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Clinical Significance of Upregulation of EZH1 Expression in Hepatocellular Carcinoma Tissues

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ABSTRACT

Background & Aims: The incidence and mortality of hepatocellular carcinoma (HCC) are increasing. It is urgent to develop more effective HCC biomarkers for diagnosis and treatment. This project intends to verify the expression of enhancer of zeste 1 polycomb repressive complex 2 subunit (EZH1) and its mechanism in HCC.
Methods: This study integrates global microarray and high-throughput sequencing datasets, combined with internal immunohistochemistry, to analyze the expression and prognostic value of EZH1 in HCC. Functional enrichment analysis was conducted to investigate transcriptional targets, which were achieved by intersecting HCC over-expressed genes, EZH1 co-expressed genes and putative transcriptional targets. The relationship between EZH1 and anticancer drugs was detected by drug sensitivity analysis.
Results: In this study, 84 datasets from 40 platforms (3,926 HCC samples and 3,428 non-cancerous liver tissues) were included to show the high expression of EZH1 in HCC. Immunohistochemistry with 159 HCC samples and 62 non-HCC samples confirmed the high expression level. HCC patients with high EZH1 expression had worse survival prognoses. Gene ontology and Reactome analysis revealed that metabolism-related pathways, including autophagy, are critical for HCC. Interestingly, as one of the EZH1 potential transcriptional targets, autophagy-related 7 (ATG7) appeared in the above pathways. ATG7 was positively correlated with EZH1, upregulated in HCC, and mediated poor prognosis. Upregulation of EZH1 was found to be in contact with HCC anti-tumor drug resistance.
Conclusions: The upregulation of EZH1 expression can promote the occurrence of HCC and lead to poor clinical progression and drug resistance; these effects may be mediated by regulating ATG7.

Key words: EZH1 – hepatocellular carcinoma – prognosis – transcription factor – ATG7.

Abbreviations: ATG7: autophagy-related 7; EZH1: enhancer of zeste 1 polycomb repressive complex 2 subunit; GEO: Gene Expression Omnibus; GO: Gene ontology; HCC: hepatocellular carcinoma; SMD: standardized mean difference; ROC: receiver operating characteristic; sROC: summary ROC; tROC: time-dependent ROC; TCGA: the Cancer Genome Atlas; THPA: the Human Protein Atlas.

INTRODUCTION

Liver cancer is increasingly becoming one of the major diseases that endangers human life and health worldwide. Liver cancer is the sixth most common cancer in the world, but the mortality rate ranks third among all tumors [1], which has brought a heavy burden to the finances of countries around the world. In the new Global Cancer Report 2020, Asian liver cancer cases accounted for 72.5% of the world's cases, and the number of cases in China ranks first in liver cancer cases of Asia [2]. Hepatocellular carcinoma (HCC) is the principal type of liver cancer diagnosis and death [3]. Due to its high heterogeneity, most patients are diagnosed in the late stage, and the surgical treatment effect is not ideal, systemic treatment becomes extremely important for advanced HCC patients [4]. However, although the emergence of targeted drugs and immunotherapy has significantly improved the survival rate of patients receiving systemic drug therapy [5, 6], the multi-drug resistance of HCC to systemic drugs seriously restricts the prognosis of patients [7]. The 5-year survival rate of HCC patients is only 18%, and the incidence and mortality of HCC are still rising sharply in recent years [8, 9], thus more effective biomarkers for early diagnosis and prognosis of HCC are urgently needed. Therefore, elucidating the pathogenesis of

HCC, finding new potential biomarkers, and improving clinical treatment strategies are crucial for HCC patients.

The mechanism of enhancer of zeste 1 polycomb repressive complex 2 subunit (EZH1) in HCC needs further study. EZH1 has the function of histone methyltransferase and is the catalytic component of polycomb repressive complex2 (PRC2). It regulates the methylation of histone H3K27 and plays a role in the development of stem cells and the regulation or maintenance of tissue homeostasis, and epigenetic changes are closely related to cancer recoding [10, 11]. There are reports suggesting that overexpression of EZH1 is associated with inhibition of apoptotic proteins and induction of cyclins [12]. EZH1 has been shown to be associated with tumors. For example, EZH1 is highly expressed in T-cell lymphoma and breast cancer [13]. The elevation of EZH1 is reported to be linked to tumor diagnosis and prognosis. For example, high expression of EZH1 improves the overall survival and progression-free survival of T lymphoma cells [14]. In recent years, the targeted inhibition of EZH1 has shown beneficial effects in tumor therapy. However, the specific mechanism and transcriptional regulatory targets of EZH1 in HCC are still unclear, exploring the expression status, transcriptional targets and clinical significance of EZH1 in HCC could bring wonderful benefits to HCC patients.

METHODS

Microarrays and High-Throughput Sequencing Datasets

Gene microarrays and high-throughput sequencing datasets of HCC tissue samples were searched and downloaded from Gene Expression Omnibus (GEO), ArrayExpress, SRA, and The Cancer Genome Atlas (TCGA). To enlarge the sample size of the normal liver, GTEx was combined with the RNA sequencing data from TCGA. Datasets in the same platform were integrated, and the batch effects were removed using sva (https://bioconductor.org/packages/sva/) and limma (https://bioconductor.org/packages/limma/) package. Integrated dataset information is shown in Table I.

mRNA Expression Analysis

The expression status of EZH1 was comprehensively analyzed by drawing a forest plot of the standardized mean difference (SMD) and summary receiver operating characteristic (sROC) curves. The prognostic value of EZH1 in HCC patients was explored using Kaplan-Meier (K-M) survival analysis. After using the survminer package (https://github.com/kassambara/survminer) to find the best survival analysis threshold, time-dependent receiver operating characteristic (tROC) curve was used to detect the performance of survival prediction.

Immunohistochemistry (IHC)

With patient consent, 159 samples from HCC patients and 62 samples from non-HCC patients were harvested from the First Affiliated Hospital of Guangxi Medical University (the study was authorized by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University and obtained the informed consent of patients or their families). Expression levels of EZH1 protein were detected using the IHC staining technique with anti-EZH1 antibody (1/50 dilution, rabbit, polyclonal antibody, ab64850). IHC scores were performed by professional pathologists. Student's t-test in SPSS 23.0 was used to compare the difference of EZH1 protein expression between HCC and non-cancerous tissues, and to explore the relationship between EZH1 protein expression and corresponding clinicopathologic features in HCC tissues. We also used the Human Protein Atlas (THPA), a database that includes immunohistochemical images of 20 common malignancies, to investigate EZH1 protein levels in HCC and normal liver tissue.

