



## Current Topic

## Effect of 14-3-3 tau protein on differentiation in BeWo choriocarcinoma cells

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## ABSTRACT

This study aimed to investigate the location and function of tau isoform of 14-3-3 proteins in human trophoblast. 14-3-3 tau was localized in human cytotrophoblast cells, but not in syncytiotrophoblast cells in both first trimester and term placenta by immunohistochemistry stain. Forskolin-induced cell fusion (BeWo cells) confirmed that 14-3-3 tau was decreased during trophoblast differentiation. Forskolin-induced differentiation was stimulated by small-interfering (si) RNA induced down-regulation of 14-3-3 tau, contrarily, it was suppressed by plasmid induced upregulation of 14-3-3 tau in BeWo cells. When BeWo cells were treated with 14-3-3 tau siRNA, an increase in protein concentration of cell cycle inhibitor p27kip1 and a decrease in protein concentration of proliferating cell nuclear antigen, as well as activation of the extracellular signal-regulated kinase (ERK) pathway, were also noticed. These findings suggest that 14-3-3 tau might be mediated trophoblast differentiation through cell cycle regulation.

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## 1. Introduction

Disturbed function of trophoblast may lead to a variety of pregnancy-associated pathologies, but functional regulation of trophoblast differentiation and proliferation is not fully understood. Recent studies have suggested that 14-3-3 proteins which are required for signal transduction pathways, cell division and apoptosis [1,2], probably play a key role in trophoblast function.

The 14-3-3 proteins are 30-kDa homodimers and have seven isoforms [3]. Several isoforms of 14-3-3 were considered relevant in functional regulation of trophoblast. For example, 14-3-3  $\epsilon$  was detected in the first trimester human cytotrophoblast and hypoxia altered its subcellular localization, suggesting that 14-3-3  $\epsilon$  played a functional role in cellular responses to reduced oxygen levels [4]. In the preeclamptic placentas, the levels of 14-3-3  $\zeta$  were significantly higher than that in normal placentas, preeclampsia was also associated with a weak interactions between 14-3-3  $\zeta$  and Bax and a strong interaction between 14-3-3  $\zeta$  and protein kinase C- $\delta$  (PKC- $\delta$ ) [5]. Recently, we found a decreased expression of another 14-3-3 protein isoform, 14-3-3 tau, in hypoxia-treated human trophoblast cell line BeWo by proteomic analysis [6], which suggests that 14-3-

3 tau plays a role in hypoxia-induced responses in the syncytialization of trophoblast.

The 14-3-3 proteins modulate protein activity through a variety of mechanisms. Binding of 14-3-3 to apoptosis-associated proteins such as BAD and Bcl2 affected their interaction with other proteins [7]. Alternatively, 14-3-3 proteins may act as a scaffold to bring proteins together as in the case of Raf and PKC [8]. Probably the most commonly identified mode of action of 14-3-3 proteins is to sequester proteins into inappropriate cell compartments, thereby inhibiting their function. A corollary to this mechanism is that 14-3-3 frequently binds to phosphorylated proteins. Dephosphorylation of the protein results in release of 14-3-3, releasing the protein which is then translocated it or otherwise activated, an example of which is KSR1 and Raf-1 [9].

In humans, 14-3-3 tau is widely expressed in brain neurons and T cells [10], which is induced by DNA damage and is required for regulation of G<sub>2</sub>/M-transition and G<sub>1</sub>/S-transition in cells [11,12]. But, there are no data about the potential role of 14-3-3 tau in trophoblast. To further investigate its potential role, we examined the effects of 14-3-3 tau on human trophoblast differentiation.

## 2. Materials and methods

## 2.1. Tissue collection

Human placentas were collected from 7 to 40 weeks of gestation (GW). Placentas from 6 to 11 GW were obtained from elective terminations of pregnancies ( $n = 6$ ). Term human placentas (38–40 GW) were obtained from uncomplicated pregnancies after

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elective caesarean section ( $n = 6$ ). Collection and processing of human placentas were approved by the Human Ethics Committee of the Obstetrics and Gynecology Hospital of Fudan University and informed consent was obtained from all patients participating in this study. Placental samples were immediately fixed in 4% paraformaldehyde dissolved in PBS, dehydrated, and embedded in paraffin. Sections (4  $\mu\text{m}$ ) were placed on poly-L-lysine-coated glass slides.

## 2.2. Immunohistochemistry

Deparaffinized tissue sections were pretreated with 3%  $\text{H}_2\text{O}_2$  for 15 min to inhibit endogenous peroxidase activity. The anti 14-3-3 tau polyclonal rabbit antibody (1:200, Santa Cruz Biotechnology, CA) diluted in PBS was applied onto the sections, and these sections were maintained overnight at 4 °C in a humidified chamber. Sections then reacted with a poly-HRP labeled anti-rabbit IgG polymer for 30 min at room temperature. After washing, sections were developed in a staining solution containing DAB for 5 min at room temperature. Sections were examined under a light microscope (Olympus D70, Tokyo, Japan) for satisfactory developed staining. To facilitate cytoplasmic visualization of the immunostained product, hematoxylin was used for counterstaining. As negative immunohistochemical controls, sections were incubated with normal rabbit serum.

