High Expression of H2BC12 Predicts Poor Survival Outcome of Low-Grade Gliomas: A Study Based on TCGA Data

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Research Article

Keywords: H2BC12, TCGA, diagnosis, prognosis, low-grade glioma (LGG)

Posted Date: December 22nd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1184050/v1>

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Abstract

Purpose

Low-grade gliomas (LGG) have highly variable clinical behaviors, with a high incidence of disease progression as 70% within ten years. Regardless of treatment combining surgery and radiotherapy or chemotherapy, LGG is still associated with adverse survival outcomes. Therefore, our study was performed to satisfy the increasing demand of novel sensitive biomarkers and therapeutic targets in treatment and diagnosis of LGG.

Methods

The TCGA data set was used to examine the relationship between H2BC12 expression and clinical pathologic characteristics. The significance of H2BC12 expression in prognosis was also investigated. In addition, H2BC12 expression-related pathways were enriched by gene set enrichment analysis (GSEA). Association analysis of H2BC12 gene expression and immune infiltration was performed by single sample gene set enrichment analysis (ssGSEA).

Results

Significantly up-regulated expression of H2BC12 mRNA was found in LGG tissue when compared to normal tissue and was proven to be diagnostic (have diagnostic significance) for LGG. In the meantime, high H2BC12 levels were associated with WHO grade, IDH status, 1p/19q codeletion, primary therapy outcome and histological type of LGG, and additionally, prognostic for adverse survival outcomes. In the multivariate analysis, high H2BC12 levels were identified to be an independent predictor for poor survival outcomes of LGG patients. Pathways in cancer, signaling by Wnt or PI3K-AKT signaling pathway, DNA repair, cellular senescence and DNA double strand break repair were differentially activated in the phenotype that positively associated with H2BC12.

Conclusion

H2BC12 is a promising biomarker for the diagnosis and prognosis of LGG.

Introduction

Gliomas are tumors occurred at glial cells, which are important for the function of cerebral nerve cells, and the most prevalent primary malignancy in brain tumors (1). According to the World Health Organization (WHO) classification, pathologically confirmed gliomas can be categorized into four grades: I, II, III, and IV. Low-grade gliomas (LGG) contain grades II-III gliomas characterized by highly variable clinical behaviors (2). It is known that LGG, as compared to high-grade gliomas (grade IV), correlates to a more favorable survival outcome, while it was reported to have a 70% risk of disease progression in 10 years (1). Aggressive high-grade gliomas have poor outcomes, even when managed by surgical resection and radiotherapy or chemotherapy (3, 4). Due to the highly offensive property, it is impossible to achieve a complete cure in LGG. In this context, delaying tumor

onset and reducing tumor progression are the most challenging issues, urgently prompting us to search for new sensitive biomarkers and new targets for treatments and diagnosis of LGG.

It is known that genetic abnormality predisposing to tumorigenesis is always accompanied by epigenetic alterations. Aberrant histone modifications, for example, can potentially enhance the effects of oncogenic drivers in disease progression, metastatic potential, and resistance to therapy (5). Structurally, histone modification-related proteins are responsible for the compact structure of chromatin in nucleosomes and can be modified via diverse enzymes, including histone family genes (H2A, H2B, H3 and H4), two heterodimers (H2A, H2B), and one DNA-associated H3/H4 tetramer. Heterodimers H2A/H2B are important in chromatin-related processes involved in transcription, DNA replication and repair. It is established that H2B monoubiquitination (H2Bub1) at lysine 120 is vitally significant in proper DNA repair, and the absence of H2Bub1 is associated with abnormal H2AX phosphorylation resulting in durable DNA damage response (6, 7). Notably, RNF20/40, ubiquitin ligases indispensable for H2Bub1, were also reported to be a part of tumorigenesis. Recent research indicated that low levels of H2Bub1 were prognostic for disease progression, which supported the role of H2Bub1 as a tumor suppressor (8). Combining the above findings, histone genes play a crucial role in tumorigenesis and progression.