Differential Expression Analysis

Over-expressed genes were identified from HCC tissue compared with normal liver tissue. The HCC over-expressed genes were computed by using the following criteria: (1) number of datasets >3; (2) standardized mean difference (SMD) >0; and (3) $p<0.05$.

Co-Expression Analysis

EZH1 co-expressed genes were identified from HCC tissue by using the following criteria: (1) Pearson correlation coefficient ≥0.30, and (2) $p<0.05$.

Chromatin Immunoprecipitation Followed by Sequencing Analysis

Putative transcriptional targets of the EZH1 factor were downloaded from Cistrome DB datasets. After intersecting with HCC over-expressed genes and EZH1 co-expressed genes, putative transcriptional targets of EZH1 were filtered by a score ≥1.

Enrichment Analysis

Gene ontology (GO) and Reactome analysis were conducted to annotate putative transcriptional targets of EZH1. Protein-to-protein interactions were built using EZH1 factors and its transcriptional targets.

Verification of EZH1 Transcriptional Targets

The transcriptional activity of EZH1 was visualized by analyzing the binding peaks in the promoter regions of the targets. We assessed the expression level and prognostic value of EZH1 transcriptional targets.

Drug Susceptibility Analysis

Finally, the association between EZH1 mRNA and the sensitivity of anti-cancer drugs was determined by calculating the Pearson correlation coefficient between EZH1 and IC50.

RESULTS

EZH1 was Upregulated and Mediated Poor Prognosis in HCC

A total of 3,926 HCC samples and 3,428 non-HCC samples from 84 datasets (Table I) were obtained by integrating the GEO, ArrayExpress, SRA, TCGA, and GTEx databases.

Table I. HCC data matrices included

ID	Platform	Public year	Country	HCC samples	Non-HCC samples
E-MTAB-8887	-	2021	Switzerland	23	17
GSE31370	GPL10558	2012	South Korea	15	5
GSE36376	GPL10558	2020	South Korea	240	193
GSE36411	GPL10558	2012	South Korea	42	42
GSE39791	GPL10558	2014	USA	72	72
GSE57957	GPL10558	2014	Singapore	39	39
GSE76427	GPL10558	2017	Singapore	115	52
GSE114564	GPL11154	2020	South Korea	63	45
GSE148355	GPL11154	2021	South Korea	0	45
GSE63863	GPL11154	2015	China	12	12
GSE65485	GPL11154	2021	China	50	5
GSE73708	GPL11154	2017	USA	8	4
GSE81550	GPL11154	2016	USA	3	3
GSE87592	GPL11154	2018	China	27	26
GSE57727	GPL14951	2015	Spain	57	5
GSE98617	GPL14951	2019	Spain	36	13
GSE113996	GPL16043	2021	China	20	20
GSE74656	GPL16043	2015	China	5	5
GSE104310	GPL16791	2017	China	12	8
GSE63018	GPL16791	2017	USA	8	9
GSE77509	GPL16791	2017	China	20	20
GSE94660	GPL16791	2018	USA	21	21
GSE97214	GPL16791	2017	China	9	9
GSE140845	GPL16791	2019	India	5	5
GSE112221	GPL16791	2019	USA	4	6
GSE101728	GPL21047	2019	China	7	7
GSE98269	GPL21047	2017	China	3	3
GSE12941	GPL5175	2010	Japan	10	10
GSE84005	GPL5175	2017	China	38	38
GSE101685	GPL570	2019	China	24	8
GSE102079	GPL570	2018	Japan	152	105
GSE107170	GPL570	2018	Italy	118	189
GSE112790	GPL570	2019	Japan	183	15
GSE121248	GPL570	2018	Singapore	70	37
GSE17548	GPL570	2013	Turkey	17	20
GSE19665	GPL570	2010	Japan	10	10
GSE29721	GPL570	2011	Canada	10	10
GSE33006	GPL570	2012	China	3	3
GSE41804	GPL570	2013	Japan	20	20
GSE45436	GPL570	2014	China	93	41
GSE6222	GPL570	2008	China	10	2
GSE62232	GPL570	2014	France	81	10
GSE6764	GPL570	2007	USA	35	40
GSE99807	GPL570	2020	China	4	4
GSE84402	GPL570	2017	China	14	14
GSE14323	GPL571	2009	USA	55	60
GSE14520	GPL571	2010	USA	22	21
GSE17967	GPL571	2009	USA	16	47
GSE9839	GPL571	2008	Switzerland	3	3

Table I (continued)

GSE45050	GPL6244	2017	USA	6	10
GSE64041	GPL6244	2016	Switzerland	60	65
GSE117361	GPL6480	2021	China	2	2
GSE54236	GPL6480	2020	Italy	81	80
GSE87630	GPL6947	2017	South Korea	64	30
GSE89377	GPL6947	2017	South Korea	40	67
GSE25599	GPL9052	2013	China	10	10
GSE77314	GPL9052	2016	China	50	50
GSE10143	GPL5474	2008	USA	80	307
GSE114783	GPL15491	2019	China	10	26
GSE115018	GPL20115	2018	China	12	12
GSE124535	GPL20795	2019	China	35	35
GSE125469	GPL20301	2019	China	3	3
GSE128274	GPL18573	2019	China	4	4
GSE14520	GPL3921	2010	USA	225	220
GSE166163	GPL23126	2021	China	3	3
GSE20140	GPL18461	2011	USA	35	34
GSE22058	GPL6793	2010	USA	100	97
GSE22405	GPL10553	2014	USA	24	24
GSE25097	GPL10687	2011	USA	268	289
GSE33294	GPL10999	2013	China	3	3
GSE46408	GPL4133	2019	China	6	6
GSE46444	GPL13369	2014	USA	88	48
GSE50579	GPL14550	2013	Germany	67	10
GSE54238	GPL16955	2014	USA	26	30
GSE55048	GPL9115	2014	China	4	4
GSE56545	GPL15433	2016	USA	21	21
GSE57555	GPL16699	2015	Japan	5	5
GSE59259	GPL18451	2015	Italy	8	8
GSE60502	GPL96	2015	China	18	18
GSE63898	GPL13667	2015	USA	228	168
GSE67764	GPL17077	2015	China	3	6
GSE76311	GPL17586	2017	USA	62	59
TCGA-GTEx	-	-	-	371	276

First, Begg's test showed no significant publication bias in the datasets (Fig. 1a, p=0.8521). The SMD forest plot showed that EZH1 was significantly upregulated in HCC tissues compared with non-HCC tissues [SMD=0.33, 95% confidence interval (CI): 0.14 – 0.52; Fig. 1b). The sROC curve showed that EZH1 had moderate discriminant ability and weak specificity and sensitivity (Fig. 1c–e, Supplementary file: Fig. S1a–b). Survival analysis showed that patients with a high expression of EZH1 showed worse survival in HCC (HR=2.157, p<0.05, Fig. 2a). tROC curve shows normal performance (Fig. 2b), and the area under the curve (AUC) values were all greater than 0.5 (AUC at 3, 5, and 7 years).

