Identification of Prognostic miRNAs Targeting EZH2 in Hepatocellular Carcinoma Using The Cancer Genome Atlas Database

Tianyu Lin (linty@zju.edu.cn)
Zhejiang University School of Medicine Sir Run Run Shaw Hospital
Xinli Guo
Zhejiang University School of Medicine Sir Run Run Shaw Hospital
Qian Du
Zhejiang University School of Medicine
Wei Liu
Zhejiang University School of Medicine Sir Run Run Shaw Hospital
Xin Zhong
Zhejiang University School of Medicine Sir Run Run Shaw Hospital
Suihan Wang
Zhejiang University School of Medicine Sir Run Run Shaw Hospital
Liping Cao
Zhejiang University School of Medicine Sir Run Run Shaw Hospital

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Abstract

Background: Enhancer of zeste homolog 2 (EZH2) gene have a prognostic role in hepatocellular carcinoma (HCC). This study aimed to identify the prognostic microRNAs (miRNAs) targeting EZH2 in HCC.

Methods and Results: We downloaded the gene and miRNA RNA-seq data from The Cancer Genome Atlas (TCGA) database. Differences in EZH2 expression between tumor and control samples and those between tumors with different clinical variables were analyzed using the Mann-Whitney U test. Association of *EZH2* expression with prognosis in HCC patients was detected using Cox regression analysis. We also identified miRNAs targeting *EZH2* with negative correlations, compared the miRNA expression profiles between tumor and control tissues, and identified pathways and protein-protein interaction pairs related to *EZH2*. The miRNA-*EZH2*-pathway network was constructed accordingly. *EZH2* was significantly upregulated in HCC tumors compared with control samples ($p<0.0001$) and in tumors with advanced T classifications (3/4 vs. 1/2, $p=0.0039$) and stages (III/IV vs. I/II, $p=0.0028$). The Cox regression analysis showed that TCGA HCC patients who had high *EZH2* expression levels showed a short survival time (HR=1.677, 95% CI 1.316-2.137; $p<0.0001$). Among miRNAs targeting EZH2, seven miRNAs, including *hsa-let-7c-5p*, were negatively correlated with *EZH2* expression and were significantly downregulated in HCC tumor samples compared with controls ($p<0.0001$). The miRNA-*EZH2*-pathway network included seven downregulated miRNAs and four pathways, including hsa00310: Lysine degradation. *Hsa-let-7c-5p* was associated with prognosis in HCC (HR=0.849 95% CI 0.739-0.975; $p=0.021$).

Conclusions: *EZH2-hsa-let-7c-5p* has a significant association with HCC prognosis and the mechanism worth investigating.

Introduction

Hepatocellular carcinoma (HCC) is the most frequent type of primary liver cancer (comprising 75%-85% of cases) and has a high global incidence and mortality rate globally. HCC-related death is estimated to approximately 662,000 annually [1] and is approximately 781,000 in 2018 [2]. HCC is the second or third most common cause of cancer-related death worldwide [2]. The main risk factors for HCC are infections of hepatitis B virus (HBV), hepatitis C virus (HCV), heavy alcohol intake, smoking, and obesity [2, 3]. However, the clinical prognosis of advanced HCC remains poor and had high incidence rates of tumor recurrence and metastasis. Accordingly, identification of diagnostic and prognostic factors for HCC remains necessary.

The enhancer of zeste homolog 2 (*EZH2*) gene is a negative prognostic biomarker in HCC [4]. EZH2 is a core component of the polycomb-repressive complex 2 (PRC2) and an essential element for histone methyltransferase activity [5]. EZH2 is an important epigenetic regulator repressing transcription [6, 7]. Interplay between EZH2 and other PRC2 components, including DNA methyltransferase 1 (DNMT1),

embryonic ectoderm development (EED), and suppressor of zeste 12 (SUZ12), results in gene transcriptional repression and DNA hypermethylation [6, 7]. EZH2, interplaying with PRC2 complex, drives hypermethylation of Lys-27 in histone 3 (H3K27me3) and promotes tumorigenesis, metastasis, and progression of many cancers, including breast cancer and HCC [8–11]. Also, the *EZH2* gene has been associated with poor prognosis in several human malignancies and might be a novel target for cancer treatment [12–14].

Genetic factors, including microRNAs (miRNAs), genes, circular RNAs, and long non-coding RNAs (lncRNAs), have been associated with tumorigenesis, drug resistance, invasion, and metastasis of human tumors. The interactions between non-coding RNAs and the *EZH2* gene in cancers have been identified [5]. Non-coding RNAs, including *hsa-miR-26a*, *hsa-let-7b*, *hsa-miR-101*, *hsa-miR-26a*, *lncRNA MEG3*, and *hsa_circ_0008450*, regulate *EZH2* and the proliferation, invasion, and tumor growth of HCC [5, 11, 12, 15, 16]. There have been too many *EZH2*- and HCC-related non-coding RNAs associated with tumor cell proliferation, invasion, and migration up to now [17–22], but the ones that are actually associated with HCC prognosis have yet to be carefully considered.

The aim of this study was to identify prognostic miRNAs interplaying with *EZH2* in HCC. Association of *EZH2* expression with prognosis in HCC patients was investigated. MiRNAs targeting *EZH2* were screened from online databases and prognostic miRNAs negatively correlated with *EZH2* in HCC patients were then identified using integrated bioinformatics analysis. This study provided a novel and important molecular mechanism of HCC development and prognosis.

Materials And Methods

Data materials

The Cancer Genome Atlas Program (TCGA) hepatocellular carcinoma (HCC) gene and miRNA expression profiles (in log2[RPM + 1] format) by RNA-seq (Illumina HiSeq 2000 RNA Sequencing platform) were downloaded from the University of California, Santa Cruz (UCSC) Xena (https://xenabrowser.net/datapages/) on Dec 20, 2020. Clinical information was extracted from the TCGA (https://portal.gdc.cancer.gov/). The gene expression profiles (in log2[x + 1] transformed RSEM normalized count) were extracted from 371 tumor biospecimens with vital status information and 50 control samples. The miRNA expression profiles (in log2[RPM + 1]) were extracted from 366 tumors and 49 control samples. Clinical variables, including survival time, pathologic stage, gender, age, race, vital status (dead or alive), prior malignancy and treatment history, were extracted and used for further analyses.

