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OPEN

Transcriptomic Profiling Revealed Lnc-GOLGA6A-1 as a Novel Prognostic Biomarker of Meningioma Recurrence

BACKGROUND: Meningioma is the most common primary central nervous system neoplasm, accounting for about a third of all brain tumors. Because their growth rates and prognosis cannot be accurately estimated, biomarkers that enable prediction of their biological behavior would be clinically beneficial.

OBJECTIVE: To identify coding and noncoding RNAs crucial in meningioma prognostication and pathogenesis.

METHODS: Total RNA was purified from formalin-fixed and paraffin-embedded tumor samples of 64 patients with meningioma with distinct clinical characteristics (16 recurrent, 30 nonrecurrent with follow-up of >5 years, and 18 with follow-up of <5 years without recurrence). Transcriptomic sequencing was performed using the HiSeq 2500 platform (Illumina), and biological and functional differences between meningiomas of different types were evaluated by analyzing differentially expression of messenger RNA (mRNA) and long noncoding RNA (lncRNA). The prognostic value of 11 differentially expressed RNAs was then validated in an independent cohort of 90 patients using reverse transcription quantitative (real-time) polymerase chain reaction.

RESULTS: In total, 69 mRNAs and 108 lncRNAs exhibited significant differential expression between recurrent and nonrecurrent meningiomas. Differential expression was also observed with respect to sex (12 mRNAs and 59 lncRNAs), World Health Organization grade (58 mRNAs and 98 lncRNAs), and tumor histogenesis (79 mRNAs and 76 lncRNAs). Lnc-GOLGA6A-1, ISLR2, and AMH showed high prognostic power for predicting meningioma recurrence, while lnc-GOLGA6A-1 was the most significant factor for recurrence risk estimation (1/hazard ratio = 1.31; P = .002).

CONCLUSION: Transcriptomic sequencing revealed specific gene expression signatures of various clinical subtypes of meningioma. Expression of the lnc-GOLGA61-1 transcript was found to be the most reliable predictor of meningioma recurrence.

KEY WORDS: Meningioma, Recurrence, mRNA, lncRNA, Prognosis

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Meningiomas are among the most common intracranial tumors and are believed to arise from the highly metabolically active arachnoid cap cells of the leptomeninges. According to Kalamarides et al,[1] meningiomas originate in meningeal precursor cells with high expression of prostaglandin D synthase. Many aspects of these tumors are not fully explained, including their hormone dependence, higher incidence in female patients,[2] and the fact that some meningiomas recur after total resection despite having benign histopathological features.[3] To help explain these observations, we here report transcriptomic differences between various clinical types of meningioma and simultaneous differential analysis of messenger RNA (mRNA) and long noncoding RNA (lncRNA) transcripts from formalin-fixed and

ABBREVIATIONS: FFPE, formalin-fixed and paraffin-embedded; **HR,** hazard ratio; **lncRNA,** long noncoding RNA; **mRNA,** messenger RNA; **NC,** neural crest; **RNA-seq,** RNA/transcriptomic sequencing; **RT-qPCR,** reverse transcription quantitative (real-time) polymerase chain reaction; **TTR,** time to recurrence; **WHO,** World Health Organization.

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paraffin-embedded (FFPE) tissue samples. In addition, we use quantitative reverse transcription polymerase chain reaction (RT-qPCR) to validate selected mRNA and long noncoding RNA (lncRNA) transcripts as potential biomarkers of prognosis in patients with meningioma. There is a growing body of evidence that products of the noncoding genome are important in tumor prognosis and biology.[4] However, little is known about the certain functions of particular lncRNAs.[5] We, therefore, believe that simultaneous analysis of mRNA and lncRNA can provide clearer biological insight and reveal higher numbers of clinically relevant biomarkers, as previously reported.[6,7]

METHODS

Patients

This study was approved by the Institutional Research Ethics Committee. Comprehensive clinical-pathological data were mined for the study's participants, all of whom signed informed consent forms. Recurrence after total or gross total (Simpson grade I, II, or III) and incomplete (Simpson grade >III) resection was defined as reappearance of the meningioma or any growth of remaining meningiomal tissue detected during follow-up imaging after primary surgery. Patients with no such events after at least >5 years of follow-up were considered nonrecurrent. The lesions originating from dura mater at the skull base and around the brainstem and spinal cord were assigned to be of mesoderm origin, while those located on the convexity or at the convexity/skull base borderline were identified as of neural crest (NC) origin. In total, 64 tumor samples were subjected to transcriptomic sequencing (RNA-seq), and 90 samples were used for RT-qPCR validation. Detailed information on the cohorts is presented in Table 1 and Figure 1A.

RNA Purification and Quality Assessment

Total RNA was purified from FFPE tumor samples in the same way as we previously reported.[8] RNA concentration and quality were assessed using a Nanodrop ND 1000 instrument (Thermo Fisher Scientific) and a Bioanalyzer 2100 using RNA Pico Kit and Chips (Agilent) according to the manufacturer's instructions. Only samples with DV_{200} (%) ≥30 were selected for the subsequent RNA-seq analysis.

Transcriptomic Sequencing by Next-Generation Sequencing (RNA-seq)

Prepared cDNA libraries (TruSeq Stranded Total RNA Library Prep Kit with Ribo-Zero Gold—Set A, Illumina) were denatured, pooled, and sequenced on a HiSeq 2500 instrument using the Illumina TruSeq SR Cluster Kit v3—cBot—HS and TruSeq SBS Kit v3—HS (50-cycles) kits (**Supplementary Methods S1**, http://links.lww.com/NEU/D171).

