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Research Article

Remifentanil induces apoptosis and G1-phase cell cycle arrest in human MCF-7 breast cancer cells

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Abstract

Objectives: Remifentanil, a fentanyl-derivative opioid analgesic acting as a µ-opioid receptor agonist, is a crucial drug in anesthesia due to its numerous benefits during surgical procedures. This study aimed to explore whether remifentanil effectively induced apoptosis in MCF-7 breast cancer cells via possible mechanisms.

Methods: Flow cytometry was performed for Annexin V/7-aminoactinomycin (7-AAD) and DAPI staining, cell cycle assays, and to measure reactive oxygen species (ROS) levels. Immunoassays for lactate dehydrogenase (LDH) and interleukin (IL)-6, as well as a chorioallantoic membrane (CAM) test, were also performed.

Results: Remifentanil effectively suppressed cell proliferation and led to the induction of cell cycle arrest at the G1 phase in MCF-7 cells. Compared with the control group, MCF-7 cells treated with remifentanil had a higher apoptotic rate with nuclear fragmentation, increased LDH release, and lower IL-6 concentrations. Overgeneration of ROS and decreased angiogenic activity were also observed in remifentanil-treated MCF-7 cells.

Conclusion: Remifentanil led to G1-phase arrest and apoptosis in MCF-7 cells. The mechanism of action of remifentanil likely involves the suppression of IL-6 production and angiogenesis, along with enhanced ROS levels and LDH generation. This preliminary study highlighted the need for further experimental evidence from future research to clearly support the significant potential of remifentanil as an anticancer agent for breast cancer.

Keywords: Anticancer effect, apoptosis, MCF-7 breast cancer cells, oxidative stress, remifentanil

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Breast cancer represents one of the most prevalent female malignant tumors globally [1]. According to a recently published statistic in 2023, thirteen percent of malignancies in women are caused by breast cancer, and the greatest number of deaths from breast cancer among women has been reported [1]. However, the global mortality of breast cancer is falling as a better indicator of progress due to early diagnosis through the established system of mammography screening, increased awareness, and improvements in comprehensive treatment options such as adjuvant chemotherapies [1]. The available options include surgery, radiation, chemotherapy, immunotherapy, or hormonal therapies, depending on the stage and subtype of breast cancer [2]. Despite significant advancements in diagnostic and therapeutic approaches against breast cancer, the treatment still remains a challenging endeavor due to the high relapse and lethality rate of the disease, significant side effects, and the lower effectiveness of therapeutic strategies [3].

Patients with breast cancer are most commonly diagnosed as hormone receptor-positive [4]. The MCF-7 (Michigan Cancer Foundation-7) cell line is characterized as the Luminal A subtype of breast cancer, which is an estrogen and progesterone receptor-positive tumor [5]. A primary care approach for the

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majority of solid organ malignancies is surgery [6]. Nonetheless, it is typical for cancer to return following surgery intended to be curative; this frequently results in refractory illness and patient mortality [6]. The biological milieu created by surgery and perioperative care encourages the survival and proliferation of cancer cells that are still present in the primary tumor after the procedure, which may raise the risk of metastasis [6]. Analgesic and anesthetic therapies may influence these processes and, in turn, either positively or negatively increase the likelihood of cancer recurrence, according to mounting data [6].

Fentanyl and its derivative remifentanil are potent opioid analgesics [7]. Remifentanil, as a selective μ-opioid receptor agonist, is used extensively in surgeries for the induction of general anesthesia and the preservation of clinical analgesia [8]. Remifentanil is a valuable agent in anesthetic practice due to its several advantages in surgeries. It has significant advantages, such as its analgesic effect, which is about 100–200 times higher than that of morphine [9]. It metabolizes quickly by non-specific esterases in the blood and tissues and acts swiftly, leading to an abrupt cessation of effects once the infusion stops [8]. This allows precise control over anesthesia depth and quick recovery after surgery [8]. Remifentanil aids in preserving a steady heart rate and blood pressure during surgery, reducing the risk of hemodynamic fluctuations, which is particularly beneficial for high-risk patients and complex procedures [10]. Its pharmacokinetic profile enables easy and precise titration to the desired effect, allowing anesthesiologists to adjust the dose in real time [10]. Due to its rapid metabolism and high plasma clearance rate, remifentanil does not accumulate in the body, even with prolonged use, making it ideal for long surgeries or extended analgesia [10]. Remifentanil can be used in various surgical settings, including general anesthesia, procedural sedation, as an adjunct in regional anesthesia, and even in the intensive care unit for optimal analgesia and sedation [11, 12]. Due to its advantages, remifentanil is often favored over other short-acting opioids and has become the primary choice of therapy in many intensive care units [11, 12]. These advantages make remifentanil a preferred choice for anesthesiologists, particularly where precise control over anesthesia and rapid recovery are critical.

