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The PI3 kinase-Akt pathway mediates Wnt3a-induced proliferation

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Abstract

Wnt3a activates proliferation of fibroblasts cells via activation of both extracellular signal-regulated kinase (ERK) and Wnt/β-catenin signaling pathways. In this study, we show that the phosphatidyl inositol 3 kinases (PI3K)-Akt pathway is also involved in the Wnt3a-induced proliferation. Akt was activated within 30 min by Wnt3a in NIH3T3 cells. By Wnt3a treatment, activated Akt was transiently accumulated in nucleus although β-catenin was accumulated in the nucleus of cells in a prolonged manner. The Wnt3a-induced Akt activation was not affected by siRNA-mediated reduction of β-catenin, indicating that Wnt3a-induced Akt activation may occur independently of β-catenin. The Wnt3a-induced Akt activation was abolished by pre-treatment with PI3K inhibitor, LY294002 and Wortmanin, but not by MEK inhibitor, U0126, indicating that Wnt3a activates Akt via PI3K. The growth and proliferation induced by Wnt3a were blocked by treatments of the PI3K inhibitors. Furthermore, Wnt3a-induced proliferation was blocked by *Akt* siRNA. These results reveal that the PI3K-Akt pathway mediates the Wnt3a-induced growth and proliferation of NIH3T3 cells.

Keywords: Wnt3a; Akt; Proliferation; β-catenin; PI3 kinase

1. Introduction

The Wnt family proteins play key roles in development and cell growth [1,2]. Wnts bind to different Frizzled (Fz) receptors of the serpentine family and act through distinct pathways [3,4]. The Wnt signaling transmitted via regulation of destruction complex, including GSK3β, Axin, CK1α, and adenomatous polyposis coli (APC) [5–7]. In the resting states forming this complex, β-catenin is phosphorylated by GSK3β and CK1α leading to the rapid proteosomal degradation of β-catenin [8,9]. Upon Wnt stimulation, the destruction complex becomes dissociated and β-catenin accumulates in the cytoplasm. The accumulated β-catenin translocates into nucleus for activation of the Tcf-4/β-catenin target genes involving development of various human cancers such as colonic cancer and melanomas [10,11].

Wnt3a is known to be involved in development [12,13] and cytoskeletal reorganization [14]. Wnt3a has been shown to activate transcription of the *LEF-1* promoter through a β-catenin-dependent and LEF-1-independent mechanism [15]. Wnt3a activates the Wnt/β-catenin signaling pathway in several cell lines including mouse mammary epithelial cell line C57MG [14,16] and NIH3T3 cells [17]. The Wnt3a protein was recently purified and recombinant Wnt3a-induced self-renewal of hematopoietic stem cells [18]. The ERK pathway was immediately and transiently activated by Wnt3a within NIH3T3 cells, and that related with proliferation [19]. The Wnt3a-induced immediate ERK pathway activation occurs by a mechanism independently of β-catenin. Nevertheless, the ERK pathway was still activated by *β-catenin* transfection [19]. In addition, the ERK pathway activation by *β-catenin* transfection was lowered by co-transfection of dominant negative form of *Tcf-4* [19]. These results indicate that Wnt3a may regulate the ERK pathway via multiple mechanisms, including immediate activation by direct signaling and posttranscriptional activation by the β-catenin/Tcf-4 complex. Whether any pathway(s) other than the Wnt/β-catenin and ERK pathways are involved in Wnt3a-induced growth regulation is currently unknown.

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The phosphatidyl inositol 3 kinases (PI3K) pathway is one of the major signaling pathways regulating cellular growth and transformation [reviewed in Ref. 20]. The oncoprotein, Akt is a key component of the PI3K-Akt signaling pathway [21]. The binding of PI3K-generated phospholipids to Akt results in the translocation of Akt from the cytoplasm to the inner surface of the plasma membrane where Akt is phosphorylated [22]. Activation of Akt results in the phosphorylation of a number of downstream substrates involved in the regulations of the movement, survival and growth of cells [20,23]. Here we show that Akt is activated by Wnt3a conditioned medium (Wnt3a-CM) and purified recombinant Wnt3a in NIH3T3 cells. Involvement of PI3K in the Wnt3a-induced Akt activation was proven by measuring an effect of the PI3K inhibitors, LY294002 and Wortmanin, in the Wnt3a-induced Akt activation. The role of PI3K-Akt pathway in the Wnt3a-induced growth regulation was investigated by measurement of effects of the PI3K inhibitors on the Wnt3a-induced growth stimulation, and bromodeoxyuridine (BrdU) incorporation. The role of PI3K-Akt pathway in growth stimulation by Wnt3a was further

