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ARTICLE *in* JOURNAL OF PROTEOME RESEARCH · NOVEMBER 2009

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Published in final edited form as:

J Proteome Res. 2010 January ; 9(1): 485–494. doi:10.1021/pr900834h.

Comparative protein profiling reveals minichromosome maintenance (MCM) proteins as novel potential tumor markers for meningiomas

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Abstract

Meningiomas are among the most frequent tumors of the brain and spinal cord accounting for 15% to 20% of all central nervous system tumors and frequently associated with neurofibromatosis type 2. In this study, we aimed to unravel molecular meningioma tumorigenesis and discover novel protein biomarkers for diagnostic and/or prognostic purposes and performed in-depth proteomic profiling of meningioma cells compared to human primary arachnoidal cells. We isolated proteins from meningioma cell line SF4433 and human primary arachnoidal cells and analyzed the protein profiles by Gel-nanoLC-MS/MS in conjunction with protein identification and quantification by shotgun nanoLC tandem mass spectrometry and spectral counting. Differential analysis of meningiomas revealed changes in the expression levels of 281 proteins ($P < 0.01$) associated with various biological functions such as DNA replication, recombination, cell cycle, and apoptosis. Among several interesting proteins, we focused on a subset of the highly significantly up-regulated proteins, the minichromosome maintenance (MCM) family. We performed subsequent validation studies by qRT-PCR in human meningioma tissue samples (WHO grade I: 14 samples, WHO grade II: 7 samples and WHO grade III: 7 samples) compared to arachnoidal tissue controls (from fresh autopsies; 3 samples) and found that MCMs are highly and significantly up-regulated in human meningioma tumor samples compared to arachnoidal tissue controls. We found a significant increase in MCM2 (8 fold) and MCM3 (5 fold), MCM4 (4 fold), MCM5 (4 fold), MCM6 (3 fold), MCM7 (5 fold) expressions in meningiomas. This study suggests that MCM family proteins are up-regulated in meningiomas and can be used as diagnostic markers.

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INTRODUCTION

Meningioma is one of the most common central nervous system tumors and accounting for 32.1% of all reported brain tumors.¹ They are derived from meningotheial (arachnoid cap) cells. These cells are most common within the arachnoid villi but may be present throughout the craniospinal arachnoid space.² According to the WHO grading system, these tumors are classified as typical WHO grade I (approximately 91% of meningiomas), atypical WHO grade II (5%), and anaplastic/malignant WHO grade III (4%).^{2,3} They are most likely to be diagnosed in adults between 40–70 years of age and significantly more common in women than in men with a greater than 2:1 ratio.⁴ Surgery is the primary and often only choice of treatment for WHO I grade tumors. Complete resection can be achieved in 38–80% of patients, depending on tumor localization. However, despite complete resection, a radiological recurrence or a second primary tumor (SPT) develops in approximately 19% of all cases.^{6–7} Radiotherapy is used as an additional post-operative treatment in WHO II and III meningiomas,⁸ and as primary treatment for recurrent or inoperable WHO I meningiomas. Additional radiotherapy could be considered for WHO I tumors if biomarkers were available to identify WHO I meningiomas at risk for recurrence.

As for all cancers, meningioma tumorigenesis is driven by the accumulation of genetic aberrations of which an overview is presented by Riemenschneider *et al.*⁸ The deletion in chromosome 22q associated with loss of the neurofibromatosis 2 (NF2) gene has been one of the most common events associated with meningioma tumorigenesis.^{9,10} The NF2 gene encodes a tumor suppressor protein, merlin, also called schwannomin, which is related to ezrin-radixin-moesin (ERM) proteins of the band 4.1 superfamily of membrane-cytoskeletal linkers.^{11,12} One of our recent studies provided evidence that miRNAs could also contribute to the tumorigenesis of meningiomas; down-regulated microRNA-200a in meningiomas was found to promote tumor growth by reducing E-cadherin and activating the Wnt/ β -catenin signaling pathway.¹³ Several other genetic events such as mutation of TP53, PTEN¹⁴ or chromosomal deletions in 1p, 3p, 6q, 10q and 14q^{15,16} and chromosomal gains in 12q, 15q, 17q and 20q¹⁷ have also been pointed.

Apart from genetic aberrations, protein expression changes have been reported. Increased expression of the proliferation marker Ki-67 was described to correlate with a higher risk for recurrence.^{18,19} Also progesterone receptor expression was found to be inversely related with WHO grade.^{20–22}

Still, knowledge of the biological principles underlying meningioma tumorigenesis is scarce and none of the genetic or protein aberrations has demonstrated sufficient potential to be implemented in routine diagnostics, so as of yet, no markers are available for the prediction of recurrence after resection.

All types of cancer as well as meningioma constitute a major public health problem that presents several challenges to researchers such as identification of biomarkers for improved and early diagnosis, classification of tumors and the definition of targets for more-effective therapeutic precautions.²³ Since proteins are the functional output of the genome, responsible for the (tumor) cell's biology and behavior, and can easily be measured in clinical samples by straightforward antibody-based detection, they can possibly be very suitable biomarkers. Recently, studies on protein profiling of different types of tumor tissue provided crucial information on the pathogenesis of cancer at the molecular level and additionally supplied multiple protein biomarker candidates for various applications. Proteomics technology enables the simultaneous quantitative investigation of thousands of proteins allowing differential expression profiling of multiple different samples.²⁴

In this study, to unravel molecular meningioma tumorigenesis and discover novel protein biomarkers for diagnostic and/or prognostic purposes, we performed in-depth proteomic profiling of meningioma cells compared to normal human primary arachnoidal cells, the cell origin of meningioma tumors. We identified 281 proteins significantly deregulated in meningiomas ($p < 0.01$). For validation of the differential expression, we focused on a subset of the highly significantly up-regulated proteins, i.e. the minichromosome maintenance (MCM) family. MCM proteins includes six highly conserved DNA binding members, MCM2 through MCM7.²⁵ They are considered to function as licensing components for S-phase of cell cycle.²⁶ The expression of all family members was validated by qRT-PCR in human meningioma tumor samples ($n=28$) and further western blot demonstrated that MCM proteins are up-regulated in meningiomas compared to arachnoidal controls. All MCM family members exhibited a striking up-regulation as most were undetected in arachnoidal cells and highly expressed in meningioma cells. Interestingly, recent studies have pointed out the role(s) of MCM family members as diagnostic and/or prognostic markers for several malignancies including colon cancer,²⁷ breast and prostate cancers.^{28,29}

MATERIALS AND METHODS

Tumor and Tissue samples

Histopathological primary tumor samples were obtained from the tissue discarded during resection, normal arachnoidal tissues were obtained from autopsies within 5–7 hour of death. Human meningioma samples were collected and de-identified by the Neuro-oncology Tumor Repository, fresh-frozen and stored at -80°C under IRB protocols approved by Massachusetts General Hospital Committee on Human Research.

