Genomic and transcriptome analysis revealing an oncogenic functional module in meningiomas

XIAO CHANG, PH.D.,¹ LINGLING SHI, PH.D.,² FAN GAO, PH.D.,¹ JONATHAN RUSSIN, M.D.,³
LIYUN ZENG, PH.D.,¹ SHUHAN HE, B.S.,³ THOMAS C. CHEN, M.D.,³
STEVEN L. GIANNOTTA, M.D.,³ DANIEL J. WEISENBERGER, PH.D.,⁴ GABRIEL ZADA, M.D.,³
KAI WANG, PH.D.,^{1,5,6} AND WILLIAM J. MACK, M.D.^{1,3}

¹Zilkha Neurogenetic Institute, Keck School of Medicine, University of Southern California, Los Angeles, California; ²GHM Institute of CNS Regeneration, Jinan University, Guangzhou, China; ³Department of Neurosurgery, Keck School of Medicine, University of Southern California, Los Angeles, California; ⁴USC Epigenome Center, Keck School of Medicine, University of Southern California, Los Angeles, California; ⁵Department of Psychiatry, Keck School of Medicine, University of Southern California, Los Angeles, California; and ⁶Division of Bioinformatics, Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, California

Object. Meningiomas are among the most common primary adult brain tumors. Although typically benign, roughly 2%–5% display malignant pathological features. The key molecular pathways involved in malignant transformation remain to be determined.

Methods. Illumina expression microarrays were used to assess gene expression levels, and Illumina single-nucleotide polymorphism arrays were used to identify copy number variants in benign, atypical, and malignant meningiomas (19 tumors, including 4 malignant ones). The authors also reanalyzed 2 expression data sets generated on Affymetrix microarrays (n = 68, including 6 malignant ones; n = 56, including 3 malignant ones). A weighted gene coexpression network approach was used to identify coexpression modules associated with malignancy.

Results. At the genomic level, malignant meningiomas had more chromosomal losses than atypical and benign meningiomas, with average length of 528, 203, and 34 megabases, respectively. Monosomic loss of chromosome 22 was confirmed to be one of the primary chromosomal level abnormalities in all subtypes of meningiomas. At the transcriptome level, the authors identified 23 coexpression modules from the weighted gene coexpression network. Gene functional enrichment analysis highlighted a module with 356 genes that was highly related to tumorigenesis. Four intramodular hubs within the module (*GAB2*, *KLF2*, *ID1*, and *CTF1*) were oncogenic in other cancers such as leukemia. A putative meningioma tumor suppressor *MNI* was also identified in this module with differential expression between malignant and benign meningiomas.

Conclusions. The authors' genomic and transcriptome analysis of meningiomas provides novel insights into the molecular pathways involved in malignant transformation of meningiomas, with implications for molecular heterogeneity of the disease.

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KEY WORDS • meningioma • copy number alteration • WGCNA • hub genes

MENINGIOMAS are common intracranial tumors that arise from the meninges, the membranous covering of the brain and spinal cord. They constitute 25%–30% of all primary brain tumors, with an annual incidence of 4–6 per 100,000 persons.^{43,53} Most meningiomas are considered benign and grow slowly.⁵¹ The WHO classifies meningiomas into 3 subtypes: Grade I (benign meningioma), Grade II (atypical meningioma), and Grade III (malignant/anaplastic meningioma). The

prevalence rates of these subtypes are roughly 80%–90%, 6%–15%, and 2%–5%, respectively, based on several reports.^{28,35} Malignant meningiomas are typically characterized by aggressive behavior, with infiltration into the adjacent brain, focal necrosis, and increased vascularity.

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Abbreviations used in this paper: SNP = single-nucleotide polymorphism; UCLA = University of California, Los Angeles; WGCNA = weighted gene correlation network analysis.

Atypical and malignant meningiomas are often associated with high recurrence rates, even following complete resection, and are associated with shorter progression-free and overall survival compared with their benign counterparts.⁴³ Currently, the mechanisms underlying malignant transformation in meningiomas are poorly understood. Although several genetic studies have been performed on meningiomas to identify disease-susceptibility genes, few have focused on genetic and chromosomal alterations that may specifically contribute to the malignant transformation of these tumors.

Prior investigations have demonstrated an association between monosomic loss of chromosome 22 and meningioma formation.⁴⁰ Other chromosome losses have been reported on 1p, 3q, 6q, 9p, 10q, 14q, 17p, 18p, 18q, and 22q.²² Relative levels of chromosomal abnormalities have been noted to correlate with higher meningioma grade.²³ A recent study indicated that benign meningiomas with alterations in chromosome 14 may have a predilection for aggressive behavior and recurrence.³⁶ A previous genome-wide association study revealed that a common variant at 10p12.31 near *MLLT10* influences the risk of meningioma. This was the first genome-wide association study signal reporting on this disease and expanded our knowledge of genetic events to that initiate tumorigenesis.⁸ Studies on the *NF2* gene in sporadic meningiomas suggest that approximately one-third to one-half of these tumors have an inactivating mutation, often accompanied by loss of the other allele.⁴⁰ Other candidate tumor suppressor genes that have been suggested to promote meningioma formation include *DAL-1*, *BAM22*, *MNI*, and *LARGE*.⁴³ However, current knowledge is insufficient to fully understand the genetic underpinnings of this disease.

Genome-wide expression profiling analysis has also identified differentially expressed genes and pathways in meningiomas.^{10,18,23} Several tumor suppressor genes are downregulated in malignant meningiomas, compared with benign and atypical ones.¹⁰ Furthermore, a specific deregulated pathway with enrichment of underexpressed genes regulated by the transcription factors *Sp1* and *AGP/EBP* was identified in fibroblastic meningiomas compared with normal dura.¹⁸ A recent study suggested that meningiomas can be classified into 5 groups based on gene expression profiles.²³

In the current study, we collected a sample set of 19 resected meningiomas comprising all 3 WHO grades, and we performed DNA copy number analysis using single-nucleotide polymorphism (SNP) genotyping arrays. In parallel, we also performed transcriptome analysis using gene expression microarrays to complement the copy number analysis. Considering the relatively small sample size, we also reanalyzed 2 publicly available meningioma expression data sets from the GEO database, totaling 9 additional malignant samples. Finally, we take advantage of weighted gene coexpression network analysis to identify dense coexpression subnetworks in meningiomas and detect oncogenic modules that are associated with malignant meningiomas. Our integrative genomics analysis yielded novel insights into the malignant transformation of meningiomas.

Methods

Gene Expression Microarray

The study was approved by the University of Southern California institutional review board. Written informed consent was obtained from all patients prior to collection of surgical tumor specimens. Following selection of benign, atypical, and malignant meningioma case samples with confirmed histopathology, total RNA samples were extracted from fresh-frozen meningioma tissue following a Qiagen RNA extraction protocol. A liquid nitrogen grinding method was used to prepare tissue samples for extraction. First, tissue samples were removed from the -80°C refrigerator, cut into fragments, and weighed immediately to ensure that no more than 100 mg tissue was present in every 1.5-ml tube. Next, liquid nitrogen was added into the tube as soon as possible, followed by tissue grinding. Next, 900 μl of QIAzol Lysis Reagent is added to each tube, vortexed for at least 60 seconds, and stored at room temperature (15°C – 25°C) for 5 minutes. The standard protocol of the RNeasy Kit is then applied to each tissue sample, with the entire process being performed on ice (RNeasy Plus Universal Mini Kit, Cat. no. 73404). The quality of total RNA was assessed using an Experion RNA StdSens Chip on a Bio-Rad bioanalyzer. Genome-wide expression profiles of approximately 47,000 transcripts were quantified using an Illumina HumanHT-12 v4 Expression BeadChip. Raw data were processed and normalized without background subtraction using the Illumina GenomeStudio software suite. Among the 19 samples, mRNA from 11 samples (5 benign, 2 atypical, and 4 malignant meningiomas) were successfully extracted with an RNA integrity number value over 7. Considering the small sample size, we added 1 more malignant sample to perform the DNA SNP microarray assay.

