Inhibition of Akt/FOXO3a signaling by constitutively active FOXO3a suppresses growth of follicular thyroid carcinoma

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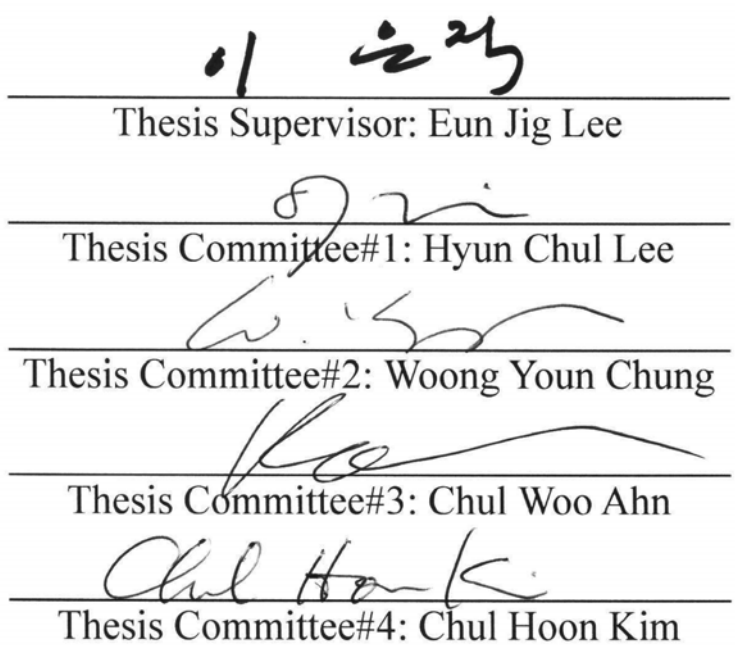
This certifies that the Doctoral Dissertation of ZhenYu Hong is approved.



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ABSTRACT

Inhibition of Akt/FOXO3a signaling by constitutively active FOXO3a suppresses growth of follicular thyroid carcinoma

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Akt-dependent FOXO3a cytoplasmic translocation is an important tumorigenic mechanism for escaping from apoptosis in cancer cells. In the present study, we examined whether non-phosphorylatable FOXO3a can inhibit cell growth of distinct follicular thyroid carcinoma(FTC) cell lines. Adenovirus carrying the FOXO3a-triple mutant (TM) sequence including point mutations at three Akt phosphorylation sites (Ad-FOXO3a-TM) was generated and transduced to the cells to mimic inhibition of Akt/FOXO3a signal. Transduction of Ad-FOXO3a-TM to FTC cells induced cell cycle arrest and apoptosis. Intratumor injection of Ad-FOXO3a-TM suppressed the growth of xenograft tumors induced in athymic mice by FTC133 cells. Consequently, our results indicate that gene therapy

based on Ad-FOXO3a-TM resulting in constitutively activated FOXO3a signaling has therapeutic potential for FTC.

Key words: FOXO3a, follicular thyroid cancer, gene therapy.

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Ⅰ. INTRODUCTION

Follicular carcinoma, the second most common type of thyroid tumor, accounts for approximately 15% of all thyroid malignancies. Although follicular thyroid carcinoma (FTC) is usually associated with an optimistic prognosis, effective management of invasive and metastatic FTC has yet to be achieved. The development of new therapeutics or anticancer drugs is essential to improve the treatment of patients with metastatic FTC that is not susceptible to chemotherapy and radiotherapy [1].

Akt activation appears to be the principal mediator of growth factor activation for thyroid cell growth and inhibition of apoptosis. Interestingly, overexpression of a constitutively active form of Akt in thyroid cells results in serum-independent growth[2,3]. Driven by genetic alterations, the PI3K/Akt pathway is frequently overactivated in human cancers, including thyroid

cancer[4-6]. Activated Akt phosphorylates a multitude of downstream targets, leading to changes in cellular function. An important down-stream target of Akt is FOXO3a, which plays a critical role in cellular proliferation and apoptosis[7].

FOXO3a is a member of the forkhead transcription factor family, which is emerging as a key factor in pathways that regulate the differentiation, metabolism, proliferation and survival of cells[8]. In response to growth factor stimulation, FOXO3a is inhibited by Akt-mediated phosphorylation. The phosphorylated FOXO3a leads to inactivation through translocation from the nucleus to the cytoplasm[9-14]. Previous studies have shown that the key actions of forkhead family transcription factors are cell cycle arrest[13-16] and apoptosis[6]. FOXO3a induces apoptosis through its localization to the nucleus and enhances subsequent transcription of several genes involved in the apoptotic pathway, such as BCL6, a Bcl-2-interacting mediator of cell death (BIM), and Fas ligand[17]. In addition, activated Foxo3a also induces cell cycle arrest via transcriptional activation of the $p27^{kip1}$ gene[16,18].

Under normal conditions, FOXO3a is constantly shuttled in and out of the nucleus, thereby contributing to the maintenance of homeostasis of the cell. The FOXO3a triple mutant (FOXO3a-TM) is not phosphorylatable and constitutively activated because three Akt phosphorylation sites, Thr32, Ser253, and Ser315, were replaced by alanine residues[13]. Previous studies have shown that PI3K-dependent FOXO3a inactivation may be an important mechanism by which thyroid cancer cells can avert apoptosis, particularly in

FTC[19]. Furthermore, a marked cytoplasmatic FOXO3a localization and active Akt was found in FTC tissues[19]. However, limited information is currently available on the role of FOXO3a in FTC cell biology.

