Global expression profile in low grade meningiomas and schwannomas shows upregulation of *PDGFD*, *CDH1* and *SLIT2* compared to their healthy tissue

MIGUEL TORRES-MARTIN[1], LUIS LASSALETTA[2], ALBERTO ISLA[3], JOSE M. DE CAMPOS[4],
GIOVANNY R. PINTO[5], ROMMEL R. BURBANO[6], JAVIER S. CASTRESANA[7],
BARBARA MELENDEZ[8] and JUAN A. REY[1]

[1]Molecular Neuro-Oncogenetics Laboratory, Research Unit, and Departments of [2]Otolaryngology and [3]Neurosurgery,
Hospital Universitario La Paz, IdiPAZ, Madrid; [4]Department of Neurosurgery, Fundacion Jimenez Diaz, Madrid, Spain;
[5]Genetics and Molecular Biology Laboratory, Federal University of Piau, Parnaiba; [6]Human Cytogenetics Laboratory,
Institute of Biological Sciences, Federal University of Para, Belem, Brazil; [7]Department of Biochemistry and Genetics,
University of Navarra School of Sciences, Pamplona; [8]Molecular Pathology
Research Unit, Virgen de la Salud Hospital, Toledo, Spain

Abstract. Schwannomas and grade I meningiomas are non-metastatic neoplasms that share the common mutation of gene *NF2*. They usually appear in neurofibromatosis type 2 patients. Currently, there is no drug treatment available for both tumors, thus the use of wide expression technologies is crucial to identify therapeutic targets. Affymetrix Human Gene 1.0 ST was used to test global gene expression in 22 meningiomas, 31 schwannomas and, as non-tumoral controls, 3 healthy meningeal tissues, 8 non-tumoral nerves and 1 primary Schwann cell culture. A non-stringent P-value cut-off and fold change were used to establish deregulated genes. We identified a subset of genes that were upregulated in meningiomas and schwannomas when compared to their respectively healthy tissues, including *PDGFD*, *CDH1* and *SLIT2*. Thus, these genes should be thoroughly studied as targets in a possible combined treatment.

Introduction

Schwannomas are benign tumors that arise from Schwann cells. They typically appear in the vestibulocochlear nerve and are considered to be grade I tumors; approximately 95% are unilateral and present sporadically, whereas 5% are associated with neurofibromatosis type 2 syndrome (NF2). Patients with NF2 present with bilateral schwannomas and other tumors, frequently meningiomas, which originate from arachnoid cells, and account for 20% of all primary intracranial tumors. The current classification of meningiomas by the World Health Organization (WHO) includes three grades: 90% are classified as grade I tumors; ~8-9% are atypical grade II tumors; and 1-2% are anaplastic/malignant grade III tumors (1). Meningiomas have a recurrence rate of 18, 40 and 80% for grade I, II and III, respectively.

Preliminary cytogenetic studies have demonstrated the absence of one chromosome 22 in both neoplasms (2,3), thus suggesting a common genetic origin for at least some subgroups of these neurogenic tumors. Subsequently, *NF2* gene (located at 22q12.2) inactivation was found to be due to several mechanisms, such as mutations or allelic loss due to monosomy or deletion of chromosome 22, accounting for up to 66% in schwannomas (4) and 18-50% in sporadic meningiomas, depending on the histopathological subtypes (5). In addition to the characteristic chromosome 22 loss, secondary alterations such as 1p deletions have been described in both tumor types, and these alterations appear to be related to tumor progression in meningiomas (6-8). Although DNA methylation studies on these neurogenic tumors have revealed the non-random involvement of this mechanism in the inactivation of some tumor-related genes (9-11), controversial data are available concerning the epigenetic (through CpG island aberrant methylation) *NF2* inactivation in both neoplasms (12-16). Indeed, recent studies on genome-wide methylation suggest that this mechanism is associated with malignant transformation in meningiomas, and allows for the epigenetic subclassification of this tumor (17,18).

Global exome sequencing in meningiomas showed that, in grade I tumors, *NF2* gene alteration (by mutation and/or

Correspondence to: Dr Juan A. Rey or Miguel Torres-Martin, Molecular Neuro-Oncogenetics Laboratory, Research Unit, Hospital Universitario La Paz, IdiPAZ, 261 Paseo de la Castellana, 28046 Madrid, Spain
E-mail: jreyh@salud.madrid.org
E-mail: migtorres.martin@gmail.com

loss of chromosome 22) is mutually exclusive with other gene mutations such as *AKT1, TRAF7, KLF4* and *SMO* (19), but not with others such as *NF1* and *NEGR1* (20). In schwannomas, no alternative mutation has been found for those samples lacking hits over *NF2* and, however, Merlin (the NF2 protein) does not seem to be present in the cases analyzed to date (21).

The expression analysis of tumor-related genes in meningiomas and schwannomas suggests a possible molecular subgroup classification in both tumors (22) with the involvement of differential regulatory pathways (23,24) related to the allelic losses at 1p and 14q in meningiomas (25). Whole genome expression analysis has been performed on schwannomas (26-28) and meningiomas (29-32). Whereas meningiomas have shown differential expression patterns based on progression and recurrence, but not strictly supported by grade (31), in schwannomas no distinctive pattern has been found using clinical correlations (28). However, a critical deregulation of microRNAs, including the upregulation of those located at the 14q32 chromosomal region, was a characteristic feature of vestibular tumors (33).

Intracranial non-recurrent WHO grade I meningiomas and schwannomas represent similar problems for patients, depending on the brain structures affected by their non-invasive growth. Currently, treatment options for patients with grade I meningiomas or schwannomas include surgery resection, radio-surgery and a 'wait and see' strategy. Thus, there is no available chemotherapeutic treatment for these tumors besides surgery, a situation especially traumatic for patients suffering bilateral vestibular schwannomas and several meningiomas such as those affected by NF2. Due to the common genetic origin of these tumors (*NF2* inactivation), previous studies have attempted to identify targets with which to inhibit both schwannoma and meningioma progression. AR42, a histone deacetylase inhibitor, was found to suppress the proliferation of meningioma and schwannoma cell lines *in vitro* (34), and the same effect was shown by cucurbitacin D and goyazensolide in primary cultures (35).