## 2.3. Cell culture

BeWo choriocarcinoma cells were purchased from the China Center for Type Culture Collection (CCTCC, Wuhan, China) and were maintained as monolayers in Kaighn's modification of Ham's F-12 medium (Sigma–Aldrich, St.Louis, USA) supplemented with 10% fetal bovine serum (FBS) under standard culture conditions of 5%  $\text{CO}_2$  in air at 37 °C with medium renewal every 2–3 days.

## 2.4. Induction of differentiation using forskolin

BeWo cells were incubated with 20  $\mu\text{M}$  forskolin or vehicle (dimethyl sulfoxide, DMSO) (Sigma–Aldrich, St.Louis, USA) in Ham's F-12 medium supplemented with 1% FBS at 37 °C for 2–3 days to induce enhanced hCG secretion and cell fusion, which are indicators of cell differentiation. The medium was replaced every day. Following addition of forskolin, cells were harvested at specific time-points for isolation of RNA and protein. BeWo cells were stained with anti E-cadherin monoclonal mouse antibody (1:400, BD biosciences, Mississauga, ON, Canada) along with an FITC-labeled goat anti-mouse IgG (Invitrogen, Carlsbad, CA) to distinguish cell border. The use of E-cadherin immunostaining has been utilized in the past for such assessments of trophoblast fusion [13]. Syncytialization was considered genuine when at least 3 or more nuclei were present in the same cytoplasm. The nuclei were counterstained with DAPI. The number of multinuclear cells in the five areas under microscope which were randomly selected and counted. The data are expressed as the ratio of each control, and the effect of 14-3-3 tau on forskolin-induced cell fusion was evaluated from three independent experiments.

## 2.5. Regulation of 14-3-3 tau expression in BeWo cells

The BeWo cells with 30% confluency in 12-well culture plates were transfected with a target small-interfering RNA (siRNA, 80 nmol/L) and a Negative control siRNA using Lipofectamine™ 2000 transfection reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. After treatment for 6 h with siRNA, the medium containing siRNA and transfection reagents was removed, and after washing, the cells were cultured for 24–96 h with fresh culture medium.

To up-regulate 14-3-3 tau, we constructed an expression plasmid using a full-length 14-3-3 tau cds (Invitrogen, Carlsbad, CA) which was cloned into the expression vector pcDNA\_HA. The resultant construction was verified by sequencing and was called HA tagged 14-3-3 tau plasmid. The BeWo cells with 80% confluency in 12-well culture plates were transfected with 14-3-3 tau plasmid (1.6  $\mu\text{g}/\text{mL}$ ) and pcDNA\_HA vector (as vector control) using Lipofectamine 2000 according to the manufacturer's instruction. The cells were cultured for 24–72 h, protein and transcript expression determined by Western Blot and RT-PCR, respectively.

**Table 1**

Details of the primers employed in the study.

Name of gene	Primer sequence	Annealing temperature (°C)	Amplicon length (bp)	Cycle
$\beta$ -hCG	F 5-GTGAACCCGGTCTCCTA-3 R 5-CTTTGAGGAAGAGGAGTCTGG-3	60	141	25
14-3-3 tau	F 5-AGCCAATGCAACTAATCCAGAGAG-3 R 5-AAGCCGTTTTAGCCAGCGTG-3	60	269	30
syncytin	F 5-AGGAGCTTCGAAACTGTGA-3 R 5-GTGAGCTAAGTTGCAAGCCC-3	56	494	22
$\beta$ -actin	F 5-AACTCCATCATGAAGTGTGACG-3 R 5-GATCCACATCTGCTGGAAGG-3	60	234	25

All the primers were synthesized by ShengGong, Shanghai, China.

## 2.6. Human chorionic gonadotropin (hCG) secretion

Conditioned media were collected at the time intervals specified and were kept at  $-80$  °C. Protein concentration in culture media was quantified. hCG secretion was determined by measuring its concentrations in the conditioned media using an enzyme immunoassay kit which specifically detects  $\beta$ -chain of hCG. Then  $\beta$ -hCG values were normalized to the protein concentration in culture media.

