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Original Article

CDC37 promotes the proliferation and invasion of human hepatocellular carcinoma cells through AKT1 signaling pathway

Xiwei Guo[1*], Xiaoqin Wang[1*], Yuanmin Pei[1], Huashan Yi[1], Xiaohan Yue[2]

[1]*Yidu Central Hospital of Weifang, Weifang, Shandong, People's Republic of China;* [2]*Weifang Medical University, Weifang, Shandong, People's Republic of China.* [*]*Equal contributors.*

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Abstract: The molecular co-chaperone CDC37 (co-chaperone cell division cycle 37) is a co-chaperone of HSP90. It has been shown to form complex with a variety of protein kinases including AKT1 and then stabilize them. Moreover, CDC37 is upregulated in human hepatocellular carcinoma cells. Here, we found that the expression levels of CDC37 were upregulated in human hepatocellular carcinoma cell lines with highly invasive potential. Overexpression of CDC37 in hepatocellular carcinoma cells could increase cell proliferation and invasion. Meanwhile, overexpression of CDC37 could increase the protein levels of AKT1. PINK1 (PTEN Induced Putative Kinase 1), a CDC37 target kinase, was also found to be upregulated by the overexpression of CDC37. Furthermore, overexpression of PINK1 could increase the activity of AKT1. Importantly, overexpression of CDC37 could not increase the cell proliferation and invasion of human hepatocellular carcinoma cells in the presence of AKT1 inhibitor A-674563. These results demonstrate that CDC37 promotes cell proliferation and invasion through stabilizing and activating AKT1. Thus, our findings support that CDC37 may be a useful therapeutic target in human hepatocellular carcinoma.

Keywords: CDC37, human hepatocellular carcinoma, proliferation, invasion, AKT1, PINK1

Introduction

Human hepatocellular carcinoma (HCC) is one of the most common adult liver malignancy worldwide [1]. HCC is the sixth most prevalent cancer and the third most common cause of cancer mortality [2]. Thus, the understanding of the key molecular drivers of HCC is important to the identifications of treatments.

AKT1, which is aberrant activated in HCC, plays critical roles in the development of human cancer [3-5]. There are various factors contributing to activation of the AKT1 signal transduction pathway in human cancer. Overexpression or amplification of AKT1 was found in several types of cancers [6-8]. Aberrant activation of AKT1 was also found in human cancers, including breast carcinoma, lung carcinoma, ovarian carcinoma, prostate carcinoma and hepatocellular carcinoma [9-11]. Aberrant activated AKT1 can mediate cell proliferation, anti-apoptotic growth, invasion and metastasis by phosphorylating and activating the downstream kinases [11]. For example, glycogen synthase kinase 3 Beta (GSK-3β) can be phosphorylated by AKT1 to protect cyclin D1 from degradation [12, 13]. AKT1 also directly phosphorylates cyclin-dependent kinase inhibitor p27 to allow its cytoplasmic localization and degradation [14]. Moreover, the activity or stability of AKT1 can be regulated by other factors. AKT1 acetylation by p300/CBP-associated factor (PCAF) enhances the phosphorylation of AKT1 and promotes its activity [15, 16]. The molecular co-chaperone CDC37, a co-chaperone of HSP90, has been shown to form complex with AKT1 and stabilize it [17, 18].

CDC37 is upregulated in human hepatocellular carcinoma cells [19-21]. Inhibition of the expression of CDC37 could inhibit the cell cycle progression and cell growth of hepatocellular carcinoma cells [20, 21]. Here, we investigate whether CDC37 is involved in the regulation to the cell growth and invasion in human hepatocellular carcinoma cells.

Material and methods

Cell culture and reagents

The human hepatocellular carcinoma cells lines HCC-LM3, HepG2, Hep3B, SMMC7721, Hub7 and the human embryonic kidney cells (HEK-293T) were cultured in Dulbecco's modified Eagle's medium (DMEM) (GIBCO, Scotland, UK), supplemented with 10% fetal calf serum (GIBCO, Scotland, UK) at 37°C and 5% CO_2 in a humidified atmosphere. Selective AKT1 inhibitor A-674563 was from Santa Cruz Biotechnology (Santa Cruz, CA).

RNA isolation and real-time RT-PCR

Total RNA were extracted from cells using Trizol (Invitrogen, California, USA) according to the manufacturer's protocol. SuperScript III Reverse Transcriptase (Invitrogen, California, USA) was used to perform the reverse transcription and cDNA synthesis. The qRT-PCR assays were performed to evaluate the expression of target genes and the primers were designed as follows: 5'-GGAGCAGAAACACAAGACCTTCG-3' (CDC37, sense); 5'-GACCAGGTAATTGGCTGTCTCC-3' (CDC37, antisense), 5'-GTCTCCTCTGACTTCAACAGCG-3' (GAPDH, sense); 5'-ACCACCCTGTTGCTGTAGCCAA-3' (GAPDH, antisense), 5'-TGGACTACCTGCACTCGGAGAA-3' (AKT1, sense); 5'-GTGCCGCAAAAGGTCTTCATGG-3' (AKT1, antisense). The mRNA expression levels were standardized to GAPDH mRNA by the ΔΔCt method. All experiments were performed at least three times.