The aim of this study was to examine whether the inhibition of Akt signaling by constitutively active FOXO3a could have potential for treatment of human FTC.

Ⅱ. MATERIALS AND METHODS

1. Cell line and reagents

Human thyroid cancer cell lines derived from primary thyroid cancer (FTC 133), lymph node metastases (FTC236) were cultured in DMEM and lung metastases (FTC 238) were cultured in DME/F-12. These media were all supplemented with 10% fetal bovine serum and 100 U/ml penicillin and streptomycin (Life Technologies, Invitrogen). The Akt1/2 kinase inhibitor was purchased from Sigma (Sigma, St. Louis, MO) and the cells were treated with the indicated doses.

2. Recombinant adenovirus

An E1/partial E3–deleted recombinant adenovirus was used for gene transfer in these experiments. Essentially, the FOXO3a-TM, driven by the human cytomegalovirus(CMV) immediate early promoter, was cloned into the E1 region of the adenovirus (Ad-FOXO3a-TM). Negative control viruses were created in a similar manner; we encoded bacterial -galactosidase (Ad-LacZ) in place of the FOXO3a-TM coding sequence. Deletion of the E1 region of adenovirus renders the virus replication deficient(Fig. 2A). Details of the construction of the recombinant adenovirus are published elsewhere[20]. Recombinant viruses were grown and propagated in the human embryonic kidney cell line 293 and purified using standard protocols[20].

3. Immunofluorescence staining

FTC133 cells were plated on the cover slips. When cells had grown to 20-30% confluence, they were cultured without serum for 24 hours. The cells were then fixed with methanol for 10 min and immersed in 1.0% Triton X-100 for 20 min and blocked with 5% goat serum for 30 min. The cover slips were incubated with phospho-Akt (#9270, Cell Signaling Technology, Danvers, MA, US) and FOXO3a (Sigma, St. Louis, MO) antibodys at 4 °C overnight. They were then exposed to biotinylated anti-rabbit IgG(H+L) and fluorescein streptavidin. After being stained with propium idodie (PI, Vector Laboratoies, Burlingame,CA), the cover slip was observed under a laser scanning confocal microscope (LSM700).

4. *In vitro* cell viability/MTS assay

Cell viability was measured by The Cell Titer 96 AQueous Non-Radioactive Cell Proliferation Assay Kit (Promega,Madison,WI) according to the manufacturer's instructions. Briefly, cells were plated in a 96-well plate (1x 10^3 per well, 100μl cell suspension per well) and cultured for 24 hours to allow for exponential cell growth. After reaching 20%~30% confluence, cells were then transduced with Ad-FOXO3a-TM and Ad-LacZ adenovirus under various concentrations (multiplicity of infection, MOI 2, 4, 8 and 16) for 1, 2, 3, 4, and 5 days respectively. Following the additions, 100μl of MST/PMS solution was added to each well and incubated for another 4 hours. The absorbance value of each well was read on an ELIZA at 490 nm.

5. Immunoblot analysis

Protein extraction and immunoblot analysis were done as described[21]. Briefly, after cells were treated with various reagents or adenovirus, FTC cells were washed with ice-cold PBS and lysed in cell lysis buffer [10mM Tris (Ph 7.7), 150mM NaCl, 7mM EDTA, 0.5% NP-40, 0.2mM PMSF (phenylmethylsulfonyl fluoride) and 0.5μg/ml leupeptin], the lysate was centrifuged at 14,000rpm for 30min at +4◦C and the supernatant was collected. The protein concentration was determined using the BCA™ Protein Assay Kit (Pierce, Rockford, IL, USA). The samples were then stored at −20◦C. Samples(20μg of protein/sample) were subjected to SDS/PAGE (10% polyacrylamide). The proteins were transferred onto nitrocellulose membrane(Schleicher & Schuell, Dassel, Germany) by semi-dry blotting. Western blot analysis was performed using specific antibodies to the indicated proteins. The secondary antibodies used were horseradish peroxidase-conjugated anti-mouse and anti-rabbit antibodies. The proteins were detected by enhanced chemiluminescence. For loading controls, membranes were washed with 1M Tris (pH 7.6), stripped for 20 min in 0.1Mglycin (pH 2.5) and subjected to anti-ß-actin antibody.). Bands were detected with Kodak (Rochester, NY) X-Omat film. The following antibodies were used in the experiments: FOXO3a (F2178), purchased from SIGMA; cyclin D1 (sc-718), β-actin (sc-47778), $p27^{kip1}$ (sc-1641) and p-FOXO3a (ser253) (sc-101683) from Santa Cruz Biotechnology; and cleaved caspase-3 (#9661), phospho-Akt (#9270), and Akt (#4691) from Cell Signaling

Technology (Danvers, MA, US).

