HKDC1 Promotes the Tumorigenesis and Glycolysis in Lung Adenocarcinoma via AMPK/mTOR Signaling Pathway

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Abstract

Background: Hexokinase domain component 1 (HKDC1) belongs to the fifth hexokinase, which plays an oncogenic role in lymphoma, liver cancer, and breast cancer, as reported. However, its biological functions in lung adenocarcinoma (LUAD) has not been studied.

Methods: We applied bioinformatics analysis, quantitative real-time polymerase chain reaction (qRT-PCR), western blotting, immunohistochemistry, and a series of functional assays in vitro and in vivo to investigate the roles of HKDC1 in LUAD.

Results: We discovered that HKDC1 was highly expressed in LUAD tissues and cell lines, and the positive expression of HKDC1 was correlated with aberrant clinicopathological characteristics in LUAD patients. Besides, HKDCI could be served as a prognostic predictor for LUAD patients. Overexpression of HKDC1 promoted the proliferation, migration, invasion, glycolysis, EMT and tumorgenicity, whereas knockdown of HKDC1 produced the opposite functional effect. Mechanistically, HKDC1 could regulate the AMPK/mTOR signaling pathway to perform its biological function.

Conclusions: Our findings suggested that HKDC1 plays an oncogenic role in LUAD. Targeting this gene may provide a promising therapeutic target to delay LUAD progress.

Background

Lung cancer has been ranking the first of all malignant tumors in the aspect of morbidity and mortality, posing colossal threat to human health [1,2]. Lung adenocarcinoma (LUAD) is one of the most commonly subtype of lung cancer, accounting for 30–35% of the primary lung cancers [3]. Although tremendous efforts have been devoted to fighting against lung cancer, limited improved survival are achieved. Recently, molecular targeted therapy has showed inspiring performance in treating LUAD [4–6], urging researchers to explore some new molecular alterations in LUAD.

Glycolysis, also known as Warburg effect, refers to the converts of glucose into lactate in cancer cells even under the sufficient oxygen conditions [7]. Accumulating evidences have demonstrated that glycolysis plays contributing roles in tumor growth and metastasis through such unique metabolic pathway. Herein, it is a new strategy to delay tumor progress by inhibiting the glycolysis of cancer cells [8].

Hexokinases (HK) are a family of enzymes that catalyze the first step in glucose metabolism by phosphorylating glucose to glucose–6-phosphatase of glucose utilization [9]. There are five HK isozymes found in mammals until now, from HK I to HK V. Among five isoforms of hexokinases, HK2 is most widely studied and a key player for aerobic glycolysis in cancers. Overexpression of HK2 is also observed in liver cancer [10], colorectal cancer [11], prostate cancer [12], and esophageal squamous cell carcinoma [13], et al, and is associated with poor prognosis in patients. Meanwhile, HK2 is also reported up-regulated and functions as a novel oncogene in lung cancer [14,15].

Hexokinase domain component 1 (HKDC1) is a recently discovered protein, which is being categorize as the fifth hexokinase [16]. In physiological condition, HKDC1 is extremely critical to maintain whole-body glucose homeostasis [17–19]. However, aberrant expressions of HKDC1 contributes to the progression of certain types of disease and cancer. For example, overexpression of HKDC1 could lead to the metabolism dysfunction of hepatocytes, which may be associated with nonalcoholic fatty liver disease [20]. In addition, increasing evidence shows that HKDC1 may play oncogenic roles in cancers, such as lymphoma [21], liver cancer [22], breast cancer [23] and colorectal cancer [24], indicating HKDC1 may be served as a therapeutics target in cancers.

Previous bioinformation analyzation predicted that HKDC1 could be a promising therapeutics target for lung cancer [25]. However, this hypothesis remains to be validated, and the downstream mechanism of HKDC1 in lung cancer needs to be explored. In this study, we performed a series of functional assays in vitro and in vivo to investigate the roles of HKDC1 in LUAD.

Methods

Cell culture

LUAD cell lines included A549 and H1299, and the normal human bronchial epithelial cell line HBE were obtained from Chinese Academy of Sciences Cell Bank (Shanghai, China) and cultured in Dulbecco's modified Eagle medium (DMEM) (Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum (FBS) (Invitrogen) at 37°C in a humidified atmosphere of 5% CO2.

Clinical tissue and specimens

A total of 20 fresh primary LUAD tissues and paired adjacent normal tissues were obtained from operation in Changhai Hospital, Second Military Medical University (Shanghai, China). 75 paired of paraffin-embedded LUAD specimens used in this study were collected from patients in 2013 who were diagnosed with primary LUAD and none of them received preoperative chemotherapy or radiotherapy. Overall survival (OS) was defined as the interval between surgery and death or last observation. This study was approved by the Ethics Committee of Changhai Hospital. All patients provided written informed consent when admitted.

Construction of overexpression and knockdown of genes

We constructed lentiviral vectors encoding the human HKDC1 gene or green fluorescent protein (GFP) in the pLenti-EF1a-EGFP-P2A-Puro-CMV-MCS–3Flag vector (HeYuan Bio-technology Co., Shanghai, China) and designated them as ov-HKDC1 or ov-NC. Stable LUAD cells knockdown of HKDC1 were generated using lentiviral constructs expressing sh-HKDC1 (sh-HKDC1#1: GGTGGACAGGTTCCTGTAT), sh-HKDC1#2: GGTCAGTGCGAATGTACAA) or sh-NC. Cells were harvested at 48h after transfection for RNA analysis and 72h after transfection for protein analysis.

Immunohistochemistry (IHC)

The LUAD tissue slides were incubated with HKDC1 (1:200, Abcam, ab228729) primary antibodies. IHC scoring was performed using a modified Histo-score (H-score) by two independent pathologists. Briefly, the proportion of positively stained cells was scored as 0–100% (<25% scores 0, 25–50% scores 1, 50–75% scores 2, 75–100% scores 3) and the intensity score were scored as 0 (negative), 1+(weakly positive), 2+(moderately positive), or 3+ (strongly positive). A final score was then calculated by multiplying these two scores.