RT-qPCR Validation

Reverse transcription was performed separately before qPCR analysis (**Supplementary Methods S1**, http://links.lww.com/NEU/D171). The qPCR analyses were performed on a LightCycler 480 thermal cycler (Roche) using TaqMan Gene Expression Assays (Thermo Fisher Scientific) according to the manufacturer's instructions in 10 µL volumes.

Data Processing and Statistical Methods

All sequencing data were processed using the bioinformatics pipeline outlined in **Supplementary Methods S1**, http://links.lww.com/NEU/D171. Connections between mRNAs based on protein homology, coexpression, and interactions were visualized using the free web tool String version 11.0.[9] Connections between mRNAs and lncRNAs based on their chromosomal coordinates, reflecting potential lncRNA *cis* regulatory targets,[5] are also shown in the presented networks. Gene expression data from the RT-qPCR validation phase were processed using the ΔCt method and further analyzed using univariate and multivariate Cox regression models of time to recurrence (TTR) implemented in the R software package (www.r-project.org).

RESULTS

Differentially expressed coding and noncoding transcripts with $\log_2$ fold change >2 or < −2 and adjusted *P*-value (q-value) <0.05 were analyzed further. The numbers of differentially expressed transcripts and their overlaps between the studied comparisons (recurrent vs nonrecurrent, NC vs mesoderm, male patients vs female patients, and World Health Organization [WHO] grades II and III vs I) are presented in Figure 1B, and more detailed information on their expression is presented in **Supplementary Results S2**, http://links.lww.com/NEU/D172. Principal component analysis is presented for each selected subgroup in **Supplementary Figure S3**, http://links.lww.com/NEU/D173. Although PANTHER pathway analyses[10,11] yielded no statistically significant results, we considered a pathway to exhibit potential differential activity with respect to a given comparison if at least 2 genes belonging to that pathway were differentially expressed within that comparison (**Supplementary Results S2**, http://links.lww.com/NEU/D172). The differentially expressed RNAs considered to be most prognostic relevant (selected according to q-value and $\log_2$ fold change concerning recurrence and/or WHO grade) were validated in an independent cohort. All transcriptomic data are publicly available at https://www.ncbi.nlm.nih.gov/bioproject/PRJNA705586 (BioProject ID: PRJNA705586).

Recurrence

We identified 69 mRNAs and 108 lncRNAs that were differentially expressed in primary tumors of recurrent and nonrecurrent patients (Figure 2A and **Supplementary Figure S4**, http://links.lww.com/NEU/D174). Most of the corresponding genes lie on chromosomes 1 to 8, but there were also 5 X-chromosomal lncRNAs and 1 X-chromosomal mRNA (XPNPEP2) (Figure 2B). Based on functional annotation clustering, the coding genes were divided into 10 clusters representing various biological functions and roles (Figure 2C). Interestingly, developmental, immunoglobulin-like, and ATP-binding genes were also differentially expressed in tumors of different histogenetic origin. Pathway analysis indicated that only a few of the significantly differentially expressed mRNAs belong to common pathways. These are the angiogenesis, the Wnt signaling and purine metabolism pathways. However, only the angiogenesis and

TABLE 1. Clinical–Pathological Features of All Meningioma Patients Included in the Data Set

	RNA-seq cohort (n = 64)				RT-qPCR validation cohort (n = 90)			
Cohort (n)	Nonrecurrent	Recurrent	*P*-value	Total[a]	Nonrecurrent	Recurrent	*P*-value	Total[a]
Female/male, n (%)	25/5 (83.3/16.7)	12/4 (75/25)	.698	46/18 (71.9/28.1)	20/13 (60.6/39.4)	21/12 (63.6/36.4)	1	57/33 (63.3/36.7)
WHO grade I/II-III, n (%)	23/7 (76.7/23.3)	9/7 (56.2/43.8)	.189	44/20 (68.8/31.2)	26/7 (78.8/21.2)	18/15 (54.5/45.5)	.068	57/33 (63.3/36.7)
Skull base/ convexitary meningioma, n (%)	16/14 (53.3/46.7)	7/9 (43.8/56.2)	.757	32/32 (50/50)	16/17 (48.5/51.5)	10/23 (30.3/69.7)	.208	45/45 (50/50)
Simpson grade I-III/IV-V, n (%)	27/3 (90/10)	8/7 (53.3/46.7)	.009	52/11 (82.5/17.5)	28/3 (90.3/9.7)	23/8 (74.2/25.8)	.184	65/18 (78.3/21.7)
Mesoderm/neural crest, n (%)	5/25 (16.7/83.3)	4/12 (25/75)	.698	13/51 (20.3/79.7)	8/25 (24.2/75.8)	6/26 (18.8/81.2)	.813	18/71 (20.2/79.8)
Age (y), median (IQR)	57.5 (47.25-65)	47.5 (38-64.25)	.235	59.5 (45.75-69.25)	57 (50-62)	54 (47-64)	.653	57 (47.25-64)
Follow-up (mo), median (IQR)	82.9 (67.87-104.79)	47.8 (29.46-74.15)	.003	65.9 (38.03-86.84)	95.4 (77.37-127.13)	40 (19.98-85.55)	<.001	69.4 (33.05-97.97)
Time to recurrence[b], surv ± SE (%)				68 ± 5.6				80 ± 5.7

surv ± SE, 5-year survival ± SE; WHO, World Health Organization.
[a]Including patients with follow-up of <5 years without recurrence.
[b]Kaplan-Meier estimate of survival at the time of 5 years after surgery.
IQR (first quartile—third quartile).

