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Comprehensive Analyses of Prognostic Values and Immune Infiltration of KDM3 Gene Family in Hepatocellular Carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is one of the most commonly diagnosed malignancy globally with a pessimistic prognosis. Previous studies have demonstrated that abnormal expression of genes in the lysine-specific histone demethylase 3 (KDM3) family with epigenetic changes and dysregulation of enzymes promotes cancer progression. In this study, multiomics analyses were utilized to analyze differential expression, prognostic value, genetic alteration, protein–protein interaction, associated biological pathways and immune cell infiltration of KDM3s in patients with HCC. KDM3A-C were significantly upregulated to different extents based on pathologic and tumor grades in patients with HCC compared to normal tissue. Of note, higher KDM3A expression was associated with poor survival in HCC patients, whereas KDM3B and KDM3C were not associated with survival. Furthermore, KDM3A-B genetic alterations had significant effects on survival in patients with HCC. Analyses of the KEGG pathway and miRNAs targets of KDM3A and KDM3B in HCC may provide potential value in tumor behaviors and treatment. The differential expression of the KDM3 family has a strongly significant correlation with the infiltration of the abundance of immune cells, including B cells, CD8$^+$ T cells, CD4$^+$ T cells, macrophages, neutrophils, and dendritic cells in HCC. This study indicates that KDM3A may have the potential to be a promising molecular target in terms of prognostic biomarkers or therapeutic targets for HCC treatment.

Keywords KDM3 · Bioinformatics · Hepatocellular carcinoma · Survival · Immune infiltration

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Introduction

Hepatocellular carcinoma (HCC) remains a global health challenge with growing incidence and is the seventh most commonly diagnosed malignancy (4.7% of 36 cancers in 2020) in the world [1]. Hepatitis B virus and C viruses are strongly associated with the development of HCC, whereas hepatitis B has a higher prevalence in Asia and Africa, and hepatitis C is more prevalent in Japan, Europe, and the USA [2, 3]. The other main risk factors for HCC include alcohol, tobacco, type 2 diabetes, obesity, nonalcoholic steatohepatitis (NASH), and aflatoxin, a contaminant in maize and nuts, which is a potent carcinogen produced by the fungi Aspergillus [4]. HCC stands the third leading cause of cancer death worldwide in 2020 [1]. This phenomenon is due to the substantial heterogeneity of HCC, which impedes the development of effective therapies. Multiple clinical aspects of the complex pathophysiology of HCC, including the relationship with cirrhosis and chronic inflammation, arterial angiogenesis, and portal vein invasion, are critical when considering the diagnosis and treatment of this relatively

unique malignancy [4]. In addition, with strikingly fast advancement of molecular biology and genetic profiling in malignancy of neoteric era, a number of different pathogenic mechanisms appears to be involved in the progression of HCC, which is still not completely understood. Since HCC is a highly lethal cancer, it is necessary to discover new and particular molecular targets that can improve treatment or predict the prognosis of patients with HCC.

Lysine-specific histone demethylase 3 (KDM3) family proteins encompass four members: KDM3A, KDM3B, KDM3C, and KDM3D. They normally function in energy metabolism, homeostasis, obesity, sex determination, hematopoiesis, hormone nuclear receptor signaling, spermatogenesis, and cell signaling pathways [5]. These proteins regulate gene expression and chromatin dynamics mainly by demethylating histone H3 lysine 9 (H3K9me1/me2). Numerous studies have shown that the expression of abnormal KDM3 genes (KDM3s) with epigenetic changes promotes cancer progression mainly due to dysregulation of enzymes [6]. Because the KDM3A level in HCC is higher than in normal tissue, studies have shown that KDM3A knockdown inhibits HCC cell growth, invasion, and epithelial-to-mesenchymal transition within the tumor microenvironment under hypoxia and nutrient starvation [5]. KDM3B was first reported as a tumor suppressor of hematopoietic malignancies by demethylation of H4R3me2s and H3K9me2s in promoter regions correlated with active gene expression [7] and also shown to be upregulated in human hepatic carcinoma. KDM3B knockout reduces cell proliferation by delaying the cell cycle due to the malformation of mitotic spindles [8]. KDM3C has been demonstrated to control the proliferation of esophageal cancer via regulation of H3K9me2 activity, target mRNA levels of YAP1 gene expression and function as a tumor suppressor in esophageal cancer [9], and regulate colorectal cancer metastasis by modulation of H3K9me2 activity-related mRNA levels of the ATF2 pathway [10]. KDM3D, an uncommonly used alias name of the hairless protein, has also recently been categorized into the KDM3 family of proteins. KDM3D has the demethylation activity of H3K9 involved mainly in hair growth and functions as a transcriptional corepressor of multiple nuclear receptors [11]. In general, the KDM3 family predominantly incorporates KDM3A, KDM3B, and KDM3C.

Currently, few studies have demonstrated the roles and impacts of KDM3s on HCC [6, 12]. KDM3 proteins have been demonstrated to be associated with alteration of the transcriptome and subsequent effects of tumorigenesis via upregulation of oncogenes, including MYV, CCND1, and JUN. The correlation between KDM3s and prognosis in patients with HCC remains unclear. Therefore, this study extends the research field directly to HCC based on a variety of large databases to determine the potential oncogenic values of distinct members of the KDM3 family in HCC, which

could help develop novel targeting strategies, thus improving clinical outcomes. This study demonstrates for the first time that KDM3 family members, chiefly consisting of KDM3A, KDM3B, and KDM3C, play critical roles in HCC progression via genetic alteration.

Methods

Cancer Cell Line Encyclopedia (CCLE) Database (https://sites.broadinstitute.org/ccle/)

CCLE database, one of the comprehensively characterized human cancer models that parallel sequencing assessments from 1019 human cancer cell lines, provides a rigorous framework on assessing genetic variants, cell targets, and small-molecule and biological therapeutics and identifies new marker-driven cancer dependencies. In this study, mRNA expression [RNA sequencing (RNAseq)] data of the KDM3 family in a variety of cell lines was used.

The Cancer Genome Atlas (TCGA) Database (https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga)

TCGA is a comprehensive and coordinated project designed to improve diagnostic methods and treatment standards and ultimately prevent cancer. The TCGA Consortium, which has obtained > 30 kinds of human tumors, leads to a tremendous surge in generation genomics and transcriptomic data and further uses recently advanced sequencing technologies with a wide variety of cancers. In this study, KDM3s mRNA expression levels in 371 patients with HCC were analyzed.

UALCAN Database (http://ualcan.path.uab.edu/)

UALCAN is an integrated data-mining platform that facilitates the comprehensive analysis of cancer transcriptome based on RNAseq and clinical data of 33 cancer types from the TCGA database. This web-based platform's user-friendly features promote relative expression analysis of a query gene across tumor tissue and normal samples, understand the combined impact of gene expression levels and clinicopathologic characteristics on patient survival, and analyze the identification of dysregulated genes in individual cancer types. In this study, UALCAN was used to analyze the associations of mRNA expression levels of KDM3 family members with clinicopathologic parameters in normal and primary tumor tissue.