Analysis of EZH2 expression

Differences in *EZH2* expression levels between tumor samples and controls, as well as between samples had different pathologic stages (I/II vs. III/IV), pathologic T classifications (1/2 vs. 3/4), ages (< 65 yrs vs.

≥ 65 yrs), genders (male vs. female), races (Asian, White, or Black), without and with prior malignancy were analyzed using the non-parametric Mann-Whitney U test or the Kruskal-Wallis H test.

Cox regression analysis for EZH2 expression

The association of *EZH2* expression with prognosis in TCGA HCC patients was analyzed using the Cox regression analysis. Also, associations of *EZH2* expression profile and clinical factors (including pathologic stage, pathologic T classification, age, gender, race, and prior malignancy) with prognosis in TCGA HCC patients were analyzed using univariate and multivariate Cox regression analysis. We also performed the Cox regression survival analysis to investigate the difference in survival percent between patients with high and low *EZH2* expression levels, which were divided using the median expression value.

Validation of the association of EZH2 with HCC prognosis

Association of *EZH2* expression with prognosis in HCC patients was validated using three online public databases: Oncolnc (http://www.oncolnc.org/), Gene Expression Profiling Interactive Analysis (GEPIA; http://gepia.cancer-pku.cn/index.html), and University of Alabama Cancer Database (UALCAN; http://ualcan.path.uab.edu/index.html). All databases performed the log-rank test of Kaplan-Meier (KM) analysis.

Identification of miRNAs targeting EZH2

MiRNAs targeting *EZH2* were identified from three databases: miRTarbase (2019 update; http://mirtarbase.cuhk.edu.cn/php/index.php), starBase (http://starbase.sysu.edu.cn/panCancer.php), and TargetScanHuman 7.2 (http://www.targetscan.org/vert_72/). Predicted miRNA-*EZH2* pairs in at least two databases were retained and used to screen miRNAs correlated with *EZH2* negatively. Spearman correlation coefficient (r) was used for screening miRNAs, with the criteria of $r < 0$ and $p < 0.05$. Negative miRNA-*EZH2* pairs were used to construct the miRNA-*EZH2* regulatory network.

Expression of miRNAs in HCC tumor samples

Differences in miRNA expression levels between tumor and control samples were analyzed using the non-parametric Mann-Whitney U test. We also analyzed the correlations of miRNAs with HCC prognosis using Cox regression analysis.

Construction of the miRNA-EZH2-pathway network

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways associated with EZH2 were identified from the KEGG database (https://www.kegg.jp/) and the Comparative Toxicogenomics Database (CTD, 2020 update; http://ctdbase.org/). We identified protein-protein interaction (PPI) pairs of *EZH2* (score > 0.4) from the STRING database (version 11.0; https://string-db.org/cgi/input?sessionId=btC19KpbQESh&input_page_show_search=on). The miRNA-*EZH2*-pathway consisting of miRNA-*EZH2* pairs, PPI pairs of *EZH2*, and *EZH2*-associated pathways was constructed using the Cytoscape (version 3.8.0; http://apps.cytoscape.org/).

Statistical analysis

All the statistical analyses were performed in the SPSS 22.0 software (IBM, Chicago, USA). Differences in patients' age, male ratio, and race were analyzed using the t-test, Chi-square test, and Wilcoxon rank sum test, respectively. Differences in miRNA and *EZH2* expression levels between groups were analyzed using the non-parametric Mann-Whitney U test, and those across more than three groups were analyzed using the non-parametric Kruskal-Wallis H test, respectively. We used the Cox regression analysis to identify the association of miRNAs, *EZH2*, and clinical variables with prognosis in HCC patients. Spearman correlation analysis was conducted to identify miRNAs negatively correlated with *EZH2* expression. Hazard ratio (HR) and 95% confident interval (CI) were calculated following Cox regression and KM survival analyses. For all analyses, the significant criterion was set at $p = 0.05$.

Results

Demographics of TCGA HCC patients

The demographics of TCGA HCC patients (n = 371) and controls (n = 50) are shown in Table 1. The average age of HCC patients and controls were 59.44 ± 13.51 and 61.68 ± 16.12 years ($p = 0.284$). Most individuals were male (67.39 vs. 56.00%, $p = 0.115$). Most HCC patients were Asian and White (342, 92.18%), the ratio was high than controls (80.00%, $p < 0.0001$; Table 1). Among tumor samples, most were at early T, N, and M classifications, without prior treatment (n = 369) and malignancy (n = 336). The median overall survival time was 598.5 (6.00-3675.0) days.

Table 1
Demographics of The Cancer Genome Atlas hepatocellular carcinoma patients.

Variables	HCC (n = 371)	Control (n = 50)	P value
Age (year)	59.44 ± 13.51	61.68 ± 16.12	0.284
Male ratio	250 (67.39%)	28 (56.00%)	0.115†
Race (Asian/White/Black/NR)	158/184/19/8	6/34/7/3	< 0.0001‡
Vital status (Live/Dead)	241/130		
Pathologic_M (0/1/NR)	266/4/101		
Pathologic_N (0/1/NR)	251/4/115		
Pathologic_T (1/2/3/4/NR)	181/94/80/13/3		
Pathologic_stage (I/II/III/IV/NR)	171/86/85/5/24		
Overall survival (days)	598.5 (6.00-3675.0)		
Prior_malignancy (Yes/No)	35/336		
Prior_treatment (Yes/No)	2/369		

, for t-test. † for Chi-square test. ‡ for Wilcoxon rank sum test. NR, not reported.

EZH2 is upregulated in HCC tumors

Comparative analysis showed that HCC tumors had a significantly higher level of *EZH2* compared with controls ($p < 0.0001$; Fig. 1A). We also observed a significantly higher level of EZH2 in tumors with advanced T classifications (3/4 vs. 1/2, $p = 0.0039$; Fig. 1B) and pathologic stages (III/IV vs. I/II, $p = 0.0028$, Fig. 1C) compared with corresponding controls.

Different EZH2 expression levels in HCC tumors from older and Asian patients

We also investigated the expression level of *EZH2* in tumors from HCC patients of different ages, races, and genders. Results showed that *EZH2* had a higher expression level in tumors from patients aged < 65 years compared with patients > 65 years ($p = 0.0277$; Fig. 2A) and from Asian patients compared with White/Black patients ($p = 0.0059$; Fig. 2C). There was no difference in *EZH2* expression level between patients with and without prior malignancy ($p = 0.1931$; Fig. 2C) and between female and male patients ($p = 0.4084$; Fig. 2D). These results might indicate that *EZH2* expression is related to age and race in HCC patients.

