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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 (Lonergan and Tindall, 2011).

Androgen, a hormone primarily produced in the testis, is metabolized by 5α-reductase to dihydrotestosterone (DHT), a molecule that binds to androgen receptor (Randall, 1994). DHT binding to androgen receptor induces androgen receptor homodimerization, thereby facilitating 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% CO_2 and 95% air.

2.2. Reagents and chemicals

Propofol (2,6-diisopropylphenol) (PubChem CID: 4943) and 5α-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 (O_2), 5% carbon dioxide (CO_2), and 74% nitrogen (N_2) was acquired from Taiyo Nippon Sanso (Tokyo, Japan). CO_2 and N_2 gas were obtained from Kist Co. Ltd. (Kyoto, Japan).

2.3. Hypoxia exposure

Hypoxia was induced using a CO_2 multi-gas incubator (APM-30D; Astec, Fukuoka, Japan) flushed with 1% O_2, 5% CO_2, and 94% N_2 at 37 °C. The cell lines were maintained in an atmosphere with 1% O_2 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′-ACCTICTGAGG-GTGAACTTGCG-3′ (reverse); *FKBP5*, 5′-GAATACACCAA-AGCTGTTGA-3′ (forward) and 5′-CTCTTCCTTGGCATCCT-3′ (reverse); *TMPRSS2*, 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 1100*g* 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 20,400*g* 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 μg) 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,