6. Reverse transcription-polymerase chain reaction (RT-PCR) analysis

The total RNA was isolated from FTC133 cells, and RT-PCR was performed as indicated by Lee et al. with minor modifications[22]. The primers used for amplification were: GAPDH (forward primer: 5'-CCATGGAGAAGGCTGGGG -3', reverse primer: 5'-CAAAGTTGTCATGGATGACC-3'); BIM (forward primer: 5'-GCCCCTACCTCCCTACAGAC-3', reverse primer: 5'-ATGGTGGTGGCCATACAAAT-3'); TRAIL(forward primer: 5'-GGAACCCAAGGTGGGTAGAT-3', reverse primer: 5'-TCTCACCACACTGCAACCTC-3'); The cycling conditions were as follows: initial denaturation at 96 ℃ for 5 min, followed by 30 cycles at 94 ℃ for 30 s, 57 ℃ for 30 s, and 72℃ for 30 s.

7. Cell cycle analysis

FTC133 cells were transduced with 8pfu/cell of Ad-LacZ and Ad-FOXO3a-TM. Cells were trypsinized 48 hours later and fixed with 70% ethanol/PBS. Cell cycle analysis was carried out by staining DNA with PI in preparation for flow cytometry with the FACScan/CellFIT system (Becton-Dickinson, San Jose, CA, USA).

8. Terminal deoxynucleotidyl transferase-mediated dUTP nick-end

labeling (TUNEL) assay

FTC133 cells were seeded on cover glass, and then transduced with 8pfu/cell of Ad-LacZ and Ad-FOXO3a-TM for 48hours. Cells were fixed with 4% neutralized buffered formalin and permeabilized with 0.1% Triton X-100 in 0.1% sodium acetate for 2 min on ice, and then the cells were stained using an apoptosis in situ detection kit (Roche Molecular Biochemical, Indianapolis IN) according to the manufacture's protocol.

9. Treatment of tumor-bearing mice with adenoviral vectors *in vivo*

All studies involving the use of nude mice were approved by the Yonsei University Medical School animal care and use committee. FTC133 cells (1×10^7) were injected into the flank area of adult (7-week old) athymic male nude mice (Harlan-Sprague-Dawley, Indianapolis, IN). When tumors of about 5-7mm in diameter developed, 100 ul (1×10^8 PFUs) of Ad-FOXO3a-TM (n=5) and Ad-lacZ (n=5) were injected into the tumors for two successive days. The tumor was measured with calipers in three dimensions every two days for 15 days. Tumor size (mm^3) was calculated using the following formula: (πx length x width x depth)/6.

10. Statistical analysis

Statistical analysis was performed using SPSS 10.0 software (SPSS Inc, Chicago, IL, USA) and $p < 0.05$ was considered statistically significant. Data are presented as the mean ± SD.

Ⅲ. RESULTS

1. Constitutively active Akt signaling in FTC

To investigate the expression of Akt and localization of FOXO3a in FTC cells, immunofluorescence staining was performed. Phosphorylated Akt was detected in FTC133 cells although they had been cultured without serum for 24 hours while FOXO3a was localized in the cytoplasm (Fig.1A). The Western blot analysis also revealed the phosphorylated Akt and FOXO3a under the same conditions (Fig.1B). Under stimulation of 100 ng/ml EGF, the phosphorylation of Akt and FOXO3a was further increased at 30 min, 2 hours, and 24 hours. However, it was inhibited by the treatment of 10 μM or 20 μM of Akt inhibitor (Fig.1B). Similar results were observed in FTC236 and FTC238 cells(Fig.1C). These results demonstrate that the inhibition of FOXO3a is strictly correlated with phosphorylation of Akt.

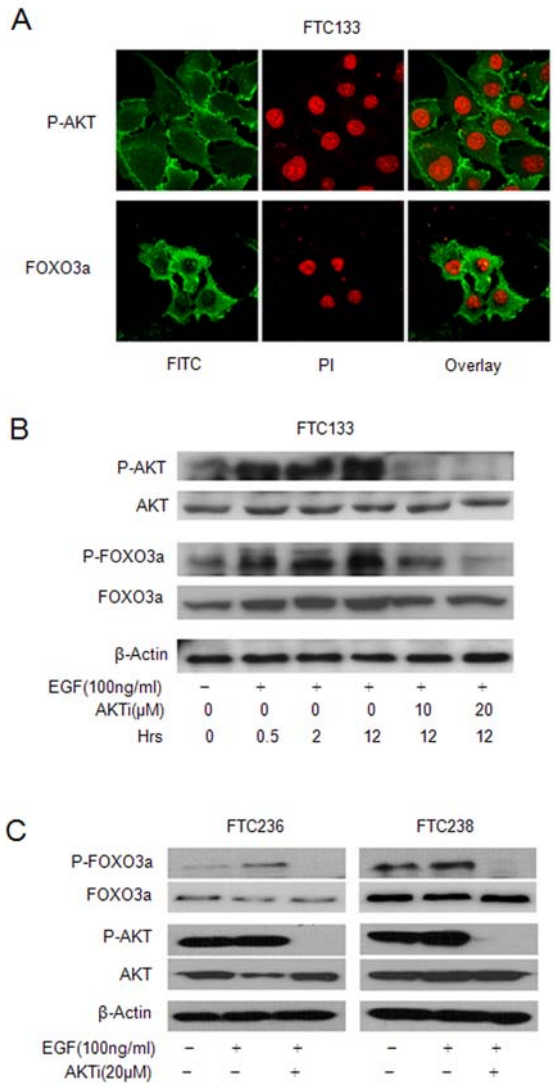

Figure 1. Akt/FOXO3a expression in FTC cell line. **(A)** FTC133 cells were cultured in the serum-starved condition for 24 hours and fixed to perform immunofluorescence staining and observed under a laser scanning confocal microscope, X200 magnification. **(B)** FTC133 cells were cultured in the serum free media for 24 hours and stimulated with EGF (100ng/ml) in the presence or absence of Akt1/2 inhibitor (10μM or 20μM). Akt1/2 inhibitor

