashiyama H, Gill JH, Pitt E, Kennedy W, Knowles MA. Negative regulation of G1/S transition by the candidate bladder tumour suppressor gene DBCCR1. Oncogene. 2001; 20(23):2956–64. Epub 2001/06/23. https://doi.org/10.1038/sj.onc.1204432 PMID: 11420708.

Izumi H, Inoue J, Yokoi S, Hosoda H, Shibata T, Sunamori M, et al. Frequent silencing of DBC1 is by genetic or epigenetic mechanisms in non-small cell lung cancers. Human molecular genetics. 2005; 14 (8):997–1007. Epub 2005/03/05. https://doi.org/10.1093/hmg/ddi092 PMID: 15746151.

Beetz C, Brodoehl S, Patt S, Kalff R, Deufel T. Low expression but indifferent genomic loss of the putative tumour suppressor DBCCR1 in astrocytoma. Oncology reports. 2005; 13(2):335–40. Epub 2005/01/12. PMID: 15643521.
States and Canadian Academy of Pathology, Inc. 2008; 21(5):632–8. Epub 2008/02/12. https://doi.org/10.1038/modpathol.2008.27 PMID: 18264085.

69. Shim UJ, Lee IS, Kang HW, Kim J, Kim WT, Kim YJ, et al. Decreased DBC1 Expression is Associated With Poor Prognosis in Patients With Non-Muscle-Invasive Bladder Cancer. Korean journal of urology. 2013; 54(9):631–7. Epub 2013/09/18. https://doi.org/10.4111/kjui.2013.54.9.631 PMID: 24044099; PubMed Central PMCID: PMC3773595.

70. Glumac PM, LeBeau AM. The role of CD133 in cancer: a concise review. Clinical and Translational Medicine. 2018; 7(1):18. Epub 2018/07/10. https://doi.org/10.1186/s40169-018-0198-1 PMID: 29984391; PubMed Central PMCID: PMC6035906.

71. Kemper K, Sprick MR, de Bree M, Scopelliti A, Vermeulen L, Hoek M, et al. The AC133 epitope, but not the CD133 protein, is lost upon cancer stem cell differentiation. Cancer research. 2010; 70(2):719–29. Epub 2010/01/14. https://doi.org/10.1158/0008-5472.CAN-09-1820 PMID: 20068153.

72. Geddert H, Braun A, Kayser C, Dimmler A, Faller G, Agaimy A, et al. Epigenetic Regulation of CD133 in Gastrointestinal Stromal Tumors. American journal of clinical pathology. 2017; 147(5):515–24. Epub 2017/04/12. https://doi.org/10.1093/ajcp/aqx028 PMID: 28398518.

73. Sun Y, Sakaiassiani PO, Tsinkavoloski O, Immervoll H, Boe SO, Svendsen A, et al. CD133-negative glioma cells form tumors in nude rats and give rise to CD133-positive cells. International journal of cancer. 2008; 122(4):761–8. Epub 2007/10/24. https://doi.org/10.1002/ijc.23130 PMID: 17955491.

74. Joo KM, Kim SY, Jin X, Song SY, Kong DS, Lee JI, et al. Clinical and biological implications of CD133-positive and CD133-negative cells in glioblastomas. Laboratory investigation; a journal of technical methods and pathology. 2008; 88(8):808–15. Epub 2008/06/19. https://doi.org/10.1038/labinvest.2008.57 PMID: 18560366.

75. Wang J, Sakaiassiani PO, Tsinkavoloski O, Immervoll H, Boe SO, Svendsen A, et al. CD133 negative glioma cells are clonogenic and tripotent. PloS one. 2009; 4(5):e5498. Epub 2009/05/12. https://doi.org/10.1371/journal.pone.0005498 PMID: 19405332; PubMed Central PMCID: PMCPMC2676510.

76. Gopisetty G, Xu J, Sampath D, Colman H, Puduval VK. Epigenetic regulation of CD133/PROM1 expression in glioma stem cells by Sp1/myc and promoter methylation. Oncogene. 2013; 32(26):3119–29. Epub 2012/09/05. https://doi.org/10.1038/onc.2012.331 PMID: 22949648; PubMed Central PMCID: PMCPMC3820114.

77. McCarth D, Bolger J, Fagan A, Byrne C, Hao Y, Qin L, et al. Global characterization of the SRC-1 transcriptome identifies ADAM22 as an ER-independent mediator of endocrine resistant breast cancer. Cancer research. 2012; 72(1):220–9. https://doi.org/10.1158/0008-5472.CAN-11-1976 PMID: 22072566; PubMed Central PMCID: PMC3681815.

78. Bolger JC, Young LS. ADAM22 as a prognostic and therapeutic drug target in the treatment of endocrine-resistant breast cancer. Vitamins and hormones. 2013; 93:307–21. Epub 2013/07/03. https://doi.org/10.1016/B978-0-12-416673-8.00014-9 PMID: 23810013.

