By regulating miR-182-5p/*BCL10*/*CYCS*, sufentanil reduces the apoptosis of umbilical cord mesenchymal stem cells caused by ropivacaine

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Summary   **Sufentanil is a type of opioid analgesic and is usually used to facilitate painless labor in combination with the local anesthetic ropivacaine. One aim of the current study was to investigate the effects of sufentanil and ropivacaine on umbilical cord mesenchymal stem cells (UCMSCs). A second aim of this study was to determine whether sufentanil attenuated the cytotoxicity of ropivacaine in vitro. UCMSCs were divided into 3 groups: one was treated with ropivacaine at a concentration of 50, 100, 200, or 400 μg/mL, another was treated with sufentanil at a concentration of 0.5, 5, 50, or 500 nmol/L, and a third was treated with a combination of ropivacaine at a concentration of 200 μg/mL and sufentanil at a concentration of 0.5, 5, 50, or 500 nmol/L. Results indicated that cell proliferation decreased in cells treated with ropivacaine while it increased in cells treated with sufentanil. In addition, sufentanil limited the inhibitory effect of ropivacaine on UCMSC growth in a dose- and time-dependent manner. Combined treatment with ropivacaine at a concentration of 200 μg/mL and sufentanil at a concentration of 500 nmol/L decreased the proportion of dead and apoptotic UCMSCs, and fewer cells were arrested in the S phase compared to cells treated with ropivacaine. Sufentanil inhibited the apoptosis induced by ropivacaine by increasing miR-182-5p, which regulated the expression of mRNA of the pro-apoptotic genes B-cell lymphoma/leukemia 10 (*BCL10*) and cytochrome c, somatic (*CYCS*). Sufentanil also increased the expression of mRNA of anti-apoptotic genes. In short, ropivacaine inhibits the cell viability and induces the apoptosis of UCMSCs in vitro while sufentanil attenuates this apoptosis by regulating miR-182-5p/*BCL10*/*CYCS*.**

Keywords: Sufentanil, ropivacaine, umbilical cord mesenchymal stem cells, cell cycle arrest, apoptosis, miR-182-5p, BCL10, CYCS

1. Introduction

The pain of childbirth can cause a series of neuroendocrine

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responses, reduce the placenta blood flow, and cause vasoconstriction and acidosis; these adverse events negatively affect the fetus and mother. Effective labor analgesia can reduce the incidence of these adverse events, shorten the birth process, and reduce energy consumption and labor pains (*1,2*). Neuraxial anesthesia is considered to be the gold standard for the control of labor pain. Epidural analgesia is considered to be the most effective method of labor analgesia. It is ideal as analgesia and can also be used as anesthesia for a caesarean section in the event that a vaginal delivery is not possible; it also beneficial to the fetus because it increases blood flow through the placenta and it allows a pregnant woman to remain awake so that she can

cooperate with the physician (*3,4*).

Synergy between opioids and local anesthetics further increases the quality of analgesia and decreases the dose requirement of both local anesthetics and opioids (*5*). Ropivacaine, as a new local anesthetic, has little cardiac toxicity and no obvious effect on the blood flow of the uterus and placenta. It has a direct role on the nerve roots or spinal cord, it does not cause fetal respiratory inhibition, and it is an effective analgesic (*6*). Sufentanil is a strong opioid analgesic that is quick-acting, it has a short half-life of elimination, and it has little effect on hemodynamics (*7*). A combination of ropivacaine and sufentanil is a highly effective form of labor analgesia that can effectively relieve pain, shorten the course of labor, reduce postpartum hemorrhage, and reduce the need to perform a cesarean section (*8*). However, whether these drugs have an adverse effect on newborn's prognosis is not known.

On the one hand, anesthesia can inhibit the stress reaction caused by surgery and pain, but on the other hand it can also directly or indirectly affect cell function. In clinical settings, studies have found that drugs have no adverse effect on physical and mental development (*9,10*), but other studies with different concentrations of anesthetics have reported that residual levels of those drugs are detectable in the cord blood and breast milk (*11*).

Umbilical cord mesenchymal stem cells (UCMSCs) are multi-functional stem cells located in the umbilical cord tissue of the newborn. These cells can differentiate into a variety of tissue cells and they offer promise for their clinical use. In the process of pregnancy and childbirth, the umbilical cord plays an important role in metabolism; substances in the umbilical cord bloodstream play an important role in the function of the umbilical cord (*12,13*). The effects of sufentanil and ropivacaine on human UCMSCs alone or in combination is still not known.

The current study investigated the cytotoxicity of sufentanil and ropivacaine in UCMSCs alone or in combination, and this study also examined the functions of those drugs. Those findings were analyzed to determine if a combination of sufentanil and ropivacaine could serve as a potential form of anesthesia that reduces cell damage.

2. Materials and Methods

2.1. *Cell culture*

Human UCMSCs were purchased from the Shanghai Branch of the Chinese Academy of Science and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 mg/mL streptomycin at 37°C in a 5% CO_2 atmosphere.