European Bioinformatics Institute (EBI): Expression Atlas (https://www.ebi.ac.uk/)

The EBI, as part of the European Molecular Biology Laboratory (EMBL), provides freely available bioinformatic resources to promote basic research in academic and industrial fields. In this study, the expression atlas database was used to further verify KDM3s expression in HCC cell lines.

Genotype-Tissue Expression (GTEx) Database (https://gtexportal.org/home/)

The GTEx project establishes a resource database and associated tissue bank for researchers to unravel the complex relationships of genetic variation and gene expression across various human tissue types. In this study, GTEx was used to analyze KDM3s transcription levels in multiple normal tissues.

HCCDB Database (http://lifeome.net/database/hccdb/)

HCCDB, a database of HCC expression atlas, was developed by curating 15 public HCC expression datasets with up to 4000 clinical samples, serving as a one-stop online resource for exploring HCC gene expression. In this study, KDM3s mRNA expression levels in different HCC datasets of the HCCDB database were analyzed.

Kaplan–Meier Plotter Analysis (https://kmplot.com/analysis/)

The Kaplan–Meier plotter can assess the effects of 54,000 genes [mRNA, microRNA (miRNA), and protein] or gene combination on survival in various cancer types based on gene arrays, RNAseq, or next-generation sequencing. The primary purpose of the tool is to serve as a meta-analysis-based discovery and validation of survival biomarkers based on gene expression data, disease-free survival (DFS), and overall survival (OS) information from GEO, EGA, and TCGA. The Kaplan–Meier survival plot was used to compare two groups of high and low genetic expression. The hazard ratio of 95% confidence intervals and log-rank test with P value were calculated to compare differences between groups. A statistically significant difference was considered when $P < 0.05$. In this study, the correlation between mRNA levels of KDM3 family members and survival in patients with HCC was analyzed by Kaplan–Meier plotter.

cBioPortal (https://www.cbioportal.org/)

The cBioPortal for Cancer Genomics is an open-source resource for the interactive exploration of multidimensional cancer genomics, which provides rapid, intuitive, and high-quality access to complex molecular profiles and clinical attributes from large-scale cancer genomics projects and empowers the translation of these abundant datasets into biological insights and clinical applications. In this study, genetic mutations in KDM3s and their association with OS and DFS of patients with HCC were displayed as Kaplan–Meier plots. The log-rank test with P value was calculated to identify significant differences between survival curves.

STRING Network (https://string-db.org/)

STRING is a database of known and predicted protein–protein interactions, including direct and indirect associations, stemming from computational prediction and primary databases. In this study, the analysis was performed to integrate the different expression of KDM3s and potential protein–protein interactions.

GeneMANIA (https://genemania.org/)

GeneMANIA is designed to identify the most related genes to a query gene set using a database of functional interaction networks and speculate gene functions using a highly accurate prediction algorithm. In this study, GeneMANIA was used to predict the functions of KDM3s and their associated genes.

LinkedOmics (http://www.linkedomics.org/login.php)

LinkedOmics is a public portal that includes multiomics data from the TCGA database and cancer cohorts of the Clinical Proteomics Tumor Analysis Consortium. This web application has three analytical modules, including LinkFinder, LinkInterpreter, and LinkCompare. The LinkFinder module was used to analyze differential expression of genes related to KDM3s in HCC using a Pearson test for correlation analysis, and the results were demonstrated by scatter plots and heat maps. The LinkInterpreter module was used to interpret coexpressed genes of KDM3s in HCC and also showed the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and miRNA targets of KDM3s in HCC by performing Gene Set Enrichment Analysis (GSEA) based on gene ontology and biological pathways. Criteria were ranked with a false discovery rate of < 0.05.

TIMER web Resource (http://cistrome.org/TIMER/)

TIMER is a web resource used for systematical evaluations of the clinical impact of six immune cells (B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic

cells) in diverse cancer types. TIMER was used to identify correlations between KDM3 expression and infiltration levels of six types of immune cells in patients with HCC.

Statistical Analysis

Expression of KDM3s was analyzed and displayed with HR and P values from a log-rank test. In this study, Pearson's correlation analysis and the 'ClusterProfiler' R package (version 3.6.3) were applied. P values less than 0.05 were considered statistically significant.

Results

KDM3 mRNA Expression Levels in Liver Tissues and Various Common Cancer Cell Lines from Different Databases

KDM3 transcription levels in multiple cancer cell lines were obtained using the CCLE database. Upon assessing a series of cancer cell lines, the boxplot of RNAseq results demonstrated that KDM3A mRNA expression levels in HCC cell lines ranked 7, whereas KDM3B and KDM3C ranked 20 and 28, respectively, among 34 cancer cell lines (Fig. 1a–c).

The KDM3s mRNA expression data were analyzed using the GTEx database, and lower levels of KDM3s expression were found in multiple non-disease tissue derived from different organs, as shown in the violin plot (Fig. S1a–c). In addition, RNAseq levels of KDM3A-C in liver cancer were extremely low across 17 human cancer types from the TCGA database (Fig. S2a–c).

Primary HCC Tumor Tissue Express Higher mRNA Levels of KDM3s, Compared to Normal Tissue

The EBI: Expression Atlas database was used to assess KDM3s expression in HCC cells. The heatmap illustrated that KDM3B expression was higher than KDM3A and KDM3C in most HCC lines (Fig. 1d). Furthermore, mRNA expression data for KDM3A-C in primary HCC tumors compared to normal liver tissue were extracted from the TCGA database through UALCAN. Boxplot showed remarkable upregulated KDM3A-C mRNA levels in primary HCC tumors compared to normal tissue with statistical significance (Fig. 2a). In addition, KDM3A-C were confirmed to be upregulated in HCC tissue compared to adjacent normal tissue in the majority of HCC datasets (Fig. 2b). KDM3A-C mRNA expression in HCC based on pathologic stage and tumor grade was also analyzed from the TCGA database through UALCAN. Among them, KDM3A was significantly increased in pathological stages I to III and tumor grades 1 to 3 HCC compared to normal tissue, and KDM3B was significantly increased in pathologic stages I to IV and tumor grades 1 to 4 HCC compared to normal tissue. As for KDM3C, only pathologic stages II and III and tumor grade 3 HCC showed statistical significance compared to normal tissue (Fig. 3a and b). Furthermore, KDM3A-C mRNA expression in various tumors and adjacent tissue was acquired from the TCGA database through the HCCDB web server. KDM3A and KDM3B were upregulated in HCC tissue compared to adjacent tissue, whereas KDM3C had the opposite result (Fig. S3a–c). Moreover, KDM3A-C mRNA expression levels in HCC, adjacent normal tissue, and cirrhotic and healthy samples were also obtained from different HCC datasets (a total of 3917 samples) of the HCCDB database.

