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Propofol inhibits Wnt/ β -catenin SignalingPathway by Up-regulating mir-219-5b and Its Effect on the Biological Behavior of HepatomaCells

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Abstract

Objective: The purpose of this study was to analyze the effect of propofol on the behavior related to the biological of hepatoma cells by up-regulating the signal pathway of Wnt/ β -Catenin in wingless MMTV integrated website family members (Wnt) by microrna-219-5b (mir-219-5b).

Methods: hepatoma cell lines Huh7 and SMMC7721 of human were collected. Some hepatoma cell lines Huh7 of human and SMMC7721 were casually divided into the blank control group (treated with 0 μ M/ml propofol), the low propofol concentration group (treated with 2 μ M/ml propofol), the medium propofol concentration group (treated with 5 μ M/ml propofol), and the high propofol concentration group (treated with 10 μ M/ml propofol). The expression level of mir-219-5b protein in each group was compared, and propofol concentration closest to clinical treatment was taken for follow-up experiment. The remaining human hepatoma cell lines, Huh7 and SMMC7721, were randomly divided into three groups: negative control group (transferred into empty corpuscle), propofol group (treated with 5 μ M/ml propofol), mir-219-5b-shrna group (decreased the expression level of mir-219-5b and treated with 5 μ M/ml propofol). Cell invasion (cell invasion number), migration ability (number of transmembrane cells), the expression of proteins linked to the Wnt / β -catenin signaling pathway (β -catenin, c-myc) and the proliferation potential (OD value) were compared at day 1, day 2, day 3 and day 4.

Results: In Huh7 and SMMC7721 cells the mean concentration propofol and the high concentration propofol group expression level of mir-219-5b was meaningfully higher than the control group of the blank. So Propofol group concentration is similar to clinical treatment concentration. There was no substantial difference in miR-219-5b expression in Huh7 and SMMC7721 cell lines between the low concentration propofol group and the blank control group. At 2D , 3D and 4D, the 0d values in the propofol group for Huh7 and SMMC7721 cells were meaningfully lower than the negative control group ($P<0.05$). At 1D , 2D, 3D and 4D, there was no substantial difference between mir-219-5b-shrna group as well as negative control group in the 0d value of Huh7 and SMMC7721 cells. The number of propofol group Huh7 and SMMC7721 invasion cells and transmembrane cells was significantly lower than those in the negative control group. In propofol therapy, Huh7 and SMMC7721 cells had meaningfully lower levels of protein in β -Catenin and c-myc than the negative control group. In addition, no noteworthy difference in protein levels of Huh7, SMMC7721 β -Catenin, and c-myc between the mir-219-5b-shrna group as well as negative control group. Conclusion: propofol can inhibit Wnt / β -catenin's signaling pathway by up-regulating the expression of mir-219-5b and then hinder the proliferation, along with migration, as well as invasion of hepatoma cells.

Keywords: Propofol, mir-219-5b, Wnt, β -Catenin.

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1. Introduction

Liver cancer occurs in Asia and Africa. In recent decades, however, the incidence of liver cancer has increased year by year in western developed countries, especially in the United States and Canada. Liver cancer has become one of the most common diseases in China; its mortality accounts for about 51% of the deaths worldwide, with an extremely highmalignancy (Cai et al., 2018). With the rapid development of society and economy, the medical technology has also been improved accordingly, and surgery and liver transplantation are used in the clinical treatment of liver cancer patients. However, metastasis or recurrence occurs in more than 70% of patients after treatment (Ketevan Mazmishvili. et al., 2018). At present, clinicopathologic features such as tumor differentiation and tumor diameter are known to correlate with prognosis. Nevertheless, due to perioperative surgery and immunosuppressive reasons, it has become a critical moment for tumor spread and metastasis; in addition, other anti-tumor treatments cannot be used after surgery, resulting in an increased risk of recurrence and metastasis (Meng-Xi Liu et al., 2018)(Zhihu (Jeff) Ding et al., 2018). As a result, there is an urgent need to investigate the molecular mechanisms involved in liver cancer and its recurrence and metastasis, so as to improve the prognosis and 5-year survival rate of patients.As an intravenous anesthetic, propofol has the advantages of good sedative effect, rapid hypnotic and forgetful function, short duration of action and few adverse reactions, so it has been widely used in clinical treatment (David A. et al., 2019). It has been reported that patients with propofol anesthesia habe better prognosis and longer survival time, but the mechanism of its action on tumors is not yet clear (Jing Wang et al., 2019). Clinical studies have suggesting that propofol has the role of inhibiting tumor invasion and metastasis in the development and occurrence of hepatic cancer (Takahiro Yoshida & Nikolai A., 2018). MicroRNA-219-5b (miR-219-5b) is part of a family of miRNAs. The past literature have confirmed that miR-219-5b can inhibit ovarian cancer cell growth-based metastasis by controlling high mobility protein A2 (Zhou et al., 2018). Hence, the aim of this study was to investigate the effect and mechanism of propofol on the inhibition of hepatoma cells' biological activity by up-regulating the Wnt/β-catenin signaling pathway via miR-219-5b.