EZH2 associates with prognosis in HCC patients

We analyzed the association of *EZH2* with prognosis in TCGA HCC patients using the stepwise Cox regression analysis. Univariate Cox regression analysis showed that pathologic T, pathologic stage, and *EZH2* expression were associated with overall survival in HCC patients ($p < 0.0001$; Table 2) after adjusting for patient's age, prior malignancy, and race. Multivariate Cox regression analysis indicated that *EZH2* expression level was the only variable contributing to a poor prognosis in HCC patients (HR = 1.677, 95% CI 1.316–2.137; $p < 0.0001$; Table 2). Cox regression survival analysis showed that HCC patients with a high level of *EZH2* had a lower survival percent compared with patients who had a low level of *EZH2* (Fig. 3). We also observed the association of *EZH2* with HCC prognosis in online databases: GEPIA (logrank p = 5.6e-05; Fig. 4A), UALCAN (logrank $p < 0.0001$; Fig. 4B), and Oncolnc (logrank p = 5.87e-05; Fig. 4C). The results indicated that a high level of *EZH2* was correlated with a poor prognosis in HCC patients.

Table 2
Cox regression analysis of variables related to death.

Variables	Univariate		Multivariate	
	HR (95% CI)	P	HR (95% CI)	P
Age	1.013 (0.999–1.027)	0.073		
Gender (Male/female)	0.815 (0.572–1.161)	0.258		
Race (Asian/White/Black)	0.884 (0.384–2.036)	0.772		
Prior malignancy (Yes/No)	0.869 (0.489–1.545)	0.633		
Pathologic T (1/2/3/4)	1.626 (0.170–1.929)	< 0.0001	1.357 (0.685–2.686)	0.381
Pathologic stage (I/II/III/IV)	1.555 (1.308–1.848)	< 0.0001	1.097 (0.571–2.107)	0.780
EZH2 expression	1.814 (1.445–2.277)	< 0.0001	1.677 (1.316–2.137)	< 0.0001

HR, Hazard ratio. CI, 95% confident interval.

Identification of miRNAs related to EZH2

We identified 24 miRNAs targeting *EZH2* in miRTarbase, starBase, and TargetScanHuman 7.2 (Table 3). Spearman correlation analysis showed that seven miRNAs had significant and negative correlations with *EZH2* ($r < 0$ and $p < 0.05$), including *hsa-let-7b-5p* (r = -0.172, $p < 0.0001$), *hsa-let-7c-5p* (r = -0.514, $p < 0.0001$), *hsa-miR-101-3p* (r = -0.360, $p < 0.0001$), *hsa-miR-144-3p* (r = -0.306, $p < 0.0001$), *hsa-miR-150-5p* (r = -0.098, p = 0.046), *hsa-miR-26a-5p* (r = -0.213, $p < 0.0001$), and *hsa-miR-26b-5p* (r = -0.312, $p < 0.0001$; Table 3). Seven miRNAs were significantly downregulated in HCC tumor tissues compared with controls (Fig. 5; $p < 0.0001$ for all miRNAs, by Mann-Whitney U test). The seven miRNAs were retained and used for the construction of the miRNA-EZH2-pathway regulatory network.

Table 3
Correlation of miRNAs with *EZH2* expression in hepatocellular carcinoma samples.

miRNAs	Databases	r	P
hsa-let-7a-5p	miRTarbase; starBase	-0.044	0.376
hsa-let-7b-5p	miRTarbase; starBase	-0.172	< 0.0001
hsa-let-7c-5p	miRTarbase; starBase	-0.514	< 0.0001
hsa-let-7e-5p	miRTarbase; starBase	0.134	0.006
hsa-miR-101-3p	miRTarbase; starBase; TargetScan 7.2	-0.360	< 0.0001
hsa-miR-144-3p	miRTarbase; starBase; TargetScan 7.2	-0.306	< 0.0001
hsa-miR-150-5p	miRTarbase; starBase	-0.098	0.046
hsa-miR-217	miRTarbase; starBase; TargetScan 7.2	0.085	0.082
hsa-miR-26a-5p	miRTarbase; starBase; TargetScan 7.2	-0.213	< 0.0001
hsa-miR-26b-5p	miRTarbase; starBase; TargetScan 7.2	-0.312	< 0.0001
hsa-miR-27a-3p	miRTarbase; starBase	0.007	0.883
hsa-miR-32-5p	starBase; TargetScan 7.2	0.047	0.338
hsa-miR-363-3p	starBase; TargetScan 7.2	0.078	0.113
hsa-miR-92a-3p	starBase; TargetScan 7.2	0.244	< 0.0001
hsa-miR-92b-3p	miRTarbase; starBase; TargetScan 7.2	0.234	< 0.0001
hsa-miR-93-5p	miRTarbase; starBase	0.620	< 0.0001
hsa-miR-98-5p	miRTarbase; starBase	0.066	0.178
hsa-miR-25-3p	miRTarbase; starBase; TargetScan 7.2	0.346	< 0.0001
hsa-miR-367-3p	starBase; TargetScan 7.2	/	/
hsa-miR-4465	starBase; TargetScan 7.2	0.564	0.090
hsa-miR-506-3p	starBase; TargetScan 7.2	0.071	0.448
hsa-miR-124-3p	miRTarbase; starBase	0.619	0.102
hsa-miR-137	miRTarbase; starBase; TargetScan 7.2	0.420	< 0.0001
hsa-miR-138-5p	miRTarbase; starBase; TargetScan 7.2	0.120	0.088

r, the Spearman correlation coefficient.

Construction of the miRNA-EZH2-pathway regulatory network

Before the construction of the miRNA-*EZH2*-pathway regulatory network, we firstly identified 10 genes related to *EZH2* from STRING (including *DNMT1*; *EED*; *SUZ12*; RB binding protein 4, chromatin remodeling factor, *RBBP4*; *PBBP7*; histone deacetylase 1, *HDAC1*; and *HDAC2*) and three *EZH2*-associated pathways overlapping between KEGG and CTD databases, including microRNAs in cancer (hsa05206), metabolic pathways (hsa01100), and Lysine degradation (hsa00310; Table 4). The miRNA-*EZH2*-pathway regulatory network composed 11 genes, seven miRNAs, and three pathways (Fig. 6).