Single-Nucleotide Polymorphism Array

DNA extraction was performed from 10 to 20 mg of tumor fragments using the DNeasy Blood & Tissue kit from (Qiagen), according to the supplied protocol. Genomic DNA (250 ng) was assayed by the HumanOmni-Express BeadChip SNP array (Illumina) with 730,525 markers, including 392,197 SNP markers. All microarray analysis was performed at the USC Epigenome Center. Raw microarray data were processed by the GenomeStudio Software (Illumina) to generate log R ratio and B allele frequency values for each marker on the array.

Data Analysis

Copy number alternations in 19 meningioma genomes were detected with a computational tool called OncoSNP from the SNP genotyping data.⁵⁶ Both stromal contamination and intratumor heterogeneity models were activated in OncoSNP to perform a joint analysis and estimate the baseline level of stromal contamination and intratumor heterogeneity. This is the most accurate analysis at the expense of computing resources. The SNP genotyping arrays show “genomic wave patterns” in which signal intensity is correlated to local guanine-cytosine content,

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so the signal intensity value was adjusted by the genomic_wave.pl program in the PennCNV package.^{7,50} We used an R package LIMMA in Bioconductor to performed pairwise class distinction to identify genes differentially expressed among the benign, atypical, and malignant meningiomas (false discovery rate adjusted p value < 0.01).⁴⁷ Since no differentially expressed genes were found in atypical and benign samples, we reanalyzed the microarray data by combining atypical and benign samples and comparing these to malignant samples. Two hundred eighty-eight differentially expressed genes were finally identified between malignant and benign-atypical samples. Copy number alternations and gene expression data were clustered using MultiExperiment Viewer software suite v.4.7.²⁹ We used DAVID web server (<http://david.abcc.ncifcrf.gov/>) to test enrichment in gene sets with GO, SwissProt, and InterPro terms compared with the background list of all genes.¹⁵

We also downloaded raw data (as CEL files) for 2 meningioma whole-genome gene expression studies from the GEO database (accession nos. GSE16581 and GSE4780, respectively). These samples were assayed on the Affymetrix Human Genome U133 Plus 2.0 platform, so we used the Affymetrix Expression Console software to preprocess the CEL files with Robust MultiChip Analysis protocol and then exported the probeset-level expression levels as a tab-delimited text file. The LIMMA package in R⁴⁷ was used to identify differentially expressed genes.

Weighted Gene Coexpression Network Analysis

To construct a network from gene expression data, we selected 3600 of the most varying genes and calculated the Pearson correlation for all pairs of selected genes. The correlation matrix was converted into an adjacency matrix with a power function, so that the connection strength between 2 genes x_i and x_j was defined as $a_{ij} = |cor(x_i, x_j)|^\beta$. The parameter β was determined by the criterion that the resulting adjacency matrix approximately fit a scale-free topological feature according to a previously proposed

model-fitting index.⁵⁷ The model-fitting index of a perfect scale-free network is 1. Here, we chose the smallest value of β ($\beta = 9$), which is the minimum value required to make the model-fitting index 0.85 (Fig. 1).^{9,57}

The adjacency matrix was further transformed into a topological overlap matrix, which captures not only the direct interaction between 2 genes but also their indirect interactions through all the other genes in the network. A similarity measure was defined:

$$TOM_{ij} = \frac{\sum_u a_{iu} a_{uj} + a_{ij}}{\min(k_i, k_j) + 1 - a_{ij}},$$

where

$$k_i = \sum_u a_{iu}$$

is the node connectivity.^{42,57} Subsequently, $1 - TOM_{ij}$ was used as a distance matrix in the hierarchical clustering of the transcript units for module detection.⁴²

Oncogenic Module Analysis

In the oncogenic module, member genes pairs with a Mutual Rank (<http://coxpresdb.jp/help/mr.shtml>) < 200 or Pearson correlation coefficient > 0.4 are considered connected.³³ The core region was identified with MCODE, a plugin planted in Cytoscape.^{1,44} Protein-protein interaction information was queried from Michigan Molecular Interactions (MiMI).¹⁷ The network topology was visualized with Cytoscape.⁴⁴

Results

Chromosomal Profiles of Genetic Alternations

We assayed copy number alteration profiles in 19 histopathologically verified human meningioma tumor

Fig. 1. Analysis of network topology for various soft-thresholding powers. **Left:** The scale-free fit index (y axis) as a function of the soft-thresholding power (x axis). **Right:** The mean connectivity (in degree, y axis) as a function of the soft-thresholding power (x axis).

samples: 10 benign (WHO Grade I), 5 atypical (WHO Grade II), and 4 malignant (WHO Grade III) meningiomas (Fig. 2). The average length of copy number gains was 423, 701, and 270 megabases in benign, atypical, and malignant meningiomas, respectively. In comparison, the average length of copy number loss was 34, 203, and 528 megabases in benign, atypical, and malignant meningiomas, respectively. In general, hierarchical clustering analysis classified the samples into 2 groups. Three malignant samples and 1 atypical sample with significant gain or loss of chromosomes were clustered into a small group. One malignant, 2 atypical, and all 10 benign samples with fewer chromosomal abnormalities were clustered into a separate larger group. The larger group could further be divided into 2 additional subgroups based on the relative degree of copy number amplification. The chromosomal copy number patterns suggested that higher grades of meningiomas demonstrate more variability in their genomic structures, especially with regard to genomic losses.

High Chromosomal Arm-Level Abnormality Occurrences in High-Grade Meningiomas

Previous studies have suggested that a strong association between increased frequency of chromosomal losses with increased histological grades exists in meningiomas.³⁴ Our copy number variation analysis corroborated the findings that malignant and atypical meningiomas present more chromosomal arm-level abnormalities than benign meningiomas (Fig. 2). Obvious deletion or amplification events in chromosomal arms were found in 2 of 4 malignant samples (Sample ID 249: amplification on 3 chromosomal arms, deletion on 9 chromosomal arms; Sample ID 255: deletion on 3 chromosomal arms) and 2 of 4 atypical samples (Sample ID 351: amplification on 17 chromosomal arms; Sample ID 678: amplification on 4 chromosomal arms, deletion on 6 chromosomal arms),

whereas only 1 arm-level deletion occurred in 1 of 15 benign samples. Three of 4 malignant samples showed a monosomic loss of chromosome 4, but this event was not observed in atypical and benign samples. However, we also found a malignant sample (Sample ID 254) and an atypical sample (Sample ID 489) that showed a significant repression of chromosomal alternations compared with other samples from the same WHO grade (Fig. 2). Thus, other mechanisms may be involved in the development and malignant transformation of meningiomas.

Loss of Chromosome 22

Our results also showed that nearly half of meningioma samples have significant deletions in chromosome 22q, including 2 of 4 malignant samples, 2 of 4 atypical samples, and 5 of 15 benign samples (Fig. 2). To confirm this observation, we applied a statistical framework to distinguish genuine chromosomal aberrations from random background events.⁴ The results showed that loss of chromosome 22q was clearly statistically significant (Q value = 0; Table 1). In addition, loss of chromosome 1p was also detected with high statistical significance (Q value = 0.0484; Table 1). Loss of chromosome 22q may be a primary event with respect to chromosomal abnormalities in meningiomas, as demonstrated by the few benign samples with monosomy of chromosome 22 and loss of chromosome 22 in most high-grade meningiomas.²³

Gene Expression Analysis

Hierarchical clustering analysis indicated that 5 benign and atypical samples were represented by similar whole-genome expression patterns and were grouped closely together. The malignant samples, however, showed varying patterns and were not clustered together. These observations imply that gene expression profiles of malignant meningiomas are likely to be disordered compared

FIG. 2. Chromosomal profiles of genomic copy number alternations and hierarchical clustering results of 19 meningioma samples. The 2 major clusters are colored by red and blue. Copy numbers of the genomic regions are marked from green (loss), black (normal), and red (gains). The regions of 13p, 14p, 15p, 21p, and 22p are ignored here, since they are not well covered by the SNP array data. The malignant samples are separated from benign/atypical samples, except for one atypical sample (Sample ID 351). A = atypical; B = benign; M = malignant.