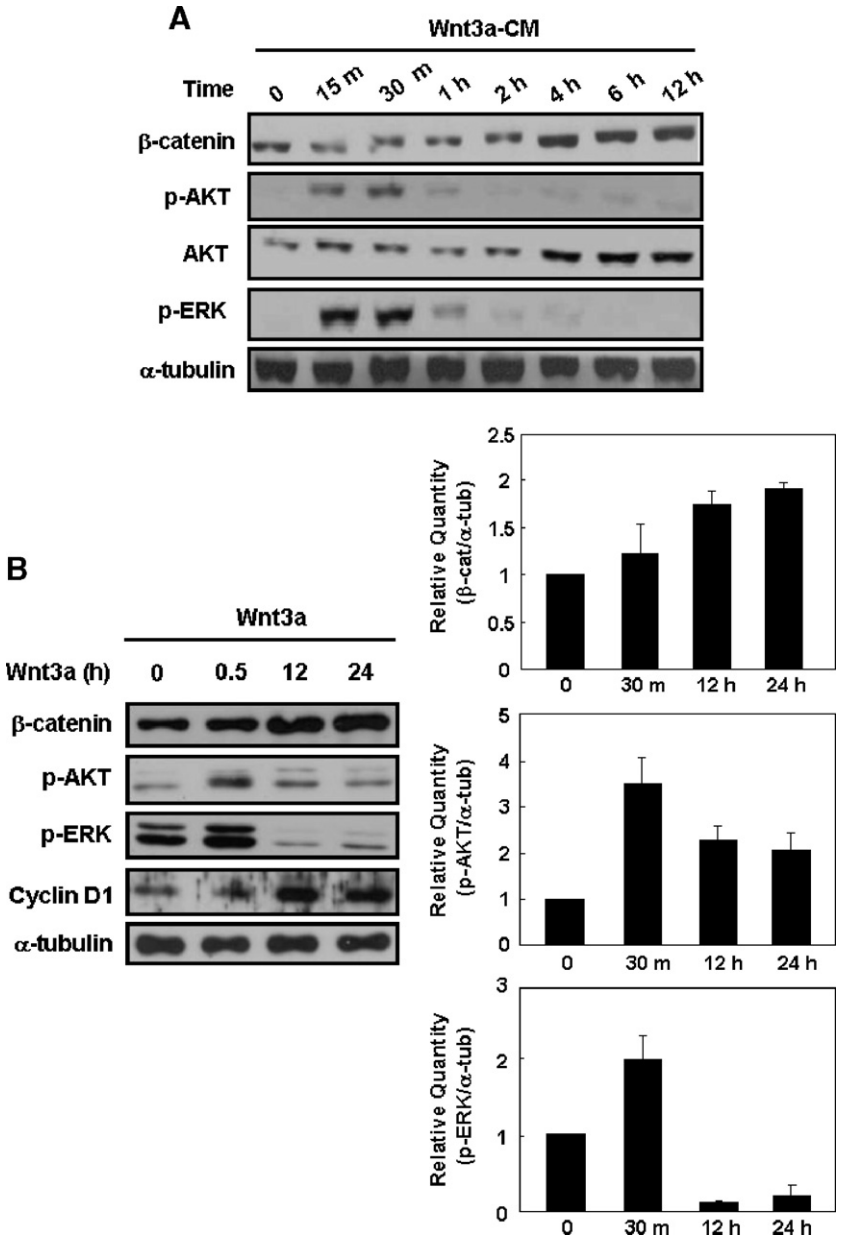

Fig. 1. Effects of Wnt3a on Akt activation. (A) NIH3T3 cells were treated with 100 μl of Wnt3A-CM [19] and harvested at intervals up to 12 h later. Western blot analysis for β-catenin, p-Akt, Akt, p-ERK and α-tubulin proteins was then performed as described in Materials and methods. (B) NIH3T3 cells were treated with 150 ng/ml of recombinant Wnt3a and harvested at 30 min, 12 h, and 24 h after treatment. Western blot analysis for β-catenin, p-Akt, p-ERK, cyclin D1, and α-tubulin proteins was then performed as described in Fig. 1A. The right panel shows quantification data for the left panel. The experiments were performed at least three times. The error bars are standard deviations of three independent experimental results.

confirmed by inhibition of the Wnt3a-induced proliferation by *Akt* siRNAs. Our data suggest that PI3K-Akt pathway regulates the Wnt3a-induced proliferation of fibroblasts.

2. Materials and methods

2.1. Reagents

Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), antibiotics, and Lipofectamine plus reagent were purchased from Invitrogen (Grand Island, NY). ERK, Phospho-ERK (p-ERK), p-Akt, Akt, β-catenin, and cyclin D1 antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Horseradish peroxidase (HRP)-conjugated anti-mouse IgG antibodies were obtained from Cell Signaling Biotechnology (Beverly, MA) and α-tubulin antibodies were obtained from Oncogene Research Products (San Diego, CA). Recombinant mouse Wnt3a expressed in Chinese hamster ovary cells was purchased from R&D systems (Minneapolis, MN). An enhanced chemiluminescence (ECL) system was obtained from Amersham Pharmacia (Uppsala, Sweden). A *Silencer*™ siRNA construction kit was obtained from Ambion (Austin, TX). U0126, Wortmannin and LY294002 were obtained from Biomol Research Laboratories (Plymouth Meeting, PA). 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt (WST-8) cell counting kit was purchased from Wako Pure Chemicals (Tokyo, Japan). Wnt3a producing L cells and control L cells [14] are described in a previous study [19].

2.2. Cell culture and production of a Wnt3a-conditioned medium

NIH3T3, Wnt3a producing L cells, and control L cells were cultured in DMEM supplemented with 10% FBS, 100 units/ml of penicillin, and 100 μg/ml of streptomycin in 5% CO2 at 37 °C. Wnt3a producing cells were maintained in the presence of G418 [17,24]. Concentrated Con-CM and Wnt3a-CM were prepared as described previously [19].