Here, H2B Clustered Histone 12 (H2BC12) was studied from aspects of expression in LGG tissue, associations with clinical characteristics, and the most involved biological pathways. Our findings suggested that H2BC12 can be recognized as a promising biomarker for LGG diagnosis and prognosis.

Materials And Methods

Data acquisition

Target RNA-seq data in TPM format, which were documented in TCGA and GTEx databases, were jointly processed by the Toil workflow software (9) and then downloaded from UCSC XENA (<https://xenabrowser.net/datapages/>). The TCGA database was searched for LGG tissue samples (n=523), and the GTEx database was consulted to obtain matched normal tissue samples (n=1,152). The RNA-seq data were log2 transformed. Corresponding clinical data of LGG were obtained.

Gsea Analysis

Hallmark gene set collections, including C2.cp.v7.2.symbols.gmt [Curated] and h.all.v7.2.symbols.gmt [Hallmarks], were retrieved from the Molecular Signatures Database (MSigDB) and chosen as the target sets. Correlations between the H2BC12 expression and all genes were characterized by R (v.3.6.3), followed by GSEA analysis using the R package clusterProfiler (10). Significance threshold was set to $|ES| > 1$, $p.adjust < 0.05$, and $FDR < 0.25$.

Analysis Of Immune Infiltration

From Bindea's investigation (11), the marker gene of 24 immune cells was retrieved. Based on LGG mRNA TPM data, single-sample GSEA (ssGSEA) (12) was utilized to quantify the number of tumor-infiltrating immune cells.

Spearman correlation was used to determine the relationship between H2BC12 and these 24 types of cells. The ggplot2 package was used to create the figures.

Statistical analysis

R (v.3.6.3) was run to complete all statistical analyses of the study. Diagnostic receiver operating characteristic (ROC) curve was generated using package pROC, while time-dependent ROC (tROC) curve was plotted assisted by package timeROC. Differential expression of H2BC12 in tumor versus normal was statistically analyzed via Wilcoxon rank sum tests. For correlational analysis between the H2BC12 mRNA and clinicopathologic characteristics of LGG, the tumor samples were assigned into two cohorts representative of high and low H2BC12 expression, respectively, with the cutoff value as the median H2BC12 expression of all samples. Chi-square test was implemented to identify the significance. Comparisons between two sets of data were completed by Wilcoxon rank sum test upon two groups or Kruskal-Wallis test when there were three groups or more. Prognostic significance of H2BC12 mRNA expression and clinicopathologic characteristics for overall survival (OS) of LGG patients were identified by univariate and multivariate Cox regression analysis. The survival significance of H2BC12 mRNA expression in subgroups of clinicopathologic characteristics was investigated by stratification and Kaplan-Meier analysis.

Results

Clinical characteristics

In total 523 primary tumor samples were obtained from the TCGA-LGG dataset. Matched clinical data were retrieved, involving WHO grade, IDH status, 1p/19q codeletion, primary therapy outcome, gender, race, age, histological type, laterality and OS event (Table 1), which revealed that LGG is much common among the youth and the elderly.

Table 1
Characteristics of patients with LGG based on TCGA

Characteristic	levels	Overall
n		528
WHO grade, n (%)	G2	224 (48%)
	G3	243 (52%)
IDH status, n (%)	WT	97 (18.5%)
	Mut	428 (81.5%)
1p/19q codeletion, n (%)	codel	171 (32.4%)
	non-codel	357 (67.6%)
Primary therapy outcome, n (%)	PD	110 (24%)
	SD	146 (31.9%)
	PR	64 (14%)
	CR	138 (30.1%)
Gender, n (%)	Female	239 (45.3%)
	Male	289 (54.7%)
Race, n (%)	Asian	8 (1.5%)
	Black or African American	22 (4.3%)
	White	487 (94.2%)
Age, n (%)	<=40	264 (50%)
	>40	264 (50%)
Histological type, n (%)	Astrocytoma	195 (36.9%)
	Oligoastrocytoma	134 (25.4%)
	Oligodendroglioma	199 (37.7%)
Laterality, n (%)	Left	256 (48.9%)
	Midline	6 (1.1%)
	Right	261 (49.9%)
OS event, n (%)	Alive	392 (74.2%)
	Dead	136 (25.8%)
DSS event, n (%)	Alive	397 (76.3%)
	Dead	123 (23.7%)