2.2. *Drug treatments*

UCMSCs in the logarithmic growth phase were randomly divided into 4 groups: a blank control group (C), a group of cells treated with ropivacaine (Ro), a group of cells treated with sufentanil (Su), and a group of cells treated with a combination of ropivacaine and sufentanil (Ro + Su). Cells treated with ropivacaine were divided into 4 groups (Ro 50, Ro 100, Ro 200, and Ro 400) depending on the concentration of ropivacaine (50, 100, 200, and 400 μg/mL). Cells treated with sufentanil were divided into 4 groups (Su 0.5, Su 5, Su 50, and Su 500) depending on the concentration of sufentanil (0.5, 5, 50, and 500 nmol/L). Cells treated with a combination of ropivacaine and sufentanil were also divided into 4 groups treated with ropivacaine at a concentration of 200 μg/mL along with different concentrations of sufentanil (0.5, 5, 50, and 500 nmol/L) (Ro 200 + Su 0.5, Ro 200 + Su 5, Ro 200 + Su 50, and Ro 200 + Su 500).

2.3. *Cell transfection*

UCMSCs were seeded in 6-well plates overnight, treated with different drugs, and then transiently transfected with miR-182-5p inhibitors or mimics with Lipofectamine 2000 (Invitrogen, CA, USA). miR-182-5p mimics (mimics), an miR-182-5p-negative control (miR-NC), an anti-miR-182-5p inhibitor (anti-miR), and a negative control (anti-NC) were synthesized by GenePharma (Shanghai, China). The effects of gene silencing or overexpression were measured using real-time PCR 48 h after transfection.

2.4. *Real-time cell analyzer (RTCA) system*

The xCELLigence RTCA DP System (ACEA Biosciences, San Diego, California, USA) allows label-free and real-time monitoring of cellular processes, such as cell proliferation, cytotoxicity, adhesion, viability, invasion, and migration using electronic cell sensor array technology. The electrode impedance, displayed as cell index (CI) values, provides quantitative information about the biological status of cells, including their number, viability, and morphology. In brief, 50 μL of cell culture medium at room temperature was added into each well of the E-plate 16 of the xCELLigence RTCA DP System. The E-plate 16 was then connected and checked in the cell culture incubator for proper electrical contacts, and the background impedance was measured for 24 h. The UCMSCs were resuspended in cell culture medium and adjusted to 5,000 cells/well. The cell suspension (100 μL) was added to 50 μL of medium containing wells on the E-plate 16 in order to determine the optimum cell concentration. After incubation at room temperature for 30 min, the E-plate 16 was placed into the cell culture

incubator. Cell adhesion, growth, and proliferation were monitored every h for a period of up to 24 h *via* the incorporated sensor electrode arrays of the E-Plate 16. After 24 h, different concentrations of sufentanil or ropivacaine were added to 200 μL of cell culture medium, and live cells were monitored every 15 min for a period of up to 96 h. The electrical impedance was measured using the RTCA-integrated software of the xCELLigence System as a dimensionless parameter termed CI.

2.5. *RNA extraction and real-time RT-PCR*

For PCR analysis, total RNA was isolated with an RNA extraction Kit (Axygen, CA, USA) according to the manufacturer's protocol. RNA purity and concentrations were measured with a NanoDrop 2000c (Thermo, Fisher, MA, USA). RNA (1 μg) was transcribed into cDNA using reverse transcriptase (Promega, Madison, USA). Normalization controls for mRNA and miRNA were GAPDH and U6 RNA, respectively. Threshold cycle (Ct) values were calculated using the software supplied with the Applied Biosystems 7900 Real-time PCR system. Primer sequences and gene abbreviations are listed in Table 1.

2.6. *Measurement of apoptosis*

After treatment with ropivacaine at a concentration of 200 μg/mL, sufentanil at a concentration of 500 nmol/L, or a combination of the two, apoptotic cells were identified using the Annexin V-FITC Apoptosis Detection Kit according to the manufacturer's instructions. UCMSCs were treated with different drugs for 48 h, about 1×10^6 cells from each group were stained with Annexin V-FITC for 30 min at 4oC in the dark, cells were stained with propidium iodide for 10 min, and then cells were analyzed with a CyAN ADP flow cytometer (Beckman Coulter, Brea, CA, USA). Each sample was examined twice.

2.7. *Cell cycle analysis*

Briefly, UCMSCs were treated with ropivacaine at a

concentration of 200 μg/mL, sufentanil at a concentration of 500 nmol/L, or a combination of the two for 48 h, washed twice with ice-cold PBS, treated with trypsin, and finally fixed in cold 70% ethanol for 30 min at 4˚C. The following procedures were carried out in accordance with the manufacturer's instructions for the Cell Cycle and Apoptosis Analysis Kit. Flow cytometry was used to analyze the treated cells.

2.8. *Real-time PCR arrays*

Each PCR array was a 384-well plate containing gene-specific optimized real-time PCR primer sets for 84 genes related to the induction and inhibition of apoptosis. The PCR array includes primer pairs to quantify RNA18s as a positive control (an invariant highly expressed endogenous control) and genomic DNA as a negative control to detect the presence of genomic DNA contamination. Gene expression profiles were analyzed according to the manufacture's protocol (Wcgene Biotech, Shanghai, China), and data was analyzed using software from Wcgene Biotech. Genes with fold-changes more than or less than 2.0 were considered to have biological significance.