2.3. Western blot analysis

NIH3T3 cells grown in DMEM with 10% FBS were treated with 100 μl of either Wnt3a-CM or Con-CM. For analyses using recombinant proteins, cells were treated with 150 ng/ml of Wnt3a, and harvested at different time points after treatment for Western blot analysis [25]. Where required, a treatment with 10 μM U0126, 20 μM LY294002 or 10 μM Wortmanin was applied 30 min before treatment with 150 ng/ml of recombinant Wnt3a. For preparation of proteins, attached cells were rinsed twice in ice-cold PBS, harvested, and lysed directly in Laemmli sodium dodecyl sulfate (SDS) sample buffer. Samples were then boiled and separated on an 8–12% SDS-polyacrylamide gel electophoresis (SDS-PAGE) followed by Western blot analysis using anti-p-ERK, -p-Akt, -Akt, -cyclin D1, -β-catenin, or -α-tubulin antibodies, followed by incubation with an appropriate horse radish peroxidase conjugated secondary antibody. Protein bands were visualized using an ECL.

2.4. siRNA preparation and treatment

Mouse β-catenin (Gene bank accession number NM_007614) and Akt1 (Gene bank accession number NM_009652) mRNA target sequences were designed using a small interfering RNA fragment (siRNA) template design tool (Ambion). The designed β-catenin mRNA target sequences were 5′-AAGGCTTTTCC CAGTCCTTCA-3′ (300–320) and 5′-AAGATGATGGTGTGCCAAGTG-3′ (1400–1420). The Akt1 mRNA target sequences were 5′-AAGAGACGATG GACTTCCGAT-3′ (627–647) and 5′-AAGGACGGGCACATCAAGATA-3′ (1133–1153). Each siRNA was synthesized using a Silencer™ siRNA construction kit (Ambion). The resulting siRNAs were transfected into NIH3T3 cells using Lipofectamine plus reagent at a concentration of 1.68 μg per 3.5 cm dish [26]. Transfected cells were grown for 48 h at 37 °C in a 5% CO_2 incubator, and harvested for Western blot analysis.

2.5. Cell number counting

NIH3T3 cells were plated at a density of 3×10^4 cells per well and incubated confluence up to 50%, and 100 μl of concentrated Con-CM or Wnt3-CM was treated for 24 h. In cases required cells were co-treated with 20 μM LY294002 or 10 μM Wortmannin. The cells were stained with 0.5% crystal violet in 20% ethanol for 5 min, and washed with double-distilled water 5 times, and photographed under a Zeiss Axiovert LSM510 inverted microscope fitted with phase contrast optics. The number of attached cells was determined by mounting 10 μl of a 0.4% trypan blue/cell mixture (1:1) onto a Tiefe Depth Profondeur 0.0025 mm^2 cell counting plate (Superior Co., Germany) and counting under a microscope.

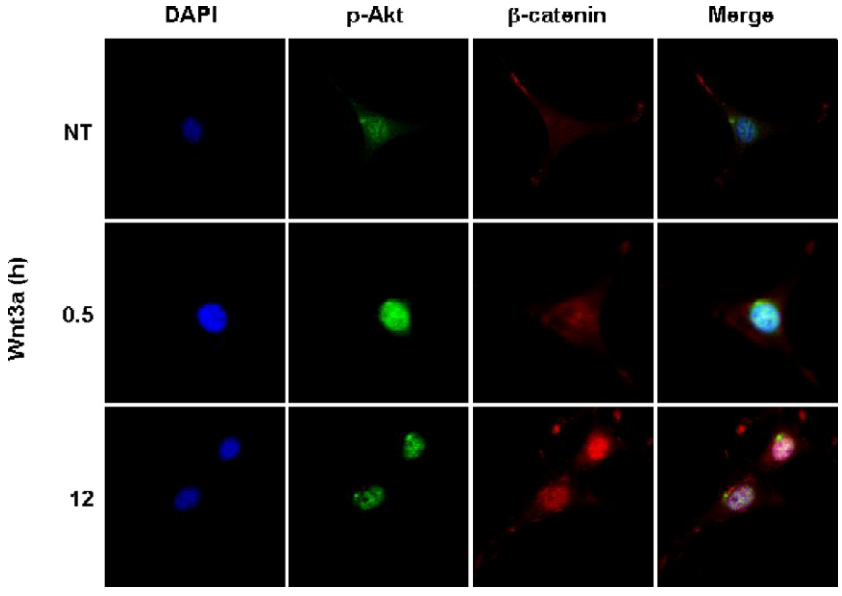

Fig. 2. Effects of Wnt3a on the nuclear accumulation of p-Akt. NIH3T3 cells were grown on cover slides in DMEM and treated with 150 ng/ml of recombinant Wnt3a. Cells were subjected to the immunocytochemical analysis for p-Akt (green) and β-catenin (red) at 0, 30 min, and 12 h after Wnt3a treatment. Cell nuclei were stained with DAPI.

2.6. Cell growth assay

NIH3T3 cells were grown to 30% confluence in DMEM with 10% FBS, and were treated with 150 ng/ml of purified Wnt3a in 2% FBS containing DMEM every 24 h. In some cases, the cells were treated with 10 μM LY294002 or 10 μM Wortmanin for 30 min before Wnt3a treatment every 24 h. After 96 h, the cells were treated WST-8 solution for 2 h, and the absorbance of the cell media at 450 nm was measured.