Cell lines

Human benign meningioma cell line SF4433, SF4068, and SF3061 were provided by Dr. Anita Lal (University of California, San Francisco) and were immortalized by transduction with expression cassettes for human telomerase and papillomavirus E6/E7.³³ Cell lines were cultured at 37°C and in 5% CO_2 in Dulbecco's Modification of Eagle's Medium (DMEM) supplemented with 10% FBS (JRH Biosciences, Kansas, USA), 100 U penicillin, and 0.1 mg/ml streptomycin. Primary arachnoidal cells were obtained from Dr. Marianne James (Molecular Neurogenetics Unit, Center for Human Genetic Research, Massachusetts General Hospital, Boston) and cultured in IMEM (Mediatech, Inc. VA, USA) complemented with 15% FBS (JRH Biosciences, Kansas, USA), 2 mg/ml insulin (Sigma-Aldrich, St. Louis, MO, USA), 100 U penicillin and 0.1 mg/ml streptomycin.

Cell lysis and SDS-PAGE

Meningioma cells, SF4433, and primary arachnoidal cells, AC030, were seeded in 10 cm^2 diameter tissue culture plates and cultured for two days until they were full-confluent (4×10^6 cells/plate). Cells were subsequently washed with 1X PBS for two times and trypsinized, centrifuged and washed with water for one times to get rid of the excess salts. Cells were scraped in RIPA buffer containing proteinase inhibitor. Each cell line sample was analyzed in duplo. Equal amount of total protein (50 μg) was separated in NuPAGE Novex Bis-Tris Mini Gel (Invitrogen). Gel was stained with Coomassie brilliant blue G-250 (Pierce), washed and each lane was sliced into ten bands using band pattern to guide the slicing. The whole process was performed in keratin-free conditions.

Gel digestion

Before MS analysis, separated proteins were in-gel digested according to the method described by Shevchenko *et al.*⁵³ Gel lanes corresponding to the different protein samples were sliced

into ten bands. The bands were washed/dehydrated three times in 50 mM ABC (ammonium bicarbonate pH 7.9)/50 mM ABC + 50% ACN (acetonitrile). Subsequently, cysteine bonds were reduced with 10 mM dithiothreitol for 1 h at 56 °C and alkylated with 50 mM iodoacetamide for 45 min at RT in the dark. After two subsequent wash/dehydration cycles the bands were dried 10 min in a vacuum centrifuge and incubated overnight with 0.06 µg/µl trypsin at 25 °C. Peptides were extracted once in 1% formic acid and subsequently two times in 50% ACN in 5% formic acid. The volume was reduced to 50 µl in a vacuum centrifuge prior to LC-MS/MS analysis.

NanoLC-MS/MS analysis

Peptides were separated by an Ultimate 3000 nanoLC system (Dionex LC-Packings, Amsterdam, The Netherlands) equipped with a 20 cm × 75 µm ID fused silica column custom packed with 3 µm 120 Å ReproSil Pur C18 aqua (Dr Maisch GMBH, Ammerbuch-Entringen, Germany). After injection, peptides were trapped at 30 µl/min on a 5 mm × 300 µm ID Pepmap C18 cartridge (Dionex LC-Packings, Amsterdam, The Netherlands) at 2% buffer B (buffer A: 0.05% formic acid in MQ; buffer B: 80% ACN + 0.05% formic acid in MQ) and separated at 300 nl/min in a 10–40% buffer B gradient in 60 min. Eluting peptides were ionized at 1.7 kV in a Nanomate Triversa Chip-based nanospray source using a Triversa LC coupler (Advion, Ithaca, NJ). Intact peptide mass spectra and fragmentation spectra were acquired on a LTQ-FT hybrid mass spectrometer (Thermo Fisher, Bremen, Germany). Intact masses were measured at resolution 50,000 in the ICR cell using a target value of 1×10^6 charges. In parallel, following an FT pre-scan, the top 5 peptide signals (charge-states 2^+ and higher) were submitted to MS/MS in the linear ion trap (3 amu isolation width, 30 ms activation, 35% normalized activation energy, Q value of 0.25 and a threshold of 5000 counts). Dynamic exclusion was applied with a repeat count of 1 and an exclusion time of 30s.

Database searching, statistics and Ingenuity Pathway Analysis

MS/MS spectra were searched against the human IPI database 3.31(67511 entries) using Sequest (version 27, rev 12), which is part of the BioWorks 3.3 data analysis package (Thermo Fisher, San Jose, CA). MS/MS spectra were searched with a maximum allowed deviation of 10 ppm for the precursor mass and 1 amu for fragment masses. Methionine oxidation and cysteine carboxamidomethylation were allowed as variable modifications, two missed cleavages were allowed and the minimum number of tryptic termini was 1. After database searching the DTA and OUT files were imported into Scaffold 2.01.01 (Proteome software, Portland, OR). Scaffold was used to organize the gel-band data and to validate peptide identifications using the Peptide Prophet algorithm⁵⁴ only identifications with a probability > 95% were retained. Subsequently, the Protein Prophet algorithm⁵⁵ was applied and protein identifications with a probability of > 99% with 2 peptides or more in at least one of the samples were retained. Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped. For each protein identified, the number of spectra was exported to Excel. The number of spectra per protein per sample was normalized against the total number of measured spectra. The beta-binomial test was performed to identify differentially expressed proteins. The list of differentially expressed proteins, including p-values and fold changes was imported in the online software package Ingenuity (Ingenuity IPA, version 7.6) and pathway and network analysis was performed with only direct relationships.

Quantitative RT-PCR

Quantitative RT-PCR Analysis was performed to determine the mRNA expression levels of MCM proteins. Total RNA was isolated from tissue specimens by using Trizol (Invitrogen) according to the manufacturer instructions and quantified by NanoDrop ND-1000

Spectrophotometer (Thermo Scientific) and stored – 80 °C. Equal amounts (1 µg) of RNA were converted into cDNAs by using Omniscript reverse transcription kit (Qiagen) in accordance with the protocol provided by manufacturer. qRT-PCR was performed in triplicate using ABI PRISM 7000 Sequence Detection system (Applied Biosystems) with the SYBR Green PCR kit from Applied Biosystems and the primers as follows: MCM2: 5'-AGACGAGATAGAGCTGACTG-3' (F) and 5'-CACCACGTACCTTGTGCTTG-3' (R);⁵⁶ MCM3: 5'-CGAGGAAGACCAGGGAATTT-3' (F) and 5'-AGGCAACCAGCTCCTCAAAG-3' (R);⁵⁷ MCM4 (www.genomecenter.ucdavis.edu/.../Human%20E2Fprimerseq.doc): 5'-CCACCACCTCCCGTCCTTAA3' (F) and 5'-AATCACAGCGGCGCTCGTAC-3' (R); MCM5: 5'-CCCATTTGGGGTATACACGTC-3' (F) and 5'-ACGGTCATCTTCTCGCATCT-3' (R);⁵⁷ MCM6: 5'-ACTAGACAGAAGCGGCTTACTC-3' (F) and 5'-CTTTTTTCGCTGAACACCGCCAGCT-3' (R);⁵⁷ MCM7: 5'-TCAATTTGTGAGAATGCCAGGCGC-3' (F) and 5'-CACAGTTACCAACTTCCCCACAGA-3' (R);⁵⁸ GAPDH mRNA was used for normalization as described.⁵⁹

