Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Propofol Regulates HIF-1 α Effect of Expression of Targeted SIRT1 Signal pathway on Kidney Renal Clear Cell Carcinoma

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ARTICLE INFO

Original paper

Article history:
Received: February 9, 2023
Accepted: March 20, 2023
Published: March 31, 2023

Keywords:

Hypoxia-inducible factor-1α, Kidney clear cell carcinoma, Propofol, Signal pathway, Silence signal regulator 1

ABSTRACT

The objective of this study was to investigate the effect of propofol on kidney renal clear cell carcinoma (KIRC) by regulating hypoxia-inducible factor-1α (HIF-1α) expression and silencing signal regulatory factor 1 (SIRT1) signal pathway. In this regard, human KIRC cell line RCC4 was added into 0, 5 and 10 μ G/ml propofol treatment and was divided into a control group (CG), low dose group (LG) and high dose group (HG). CCK8 was used to detect the proliferative ability of the three groups of cells, ELISA was used to detect the level of inflammatory factors in the cells, Western blot was used to detect the protein expression, qPCR was used to detect the related mRNA expression level, and Transwell method was used to detect the invasive ability of the cells in vitro. The experimental results showed that propofol decreased the proliferation and invasion ability of KIRC cells, up-regulated the expression of TGF- β 1, IL-6, TNF- α, HIF-1 α, Fas, bax and FasL, and down-regulated the expression of SIRT1 in a dose-dependent manner. It was concluded that propofol can inhibit the SIRT1 signal pathway by up-regulating the level of HIF-1α in KIRC, which can significantly decrease the proliferation and invasion ability of KIRC cells, induce apoptosis and increase the release of intracellular inflammatory factors.

Doi: http://dx.doi.org/10.14715/cmb/2023.69.3.21

Introduction

Renal cell carcinoma (RCC) is a common malignant tumor in the urinary system (1). As one of the ten most common cancers in the world, the incidence of RCC is increasing at an average annual rate of 2% and 4% (2). Kidney renal clear cell carcinoma (KIRC) is the most common pathological subtype of RCC, accounting for about 75% of all RCCs (3). Surgical resection is an important method for the clinical treatment of KIRC, including radical nephrectomy, partial nephrectomy and so on. In the process of complex renal surgery, patients need to inject a higher dose of propofol for a long time to complete anesthesia to ensure a safe and smooth operation. As one of the commonly used intravenous general anesthesia drugs, propofol has not only strong sedative and anesthetic effects but also strong antioxidant stress and anti-anxiety effects. Propofol also has the effect of inhibiting cancer cell invasion, proliferation, adhesion and inducing cancer cell apoptosis (4). Hypoxia-inducible factor-1 α (HIF-1 α) is a transcription factor that can induce epithelial-mesenchymal transformation of solid tumors, which in turn can promote tumor cell infiltration and metastasis (5). It has been found that propofol may affect the process of cell energy metabolism through targeted regulation of HIF-1 α (6). In addition, signal regulator 1 (SIRT1) as the most important member of the silent information regulator family, which can regulate apoptosis and autophagy and oxidative stress by acetylating histone and non-histone proteins (7). The purpose of this study was to investigate the effect of propofol on renal clear cell carcinoma (RCC) by regulating the SIRT1 signal pathway targeting HIF-1 α expression, so as to clarify the molecular mechanism of the effect of propofol on renal clear cell carcinoma.

Materials and Methods

Human KIRC cell line RCC4 was purchased from the American ATCC cell bank. 0.25% EDTA trypsin, penicillin-streptomycin double antibody, fetal bovine serum and DMEM medium were all purchased from ThermoFisher-Scientific Company of the United States. HIF-1 α, SIRT-1mRNA primers and β-actin primers (Sigma Co., Ltd.); qPCR detection kit, immunohistochemical sheep anti-rabbit second antibody, CCK-8 detection kit (Shanghai Biyuntian Company); IL-6, TNF- α, IL-1β enzyme-linked immunosorbent assay kit (Shanghai Biyuntian Co., Ltd.). HIF-1 α, SIRT1, Fas, bax, FasL, bcl- 2 antibodies (British Abcam Biotechnology Co., Ltd.); rat DUSP5 gene overexpression lentivirus needed in this study (Guangzhou Saiye Biotechnology Co., Ltd.).