Wnt signaling pathways were associated with transcripts exhibiting differential expression in other comparisons. Six exceptionally strongly differentially expressed (q < 0.001) RNAs were selected for further validation and evaluation of their prognostic value (HEPACAM2, lnc-FAT1-3, lnc-MAST4-5, TDRD1, ISLR2, and lnc-GOLGA6A-1).

Histogenesis

We identified 79 mRNAs and 76 lncRNAs exhibiting differential expression between mesodermal lesions and those arising from the NC, most of which were closely connected (Figure 3 and **Supplementary Figure S5**, http://links.lww.com/NEU/D175). The only significantly upregulated group of RNAs in mesodermal tumors were homeobox-related transcripts; most of the remaining transcripts were downregulated. However, a few non–homeobox-related transcripts (4 mRNAs and 11 lncRNAs) were upregulated in mesodermal tumors. Chromosomes 1, 7, and 17 had the greatest numbers of mapped transcripts exhibiting differential expression with respect to histogenesis; in addition, there were 3 differentially expressed X-chromosomal mRNAs (**Supplementary Figure S6, panel A**, http://links.lww.com/NEU/D176). Functionally, these transcripts were linked to angiogenesis, blood coagulation, neural development, and general development, and 4 were associated with Wnt signaling.

Sex

There were 59 noncoding and 12 coding transcripts that were expressed differentially in male patients and female patients (**Supplementary Figure S7**, http://links.lww.com/NEU/D177, and **Supplementary Figure S8**, http://links.lww.com/NEU/D178). As expected, most of these transcripts were localized to the Y chromosome. However, 2 autosomal coding genes, S100B and NTM, were also identified. Both of them are associated with neural development, especially with neurite outgrowth (**Supplementary Figure S7**, http://links.lww.com/NEU/D177). S100B also exhibited differential expression with respect to WHO grade and tumor recurrence and was therefore selected for further validation. In addition, 7 autosomal and 5 X-chromosomal lncRNAs were differentially expressed between male patients and female patients (**Supplementary Figure S8**, http://links.lww.com/NEU/D178).

WHO Grades

Because of the low number of WHO grade III tumors (3 patients) in our cohort, the WHO grade II (17 patients) and WHO grade III groups were merged and compared with the WHO grade I group (44 patients) because it was performed in recently published genomic[12,13] and proteomic[14] original studies and reviews.[15] In total, 58 mRNAs and 98 lncRNAs exhibited differential expression with respect to tumor grade (Figure 1 and **Supplementary Figure S9**, http://links.lww.com/NEU/D179). Most of them were mapped to chromosomes 2, 5, and 12 (**Supplementary Figure S6, panel B**, http://links.lww.com/NEU/D176). Functional annotation clustering revealed 11 common functional patterns among the differentially expressed mRNAs (Figure 4). Three of the differentially expressed RNAs (AMH, ECEL1, and CCAT2) were selected for further validation. Moreover, CPE was also selected for validation because it was specifically downregulated in grade III tumors (q < 0.001).