## 2.7. Cell proliferation assay

To determine the effect of 14-3-3 tau suppression on BeWo cells proliferation, we used a tetrazolium reagent, 2-(4-indophenyl)-3-(4-nitrophenyl)-5-(2,4-disulphophenyl)-2H-tetrazolium monosodium salt (WST-1, Cell Counting Kit, Beyotime, Beijing, China). This assay is based on the measurement of the formazan dye that is liberated after the cleavage of this reagent by mitochondrial dehydrogenase activity in viable cells. BeWo cells were seeded in 96-well culture plates in the media supplemented with 10% FBS, transfected with 14-3-3 tau or negative control siRNA for 48 h–96 h. The cells were then incubated with WST-1 reagent for 1 h at 37 °C. The staining intensity in the medium was measured by determining the absorbance at 450 nm, and the data were expressed as ratios of the control value.

## 2.8. RNA extraction and semi-quantitative reverse transcription polymerase chain reaction (RT-PCR)

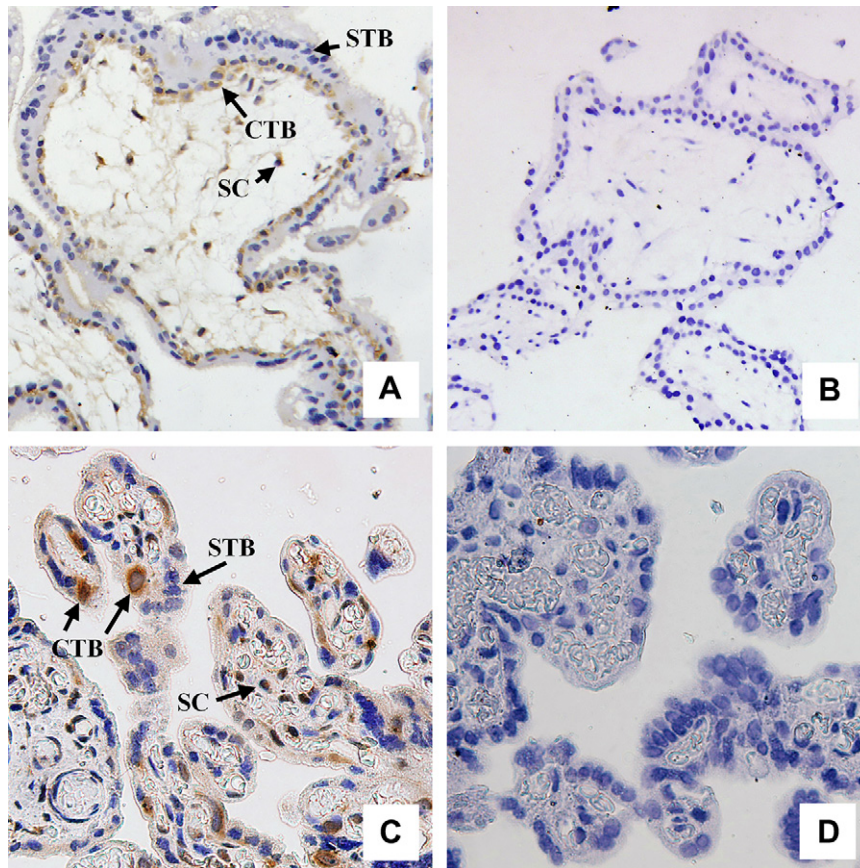
Total RNA was extracted from the cell culture dishes using Trizol (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The cDNA was synthesized from 5  $\mu\text{g}$  total RNA by reverse transcription kit (Takara, Dalian, China) as described in manufacturer's instructions. Relative transcript abundance of 14-3-3 tau, syncytin,  $\beta$ -hCG was analyzed by semi-quantitative RT-PCR using  $\beta$ -actin (ACTB) as an internal standard. The primers, expected sizes of the PCR products and amplified conditions used in the subsequent RT-PCR were shown in Table 1. The amount of template cDNA and the number of cycles were determined experimentally so that quantitative comparison could be made during the exponential phase of the amplification process for both target and reference gene. PCR products were separated on a 2% agarose gel. Gels were stained with ethidium bromide. A single band for each gene was observed at the expected size. The intensity of either the target or  $\beta$ -actin band for each sample was quantified using a gel documentation and analysis system and the ratio of the two was used as a normalized value for expression of each target gene. All assays were conducted in triplicate.

## 2.9. Western blot analysis

Cell lysates were prepared by radioimmunoprecipitation assay (RIPA) buffer. Protein samples were resolved by polyacrylamide gel electrophoresis on SDS –12% polyacrylamide gels and electrotransferred to PVDF membranes. After blocking in PBS/Tween-20 with 5% dry milk for 1 h at room temperature, membranes were incubated with specific primary antibodies overnight at 4 °C. The following antibodies were used in this study: the anti 14-3-3 tau polyclonal rabbit antibody (1:800, Santa Cruz Biotechnology, CA), the anti PCNA monoclonal mouse antibody (1:400, Santa Cruz Biotechnology, CA), the anti p27kip1 polyclonal rabbit antibody (1:1000, Cell Signal, US), the anti phospho-ERK1/2 monoclonal mouse antibody and the anti-ERK1/2 polyclonal rabbit antibody (1:1000, KangChen-Biotech, China). The immunoreactive bands were detected by a chemiluminescence detection kit (Tiangen, Beijing, China) after incubation with horseradish peroxidase-labeled mouse or rabbit IgG antibody (1:10,000, Jackson, USA). The membrane was re-probed with anti- $\beta$ -actin (ACTB)(1:10,000; KangChen-Biotech, China) as a loading control. All blotting experiments were repeated at least three times, and representative data are shown.

