

miRNA-195: The New Target of Inhibiting the Proliferation, Migration and Invasion of Gastric Cancer Cells via Propofol

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Abstract: Objective: To explore the effect of propofol (Prof) on the proliferation, migration and invasion of human gastric cancer cell MGC-803 and its molecular mechanism. **Methods:** The MTT method was used to study the effects of Prof with different doses and durations on the viability of MGC-803 cells. Hoechst 33258 staining and electron microscopy were used to detect the effects of Prof on MGC-803 cell apoptosis. Transwell experiments were used to detect the effects of Prof on the migration and invasion of MGC-803 cells. RT-PCR detects the effect of Prof on the expression of miR-195 in MGC-803 cells, and Western Blot detects the effect of Prof on the protein expression of JAK/STAT signaling pathway. **Results:** Compared with 0 μ g/ml Prof, 5 μ g/ml, 10 μ g/ml and 20 μ g/ml Prof treatment with 24h, 48h and 72h can significantly reduce cell viability ($P < 0.05$). Compared with the Control group, the percentage of Hoechst 33258 staining positive cells in the Prof group and the apoptosis rate under the electron microscope were significantly increased ($P < 0.05$). Compared with the Control group, the cell migration rate and invasion rate of the Prof group were significantly reduced ($P < 0.05$). Compared with the Control group, the expression of miRNA-195 in the Prof group cells was increased significantly ($P < 0.05$). Compared with the Control group, the activity of p-Jak1 and p-STAT3 proteins in the Prof group were significantly reduced ($P < 0.05$). **Conclusion:** Prof can reduce

the cell viability, migration and invasion of gastric cancer cell MGC-803, and promote its apoptosis. Its mechanism may be related to the promotion of miR-195 expression and inhibition of JAK/STAT signal pathway activity.

Key words: Propofol; miRNA-195; Gastric cancer cells; Proliferation; Migration; Invasion

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1 Introduction

Worldwide, cancer is still second only to cardiovascular disease as a major health problem^[1]. Gastric cancer is one of the most common and deadly cancers in the human digestive system. It is a malignant tumor with a high morbidity and mortality rate, and about 300,000 patients die of gastric cancer in China every year^[2]. Gastric cancer is caused by many factors, mainly including incorrect eating habits and lifestyle, gastric inflammation and family history of gastric cancer^[3]. The early symptoms of gastric cancer are not obvious, while the clinical symptoms of advanced gastric cancer are stomachache, abdominal distension and discomfort, nausea, vomiting, weight loss and fever^[4]. Although the diagnosis and treatment of gastric cancer have

been improved in recent years, the 5-year survival rate of patients is still only 30%^[5]. Therefore, finding novel and more effective drugs will contribute to the treatment of gastric cancer.

Propofol (Prof) is a common clinical intravenous anesthetic^[6]. Compared with other anesthetics, Prof has many characteristics such as quick onset, short action and less side effect^[7]. In recent years, studies have shown that Prof has anti-tumor effects in various cancers such as breast cancer, lung cancer, pancreatic cancer, ovarian cancer, liver cancer and stomach cancer^[8]. In terms of gastric cancer, Yang et al. showed that Prof inhibited the proliferation of SGC-7901 and MGC-803 cells by upregulation of growth inhibitor 3 (InG3)^[9]. Peng et al. demonstrated that Prof inhibits proliferation and induces apoptosis in human gastric cancer SGC-7901 cells by regulating matrix metalloproteinase-2 (MMP-2)^[10]. However, more experiments and clinical studies are needed to further explore the deeper molecular mechanisms of Prof's inhibition of gastric cancer growth and invasion. MicroRNAs (miRNAs) are small non-coding RNA transcripts that co-regulate the expression of one third of genes in the genome and are involved in the pathogenesis of many diseases, including cancer^[11]. Prof plays an anti-tumor role by regulating miRNA expression in cancer cells^[12]. MiRNA 195 (miR-195) is a tumor suppressor that is down-regulated in a variety of cancer tissues and cells, including gastric cancer^[13]. However, there has been no study on the effect of Prof on the expression of miR-195 in gastric cancer cells, and it is not clear whether Prof plays an anti-tumor role by regulating the expression of miR-195 in gastric cancer cells, so more studies are still needed to confirm this. This study mainly explored the effects of Prof. On the viability, apoptosis, migration and invasion of gastric cancer cells, as well as the regulation of Prof. On miR-195 expression and its mechanism,