Western Blot

Western blot analysis—Meningioma cells and primary arachnoidal cells were harvested and total protein was separated on a SDS–8% polyacrylamide gel and blotted onto nitrocellulose. The membrane was blocked with 5% nonfat dry milk in TBS-T (TBS containing 0.05% Tween-20) for 2 hrs at 37°C and then rinsed once with TBS-T and washed twice for 15 min and twice for 5 min at room temperature with TBS-T.¹³ The primary antibodies used were MCM3 (Cell Signaling Technology, #4012) in 1:1000 ratio, and β-actin (Sigma, #A5441) in 1:1000 dilution.

RESULTS

Proteomics protein profiling reveals a set of differential proteins in meningiomas

We performed a proteomics study to investigate the protein expression profile in meningioma cells as compared to human primary arachnoidal cells. Based on the findings of others, arachnoidal cells are believed to be the cell of origin of meningioma tumors.^{30,32} A flow chart of our experimental procedures is shown in Fig. 1. We isolated proteins from meningioma cell line SF4433³³ and primary arachnoidal cells³⁰ and analyzed, in duplicate, the protein profiles by Gel-nanoLC-MS/MS, i.e. protein separation by 1D SDS-PAGE in conjunction with protein identification and quantification by shotgun nanoLC tandem mass spectrometry and spectral counting. Our approach permitted the simultaneous identification and quantification of over 2,800 proteins; Differential analysis of meningiomas revealed changes in the expression levels of 281 proteins (Supplementary Table 1; $P < 0.01$) associated with various biological functions such as DNA replication, recombination, cell cycle, and apoptosis. Of these 281 differential proteins 103 were from the cytoplasm, 91 from the nucleus, 37 from the plasma membrane, 19 extracellular and 31 had unknown cellular location. For 133 of these proteins, expression was exclusively detected in either arachnoidal or meningioma cells (on/off regulation). Ninety-seven proteins were found to be detected only in meningioma cells (Table 1), whereas 36 proteins were solely found in arachnoidal cells (Table 2). Within the group of on/off regulated proteins Ingenuity Pathway Analysis (IPA) revealed a significant overrepresentation of proteins involved in cancer (65 out of 133), cellular growth and proliferation (31 out of 133) and DNA replication, recombination and repair (28 out of 133). Network analysis exposed a network of direct relationships between the proteins involved in these cellular processes as depicted in Figure 1. Representative protein families in this network of on/off regulated proteins are the minichromosome maintenance (MCM) family, the replication factor C subunits (RFC

proteins) and the structural maintenance of chromosomes (SMC) family (see Figure 2). All three protein families are present in the nucleus and involved in DNA replication. Additionally several other proteins were found to be up-regulated (4–12-fold) such as replication protein A (RPA1) Epiplakin 1 (EPPK1), Flap endonuclease 1 (FEN1), BAG family molecular chaperone regulator 3 (BAG3), apoptosis inhibitor isoform 5 (API5), protein tyrosine kinase 7 (PTK7), DNA mismatch repair proteins, MSH2 and MSH6, and double-strand break repair protein MRE11, and DNA repair protein RAD50. Proteins such as AP-1 complex subunit beta-1 (APIB1), G1/S-specific cyclin-D1 (CCND1), cell division protein kinase 6 (CDK6), were found to be detected only in arachnoidal cells (Table 2).

Validation studies: the possible role(s) of the MCMs in meningioma diagnosis

MCM2, MCM3, MCM4, MCM5 and MCM7 were exclusively detected in meningioma cells and MCM6 was found to be over 30-fold upregulated (Table 1). Based on studies emphasizing the functional importance of the MCM family in diagnosis of several malignancies such as MCM2 and MCM5 in colon cancer,²⁷ MCM2 in breast cancer,²⁸ and MCM7 in prostate cancer,²⁹ we decided to follow-up on the differential expression of the MCM family. To validate our proteomics discovery data, we first performed qRT-PCR reactions in meningioma tumor tissue samples WHO grade I, half of which were deleted for the *NF2* locus and half were not as determined by comparative genomic hybridization¹⁵ (14 samples), WHO grade II (7 samples) and WHO grade III (7 samples) compared to arachnoidal tissue controls (from fresh autopsies; 3 samples). WHO Grade I meningiomas are by far the most common type of meningiomas, representing an initial stage in tumor development, and as such the normal arachnoidal tissue of origin was deemed the best control to look for changes in proteins related to tumorigenesis. As shown in Figure 3, we found a significant increase in *MCM2* (8 fold) and *MCM3* (5 fold), *MCM4* (4 fold), *MCM5* (4 fold), *MCM6* (3 fold), *MCM7* (5 fold) expressions in meningiomas compared to arachnoidal controls. It is possible that MCM family might be also expressed in arachnoidal cells and tissue but their expression levels are below detection limit by MS. However, we did not observe a significant change between meningioma Grade I, II, and III samples. These data suggested that the MCM family proteins are up-regulated in meningiomas and might serve as diagnostic markers. We further evaluated the expression of the MCM family in meningioma cells in western blots. We have chosen MCM3 as a representative member of the family and compared its expression profiles in three different meningioma cell lines, SF4433, SF4068, and SF3061 to primary arachnoidal cells. As shown in Fig. 4, MCM3 proteins were detected in all meningioma cell lines, whereas no expression was found in arachnoidal cells. We have also performed qRT-PCRs for determination of *MCM3* expression levels in those cells. As shown in Fig. 4B and D, we found a significant increase in *MCM3* expression in all three meningioma cell lines compared to primary arachnoidal cells. Moreover, low level of *MCM3* expression was observed in arachnoidal cells compared to SF4433, SF4068 and SF3061 cells.

DISCUSSION

To date, to our knowledge there is no report on proteomics based protein profiling in meningiomas compared to arachnoidal tissues, the origin of this tumor. In the present study, we define a meningioma protein signature by Gel-NanoLC-MS/MS profiling of a meningioma cell line. This signature includes 281 differential proteins in meningioma cells as compared with control primary arachnoidal cells. Out of these 281 proteins, 97 were found to be exclusively expressed in meningiomas; whereas 36 proteins were only detected in arachnoidal cells. Because of the striking exclusive expression of all MCM family members, we focused on this family of proteins. We performed subsequent validation studies by qRT-PCR in tissue samples and western blot on the cell lines and found that MCMs are highly and significantly up-regulated in human meningioma tumor samples compared to arachnoidal tissue controls.