## 2.10. Statistical analysis

Data were presented as mean  $\pm$  S.D. of at least three independent experiments performed in triplicate. Differences between groups were analyzed using an ANOVA and a  $P$ -value less than 0.05 was considered to be statistically significant.



**Fig. 1.** Expression of 14-3-3 tau protein in human placentas. Shown are examples of the first trimester villi (A)–(B) and term placenta (C)–(D) that were immunostained to determine 14-3-3 tau expression. (B) and (D): Negative control. Representative images were taken at  $\times 40$  magnification. CTB, cytotrophoblasts; STB, syncytiotrophoblasts; SC, stromal cells.

### 3. Results

#### 3.1. Localization of 14-3-3 tau protein in placenta

In first trimester placenta tissue, 14-3-3 tau protein was strongly stained in the cytotrophoblast layer, the syncytiotrophoblast layer was poorly stained (Fig. 1A). In term placenta tissue, 14-3-3 tau protein was also strongly stained in cytotrophoblast which is scattered in the terminal villi (Fig. 1B). In addition, stromal cells were also frequently stained in first trimester and term placenta tissue.

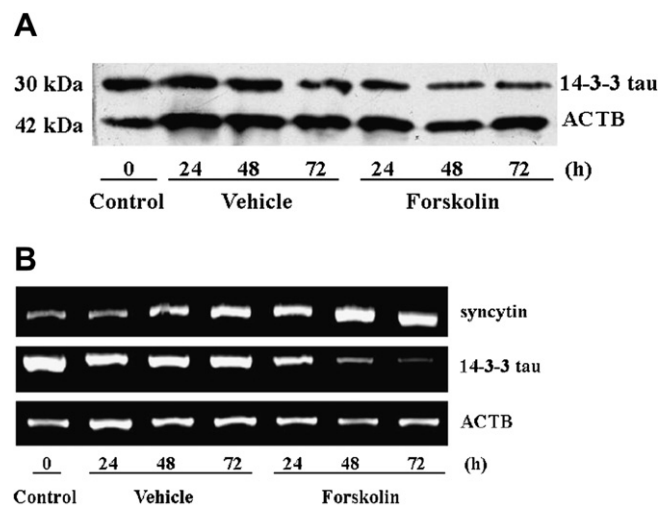
#### 3.2. Effects of forskolin-induced differentiation on 14-3-3 tau expression

Immunohistochemistry results revealed that differentiated syncytiotrophoblasts in human first trimester villi and term placenta tissue show very low level of 14-3-3 tau expression. Thus, we examined how 14-3-3 tau expression in normal BeWo cells changes during their forskolin-induced differentiation. Forskolin-induced cell fusion resulted in significantly increased syncytin transcript, but 14-3-3 tau was significantly reduced by about 63% and 93% of transcript level and 36% and 39% of protein level after forskolin treatment for 48–72 h (Fig. 2). Thus, endogenous 14-3-3 tau expression is down-regulated during trophoblast differentiation.