Cell culture and treatment

After resuscitation, the cells were inoculated in a T25 culture bottle with DMEM medium containing 10% fetal bovine serum and cultured in a constant temperature incubator (37 ℃, 5% CO_2). Trypsin digestion, passage and cryopreservation were carried out according to cell growth.

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Set up a control group (CG), low-dose group (LDG) and high-dose group (HDG). The cells in the CG were cultured routinely without any treatment, and the cells in LDG and HDG were treated with propofol at 5 and 10 μg/mL, respectively.

The proliferation of cells was detected by CCK8

Cells were treated with propofol for 48 hours. 10 μ l CCK8 solution was added to each well. After being cultured in a constant temperature incubator (37 ℃, 5% CO_2) for 4 hours, the proliferation of cells in each group was detected by enzyme labeling (OD450nm).

Detection of inflammatory cytokines in cells by ELISA method

The cell density was adjusted to 1×10^6/well and inoculated in a 6-well plate and cultured in a constant temperature incubator (37 ℃, 5% CO_2). After 48 hours of treatment with propofol in the low-dose group and high-dose group, the levels of IL-6, TNF-α and IL-1β were detected at OD 450nm.

Detection of protein expression by Western blot

The cell density was adjusted to 1×10^6/well and inoculated in a 6-well plate and cultured in a constant temperature incubator (37 ℃, 5% CO_2). The LDG and HDG were treated with propofol for 48 h, then the cell protein lysate was homogenized at 4 ℃ to make 10% homogenate, then the supernatant was centrifuged to determine the protein concentration, gel preparation, electrophoresis 90min, gel cutting, membrane transfer 90min, milk sealing. After cleaning, they were incubated with HIF-1 α, SIRT1, Fas, bax, FasL, bcl-2 primary and secondary antibodies, and developed, and the results were analyzed by Bio-Rad image laboratory software.

qPCR to detect the expression level of related mRNA

The cell density was adjusted to 1×10^6/well and inoculated in a 6-well plate and cultured in a constant temperature incubator (37 ℃, 5% CO_2). After 48 hours of treatment with propofol in LDG and HDG, RNA was extracted by RNA extraction kit, miRNA was reverse transcribed into cDNA by One Step Prime Script miRNA cDNA synthesis kit, quantitative real-time PCR was performed by miRNA fluorescence quantitative PCR detection kit, and the cycle was completed according to the kit instructions. After the reaction was completed, the relative expressions of HIF-1 α and SIRT1mRNA were calculated in the software.

Detection of invasive ability of cells *in vitro* by Transwell method

Adjust the cell density to 5×10^5 cells per well and inoculate it in the upper chamber of Transwell and the complete medium of 10% fetal bovine serum in the lower chamber. The LDG and HDG were treated with propofol for 48 hours, while the control group was given the same amount of DMEM culture medium, fixed and stained, and the purple-stained transmembrane cells were counted under the microscope.

Statistical method

Statistical methods: statistical software SPSS 22.0 was used for data processing and analysis, measurement data were expressed by ($\bar{x}\pm s$), F-variance analysis was used for multi-group comparison, and LSD-t test was used for pairwise comparison between multiple groups. Compared with CG: $^aP<0.05$; compared with the LDG: $^bP<0.05$.

Results

Effects of propofol on proliferation and invasion of KIRC cells

The activity of cell proliferation and the number of cell invasions in the LDG were reduced than those in CG ($P<0.05$), and those were reduced in HDG than in LDG and CG ($P<0.05$) (Table 1).

Effect of propofol on the expression of the apoptotic protein in KIRC cells

The expression of apoptotic protein Fas, bax and FasL in the LDG was higher, while the expression level of bcl-2 was reduced than that in CG. The expression level of apoptotic protein Fas, bax and FasL in HDG was raised than that in LDG and CG, while the expression level of bcl-2 was reduced than that in LDG and CG ($P<0.05$) (Table 2).

Effect of propofol on the level of inflammatory factors in KIRC cells

The levels of TGF-β1, IL-6 and TNF-α in LDG were raised than those in CG, while those were higher in HDG

Table 1. Effects of propofol on proliferation and invasion of KIRC cells.