2.9. *Statistical analysis*

Each experiment was performed in sextuplicate and repeated at least three times to ensure replicability. Results were analyzed *via* one-way analysis of variance (ANOVA) using the software GraphPad Prism (version 5.0, GraphPad Software, La Jolla, CA, USA), followed by Dunnett's *t*-test. $P < 0.05$ was considered to be significant.

3. Results

3.1. *Sufentanil limited the inhibitory effect of ropivacaine on UCMSC growth in a time- and concentration-dependent manner*

This study examined the effects of ropivacaine and sufentanil on the growth of UCMSCs. UCMSCs were treated with different concentrations of ropivacaine,

Table 1. The primers used in the study

Gene	Forward Primer	Reverse Primer
miR-182-5p	TGCGGTTTGGCAATGGTAGAAC	CCAGTGCAGGGTCCGAGGT
BCL10	GGAGTGTGAGCCACCTAAG	CTGGGCGATAGAGCAAGAC
CYCS	AAGGCATCACCTGGGGAGAG	ACAGAAACATTCCATCAGCCAT
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
GAPDH	AATCCCATCACCATCTTC	AGGCTGTTGTCATACTTC

Abbreviations: *APAF1*: apoptotic peptidase-activating factor 1; *BAK1*: Bcl-2 homologous antagonist/killer; *BCL2L2*: BCL2-like protein 2; *BCL10*: B-cell CLL/lymphoma 10; *BID*: BH3-interacting domain death agonist; *BIRC3*: Baculoviral IAP repeat-containing protein 3; *BNIP3*: BCL2-interacting protein 3; *CFLAR*: CASP8 and FADD-like apoptosis regulator; *CYCS*: cytochrome c, somatic; *FADD*: Fas-associated protein with a novel death domain; IGF1R: insulin-like growth factor 1 receptor; *NOD1*: nucleotide-binding oligomerization domain-containing protein 1; *TNFRSF9*: tumor necrosis factor receptor superfamily member 9; *TNFRSF11B*: TNF receptor superfamily member 11b; *TNFRSF21*: TNF receptor superfamily member 21;*TP53BP2*: tumor protein p53-binding protein 2; *TRAF3*: TNF receptor-associated factor 3

sufentanil, or a combination of the two, and then the xCELLigence RTCA DP System was used to monitor cell proliferation in real time. Ropivacaine was found to inhibit the proliferation of UCMSCs (Figure 1A) while sufentanil promoted the proliferation of UCMSCs (Figure 1B). Sufentanil markedly limited the inhibitory effect of ropivacaine on UCMSC growth in a time- and concentration-dependent manner in the cells treated

with a combination of ropivacaine and sufentanil (Figure 1C).