Western blot

Cells were harvested, washed twice in PBS, and lysed in lysis buffer (protease inhibitors were added immediately before use) for 30 min on ice. The protein concentration was determined using BCA assay kit (Merck Millipore Bioscience, Germany). Then the samples were electrophoresed by 10% SDS-PAGE gel, and transferred to a polyvinylidine difluoride (PVDF) membrane (Merck Millipore Bioscience, Germany). The membranes were blocked with 5% BSA for 1 hour at room and then the primary antibodies were added for incubation overnight at 4°C. After being washed twice with TBST for 15 min, peroxidase conjugated secondary antibodies were incubated with the membrane for 30 min. Followed by washing twice with TBST for 15 min, the membranes were visualized by an enhanced chemiluminescence system (ECL, Amersham Pharmacia Biotech). The primary antibodies: rabbit anti-human CDC37 (1:1000) and rabbit anti-human GAPDH antibody (1:5000) were from Abcam (Cambridge, MA, USA); Rabbit anti-human AKT1 (1:1000), mouse anti-human phospho-AKT1 (S473, 1:1000) and rabbit anti-human PINK1 (1:1000) were from Cell Signaling Technologies (Beverly, MA, USA). Goat anti-Mouse IgG peroxidase conjugated secondary antibodies (1:10000) and Goat anti-Rabbit IgG peroxidase conjugated secondary antibodies (1:10000) were from GeneTex (Irvine, CA, USA).

Lentiviral vector production and cell transduction

The lentiviral expression vectors pBoBi-puro-CDC37 (NM_007065) and pBoBi-puro-PINK1 (NM_032409) were purchased from Genesent (Shanghai, China). Lentivirus plasmid vectors pLKO.1-puro with shRNA specific for CDC37 and non-target shRNA control were purchased from Sigma-Aldrich (St. Louis, MO, USA). The target sequences to CDC37 were: sh-a, GCCCATTCAAGTCTCTGCTTT; sh-b, CCAGACAATCGTCATGCAATT. The target sequences of non-target shRNA control: CCGGCAACAAGATGAAGAG. Lentiviral vectors were produced in HEK 293T cells with packaging plasmids. The recombinant viral supernatants were harvested from HEK 293T cells and then used to infect target cells in the presence of 8 μg/ml polybrene. After selected with puromycin-containing media for 72 hours, then the expression levels of target genes were analyzed by western blot.

Plate colony formation assay

The cells were seeded at a density of 2000 cells per well in 6-well plates. After 2 weeks, cells were washed with PBS, fixed in 10% methanol for 15 min, and stained with Giemsa for 20 min. Visualized colonies were then photographed and scored. Each plate colony formation experiment was repeated at least three times.

In vitro matrigel invasion assay

Cells were added onto BD Falcon Cell Culture Inserts coated with Matrigel inside BD BioCoat Matrigel Invasion Chambers (Becton Dickinson, Bedford, MA, USA). After cultured at 37°C for 48 h, cells invaded through Matrigel to the bottom side of the inserts were stained with hematoxylin-eosin (H&E), photographed under microscope and quantified.

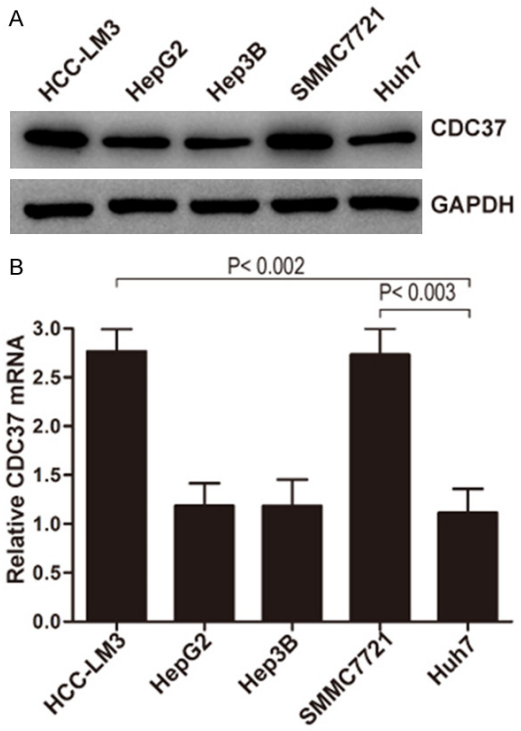

Figure 1. The expression levels of CDC37 in human hepatocellular carcinoma cell lines. A. CDC37 and GAPDH protein expression levels were determined by western blot analysis in five human hepatocellular carcinoma cell lines as indicated. B. The mRNA expression levels of CDC37 in five human hepatocellular carcinoma cell lines were determine by real-time RT-PCR analysis. GAPDH was used as an internal quantitative control.

Statistical analysis

Data are presented as means ± SE of the indicated number of experiments, and statistical analyses were performed using Student's t test using GraphPad prism 5. A P value of < 0.05 was considered statistically significant. The results were reproduced in three repeated experiments.

Results

CDC37 is overexpressed in human hepatocellular carcinoma cell lines with highly invasive potential

Previous studies have showed that CDC37 is upregulated in several types of cancers, including human hepatocellular carcinoma cells [21-23]. Here, we examined the expression levels of CDC37 in five human hepatocellular carcinoma cell lines. The expression of CDC37 was detected in all of these cell lines (**Figure 1A**). In addition, western blot analysis indicated that HCC-LM3 and SMMC7721 cells with highly invasive potential express higher levels of CDC37 compared to other lowly highly invasive cell lines (**Figure 1A**). We then performed real-time PCR to examine the expression levels of CDC37 mRNA. As shown in **Figure 1B**, the expression levels of CDC37 mRNA were consistent with the protein expression in the same human hepatocellular carcinoma cell lines. These results indicated that CDC37 was abundantly overexpressed inhuman hepatocellular carcinoma cell lines, especially in the highly invasive cell lines.