Characteristic	levels	Overall
PFI event, n (%)	Alive	318 (60.2%)
	Dead	210 (39.8%)

High Expression Of H2bc12 Mrna In Lgg Tumor Tissue

Apart from the tumor samples acquired, matched normal samples (n=1,152) were obtained from the GTEx database. Expression of the H2BC12 mRNA was examined in the two cohorts, showing a significant upward trend in primary tumor tissue (Figure 1A, $p<0.001$). Besides, H2BC12 protein expression was also detected by immunohistochemistry using the HPA database (<https://www.proteinatlas.org/>), indicating that the H2BC12 proteins were in high levels in both LGG tissue and normal tissue (Figure 1B). Together, H2BC12 might be an oncogene in LGG and play a vital role in the normal development of the brain.

Roc Analysis For H2bc12 In The Diagnosis Of Lgg

ROC curve was plotted to evaluate the diagnostic significance of H2BC12 mRNA expression for LGG. The area under the curve (AUC) was 0.823 (Figure 2A), indicating significance in distinguishing between normal and tumor samples with certain accuracy. Besides, tROC curves were drawn to identify the predictive ability of H2BC12 mRNA for OS of LGG patients. The AUC values for 1-, 2-, and 3-year OS were 0.766, 0.702, and 0.677, respectively (Figure 2B). It was suggested that H2BC12 is equipped with a good prognostic performance.

Correlations Between H2bc12 Mrna And Clinicopathologic Characteristics Of Lgg

The correlational analysis demonstrated that there were significant associations between the H2BC12 mRNA and clinicopathologic characteristics including WHO grade, IDH status, 1p/19q codeletion, primary therapy outcome and histological type (Figure 3A-E). In addition, tumor samples of each clinicopathologic subgroup were divided into two groups according to medium H2BC12 mRNA. Further analysis revealed that high H2BC12 mRNA expression was significantly associated with WHO grade, IDH status, 1p/19q codeletion, primary therapy outcome, histological type, OS event, disease-specific survival (DSS) event and progress-free interval (PFI) event (Table 2, $p<0.001$), which was statistically determined by the Chi-square test. Collectively, the H2BC12 mRNA expression is intimately correlated with clinicopathologic features of LGG patients, suggesting that H2BC12 might be involved in LGG progression.

Table 2
Relationship between H2BC12 mRNA expression and clinical characteristics in LGG

Characteristic	levels	Low expression of H2BC12	High expression of H2BC12	p
n		264	264	
WHO grade, n (%)	G2	138 (29.6%)	86 (18.4%)	< 0.001
	G3	95 (20.3%)	148 (31.7%)	
IDH status, n (%)	WT	13 (2.5%)	84 (16%)	< 0.001
	Mut	250 (47.6%)	178 (33.9%)	
1p/19q codeletion, n (%)	code1	148 (28%)	23 (4.4%)	< 0.001
	non-code1	116 (22%)	241 (45.6%)	
Primary therapy outcome, n (%)	PD	33 (7.2%)	77 (16.8%)	< 0.001
	SD	76 (16.6%)	70 (15.3%)	
	PR	36 (7.9%)	28 (6.1%)	
	CR	84 (18.3%)	54 (11.8%)	
Gender, n (%)	Female	117 (22.2%)	122 (23.1%)	0.727
	Male	147 (27.8%)	142 (26.9%)	
Race, n (%)	Asian	4 (0.8%)	4 (0.8%)	0.230
	Black or African American	7 (1.4%)	15 (2.9%)	
	White	245 (47.4%)	242 (46.8%)	
Age, n (%)	<=40	131 (24.8%)	133 (25.2%)	0.931
	>40	133 (25.2%)	131 (24.8%)	
Histological type, n (%)	Astrocytoma	61 (11.6%)	134 (25.4%)	< 0.001
	Oligoastrocytoma	69 (13.1%)	65 (12.3%)	
	Oligodendroglioma	134 (25.4%)	65 (12.3%)	
Laterality, n (%)	Left	122 (23.3%)	134 (25.6%)	0.412
	Midline	2 (0.4%)	4 (0.8%)	
	Right	137 (26.2%)	124 (23.7%)	