Group	Sample	Proliferation ability	Number of cell invasion
CG	6	0.22±0.04	121.05±20.26
LDG	6	0.18±0.03[a]	103.12±19.35[a]
HDG	6	0.10±0.01[ab]	51.72±10.31[ab]
F		25.846	26.157
P		0.000	0.000

Table 2. The expression of the apoptotic protein in KIRC cells.

Group	Sample	Fas	bax	FasL	bcl-2
CG	6	0.35±0.14	0.37±0.09	0.41±0.14	0.54±0.03
LDG	6	0.63±0.17[a]	0.63±0.14[a]	0.64±0.24[a]	0.39±0.04[a]
HDG	6	0.84±0.18[ab]	0.86±0.16[ab]	0.78±0.12[ab]	0.31±0.03[ab]
F		13.446	20.294	18.641	72.177
P		0.001	0.000	0.000	0.000

than in LDG and CG (P<0.05) (Table 3).

Effect of propofol on the relative expression of HIF-1 α and SIRT1mRNA in KIRC cells

The HIF-1α mRNA expression in LDG was raised than that in the CG, while the relative expression of SIRT1 mRNA in HDG was higher than that in the LDG and CG, while the relative expression of SIRT1mRNA in HDG was higher than that in LDG and CG (P<0.05) (Table 4).

Effect of propofol on HIF-1 α and SIRT1 protein expression in KIRC cells

The HIF-1α protein expression was raised and SIRT1 protein expression was reduced in LDG than those in CG, while the HIF-1α protein expression was reduced and SIRT1 protein expression was raised in HDG than those in LDG and CG (P<0.05) (Table 5).

Discussion

KIRC has a high degree of malignancy, rapid progress, and strong invasiveness, and is not sensitive to radiotherapy and chemotherapy (8). During the perioperative period, patients undergoing radical nephrectomy are easily affected by immune, endocrine system dysfunction and other factors, resulting in abnormal tumor spread and seriously affecting the prognosis of patients. According to recent studies, it has been found that narcotic drugs can have a certain effect on the malignant potential of tumor cells. Propofol is a commonly used intravenous anesthetic in the clinic, which is widely used to maintain and induce anesthesia during radical nephrectomy. Studies have found that propofol is especially suitable for perioperative anesthesia in cancer surgery. It can affect the proliferation, invasion, and apoptosis of many kinds of tumor cells through different signal pathways or gene fragments (9). In the process of complex renal surgery, patients need to inject a high dose of propofol for a long time to complete anesthesia and ensure a safe and smooth operation. However, there are few reports on whether propofol affects the proliferation, invasion, apoptosis, and specific mechanism of human KIRC cells (10). This study investigated the propofol effect on KIRC by regulating the SIRT1 signal pathway targeting HIF-1 α expression, so as to clarify the molecular mechanism of the effect of propofol on KIRC.

The results showed that the cell proliferative activity and the number of cell invasions in the LDG were lower than those in the CG, while those in the HDG were lower than those in the LDG and CG. It is suggested that propofol can significantly decrease the proliferation and invasion of KIRC cells in a dose-dependent manner. The higher the concentration of propofol, the stronger the proliferation and invasion ability of KIRC cells. It has been found that anesthetics can participate in the pathological and physiological processes of tumor cells by affecting angiogenesis and mediating cell proliferation and invasion (11). Other studies have found that the abnormal expression of Fas pathway (exogenous apoptosis pathway) is an important factor in inhibiting apoptosis. Fas and its ligand FasL belong to the cell surface receptors of the tumor necrosis factor family, and their interaction leads to ligand-mediated cell death. The down-regulation of Fas and its ligand FasL or the weakening of signal transduction are related to the development of tumors (12,13). Bax is one of the most representative pro-apoptotic molecules, and bcl-2 is an anti-apoptotic molecule, which is closely related to tumor cell apoptosis. Bcl-2/Bax is often used to evaluate the level of tumor cell apoptosis (14,15). The expression levels of apoptotic protein Fas, bax and FasL were raised and bcl-2 was reduced in LDG than those in CG, while the expression

Table 3. Inflammatory factors level in KIRC cells ($pg \cdot L^{-1}$).