Table 4
Genes and pathways associated with EZH2.

Gene symbol	**Description**	**Resource**
AEBP2	AE binding protein 2	STRING
DNMT1	DNA methyltransferase 1	STRING
EED	embryonic ectoderm development	STRING
HDAC1	histone deacetylase 1	STRING
HDAC2	histone deacetylase 2	STRING
PHF19	PHD finger protein 19	STRING
RBBP4	RB binding protein 4, chromatin remodeling factor	STRING
RBBP7	RB binding protein 7, chromatin remodeling factor	STRING
SUZ12	SUZ12 polycomb repressive complex 2 subunit	STRING
YY1	YY1 transcription factor	STRING
Pathway ID	**Description**	**Resource**
hsa00310	Lysine degradation	CTD, KEGG
hsa01100	Metabolic pathways	CTD, KEGG
hsa05206	MicroRNAs in cancer	CTD, KEGG
CTD, Comparative Toxicogenomics Database. KEGG, Kyoto Encyclopedia of Genes and Genomes.		

Screening of miRNAs associated with HCC prognosis

At last, we screened out HCC prognosis-associated miRNAs among the seven downregulated miRNAs using Cox regression analysis. Univariate Cox regression analysis identified that only *hsa-let-7c-5p* was associated with prognosis in HCC patients (HR = 0.849, 95% CI 0.739–0.975; p = 0.021; Table 5). These results showed that *hsa-let-7c-5p-EZH2* might be an important miRNA-mRNA axis in HCC.

Table 5
Univariate Cox regression survival analysis of miRNAs in hepatocellular carcinoma.

miRNAs	Univariate	
	HR (95% CI)	P
hsa-let-7b-5p	1.057 (0.872–1.280)	0.572
hsa-let-7c-5p	0.849 (0.739–0.975)	**0.021**
hsa-miR-101-3p	0.961(0.756–1.221)	0.746
hsa-miR-144-3p	0.943(0.843–1.055)	0.303
hsa-miR-150-5p	0.913(0.819–1.019)	0.105
hsa-miR-26a-5p	1.188(0.909–1.552)	0.208
hsa-miR-26b-5p	1.188(0.942–1.499)	0.146
HR, hazard ratio. CI, 95% confident interval.		

Discussion

Our present study confirmed that the *EZH2* gene had a higher expression level in tumors compared with controls and in advanced tumors compared with early-stage tumors. *Hsa-let-7c-5p* was upregulated in HCC tumors compared with controls and its expression was associated with a good prognosis in HCC patients. These results showed that the *hsa-let-7c-5p-EZH2* axis might have a crucial role in the progression of HCC.

EZH2 is a core component of the PRC2 and trimethylates H3K27. Deregulation of *EZH2* is associated with gene expression repression, tumorigenesis, development, and tumor cell radiosensitivity [5, 23–25]. EZH2 regulates downstream genes and signalings in a PRC2-independent manner [13]. *EZH2* functions as both an oncogenic gene or as a tumor suppressor gene by activating its downstream target genes and signalings through a PRC2-independent way [13]. For instance, EZH2 directly binds to the promoter of the large tumor suppressor 2 (*LATS2*) gene and induces its H3K27me3 in gallbladder cancer cells [26]. EZH2 also decreases forkhead box C1 (*FOXC1*) expression by promoting H3K27me3 in breast cancers [27]. *LATS2* is a member of the large tumor suppressor family [28, 29] and the *FOXC1* transcription factor also as an oncogenic gene by regulating cell proliferation, senescence, angiogenesis, and metastasis [30, 31]. This evidence reveals the important role of EZH2 in human cancers.

Recent evidence shows that *EZH2* regulates immune responses in human cancers, including HCC [4, 13, 32, 33]. Elevated *EZH2* level was positively correlated with immunosuppression in HCC and was negatively correlated with the contents of Class I major histocompatibility complex molecules [4]. However, loss or knockdown of *EZH2* in regulatory T cells and natural killer cells enhance their

recruitment and anti-tumor immunity [34, 32]. Therefore, many efforts have been made to inhibit EZH2 methyltransferase activity, break PRC2' structure, suppress EZH2 expression, or develop EZH2 inhibitors with low toxicity, high efficiency, and high selectivity in cancer treatment.

EZH2 and H3K27me3 levels could be regulated by non-coding RNAs [5, 35]. Many EZH2-related miRNAs, including tumor suppressor miRNAs *hsa-let-7b*, *hsa-miR-101*, and *hsa-miR-26a* have been identified [5, 36]. These miRNAs have tumor suppressive roles by inhibiting *EZH2* and H3K27me3 levels and then abrogating the aggressive type of cancers [37, 5]. Xu et al [38] showed that *hsa-miR-101* inhibited human HCC progression and promoted cytostatic drug sensitivity by suppressing *EZH2*. Also, *miR-101-3p* induces autophagy by targeting *EZH2* [39]. We observed that *EZH2* was negatively targeted by seven downregulated miRNAs in HCC, including *hsa-let-7b-5p*, *hsa-let-7c-5p*, *hsa-miR-101-3p*, *hsa-miR-144-3p*, *hsa-miR-150-5p*, *hsa-miR-26a-5p*, and *hsa-miR-26b-5p*. All miRNAs are associated with the proliferation and metastasis in HCC cells and prognosis in HCC patients [40–45]. Although the regulation of other miRNAs could regulate HCC cell proliferation, migration, and metastasis, only *hsa-let-7c-5p* was associated with prognosis in HCC patients in the TCGA database, showing that the role of the *hsa-let-7c-5p-EZH2* axis in the HCC might be very important. Also, the involvement of EZH2 in microRNAs in cancer (hsa05206) showing miRNA-mediated EZH2 might play important roles in HCC development.