3.2. Sufentanil inhibited the apoptosis of UCMSCs caused by ropivacaine and it influenced the cell cycle

To determine whether ropivacaine or sufentanil would induce the proliferation of UCMSCs, whether the drugs
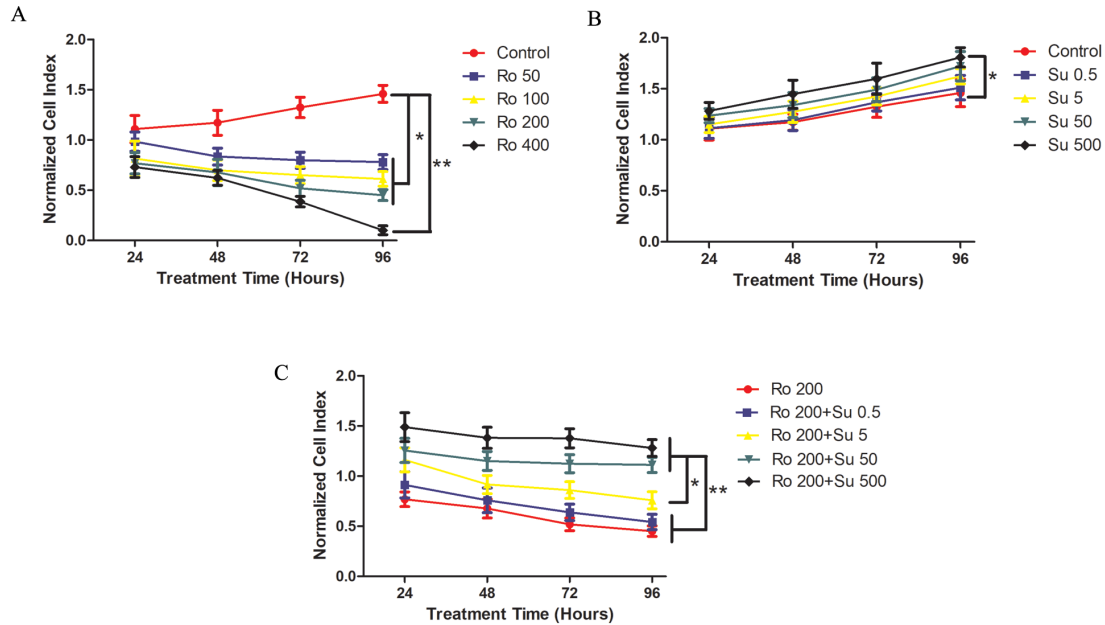
Figure 1. Effect of different concentrations of ropivacaine or sufentanil or a combination of the two on cell proliferation of cultured UCMSCs after treatment for 24, 48, 72, or 96 h. (A) Ropivacaine inhibited the proliferation of UCMSCs in a time- and concentration-dependent manner. **(B)** Sufentanil promoted the proliferation of UCMSCs in a time- and concentration-dependent manner. **(C)** Sufentanil limited the inhibitory effect of ropivacaine on UCMSC growth in a time- and concentration-dependent manner. *$p < 0.05$. All results are expressed as the mean ± SEM, and data are representative of at least three experiments.
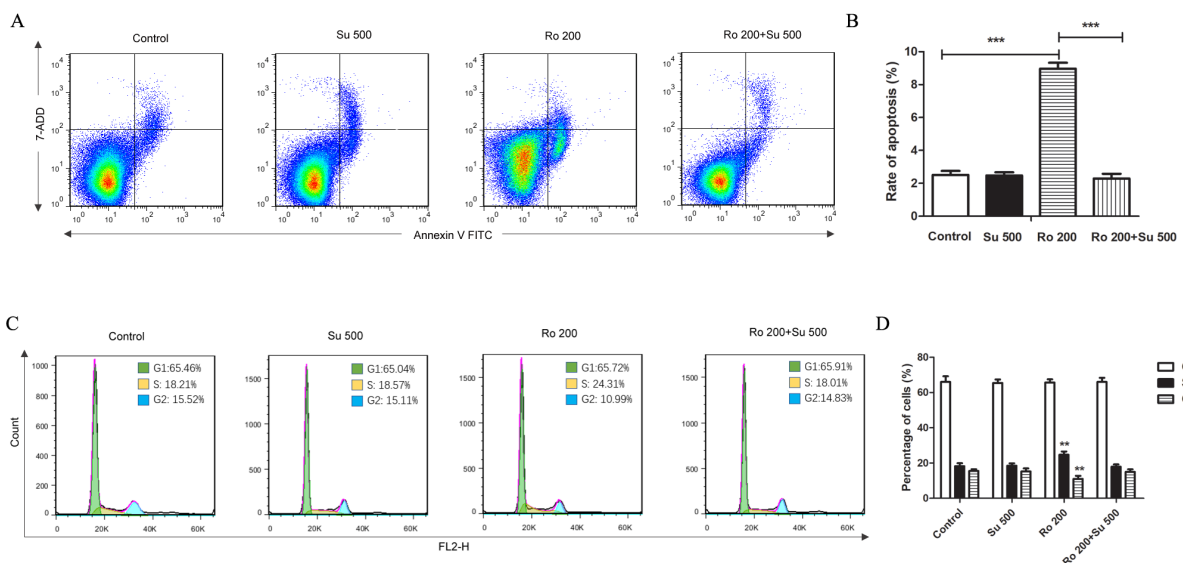

Figure 2. Effect of ropivacaine, sufentanil, or a combination of the two on cell apoptosis and the cell cycle of cultured UCMSCs after treatment for 48 h. (A) Flow cytometry dot plots indicating the binding of Annexin V-FITC (FL-1) and PI uptake (FL-3) of UCMSCs treated with different drugs for 48 h. **(B)** Rates of apoptotic cells as shown in A. **(C)** Cell cycle analysis of UCMSCs treated with different drugs for 48 h. The G1, S, and G2 phases are displayed in three different colors in each chart. **(D)** The percentage of UCMSCs in the G1, S, and G2 phases as shown in **(C)**.*$p < 0.05$. All results are expressed as the mean ± SEM, and data are representative of at least three experiments.

would affect the apoptosis of those cells was determined. As shown in Figure 2A and 2B, the percentage of apoptotic cells increased in cells treated with ropivacaine at a concentration of 200 μg/mL while treatment with sufentanil at a concentration of 500 nmol/L did not affect apoptosis. A combination of the two drugs decreased the apoptosis of UCMSCs in comparison to cells treated with ropivacaine. Flow cytometry was used to determine whether the promotion of UCMSC proliferation by sufentanil was mediated, at least in part, by affecting cell cycle progression. As shown in Figure 2C and 2D, treatment with ropivacaine at a concentration of 200 μg/mL significantly increased the percentage of UCMSCs in the S phase and decreased the percentage in the G2 phase in comparison to the control group and the cells treated with sufentanil at a concentration of 500 nmol/L. In contrast, treatment with a combination of ropivacaine and sufentanil decreased the number of cells in the S phase and increased the number in the G2 phase compared to cells treated with ropivacaine. These findings indicated that ropivacaine arrested the cell cycle in the S phase and that sufentanil halted that arrest, suggesting that sufentanil modulates the inhibition of cell proliferation by ropivacaine by modulating the S phase of the cell cycle.

