BBA - Molecular Basis of Disease 1865 (2019) 2464–2474

Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

journal homepage: www.elsevier.com/locate/bbadis

# Human steroid sulfatase enhances aerobic glycolysis through induction of HIF1α and glycolytic enzymes

Sangyun Shin[a], Yeo-Jung Kwon[a], Dong-Jin Ye[a], Hyoung-Seok Baek[a], Tae-Uk Kwon[a], Donghak Kim[b], Young-Jin Chun[a,*]

[a] College of Pharmacy, Chung-Ang University, Seoul, Republic of Korea
[b] Department of Biological Sciences, Konkuk University, Seoul, Republic of Korea

ARTICLE INFO

*Keywords:*
Steroid sulfatase
Dehydroepiandrosterone
Aerobic glycolysis
Hypoxia inducing factor 1α
Hexokinase 2
Pyruvate kinase M2

ABSTRACT

Human steroid sulfatase (STS) has been linked with poor prognosis in steroid-associated tumors and represents an important clinical target in cancers, yet the mechanism of STS-induced carcinogenesis remains unclear. To correlate STS with cancer metabolism, we determined the effects of STS on aerobic glycolysis. STS overexpression increased cellular levels of lactic acid, the final product of aerobic glycolysis. Moreover, STS suppressed the oxygen consumption rate (OCR), which represents mitochondrial respiration. Inhibition of STS by the specific inhibitor STX064 recovered STS-induced OCR repression and lactic acid over-production. DHEA, but not DHEA-S, suppressed the OCR level and enhanced lactic acid production. To understand the molecular mechanism of STS-induced cancer metabolism, we measured the expression of glycolytic enzymes hexokinase 2 (HK2) and pyruvate kinase M2 (PKM2), which was highly upregulated by STS and DHEA at both protein and mRNA levels. HIF1α is a key mediator of aerobic glycolysis, and STS enhanced HIF1α promoter activity, mRNA expression, and protein expression. Down-regulation of HIF1α by siRNA suppressed the HK2 and PKM2 expression induced by both STS and DHEA. HIF1α siRNA also recovered the OCR repression and lactic acid over-production induced by both STS and DHEA. To explore the mechanism *in vivo*, we produced transgenic mice overexpressing STS and found that STS expression was particularly enhanced in the lung. Consistent with our *in vitro* results, the expression of HIF1α, HK2, and PKM2 was also increased in mouse lung tissues. In conclusion, we suggest that STS may induce aerobic glycolysis through enhancing HIF1α expression.

## 1. Introduction

High expression of the steroid sulfatase (STS) enzyme has been correlated with poor prognosis in patients with steroid-associated tumors such as breast, cervix, and prostate cancers [1–4]. As STS is expected to be one of the major potential clinical targets in estrogen-mediated carcinogenesis, potent STS inhibitors have been developed and studied in patients with metastatic breast cancer [5–9]. Although STS activity is increased in malignant diseases, the molecular mechanisms of STS-mediated carcinogenesis remain unclear.

STS is known to mediate the production of biologically active hormones in cancers [4,6]. STS hydrolyzes the steroidal substrates, estrone sulfate and dehydroepiandrosterone sulfate (DHEA-S), to their unconjugated forms, estrone and dehydroepiandrosterone (DHEA), respectively [10]. DHEA and estrone are precursors for the biosynthesis of estrogens and androgens, which are known to stimulate certain tumor types [11]. Numerous clinical and experimental studies have demonstrated the association of DHEA with cancer among other diseases [12–14].

Because of the limitations of mitochondrial respiration, cancer cells often need to alter their metabolism to produce sufficient energy for their survival and proliferation [15–17]. Although normal cells catabolize glucose by mitochondrial oxidative phosphorylation, cancer cells use aerobic glycolysis to generate ATP by converting glucose to lactic acid [18–20]. Various studies have measured increases in lactic acid production and oxygen consumption rate (OCR) suppression as measures of aerobic glycolysis induction in cancer cells [21].

The glycolysis pathway includes many proteins known for their

*Abbreviations:* STS, steroid sulfatase; DHEA, dehydroepiandrosterone; HIF1α, hypoxia inducing factor 1α; HK2, hexokinase 2; PKM2, pyruvate kinase M2; OCR, oxygen consumption rate; GLUT-1, glucose transporter 1; VEGF, vascular endothelial growth factor; hCG, human chorionic gonadotropin; PMSG, pregnant mare's serum gonadotropin; MEM, minimum essential medium; PMSF, phenylmethylsulfonyl fluoride; dNTPs, deoxynucleoside triphosphate

* Corresponding author at: College of Pharmacy, Chung-Ang University, 84 Heukseok-Ro, Dongjak-Gu, Seoul 06974, Republic of Korea.
*E-mail address:* yjchun@cau.ac.kr (Y.-J. Chun).

https://doi.org/10.1016/j.bbadis.2019.06.005
Received 20 February 2019; Received in revised form 10 May 2019; Accepted 5 June 2019
Available online 11 June 2019

pivotal roles in cancer. Although most of the glycolytic enzymes participate in both glycolysis and gluconeogenesis, only a few are specifically involved in glycolysis [22]. Pivotal mediators of aerobic glycolysis include hexokinase 2 (HK2) and pyruvate kinase isotype M2 (PKM2), both of which are highly expressed in malignant tumors. Crucially, enhanced expression of both HK2 and PKM2 is related to poor prognosis of cancer patients [23–25].

Hypoxia inducing factor 1 (HIF1) is a heterodimeric basic helix-loop-helix structure composed of HIF1α and the nuclear translocator HIF1β [26,27]. HIF1 expression is regulated by the surrounding microenvironment and is particularly correlated with cancer progression [28,29]. HIF1α is recognized as a master regulator of the hypoxic response, activating many genes crucial for adaptation to hypoxia [30]. Many studies have shown that enhanced HIF1α expression is highly associated with cancer initiation [31–34]. As a key mediator of glycolytic signaling, HIF1α plays an important role in carcinogenesis through regulation of HK2 and PKM2 [35].

In this study, we investigated the effect of STS and DHEA on aerobic glycolysis to elucidate how STS regulates cancer cell metabolism in human cervical cancer cells.

## 2. Materials and method

### 2.1. Materials

Rabbit polyclonal antibody for STS was purchased from Abcam (Cambridge, UK). Antibodies against HIF1α, HK-2, PKM2, GLUT-1, VEGF and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibody against phospho-PKM2 (pY105) was from Cell Signaling (Danvers, MA, USA). Enhanced chemiluminescence detection reagents were obtained from Thermo Scientific (Rockford, IL, USA). DHEA and DHEA-S were purchased from Sigma-Aldrich (St. Louis, MO, USA). The lactate assay kit was purchased from BioVision (San Francisco, CA, USA). The Dual-Luciferase® Reporter (DLR™) assay, M-MLV reverse transcriptase, and RNase inhibitor were obtained from Promega (Madison, WI, USA). Fetal bovine serum (FBS) and RPMI 1640 medium were obtained from HyClone (Logan, UT, USA). The Neon transfection system was purchased from Invitrogen (Carlsbad, CA, USA). Ex *Taq* polymerase was obtained from TaKaRa Bio (Shiga, Japan). SYBR green was purchased from Qiagen (Hilden, Germany). Other chemicals and reagents were of the highest quality commercially available.

### 2.2. Cell culture

Human cervical cancer HeLa cells were obtained from the Korean Cell Line Bank (Seoul, Korea). Cells were cultured in MEM supplemented with 10% (v/v) heat-inactivated FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were maintained at 37 °C in a humidified atmosphere with 5% $CO_2$. After incubation, the cells were harvested by scrapping and were solubilized in 50 mM Tris-HCl (pH 8.0) containing 150 mM NaCl, 1% nonidet P-40, 1 mM PMSF, 1 μg/ml aprotinin, and 1 μg/ml leupeptin. Cells ($1 \times 10^6$) were maintained at 37 °C in a humidified atmosphere with 5% $CO_2$. After 24 h, the media was changed to MEM supplemented with 10% (v/v) charcoal-stripped FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin.

### 2.3. Transient transfection of plasmid DNA and siRNA

Cells were seeded at a density of $1 \times 10^6$ cells per 100 mm-dish. The Neon™ transfection system (Invitrogen) was used to transfect cells with 8 μg of plasmid DNA or 37.5 nM of siRNA, according to the manufacturer's instructions. Transfected cells were cultured in 100 mm dishes with antibiotic-free MEM for 24 h.

### 2.4. Western blotting

Whole cells were harvested by scraping and lysed in 50 mM Tris-HCl (pH 8.0) containing 150 mM NaCl, 1% nonidet P-40, 1 mM PMSF, 1 μg/ml aprotinin, and 1 μg/ml leupeptin for 30 min followed by centrifugation at 22,000*g* for 15 min at 4 °C. Protein concentration was measured using BCA Protein Assay Reagents (Thermo). Extracted proteins (20 μg) were separated by SDS-PAGE on 10–12% polyacrylamide gels and electrophoretically transferred onto PVDF membranes. Membranes were blocked with 5% nonfat milk in Tris-buffered saline containing 0.1% Tween-20 for 1 h at 4 °C, and then incubated overnight with specific antibodies. The next day, membranes were incubated with secondary antibodies for 2 h, and then proteins were visualized using enhanced chemiluminescence reagents (Thermo). Quantitative data were obtained using Quantity One software (Bio-Rad, Rockford, IL, USA).

### 2.5. Quantitative PCR

Total RNA was extracted using Ribospin™ (GeneALL, Seoul, Korea), according to the manufacturer's instructions. Total RNA (500 ng) was reverse transcribed at 37 °C for 1 h in a 20 μl total volume containing 5× RT buffer, 10 mM dNTPs, 40 U RNase inhibitor, 200 U Moloney murine leukemia virus reverse transcriptase, and 100 pmol oligo-dT primer. Quantitative PCR (qPCR) was performed using the Qiagen Rotor-Gene SYBR® PCR Kit (Hiden, Germany), as recommended by the manufacturer, followed by analysis using Qiagen Rotor-Gene Q Series software. Each reaction contained 12.5 μl of 2× SYBR® Green PCR Master Mix, 1 μM oligonucleotide primers, and 2 μl cDNA in a final volume of 25 μl. Amplification was conducted as follows: one cycle at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 5 s and annealing/extension at 60 °C for 10 s. Primer sequences are listed in Supplementary Table 1.