Prognostic Values of the KDM3s Expression Levels in patients with HCC from TCGA

Kaplan–Meier plotter datasets and log-rank P value showed a correlation between KDM3A-C expression levels and survival in patients with HCC. Higher expression of KDM3A was associated with shorter OS and recurrence-free survival (RFS) in HCC patients (log-rank $P = 0.0013$, median OS = 33.5 months in patients with high expression of KDM3A and 71 months in patients with low KDM3A expression; log-rank $P = 0.0038$, median RFS = 12.87 months in high expression of KDM3A and 36.1 months in low KDM3A expression; Fig. 4a), but there was no statistically significant correlation between KDM3B-C expression and OS in patients with HCC (Fig. 4b and c). KDM3B expression was slightly significantly associated with DFS (log-rank $P = 0.049$). Survival analyses are summarized in Fig. 4d.

Genetic Mutations, Expression, and Interaction Analyses of KDM3s and Their Associations with OS and DFS in HCC Patients

Mutations in KDM3A-C genes in HCC patients were assessed with the cBioPortal analysis. Among 440 HCC patients with sequencing data, 29 had genetic alterations, with a mutation rate of 2.7%. Overall, two or more alterations were detected, and high mRNA-level alterations were most common in HCC samples. Among them, KDM3A, KDM3B, and KDM3C had similar genetic alterations, with mutation rates of 7%, 7%, and 6%, respectively (Fig. 5a). Kaplan–Meier plotter and log-rank P value in the cBioPortal analysis revealed that genetic alterations in KDM3 (including all genetic variants of KDM3A-C) had effects on OS and DFS in HCC patients (OS: $P = 2.558e^{-5}$, Fig. 5b; DFS: $P = 0.0145$, Fig. 5d). Further analysis demonstrated that genetic alterations in KDM3A and KDM3B were significantly associated with OS in patients with HCC

Fig. 1 KDM3 transcription levels in the liver and various common cancer cell lines. **a–c** Boxplot showing KDM3A, KDM3B, and KDM3C mRNA expression in multiple cancer cell lines (CCLE database). **d** mRNA expression of KDM3s in liver cell lines (EMBL-EBI: Expression Atlas)
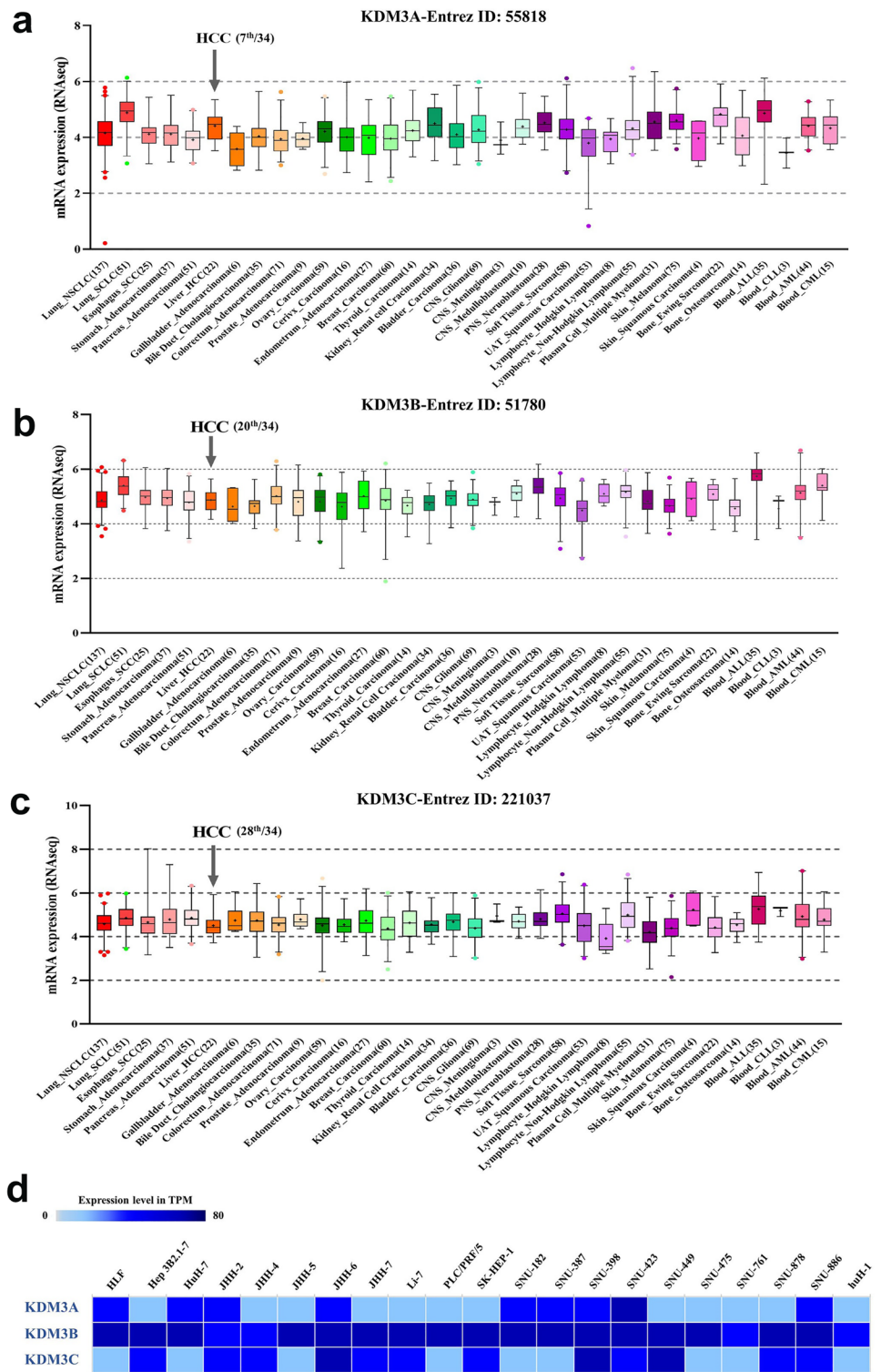
($P < 0.001$; Fig. 5c), whereas only genetic alterations in KDM3A had a statistically significant correlation with DFS ($P < 0.001$; Fig. 5e). Furthermore, by mapping to the search tool to retrieve interacting genes with the STRING network, protein–protein interaction analysis of KDM3s was performed to explore the potential interactions and