RNA extraction and quantitative RT-PCR (qRT-PCR)

Total RNA was extracted from cultured LUAD cell lines or tissue specimens using Trizol (Invitrogen, Grand Island, NY) according to the manufacturer's instruction. The cDNA was synthesized using the PrimeScript RT Reagent Kit (TaKaRa Bio, Shiga, Japan) following the manufacturer's instructions. Real-time PCR was performed on a Roche Light Cycler 480 (Roche) using SYBR Green PCR Master Mix (TaKaRa Bio, Shiga, Japan). Fold change relative to mean value was determined by 2-△△Ct. All experiments were performed in triplicate. Primer sequences are listed as followed.

HKDC1:

5'-ATCCTGGCAAGCAGAGATACG–3'(forward)

5'-GACGCTCTGAAATCTGCCCT–3'(reverse);

GAPDH:

5'-GGAGCGAGATCCCTCCAAAAT–3'(forward)

5'-GGCTGTTGTCATACTTCTCATGG–3'(reverse);

Western blotting

Whole cultured cells were homogenized in 0.1% SDS and 1 mM PMSF. Protein extracts were subjected to SDS-PAGE and analyzed using the following primary antibodies: HKDC1(Abcam, ab228729), Phospho-AMPKα(Cell Signaling Technology, 2535), Phospho-mTOR(Cell Signaling Technology, 5536), Phospho-p70S6(Cell Signaling Technology, 9234), Vimentin(Santa Cruz, sc–6260), Snail(CST, 3879), E-cadherin (Abcam, ab40772), N-cadherin(Abcam, ab18203) and GADPH antibody(Abcam, ab8245). Then, the membranes were incubated with secondary antibodies (CST, 7076, 7074) at room temperature for 1 hours. All experiments were performed in triplicate.

Reagents

Rapamycin (Abcam, ab120224) was used in LUAD cells to inactive mTOR signaling pathway.

Bioinformatics analysis.

we utilized the Oncomine (https://www.oncomine.org/resource/login.html) and UALCAN (http://ualcan.path.uab.edu/analysis.html) database for detecting the expression level of HKDC1 genes.

Measurement of glucose and lactate

Transfected A549 and H1299 cells were seed in 6-well plates (5×10^5) and the culture media were harvested 48 h after transfection. The glucose and lactate levels were measured using a Glucose Assay Kit (Sigma-Aldrich, USA) and a Lactic Acid Assay Kit (Sigma-Aldrich, USA), respectively, according to the manufacturer's protocol. The values were normalized to total protein concentration. All experiments were performed in triplicate.

Cell proliferation assays

Cell viability was measured by Cell Counting Kit–8 (CCK–8, bimake, USA). Briefly, transfected cells were seeded in 96-well plates (5000 cells/well) and cultured for 3 days to assess proliferation. The absorbance was measured at 450 nm. All experiments were performed in triplicate.

Cell migration and invasion assay.

Cell migration and invasion ability was assessed by 24-well transwell chambers (Corning) with Matrigel (Corning, Bedford, MA, USA) coating or not. Briefly, approximately $1*10^5$ cells were resuspended in 300-uL serum-free DMEM and seeded into the upper chambers, whereas the bottom chamber was filled with 500-uL 10% FBS medium. Twenty-four hours later, the migrated/invaded cells in lower chamber were fixed with 4% paraformaldehyde and stained with 1% crystal violet. All experiments were performed in triplicate. The counting of cells was accomplished by Image J software (Rawak Software Inc, Stuttgart, Germany).

Xenograft Mice

Five-week-old male BALB/c nude mice were purchased from Shanghai Experimental Center (Shanghai, China). Xenograft tumor models were established by subcutaneous injection of ovHKDC1 or shHKDC1 (5×10^6) transfected A549 cells into the right dorsal flank. One week after injection, the mice were tested once a week for a total of 4–5 weeks. Tumor volume (V, cm^3) was evaluated based on tumor length (L) and width (W) with the following formula: $V = 1/2\times L\times W^2$. All manipulations were performed in accordance with currently prescribed guidelines and under a protocol approved by SMMU Ethical Review Committee (Shanghai, China).

Statistical analysis.

Data analysis was carried out using IBM SSPS 24 (IBM Corp., Armonk, NY, USA) and data were reported as mean±SD. Student's t-test was used to determine differences between groups and two-tailed ANOVA

test was applied in case of multiple groups. The association between gene and clinicopathological features was analyzed by chi- square test or Fisher's exact test. Kaplan-Meier curves were used to compare the OS between groups. Multivariate analysis was employed to determine independent factors affecting the prognosis of patients. Differences were considered statistically significant when $P < 0.05$.

Results

HKDC1 was up-regulated in LUAD tissues and cells

First of all, we measured the expression of HKDC1 in two public databases, Oncomine and UALCAN. The data of Okayama lung and Selamat lung samples from Oncomine database and TCGA samples from UALCAN database showed that the expression level of HKDC1 was significantly elevated in LUAD tissues compared with that in the normal lung tissues (Fig. 1A, 1B).

To further confirm the expression of HKDC1 in LUAD, we examined the expression of HKDC1 mRNA in 20 fresh LUAD tissues and their corresponding adjacent normal tissues. The HKDC1 mRNA expression level was significantly elevated in LUAD tissues in comparison to normal lung tissues ($p<0.01$, Fig. 1C). Simultaneously, we examined HKDC1 expression in cell lines by qRT-PCR analysis and western blotting. The HKDC1 expression level was obviously upregulated in the LUAD cell lines compared with the normal human lung epithelial cell line HBE (Fig. 1D).

HKDC1 was associated with aggressive features and poor prognosis in LUAD

To explore the expression pattern of HKDC1 in LUAD, we performed IHC in 75 paired of LUAD specimens. As shown in Fig. 2A, the expression level of HKDC1 in LUAD was graded from 0 to 3 by intensity of IHC staining. The strong staining of HKDC1 was far more frequently observed in LUAD tissues (53/75, 70.7%) than in adjacent normal tissues (16/75, 21.3%, Fig. 2B). Table 1 showed the correlation between HKDC1 expression and clinicopathological features of LUAD patients. The results demonstrated that the expression of HKDC1 was associated closely with histologic differentiation ($p = 0.003$), pN stage ($p = 0.009$) while there was no association between HKDC1 expression and age ($p = 0.681$), sex ($p = 0.302$), tumor location ($p = 0.428$), pT stage ($p = 0.627$) or TNM stage ($p = 0.843$).