In the present study, we used microarray technology to compare gene-expression patterns and identify genes and pathways of potential interest as key targets for the combined treatment of vestibular schwannomas and grade I meningiomas.

Materials and methods

Statement of ethics and samples. The local Ethics Review Board of La Paz University Hospital approved the study protocol according to the principles of the Declaration of Helsinki. All patients received detailed information concerning the study and provided their written informed consent prior to their inclusion. In this study, we used RNA from 22 meningiomas, 31 schwannomas and, as non-tumoral controls, 3 healthy meningeal tissues, 8 non-tumoral nerves and 1 primary Schwann cell culture. The three control non-tumoral meningeal RNAs derived from two healthy males and one female and were purchased from BioChain® (cat. no. R1234043-10-D03; lot nos. B108134, A602330 and B501146).

RNA extraction and microarray experiments. The RNA was extracted with the RNeasy® Mini kit (Qiagen, Valencia, CA,

USA) as indicated previously (28). For global gene expression, the Affymetrix Human Gene 1.0 ST was used. The expression profile of the meningiomas and the meninges samples can be accessed at the gene expression Omnibus (GEO) database GSE54934. The arrays of schwannomas and control nerves were previously published (28) and are available at the GEO database GSE39645. The arrays were processed at the Institute for Research in Biomedicine (IRB), Barcelona, Spain.

Statistical analysis. The normalization and summarization were performed using the robust multichip average (RMA). In order to reduce the batch effect among tumors (schwannomas and meningiomas) and controls (healthy nerves and meninges), a critical aspect for our analysis, we used ComBat (36). For data analysis, the genes were considered deregulated between groups when at least a 2-fold change in expression and a $P<0.05$ cut-off (ANOVA) was identified, as previously recommended by the MAQC consortium (37). For the comparison between schwannomas and meningiomas in order to obtain a list of genes with no changes among both tumor types, we used a more restrictive fold (<1.5) exclusively for this purpose, since the ComBat effect could have lowered these values and false-positives could appear. For comparative purposes, a list of differentially expressed genes and fold-change was obtained with the Geo2R web tool (http://www.ncbi.nlm.nih.gov/geo/geo2r/) in the series GSE43290, which includes 4 meninges as controls and 47 tumors (29). As our meningioma series mainly included grade I tumors, only the 33 WHO grade I meningiomas and the 4 controls included in this report were used for comparison.

DNA extraction. The DNA was extracted by standard methods, as previously described (22). The data regarding the *NF2* status, including loss of heterozygosity of 22q (LOH), Multiplex ligation-dependent probe amplification (MLPA) of *NF2* (SALSA P044) and sequence analysis by dHPLC were reported previously in detail (22,28), and were performed as described (22). Clinical and *NF2* status data from the meningiomas correspond to cases M02, M04, M05, M07, M09, M10, M12, M14, M24, M25, M28, M29, M30, M31, M32, M33, M34, M38, M39, M40, M41 and M42, as previously reported (22). The complete case series of schwannomas from our previous report (28) was included.

Results and Discussion

Comparison with respect to previous analyses of meningiomas and the summary of results in schwannomas. Meningioma profiling was analyzed extensively in previous studies, and up to five expression subgroups were characterized (31), although this classification did not represent the actual WHO classification. Recurrence and progression appear to play a relevant role in the expression pattern of these tumors (32), and two meningioma groups were identified showing different clinical and pathological behaviors, more related to clinical outcome than to WHO grade *per se*. Furthermore, depending on the cytogenetic aberrations, differential expression patterns have been described (25,29). Tumors that presented monosomy of chromosome 22 and cases with multiple karyotype alterations had a differential expression pattern, whereas those cases with

deletion of chromosome 1 alone showed random behavior (29). In summary, previous analyses of gene-expression patterns in meningiomas do not seem to accurately represent the current WHO classification, although recurrence and progression status might be reflected in these studies. We used 20 grade I meningiomas, 2 grade II meningiomas and 3 healthy meninges. Practically the same values were obtained when meningiomas grade II were removed from the study (data not shown). When we compared our results in meningiomas with those obtained from the dataset GSE43290 (29), we found a high consistency in our results, such as the downregulation of diverse genes such as *SNAP25, MBP, TTR* and *VSNL1*, and the upregulation of *FBN2, FGF9* and *SULF1* (full data available upon request). As in previous studies, our 2 cases of meningioma grade II did not show a different trend. The schwannoma expression profile was previously explained (28). In brief, the upregulation of *SPP1, MET* and associated genes or *LATS2* was reported, whereas the downregulation of *CAV1, AR* and *PAWR* was found. In general, myelinization genes were overexpressed, suggesting that schwannoma cells could resemble a previous state of mature Schwann cells.