### 2.6. Luciferase reporter assay

Cells ($1.5 \times 10^4$/well) were transfected with GLUT-1 or HIF1α reporter plasmids using the Neon™ transfection system (Invitrogen) according to the manufacturer's protocol. pRL-renilla (Promega, Madison, WI, USA) was co-transfected as a control. After 24 h, cells were lysed using lysis buffer and luciferase activities were measured with FilterMax F3 (Molecular Devices, USA) using the Dual-Luciferase® Reporter system (Promega).

### 2.7. Lactic acid production

Cells ($1 \times 10^4$/well) were seeded into 96-well plates in glucose-free MEM medium and incubated at 37 °C. After 24 h, the media was collected and diluted 1:100 in lactate assay buffer. The amount of lactate present in the media was then estimated using the lactate assay kit (BioVision) according to the manufacturer's instructions. Absorbance was measured at 450 nm using a Tecan Sunrise microplate reader (Groedig, Austria). The amount of lactate produced by the cells was calculated by subtracting the amount of lactate in cell-free media from the amount of lactate in the media of each sample.

### 2.8. Oxygen consumption rate

Oxygen consumption rate was measured using the Extracellular Flux (XF) analyzer (Seahorse Bioscience, MA, USA), according to the manufacturer's instructions. Cells ($2 \times 10^4$/well) were incubated in MEM medium (pH 7.4) supplemented with glucose (5.5 mM) and pyruvate (1 mM). After taking basal measurements, mitochondrial respiration inhibitor, including oligomycin (2 μg/ml), rotenone (2 μM), and carboxylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP, 0.375 μM), was auto-injected into the experimental wells, followed by another

three measurement cycles. Each average basal or post-oligomycin respiration rate was measured after the readings achieved a steady state.

### 2.9. Generation of STS transgenic mouse

A portion of the human STS gene (promoter and entire 5′-untranslated region) was synthesized and cloned into the $Amp^R$ site of pcDNA3.1 vector (Invitrogen) to generate plasmid of STS. STS DNA for microinjection is a 7088-bp fragment containing CMV promoter. *Pvu*I and *Avr*II enzymes, which is each located at the beginning and outside part of the promoter, were used for restriction enzyme digestion to secure the fragment. 4.4-kb backbone fragment was deleted through gel-extraction, and 2.2-kb fragment was used. STS transgenic mice were generated, interbred and maintained in pathogen-free condition at Macrogen, Inc. (Seoul, Korea). All manipulations were conducted with the Macrogen Institutional Animal Care and Use Committee approval. Briefly, PMSG and hCG were injected to C57BL/6N female mice for superovulation. PMSG (7.5 IU) and hCG (5 IU) were i.p. injected 48 h interval to the female mouse of 5–8 weeks. After hCG injection, these female mice were mated with C57BL/6N stud male mice. Next day, virginal plug checked female mice were sacrificed and harvested fertilized embryo. STS DNA was microinjected into a cell embryo. For microinjection, 4 ng/μl of DNA was directly injected into the male pronucleus of zygote by using micromanipulator. Microinjected embryos were then incubated at 37 °C during 1–2 h. Fourteen to sixteen embryos injected at one-cell stage were transplanted into oviducts of the pseudopregnant recipient female mice. After F0 were born, genotyping test using tail cut samples to confirm the presence of STS gene were performed by PCR analysis (forward primer for CMV promoter; 5′-GTGG ATAGCGGTTTGACTCAC-3′, reverse primer for human STS; 5′-GTCTT GCTGTGACAGCTCATC-3′).

### 2.10. Immunohistochemistry

Mouse lung tissues were fixed in 4% paraformaldehyde in phosphate buffed saline, then embedded in paraffin and sectioned. For immunohistochemistry, sections were deparaffinized and incubated for 10 min with 3% $H_2O_2$. Slides were blocked with 5% nonfat milk in Tris-buffered saline and incubated with the relevant primary antibodies (STS and HIF1α) overnight at 4 °C. Secondary antibody (1:200) was applied to sections for 1 h at 20 °C.

### 2.11. Statistical analysis

Statistical analysis was performed using Graph-Pad Prism software (San Diego, CA, USA). One-way analysis of variance was performed, followed by Dunnett's multiple comparison *t*-test when appropriate. Differences were considered statistically significant at $p < 0.05$.

## 3. Results

### 3.1. Steroid sulfatase modulates cell metabolism in HeLa cells

To determine whether STS expression induces aerobic glycolysis, we measured both the production of lactic acid and the oxygen consumption rate (OCR) in HeLa cells. STS was overexpressed by transfection with pcDNA3.1-STS or inhibited using the specific STS inhibitor STX064 (2 μM) [36,37]. Overexpression of STS caused a significant increase in lactate level compared to control cells, and treatment with STX064 inhibited STS-induced lactic acid production (Fig. 1A). Moreover, the OCR level was significantly reduced in STS-overexpressing cells. In addition, STX064 treatment elevated STS-reduced OCR levels (Fig. 1B). Treatment with DHEA (100 nM), a major product of STS activity, also enhanced lactic acid production in HeLa cells. However, treatment with DHEA-S caused no significant change in lactic acid production compared to control cells (Fig. 1C). Furthermore, DHEA-S had no effect on mitochondrial respiration, whereas DHEA significantly suppressed the OCR level (Fig. 1D). These results suggest that STS may promote lactic acid production *via* induction of aerobic glycolysis and prevention of mitochondrial respiration in HeLa cells.

### 3.2. STS and its major product DHEA induce expression of glycolytic enzymes

To investigate how STS modulates aerobic glycolysis, we measured the mRNA and protein levels of glycolytic enzymes using qPCR and western blotting, respectively. Overexpression of STS increased HK2 and PKM2 expression at both the mRNA and protein levels (Fig. 2A and B). Treatment with DHEA (0, 25, 50, and 100 nM) also strongly induced HK2 and PKM2 expression at both the mRNA and protein levels (Fig. 2C and D). These results demonstrate that STS and DHEA may activate aerobic glycolysis through induction of glycolysis-related factors such as HK2 and PKM2.

### 3.3. Inhibition of STS prevents induction of glycolytic enzymes

To further examine the potential role of STS in inducing aerobic glycolysis, cells were treated with STX064. STX064 treatment significantly suppressed STS-induced HK2 and PKM2 mRNA levels, as shown by qRT-PCR (Fig. 3A). The enhanced protein levels of glycolytic enzymes induced by STS overexpression were also reduced by STX064 treatment (Fig. 3B). These results show that STX064 strongly suppresses STS-induced aerobic glycolysis.

### 3.4. STS and DHEA induce HIF1α expression

To identify the mechanism by which STS influences glycolytic enzyme expression, we assessed the mRNA and protein levels of HIF1α, an important mediator of glycolysis. STS overexpression strongly induced mRNA and protein levels of HIF1α (Fig. 4A and B). In addition, a higher level of HIF1α promoter activity was observed in STS-transfected cells than in control cells, and this increase was suppressed by STX064 treatment (Fig. 4C). Moreover, induction of HIF1α mRNA in STS overexpressing cells was decreased by transfection with STS siRNA (Supplementary Fig. 1). DHEA also increased the promoter activity and mRNA level of HIF1α, whereas DHEA-S had no effect (Fig. 4D and E). Following treatment with DHEA (0, 25, 50, or 100 nM), HIF1α mRNA and protein expression were increased in concentration-dependent manners (Fig. 4F and G). DHEA also induced HIF1α mRNA and protein expression in a time-dependent manner (Fig. 4H and I). We next investigated whether STS induces downstream targets of HIF1α, such as vascular endothelial growth factor (VEGF) and glucose transporter 1 (GLUT-1). Previous reports have indicated that the expression of VEGF and GLUT-1 is transcriptionally increased by HIF1α induction [38,39], and we found that STS enhanced VEGF and GLUT-1 expression at both the mRNA and protein levels (Fig. 5A and B). The promoter activity of GLUT-1 was also increased by STS (Fig. 5C). Furthermore, DHEA, but not DHEA-S, induced VEGF and GLUT-1 mRNA expression (Fig. 5D). These results suggest that STS increases HIF1α mRNA and protein expression through promoter activation.