2 Materials and methods

2.1 Cell culture

The human gastric cancer cell line MGC-803 was purchased from the cell resource center, Institute of basic medicine, Chinese Academy of Medical Sciences, resource number: 3111C0001CCC000227. The suspension of fetal cells was thawed in 20% DMS at 37°C for 2 days, and then the supernatant was

changed to 37% DMS. When the confluence of cells reached 85%, the cells were digested with 0.25% trypsin, and the single cell suspension was prepared, counted and inoculated in the culture plate. All operations were performed under aseptic conditions.

2.2 Propofol intervention

The cells were seeded on 96 well plates at a density of 5×10^4 cells / ml, 100 μ l per well, about 5000 cells. Propofol solutions of 1 μ g/ml, 5 μ g/ml, 10 μ g/ml and 20 μ g/ml were added to each well respectively. Propofol was purchased from sigma Aldrich company in USA. The cells were cultured at 37°C for 24h, 48h and 72h in a 5% CO₂ incubator. The well with the same volume of culture medium was used as the control. Six multiple wells were set for each concentration gradient.

2.3 Cell viability test

MTT assay was used to detect the effect of propofol on the viability of MGC-803 cells. After the cells were intervened according to part 2.2, 20 μ l of 5 mg/ml MTT solution was added to each well. After 4 h of continuous incubation at 37 °C, 150 μ l of dimethyl sulfoxide (DMSO) was added to each well, and the cells were vibrated fully until the purple crystal was completely dissolved. The culture plate was placed in the microplate reader, and the OD of each well was measured at 57nm. The blank hole is a hole with only medium but no cells.

$$\text{Cell vitality(\%)} = \frac{(\text{OD experiment hole} - \text{OD blank hole})}{(\text{OD controlled hole} - \text{OD blank hole})} \times 100\%.$$

MTT cell proliferation and cytotoxicity test kit was purchased from Shanghai biyuntian company.

2.4 Detection of apoptosis

Hoechst 33258 staining was used to detect the effect of propofol on the apoptosis of MGC-803 cells. The cells were divided into two groups. Control group and propofol group. According to the intervention method in part 2.2, the propofol group cells were cultured in the medium with 10 μ g/ml propofol solution for 24 hours, and the control group cells were cultured in the medium with the same volume of medium for 24 hours. Each group had 6 replicates. After that, the medium was discarded, the cells were washed twice with PBS, stained with 10 μ m Hoechst 33258 for 10 min at 37 °C, washed twice with PBS, and the number of Hoechst 33258 positive staining cells per 1000 cells was observed and counted by

inverted fluorescence microscope (Leica dmi3000b, Germany). Five fields of vision were selected for each film and 6 repeated films were made for each group. The final result was taken as the mean value.

The apoptosis of single cell was observed by electron microscope. The cells treated and cultured according to the grouping method were washed twice with culture medium, centrifuged at 2000 rpm for 10 min, the supernatant was discarded, and the cell precipitate was put into 3% glutaraldehyde and fixed at 4 °C for 2 h. PBS was washed three times for 2 min. After fixation with 1% osmium tetroxide for 2 hours, PBS was used for cleaning. The cells were dehydrated with different levels of ethanol and embedded with epon618 resin. The embedded cells were sliced into ultrathin sections after 500 nm with a microtome. After that, 1% uranyl acetate was used for primary dyeing and lead citrate was used for secondary dyeing. Use JEOL Japanese JEM - 3200 FS biopsy field emission transmission electron microscopy (tem) observation, with Japan's push around Soft Imaging Solutions GmbH photograph Software, magnification of 30000×. The apoptotic rate was counted as the number of apoptotic cells per 100 cells. Five fields of vision were selected for each film and 6 repeated films were made for each group. The final result was taken as the mean value.