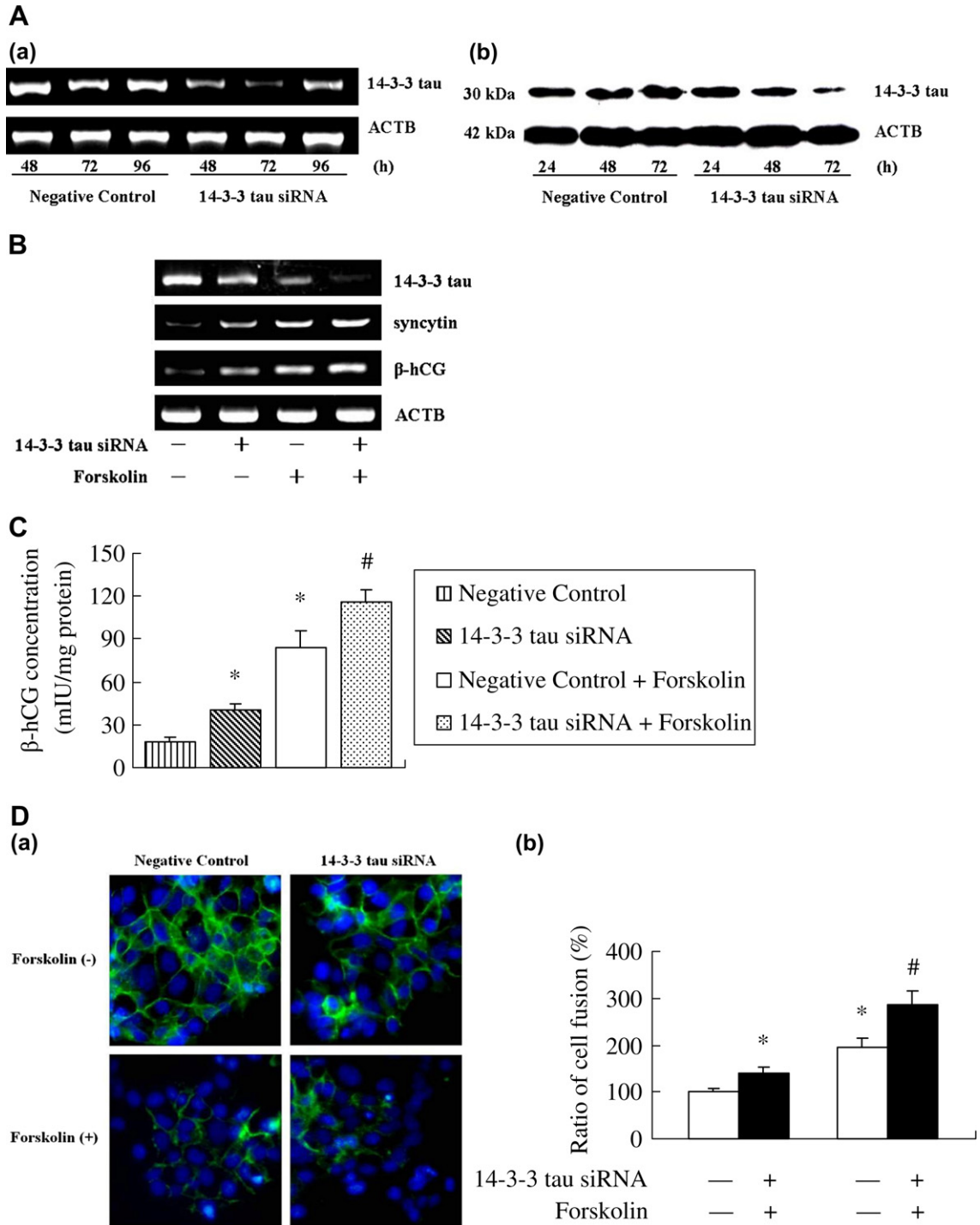
#### 3.3. Effects of 14-3-3 tau on forskolin-induced BeWo cell differentiation

To determine whether 14-3-3 tau regulates trophoblast differentiation, the effect of 14-3-3 tau knockdown and over expression

on forskolin-induced  $\beta$ -hCG and syncytin expression was evaluated in BeWo cells. siRNA transfection effect in BeWo cells is more than 95% (data not shown), we determined both the transcript and protein levels of 14-3-3 tau, and observed a knockdown about 80% at transcript levels and 65% at protein levels at 72 h after transfection (Fig. 3A). We observed that 14-3-3 tau siRNA treatment