CDC37 promotes the proliferation of human hepatocellular carcinoma cells

To examine the role of CDC37 in human hepatocellular carcinoma cells, stable CDC37 overexpressing HepG2 or Hep3B cells were established using lentiviral expression system. Western blot analysis showed that the expression levels of CDC37 protein were dramatically increased in CDC37 stably overexpressing cells compared to the empty vector controls (**Figure 2A**). We then investigated the function of CDC37 to the proliferation of human hepatocellular carcinoma cells. Our findings showed that stably overexpressing CDC37 in HepG2 or Hep3B cells significantly promoted the cell proliferation compared to the empty vector controls (**Figure 2A**). Moreover, stable CDC37 knock-down HCC-LM3 cells were also established using lentivirus-mediated RNA interference. Western blot analysis showed that the expression levels of CDC37 protein were dramatically decreased in CDC37 stably knockdown HCC-LM3 cells compared to the scramble controls (**Figure 2B**). In CDC37 stably knockdown HCC-LM3 cells, cell proliferation is greatly reduced compared to the scramble controls (**Figure 2B**). Taken together, these results indicated that CDC37 was essential to the proliferation of human hepatocellular carcinoma cells.

CDC37 promotes the cell invasion and the activity of AKT1 of human hepatocellular carcinoma cells

To investigate whether CDC37 affects the cell invasion of human hepatocellular carcinoma cells, we performed cell invasion assay to assess the invasive potential between CDC37

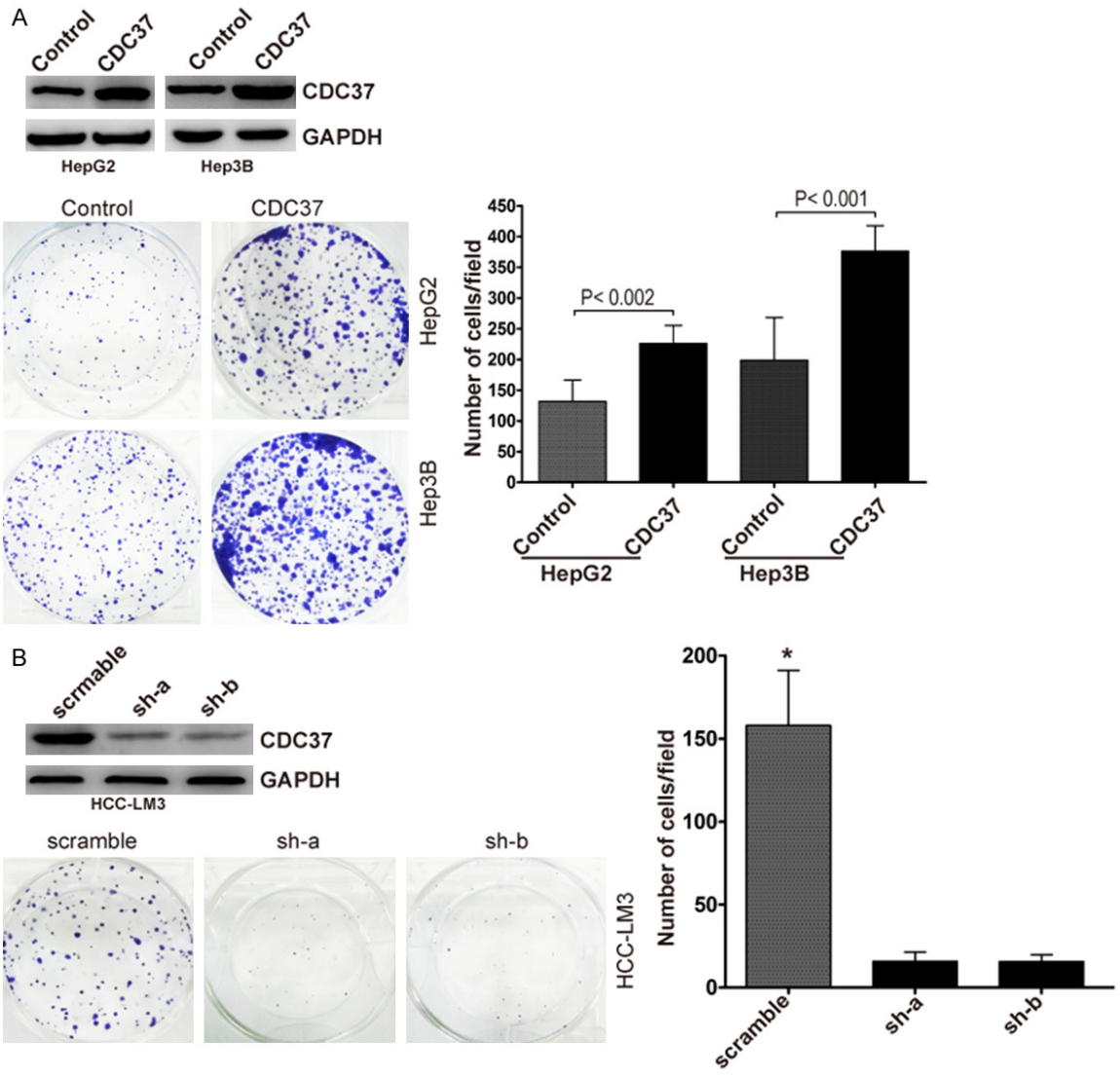

Figure 2. CDC37 promotes the proliferation of human hepatocellular carcinoma cells. A. CDC37 protein levels in vector control and CDC37 stably over-expressed HepG2 or Hep3B cells by western blot analysis (upper panel). GAPDH was used as an internal quantitative control. Representative photographs of colony formation in vector control and CDC37 stably over-expressed HepG2 or Hep3B cells (down panel). Representative data are shown as the mean ± SD. n=3; $P < 0.002$ or $P < 0.001$, vs. each control group. B. CDC37 protein levels in scramble control and CDC37 stably knock-down (sh-a/sh-b) HCC-LM3 cells by western blot analysis (upper panel). GAPDH was used as an internal quantitative control. Representative photographs of colony formation in scramble control and CDC37 stably knock-down HCC-LM3 cells (down panel).Representative data are shown as the mean ± SD. n=3; $^{*}P < 0.0001$, vs. each knock-down group.