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TABLE 1: Significance of chromosomal arm abbreviations predicted by GISTIC software*

Arm	No. of Genes	Amplification			Deletion		
		Frequency	z-Score	Q Value	Frequency	z-Score	Q Value
1p	1423	0	-1.17	0.887	0.26	2.68	0.0484†
1q	1274	0	-1.21	0.887	0.16	1.16	0.433
2p	590	0	-1.03	0.887	0.16	1.74	0.202
2q	1017	0	-1.18	0.887	0.11	0.488	0.623
3p	661	0.06	-0.0907	0.887	0.11	0.812	0.508
3q	732	0.11	0.76	0.887	0.06	-0.127	0.77
4p	291	0	-0.94	0.887	0.16	2.07	0.152
4q	667	0	-1.05	0.887	0.16	1.66	0.21
5p	169	0.05	0.0851	0.887	0	-0.957	0.887
5q	1009	0.05	-0.376	0.887	0	-1.21	0.887
6p	768	0.06	-0.204	0.887	0.06	-0.204	0.77
6q	550	0.06	-0.0317	0.887	0.11	0.897	0.481
7p	390	0	-1.03	0.887	0.05	-0.0551	0.77
7q	815	0	-1.16	0.887	0.05	-0.284	0.77
8p	370	0.11	0.964	0.887	0	-0.994	0.887
8q	554	0.11	0.811	0.887	0	-1.05	0.887
9p	275	0	-1.02	0.887	0	-1.02	0.887
9q	731	0	-1.13	0.887	0.05	-0.242	0.77
10p	248	0	-0.956	0.887	0.11	1.08	0.458
10q	817	0	-1.12	0.887	0.11	0.619	0.615
11p	578	0	-1.02	0.887	0.16	1.75	0.202
11q	1048	0	-1.19	0.887	0.11	0.469	0.623
12p	374	0.05	-0.0455	0.887	0	-1.02	0.887
12q	943	0.05	-0.345	0.887	0	-1.19	0.887
13q	400	0	-1.06	0.887	0	-1.06	0.887
14q	874	0	-1.03	0.887	0.26	3.26	0.0109
15q	776	0.06	-0.208	0.887	0.06	-0.208	0.77
16p	585	0.06	0.012	0.887	0.17	1.85	0.202
16q	456	0.06	0.0209	0.887	0.11	0.973	0.46
17p	420	0.06	0.0417	0.887	0.11	1	0.46
17q	1086	0.06	-0.293	0.887	0.11	0.527	0.623
18p	93	0	-0.932	0.887	0.05	0.138	0.755
18q	265	0	-0.989	0.887	0.05	0.022	0.77
19p	721	0	-1.03	0.887	0.21	2.53	0.0552
19q	1055	0	-1.15	0.887	0.16	1.33	0.36
20p	249	0.11	1.16	0.887	0.06	0.147	0.755
20q	528	0.11	0.914	0.887	0.06	-0.0196	0.77
21q	324	0.17	2.14	0.636	0.06	0.162	0.755
22q	598	0	-0.773	0.887	0.53	8.38	0†

* GISTIC = Genomic Identification of Significant Targets in Cancer.

† Values are below 0.05, indicating that the copy number variations in this chromosome are significantly associated with disease.

with benign and atypical tumors (Fig. 3). We identified 260 differentially expressed genes between malignant and benign samples and 59 differentially expressed genes between malignant and atypical samples. Interestingly, we did not identify any differentially expressed genes between benign and atypical samples (Fig. 4). In a previous study, we also demonstrated that genome-wide methylation patterns cannot differentiate benign and atypical meningiomas but can easily differentiate malignant from benign/atypical samples.¹¹ Considering the small sample size for the atypical group and the low power to detect differential expression, we combined the benign and atypical samples and subsequently identified 288 differentially expressed genes between the malignant and benign/atypical sample groups. Hierarchical clustering analysis confirmed that the differentially expressed genes were clearly divided into these malignant and nonmalignant groups (Fig. 5).

Notably, a probable tumor suppressor gene, *MNI*, showed significant repression in all the malignant samples analyzed in our study (Table S1, http://bionas.usc.edu/meningioma/Table_S1.xlsx). Previous research also suggests that inactivation of the *MNI* gene in meningiomas may contribute to their pathogenesis.²⁴ We also found that the *IGFBP5* gene (whose promoter transcription can be activated by the *MNI* gene product) was significantly suppressed (Table S1).³¹ Interestingly, *MNI* can both stimulate and inhibit transcription of this gene. Overexpression of *MNI* was also reported in the development of acute myeloid leukemia.³⁰

Considering the relatively small sample size, to further validate our gene expression result, we downloaded the raw data from 2 meningioma gene expression studies from GEO, hereafter referred to as the University of California, Los Angeles (UCLA) study (GSE16581, 63 samples)²³ and

FIG. 4. Venn diagram of differentially expressed genes among 3 classifications of meningiomas. Red circle shows the number of differentially expressed genes between malignant and benign meningiomas, purple circle shows the number of differentially expressed genes between malignant and atypical meningiomas, and green circle shows the number of differentially expressed genes between benign and atypical meningiomas.

the Scheck study (GSE4780, 56 samples). We identified 410 and 465 genes differentially expressed between benign and malignant samples from the UCLA and Scheck study, respectively. However, the number of overlapping genes among the 3 data sets is low: only 3 common genes were identified: *LEPR*, *FXYD5*, and *KCNMA1*. *LEPR* was

FIG. 3. Hierarchical clustering results of transcriptome data using the 1000 most variably expressed genes. Genes with high, medium, and low expression values are colored by red, black, and green, respectively. The malignant samples are clearly separated from benign/atypical samples.

Fig. 5. Hierarchical clustering of 288 genes with differential expression between 5 malignant groups and 5 benign and 2 atypical groups. The cluster is color coded using *red* for upregulation, *green* for downregulation, and *black* for median expression.

associated with pituitary adenoma,^{12,20,46} *FXRD5* was associated with a broad spectrum of cancers, and *KCNMA1* was also associated with glioma.^{3,19,27,41} The number of overlapping genes between each pair of the 3 data sets was higher: 26 between the UCLA and USC studies, 34 between the Scheck and USC studies, and 35 between the UCLA and Scheck studies. Interestingly, we found that the *MNI* gene was also differentially expressed in the UCLA data set. We next mapped the identified differentially expressed genes from the 3 data sets on the Cancer Genome Anatomy Project by DAVID and extracted the top 10 significantly enriched pathways (Table S2, http://bionas.usc.edu/meningioma/Table_S2.xlsx). For our data set, the top 4 pathways were all relevant to meningiomas, indicating a strong correlation of our data set and the Cancer Genome Anatomy Project. The first and third most significantly enriched pathways in the Scheck study were also related to meningiomas. However, no pathway associated with meningiomas was detected in the UCLA study.

Association Between DNA Copy Number and Gene Expression Levels

We calculated the Pearson correlation coefficient between DNA copy number and gene expression level. Our results showed that a certain proportion of genes were closely correlated (Pearson correlation coefficient > 0.5; Fig. 6). Gene ontology enrichment analysis suggested that genes related to mitochondrial function were significantly enriched in DNA copy number and correlation to high gene expression (Table S3, http://bionas.usc.edu/meningioma/Table_S3.xlsx). Analysis using Fisher's exact test also lent support to the hypothesis that these genes are enriched in chromosome regions with clear copy number abnormalities (chromosome 22q13.1 [$p = 2.6e-5$], chromosome 4p16.3 [$p = 1.15e-8$]; Table S3).