Characteristic	levels	Low expression of H2BC12	High expression of H2BC12	p
OS event, n (%)	Alive	237 (44.9%)	155 (29.4%)	< 0.001
	Dead	27 (5.1%)	109 (20.6%)	
DSS event, n (%)	Alive	240 (46.2%)	157 (30.2%)	< 0.001
	Dead	23 (4.4%)	100 (19.2%)	
PFI event, n (%)	Alive	196 (37.1%)	122 (23.1%)	< 0.001
	Dead	68 (12.9%)	142 (26.9%)	

Role Of H2bc12 In Lgg Patient Survival

In survival analysis, the OS of patients with high H2BC12 expression was much poorer as compared to those with low H2BC12 expression (Figure 4A, $p<0.001$), and similar results were observed as regards DSS and PFI (Figure 4B-C, $p<0.001$). To identify the prognostic factors for OS of LGG patients, univariate regression analysis was performed using a Cox model, demonstrating significant prognostic significance of H2BC12 mRNA, WHO grade, 1p/19q codeletion, primary therapy outcome, TP53, IDH status, age and histological type for OS (Table 3, $p<0.01$). Additionally, a further multivariate model was established, and it was found that, H2BC12 mRNA, WHO grade, primary therapy outcome, IDH status, age and histological type have independent prognostic significance for OS of LGG (Table 3, $p<0.05$).

Table 3

Correlations between overall survival and mRNA expression of H2BC12 analyzed by univariate and multivariate Cox regression

Characteristics	Total(N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
WHO grade (G3 vs. G2)	466	3.059 (2.046-4.573)	<0.001	1.845 (1.147-2.967)	0.012
1p/19q codeletion (non-codel vs. codel)	527	2.493 (1.590-3.910)	<0.001	1.293 (0.670-2.496)	0.443
Primary therapy outcome (PR&CR vs. PD&SD)	457	0.202 (0.113-0.359)	<0.001	0.281 (0.148-0.532)	<0.001
TP53 (High vs. Low)	527	1.689 (1.189-2.400)	0.003	1.352 (0.874-2.091)	0.175
IDH status (Mut vs. WT)	524	0.186 (0.130-0.265)	<0.001	0.455 (0.281-0.735)	0.001
Gender (Male vs. Female)	527	1.124 (0.800-1.580)	0.499		
Age (>40 vs. ≤40)	527	2.889 (2.009-4.155)	<0.001	3.491 (2.191-5.561)	<0.001
Histological type (Oligoastrocytoma&Oligodendroglioma vs. Astrocytoma)	527	0.606 (0.430-0.853)	0.004	1.018 (0.642-1.615)	0.939
H2BC12 (High vs. Low)	527	4.415 (2.885-6.756)	<0.001	2.267 (1.252-4.104)	0.007

Table 4
Gene sets enriched in positively correlated with H2BC12 mRNA expression phenotype high

MSigDB collection	Gene set name	NES	p.adj	FDR
c2.cp.v7.2.symbols.gmt [Curated]	KEGG_PATHWAYS_IN_CANCER	1.622	0.009	0.006
	REACTOME_SIGNALING_BY_WNT	1.441	0.009	0.006
	WP_PI3KAKT_SIGNALING_PATHWAY	1.634	0.009	0.006
	REACTOME_DNA_REPAIR	1.620	0.009	0.006
	REACTOME_CELLULAR_SENESCENCE	1.963	0.009	0.006
	REACTOME_DNA_DOUBLE_STRAND_BREAK_REPAIR	1.759	0.009	0.006
h.all.v7.2.symbols.gmt [Hallmarks]	HALLMARK_KRAS_SIGNALING_UP	1.754	0.003	0.001
	HALLMARK_TNFA_SIGNALING_VIA_NFKB	2.189	0.003	0.001
	HALLMARK_G2M_CHECKPOINT	1.996	0.003	0.001
	HALLMARK_GLYCOLYSIS	1.603	0.003	0.001
	HALLMARK_HYPOXIA	1.726	0.003	0.001
	HALLMARK_P53_PATHWAY	1.483	0.003	0.001
NES, normalized enrichment score; p.adj, adjust p value; FDR, false discovery rate.				

