

Expression and prognostic value of microRNAs in lower-grade glioma depends on IDH1/2 status

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Abstract Histological and genomic characteristics are widely used in glioma management and research. This study investigated their relationship to the expression and prognostic value of microRNAs (miRNAs) in lower-grade glioma (LGG). A total of 447 LGG samples with available clinical and genomic information from The Cancer Genome Atlas database were reviewed. Samples with isocitrate dehydrogenase (IDH) 1/2 mutations (n=366) were randomly divided into training and validation sets to establish and confirm a four-miRNA-based risk classifier. We found that IDH1/2 mutation status had greater impact than histological and other genomic features on miRNA expression patterns; 361/487 (74%) of miRNAs were differentially expressed according to IDH1/2 mutation status. Importantly, there were no miRNAs with the same prognostic significance among groups with different IDH1/2 mutation status. For IDH1/2-mut LGG, a four-miRNA risk classifier (miR-10b, miR-130b, miR-1304, and miR-302b) was established that could independently distinguish cases as high or low risk of poor prognosis in both training and validation sets. The risk classifier outperformed individual

miRNAs and traditional prognostic factors in terms of sensitivity and specificity. Bioinformatic analyses indicated that high-risk samples were more mitotically active than low-risk samples. Taken together, IDH1/2 mutation status had a significant influence on miRNA expression and prognostication in LGG. The four-miRNA-based risk classifier can be used for risk stratification of IDH1/2-mut LGG.

Keywords Lower-grade glioma · Genomic aberration · IDH1/2 mutation · MicroRNA profile · Prognostic classifier

Introduction

Glioma is the most common type of tumor in the central nervous system. The World Health Organization classifies glioma as grade II–IV according to malignancy [1]. Lower-grade glioma (LGG), corresponding to grades II and III, is infiltrative and tends to recur or progress to grade IV glioblastoma. In addition to the histological classification (astrocytoma, oligodendroglioma, and oligoastrocytoma), advances in genomics are increasingly being used in LGG stratification [2–4]. Mutations in isocitrate dehydrogenase (IDH)1 or 2 (IDH1/2) are the most frequent genomic aberration in LGG, and are associated with a distinct glioma cell metabolic profile, hypermethylated phenotype, and better prognosis [5]. Additional genomic aberrations—including 1p/19q co-deletion and mutations in the telomerase reverse transcriptase (TERT) promoter as well as in tumor protein (TP)53 and alpha thalassemia/mental retardation syndrome X-linked (ATRX)—also play critical roles in gliomagenesis and serve as prognostic indicators [6–9]. The latest edition of glioma classification integrates IDH1/2 mutational and 1p/19q co-deletion statuses into

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current glioma classification further highlighting the role of genomic aberrations in glioma development and management [10].

MicroRNAs (miRNAs) are a class of non-coding RNAs that post-transcriptionally regulate a broad range of biological processes. Many miRNAs are dysregulated in glioma and have been linked to its development and progression [11, 12]. Recently, the detection of stable miRNA expression in cerebrospinal fluid, blood serum, and other body fluids has suggested the possibility of using miRNAs as a non-invasive biomarker for clinical applications [13, 14]. Even multiple genomic events have been well established as important drivers enabling glioma development, lim-

Establishment of a miRNA-based risk classifier

miRNA-seq data were normalized by transformation using a scaled model. To develop a miRNA-based risk classifier, we firstly analyzed the association between OS and expression levels of individual miRNAs by univariate Cox regression analysis in the training set. miRNAs that were significant in predicting survival ($P < 0.01$, $n = 7$) were used to develop the miRNA-based risk classifier, which was formulated according to a linear combination of miRNA expression levels that was weighted with regression coefficients (β) from the univariate Cox regression analysis [15]. The risk value for each patient was calculated as follows:

$$\text{Risk value} = \text{expression}_{\text{miRNA}(1)} \times \beta_{\text{miRNA}(1)} + \text{expression}_{\text{miRNA}(2)} \times \beta_{\text{miRNA}(2)} + \text{expression}_{\text{miRNA}(3)} \times \beta_{\text{miRNA}(3)} + \dots + \text{expression}_{\text{miRNA}(n)} \times \beta_{\text{miRNA}(n)}$$

ited studies have incorporated these genomic features into miRNA analysis. Accordingly, it is urgent to explore the role of genomic events in influencing miRNA expression and prognostic profile. Here, we analyzed 447 LGG cases with available clinical and molecular data from the cancer genome atlas (TCGA) database. Expression and prognostic value of miRNAs were profiled with respect to histological and genomic features. IDH1/2 mutation status exerted an overwhelming influence on miRNA expression and prognostic significance. A four miRNA-based risk classifier was established with superior prognostic value for IDH1/2-mut LGG.

