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Effect of propofol on androgen receptor activity in prostate cancer cells

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ABSTRACT

Androgen receptor is a nuclear receptor and transcription factor activated by androgenic hormones. Androgen receptor activity plays a pivotal role in the development and progression of prostate cancer. Although accumulating evidence suggests that general anesthetics, including opioids, affect cancer cell growth and impact patient prognosis, the effect of those drugs on androgen receptor in prostate cancer is not clear. The purpose of this study was to investigate the effect of the general anesthetic propofol on androgen receptor activity in prostate cancer cells. An androgen-dependent human prostate cancer cell line (LNCaP) was stimulated with dihydrotestosterone (DHT) and exposed to propofol. The induction of androgen receptor target genes was investigated using real-time reverse transcription polymerase chain reaction, and androgen receptor protein levels and localization patterns were analyzed using immunoblotting and immunofluorescence assays. The effect of propofol on the proliferation of LNCaP cells was analyzed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays. Propofol significantly inhibited DHT-induced expression of androgen receptor target genes in a dose- and time-dependent manner, and immunoblotting and immunofluorescence assays indicated that propofol suppressed nuclear levels of androgen receptor proteins. Exposure to propofol for 24 h suppressed the proliferation of LNCaP cells, whereas 4 h of exposure did not exert significant effects. Together, our results indicate that propofol suppresses nuclear androgen receptor protein levels, and inhibits androgen receptor transcriptional activity and proliferation in LNCaP cells.

1. Introduction

Prostate cancer is one of the most frequently diagnosed cancers and ranks as the second leading cause of male cancer-related death in the United States (Siegel et al., 2016). One of the hallmarks of prostate cancer is its dependency on androgen and androgen receptor. Several reports have demonstrated that androgen and androgen receptor activation are essential not only for normal prostate growth and maintenance but for the development and progression of prostate cancer (Kim and Coetzee, 2004; Zhao et al., 2014). Androgen ablation therapies, such as medical or surgical castration, effectively treat most cases of primary prostate cancer. Unfortunately, however, most patients ultimately progress to a castration-resistant state (Loneragan and Tindall, 2011).

Androgen, a hormone primarily produced in the testis, is metabolized by 5 α -reductase to dihydrotestosterone (DHT), a molecule that binds to androgen receptor (Randall, 1994). DHT binding to androgen receptor induces androgen receptor homodimerization, thereby facil-

itating the translocation of the androgen receptor complex into the nucleus (Quigley et al., 1995). In the nucleus, the androgen receptor complex binds to specific DNA sequences referred to as androgen response elements, thereby promoting the transcription of androgen-responsive genes such as *prostate-specific antigen (PSA)*, *FK506 binding protein 5 (FKBP5)*, and *transmembrane protease serine 2 (TMPRSS2)* (Girling et al., 2007; Mostaghel et al., 2007). PSA is strongly associated with total prostate cancer volume and is regarded as the most reliable biomarker for monitoring the presence and progression of prostate cancer (Heinlein and Chang, 2004).

A number of studies evaluating the impact of anesthetics and anesthetic techniques on cancer have recently been reported (Byrne et al., 2017; Sekandarzad et al., 2016). Although surgical resection remains a standard treatment for cancer, surgical manipulation can release tumor cells into the circulation (Pesta et al., 2013). In addition, surgery itself and various secondary factors, including blood loss, acid-base balance disturbance, temperature change, and pain, could induce metabolic, neuroendocrine, and inflammatory responses, and those

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changes can modify tumor-specific immune responses (Wigmore et al., 2016). As these changes can promote metastasis, perioperative clinical management aimed at preventing cancer progression is considered an important component of cancer treatment. Previous studies evaluating the effects of anesthetics and opioids on cancer progression have reported conflicting results. For example, in vitro studies demonstrated that volatile anesthetics (Santamaria et al., 2010) and opioids (Forget et al., 2010) exert a suppressive effect on the activity of natural killer (NK) cells, cytotoxic lymphocytes that are critical for tumor cell immunity. In contrast, local anesthetics are reported to preserve NK cell activity and the T helper 1/T helper 2 (TH1/TH2) cell ratio in vitro (Wada et al., 2007). Consistent with these studies, local anesthesia combined with general anesthesia showed superior cancer-free survival rates compared with general anesthesia alone in prostate (Biki et al., 2008), breast (Exadaktylos et al., 2006), and ovarian cancer patients (de Oliveira et al., 2011). These findings suggest that anesthetics impact disease prognosis in multiple types of cancer. However, large-scale, prospective studies are required to determine which anesthetics and anesthetic techniques are optimal for different types of cancer.

In the current investigation, we studied the effect of propofol on prostate cancer cells. Propofol is one of the most commonly used drugs in the critical care setting and for the induction of general anesthesia and moderate and deep sedation intraoperatively. Recent studies indicated that propofol exerts antitumor effects in some cancers. For example, propofol induces apoptosis in cervical cancer cells via the mammalian target of rapamycin (mTOR) pathway (Zhang et al., 2015). However, another study demonstrated that propofol induces proliferation in gallbladder cancer cells (Zhang et al., 2012). Therefore, the effect of propofol appears to vary according to cancer cell type. Although the effect of propofol in prostate cancer remains unclear, one study demonstrated that propofol modulated the malignancy of PC3 prostate cancer cells (Huang et al., 2014). Androgen receptor is essential for prostate cancer progression, but the influence of general anesthetics on androgen receptor activity remains unknown. Therefore, we explored the effects of propofol in prostate cancer cells, with a specific focus on androgen receptor activity.

2. Material and methods

2.1. Cell lines

The androgen-dependent human prostate cancer cell line LNCaP (ATCC, Manassas, VA, USA) was maintained in Roswell Park Memorial Institute (RPMI) 1640 (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 0.1 mg/ml streptomycin, 100 U/ml penicillin, and 10% fetal bovine serum (FBS). Another androgen-dependent human prostate cancer cell line, VCaP, was generously provided to us by Dr. Makino (Department of Urology, Kyoto University Hospital). It was maintained in Dulbecco's modified Eagle's medium (Sigma-Aldrich) supplemented with streptomycin, penicillin, and 10% FBS. The media were changed after the first 3 days, and changed twice weekly thereafter. A few days before the experiments, the cells were cultured overnight in medium supplemented with 10% charcoal-stripped FBS. All cell cultures were maintained at 37 °C in a humidified incubator with 5% CO₂ and 95% air.