stably overexpressing cells and the empty vector controls. As shown in **Figure 3A**, overexpression of CDC37 promoted the cell invasion in HepG2 or Hep3B cells compared to their empty vector controls. CDC37 has been shown to form complex with AKT1 and then stabilize it [21]. Moreover, active AKT1 was reported to enhance cell invasion and migration in several types of cancer cells [24-26]. We then assessed the effect of CDC37 on the activity of AKT1. AKT1 is activated by phosphorylation at Thr308 and Ser473 [27]. The phosphorylation at Ser-473 of AKT1 can represent the active form of AKT1 [28]. We then detected the change of the levels of AKT1 total and phosphorylated protein at Ser473 after the overexpression of CDC37 in in HepG2 or Hep3B cells. As shown in **Figure 3B**, overexpression of CDC37 resulted

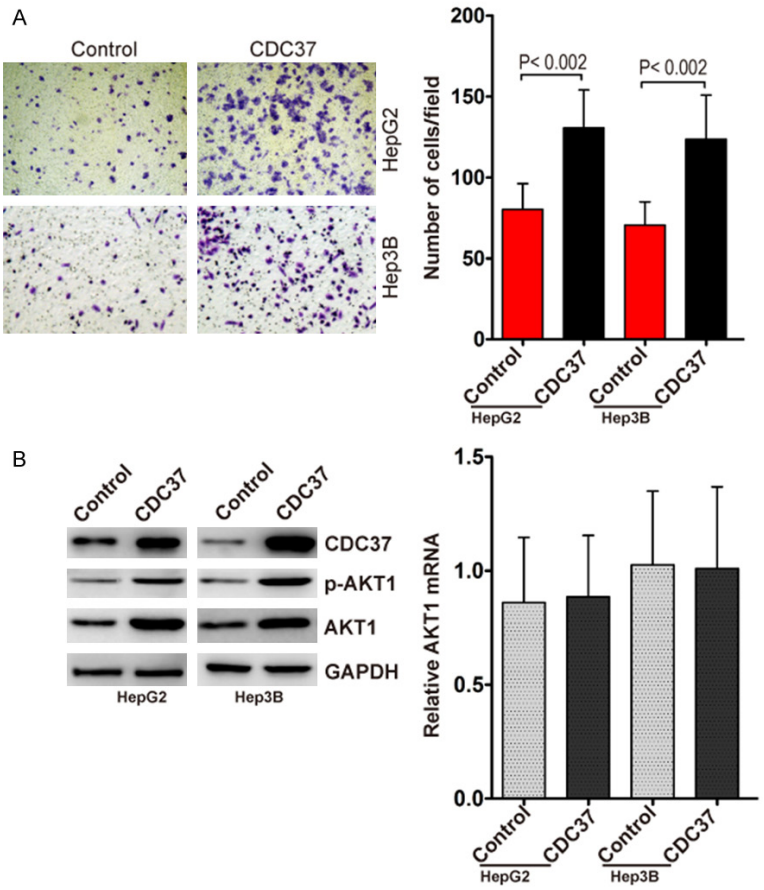

Figure 3. CDC37 promotes the cell invasion and the activity of AKT1. A. Invade cells in invasion assay were stained and counted in vector control and CDC37 stably over-expressed HepG2 or Hep3B cells. P < 0.002 compared with the control group. B. CDC37, AKT1, p-AKT1 (phosphorylation at Ser473) and GAPDH expression levels were determined by western blot analysis in vector control and CDC37 stably over-expressed HepG2 or Hep3B cells (left panel). The mRNA expression levels of AKT1 in vector control and CDC37 stably over-expressed HepG2 or Hep3B cells were determine by real-time RT-PCR analysis (right panel). GAPDH was used as an internal quantitative control.

in an increase in the levels of phosphorylated AKT1 protein at Ser473 and total AKT1 proteins. Moreover, the expression levels of AKT1 mRNA were not changed by the overexpression of CDC37 (**Figure 3B**). Our results revealed that overexpression of CDC37 could promote the invasion in human hepatocellular carcinoma cells. Therefore, we hypothesized that the increase of cell invasion by overexpression of CDCD37 might be due to the increased protein levels and, hence, the levels of active AKT1.

CDC37 increases the stability of PINK1 kinase

PTEN-induced kinase 1 (PINK1) was reported to increase the phosphorylation of AKT1 at Ser473 and promote the activity [29]. Moreover, the stability of PINK1 protein was enhanced by the direct interaction with CDC37 [30]. Here, we found that overexpression of CDC37 increase the protein levels of PINK1 in HepG2 or Hep3B cells (**Figure 4A**). Furthermore, we performed cycloheximide (CHX) chase experiments in vector control and CDC37 stably overexpressing HepG2 cells to determine endogenous PINK1 protein stability. In the vector control HepG2 cells, we found endogenous PINK1 destabilized at 4 h after the treatment with CHX. At 6 h, the protein levels of endogenous PINK1 continued to decrease (**Figure 4B**). In CDC37 stably overexpressing HepG2 cells, endogenous PINK1 was found to be slightly destabilized at 4 h. At 6 h, the protein levels of endogenous PINK1 did not changed compared to the levels at 4 h (**Figure 4B**). The stability of AKT1 protein was found to be consistent with PINK1 protein in CHX chase experiments. Additionally, overexpression of PINK1 in HepG2 or Hep3B cells was found to increase the phosphorylation of AKT1 at Ser473. These findings suggest that overexpression of CDC37 could stabilize AKT1 and activate AKT1 by stabilizing PINK1.