MCMs were first discovered in yeast *Saccharomyces cerevisiae* mutants that had defects in maintaining a simple minichromosome.³⁴ Recent data suggested that these proteins are implicated not only in DNA maintenance but also in many other chromosome processes such as transcription, chromatin remodeling and genome stability.^{35,36} They are activated by cyclin-dependent kinases, such as Cdc6, Cdt1 and Dbf4/Cdc7 in the early G₁ phase of the cell cycle to form the origin complex called the pre-replication complex (pre-RC).^{37,39} The hexameric MCM component of the pre-RC shows helicase activity that may provide DNA unwinding services during replication.⁴⁰ Thus, MCM proteins allows the DNA replication machinery to access binding sites on the DNA.⁴⁰ MCMs are expressed in abundance in all phases of the cell cycle and degraded in quiescence, senescence and differentiation steps thus they can be used as a specific markers of the cell cycle state in tissues.⁴¹ This feature of MCM proteins in proliferating cells has led to their potential clinical application as a marker for cancer screening.⁴² Several studies suggested that increased levels of MCMs can identify not only malignant cells^{43–48} but also precancerous cells and recurrence of the tumor^{49–51} indicating that they might also serve as a prognostic tumor marker. Further validation studies need to be performed to resolve whether MCMs might be also prognostic marker for meningiomas.

We found that the combination of 1D SDS-PAGE and shotgun nanoLC-MS/MS was a very valuable approach for proteome analysis, enabling the synchronous identification and quantification of over 2,800 proteins. Besides the MCM family proteins, this analysis has proposed many other proteins that might contribute to meningioma tumorigenesis or be potential biomarkers for diagnosis, prognosis and treatment prediction, such as the RFC proteins, the SMC family proteins and MRE11, MSH6, HDAC2, FEN1, RAD50, and STAT2. To unravel the possible role(s) of these proteins in meningioma tumorigenesis, further investigations are needed.

So far, there has been no biomarker for meningiomas. In this study, we provided evidence that MCMs can serve as diagnostic biomarkers for meningiomas. Further validation is necessary to be able to use the expression of these proteins to predict the change of regrowth after surgery in order to improve medical care in meningioma patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported by the Children's Tumor Foundation 2007-01-043 (O.S.) and NINDS NS24279 (O.S.). We thank Ms. Silvina A. Fratanoni for assistance with the SDS-PAGE and in-gel digestion of the samples and Marianne F. James for human primary arachnoidal cells (Massachusetts General Hospital), and Dr. Xandra O. Breakefield for providing laboratory facilities for this work. The VUmc-Cancer Center Amsterdam is acknowledged for financial support for the proteomics infrastructure, TVP and CRJ.

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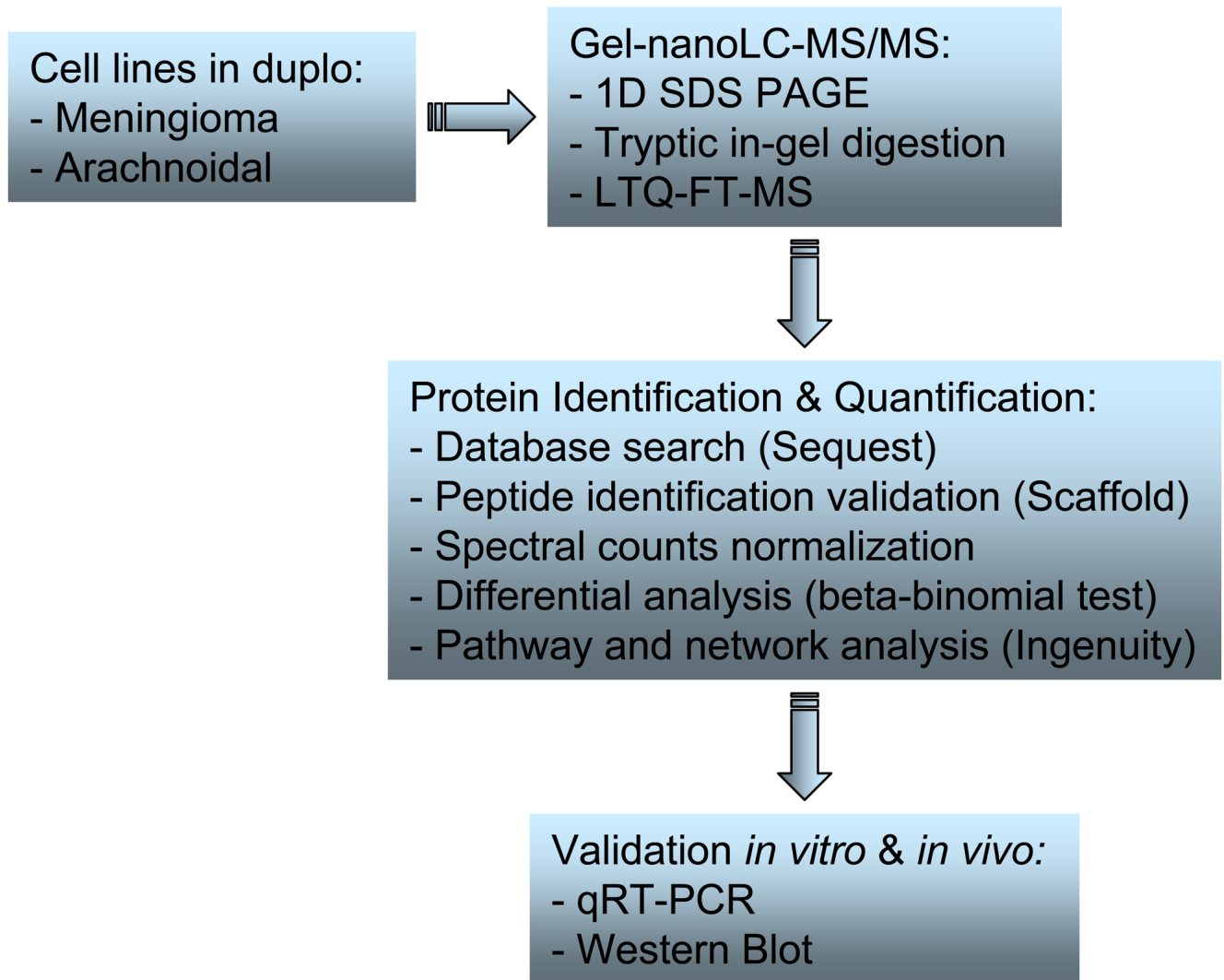


Fig. 1. A schematic presentation of the proteomics workflow applied to the analysis of meningioma and arachnoidal cells

Proteins from cell lysates were isolated by centrifugation and separated by SDS-PAGE. Separated proteins were digested and peptide extracts are injected to LC column and second separation was performed by chromatography. The samples eluted from the LC column were analyzed by mass spectrometry. The mass spectrum obtained MS/MS are compared with the theoretical spectrums provided by databases. The candidate proteins were validated *in vitro* and *in vivo* by qRT-PCR and western blots.