Materials and methods

Patient samples

Records of 447 patients with LGG—including clinical information and genomic data—were obtained from TCGA database (<http://cancergenome.nih.gov>). Study protocol was approved by the ethics committees of participating institutions. Patients' miRNA and mRNA expression profiles were tested by miRNA-seq and mRNA-seq, respectively. Overall survival (OS)—i.e., the time interval from the date of diagnosis until death or the last follow-up—was used as the clinical end point. Patients with mutations in IDH1 or IDH2 were classified as the mutant (IDH1/2-mut) group ($n = 366$; Table S1). Patients with wild-type IDH1 and IDH2 were classified as the wild-type (IDH1/2-wt) group ($n = 81$; Table S1). The IDH1/2-mut group was randomly divided into training and validation sets ($n = 183$ each; Table S2).

A total of six miRNA-based classifiers were formed based on the top two to seven miRNAs. A univariate Cox regression analysis was then carried out to compare the prognostic values of the six classifiers, yielding a four miRNA-based classifier with prognostic significance in the training set.

Bioinformatics analysis

A total of 364 patients in the IDH1/2-mut group with paired miRNA and mRNA expression data were analyzed to determine the basis for differences in miRNA-associated risk among IDH1/2-mut LGG patients. Significance analysis of microarrays (SAM) was used to identify genes that were differentially expressed between patients with risk values in the top and bottom quarters.

Gene ontology (GO) analysis was performed using the database for annotation, visualization, and integrated discovery (<http://david.abcc.ncifcrf.gov/home.jsp>) functional annotation tool [16]. Gene network data were analyzed online with STRING 10.0 (<http://www.string-db.org/>) [17]. Biological functions of interest were validated by gene set enrichment analysis (<http://www.broadinstitute.org/gsea/index.jsp>) [18].

Statistical analysis

SPSS (SPSS Inc., Chicago, IL, USA), GraphPad Prism 6 (GraphPad Inc., La Jolla, CA, USA), and R 3.1.2 software (<https://www.r-project.org/>) were used for statistical analyses. Differences in clinical and genomic characteristics between groups were evaluated using the Student's t or χ^2 test. The Student's t test and Bonferroni correction were used to assess differences in miRNA expression profiles.

Principal components analysis was conducted to access expression patterns of grouped patients. A Kaplan–Meier survival analysis was used to estimate survival distribution, and the log-rank test was used to assess differences between stratified groups. Uni- and multivariate Cox regression analyses were used to identify independent prognostic factors.

Results

Clinical and genomic characteristics

We analyzed the records of 447 LGG patients (grade II, $n=217$; grade III, $n=230$), including cases of astrocytoma ($n=195$), oligodendroglioma ($n=138$), and oligoastrocytoma ($n=114$). Seizure history data were available for 430 cases; of these, 272 suffered a seizure and 158 did not. Genomic aberrations, including mutations in IDH1 (348/447, 78%), IDH2 (18/447, 4%), the TERT promoter (115/255, 45%), ATRX (171/447, 38%), TP53 (218/447, 49%), epidermal growth factor receptor (EGFR) (34/447, 8%), and phosphatase and tensin homolog (PTEN) (22/447, 5%) and 1p/19q co-deletion (75/251, 30%) were detected. Histological grade and IDH1/2 mutation status were significantly associated with patient outcome (Fig. 1a, b). The prognostic value of histological classification, seizure history, and genomic aberrations was investigated (Fig. S1); these features were compared according to IDH1/2 mutation status (Table S1).

Relationship between IDH1/2 mutation status and miRNA expression profile

Differences in miRNA expression between groups were evaluated. Differential miRNA expression was defined as a false discovery rate (FDR) <0.05 . miRNA expression profiles were broadly affected by genomic aberrations (Fig. S2), of which IDH1/2 mutation status was the most significant (Fig. 1c). The overall miRNA expression patterns associated with IDH1/2 mutation were profiled in Fig. S3A. Of 487 miRNAs, 361 (74%) were differentially expressed as a result of IDH1/2 mutation, including 10 and 351 that were upregulated in IDH1/2-mut and IDH1/2-wt groups, respectively. These differentially expressed miRNAs showed significant overlap with those associated with other genomic aberrations (Additional File 1). When we stratified cases based on the histological grading system, the effect of IDH1/2 mutation status on miRNA expression remained significant, with 272 (55%; four upregulated in IDH1/2-mut vs. 268 upregulated in IDH1/2-wt) and 310 (64%; 12 upregulated in IDH1/2-mut vs. 298 upregulated in IDH1/2-wt) miRNAs identified as differentially expressed in grades II and III, respectively (Fig. 1d, e).

We investigated miRNA expression patterns associated with glioma grade. The overall miRNA expression patterns associated with glioma grade were profiled in Fig. S3B. A total of 74 miRNAs (15%) were identified as differentially expressed according to histological criteria, including 14 and 60 that were upregulated in grades II and III, respectively (Fig. 1f). Most of these (63/74, 85%) overlapped with miRNAs that were influenced by IDH1/2 mutation status. This dataset was further shrunk when we grouped patients based on IDH1/2 mutation status: eight (2%, three upregulated in grade II vs. five upregulated in grade III) were differentially expressed in IDH1/2-mut patients as compared to none in IDH1/2-wt patients (Fig. 1g, h). Principal component analysis was further performed based on the overall miRNA expression data. We found that IDH1/2 mutation status clearly divided patients into two sections (Fig. 1l) as compared to glioma grade (Fig. 1m). These results indicate that IDH1/2 mutation status extensively affects miRNA profile in LGG.