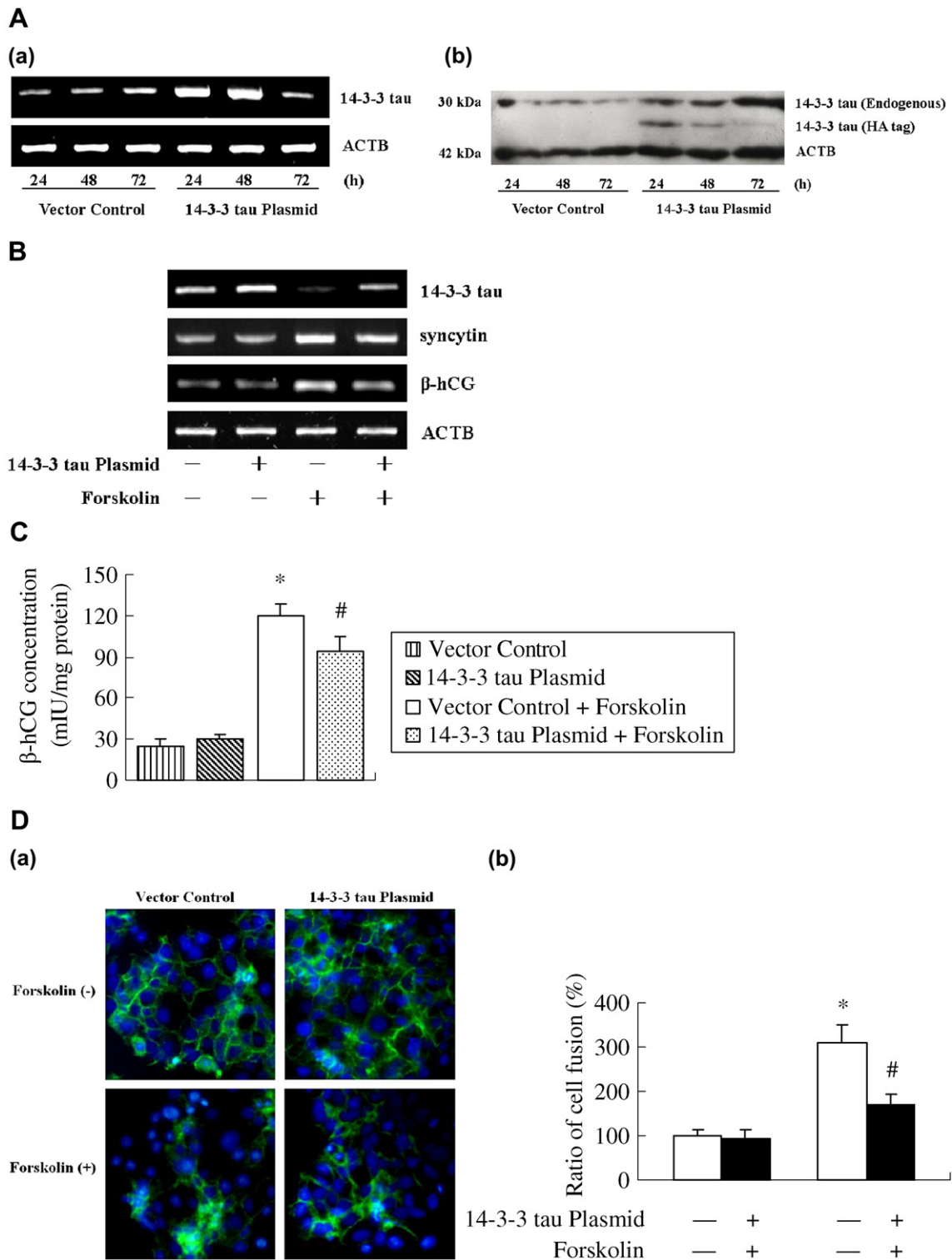
**Fig. 2.** Effects of forskolin-induced differentiation on 14-3-3 tau expression in BeWo cells. (A) Forskolin treated BeWo cells for 0, 24, 48 and 72 h were electrophoresed on 12% SDS-PAGE gel and probed with rabbit polyclonal antibody to 14-3-3 tau. (B) The relative abundance of syncytin transcript, 14-3-3 tau transcript were analyzed by RT-PCR.



**Fig. 3.** Effects of 14-3-3 tau knockdown on forskolin-induced differentiation of BeWo cells. BeWo cells transfected with negative control or 14-3-3 tau siRNA for 24 h and then cultured without or with forskolin (20  $\mu$ M) for 2 days. (A) Knockdown effect of 14-3-3 tau siRNA. a: RT-PCR. b: Western blot. 14-3-3 tau siRNA had a maximal inhibition effect on both transcript and protein level at 72 h after transfection. (B) Total RNA was subjected to semi-quantitative RT-PCR to determine 14-3-3 tau,  $\beta$ -hCG, syncytin and ACTB expression. ACTB served as an internal control. (C)  $\beta$ -hCG secretion in the culture media was detected using an enzyme immunoassay kit and normalized to culture media protein. \* $P < 0.05$ , vs. negative control without forskolin; # $P < 0.05$ , vs. negative control with forskolin. (D) a: BeWo cells were stained with DAPI (blue) and anti-E-cadherin protein (green) to indicate cell fusion. Representative pictures are shown. b: The number of multinuclear cells in five randomly selected areas was counted and is represented as ratios relative to the control [Negative control/forskolin (-)]. The data from three independent experiments are shown as mean  $\pm$  S.D. \* $P < 0.05$ , vs. negative control without forskolin; # $P < 0.05$ , vs. negative control with forskolin.

increased the expression of  $\beta$ -hCG and syncytin mRNA in the absence of forskolin. Forskolin elevated the levels of  $\beta$ -hCG and syncytin mRNA in control siRNA-treated BeWo cells. When 14-3-3 tau expression was knocked down, the forskolin-induced expression of  $\beta$ -hCG and syncytin mRNA was further elevated (Fig. 3B).

