Distinct roles of Akt1 in regulating proliferation, migration and invasion in HepG2 and HCT 116 cells

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Abstract. Elucidating the effects of genes involved in tumors may improve therapeutic strategies for human cancer. Recently, several studies discovered that Akt1 plays a dual role in mediating cell proliferation, migration and invasion, depending on the cell type. However, the pathophysiological role of Akt1 in hepatocellular carcinoma (HCC) and colorectal carcinoma cells remains poorly understood. In the present study, we transfected the Akt1-expressing plasmids into the tumor cells that expressed only low levels of Akt1. The migration and invasion abilities were analyzed in 24-well Boyden chambers. The expression of proteins was detected using western blot analysis. Our results demonstrated that overexpression of Akt1 significantly enhanced the proliferation rates and promoted the colony formation in both HepG2 and HCT 116 cells. When treated with wortmannin, the ability to form colonies was significantly attenuated in both cell lines. Of note, enforced expression of Akt1 induced HepG2 cell migration and invasion; by contrast, upregulation of Akt1 expression suppressed the migration and invasion of HCT 116 cells. Subsequent mechanistic investigations revealed that upregulation of Akt1 markedly induced the expression of Bcl-2 and NF-κB in both types of tumor cells. Notably, we observed a similar increase of MMP2, MMP9, HIF1α and VEGF in HCC cells, whereas Akt1 significantly suppressed the expression of these molecules in colorectal carcinoma cells. These data suggest a dual role for Akt1 in tumor cell migration and invasion and highlight the cell type-specific actions of Akt1 kinases in the regulation of cell motility.

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Introduction

The phosphoinositide 3-kinase (PI3K) signaling pathway regulates a multitude of cellular processes including cell survival, proliferation, migration and invasion. Deregulation of the PI3K signaling pathway is often detected in various types of human cancer. The serine/threonine kinase Akt, also known as protein kinase B (PKB), which was initially identified as a proto-oncogene Akt8, from a spontaneous thymoma of an AKR mouse (1), has a strong oncogenic function and is the primary downstream mediator of PI3K pathway function (2). In mammals, there are three different isoforms of Akt, termed Akt1, Akt2 and Akt3, which share a high degree of homology, while the three Akt isoforms have some different functions (3).

Amplification of Akt1 was first discovered in a primary human gastric adenocarcinoma (1). Currently, increasing evidence indicates that Akt1 has been implicated in the control of various biological processes, including cell proliferation, survival and tumor formation. Menges *et al* (4) demonstrated that Akt1 is positively correlated with tumor cell proliferation and survival. Downregulation of Akt1 expression inhibits K562 cell proliferation (5). Furthermore, by using bitransgenic MMTV-c-ErbB2, MMTV-myr-Akt1 mouse models, constitutively active Akt1 markedly accelerated MMTV-c-ErbB2 mammary tumorigenesis (6). However, the role of Akt1 in cell proliferation remains controversial. For instance, a recent article indicated that constitutive Akt1 signals attenuate B-cell receptor signaling and proliferation (7). Therefore, it is essential to investigate whether ectopic expression of Akt1 promotes cell proliferation or not in other cell types, including hepatocellular carcinoma (HCC) and colorectal cancer cells.

Cell migration and invasion are two of the most important steps involved in cancer metastasis, which accounts for >90% of all cancer-related deaths (8,9). Growing evidence indicates that activation of Akt1 is associated with cancer cell migration, invasion and metastasis. For example, selective activation of Akt1 not only positively regulates IGF-1-induced SKOV-3 cell migration and invasion, but also markedly promotes tumor metastasis (10). Previous studies showed that Akt isoform Akt1 limits breast cancer cell motility and invasion (11,12) and several other independent studies have validated and extended these observations (13,14). Moreover,

these data have been validated in transgenic mouse models, in which AKT1 was shown to have an inhibitory effect on breast cancer invasiveness (15,16). Although overwhelming evidence has demonstrated that the PI3K/Akt signaling cascade plays a crucial role in the regulation of the malignant behaviors, including migration and invasion in HCC and colorectal carcinomas, the role of Akt1 and its precise molecular mechanisms in these cancer cells remain largely unknown.

In the present study, we demonstrated that upregulation of Akt1 significantly increased cell proliferation and enhanced the ability to form colonies in both HepG2 and HCT 116 cells. Furthermore, we also discovered that enforced expression of Akt1 significantly enhanced the ability of migration and invasion in HepG2 cells, while it reduced HCT 116 cell migration and invasion. These data demonstrate a dual role for Akt1 in tumor cell migration and invasion and highlight the cell type-specific actions of Akt1 kinases in the regulation of cell motility.

Materials and methods

Materials. 3-(4, 5-dimethylthiazol)-2,5-diphenyltetrazolium bromide (MTT), G418 and wortmannin were purchased from Sigma (St. Louis, MO, USA). RPMI-1640 and fetal calf serum (FCS) were purchased from Gibco (Grand Island, NY, USA). The sources of primary antibodies used for western blot analysis, Akt1, Bcl-2, NF-κB, MMP2, MMP9, HIF1α, VEGF, as well as horseradish peroxidase-conjugated anti-mouse and anti-rabbit antibodies were all purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). All other chemicals used in the experiments were commercial products of reagent grade.

