The Hedgehog (Hh) signaling pathway has an important role during embryogenesis and in adult life, regulating proliferation, angiogenesis, matrix remodeling and stem-cell renewal. Deregulation of the Hh pathway is involved in tumor development, since mutations in several components of this pathway were found in patients with basal cell carcinoma, medulloblastoma and other tumors; however, the role of Hh in meningiomas has not been studied yet. Meningiomas represent 30% of primary cranial tumors, are mostly benign and prevail in the second half of life. Novel therapies for meningiomas such as targeted molecular agents could use Hh pathway components. To provide information concerning molecular alterations, by use of real-time RT-PCR, we studied expression at the mRNA level of 32 Hh pathway and target genes in 36 meningioma specimens of different grades. mRNA levels of 16 genes, involved mainly in Hh pathway activation and cell proliferation, increased in meningiomas in comparison with normal tissue, whereas those of 7 genes, mainly related to Hh pathway repression, decreased. The most significant changes occurred in signal transduction (SMO) and GLI-transcription factor genes, and the target FOXM1 mRNA attained the highest values; their overexpression was found in aggressive and in benign tumors. Some proliferation-related genes (SPP1, IGF2) were overexpressed in higher meningioma grades. A correlation in expression between genes with a similar function was also found. Our results show a marked activation of the Hh pathway in meningiomas, which may be important for their biological and clinical characterization and would be useful for gene therapy.

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INTRODUCTION

The study of genetic alterations involved in tumorigenesis is important for molecular characterization of tumors and offers useful targets for therapy. The Hedgehog (Hh) signaling pathway is closely related to processes such as cell proliferation, differentiation, angiogenesis, cellular matrix remodeling and stem-cell homeostasis, all of which are involved in tumorigenesis and metastasis (1). Deregulation of this pathway was found in different tumors such as basal cell carcinoma, medulloblastoma, rhabdomyoma, rhabdomyosarcoma and other tumors (2–4), but there have been no reports on meningiomas so far.

The Hedgehog pathway plays a fundamental role in multiple developmental processes as well as in adult tissues and is conserved across the animal kingdom from insects to vertebrates (1,5,6). Hh ligands have evolved from one in Drosophila melanogaster to three in vertebrates: Sonic hedgehog (SHH), the most widely expressed in mammalian tissues; Indian hedgehog (IHH), specific for bone development; and Desert hedgehog (DHH), restricted to gonads (1). Hh proteins are synthesized, palmitylated by a palmitoylacyltransferase specific for SHH (HHAT), cholesterol-modified and released by means of the transmembrane protein Dispached (DISP) (5–7). They act at short or long distance, and their gradient determines the development and differentiation of cells (7,8).

The Hh signaling function is accomplished through the process of ligand binding to the receptor Patched (PTCH), transduction of the signal mediated by the transmembrane protein Smoothed (SMO) and activation of specific transcription factors regulating the Hh target gene expression. The PTCH receptor is suppressed in response to ligand binding, something unusual for a signal receptor, whereas SMO is activated (1,5–7). Other proteins that modulate the Hh function are Hh interacting protein (HHIP), involved in a negative feedback;
the growth arrest specific 1 (GASI), regulating the range and level of signaling; and Rab23, a small G-protein having a major role in the subcellular localization of Hh pathway components (1,9,10).

Activation of SMO initiates a signal transduction cascade involving several intermediate proteins such as the Kinesin family (Kif7, Kif27) that interact with microtubules and other proteins; a Fused homolog, the serine-threonine kinase 36 (STK36) not essential for mammals; the Suppressor of fused (SUFU) a member of the Gli family of transcription factors, which are named GLI after glioblastoma); and a β-transducin-repeat–containing E3 ligase (BTRC), which binds the phosphorylated GLI2 and GLI3 for their processing into a repressor (GLI3) or to ubiquitine-proteosome-mediated degradation (GLI2) (1,5,6,11). The relative levels of GLI1 transcription factors GLI1/GLI2 (activators) and GLI3 (repressor) determine which target genes are expressed; another member, GLI4, and GLI-similar 1 and 2 (GLIS1/GLIS2) are additional transcription factors (7,12–15).