Clinical Stratification

As proven in the multivariate Cox regression analysis, WHO grade, primary therapy outcome, IDH status, age and histological type were independent prognostic factors for the OS in LGG. In subsequent work, clinical stratification was conducted based on the TCGA-LGG dataset, and it was found that in subgroups of WHO grade: G2, WHO grade: G3, primary therapy outcome: PD&SD, primary therapy outcome: PR&CR, IDH status: Mut, age<=40 and age>40, patients with low H2BC12 expression had better survival outcomes than those with highly expressing H2BC12 (Figure 5, $p<0.001$). This reflected that H2BC12 had independent prognostic significance for OS of LGG patients and increased H2BC12 level is associated with poorer OS.

H2bc12 - Related Signaling Pathways Based On Gsea

GSEA was performed to find the activated signaling pathways related to H2BC12 in LGG. Based on the Curated collection, there were six signaling pathways activated in H2BC12 overexpressed phenotype, including pathways in cancer, signaling by Wnt or PI3K-AKT signaling pathway, DNA repair, cellular senescence, and DNA double strand break repair (Figure 6). Based on the Hallmarks collection defined by MSigDB, other than the six pathways above, KRAS signaling up, TNFA signaling via NFKB, G2M checkpoint, glycolysis, hypoxia and p53 pathway also presented with significant enrichment in H2BC12 overexpressed phenotype (Figure 7).

Collectively, the H2BC12 mRNA expression may serve as an important player in the initiation and development of LGG.

H2BC12 expression is linked to the level of immune infiltration

Tumor-infiltrating lymphocytes are independent indicators of cancer survival. As a result, we evaluated whether H2BC12 expression is related to the level of immune infiltrate in LGG. According to our findings, H2BC12 showed a strong positive correlation with macrophages, eosinophils, neutrophils, and T cells; H2BC12 exhibited a strong inverse relationship with pDC, NK CD56bright cells, TReg, and DC (Figure 8A). Further analysis showed that compared with the low-H2BC12 group, the infiltration level of Neutrophils and T cells in the high-H2BC12 group was significantly increased (Figure 8B). The infiltration levels of pDC, NK CD56bright cells, TReg and DC was significantly reduced in the high-H2BC12 group (Figure 8C).

Discussion

Gliomas are fetal tumors most prevalent in the central nervous system (CNS). LGG (WHO II, III) are low-grade tumors that grow slowly with lesser malignant properties than high-grade tumors. However, there is a high risk of disease progression to advanced glioma in most LGG patients (1). Besides, there was research reporting that patients with LGG receiving chemotherapy would experience a poor outcome (13). According to bioinformatics, the WHO included several molecular markers, such as IDH mutation status, chromosome 1p or 19q codeletion (1p/19q codeletion) status, into the guideline for diagnosis of glioma, to raise the accuracy in disease diagnosis and further treatment (14). In this context, the demand of biomarkers with early diagnostic value is increasing, which will be of vital significance for the treatment and prognosis of LGG patients.