Gene co-overexpression in meningiomas and schwannomas. Using the ANOVA test at P<0.05 significance across the four groups (all meningiomas, schwannomas, control healthy meninges and control nerves), we obtained a list of 12,395 genes with differential expression among these four groups. Of those, 346 (data not shown; available upon request) did not meet the criteria established for accepting deregulation differences between both tumor groups, which was ≤1.5-fold of the differential expression between the schwannomas and the meningiomas, a limit value selected as deregulated between these two groups due to the correction effect of ComBat. Among those 346 genes with similar expression in tumors, 47 showed co-overexpression in schwannomas and meningiomas when compared with their respective controls at 2-fold (as ComBat correction would only be based on batch effect) (Table I). These genes included E-cadherin (*CDH1*), which is usually silenced by several mechanisms, such as the Wnt signaling pathway in human cancer, including meningiomas (38), and platelet-derived growth factor D (*PDGFD*), an activator for PDGFR-β (39). This pathway has been reported as overexpressed in multiple cancer types such as pancreatic cancer and brain tumors, including schwannomas (39). Another gene reported as expressed (and protein present) in meningiomas and schwannomas is tyrosine kinase receptor *MET* (40), which is responsible for cell migration, anchorage-independent growth and many other functions. High levels of this receptor have been found in a wide variety of tumors, such as breast cancer, renal cell carcinoma and head and neck tumors (41). Mechanisms such as point mutations, alternative splicing, genomic amplification and transcript amplification appear to participate in overexpression of *c-MET* (reviewed in ref. 42). Accordingly, we found *MET* upregulation in both neoplasms compared with their respective control tissues, and again, a similar level of expression between both tumor types was detected. *SLIT2* is a member of the Slit family that modulates cell migration by binding with the Robo family. This gene has been found expressed in the development of several malignancies such as colorectal epithelial cell

carcinogenesis (43). The findings in this report (43), suggest that Slit2-Robo1 causes E-cadherin degradation, and although our results show an upregulation of the E-cadherin gene, the former mechanism should not be ruled out in the tumors we studied. In other neoplasms, although expressed, *SLIT2* does not seem to play any role (44). In agreement with the data from the study selected for validation (29), several genes, including *CDH1, PDGFD, CX3CR1, CCND1* and *SLIT2*, were also upregulated, as shown in data obtained from meningioma dataset GSE43290 (29); in contrast, *MET* showed a trend of upregulation but did not reach 2-fold. Functional annotation using DAVID showed enrichment in inflammatory response, cell migration and defense response (data not shown; available upon request).

Gene co-infraexpression in meningiomas and schwannomas. A total of 35 genes (Table II) with no difference in expression between schwannomas and meningiomas were underexpressed when compared with their respective controls in both neoplasms. Among them are selectin E (*SELE*) and Rho family GTPase 1 (*RND1*), which is linked to semaphorins (45,46) and cytoskeleton organization in axons. The chemokine (C-X-C motif) ligand 2 (*CXCL2*) was significantly downregulated in schwannomas and meningiomas, whereas the opposite trend has been shown in malignant neoplasms such as ovarian and endometrial cancer and oral squamous cell carcinoma (47). As schwannomas and meningiomas are usually non-invasive, this fact could explain the different trend in deregulation of *CXCL2*. Stathmin-like 2 (*STMN2*) showed the same pattern: upregulation in hepatoma cells but downregulation in schwannomas and meningiomas. Notably, *STMN2* interacts with Rho family GTPase 1 (*RND1*) in axon extension (48), another gene that was downregulated in both tumors. Other downregulated genes in both tumors were E-selectin (*SELE*) and vascular adhesion protein 1 (*AOC3*), related to the tethering and rolling of leukocytes (49); thus, the non-invasive nature of grade I meningiomas and schwannomas could explain the downregulation of these genes. Validation with the dataset GSE43290 was performed, and included, among others, downregulation of *AOC3, STMN2, SELE, RGS4, THBS4* and *RND1*. Functional analysis with DAVID included leukocyte and cell migration, heparin binding or membrane fraction (data available upon request).

Gene expression differences between meningiomas and schwannomas. The main goal of our study was to test gene expression profiles common to schwannomas and meningiomas in regard to their respective controls, and having taken into account their relative expression. However, we also studied the gene expression differences between both neurogenic neoplasms. As samples were processed in various batches, we used a Bayesian method to reduce the batch effect. Because of this effect, the differential expression of certain genes in schwannomas and meningiomas could have decreased. This issue, although it limits our information, is vital to our study since the batch effect was very marked; 192 genes were upregulated at 1.5-fold differences and P<0.05 (data available upon request) in schwannomas as compared to meningioma expression. Most of these genes are related to neuron migration and the myelin sheath, such as the following: peripheral

Table I. Genes overexpressed in meningioma and schwannoma when compared with their respective control tissue.