Song et al [46] confirmed the downregulation of *hsa-let-7c-5p* in HCC tumor tissues. They showed that a high level of *hsa-let-7c-5p* was correlated with a long overall survival period. Li et al. [47] confirmed that *hsa-let-7c-5p* and EZH2 were downregulated and upregulated in breast cancer tissues compared with controls, respectively. They also showed that the inhibition of *hsa-let-7c-5p* increased *EZH2* expression in MDA-MB-231 breast cancer cells. However, there is poor information on the regulation of *hsa-let-7c-5p* on *EZH2* and the axis in HCC. Therefore, identification of the *hsa-let-7c-5p-EZH2* axis might provide a novel and reference to HCC mechanism. Also, the strategy focusing on inhibiting *EZH2* might provide a reference for the treatment of HCC.

Conclusions

Our study showed that the *EZH2* gene was upregulated in HCC tumor samples and its level was correlated with prognosis in HCC patients negatively. Also, *EZH2* was negatively targeted by *hsa-let-7c-5p*, which had a lower level in HCC tumors compared with control and a positive correlation with overall survival in HCC patients. However, important roles of the *hsa-let-7c-5p-EZH2* axis in HCC development and prognosis should be validated by experiments.

Abbreviations

CI, confident interval; CTD, the Comparative Toxicogenomics Database; DNMT1, DNA methyltransferase 1; EED, embryonic ectoderm development; EZH2,enhancer of zeste homolog 2; FoxO1, forkhead box O1; GEPIA, Gene Expression Profiling Interactive Analysis; HCC, hepatocellular carcinoma; H3K27me3, Lys-27 in histone 3; HBV, hepatitis B virus; HCV, hepatitis C virus; HR, Hazard ratio; KEGG, Kyoto Encyclopedia of

Genes and Genomes; KM, Kaplan-Meier; LATS2, Large Tumor Suppressor 2;lncRNAs, long non-coding RNAs; miRNAs, microRNAs; PPI, protein-protein interaction; PRC2, polycomb-repressive complex 2; STAT3, signal transducer and activator of transcription 3; SUZ12, suppressor of zeste 12; TCGA, The Cancer Genome Atlas; UCSC, University of California, Santa Cruz. UALCAN, University of Alabama Cancer Database.

Declarations

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Not applicable.

Ethical approval

This article does not contain any studies with human participants performed by any of the authors.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analyzed during this study are included in this published article. The original miRNA and gene expression profiles were downloaded from the UCSC Xena (https://xenabrowser.net/datapages/).

Competing interests

The authors declare that they have no competing interests.

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Figures

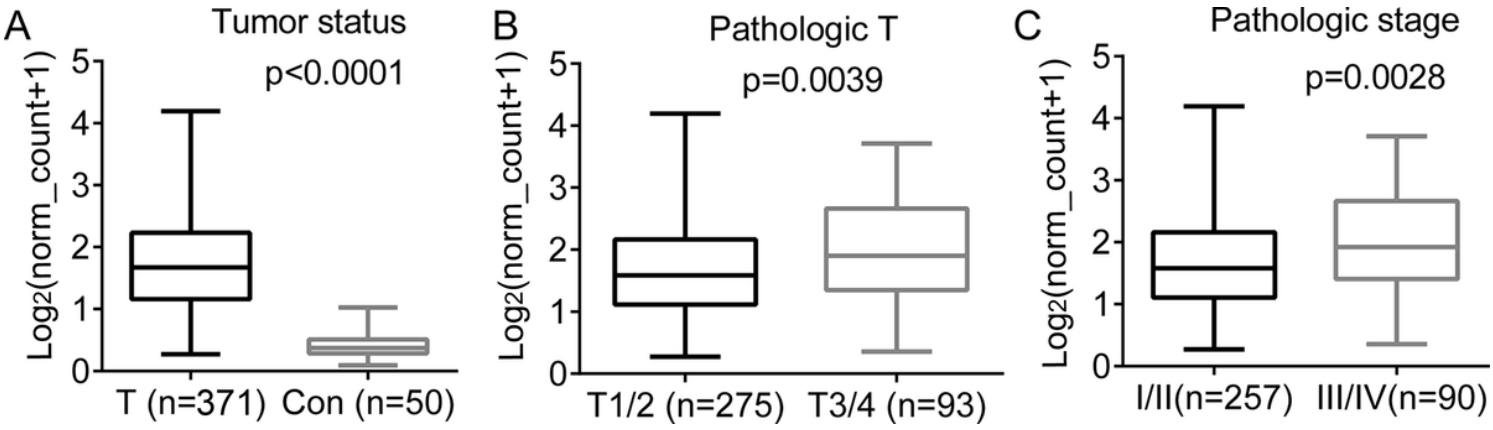

Figure 1

The expression profile of EZH2 in hepatocellular carcinoma tumors. A, difference in EZH2 level between tumor and normal control tissues. B-C, differences in EZH2 levels between tumor tissues with different pathologic T classifications and stages, respectively. Differences were analyzed using the non-parametric Mann-Whitney U test.