Prognostic value of miRNAs with respect to IDH1/2 mutation status

A univariate Cox regression analysis was carried out to assess the prognostic value of specific miRNAs. A P value of 0.01 was used as the threshold for evaluating statistical significance. We found that 96 of the miRNAs (%) had prognostic value (Fig. 1l) in all LGG patients. Of these, 94 (98%) were defined as risk-associated with a hazard ratio (HR) >1 for death whereas only two (2%) were defined as protective with an HR <1 . An additional 60 (12%) and 3 (1%) miRNAs with prognostic significance were identified in the IDH1/2-mut and IDH1/2-wt groups, respectively (Fig. 1j, k), emphasizing a prognostic role of miRNAs in IDH1/2-mut group than in IDH1/2-wt group. It is worth noting that none of these miRNAs overlapped and were all risk-associated for the corresponding group.

Development of a miRNA risk classifier for cases of IDH1/2 mutation

Patients were randomly assigned to training and validation sets that did not differ in terms of clinical and molecular characteristics (Table S2). The prognostic value of miRNAs in the training set was evaluated by univariate Cox regression analysis; seven miRNAs were found to have prognostic significance ($P < 0.01$; Table S3) and were used to develop six risk classifiers. A univariate Cox regression analysis indicated that the four miRNA-based risk classifier had the greatest prognostic value and highest HR for death in the training set (Table S3). We therefore selected this classifier—which included miR-10b, miR-130b, miR-1304, and miR-302b—for further analysis. The expression

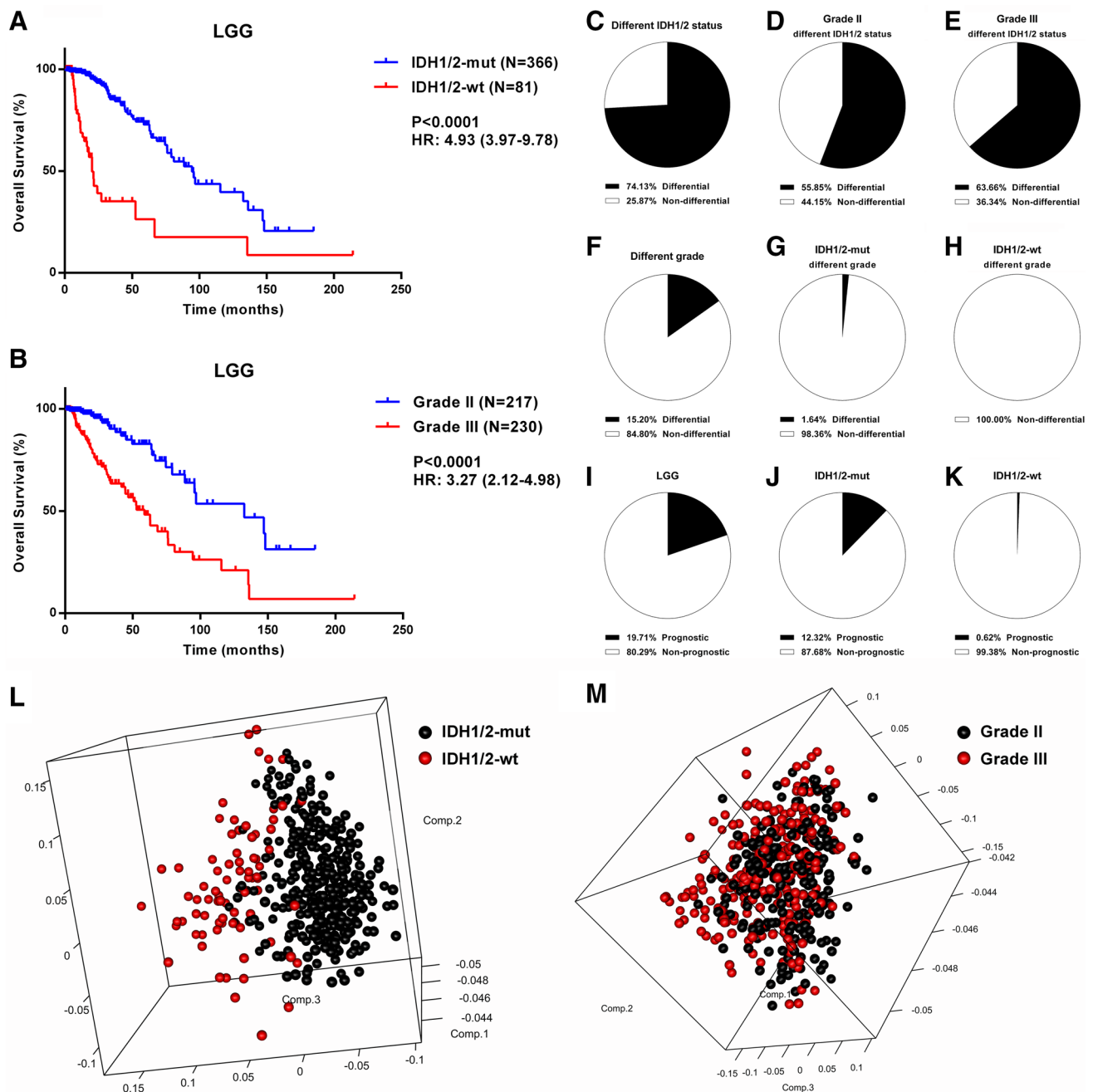


Fig. 1 IDH1/2 status has an extensive impact on miRNA expression and prognostication. **a, b** Prognostic value of IDH1/2 mutation and histological grade, as assessed by Kaplan–Meier curves and the log-rank test. **c–e** miRNAs differentially expressed according to IDH1/2 mutation status. **f–h** miRNAs differentially expressed according to