2.5 Detection of cell migration and invasion

Transwell cell invasion: The dissolved Matrigel was mixed with serum-free medium, and then spread on the bottom of Transwell chamber, and placed in the incubator for 3 h. After 24 hours of propofol intervention, the cells were digested with trypsin to prepare cell suspension. 200 µl cell suspension was added into Transwell chamber, and the cell concentration was 2×10^5 /ml. DMEM medium containing 10% fetal bovine serum was added into the lower chamber and cultured in the incubator with 5% CO₂ at 37 °C for 30 h. the chamber was removed and fixed with methanol. The membrane was stained with hematoxylin. The lower chamber surface of the membrane was observed under the microscope and the number of transmembrane cells in each well was counted.

Cell migration: The detection of cell migration was the same as that of cell invasion, except that Matrigel was not pre coated.

2.6 RT-PCR detection

Total RNA was extracted from 2.4 parts of cells by Trizol lysate. The absorbance value a at 260 nm and

280nm of total RNA was detected, and the total RNA was quantified according to the ratio of a 260 / A280. After that, 1µg of the total RNA template was used to synthesize the first strand cDNA with the reverse transcription Kit (m-mlv). SYBR mix, specific primers and water were used to amplify the cDNA. GAPDH was used as internal reference. The relative expression of the target gene was calculated by $2^{-\Delta\Delta Ct}$. Primers were designed by primer designing tool software and synthesized by Shanghai Shenggong company.

2.7 Western blot

The Ripa lysate was purchased from Shanghai biyuntian company. The BCA protein concentration assay kit was purchased from Shanghai biyuntian company. The total protein of 20 µg was added into the sample buffer and separated by 10% SDS-PAGE gel. The electrophoresis voltage was 100V and the electrophoresis time was 90min. At the end of electrophoresis, the total protein was transferred to PVDF membrane under the condition of current 300 mA and time 60 min. Bovine serum albumin (BSA) was transferred to the shaker at room temperature for 45 minutes. The first antibody was added, incubated at 4 °C overnight, and washed with TBST three times for 5 minutes each time. Add horseradish peroxidase labeled second antibody, incubate in room temperature shaker for 1 h, and wash with TBST for 3 times, 5 min each time. Add the super sensitive ECL luminous solution and emit light in the dark room. The high sensitive ECL chemiluminescence kit was purchased from Shanghai biyuntian company, Article NoP0018M. GAPDH was the internal reference. Phospho-jak1 (tyr1022 / 1023) rabbit polyclonal antibody, phospho-stat3 (tyr705) rabbit polyclonal antibody, horseradish peroxidase labeled Goat anti rabbit IgG and GAPDH rabbit monoclonal antibody were purchased from Shanghai biyuntian company. The target strip was photographed with the gel imaging system of Bio-rad company of America, and the gray value of the target strip was statistically analyzed by Quantity One 4.6.2 software. Relative expression

$$\text{of the target protein} = \frac{\text{Objective Protein gray value}}{\text{GAPDH gray value}}$$

2.8 Statistical analysis

SPSS 22.0 software was used for statistical analysis. T test was used for comparison between the two groups.

One way ANOVA and LSD multiple comparison were used for comparison between multiple groups. The difference was statistically significant ($P < 0.05$).

3 Results

3.1 Effect of propofol on the viability of MGC-803 cells

MGC-803 cells were treated with different doses of Prof for 24 h, 48 h and 72 h. The results showed that compared with 0 $\mu\text{g} / \text{ml}$ of Prof, 5 $\mu\text{g} / \text{ml}$, 10 $\mu\text{g} / \text{ml}$ and 20 $\mu\text{g} / \text{ml}$ of Prof could significantly reduce the cell viability for 24 h, 48 h and 72 h ($P < 0.05$). The follow-up experiments were carried out with 10 $\mu\text{g} / \text{ml}$ of Prof for 24 hours.