achieve a protein–protein interaction network of 13 nodes and 46 edges with enrichment (Fig. 6a). These differentially expressed KDM3s were associated with cell–cell communication, multiple signaling pathways, and developmentally biological processes that regulate tissue specification and organ development. Furthermore, GeneMANIA results
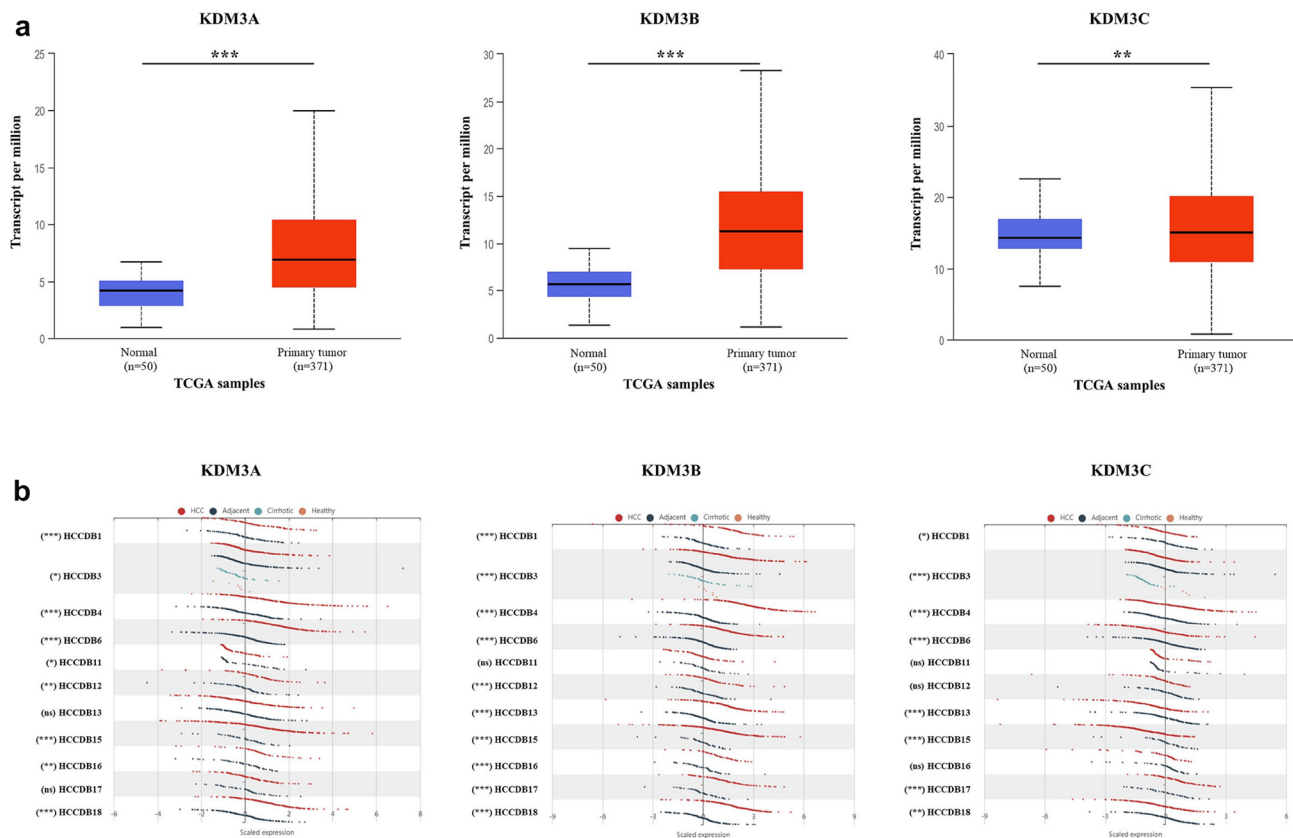
Fig. 2 Relative KDM3s mRNA expression in clinical samples. **a** Boxplots showing KDM3s mRNA expression in HCC ($n=371$) and corresponding normal tissue ($n=50$) using the UALCAN TCGA database. **b** The mRNA expression levels of KDM3s in different HCC datasets (a total of 3917 samples). TPM, transcripts per million. *$P<0.05$; **$P<0.01$; ***$P<0.001$

also identified differentially expressed KDM3s and multiple related molecules (androgen receptor (AR), NKX3-1, TMPRSS2, RPS6KA5, etc.) primarily connected to histone demethylation, protein demethylation, protein dealkylation, chromatin binding, and hormone receptor binding (Fig. 6b).

Differentially Expressed Genes Related to KDM3s and KEGG Pathway Analysis of KDM3s Related to Differentially Expressed Genes Associated with HCC

Both KDM3A and KDM3B were significantly associated with OS and/or DFS; therefore, these genes were further analyzed using LinkedOmics. The volcano plot showed differential expression of genes either negatively or positively related to KDM3A and KDM3B in patients with HCC using a Pearson test for correlation analysis (Fig. 7a; Fig. S4a). The heat map presented the top 50 positively correlated and top 50 negatively correlated significant KDM3A (Fig. 7b) and KDM3B (Fig. S4b). LinkedOmics was also used in correction between KDM3A-C in HCC patients, as KDM3A was positively correlated with

KDM3B (Pearson correlation $=0.5992$; $P=1.559e^{-37}$) and KDM3C (Pearson correlation $=0.5412$; $P=1.281e^{-29}$), whereas KDM3B was positively correlated with KDM3C (Pearson correlation $=0.6302$; $P=1.811e^{-42}$; Fig. 7c; Fig. S4c).