histological grade. **i–k** Proportion of miRNAs with prognostic significance in LGG (**i**), IDH1/2-mut group (**j**), and IDH1/2-wt group (**k**). **l, m** Principal component analysis based on overall miRNA expression data among patients grouped by IDH1/2 mutation status (**l**) and glioma grade (**m**)

levels of these miRNAs in association with glioma grade and IDH1/2 mutation are shown in Fig. S4.

Patients in the training set were divided into high- (n=92) and low-risk (n=91) groups using the median risk value as a cutoff (−0.5524). A Kaplan–Meier analysis

revealed that OS was reduced to a greater degree in high-risk as compared to low-risk patients (Fig. 2a). The four miRNA-based classifier was also applied to patients in the validation set using the same cutoff value to classify them into high- (n=98) and low-risk (n=85) groups. High-risk

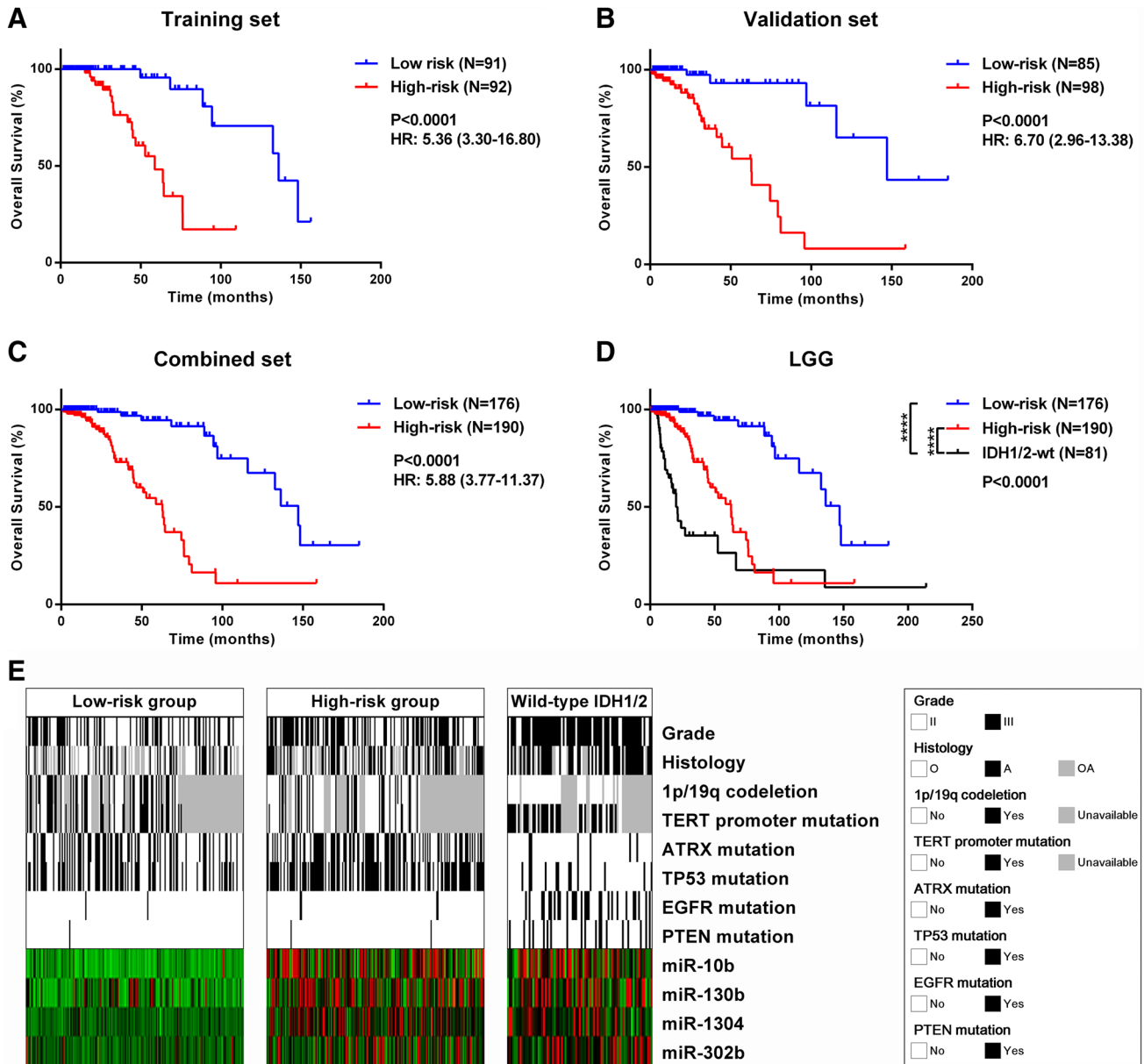


Fig. 2 A four miRNA-based risk classifier for IDH1/2-mut LGGs. **a–c** Comparison of OS of different risk groups in training (**a**), validation (**b**) and combined (**c**) sets. **d** Comparison of OS among low-