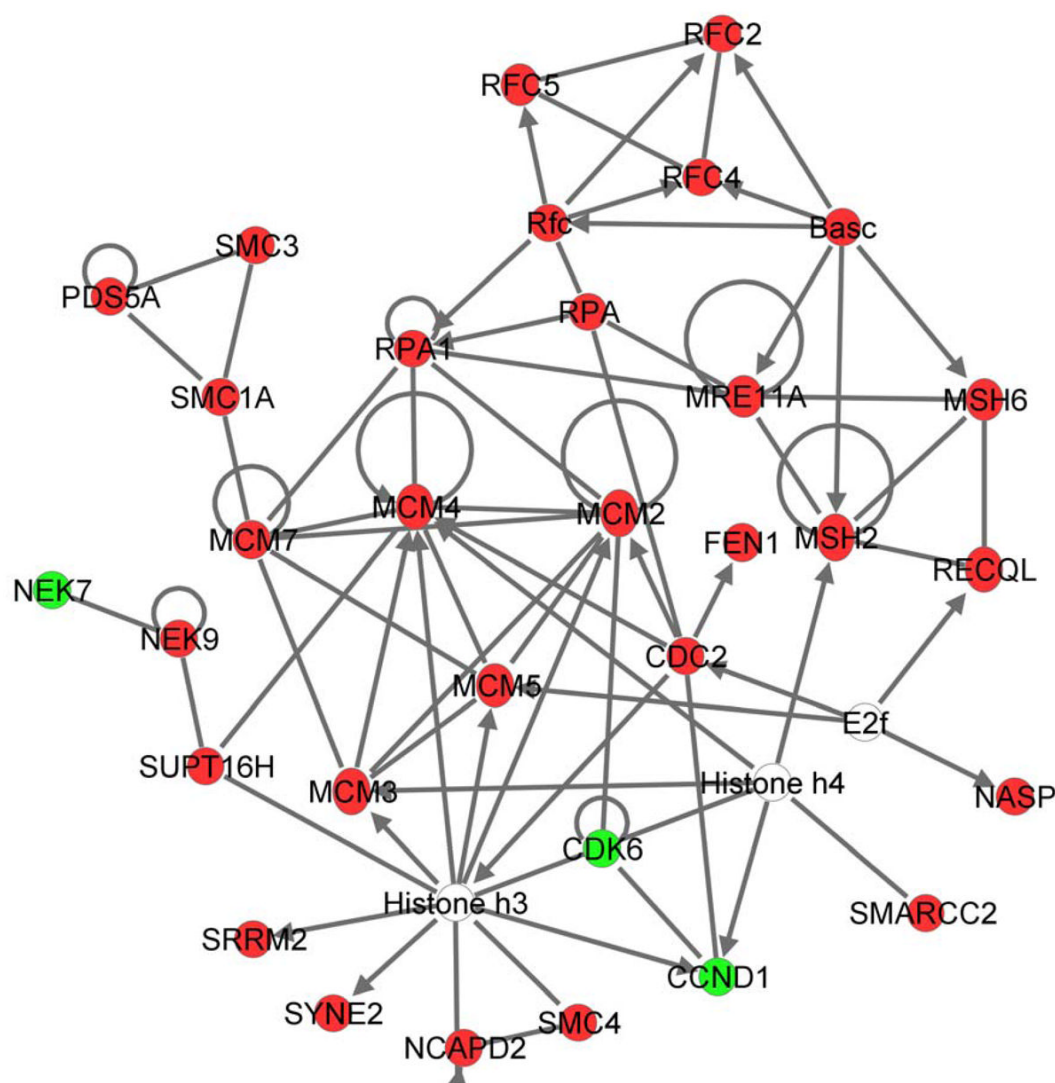


Fig. 2. Most significant network of direct relationship between on/off regulated differential proteins Ingenuity Pathway Analysis annotates the differential proteins with biological and cellular functions and then calculates which of these biological and cellular processes are significantly overrepresented within the list of differential proteins. Further, Ingenuity constructs a network of all the proteins involved in the overrepresented biological functions. Figure 2 presents an overview of the proteins exclusively expressed in either meningioma (in red) or arachnoidal (in green) cells and involved in the most significant overrepresented processes namely DNA replication, Recombination, and Repair, Cancer and Cell Cycle; and shows the direct relationships between the proteins as described in literature. Ingenuity constructed a highly significant functional network involved in DNA replication, Recombination, and Repair, Cancer, Cell Cycle of protein exclusively expressed in either meningioma (in red) or arachnoidal (in green) cells.