Several genes are regulated by the Hh signal: (a) MTSS1 (metastasis suppressor 1)—a regulator of actin filaments assembly—which is expressed in embryonic tissues including the developing central nervous system and downregulated in metastatic cancer cell lines (16); (b) the Forkhead family of transcription factors, which are associated with cell growth and proliferation, such as FOXE1, FOXA2 and FOXM1 (a regulator of cell-cycle gene-expression), and all have been found in human cancers (17–19); (c) SPP1 (osteopontin) and OSF (periostin)—secreted extracellular matrix-associated proteins—which have a crucial role in cell adhesion, prevention of apoptosis, invasion and migration (20,21); (d) IGF2 (insulin-like growth factor 2) and CCND2, a cyclin required for cell cycle G1/S transition, both of which are overexpressed in tumors (22,23); (e) PTHR1, a parathyroid hormone receptor, which is overexpressed in osteosarcoma and bone metastases of other tumors (24).

Activation of the Hh pathway, therefore, stimulates several processes related to cancer such as cell-cycle machinery, expression of angiogenic and anti-apoptotic factors and stem-cell renewal, which may involve transformation into cancer stem cells (6,20,21,23).

The most common mutations that activate the Hh pathway occur in (a) PTCH or SUIFL, causing the loss of their function; (b) SMO, activating it; or (c) GLI1, amplified in 15% of glioblastomas. The nevoid basal cell carcinoma syndrome (NBCC) caused by PTCH mutations is associated with development of tumors such as basal cell carcinoma, medulloblastoma and meningioma (1,2).

Specific inhibitors of Hh signaling will provide an effective therapy for a wide range of malignancies. The most common ways to target this pathway are (a) modulating SMO by its antagonists, the plant alkaloid cyclopamine or the derived synthetic compounds, which have been used in a phase 1 clinical trial (25–29); (b) using SHH-neutralizing antibodies (30); and (c) inhibiting GLI1 with a small interfering RNA (siRNA) (31).

Meningiomas represent 30% of primary cranial tumors (Central Brain Tumor Registry of the United States [CBTRUS]) prevailing in the second half of life. They are mostly sporadic and often considered benign; however, three malignancy grades are recognized by WHO: grade I or benign (MGI); grade II or atypical (MGII), associated with a high rate of recurrence; and grade III or anaplastic (MGIII), highly aggressive with a short survival time.

Emerging therapies for meningiomas are also being developed, and the components of the Hh pathway are candidates for targeting. Herein we investigated the expression of a large panel of components and related molecules involved in the Hh pathway. Using the real-time quantitative RT-PCR, we quantified the expression of 32 genes at the mRNA level in 36 meningioma specimens of different grades and attempted to determine a link between gene expression and pathological parameters.

**MATERIALS AND METHODS**

**Patients and Samples**

Samples of 36 meningiomas were obtained by surgical excision from patients with sporadic tumors at the Neurosurgery departments of Brussels (26 patients) and Buenos Aires (10 patients) from 2003 to 2005. They included 23 MGI tumor samples with a similar number of meningothelial and transitional meningiomas, nine MGII and four MGIII. Specimens of adjacent normal meningeal tissue from five patients and of normal cerebellum from one patient were used as sources of normal RNA. Immediately after surgery, tumors and normal tissue samples were stored in liquid nitrogen. The patients were 21 women and 15 men with a mean age of 63 (range 41–80) years. This study received ethical approval from the Ethical Committee of “Hospital de Clinicas Jose de San Martin.” The patients had given a written consent to use their biopsy samples for this study.

**RNA Extraction and cDNA Synthesis**

Total RNA was extracted from meningioma specimens using the acid–phenol guanidinium method. Frozen tissues were triturated in liquid nitrogen in a mortar, and the powder was homogenized in the extraction solution, vortexed and treated with chloroform on ice. Isopropanol was added to the upper phase, and the precipitated total RNA was washed, dissolved in sterile water, quantified by UV absorbance at 260 and 280 nm and assessed qualitatively by gel electrophoresis to check the presence of 18S and 28S RNA bands. RNA was reverse transcribed to cDNA as previously reported (32).

**Real-Time Quantitative PCR**

The theoretical bases and PCR reaction conditions have been described (32). Briefly, quantitative values were obtained from the threshold cycle number.
at which the increase in the signal associated with the exponential growth of PCR product begins to be detected. The amount of RNA added to reaction mix was determined by normalization of each sample to an endogenous RNA control (TBP [TATA box-binding protein]). The results, presented as N-fold differences in target gene expression, were negligible. Table 1 lists the nucleotide sequences of the primers.