The KEGG pathway analysis of KDM3A coexpression genes in HCC was performed using GSEA to shed further light on their biological functions. Differentially expressed genes related to KDM3A were mainly enriched in mechanisms, including adherens junction, miRNAs in cancer, structural constituent of ribosome, nonalcoholic fatty liver disease (NAFLD), proteasome, etc. (Fig. 7d). Moreover, the LinkInterpreter module of LinkedOmics was used to investigate the enrichment analysis of miRNAs for the potential molecular mechanism of KDM3A in HCC. Results showed that KDM3A was associated with (ATATGCA) MIR-448, (GCAAAAA) MIR-129, (ATGTTAA) MIR-302C, (TGAATGT) MIR-181A-D, and (CGGTGTG) MIR-220 (Fig. 7e). Furthermore, the KEGG pathway and miRNA targets of KDM3B were also analyzed, revealing differentially expressed genes related to several pathways in HCC patients (Fig. S4d and e).
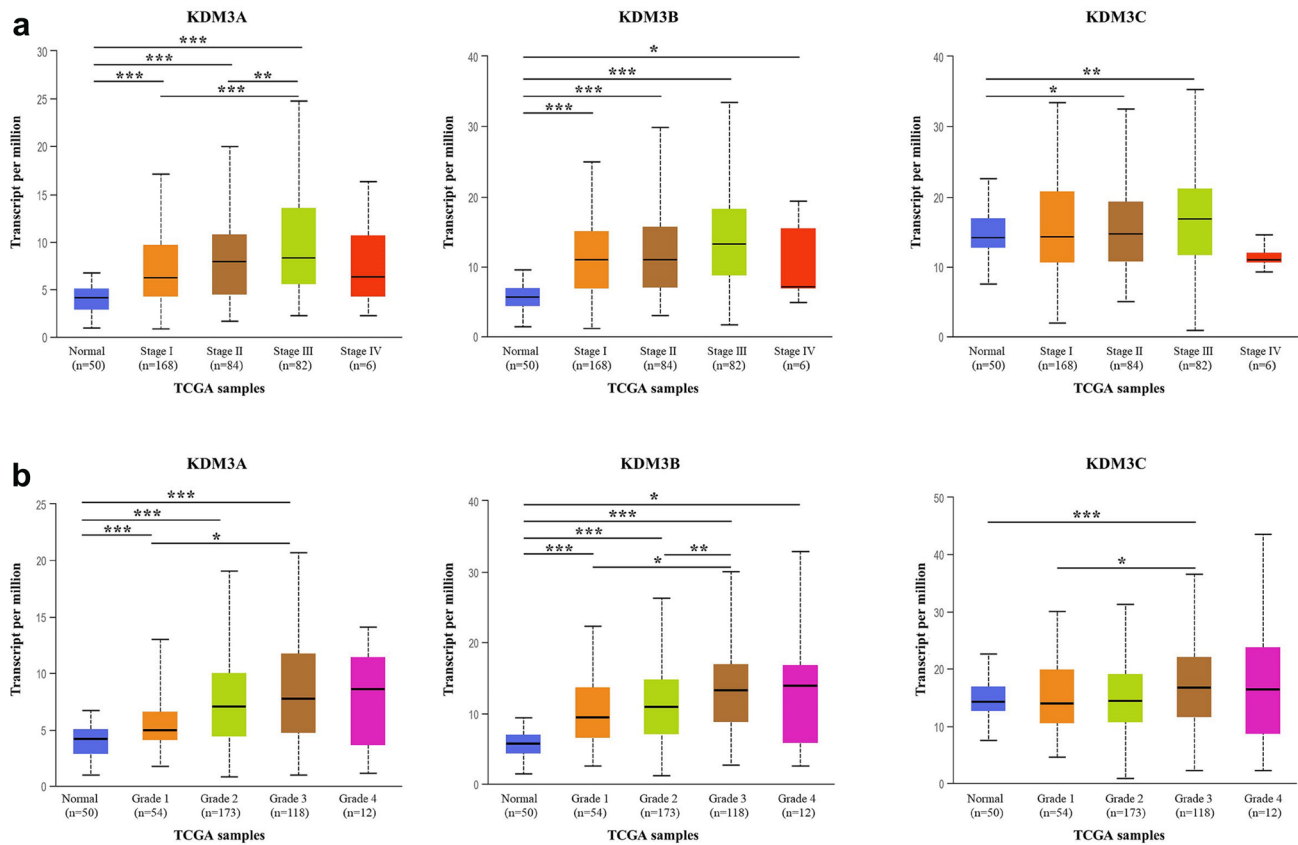
Fig. 3 **a** Boxplots showing KDM3s mRNA expression in HCC based on pathologic stages. **b** Boxplots showing KDM3s mRNA expression in HCC based on tumor grades. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Immune Cell Infiltration of KDM3s in Patients with HCC

The immune cell level is viewed as the central focus for engaging the immune system against cancer. The TIMER database was used to explore the relationship between the KDM3 family and immune cell infiltration. The differential expression of the KDM3A-C family had a strongly significant correlation with the infiltration of the abundance of host immune cells, including B cells, CD8^{+} T cells, CD4^{+} T cells, macrophages, neutrophils, and dendritic cells in HCC (Fig. 8).

Discussion

The incidence of HCC has arisen worldwide over the last 20 years and is expected to increase until 2030 [13]. The prognosis of patients with advanced HCC is prominently poor despite emerging targeted and immunotherapies. Early detection of HCC is of critical importance, as it is associated with improved survival and the possibility of curative treatment. The leading-edge sequencing technology in

bioinformatic fields may facilitate the global and systematic evaluation of molecular landscapes in HCC. Subsequently, it might provide promising and independent prognostic molecular targets for survival and treatment.

KDM3 family proteins are also known as Jumonji domain-containing 1 (JMJD1) and Jumonji C domain-containing histone demethylase 2 (JHDM2) proteins. The KDM3 family proteins play important roles in the epigenetic modification of gene expression without altering the primary DNA sequence via their histone lysine demethylase activity. KDM3 genes potentially contribute to malignant cellular transformation, carcinogenesis, and tumor progression by influencing multiple mechanisms, including gene transcription, chromosomal stability, cell differentiation, and cell cycle [6, 14]. To further explore the roles of KDM3 genes in the prognosis and tumorigenesis of HCC, data from multiple public datasets were used and analyzed.

In this study, KDM3A-C genes were expressed in multiple cancer cell lines, including liver cancer cells. Of note, KDM3A in HCC was higher than in most other cancer cell lines, whereas KDM3B and KDM3C in HCC were lower than those in at least half of the other cancer types. Furthermore, KDM3A and KDM3C expression levels varied

Fig. 4 OS and RFS analysis of KDM3s expression in HCC patients (Kaplan–Meier plotter datasets). **a** KDM3A. **b** KDM3B. **c** KDM3C. **d** Summary in tables