Previous studies on meningiomas revealed several aberrant signaling pathways that may be involved in tumorigenesis.³⁹ These pathways include membrane-associated protein 4.1 family-relevant pathways, vascular endothelial growth factor-involved angiogenic pathways, Hedgehog signaling pathway, MAPK and PI3K signaling pathways, Notch signaling pathway, and growth factor- and cytokine-induced pathways. We next examined our copy number variation and gene expression data with respect to the candidate meningioma pathways. We found

that copy number alterations disrupt genes in some of these pathways and may result in gene expression changes. Besides *MNI*, a few genes in the PI3K/Akt pathway and some bone morphogenetic proteins were also differentially expressed between the benign/atypical and malignant samples. These results suggested that these pathways may play a role in tumor progression toward malignancy (Table S4, http://bionas.usc.edu/meningioma/Table_S4.xlsx).

An Oncogenic Module Identified by Weighted Gene Coexpression Networks

We further analyzed the gene expression data with the weighted gene correlation network analysis (WGCNA) algorithm, which has been widely applied in gene coexpression network construction and module detections.^{5,16,21,32,57} Twenty-three coexpression modules were identified (Fig. 7). Functional annotations of these modules were performed by analyzing the gene compositions within each module. Three modules were significantly enriched in genes with specific functional categories—Module I: glycoprotein/membrane/blood vessel development/cell migration, 356 genes; Module II: cell cycle, 97 genes; and Module III: synapse/membrane, 1289 genes. Of these, Module I is considered an oncogenic module since it is enriched in cancer-related functional processes. Not surprisingly, the differentially expressed gene *MNI*, as well as its target gene *IGFBP5*, was also contained within this oncogenic module.

In a biological network, a few highly connected

Fig. 6. Distribution of DNA number and expression correlation. The x axis represents the Pearson correlation coefficient, and the y axis represents the frequency. The average is significantly higher than zero ($p = 0$).

Fig. 7. Weighted gene coexpression network identified multiple functional modules. The upper portion of the figure is the cluster dendrogram of genes, and the lower section shows the modules of coexpressed genes. Genes within the same module are colored in the same color.

nodes that hold the whole network together are referred to as hubs.⁴⁸ Similarly, genes with a high number of interactions are believed to play an important role in organizing the biological process of functional modules.^{2,13,48} We therefore zoomed in to identify hub genes in the oncogenic module. Unlike classic gene coexpression networks or protein-protein interaction networks, WGCNA defined a whole network connectivity measure (k_{Total}) for each gene, based on its Pearson correlation coefficient with all of the other genes, and an intramodular connectivity measure (k_{Within}) when only considering the connection strength of each gene with all the other genes within the specific module.⁵⁷ In this study, the intramodular connectivity is far more meaningful than whole network connectivity in the oncogenic module. We hypothesized that intramodular hub genes may be associated with meningioma tumorigenesis. As expected, 2 intramodular hub genes (*GAB2* and *KLF2*) with the highest values of connectivity were related to tumorigenesis, and 2 additional intramodular hub genes (*ID1* and *CTF1*) are known oncogenes (Table 2). These 4 hub genes were also significantly differentially expressed between malignant and benign meningiomas (Table S1). Interestingly, the 4 hub genes are also related to leukemia.^{25,37,52,54} As described above, the meningioma tumor suppressor *MNI* in this module was also associated with leukemia.^{26,30}

Validation of the Oncogenic Module With Other Biological Networks

We further validated the oncogenic module with a coexpressed gene database, COXPRESdb, which collected large mammalian coexpression information from

publicly available GeneChip data.³³ We created a subnetwork by mapping the genes of the oncogenic module onto the COXPRESdb database (Fig. 8). To further explore the oncogenic module in detail, we identified a highly connected core region of 27 genes with MCODE from the oncogenic module (Fig. 9; see *Methods*). The 27 core genes containing *GAB2* and *KLF2* are mostly related to cancer. *ID1* and *CTF1* are also directly connected with members of the core region (Fig. 7). Although *MNI* is not directly connected to the core region, the protein-protein interaction networks show that *MNI* can connect to *KLF2* through *EP300*. These results show a layered structure in which *GAB2* and *KLF2* function as central hubs. Genes or gene products interacting with them can form a core region in the oncogenic module. Most of the genes in the module can associate with *GAB2* and *KLF2* through those in the core region.

Discussion

In this study, we compared different subtypes of meningiomas at both the genomic and transcriptome levels. Our results identified chromosomal level alternations and differentially expressed genes between benign and malignant meningiomas. Gene coexpression network analysis revealed a module enriched with oncogenes that may represent a potential developmental pathway in meningioma pathogenesis and malignant transformation.

At the genomic level, copy number alteration analysis revealed that the degree of chromosomal abnormality correlates with the WHO meningioma grade. Monosomic loss of chromosome 22 may be considered a significant

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TABLE 2: Connectivity strength of genes in the oncogenic module*

Gene	k _{Total}	k _{Within}	k _{Out}	k _{Diff}
GAB2†	91.937226	61.3776024	30.559624	30.8179788
KLF2†	88.975097	61.065278	27.909819	33.15545911
SNORA59A	93.758325	60.5207121	33.237613	27.28309894
PTGDR	87.801063	59.7878167	28.013246	31.77457035
SNORA59B	89.138849	57.4173046	31.721544	25.69576019
SLC47A1	93.172951	57.3408979	35.832053	21.50884502
SCARA5	92.73639	57.2757312	35.460659	21.81507259
CLIC2	84.49493	57.0510458	27.443885	29.60716128
PDZRN3	79.200217	55.7714971	23.42872	32.34277751
ENPP6	81.056553	53.4198296	27.636723	25.78310628
FGL2	80.553559	53.1927761	27.360783	25.83199294
ID1†	93.686755	52.1946409	41.492114	10.70252651
THSD4	84.486848	51.3173816	33.169466	18.14791539
PDE9A	77.034959	50.9068069	26.128152	24.77865462
CTF1†	79.880531	50.8034799	29.077051	21.72642927
SULF2	81.416589	50.291748	31.124841	19.16690698
SLC26A2	79.16335	50.0251871	29.138163	20.88702448
KCNMA1	81.470279	50.0093559	31.460924	18.54843236
LRRN4CL	75.794449	49.2979355	26.496514	22.8014219
LOC100131277	70.845041	48.6058186	22.239222	26.36659624
HSPB2	79.865166	48.3246837	31.540482	16.78420127
EPHX1	79.476038	47.937519	31.538519	16.399
ST6GALNAC2	67.865026	47.9022072	19.962819	27.93938834
CTXN3	68.096972	47.4314341	20.665538	26.76589649
NHEDC2	71.413542	47.234591	24.178951	23.05563971
WNK4	72.822522	46.761346	26.061176	20.70016994
ST6GAL1	72.57842	46.6197313	25.958689	20.66104219
TMEM100	72.370632	46.0702591	26.300373	19.76988595
PGR	69.890521	45.9499607	23.940561	22.00940005
SEMA6C	72.651289	45.7996648	26.851624	18.94804063
SLC7A2	88.093278	45.5681683	42.52511	3.043058586
PENK	60.904288	45.1546739	15.749614	29.40506027
DHRS3	71.615595	45.0212782	26.594317	18.426961
PGM5	66.511249	44.4179815	22.093267	22.32471413
SLPI	83.390389	43.4470172	39.943371	3.503645876
TMOD1	72.801692	42.8815547	29.920138	12.96141702
SMAD9	61.961535	42.6894245	19.272111	23.41731393
MSRB3	73.598286	42.3308705	31.267416	11.06345497
PHGDH	74.777432	42.2953639	32.482069	9.81329536
MAL2	58.90444	42.051725	16.852715	25.19901013
IGFBP5	64.650266	41.8840948	22.766171	19.11792343
PLEKHA6	75.35317	41.6674635	33.685706	7.981757175
C9ORF125	75.376087	40.8383805	34.537707	6.300673862
PODN	63.918771	40.6111519	23.307619	17.30353258
PLCD4	70.390593	40.603878	29.786715	10.81716307
AFAP1L1	68.033303	40.441747	27.591556	12.8501905
LOC646347	61.70555	40.1098155	21.595734	18.514081
SLC7A1	59.581774	39.9054968	19.676277	20.22921977
HS.569162	52.549036	39.8293081	12.719727	27.10958066