risk, high-risk, and IDH1/2-wt groups. **e** Clinical characteristics and miRNA expression patterns of low-risk, high-risk, and IDH1/2-wt groups. ***P<0.0001

patients had shorter survival time than those in the low-risk group (Fig. 2b). When we combined the training and validation sets, the four-miRNA-based classifier retained prognostic significance, with high risk reflecting poor outcome (Fig. 2c). High-risk patients in the IDH1/2-mut group had longer OS than IDH1/2-wt patients (Fig. 2d); moreover, the four miRNA-based classifier had no prognostic value in IDH1/2-wt group (Fig. S5). These results indicate that the four miRNA-based classifier is prognostic for LGG with IDH1/2 mutation.

Correlation of LGG patient characteristics with the risk classifier

Considering the distinct outcomes of high- and low-risk groups, we compared their clinical and molecular features (Table S4). A higher risk was associated with grade III glioma, astrocytoma, and patients receiving radiation. Genomic aberration profiles also differed between high- and low-risk groups. The latter included more cases with 1p/19q co-deletion and TERT promoter mutation. In contrast, the high-risk group had more cases with ATRX

and TP53 mutations. Moreover, expression profiles of the four classifier miRNAs differed between risk groups, with higher levels detected in the high-risk group (all $P < 0.0001$; Fig. 2e). Similar expression patterns were observed in the high-risk and IDH1/2-wt groups (all $P > 0.05$; Fig. 2e and S6).

Independent and accurate prediction of prognosis with the four miRNA-based classifier

Given the higher risk value associated with other prognostic indicators, we investigated whether the four miRNA-based risk classifier could serve as an independent predictor of prognosis in IDH1/2-mut LGG. Several clinical factors were examined by uni- and multivariate Cox regression analysis. The four miRNA-based classifier was an independent risk factor in the training, validation, and combined sets (Table 1). Interestingly, none of the genomic aberrations reached prognostic significance when tested by univariate Cox regression analysis in the combined set (Table S5).

OS > 3 years or ≤ 3 years were designated as long or short survival, respectively. Receiver operating characteristic (ROC) curves were used to assess the prognostic validity of our miRNA classifier and other variables. The area under the ROC curve was greater for the four miRNA-based classifier than for the constituent miRNAs or other clinical factors (Table S6), indicating its higher predictive sensitivity and specificity.

Stratification analysis of the four miRNA-based risk classifier

Survival analysis was carried out according to the four miRNA-based classifier in patients stratified by clinical and genomic variables. When stratified by clinical factors (age, grade, histology classification, seizure history, and radiation treatment), the classifier still distinguished between cases with good and poor prognosis (Fig. 3a–h, S7). Furthermore, IDH1/2-mut LGG patients with similar genomic aberrations were sorted into subgroups of good and poor prognosis with the classifier (Fig. 3i–p). These results indicate that the four miRNA-based classifier can enhance the prognostic value of clinical and genomic factors.

Correlation between the miRNA classifier and gene expression and biological process profiles

To identify the biological mechanisms that distinguish LGG patients with good versus poor prognosis, we examined paired miRNA and mRNA expression data from 364 cases. Gene expression profiles of patients in the top and bottom quarters of risk value were compared by SAM

analysis. We identified 479 genes with different expression patterns, including 449 that were upregulated in high-risk patients and 30 that were downregulated in low-risk patients (> 2 -fold change, permutation number of 1000, $FDR < 0.01$) (Fig. 4a and Online Resource 2). It is worth noting that numerous homeobox (HOX) genes were among the upregulated gene list, including even-skipped HOX 2, HOXA10, HOXA11, HOXA13, HOXA5, HOXA6, HOXA7, HOXA9, HOXC4, HOXC5, HOXD10, HOXD3, HOXD4, HOXD8, and HOXD9.

To investigate the biological implications for the high-risk group, a GO analysis was carried out. We found that upregulated genes were significantly associated with mitosis and the cell cycle (Fig. 4b). The latter was identified as the pathway most highly represented among upregulated genes (Kyoto Encyclopedia of Genes and Genomes: hsa04110; $P = 1.24E-09$). Moreover, upregulated genes associated with the top ten biological processes formed a close network according to search tool for the retrieval of interacting genes/proteins results (Fig. 4c). Gene set enrichment analysis confirmed that genes related to mitosis and the cell cycle were significantly enriched in the high-risk cohort (Fig. 4d, e).