By Kaplan-Meier analysis, overall survival (OS) was more unfavorable in high expression of HKDC1 than those with low expression ($p = 0.007$, Fig. 2C). Further subgroup analysis demonstrated that patients with high expression of HKDC1 had unfavorable OS in pT1-T2 stage patients (Fig. 2D). By univariate Cox analysis, differentiation, pT stage, pN stage and HKDC1 expression showed statistically significant associations with OS ($p = 0.001$; $p<0.001$; $p<0.001$; $p = 0.010$; respectively). Multivariate Cox analysis revealed that HKDC1 expression was still an independent factor affecting the OS ($p = 0.018$, Table 2).

Table 1
Correlations Between HKDC1 Expression and Clinicopathologic Features

	HKDC1 expression		
Characteristic	Low expression (%)	High expression (%)	P value
Age			0.681
>55	16 (30.77%)	36 (69.23%)	
≤55	6 (26.09%)	17 (73.91%)	
Sex			0.302
Male	10 (24.39%)	31 (75.61%)	
Female	12 (35.29%)	22 (64.71%)	
Tumor location			0.428
Left lung	9 (25.00%)	27 (75.00%)	
Right lung	13 (33.33%)	26 (66.67%)	
Histologic differentiation			**0.003**
Well+Moderate	20 (40.82%)	29 (59.18%)	
Poor	2 (7.69%)	24 (92.31%)	
Pathological T stage			0.627
T1-2	15 (31.25%)	33 (68.75%)	
T3-4	7 (25.93%)	20 (74.07%)	
Pathological N stage			**0.009**
N0	16 (43.24%)	21 (56.76%)	
N+	6 (15.79%)	32 (84.21%)	
TNM stage			0.843
I+II	13 (30.23%)	30 (69.77%)	
III+IV	9 (28.13%)	23 (71.87%)	

Table 2
Univariate and Multivariate Survival Analysis for Patients With LUAD

Characteristic	HR	95% CI	P value
Univariate analysis			
Age (≤55 vs >55)	1.277	0.697-2.338	0.428
Sex (Male vs Female)	1.677	0.930-3.024	0.086
Location (Left vs Right)	1.582	0.891-2.811	0.118
Differentiation (Poor vs Well+Moderate)	2.682	1.501-4.794	**0.001**
pT stage (T3-4 vs T1-2)	3.259	1.820-5.836	**<0.001**
pN stage (N+ vs N0)	5.835	3.007-11.323	**<0.001**
HKDC1 expression (High vs Low)	2.625	1.265-5.448	**0.010**
Multivariate analysis			
Differentiation (Poor vs Well+Moderate)	1.276	0.644-2.528	0.485
pT stage (T3-4 vs T1-2)	2.245	1.185-4.253	**0.013**
pN stage (N+ vs N0)	4.561	2.062-10.089	**<0.001**
HKDC1 expression (High vs Low)	2.598	1.176-5.739	**0.018**

95% CI = 95% confidence interval; HR = Hazard Risk.

HKDC1 promoted proliferation, invasion, migration in vitro and LUAD cells tumorigenesis in vivo

To investigate the biological behaviors of HKDC1 in LUAD cells, we established two LUAD cell lines stably expressing HKDC1, and the expression level of HKDC1 was examined by qRT-PCR and western blotting. The result showed that HKDC1 expression was obviously upregulated in ov-HKDC1 cells compared to its control cells (Fig. 3A, 3B).

To determine the effect of HKDC1 on cell proliferation, we performed the CCK–8 assay. The results indicated that HKDC1 overexpression promoted the proliferative ability of LUAD cells (Fig. 3C). Subsequently, transwell assay was employed to investigate the impact of HKDC1 on cell migration and invasion. The results revealed that HKDC1 overexpression in A549 and H1299 cells increased the migratory and invasive abilities compared to the corresponding cell group (Fig. 3D, 3E). To investigate the effect of HKDC1 on LUAD tumor growth in vivo, we performed the xenograft growth assays in nude mice. As a result, the tumors in HKDC1 overexpression group exhibited larger sizes than those in control group (Fig. 3F).

Silencing HKDC1 inhibited proliferation, invasion, migration of LUAD

Then we constructed the lentivirus-mediated HKDC1 knockdown LUAD cells, which was validated by qRT-PCR and western blotting (Fig. 4A, 4B). On the contrary, knockdown of HKDC1 inhibited the proliferation of LUAD cells and decreased the migratory and invasive abilities compared to those in the corresponding groups (Fig. 4C-E). In addition, animal experiments showed that tumors of HKDC1 knockdown groups were significantly smaller than their control group, as expected (Fig. 4F).

HKDC1 regulated glycolysis and epithelial-mesenchymal transition in LUAD cells

HK enzymes are rate-limiting enzyme of glycolysis, which is involved in regulating glycolysis in multiple cancers [26]. As a member of HK family, it is necessary to investigate the effects of HKDC1 on glycolysis. Cancer cells convert glucose into lactate, thus glucose consumption and lactate production were two common measures to reflect glycolysis level [27]. Firstly, we detected the glycolysis levels by overexpressing HKDC1 in LUAD cells. The results showed that both glucose consumption and lactate production were significantly increased in ov-HKDC1 A549 and H1299 cells compared with control cells (Fig. 5A). On the contrary, silencing HKDC1 reduced the glucose consumption and lactate production in both A549 and H1299 cells (Fig. 5B). Taken together, these data demonstrated that HKDC1 promoted aerobic glycolysis and lactate production in LUAD cells.

Increasing studies have shown that aberrant cancer metabolism can regulate EMT through certain pathological pathways [28], and in turn, EMT also exacerbates dysregulation of glucose metabolism. Therefore, we evaluated the impact of HKDC1 on the EMT in LUAD cells. The results of western blotting revealed decreased levels of epithelial marker, E-cadherin and increased levels of mesenchymal markers such as N-cadherin, Vimentin, and Snail in the ov-HKDC1 group (Fig. 5A). On the contrary, increased epithelial marker and decreased mesenchymal marker were observed in the sh-HKDC1 group (Fig. 5B), indicating that HKDC1 could enhance EMT capacity of LUAD cells.