(continued)

TABLE 2: Connectivity strength of genes in the oncogenic module* (continued)

Gene	k _{Total}	k _{Within}	k _{Out}	k _{Diff}
SLC38A2	79.247877	39.5546778	39.693199	-0.138521551
ALPL	69.246473	39.4975812	29.748892	9.748689668
LPAR5	61.829622	39.2725587	22.557063	16.71549586
ADRB2	67.238236	39.2556038	27.982632	11.27297214
A4GALT	78.239733	39.0776285	39.162105	-0.084476175
C10ORF116	61.504405	38.5604319	22.943973	15.61645855
SOD3	55.24492	38.5499784	16.694941	21.85503705
LOC644348	53.888645	38.2882185	15.600427	22.68779164
LOC728715	65.672533	38.23127	27.441263	10.7900071
LOC642590	58.959018	38.1160477	20.84297	17.27307792
HS.525922	54.111846	38.0848143	16.027032	22.05778262
HCG4	52.02505	38.0736661	13.951384	24.12228233
MN1	69.20248	38.0478432	31.154637	6.893206166
CLEC1A	71.985292	37.7632783	34.222014	3.541264242
FAM20A	77.820807	37.729718	40.091089	-2.361371218
ALDH2	66.540582	37.334502	29.20608	8.128422392
EHD1	52.965111	37.1110976	15.854014	21.257084
NMNAT2	63.619487	36.2615548	27.357932	8.903622913
KIAA1199	66.223303	35.8189545	30.404349	5.414605574
KLHL13	60.59826	35.6899059	24.908354	10.78155167
LEPR	55.77418	35.6854562	20.088724	15.59673201
SMS	54.925611	35.6405658	19.285045	16.35552082
JAM2	55.792434	35.5409367	20.251497	15.28943996
TOB1	74.265798	35.5301485	38.735649	-3.205500765
ACSL5	60.50137	35.3709656	25.130404	10.24056162
SFRP2	75.13541	35.116726	40.018684	-4.9019577
UGT3A2	49.861588	35.060084	14.801504	20.25858018
HNMT	54.075316	34.9770918	19.098224	15.87886739
PHLDB2	68.831888	34.4682469	34.363641	0.104606126
HS.286666	56.068406	34.3583383	21.710068	12.64827032
SELENBP1	66.808431	34.2560299	32.552401	1.703628871
TBC1D8	63.331815	34.0514957	29.28032	4.771176193
GPR4	54.442049	34.0008219	20.441227	13.55959473
ARL4D	46.539182	33.3520272	13.187155	20.164872
SERPINB1	59.59758	33.2218756	26.375704	6.846171609
TCN2	57.207409	33.0428522	24.164557	8.878295542
FGFRL1	53.029408	32.958395	20.071013	12.88738164
MAN1C1	55.226112	32.514126	22.711986	9.802139895
OXTR	62.350785	32.0453568	30.305428	1.739928946
C18ORF1	53.011275	31.9067125	21.104562	10.80215021
FXYD5	47.9475	31.5981761	16.349324	15.24885214
TSC22D1	56.535991	31.5693995	24.966591	6.602808354
MBOAT1	46.95168	31.287494	15.664186	15.62330761
PDE6A	45.616977	31.2342021	14.382775	16.85142689
HMHA1	57.928421	30.8650788	27.063342	3.801736731
SULF1	47.61938	30.7694661	16.849914	13.91955223
CLCNKB	43.962685	30.754402	13.208283	17.54611929
FAT4	46.928609	30.740171	16.188438	14.55173324
CALML4	46.71526	30.7183496	15.99691	14.72143933

(continued)

An oncogenic functional module in meningioma

TABLE 2: Connectivity strength of genes in the oncogenic module* (continued)

Gene	k _{Total}	k _{Within}	k _{Out}	k _{Diff}
TMEM20	51.910543	30.521065	21.389478	9.131586859
CYP4F12	47.644483	30.0807017	17.563781	12.51692053
SLC16A9	55.309621	29.9600739	25.349547	4.610526902
TP53I11	48.91971	29.9241109	18.995599	10.92851171
CHMP1B	50.517347	29.6498198	20.867527	8.782292372
EYA1	50.156311	29.0398559	21.116455	7.923400575
RIPK4	41.638271	28.8288445	12.809427	16.01941788
SPTLC3	68.877063	28.7206109	40.156452	-11.43584107
GADD45B	56.387845	28.5303764	27.857468	0.672908301
LZTS1	74.164723	28.0142455	46.150477	-18.13623175
PIK3C2B	50.789735	27.9914468	22.798289	5.193158155
MET	55.95372	27.8153185	28.138402	-0.32308342
UNC13B	50.005877	27.6928691	22.313008	5.379861043
ACSF2	42.346233	27.6105974	14.735635	12.87496221
C15ORF52	47.799629	27.5488835	20.250745	7.298138423
PLCE1	53.351366	27.3755524	25.975814	1.39973842
SIX2	49.809054	27.2042265	22.604828	4.599398799
TMEM195	41.568529	26.8311964	14.737333	12.09386376
NR4A2	42.088573	26.8063421	15.282231	11.52411149
REEP1	52.473201	26.7233188	25.749882	0.973436371
SHB	44.159135	26.6600985	17.499036	9.161062391
ADCY3	60.796418	26.6581086	34.13831	-7.480200999
ASTN2	47.361492	26.5419923	20.8195	5.722492236
PGF	38.778386	26.3195466	12.45884	13.86070701
TNFAIP8L3	46.174732	26.2606133	19.914119	6.346494281
CXCR7	52.394703	25.8960562	26.498647	-0.602590624
SLC20A2	53.499574	25.6518708	27.847703	-2.195832492
HS6ST2	41.850442	25.5123381	16.338104	9.174234209
EYA2	57.18162	25.2882305	31.893389	-6.605158749
AFAP1	45.73445	25.0514673	20.682983	4.368484348
PDE1A	45.411184	25.0329288	20.378255	4.654673372
MYO5B	40.078825	24.814022	15.264803	9.549219448
HMGCLL1	38.510179	24.6394778	13.870701	10.7687769
SEMA3C	47.946028	24.3910219	23.555006	0.836016004
NBL1	65.594206	24.2754579	41.318748	-17.04329014
BCAT2	53.43673	23.9011265	29.535604	-5.63447704
TEKT3	52.68643	23.6896794	28.996751	-5.307071562
CCDC34	48.151283	23.6472239	24.504059	-0.856835355
APLP2	47.080971	23.36547	23.715501	-0.350030569
C8ORF34	36.91195	23.2162621	13.695688	9.52057455
SLC26A7	37.305344	23.166268	14.139076	9.027191859
C10ORF26	44.129558	23.131567	20.997991	2.133576376
C2ORF40	47.348227	22.7171508	24.631076	-1.913925259
ALX3	40.123675	22.5446289	17.579046	4.965582441
ADI1	37.446839	22.4786302	14.968209	7.51042108
RFTN1	42.389419	22.4401878	19.949231	2.490957076
LOC54103	42.858358	22.3174184	20.54094	1.776478254
RRAD	37.159657	21.9635591	15.196098	6.76746107
CYP4X1	34.623603	21.8296302	12.793973	9.035657623

(continued)