2.7. Immunocytochemistry and BrdU incorporation

For immunocytochemical analysis, NIH3T3 cells were plated onto coverslips at a 30% confluence, then placed into 6-well plates and grown in DMEM supplemented with 10% FBS. The cells were not or treated with 150 ng/ml of recombinant Wnt3a for 30 min or 12 h in DMEM containing 10% FBS [25,27].

For immunocytochemical analysis, the cells were fixed in a methanol/formaldehyde (99:1) mixture at −20 °C for 20 min, permeabilized with 0.2% Triton X-100 at 4 °C for 30 min, and blocking with PBS containing 1% bovine serum albumin (BSA). The cells were incubated with anti-β-catenin or anti-p-Akt antibody (1:100) for 2 h and incubated with goat-anti-mouse-Cy ™2-conjugated or goat-anti-rabbit-Rhodamine Red ™-X-conjugated secondary antibody (1:200) for 1 h. Cells were then treated with 1 μM 4′,6′-diamidine-2-phenylindole dihydrochloride (DAPI) in PBS for 10 min. For a BrdU incorporation study, cells were plated onto coverslips at 40% confluent. Cells were then treated with 150 ng/ml of recombinant Wnt3a in DMEM supplemented with 2% FBS. Where required, 20 μM LY294002 or 10 μM Wortmanin is added for 30 min before recombinant Wnt3a treatment. For siRNA experiments, cells were transfected with 1.68 μg of siRNA in a 3.5 cm dish. Twenty-four hours after transfection, cells were treated with 150 ng/ml of recombinant Wnt3a in DMEM supplemented with 2% FBS for 24 h. Cells were grown for 5 h in DMEM containing 20 μM BrdU before immunocytochemical

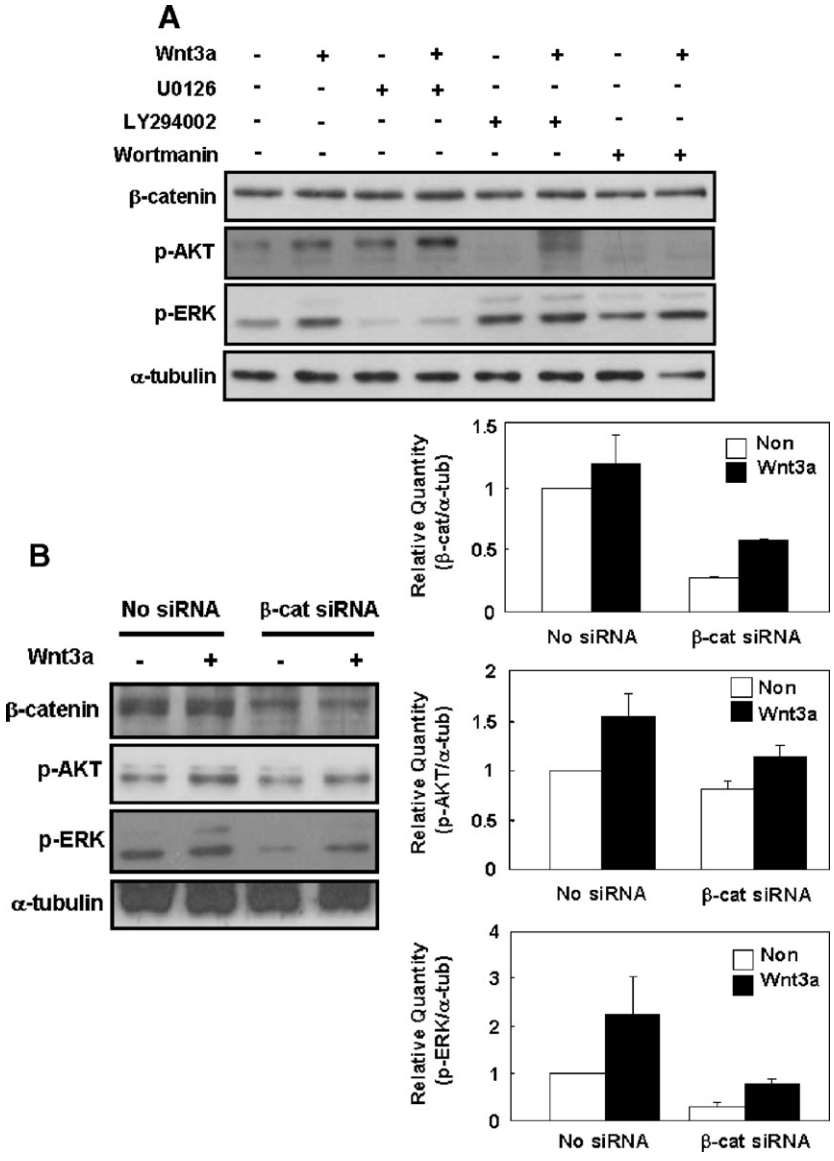

Fig. 3. Effects of the PI3K inhibitors and *β-catenin* siRNA on Wnt3a-induced Akt activation. (A) NIH3T3 cells were untreated or treated with 150 ng/ml of recombinant Wnt3a for 30 min. Where indicated, cells were co-treated with 20 μM LY294002, 10 μM Wortmanin, or 10 μM U0126. Western blot analysis for β-catenin, p-Akt, p-ERK, and α-tubulin was then performed as described in Materials and methods. (B) NIH3T3 cells were transfected with β-catenin siRNA, and harvested 48 h later. Where indicated, cells were treated with 150 ng/ml recombinant Wnt3a for 30 min before harvesting. The right panel shows quantification data for the left panel. The experiments were performed at least three times. The error bars are standard deviations of three independent experimental results.