2.2. Reagents and chemicals

Propofol (2,6-diisopropylphenol) (PubChem CID: 4943) and 5 α -dihydrotestosterone (DHT) (PubChem CID: 10635) were obtained from Sigma (St. Louis, MO, USA). Isoflurane (PubChem CID: 3763) was purchased from Abbvie (Tokyo, Japan). Sevoflurane (PubChem CID: 5206) was purchased from Mylan Pharmaceutical Co. Ltd. (Osaka, Japan) and Desflurane (PubChem CID: 42113) from Baxter (Tokyo, Japan). The gas mixture composed of 21% oxygen (O₂), 5% carbon dioxide (CO₂), and 74% nitrogen (N₂) was acquired from Taiyo

Nippon Sanso (Tokyo, Japan). CO₂ and N₂ gas were obtained from Kist Co. Ltd. (Kyoto, Japan).

2.3. Hypoxia exposure

Hypoxia was induced using a CO₂ multi-gas incubator (APM-30D; Astec, Fukuoka, Japan) flushed with 1% O₂, 5% CO₂, and 94% N₂ at 37 °C. The cell lines were maintained in an atmosphere with 1% O₂ for 4 h immediately after propofol was administered.

2.4. Real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA was isolated using a NucleoSpin[®] RNA II kit (Macherey-Nagel, Düren, Germany). First-strand cDNA synthesis was conducted using a One Step SYBR[™] PrimeScript[™] RT-PCR Kit II (Takara Bio, Shiga, Japan) according to the manufacturer's instructions. qRT-PCR assays were conducted using the 7300 Real-Time PCR System (Applied Biosystems, CA, USA). The PCR primers used to amplify *glucose transporter 1* (*GLUT1*) and *lactate dehydrogenase* (*LDHA*) were obtained from Qiagen (Valencia, CA, USA). All other PCR primers were purchased from Invitrogen (Carlsbad, CA, USA). The sequences of the PCR primers were as follows: *PSA*, 5'-CCCACACCCGCTCTACGATA-3' (forward) and 5'-ACCTCTGAGG-GTGAACITGCG-3' (reverse); *FKBP5*, 5'-GAATACACCAA-AGCTGTTGA-3' (forward) and 5'-CTCTTCCTTGGCATCCT-3' (reverse); *TPRSS2*, 5'-CTGCCAAGGTGCTTCTC-3' (forward) and 5'-TTAGCCGTCTGCCCTC-3' (reverse); and *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*), 5'-GGCCTCCAAGGAGGAAGACC-3' (forward) and 5'-AGGGGTCTACATGGCAACTG-3' (reverse). *GAPDH* was used as an internal control to calculate corrected C_t values. All of the PCR assays were conducted in triplicate.

2.5. PSA enzyme-linked immunosorbent assay (ELISA)

PSA levels in supernatants from LNCaP cells were measured using the RayBio[®] Human PSA-total ELISA Kit (Ray Biotech, Norcross, GA, USA) according to the manufacturer's instructions.

2.6. Immunoblotting assay

Whole-cell lysates were isolated as previously described (Tanaka et al., 2011). LNCaP cells were harvested and washed with phosphate-buffered saline (PBS) and centrifuged at 1100g for 5 min. Cell pellets were resuspended in ice-cold lysis buffer composed of 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.1% sodium dodecyl sulfate (SDS), 1% Nonidet P-40, 2 mM DTT, 5 mM EDTA, 1 mM sodium orthovanadate, and cOmplete[™] Protease Inhibitor Cocktail (Roche Diagnostics, Tokyo, Japan). The cell lysates were centrifuged at 20,400g for 10 min at 4 °C, and the supernatants containing the whole-cell lysates were subsequently collected. Nuclear and cytoplasmic LNCaP cell extracts were isolated using a Nuclear Extraction Kit (Active Motif, Carlsbad, CA, USA). Proteins (100 μ g) were resolved using 7.5% SDS-polyacrylamide gel electrophoresis (SDS/PAGE), and the separated proteins were electrotransferred to polyvinylidene difluoride membranes in transfer buffer. The membranes were probed with the following primary antibodies: rabbit monoclonal anti-androgen receptor (#5153; Cell Signaling, Stockholm, Sweden), mouse monoclonal anti-hypoxia-inducible factor (HIF)-1 α (#610959; BD Bioscience, San Jose, CA, USA), mouse monoclonal anti-HIF-1 β (#611078; BD Bioscience), mouse monoclonal anti- β -actin (A5316; Sigma-Aldrich), and rabbit polyclonal anti-lamin A/C (#2032; Cell Signaling). The membranes were probed with antibodies diluted in Tris buffered saline with Tween-20 (TBS-T) supplemented with 5% non-fat dry milk overnight at 4 °C, and subsequently incubated with horseradish peroxidase (HRP)-conjugated anti-rabbit or anti-mouse secondary antibodies (GE Healthcare,

Piscataway, NJ) for 1 h. The signals were detected using an enhanced chemiluminescence reagent (GE Healthcare).

2.7. Cell viability assay

Cell viability was quantified using a Cell Proliferation Kit I (Roche Diagnostics). The cells were seeded in a 96-well plate. After 48 h, the cells were treated with DHT and propofol. Ten μ l of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) labeling reagent was added to each well at the indicated time points, and the samples were further incubated for 4 h in a humidified atmosphere. Then, solubilization solution was added to each well, and the plate was further incubated overnight at 37 °C. A plate reader was used to read the absorption at 590 nm using a reference wavelength of 650 nm. The assay was conducted in triplicate.

2.8. Immunofluorescence assay

LNCaP cells were fixed in 4% paraformaldehyde for 10 min. Fixed cells were permeabilized with PBS supplemented with 0.1% Tween-20 for 10 min and washed 2 times (5 min per wash). Then, the cells were blocked with serum-free protein-blocking solution supplemented with 0.1% cold fish skin gelatin and subsequently incubated with the primary antibody (1:600) overnight at 4 °C. Then, the cells were incubated with Alexa Fluor® 488-conjugated secondary antibody (1:1000) (ThermoFisher Scientific, Yokohama, Japan) for 1 h at room temperature. After the cells were washed twice with PBS (5 min per wash), DNA was stained with 4',6-diamidino-2-phenylindole (DAPI). The stained cells were visualized under a Nikon TE300 microscope (Nikon, Tokyo, Japan).

2.9. Statistical analysis

All data associated with continuous variables are presented as the mean $\pm$ standard deviation (S.D.). We have used data from our recent similar studies and confirmed normal distribution. Differences between groups were analyzed using one-way analysis of variance followed by the Student-Newman-Keuls test if appropriate, and otherwise we used Wilcoxon *t*-test with Bonferroni correction. Statistical significance was defined as $P < 0.05$.

3. Results

3.1. Propofol inhibits DHT-induced upregulation of PSA expression in LNCaP cells

To analyze the effect of propofol on PSA expression, LNCaP cells were exposed to propofol in the presence or absence of DHT. PSA expression levels significantly increased in DHT-treated cells, and propofol inhibited this effect in a concentration-dependent manner (Fig. 1A). The inhibitory effect of propofol was observed at a concentration as low as 10 μ M (1.8 μ g/ml), which corresponds to a clinically relevant concentration (Siddiqui et al., 2005). Next, we conducted a time course analysis of the effect of propofol on PSA expression. PSA expression in DHT-treated cells in a time-dependent manner, and propofol significantly suppressed this effect (Fig. 1B). We verified the effect of propofol on PSA protein levels in LNCaP cells using ELISA. PSA protein concentration significantly increased after 24 h of DHT exposure, and propofol suppressed this effect (Fig. 1C).