2. Materials and Methods

2.1. Experimental Materials

We obtained human hepatoma cells Huh7 and SMMC7721 from Shanghai Honsun Biological Technology Co., Ltd.). All cell lines were put in a medium DMEM and grown in a constant incubator at 37 ° C with a concentration of 5% carbon dioxide. The cells were taken out for testing while they were in good health.

2.2. Methods

(1) Some human hepatoma cells Huh7 and SMMC7721 were taken out and randomly divided into the blank control group (treated with the 0μm/mLpropofol), the low-concentration propofol group (treated with the 2μm/mLpropofol), the medium-concentration propofol group (treated with the 5μm/mLpropofol), and the high-concentration propofolgroup (treated with the 10μm/mLpropofol); western blot was used to detect the miR-219-5bexpression level in each group of cells, and thepropofol closest to the clinical treatment concentrationwas taken out for subsequent experiments.

(2) The remaining human hepatoma cells Huh7 and SMMC7721 were taken out and randomly divided into the negative control group (transferred into the empty vector plasmid), the propofol group (treated with the 5μm/mLpropofol), the miR-219-5b-shRNA group (down-regulation of the miR-219-5b expression level and treated with the 5μm/mLpropofol).

(3) Transwell experiment was used to test and compare the invasion and migration of the negative control group, thepropofol group and the miR-219-5b-shRNA group cells, respectively.

(4) MTT method was used to test and compare the cell proliferation (OD value) of the negative control group, the propofol group andthe miR-219-5b-shRNA group at 1d, 2d, 3dand 4d.

(5) Western blot was used to test and compare the white expression levels of Wnt/β-catenin signaling pathway related protein (β-catenin and C-Myc) in the negative control group, the propofol group and miR-219-5b-shRNA group.

2.3. Statistical methods

($\bar{x}\pm s$) is used to express the OD values of Huh7 and SMMC7721 cells in each group, the number of invasion cells and the number of transmembrane cells. The *t* test was used for comparison between the two groups, and the one-way varianceanalysis was used for comparison among multiple groups. In this study, all data were analyzed using SPSS19.0. If $P<0.05$, the difference was considered noteworthy.

3. Result

3.1. Effects of Different Concentrations of Propofol on the miR-219-5b Expression Level

The expression levels of miR-219-5b in the medium-concentration propofol group Huh7 and SMMC7721 cells, the high-concentration propofol group, were considerably higher than the blank control group (P<0.05). Additionally, the concentration of the propofol group of medium concentration was similar to the dose used in clinical practice. The levels of expression of miR-219-5b in the low-concentration propofol group Huh7 and SMMC7721 cell lines were not meaningfully different from those of the blank control group (P>0.05). See Table 1.

3.2. Comparison of the Proliferation of Huh7 and SMMC7721 Cells in Each Group

At 2d, 3d and 4d, the OD values of propofol group Huh7 and SMMC7721 cells were meaningfully lower than the negative control group. At 1d, 2d, 3d, and 4d, there was no noteworthy difference in the OD values of Huh7 and SMMC7721 cells in miR-219-5b-shRNA group compared with the negative control group. See Table 2.

Table 1: **Effects of Different Concentrations of Propofol on the miR-219-5b Expression Level ($\bar{x}\pm s$)**

Group	Huh7 Cell	SMMC7721 Cell
Blank Control Group	1.00±0.001	1.01±0.02
Low-concentration Propofol Group	1.23±0.13	1.21±0.16
Medium-concentration Propofol Group	2.98±0.25*	3.55±0.24*
High-Concentration Propofol Group	3.84±0.26*	3.85±0.30*

Note:* represents that compared with the blank control group, P＜0.05

Table 2: **Comparison of the OD Values of Huh7 and SMMC7721 Cells in Each Group(x±s)**