Cell culture and treatment. Cell lines from human HCC (HepG2) and colorectal carcinoma (HCT 116) were purchased from the Cell Bank of the Chinese Academy of Science (Shanghai, China). These cells were maintained in RPMI-1640 medium supplemented with 10% FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37˚C in a humidified incubator containing 5% CO_2.

Plasmid construction and transfection. The full-length coding region (1443 bp) of Akt1 was amplified from human genomic DNA by reverse transcription-polymerase chain reaction (RT-PCR) using the following primers: Akt1-sense 5'-CCG CTC GAG ACC ATG AGC GAC GTG GCT ATT GTG AAG-3', and antisense 5'-CGG GAT CCG CAG TCC ACC GCC GCC TCAG-3', and the PCR products were then digested with *Xho*I/*Bam*HI and were inserted into the pIRES2-EGFP vector (Clontech Laboratories, Inc., Palo Alto, CA, USA) carrying neomycin resistance gene. The recombinant construct was verified by direct DNA sequencing.

For transfection, HepG2 and HCT 116 cells were seeded in 24-well plates at $5x10^4$/well and incubated at 37˚C in a humidified incubator containing 5% CO_2. The next day, when these cells were ~80% confluent, cells were transfected with TurboFect™ *in vitro* transfection reagent according to the manufacturer's instructions. Briefly, recombinant plasmid (1 μg) was mixed with TurboFect™ reagent and pre-incubated for 20 min at room temperature in 100 μl of serum-free RPMI-1640. HepG2 and HCT 116 cells transfected with pIRES2-EGFP-Akt1 and pIRES2-EGFP vector were termed as HepG2-Akt1 and HepG2-Vec, HCT 116-Akt1 and HCT 116-Vec, respectively. These transfected cells were selected in the presence of G418 (100 μg/ml) for 2 weeks, and then the stable plasmid-transfected clones were generated by using limiting dilution analyses in 96-well plates.

Cell growth curve analysis. The MTT assay was used to detect the proliferation rate of cells transfected with empty vector or Akt1 plasmid. The process was performed as previously described with some modifications (17). Briefly, 2,000 cells/well were plated in 96-well plates and incubated for 1, 2, 3, 4, 5, 6 and 7 days, respectively. Then, 50 μl MTT reagents (1 mg/ml) was added at indicated time-points and cells were incubated for 4 h at 37˚C. Supernatants were removed from the wells and 100 μl DMSO was pipetted to solubilize the crystal product for 10 min at room temperature. The absorbance (OD) of each well was measured with a microplate reader (Bio-Rad Laboratories) at a wavelength of 570 nm.

RNA isolation and RT-PCR. Total RNA was extracted using TRIzol reagent (Invitrogen). Briefly, 2 μg of total RNA was subjected to DNase I digestion (1 U/μl; Fermentas, Hanover, MD, USA) at 37˚C for 30 min and then to heat inactivation of DNase I at 70˚C for 15 min, followed by reverse-transcription using Moloney murine leukemia virus reverse transcriptase (Promega). The PCR primers and regimen were: 5'-ATG AGC GAC GTG GCT ATT GTG AAG-3' and 5'-GAG GCC GTC AGC CAC AGT CTG GATG-3' for Akt1 (330 bp); 5'-CGG AGT CAA CGG ATT GGT CGT AT-3', and 5'-AGC CTT CTC CAT GGT GG TGA AGAC-3' for GAPDH (307 bp). All PCR reactions were performed using standard PCR conditions: 95˚C for 5 min, 95˚C for 45 sec, annealing at different temperatures for each gene respectively for 45 sec, extension at 72˚C for 1 min for 30 cycles, and a final extension at 72˚C for 10 min. PCR products were separated on 1.0% agarose gel. The gel was then digitally photographed and scanned with UVI Gel Analyzing System (UVI Tech, Cambridge, UK).

Colony formation assay. Cells were plated in a fresh 24-well plate at a density of 200 cells/well and maintained in RPMI-1640 containing 10% FCS. The medium was changed every 3 days for 14 days until visible colonies formed. Colonies were fixed and stained with 0.1% crystal violet in 20% methanol for 15 min. Individually stained colonies (>50 cells) were counted in each well. The colony formation assay was performed as previously reported with some modifications (18).

Wound scratch assay. The wound scratch assay was performed as previously described (19). Briefly, cells were grown to confluence overnight prior to serum starvation for 24 h. The confluent cell monolayer was then scratched with a pipette tip (20 μl) and washed thrice with PBS to remove floating cells. After the line scratch, the width of the wound was measured and recorded as t=0. The cells were then allowed to migrate back into the wounded area and the closing of the wound was measured at 24 h. The migration distance (in μm) was determined as the reduction of the width of the open area.