3.2. Propofol inhibits the expression of androgen-dependent genes in LNCaP cells

Next, we examined the effect of propofol on the expression of other androgen-dependent genes. *FKBP5* and *TMPRSS2* are androgen-dependent genes associated with prostate cancer progression (Girling

et al., 2007; Mostaghel et al., 2007). Propofol significantly inhibited the expression of both *FKBP5* (Fig. 2A) and *TMPRSS2* (Fig. 2B) in LNCaP cells exposed to DHT for 4, 8, and 24 h.

3.3. Propofol inhibits the expression of androgen-dependent genes in VCaP cells

To exclude the possibility that the inhibitory effect of propofol on androgen-dependent genes is limited to LNCaP cells, we performed the same series of experiments in another prostate cancer cell line, VCaP. In VCaP cells, DHT exposure significantly increased PSA expression levels and propofol significantly suppressed PSA induction, although the difference was not so distinct compared with LNCaP cells (Fig. 3A). In addition, *TMPRSS2* expression levels were significantly inhibited with propofol for 4 h but not *FKBP5* expression levels (Fig. 3B and C).

3.4. Propofol inhibits LNCaP cell viability

To evaluate the effect of propofol on LNCaP cell viability, we conducted MTT assays. Cell viability was not inhibited in LNCaP cells exposed to propofol for 4 h (Fig. 4A), whereas cell viability decreased in a concentration-dependent manner in LNCaP cells exposed to propofol for 24 h (Fig. 4B).

3.5. Volatile anesthetics inhibit androgen-dependent gene expression in LNCaP cells

Similar to propofol, volatile anesthetics are commonly employed during general anesthesia in the clinical setting. To determine if the inhibitory effect of propofol on androgen-dependent gene expression in prostate cancer cells is a common feature of other general anesthetics, we evaluated the effects of the volatile anesthetic isoflurane on androgen-dependent gene expression. As shown in Fig. 5A, isoflurane significantly suppressed DHT-induced upregulation of PSA expression in LNCaP cells. In addition, *FKBP5* and *TMPRSS2* expression levels were inhibited in DHT-exposed LNCaP cells treated with isoflurane for 8 h, but not in cells treated with isoflurane for 4 h (Fig. 5B and C). Currently, sevoflurane and desflurane are more commonly used as they show more rapid recovery than isoflurane. Next, we investigated the effect of sevoflurane and desflurane on androgen-dependent gene expression. Both drugs significantly suppressed PSA and *FKBP5* expression when exposed to DHT for 4 h (Fig. 5D and E). *TMPRSS2* expression was suppressed in LNCaP cells treated with desflurane but not with sevoflurane (Fig. 5F).

3.6. Propofol and isoflurane attenuate nuclear androgen receptor protein levels in LNCaP cells

After binding to androgenic hormones such as DHT, androgen receptor translocates to the nucleus where it functions as a transcriptional factor. To identify potential mechanisms mediating the inhibitory effect of anesthetics on DHT-induced androgen receptor-dependent gene expression, we assessed androgen receptor protein levels and localization patterns using immunoblotting and immunofluorescence assays. As shown in Fig. 6A, Total androgen receptor protein levels did not increase in whole-cell extracts of DHT-treated cells compared with untreated cells, and they were not suppressed with propofol or isoflurane. However, nuclear androgen receptor protein levels increased in DHT-treated cells, and both propofol and isoflurane significantly inhibited this effect. Consistent with the results of immunoblotting assay, immunofluorescence assays showed that propofol and isoflurane inhibited nuclear androgen receptor protein levels in DHT-treated cells (Fig. 6B).