3.3. *Sufentanil attenuated ropivacaine cytotoxicity by regulating apoptosis-related genes*

PCR arrays were used to study the expression of 84 key genes involved in programmed cell death. These 84 genes included those that induced apoptosis, those that inhibited apoptosis, and those that coded for death domain proteins, caspases, and regulators (Figure 3A). Treatment with ropivacaine at a concentration of 200 μg/mL was found to significantly increase the expression of mRNA of most pro-apoptotic genes such as *BCL10*, *CYCS*, *FADD*, and the caspase family (Figure 3B) while treatment with sufentanil at a concentration of 500 nmol/L mainly decreased the expression of mRNA of pro-apoptotic genes and increased the expression of mRNA of anti-apoptotic genes such as *BIRC3*, *BNIP3*, and *BNIP3L* (Figure 3C). Combined treatment with ropivacaine and sufentanil attenuated apoptosis caused by ropivacaine by markedly decreasing the expression of mRNA of pro-apoptotic genes such as *BAK1*, *BCL10*, *CASP1*, *CASP4*, *CYCS*, and *FADD* and by increasing the expression of mRNA of anti-apoptotic genes such as *BIRC3* and *BNIP3* (Figure 3D).

3.4. *MiR-182-5p participated in regulating cell proliferation induced by sufentanil and ropivacaine*

Based on the apoptosis-regulating genes expressed in cells treated with a combination of ropivacaine and sufentanil, several micro-RNAs that may participate in modulating apoptosis were identified. Results indicated
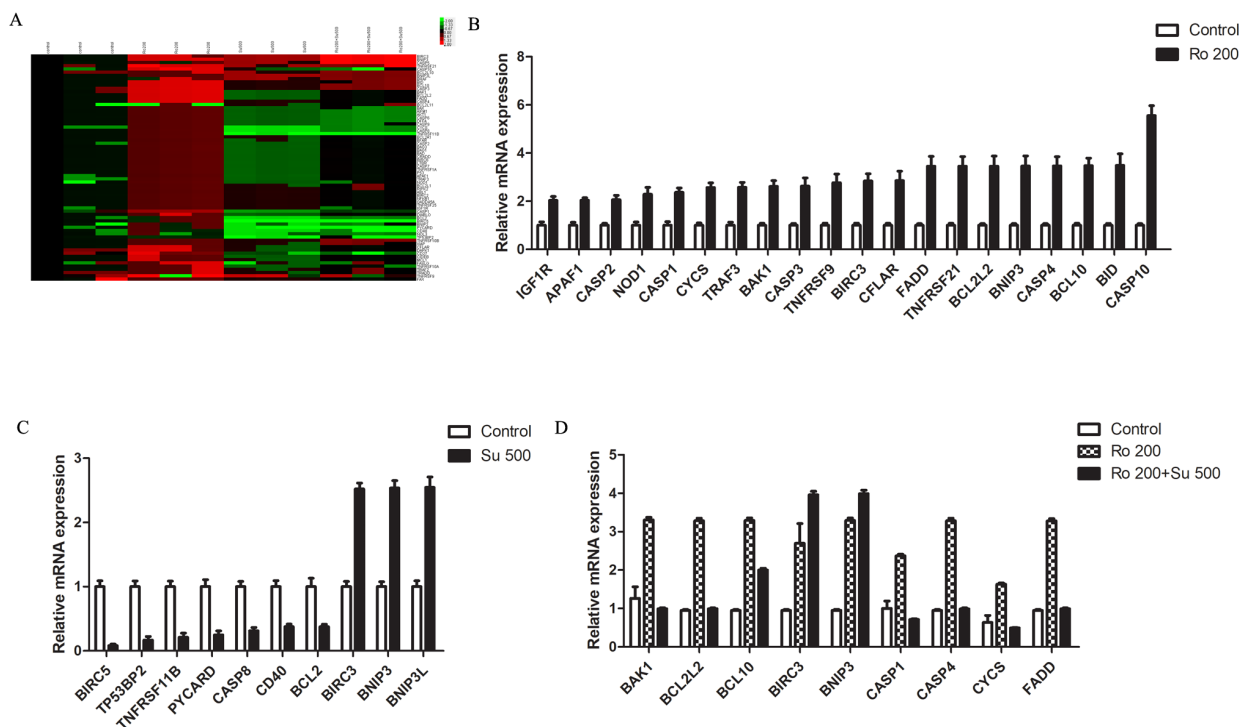