Experiments were performed with duplication for each data point. Positive controls for all genes were obtained by performing an RNA pool control, which was prepared by mixing identical amounts of RNA from various human normal and tumor tissues. PCR amplification was performed in an ABI Prism 7700 Sequence detection system using the SYBR® Green PCR Core Reagent kit (Perkin-Elmer Applied Biosystems, Oak Brook, IL).

**Statistical Analyses**

Because the mRNA levels did not fit a Gaussian distribution, they were charac-

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**Table 1. Oligonucleotide primer sequences used**

| Gene   | Primer forward                   | Primer reverse                   |
|--------|----------------------------------|----------------------------------|
| IHH    | 5' AGG CCC GGC TCTG ACT GGG TGT ATT 3' | 5' GCG GCC GAG TGC TCG GAC TT 3'  |
| SHH    | 5' CCG GCT TCG ACT GGG TGT ACT A 3' | 5' GAC CGC CAG TGA GGT ATC AGC TT 3' |
| DHH    | 5' CCG GCT TCG ACT GGG TCT AC 3'   | 5' GCT CCT GCT GCT GCA TCT GT 3'  |
| HHA    | 5' CCT GGA GCA CCA TGG CT 3'       | 5' AGC AAT CAG CTG GGA ATA CTG CT 3' |
| DISP1  | 5' ACT TCT CTG ATC TGG TCG GT 3'   | 5' AAC GAA CAT CAC TAC CGC GGC TCT 3' |
| DISP2  | 5' TGG CCA GCA CCA TGG CA 3'       | 5' GGC GCA GCA TGG CTT GCT 3'     |
| PTCH1  | 5' CCC CTG TAC GAA GTG GAC ACT TCC 3' | 5' GC GCG AAA GAA GTA CCT TAC A 3' |
| PTCH2  | 5' GAT GGG GCC ATC TCC ACA TT 3'   | 5' GGC GCA GCA TGG CTT GCT 3'     |
| SMO    | 5' GCT ACT TCC TCA TCC GAG GAC TCA 3' | 5' CGG AGC GTC ACC ACC TGT 3'     |
| HHP    | 5' GGG CGC CTG GAG AAT AAG ATA TTT 3' | 5' GCT TCT CGC AAA TTC ACC ACC TTA 3' |
| GAS1   | 5' CGT CAT TGA GGA CAT GCT GGC TAT 3' | 5' CAT TGC CAT TGT GCG CTT CAT 3' |
| RAB23  | 5' GAA AGT AGT AGC CGA AGG AGA T 3' | 5' TTC TGC TGT ACC GAC TCG CAT 3' |
| KIF7   | 5' GTG CCA GTG CGA GAT GCA CTT 3'  | 5' AGT GCC TCA GCT TCC TCA TTTC T 3' |
| KIF27  | 5' AGC TCT CAG TGC TGT AGA TAT 3'  | 5' GCT CCA GTC ACC CAT ACC TCT 3' |
| STK36  | 5' ACC CCA GAT TGT GAA CGA GTA C 3' | 5' GGC GAT GGA AGG CTC ACC TCT 3' |
| SUI7   | 5' CGT GCT GAC AGA GGA CCC ACA A 3' | 5' CGG GAG CCA CCA GTC ACC TTA 3' |
| BTRC   | 5' GTC TAC GGA CCC TTC TCG AGA AT 3' | 5' CAT CAC TCA TCC ACC TAC TCT 3' |
| GLI1   | 5' CCA ACC CCA GAC GCA TAC AGG AT 3' | 5' CAC AGA TTC GTC ACC TCT C 3' |
| GLI2   | 5' AAG TCA CTC AAG GAT TCC TCG TCA 3' | 5' GCT CTC AG CAG GAG ACC TCT 3' |
| GLI3   | 5' CGC GAC TGA ACC CCA TTC TAC 3'  | 5' GTG TGG TGG GTG GTG CCA TCT 3' |
| GLI4   | 5' CCA TGG GCA TCG ACA TGG CT 3'   | 5' TCC TCT AGC TCT TCG AGA TCC AGG T 3' |
| GLI5   | 5' CTG TCG TCG GCG CAA GGT TAA 3'  | 5' CGG GCG TCA GTG CAT AAT CTT 3' |
| GLI6   | 5' CCA GAC CCA CAA GGT TAC CA 3'   | 5' CAT CCG GTA GCA TGC ACC ATG 3' |
| MTSS1  | 5' CGT CCG CCA GTG ATT GAA GAA 3'  | 5' GGG GAG CAG TTT GTG AGG GAC TCT 3' |
| FOXE1  | 5' GGC GCG CTG TGG GAC TAC AA 3'   | 5' TGG CGG ACA GCA AGT CAT AAT 3' |
| FOXA2  | 5' TGG GAG CCG AGA TAGT GGA AA 3'  | 5' GAG GAG TGG CCC TGG GGC TCT 3' |
| FOXM1  | 5' GGG GAC GGT CAT ATG GTG A 3'    | 5' TGA TCG ACA GTG GGG AAA T 3'   |
| SPP1   | 5' TCG CAG ACC TGA CAT CCA GTA C 3' | 5' TCG CTC AAC TCC TCG CTT TCC AT 3' |
| OFD    | 5' GTC CTA ATT CCT GAC TGT GCT GCA AAA 3' | 5' GGC CAA CCA GAT CGG TGA A 3' |
| IG52   | 5' CGA CCG TGC TCC CGG ACA AC 3'   | 5' AGG CGC TGG GTG GAC TGG CT 3' |
| CCND2  | 5' GCT GTG TCT GTG ATC CGC AAG A 3' | 5' GCC AAA CTA AAA GTC GGT GGG A 3' |
| PTHR1  | 5' ACA ACA GGA CGT GGG CCA ACC ATC 3' | 5' CGG TCA AAC ACC TCC TCA TT 3' |
| MKI6    | 5' ATT GAA CCT GCG GAA GAG GAT C 3' | 5' GGA CGC GCG GAG TAT TCC CTT A 3' |
characterized in each group by their median values and ranges. Comparisons between the medians of target gene mRNA levels and histopathological parameters were checked by the nonparametric Kruskal–Wallis test. To evaluate the significance of multiple analyses, pair confrontations between the means of group ranges were performed by Conover’s method (33).