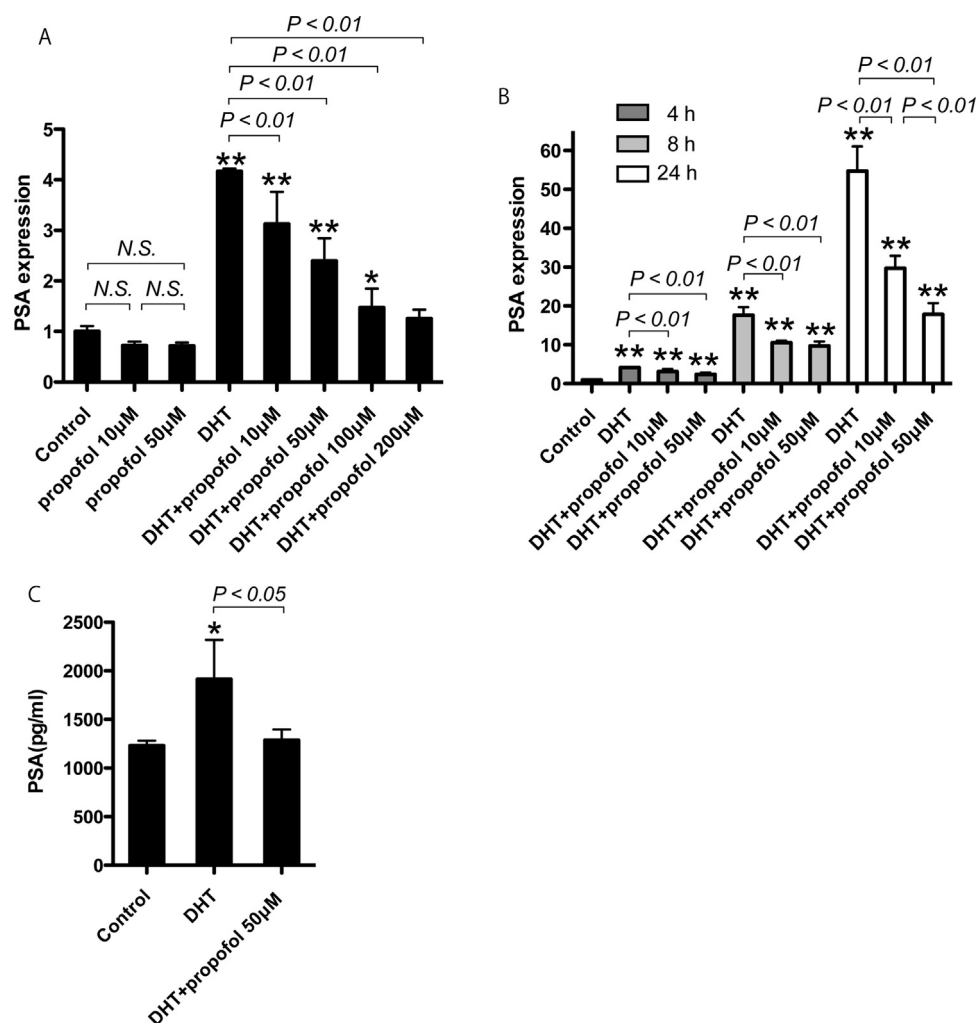


Fig. 1. Effect of propofol on the mRNA and protein expression of PSA in LNCaP cells. LNCaP cells were exposed to dihydrotestosterone (DHT) (10 nM) and propofol at various concentrations for (A) 4 h and (B) 4–24 h. PSA mRNA was assayed using real-time quantitative polymerase chain reaction (qRT-PCR). The expression levels of *GAPDH* mRNA were used as an internal standard to normalize the expression levels of *PSA*. (C) LNCaP cells were exposed to DHT with or without 50 μM propofol for 24 h. The concentration (pg/ml) of PSA proteins that were obtained from the culture media was measured using enzyme-linked immunosorbent assay (ELISA). Data are shown as mean ± S.D. (n = 3); ***P* < 0.01; **P* < 0.05 vs control; N.S., not significant.

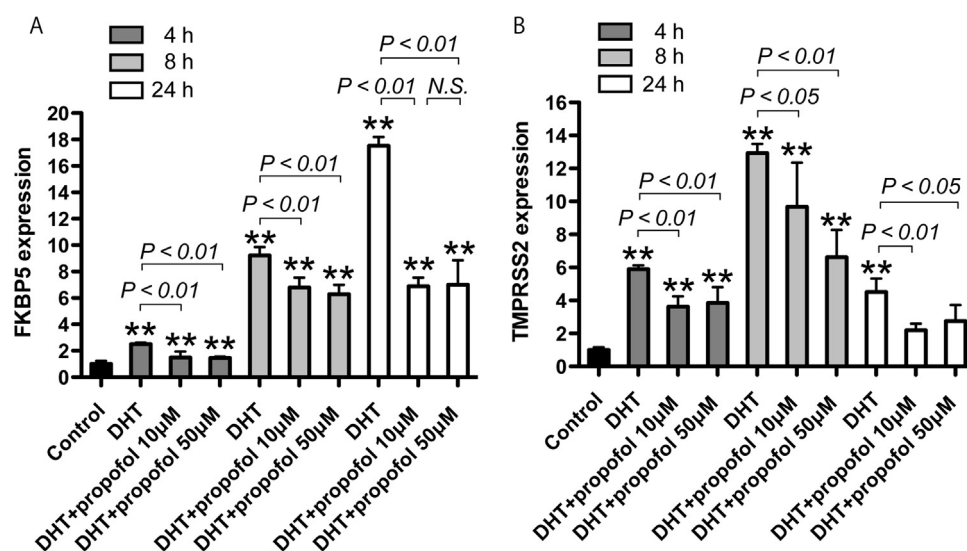


Fig. 2. Effect of propofol on the mRNA expression of androgen receptor target genes in LNCaP cells. LNCaP cells were exposed to DHT (10 nM) and propofol at indicated concentrations for 4–24 h. (A) *FKBP5* and (B) *TMPPSS2* mRNA were assayed using real-time qRT-PCR. The expression levels of *GAPDH* mRNA were used as an internal standard to normalize the expression levels of *FKBP5* and *TMPPSS2*. Data are shown as mean ± S.D. (n = 3); ***P* < 0.01 vs control; N.S., not significant.