Discussion

In addition to time honored histological classification, glioma subtypes can be distinguished by genomic status [2]. miRNAs are known key players in downstream oncogenic pathways. However, little is known about their relationship to histological grade and genomic status. In the present study, we found that IDH1/2 mutation status, rather than other genomic aberrations, was closely associated with overall miRNA expression profile. We speculate that IDH1/2 has a broad range of functions in miRNA regulation; indeed, previous studies reported IDH1/2 mutation as an early event triggering other oncogenic aberrations that cooperatively induce gliomagenesis [19]. Considering the great overlap between IDH1/2 mutation related and other genomic aberrations related differential miRNAs, IDH1/2 status could be a leading aberration for the distinct miRNA profile. The fact that tumors are initiated by the clonal expansion of cells with distinct IDH1/2 mutation status [20] along with the tissue specificity of miRNAs [21] raises our hypothesis that distinct miRNA profile is resulted from diverse cell origins of glioma which is related to IDH1/2 status.

On the other hand, we found that histological malignancy progression had limited impact on miRNA expression patterns. Recent studies have reported miRNA profiles associated with glioma progression [11, 22, 23]; we found

Table 1 Univariate and multivariate cox regression analysis in training, validation and combined sets

Variable	Training set			Validation set			Combined set				
	Univariate		Multivariate	Univariate		Multivariate	Univariate		Multivariate		
	HR	P value	HR	P value	HR	P value	HR	P value	P value		
Age											
Increasing years	1.0577	0.0011	1.0398	0.1232	1.0436	0.0159	1.0292	1.0484	0.0001	1.0298	0.0534
Gender											
Male vs. female	1.6928	0.1846			1.0297	0.9388		1.2738	0.3771		
KPS											
Increasing scores	0.9213	0.0001	0.9293	0.0029	0.9621	0.0428	0.9686	0.9511	0.0003	0.9646	0.0123
Grade											
II vs. III	2.5701	0.0261	1.3797	0.5862	3.1920	0.0038	2.6806	3.0147	0.0002	2.6566	0.0111
Histology											
Astrocytoma	Ref				Ref			Ref			
Oligoastrocytoma	0.5685	0.3246			0.3849	0.0968		0.4743	0.0608		
Oligodendroglioma	0.7715	0.5695			0.4651	0.0667		0.5777	0.0726		
Seizure											
Yes vs. no	1.7163	0.1897			1.0219	0.9567		1.3119	0.3349		
Radiation											
Yes vs. no	0.6620	0.5897			0.7912	0.6536		0.8193	0.6349		
Four-miRNA signature											
Higher risk	2.4701	3.60E-08	2.0812	2.82E-04	2.0352	6.81E-08	1.7637	2.0752	7.25E-14	1.7594	2.01E-06