The relationships between mRNA levels of the various target genes were performed by the nonparametric Spearman rank correlation test. Differences between two populations were judged significant at confidence level greater than 95% ($P < 0.05$).

RESULTS

Hedgehog Pathway mRNA Levels in Meningiomas

Figure 1 shows the median mRNA levels of 32 Hedgehog pathway genes, target genes and the proliferation marker MKI67 in different meningioma grades (MGI, MGII and MGIII). Median standardized mRNA levels in meningiomas were determined relative to the median mRNA level in normal meninges, which has an assigned value of 1 and is indicated by a horizontal line. Statistically significant differences in mRNA levels between meningiomas and normal tissue are denoted by asterisk. The tests used and $P$ values are as in Table 2.

Figure 1. mRNA levels of 32 Hedgehog pathway genes, target genes and the proliferation marker MKI67 in different meningioma grades (MGI, MGII and MGIII). Median standardized mRNA levels in meningiomas were determined relative to the median mRNA level in normal meninges, which has an assigned value of 1 and is indicated by a horizontal line. Statistically significant differences in mRNA levels between meningiomas and normal tissue are denoted by asterisk. The tests used and $P$ values are as in Table 2.
mostly in MGI and MGIII, the more aggressive tumors.

A nonsignificant increase in median mRNA level of five additional genes was found; however, they showed values higher than normal in a considerable amount of meningiomas: 22%–75% (GAS1, STK36, BTRC, FOXA2 and OSF).

Decreased mRNA levels (underexpressed genes). A significant decrease in the mRNA level of the following genes was found in meningiomas in comparison with normal tissue: (a) DHH, a gonad-specific Hh ligand, was underexpressed in 50% to 71% of meningiomas regardless of grade; (b) PTCH1, a Hh ligand receptor that represses the Hh pathway, was underexpressed in 11% to 43% of tumors; (c) PTHR1, a parathyroid receptor participating, coupled with Hh ligand IHH, in the regulation of chondrocyte differentiation, showed reduced mRNA levels in 22% to 35% of meningiomas regardless of grade.