### 3.5. HIF1α is required for STS-induced aerobic glycolysis

To further explore the molecular mechanism for STS-induced aerobic glycolysis, we used siRNA to knock down HIF1α expression. After co-transfection with the STS expression vector and HIF1α siRNA, the expression of glycolytic enzymes was assessed by qRT-PCR and western blotting. As seen previously, both the mRNA and protein level of HK2 and PKM2 were significantly increased in STS-overexpressed cells. However, little change in glycolytic enzyme expression was observed in cells co-transfected with the STS expression vector and HIF1α siRNA (37.5 nM) compared to control cells (Fig. 6A and B). We also co-

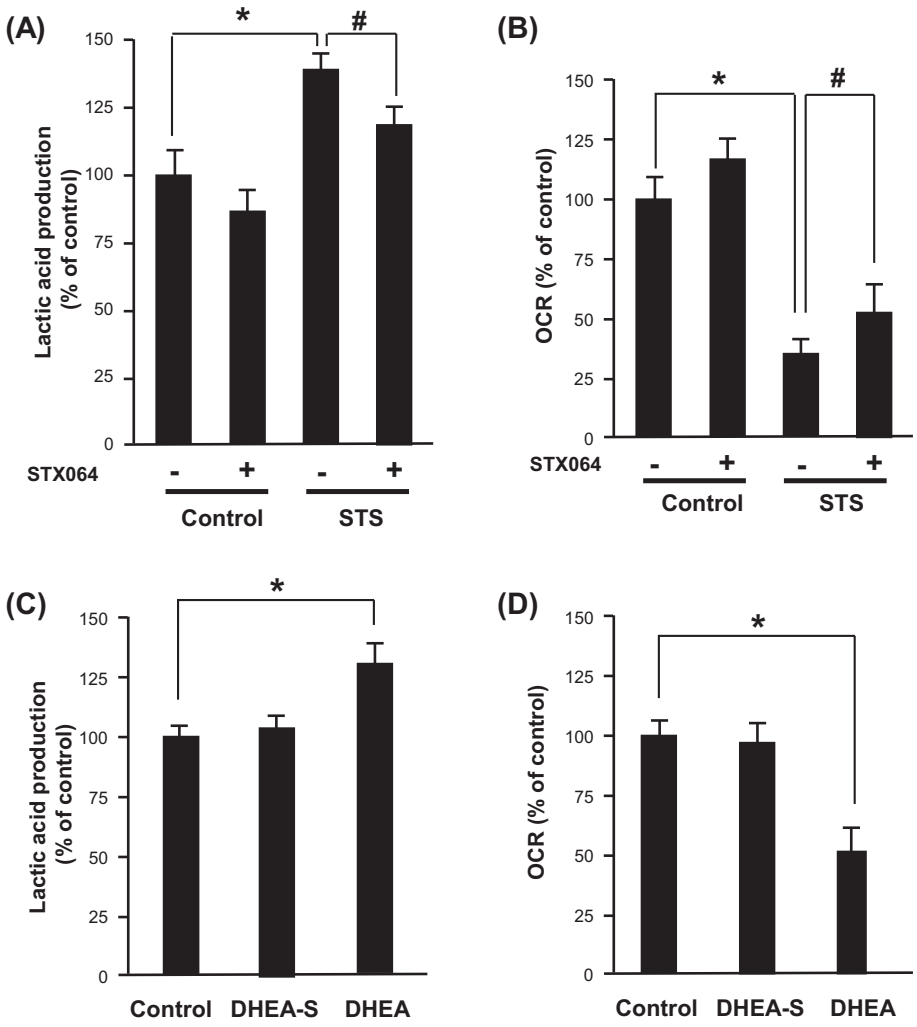


**Fig. 1.** Steroid sulfatase modulates cell metabolism in HeLa cells.
(A) Lactic acid production assay. HeLa cells were transfected with pcDNA3.1-STS and then treated with STX064 (2 μM) for 24 h. Lactic acid concentration was measured using a microplate reader at 450 nm. (B) Measurement of oxygen consumption rate. Cells were transfected with pcDNA3.1-STS and then treated with STX064 (2 μM) for 24 h. Oxygen consumption rate was measured using XF analyzer. (C) Lactic acid production assay. DHEA-S (100 nM) or DHEA (100 nM) were treated in HeLa cells. Lactic acid concentration was measured using the microplate reader at 450 nm. (D) Measurement of oxygen consumption rate. DHEA-S (100 nM) or DHEA (100 nM) were treated in HeLa cells. Oxygen consumption rate was measured using the XF analyzer. Values are shown as means ± S.D. (n = 3). $^{*}p < 0.05$ compared with control cells. $^{\#}p < 0.05$ compared to STS-overexpressed cells.

treated cells with DHEA and HIF1α siRNA to examine whether HIF1α mediates DHEA-induced expression of glycolytic enzymes. As seen previously, DHEA, but not DHEA-S, induced both the mRNA and protein expression of HK2 and PKM2. However, this DHEA-induced glycolytic enzyme expression was effectively suppressed by HIF1α knockdown at both the mRNA and protein level (Fig. 6C and D). Expression of PKM2 mRNA induced by STS overexpression was significantly inhibited by HIF1α knockdown. However, PKM1 mRNA level which was downregulated by STS overexpression was restored by HIF1α siRNA (Supplementary Fig. 2A). Moreover, knockdown of c-Myc by siRNA also prevented isoform switching from PKM1 to PKM2 promoted by STS expression (Supplementary Fig. 2B). We next investigated the effect of STS-induced HIF1α signaling on cellular energy metabolism using lactic acid production and OCR assays. As seen previously, overexpression of STS increased the lactic acid level and suppressed the OCR level compared to normal cells. However, knockdown of HIF1α suppressed the STS-induced lactic acid production and restored the OCR to control level (Fig. 7A and B). In addition, HIF1α siRNA recovered both the increase in lactic acid production and the suppression of OCR induced by DHEA (Fig. 7C and D). These results demonstrate that STS, and its major product DHEA, may alter mitochondrial respiration to aerobic glycolysis through induction of HIF1α in HeLa cells.

### 3.6. STS induces glycolytic enzymes in transgenic mice

To demonstrate whether STS induces aerobic glycolysis *in vivo*, STS-overexpressing transgenic mice were produced using the C57BL/6 mouse model. We confirmed that STS mRNA expression was significantly increased in almost every organs of the transgenic mouse (Fig. 8A). Crucially, HIF1α mRNA expression was enhanced in the lung tissues of STS transgenic mouse. The increased expression of STS and HIF1α was also confirmed at the protein level by immunohistochemistry (Fig. 8B and C). In addition, the glycolytic enzymes HK2 and PKM2 were increased in the lung tissues of the STS transgenic mouse (Fig. 8D). These results indicate that STS may promote aerobic glycolysis through inducing HIF1α and glycolytic enzymes *in vivo*.

## 4. Discussion

In order to obtain sufficient cellular energy for their growth and survival, cancer cells modify their metabolism [40]. In contrast to the mitochondrial oxidative phosphorylation pathway used by normal cells, most cancer cells primarily catabolize glucose by aerobic glycolysis [41]. This induction of aerobic glycolysis has long been described as an important hallmark of cancer [18]. To our knowledge, our study is the first to show the relationship between STS and aerobic glycolysis. STS increased lactic acid production and suppressed oxygen consumption in HeLa cells. This data is consistent with results of previous studies on the activation of aerobic glycolysis in cancer cells, wherein the level of lactic acid increased and mitochondrial oxidation was suppressed [19,41–43]. Induction of HIF1α expression by STS mediates increased levels of glycolytic enzymes, including HK2 and PKM2. These data show that HIF1α is an essential transcription factor for the activation of

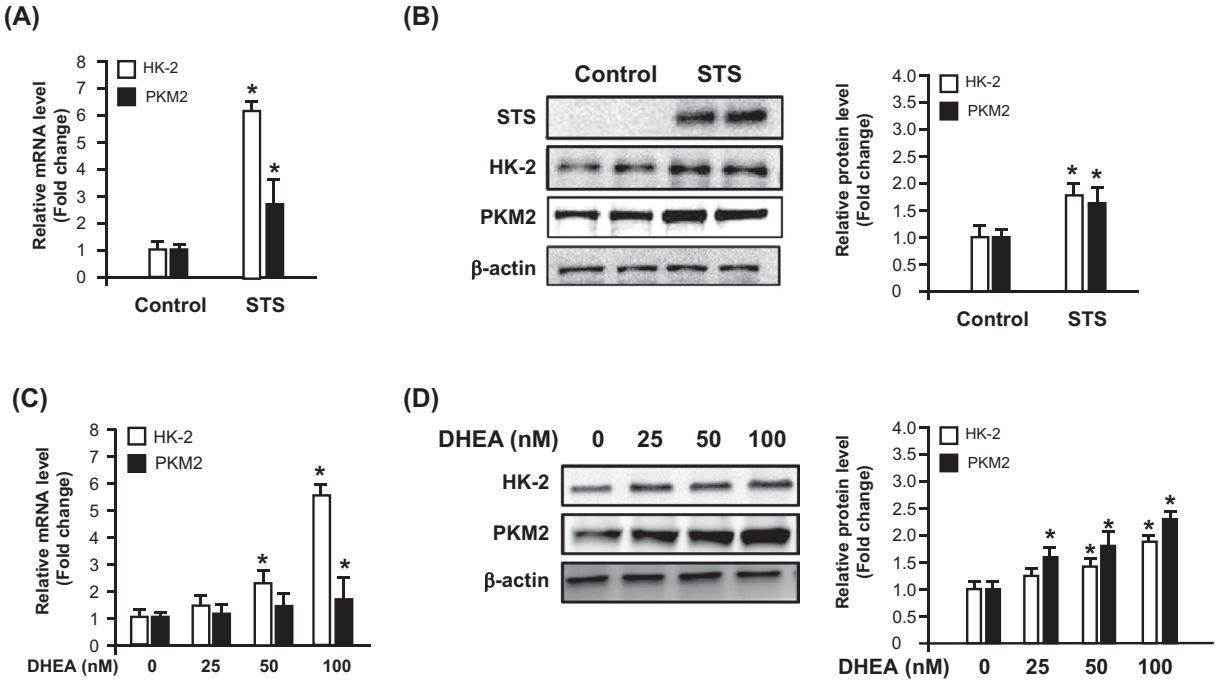


**Fig. 2.** Induction of glycolytic enzyme expression by STS and DHEA in HeLa cells.
(A) Cells were transfected with pcDNA3.1-STS for 24 h. Total RNA was isolated, and HK2 or PKM2 mRNA levels were quantified by qPCR with specific primers. Values are shown as means ± S.D. (n = 3). (B) Cells were transfected with pcDNA3.1-STS for 24 h. Total cellular protein (20 μg) was subjected to western blotting analysis with HK2 or PKM2 antibodies. β-Actin was used as a loading control. (C) Cells were treated with DHEA (0, 25, 50, or 100 nM), followed by total RNA isolation. HK2 or PKM2 mRNA levels were quantified by qPCR with specific primers. Values are shown as means ± S.D. (n = 3). (D) Cells were treated with DHEA (0, 25, 50, or 100 nM). Total cellular protein (20 μg) was subjected to western blotting analysis with HK2 or PKM2 antibodies. β-Actin was used as a loading control. $^{*}p < 0.05$ compared with control cells.