The Expression Level of EZH1 Protein in HCC

To verify the expression of EZH1 in tissues, we detected EZH1 protein levels in 159 HCC and 62 non-HCC tissues. The IHC staining scoring criteria are shown in Table II. IHC staining showed that EZH1 was significantly upregulated in HCC tissues compared with the control group (p<0.001, Table III, Fig. 2c); the AUC also shows its superiority in distinguishing cancer and non-cancer ability (AUC=0.9524, Fig. 2d), and EZH1 was strongly stained in the nucleus (Fig. 2e–f). The above results indicate that the EZH1 protein level is upregulated in HCC tissues. In addition, the results in the THPA database also verify this (Fig. 2g–h). Other pathological parameters were not statistically significant (Table III).

Exploration of EZH1 Transcriptional Targets

Through the intersection of HCC overexpression genes, EZH1 co-expression genes, and transcription factor (TF) targets in chromatin immunoprecipitation followed by sequencing (ChIP-seq), 566 potential transcriptional targets were finally obtained (Fig. 3a).

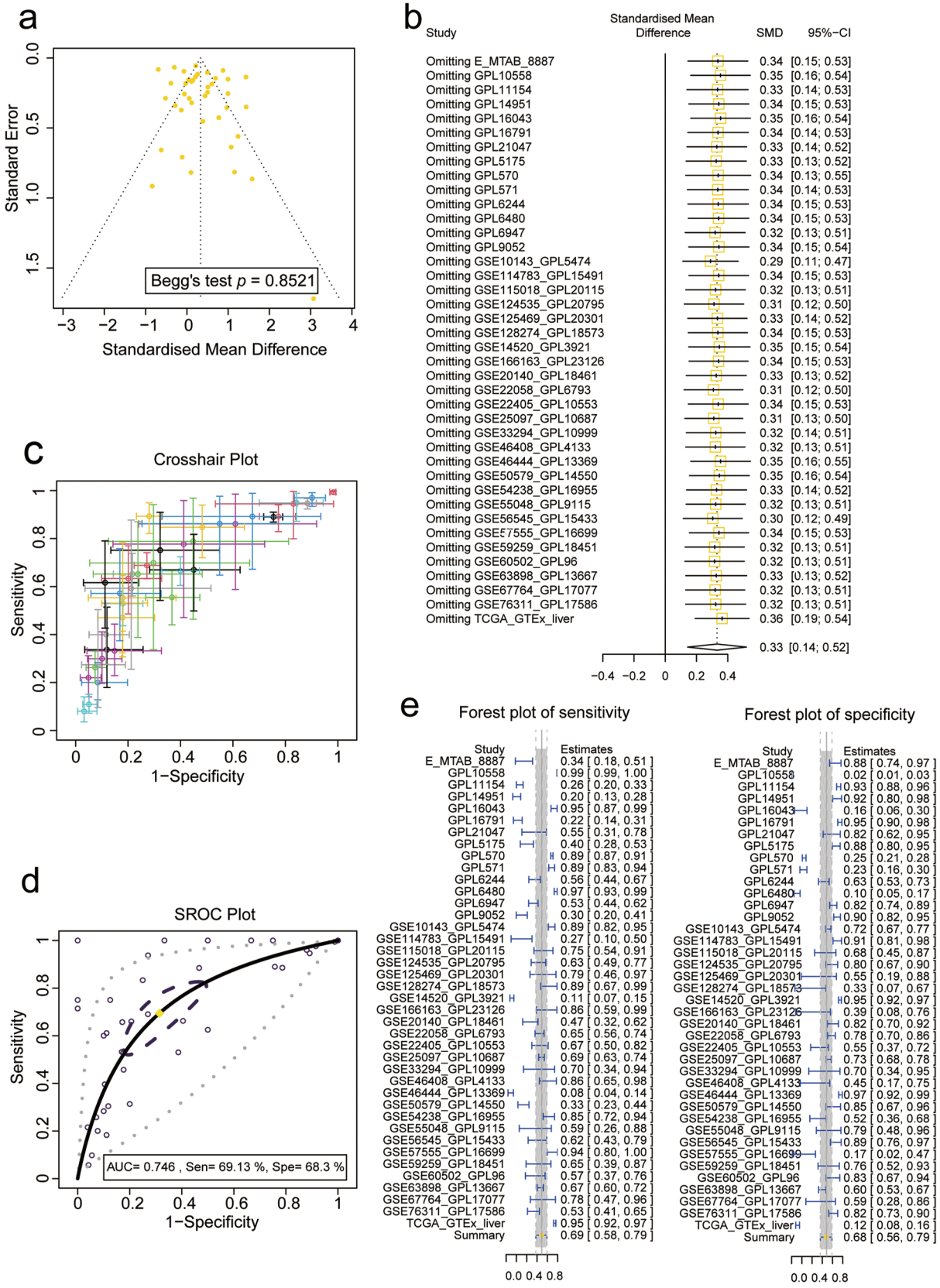

Fig. 1. High expression of EZH1 based on global HCC data. Totally, we collected 37 platform matrices to explore the expression data of EZH1 in HCC. (a) There was a high stability of the quantitative synthesis result, for the insignificant publication bias suggested from the funnel plot (Begg's test: p=0.8521). (b) EZH1 was upregulated for the investigation in 3926 HCC tissues and 3428 non-HCC tissues showed in the standard mean difference forest plot. (c-d) The result showed a moderate discriminatory ability of EZH1 from the summary characteristics operating curve, with weak sensitivity and moderate specificity (e).