analysis. The cells were fixed in a methanol/formaldehyde (99:1) mixture, and permeabilized with PBS containing 0.2% Triton X-100. The cells were then fixed in 3.7% formaldehyde for 10 min before being incubated with 2 N HCl for 30 min. After blocking with PBS containing 1% BSA, the cells were incubated with anti-BrdU monococlonal antibody (1:20) for 2 h, and followed by incubation with Goat anti-mouse-Cy™2-conjugated secondary antibody (1:200) for 1 h. DAPI was then applied at a final concentration of 1 μM in PBS for 10 min, and the cells were washed in distilled water. Samples were mounted for photographyon a Radiance 2100 Laser Scanning System (Bio-Rad, UK). Each analysis was performed at least three times.

3. Results

3.1. Wnt3a transiently activates Akt independently of β-catenin

Wnt3a-induced proliferation and G1 to S phase cell cycle progression is partially blocked by MEK inhibitor, U0126, whereas Wnt3a-induced ERK activation is almost abolished [19]. This suggests that other pathway(s) may mediate Wnt3a-induced proliferation. To address involvement of the PI3K-Akt pathway in Wnt3a-induced proliferation, we examined whether Wnt3a could activate the PI3K-Akt pathway within NIH3T3 cells. The Akt activity, which was indirectly measured through Western blot analysis of phosphorylated Akt (p-Akt) level, was significantly increased within 15 min after treatment of Wnt3a conditioned medium (Wnt3a-CM). The immediately increased Akt activities start to re-decrease since after 1 h (Fig. 1A). The profile of the Akt activation was highly similar to that of ERK activation. The β-catenin protein levels were also increased by Wnt3a treatment, but these increases were observed at 4 h after treatment with Wnt3a-CM. Akt protein levels were also increased in a similar profile with the β-catenin level change by Wnt3a-CM treatment (Fig. 1A). The transient Akt activation by Wnt3a was further confirmed by measuring effects of purified recombinant Wnt3a on the Akt activation (Fig. 1B, right panel shows the quantification data). The cyclin D1 protein which is one of the major targets of both Wnt/β-catenin and Ras-ERK pathway [28] was significantly increased with the increase of β-catenin at 12 and 24 h after treatment of Wnt3a (Fig. 1B). The Wnt3a-induced Akt activation was further investigated by immunocytochemical analysis of NIH3T3 cells stimulated with recombinant Wnt3a. The p-Akt was significantly enriched in nucleus of cells at 30 min after treatment of Wnt3a, and the nuclear p-Akt levels were significantly reduced at 12 h after Wnt3a stimulation (Fig. 2). On the other hand, β-catenin was weakly increased in nucleus of cells at 30 min after treatment of Wnt3a, and nuclear β-catenin was significantly increased at 12 h after Wnt3a treatment (Fig. 2) [19].

3.2. PI3K mediate Wnt3a-induced Akt activation independently of β-catenin

To identify involvement of PI3K in Wnt3a-induced Akt activation, we measured an effect of the PI3K inhibitors, LY294002 and Wortmanin, on the Wnt3a-induced Akt activation. The immediate Akt activation by Wnt3a was mostly blocked by pre-treatment of LY294002 and Wortmanin, whereas pre-treatment of MEK inhibitor, U0126, had no effect on Wnt3a-induced Akt activation (Fig. 3A). On the other hand, the ERK activation by Wnt3a was reduced by U0126, but not by LY294002 or Wortmanin (Fig. 3A), indicating that independence of ERK in the Wnt3a-induced Akt activation. Therefore, PI3K mediates Wnt3a-induced Akt activation. The Akt activities were reduced by siRNA-mediated β-catenin reduction, but the Wnt3a-induced immediate Akt activation was remained in cells reduced β-catenin (Fig. 3B, right panel shows the quantification data) [19], indicating that Akt activation by Wnt3a-induced direct signaling occurs independently of β-catenin as does the Wnt3a-induced ERK activation.