Gene	Database	Chromosome	C-M	N-S	M-S	P-value
CDH1	NM_004360	16q22.1	5.4	5.8	-1.0	8.35E-09
PDGFD	NM_025208	11q22.3	4.4	6.2	-1.1	7.48E-13
SLIT2	NM_004787	4p15.2	3.6	5.8	-1.1	2.02E-13
HLA-DPA1	NM_033554	6p21.3	2.9	3.9	-1.1	5.24E-07
PAPPA	NM_002581	9q33.2	2.8	3.8	-1.1	3.68E-06
TREM2	NM_018965	6p21.1	2.7	5.5	-1.3	2.8E-12
HLA-DPA1	NM_033554	6p21.3	2.6	4.1	-1.2	5.24E-07
HPGDS	NM_014485	4q22.3	2.6	2.4	-1.1	3.83E-07
GPR34	NM_001097579	Xp11.4	2.6	12.0	-1.5	9.9E-11
CX3CR1	NM_001337	3p21|3p21.3	2.5	5.7	-1.2	2.15E-07
ANKRD22	NM_144590	10q23.31	2.5	7.3	-1.4	1.04E-06
C3	NM_000064	19p13.3-p13.2	2.4	2.6	-1.2	5.77E-05
CYBB	NM_000397	Xp21.1	2.4	4.1	-1.2	1.11E-07
LGALS3BP	NM_005567	17q25	2.4	2.9	-1.1	4.67E-13
WIPI1	NM_017983	17q24.2	2.4	2.0	-1.0	2.55E-12
APOBEC3C	NM_014508	22q13.1	2.4	2.5	-1.1	3.35E-08
C3AR1	NM_004054	12p13.31	2.4	4.8	-1.2	4.43E-09
FCGBP	NM_003890	19q13.1	2.3	9.7	-1.3	1.03E-11
FRAS1	NM_025074	4q21.21	2.3	3.2	-1.2	7.42E-08
FLRT3	NM_198391	20p11	2.3	3.8	-1.2	1.76E-05
FCGR1A	NM_000566	1q21.2-q21.3	2.3	4.3	-1.3	1.66E-08
MET	NM_001127500	7q31	2.3	4.9	-1.3	1.22E-08
ITPR3	NM_002224	6p21	2.3	3.7	-1.1	3.69E-12
FCGR1B	NM_001017986	1p11.2	2.3	2.9	-1.2	4.14E-08
ALCAM	NM_001627	3q13.1	2.2	2.5	-1.0	4.26E-09
HLA-DPB1	NM_002121	6p21.3	2.2	4.6	-1.3	1.75E-07
LAMB1	NM_002291	7q22	2.2	2.0	-1.2	4.57E-07
C8orf84	NM_153225	8q21.11	2.2	2.6	-1.2	8.11E-06
SLFN12	NM_018042	17q12	2.2	2.3	-1.2	4.14E-10
FCGR1A	NM_000566	1q21.2-q21.3	2.2	3.1	-1.2	1.66E-08
LHFPL2	NM_005779	5q14.1	2.1	2.3	-1.1	8.13E-09
MS4A6A	NM_152852	11q12.1	2.1	4.5	-1.3	4.8E-08
CD84	NM_001184879	1q24	2.1	2.7	-1.2	3.38E-09
TRIM22	NM_006074	11p15	2.1	2.2	-1.1	2.09E-09
CD4	NM_000616	12pter-p12	2.1	2.5	-1.1	1.69E-07
CSF1R	NM_005211	5q32	2.1	3.8	-1.2	5.21E-08
GFRA1	NM_005264	10q26.11	2.1	4.9	-1.4	1.34E-07
HLA-DPB1	NM_002121	6p21.3	2.1	4.5	-1.3	1.75E-07
CD86	NM_175862	3q21	2.1	2.9	-1.3	1.03E-06
C1QA	NM_015991	1p36.12	2.1	4.3	-1.2	1.24E-07
TLR7	NM_016562	Xp22.3	2.0	3.6	-1.3	7E-08
CCND1	NM_053056	11q13	2.0	2.7	-1.1	1.48E-11
HLA-DQA1	NM_002122	6p21.3	2.0	2.6	-1.2	4.19E-05
FAM105A	NM_019018	5p15.2	2.0	2.6	-1.1	9.2E-08
C6orf138	NM_001013732	6p12.3	2.0	3.4	-1.2	1.99E-10
P2RY13	NM_176894	3q24	2.0	2.4	-1.2	3.08E-06
PROS1	NM_000313	3q11.2	2.0	5.0	-1.4	9.1E-14

Official gene symbol is shown for every gene. C-M values correspond to the fold-change value of control healthy meninges (C) minus meningioma (M). In the case of N-S, N refers to nerve healthy tissue minus schwannoma (S). In the column M-S, meningioma (M) minus schwannoma (S) is performed. Only those genes with ≤1.5 fold-change between both tumors are shown.

Table II. Genes infraexpressed in meningioma and schwannoma when compared with their respective control tissue.

Gene	Database	Chromosome	C-M	N-S	M-S	P-value
SAA1	NM_000331	11p15.1	-2.0	-2.7	1.1	0.000414
INHBA	NM_002192	7p15-p13	-2.0	-4.1	1.2	2.42E-09
PCDH18	NM_019035	4q31	-2.0	-2.6	1.2	0.000183
PTGIS	NM_000961	20q13.13	-2.1	-3.4	1.3	1.72E-06
HHIP	NM_022475	4q28-q32	-2.1	-2.9	1.1	1.44E-06
AQP9	NM_020980	15q	-2.1	-4.5	1.0	2.78E-08
TCEAL2	NM_080390	Xq22.1-q22.3	-2.1	-2.4	1.4	0.000135
S100A12	NM_005621	1q21	-2.2	-5.6	1.1	9.51E-07
PDE3A	NM_000921	12p12	-2.2	-2.0	1.2	2.03E-08
S100A9	NM_002965	1q21	-2.2	-4.6	-1.0	2.63E-05
PAK3	NM_002578	Xq23	-2.3	-4.1	1.3	5.36E-11
SLC16A7	NM_004731	12q13	-2.3	-2.2	1.1	4.89E-12
PI16	NM_153370	6p21.2	-2.3	-5.1	1.2	1.76E-09
MGST1	NM_145792	12p12.3-p12.1	-2.3	-3.6	1.1	0.001079
FGFR2	NM_000141	10q26	-2.3	-2.6	1.3	1.69E-09
TRPM3	NM_206946	9q21.12	-2.4	-2.0	1.1	3.65E-11
PDZRN4	NM_013377	12q12	-2.5	-3.0	1.1	1.14E-08
THBS4	NM_003248	5q13	-2.5	-3.5	1.0	1.71E-09
STEAP4	NM_024636	7q21.12	-2.7	-4.2	1.1	1.38E-07
DCLK1	NM_004734	13q13	-2.8	-2.4	1.1	2.22E-07
ZNF385D	NM_024697	3p24.3	-3.0	-2.0	1.1	1.34E-05
CXCL2	NM_002089	4q21	-3.1	-3.3	-1.0	7.52E-09
FABP4	NM_001442	8q21	-3.1	-13.5	1.2	1.29E-12
IL6	NM_000600	7p21	-3.1	-4.6	-1.0	0.000397
SELE	NM_000450	1q22-q25	-3.3	-6.6	1.0	2.29E-09
SLC14A1	NM_001128588	18q11-q12	-4.0	-3.3	1.0	2.7E-09
APLNR	NM_005161	11q12	-4.8	-3.1	-1.0	8.05E-11
ADH1B	NM_000668	4q23	-4.9	-3.6	-1.1	6.95E-07
ADCYAP1R1	NM_001118	7p14	-5.1	-3.3	1.0	6.98E-11
RND1	NM_014470	12q12	-5.4	-3.1	-1.0	3.5E-09
ADAMTS1	NM_006988	21q21.2	-6.0	-2.4	-1.1	4.64E-11
HSPB8	NM_014365	12q24.23	-6.0	-3.5	1.0	5.3E-07
AOC3	NM_003734	17q21	-6.3	-2.5	-1.1	3.64E-11
RGS4	NM_001102445	1q23.3	-6.7	-2.1	-1.1	2.55E-10
STMN2	NM_007029	8q21.13	-9.2	-2.9	-1.1	4.92E-11