was pretreated for 2 hours before EGF treatment. Duration of EGF treatment is indicated as hours at the bottom of panel. **(C)** FTC236 and FTC238 cells were cultured in the serum free media for 24 hours and stimulated with EGF (100ng/ml) for 12 hours in the presence or absence of 20μM Akt1/2 inhibitor. Akt1/2 inhibitor was pretreated for 2 hours before EGF treatment. Cell lysates were extracted and the Western blots were performed. Similar results were obtained in three independent experiments.

2. Constitutively active FOXO3a suppressed FTC cell growth

To determine whether inhibition of the Akt signaling pathway has any effect on cell growth of FTC, the constitutively active form of FOXO3a was transduced by adenovirus (Ad-FOXO3a-TM) (Fig. 2A). Cells were left untransduced or transduced with Ad-LacZ or Ad-FOXO3a-TM with various MOI. Untransduced cells or cells transduced with Ad-LacZ showed a similar increase in growth rate during the experimental period; whereas cells transduced with Ad-FOXO3a-TM exhibited a significant reduction in growth rate (Fig. 2B,2C).

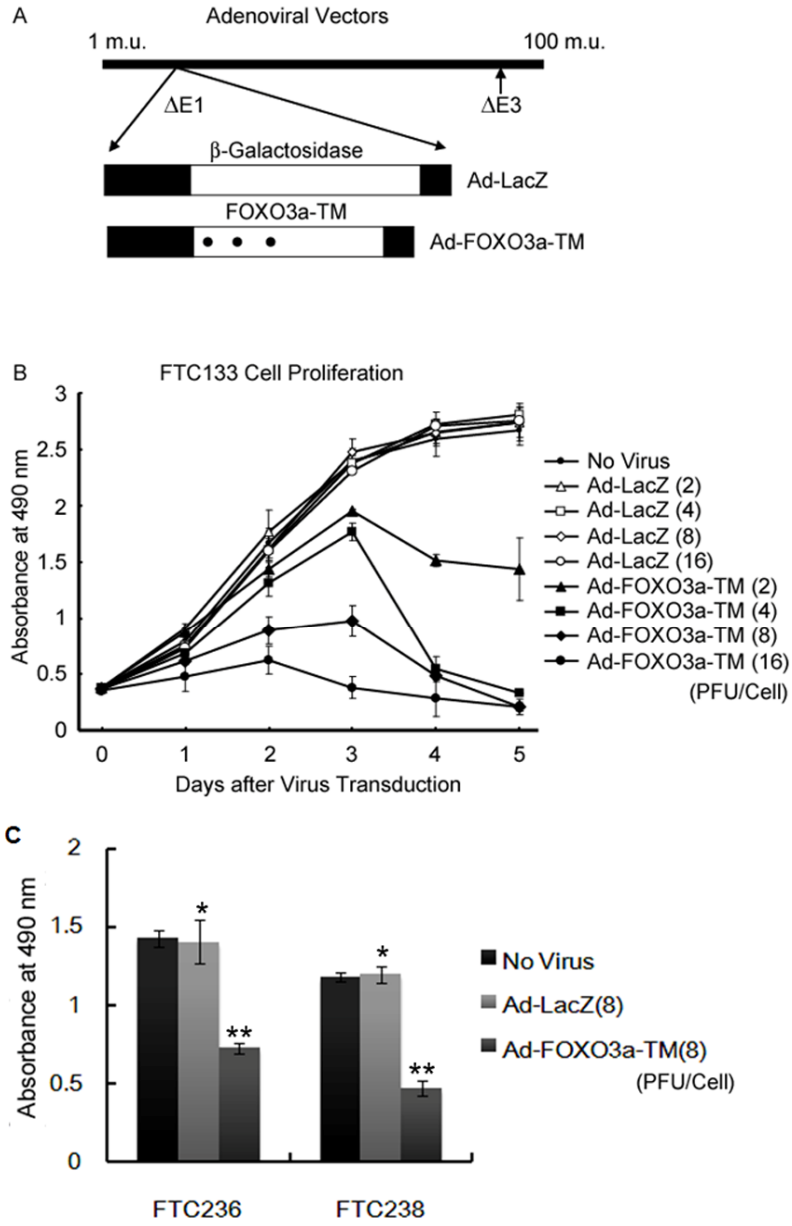

Figure 2. The effect of Ad-FOXO3a-TM on FTC cell growth. **(A)** Structure of recombinant adenoviral vectors. Adenoviral vectors carrying the β-galactosidase gene (Ad-LacZ) or FOXO3a-TM gene (Ad-DOXO3a-TM) are controlled by the CMV promoter. **(B)** Transduction of Ad-FOXO3a-TM gene