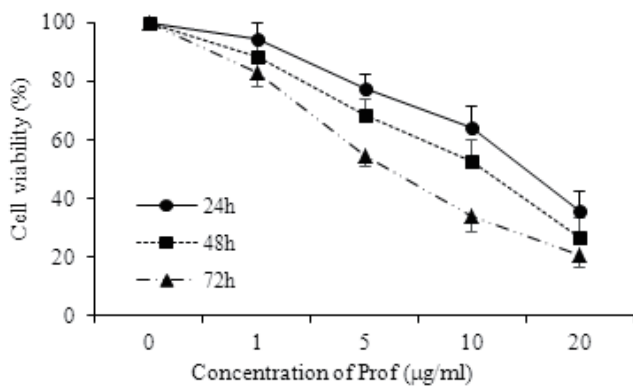


Figure 1. Effect of Prof on MGC-803 cell viability
Note: *05, compared with 0 g / ml; #P 0.05, compared with 24 hours.

3.2 Effect of propofol on apoptosis of MGC-803 cells

Hoechst 33258 staining was used to evaluate the effect of Prof on the apoptosis of MGC-803 cells. Green cells were positive for Hoechst 33258 staining and were in the process of apoptosis. As shown in Figure 2, compared with control group, the percentage of Hoechst 33258 positive cells in Prof group was significantly increased ($P < 0.05$).

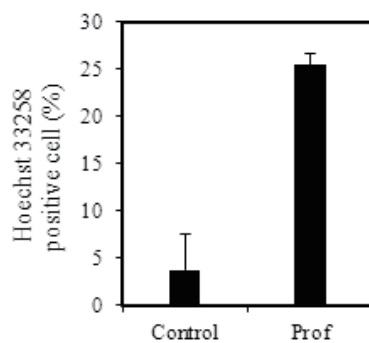


Figure 2. Comparison of Hoechst 33258 staining results of two groups of cells
Note: * $P < 0.05$, compared with control group

As shown in Figure 3, under the electron microscope, contraction of nucleus and cytoplasm, condensation and marginalization of chromatin on nuclear membrane, and formation of apoptotic bodies were observed in the cells of Prof group, indicating that the cells of Prof group were apoptotic. In control group, nucleus and cytoplasm were intact, chromatin structure was normal, and apoptotic bodies were not found. The statistical results of apoptotic cells under electron microscope showed that the apoptotic rate of Prof group was significantly higher than that of control group ($P < 0.05$).

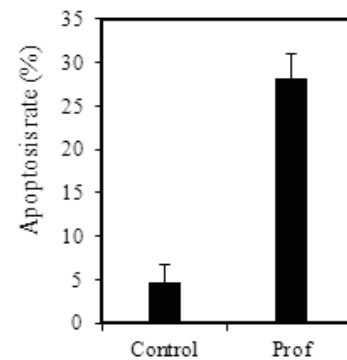


Figure 3. Comparison of electron microscopic results of two groups of cells
Note: * $P < 0.05$, compared with control group

3.3 Effects of propofol on the migration and invasion of MGC-803 cells

The results of cell migration experiment are shown in Figure 4. Compared with control group, the cell migration rate of Prof group was significantly lower ($P < 0.05$). The results of cell invasion experiment are shown in Figure 5. Compared with control group, the invasion rate of Prof group was significantly lower ($P < 0.05$).

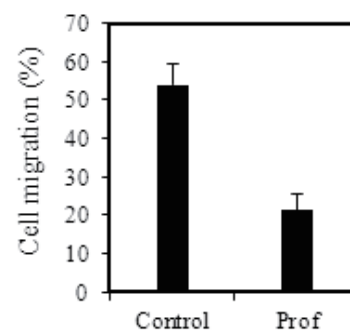


Figure. 4 Comparison of cell migration between the two groups
Note: * $P < 0.05$, compared with control group

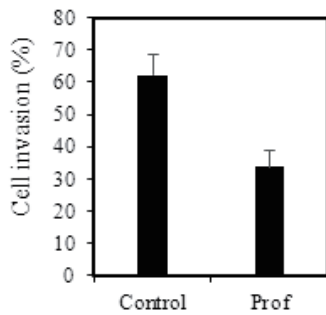


Figure 5. Comparison of cell invasion results between the two groups
 Note: * $P < 0.05$, compared with control group

3.4 Effect of propofol on the expression of miR-195 in MGC-803 cells

The detection results of miR-195 expression in two groups of cells are shown in Figure 6. Compared with control group, the expression of miR-195 in Prof group was significantly increased ($P < 0.05$).