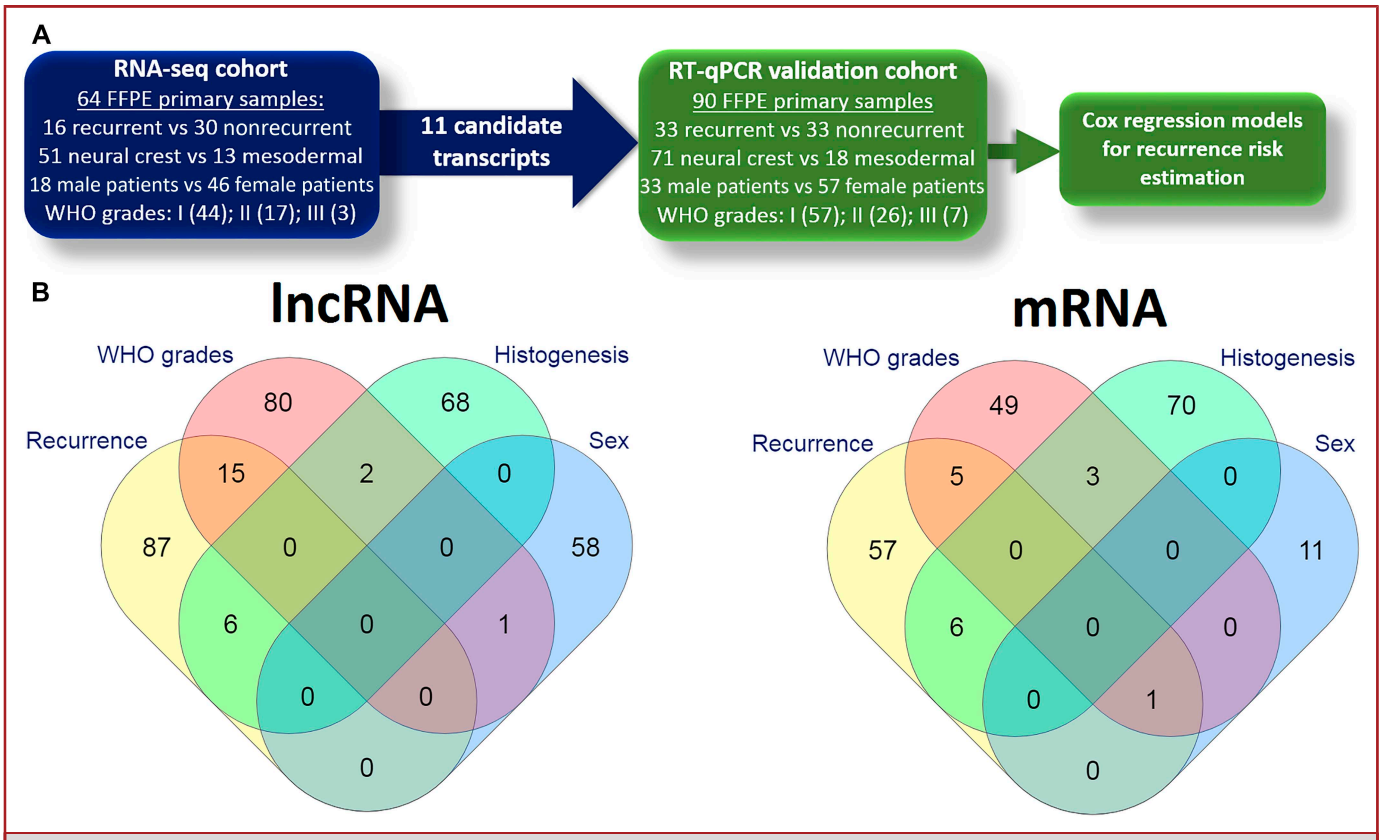


FIGURE 1. *Study summary:* **A**, *Overview of the study's workflow and the cohorts of patients with meningioma.* **B**, *Venn diagrams of differentially expressed genes with log_2 fold changes >2 or < −2 and adjusted P-values (q-values) <0.05 within each patient subgroup. Differential expression is analyzed with respect to recurrence (primary recurrent vs nonrecurrent patients), WHO grade (grades II and III vs grade I), histogenesis (neural crest vs mesodermal tumors), and sex (male vs female patients). FFPE, formalin-fixed and paraffin-embedded; lncRNA, long noncoding ribonucleic acid; mRNA, messenger ribonucleic acid; RNA-seq, RNA/transcriptomic sequencing; WHO, World Health Organization.*

RT-qPCR Validation of Selected Hits

S100B exhibited qualitatively different expression between male patients and female patients (Pearson test, $P = .013$), but there was no significant quantitative difference. Female patients' samples were found to be S100B positive more frequently (78.9%) than samples from male patients (51.5%). Lnc-MAST4-5 exhibited qualitatively reduced expression in groups with unfavorable prognosis (Figure 5A). Three other validated transcripts also exhibited significantly higher expression in male patients than in female patients, namely AMH (Student *t*-test, $P = .004$), ISLR2 (Student *t*-test, $P = .003$), and lnc-GOLGA6A-1 (Wilcoxon exact test, $P = .008$).

The only validated transcript exhibiting significant qualitative (Pearson test, $P = .036$) and quantitative (Student *t*-test, $P = .045$) differences in expression between samples of different WHO grades was lnc-MAST4-5 (Figure 5A and 5B). However, it should be noted that grading of meningiomas is often burdened with high subjective error. This may explain why only lnc-MAST4-5 showed any significant correlation with WHO grades.

Among recurrent patients, ISLR2, lnc-GOLGA6A-1, and AMH were strongly upregulated and their expression patterns were correlated across the entire cohort (the correlation coefficient r varied from 0.72 to 0.85) with high significance ($P < .001$; Figure 5C). ISLR2 and lnc-GOLGA6A-1 were mapped to the same locus and are both controlled by the regulatory sequence GH15J074130. In addition, ISLR2, lnc-GOLGA6A-1, and AMH are regulated by many common transcription factors including KLF4 (Figure 5C). *KLF4* was previously reported to carry activating mutations in meningiomas.[16]

Cox regression models with adjustments for clinical factors revealed ISLR2, lnc-GOLGA6A-1, and AMH all significantly influenced time to recurrence (TTR) survival (**Supplementary Table S10**, http://links.lww.com/NEU/D180). The final multivariate model was created by stepwise selection and featured lnc-GOLGA6A-1 as the sole significant recurrence risk factor, with 1/hazard ratio = 1.31 and $P = .002$. A model in which the clinical factors were fixed was identical to the adjusted univariate model for lnc-GOLGA6A-1 (**Supplementary Table S10**, http://links.lww.com/NEU/D180). The modeling procedure and final models are presented in Figure 5D.

TTR survival was analyzed separately for patients expressing lnc-GOLGA6A-1 at low and high levels. High expression was