inhibits FTC133 cell growth. FTC133 cells were treated with normal medium, Ad-FOXO3a-TM and Ad-LacZ with various MOI in the range 2-16pfu/cell for indicated time. Cell proliferation was evaluated by MTS assay. (C) FTC236 and FTC238 cells were untreated or treated with Ad-FOXO3a-TM and Ad-LacZ with MOI of 8 pfu/cell for 4 days. Cell proliferation was evaluated by MTS assay. *$p>0.05$ versus no virus: **$p<0.01$ versus Ad-LacZ.

3. Constitutively active FOXO3a inhibited cell growth via cell cycle arrest

To examine the mechanisms of Ad-FOXO3a-TM induced growth suppression in FTC133 cells, flow cytometry for the cell cycle analysis and Western blot for the proteins involved in the cell cycle were performed. Ad-FOXO3a-TM transduction exhibited an increase in cell numbers in the G0/G1 phase and a decrease in the S phase compared with Ad-lacZ transduced or untransduced cells (Fig.3A). Ad-FOXO3a-TM transduction suppressed the expression of cyclin D1 and stimulated the expression level of $p27^{kip1}$, whereas these were not observed in Ad-LacZ-transduced or untransduced cells (Fig. 3B).

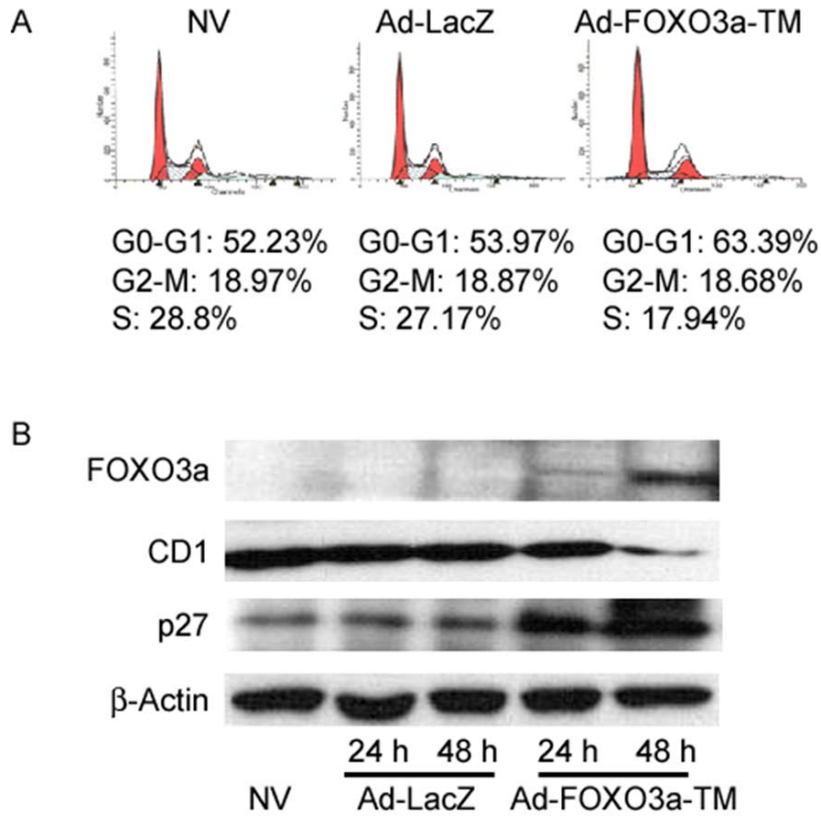

Figure 3. The effect of Ad-FOXO3a-TM on cell cycle. **(A)** Ad-FOXO3a-TM effects on cell cycle progression. Cell cycle analyses were done on untransduced FTC133 cells, cells transduced with Ad-LacZ or Ad-FOXO3a-TM with 8pfu/cell for 48 hours as described in Materials and Methods. **(B)** Western blot analysis of $p27^{kip1}$ and cyclin D1 in adenovirus-transduced FTC133. The cells were transduced with Ad-LacZ or Ad-FOXO3a-TM with 8pfu/cell and whole-cell lysates were prepared at 24 or 48 hours. Results are representative of three independent experiments.

4. Constitutively active FOXO3a induced apoptosis in FTC133 cells

To examine whether Ad-FOXO3a-TM-induced inhibition of cell growth is caused by apoptosis, a TUNEL assay was performed. The untransduced and Ad-LacZ transduced FTC133 cells did not show positive TUNEL staining; however, FTC133 cells transduced with Ad-FOXO3a-TM did show positive TUNEL staining (Fig.4A). RT-PCR analysis revealed that mRNA expression for BIM and TRAIL was higher in Ad-FOXO3a-TM transduced cells than in Ad-LacZ transduced or untransduced cells (Fig.4B). Casepase 3 activation was detected at 48 hours in Ad-FOXO3a-TM transducted cells but not in Ad-LacZ-transduced or untransduced cells (Fig.4C).