79. Andreade LC, Peukert D, Lumsden A, Gilthorpe JD. Analysis of Lrrn1 expression and its relationship to neuromeric boundaries during chick neural development. Neural Development. 2007; 2:22. https://doi.org/10.1186/1749-8104-2-22 PMID: 17973992; PubMed Central PMCID: PMCPM2225406.

80. Okamoto M, Miyata T, Konno D, Ueda HR, Kasukawa T, Hashimoto M, et al. Cell-cycle-independent transitions in temporal identity of mammalian neural progenitor cells. Nature communications. 2016; 7:11349. Epub 2016/04/21. https://doi.org/10.1038/ncomms11349 PMID: 27094546; PubMed Central PMCID: PMCPMC4842982.

81. Cai YN, Dai XH, Zhang QH, Hu R, Dai ZM. Gene expression profiling of somatic and pluripotent cells reveals novel pathways involved in reprogramming. Genetics and molecular research: GMR. 2015; 14(4):12085–92. Epub 2015/10/28. https://doi.org/10.4238/2015.October.5.21 PMID: 26505355.

82. Hossain S, Takatori A, Nakamura Y, Suena ga Y, Kamijo T, Nakagawa R. NLRR1 enhances EGF-mediated MYCN induction in neuroblastoma and accelerates tumor growth in vivo. Cancer research. 2012; 72(17):4587–96. Epub 2012/07/21. https://doi.org/10.1158/0008-5472.CAN-12-0943 PMID: 22815527.

83. Miljkovic-Licina M, Hammel P, Garrido-Urbani S, Lee BP, Meguenani M, Chaabane C, et al. Targeting olfactomedin-like 3 inhibits tumor growth by impairing angiogenesis and pericyte coverage. Molecular cancer therapeutics. 2012; 11(12):2588–99. Epub 2012/09/25. https://doi.org/10.1158/1535-7163.MCT-12-0245 PMID: 23002094.

84. Ozdemir BC, Hensel J, Secondini C, Wetterwald A, Schwaninger R, Fleischmann A, et al. The molecular signature of the stroma response in prostate cancer-induced osteoblastic bone metastasis highlights expansion of hematopoietic and prostate epithelial stem cell niches. PloS one. 2014; 9(12):e114530. Epub 2014/12/09. https://doi.org/10.1371/journal.pone.0114530 PMID: 25485970; PubMed Central PMCID: PMC4259356.
85. Miller-Delaney SF, Lieberam I, Murphy P, Mitchell KJ. Pldc2 is a mitogen for neural progenitors. PloS one. 2011; 6(1):e14565. Epub 2011/02/02. https://doi.org/10.1371/journal.pone.0014565 PMID: 21283688; PubMed Central PMCID: PMCPMC3024984.

86. Ikeda M, Chiba S, Ohashi K, Mizuno K. Furry protein promotes aurora A-mediated Polo-like kinase 1 activation. The Journal of biological chemistry. 2012; 287(33):27670–81. Epub 2012/07/04. https://doi.org/10.1074/jbc.M112.378968 PMID: 22753416; PubMed Central PMCID: PMCPMC3431660.

87. Lehembre F, Yilmaz M, Wicki A, Schomber T, Strittmatter K, Ziegler D, et al. NCAM-induced focal adhesion assembly: a functional switch upon loss of E-cadherin. The EMBO journal. 2008; 27(19):2603–15. Epub 2008/09/06. https://doi.org/10.1038/emboj.2008.178 PMID: 18772882; PubMed Central PMCID: PMCPMC2567408.

88. Min H, Hong J, Cho IH, Jang YH, Lee H, Kim D, et al. TLR2-induced astrocyte MMP9 activation compromises the blood brain barrier and exacerbates intracerebral hemorrhage in animal models. Molecular Brain. 2015; 8:23. Epub 2015/04/17. https://doi.org/10.1186/s13041-015-0116-z PMID: 25879213; PubMed Central PMCID: PMCPMC4397689.

89. Ifuku M, Buonfiglioli A, Jordan P, Lehnardt S, Kettenmann H. TLR2 controls random motility, while TLR7 regulates chemotaxis of microglial cells via distinct pathways. Brain, behavior, and immunity. 2016; 58:338–47. Epub 2016/08/25. https://doi.org/10.1016/j.bbi.2016.08.003 PMID: 27554518.

90. Shivapathasundram G, Wickremesekera AC, Tan ST, Itinteang T. Tumour stem cells in meningioma: A review. Journal of clinical neuroscience: official journal of the Neurosurgical Society of Australasia. 2018; 47:66–71. Epub 2017/11/09. https://doi.org/10.1016/j.jocn.2017.10.059 PMID: 29113852.