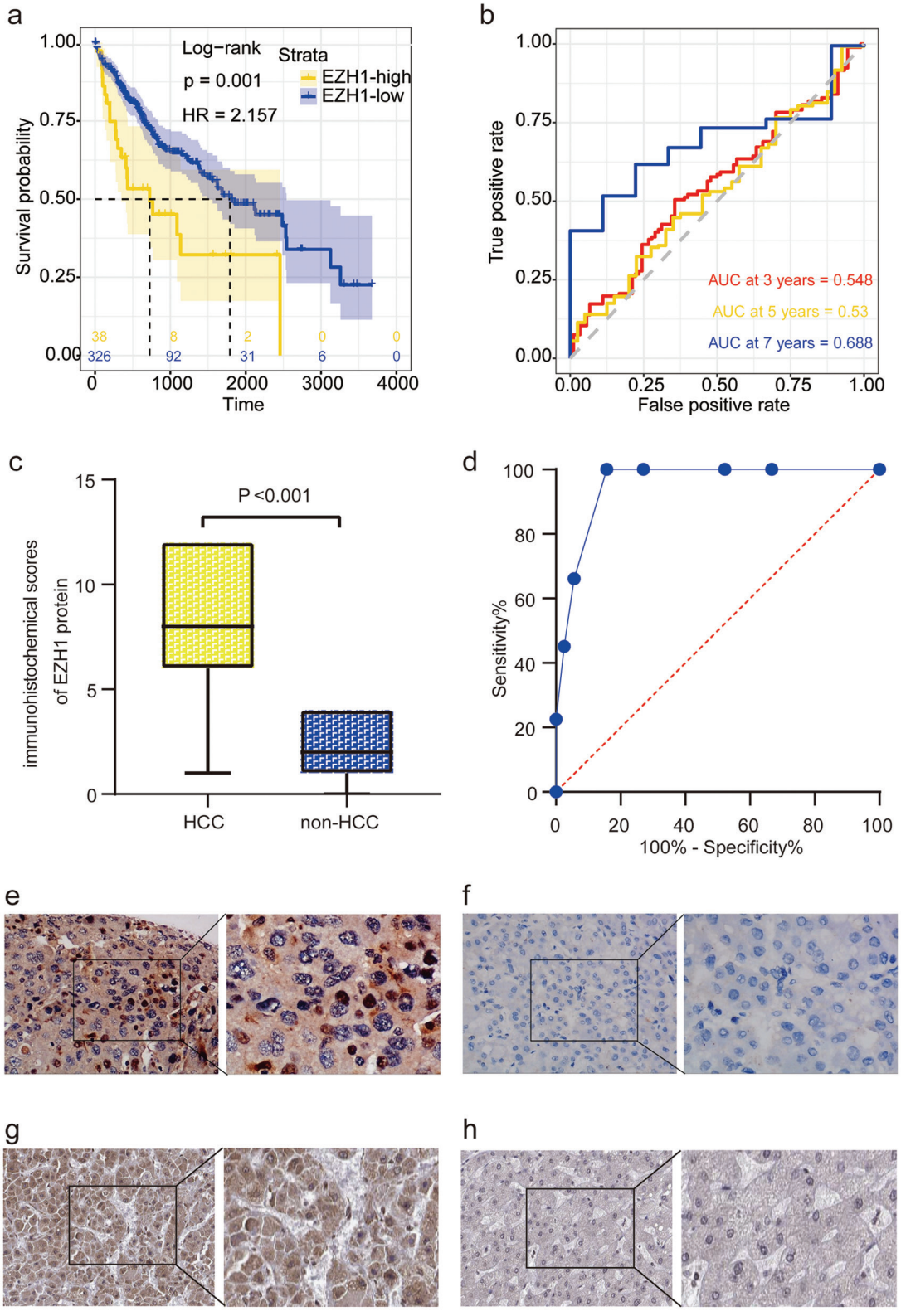

Fig. 2. Survival analysis of EZH1 mRNA and EZH1 protein levels in HCC. (a) Survival analysis curve showed that the patients with overexpression of EZH1 had shorter survival time. (b) tROC curve of 3-, 5-, and 7-years overall survival suggested the ability of the risk model to predict the prognosis effectively. Boxplot showed the high expression of EZH1 protein in HCC (c) and the increased protein expression displayed a strong ability in differentiating HCC from non-HCC tissue samples (d). HCC tissue showed high EZH1 expression (e) compared with paracancer liver tissue (f) by internal immunohistochemistry (magnification, ×200 and ×400). The same result showed in tumor tissue specimens (g) and normal liver tissue specimens (h) via THPA website analysis.

Table II. Details of the IHC staining scoring criteria

Staining intensity	Total score (staining intensity * percentage of positive cells)				
	0 (< 5%)	1 (5%–25%)	2 (26%–50%)	3 (51–75%)	4 (76–100%)
0 (no staining)	0	0	0	0	0
1 (light staining)	0	1	2	3	4
2 (medium staining)	0	2	4	6	8
3 (strong staining)	0	3	6	9	12

And we performed functional enrichment analysis of these potential transcriptional targets. The GO analysis revealed that the potential transcriptional targets were significantly enriched in macroautophagy, regulation of autophagy, negative regulation of catabolic process, protein catabolic process, ATP-dependent activity, acting on RNA and ATP hydrolysis activity. RHOU GTPase cycle was reflected in the Reactome enrichment (Fig. 3b–c). Taken together, the results showed the importance of metabolic-related pathways, including autophagy, in HCC.

Verification of EZH1 Transcriptional Targets

To further clarify the EZH1 transcriptional targets, we performed PPI analysis on the genes in the two autophagy pathways and EZH1, respectively (Fig. 4a–b). Combined with the binding peak of the target promoter region in the ChIP-seq data, multiple genes in the two autophagy pathways were predicted to be potential transcriptional targets of EZH1. Interestingly, autophagy-related 7 (ATG7) appears not only in autophagy-related pathways, including macroautophagy, regulation of autophagy, but also other metabolism-related pathways, including negative regulation of catabolic process

Table III. The clinicopathologic parameters of 159 HCC samples and 62 cases of normal samples by IHC

Clinicopathological parameter	Number	Mean ± SD	T value	p
Tissue				
HCC	159	8.484±3.119		
Non-HCC	62	2.000±1.589	20.310	<0.001
Gender				
Male	144	8.389±3.102		
Female	8	9.125±2.799	-0.656	0.513
Age(years)				
≥60	21	8.238±2.862		
<60	131	8.458±3.126	0.303	0.763
Stage				
I	9	8.444±3.539		
II-III	143	8.426±3.066	0.017	0.987
T				
T3	36	8.472±3.753		
T2	116	8.414±2.862	0.086	0.932