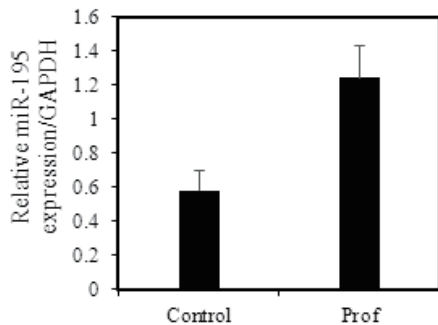


Figure 6. Comparison of miR-195 expression between the two groups
 Note: * $P < 0.05$, compared with control group

3.5 Effect of propofol on JAK / STAT signaling pathway in MGC-803 cells

The expression of JAK / STAT signaling pathway molecules in the two groups were shown in Figure 7 and Figure 8. Compared with control group, the activity of p-jak1 and p-STAT3 protein in Prof group was significantly decreased ($P < 0.05$).

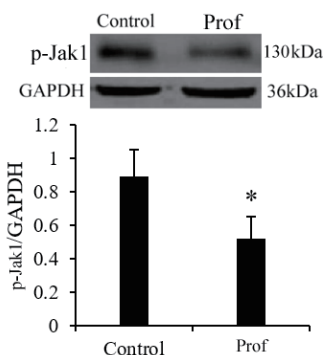


Figure 7. Comparison of p-jak1 protein expression between the two groups
 Note: * $P < 0.05$, compared with control group

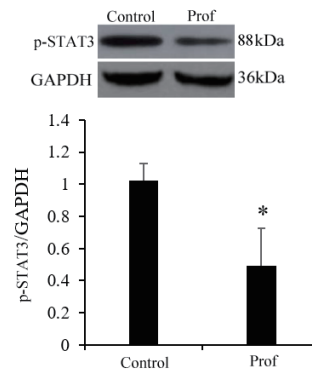


Figure 8. Comparison of p-STAT3 protein expression between the two groups
 Note: * $P < 0.05$, compared with control group

4 Discussion

In this study, we found that the commonly used intravenous anesthetic drug Prof significantly inhibited the viability, migration and invasion of gastric cancer MGC-803 cells, and significantly promoted cell apoptosis. In addition, the expression level of miR-195 in MGC-803 cells after Prof treatment was increased and the JAK/STAT signaling pathway was inactivated.

As one of the widely used intravenous anesthetics, Prof has many advantages in clinical anesthesia^[14]. In addition, in addition to its anesthetic effect, Prof also has a variety of non-anesthetic effects, such as neuroprotective effect, antioxidant effect, anti-anxiety effect, and anti-tumor effect^[15]. Investigations into the multiple efficacy of known drugs will be of great value to patients and drug production, especially in terms of cost savings in clinical trials testing drug safety, as is the case with prednisone and sildenafil citrate^[16,17]. In terms of anti-tumor, considering that Prof. Has almost no side effects on patients' health, the search for potential targets of Prof.'s anti-tumor action will provide possible chemotherapy drugs for cancer treatment. For gastric cancer, Yang et al.^[9] and Peng et al.^[10] have demonstrated that Prof inhibates the proliferation of gastric cancer SGC-7901 and MGC-803 cells in a dose-dependent manner. Consistent with previous studies, we found that Prof could significantly inhibit the viability of gastric cancer MGC-803 cells, while in this study, it was also found that Prof could significantly induce apoptosis of MGC-803 cells. These findings further confirm that Prof plays an anti-tumor role in gastric cancer by inhibiting cell proliferation.