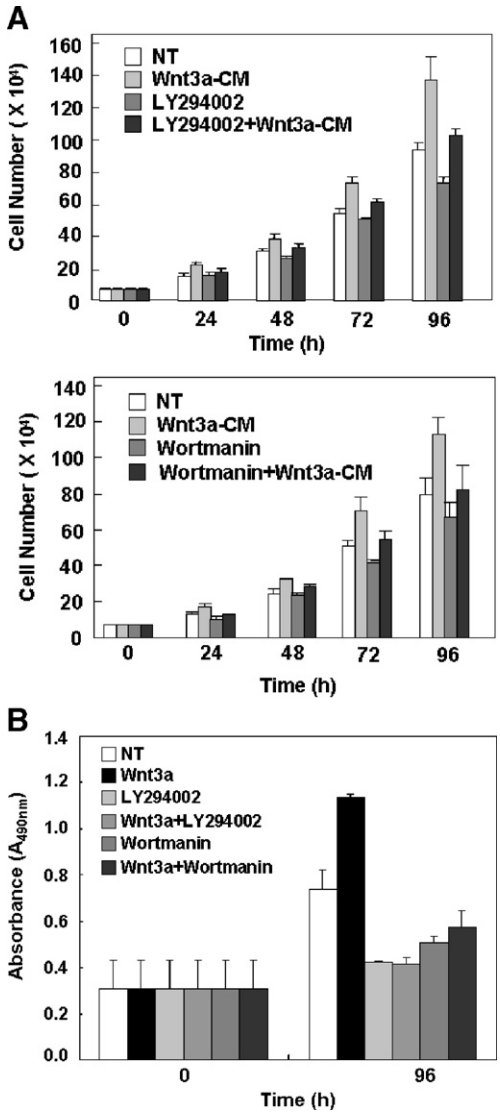

Fig. 4. Effects of the PI3K inhibitors, LY294002 and Wortmanin, on Wnt3a-induced growth stimulation. (A) NIH3T3 cells were grown to 50% confluence in DMEM, and 100 μl of concentrated control Con-CM or Wnt3A-CM [19] was treated for different time periods (0, 24, 48, 72 and 96 h) in 2% FBS containing medium, and the number of attached cells was counted. (B) NIH3T3 cells were grown to 30% confluence in DMEM, and 150 ng/ml of recombinant Wnt3a was treated or non-treated for 96 h in 2% FBS containing medium. Where indicated, cells were co-treated with 10 μM LY294002 or 10 μM Wortmanin, and cell growth was determined by measurement of Absorbance 5 nm.

3.3. PI3K and Akt mediate the Wnt3a-induced growth and proliferation

To determine whether the PI3K-Akt pathway regulates Wnt3a-induced growth, we measured an effect of LY294002 and Wortmanin on the growth stimulation by Wnt3a. The cell numbers were increased around 30% by 96 h after Wnt3a-CM treatment. The Wnt3a-induced cell number increase was almost completely blocked by co-treatment of LY294002 (Fig. 4A, upper panel). The cell numbers acquired by LY294002 alone was lower than that acquired without any treatment. The similar effects were observed by treatments of the alternative inhibitor of PI3K, Wortmanin (Fig. 4A, lower panel). We also observed similar effects of LY294002 and Wortmanin on the growth stimulation by recombinant Wnt3a (Fig. 4B). These results demonstrate that PI3K is involved in the regulation both basal and Wnt3a-induced cellular growth.

Next, we examined the effect of LY294002 and Wortmanin on Wnt3a-induced proliferation. Treatment with recombinant Wnt3a increased the percentage of BrdU positive cells from 20% to 50% (Fig. 5A, representative results shown in lower panel). The Wnt3a-induced cell proliferation was significantly abolished by co-treatment with LY294002 (from 50% to 27%) or Wortmanin (from 50% to 30%) (Fig. 5A, lower panel shows the representative data). We confirmed these results using Akt siRNA. The BrdU incorporation was increased from 20% to 50% by Wnt3a treatment. Both basal and Wnt3a-induced BrdU incorporation were reduced from 20 to 15% and 50% to 27%, respectively, by reduction of Akt treatment of Akt siRNA (Fig. 6A, lower panel shows the representative data). The Wnt3a-