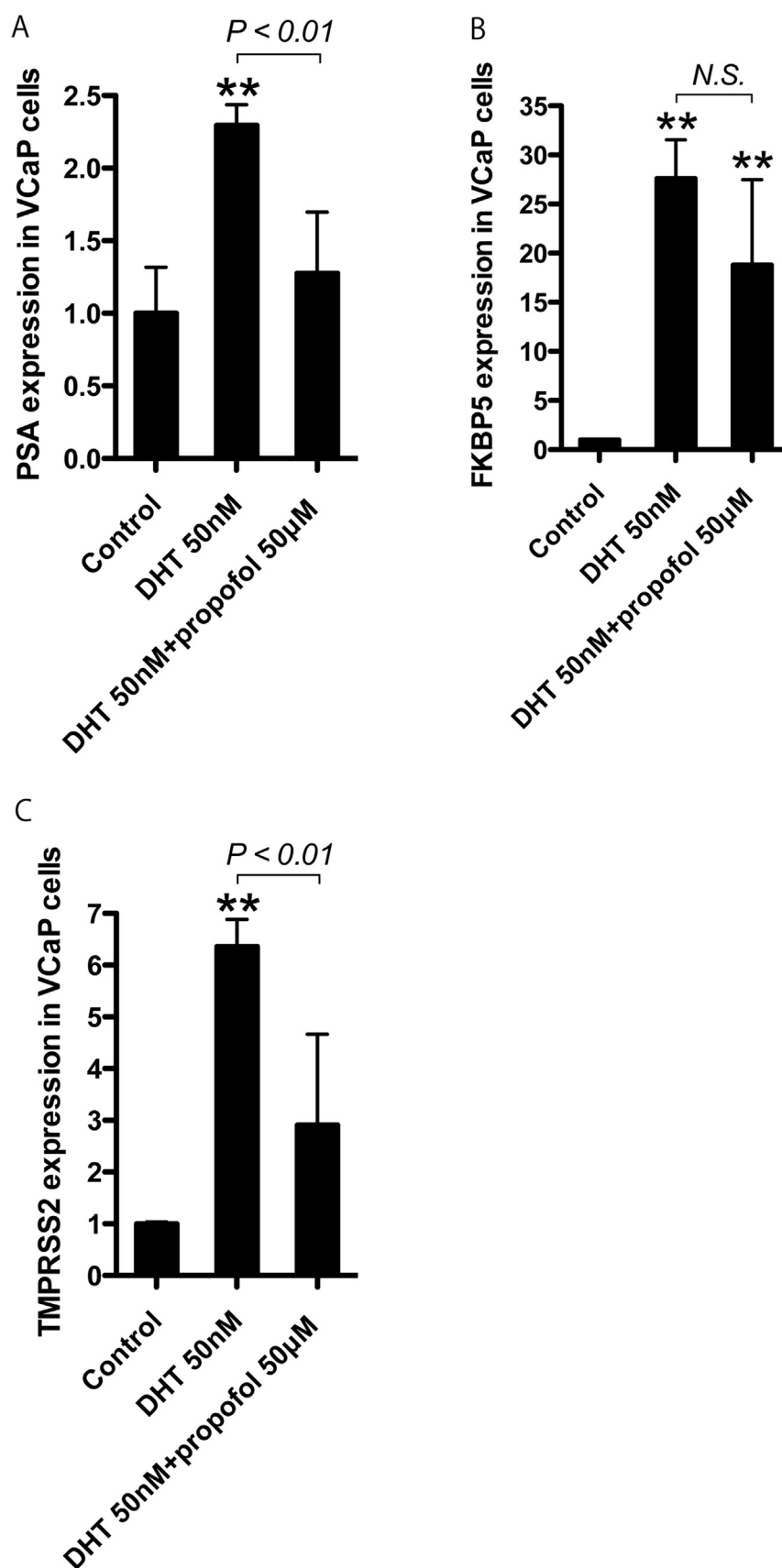


Fig. 3. Effect of propofol on the mRNA expression of androgen receptor target genes in VCaP cells. VCaP cells were exposed to DHT (50 nM) and propofol (50 μM) for 4 h. (A) PSA, (B) FKBP5, and (C) TMPRSS2 mRNA were assayed using real-time qRT-PCR. The expression levels of GAPDH mRNA were used as an internal standard to normalize the expression levels of PSA, FKBP5, and TMPRSS2. Data are shown as mean ± S.D. (n = 3); ** $P < 0.01$ vs control; N.S., not significant.

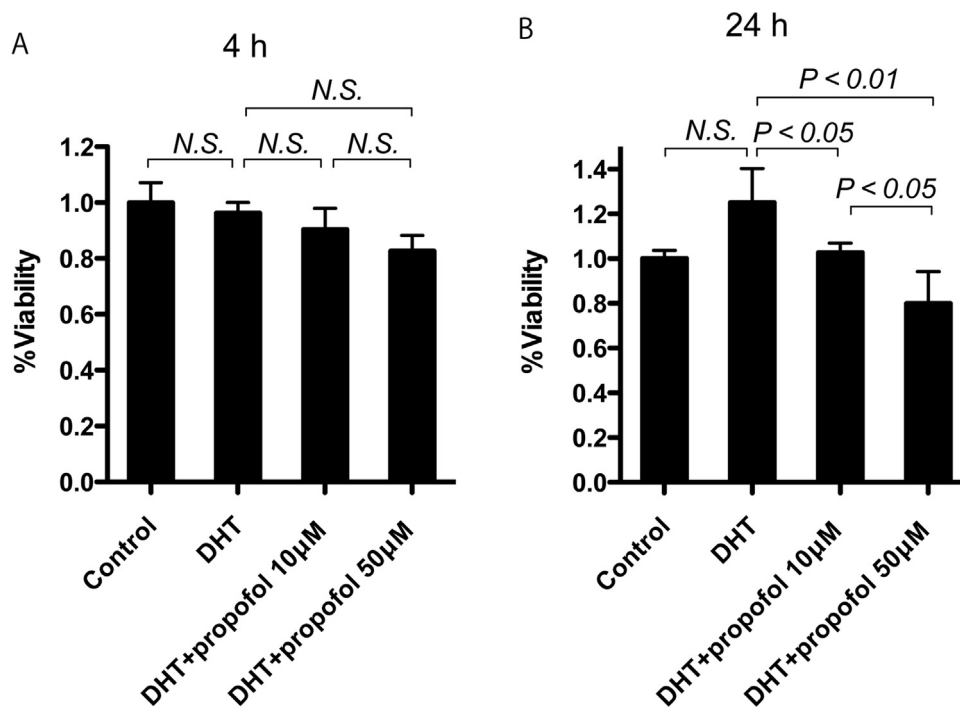


Fig. 4. Effect of propofol on cell viability. Cultured cells were treated with DHT (10 nM) and propofol (10 and 50 μM) for 4 h (A) or 24 h (B). Cell death was determined using the MTT assay. The result was shown as the relative ratio of control. Data are shown as mean $\pm$ S.D. (n = 3); N.S., not significant.

3.7. Propofol inhibits the expression of HIF-dependent genes and nuclear HIF protein levels in LNCaP cells under hypoxic conditions

Over the course of disease progression, regions of solid tumors often become hypoxic due to insufficient blood flow. By adapting to the hypoxic environment, tumors can progress and become more malignant (Greco et al., 2003; Hockel and Vaupel, 2001). Consistent with these observations, hypoxia is associated with a poor prognosis in multiple cancers, including prostate cancer (Milosevic et al., 2012). The transcription factor HIF is a key regulator of cellular adaptation to hypoxic conditions, and it regulates multiple genes involved in angiogenesis, metabolism, survival, and invasion (Semenza, 2010). To evaluate the effect of propofol on hypoxic prostate cancer cells, we exposed LNCaP cells to propofol under hypoxic conditions. As shown in Fig. 7A, propofol suppressed DHT-induced PSA upregulation in LNCaP cells under hypoxic conditions. In addition, hypoxia induced the expression of the HIF-dependent genes *GLUT1* and *LDHA* in the presence or absence of DHT, and propofol significantly inhibited this effect (Fig. 7B and C). HIF is a heterodimeric transcription factor composed of α and β subunits (Semenza, 2003). HIF-1 α is predominantly expressed under hypoxic conditions. Under these conditions, HIF-1 α translocates to the nucleus and binds to HIF-1 β , thereby becoming transcriptionally active (Semenza, 2003). Immunoblotting assays revealed that propofol suppressed nuclear levels of both HIF1 α and HIF-1 β under hypoxic conditions (Fig. 8).

4. Discussion

In the current study, we found that propofol significantly inhibited DHT-induced androgen receptor -dependent gene expression and suppressed nuclear androgen receptor protein levels in LNCaP cells. The development and proliferation of prostate cancer is strongly influenced by the transactivation of androgen receptor (Kim and

Coetzee, 2004; Zhao et al., 2014). Therefore, inhibiting androgen receptor activity is predicted to attenuate prostate cancer progression. We demonstrated that the viability of LNCaP cells was significantly suppressed by long-term exposure to propofol. Previous studies reported that propofol exerts antitumor effects in vitro in various types of cancer cells including osteosarcoma (Xu et al., 2016), pancreatic (Chen et al., 2017), lung (Cui et al., 2014), and cervical cancer cells (Zhang et al., 2015). However, the mechanism mediating the anti-cancer effects of propofol differed in each context. In contrast, volatile anesthetics have been reported to promote tumor cell progression. For example, isoflurane increases the malignant potential of ovarian cancer (Luo et al., 2015) and glioblastoma cells (Zhu et al., 2016). However, in the present study, both isoflurane and propofol suppressed the expression of androgen receptor-dependent genes and nuclear androgen receptor protein levels in LNCaP cells, suggesting that both types of general anesthetics suppress prostate cancer proliferation by inhibiting androgen receptor activity.