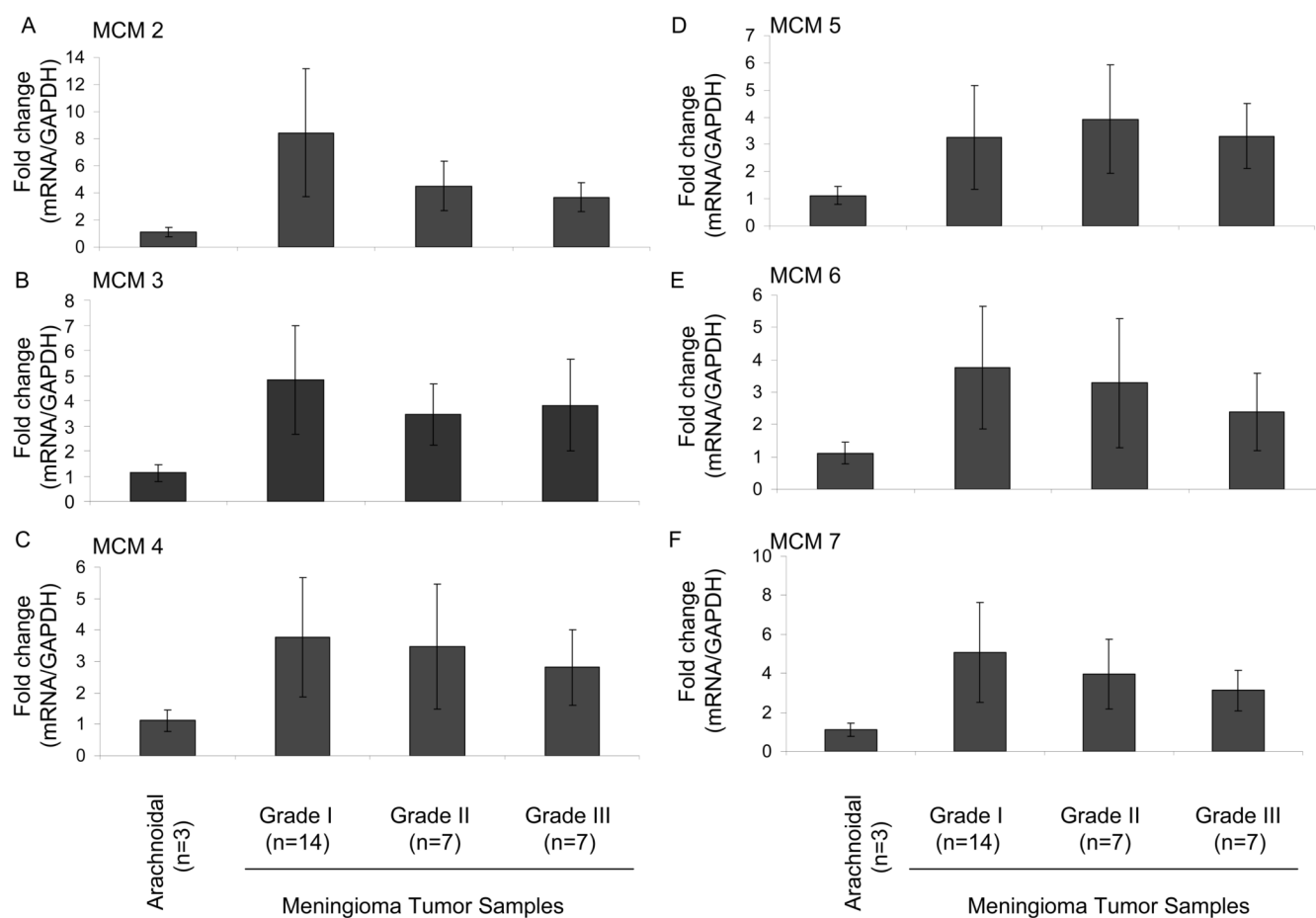


Fig. 3. Validation of the MCM family proteins in meningiomas by qRT-PCR

RNAs were isolated from arachnoidal and human meningioma tumor samples and qRT-PCR reactions were performed for *MCM2* (A), *MCM3* (B), *MCM4* (C), *MCM5* (D), *MCM6* (E), and *MCM7* (F) and normalized to *GAPDH* levels. The expression levels of the *MCM* transcripts in meningioma tumor samples were compared to human arachnoidal tissues. These experiments were performed in triplicate and the values are expressed as mean \pm S.D. P value for every pair-wise comparison in this experiment is below 0.0001 (***) $p < 0.0001$.

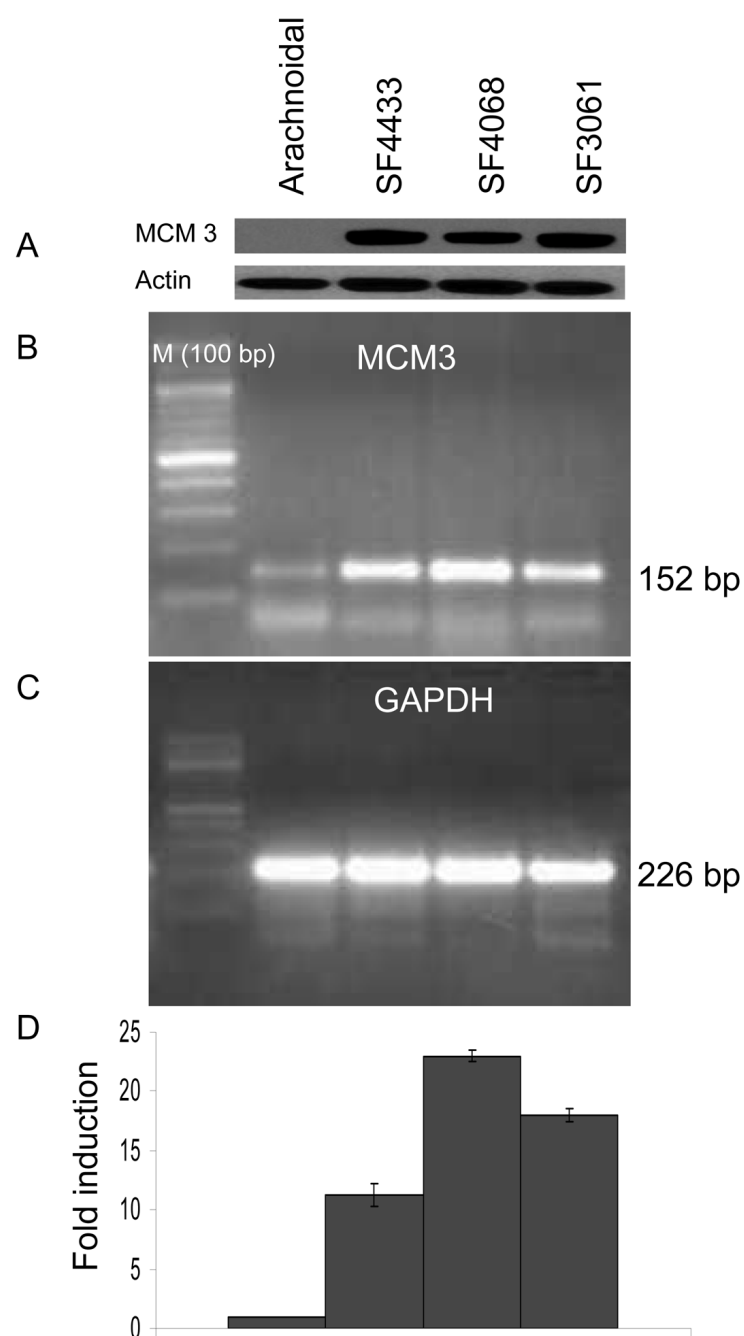


Fig. 4. MCM3 protein and mRNA expression levels in meningiomas

MCM3 protein (A) and mRNA levels (B, C, D) of meningioma cell lines, SF4433, SF4068, and SF3061 were compared to primary arachnoidal cells. A: Western blot analysis was performed with antibodies to MCM3 and anti-actin. B, C, and D: In a parallel experiment, total RNA was extracted from human primary arachnoidal cells and all three meningioma cell lines and qRT-PCR reactions were performed for *MCM3* and *GAPDH* mRNAs. The data were normalized to *GAPDH* mRNA in each sample.