A nonsignificant decrease in the median mRNA level of four additional genes (HHIP, SUFU, MTSS1 and GLIS1) was found in meningiomas; some of them, such as the negative Hh pathway regulators HHIP and SUFU, showed values lower than normal in 13%–50% of tumors.

Figure 2 shows an example of the mRNA levels of PTCH1, SMO, GLI2 and FOXM1 genes in each of the six normal tissues (N), 23 grade I meningiomas (MGI), 9 grade II meningiomas (MGII) and 4 grade III meningiomas (MGIII). Median values are indicated for normal meninges and each tumor group by horizontal bars. P values for the three tumor grades versus normal tissue are as in Table 2.

Comparison of the Hedgehog Pathway and Related mRNA Levels with the Grades of Meningiomas

mRNA levels of 9 of 23 Hh pathway genes and 5 of 9 Hh-inducible genes, related to cell proliferation/invasion, differed significantly as regards total or each grade of meningiomas and normal tissue (Table 2), although the changes were not always statistically significant for the MGIII group, probably owing to the small sample size. It should be noted that the PTCH1 mRNA level decreased only in MGI compared with normal tissue, whereas SPP1 and IGF2 mRNA levels increased in the intermediate grade (MGII) or in MGII and MGIII, respectively, but not in the benign MGI grade.
levels, reduced in meningiomas, correlated with those of a membrane protein ligand-secretion receptors, correlated (b) Hh ligand receptors GAS1 thus supporting the actual increase of 

with those of a membrane protein ligand-secretion receptors, correlated (a) Hh ligand receptors GAS1 thus supporting the actual increase of 

mRNA levels, encoding the Hh activators and repressors levels; (c) FOXM1, SP1 and IG2, increased in the intermediate grade (MGI) compared with MGI; (c) PTCH1, decreased in MGI in comparison with MGI and MGI III (Table 2).

### Relationship between mRNA Values of Hh Pathway and Related Genes in Meningiomas

Table 3 lists the most relevant correlations (P < 0.01) between mRNA levels of Hh pathway genes as well as between them and those of their target genes in meningiomas. The most significant values were found for the following genes: (a) DISP1 mRNA levels, encoding the Hh ligand-secretion receptors, correlated with those of a membrane protein GAS1 (r = 0.65), both increased in meningiomas thus supporting the actual increase of GAS1 mRNA despite its nonsignificance; (b) Hh ligand receptors PTCH1/2 mRNA levels, reduced in meningiomas, correlated with those of RAB23 (r = 0.54) a regulator of GLI2/GLI3 activities, as well as with those of GLI1/GLI2 (r = 0.68, 0.53) which increased in meningiomas, data that have no logical explanation and might reflect the importance of relative activators and repressors levels; (c) SMO expression correlated with that of a high number of Hh pathway and target genes such as GAS1 (r = 0.57), KIF7 (r = 0.73); the transcription factors GLI2/ GLIS2, which showed the highest correlations, r = 0.85 and 0.75, respectively; and a target gene, the growth factor IGF2 (r = 0.52) in full agreement with the role of SMO as a first transducer of the Hh cascade; (d) KIF7 mRNA levels correlated with those of other positive Hh pathway regulators such as SMO and STK36 (r = 0.66) and more closely with those of transcription factors GLI2/ GLIS2 (r = 0.76 and 0.78, respectively); (e) SUFU mRNA levels correlated with those of BTSC (r = 0.54), both Hh pathway repressors in mammals; (f) the expression of transcription factors GLI2 and GLIS2 showed a correlation between them (r = 0.70) and with other positive Hh pathway regulators in meningiomas such as GAS1 and STK36 (r = 0.56, 0.64), more strongly with KIF7 (r = 0.76, 0.78) and SMO (r = 0.85, 0.75) as stated before and with the GLI2 target IGF2 (r = 0.52); (g) MTSS1 mRNA levels correlated with those of PTHR1 (r = 0.57), in full agreement with the underexpression of both genes in meningiomas but not with the expression of PTHR1 in other tumors (sarcomas and bone metastases); (h) IGF2 mRNA levels correlated mainly with those of SMO and GL2 as stated before, consistent with the association of the Hh pathway activation and cell proliferation, and with those of FOXM1 (r = 0.58) and CCND2 (r = 0.66), as expected from the close association between progression to DNA replication and cell proliferation.