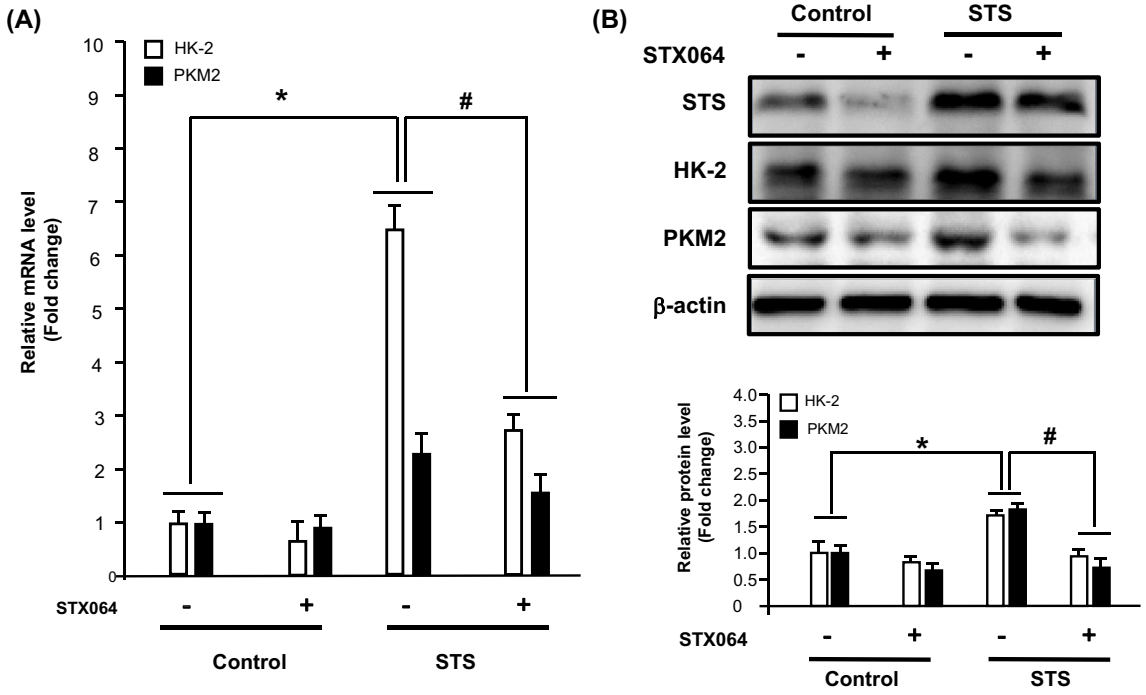


**Fig. 3.** Inhibition of STS suppresses glycolytic enzyme expression in HeLa cells.
Cells were transfected with pcDNA3.1-STS and then treated with STX064 (2 μM) for 24 h. (A) Total RNA was isolated, and HK2 or PKM2 mRNA levels were quantified by qPCR with specific primers. Values are shown as means ± S.D. (n = 3). (B) Total cellular protein (20 μg) was subjected to western blotting analysis with HK2 or PKM2 antibodies. β-Actin was used as a loading control. Values are shown as means ± S.D. (n = 3). $^{*}p < 0.05$ compared with control cells. $^{\#}p < 0.05$ compared to STS-overexpressed cells.

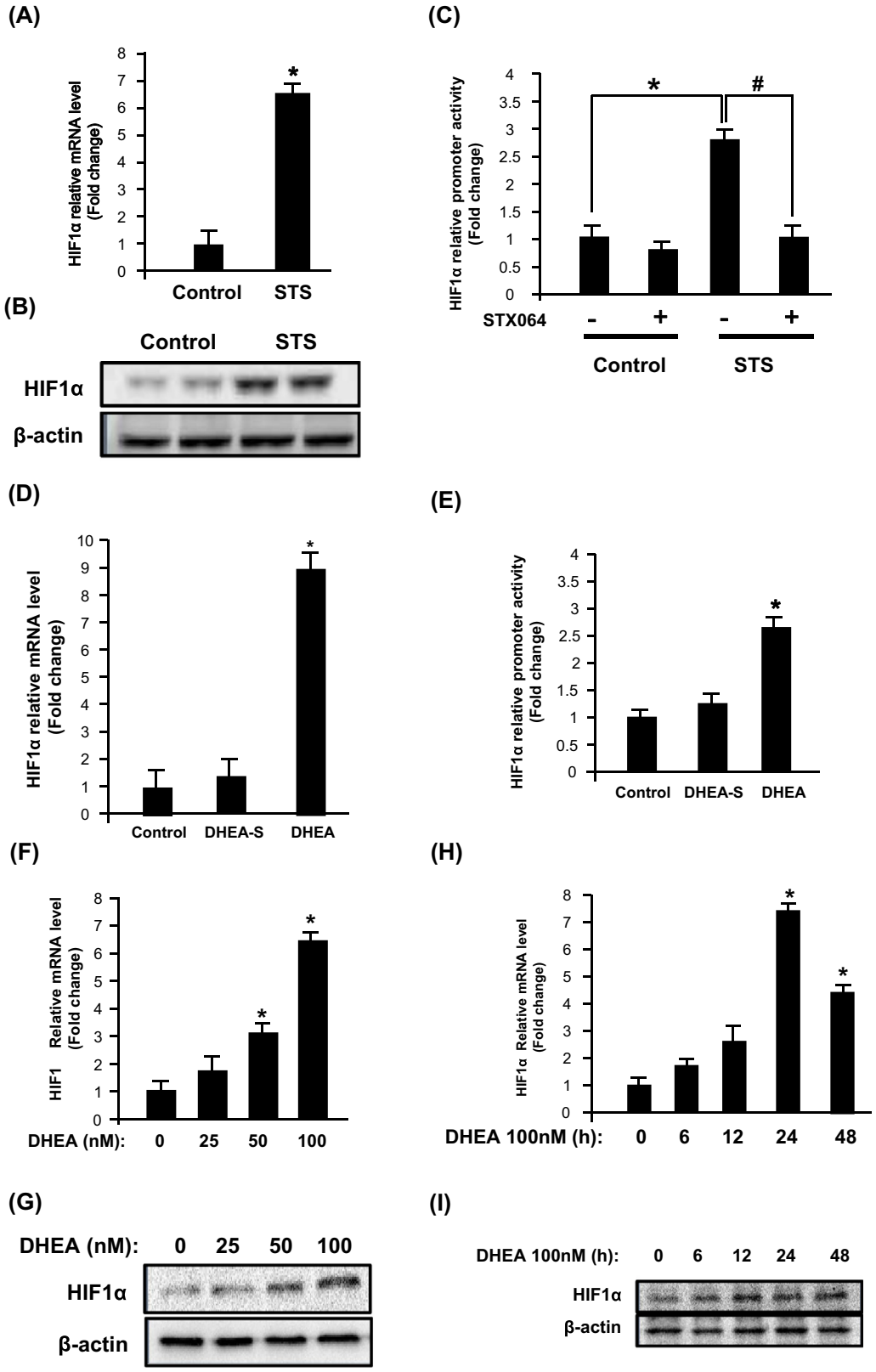


**Fig. 4.** STS and DHEA induce HIF1α expression in HeLa cells.
(A) Cells were transfected with pcDNA3.1-STS for 24 h. Total RNA was isolated, and HIF1α mRNA levels were quantified by qPCR with specific primers. Values are shown as means ± S.D. (n = 3). (B) Cells were transfected with pcDNA3.1-STS for 24 h, and total cellular protein (20 μg) was subjected to western blotting analysis with HIF1α antibody. β-Actin was used as a loading control. (C) Cells were transiently co-transfected with pcDNA3.1-STS, HIF1α luciferase vector, and renilla luciferase vector and then treated with STX064 (2 μM) for 24 h, followed by dual-luciferase assay. The relative firefly luciferase activity, normalized to the renilla luciferase activity, is shown. (D) Cells were treated with DHEA-S (100 nM) or DHEA (100 nM). Values are shown as means ± S.D. (n = 3). (E) Cells were treated with DHEA-S (100 nM) or DHEA (100 nM), and transiently transfected with HIF1α luciferase vector and renilla luciferase vector for 24 h, followed by dual-luciferase assay. The relative firefly luciferase activity, normalized by the renilla luciferase activity, is shown. Values are shown as means ± S.D. (n = 3). (F) Cells were treated at different concentrations of DHEA (0, 25, 50, and 100 nM). Total RNA was isolated, and HIF1α mRNA levels were quantified by qPCR with specific primers. Values are shown as means ± S.D. (n = 3). (G) Cells were treated at different concentrations of DHEA (0, 25, 50, or 100 nM). Total cellular protein (20 μg) was subjected to western blotting analysis with HIF1α antibody. β-Actin was used as a loading control. (H) Following treatment of cells with 100 nM DHEA, total RNA was isolated at various time-points (0, 6, 12, 24, or 48 h), and then HIF1α mRNA levels were quantified by qPCR with specific primers. Values are shown as means ± S.D. (n = 3). (I) Following treatment of cells with 100 nM DHEA, total cellular protein (20 μg) was extracted at various time-points (0, 6, 12, 24, or 48 h) and analyzed by western blotting with HIF1α antibody. β-Actin was used as a loading control. $^{*}p < 0.05$ compared with control cells. $^{\#}p < 0.05$ compared to STS-overexpressed cells.

aerobic glycolysis.