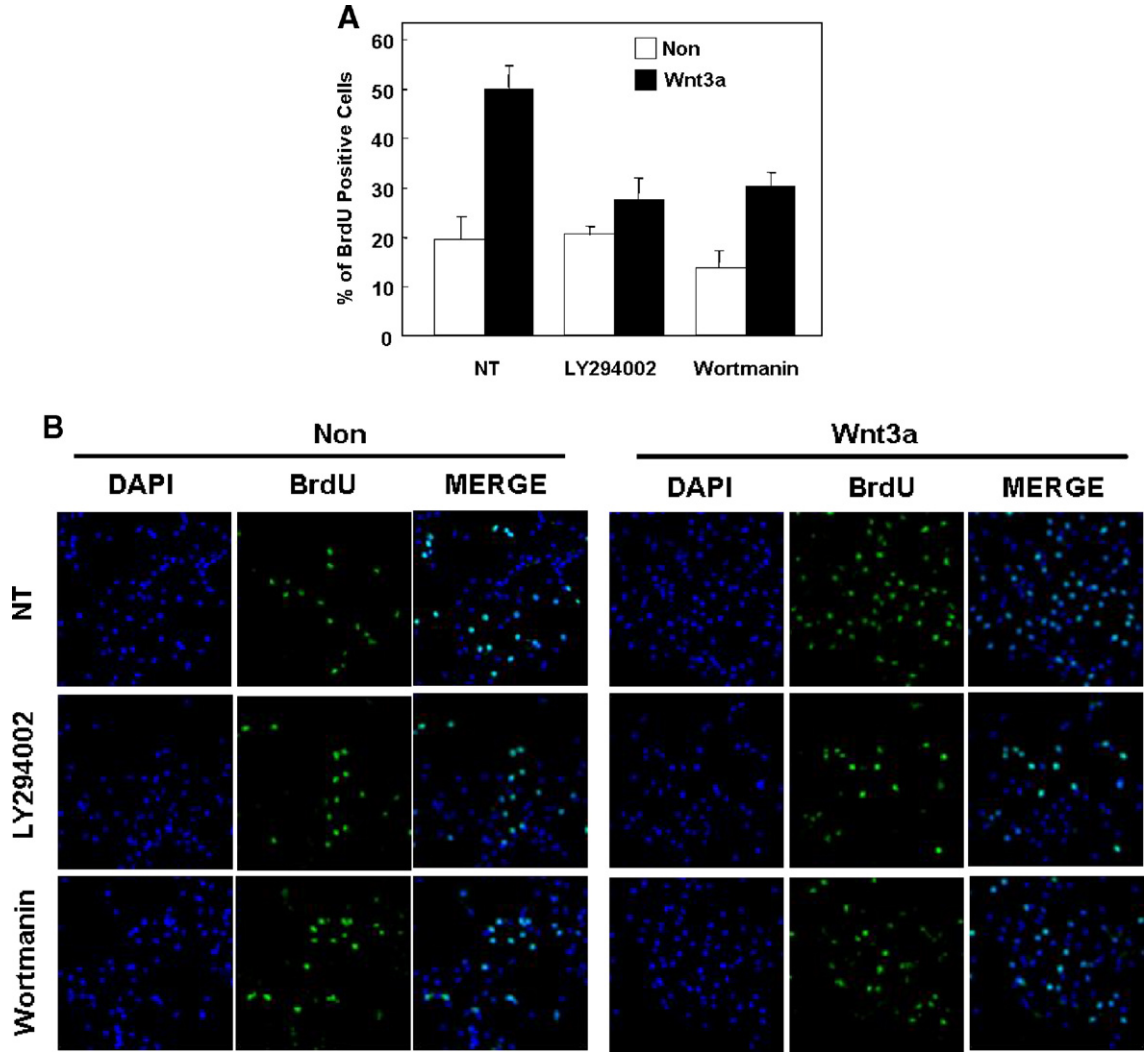

Fig. 5. Effects of LY294002 and Wortmanin on Wnt3a-induced proliferation. (A) NIH3T3 cells were grown to 40% confluence on cover slide in DMEM, and treated for 24 h with 150 ng/ml of recombinant Wnt3a in DMEM containing 2% FBS. Where indicated, cells were co-treated with 20 μM of LY294002 or 10 μM of Wortmanin. Cells were labeled with 20 μM BrdU for 5 h prior to immunocytochemical analysis using an anti-BrdU antibody. Cell nuclei were stained with DAPI. Cells containing BrdU incorporated into the nucleus were scored as BrdU positive cells, and the relative percentage of BrdU positive cells was determined. Analyses were performed at least three times and 100 cells were counted in each case. Error bars indicate the standard deviations of three independent analyses. (B) Representative images of BrdU staining are shown.