Official gene symbol is shown for every gene. C-M values correspond to the fold-change value of control healthy meninges (C) minus meningioma (M). In the case of N-S, N refers to nerve healthy tissue minus schwannoma (S). In the column M-S, meningioma (M) minus schwannoma (S) is performed. Only those genes with ≤1.5 fold-change between both tumors are shown.

myelin protein 2 (PMP2), expressed in the cytoplasmic side of myelin in the peripheral nervous system (50); myelin protein zero (MPZ), representing 50% of the total myelin protein in the peripheral nervous system (51); neurexin 1 (NRXN1), which mediates formation and maintenance of synaptic junctions (52); and neural cell adhesion molecule 2 (NCAM2), which is involved in axonal projection (53).

Upregulation in meningiomas compared with schwannomas gave us 88 genes (data available upon request) and included cellular retinoic acid binding protein 2 (CRABP2),

a chaperon downregulated in high-grade gliomas (54), and secreted frizzled-related protein 2 gene (SFRP2), a gene identified as a tumor suppressor in a renal cell carcinoma cell line (55).

Another comparison concerned those genes that were upregulated in schwannomas with respect to nerves, and downregulated in meningiomas with respect to healthy meninges. These findings included genes such as hepatocyte cell adhesion molecule (HEPACAM), neuritin 1 (NRN1) and kinesin family member 1A (KIF1A).
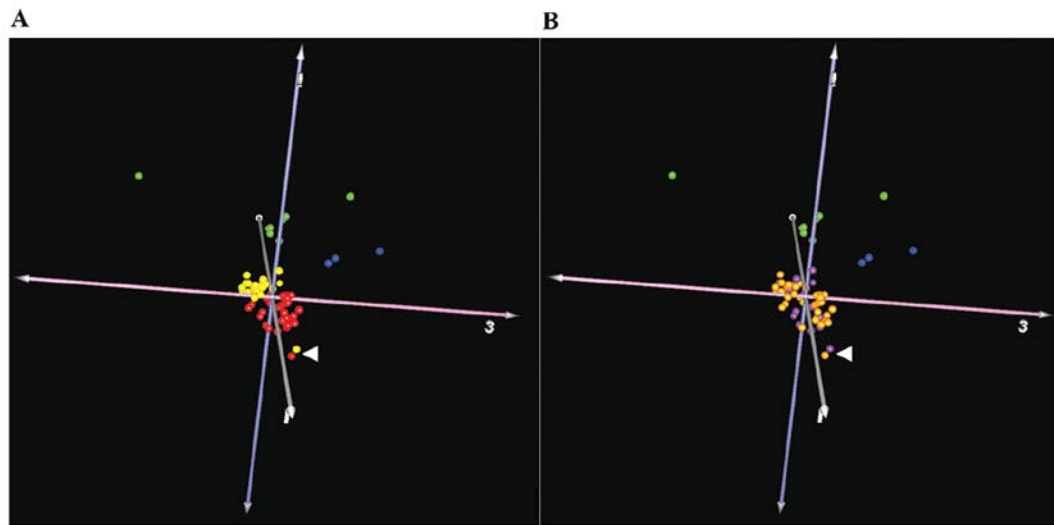
Figure 1. Principal component analysis (PCA) of all samples studied. Green dots represent non-tumoral healthy nerves and the blue dots non-tumoral meninges samples. (A) Red dots are all the schwannomas studied, while yellow dots are the meningioma samples. Both entities are clearly separated, except for two samples (white arrow), one corresponding to a schwannoma from an NF2 patient and the other to a grade I meningioma. The most remote green dot corresponds to the cultured Schwann cells used as controls. (B) Violet dots are samples without alteration in the *NF2* gene, and gold dots are tumors carrying such an alteration. The same point of view is shown in both images A and B, and no clear distinction between those two groups was found.

The *NF2* mutation rate (determined by sequencing, MLPA and chromosome 22q LOH analyses) in this series was 74% for schwannomas and 68% for meningiomas. We compared the expression patterns in samples from both tumor types, and with respect to the presence or lack of any alteration in the *NF2* gene (38 samples with alteration and 15 without any). Using these groups, we identified 2 genes with differential expression levels. The natriuretic peptide receptor C/guanylate cyclase C (atrionatriuretic peptide receptor C) (*NPR3*) was downregulated in those samples without *NF2* alterations. This gene codes for a receptor coupled to various signaling transduction cascades in several tissues such as cardiac myocytes and fibroblasts (56). On the other hand, the G antigen 12J (*GAGE12J*) gene, transcribed in human fetal and tumoral tissues (57), was also downregulated, but on this occasion in tumors with *NF2* alteration. As only 2 genes were detected, based on our microarray results in both neoplasms, it would seem that there is no differentiated subset of expression profiles of genes between samples with or without alteration over *NF2* in grade I meningiomas and schwannomas (Fig. 1). Nevertheless, single genes could be altered in tumors with or without *NF2* alteration, although such a reduced number could be due to outlier values.