Inhibition of AKT1 attenuates CDC37 induced cell proliferation and invasion

In order to test the hypothesis that enhanced cell proliferation and invasion induced by overexpression of CDCD37 might be due to the increased activity of AKT1, selective AKT1 inhibitor A-674563 was used. In HepG2 or Hep3B cells, CDC37 overexpression induced upregulation of phosphorylation of AKT1 at Ser473 was inhibited (**Figure 5A**). Moreover, we performed cell invasion assay to assess whether A-674563 affect the cell invasion in CDC37 stably overexpressing human hepatocellular carci-

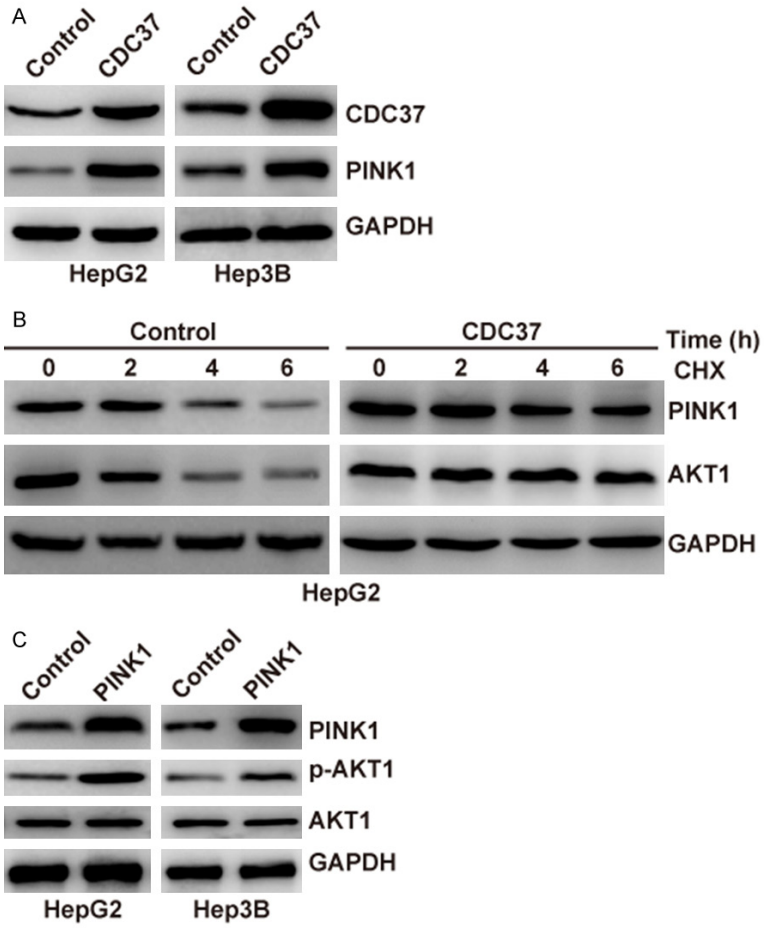

Figure 4. CDC37 increases the stability of PINK1 kinase. A. CDC37, PINK1 and GAPDH expression levels were determined by western blot analysis in vector control and CDC37 stably over-expressed HepG2 or Hep3B cells. B. The vector control and CDC37 stably over-expressed HepG2 cells were treated with 50 μg/mL of cycloheximide (CHX) for indicated times. Endogenous PINK1, AKT1 and GAPDH protein levels were determined by western blot analysis. C. PINK1, p-AKT1, AKT1 and GAPDH expression levels were determined by western blot analysis in vector control and PINK1 stably over-expressed HepG2 or Hep3B cells.

noma cells. As shown in **Figure 5B**, AKT1 inhibitor A-674563 significantly inhibited the increase of invasion induced by the overexpression of CDC37. In addition, enhanced cell proliferation by the overexpression of CDC37 was also be inhibited by A-674563 (**Figure 5C**). These results indicate that increased cell proliferation and invasion induced by the overexpression of CDC37 was mediated by AKT1 signaling pathway.

Discussion

In this study, we demonstrate that expression levels of CDC37 were upregulated in human hepatocellular carcinoma cell lines with highly invasive potential. Overexpression of CDC37 promotes the cell proliferation and invasion of human hepatocellular carcinoma cells. Meanwhile, overexpression of CDC37 increases the levels and the activity of AKT1, which could enhance cell invasion and migration in several types of cancer cells. Importantly, a selective AKT1 inhibitor A-674563 could attenuate CDC37 induced cell proliferation and invasion of human hepatocellular carcinoma cells. These results suggest that increased cell proliferation and invasion induced by the overexpression of CDC37 was mediated by AKT1 signaling pathway.

Overexpression or aberrant activation of AKT1 was found in several types of cancers, including breast carcinoma, lung carcinoma, ovarian carcinoma, prostate carcinoma and hepatocellular carcinoma [11]. Aberrant activated AKT1 can mediate cell proliferation [31], anti-apoptotic growth [32], invasion and metastasis [33] by phosphorylating and activating the downstream kinases. For example, Glycogen Synthase Kinase 3 Beta (GSK-3β) [34], cyclin-dependent kinase inhibitor p27 [14], B-Cell CLL/Lymphoma 10 (BCL10) [35], SMAD Family Member 3 (SMAD3) and BCL2-Associated Agonist of Cell Death (BAD) can be phosphorylated by AKT1 [36, 37]. Importantly, AKT activation was necessary for the migration and invasion for the tumor cells [38]. It was reported that AKT1 together with the Inositol Polyphosphate 5-Phosphatase PIPP regulates AKT1-dependent breast cancer cell migration and invasion [39]. Thus, understanding the regulatory mechanisms of AKT1 activity and expression is necessary for developing effective AKT1-targeting therapeutic strategies.