Figure 3. Sufentanil attenuated ropivacaine cytotoxicity by regulating apoptosis-related genes. (A) Eighty-four apoptosis-related genes were analyzed using a PCR array. **(B)** The levels of expression of mRNA of apoptosis-related genes increased as a result of treatment with ropivacaine at a concentration of 200 μg/mL. **(C)** The levels of expression of mRNA of apoptosis-related genes were modulated by sufentanil at a concentration of 500 nmol/L. **(D)** The levels of expression of mRNA of pro-apoptotic and anti-apoptotic genes were modulated by ropivacaine and altered by a combination of ropivacaine and sufentanil. Significance is indicated in all of the bar graphs with $*p < 0.05$. All results are expressed as the mean ± SEM, and data are representative of at least three experiments.
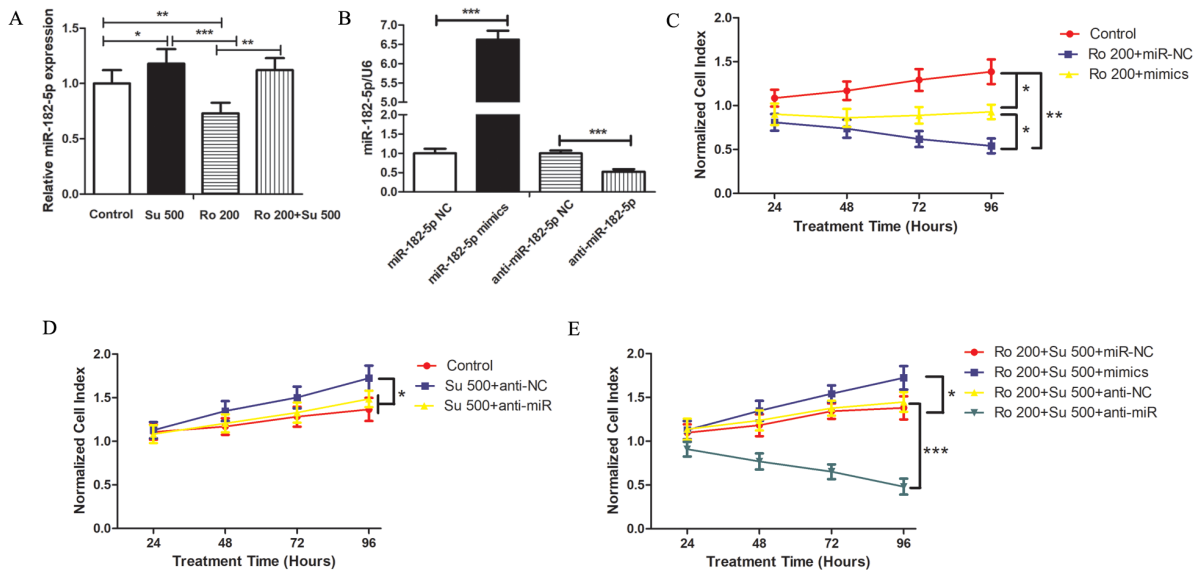
Figure 4. Sufentanil or ropivacaine modulated miR-182-5p expression to regulate cell proliferation (A) The levels of expression of miR-182-5p mRNA in each treatment group after treatment for 48 h. **(B)** The levels of expression of miR-182-5p mRNA in UCMSCs 48 h after transfection with miR-182-5p mimics, inhibitors, or a negative control (NC). **(C)** MiR-182-5p mimics attenuated the inhibition of cell proliferation by ropivacaine. **(D)** MiR-182-5p inhibitors attenuated the promotion of cell proliferation by sufentanil. **(E)** MiR-182-5p mimics improved cell proliferation while inhibitors attenuated cell proliferation in cells treated with a combination of ropivacaine and sufentanil. *$p < 0.05$. All results are expressed as the mean ± SEM, and data are representative of at least three experiments.

that treatment with sufentanil at a concentration of 500 nmol/L increased the level of miR-182-5p which had decreased as a result of treatment with ropivacaine at a concentration of 200 μg/mL (Figure 4A). miR-182-5p mimics or miR-182-5p inhibitors (Figure 4B) were used to determine cell proliferation in response to treatment with different drugs. Transfection with miR-182-5p mimics was found to improve cell proliferation in cells treated with ropivacaine at a concentration of 200 μg/mL (Figure 4C) and cells treated with a combination of ropivacaine and sufentanil (Figure 4E), while transfection with miR-182-5p inhibitors was found to attenuate cell proliferation in cells treated with sufentanil at a concentration of 500 nmol/L (Figure 4D) and cells treated with a combination of ropivacaine and sufentanil (Figure 4E).

3.5. Sufentanil modulated miR-182-5p to inhibit apoptosis caused by ropivacaine by targeting BCL10 and CYCS

To look for the target genes of miR-182-5p in cells rendered apoptotic by drug treatment, online prediction software (TargetScan 7.0 and miRBase) was used to search for the most likely target genes. Results indicated that *BCL2*, *BCL10*, and *CYCS* were the likely target genes of miR-182-5p (Figure 5A). Since *BCL2* is a proven target of miR-182-5p, the current results verified that *BCL2* increased in cells treated with sufentanil at a concentration of 500 nmol/L and transfected with inhibitors (Figure 5C). Expression of *BCL10* and *CYCS* mRNA decreased in cells treated with

ropivacaine at a concentration of 200 μg/mL (Figure 5B) and cells treated with a combination of ropivacaine and sufentanil (Figure 5D) while it increased in cells treated with sufentanil at a concentration of 500 nmol/L (Figure 5C) and cells treated with a combination of ropivacaine and sufentanil (Figure 5D). miR-182-5p mimics were found to attenuate apoptosis induced by ropivacaine while miR-182-5p inhibitors increased the percentage of apoptotic cells (Figure 5E).

4. Discussion

The current results indicated that treatment with ropivacaine alone induced cytotoxicity in UCMSCs by inhibiting proliferation, impacting the cell cycle, and promoting apoptosis, while treatment with sufentanil had the opposite effect. A combination of ropivacaine and sufentanil attenuated that cytotoxicity by modulating apoptosis-related genes. Several studies on use of a combination of ropivacaine and sufentanil to facilitate painless labor or a cesarean section have found no abnormalities in the clinical assessment of the fetus, liver function, renal function, or routine blood results, but the possible effects of a small amount of the drugs on the newborn over the long-term remain unclear. The current results warrant re-examination of the effects of supplementing narcotics with local anesthetics for labor analgesia.