Table 1

List of proteins exclusively expressed in meningioma cells

Gene symbol and protein name	Accession number	Normalized spectral counts			P-value
		Arachnoidal cells in duplo	Meningioma cells in duplo	Meningioma cells in duplo	
MX1 Interferon-induced GTP-binding protein Mx1	IP100167949	0	0	52	0.00005
MCM2 DNA replication licensing factor MCM2	IP100184330	0	0	23	0.00017
MCM3 DNA replication licensing factor MCM3	IP100013214	0	0	21	0.00019
MCM7 Isoform 1 of DNA replication licensing factor MCM7	IP100299904	0	0	21	0.00026
MCM4 DNA replication licensing factor MCM4	IP100018349	0	0	19	0.00027
SAMHD1 SAM domain and HD domain-containing protein 1	IP100294739	0	0	14	0.00035
MCM5 DNA replication licensing factor MCM5	IP100018350	0	0	16	0.00037
RECQL ATP-dependent DNA helicase Q1	IP100178431	0	0	11	0.00047
OAS3 2'-5'-oligoadenylate synthetase 3	IP100002405	0	0	10	0.00054
SYNE2 Isoform 1 of Nesprin-2	IP100239405	0	0	20	0.00055
EPPK1 Epiplakin	IP100010951	0	0	16	0.00056
CDC2 Hypothetical protein DKFZp686L20222	IP100026689	0	0	10	0.00058
HMG2 High mobility group protein B2	IP100219097	0	0	9	0.00064
RPA1 Replication protein A 70 kDa DNA-binding subunit	IP100020127	0	0	10	0.00074
CLDN11 Claudin-11	IP100026053	0	0	9	0.00080
SRRM2 Isoform 1 of Serine/arginine repetitive matrix protein 2	IP100782992	0	0	13	0.00096
NASP Isoform 1 of Nuclear autoantigenic sperm protein	IP100179953	0	0	7	0.00097
SMC4 Isoform 1 of Structural maintenance of chromosomes protein 4	IP100411559	0	0	7	0.00107
MCAM Isoform 1 of Cell surface glycoprotein MUC18 precursor	IP100016334	0	0	6	0.00122
FEN1 Flap endonuclease 1	IP100026215	0	0	6	0.00122
RRM1 Ribonucleoside-diphosphate reductase large subunit	IP100013871	0	0	6	0.00127
FXR1 Isoform 1 of Fragile X mental retardation syndrome-related protein 1	IP100016249	0	0	7	0.00130
CRABP2 Cellular retinoic acid-binding protein 2	IP100216088	0	0	6	0.00142
RIF1 Isoform 1 of Telomere-associated protein RIF1	IP100477805	0	0	5	0.00166
SMARCC2 Isoform 2 of SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily C member 2	IP100150057	0	0	5	0.00173
TMPO Lamina-associated polypeptide 2 isoform alpha	IP100216230	0	0	8	0.00189

Gene symbol and protein name	Accession number	Normalized spectral counts			P-value
		Arachnoidal cells in duplo	Meningioma cells in duplo		
TCEA1 Isoform 1 of Transcription elongation factor A protein 1	IP100333215	0	0	5	0.00192
EFTUD2 116 kDa U5 small nuclear ribonucleoprotein component	IP100003519	0	0	6	0.00195
PGM2 Phosphoglucomutase-2	IP100550364	0	0	5	0.00200
RFC4 Replication factor C subunit 4	IP100017381	0	0	5	0.00200
VPS13A 351 kDa protein	IP100251344	0	0	9	0.00217
PDS5A SCC-112 protein	IP100303063	0	0	4	0.00243
OAS2 Isoform p69 of 2'-5'-oligoadenylate synthetase 2	IP100218185	0	0	4	0.00253
GALE UDP-glucose 4-epimerase	IP100553131	0	0	4	0.00253
NNMT Nicotinamide N-methyltransferase	IP100027681	0	0	6	0.00257
MRE11A Isoform 1 of Double-strand break repair protein MRE11A	IP100029159	0	0	5	0.00265
TMEM137;RBM14 Isoform 1 of RNA-binding protein 14	IP100013174	0	0	5	0.00265
UBE2C Ubiquitin-conjugating enzyme E2 C	IP100013002	0	0	5	0.00265
PSMB9 Isoform LMP2.L of Proteasome subunit beta type 9 precursor	IP100000787	0	0	4	0.00284
TPRKB Isoform 3 of TPS3RK-binding protein	IP100217362	0	0	4	0.00313
NCAPD2 Condensin complex subunit 1	IP100299524	0	0	4	0.00313
RCC2 Protein RCC2	IP100465044	0	0	4	0.00313
SMCHD1 similar to SMC hinge domain containing 1	IP100465022	0	0	8	0.00320
DYSF Dysferlin_v1	IP100020210	0	0	4	0.00341
IFIT1 Interferon-induced protein with tetratricopeptide repeats 1	IP100018300	0	0	3	0.00399
DNAJB1 DnaJ homolog subfamily B member 1	IP100015947	0	0	3	0.00399
TAP1 transporter 1, ATP-binding cassette, sub-family B	IP100646625	0	0	3	0.00399
GGH Gamma-glutamyl hydrolase precursor	IP100023728	0	0	3	0.00399
PTK7 PTK7 protein tyrosine kinase 7 isoform c precursor	IP100168813	0	0	3	0.00413
ANP32E Acidic leucine-rich nuclear phosphoprotein 32 family member E	IP100165393	0	0	3	0.00413
H1FO Histone H1.0	IP100550239	0	0	3	0.00413
SUPT16H FACT complex subunit SPT16	IP100026970	0	0	5	0.00421
SCD Acyl-CoA desaturase	IP100299468	0	0	5	0.00421
C6orf211 UPF0364 protein C6orf211	IP100002270	0	0	5	0.00421
SPC24 Kinetochore protein Spc24	IP100168317	0	0	5	0.00421

Gene symbol and protein name	Accession number	Normalized spectral counts			P-value
		Arachnoidal cells in duplo	Meningioma cells in duplo		
PPIF Peptidyl-prolyl cis-trans isomerase, mitochondrial precursor	IP100026519	0	0	3	0.00441
PYGL Glycogen phosphorylase, liver form	IP100470525	0	0	3	0.00441
C20orf77 Uncharacterized protein C20orf77	IP100009659	0	0	3	0.00441
DNMT1 Isoform 1 of DNA (cytosine-5)-methyltransferase 1	IP100031519	0	0	4	0.00452
DEK Protein DEK	IP100020021	0	0	4	0.00452
GNL1 guanine nucleotide binding protein-like 1	IP100396387	0	0	4	0.00452
C7orf50 MGC11257 protein	IP100031651	0	0	4	0.00452
MVK Mevalonate kinase	IP100010717	0	0	4	0.00452
SMC3 Structural maintenance of chromosomes protein 3	IP100219420	0	0	3	0.00576
SMC1A Structural maintenance of chromosomes protein 1A	IP100291939	0	0	3	0.00576
IFIT3 Interferon-induced protein with tetratricopeptide repeats 3	IP100024254	0	0	3	0.00583
FAM129A Niban protein	IP100328350	0	0	3	0.00583
UBE2E1;UBE2E2 Ubiquitin-conjugating enzyme E2 E1	IP100021346	0	0	3	0.00583
FAM128B hypothetical protein LOC80097	IP100410094	0	0	3	0.00583
MSH2 DNA mismatch repair protein Msh2	IP100017303	0	0	2	0.00800
IGF2BP1 Insulin-like growth factor 2 mRNA-binding protein 1	IP100008557	0	0	2	0.00800
RFC2 Isoform 1 of Replication factor C subunit 2	IP100017412	0	0	2	0.00800
RFC5 Replication factor C subunit 5	IP100031514	0	0	2	0.00806
PLSCR1 Phospholipid scramblase 1	IP100005181	0	0	4	0.00837
C10orf35 Uncharacterized protein C10orf35	IP100060546	0	0	4	0.00837
SDC1 Syndecan-1 precursor	IP100002441	0	0	5	0.00872
MSH6 Isoform GTBP-N of DNA mismatch repair protein MSH6	IP100384456	0	0	2	0.00930
TK1 Thymidine kinase, cytosolic	IP100299214	0	0	2	0.00930
DDX58 Isoform 1 of Probable ATP-dependent RNA helicase DDX58	IP100654731	0	0	2	0.00974
CBFB core-binding factor, beta subunit isoform 1	IP100024871	0	0	2	0.00974
C16orf61 UPF0287 protein C16orf61	IP100024619	0	0	2	0.00974
ATG3 Isoform 1 of Autophagy-related protein 3	IP100022254	0	0	2	0.00974
NEK9 Serine/threonine-protein kinase Nek9	IP100301609	0	0	2	0.00974
SFRS10 Isoform 1 of Splicing factor, arginine/serine-rich 10	IP100301503	0	0	2	0.00974