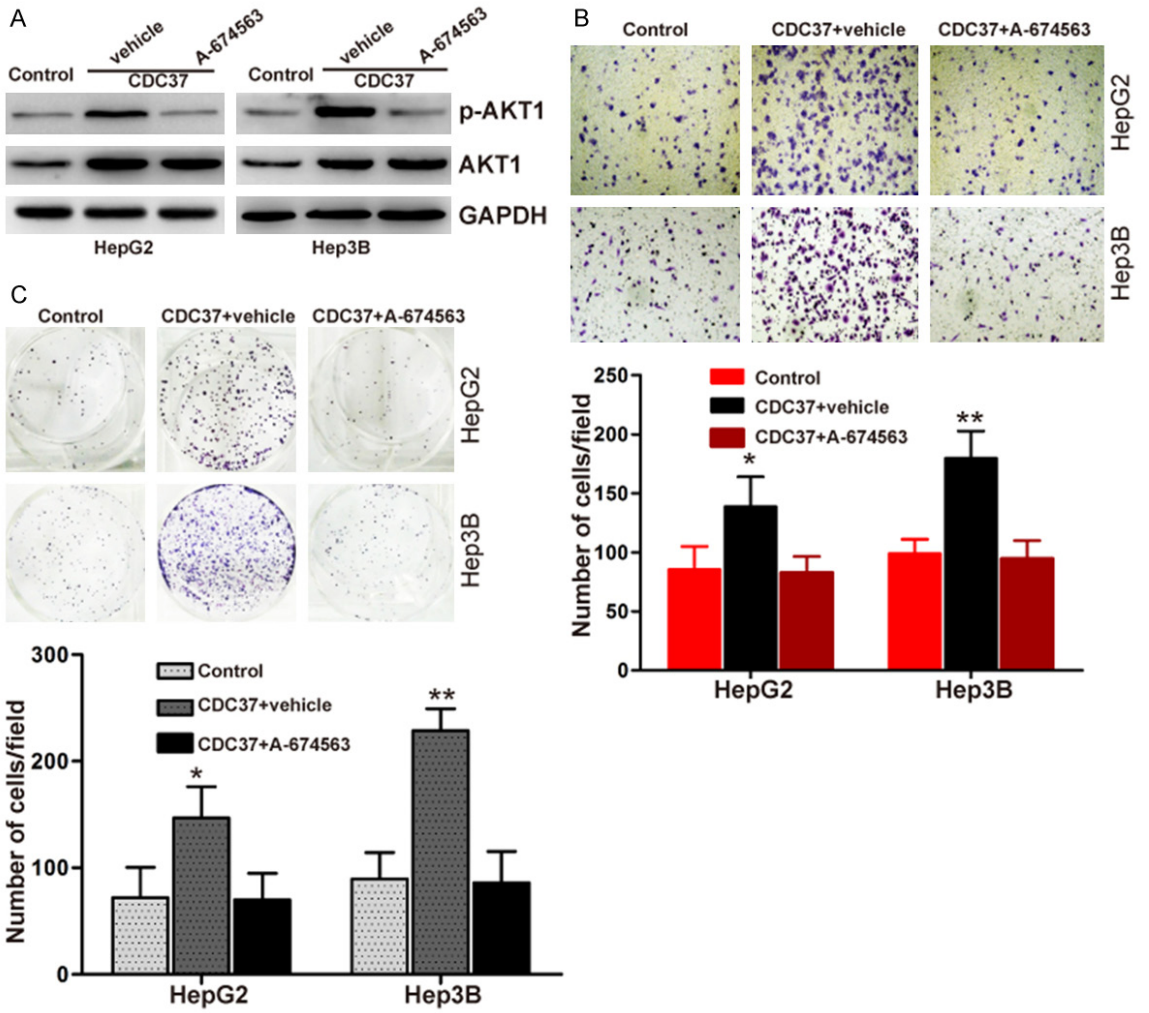

Figure 5. The effect of AKT1 inhibitor to the regulation of CDC37 induced cell proliferation and invasion. A. The vector control and CDC37 stably over-expressed HepG2/Hep3B cells treated with vehicle (DMSO) or 50 nM of A-674563 as indicated. AKT1, p-AKT1 and GAPDH expression levels were determined by western blot analysis. B. Invade cells in invasion assay were stained and counted in vector control and CDC37 stably over-expressed HepG2/Hep3B cells treated with vehicle (DMSO) or A-674563. $^{*}P < 0.003$, $^{**}P < 0.002$ compared with other group. C. The representative photographs of colony formation in vector control and CDC37 stably over-expressed HepG2/Hep3B cells treated with vehicle (DMSO) or A-674563. Representative data are shown as the mean ± SD. n=3; $^{*}P < 0.002$, $^{**}P < 0.001$ compared with other group.

CDC37 is upregulated in human hepatocellular carcinoma cells [21]. Inhibition of the expression of CDC37 could inhibit the cell cycle progression and cell growth of hepatocellular carcinoma cells [18, 21]. Here, we found that the expression of CDC37 was detected in several hepatocellular carcinoma cell lines. CDC37 was also found overexpressed in hepatocellular carcinoma cell lines with highly invasive potential. Stable overexpression of CDC37 could promote the cell proliferation and invasion in human hepatocellular carcinoma cells. Stable knockdown of CDC37 greatly reduced the proliferation compared to the scramble controls in human hepatocellular carcinoma cells. These results indicated that CDC37 was essential to the proliferation of human hepatocellular carcinoma cells. Moreover, stable overexpression of CDC37 was found to promote the cell invasion of human hepatocellular carcinoma cells. Meanwhile, an increase of the activity of AKT1 was also found in CDC37 stably overexpressing human hepatocellular carcinoma cells. This imply that the increase of cell invasion by overexpression of CDCD37 might be due to the increased activity of AKT1.