Immunoblot analysis of the conditioned media of BeWo cells confirmed that 14-3-3 tau siRNA stimulated their  $\beta$ -hCG secretion in the absence or presence of forskolin (Fig. 3C). Further, we examined the effect of 14-3-3 tau knockdown on forskolin-induced cell fusion of BeWo cells. Forskolin increased the number of



**Fig. 4.** Effect of 14-3-3 tau over expression on forskolin-induced differentiation of BeWo cells. BeWo cells transfected with vector control or 14-3-3 tau plasmid were cultured without or with forskolin (20  $\mu$ M) for 2 days. (A) Over expression effect of 14-3-3 tau plasmid. a: RT-PCR. b:Western blot. The HA tagged 14-3-3 tau and the endogenously expressed 14-3-3 tau bands could easily be distinguished, since the HA tagged protein migrated slightly slower than the endogenous protein. (B) Total RNA was subjected to semi-quantitative RT-PCR to determine 14-3-3 tau,  $\beta$ -hCG, syncytin and ACTB expression. ACTB served as an internal control. (C)  $\beta$ -hCG secretion in the culture media was detected using an enzyme immunoassay kit and normalized to culture media protein. \* $P < 0.05$ , vs. vector control without forskolin; # $P < 0.05$ , vs. vector control with forskolin. (D) a: BeWo cells were stained with DAPI (blue) and anti-E-cadherin protein (green) to indicate cell fusion. Representative pictures are shown. b: The number of multinuclear cells in five randomly selected areas was counted and is represented as ratios relative to the control [vector control/forskolin (-)]. The data from three independent experiments are shown as mean  $\pm$  S.D. \* $P < 0.05$ , vs. vector control without forskolin; # $P < 0.05$ , vs. vector control with forskolin.

multinuclear cells that showed cell fusion, furthermore in 14-3-3 tau siRNA-treated cells, forskolin-induced cell fusion was significantly increased (Fig. 3D).

To further confirm the role that 14-3-3 tau plays in BeWo cell differentiation, BeWo cells were transiently transfected with pcDNA\_HA-14-3-3 tau plasmid and then cultured with or without forskolin for 48 h. Fig. 4A showed the upregulation effect of 14-3-3 tau plasmid on BeWo cells. In the absence of forskolin treatment, 14-3-3 tau plasmid treatment did not affect  $\beta$ -hCG and syncytin expression (Fig. 4B). Forskolin treatment elevated  $\beta$ -hCG and syncytin expression in these cells, however, 14-3-3 tau plasmid suppressed forskolin-induced  $\beta$ -hCG and syncytin mRNA expression in BeWo cells (Fig. 4B and C). Fusion assay confirmed that 14-3-3 tau plasmid treatment reduced the forskolin-stimulated cell fusion compared with vector control (Fig. 4D).

#### 3.4. Effects of 14-3-3 tau on BeWo cell proliferation

BeWo cells showed a decrease in cell density after 72 h transfection of 14-3-3 tau siRNA, it was most evident at the 72 h time point after transfection, where the proliferation of BeWo cells was decreased by  $30.3 \pm 4.5\%$  (Fig. 5A). Expression of cell cycle promoter PCNA was decreased and cell cycle inhibitor p27kip1 was increased, the extracellular signal-regulated kinase (ERK) pathway was also activated (Fig. 5B).

## 4. Discussion

The differentiation of proliferating mononuclear cytotrophoblast into non-proliferative multinucleated but highly functional syncytiotrophoblast has not yet been clearly documented. In the present study, our results suggest that 14-3-3 tau may be an intracellular regulatory factor associated with the proliferation and differentiation of human trophoblastic cell line, BeWo.

14-3-3 tau was markedly expressed in cytotrophoblast cells (not syncytiotrophoblast cells) in placenta villus and decreased with

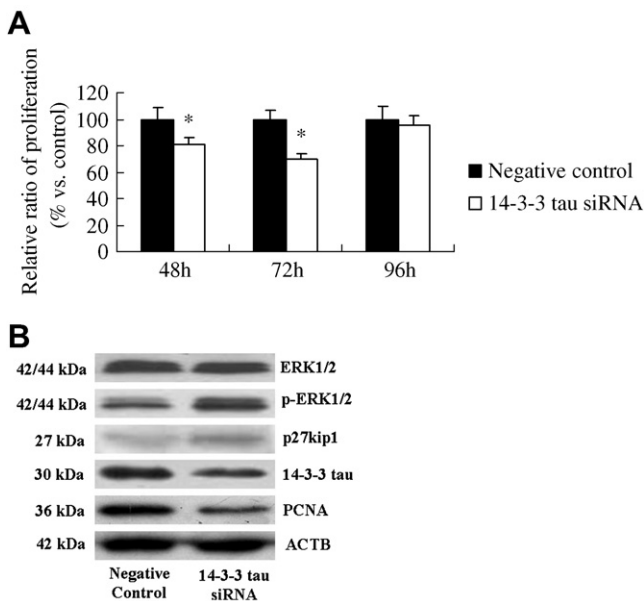
cytotrophoblast cells during gestation process. We then mimicked these observations in vitro through inducing BeWo cells differentiation by forskolin, 14-3-3 tau transcript and protein level were decreased following trophoblast cells fusion. Thus different status of 14-3-3 tau in the cytotrophoblast and syncytiotrophoblast suggests that 14-3-3 tau involved in cytotrophoblast cell proliferation and/or differentiation ability.