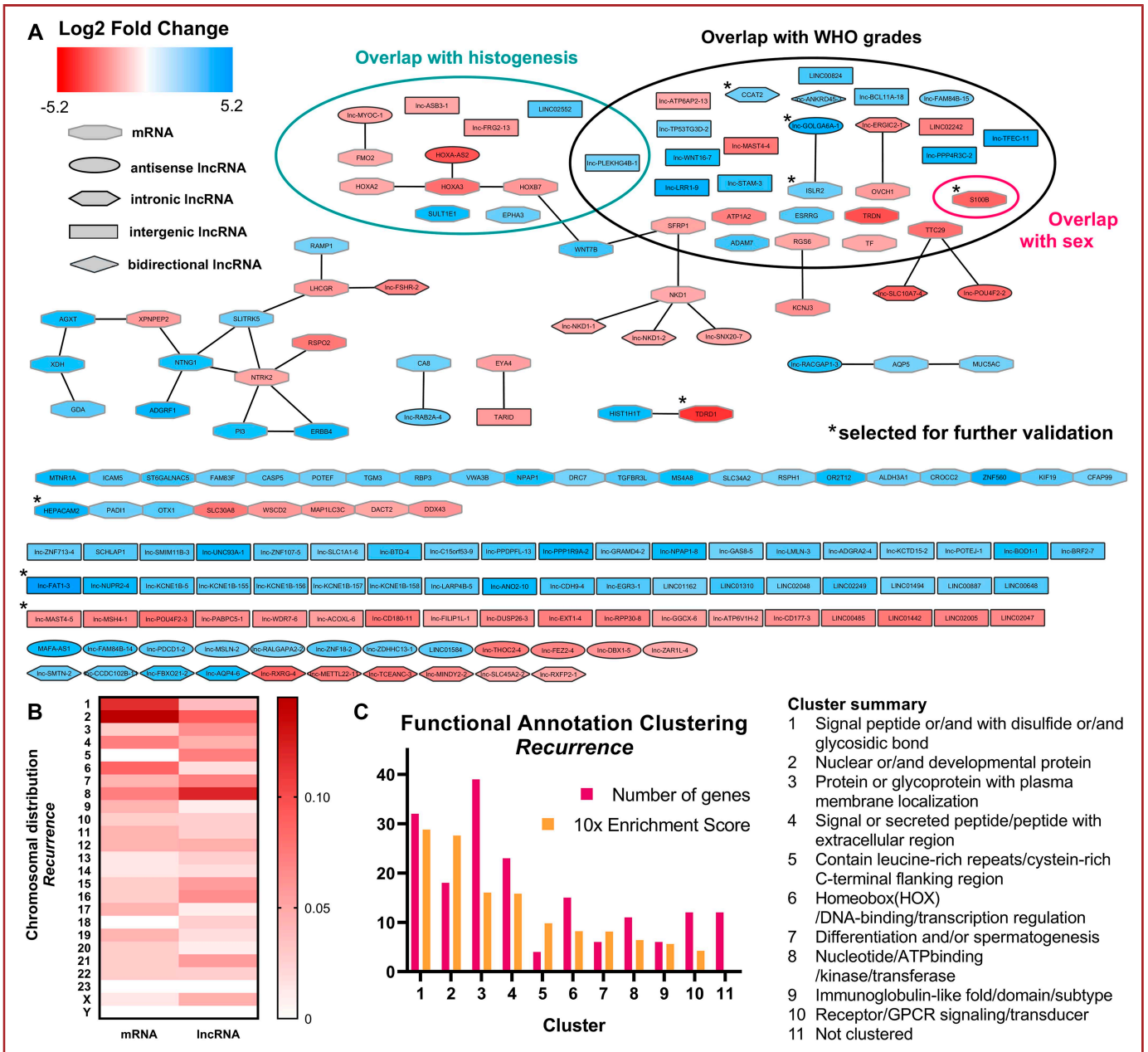


FIGURE 2. *Differentially expressed genes among primary tumors in recurrent and nonrecurrent patients. Transcripts upregulated in recurrent patients are shown in blue.* **A**, *Network showing the fold changes, overlaps, transcript types, and connections of differentially expressed transcripts.* **B**, *Chromosomal distribution of differentially expressed transcripts.* **C**, *Overview of functional annotation clustering of mRNAs in which the biological significance of each cluster is quantified using enrichment scores. GPCR, G-protein-coupled receptor; lncRNA, long noncoding ribonucleic acid; mRNA, messenger ribonucleic acid; WHO, World Health Organization.*

determined based on a ΔCt cut-off value computed using the maximally selected rank statistics method implemented in the survminer R package. Patients with meningioma whose expression of lnc-GOLGA6A-1 was below the cut-off (ΔCt > 2.34) had significantly longer TTR survival (P = .001; Figure 5E).

DISCUSSION

Despite the considerable diagnostic and therapeutic potential of strategies targeting epigenetic factors, only a few studies have examined the relationship between lncRNA expression and meningioma.[16] The importance of lncRNAs and Wnt signaling in