PINK1 was reported to increase the phosphorylation of AKT1 at Ser473 and promote the activity of AKT1 [29]. The data presented herein indicate that overexpression of PINK1 can increase the phosphorylation of AKT1 at Ser473. Moreover, the stability of PINK1 or AKT1 protein was enhanced by the overexpression of CDC37. In addition, selective AKT1 inhibitor A-674563 could attenuate CDC37 induced cell proliferation and invasion of human hepatocellular carcinoma cells. These results suggest that increased cell proliferation and invasion induced by the overexpression of CDC37 was mediated by AKT1 signaling pathway.

Disclosure of conflict of interest

None.

Address correspondence to: Yuanmin Pei, Yidu Central Hospital of Weifang, Weifang, Shandong, People's Republic of China. Tel: +86-536-3276-999; E-mail: yuanmpei@126.com

References

[1] Ye J, Wang J, Tan L, Yang S, Xu L, Wu X, Deng H and Tan H. Expression of protein TARBP1 in human hepatocellular carcinoma and its prognostic significance. Int J Clin Exp Pathol 2015; 8: 9089-9096.

[2] Herszenyi L and Tulassay Z. Epidemiology of gastrointestinal and liver tumors. Eur Rev Med Pharmacol Sci 2010; 14: 249-258.

[3] Grabinski N, Ewald F, Hofmann BT, Staufer K, Schumacher U, Nashan B and Jucker M. Combined targeting of AKT and mTOR synergistically inhibits proliferation of hepatocellular carcinoma cells. Mol Cancer 2012; 11: 85.

[4] Wang YW, Lin KT, Chen SC, Gu DL, Chen CF, Tu PH and Jou YS. Overexpressed-eIF3I interacted and activated oncogenic Akt1 is a theranostic target in human hepatocellular carcinoma. Hepatology 2013; 58: 239-250.

[5] Liu K, Shi Y, Guo XH, Ouyang YB, Wang SS, Liu DJ, Wang AN, Li N and Chen DX. Phosphorylated AKT inhibits the apoptosis induced by DRAM-mediated mitophagy in hepatocellular carcinoma by preventing the translocation of DRAM to mitochondria. Cell Death Dis 2014; 5: e1078.

[6] Liu LZ, Zhou XD, Qian G, Shi X, Fang J and Jiang BH. AKT1 amplification regulates cisplatin resistance in human lung cancer cells through the mammalian target of rapamycin/p70S6K1 pathway. Cancer Res 2007; 67: 6325-6332.

[7] Dobashi Y, Kimura M, Matsubara H, Endo S, Inazawa J and Ooi A. Molecular alterations in AKT and its protein activation in human lung carcinomas. Hum Pathol 2012; 43: 2229-2240.

[8] Cui XW, Zhao FJ, Liu J, Song XM, Xu P, Zhou X, Zhang J, Wang T, Kang CS and Zhang QY. Suppression of Akt1 expression by small interference RNA inhibits SGC7901 cell growth in vitro and in vivo. Oncol Rep 2009; 22: 1305-1313.

[9] Cai J, Zhao J, Zhang N, Xu X, Li R, Yi Y, Fang L, Zhang L, Li M, Wu J and Zhang H. MicroRNA-542-3p Suppresses Tumor Cell Invasion via Targeting AKT Pathway in Human Astrocytoma. J Biol Chem 2015; 290: 24678-24688.

[10] Madhunapantula SV and Robertson GP. Therapeutic Implications of Targeting AKT Signaling in Melanoma. Enzyme Res 2011; 2011: 327923.

[11] Altomare DA and Testa JR. Perturbations of the AKT signaling pathway in human cancer. Oncogene 2005; 24: 7455-7464.

[12] Shimura T, Noma N, Oikawa T, Ochiai Y, Kakuda S, Kuwahara Y, Takai Y, Takahashi A and Fukumoto M. Activation of the AKT/cyclin D1/Cdk4 survival signaling pathway in radioresistant cancer stem cells. Oncogenesis 2012; 1: e12.

[13] Guo X, Li W, Wang Q and Yang HS. AKT Activation by Pdcd4 Knockdown Up-Regulates Cyclin D1 Expression and Promotes Cell Proliferation. Genes Cancer 2011; 2: 818-828.

[14] Rodier G, Montagnoli A, Di Marcotullio L, Coulombe P, Draetta GF, Pagano M and Meloche S. p27 cytoplasmic localization is regulated by phosphorylation on Ser10 and is not a prerequisite for its proteolysis. Embo Journal 2001; 20: 6672-6682.

[15] Zhang S, Sun G, Wang Z, Wan Y, Guo J and Shi L. PCAF-mediated Akt1 acetylation enhances the proliferation of human glioblastoma cells. Tumour Biol 2015; 36: 1455-1462.

[16] Zheng X, Gai X, Ding F, Lu Z, Tu K, Yao Y and Liu Q. Histone acetyltransferase PCAF up-regulated cell apoptosis in hepatocellular carcinoma via acetylating histone H4 and inactivating AKT signaling. Mol Cancer 2013; 12: 96.

[17] Basso AD, Solit DB, Chiosis G, Giri B, Tsichlis P and Rosen N. Akt forms an intracellular complex with heat shock protein 90 (Hsp90) and Cdc37 and is destabilized by inhibitors of Hsp90 function. J Biol Chem 2002; 277: 39858-39866.

[18] MacLean M and Picard D. Cdc37 goes beyond Hsp90 and kinases. Cell Stress Chaperones 2003; 8: 114-119.