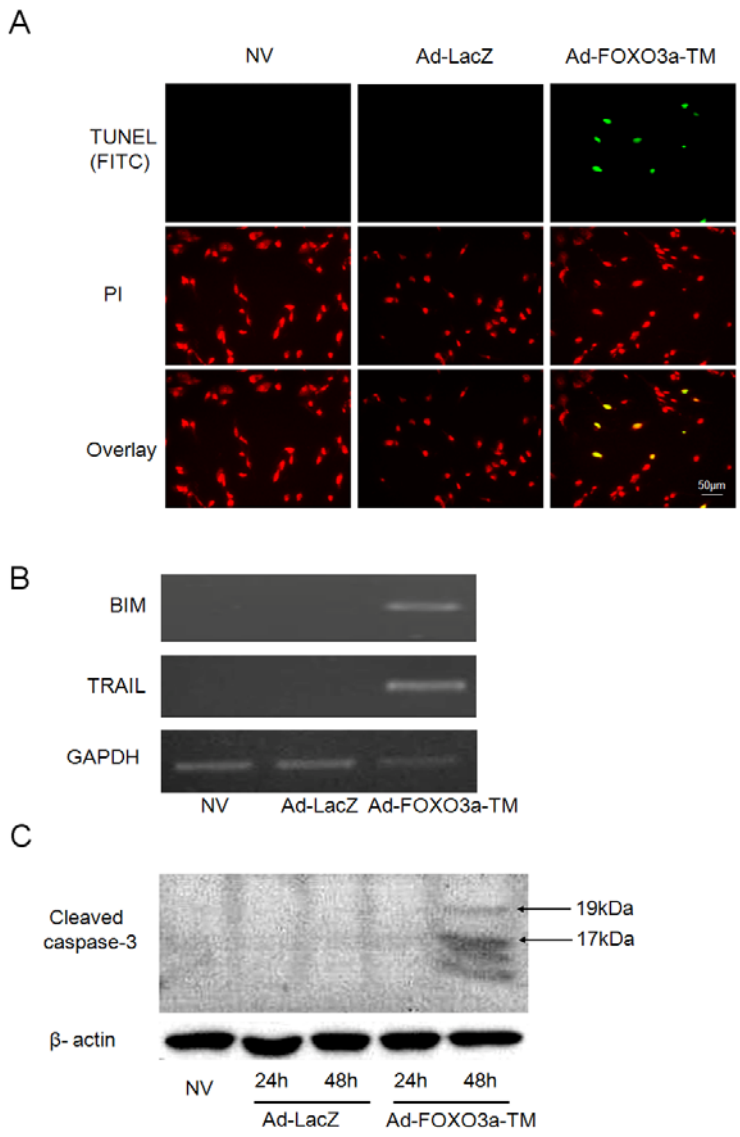

Figure 4. The effect of Ad-FOXO3a-TM on apoptosis. **(A)** TUNEL assay was performed to detect apoptosis of untransduced FTC133 cells, cells transduced with Ad-LacZ or Ad-FOXO3a-TM with 8pfu/cell for 48hours. **(B)** RT-PCR was performed with total mRNA of cells untransduced or cells transduced with Ad-FOXO3a-TM and Ad-LacZ with 8pfu/cell for 48hours. **(C)** Whole cell lysates were prepared from untransduced FTC133 cells, cells

were transduced with Ad-LacZ or Ad-FOXO3a-TM with 8 pfu/cell for 24 hours or 48 hours, respectively. Similar results were obtained in three independent experiments.

5. Constitutively active FOXO3a inhibited tumor growth in FTC xenografts

The effects of Ad-FOXO3a-TM on FTC133 tumor growth were evaluated using FTC xenografts in nude mice. When the established tumors reached 5–7 mm in diameter, adenovirus or PBS were injected into the tumors. As indicated in Fig. 5, there was no difference in average tumor volumes between the PBS treated group (tumor volume 1625.473 ± 437.7 mm^3) or Ad-LacZ treated group (1781.5 ± 949.8 mm^3) 15 days after the injection ($p>0.05$). In contrast, tumor volume in the Ad-FOXO3a-TM treated group (195.5 ± 232.3 mm^3) decreased significantly ($P < 0.05$), and one tumor completely disappeared.