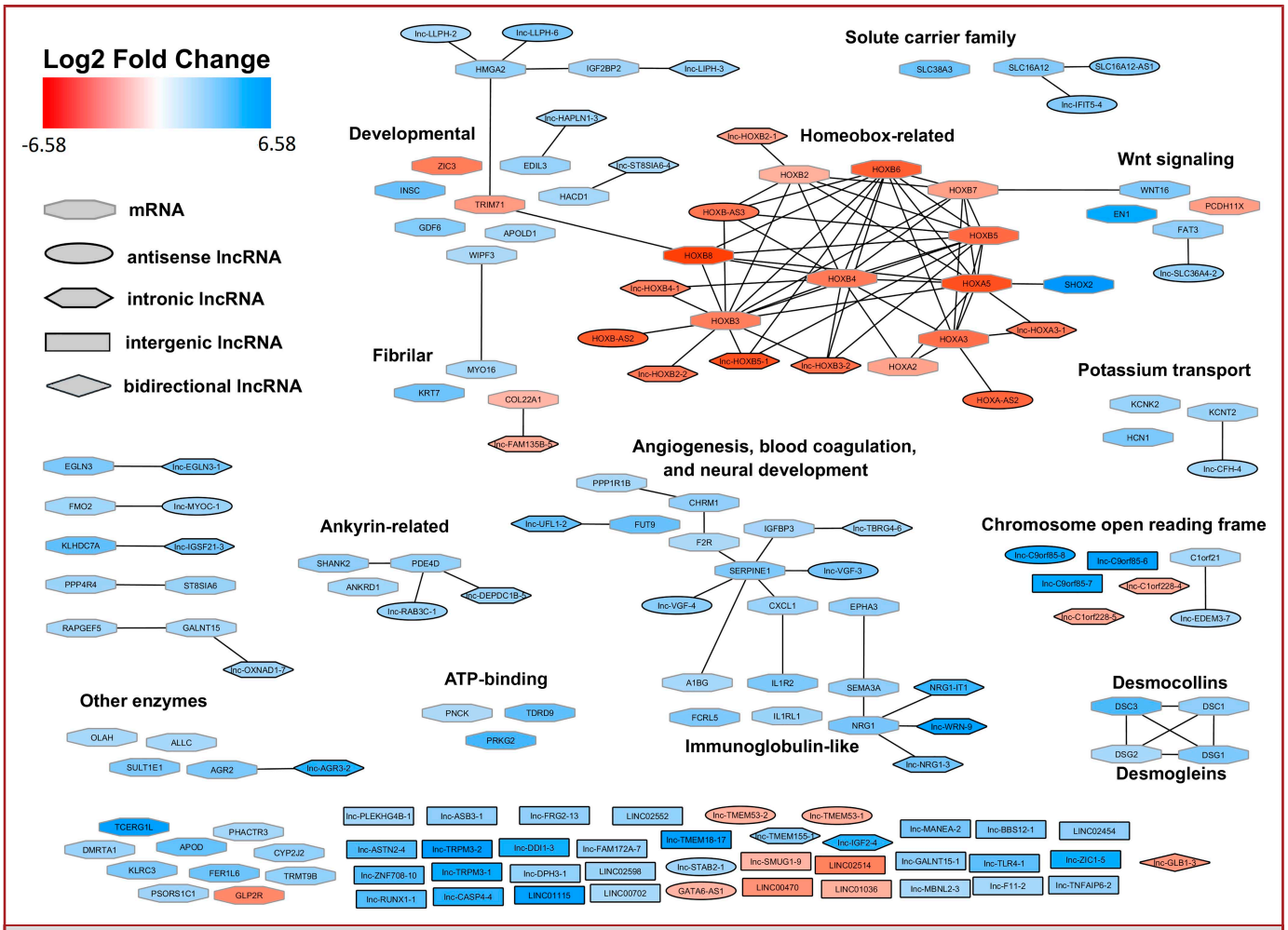


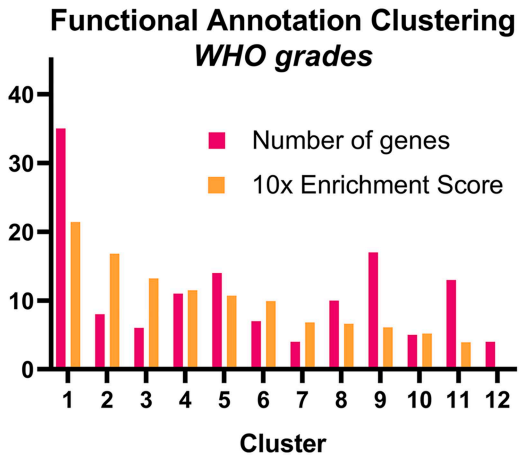
FIGURE 3. *Differentially expressed genes among tumors arising from neural crest and mesodermal cells showing their fold changes, common biological roles, transcript types, and connections. Transcripts upregulated in neural crest tumors are shown in blue. lncRNA, long noncoding ribonucleic acid; mRNA, messenger ribonucleic acid.*

meningiomas was previously highlighted.[17,18] Another study that applied RNA-seq to FFPE canine samples confirmed that angiogenesis and Wnt signaling are crucial in meningioma formation.[19] Our results also indicate that genes associated with Wnt signaling and angiogenesis are expressed differentially in recurrent and nonrecurrent patients.

The 11 transcripts selected according to q-value and $\log_2$ fold change concerning recurrence and/or WHO grade were validated in an independent cohort. Eight of them were successfully analyzed. CCAT2 and S100B did not exhibit significant differences in expression between primary recurrent and nonrecurrent tumors in the RT-qPCR validation cohort. The S100B serum level was previously associated with poor outcomes in patients after meningioma resection.[20] However, its expression is also affected by brain injury and the course of surgery, which may introduce bias when comparing results across different patient cohorts.

ISLR2 and lnc-GOLGA61-1 showed similar expression patterns in both the RNA-seq and RT-qPCR validation cohorts; in both cases, their levels were higher in groups with unfavorable prognosis. This phenomenon was previously observed in neuroblastomas, where *ALK* mutation and *MYCN* amplification were both associated with elevated lnc-GOLGA61-1 levels.[21] In addition, hypermethylation of the promotor region for lnc-GOLGA61-1 was associated with improved survival of patients with IDH1 wild-type glioblastoma.[22] Our results provided clearer biological insight into meningiomas, where lnc-GOLGA61-1 showed the strongest prognostic power in the estimation of their recurrence. Altogether, these findings strongly support the importance of lnc-GOLGA61-1 oncogenic features in brain tumors.