Gene symbol and protein name	Accession number	Normalized spectral counts			P-value
		A rachnoidal cells in duplo	Meningioma cells in duplo		
SDHC Succinate dehydrogenase cytochrome b560 subunit, mitochondrial precursor	IP100016968	0	0	2	0.00974
GNPDA2 Glucosamine-6-phosphate isomerase SB52	IP100550894	0	0	2	0.00974
TPD52 Tumor protein D52	IP100218323	0	0	2	0.00974
GDA Guanine deaminase	IP100465184	0	0	2	0.00974
DDX42 Isoform 1 of ATP-dependent RNA helicase DDX42	IP100409671	0	0	3	0.00991
FLJ14668 Hypothetical protein FLJ14668	IP100303722	0	0	3	0.00991
HSPA4L Heat shock 70 kDa protein 4L	IP100295485	0	0	3	0.00991
IFIT2 Interferon-induced protein with tetrapeptide repeats 2	IP100018298	0	0	3	0.00991
POLR2J RPB11a protein	IP100003310	0	0	3	0.00991
CTPS2 CTP synthase 2	IP100645702	0	0	3	0.00991
LEPREL1 Prolyl 3-hydroxylase 2 precursor	IP100217055	0	0	3	0.00991
EXOSC1 3'-5' exoribonuclease CSL4 homolog	IP100032823	0	0	3	0.00991
TMPO Isoform Beta of Lamina-associated polypeptide 2, isoforms beta/gamma	IP100030131	0	0	3	0.00991

Table 2

List of proteins exclusively expressed in arachnoidal cells

Gene symbol and protein name	Accession number	Normalized spectral counts			P-value
		Arachnoidal cells in duplo	Meningioma cells in duplo		
GLS Isoform KGA of Glutaminase kidney isoform, mitochondrial precursor	IP100289159	17	0	0	0.00025
SDCBP Syntenin isoform 3	IP100479018	12	0	0	0.00050
NEK7 Serine/threonine-protein kinase Nek7	IP100152658	12	0	0	0.00050
GALNT1 Isoform 1 of Polypeptide N-acetylgalactosaminyltransferase 1	IP100025818	10	0	0	0.00060
ITGA2 Integrin alpha-2 precursor	IP100013744	10	0	0	0.00070
SERPINE1 Plasminogen activator inhibitor 1 precursor	IP100007118	10	0	0	0.00084
AKR1C3 Aldo-keto reductase family 1 member C3	IP100291483	7	0	0	0.00106
HSPB6 Heat-shock protein beta-6	IP100022433	10	0	0	0.00110
KCTD12 BTB/POZ domain-containing protein KCTD12	IP100060715	8	0	0	0.00136
ATP6V0A1 Isoform 1 of Vacuolar proton translocating ATPase 116 kDa subunit a isoform 1	IP100465178	6	0	0	0.00155
QPRT Nicotinate-nucleotide pyrophosphorylase	IP100300086	7	0	0	0.00186
TM9SF2 Transmembrane 9 superfamily protein member 2 precursor	IP100018415	5	0	0	0.00192
TMEM173 Transmembrane protein 173	IP100257059	4	0	0	0.00274
LOX Protein-lysine 6-oxidase precursor	IP100002802	6	0	0	0.00275
IKIP IKIP2	IP100401791	4	0	0	0.00289
ALDOC Fructose-bisphosphate aldolase C	IP100418262	5	0	0	0.00290
CRABP1 Cellular retinoic acid-binding protein 1	IP100219930	5	0	0	0.00290
TOM1 Target of myb1	IP100023191	4	0	0	0.00350
MICA:HLA-A:HLA-A29.1;LOC730410:HLA-B:HLA-C HLA class I histocompatibility antigen, A-23 alpha chain precursor	IP100472151	3	0	0	0.00455
SLC12A2 Isoform 1 of Solute carrier family 12 member 2	IP100022649	5	0	0	0.00458
COL18A1 Isoform 2 of Collagen alpha-1(XVIII) chain precursor	IP100022822	3	0	0	0.00503
CCND1 G1/S-specific cyclin-D1	IP100028098	3	0	0	0.00503
CDK6 Cell division protein kinase 6	IP100023529	4	0	0	0.00505
SIL1 Nucleotide exchange factor SIL1 precursor	IP100296197	4	0	0	0.00505
RND3 Rho-related GTP-binding protein RhoE precursor	IP100001437	4	0	0	0.00505
ITIH3 Inter-alpha-trypsin inhibitor heavy chain H3 precursor	IP100028413	7	0	0	0.00547

Gene symbol and protein name	Accession number	Normalized spectral counts			P-value
		Arachnoidal cells in duplo	Meningioma cells in duplo		
COMMD7 COMMD7 protein	IP100641139	3	3	0	0.00673
HCCS Cytochrome c-type heme lyase	IP100023406	3	3	0	0.00673
KIAA1199 Isoform 1 of Protein KIAA1199 precursor	IP100376689	3	3	0	0.00673
DNAJC5 Isoform 2 of DnaJ homolog subfamily C member 5	IP100023780	3	3	0	0.00673
EHD3 EH domain-containing protein 3	IP100021458	3	3	0	0.00673
ETFDH Isoform 1 of Electron transfer flavoprotein-ubiquinone oxidoreductase, mitochondrial precursor	IP100032875	3	3	0	0.00673
SEC24B Protein transport protein Sec24B	IP100030851	2	4	0	0.00926
FAH Fumarylacetoacetase	IP100031708	4	2	0	0.00930
CLU clusterin isoform 1	IP100400826	5	2	0	0.00936
RABGGTA Geranylgeranyl transferase type-2 alpha subunit	IP100022664	5	2	0	0.00936