Boyden chamber assay. The migration and invasion assays were performed in 24-well Boyden chambers with 8 μm pore size polycarbonate membranes (Corning, Corning, NY, USA), as previously described (20). For the invasion assay, the membrane was coated with 15 μg Matrigel (R&D Systems) to form a matrix barrier. Serum-starved cells ($1x10^5$ cells) were seeded into the upper compartment of the chamber in serum-free medium, supplemented with 100 nM wortmannin or not, while the lower compartment was filled with 600 μl of DMEM containing 10% FBS. Following incubation at 37˚C for 24 h, tumor cells remaining on the upper surface of the membrane were removed with cotton swabs. The cells on the lower surface of the membrane were fixed, stained with crystal violet and then counted under a light microscope. For the migration assay, only one third of cells was applied to the Transwell chamber without Matrigel.

Western blot analysis. After treatment with 100 nM wortmannin or not for 24 h, cells were harvested and washed with PBS. Cell lysates were prepared in the protein extraction buffer containing 150 mM NaCl, 10 mM Tris (pH 7.2), 5 mM EDTA, 0.1% Triton X-100, 5% glycerol and 2% SDS. Western blot analysis was performed as previously described (21). The total protein concentration was determined using the protein assay kit (Beyotime, China). Cell lysates in 5X SDS-sample buffer were boiled for 5 min and then equal amounts of total proteins were separated using 10 or 12% SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes (Immobilion Millipore). Membranes were then blocked in PBST containing 5% dried skimmed milk for 1 h at room temperature. The blots were incubated with corresponding primary antibodies at 4˚C overnight. After washing three times with PBST, the membranes were incubated with corresponding horseradish peroxidase-conjugated goat anti-mouse or anti-rabbit secondary antibody, and then washed three times with PBST. Proteins were detected using the ECL plus reagents (Beyotime, China).

Statistical analysis. Data are expressed as the means ± SD from at least three independent experiments. Unless otherwise noted, the differences between groups were assessed by ANOVA. All the tests performed in the present study were two-sided using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). Differences were considered statistically significant at values of $P<0.05$.

Results

Akt1 is stably overexpressed in HepG2 and HCT 116 cells. After stably transfected cells were individually selected, the Akt1 and GAPDH mRNA levels were measured using RT-PCR. Compared with the control group, the pIRES2-EGFP-Akt1 transcripts were strongly upregulated (Fig. 1A and B). Consistent with our RT-PCR data, western blot analysis revealed that Akt1 protein levels were also clearly increased in pIRES2-EGFP-Akt1-transfected cells (Fig. 1C and D). The pIRES2-EGFP empty vector did not substantially affect the endogenous Akt1 expression in both mRNA and protein levels. Collectively, these findings indicate that Akt1 is stably overexpressed in HepG2 and HCT 116 cells.

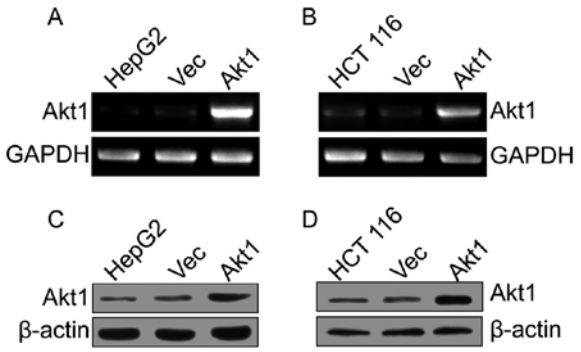

Figure 1. Overexpression of Akt1 detected in transfections. Construction of the recombinant eukaryotic expression vector pIRES2-EGFP containing the Akt1 gene was used in the studies. Transfection of the recombinant plasmid pIRES2-EGFP-Akt1 or pIRES2-EGFP empty vector into HepG2 and HCT 116 cells, respectively. (A and B) Semi-quantitative RT-PCR analysis was used to identify the Akt1 mRNA level in tumor cells. GAPDH was used as a loading control. (C and D) Western blot analysis was used to determine the Akt1 protein level in various tumor cells; β-actin was used as an internal control.

Akt1 upregulation promotes cell proliferation and colony formation in HepG2 and HCT 116 cells. We then determined the functional consequences of Akt1. As shown in Fig. 2A and B, compared with the controls, the proliferation rates of HepG2-Akt1 and HCT 116-Akt1-transfected cells were increased significantly ($P<0.01$); then, the capacity of colony formation was evaluated on these cell lines. Our results showed that the number of colonies of HepG2-Akt1 and HCT 116-Akt1 cells were 66.7±9.6 and 71.7±5.9, respectively; however, the number of colonies was only 29.3±4.7 and 29.0±6.0 for HepG2-Vec and HCT 116-Vec cells, respectively. There was a statistically significant increase in the number of colonies of HepG2-Akt1 and HCT 116-Akt1 cells compared to that of the individual control groups (Fig. 2C and D; $P<0.01$, $P<0.001$), while wortmannin significantly attenuated the colonies of the tumor cells with upregulation of Akt1. These results indicate that Akt1 promotes tumor cell proliferation and colony formation.

Upregulation of Akt1 expression promotes HepG2 cell migration and invasion. Next, we examined the effect of Akt1 on HepG2 cell migration and invasion. As shown in Fig. 3A, a significant difference between wound distance of HepG2-Akt1 cells compared to HepG2-Vec cells was observed. Forced expression of Akt1 markedly stimulated wound closure compared with the empty vector-transfected cells, while this effect was significantly decreased after treatment with wortmannin (Fig. 3A). Boyden chamber Transwell assay also showed that the migration of HepG2 cells was increased by >2-fold due to Akt1 overexpression, but was significantly decreased when treated with wortmannin (Fig. 3C).