Group	Time	Huh7 Cell Line	SMMC7721 Cell Line
Negative Control Group	1d	0.11±0.02	0.28±0.03
	2d	0.20±0.03	0.43±0.01
	3d	0.52±0.03	0.71±0.05
	4d	0.78±0.05	0.89±0.10
Propofol Group	1d	0.10±0.01	0.21±0.02
	2d	0.12±0.01#	0.28±0.02#
	3d	0.22±0.03#	0.34±0.02#
	4d	0.38±0.04#	0.40±0.01#
miR-219-5b-shRNA Group	1d	1.03±0.01	0.22±0.01
	2d	0.22±0.15	0.41±0.02
	3d	0.40±0.05	0.60±0.09
	4d	0.60±0.06	0.75±0.10

Note: # represents that compared with the negative control group, P＜0.05

3.3. Comparison of Invasion and Migration of Huh7 and SMMC7721 Cells in Each Group

In the Propofol group, the number of invasion cells and the number of Huh7 and SMMC7721 transmembrane cells were meaningfully lower than the negative control group. No major differences were found in the number of invasion cells and the number of Huh7 and SMMC7721 transmembrane cells in the miR-219-5b-shRNA community compared to the negative control group (P>0,05). See Table 3.

3.4. Comparison of Wnt/β-cateninSignaling Pathway Related Protein Expression Levels in Huh7 and SMMC7721 Cells in Each Group

The Huh7 and SMMC7721 cells in the Propofol

group were meaningfully lower in β-cateninand C-Myc protein level than those in the negative control group. There were no significant differences in Huh7 and SMMC7721 cells' β-catenin and C-Myc protein expression levels in the miR-219-5b-shRNA group as compared to the negative control group (P>0.05). See Table 4 and Figure 1.

Table 3: **Comparison of Invasion and Migration of Huh7 and SMMC7721 Cells in Each Group ($\bar{x}$±s)**

Group	Cell Line	Number of invasion cells	Number of TransmembraneCells
Negative Control Group	Huh7 Cell	60.10±10.78	101.11±20.45
	SMMC7721 Cell	90.45±11.56	130.68±19.12
Propofol Group	Huh7 Cell	23.97±5.67#	32.94±10.39#
	SMMC7721 Cell	32.76±3.76#	49.45±12.56#
miR-219-5b-shRNA Group	Huh7 Cell	61.78±13.98	98.75±15.86
	SMMC7721 Cell	98.56±8.18	133.17±15.56

Note: # represents that compared with the negative control group, P＜0.05

Table 4: **Comparison of β-catenin and C-Myc Protein Expression Levels of Huh7 and SMMC7721 Cells in Each Group ($\bar{x}$±s)**

Group	Cell Line	β-catenin Expression	C-Myc Expression
Negative Control Group	Huh7 Cell	1.02±0.03	1.02±0.03
	SMMC7721 Cell	1.00±0.01	1.00±0.02
Propofol Group	Huh7 Cell	0.23±0.01#	0.26±0.01#
	SMMC7721 Cell	0.34±0.03#	0.45±0.02#
miR-219-5b-shRNA Group	Huh7 Cell	0.98±0.02	1.01±0.03
	SMMC7721 Cell	0.97±0.03	0.96±0.02

Note: # represents that compared with the negative control group, *P*＜0.05

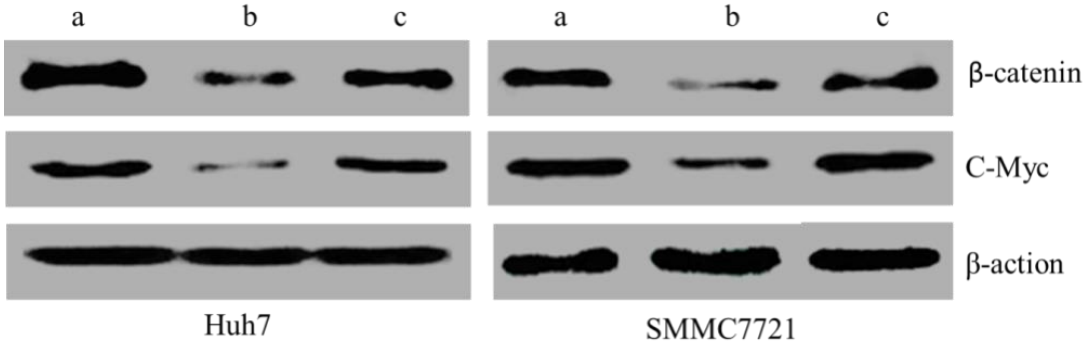

Figure 1: **Comparison of β-catenin and C-Myc Protein Expression Levels of Huh7 and SMMC7721 Cells in Each Group**

Note: a represents the negative control group; b represents the propofol group; c represents the miR-219-5b-shRNA group