Clinical studies comparing general anesthesia plus epidural anesthesia with general anesthesia alone have reported conflicting results. For example, Scavonetto et al. reported that general anesthesia alone was associated with an increased risk of systemic progression (hazard ratio [HR] = 2.81) and higher overall mortality (HR = 1.32) compared with the combination of general and epidural anesthesia in more than 3000 patients with prostate cancer (Scavonetto et al., 2014). In contrast, another study reported that outcomes in prostate cancer patients were not affected by adjunctive epidural anesthesia (Tsui et al., 2010). Furthermore, a recent study detected no significant difference between general anesthesia and epidural anesthesia in prostatectomy outcomes (Sprung et al., 2014). Therefore, the differential effects of various anesthesia techniques in prostate cancer patients remains controversial, despite evidence from multiple studies demonstrating that local anesthesia inhibits cancer progression in vitro (Kim et al., 1997; Werdehausen et al., 2009). The anti-androgen receptor activity

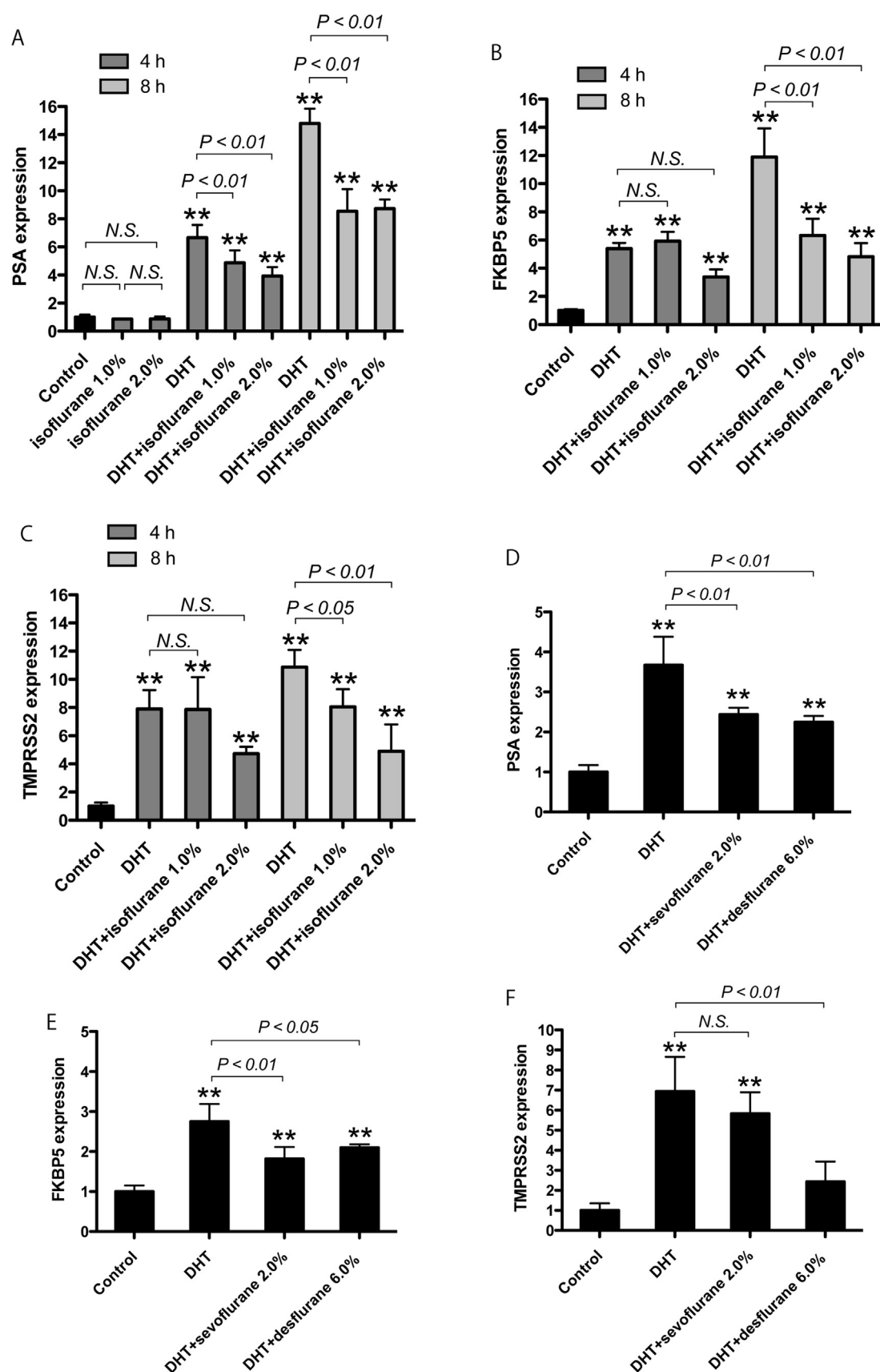


Fig. 5. Effect of volatile anesthetics on the mRNA expression of androgen receptor target genes in prostate cancer cells. LNCaP cells were exposed to DHT (10 nM) and volatile anesthetics (isoflurane, sevoflurane, and desflurane) for indicated concentrations. Sevoflurane and desflurane were administered for 4 h. (A, D) PSA, (B, E) FKBP5, and (C, F) TMPRSS2 mRNA was assayed using real-time qRT-PCR. The expression levels of GAPDH mRNA were used as an internal standard. Data are shown as mean $\pm$ S.D. (n = 3); ** P < 0.01 vs control; N.S., not significant.