To determine whether Akt1 also regulates HepG2 cell invasion, the Transwell assay was repeated. As shown in Fig. 4A, upregulation of Akt1 expression markedly induced cell invasion compared with the control group, whereas cell invasion was strongly suppressed by wortmannin. Taken together, these results demonstrate that Akt1 is correlated with cell migration and invasion potential of liver cancer HepG2 cells.

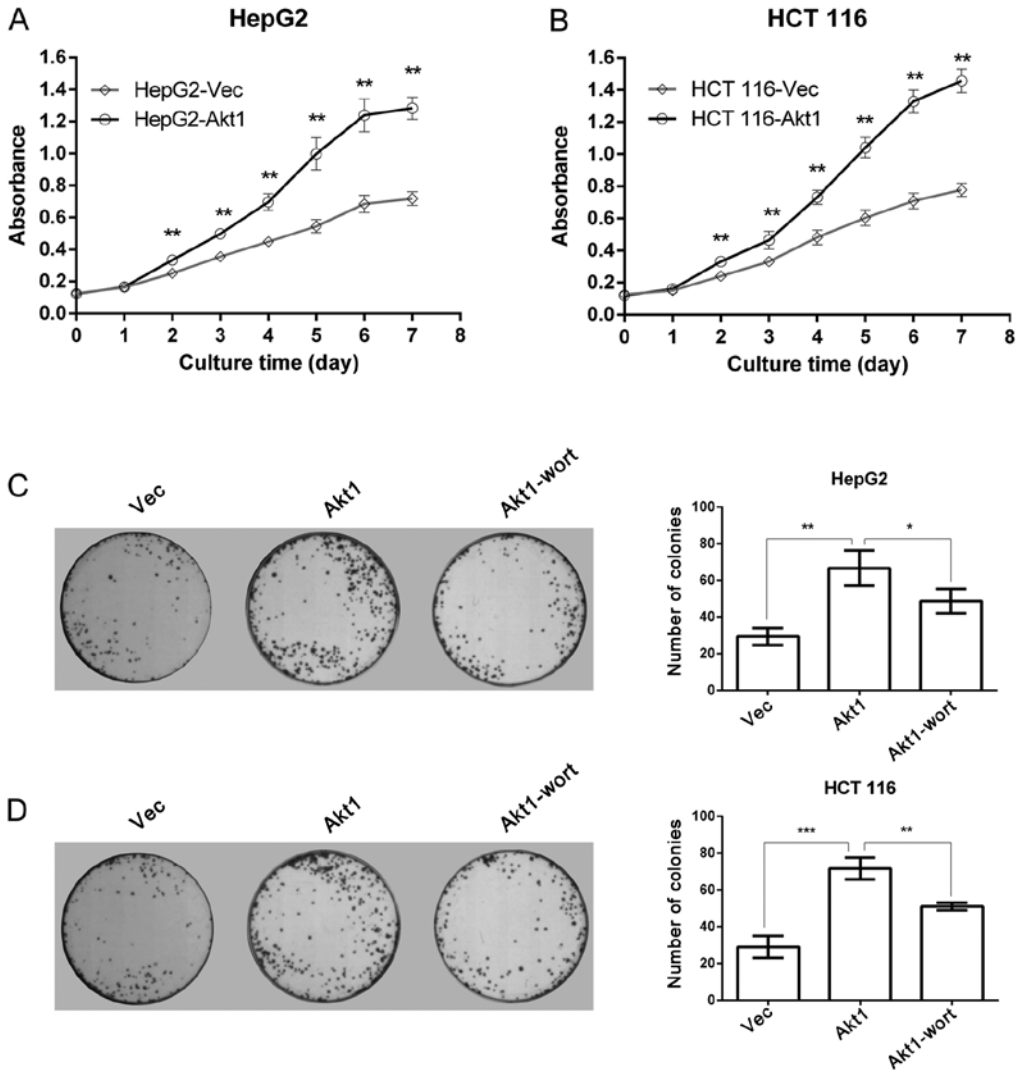

Figure 2. Upregulation of Akt1 promotes tumor cell proliferation and colony formation. (A and B) MTT assay showed that Akt1 overexpression promoted the cell proliferation rates in both HepG2 and HCT 116 cell lines. (C and D) Upregulation of Akt1 expression increased the number of colony formation in HepG2 and HCT 116 cells. Cells were incubated in the absence or presence of 100 nM wortmannin (wort) for 24 h, which was then changed with fresh medium. Colony numbers (>50 cells/colony) were counted at day 15. These results were reproducible in three independent experiments. *P<0.05; **P<0.01, ***P<0.001.