Metastatic inhibition of gastric cancer is also considered as an important strategy for the treatment of gastric cancer^[18]. Our results showed that Prof could significantly inhibit the migration and invasion of gastric cancer MGC-803 cells. Studies have speculated that the mechanism by which Prof inhibits the migration and invasion of gastric cancer cells may be related to its reduction of the expression levels of MMP-2, MMP-9 and vimentin, but the specific mechanism remains to be further confirmed^[19]. These findings provide further evidence that Prof also exerts an anti-tumor effect on gastric cancer by inhibiting the migration and invasion of gastric cancer cells. In order to balance the anesthetic effect and anti-tumor effect of Prof in gastric cancer, we speculated that the anti-tumor effect of Prof might inhibit the growth and metastasis of gastric cancer during resection surgery, while the anesthetic effect of Prof could relieve the pain of patients during chemotherapy, but this speculation still needs to be confirmed by further animal and clinical studies.

MiRNAs do not encode proteins, but are involved in the regulation of various gene expressions at the post-transcriptional level^[20]. One of the most important findings in this study is that Prof can up-regulate the expression of miR-195 in MGC-803 cells. As a tumor suppressor in cells, miR-195 is down-regulated in a variety of cancer tissues and cancer cells^[13], suggesting that inhibition of miR-195 expression may be an important cause of cancer development. In this study, we found that Prof could significantly up-regulate the expression level of miR-195 in MGC-803 cells, suggesting that Prof played an anti-tumor role at least in part by up-regulating miR-195 in gastric cancer MGC-803 cells. However, the specific effects of miR-195 blocking on the migration and invasion of gastric cancer cells remain to be confirmed by subsequent experiments.

JAK/STAT signaling pathway is one of the important signaling pathways in cells, and plays a crucial regulatory role in many cell functions, such as cell growth, cell cycle transformation, cell migration, cell invasion and cell apoptosis^[21]. Activation of the JAK/STAT pathway has been found in various cancer cells such as gastric cancer cells^[22]. Ouyang et al. demonstrated that down-regulation of the JAK/STAT pathway can reduce the proliferation and invasion of gastric cancer cells^[23]. In this study, we found that Prof treatment significantly inactivated the Jak/Stat

signaling pathway in gastric cancer MGC-803 cells. These results further suggest that Prof can inhibit cell proliferation, migration and invasion and induce cell apoptosis by up-regulating miR-195 and inactivating the JAK/STAT signaling pathway, thus playing an anti-tumor role in gastric cancer MGC-803 cells.

In conclusion, our study further confirmed the antitumor effect of Prof on the growth and metastasis of gastric cancer MGC-803 cells. Prof can significantly inhibit the proliferation, migration and invasion of gastric cancer MGC-803 cells and promote apoptosis by up regulating the expression of miR-195 and inactivating JAK / STAT signaling pathway. This finding provides a new theoretical basis for further study of Prof in the treatment of gastric cancer.

References

- [1] Siegel R.L., Miller K.D., Jemal A. Cancer statistics, 2017 [J]. *CA Cancer J Clin*, 2017, 67(1): 7-30.
- [2] Cheng AP, Chen SL, Progress in diagnosis of peritoneal metastasis of gastric cancer [J]. *Diagnostics Theory and Practice*, 2014, 13 (5): 1550-552.
- [3] Ye Y, Wu Y, Xu J, *et al.* Association between dietary carbohydrate intake, glycemic index and glycemic load, and risk of gastric cancer [J]. *Eur J Nutr*, 2017, 56(3): 1169-1177.
- [4] Waddell T, Verheij M, Allum W, *et al.* Gastric cancer: ESMO-ESSO-ESTRO Clinical Practice Guidelines for diagnosis, treatment and follow-up [J]. *Radiother Oncol*, 2014, 110(1): 189-194.
- [5] Liu H, Gao Y, Song D, *et al.* Correlation between microRNA-421 expression level and prognosis of gastric cancer [J]. *Int J Clin Exp Pathol*, 2015, 8(11): 15128-15132.
- [6] Chidambaran V, Costandi A, D'Mello A. Propofol: A review of its role in pediatric anesthesia and sedation [J]. *CNS Drugs*, 2015, 29(7): 543-563.
- [7] Oren-Ziv A, Hoppenstein D, Shles A, *et al.* Sedation methods for intra-articular corticosteroid injections in Juvenile Idiopathic Arthritis: A review [J]. *Pediatr Rheumatol Online J*, 2015, 1328.
- [8] Wang J, Cheng CS, Lu Y, *et al.* Novel findings of anti-cancer property of propofol [J]. *Anticancer Agents Med Chem*, 2018, 18(2): 156-165.
- [9] Yang C, Gao J, Yan N, *et al.* Propofol inhibits the growth and survival of gastric cancer cells in vitro through the upregulation of ING3[J]. *Oncol Rep*, 2017, 37(1): 587-593.
- [10] Peng Z, Zhang Y. Propofol inhibits proliferation and accelerates apoptosis of human gastric cancer cells by