HKDC1 regulates AMPK/mTOR signaling pathway

To gain further insight into the molecular mechanism by which HKDC1-mediated oncogenic roles in LUAD, we tried to make a connection between HKDC1 and its potential signaling pathway. AMPK and mTOR are two protein kinases that highly sensitive to cellular energy status, and they play a crucial role in glycolysis and protein synthesis [29]. Thus, the influence of HKDC1 on AMPK/mTOR signal pathway was investigated subsequently. In our study, we observed that phosphorylation level of AMPKα at Thr172 was markedly decreased and phosphorylation level of mTOR at Ser2448 was significantly increased in ov-HKDC1 LUAD cells, whereas p-AMPK level was increased and p-mTOR level was decreased in sh-HKDC1 LUAD cells, indicating that HKDC1 acted as an important regulator on AMPK/mTOR signal pathway (Fig. 6A).

To further verify whether the oncogenic role of HKDC1 was mediated by AMPK/mTOR signal pathway in LUAD, we applied a rescue strategy. Rapamycin, an inhibitor of AMPK/mTOR signal pathway, was

incubated in ov-HKDC1 LUAD cells. According to the results of a series of functional experiments, the proliferation (Fig. 6B), migration, invasion (Fig. 6C, 6D), glycolysis (Fig. 6E) and EMT (Fig. 6F) capacity could be compromised after the treatment of rapamycin.

Collectively, these results showed that rapamycin could significantly decrease HKDC1-induced oncogenic functions of LUAD cells, indicating that HKDC1 executes biological behaviors via regulating AMPK/mTOR pathway.

Discussion

In this study, we discovered that HKDC1 was highly expressed in LUAD tissues and cell lines, and the positive expression of HKDC1 was correlated with aberrant clinicopathological characteristics in LUAD patients. Besides, HKDCI could be served as an OS predictor for LUAD patients. Overexpression of HKDC1 promoted the proliferation, migration, invasion, glycolysis, EMT and tumorgenicity in vitro and in vivo, whereas knockdown of HKDC1 produced the opposite functional effect. Mechanistically, HKDC1 could regulate AMPK/mTOR signaling pathway to perform its biological function. Targeting HKDC1 may provide a new therapeutic strategy for LUAD treatment.

Li and colleagues screened the top 20 genes with a possibility of being developed into potential therapeutic targets of lung cancer, among which 19 genes were targeted by approved drugs or drugs used in clinical trials [25]. HKDC1 was the only one that had not been included in these 20 candidate targets, which attracted our interests. Besides, the role as an oncogenic gene of HKDC1 in liver cancer and colorectal cancer prompts us to investigate the its functional effect in LUAD. In the present study, we found that HKDC1 was highly expressed in LUAD tissues while was not expressed or lowly expressed in adjacent normal lung epithelial tissues, and its expression was correlated with aberrant clinicopathological characteristics and prognosis. These data preliminarily revealed that HKDC1 displays oncogenic function in LUAD and these unique properties make it to be a perfect therapeutic target for LUAD.

Metabolism reprogramming, such as glycolysis under the aerobic conditions, has been increasingly deemed as a hallmark for tumor progression in multiple cancers [30]. Elevated glycolysis in cancer cells promotes glucose uptake and lactate production to fulfill their metabolic demands, followed by the increased ability of invasion and metastasis. It has been reported recently that HKDC1 catalyzes glucose phosphorylation and the cellular energy metabolism involving cancer growth and metastasis [23]. In our study, we observed that HKDC1 increased the glucose consumption and the lactate production, indicating HKDC1 could regulate the aerobic glycolysis in LUAD. Chen et al found that HKDC1 was located on the mitochondrial membrane and regulated the permeability transition pore opening, which gave a reasonable mechanism for metabolic effect of HKDC1[23]. Moreover, HKDC1 could promote the proliferation, invasion and EMT capacity in vitro and tumor growth in vivo. These findings have demonstrated that HKDC1 could function as an oncogenic gene in LUAD, which provides more evidence that it may serve as a therapeutic target for LUAD.

AMPK is a sensor of energy status that maintains cellular energy homeostasis, which contributes to regulating mitochondrial biogenesis and disposal, cell polarity, cell growth and proliferation [31]. mTOR, a central integrator of nutrient and growth factors, is regulated negatively by AMPK, which in turn promotes the processes including cell cycle, cell growth and angiogenesis [32]. AMPK/mTOR signaling pathway is dysregulated in most human cancers and has been considered as a promising therapeutic target against cancers [33,34]. On the ground that HKDC1 could promote the aerobic glycolysis in LUAD, we conjectured that AMPK/mTOR pathway may mediate the oncogenic functions of HKDC1. To test the effect of HKDC1 on this pathway, western blotting was used to examine the expression of the key factors of the signaling pathway. As expected, HKDC1 overexpression resulted in the decreased p-AMPK expression and the increased p-mTOR expression. More importantly, the blockage of AMPK/mTOR pathway by rapamycin could attenuate the biological effect of HKDC1 on LUAD cells, indicating biological role of HKDC1 was mediated by AMPK/mTOR signal pathway.

In conclusion, our study demonstrates that HKDC1 plays an oncogenic role in LUAD. Targeting this gene may provide a promising therapeutic target to delay LUAD progress.

Conclusions

HKDC1 is highly expressed in lung adenocarcinoma (LUAD) and it could be served as a prognostic predictor for LUAD patients. Overexpression of HKDC1 promotes the proliferation, migration, invasion, glycolysis, EMT and tumorgenicity of LUAD via activating the AMPK/mTOR signaling pathway. Thus, HKDC1 may be a promising therapeutic target of LUAD.

Declaration

Ethics approval and consent to participate

Specimen collection and animal experiments were approved by the Ethics Committee of Changhai Hospital.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analyzed during this study are included in this published article and its additional fles.

Competing interests

None

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Authors' contributions

Xinyu Wang: performed the experiments, conduct the statistical analysis, conception and design, data analysis, manuscript writing. Qijue Lu, Yue Zhao: data analysis, manuscript revision, final approval of manuscript. Jianglong Chen, Xiang Fei, Chaojing Lu: conduct the statistical analysis, help data analysis. Chunguang Li, Hezhong Chen: conception and design, financial support.