Overexpression of Akt1 suppresses the migration and invasion of HCT 116 cells. We determined the role of Akt1 in HCT 116 cell migration. Upregulation of Akt1 expression markedly suppressed wound closure compared with HCT 116-Vec cells, while wortmannin treatment significantly reversed the effects of Akt1 on HCT 116 cells (Fig. 3B). The Transwell assay also indicated that ectopic expression of Akt1 noticeably decreased HCT 116-Akt1 cell migration by ~50% in comparison with the control group (Fig. 3D). The addition of wortmannin significantly attenuated the inhibitory effect of Akt1 on HCT 116 cell migration (Fig. 3D). We then examined whether Akt1 also regulates HCT 116 cell invasion. Akt1 overexpression significantly inhibited invasion compared to control cells transfected with empty vector (Fig. 4B), while wortmannin partially antagonized the inhibitory effect of Akt1 on migration and invasion of HCT 116 cells (Fig. 4B). These findings suggest that Akt1 suppresses the cell migration and invasion of HCT 116 cells.

Cellular signaling pathways involved in Akt1 action. To identify the cellular signaling pathways involved in Akt1 action, we first examined the pro-survival and proliferative molecules downstream of Akt1, including Bcl-2 and nuclear factor-κB (NF-κB). Overexpression of Akt1 significantly induced the expression of Bcl-2 and NF-κB in both HepG2 (Fig. 5A) and HCT 116 cells (Fig. 5B). However, wortmannin partly reversed Akt1-mediated effects.

Overexpression of MMPs is associated with tumor invasion and metastasis and, in particular, MMP2 and MMP9, which are well known to play a pivotal role in tumor invasion and metastasis development in various types of cancer including hepatocellular and colorectal carcinomas (22,23). Our results also showed that Akt1 overexpression significantly increased the expression of MMP2 and MMP9 in HepG2 cells, while there was an opposing effect of Akt1 in HCT 116 cells. Of note, wortmannin significantly attenuated these effects of Akt1 in both types of tumor cells (Fig. 5). Our results also demonstrated that upregulation of Akt1 also enhanced the expression of HIF-1α and VEGF in HepG2 cells, while it attenuated the levels of these two types of proteins in HCT 116 cells.

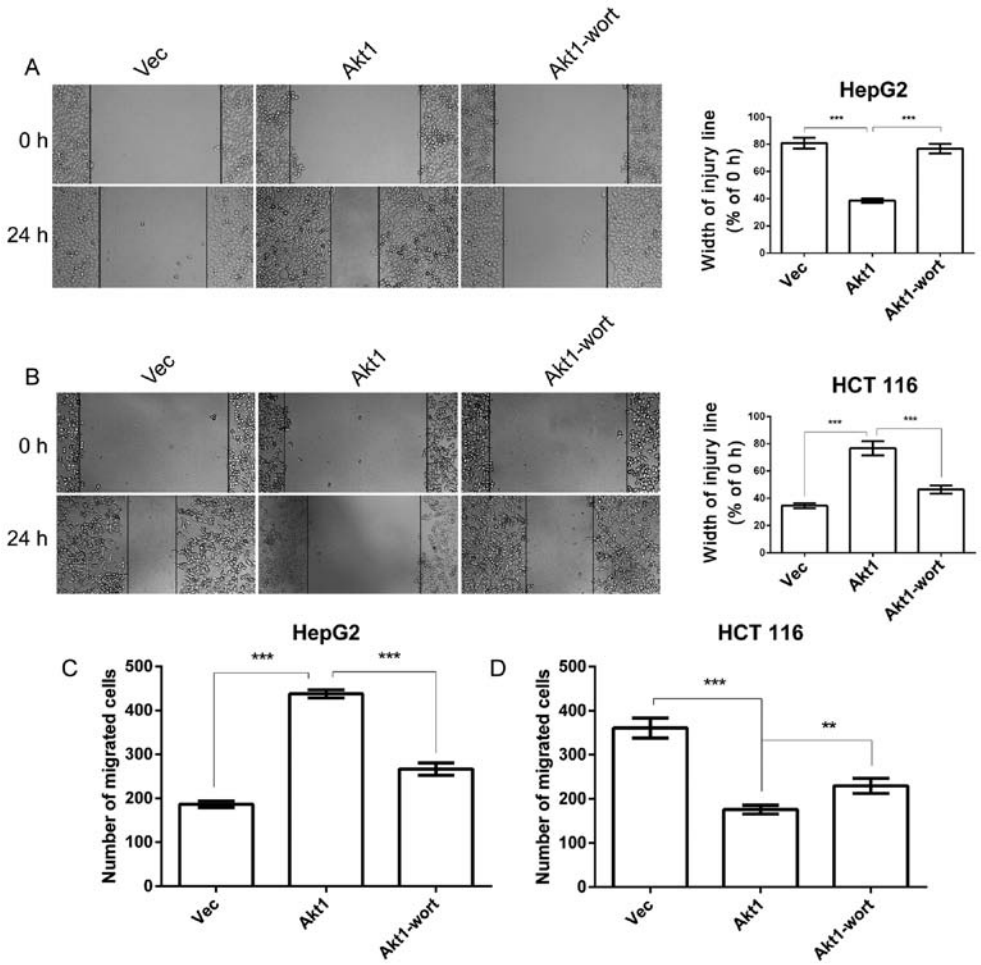

Figure 3. The effects of Akt1 on cancer cell migration. (A and C) Effect of Akt1 on HepG2 cell migration was detected by wound scratch assay and Transwell chamber without Matrigel. Representative results of migration of Vec-transfected and Akt1-transfected HepG2 cells that were treated with 100 nM wortmannin (wort), or that were left untreated for 24 h, and then photographed. (B and D) Effect of Akt1 on HCT 116 cell migration was performed using wound scratch assay and Transwell chamber without Matrigel. Representative results of migration of the HCT 116 cell lines that were transfected with empty vector or Akt1. These cells were incubated with or without 100 nM wort for 24 h. $^{**}P<0.01$; $^{***}P<0.001$.