- regulation of microRNA-451 and MMP-2 expression [J]. *Genet Mol Res*, 2016, 15(2).
- [11] Hammond SM. An overview of microRNAs [J]. *Adv Drug Deliv Rev*, 2015, 873-14.
- [12] Zhang J, Wu GQ, Zhang Y, *et al.* Propofol induces apoptosis of hepatocellular carcinoma cells by upregulation of microRNA-199a expression [J]. *Cell Biol Int*, 2013, 37(3): 227-232.
- [13] Deng H, Guo Y, Song H, *et al.* MicroRNA-195 and microRNA-378 mediate tumor growth suppression by epigenetical regulation in gastric cancer [J]. *Gene*, 2013, 518(2): 351-359.
- [14] Singh Bajwa SJ, Bajwa SK, Kaur J. Comparison of two drug combinations in total intravenous anesthesia: Propofol-ketamine and propofol-fentanyl [J]. *Saudi J Anaesth*, 2010, 4(2): 72-79.
- [15] Lundstrom S, Twycross R, Mihalyo M, *et al.* Propofol [J]. *J Pain Symptom Manage*, 2010, 40(3): 466-470.
- [16] Bergmann TK, Barraclough KA, Lee KJ, *et al.* Clinical pharmacokinetics and pharmacodynamics of prednisolone and prednisone in solid organ transplantation[J]. *Clin Pharmacokinet*, 2012, 51(11): 711-741.
- [17] Patankar M. Sildenafil citrate[J]. *J Indian Med Assoc*, 2000, 98(12): 800-803, 815.
- [18] Dulskas A, Al Bandar M, Choi YY, *et al.* A case of gastric cancer metastasis to the breast in a female with BRCA2 germline mutation and literature review [J]. *Acta Chir Belg*, 2019, 119(1): 59-63.
- [19] Zhang L, Chen X, Liu B, *et al.* MicroRNA-124-3p directly targets PDCD6 to inhibit metastasis in breast cancer[J]. *Oncol Lett*, 2018, 15(1): 984-990.
- [20] Fabian MR, Sundermeier TR, Sonenberg N. Understanding how miRNAs post-transcriptionally regulate gene expression [J]. *Prog Mol Subcell Biol*, 2010, 501-20.
- [21] Pencik J, Pham HT, Schmoellerl J, *et al.* JAK-STAT signaling in cancer: From cytokines to non-coding genome [J]. *Cytokine*, 2016, 8726-36.
- [22] Khanna P, Chua PJ, Wong BSE, *et al.* GRAM domain-containing protein 1B (GRAMD1B), a novel component of the JAK/STAT signaling pathway, functions in gastric carcinogenesis [J]. *Oncotarget*, 2017, 8(70): 115370-115383.
- [23] Ouyang J, Pan X, Lin H, *et al.* GKN2 increases apoptosis, reduces the proliferation and invasion ability of gastric cancer cells through down-regulating the JAK/STAT signaling pathway [J]. *Am J Transl Res*, 2017, 9(2): 803-811.