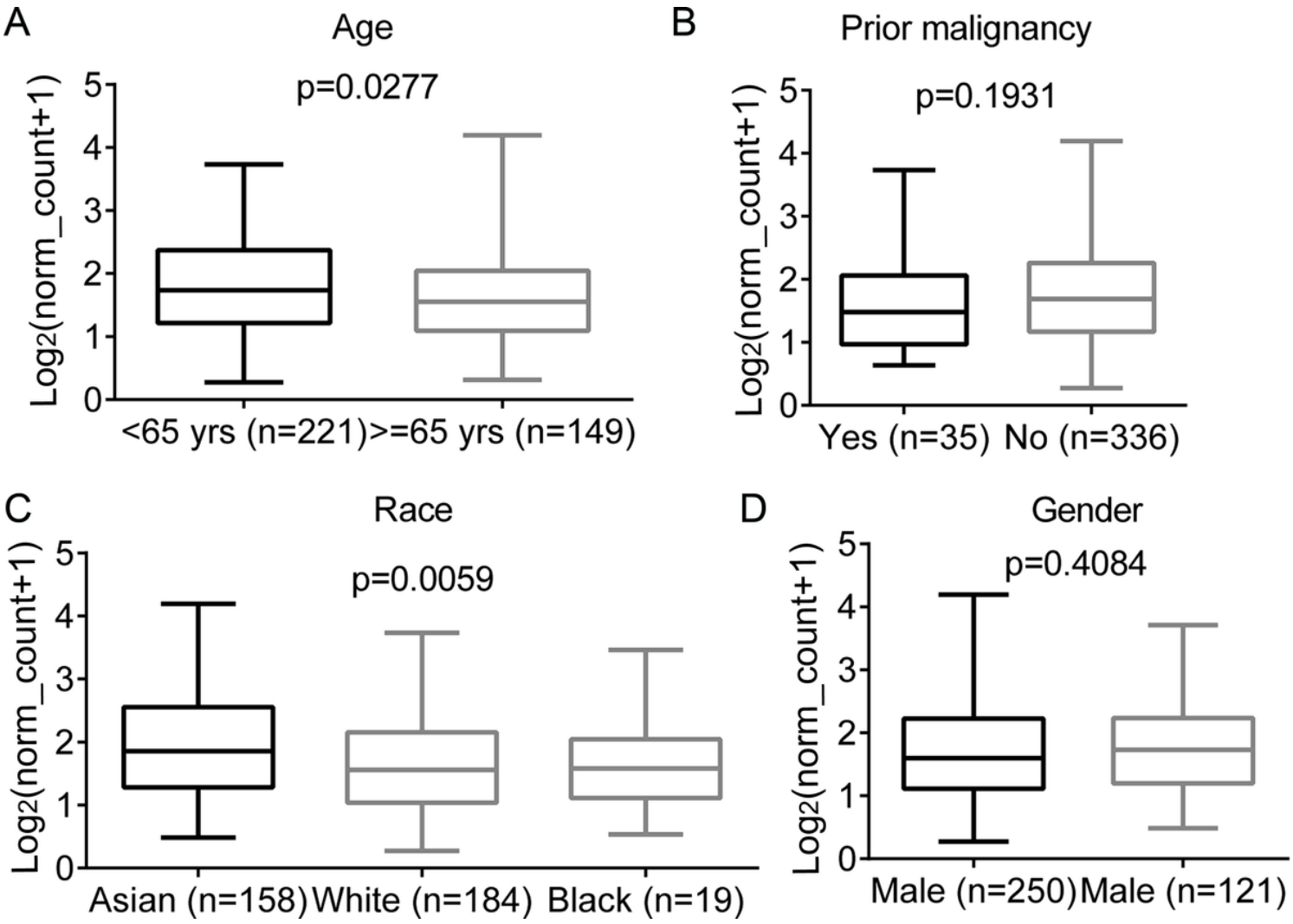

Figure 2

Expression profile of EZH2 in hepatocellular carcinoma tumors from patients with different clinical variables. The difference in EZH2 expression level tumors samples from patients aged over and younger 65 years (A), with and without prior malignancy (B), Asian, White, and Black (C), and male and female (D). Differences were analyzed using non-parametric Mann-Whitney U test (in figure A, B and D) and the Kruskal-Wallis H test (in figure C).

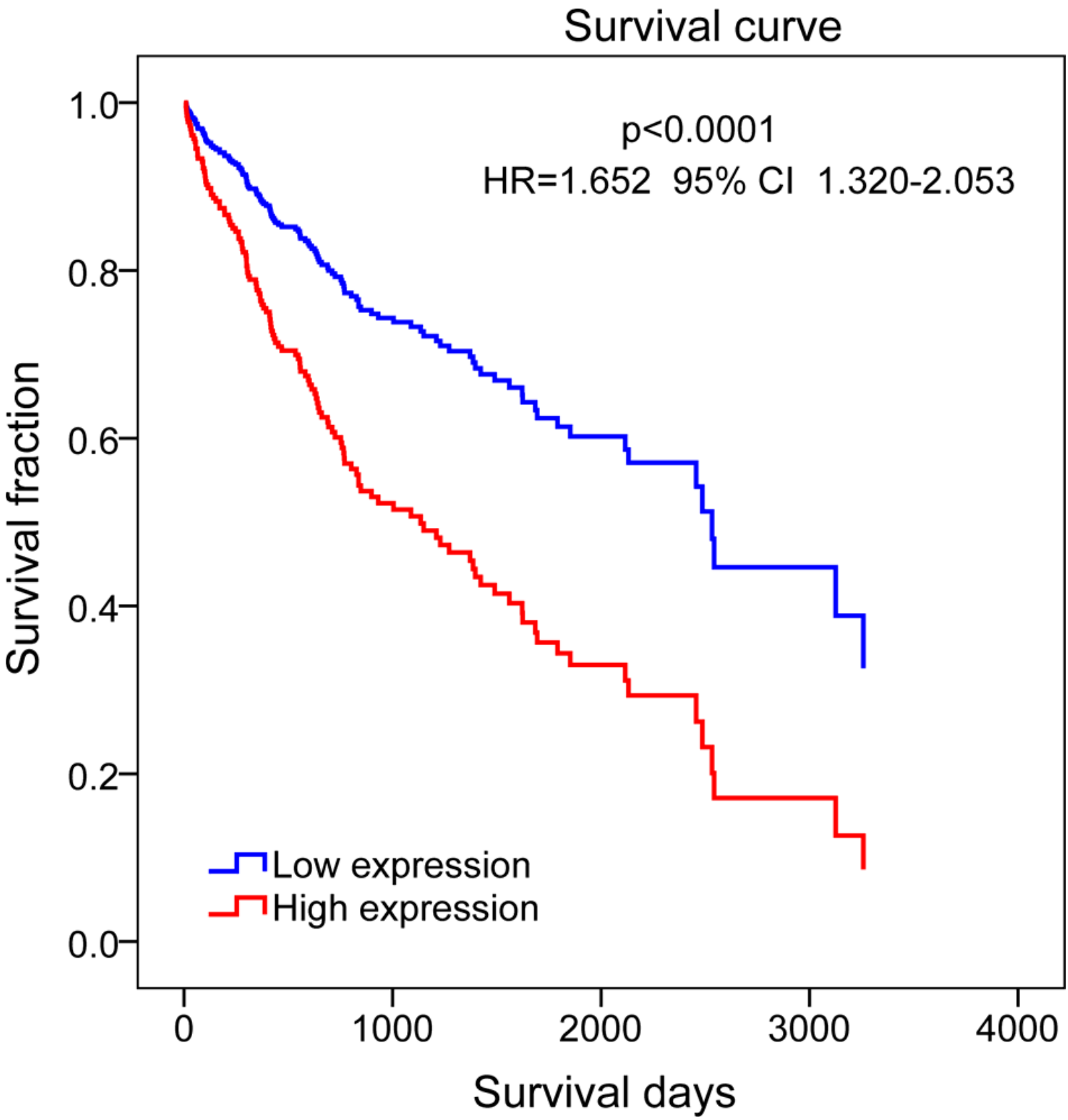

Figure 3

Cox regression survival analysis for EZH2 expression in hepatocellular carcinoma. The TCGA cohort (n=371) was used for this analysis. HR, Hazard ratio. CI, 95% confident interval.