Interestingly, AMH expression also correlated with that of ISLR2 and lnc-GOLGA61-1 in the RT-qPCR validation cohort; one of the potential sources of this correlation is the fact that those 3



Cluster summary

1 Membrane component or/and with disulfide or/and with glycosidic bond
2 Ion (esp. K+/voltage dependent/channel) transport with BTB/POZ domain
3 C-type lectin-like and carbohydrate bonding
4 Signal-anchor/Golgi apparatus (membrane)/transferase
5 Lipoprotein/chemical synaptic transmission/palmitate
6 Activator/Homeobox (HOX) - DNA-binding region
7 Chemical synaptic transmission/cell junction
8 Secreted and extracellular protein
9 Transcription regulation (esp. from RNA pol II promoter)/HOX/activator
10 Oxidation-reduction process in mitochondrion
11 Metal-binding (esp. Zn)/Zinc-finger
12 Not clustered

FIGURE 4. *Differentially expressed genes in tumors of different WHO grades (WHO grade II + III vs WHO grade I). Overview of functional annotation clustering of mRNAs in which the biological significance of each cluster is quantified with an enrichment score. BTB/POZ, Broad-Complex, Tramtrack, and Bric a Brac/ Poxvirus and Zinc finger structural domain; WHO, World Health Organization.*

genes are regulated by many common transcription factors, as we showed in Figure 5C.

To the best of our knowledge, this is the first study to examine differences in expression profiles between meningiomas of different histogenic origin. Regional variability in meningeal histogenesis with various meningeal progenitors suggests playing a role in meningioma development and their variable behavior.[1] The precursor is of mesoderm origin at the skull base and NC-derived at the convexity.[1] During the early prenatal stage, there is a NC-mesodermal interface where the frontal NC-derived and parietal mesoderm-derived bones meet. When the telencephalon begins to expand caudally, it carries this borderline with it.[23] NC-derived meninges thus extend from the convexity to the posterior/caudal edge of cerebral hemispheres, whereas the meningeal layers of the posterior cranial fossa, around the brainstem and spinal cord, arise from the mesoderm.[24] Previous studies have suggested a link between meningioma location and WHO grading, with nonskull base meningiomas being more aggressive.[25-27] Interestingly, the proportion of WHO grade II meningiomas was higher among lesions originating from the NC compared with paraxial mesoderm.[28] In general, tumors arising from the NC are more likely to be aggressive and malignant because NC-derived cells have a higher capacity for migration, proliferation, and differentiation.[29] Consequently, it is unsurprising that developmental and homeobox-related transcripts, which are also involved in ontogenetic development, were differentially expressed among tumors with different proposed histogenesis in our study (Figure 3). This together with the high interconnectedness of the differentially expressed genes supports the theory that the ontogenetic origin of the tissue from which a meningioma arises is biologically and clinically relevant.

The final aspect value to mention is the sex distribution of meningiomas. Nonmalignant meningiomas occur more frequently in women (2.3:1).[30] In addition, WHO grade I meningiomas were significantly more frequent in female patients,[12] whereas WHO grade II and III lesions were observed more frequently in male patients.[31] The greater frequency of higher-grade lesions in men was reflected in sex-specific differences in DNA methylation profiles.[32] In keeping with these results, our data indicated that the prognostically unfavorable markers ISLR2, lnc-GOLGA61-1, and AMH were expressed more strongly in male patients while the prognostically favorable S100B was expressed more strongly in female patients. Regarding this confounding effect in recurrence risk estimation, univariate and multivariate models were adjusted with sex as an input parameter in our validation cohort.

Limitations

This study is burdened by its retrospective nature. Availability and quality of biological material and a paucity of detailed information, such as loss of patients in follow-up or follow-up period less than 5 years in a significant number of cases, have been reasons for the relatively small numbers of patients in our cohorts. Moreover, because various tumor locations or the extent of tumor involvement of surrounding structures were not adjusted for in RNA-seq cohort (**Supplementary Table S11**, http://links.lww.com/NEU/D181), prospective analyses are warranted to validate the role of lncRNAs after adjustment of these factors.

CONCLUSION

Profiling of coding and noncoding RNA in meningiomas among clinically relevant subgroups revealed the long noncoding RNA lnc-GOLGA61-1 to be of strong prognostic relevance. Moreover, distinct transcriptomic signatures of meningiomas in