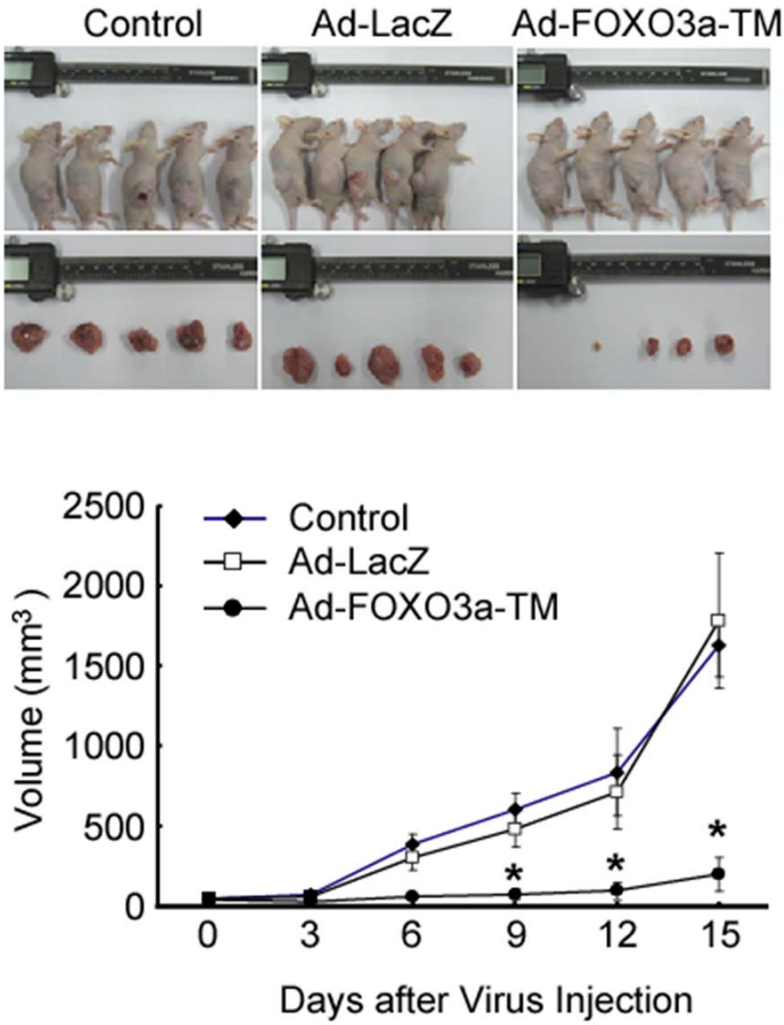

Figure 5. *In vivo* anti-tumor effect of Ad-FOXO3a-TM on FTC xenografts. FTC133 cells (1 x 10^7) were injected into the flank area of adult (7-week old) athymic male nude mice (Harlan-Sprague-Dawley, Indianapolis, IN). When tumors of about 5-7 mm in diameter developed, 100 ul PBS, 100 ul (1 x 10^8 PFUs) of Ad-FOXO3a-TM (n= 5) and Ad-LacZ (n=5) were injected into the tumors for two successive days, and tumor size was measured with calipers in three dimensions every three days. Tumor size (mm^3) was calculated using

the formula: (π x length x width x depth)/6. This experiment was repeated twice, and similar results were obtained in each trial.

Ⅳ. DISCUSSION

Overactivation of PI3K/Akt signaling is a hallmark of many human cancers. FOXOs have emerged as a critical effector arm of PI3K/Akt signaling. In this study we have demonstrated that inhibition of Akt/FOXO3a signaling by the usage of constitutively active FOXO3a, suppressed growth of FTC.

Genetic alterations found in thyroid follicular carcinomas include mutations of RAS genes, the PIK3CA gene, the PTEN gene and PAX8-PPARγ rearrangement[23]. Theoretically, all of these genetic alterations except for the PAX8-PPARγ rearrangement could possibly induce activation of Akt, resulting in the persistent inhibition of FOXO3a.

It was previously reported that immunohistochemical staining performed in human follicular thyroid cancer tissues displayed strong p-Akt staining intensity in the 65% of the investigated FTC. Most cases of thyroid carcinoma with cytoplasmatic FOXO3a expression simultaneously showed increased Akt phosphorylation. In contrast, p-Akt expression was very faint and present in less than 13% of follicular adenoma tissue and less than 5% of normal thyroid tissue. This was correlated with an exclusive nuclear FOXO3a localization in these tissues[19]. In agreement with this observation, we provided evidence that in the FTC cell lines, Akt is constitutively activated in a serum free condition and this activation could be enhanced by stimulation of the epidermal growth factor. Furthermore, overexpression of a

constitutively activated form of Akt 1 (myristoylated Akt) in thyroid cells results in serum-independent growth and leads to resistance to cell death, although it appears to be insufficient to transform the thyroid cells[32,33]. Therefore, Akt is critical in follicular thyroid cancer growth. In addition, we observed that the phosphorylation level of FOXO3a strictly correlated with the p-Akt level. Data from the application of Akt1/2 inhibitor indicated that Akt inhibition in FTC cells resulted in reduction of FOXO3a phosphorylation. Our findings agree with the generally accepted model of the Akt/FOXO3a pathway in which Akt plays an important upstream regulatory role in FOXO3a inhibition[9-11,13]. Taken together, driven by genetic alterations and stimulation of growth factors, akt is constitutively activated which resulted in persistent inhibition of FOXO3a in FTC.

Based on these findings, we hypothesized that inhibition of Akt/FOXO3a signaling might be a good alternative approach to achieve follicular thyroid cancer therapy. To date, there is no effective inhibitor of FOXO3a currently available. Therefore, in order to block Akt signaling, we chose an adenovirus carrying FOXO3a-TM as the gene delivery vehicle. As expected, inhibition of Akt signaling by the usage of the constitutively activated form of FOXO3a significantly suppressed FTC cell growth.