At present, there is no chemotherapeutic treatment available for either meningiomas or schwannomas, thus research for a combined solution could be of great value to those patients affected with both tumor types, primarily patients with neurofibromatosis type 2. In this study, we found a set of genes with aberrant expression in both entities compared with their respective control tissue, but with similar expression levels between these tumors, including PDGF, c-Met or Slit2 pathways. Thus, these and the other genes identified in this study, and their regulatory pathways, might be of interest for further experiments in the search for common solutions for patients affected by schwannomas and meningiomas.

References

1. Louis DN, Ohgaki H, Wiestler OD and Cavenee WK (eds): WHO Classification of Tumors of the Central Nervous System. IARC Press, Lyon, 2007.
2. Zankl H and Zang KD: Cytological and cytogenetical studies on brain tumors. 4. Identification of the missing G chromosome in human meningiomas as no. 22 by fluorescence technique. Humangenetik 14: 167-169, 1972.
3. Rey JA, Bello MJ, De Campos JM, Kusak ME and Moreno S: Cytogenetic analysis in human neurinomas. Cancer Genet Cytogenet 28: 187-188, 1987.
4. Hadfield KD, Smith MJ, Urquhart JE, Wallace AJ, Bowers NL, King AT, Rutherford SA, Trump D, Newman WG and Evans DG: Rates of loss of heterozygosity and mitotic recombination in NF2 schwannomas, sporadic vestibular schwannomas and schwannomatosis schwannomas. Oncogene 29: 6216-6221, 2010.
5. Hansson CM, Buckley PG, Grigelioniene G, Piotrowski A, Hellström AR, Mantripragada K, Jarbo C, Mathiesen T and Dumanski JP: Comprehensive genetic and epigenetic analysis of sporadic meningioma for macro-mutations on 22q and micro-mutations within the NF2 locus. BMC Genomics 8: 16, 2007.
6. Leone PE, Bello MJ, de Campos JM, Vaquero J, Sarasa JL, Pestaña A and Rey JA: *NF2* gene mutations and allelic status of 1p, 14q and 22q in sporadic meningiomas. Oncogene 18: 2231-2239, 1999.
7. Leone PE, Bello MJ, Mendiola M, Kusak ME, De Campos JM, Vaquero J, Sarasa JL, Pestana A and Rey JA: Allelic status of 1p, 14q and 22q and NF2 gene mutations in sporadic schwannomas. Int J Mol Med 1: 889-892, 1998.
8. Bello MJ, de Campos JM, Kusak ME, Vaquero J, Sarasa JL, Pestaña A and Rey JA: Allelic loss at 1p is associated with tumor progression of meningiomas. Genes Chromosomes Cancer 9: 296-298, 1994.

9. Bello MJ, Martinez-Glez V, Franco-Hernandez C, Pefla-Granero C, de Campos JM, Isla A, Lassaletta L, Vaquero J and Rey JA: DNA methylation pattern in 16 tumor-related genes in schwannomas. Cancer Genet Cytogenet 172: 84-86, 2007.

10. Bello MJ, Amiñoso C, Lopez-Marin I, Arjona D, Gonzalez-Gomez P, Alonso ME, Lomas J, de Campos JM, Kusak ME, Vaquero J, Isla A, Gutierrez M, Sarasa JL and Rey JA: DNA methylation of multiple promoter-associated CpG islands in meningiomas: relationship with the allelic status at 1p and 22q. Acta Neuropathol 108: 413-421, 2004.

11. Liu Y, Pang JC, Dong S, Mao B, Poon WS and Ng HK: Aberrant CpG island hypermethylation profile is associated with atypical and anaplastic meningiomas. Hum Pathol 36: 416-425, 2005.

12. Kino T, Takeshima H, Nakao M, Nishi T, Yamamoto K, Kimura T, Saito Y, Kochi M, Kuratsu J, Saya H and Ushio Y: Identification of the cis-acting region in the NF2 gene promoter as a potential target for mutation and methylation-dependent silencing in schwannoma. Genes Cell 6: 441-454, 2001.

13. Gonzalez-Gomez P, Bello MJ, Alonso ME, Lomas J, Arjona D, de Campos JM, Vaquero J, Isla A, Lassaletta L, Gutierrez M, Sarasa JL and Rey JA: CpG island methylation in sporadic and neurofibromatis type 2-associated schwannomas. Clin Cancer Res 9: 5601-5606, 2003.

14. Lomas J, Bello MJ, Arjona D, Alonso ME, Martinez-Glez V, Lopez-Marin I, Amiñoso C, de Campos JM, Isla A, Vaquero J and Rey JA: Genetic and epigenetic alteration of the NF2 gene in sporadic meningiomas. Genes Chromosomes Cancer 42: 314-319, 2005.

15. Kullar PJ, Pearson DM, Malley DS, Collins VP and Ichimura K: CpG island hypermethylation of the neurofibromatosis type 2 (NF2) gene is rare in sporadic vestibular schwannomas. Neuropathol Appl Neurobiol 36: 505-514, 2010.

16. Koutsimpelas D, Ruerup G, Mann WJ and Brieger J: Lack of neurofibromatosis type 2 gene promoter methylation in sporadic vestibular schwannomas. ORL J Otorhinolaryngol Relat Spec 74: 33-37, 2012.

17. Kishida Y, Natsume A, Kondo Y, Takeuchi I, An B, Okamoto Y, Shinjo K, Saito K, Ando H, Ohka F, Sekido Y and Wakabayashi T: Epigenetic subclassification of meningiomas based on genome-wide DNA methylation analyses. Carcinogenesis 33: 436-441, 2012.

18. Gao F, Shi L, Russin J, Zeng L, Chang X, He S, Chen TC, Giannotta SL, Weisenberger DJ, Zada G, Mack WJ and Wang K: DNA methylation in the malignant transformation of meningiomas. PloS One 8: e54114, 2013.