4. Discussion

With changes in people's living habits and diet, the incidence of liver cancer has also increased accordingly. Due to proneness of recurrence and metastasis, the disease causes extremely poor metastasis of patients. It has been reported that nearly half of the patients develop metastases after treatment, with liver metastasis first, followed by lungs and brain, and the patients have a very low 5-year survival rate (Dong Zhiyong, 2019). Therefore, it is urgent to find a relevant drug and molecular mechanism that can effectively improve the survival rate and reduce the recurrence and metastasis of patients. Propofol is widely used in clinical anesthesia and ICU, and it has become a first-line

intravenous anesthetic in tumor resection. Multiple studies have confirmed that different types of anesthetics can cause changes in the patients' immune system, limit their immune process, and ultimately increase the risk of tumor metastasis; propofol has the functions of inhibiting tumor growth, decreasing the rate of metastasis, and resisting the inflammation and oxidation activity (David W Hewson et al., 2019). In other studies, patients were anesthetized by inhalation of sevoflurane and propofol, respectively; the results showed that the postoperative survival rate of patients in propofol group was significantly higher than that of sevoflurane, and the risk of metastasis was significantly lower than that of sevoflurane, indicating that propofol is effective in improving the prognosis of patients (Jan Gelberg et al., 2019).

Previous studies have confirmed that miR-219-5b has the function of hindering the progression of tumors, and it can exist in humans as a tumor suppressor gene (Lahdaoui et al., 2019). According to gastric cancer-related studies, the up-regulation of miR-219-5b expression level can significantly inhibit the metastasis of cancer cells and epithelial-mesenchymal transition; furthermore, the up-regulation of its level of expression can significantly hinder the up-regulation of its level of expression can significantly hinder the signaling pathway for Wnt / β-catenin and eventually achieve the function of inhibiting cancer cell proliferation , migration and invasion (Wu Yanhua et al., 2017). According to studies relating to liver cancer, miR-219-5bplays an significant role in hindering hepatomacell proliferation , migration and invasion (Bruinsma et al., 2018). Wnt / β-catenin signaling pathway belongs to one of the main branches of the Wnt signal transduction pathway, which can induce the activation of the target gene on the basis of stable β-catenin; and the obvious activation of the signal makes it easy to cause tumor (Long Jianwen et al., 2017). Clinical studies have shown that the signaling pathway of Wnt / β-catenin can participate in the epithelial-mesenchymal transition and play an important part in this cycle. According to several studies, activation of the signaling pathway for Wnt / β-catenin can promote the tumor's epithelial-mesenchymal transition cycle, leading to increased tumor malignancy and eventually tumor malignancy.

In the propofol medium-concentration group along with the propofol high-concentration group were meaningfully higher than the blank control group and the concentration of the propofol medium-concentration group. In the next experiment the medium-concentration (5μm / mL) propofol was then taken out. Next, this study carried out a series of functional experiments, while the effect of propofol on the malignant biological activity of hepatoma cells was observed. First of all, the MTT approach was used to check cell proliferation in each group at different periods of time, and the results showed that the 0D values of Huh7 and SMMC7721 cells in the propofol group at 2d , 3d and 4d were significantly along with the negative control group, indicating that propofol can inhibit hepatoma cells, but the ability of propofol to inhibit hepatoma cell proliferation was meaningfully reduced following down-regulation of the expression level miR-219-5b. Second, the Transwell method was used to check cell proliferation and migration in each group, and the results exposed about the number of invasive cells and the number of Huh7 and SMMC7721 transmembrane cells in the propofol community were significantly along with the negative control group, Indicating that propofol may significantly inhibit hepatoma cell invasion as well as migration, but the ability of propofol to inhibit hepatoma cell invasion as well as migration has been meaningfully reduced following down-regulation of the expression level miR-219-5b. So Propofol group concentration is similar to clinical treatment concentration. There was no substantial difference in miR-219-5b expression in Huh7 and SMMC7721 cell lines between the low concentration propofol group and the blank control group. To explain the mechanism by which propofol inhibits the malignant biological activity of hepatoma cells by up-regulating the expression level miR-219-5b, this study adopted the western blot to check the levels of protein associated with the expression of Wnt / β-catenin signaling pathway in each cell group; The β-catenin and C-Myc protein expression rates of Huh7 and SMMC7721 cells in the propofol community were significantly along with negative control group ($P<0.05$), suggesting that the impact of propofolinhibiting the signaling pathway function of Wnt / β-catenin can be substantially reversed by down-regulating the level of miR-219-5bexpression.

In conclusion, propofol can inhibit the Wnt/β-catenin signaling pathway by up-regulating the miR-219-5bexpression levels, thereby hindering the

biological behaviors of proliferation, migration and invasion of hepatoma cells.

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