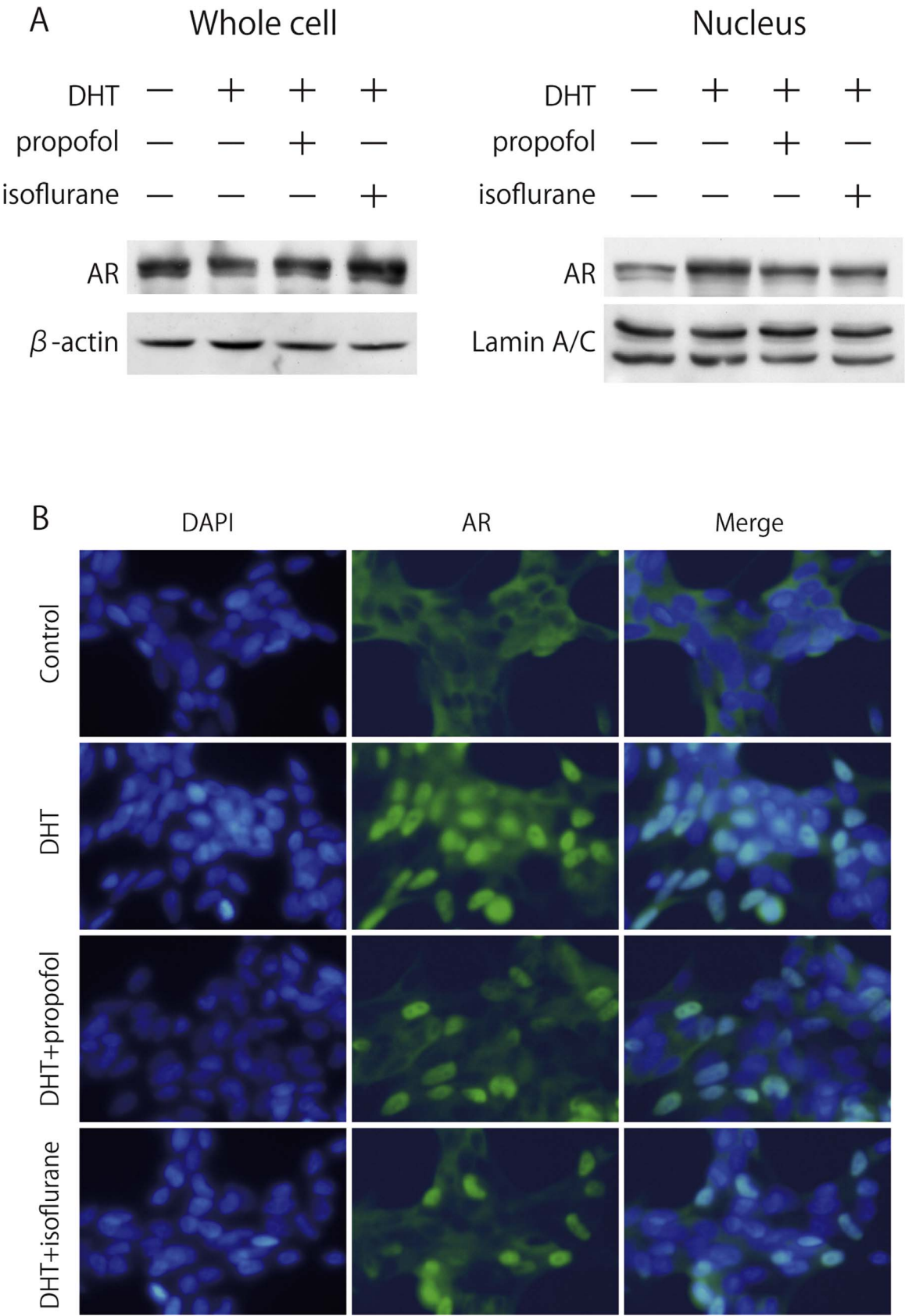


Fig. 6. Propofol and isoflurane inhibit DHT-stimulated nuclear expression of androgen receptor in prostate cancer cells. (A) LNCaP cells were treated for 8 h with 10 nM DHT in the presence or absence of 50 μ M propofol or 1% isoflurane immunoblotting for the expression levels of androgen receptor in the whole cell and the nuclear extracts was shown. (B) LNCaP cells were treated for 8 h with 10 nM DHT in the presence or absence of 50 μ M propofol or 1% isoflurane. Immunofluorescence staining was performed to investigate the intracellular distribution of androgen receptor (green) in LNCaP cells. DNA was stained with 4',6-diamidino-2-phenylindole to identify the nucleus (blue). Magnification: $\times 400$. Each experiment was repeated at least three times with comparable results. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

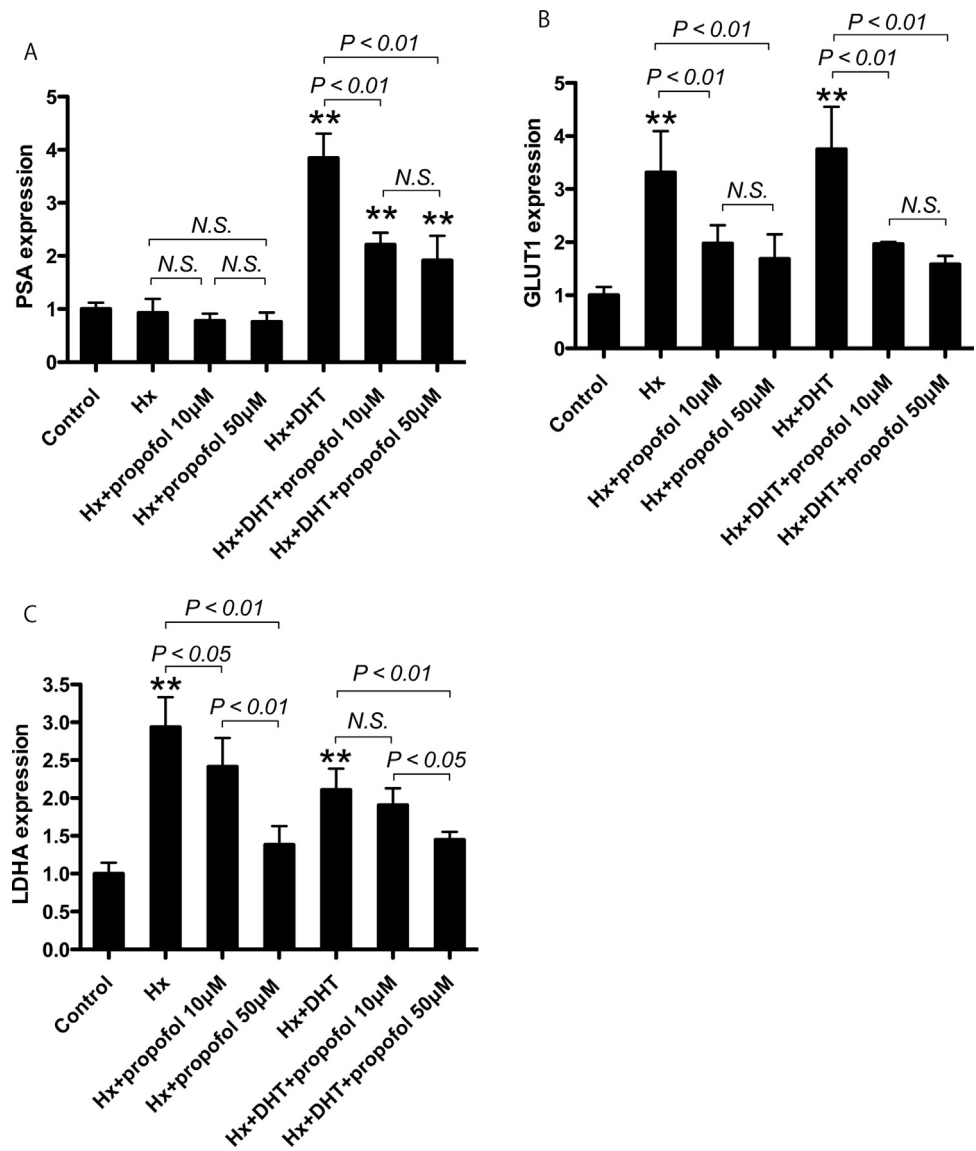


Fig. 7. Effect of propofol on the mRNA expression of PSA, GLUT1, and LDHA under hypoxic conditions. LNCaP cells were exposed to 4 h of 1% O₂ (hypoxia: Hx) and propofol with and without 10 nM DHT. In each case, controls were exposed to 4 h of 20% O₂. (A) PSA, (B) GLUT1, and (C) LDHA mRNA were assayed using real-time qRT-PCR. The expression levels of GAPDH mRNA were used as an internal standard. Data are shown as mean ± S.D. (n = 3); **P < 0.01 vs control; N.S., not significant.