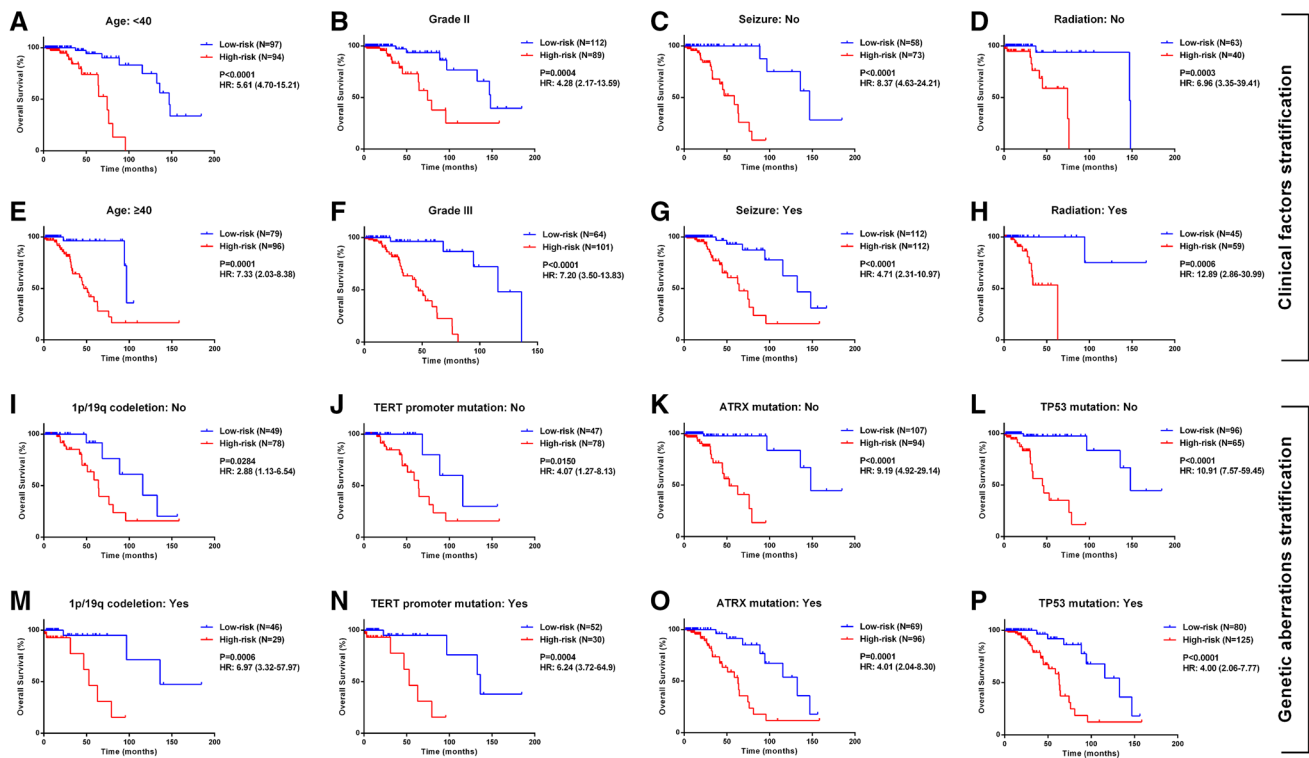


Fig. 3 The four miRNA-based classifier remains powerful in stratified cohorts. The four miRNA-based classifier for distinguishing LGG samples with different prognosis in cohorts stratified by **a–h** clinical and **i–p** genomic characteristics

a similar proportion of malignancy-related miRNAs [11]. While, they did not take genomic aberrations into consideration. Here, we found that most of the differentially expressed miRNAs were no longer significant after stratification according to IDH1/2 mutation status, implying that genomic status but not histological malignancy had a greater influence on miRNA profile.

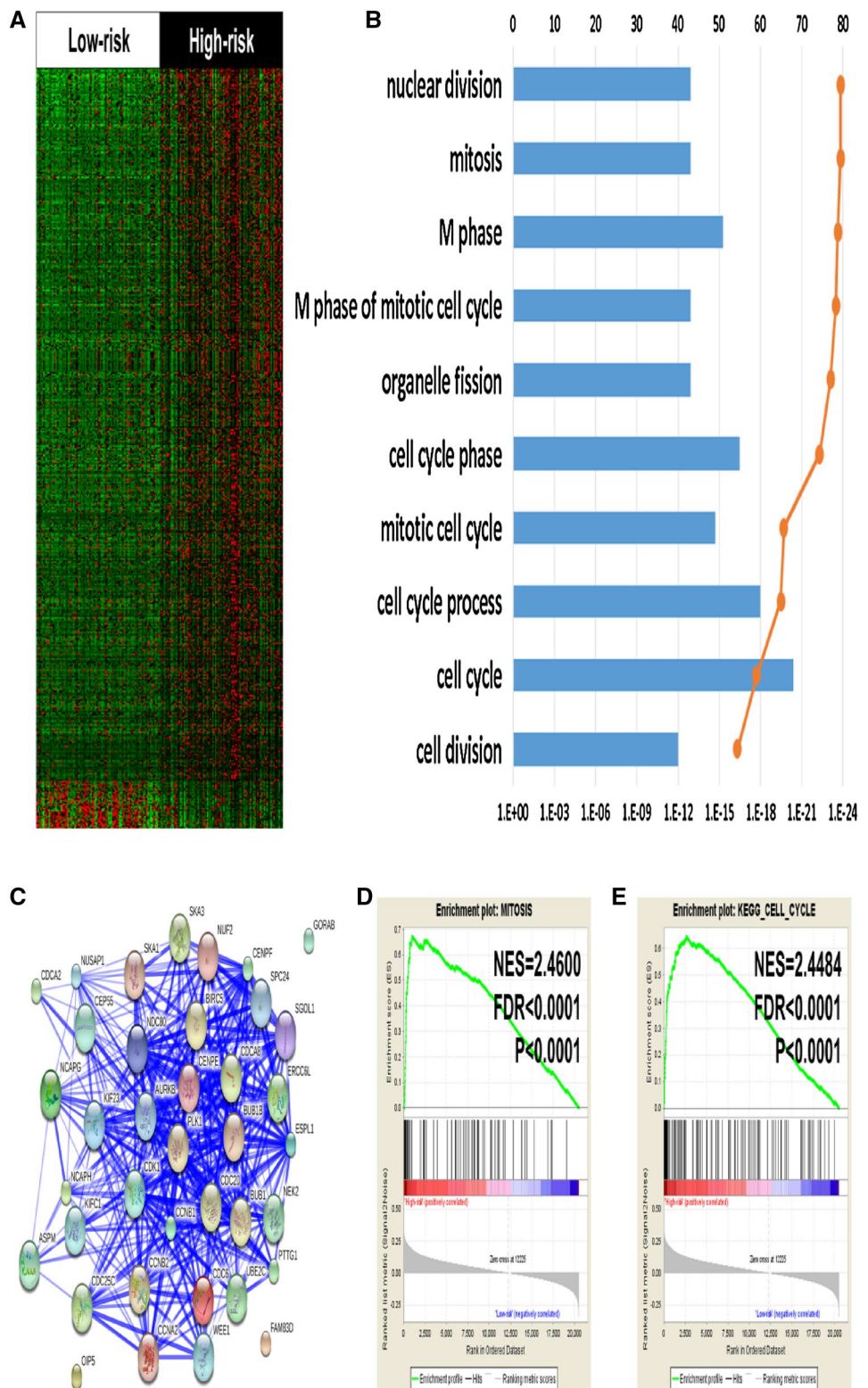
Genomic aberrations, such as IDH1/2 mutation, 1p/19q codeletion and TERT promoter mutation, have been well accepted to determine glioma prognosis in various mechanisms, which contained their miRNA regulating role as an alternative way. IDH1/2 status is the most important genomic events behaving extensive and profound biological influence in LGG. Great efforts have been made for elucidating gliomagenesis on the basis of IDH1/2 status. TERT promoter mutation has been reported to have inconsistent prognostic value with respect to IDH1/2 status [7]. We observed similar situation for miRNAs. Considering the overwhelming miRNA regulatory function of IDH1/2 status, it is reasonable to hypothesize that IDH1/2 status serve as a prominent role in regulating miRNAs' expression and prognostication. Accordingly, we suggest giving sufficient consideration of IDH1/2 status on miRNA study.