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Figures

Fig. 1

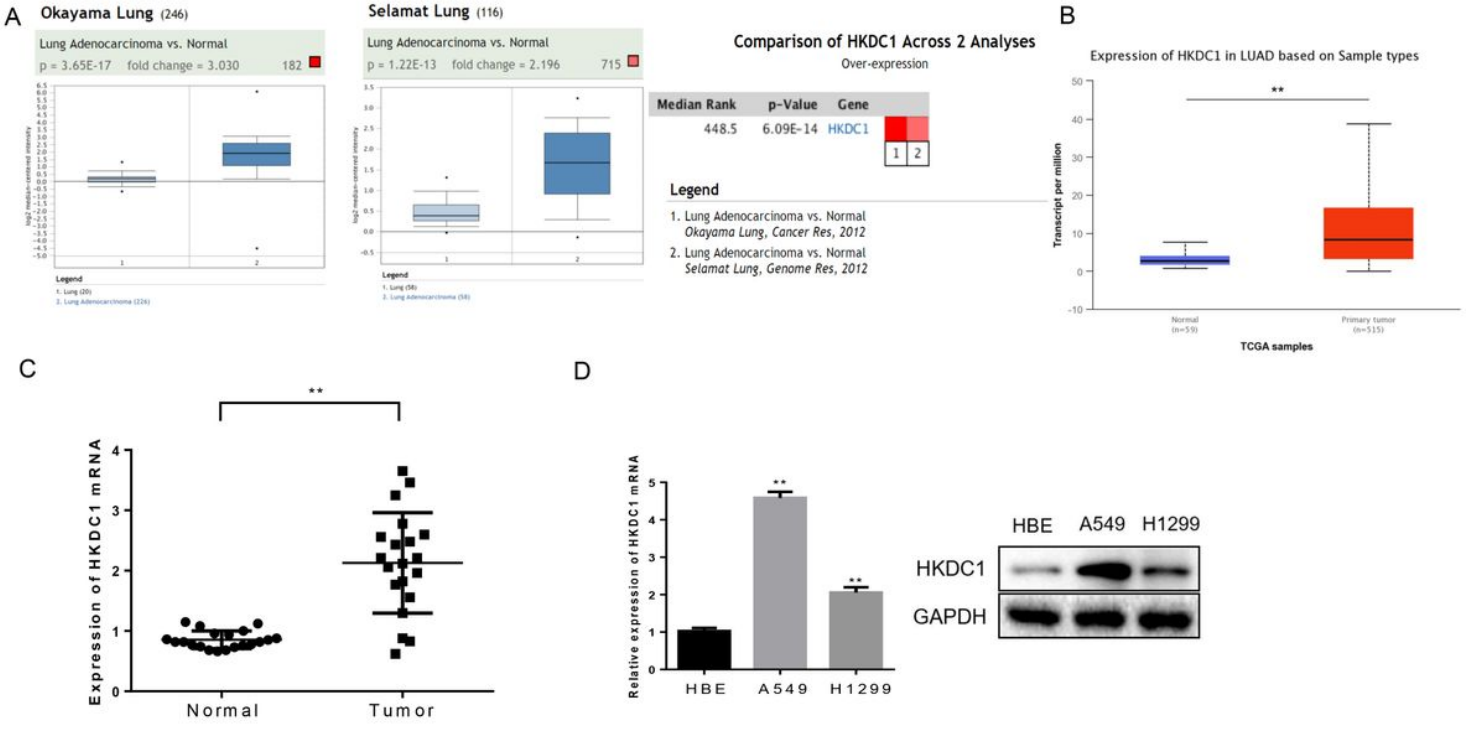

Figure 1

HKDC1 was up-regulated in LUAD tissues and cells. (A, B) Oncomine (A) and UALCAN (B) data showed that HKDC1 was highly expressed in LUAD tissues than normal tissues. (C) The HKDC1 mRNA expression level was significantly elevated in LUAD tissues than their normal lung tissues in 20 paired of fresh tissues. (D) The HKDC1 expression level was obviously highly expressed in the LUAD cell lines compared with the normal human lung epithelial cell line HBE. **P<0.01