19. Clark VE, Erson-Omay EZ, Serin A, Yin J, Cotney J, Ozduman K, Avşar T, Li J, Murray PB, Henegariu O, Yilmaz S, Günel JM, Carrión-Grant G, Yilmaz B, Grady C, Tanrikulu B, Bakircioğlu M, Kaymakçalan H, Caglayan AO, Sencar L, Ceyhun E, Atik AF, Bayri Y, Bai H, Kolb LE, Hebert RM, Omay SB, Mishra-Gorur K, Choi M, Overton JD, et al: Genomic analysis of non-NF2 meningiomas reveals mutations in TRAF7, KLF4, AKT1, and SMO. Science 339: 1077-1080, 2013.

20. Brastianos PK, Horowitz PM, Santagata S, Jones RT, McKenna A, Getz G, Ligon KL, Palescandolo E, Van Hummelen P, Ducar MD, Raza A, Sunkavalli A, Macconaill LE, Stemmer-Rachamimov AO, Louis DN, Hahn WC, Dunn IF and Beroukhim R: Genomic sequencing of meningiomas identifies oncogenic SMO and AKT1 mutations. Nat Genet 45: 285-289, 2013.

21. Stemmer-Rachamimov AO, Xu L, Gonzalez-Agosti C, Burwick JA, Pinney D, Beauchamp R, Jacoby LB, Gusella JF, Ramesh V and Louis DN: Universal absence of merlin, but not other ERM family members, in schwannomas. Am J Pathol 151: 1649-1654, 1997.

22. Martinez-Glez V, Franco-Hernandez C, Alvarez L, De Campos JM, Isla A, Vaquero J, Lassaletta L, Casartelli C and Rey JA: Meningiomas and schwannomas: molecular subgroup classification found by expression arrays. Int J Oncol 34: 493-504, 2009.

23. Torres-Martín M, Martinez-Glez V, Peña-Granero C, Isla A, Lassaletta L, De Campos JM, Pinto GR, Burbano RR, Meléndez B, Castresana JS and Rey JA: Gene expression analysis of aberrant signaling pathways in meningiomas. Oncol Lett 6: 275-279, 2013.

24. Torres-Martín M, Martinez-Glez V, Peña-Granero C, Lassaletta L, Isla A, de Campos JM, Pinto GR, Burbano RR, Meléndez B, Castresana JS and Rey JA: Expression analysis of tumor-related genes involved in critical regulatory pathways in schwannomas. Clin Transl Oncol 15: 409-411, 2013.

25. Martínez-Glez V, Alvarez L, Franco-Hernández C, Torres-Martin M, de Campos JM, Isla A, Vaquero J, Lassaletta L, Castresana JS, Casartelli C and Rey JA: Genomic deletions at 1p and 14q are associated with an abnormal cDNA microarray gene expression pattern in meningiomas but not in schwannomas. Cancer Genet Cytogenet 196: 1-6, 2010.

26. Aarhus M, Bruland O, Sætran HA, Mork SJ, Lund-Johansen M and Knappskog PM: Global gene expression profiling and tissue microarray reveal novel candidate genes and downregulation of the tumor suppressor gene CAV1 in sporadic vestibular schwannomas. Neurosurgery 67: 998-1019, 2010.

27. Cayé-Thomasen P, Borup R, Stangerup S-E, Thomsen J and Nielsen FC: Deregulated genes in sporadic vestibular schwannomas. Otol Neurotol 31: 256-266, 2010.

28. Torres-Martin M, Lassaletta L, San-Roman-Montero J, De Campos JM, Isla A, Gavilan J, Melendez B, Pinto GR, Burbano RR, Castresana JS and Rey JA: Microarray analysis of gene expression in vestibular schwannomas reveals SPP1/MET signaling pathway and androgen receptor deregulation. Int J Oncol 42: 848-862, 2013.

29. Tabernero MD, Maillo A, Gil-Bellosta CJ, Castrillo A, Sousa P, Merino M and Orfao A: Gene expression profiles of meningioma are associated with tumor cytogenetics and patient outcome. Brain Pathol 19: 409-420, 2009.

30. Keller A, Ludwig N, Backes C, Romeike BFM, Comtesse N, Henn W, Steudel W-I, Mawrin C, Lenhof H-P and Meese E: Genome wide expression profiling identifies specific deregulated pathways in meningioma. Int J Cancer 124: 346-351, 2009.

31. Lee Y, Liu J, Patel S, Cloughesy T, Lai A, Farooqi H, Seligson D, Dong J, Liau L, Becker D, Mischel P, Shams S and Nelson S: Genomic landscape of meningiomas. Brain Pathol 20: 751-762, 2010.

32. Pérez-Magán E, Campos-Martín Y, Mur P, Fiaño C, Ribalta T, García JF, Rey JA, Rodríguez de Lope A, Mollejo M and Meléndez B: Genetic alterations associated with progression and recurrence in meningiomas. J Neuropathol Exp Neurol 71: 882-893, 2012.

33. Torres-Martin M, Lassaletta L, de Campos JM, Isla A, Gavilan J, Pinto GR, Burbano RR, Latif F, Melendez B, Castresana JS and Rey JA: Global profiling in vestibular schwannomas shows critical deregulation of microRNAs and upregulation in those included in chromosomal region 14q32. PloS One 8: e65868, 2013.

34. Bush ML, Oblinger J, Brendel V, Santarelli G, Huang J, Akhmametyeva EM, Burns SS, Wheeler J, Davis J, Yates CW, Chaudhury AR, Kulp S, Chen CS, Chang LS, Welling DB and Jacob A: AR42, a novel histone deacetylase inhibitor, as a potential therapy for vestibular schwannomas and meningiomas. Neuro Oncol 13: 983-999, 2011.