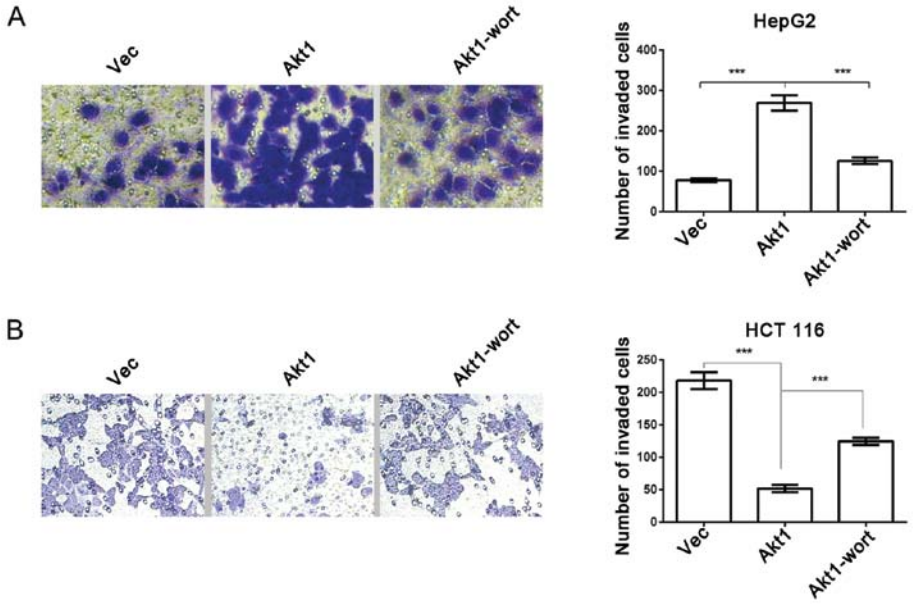

Figure 4. The effects of Akt1 on tumor cell invasion. (A) Akt1 promoted the invasion of HepG2 cells. (B) Akt1 upregulation attenuated the invasion of HCT 116 cells. Representative results of invasion of Vec-transfected and Akt1-transfected HepG2 and HCT 116 cells that were treated with 100 nM wortmannin (wort) or that were left untreated. These cells were then added to Transwell chamber coated with Matrigel and incubated for 24 h. Viability of cell invasion was expressed by the number of invading cells. $^{***}P<0.001$.

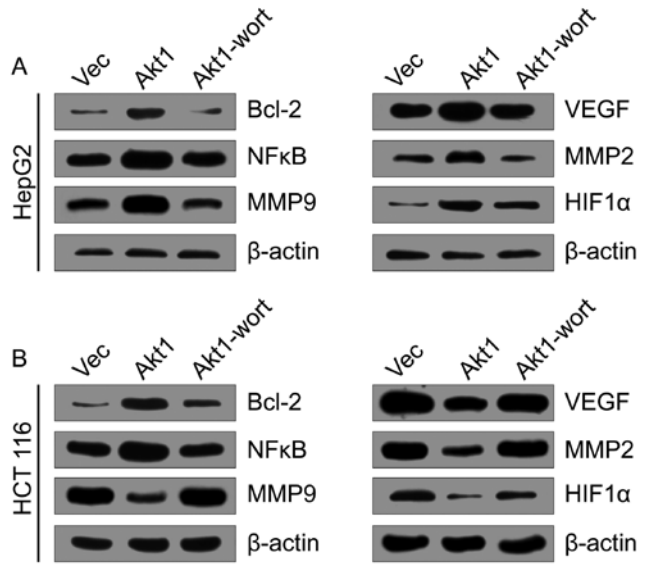

Figure 5. The effects of Akt1 on cell signaling pathways. Western blot results for the expression of Bcl-2, NF-κB, MMP2 and MMP9, HIF1α and VEGF. (A and B) Akt1 regulates the expression of various proteins. HepG2 and HCT 116 cells transfected with empty vector and Akt1 were cultured in the presence or absence of 100 nM wortmannin (wort) for 24 h, prior to immunoblotting analysis for these protein expressions. All results were repeated in three independent experiments and the representative immunoblots are shown; β-actin was used as an internal control.

Discussion

Activation of the PI3K/Akt signaling pathway has been detected in several types of cancer, and PI3K or its downstream components, including AKTs, are considered attractive targets for cancer therapy. However, several studies have highlighted that the biological outcomes obtained upon AKT inhibition are very complex (13,24,25), including the potential cell-type specific effects of AKT isoform Akt1 on cell migration and invasion (10). Therefore, it is necessary to investigate the potential role of Akt1 in various types of human cancer before its inhibitors are used as cancer therapies.