So far, the precise mechanism of HIF1α-induced aerobic glycolysis has been unclear. Previous studies have shown that increased HIF1α expression activates its DNA binding activity, enhancing the expression of downstream targets such as VEGF and glucose transporters [35]. Consistent with this, our data showed that STS induced GLUT-1 promoter activity (Fig. 5C). Although the mechanism is not fully understood yet, our results indirectly demonstrate that STS may induce HIF1α accumulation or maintenance. However, other studies may help to shed light on the potential relationship between STS and HIF1α expression. For example, a recent study showed that androgens, including DHEA, lead to the phosphorylation of mTOR and increase abnormal

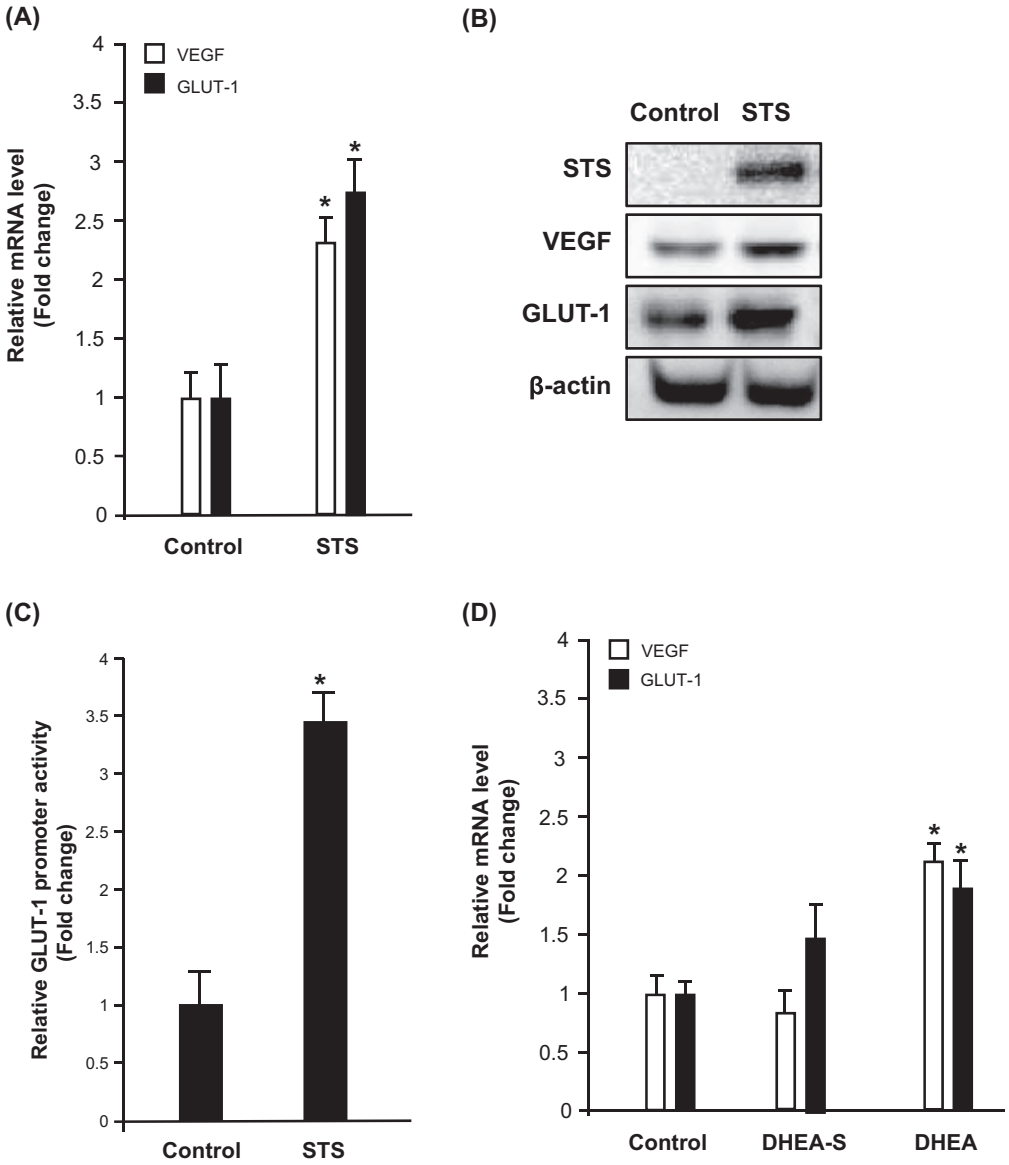


**Fig. 5.** STS and DHEA induce expression of HIF1α downstream targets in HeLa cells.
(A) Cells were transfected with pcDNA3.1-STS for 24 h. Total RNA was isolated, and VEGF or GLUT-1 mRNA levels were quantified by qPCR with specific primers. Values are shown as means ± S.D. (n = 3). (B) Cells were transfected with pcDNA3.1-STS for 24 h. Total cellular protein (20 μg) was subjected to western blotting analysis with VEGF or GLUT-1 antibodies. β-Actin was used as a loading control. (C) Cells were transiently co-transfected with pcDNA3.1-STS, GLUT-1 luciferase vector and renilla luciferase vector for 24 h, followed by dual-luciferase assay. The relative firefly luciferase activity, normalized to the renilla luciferase activity, is shown. Values are shown as means ± S.D. (n = 3). (D) Cells were treated with DHEA-S (100 nM) or DHEA (100 nM). Total RNA was isolated, and VEGF or GLUT-1 mRNA levels were quantified by qPCR with specific primers. Values are shown as means ± S.D. (n = 3). *$p < 0.05$ compared with control cells.

mitochondrial function [44]. Furthermore, it is widely known that mTOR induces HIF1α activity and regulates aerobic glycolysis in cells [45,46]. Based on these studies, we hypothesize that mTOR may mediate STS-induced aerobic glycolysis through HIF1α activation, but further investigation will be required to confirm this.

In this study, we found that HIF1α plays a crucial role in STS-induced aerobic glycolysis through regulating glycolytic proteins such as HK2 and PKM2. Recently, it has been reported that induction of HK2 enhances cancer cell proliferation by producing lactic acid [47]. Moreover, many studies have shown that translocation of HK2 to mitochondria induces apoptosis by cytochrome *c* release in various cancer cells [48–50]. In our previous study, we identified a novel mechanism of apoptosis induction through mitochondrial translocation of annexin A5 [51]. It is therefore possible that STS may prevent apoptosis through suppression of annexin A5, although more evidences are needed to confirm this.

PKM2 is a pivotal rate-limiting enzyme of aerobic glycolysis [52–54], and our study confirmed that STS overexpression enhances PKM2 expression (Fig. 6A and B). A recent study suggested that HIF1α mediates alternative splicing of PKM1 and PKM2 [55]. We also showed that STS may promote isoform switching from PKM1 to PKM2 to accelerate PKM2 expression and suppress PKM1 expression through activation of HIF1α or c-Myc. Thus, the possibility worth investigating is that STS regulates PKM alternative splicing *via* HIF1α or c-Myc, which in turn causes PKM2 induction and lactic acid production.

In addition, we found that STS and DHEA strongly activate phosphorylation of PKM2 at tyrosine 105 (Supplementary Fig. 3). Increased PKM2 phosphorylation may inhibit PKM2 activity and promote increased lactic acid production and cancer cell proliferation. Because oncogenic tyrosine kinases including BCR-ABL, FGFR1, and JAK2 mainly phosphorylate tyrosine 105 residue of PKM2, further study will be necessary to elucidate whether STS is able to activate these tyrosine kinases to enhance PKM2 tyrosine phosphorylation. Recent study showed that phosphorylation of PKM2 at tyrosine 105 induces cancer stem cell-like cell properties by enhancing nuclear translocation and activation of Yes-associated protein (YAP) [56]. Thus, the possibility that STS may modulate Hippo signaling through activation of YAP/TAZ and TEAD-mediated transcription needs to be investigated to find the novel mechanism of cancer progression by STS induction.

In our *in vivo* experiments, the expression of HIF1α and the glycolytic enzymes HK2 and PKM2 was strongly enhanced in the lung tissue of STS-overexpressing transgenic mice (Fig. 7C and D). Studies have recently reported that HIF1α induces lung injury and T-cell abundance through FoxP3 *in vivo* [57,58]. Based on these reports, STS could be