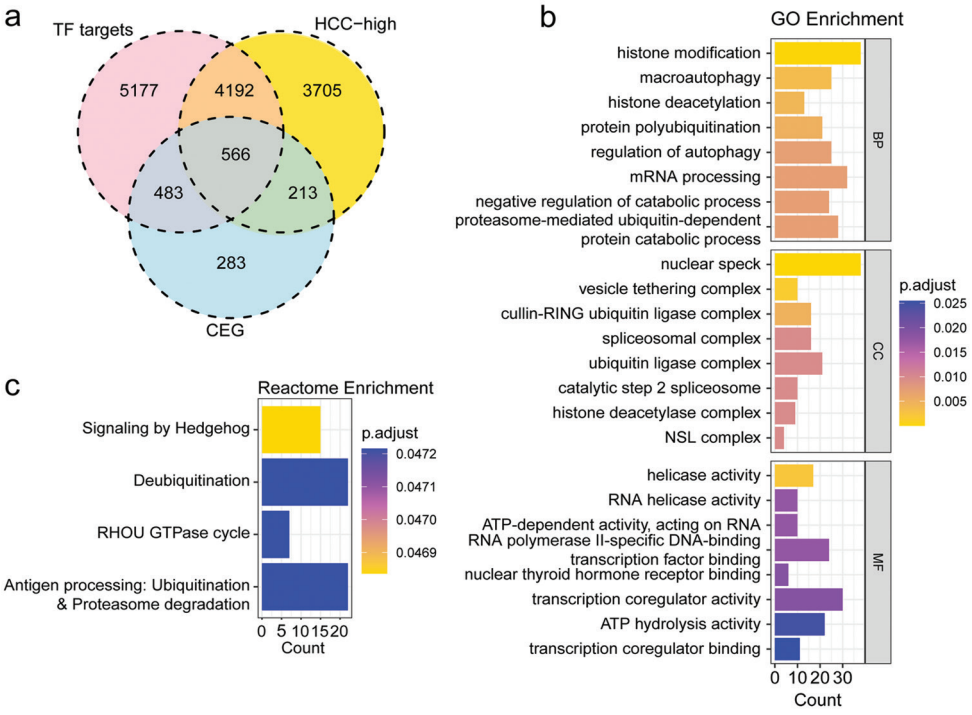

Fig. 3. Exploring transcriptional targets of EZH1. Transcriptional targets were identified through intersecting by HCC over-expressed genes and EZH1 co-expressed genes, putative transcriptional targets of EZH1 factor downloaded from Cistrome DB datasets(a). GO (b) and Reactome (c) enrichment pathways of the putative transcriptional targets showed the enrichment of autophagy and energy metabolism-related pathway.

(Fig. 4a,b,d). Moreover, in the transcription initiation region of ATG7, we found a significant signal peak of EZH1 (Fig. 4c), and the correlation analysis showed a positive correlation between EZH1 and ATG7 (R=0.44, p<0.001, Fig. 4e). The expression of ATG7 in the global HCC cohort also showed an upward trend (SMD=0.77, 95%CI: 0.57–0.96, Fig. 5a). Consistent with EZH1, the upregulation of ATG7 was found to be linked to poor prognosis (ATG7: HR=2.053, p<0.001, Fig. 5b–c).