In the present study, our findings showed that upregulation of Akt1 resulted in increased cell proliferation in both HepG2 and HCT 116 cells. Inhibition of PI3K by wortmannin efficiently reduced the colonies in tumor cells with Akt1 transfection. Consistent with our findings, ablation of AKT1 decreased the proliferation of the androgen-independent cell line PC-3 (26). Moreover, as these are *in vitro* assays, validation of these results *in vivo* is required and only few reports have already addressed these issues. Using mice bitransgenic of mammary tumorigenesis with constitutively active Akt1 and ErbB-2, Young *et al* (6) reported that overexpression of activated Akt1 resulted in increased tumor frequency. In contrast, loss of Akt1 reduced tumor proliferative function in a murine model of thyroid cancer (27). These results suggested that Akt1 expression in tumor cells is required for cell proliferation, although how ectopic expression of Akt1 promotes tumor cell proliferation remains unclear.

We investigated the potential cellular signaling pathway involved in Akt1-induced cell proliferation. Bcl-2 is a well-known pro-survival gene downstream of Akt1. It is often overexpressed in various human tumors. In the present study, our results indicated that Bcl-2 is overexpressed in both HepG2-Akt1 and HCT 116-Akt1 cells compared with their respective vector-transfected cells. Upon wortmannin treatment, the expression of Bcl-2 was significantly downregulated, suggesting that Bcl-2 may play a key role in Akt1-induced proliferation. In concordance with our observations, a recent study indicated that the Akt1/Bcl-2 signaling pathway plays a critical role in angiopoietin-2-stimulated cell survival and proliferation both *in vitro* and *in vivo* (28). Moreover, silencing of Akt1 was associated with the suppression of Bcl-2 which, in turn, resulted in the inhibition of cell proliferation, stimulation of apoptosis *in vitro* and inhibition of xenograft growth in nude mice (29).

Nuclear factor-κB (NF-κB) is also a ubiquitous transcription factor. It is often thought to contribute to malignant transformation by aberrant activation of cellular functions that are commonly associated with tumor promotion, including stimulating cell growth and proliferation (30). Furthermore, Han *et al* (31) supported the notion that constitutive activation of the PI3K/Akt1/NF-κB signaling pathway is important for cell survival and proliferation in iMyc$^{E\mu}$ B-cell lymphomas. In the present study, enforced expression of Akt1 increased the expression of NF-κB in both HepG2 and HCT 116 cells. Upon wortmannin treatment, the level of NF-κB was markedly downregulated comparable to that of HepG2-Vec and HCT 116-Vec cells, respectively. Considering that, the expression of Bcl-2 and NF-κB is in concordance with Akt1-mediated cell proliferation in both HepG2 and HCT 116 cells. Hence, we proposed that Bcl-2 and NF-κB are two important molecules involved in Akt1-regulated cell proliferation.

In addition to the effect of Akt1 on tumor cell growth, its effects on tumor cell migration and invasion are of considerable importance. In the present study, we demonstrated that upregulation of Akt1 expression in liver cancer HepG2 cells markedly promoted the wound closure and the number of migrated cells. Moreover, enforced expression of Akt1 also significantly enhanced the invasion of HepG2 cells. Treatment with wortmannin significantly attenuated the positive effect of Akt1 on HepG2 cell migration and invasion. Then, we examined whether Akt1 plays a similar role in HCT 116 cells. Notably, the results from the wound healing assay suggested that overexpression of Akt1 significantly inhibited the wound closure of HCT 116 cells, while wortmannin partially rescued the inhibitory effect of Akt1 on colorectal tumor HCT 116 cells. In addition, the results from the Transwell migration assay also verified that enforced Akt1 expression significantly reduced HCT 116 cell migration compared with the corresponding control group. Furthermore, we also discovered that upregulation of Akt1 expression significantly inhibited HCT 116 cell invasion. However, following treatment with wortmannin, the inhibitory effect of Akt1 on HCT 116 cell migration and invasion was considerably alleviated. Considering the completely inverse effect of Akt1 on the migration and invasion in HepG2 and HCT 116 cells, we propose that the potential molecular mechanisms may be different in these two types of cell lines.

Matrix metalloproteinases (MMPs) are a large family of enzymes, which play a fundamental role in various components of the extracellular matrix degradation and remodeling (32).