The current study involved real-time detection of cell proliferation, and results indicated that cell viability decreased markedly as the concentration of ropivacaine increased. However, treatment with
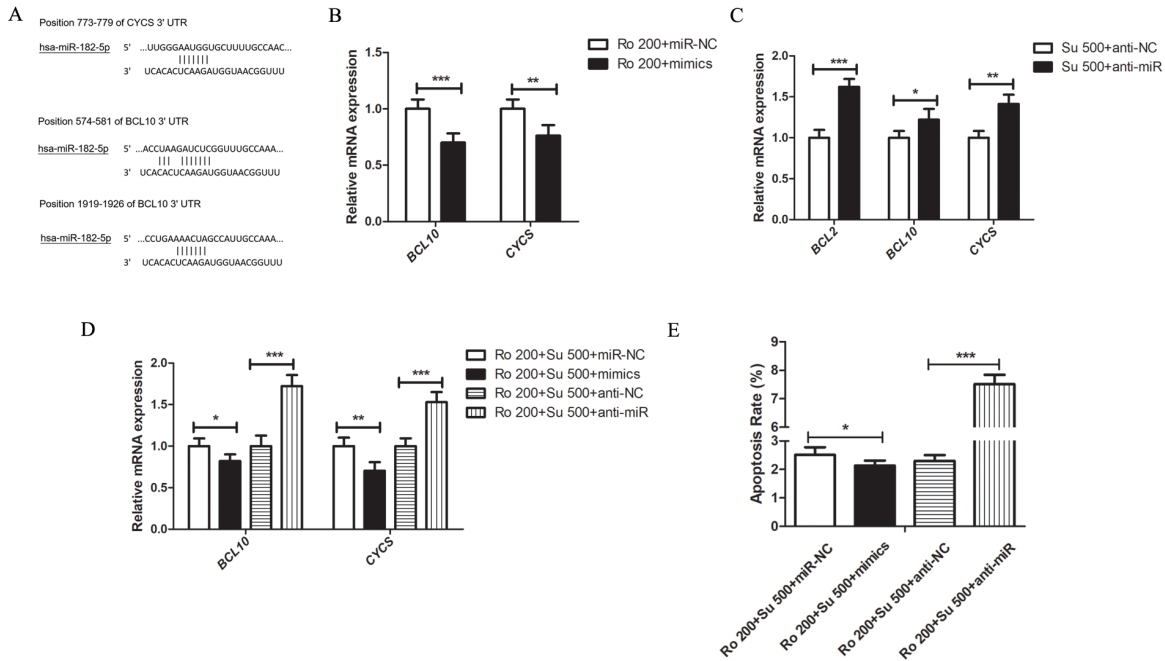
Figure 5. MiR-182-5p improved cell survival and suppressed cell apoptosis by targeting *BCL10* and *CYCS* expression. (A) *BCL10* and *CYCS* were predicted to be target genes of miR-182-5p. **(B)** The levels of expression of *BCL10* and *CYCS* mRNA decreased in cells treated with ropivacaine after transfection with miR-182-5p mimics **(C)** The levels of expression of *BCL2*, *BCL10*, and *CYCS* mRNA increased in cells treated with sufentanil after transfection with miR-182-5p inhibitors. **(D)** MiR-182-5p mimics decreased the levels of expression of *BCL10* and *CYCS* mRNA while inhibitors increased those levels in cells treated with a combination of ropivacaine and sufentanil. **(E)** MiR-182-5p mimics inhibited apoptosis while inhibitors increased apoptosis in cells treated with a combination of ropivacaine and sufentanil. *p < 0.05. All results are expressed as the mean ± SEM, and data are representative of at least three experiments.

an increasing concentration of sufentanil alone or in combination improved cell viability. In clinical studies, ropivacaine has a low toxicity when administered at a low concentration to provide a motor and sensory nerve block, but adverse reactions to ropivacaine have also been reported (*14,15*). Many studies have indicated that sufentanil inhibits the growth of cancer cells such as human breast cancer cells or colon and pancreatic cancer cells *in vitro* (*16,17*). Cord blood contains hematopoietic stem cells that can restore the human hematopoietic and immune systems. Sufentanil may improve cell viability by protecting cell or organ development when a residual amount of the drug enters the bloodstream of a fetus or baby through the umbilical cord or breastfeeding (*18*).