The human genome is predicted to have nearly 1000 miRNAs, implying it should be workable to identify reliable miRNA biomarkers. It also suggests that risk

stratification would be more robust based on multiple miRNA-based classifiers as compared to individual miRNAs [24]. In this study, miRNA expression pattern played a greater prognostic role in the IDH1/2-mut than in the IDH1/2-wt group, encouraging us to establish a multiple miRNA-based classifier for IDH1/2-mut patients. Therefore, we developed a four-miRNA-based classifier (miR-10b, miR-130b, miR-1304, and miR-302b) that outperformed the individual constituent miRNAs and traditional clinical factors in terms of prediction sensitivity and specificity. The risk grouping retained its power regardless of risk stratification, underscoring its prognostic importance and stability. Furthermore, the classifier had specificity for IDH1/2-mut patients, which was consistent with the results obtained with individual miRNAs. Importantly, high-risk cases were less likely to have protective aberrations (1p/19q codeletion and TERT promoter mutation) and more likely to have those that were risky (ATRX and TP53 mutations) than low-risk cases. However, high-risk cases showed similar miRNA expression profiles as the IDH1/2-wt group. This may explain why high-risk cases had a prognosis between low-risk and IDH1/2-wt cases even with IDH1/2 mutation.

Existing studies have partially explored the role of the constituting miRNA in glioma and other types of cancer. miR-10b is upregulated in high-grade glioma and is

Fig. 4 Active mitosis is highly associated with the four miRNA-based classifier. **a** Heatmap of genes differentially expressed according to the four-miRNA classifier. **b** Top enriched biological processes of genes upregulated in high-risk LGG. **c** Network of upregulated genes involved in biological processes shown in (b). **d** High-risk samples were enriched in genes involved in mitosis and (e) Cell cycle, as determined by gene set enrichment analysis



associated with unfavorable prognosis [23, 25]; its overexpression promotes proliferation and invasion in glioma and glioma stem cells [26–28]. miR-130b expression is correlated with malignancy progression in glioma [29]. Existing

researches have focused on its role in promoting glioma migration and invasion [30]. The stem cell-like phenotype of glioma cells is also enhanced by its inactivation of the Hippo signaling pathway [31]. In other types of cancer,

miR-130b overexpression stimulates cell proliferation via suppression of PTEN [32–34].

Further bioinformatic analyses showed that miRNAs associated active mitosis were a primary cause of poor prognosis in the IDH1/2-mut group. Even high rates of proliferation are a major feature of advanced malignancy. There remains controversy about the malignant role of proliferation in glioma with different IDH1/2 status. Adriana et al. reported that mitotic index had prognostic value in IDH1/2-wt but not IDH1/2-mut gliomas [35]. While, another study found that Ki-67, a reliable indicator of proliferation, had equal prognostic power in either IDH1/2-mut or IDH1/2-wt gliomas [36]. Existing studies have focused on universal proliferative indicator. Additional studies are still needed to identify glioma-specific proliferation markers and clarify the relationship between proliferation and IDH1/2 status.

Taken together, we took advantage of an integration of clinical, genomic and array data to systematically investigate the miRNA profile in a large samples sized LGG cohort. IDH1/2 was identified to be with an overwhelming impact on miRNA expression pattern and prognostication. We established a four-miRNA-based risk classifier serving as an accurate and independent stratification tool for IDH1/2-mut LGGs. Considering few miRNA studies have taken genomic aberrations into consideration, our study could offer novel implications for future research. While, this study had two limitations: the difficulty of collecting an external validation cohort and its retrospective nature. Even we randomly categorized cases into a set for internal validation, we hope our research will encourage high-quality data sharing and the publications of reports validating our findings. To validate the clinical applicability of our findings, a prospective study with a large independent dataset is required along with experimental research to clarify the function and mechanisms of action of the four miRNAs in the classifier, both individually and in combination. Owing to the stability of miRNA in various types of body fluid, detection of the four-miRNA-based classifier can be particularly valuable and powerful in a clinical setting.

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Compliance with Ethical Standards

Conflict of interest All authors declare that they have no conflict of interest

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards

Informed consent Informed consent was obtained from all individual participants included in the study.

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