Group	Sample	IL-6	TNF-α	TGF-β1
CG	6	97.86±11.28	50.71±8.22	9.34±1.76
LDG	6	150.37±16.09[a]	121.41±13.14[a]	16.36±2.13[a]
HDG	6	180.28±18.04[ab]	170.33±17.11[ab]	30.28±4.84[ab]
F		44.036	122.147	65.827
P		0.000	0.000	0.000

Table 4. HIF-1α and SIRT1mRNA expressions in KIRC cells.

Group	Sample	HIF-1α mRNA	SIRT1 mRNA
CG	6	1.24±0.32	2.59±0.67
LDG	6	1.65±0.40[a]	1.76±0.43[a]
HDG	6	2.47±0.54[ab]	0.83±0.35[ab]
F		12.744	18.451
P		0.000	0.000

Table 5. HIF-1 α and SIRT1 protein expression in KIRC cells.

Group	Sample	HIF-1α	SIRT1
CG	6	0.18±0.04	0.83±0.25[a]
LDG	6	0.44±0.11[ab]	0.49±0.17[ab]
HDG	6	0.85±0.27[a]	0.13±0.03
F		23.716	23.896
P		0.000	0.000

levels of apoptotic protein Fas, bax and FasL were raised and bcl-2 was reduced in HDG than that in LDG and CG. It is suggested that propofol can significantly increase the apoptosis level of KIRC cells in a dose-dependent manner. The higher the concentration of propofol is, the stronger the apoptosis-promoting ability of propofol is. In addition, activating the apoptosis pathway of tumor cells can induce the release of many inflammatory factors, which in turn activate the process of autophagy and promote the apoptosis of tumor cells (16). TGF-β1, IL-6 and TNF-α are all important inflammatory regulatory cytokines, among which TGF-β1 and TNF-α are endogenous cytokines, which can promote the release of proinflammatory factors by activating middle lymphocytes, macrophages, neutrophils, and other immune inflammatory cells, while IL-6 can induce acute inflammatory response by promoting the activation of lymphocytes in blood (17). The levels of TGF-β1, IL-6 and TNF- α in LDG were higher than those in CG, while the levels of TGF-β1, IL-6 and TNF-α in HDG were higher than those in LDG and CG. It is suggested that propofol can increase the level of inflammatory factors and further promote apoptosis in KIRC cells.

HIF-1α is a dimer transcription factor and an important cellular regulatory factor involved in the regulation of intracellular oxygen metabolism. Current studies have found that genes regulated by HIF-1α play a role in cell invasion and migration, extracellular matrix remodeling, DNA damage and repair, tumor metabolism, apoptosis and autophagy, angiogenesis, tumor proliferation and other key links in tumorigenesis and development (18). Other studies have found that there is chromosome 3p abnormality in KIRC, which leads to the abnormality of tumor suppressor gene VHL, which leads to the inability of HIF-1α to bind to E3 ligase, resulting in sustained high expression of HIF-1ct protein, cell cycle imbalance and apoptosis, resulting in the occurrence and development of tumor (19). Propofol can up-regulate HIF-1α, which mediates the response of many kinds of cells to hypoxia. SIRT1 is widely found in mammalian tissues and cells. It is an NAD^+-dependent protein deacetylase that can be modified by transcription factors, acetylated histone and other proteins (20-25). The HIF-1α expression was raised and SIRT1 expression was reduced in LDG than those in CG. It is suggested that propofol can inhibit the SIRT1 signal pathway by up-regulating the level of HIF-1α in KIRC.

To sum up, propofol can inhibit the SIRT1 signal pathway by up-regulating the level of HIF-1 α in KIRC, resulting in a decrease in the ability of proliferation and invasion of KRC, induce apoptosis and increase the release of intracellular inflammatory factors. This experimental study provides a theoretical basis for the selection of anesthetics in patients with KIRC.

Fundings

The research is supported by: This work was sponsored by the Natural Science Foundation of Fujian Province, China(No. 2022J011319) and the key Clinical Specialty Discipline Construction Program of Fuzhou, Fujian, China.

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