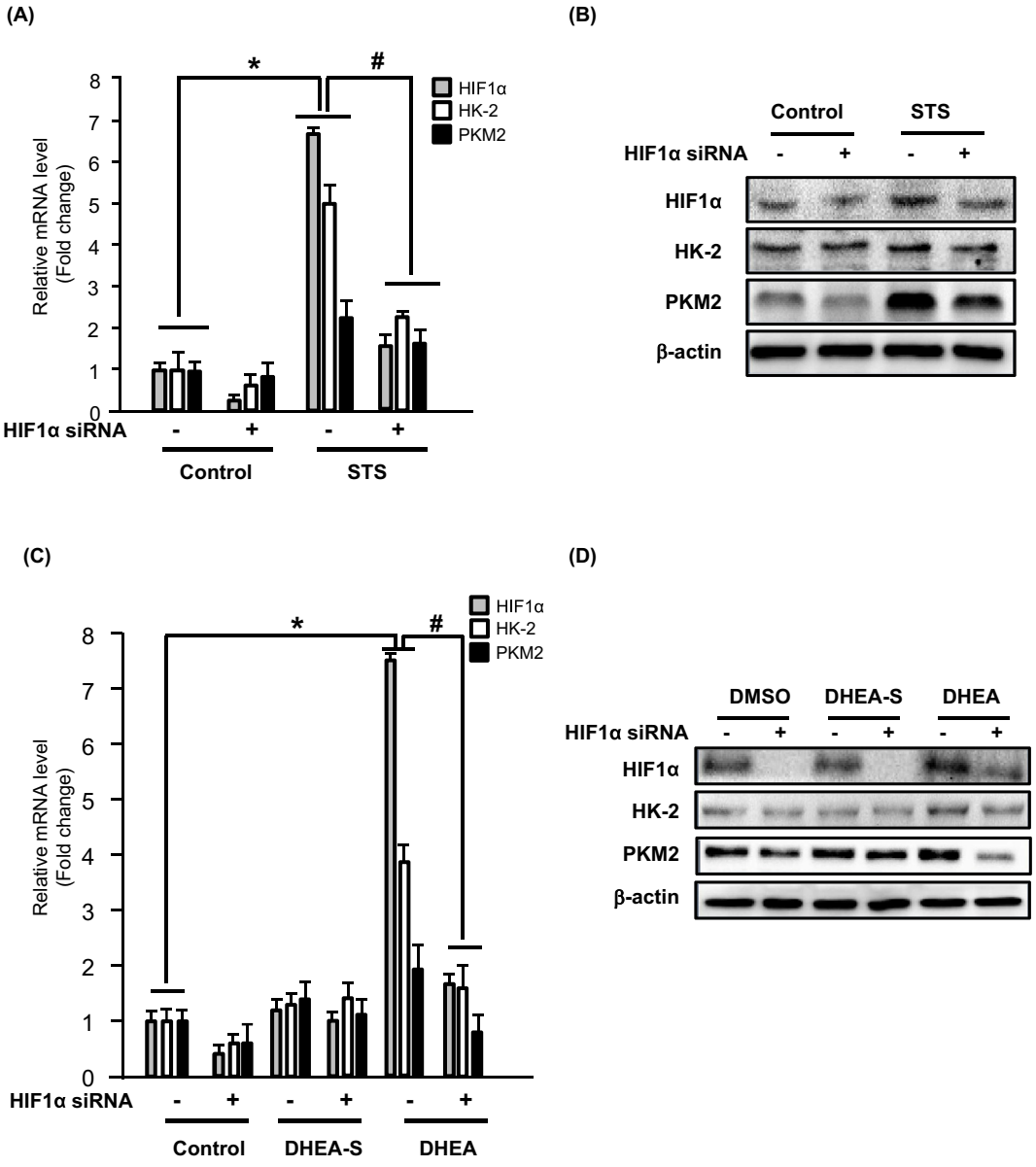


**Fig. 6.** Induction of glycolytic proteins by STS and DHEA is mediated by HIF1α in HeLa cells. (A) Cells were transfected with the pcDNA3.1-STS and HIF1α siRNA (37.5 nM). Total RNA was isolated, and HIF1α, HK2 or PKM2 mRNA levels were quantified by qPCR with specific primers. Values are shown as means ± S.D. (n = 3). (B) Cells were transfected with pcDNA3.1-STS and HIF1α siRNA (37.5 nM). Total cellular protein (20 μg) was subjected to western blotting analysis with HIF1α, HK2, or PKM2 antibodies. β-Actin was used as a loading control. (C) Cells were treated with DHEA-S (100 nM) or DHEA (100 nM) combined with HIF1α siRNA transfection (37.5 nM), as indicated. Total RNA was isolated, and HIF1α, HK2 or PKM2 mRNA levels were quantified by qPCR with specific primers. Values are shown as means ± S.D. (n = 3). (D) Cells were treated with DHEA-S (100 nM) or DHEA (100 nM) after transfection with HIF1α siRNA (37.5 nM). Total cellular protein (20 μg) was subjected to western blotting analysis with HIF1α, HK2, or PKM2 antibodies. β-Actin was used as a loading control. $^{*}p < 0.05$ compared with control cells. $^{\#}p < 0.05$ compared to STS-overexpressed cells or DHEA-treated cells.

related to lung diseases such as fibrosis, as HIF1α expression was increased 33-fold in the lung tissue of STS transgenic mice, compared to control mice. In our previous studies, we demonstrated that inflammatory cytokines such as TNF-α and IL-6 induce STS expression *via* the PI3K/AKT pathway [59,60]. TNF-α expression is highly correlated to the induction of pulmonary fibrosis in mice [61]. We therefore suggest that STS expression induced by inflammatory cytokines may exacerbate pulmonary fibrosis, as HIF1α expression is highly increased by STS both *in vivo* and *in vitro*.

In summary, our results imply that STS expression plays a pivotal role in the activation of aerobic glycolysis. We demonstrated that STS, and its major product DHEA, may induce aerobic glycolysis through enhancing the expression of HIF1α and glycolytic enzymes. In the STS transgenic mouse, we found that HIF1α and the glycolytic enzymes HK2 and PKM2 were increased most strikingly in the lung tissue. The possibility that STS, induced by inflammatory cytokines, may exacerbate pulmonary fibrosis is certainly worth further investigation. Future studies should also be directed at identifying unknown downstream targets of STS, which may mediate HIF1α expression and aerobic glycolysis, as well as potentially impact lung fibrosis. Since STS is considered a prognostic indicator of various cancers, understanding the exact mechanism of STS-induced cancer progression may also be useful in developing novel strategies for cancer treatment.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbadis.2019.06.005.

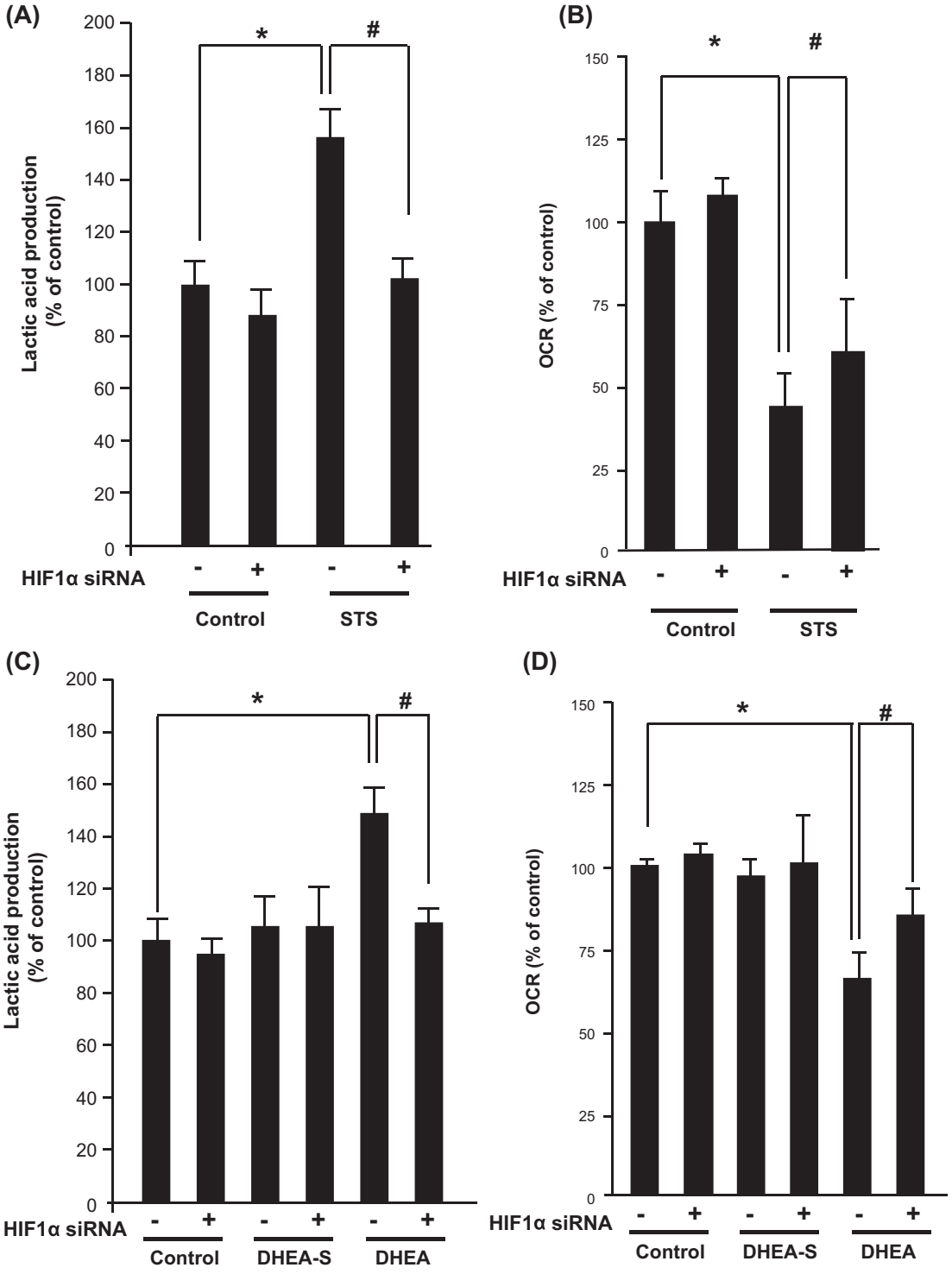


**Fig. 7.** STS-induced alterations in cell metabolism are mediated by HIF1α in HeLa cells.
(A–B) Cells were transfected with pcDNA3.1-STS and HIF1α siRNA (37.5 nM) for 24 h, followed by measurement of (A) lactic acid concentration or (B) oxygen consumption rate. Values are shown as means ± S.D. (n = 3). (C–D) Cells were treated with DHEA-S (100 nM) or DHEA (100 nM), after transfection with HIF1α siRNA (37.5 nM), followed by measurement of lactic acid concentration (C) or oxygen consumption rate (D). Values are shown as means ± S.D. (n = 3). $^{*}p < 0.05$ compared with control cells. $^{\#}p < 0.05$ compared to STS-overexpressed cells or DHEA-treated cells.

## Authors' contribution

The project was conceptualized and designed by Y.J. Chun. S Shin, Y.J. Kwon, D.J Ye, H.S. Baek, T.W. Kwon performed the experimental and analyzed data with support of Y.J. Chun. S Shin, D.J. Ye, and Y.J. Chun wrote the manuscript. All authors read and provided feedback on manuscript and figures.

## Funding

This study was supported by the National Research Foundation of Korea (NRF), funded by the Korean government (MSIP) (Grant No. NRF2017R1E1A1A01074032 and NRF2015R1A5A1008958). The funders had no role in the study design, data collection or analysis, the decision to publish, or preparation of the manuscript.