Another novel finding of this study is that 14-3-3 tau expression may suppress trophoblast differentiation. Differentiation markers,  $\beta$ -hCG and syncytin, were detected in BeWo cells. Considering  $\beta$ -hCG and syncytin expression are not necessarily directly linked or paralleled the degree of syncytialization [14,15], the loss of intracellular boundaries by using E-cadherin staining was also evaluated. Knocking down of 14-3-3 tau increased expression of the differentiation markers and induced cell fusion in BeWo cells, with or without forskolin treatment. On the other hand, over expression of 14-3-3 tau by a constructed plasmid decreased  $\beta$ -hCG and syncytin expression and also suppressed cell syncytialization in the presence of forskolin. We also noticed that in the absence of forskolin, mononucleated cells expressed a basal level of  $\beta$ -hCG and syncytin which couldn't be decreased by over expression of 14-3-3 tau. It suggested that 14-3-3 tau mostly played its role in the process of differentiation.

Although the mechanism by which 14-3-3 tau regulates trophoblast differentiation remains unknown, it is likely that 14-3-3 tau is involved in the preparation or initiation of trophoblast differentiation. This notion is based on our observation that trophoblast differentiation was significantly induced when 14-3-3 tau expression was knocked down by siRNA treatment prior to receiving the forskolin stimulus. In particular, it is likely that 14-3-3 tau-mediated modulation of cell cycle participates in trophoblast differentiation. Studies demonstrated that prevention of entry into S phase may allow trophoblasts to respond to signals that potentiate differentiation and regulation of G<sub>1</sub> phase cyclin, Cdk and Cdk inhibitors activity may be involved in the terminal differentiation process of cytotrophoblasts [16,17]. Thus, knocking down 14-3-3 tau may arrest the cell cycle and thereby induced trophoblast differentiation.

Arrest of proliferation is one of the prerequisites for differentiation of cytotrophoblasts into syncytiotrophoblasts. As a cell cycle regulator, 14-3-3 tau may function at several key points in G<sub>1</sub>/S- and G<sub>2</sub>/M-transition by binding to regulatory proteins and modulating their function. After downregulating 14-3-3 tau through transfection with siRNAs, there was a decrease in the expression of cell cycle promoter, PCNA, which is expressed late in the G<sub>1</sub> phase and early in the S phase of cell cycle [18]. Moreover, p27Kip1, a CDK inhibitor which mediates G<sub>1</sub>-arrest by inhibiting cyclin E-Cdk 2 complexes, were increased during 14-3-3 tau knockdown. Sekimoto et al. demonstrated that 14-3-3 tau suppresses p27kip1 activity by binding and sequestered it in the cytoplasm [19]. Whether p27Kip1 acts as a CDK inhibitor depends on its concentration, its distribution among different complexes and its localization. In present study, suppressed 14-3-3 tau expression may result in lower inhibition of p27kip1. These results suggest that knockdown 14-3-3 tau-induced inhibition of trophoblast proliferation is due to arrest in cell cycle from G<sub>1</sub> phase to S phase.

In addition, treatment with 14-3-3 tau siRNA activated ERK1/2 pathway. As a classical signal pathway, ERK activation mediates cell cycle arrest [20], and phosphorylation of p27Kip1 in vitro and in vivo [21]. In trophoblasts, ERK1/2 were suggested to play significant roles in initiating trophoblast differentiation and fusion. Specific inhibitors for ERK1/2 impaired differentiation and syncytialization in primary trophoblast cultures [22]. The evidence of interaction between 14-3-3 tau and ERK1/2 was limited, we supposed that 14-3-3 tau is likely a negative regulator of ERK1/2. 14-3-3 tau siRNA impaired the



**Fig. 5.** Effect of 14-3-3 tau on BeWo proliferation. BeWo were treated with negative control or 14-3-3 tau siRNA cultured for 48, 72 or 96 h. (A) Cell viability was evaluated by using the WST-1 assay. The data from three independent experiments are represented as ratios of the control levels and are mean  $\pm$  S.D. \* $P < 0.05$ , vs. negative control. (B) 14-3-3 tau, PCNA, p27kip1 and ERK1/2 protein level after 72 h treatment was analyzed by Western Blot. The results presented are from a representative experiment.

suppression effect of 14-3-3 tau on phospho-ERK1/2, which activated the pathway, and then resulted in BeWo cells arresting in G<sub>1</sub> phase.

In conclusion, we showed by using *in vitro* trophoblast models that 14-3-3 tau expression affects in the cytotrophoblast differentiation via cell cycle regulation. These multiple roles of 14-3-3 tau in trophoblasts suggest that 14-3-3 tau may be an essential regulator of placentation in early pregnancy and play a role in the development of differentiation-related gestational diseases, such as preeclampsia and abortion.

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