35. Spear SA, Burns SS, Oblinger JL, Ren Y, Pan L, Kinghorn AD, Welling DB and Chang LS: Natural compounds as potential treatments of NF2-deficient schwannoma and meningioma: cucurbitacin D and goyazensolide. Otol Neurotol 34: 1519-1527, 2013.

36. Johnson WE, Li C and Rabinovic A: Adjusting batch effects in microarray expression data using empirical Bayes methods. Biostatistics 8: 118-127, 2007.

37. MAQC Consortium, Shi L, Reid LH, Jones WD, Shippy R, Warrington JA, Baker SC, Collins PJ, de Longueville F, Kawasaki ES, Lee KY, Luo Y, Sun YA, Willey JC, Setterquist RA, Fischer GM, Tong W, Dragan YP, Dix DJ, Frueh FW, Goodsaid FM, Herman D, Jensen RV, Johnson CD, Lobenhofer EK, Puri RK, Schrf U, Thierry-Mieg J, Wang C, Wilson M, Wolber PK, et al: The MicroArray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements. Nat Biotechnol 24: 1151-1161, 2006.

38. Zhou K, Wang G, Wang Y, Jin H, Yang S and Liu C: The potential involvement of E-cadherin and beta-catenins in meningioma. PloS One 5: e11231, 2010.

39. Wang Z, Kong D, Li Y and Sarkar FH: PDGF-D signaling: a novel target in cancer therapy. Curr Drug Targets 10: 38-41, 2009.

40. Moriyama T, Kataoka H, Kawano H, Yokogami K, Nakano S, Goya T, Uchino H, Koono M and Wakisaka S: Comparative analysis of expression of hepatocyte growth factor and its receptor, c-met, in gliomas, meningiomas and schwannomas in humans. Cancer Lett 124: 149-155, 1998.

41. Cipriani NA, Abidoye OO, Vokes E and Salgia R: MET as a target for treatment of chest tumors. Lung Cancer 63: 169-179, 2009.

42. Lai AZ, Abella JV and Park M: Crosstalk in Met receptor oncogenesis. Trends Cell Biol 19: 542-551, 2009.
43. Zhou WJ, Geng ZH, Chi S, Zhang W, Niu XF, Lan SJ, Ma L, Yang X, Wang LJ, Ding YQ and Geng JG: Slit-Robo signaling induces malignant transformation through Hakai-mediated E-cadherin degradation during colorectal epithelial cell carcinogenesis. Cell Res 21: 609-626, 2011.
44. Dai CF, Jiang YZ, Li Y, Wang K, Liu PS, Patankar MS and Zheng J: Expression and roles of Slit/Robo in human ovarian cancer. Histochem Cell Biol 135: 475-485, 2011.
45. Zanata SM, Hovatta I, Rohm B and Püschel AW: Antagonistic effects of Rnd1 and RhoD GTPases regulate receptor activity in Semaphorin 3A-induced cytoskeletal collapse. J Neurosci 22: 471-477, 2002.
46. Hota PK and Buck M: Thermodynamic characterization of two homologous protein complexes: associations of the semaphorin receptor plexin-B1 RhoGTPase binding domain with Rnd1 and active Rac1. Protein Sci 18: 1060-1071, 2009.
47. Oue E, Lee JW, Sakamoto K, Iimura T, Aoki K, Kayamori K, Michi Y, Yamashiro M, Harada K, Amagasa T and Yamaguchi A: CXCL2 synthesized by oral squamous cell carcinoma is involved in cancer-associated bone destruction. Biochem Biophys Res Commun 424: 456-461, 2012.
48. Li YH, Ghavampur S, Bondallaz P, Will L, Grenningloh G and Püschel AW: Rnd1 regulates axon extension by enhancing the microtubule destabilizing activity of SCG10. J Biol Chem 284: 363-371, 2009.
49. Jalkanen S, Karikoski M, Mercier N, Koskinen K, Henttinen T, Elima K, Salmivirta K and Salmi M: The oxidase activity of vascular adhesion protein-1 (VAP-1) induces endothelial E- and P-selectins and leukocyte binding. Blood 110: 1864-1870, 2007.
50. Eylar EH, Szymanska I, Ishaque A, Ramwani J and Dubiski S: Localization of the P2 protein in peripheral nerve myelin. J Immunol 124: 1086-1092, 1980.
51. Everly JL, Brady RO and Quarles RH: Evidence that the major protein in rat sciatic nerve myelin is a glycoprotein. J Neurochem 21: 329-334, 1973.
52. Bottos A, Rissone A, Bussolino F and Arese M: Neurexins and neuroligins: synapses look out of the nervous system. Cell Mol Life Sci 68: 2655-2666, 2011.
53. Alenius M and Bohm S: Identification of a novel neural cell adhesion molecule-related gene with a potential role in selective axonal projection. J Biol Chem 272: 26083-26086, 1997.
54. Campos B, Warta R, Chaisaingmongkol J, Geiselhart L, Popanda O, Hartmann C, von Deimling A, Unterberg A, Plass C, Schmezer P and Herold-Mende C: Epigenetically mediated downregulation of the differentiation-promoting chaperon protein CRABP2 in astrocytic gliomas. Int J Cancer 131: 1963-1968, 2012.
55. Konac E, Varol N, Yilmaz A, Menevse S and Sozen S: DNA methyltransferase inhibitor-mediated apoptosis in the Wnt/β-catenin signal pathway in a renal cell carcinoma cell line. Exp Biol Med 238: 1009-1016, 2013.
56. Rose RA and Giles WR: Natriuretic peptide C receptor signalling in the heart and vasculature. J Physiol 586: 353-366, 2008.
57. Gjerstorff MF and Ditzel HJ: An overview of the GAGE cancer/testis antigen family with the inclusion of newly identified members. Tissue Antigens 71: 187-192, 2008.