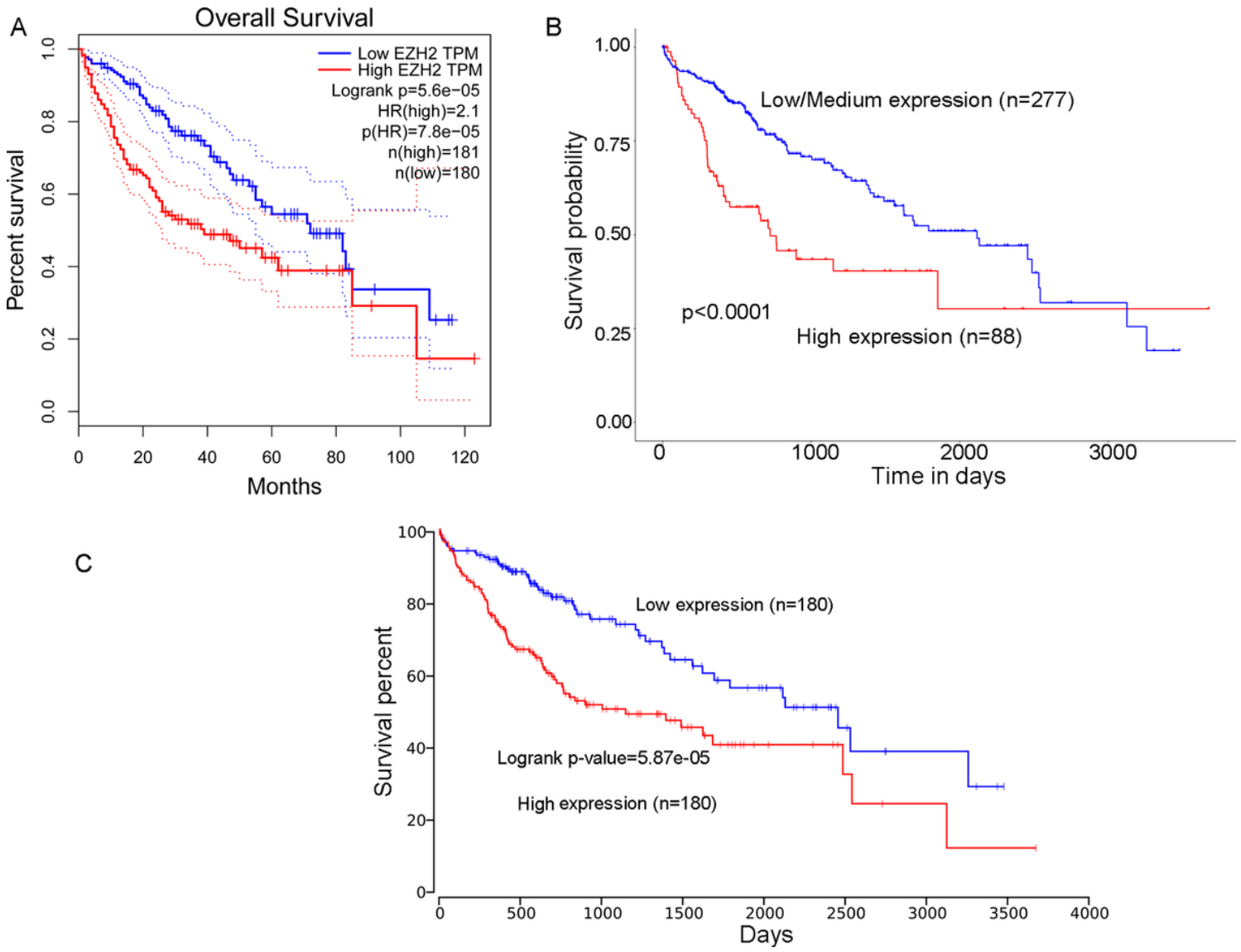

Figure 4

The correlation of EZH2 with hepatocellular carcinoma overall survival in the online databases. A, B, and C, the survival analysis in GEPIA, UALCAN, and Oncolnc database, respectively. All databases performed the log-rank test of Kaplan-Meier (KM) analysis.