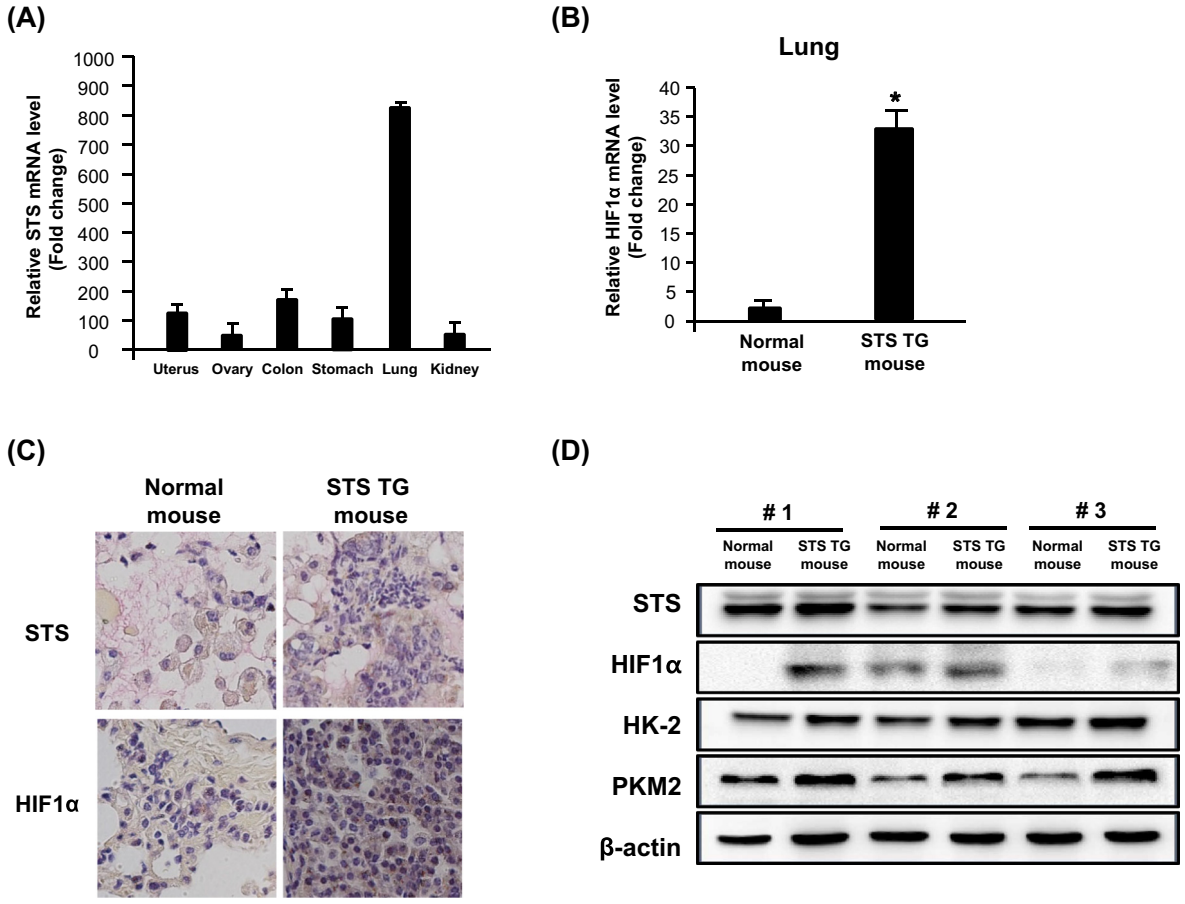


**Fig. 8.** STS induces HIF1α and glycolytic enzymes in transgenic mice.
(A) Total RNA was extracted from the indicated tissues of STS transgenic and normal mice, and STS mRNA levels were quantified by qPCR with specific primers. Relative gene expression levels between normal mouse and STS transgenic mouse was determined. Values are ratios (fold-change) of expression level of STS transgenic/normal tissues (means ± S.D.; n = 3). (B) Total RNA was extracted from mouse lung tissues of STS transgenic and normal mice, and HIF1α mRNA levels were quantified by qPCR with specific primers. Values are shown as means ± S.D. (n = 3). *$p < 0.05$ compared to normal mouse tissues. (C) Mouse lung tissues were fixed in 4% paraformaldehyde, embedded in paraffin and sectioned. Sections were deparaffinized, incubated with 3% $H_2O_2$, blocked, and analyzed by immunohistochemistry with STS or HIF1α antibodies. Magnification; ×400. (D) Total cellular protein was extracted from lung tissues of STS transgenic and normal mice (n = 3), and subjected to western blotting analysis with STS, HIF1α, HK2, or PKM2 antibodies. β-Actin was used as a loading control.

## Transparency document

The Transparency document associated with this article can be found, in online version.

## Declaration of Competing Interest

All authors declare that there are no conflicts of interest.

## References

[1] T.L. Dao, C. Hayes, P.R. Libby, Steroid sulfatase activities in human breast tumors, Proc. Soc. Exp. Biol. Med. 146 (1974) 381–384.
[2] D.P. Pare, et al., Expression of estrogen sulfotransferase 1E1 and steroid sulfatase in breast cancer, Breast Cancer (Auckl.) 3 (2009) 9–21.
[3] Y. Miyoshi, et al., High expression of steroid sulfatase mRNA predicts poor prognosis in patients with estrogen receptor-positive breast cancer, Clin. Cancer Res. 9 (2003) 2288–2293.
[4] S. Shin, H.J. Im, Y.J. Kwon, D.J. Ye, H.S. Baek, D. Kim, H.K. Choi, Y.J. Chun, Human steroid sulfatase induces Wnt/β-catenin signaling and epithelial-mesenchymal transition by upregulating Twist1 and HIF-1α in human prostate and cervical cancer cells, Oncotarget 8 (2017) 61604–61617.
[5] A. Purohit, G.J. Williams, N.M. Howarth, B.V. Potter, M.J. Reed, Inactivation of steroid sulfatase by an active site-directed inhibitor, estrone-3-O-sulfamate, Biochemistry 34 (1995) 11508–11514.
[6] A. Billich, P. Nussbaumer, P. Lehr, Stimulation of MCF-7 breast cancer cell proliferation by estrone sulfate and dehydroepiandrosterone sulfate: inhibition by novel non-steroidal steroid sulfatase inhibitors, J. Steroid Biochem. Mol. Biol. 73 (2000) 225–235.
[7] B.L. Dennefors, F. Knutson, P.O. Janson, I. Jansson, L. Hamberger, Ovarian steroid production in a woman with polycystic ovary syndrome associated with endometrial cancer, Acta Obstet. Gynecol. Scand. 64 (1985) 387–392.
[8] P.A. Foster, M.J. Reed, A. Purohit, Recent developments of steroid sulfatase inhibitors as anti-cancer agents, Anti Cancer Agents Med. Chem. 8 (2008) 732–738.
[9] M. Salah, A.S. Abdelsamie, M. Frotscher, First dual inhibitors of steroid sulfatase (STS) and 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1), J. Med. Chem. 60 (2017) 4086–4092.
[10] A. Purohit, M.J. Reed, N.C. Morris, G.J. Williams, B.V. Potter, Regulation and inhibition of steroid sulfatase activity in breast cancer, Ann. N. Y. Acad. Sci. 784 (1996) 40–49.
[11] J.M. Day, A. Purohit, H.J. Tutill, P.A. Foster, L.W. Woo, B.V. Potter, M.J. Reed, The development of steroid sulfatase inhibitors for hormone-dependent cancer therapy, Ann. N. Y. Acad. Sci. 1155 (2009) 80–87.
[12] M. Schmidt, M. Kreutz, G. Loffler, J. Scholmerich, R.H. Straub, Conversion of dehydroepiandrosterone to downstream steroid hormones in macrophages, J. Endocrinol. 164 (2000) 161–169.
[13] J.T. Arnold, X. Liu, J.D. Allen, H. Le, K.K. McFann, M.R. Blackman, Androgen receptor or estrogen receptor-β blockade alters DHEA-, DHT-, and E(2)-induced proliferation and PSA production in human prostate cancer cells, Prostate 67 (2007) 1152–1162.
[14] S.S. Tworoger, S.A. Missmer, A.H. Eliassen, D. Spiegelman, E. Folkerd, M. Dowsett, R.L. Barbieri, S.E. Hankinson, The association of plasma DHEA and DHEA sulfate with breast cancer risk in predominantly premenopausal women, Cancer Epidemiol. Biomark. Prev. 15 (2006) 967–971.
[15] E. Racker, M. Spector, Warburg effect revisited: merger of biochemistry and molecular biology, Science 213 (1981) 303–307.
[16] K.O. Alfarouk, et al., Glycolysis, tumor metabolism, cancer growth and dissemination. A new pH-based etiopathogenic perspective and therapeutic approach to an old cancer question, Oncoscience 1 (2015) 777–802.