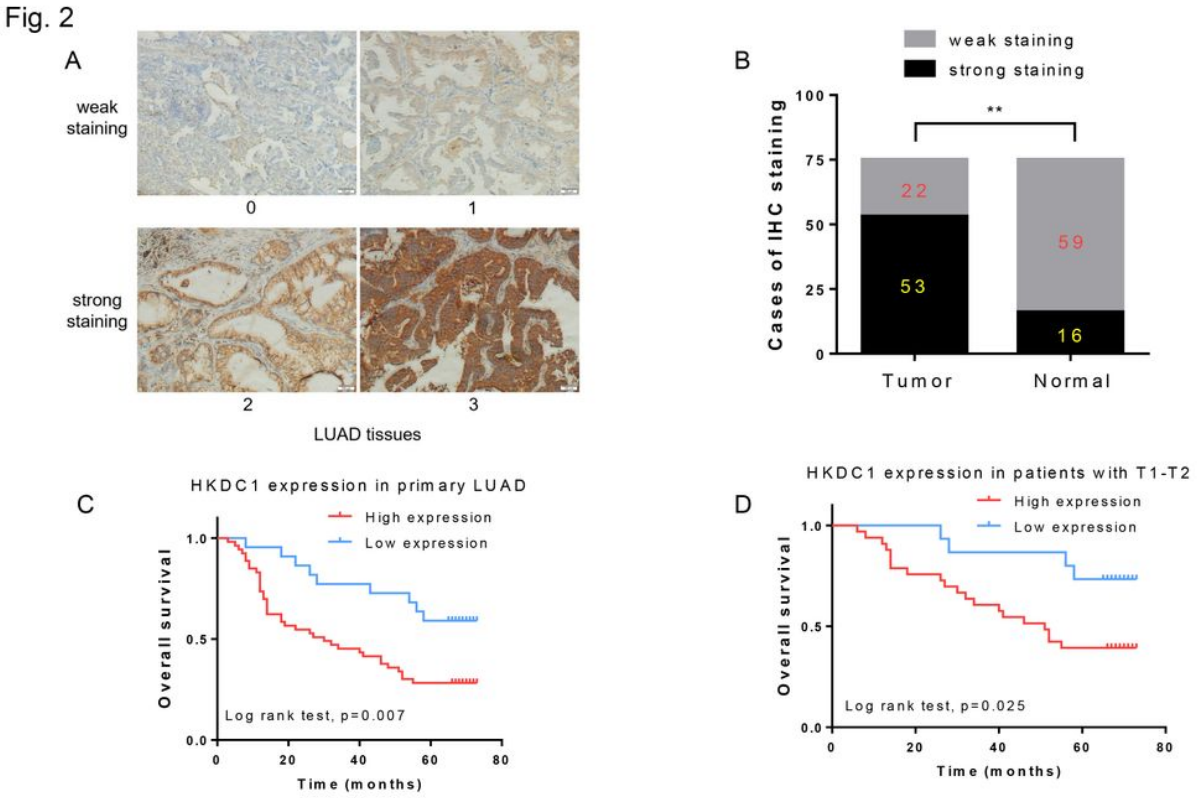

Figure 2

HKDC1 was associated with aggressive features and poor prognosis in LUAD. (A) Representative staining intensity of HKDC1 was graded from 0 to 3 by IHC analysis (Magnification: ×200). (B) The ratio for strong stating and for weak staining in 75 paired of tumor and non-tumor tissues. (C) Overall survival (OS) was more unfavorable in high expression of HKDC1 than those with low expression in 75 LUAD patients. (D) Subgroup analysis demonstrated that pT1-T2 stage patients with high expression of HKDC1 had unfavorable OS. **P<0.01

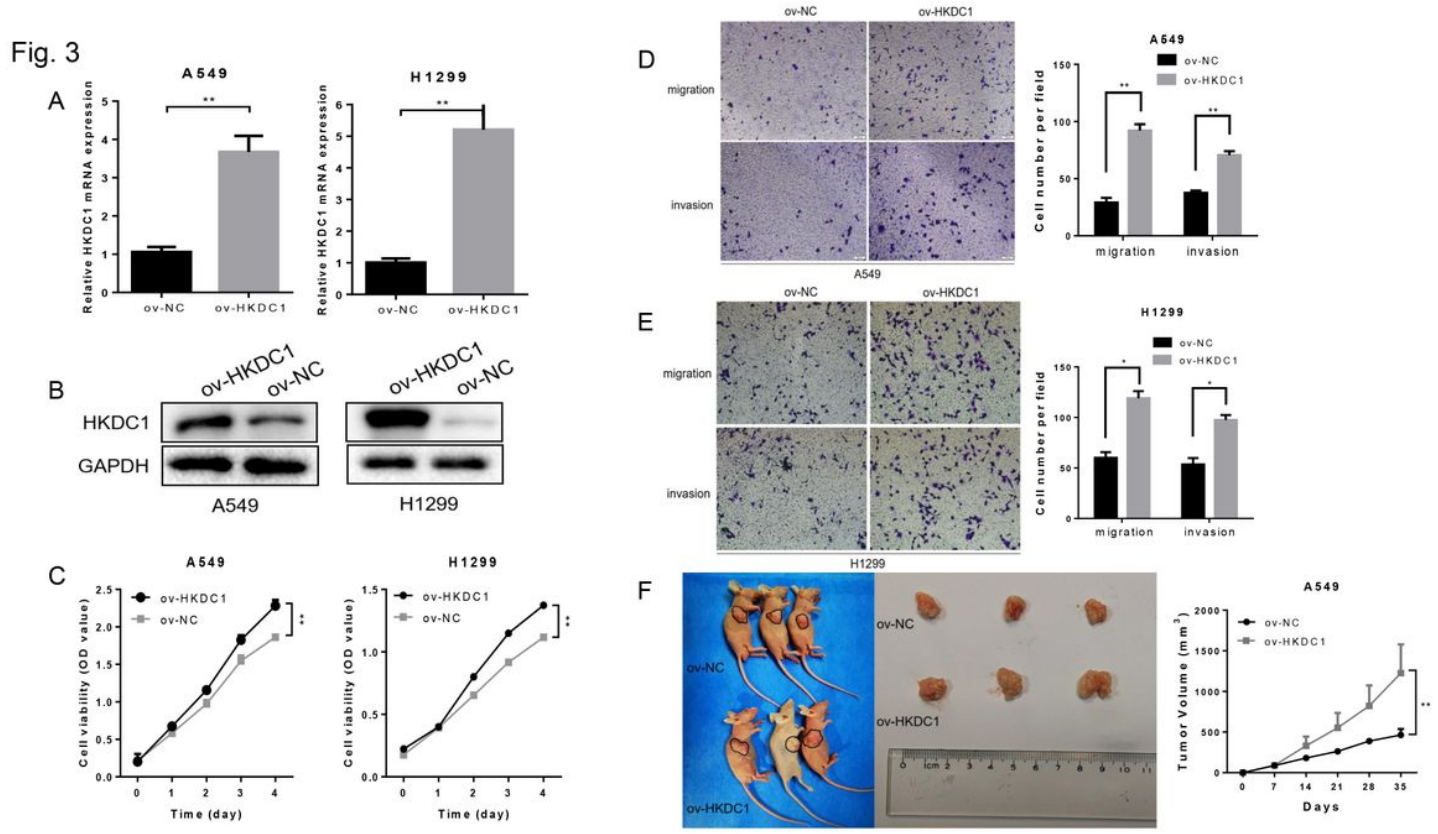

Figure 3

HKDC1 promoted proliferation, invasion, migration in vitro and tumorigenesis in vivo. (A, B) The mRNA (A) and protein level (B) of HKDC1 was detected after transfected with overexpressed HKDC1 in both A549 and H1299 cells. (C) Both A549 and H1299 cells viability was increased after transfected with overexpressed HKDC1, detected by CCK-8 assay. (D, E) Overexpression of HKDC1 promoted A549 (D) and H1299 (E) cells migration and invasion in vitro as assessed by the wound healing assay (scale bar, 100μm). (F) HKDC1 overexpression promoted tumor growth in vivo. *P<0.05, **P<0.01