TABLE 2: Connectivity strength of genes in the oncogenic module* (continued)

Gene	k _{Total}	k _{Within}	k _{Out}	k _{Diff}
KLK1	36.747332	21.7187729	15.028559	6.69021392
MARCH3	32.667551	21.4550358	11.212515	10.24252109
RSPO3	35.181944	21.4229844	13.758959	7.664025135
SLC24A3	43.296281	21.3641068	21.932174	-0.568067224
MICAL1	40.462948	21.2879353	19.175012	2.112922943
TRPC3	32.613814	21.1406533	11.473161	9.667492816
FLJ20920	33.063828	20.9677145	12.096113	8.871601067
ZNF680	45.761023	20.8070623	24.95396	-4.146897924
CLCNKA	32.411415	20.6696875	11.741727	8.927960491
ANO2	30.642068	20.6683747	9.973693	10.6946812
C10ORF33	35.261246	20.6632203	14.598025	6.065194948
PDE5A	34.443581	20.6569813	13.786599	6.870382082
SQRDL	38.790877	20.5781296	18.212747	2.365382227
CTSZ	36.013168	20.5570576	15.456111	5.100946672
PROS1	45.22208	20.3650076	24.857072	-4.492064393
LOC342979	38.78767	20.3113441	18.476326	1.835018433
NCAPD3	49.296213	20.2177589	29.078454	-8.860695375
AFAP1L2	42.80353	20.1962619	22.607268	-2.411006256
NPTX2	39.77959	20.0275871	19.752003	0.27558387
BAIAP2L2	37.365078	19.8812506	17.483828	2.397422803
C10ORF73	35.715635	19.7470922	15.968542	3.778549776
PCOLCE2	34.435991	19.6626869	14.773304	4.889382806
SLITRK4	36.202406	19.4122635	16.790143	2.622120752
TGFBR3	47.362059	19.3067906	28.055268	-8.748477346
BMP5	33.001158	19.2339653	13.767193	5.466772139
CHST4	39.852826	19.1641381	20.688687	-1.524549272
ITM2C	48.764021	19.0204147	29.743606	-10.72319115
PSAT1	45.139185	18.9106324	26.228553	-7.31792061
ELOVL6	36.392406	18.8322914	17.560115	1.272176728
IGFBP3	32.753029	18.7525297	14.000499	4.75203061
CTSK	58.11487	18.5960699	39.5188	-20.92273007
BOC	36.28372	18.2785785	18.005141	0.273437118
C10ORF11	41.198815	18.1827797	23.016035	-4.833255754
CDH1	33.942365	18.1071814	15.835183	2.271998064
ST3GAL5	33.779051	18.1022425	15.676808	2.425434052
IL34	30.49039	18.0939202	12.39647	5.697450237
NELL2	45.247855	17.9842398	27.263615	-9.279375304
GPR137C	43.825082	17.9615367	25.863546	-7.902008965
SFRP1	27.271184	17.8838074	9.387377	8.496430893
LOC728211	27.579571	17.7838233	9.795747	7.988075957
DEFB1	38.236686	17.4005093	20.836176	-3.435667055
ASAM	46.900685	17.3110555	29.58963	-12.27857443
SHF	26.368082	16.9777555	9.390326	7.587428967
COPZ2	48.25746	16.801448	31.456012	-14.65456387
PLD1	32.6877	16.7055927	15.982108	0.723485181
BTG2	31.200689	16.4685995	14.73209	1.736509694
STARD8	28.945853	16.2884431	12.65741	3.631033206
CHSY3	27.143499	16.2329674	10.910532	5.322435723
FOXO1	31.700055	16.1117571	15.588298	0.523458878

(continued)

An oncogenic functional module in meningioma

TABLE 2: Connectivity strength of genes in the oncogenic module* (continued)

Gene	k _{Total}	k _{Within}	k _{Out}	k _{Diff}
MPZL2	28.159703	16.0215742	12.138129	3.883445349
LOC388494	28.64752	15.9095065	12.738013	3.171493282
TMEM16B	22.768178	15.653603	7.114575	8.539027983
FAM63B	32.989171	15.6484054	17.340766	-1.692360696
CD55	26.193048	15.6275422	10.565506	5.062036543
AHNAK2	33.934328	15.6012845	18.333044	-2.731759213
POL3S	33.010494	15.5574662	17.453027	-1.895561148
PDGFRL	38.28427	15.3895187	22.894751	-7.505232417
C14ORF78	31.374417	15.1415776	16.232839	-1.091261493
DACT1	27.437047	14.924693	12.512354	2.412339487
COL18A1	26.598642	14.6509657	11.947677	2.703289168
PTPN13	28.144953	14.3940342	13.750919	0.643115561
FOXP1	31.035432	14.2924979	16.742934	-2.450436567
SETBP1	28.754646	14.290703	14.463943	-0.173240412
MIR185	30.751939	14.2429239	16.509015	-2.266090945
PPFIBP1	38.216457	14.1651799	24.051277	-9.886097078
PVRL3	45.43931	14.0844661	31.354843	-17.27037735
ADAMTSL1	26.517671	14.0231056	12.494565	1.528540236
EMILIN1	26.868861	13.9034047	12.965456	0.937948195
TMEM30B	28.077249	13.8631885	14.214061	-0.35087206
LOC647979	24.725424	13.8270804	10.898344	2.928736384
F10	22.97621	13.6589004	9.317309	4.341591077
CYP3A5	26.122458	13.5492804	12.573178	0.976102708
RERG	37.786428	13.3516863	24.434741	-11.08305495
KLHL14	19.19865	13.2802935	5.918357	7.361936841
GMPR	25.509252	13.2028389	12.306413	0.896425719
KLF4	37.574339	13.1700615	24.404277	-11.23421574
TMEM37	24.225877	13.0406309	11.185246	1.855385222
ZNF503	28.288625	12.9115998	15.377025	-2.465425451
FAM101A	18.584357	12.8186932	5.765664	7.053029009
C11ORF80	30.37899	12.7866418	17.592348	-4.805706326
ATOH8	19.697787	12.7577426	6.940044	5.81769829
C10ORF65	22.300592	12.6956716	9.604921	3.090751068
TAGLN	28.571186	12.6764854	15.8947	-3.218215036
EDG7	24.217116	12.6736048	11.543511	1.130093344
CTHRC1	35.16698	12.5428042	22.624176	-10.08137184
RASEF	17.300335	12.477871	4.822464	7.655406849
SALL4	21.862568	12.3921531	9.470415	2.921738591
C11ORF70	26.184607	12.2370312	13.947576	-1.710544996
AGPAT9	22.629747	12.0724255	10.557322	1.515103936
SERTAD4	21.015614	12.0376522	8.977961	3.059690825
NPNT	29.061401	11.7260254	17.335375	-5.609350123
INMT	20.773939	11.723038	9.050901	2.672137019
B4GALT1	28.03345	11.5831866	16.450263	-4.867076274
SMAD6	24.775931	11.5825681	13.193362	-1.610794394
ANLN	38.970357	11.5347278	27.43563	-15.90090182
NTN3	20.823662	11.4167512	9.406911	2.009840221
PBX3	21.974249	11.3341401	10.640109	0.694030737
C10RF133	21.208224	11.321314	9.88691	1.434403989

(continued)

TABLE 2: Connectivity strength of genes in the oncogenic module* (continued)