### DISCUSSION

The altered expression of 16 Hh pathway genes and their targets in meningiomas is in full agreement with their function as activators or inhibitors. HHAT, DISP1, SMO, STK36 and GL11/2 involved in Hh pathway activation were overexpressed, five of them with a
Hedgehog Pathway mRNA Levels in Meningiomas

A significant change in mRNA levels of several Hh pathway and target genes was observed in meningiomas. PTCH1, HHIP, and SUFU, which repress the Hh pathway, were underexpressed. In addition, the well-known inducible genes FOXA2/M1, SPP1, OSF, IGF2, and CCND2 dealing with cell proliferation, invasion/migration and DNA replication were also overexpressed. Activation of these genes may reflect a complex regulation of the Hh pathway to maintain correct cell functioning.

On the other hand, the altered expression of seven genes in meningiomas does not fully agree with the function assigned to them in the Hh pathway. The following genes, presumably negative regulators, showed an increase in mRNA levels: (a) the growth arrest–specific 1 gene (GAS1) which, despite its name, participates in cerebella cell proliferation, thus suggesting multiple functions for this gene; (b) kinesin family member KIF7, a presumed homolog of Drosophila's Hh-suppressor cos2; (c) BTRC, the E3 ligase for GLI3 processing to repressor form—however, it may also be associated with tumorigenesis through a dysregulation of the proteolysis of its substrates; (d) GLIS2, a well-known Wnt/β-catenin pathway repressor, may also have another role relative to tumorigenesis in this pathway; (e) MTSS1, a metastasis suppressor that is underexpressed in meningiomas.

A significant change in mRNA levels of several Hh pathway and target genes

Table 3. Correlations between mRNA levels of Hedgehog pathway and target genes; P values and correlation coefficients (Spearman rank correlation test).a

| DISP1/Disp2 | PTCH1/PTCH2 | SMO | KIF7 | STK36 | SUFU | GLI2 | GLI4 | GLI3 | GLI4 | GLIS2 | MTSS1 | FOXM1 | IGF2 |
|------------|-------------|-----|------|-------|------|------|------|------|------|-------|-------|-------|------|
| HHAT       | 0.01/0.44   | 1.90E03/0.48 | 2.40E03/0.47 |
| PTCH1/PTCH2| 3.50E03/0.46 | 2.40E03/0.47 |
| HHIP       | 2.30E05/0.65 | 2.70E04/0.57 | 2.60E03/0.47 | 3.50E04/0.56 | 2.10E04/0.58 |
| GAS1       | 3.20E05/0.65 | 2.70E04/0.57 | 2.60E03/0.47 | 1.30E03/0.5 | 3.50E04/0.56 | 2.10E04/0.58 |
| RAB23      | 5.90E04/0.54 | 1.80E03/0.49 | 2.80E03/0.47 |
| KIF7       | 1.10E05/0.51 | 3.10E05/0.73 | 2.80E03/0.47 |
| STK36      | 1.10E05/0.51 | 1.70E03/0.49 | 2.60E03/0.46 |
| BTRC       | 2.00E03/0.48 | 5.80E04/0.54 | 3.20E04/0.56 | 7.30E05/0.62 | 4.00E05/0.64 |
| GU1        | 1.20E05/0.68 | 3.30E03/0.46 | 0.01/0.41 |
| GU2        | 0.01/0.41 | 4.70E05/0.85 | 1.20E05/0.76 |
| GU3        | 1.70E03/0.49 | 4.10E03/0.45 |
| GU4        | 2.40E04/0.57 | 0.01/0.39 |
| GU5        | 0.01/0.44 | 7.20E04/0.53 | 6.00E06/0.78 | 8.10E06/0.7 | 2.10E05/0.66 |
| FOXM1      | 2.80E03/0.47 | 0.01/0.42 |
| SPP1       | 9.70E04/0.52 | 2.80E03/0.47 | 9.50E04/0.52 | 3.40E03/0.46 | 2.10E04/0.58 | 2.20E03/0.66 |
| CCND2      | 2.30E04/0.57 | 2.30E04/0.57 | 2.30E04/0.57 | 2.30E04/0.57 | 2.30E04/0.57 | 2.30E04/0.57 | 2.30E04/0.57 | 2.30E04/0.57 | 2.30E04/0.57 |