MMPs have been found to be overexpressed in several human tumors and correlate with advanced stage, invasion, metastatic properties and poor prognosis (33). Among secreted MMPs, MMP2 and MMP9 are well known to play a pivotal role in tumor invasion and metastasis development in several types of cancer including hepatocellular and colorectal carcinomas (22,23). Moreover, it has been shown that silencing of Akt1 is associated with reduced expression of MMP2 and MMP9 in both SGC7901 gastric adenocarcinoma and U251 glioma cells (34). Consistent with these reports, our results also showed that upregulation of Akt1 resulted in increased expression of MMP2 and MMP9 in HepG2 cells, while it inhibited the expression of MMP2 and MMP9 in colorectal carcinoma HCT 116 cells. Treatment with pharmacologic inhibitor of PI3K completely reversed the effect of Akt1 in both cell types. In addition, the protein levels of HIF1α and VEGF were also significantly increased in HepG2-Akt1 cells compared to its control; by contrast, they were degraded in HCT 116 cells. Upon wortmannin treatment, the expression of HIF1α and VEGF was strongly suppressed in HepG2 cells, whereas it was upregulated in HCT 116 cells. Consistent with these results, activation of the PI3K/Akt1 pathway in tumor cells resulted in increased VEGF secretion, both by HIF-1α-dependent and -independent mechanisms. Moreover, sustained endothelial activation of Akt1 has been shown to induce the formation of structurally abnormal blood vessels (35). Furthermore, silencing of Akt1 by siRNA markedly decreased HIF-1α translation in normoxia in the presence of dimethyloxallyl glycine and in hypoxia (36). Collectively, these findings suggested that several signaling molecules, including MMP2, MMP9, HIF1α and VEGF, may play a key role in Akt1-mediated cell migration and invasion. Meanwhile, we proposed that Akt1 upregulation may first promote the expression of MMP2, MMP9, HIF1α and VEGF, which contributed to inducing the migration and invasion of HepG2 cells. In contrast, in colorectal carcinoma HCT 116 cells, upregulation of Akt1 suppressed the expression of these genes, which resulted in reduced cell migration and invasion. However, how Akt1 regulates these multiple molecules remains to be further investigated.

In the present study, we demonstrated that upregulation of Akt1 promotes cell proliferation in both HepG2 and HCT 116 cells, while the role of Akt1 in cell migration and invasion is completely distinct in these two cell types, suggesting the role of Akt1 in cell proliferation is independent of cell migration and invasion, although all are malignant phenotypes in the process of tumor development. Consistent with our observations, Pierau *et al* (7) demonstrated that constitutive Akt1 signals attenuate B-cell receptor signaling and proliferation, but enhance B-cell migration and effector function. Moreover, our results also demonstrated that overexpression of Akt1 promoted the cell migration and invasion of human liver cancer HepG2 cells; however, it attenuated the cell migration and invasion of human colorectal carcinoma HCT 116 cells. Therefore, we proposed that the effect of Akt1 on cell migration and invasion is likely due to the difference in cell type and context. Indeed, it has been shown that human non-small cell lung cancer (NSCLC) A549 cells with an expression construct for Akt1, exhibited significantly higher invasive ability through Matrigel than those cells with a control empty vector (37). Knockdown of Akt1 expression in NSCLC cells resulted in decreased cell migration (38). Furthermore, high levels of total Akt1 were also associated with increased lymph node metastasis in human prostate cancer (39). In the present study, ectopic expression of Akt1 significantly promoted the cell proliferation, migration and invasion in human liver cancer HepG2 cells, suggesting that Akt1 is may be a promising target for human liver cancer therapy.

On the other hand, Yoeli-Lerner *et al* (12) revealed that expression of activated Akt1 potently blocked the cell migration and invasion through Matrigel in three distinct breast cancer cell lines. Irie *et al* (13) also discovered that silencing of Akt1 expression markedly enhanced cell migration induced by growth factor in MCF-10A cells. Consistent with these observations, in the present study, upregulation of Akt1 expression significantly inhibited the cell migration and invasion in colorectal carcinoma HCT 116 cells. Furthermore, *in vivo* evidence also identified the role for Akt1 in blunting breast cancer cell invasion and subsequent metastasis. Using a mouse bitransgenic assay of mammary tumorigenesis with constitutively activated Akt1 and ErbB-2, Hutchinson *et al* (15) reported that upregulation of activated Akt1 expression resulted in a decrease in the incidence of metastatic lesions compared with control animals.

Although we, and other groups, have demonstrated the inhibitory effect of Akt1 on cell migration and invasion in several types of tumor cells, the precise cell signaling mechanisms remain to be further investigated. Multiple molecular mechanisms have been shown to be involved in Akt1-mediated negative regulation of cell migration and invasion, including inhibition of NFAT transcription factor (12) or phosphorylation of paladin (11), activation of ERK signaling pathway (13) and cell surface B1-integrins (14). Of note, silencing of AKT1 in PC-3 cells also resulted in strong upregulation of VEGFR2 (14). Consistently, in the present study, we directly demonstrated that enforced expression of Akt1 significantly not only reduced the expression of VEGF, but also suppressed the expression of MMP2, MMP9 and HIF1α, which then suppressed the migration and invasion of HCT 116 cells.

In the present study, our experiments on Akt1 were limited to human hepatocellular carcinoma HepG2 cells and colorectal carcinoma HCT 116 cells. Enforced Akt1 expression significantly increased cell proliferation through induction of Bcl-2 and NF-κB in both HepG2 and HCT 116 cells. Moreover, our observations also showed that Akt1 overexpression significantly promoted the expression of MMP2, MMP9, HIF1α and VEGF, which then contributed to enhancing the migration and invasion of HepG2 cells. Upregulation of Akt1 expression markedly downregulated the levels of these proteins and suppressed the migration and invasion of HCT 116 cells. In order to verify these effects of Akt1, more types of liver cancer and colorectal carcinoma cell lines are required. Furthermore, the precise molecular mechanisms remain to be further investigated.

Although Akt1 contributes to proliferation in both types of tumor cells, specific pharmacological inhibition may have a differential impact on migration and invasion in different cell types. Understanding these differences is crucial to the implication of specific inhibitors for cancer therapies.

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