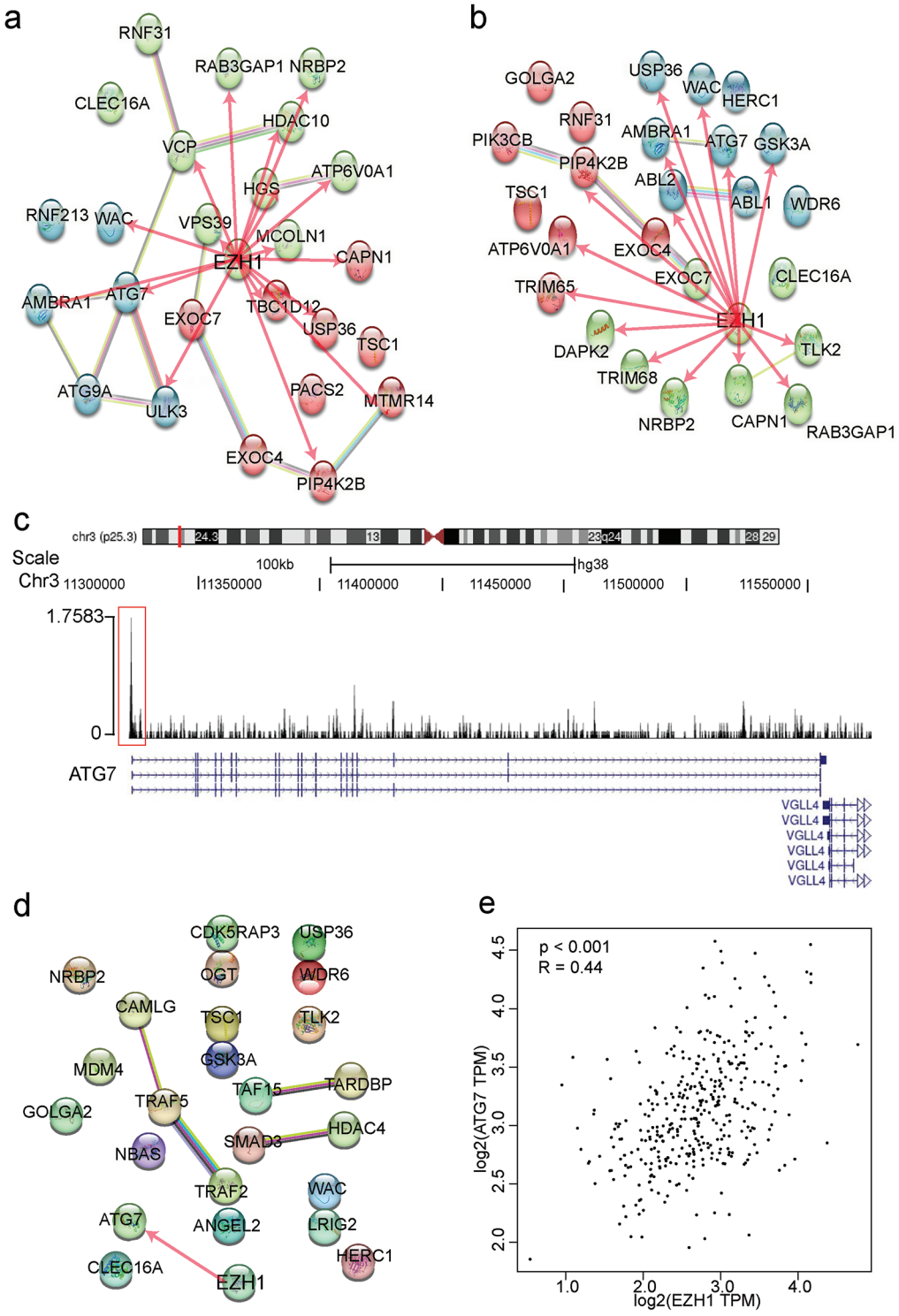

Fig. 4. Verification of EZH1 transcriptional targets. (a-b) The interplay between the EZH1 and transcriptional targets was analyzed in a protein-protein interaction network. (c) ATG7 was predicted as a transcriptional targets of EZH1 in HCC by the ChIP-seq data downloaded from Cistrome DB. (d) ATG7 appeared in the negative regulation of catabolic process pathway. (E) EZH1 had a positive correlation with ATG7.

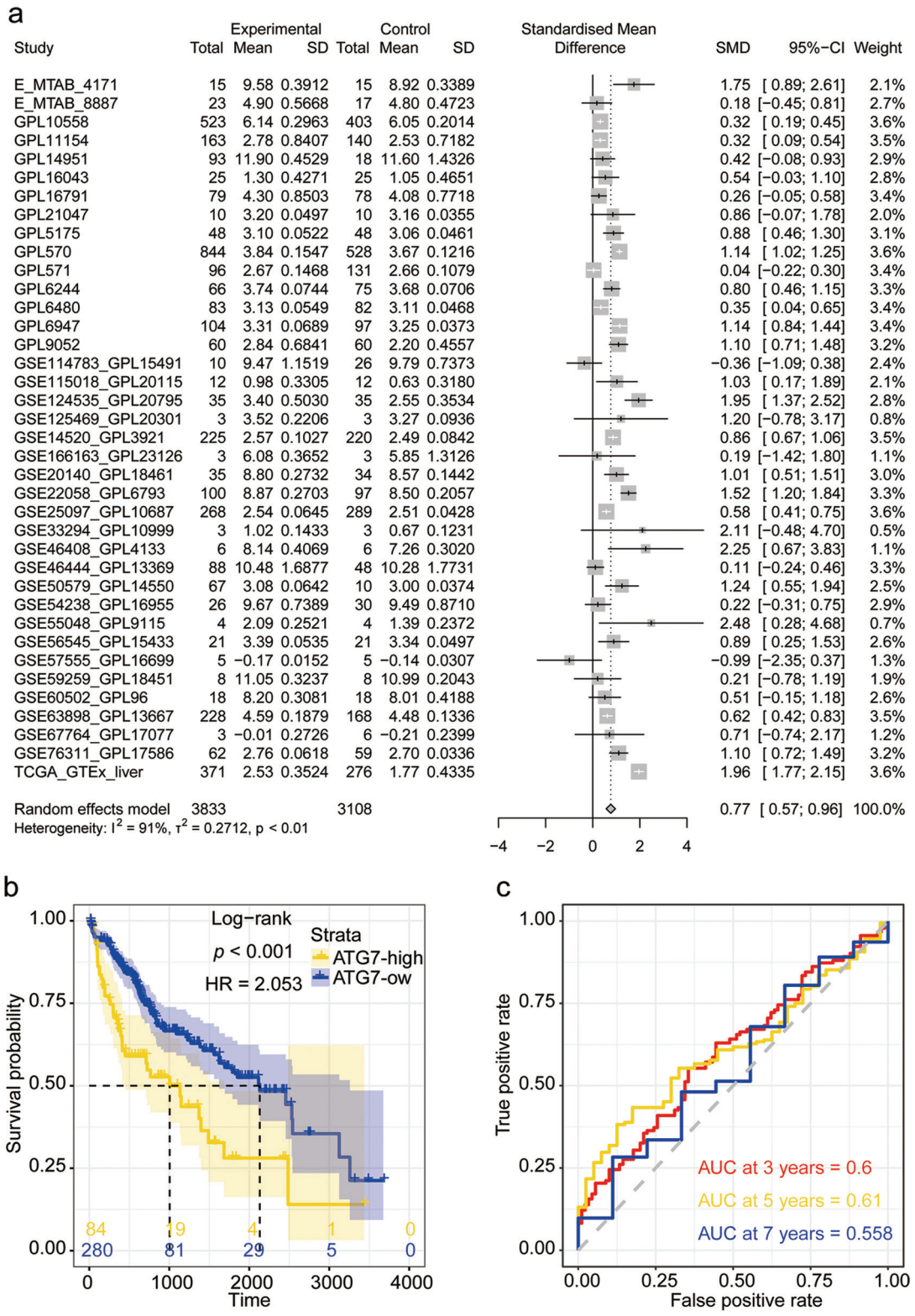

Fig. 5. The high expression of ATG7 in HCC and its significance. (a) ATG7 was highly expressed in HCC based on global data. (b) HCC patient group with high expression of ATG7 had a worse prognosis. (c) tROC curve showed a low predictive ability.

Upregulation of EZH1 Showed Positive Association with Drug Resistance

Lastly, we explored the correlation between EZH1 and multiple drug sensitivity. The results suggested that the high expression of EZH1 in HCC tissues was associated with the upregulation of IC50 in most anticancer drugs; the high expression of EZH1 may promote drug resistance in HCC (Fig. 6a–b).