Growth factors trigger various events that promote the G0-G1 transition of the cell cycle; these events include up-regulation of cyclin D expression and $p27^{kip1}$ degradation, which result in consecutive activation of the cyclin D–CDK4 complexes and progression into the S phase. As

downstream signal pathway of growth factors, PI3K/Akt/FOXO3a was thus also implicated in cell cycle regulation[24]. Expression of a constitutively active form of FOXO that cannot be phosphorylated by Akt led to cell cycle arrest or apoptosis as a result of induction or suppression of gene expressions involved in cell cycle regulation[16,25-28]. Our data indicate that transduction of Ad-FOXO3a-TM to the FTC133 cell line induced cell cycle redistribution. Transducing Ad-FOXO3a-TM to FTC133 cells resulted in cell cycle arrest at the G0/G1 phase and prevented entry of cells to S phase. To further explore FOXO3a-induced cell cycle arrest, we investigated what kinds of cell cycle-related proteins were involved. We found that transduction of Ad-FOXO3a-TM induced up-regulation of $p27^{kip1}$ and down-regulation of cyclin D1 in FTC133 cells. This is in agreement with previous findings that FOXO directly activates the transcription of the $p27^{kip1}$ gene, whose promoter contains multiple consensus FOXO binding motifs[16,26]. Furthermore, it is well known that as a cyclin-dependent kinase inhibitor, $p27^{kip1}$ can inactivate cyclin D1. On the other hand, FOXO has been implicated in a $p27^{kip1}$-independent inhibition of the cell cycle based on the observation that conditional activation of FOXO leads to reduced expression of cyclin D1 and consequent cell cycle arrest even in the absence of $p27^{kip1}$ [29,30].

In addition to cell cycle arrest, we also found that apoptosis is another cause of FTC133 cell growth suppression induced by Ad-FOXO3a-TM. It has been reported that overexpression of FOXO family members in prostate cancer cell line resulted in apoptosis and increased the expression of the

death receptor ligands, TRAIL. FOXO recognition elements (FRE) of FOXO3 exist in the TRAIL promoter, indicating that TRAIL is a direct target of FOXO3[31]. In addition to the death receptor ligands, FOXO proteins have been shown to be involved in the transactivation of the Bcl-2 family. One member of the family, BIM, contains only a protein interaction motif known as the BH3 domain, which functions in the intrinsic, mitochondrial apoptotic pathway. Overexpression of FOXO transcription factors induces BIM expression and promotes death of sympathetic neurons in a BIM-dependent manner. It was also discovered that FOXO3a directly activates the BIM promoter via two conserved FOXO binding sites[17]. Similar to the prostate cell line or the sympathetic neurons, our data indicated that Ad-FOXO3a-TM can up-regulate mRNA expression level of TRAIL and BIM in FTC133 cells. The increase of their mRNA level might be induced by direct activation of their promoters by Ad-FOXO3a-TM. Caspase-3, which is a downstream target of TRAIL and BIM was also cleaved after treatment of Ad-FOXO3a-TM for 48 hours. Conclusively, overexpression of constitutively activated form of FOXO3a might induce cell death through upregulation of BIM and TRAIL, and these events likely lead to cleavage of caspase-3 to induce apoptosis in FTC 133 cells.

Because Ad-FOXO3a-TM transduction induced apoptosis as well as cell cycle arrest of FTC133 cells *in vitro*, we explored the effect of Ad-FOXO3a-TM on the tumor growth *in vivo*. We observed that Ad-FOXO3a-TM transduction by intratumoral injections was very effective in suppressing

FTC tumor growth. The results lend support to proposals that blocking Akt signaling with Ad-FOXO3a-TM has therapeutic potential.

Ⅴ. CONCLUSION

In conclusion, the inhibition of Akt/FOXO3a signaling by constitutively active FOXO3a suppressed FTC growth. Furthermore, these data suggest that gene therapy based on Ad-FOXO3a-TM has potential in the treatment of human FTC.

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Abstract(in korean)

지속적으로 활성화된 FOXO3로 Akt/FOXO3a 신호 전달 억제시 유도되는 갑상선 난포암 성장 억제 효과

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홍진우

Akt에 의한 FOXO3a의 세포질로의 전위는 암세포가 apoptosis를 회피하는 중요한 암 발생 기전으로 알려져 있다. 본 연구에서, 인산화가 안되는 FOXO3a가 다양한 갑상선난포암 세포주에서 세포성장에 미치는 영향을 확인 하였다. 유전자 조작을 통해 FOXO3a gene에서 세개의 Akt 인산화 site가 point mutation 된 FOXO3a-triple mutant(TM)유전자를 제작하였다. 이를 포함하는 아데노바이러스(Ad-FOXO3a-TM)를 제작하고 세포에 주입하여 Akt/FOXO3a 신호전달 억제효과를 유도하였다. Ad-FOXO3a-TM를 갑상선난포암세포에 전달한 결과 세포주기의 휴면과 apoptosis가 유발 되였다. Athymic mice에 FTC133 세포주를 이종 이식하여 생성된 암 조직에 Ad-FOXO3a-TM를 주입한 결과 암의 성장이 억제 되었다. 본 연구 결과는 추후 갑상선난포암 치료에 Ad-FOXO3a-TM을 이용한 유전자 치료가 유용할 수 있음을 제시한다.

핵심되는 말: FOXO3a, 갑상선난포암, 유전자치료법.