aP values of 0.01 or lower and respective correlation coefficients (in bold) are separated by a slash. The values of DISP1 and DISP2 as well as PTCH1 and PTCH2 are enclosed in the same cell, denoting DISP2 and PTCH2 by italics. Correlation coefficient values higher than 0.7 are underlined.
was found in low-grade tumors (MGI) in comparison with normal tissue: HHAT, DISP1, SMO, KIF7, GLI2, GLI2 and FOXM1 mRNA levels increased in MGI, and those of PTCH1, the tumor suppressor, decreased (Table 2). Thus, these genes could have a relevant role in the initial steps of tumorigenesis. Moreover, PTCH1 mRNA levels decreased only in low-grade meningiomas, suggesting that this change is not significant for progression to a more malignant state, which would be attained by other genes overexpressed in MGGII and MGGIII.

A significant increase in mRNA levels of GLI1, FOXM1, SPP1 and IGF2 was found in the more invasive tumors (MGGII + MGGIII) compared with lower meningiomas grades. Therefore, these genes could be the markers for invasiveness and aggressiveness. The significant changes in expression of these genes, as well as that of PTCH1, between MGI and MGGII/MGGIII suggest different biological functions involved in the development of the three types of meningiomas. Moreover, the benign MGI tumors (95% of total meningiomas) showed a lower number of genes with an altered expression than the atypical (4.7%–7.2%) and anaplastic (1%–2.8%) tumors, both of them presenting similar alterations: an increased expression of genes involved in cell proliferation and invasiveness (FOXM1, SPP1 and IGF2).

A wide variation in the increased mRNA levels of several genes (HHAT, SMO, KIF7, GLI1, GLI2, FOXM1, SPP1 and IGF2) occurred between meningiomas and normal tissue, suggesting a strong upregulation of these genes in meningiomas. The number of MGGIII tumors was low and the differences among them resulted in no statistical significance in the expression of many genes. However, several relevant Hh pathway genes—such as SMO, GLI2, GLI2 and the target growth factor IGF2—showed a significant increase in MGGII versus normal and versus MGI (only GLI2 and IGF2).

Hh Pathway Members with Similar Function Show a Correlation in Their Expression

A positive correlation between the SMO and GLI family expression is in full agreement with the activating action of SMO on the transcription of these factors. The closest association of SMO with GLI2/GLI2 suggests that they have the most relevant function in the Hh pathway of meningiomas. This is supported by the fact that GLI2 was found mainly in neural tissues (36), mRNA levels of GLI family members correlated between them and with those of other regulating factors, showing the closest associations for GLI2. These results support a relevant role of GLI2 in meningiomas.

The KIF7 expression correlates with that of many Hh pathway–activating genes; moreover, its strong correlation with GLI2/GLI2 transcription factors suggests an important role for KIF7 in the Hh pathway of meningiomas. Similarly, the expression of GAS1 correlates with Hh pathway–activating genes, although not as strongly as KIF7, supporting its positive role in this pathway in meningiomas. Hh pathway members with an activating role such as SMO, GLI family and KIF7 also correlate in their expression with that of the growth factor IGF2, in full agreement with the association between Hh pathway activation and cell proliferation.

In conclusion, our results show, to our knowledge for the first time, an activation of the Hh pathway in meningiomas. The positive Hh pathway regulators such as HHAT, DISP, SMO, KIF7, GLI1/GLI2 and GLI2 are overexpressed in all meningioma grades, including benign tumors, whereas Hh target genes, involved in cell proliferation and invasiveness—such as IGF2 and SPP1—are overexpressed in meningioma grades II and III but not in grade I. FOXM1, the GLI target gene, shows the most marked rise in mRNA levels, 90- to 100-fold above normal values in meningioma grades II and III and a 17-fold increase in benign tumors. Our results should be a significant step toward an advance in the biological and clinical characterization of meningiomas. They may also be useful to consider the Hh pathway and related genes as targets for gene therapy.

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DISCLOSURE

The authors declare that they have no competing interests as defined by Molecular Medicine, or other interests that might be perceived to influence the results and discussion reported in this paper.

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