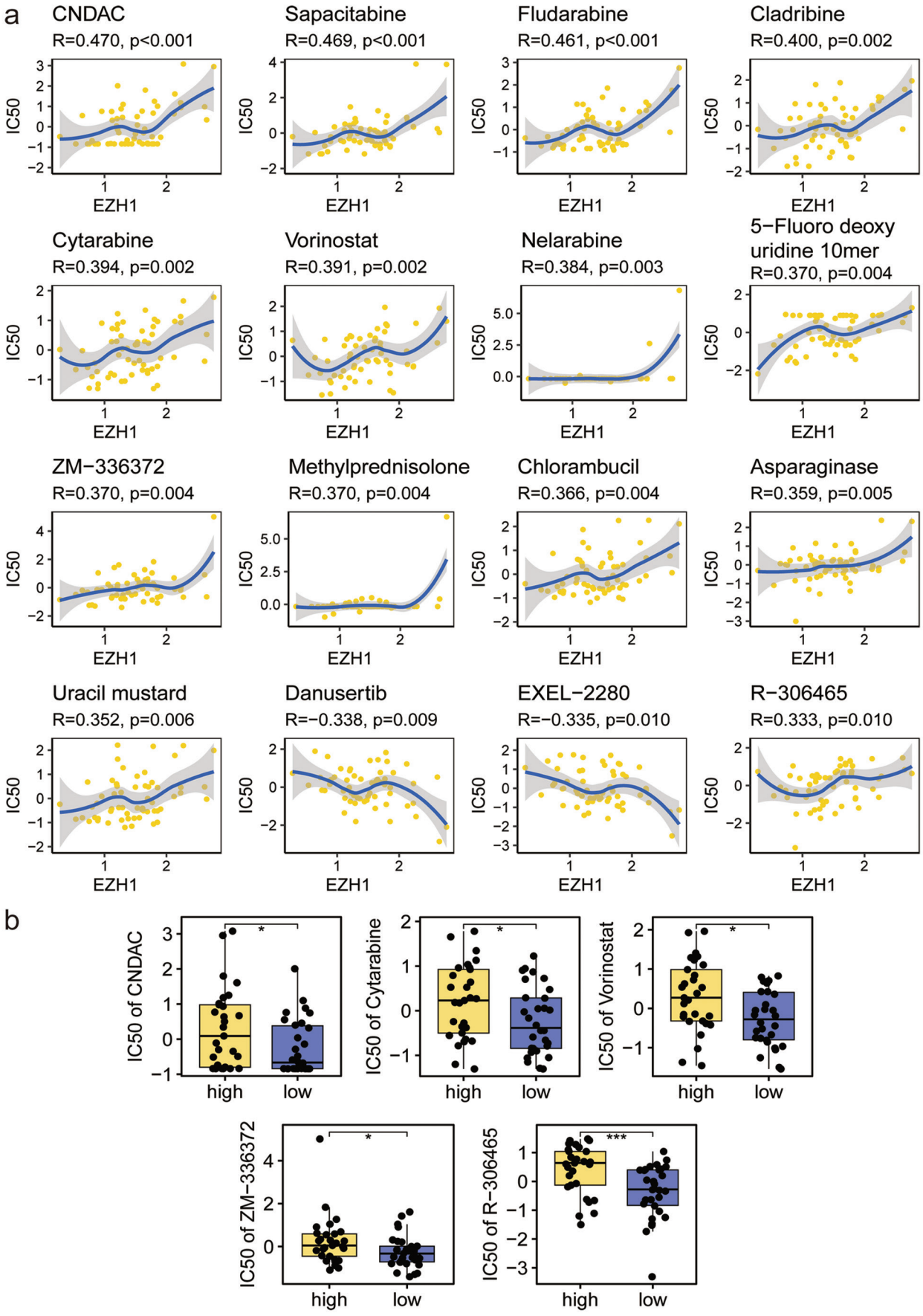

Fig. 6. The association between expression of EZH1 and drug sensitivity. Scatter plots (a) and boxplot (b) suggested that the upregulation of EZH1 was related to the resistance of antitumor drugs.

DISCUSSION

This study found that EZH1 mRNA and protein were upregulated in HCC, and its overexpression was related to poor prognoses. This study is the first to comprehensively explore the clinicopathological significance and molecular basis of EZH1 in HCC using global multicenter RNA-seq datasets and microarray data. EZH1 is deemed to be relevant to the occurrence and development of tumors. Previous studies showed that in triple-negative breast cancer, the expression levels of EZH1 protein and mRNA show a down regulation [15], while EZH1 overexpression significantly eliminates the function of TRIM21 protein to induce apoptosis of cancer cells in gastric cancer [16]. Therefore, the role of EZH1 in different tumors is complex. After the integration of multi-center microarray and sequencing data, we found that EZH1 mRNA showed a clear high expression in HCC tissues, suggesting that EZH1 was considerable for the occurrence and development of HCC. Differential gene expression assessment plays a fundamental role in exploring its effect in the development of tumors. Compared with other cancer single-gene detection, our exploration of EZH1 expression in HCC is a large sample using an advanced integration algorithm by calculating the SMD value and drawing a sROC curve, which has distinct advantages. In this study, 3926 HCC samples and 3428 normal liver tissue samples used to analyze EZH1 expression came from global public data, which was in line with the concept of evidence-based medicine. The results of the sROC curve, sensitivity, and specificity suggest the credibility of our work. In addition, IHC results also supported the high expression of EZH1 in HCC at the protein level. The K-M survival analysis implied that overexpression of EZH1 showed a lower survival rate of HCC patients compared to the control group.