more than KDM3B in 21 liver cancer cell lines. Although KDM3A-C expression in clinical HCC samples was low, further investigation revealed that KDM3A-C had higher expression in liver tumors than in adjacent tissue. KDM3A-C were highly expressed in most HCCDB datasets. Altogether, the results may indicate that KDM3s correlate with
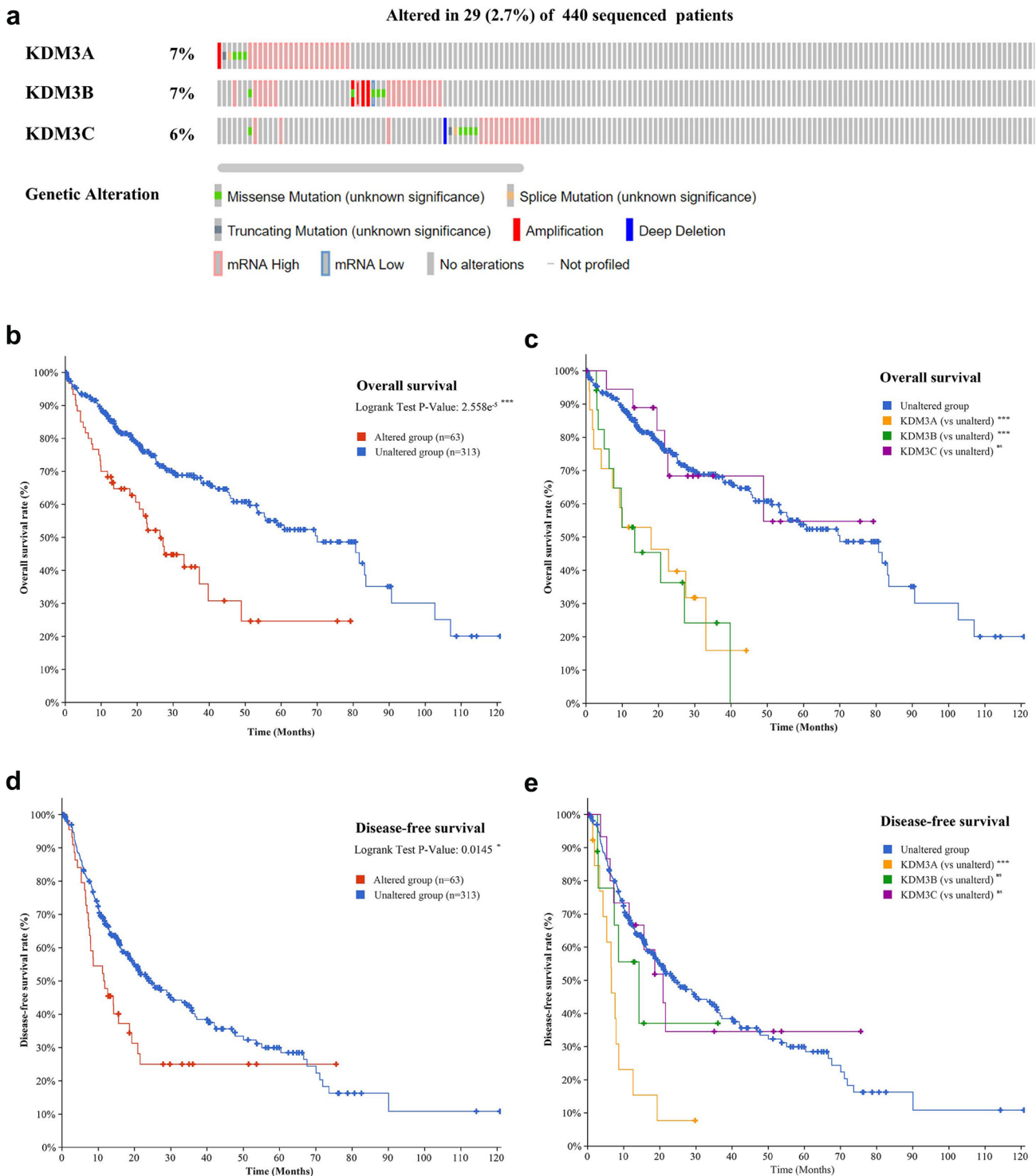
Fig. 5 Genetic alterations in KDM3s and correlation with prognosis in HCC patients (cBioPortal). **a** KDM3s genetic alterations in HCC patients were analyzed by the database. Different types of genetic alteration were denoted in different colors. OS (**b** and **c**) and DFS (**d** and **e**) analysis of KDM3s genetic alternations in HCC

tumorigenesis and behavior of HCC. Previous studies have shown that the KDM3A level plays an important role in the proliferation of human HCC cells under hypoxia. In addition,

KDM3A gene silencing abrogated hypoxia-induced adrenomedullin expression and suppressed HCC tumorigenicity [15]. The knockout experiment of KDM3B in HCC
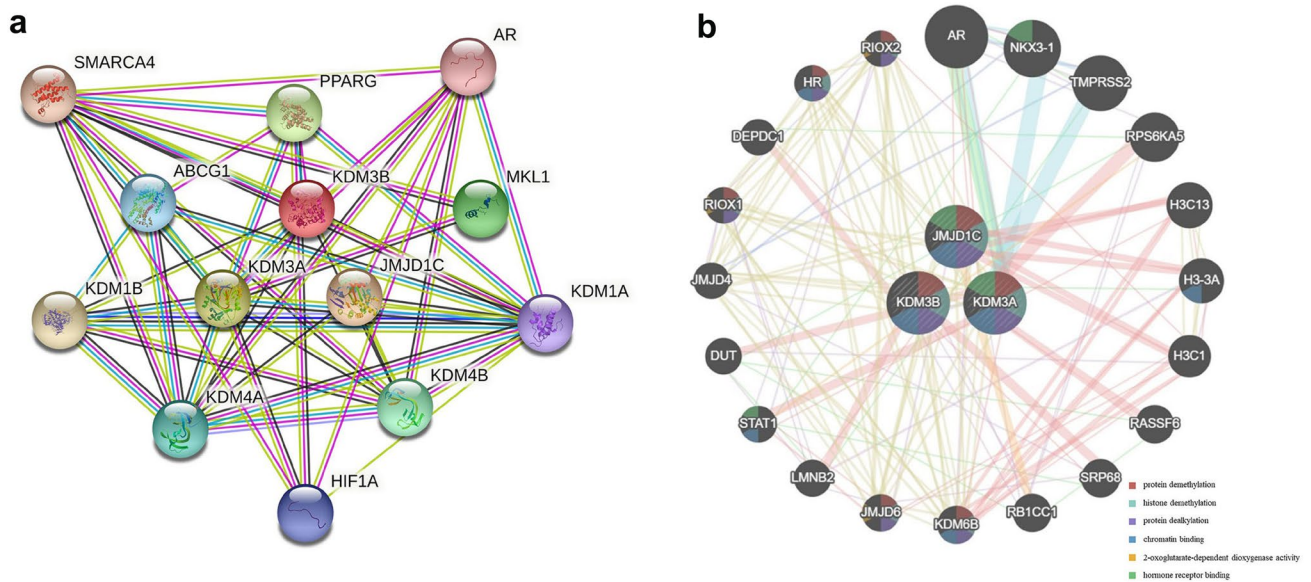
Fig. 6 **a** Protein–protein interaction network of KDM3s in HCC (STRING network). **b** Predicted functions of expressed KDM3s and their associated genes (GeneMANIA)

cells showed that cell cycle retardation was induced by the down-regulated expression of cell cycle genes, implicating KDM3B as a crucial epigenetic factor in cell cycle regulation [8]. KDM3C was reported to have an anti-inflammatory function in regulating inflammatory responses through the suppression of nuclear factor-κB signaling [16], which is involved in the development of hepatocellular injury, liver fibrosis, and HCC [17].

In this study, a survival analysis through Kaplan–Meier plotter revealed that high KDM3A expression in patients with HCC was correlated with poor OS and RFS, and KDM3B was correlated with poor DFS. However, KDM3C expression had no impact on the survival of patients with HCC. The KDM3A level in HCC was higher than in normal tissue in the previous study, and the high expression of the KDM3A protein by immunohistochemical staining was correlated with a higher rate of recurrence [12]. KDM3B was reported to have a vital role in the recurrence of castration-resistant prostate cancer [18]. KDM3C was demonstrated to promote the progression of prostate cancer, colon cancer, and acute myeloid leukemia, but no correlation with HCC was reported [19]. Altogether, the results indicated that KDM3A is a promising biomarker for survival from HCC, and KDM3B may have the potential to predict HCC recurrence.