Gene	k _{Total}	k _{Within}	k _{Out}	k _{Diff}
SRGAP1	23.163626	11.2586245	11.905002	-0.646377281
BOP1	33.917157	11.1905378	22.726619	-11.53608163
FREM2	20.97208	10.8616073	10.110473	0.751134643
RHOB	20.903824	10.8321524	10.071672	0.760480515
ANO6	41.978064	10.6445366	31.333528	-20.68899102
TJP3	23.072419	10.601802	12.470617	-1.868814754
FGF11	23.884247	10.5932122	13.291034	-2.697822252
ARHGAP10	23.397384	10.563653	12.833731	-2.270077847
HES1	44.486825	10.541474	33.945351	-23.40387648
NQO1	22.690692	10.5388687	12.151824	-1.612955021
LOC387882	28.96743	10.5362801	18.43115	-7.89486971
RPS6KA5	34.274251	10.5287587	23.745492	-13.21673371
KLK7	19.826662	10.5058015	9.320861	1.184940615
MEOX2	24.262074	10.2859715	13.976103	-3.690131443
MND1	32.638701	10.1533671	22.485333	-12.33196635
EDN1	28.699333	10.0883972	18.610936	-8.52253882
SIX1	26.219793	10.0049291	16.214864	-6.209935026
HSPB6	34.07586	9.9289733	24.146886	-14.21791312
FGR	19.339602	9.9195676	9.420034	0.499533463
SMOX	26.819625	9.9041951	16.91543	-7.01123496
DSP	19.183297	9.8944749	9.288822	0.605652459
NFKBIZ	17.576362	9.8651966	7.711165	2.154031451
CDC7	19.695647	9.8613918	9.834256	0.027136139
CDH5	15.342002	9.7681584	5.573844	4.194314517
S100P	16.638205	9.6795362	6.958669	2.720867288
MR1	23.281125	9.6457053	13.63542	-3.989714456
KCNK5	14.699684	9.6202299	5.079454	4.540775581
LPAR3	17.438678	9.5833715	7.855307	1.728064961
FZD7	19.926706	9.5367574	10.389948	-0.853190853
FABP3	22.269481	9.5310268	12.738454	-3.207427529
COL23A1	20.185272	9.4171492	10.768122	-1.350973071
ZBTB16	20.485859	9.2975164	11.188343	-1.890826658
PDZD2	35.435857	9.2492791	26.186577	-16.93729831
STOX1	35.103324	9.0969995	26.006324	-16.90932479
C10ORF105	21.291921	8.9998536	12.292067	-3.292213634
RNF24	18.454095	8.9591057	9.494989	-0.535883418
C10RF21	48.734478	8.8720507	39.862427	-30.99037668
CFB	27.560248	8.8657687	18.69448	-9.828710926
CHODL	16.820116	8.5512157	8.268901	0.282315188
GCGR	13.388728	8.3906571	4.998071	3.392586019
LOC100133551	13.771589	8.2338936	5.537696	2.696197965
RCSD1	14.070933	8.1794294	5.891504	2.287925604
GDE1	20.05449	8.1312334	11.923257	-3.792023541
HRCT1	17.205117	8.0024474	9.202669	-1.200221661
GALNTL4	18.831225	7.8499182	10.981307	-3.131388378
PRDM6	20.245629	7.836219	12.40941	-4.573191005
SLC25A29	15.692794	7.4481252	8.244669	-0.796544006
TIMP3	13.91059	7.4429861	6.467604	0.975382132
KLK8	18.009085	7.3627909	10.646294	-3.283503569

(continued)

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TABLE 2: Connectivity strength of genes in the oncogenic module* (continued)

Gene	k _{Total}	k _{Within}	k _{Out}	k _{Diff}
SLC22A18	14.091617	7.2046128	6.887005	0.317608148
CHCHD6	17.018761	6.9436167	10.075144	-3.131527701
FGF2	18.014674	6.9053371	11.109337	-4.203999999
LOC388588	15.042224	6.7182982	8.323926	-1.60562803
TCF3	19.122877	6.5475548	12.575322	-6.027767481
GALNT12	11.082285	6.4959277	4.586357	1.909570346
SLC13A4	13.149774	6.3012236	6.848551	-0.547327043
XYLT1	11.225407	6.2435902	4.981817	1.26177294
ENPP1	14.339533	6.2405762	8.098957	-1.858380392
MSLN	12.863767	6.2334341	6.630333	-0.396899349
HS.572444	13.658788	6.2107538	7.448034	-1.237280446
AOX1	17.079673	6.1897817	10.889891	-4.700109256
SLC6A20	9.677573	6.164331	3.513242	2.651088869
IL18R1	13.32097	6.1067296	7.214241	-1.107511012
PRG4	11.906875	6.0772185	5.829657	0.24756167
PLXNA2	12.0217	5.8286637	6.193036	-0.364372752
MST4	13.770683	5.7500375	8.020646	-2.270608299
CYB5R2	16.525844	5.7451343	10.78071	-5.035575398
SPATA18	14.322105	5.7384282	8.583677	-2.845248513
NXPH3	8.373418	5.6906602	2.682758	3.007902253
FMO1	9.657889	5.47014	4.187749	1.282391373
C6ORF26	10.18049	5.4453886	4.735101	0.710287393
GADL1	12.256558	5.3158141	6.940744	-1.624930024
CLDN7	9.533733	5.1559934	4.377739	0.778254026
AXUD1	10.304096	5.1110176	5.193079	-0.082061128
GINS4	26.081854	4.9081524	21.173701	-16.26554896
LHCGR	8.31711	4.884274	3.432836	1.451438378
FRZB	11.067715	4.8388184	6.228896	-1.390077976
FLJ30375	9.323856	4.7718644	4.551992	0.219872435
TNFRSF21	8.721926	4.7517213	3.970204	0.78151713
C12ORF48	20.386633	4.7285983	15.658035	-10.92943636
CCDC68	12.182918	4.6767419	7.506176	-2.829434386
FANCC	22.845453	4.4256789	18.419774	-13.99409526
HS.444785	9.280858	4.3534548	4.927403	-0.57394811
LPHN2	11.426724	4.3128621	7.113862	-2.8010001
NAPSB	8.7574	4.2768968	4.480503	-0.203605997
CPXM2	10.082331	4.2752754	5.807055	-1.531780019
HS.119933	10.118276	4.2438622	5.874414	-1.630551804
MYO1B	8.213893	4.1076496	4.106243	0.001406504
MLF1IP	20.299115	4.0235208	16.275595	-12.25207393
LYPLAL1	8.038086	3.9962114	4.041875	-0.045663261
MFAP2	16.584184	3.7779842	12.8062	-9.028215801
CLIC3	9.925067	3.7155352	6.209532	-2.493996661
ABCG2	11.450555	3.6594262	7.791129	-4.131703004
ENG	10.649239	3.5811315	7.068107	-3.486975962
COL8A2	9.139634	3.5778818	5.561753	-1.983870823
LYPD6B	6.694317	3.4947409	3.199576	0.295165126
ANGPTL7	9.985216	3.3629758	6.62224	-3.259264177
GPSM2	16.000406	3.3471303	12.653276	-9.306145687

(continued)

TABLE 2: Connectivity strength of genes in the oncogenic module* (continued)

Gene	k_{Total}	k_{Within}	k_{Out}	k_{Diff}
SIX4	21.397128	3.2923045	18.104824	-14.81251918
ZBTB7C	9.063249	3.2155378	5.847711	-2.632172957
PRRX1	6.612249	3.1330223	3.479227	-0.346204832
TMEM154	10.302565	2.9347514	7.367814	-4.433062307
COL12A1	10.96393	2.9258159	8.038115	-5.11229867
HS.575038	6.718715	2.8289754	3.88974	-1.060764291
MSX1	4.599336	2.4443386	2.154997	0.289341444
PLEKHG3	7.857878	1.9532737	5.904604	-3.951330301
ST3GAL6	8.276674	1.7560559	6.520618	-4.764561802
CD177	3.733092	1.5403244	2.192767	-0.652442683
PPP1R3C	3.524628	1.4846765	2.039952	-0.555275244
FAM107B	4.811333	1.3696313	3.441702	-2.072070786
ADAMTSL5	2.497067	0.6625621	1.834505	-1.17194317

* k_{Total} = total connectivity; k_{Within} = connectivity within the oncogenic module; k_{Out} = connectivity outside the oncogenic module; k_{Diff} = difference between k_{Within} and k_{Out} .