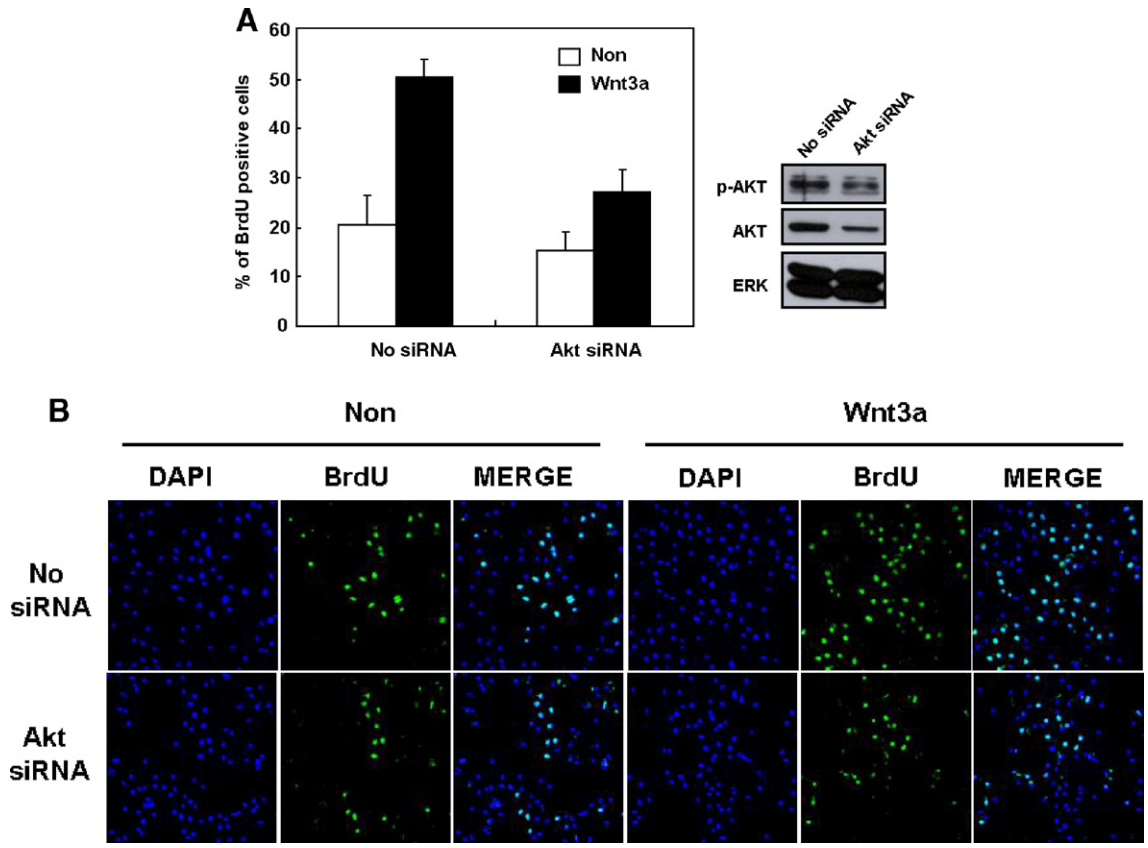

Fig. 6. Effects of Akt siRNAs on Wnt3a-induced proliferation. (A) NIH3T3 cells were grown on cover slides in DMEM and transfected with Akt siRNAs for 48 h. Where indicated, cells were treated with 150 ng/ml of recombinant Wnt3a for 24 h before immunocytochemical analysis. Cells were labeled with 20 μM BrdU 5 h prior to immunocytochemical analysis. Cell nuclei were stained with DAPI. Cells containing BrdU incorporated into the nucleus were scored as BrdU positive cells and quantified. Analyses were performed at least three times, and 100 cells were counted in each case. Error bars indicate the standard deviations of three independent analyses. The right panel shows results of Western blot analyses of Akt after transfection of NIH3T3 cells with Akt siRNA. (B) Representative images of BrdU staining are shown.

induced BrdU incorporation was more significantly reduced by Akt siRNA (Fig. 6A, representative results shown in lower panel). Taken together, these results show that both PI3K and Akt are involved in Wnt3a-induced G1 to S phase cell cycle progression.

4. Discussion

Wnt3a is involved in the proliferation of various cell types including chick somites, multiple myeloma cells, human mesenchymal and haematopoietic stem cells, and NIH3T3 cells [18,19,29–31]. Wnt3a-induced proliferation is mediated, at least partly, by the Wnt/β-catenin and ERK pathways [19].

The PI3K-Akt pathway mediates diverse cellular physiologies such as apoptosis, differentiation and intermediary metabolism and cellular proliferation, and both transcriptional and posttranscriptional mechanisms are involved [32]. There is evidence to support a connection between this pathway and Wnt signaling. For instance, Wnt-7a inhibits apoptosis of articular chondrocytes by activating of Akt [33].

We identified involvement of the PI3K-Akt pathway in the Wnt3a-induced proliferation. Akt activation, which parallels ERK activation, occurs within 15 min of Wnt3a treatment and declines rapidly thereafter. The kinetics of Akt activation is highly similar to that of ERK. Therefore, Akt and ERK activation by Wnt3a is immediate and transient [19]. The immediate Akt activation by Wnt3a was abolished by pretreatment with LY294002 or Wortmanin, but was unaffected by U0126 pretreatment, indicating that PI3K but not ERK is involved in the Wnt3a-induced Akt activation. ERK activity was increased by LY294002 and Wortmanin (Fig. 3A), suggesting that PI3K may negatively regulate the ERK pathway in fibroblasts. This finding is consistent with other studies showing that activation of the PI3K-Akt pathway inhibits the ERK pathway [34,35].

The Akt activation by Wnt3a is also maintained by siRNA-mediated reduction of β-catenin (Fig. 3B), suggesting that the mechanisms by which Wnt3a activates Akt and ERK may be the same [19]. Also this finding suggests that Wnt3a-induced Akt activation may occur independently of β-catenin.

Akt protein levels were increased after 4 h and remained elevated for up to 12 h. These changes in Akt protein levels paralleled those of β-catenin (Fig. 1A) [19]. The increase of both β-catenin and Akt by Wnt signaling indicates that protein levels of those proteins may be regulated by posttranscriptional events rather than immediate signaling involving modification of proteins. The

Akt gene is known to be regulated by Wnt/β-catenin signaling via a Tcf-4/β-catenin binding site in its promoter [36]. The auto-regulation of β-catenin by Wnt/β-catenin was not observed.

The Wnt/β-catenin, ERK, and PI3K pathways are major transforming pathways involved in growth control [37–42]. The two pathways interact with each other to regulation growth and transformation of cells, although the relationship between the ERK and Wnt/β-catenin pathways is poorly understood [19,43]. The regulation of the PI3K-Akt pathway by Wnt signaling is also poorly characterized, although a recent study revealed that the PI3K/Akt pathway is involved in the anti-apoptotic effects of Wnt3a in osteoblasts [44]. Our data reveal that PI3K-Akt pathway is involved in Wnt3a-induced growth and proliferation in fibroblasts. The growth of NIH3T3 cells stimulated by Wnt3a was abolished by co-treatment of LY294002 and Wortmanin, indicating that PI3K is involved in growth stimulation of fibroblasts by Wnt3a. The Wnt3a-induced cell proliferation was significantly reduced by pre-treatment of LY294002 and Wortmanin, indicating that the growth stimulation of NIH3T3 cells by Wnt3a occurs by stimulation of cell proliferation via the PI3K pathway. Consistent with these findings, Akt reduction using Akt siRNA abolishes Wnt3a-induced proliferation.

In summary, the PI3K-Akt pathway regulates Wnt3a-induced fibroblast growth. Our study demonstrates that there is a cross-talk between the Wnt/β-catenin and PI3K-Akt pathways in the regulation of proliferation indicate that the major transforming pathways interact in growth and transformation of cells. It is currently unclear why both the PI3K-Akt and the ERK pathways are involved in Wnt3a-induced growth regulation. However, cells may respond to a common extra-cellular growth regulatory factor via multiple signaling pathways for optimal physiological responses.

Acknowledgments

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