Genetic alterations of KDM3 genetic alterations in patients with HCC were analyzed in this study. The results showed that KDM3A and KDM3B have significant impacts on tumor characteristics related to the prognosis of patients with HCC. The biological interaction network

of KDM3s was analyzed using STRING and GeneMANIA. The functions of associated genes with KDM3 interactions were primarily demethylation and chromatin binding. The protein–protein interaction network of KDM3s revealed associated proteins, such as SMARCA4, AR, peroxisome proliferator-activated receptor γ (PPARγ), etc. SMARCA4, known as BRG1, is bound to activator protein 1 transcription sites by interaction with c-Jun protein in a KDM3A-dependent manner and contributes to hepatic tumorigenesis [20]. KDM3A exhibits hormone-dependent recruitment to AR target genes to mediate transcription activation [21]. Inhibition of AR evokes feedback activation of the target of rapamycin signaling of AKT mammals that promotes the expression of the nuclear AR protein by restricting ubiquitin-dependent degradation of AR and enhancing the nuclear localization of AR, explaining the precise role of nuclear overexpression of AR in HCC [22]. Furthermore, KDM3A mediates β-adrenergic receptor and PPARγ activation through the PRDM16-PPARγ-P-JMJD1A complex, which plays a significant role in beige adipogenesis [23].

Exosomes are membrane-bound extracellular vesicles that transport bioactive materials between cells and organs. Adipocyte-derived exosomes carry key miRNAs and long noncoding RNAs to involve the modulation of HCC host cell signaling in HCC [24]. In this study, miR-302C, miR-181A-D, miR-515-5P, miR-519E, and miR220 were significantly identified as the common miRNA targets of KDM3A and KDM3B. These results could be applied to investigate the regulatory mechanism of KDM3s in HCC.
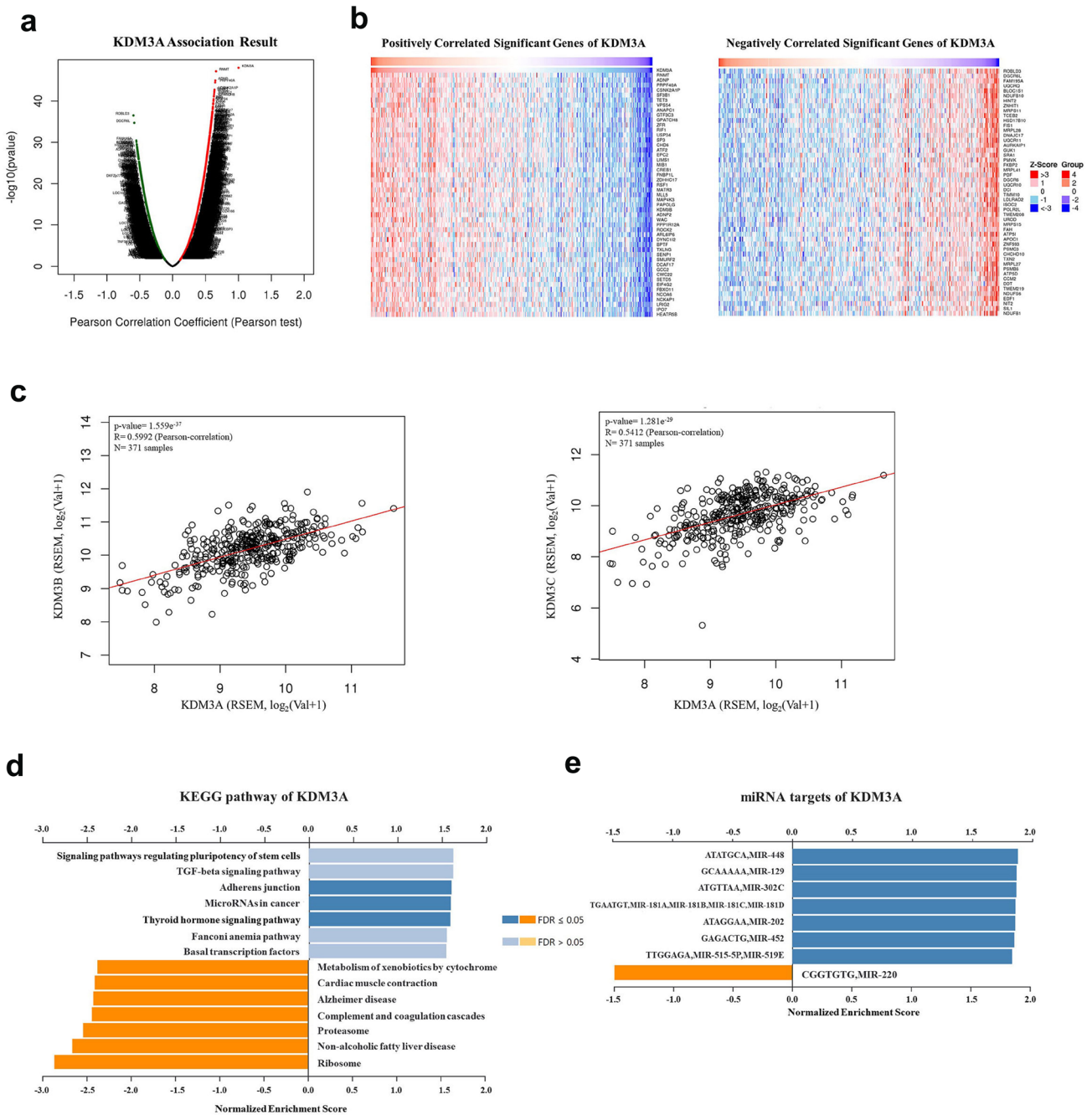
Fig. 7 Coexpressed genes of KDM3A in HCC (LinkedOmics). **a** Volcano plot showing the differential expression of genes related to KDM3A in HCC using a Pearson test for correlation analysis. **b** Heat map showing the top 50 positively and negatively correlated sig-nificant genes of KDM3A. **c** GSEA showing the KEGG pathway of KDM3A in HCC. (d) GSEA showing miRNA targets of KDM3A in HCC. FDR, false discovery rate