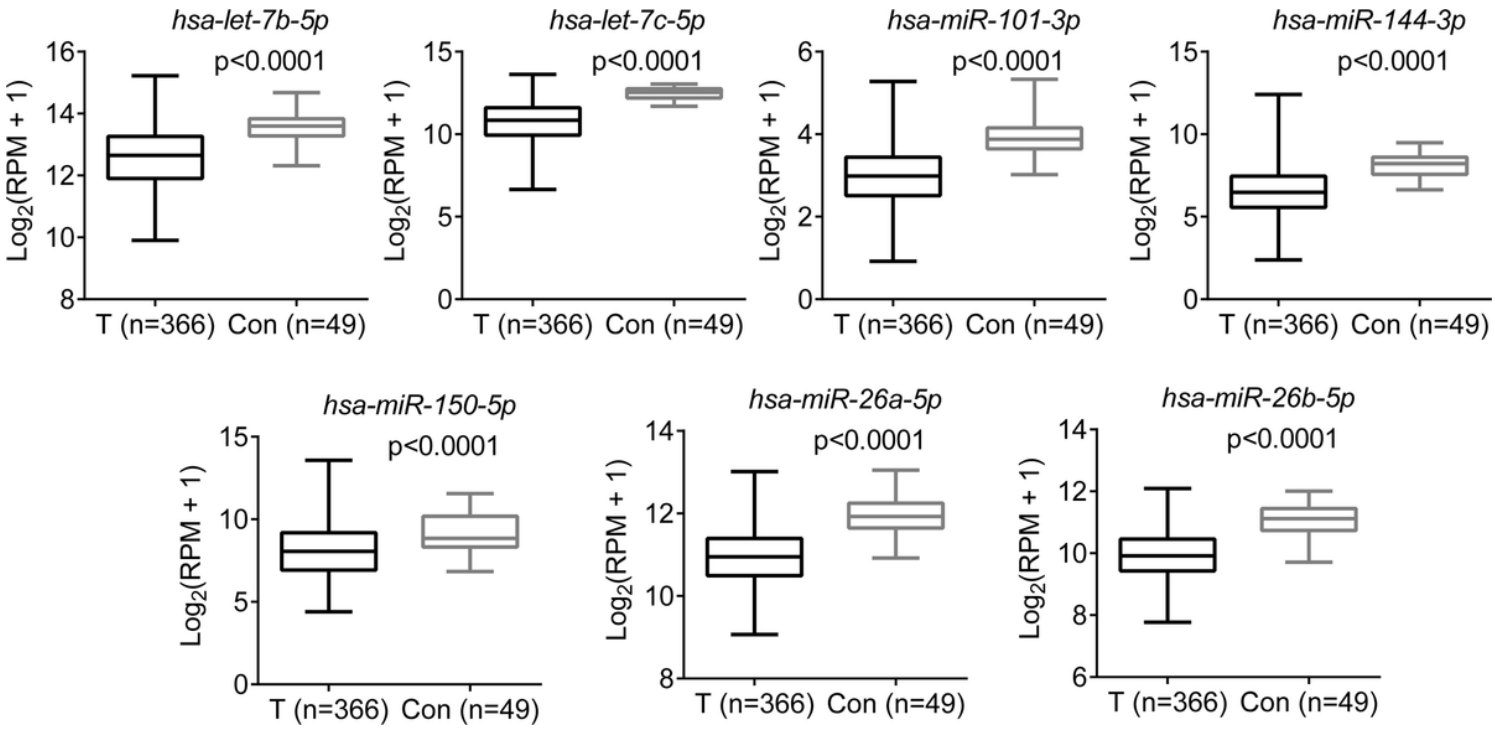

Figure 5

Expression profiles of seven miRNAs in hepatocellular carcinoma tumor and normal control tissues. The dataset was downloaded from The Cancer Genome Atlas database. Differences in EZH2 expression levels between two groups were analyzed using the non-parametric Mann-Whitney U test.

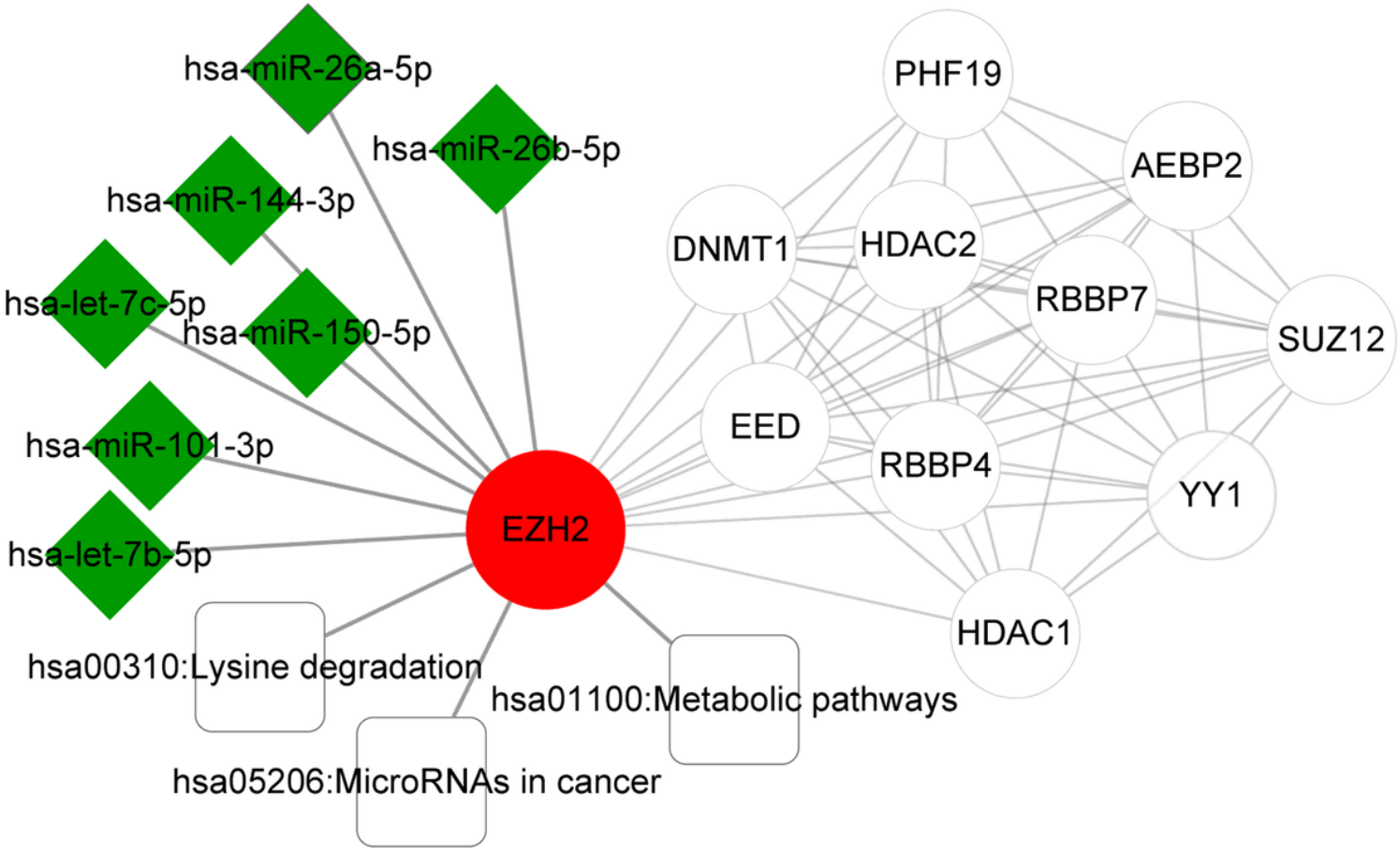

Figure 6

The miRNA-EZH2-pathway network in hepatocellular carcinoma. Upregulated EZH2 is shown by red node and downregulated miRNAs are indicated by green diamonds. Pathways and EZH2-associated genes are presented by empty squares and circles, respectively.