In this study, we firstly obtained RNA-seq data documented in TCGA-LGG and matched normal samples from GTEx in the UCSC XENA database, demonstrating that H2BC12 mRNA significantly increased in tumor tissue compared to the normal control, suggesting that H2BC12 might be active in promoting LGG initiation. Kim et al. (15) reported the top six most highly expressed genes in breast cancer, including HIST1H2BK (H2BC12), STAT3, CTSD, SREBF1, IGFBP5, and DDR1, from 49 signature genes of tumor dormancy based on cancer cell line data and microarray data, which further verified the role of H2BC12 as a potential tumor dormancy marker (15). Dormant cells are highly adaptable in chemotherapy since they can rapidly target proliferating cells. In the meantime, they can still survive for a long time and even reproduce after chemotherapy is terminated. Han et al. (16) reported that H2BC12 displayed increased expression in drug-resistant cell MDA-MB-231 in breast cancer, showing a close relationship between the H2BC12 expression and development of drug resistance. Here, we found that H2BC12 proteins presented with high expression in both the tumor and normal tissues, implying that H2BC12 might be crucial in human normal physiological activities. Research revealed that H2A/H2B are important participants in chromatin transcription, DNA replication and repair (17). Similarly, we noted good diagnostic performance of H2BC12 for LGG, characterized by an AUC of the ROC curve as 0.823. The tROC curve also validated the moderate prognostic value of H2BC12 for OS of LGG patients. There was a study which identified that the signatures based on histone gene family are potentially good indicators for the outcome of cervical cancer patients (17).

We then profiled the association between H2BC12 expression level and clinicopathologic characteristics of LGG. Notably, increased expression of H2BC12 was correlated significantly with WHO grade, IDH status, 1p/19q

codeletion, primary therapy outcome and histological type. This demonstrated that H2BC12 mRNA expression is closely related to the clinicopathologic characteristics of LGG, and H2BC12 might be involved in the disease progression. Further survival analysis was conducted to validate the association of high H2BC12 expression with adverse survival outcomes of LGG patients. We thus believe that H2BC12 serves as a high-risk factor for LGG. Previous bioinformatics analysis identified that high H2BC12 expression predicted adverse outcomes of breast, pancreatic and ovarian cancers (18–20). We next performed univariate and multivariate analyses to identify factors predicting OS with Cox regression models. The results showed that H2BC12, WHO grade, primary therapy outcome, IDH status, age and histological type all could be prognostic factors for LGG. Given this, a further clinical stratification analysis was designed to identify whether H2BC12 is an independent predictor. We found that the independent prognostic value of H2BC12 was superior to IDH status, WHO grade and histological type.

Finally, we conducted GSEA to uncover the H2BC12-related pathways in LGG. The results showed that there were six pathways, including pathways in cancer, signaling by Wnt or PI3K-AKT signaling pathway, DNA repair, cellular senescence and DNA double strand break repair, which demonstrated differential enrichment in H2BC12 high expression phenotype. Research revealed that activated PI3K-AKT could facilitate the invasiveness of glioma cells (21). It was studied that DNA repair genes are associated with the LGG (22). DNA repair damage is a main cause for the development of radio-resistance and chemo-resistance in gliomas (23). All these findings indicate the potential important role of H2BC12 in LGG progression.

In all, this research verified the significance of H2BC12 in the diagnosis and prognosis of LGG patients. Inevitably, limitations still exist which remain to be resolved. First, the study was carried out only with bioinformatics analysis, requiring further validation in clinical samples. Second, there is a need to clarify the H2BC12-mechanism of action in LGG.

Conclusion

This study identified the differentially up-regulated expression of H2BC12 in LGG tissue and proved its significant ability in predicting adverse overall survival of LGG patients. H2BC12, therefore, is promising to be applied for the diagnosis and prognosis of LGG.

Abbreviations

LGG, Low-grade gliomas; WHO, World Health Organization; GSEA, gene set enrichment analysis; ssGSEA, single sample gene set enrichment analysis; H2Bub1, H2B monoubiquitination; H2BC12, H2B Clustered Histone 12; MSigDB, Molecular Signatures Database; ssGSEA, single-sample GSEA; ROC, receiver operating characteristic; tROC, time-dependent ROC; OS, overall survival; AUC, area under the curve; DSS, disease-specific survival; PFI, progress-free interval; CNS, central nervous system.