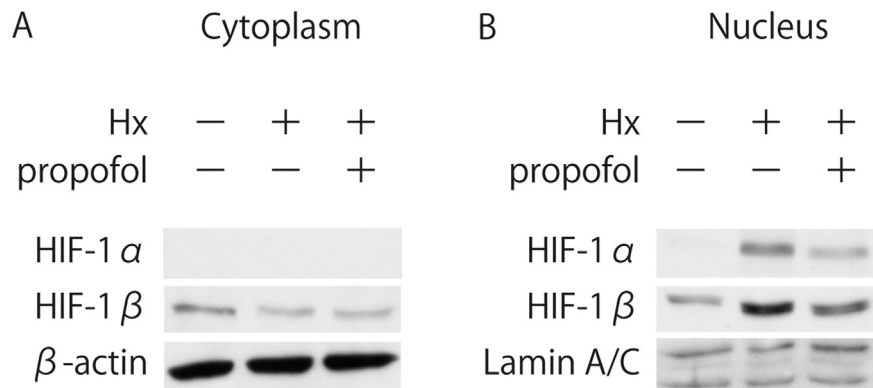


Fig. 8. Propofol inhibits the nuclear expression of HIF proteins in prostate cancer cells. Immunoblotting measured the expression levels of HIF-1α and HIF-1β in the cytoplasmic and the nuclear extracts. LNCaP cells were treated for 4 h with 1% O₂ in the presence or absence of 10 μM propofol. These experiments were repeated for at least three times, and the images in (A) and (B) signify the cytoplasmic and nuclear extract images, respectively.

of general anesthetics observed in the current study might be the reason why local anesthesia did not demonstrate significant effects in multiple clinical studies.

Similar to androgen receptor activity, hypoxia is another key mediator of prostate cancer (Fraga et al., 2015). Most locally advanced solid cancers contain hypoxic regions, and hypoxia contributes to more

aggressive and metastatic phenotypes (Greco et al., 2003; Hockel and Vaupel, 2001). Most genes essential for the adaptation of cancer cells to hypoxic conditions are regulated by the transcriptional factor HIF-1 (Semenza, 2003). HIF-1 is composed of an oxygen-sensitive α subunit (HIF-1 α) and a constitutively expressed β subunit (HIF-1 β) (Semenza et al., 1991). Under normal conditions, HIF-1 α is rapidly degraded via the ubiquitin-proteasome pathway. However, under hypoxic conditions, HIF-1 α is stabilized and transported to the nucleus where it forms a dimer with HIF-1 β (Wang et al., 1995). HIF-1 α expression is associated with tumor growth, progression, metastasis, and resistance to radiotherapy and chemotherapy (Deep et al., 2017; Lu and Kang, 2010). We found that propofol significantly suppressed nuclear HIF-1 levels and the expression of HIF-dependent genes in hypoxic conditions with or without DHT stimulation. As HIF-1 expression correlates with prostate cancer malignancy (Milosevic et al., 2012), the inhibitory effect of propofol on HIF-1 can provide therapeutic benefits. However, isoflurane has been reported to upregulate HIF-1 α protein levels in various types of cancer cells, including prostate cancer cells (Huang et al., 2014). In a retrospective study comparing volatile anesthesia and propofol in cancer-related surgery in a total of 11,395 patients, mortality was 50% greater with volatile anesthesia compared with propofol-based anesthesia (Wigmore et al., 2016). This difference might be associated with the differential effects of volatile anesthetics and propofol on HIF-1. However, a large-scale study comparing propofol-based and volatile anesthetic-based approaches is required to clarify this issue.

Androgen receptor translocates from the cytoplasm to the nucleus after binding to its ligands (Quigley et al., 1995). In the current study, although propofol did not significantly affect total androgen receptor protein levels, it significantly decreased nuclear androgen receptor levels. These findings suggest that propofol might affect the transport of androgen receptor proteins into the nucleus. A recent report demonstrated that the transport of androgen receptor proteins into the nucleus is mediated by microtubules (Thadani-Mulero et al., 2012). Androgen receptor is recruited to microtubules either directly or with the cooperation of the motor protein dynein, and is subsequently transported into the nucleus where it becomes transcriptionally active (Mistry and Oh, 2013). Microtubule-stabilizing drugs referred to as taxanes are commonly used to treat prostate cancer. Taxanes reportedly exert anti-cancer effects, at least in part, by disrupting the nuclear trafficking of androgen receptor proteins (Darshan et al., 2011). HIF-1 (Carbonaro et al., 2012) and glucocorticoid receptor (Harrell et al., 2004) are also transported into the nucleus via a microtubule-dependent system. In the current study, nuclear levels of both HIF-1 α and HIF-1 β decreased in LNCaP cells. Therefore, our results suggest that propofol might suppress nuclear levels of androgen receptor and HIF-1 by inhibiting microtubule-based transport. Microtubule-dependent transport is considered important not only for cancer progression but also for normal physiological functions such as neuronal activity or organ development (Schiavo et al., 2013; Vallee et al., 2012). Therefore, further investigation into the precise effects of propofol on microtubules is required.

As the limitation of the current study, the surgery for prostate cancer lasts for several hours, and mostly no longer than a dozen of hours. In our study, propofol attenuated the viability of LNCaP cells for 24 h and not 4 h. Isoflurane suppressed the induction of *FKBP5* and *TMPRSS* in 8 h and not 4 h. Therefore, in clinical settings, the administration time of general anesthetics may not be long enough for the anti-androgen receptor activity we observed in this study to have a practical effect. However, in the cases of critical care, general anesthetics, including propofol, could be administered for longer hours and could have a significant impact on a patient's prognosis.

In conclusion, our study demonstrated that propofol suppresses androgen-androgen receptor complex-mediated transcriptional activity and attenuates nuclear androgen receptor levels in LNCaP cells. In addition, propofol suppressed the nuclear accumulation of HIF-1 α and

HIF-1 β under hypoxic conditions. As androgen receptor and HIF transactivation is associated with prostate cancer growth and metastasis, propofol might confer substantial clinical benefits to patients with prostate cancer. However, a prospective clinical study is required to confirm this hypothesis.

Authors' contributions

K.T. performed most of the experiments and wrote the manuscript. A.H., H.D., T.M. and N.T. helped in conducting the experiments. T.T. supervised the experiment, and wrote the manuscript.

Declaration of interest

None declared.

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