[19] Pascale RM, Simile MM, Calvisi DF, Frau M, Muroni MR, Seddaiu MA, Daino L, Muntoni

MD, De Miglio MR, Thorgeirsson SS and Feo F. Role of HSP90, CDC37, and CRM1 as modulators of P16(INK4A) activity in rat liver carcinogenesis and human liver cancer. Hepatology 2005; 42: 1310-1319.
[20] Wang Z, Wei W, Sun CK, Chua MS and So S. Suppressing the CDC37 cochaperone in hepatocellular carcinoma cells inhibits cell cycle progression and cell growth. Liver Int 2015; 35: 1403-1415.
[21] Gray PJ Jr, Prince T, Cheng J, Stevenson MA and Calderwood SK. Targeting the oncogene and kinome chaperone CDC37. Nat Rev Cancer 2008; 8: 491-495.
[22] Trepel J, Mollapour M, Giaccone G and Neckers L. Targeting the dynamic HSP90 complex in cancer. Nat Rev Cancer 2010; 10: 537-549.
[23] Zhao M, Ma J, Zhu HY, Zhang XH, Du ZY, Xu YJ and Yu XD. Apigenin inhibits proliferation and induces apoptosis in human multiple myeloma cells through targeting the trinity of CK2, Cdc37 and Hsp90. Mol Cancer 2011; 10: 104.
[24] Virtakoivu R, Pellinen T, Rantala JK, Perala M and Ivaska J. Distinct roles of AKT isoforms in regulating beta1-integrin activity, migration, and invasion in prostate cancer. Mol Biol Cell 2012; 23: 3357-3369.
[25] Kim D, Kim S, Koh H, Yoon SO, Chung AS, Cho KS and Chung J. Akt/PKB promotes cancer cell invasion via increased motility and metalloproteinase production. FASEB J 2001; 15: 1953-1962.
[26] Joglekar M, Elbazanti WO, Weitzman MD, Lehman HL and van Golen KL. Caveolin-1 mediates inflammatory breast cancer cell invasion via the Akt1 pathway and RhoC GTPase. J Cell Biochem 2015; 116: 923-933.
[27] Yung HW, Charnock-Jones DS and Burton GJ. Regulation of AKT Phosphorylation at Ser473 and Thr308 by Endoplasmic Reticulum Stress Modulates Substrate Specificity in a Severity Dependent Manner. PLoS One 2011; 6: e17894.
[28] Kim E, Kim M, Woo DH, Shin Y, Shin J, Chang N, Oh YT, Kim H, Rheey J, Nakano I, Lee C, Joo KM, Rich JN, Nam DH and Lee J. Phosphorylation of EZH2 activates STAT3 signaling via STAT3 methylation and promotes tumorigenicity of glioblastoma stem-like cells. Cancer Cell 2013; 23: 839-852.
[29] Akundi RS, Zhi L and Bueler H. PINK1 enhances insulin-like growth factor-1-dependent Akt signaling and protection against apoptosis. Neurobiol Dis 2012; 45: 469-478.
[30] Weihofen A, Ostaszewski B, Minami Y and Selkoe DJ. Pink1 Parkinson mutations, the Cdc37/Hsp90 chaperones and Parkin all influence the maturation or subcellular distribution of Pink1. Hum Mol Genet 2008; 17: 602-616.
[31] Li R, Wei J, Jiang C, Liu D, Deng L, Zhang K and Wang P. Akt SUMOylation regulates cell proliferation and tumorigenesis. Cancer Res 2013; 73: 5742-5753.
[32] Green BD, Jabbour AM, Sandow JJ, Riffkin CD, Masouras D, Daunt CP, Salmanidis M, Brumatti G, Hemmings BA, Guthridge MA, Pearson RB and Ekert PG. Akt1 is the principal Akt isoform regulating apoptosis in limiting cytokine concentrations. Cell Death Differ 2013; 20: 1341-1349.
[33] Miao H, Li DQ, Mukherjee A, Guo H, Petty A, Cutter J, Basilion JP, Sedor J, Wu J, Danielpour D, Sloan AE, Cohen ML and Wang B. EphA2 mediates ligand-dependent inhibition and ligand-independent promotion of cell migration and invasion via a reciprocal regulatory loop with Akt. Cancer Cell 2009; 16: 9-20.
[34] Rossig L, Badorff C, Holzmann Y, Zeiher AM and Dimmeler S. Glycogen synthase kinase-3 couples AKT-dependent signaling to the regulation of p21Cip1 degradation. J Biol Chem 2002; 277: 9684-9689.
[35] Yeh PY, Kuo SH, Yeh KH, Chuang SE, Hsu CH, Chang WC, Lin HI, Gao M and Cheng AL. A pathway for tumor necrosis factor-alpha-induced Bcl10 nuclear translocation. Bcl10 is up-regulated by NF-kappaB and phosphorylated by Akt1 and then complexes with Bcl3 to enter the nucleus. J Biol Chem 2006; 281: 167-175.
[36] Roffe S, Hagai Y, Pines M and Halevy O. Halofuginone inhibits Smad3 phosphorylation via the PI3K/Akt and MAPK/ERK pathways in muscle cells: effect on myotube fusion. Exp Cell Res 2010; 316: 1061-1069.
[37] Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y and Greenberg ME. Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell 1997; 91: 231-241.
[38] Fan W, Zhou ZY, Huang XF, Bao CD and Du F. Deoxycytidine kinase promotes the migration and invasion of fibroblast-like synoviocytes from rheumatoid arthritis patients. Int J Clin Exp Pathol 2013; 6: 2733-2744.
[39] Ooms LM, Binge LC, Davies EM, Rahman P, Conway JR, Gurung R, Ferguson DT, Papa A, Fedele CG, Vieusseux JL, Chai RC, Koentgen F, Price JT, Tiganis T, Timpson P, McLean CA and Mitchell CA. The Inositol Polyphosphate 5-Phosphatase PIPP Regulates AKT1-Dependent Breast Cancer Growth and Metastasis. Cancer Cell 2015; 28: 155-169.