Declarations

Acknowledgments

Not applicable

Ethics Statement

Not applicable

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding

This work was supported by the Taishan Scholar Project of Shandong Province of China (grant no. tsqn202103200), the Latitudinal Research Project of Shandong University (grant no. 6010120011) and the Shandong traditional Chinese medicine science and technology project (grant no. 2021M033).

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Yilei Xiao, Zhaoquan Xing and Zhaogang Dong. The first draft of the manuscript was written by Yilei Xiao, Mengyou Li, Xin Li, Ding Wang and Zhaogang Dong, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

The data that support part of the findings of this study are openly available in TCGA at [<https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga>], GTEx at [<https://www.gtexportal.org/home/index.html>], GSEA [<http://software.broadinstitute.org/gsea/downloads.jsp>].

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Figures

Figure 1

H2BC12 is significantly up-regulated in LGG tissue versus normal or adjacent normal tissues.

(A) H2BC12 showed significantly higher expression in cancer tissue versus normal tissue ($p < 0.001$). (B) Immunohistochemistry based on the HPA database showed high H2BC12 protein expression in both normal and tumor tissues.

Figure 2

ROC curve of H2BC12 mRNA expression in diagnosis and prognosis of LGG.

(A) Diagnostic ROC curve showed the accurate discriminative capability of H2BC12 in distinguishing between normal and tumor (AUC=0.823). (B) tROC curve demonstrated AUC values for 1-, 2-, and 3-year survival as 0.766, 0.702, and 0.677, respectively.

Figure 3

Association of H2BC12 expression with clinicopathologic characteristics. H2BC12 expression was correlated significantly with WHO grade (**A**, $p < 0.001$), IDH status (**B**, $p < 0.001$), 1p/19q codeletion (**C**, $p < 0.001$), primary therapy outcome (**D**, $p < 0.01$) and histological type (**E**, $p < 0.01$).

Figure 4

High expression of H2BC12 is associated with poor OS, DSS and PFI in patients with LGG.

OS (**A**, $p < 0.001$), DSS (**B**, $p < 0.001$) and PFI (**C**, $p < 0.001$) were significantly poorer in patients with high H2BC12 expression than those with low H2BC12 expression. OS, Overall Survival; DSS, Disease Specific Survival; PFI,

Figure 5

Clinical stratification analysis of the survival difference between patients with LGG in the high- and low-H2BC12 groups by the WHO grade, primary therapy outcome, IDH status, age and histological type.

Kaplan-Meier survival curves of patients in the high- and low-H2BC12 groups within eight clinically stratified subgroups, including WHO G2 (A), WHO G3 (B), primary therapy outcome: PD&SD (C), primary therapy outcome: PR&CR (D), IDH status: WT (E), IDH status: Mut (F), age<=40 (G) and age>40 (F), respectively. Patients in the low-H2BC12 group had better survival outcomes than those in the high-H2BC12 group across all clinically stratified subgroups except IDH status of WT ($p<0.01$).

Figure 6
Enrichment plots from GSEA in c2.cp.v7.2.symbols collection.

GSEA results showed pathways in cancer (A), Wnt signaling pathway (B) PI3K-AKT signaling pathway (C), DNA repair (D), cellular senescence (E) and DNA double strand break repair (F) which were differentially enriched in

H2BC12 high expression phenotype. NES, normalized ES; p.adj, p.adjust; FDR, False Discovery Rate.

Figure 7

Enrichment plots from GSEA in h.all.v7.2.symbols collection.

GSEA results showed KRAS signaling up (A), TNFA signaling via NFKB (B), G2M checkpoint (C), glycolysis (D), hypoxia (E) and p53 pathway (F) which were differentially enriched in H2BC12 high expression phenotype. NES, normalized ES; p.adj, p.adjust; FDR, False Discovery Rate.

Figure 8

Correlation analysis between H2BC12 and immune infiltration.

(A) Association analysis between H2BC12 expression and immune cells. **(B, C)** Differences in immune cell infiltration levels between high and low H2BC12 expression groups.