[17] M. Rogalinska, The role of mitochondria in cancer induction, progression and changes in metabolism, Mini Rev. Med. Chem. 16 (2015) 524–530.
[18] M.G. Vander Heiden, L.C. Cantley, C.B. Thompson, Understanding the Warburg effect: the metabolic requirements of cell proliferation, Science 324 (2009) 1029–1033.
[19] Q. Sun, et al., Mammalian target of rapamycin up-regulation of pyruvate kinase isoenzyme type M2 is critical for aerobic glycolysis and tumor growth, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 4129–4134.
[20] S.A. Tahir, G. Yang, A. Goltsov, K.D. Song, C. Ren, J. Wang, W. Chang, T.C. Thompson, Caveolin-1-LRP6 signaling module stimulates aerobic glycolysis in prostate cancer, Cancer Res. 73 (2013) 1900–1911.
[21] M.W. Nijsten, G.M. van Dam, Hypothesis: using the Warburg effect against cancer by reducing glucose and providing lactate, Med. Hypotheses 73 (2009) 48–51.
[22] H.G. Hers, L. Hue, Gluconeogenesis and related aspects of glycolysis, Annu. Rev. Biochem. 52 (1983) 617–653.
[23] M. Werle, J. Kreuzer, J. Höfele, A. Elsässer, C. Ackermann, H.A. Katus, A.M. Vogt, Metabolic control analysis of the Warburg-effect in proliferating vascular smooth muscle cells, J. Biomed. Sci. 12 (2005) 827–834.
[24] P. Gupta, K. Jagavelu, D.P. Mishra, Inhibition of NADPH oxidase-4 potentiates 2-deoxy-d-glucose-induced suppression of glycolysis, migration, and invasion in glioblastoma cells: role of the Akt/HIF1α/HK-2 signaling axis, Antioxid. Redox Signal. 23 (2015) 665–681.
[25] J. Liu, D. Wen, X. Fang, X. Wang, T. Liu, J. Zhu, p38MAPK signaling enhances glycolysis through the up-regulation of the glucose transporter GLUT-4 in gastric cancer cells, Cell. Physiol. Biochem. 36 (2015) 155–165.
[26] G.M. Shen, Y.Z. Zhao, M.T. Chen, F.L. Zhang, X.L. Liu, Y. Wang, C.Z. Liu, J. Yu, J.W. Zhang, Hypoxia-inducible factor-1 (HIF-1) promotes LDL and VLDL uptake through inducing VLDLR under hypoxia, Biochem. J. 441 (2012) 675–683.
[27] F.S. Wang, C.J. Wang, Y.J. Chen, P.R. Chang, Y.T. Huang, Y.C. Sun, H.C. Huang, Y.J. Yang, K.D. Yang, Ras induction of superoxide activates ERK-dependent angiogenic transcription factor HIF-1α and VEGF-A expression in shock wave-stimulated osteoblasts, J. Biol. Chem. 279 (2004) 10331–10337.
[28] L. Zhang, et al., Hypoxia induces epithelial-mesenchymal transition via activation of SNAI1 by hypoxia-inducible factor1α in hepatocellular carcinoma, BMC Cancer 13 (2013) 108.
[29] D. Ryu, I.G. Ryoo, M.K. Kwak, Overexpression of CD44 standard isoform upregulates HIF-1α signaling in hypoxic breast cancer cells, Biomol. Ther. 26 (2018) 487–493.
[30] G.L. Semenza, Intratumoral hypoxia, radiation resistance, and HIF-1, Cancer Cell 5 (2004) 405–406.
[31] J.J. Lum, et al., The transcription factor HIF1α plays a critical role in the growth factor-dependent regulation of both aerobic and anaerobic glycolysis, Genes Dev. 21 (2007) 1037–1049.
[32] P. Li, et al., Genetic polymorphisms in HIF1A are associated with prostate cancer risk in a Chinese population, Asian J. Androl. 14 (2012) 864–869.
[33] S. Deb, et al., Nuclear HIF1A expression is strongly prognostic in sporadic but not familial male breast cancer, Mod. Pathol. 27 (2014) 1223–1230.
[34] B. Liu, Q. Liu, Y. Song, X. Li, Y. Wang, S. Wan, Z. Zhang, H. Su, Polymorphisms of HIF1A gene are associated with prognosis of early stage non-small-cell lung cancer patients after surgery, Med. Oncol. 31 (2014) 877.
[35] H. Lu, R.A. Forbes, A. Verma, Hypoxia-inducible factor 1 activation by aerobic glycolysis implicates the Warburg effect in carcinogenesis, J. Biol. Chem. 277 (2002) 23111–23115.
[36] L. Fusi, A. Purohit, J. Brosens, L.W. Woo, B.V. Potter, M.J. Reed, Inhibition of steroid sulfatase activity in endometriotic implants by STX64 (667Coumate): a potential new therapy, ScientificWorldJournal 8 (2008) 1325–1327.
[37] L.W. Woo, D. Ganeshapillai, M.P. Thomas, O.B. Sutcliffe, B. Malini, M.F. Mahon, A. Purohit, B.V. Potter, Structure-activity relationship for the first-in-class clinical steroid sulfatase inhibitor Irosustat (STX64, BN83495), ChemMedChem 6 (2011) 2019–2034.
[38] J. Zhang, J. Xu, Y. Dong, B. Huang, Downregulation of HIF1α inhibits the proliferation, migration and invasion of gastric cancer by inhibiting PI3K/AKT pathway and VEGF expression, Biosci. Rep. 38 (2018) BSR20180741.
[39] K. Song, M. Li, X.J. Xu, L. Xuan, G.N. Huang, X.L. Song, Q.F. Liu, HIF1α and GLUT1 gene expression is associated with chemoresistance of acute myeloid leukemia, Asian Pac. J. Cancer Prev. 15 (2014) 1823–1829.
[40] R.G. Jones, C.B. Thompson, Tumor suppressors and cell metabolism: a recipe for cancer growth, Genes Dev. 23 (2009) 537–548.
[41] C. Garrigues, N. Goupil-Feuillerat, M. Cocaign-Bousquet, P. Renault, N.D. Lindley, P.C. Loubiere, Glucose metabolism and regulation of glycolysis in Lactococcus lactis strains with decreased lactate dehydrogenase activity, Metab. Eng. 3 (2001) 211–217.
[42] P.M. Spooner, I.S. Edelman, Effects of aldosterone on $Na^+$ transport in the toad bladder. I. Glycolysis and lactate production under aerobic conditions, Biochim. Biophys. Acta 444 (1976) 653–662.
[43] A. Salimi, B.S. Talatappe, J. Pourahmad, Xylene induces oxidative stress and mitochondria damage in isolated human lymphocytes, Toxicol. Res. 33 (2017) 233–238.
[44] X. Song, Q. Shen, L. Fan, Q. Yu, X. Jia, Y. Sun, W. Bai, J. Kang, Dehydroepiandrosterone-induced activation of mTORC1 and inhibition of autophagy contribute to skeletal muscle insulin resistance in a mouse model of polycystic ovary syndrome, Oncotarget 9 (2018) 11905–11921.
[45] R.V. Pusapati, A. Daemen, C. Wilson, W. Sandoval, M. Gao, B. Haley, A.R. Baudy, G. Hatzivassiliou, M. Evangelista, J. Settleman, mTORC1-dependent metabolic reprogramming underlies escape from glycolysis addiction in cancer cells, Cancer Cell 29 (2016) 548–562.
[46] H. Harada, S. Itasaka, S. Kizaka-Kondoh, K. Shibuya, A. Morinibu, K. Shinomiya, M. Hiraoka, The Akt/mTOR pathway assures the synthesis of HIF-1α protein in a glucose- and reoxygenation-dependent manner in irradiated tumors, J. Biol. Chem. 284 (2009) 5332–5342.
[47] M. Anderson, R. Marayati, R. Moffitt, J.J. Yeh, Hexokinase 2 promotes tumor growth and metastasis by regulating lactate production in pancreatic cancer, Oncotarget 8 (2017) 56081–56094.
[48] J.G. Pastorino, J.B. Hoek, Hexokinase II: the integration of energy metabolism and control of apoptosis, Curr. Med. Chem. 10 (2003) 1535–1551.
[49] K. Machida, Y. Ohta, H. Osada, Suppression of apoptosis by cyclophilin D via stabilization of hexokinase II mitochondrial binding in cancer cells, J. Biol. Chem. 281 (2006) 14314–14320.
[50] G.Y. Gwak, J.H. Yoon, K.M. Kim, H.S. Lee, J.W. Chung, G.J. Gores, Hypoxia stimulates proliferation of human hepatoma cells through the induction of hexokinase II expression, J. Hepatol. 42 (2005) 358–364.
[51] J.J. Jeong, N. Park, Y.J. Kwon, D.J. Ye, A. Moon, Y.J. Chun, Role of annexin A5 in cisplatin-induced toxicity in renal cells: molecular mechanism of apoptosis, J. Biol. Chem. 289 (2014) 2469–2481.
[52] E.C. Ferguson, J.C. Rathmell, New roles for pyruvate kinase M2: working out the Warburg effect, Trends Biochem. Sci. 33 (2008) 359–362.
[53] O. Feron, Pyruvate into lactate and back: from the Warburg effect to symbiotic energy fuel exchange in cancer cells, Radiother. Oncol. 92 (2009) 329–333.
[54] A.S. Marsin, L. Bertrand, M.H. Rider, J. Deprez, C. Beauloye, M.F. Vincent, G. Van den Berghe, D. Carling, L. Hue, Phosphorylation and activation of heart PFK-2 by AMPK has a role in the stimulation of glycolysis during ischaemia, Curr. Biol. 10 (2000) 1247–1255.
[55] A.L. Williams, V. Khadka, M. Tang, A. Avelar, K.J. Schunke, M. Menor, R.V. Shohet, HIF1 mediates a switch in pyruvate kinase isoforms after myocardial infarction, Physiol. Genomics 50 (2018) 479–494.
[56] Z. Zhou, M. Li, L. Zhang, H. Zhao, Ö. Şahin, J. Chen, J.J. Zhao, Z. Songyang, D. Yu, Oncogenic kinase-induced PKM2 tyrosine 105 phosphorylation converts non-oncogenic PKM2 to a tumor promoter and induces cancer stem-like cells, Cancer Res. 78 (2018) 2248–2261.
[57] L.A. Shimoda, G.L. Semenza, HIF and the lung: role of hypoxia-inducible factors in pulmonary development and disease, Am. J. Respir. Crit. Care Med. 183 (2011) 152–156.
[58] E.T. Clambey, et al., Hypoxia-inducible factor-1α-dependent induction of FoxP3 drives regulatory T-cell abundance and function during inflammatory hypoxia of the mucosa, Proc. Natl. Acad. U.S.A. 109 (2012) E2784–E2793.
[59] H.J. Im, N.H. Park, Y.J. Kwon, S. Shin, D. Kim, Y.J. Chun, Bacterial lipopolysaccharides induce steroid sulfatase expression and cell migration through IL-6 pathway in human prostate cancer cells, Biomol. Ther. 20 (2012) 556–561.
[60] B.Y. Suh, J.J. Jung, N. Park, C.H. Seong, H.J. Im, Y. Kwon, D. Kim, Y.J. Chun, Induction of steroid sulfatase expression by tumor necrosis factor-α through phosphatidylinositol 3-kinase/Akt signaling pathway in PC-3 human prostate cancer cells, Exp. Mol. Med. 43 (2011) 646–652.
[61] R.S. Thrall, S.N. Vogel, R. Evans, L.D. Shultz, Role of tumor necrosis factor-α in the spontaneous development of pulmonary fibrosis in viable motheaten mutant mice, Am. J. Pathol. 151 (1997) 1303–1310.