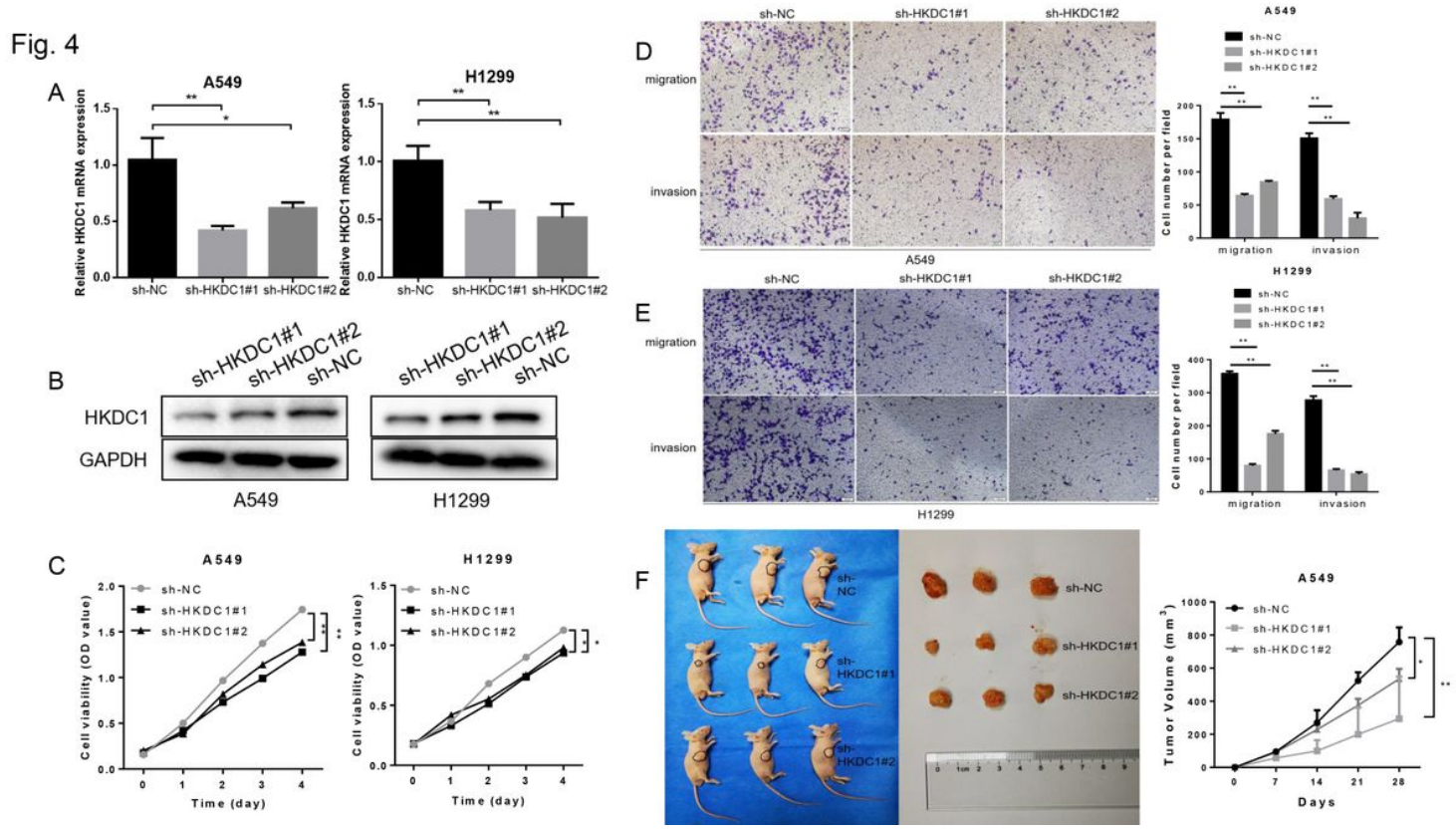

Figure 4

Silencing HKDC1 inhibited proliferation, invasion, migration in vitro and tumorigenesis in vivo. (A, B) The mRNA (A) and protein level (B) of HKDC1 was detected after transfected with HKDC1 knockdown in both A549 and H1299 cells. (C) Both A549 and H1299 cells viability was decreased after transfected with shHKDC1, detected by CCK-8 assay. (D, E) Knockdown of HKDC1 inhibited A549 (D) and H1299 (E) cells migration and invasion in vitro as assessed by the wound healing assay (scale bar, 100μm). (F) HKDC1 knockdown inhibited tumor growth in vivo. *P<0.05, **P<0.01