KEGG signaling pathway analysis for KDM3A and KDM3B revealed that some pathways involve signaling pathways regulating the pluripotency of stem cells, metabolism of xenobiotics by cytochrome and NAFLD, etc. Signaling pathways regulating the pluripotency of stem cells mainly include the self-renewal and multidirectional differentiation of stem cells. Liver cancer stem cells are currently considered a specific subpopulation with a significant tumorigenic potential to contribute to the development and recurrence of HCC [25]. The metabolism of xenobiotics has important roles in cancer via transcriptional and post-transcriptional (miRNA) regulation of cytochrome P450
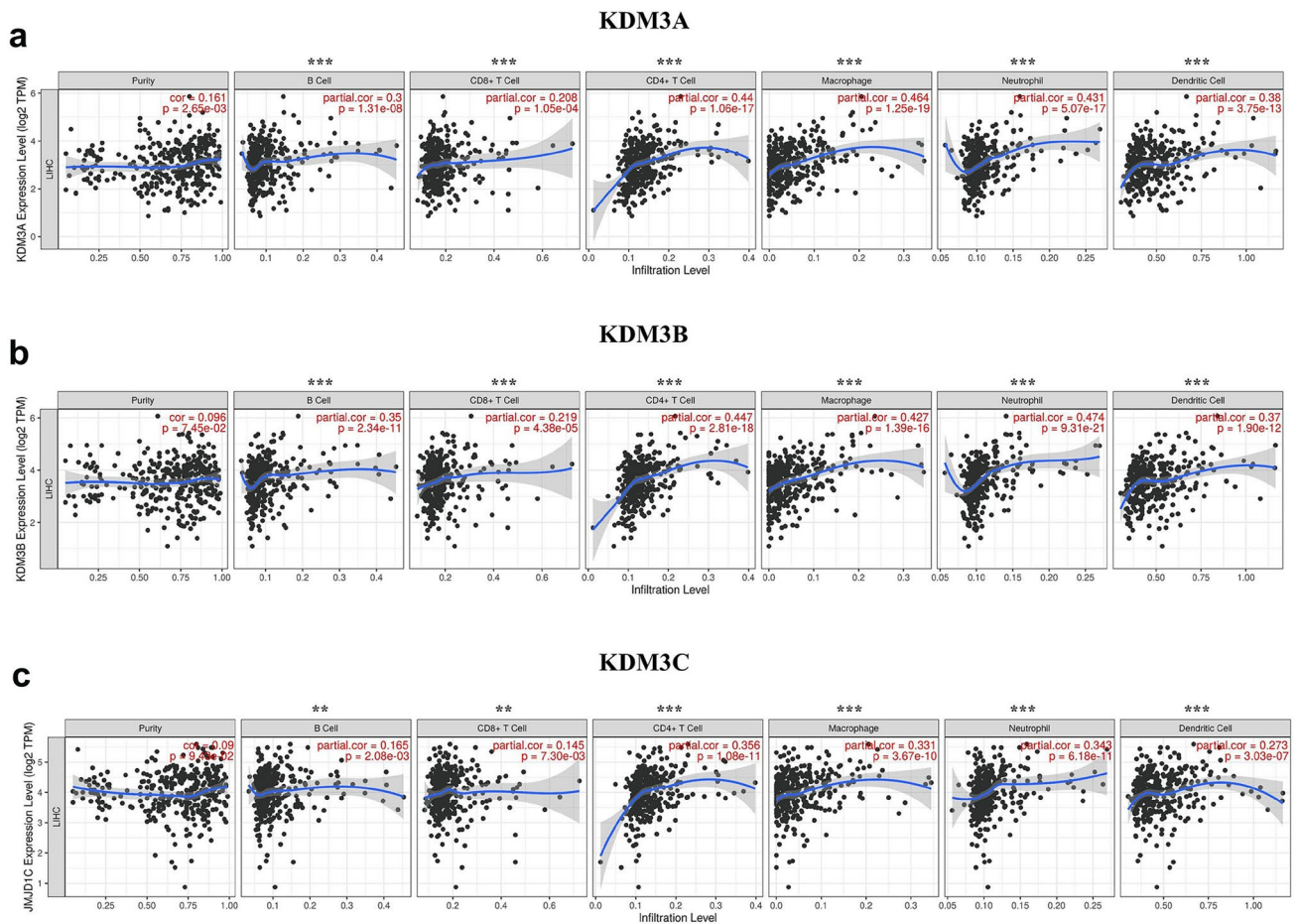
Fig. 8 Correlations between differentially expressed KDM3s and infiltration levels of six immune cell types (TIMER web resource). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

enzymes [26]. The expression of xenobiotic and steroid hormone-metabolizing enzymes that mediate signals appears to potentially promote tumor growth in HCC of the noncirrhotic liver [27]. NAFLD encompasses simple steatosis and NASH. NASH-driven HCC has been proposed, and fatty acid metabolism is involved in hepatocarcinogenesis [28]. KDM3s are directly or indirectly involved in regulating lipid metabolism through their demethylase activity [29].

The tumor microenvironment, which is collectively composed of a heterogeneous population of cancer cells, has a great variety of resident and infiltrating host cells, immune cells, secreted factors, signaling molecules, and extracellular matrix proteins, which profoundly impact tumor growth through interactions with cancer cells and lead to immune evasion, angiogenesis, hypoxia, and invasion that further shape tumor progression and determine treatment choices [30]. HCC is an immune-related tumor; the number and types of tumor-infiltrated immune cells can express biomarkers during clinical application and specialize in cancer diagnosis and prognosis [31]. At present, the most promising

immunotherapeutic approach toward HCC is the use of immune checkpoint inhibitors that show strong antitumor activity for a subclass of patients. To date, a combination of atezolizumab (anti-PDL1 antibody) and bevacizumab (vascular endothelial growth factor blocker) is viewed as the best available first-line treatment for advanced HCC [32]. This study demonstrates the value of a strongly significant correlation between KDM3s and the infiltration of the abundance of host immune cells of HCC, which stands as the cornerstone of advanced investigations of novel immunotherapy targets.

In this era of big data, innovative bioinformatics helps researchers identify novel biomarkers from public databases. Through this new disclosure, a comprehensive understanding of KDM3 bioinformatics in HCC is provided to elucidate a possible mechanism of tumorigenesis and investigate new targeted therapies or immunotherapies. Nevertheless, this study still has limitations. All data were analyzed using public datasets, although the resource is well established and reliable. Further studies are essential to obtain a deeper

and more detailed understanding of its application in clinical settings.

Conclusion

In this study, multiomics were applied to analyze and evaluate HCC data, suggesting various evidence for high KDM3A-B mRNA expression levels in HCC and their potentially independent prognostic roles in patients with HCC. Furthermore, high mRNA-level alterations and genetic variants in KDM3s were proven to affect OS and DFS in patients with HCC. Furthermore, multiple signaling pathways, developmental biological processes, and various molecules involved in tissue specification and organ development, as well as infiltration of the abundance of host immune cells are presented. The results indicate that KDM3A and KDM3B are novel prognostic biomarkers for the survival of patients with HCC. In particular, KDM3A has the potential to become a promising molecular target in drug discovery for treating patients with HCC.

Declarations

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