This study further explored the transcriptional targets of EZH1 to clarify the mechanism of EZH1 in HCC. It is worth noting that a large number of transcriptional targets are enriched in the autophagy pathway. Studies have shown that metabolic changes are signs that cancer cells are different from normal cells [17]. Two major cellular pathways are included in metabolic adaptation, one is the production of adenosine triphosphate from mitochondrial oxidative phosphorylation to glycolysis, and the other is the circulation of intracellular components through autophagy [18]. Autophagy is the process of degrading damaged organelles and misfolded proteins in cells, which is important for maintaining cell homeostasis. Autophagy may promote or inhibit tumor growth in different situations. For example, tumor-prone mouse models with RAS pathway activation show a lack of autophagy, which leads to pre-tumor lesions and increased tumor incidence, suggesting that cancer development may be prevented by autophagy, while tumor cells increase autophagy flux through a variety of mechanisms that contribute to their growth in pancreatic cancer [19]. In HCC, the expression of ATG2A and ATG14 has been shown to drive hypoxia-induced autophagy and the malignant progression of HCC [20]. Therefore, we speculate that EZH1 may play a role in the development of HCC by regulating autophagy. In addition, recent studies have shown that autophagy was associated with drug resistance in anti-tumor therapy, such as in cervical cancer [21]. And autophagy inhibitors were found to have a surprisingly effective therapeutic effect when combined with molecular targeted or chemotherapeutic drugs in HCC [22]. However, there are still many difficulties in exploring the combination of autophagy regulation and existing HCC treatment regimens. The significant enrichment of EZH1 putative transcriptional targets in the autophagy-related pathway provides a new theoretical basis for the treatment of autophagy inhibition in HCC. In addition, EZH1 transcriptional targets have also been found to be enriched in multiple pathways related to other pathways of metabolic adaptation, in which tumor cells promote cell proliferation by increasing energy supply. For example, fat can induce glucose metabolism in untransformed hepatocytes to promote liver tumorigenesis. Mitochondrial dysfunction causes mitochondrial oxidative phosphorylation defects and reactive oxygen species production to promote HCC progression [23]. As a homologue of EZH1, there are many overlaps in the functions of EZH1 and EZH2. It has been reported that the transformation of myeloproliferative tumors into leukemia can be mediated by the metabolism of branched-chain amino acids enhanced by EZH2 inactivation [24]. EZH1 may be linked to metabolic recoding. However, although research on metabolism and cancer has been accumulated for decades, the progress of targeted metabolic pathways to achieve precise treatment of cancer is slow, and the clinical benefits of cancer patients are limited [25]. More in-depth research needs to be carried out to develop effective drugs. The interaction between autophagy and energy metabolism was found to play a vital role in the tumorigenesis and development of HCC. For example, autophagy can promote metastasis and glycolysis by upregulating MCT1 expression and Wnt/β-catenin signaling pathway activation in HCC cells [26]. Clinical advanced progression will occur in HCC because of the production of reactive oxygen species, which is mediated by oxidative phosphorylation and autophagy [27]. In this study, we found that EZH1 transcriptional targets were co-enriched in autophagy and energy metabolism-related pathways. We speculated that EZH1 may promote HCC progression by regulating autophagy/energy metabolism.

ATG7 was found to be a potential transcriptional target of EZH1. Verification of potential transcriptional targets is helpful in exploring the mechanism of EZH1. Further exploration of the molecular regulatory axis is crucial for elucidating the mechanism of tumorigenesis and development and developing therapeutic targets [28, 29]. The transcriptional regulatory relationship between EZH1 and ATG7 was verified by ChIP-seq data downloaded from Cistrome DB datasets. Interestingly, ATG7 appears in both autophagy and energy metabolism-related pathways. On one hand, ATG7 itself is an autophagy-related gene and has been shown to be essential for inducing autophagy. It has been reported that acute systemic loss of ATG7 in mice induces greater regression of KRAS-driven cancer, suggesting that host autophagy promotes tumor growth [30]. Studies have shown that loss of ATG7 function can mediate severe fatty liver by impairing autophagy metabolism, and cohort analysis shows that loss of ATG7 autophagy function increases the risk of cirrhosis and HCC [31]. This again verifies the dual nature of autophagy in tumorigenesis and development. On the other hand, ATG7 has been reported

to be related to energy metabolism. Studies have shown that ATG7 can activate dendritic cells and accelerate glycolysis [32]. Moreover, decreased expression of ATG7 has also been shown to reduce autophagy and fat production at the same time [33]. ATG7 may mediate the occurrence and development of tumors by regulating autophagy and energy metabolism. This study demonstrated a positive correlation between EZH1 and ATG7, and both were significantly overexpressed. Patients with high expression showed worse survival rates in HCC. In summary, we speculate that EZH1 promotes HCC progression through the autophagy/energy metabolism pathway, and that the EZH1-ATG7 regulatory axis may play an important role in it.

Based on the fact that targeted inhibition of EZH1 treatment may bring clinical benefits to HCC patients, the role of EZH1 in HCC treatment through drug sensitivity analysis was verified. The drug sensitivity data, derived from CellMiner™ database 2.4.2, and after data processing and analysis, found that high expression of EZH1 was positively linked to the drug resistance of various anti-tumor drugs, suggesting that the high expression of EZH1 in HCC will mediate the resistance of patients to various drugs or even no response. Most patients with HCC are clinically advanced at the time of initial diagnosis and are no longer suitable for surgical treatment [34]. For advanced HCC or patients with poor liver function, surgical resection and other local treatments are not effective and are no longer suitable for use. In this case, systemic drugs are a more effective choice [35]. For example, a variety of targeted drugs, such as tyrosine kinase inhibitors, have been developed to continuously improve overall survival [36]. Immune-checkpoint inhibitors have completely changed the clinical management of HCC in the past five years [37]. However, the widespread drug resistance of HCC has seriously hindered the long-term clinical benefits of existing treatments; it is urgent to find new treatments to overcome this drug resistance [38]. High expression of EZH1 promotes the resistance of tumor cells to anti-HCC treatment. Exploring targeted inhibition of EZH1 is expected to improve the sensitivity of patients to drugs. In addition, autophagy and energy metabolism have been shown to be associated with tumor treatment resistance. In chronic myeloid leukemia, the drug 2-DG induces severe metabolic stress, including inhibition of glycolysis and mitochondrial oxygen consumption, leading to autophagic cell death in tumor cells, thereby solving the resistance of the TKI drug imatinib. Therefore, EZH1 inhibitors may contribute to overcoming HCC systemic drug resistance by regulating autophagy/energy metabolism, and the EZH1-ATG7 axis may be an effective targeting pathway.

This article has its limitations. Firstly, there are few prognostic data included in this study, and the prognostic value of EZH1 may not be comprehensively evaluated. Secondly, there are not in vivo and in vitro experiments to verify the existence and mechanism of the EZH1-ATG7 regulatory axis in HCC development and treatment. The changes of autophagy and energy metabolism in this process also need further experiments to prove.

CONCLUSIONS

This study demonstrated the high expression of EZH1 in HCC through global data and internal immunohistochemistry and shown that EZH1-ATG7 regulatory axis plays an important role in the poor clinical progression and treatment of HCC. The results of this study are expected to elucidate the molecular mechanism of HCC and to lay a theoretical foundation for the application of EZH1 inhibitors in the treatment of HCC.

Conflicts of interest: None to declare.

Authors' contribution: S.C., Y.D. conceived and designed the study; S.C., J.D.L., Z.G.H., R.H. collected data. S.C., J.D.L., J.C. performed the statistical analysis. S.C., F.C., J.J.L., J.C. drafted the manuscript. S.C., Z.Q.H., G.C., Y.D. revised critically revision the manuscript for important intellectual content. All the authors approved the final version of the manuscript.

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