Fig. 5

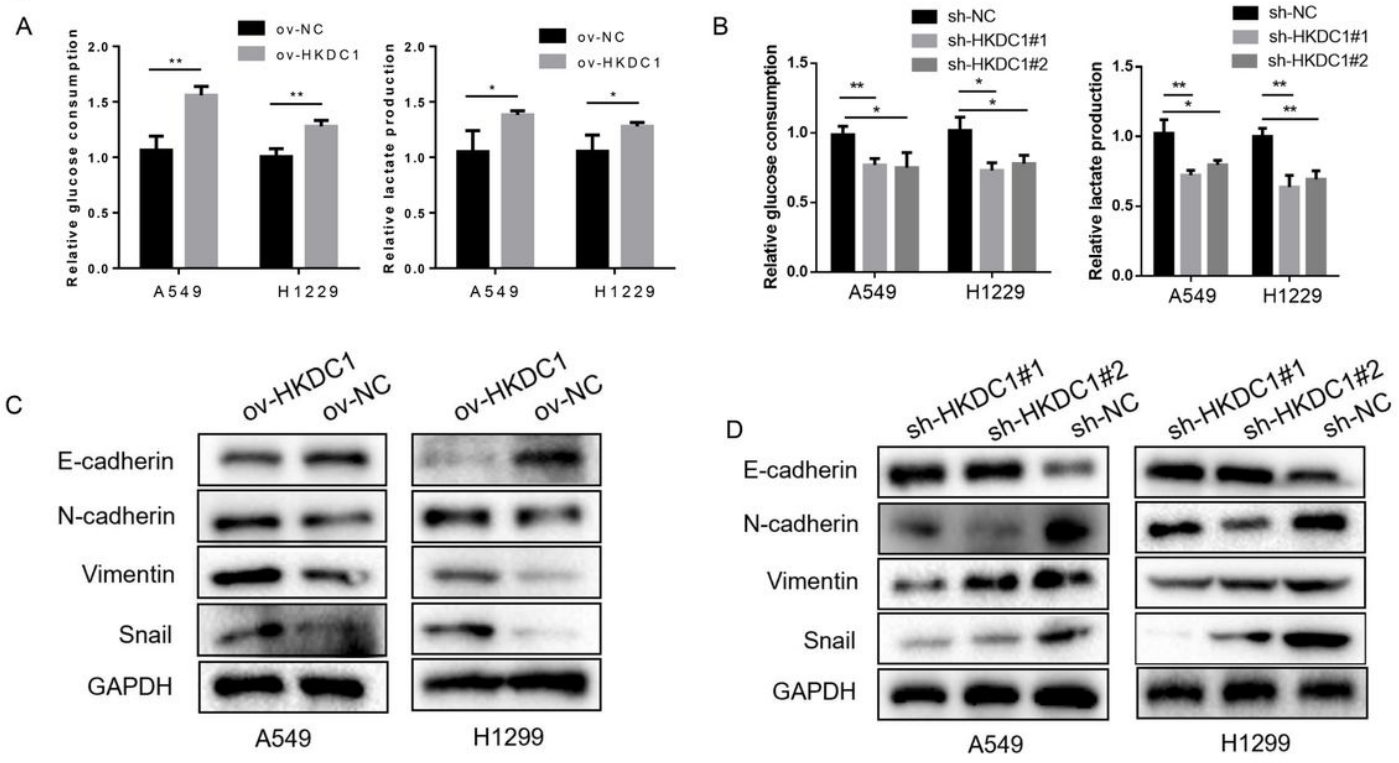

Figure 5

HKDC1 regulated glycolysis and epithelial-mesenchymal transition in LUAD cells. (A) Overexpression of HKDC1 promoted the glucose consumption and lactate production in both A549 and H1299 cells. (B) Silencing HKDC1 reduced the glucose consumption and lactate production in both A549 and H1299 cells. (C) Overexpression of HKDC1 promoted the expression of N-cadherin, Snail, Vimentin while decreased the expression of E-cadherin in LUAD cells. (D) Silencing HKDC1 decreased the expression of N-cadherin, Snail, Vimentin while increased the expression of E-cadherin in LUAD cells. *P<0.05, **P<0.01

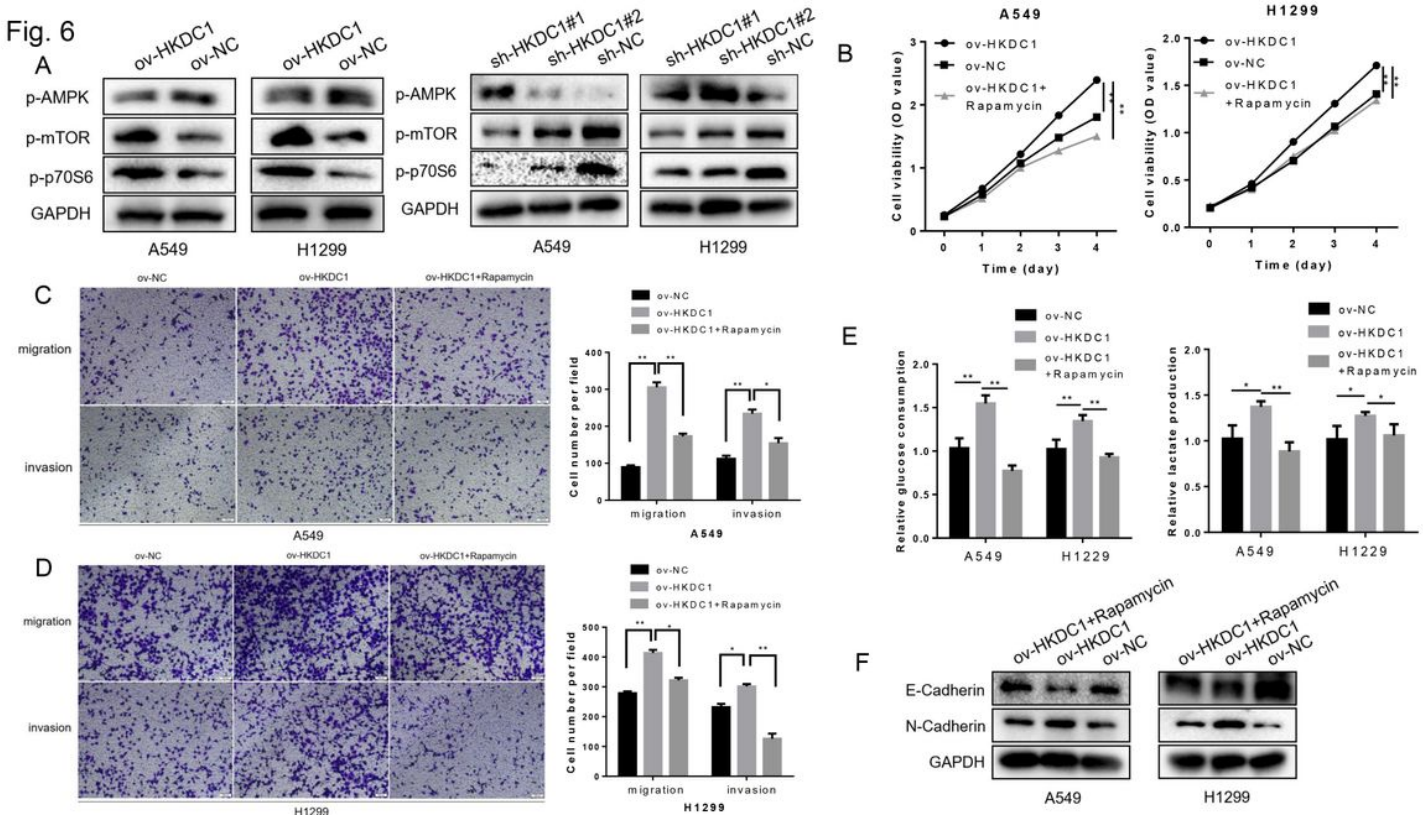

Figure 6

HKDC1 regulates AMPK/mTOR signaling pathway. (A) HKDC1 regulated the expression of p-AMPK, p-mTOR and p-p70S6 in LUAD cells. (B) Rapamycin compromised the proliferative effect of HKDC1 in LUAD cells. (C) Rapamycin compromised the migratory effect of HKDC1 in LUAD cells. (D) Rapamycin compromised the invasive effect of HKDC1 in LUAD cells. (E) Rapamycin compromised the metabolic effect of HKDC1 in LUAD cells. (F) Rapamycin compromised the EMT capacity of HKDC1 in LUAD cells. *P<0.05, **P<0.01