Apoptosis is an active process of intracellular programmed death that is caused by physiological and pathological changes to remove redundant and damaged cells (*19*). In the current study, UCMSCs were increasingly arrested in the S phase and displayed more apoptotic characteristics as the concentration of ropivacaine increased. In the tumor microenvironment, sufentanil attenuates cytotoxicity by inhibiting connexin 43-composed gap junction function in the treatment of colorectal cancer (*20*). Ropivacaine reversibly reduced the permeability of the nerve fiber membrane to sodium ions, it slowed the speed of depolarization, and it alleviated stress (*21*). Peroxides, DNA damage,

and calcium homeostasis may induce neuron apoptosis, and mitochondrial dysfunction may activate metabolic changes related to apoptosis (*22*). According to the PCR array of apoptosis-related genes, ropivacaine modulated apoptosis by significantly increasing the expression of mRNA of apoptosis-related genes. This included genes that positively regulate apoptosis such as *BAK1*, *BID*, *BNIP3*, and *CYCS*, the death domain receptors *FADD*, *NOD1*, and *TNFRSF9*, the death domain proteins *TNFRSF21* and *TRAF3*, a gene that negatively regulates apoptosis such as *BCL10*, and the FADD-like apoptosis regulator *CFLAR*. Caspases can be divided into initiators such as *CASP2*, *CASP8*, *CASP9*, and *CASP10*, and effectors such as *CASP3*, *CASP6*, and *CASP7*. *CASP1* and *CASP4* are inflammatory caspases that cleave other proteins, such as the precursors of interleukins, and thus indirectly induce apoptosis (*23-25*). Ropivacaine modulated caspases such as *CASP2*, *CASP3*, *CASP4* and *CASP10* and caspase activators such as *APAF1* and *CASP1* to induce apoptosis. It also increased anti-apoptotic genes such as *IGF1R*, *BIRC3*, and *BCL2L2*. Sufentanil inhibited apoptosis mainly by decreasing the expression of mRNA of pro-apoptotic genes such as *TP53BP2*, *TNFRSF11B*, and *CD40* and by increasing the expression of mRNA of anti-apoptotic genes such as *BIRC3* and *BNIP3*. In combination, sufentanil may inhibit the expression of mRNA of pro-apoptotic genes and promote the expression of
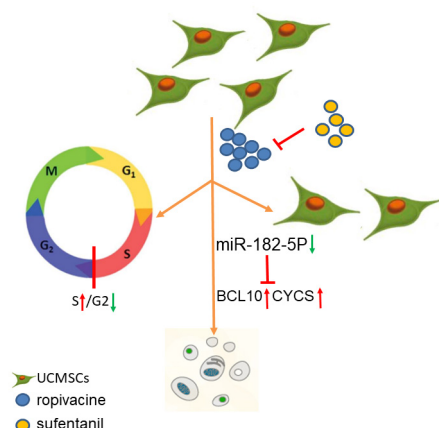
Figure 6. Schematic diagram of sufentanil reducing the apoptosis of UCMSCs caused by ropivacaine. Ropivacaine induced cytotoxicity in UCMSCs by inhibiting their proliferation, impacting the cell cycle, and promoting their apoptosis. Sufentanil improved cell viability and inhibited the apoptotic effects of ropivacaine by increasing miR-182-5p expression, which targeted the pro-apoptotic genes *BCL10* and *CYCS*.

mRNA of anti-apoptotic genes induced by ropivacaine, even though these genes were not affected in cells treated with sufentanil. This may be due to drug interactions that lead to modulation *via* different signaling pathways. Sufentanil may provide protection from myocardial ischemia *via* the PI3K/Akt-GSK-3β pathway by modulating antiapoptotic proteins (*26*), and ropivacaine may be a potential treatment for OA by down-regulating the calcineurin/NFAT1 signaling pathway (*27*). However, the specific mechanism of action of these drugs need to be explored in a future study.

Studies have increasingly indicated that miRNAs play a key role in regulating gene expression during disease progression (*28*). MiR-182-5p has been reported to modulate cell function in many cancers. Inhibiting miR-182-5p by regulating CASP9 expression has a pro-apoptotic and anti-proliferative effect in human breast cancer (*29*). miR-182-5p promotes cell proliferation and metastasis and it inhibits apoptosis by knocking down RECK and Smad4 in bladder cancer (*30*), and it promotes the progression of hepatocellular carcinoma by suppressing FOXO3a expression (*31*). BCL2 is known to be an anti-apoptotic gene in tumors (*32*), and miR-182-5p regulates *BCL2L12* and *BCL2* expression in acute myeloid leukemia (*33*). However, its role as a suppressive miRNA is not clear and needs to be studied. *BCL10* is an apoptotic regulatory molecule that is an antigen receptor-specific regulator of NF-κB, which is closely associated with the immune response (*34*). Cytochrome c (*Cyt c*), a heme-containing mitochondrial protein, has a critical function in both respiration and apoptosis. During apoptosis, cytochrome is released into the cytosol, where it subsequently participates in the process leading to caspase-9 and caspase-3 activation (*35*). The current results indicated that miR-182-5p

expression was regulated in USMSCs by sufentanil or ropivacaine treatment, and that miR-182-5p improved cell survival and suppressed cell apoptosis by targeting *BCL10* and *CYCS* expression. Those drugs may play an anti-apoptotic role by regulating mitochondrial metabolism and the immune response *via* miR-182-5p.

Sufentanil improved cell viability and inhibited the apoptotic effects of ropivacaine by increasing miR-182-5p expression, which targeted the pro-apoptotic genes *BCL10* and *CYCS* in UCMSCs (Figure 6). Further studies are needed to determine the exact mechanisms for those actions. Findings from those studies could lay the foundation for combined use of those drugs in painless labor.

Acknowledgements

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