† Important hubs that are addressed in the text.

and early step in meningioma pathogenesis. Nearly half of our samples harbored significant deletions in chromosome 22q, a finding that substantiates previous reports.^{23,40} Loss of chromosome 22, however, was not apparent in all of the malignant samples; indeed only 2 of 4 exhibited deletion in this region. Loss of chromosome 4 was evident in 3 of 4 malignant samples, suggesting a potential role in malignant transformation. One malignant sample (Sample ID 254) demonstrated only minor changes in copy number across the entire genome. The sole chromosomal abnormality noted in this tumor was the gain of chromosome 21 (Fig. 1). Although these observations

support a role for chromosomal-scale abnormalities in the development and progression of meningiomas, they reaffirm previously known challenges in extrapolating complicated mechanisms from a relatively small sample size.

We further investigated gene expression differences between nonmalignant and malignant meningiomas at the transcriptome level. The whole-genome expression profile was consistent among the nonmalignant (benign and atypical) samples. These patterns, however, were altered in the malignant samples, suggesting disruption of normal gene regulatory mechanisms. Furthermore, each

FIG. 8. Network topology of the oncogenic module. The core region is marked by yellow, hub genes within the core region are marked in green, and hub genes outside the core region are marked in white.

FIG. 9. The network topology of the core region; hub genes within the core region are marked in green.

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malignant sample presented a unique expression pattern, implying heterogeneity in the factors involved with alterations in the gene regulatory system (Fig. 2). Additionally, based on analysis of previously identified abnormal signaling pathways in meningiomas, we found that genes such as *MNI* and bone morphogenetic proteins are also differentially expressed between benign and malignant meningiomas.

We also identified some genes that displayed a high association between DNA copy number and gene expression. These genes were enriched in chromosome 1, 2, 3, 4, 16, and 22, which is consistent with regions harboring high levels of copy number alterations. As previously stated, loss of chromosome 22 was identified in the majority of samples, whereas loss of chromosome 4 was noted in 3 of 4 malignant meningiomas. Copy number alterations may therefore exert a dose-dependent effect on gene expression level.

Since biological network studies provided systematic understanding of the biological process, we also applied WGCNA to identify gene coexpression modules and potential oncogenes.^{2,6,45} The WGCNA approach has been widely used to identify potential oncogenic coexpression modules and predict the molecular targets and prognosis markers.^{14,16,38,55,58} For instance, Horvath et al. have successfully identified the gene *ASPM* as a molecular target for glioblastoma, another primary brain tumor.¹⁴ Ivliev et al. also identified transcriptional modules related to proastrocytic differentiation and sprout signaling in gliomas.¹⁶ In our study, among the 23 coexpression modules, we identified one that was strongly associated with tumorigenesis and could be considered an oncogenic module. This module contains 356 genes and is enriched in glycoprotein/membrane/blood vessel development and cell migration. We also identified the intramodular hub genes that are thought to contribute significantly to the topological architecture and biological process of functional modules. Four oncogene hubs (*GAB2*, *KLF2*, *ID1*, and *CTF1*) were detected among the top 15 most connected genes within the oncogenic module. Prior studies have suggested that these 4 genes are associated with leukemia.^{25,37,52,54} Interestingly, the *meningioma 1* gene, often abbreviated as *MNI*, was also identified in this oncogenic module. *MNI* was first discovered in 1995 from a meningioma patient with a balanced translocation that disrupts *MNI* in its first exon. In this case, no expression of *MNI* mRNA was observed, so *MNI* was considered as a candidate tumor suppressor gene, affecting meningioma formation via inactivation.²⁴ However, further research has suggested that overexpression of the *MNI* gene can induce acute myeloid leukemia. Our study demonstrated differential *MNI* gene expression between benign and malignant meningiomas. Other differentially expressed oncogenes found in this module were *IGFBP5*, *MET*, and *TGFBR3*. *IGFBP5* is also upregulated by *MNI* and linked to other cancer types, including glioblastoma multiforme.^{31,49}

We further identified a core region within the oncogenic coexpression module, consisting of the hub genes *GAB2* and *KLF2*. The additional hub genes *ID1* and *CTF1* were directly connected to these members of the

core region. Although *MNI* was not directly connected in the coexpression network, protein-protein interaction networks show that *MNI* can connect to *KLF2* via *EP300*. These results suggest that *GAB2* and *KLF2* may play an important role in the meningioma developmental pathway. Oncogenes interacting with *GAB2* and *KLF2* can function together as a small group. In turn, oncogenes indirectly connected to *GAB2* and *KLF2* via neighbors can form a large oncogenic module for meningiomas.

Considering the small sample size, we analyzed 2 relatively large gene expression data sets including 68 and 56 meningioma samples, respectively, although the number of malignant samples is still quite small (6 and 3, respectively). Functional enrichment analysis indicated that our data (the USC study) and those from the Scheck study were highly consistent with the Cancer Genome Anatomy Project database. We also applied WGCNA to these 2 data sets. However, WGCNA failed to find a significant meningioma-related module. A possible reason may be the imbalance of sample proportion from different subtypes because only 6 malignant samples of the total 68 samples are involved in the UCLA study and only 3 malignant samples of the total 56 samples are involved in the Scheck data set. This issue will lead to a bias in calculating the expression correlation between gene pairs. Therefore, the relative proportion of subtypes may be more important than sample size when applying WGCNA to construct gene coexpression networks.

Although limited by sample size, this systematic analysis of genomic and transcriptome differences between benign and malignant meningiomas revealed novel insight into the machinery of meningioma pathogenesis and transformation. If confirmed in larger studies, these alterations may serve as targets for novel diagnostic modalities and therapeutic interventions.

Conclusions

In summary, copy number alteration analysis confirmed the high frequency of chromosome 22 monosomy and the association between WHO grade and chromosomal abnormalities. Transcriptome analysis identified several genes that were highly differentially expressed between the benign and malignant groups, including a meningioma candidate gene, *MNI*. In addition, we used a weighted gene coexpression network analysis and identified an oncogenic module associated with meningiomas. Hub oncogenes in this module are also associated with the development of leukemia, implying potential common pathways between the 2 types of neoplastic diseases.

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Disclosure

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Author contributions to the study and manuscript preparation include the following. Conception and design: Mack, Zada, Wang. Acquisition of data: Gao, Russin, Zeng, He, Giannotta, Zada. Analysis and interpretation of data: Chang, Shi, Gao, Weisenberger, Zada, Wang. Drafting the article: Chang, He, Wang. Critically revising the article: Mack, Russin, Zada, Wang. Reviewed submitted version of manuscript: Mack, Zada, Wang. Approved the final version of the manuscript on behalf of all authors: Mack. Statistical analysis: Chang, Shi, Weisenberger, Wang. Administrative/technical/material support: Chen, Wang. Study supervision: Mack, Chen, Giannotta, Wang.

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Address correspondence to: William J. Mack, M.D., Department of Neurosurgery, Keck School of Medicine, University of Southern California, Los Angeles, 1200 N. State St., Ste. 3300, Los Angeles, CA 90033. email: mackw@usc.edu.

Supplemental online information: Supplementary tables are hosted on the file server in Dr. Kai Wang's lab at the University of Southern California, Los Angeles.

Supplementary Table 1 ("Expression signal value and copy number of differentially expressed genes"): http://bionas.usc.edu/meningioma/Table_S1.xlsx.

Supplementary Table 2 ("Comparison between 3 gene expression data sets"): http://bionas.usc.edu/meningioma/Table_S2.xlsx.

Supplementary Table 3 ("Genes with high correlation between copy number and expression level"): http://bionas.usc.edu/meningioma/Table_S3.xlsx.

Supplementary Table 4 ("Aberrant signaling pathways in meningiomas"): http://bionas.usc.edu/meningioma/Table_S4.xlsx.