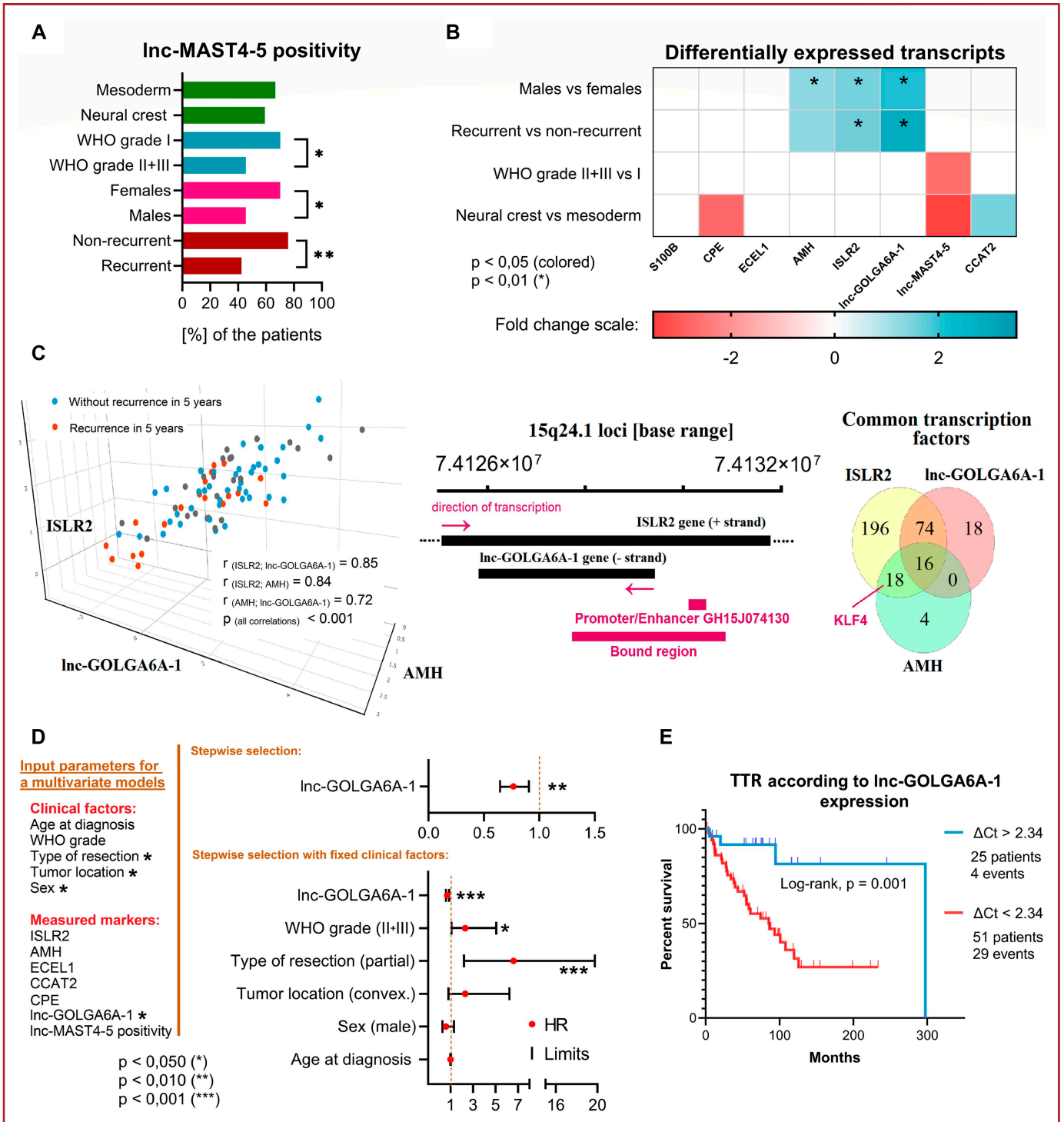


FIGURE 5. *RT-qPCR validation of selected transcripts.* **A**, *Differences in the lnc-MAST4-5 positivity percentage in selected subgroups.* **B**, *Overview of significantly deregulated transcripts.* **C**, *Transcriptional correlation and regulation of the expression of ISLR2, AMH, and lnc-GOLGA6A-1.* **D**, *Stepwise selection using the Bayesian information criterion leading to the final multivariate Cox regression models for estimation of recurrence risk.* **E**, *TTR survival analysis for patients expressing lnc-GOLGA6A-1 at low and high levels. Recurrence is considered as an event. HR, hazard ratio; RT-qPCR, reverse transcription quantitative (real-time) polymerase chain reaction; TTR, time to recurrence.*

male and female patients and signatures associated with different histogenetic origins have been revealed for the first time.

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Disclosures

Dr Hajduch is funder and shareholder of spin-off company IntellMed, Ltd, which is developing and manufacturing molecular cancer diagnostics. However, no company products were used in this study. The other authors have no personal, financial, or institutional interest in any of the drugs, materials, or devices described in this article.

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Supplementary Methods S1. Detailed description of procedures used to prepare cDNA libraries for RNA-seq, sequencing setup with basic technical outputs, procedures for preparing cDNA for RT-qPCR, and processing of all obtained data.
Supplementary Results S2. List of significantly differentially expressed genes from specific analyses including the raw outputs from PANTHER pathway analyses.
Supplementary Figure S3. Principal component analysis for each selected subgroup.
Supplementary Figure S4. Unsupervised hierarchical clustering of differentially expressed mRNAs and lncRNAs (at q-value <0.01 and 0.05) in recurrent and nonrecurrent patients based on log-transformed (log_2) RNA expression data.
Supplementary Figure S5. Unsupervised hierarchical clustering of differentially expressed mRNAs and lncRNAs (at q-value <0.01 and 0.05) in tumors arising from neural crest and mesodermal cells based on log-transformed (log_2) RNA expression data.
Supplementary Figure S6. Chromosomal distribution of differentially expressed transcripts. **A**, Chromosomal origin of differentially expressed transcripts according to the histogenetic origin of the tumors (neural crest vs mesoderm). **B**, Chromosomal origin of differentially expressed transcripts according to tumor grade (WHO grade II + III vs WHO grade I).
Supplementary Figure S7. Unsupervised hierarchical clustering of 12 differentially expressed mRNAs in male and female patients based on log-transformed (log_2) RNA expression data including chromosomal locations and function.

Supplementary Figure S8. Unsupervised hierarchical clustering of 59 differentially expressed lncRNAs in male and female patients based on log-transformed (log_2) RNA expression data including chromosomal locations.

Supplementary Figure S9. Unsupervised hierarchical clustering of differentially expressed mRNAs and lncRNAs (at q-value <0.01 and 0.05) in high-grade (WHO grades II and III) and low-grade (WHO grade I) tumors based on log-transformed (log_2) RNA expression data.

Supplementary Table S10. Univariate Cox regression models of time to recurrence (TTR) data with adjustment for clinical factors for all transcripts examined in the validation cohort. Factors shown in bold were statistically significant ($P < .05$). Hazard ratios (HR) are computed based on ΔCt unit change.

Supplementary Table S11. Relationships between individual clinical factors, expressed as *P*-values.