Reactive oxygen species (ROS) are generated during aerobic metabolism within the biological system [13]. Inflammation triggers higher amounts of ROS, which might result in oxidative stress [14]. In addition, oxidative stress is capable of producing an increased inflammatory response [14]. ROS at low levels act as signaling molecules in the regulation of cellular or physiological activities and homeostasis [13]. The overproduction of ROS due to a disturbed redox balance would result in oxidative stress under pathophysiological conditions, which causes a direct oxidizing effect and thereby damage to fundamental cellular elements, including lipids, proteins, and DNA [14].

It has been reported that remifentanil alleviates inflammation and oxidative stress in many experimental models [15, 16]. Remifentanil ameliorated myocardial ischemia and acute lung injury in rats via the inhibition of several proinflammatory cytokines [15, 17]. Oxidative stress and inflammation are crucial mechanisms in the pathogenesis of cancer [14]. In addition, previous *in vitro* studies have demonstrated controversial results that opioids could inhibit or promote the proliferation of various cancer cells [18, 19]. Morphine promoted apoptosis and caused G0/G1 and G2/M phase arrest in hepatocellular and gastric cancer cell lines, respectively [20, 21]. Moreover, a study by Li et al. [22] demonstrated the anticancer effects of fentanyl on MGC-803 gastric cancer cells via cell cycle arrest in the G0/G1 phase.

Specifically, this study examines how remifentanil influences cytotoxicity, apoptosis, cell cycle, inflammation, oxidative stress, and angiogenesis of MCF-7 cells, considering the broader context of using antioxidant and anti-inflammatory agents as an anticancer strategy. However, future research could be employed on animal models and various kinds of cancer cell lines to elucidate the *in vivo* and *in vitro* anticancer effects of remifentanil in detail. Then the preclinical data can be adopted for clinical studies.

Materials and Methods

Cell culture

MCF-7 cells (ATCC, Manassas, VA, USA) were grown in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA, USA) with 10% fetal bovine serum and 1% penicillin/streptomycin at 37°C with 5% CO_2. Adherent cells were sub-cultured every two days. At a confluency of 80%, the cells were passaged with 0.05% trypsin (Gibco, CA, USA) for further experiments. Stock solutions of remifentanil were dissolved in dimethyl sulfoxide (DMSO) or saline (0.9% NaCl) and subsequently in culture medium. Throughout treatment, the DMSO levels did not rise above 0.1%.

Cell viability

The MCF-7 cell viability was evaluated by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay (Beyotime Biotech, Haimen, China). In summary, 150 μL of cell mixture ($1{\times}10^4$ cells) was added to each well. The cells were incubated overnight and then treated for 24 and 48 h with remifentanil (0.15625, 0.3125, 0.625, 1.25, 2.5, 5, 10 μg/mL). After the removal of media, MTT solution (Sigma Aldrich, Missouri, USA) was applied to each well for 4 h at 37°C. Following the addition of 150 μL of DMSO, the absorbance of each well was determined at 550 nm using a plate reader.

Apoptosis assay by flow cytometry

The apoptosis rate was assessed using an Annexin V/7-aminoactinomycin (7-AAD) detection kit (BD Biosciences, NJ, USA). The cells were exposed to 0.44 μg/mL of remifentanil for 24 and 48 h in 6-well culture plates. Cells were then collected, rinsed with cold PBS, suspended in binding buffer, and stained with PE-Annexin V for 15 minutes. After the addition of 7-AAD (5 μg/mL) and binding buffer, tubes were incubated for 30 min in the dark. Then, a flow cytometer (BD Accuri C6, USA) was used for the quantification of apoptotic cells.

4′,6-Diamidino-2-phenylindole (DAPI) staining

After exposure to remifentanil for 24 and 48 h in 6-well plates, MCF-7 cells (2×10^4 cells/well) were collected, rinsed with PBS, and then preserved in 4% formaldehyde for 10 minutes. After centrifugation, cells were treated with 20 µL DAPI (Invitrogen, USA) in the dark for 20 min at room temperature. Afterwards, the supernatant was removed, the cell pellet was mixed with sterile PBS, and the cell suspension was placed on coverslips. The nuclei of the stained cells were visualized under confocal microscopy (Zeiss LSM 510, USA).

Cell cycle assay by flow cytometry

After 24 and 48 h of treatment with remifentanil (0.44 µg/mL), the cells were collected by 0.05% trypsin for harvesting, washed twice with assay buffer, and fixed in 70% ethanol at 4°C overnight. Following the elimination of ethanol, cells were resuspended in PBS. After staining cells with propidium iodide (PI) containing RNase (20 µg/mL) for 30 min at 37°C in the dark, a flow cytometer (BD Accuri C6, USA) with CellQuest software was used to determine cell cycle distribution by measurements of side and forward scatter.

Flow cytometric analysis of intracellular ROS generation

Dichloro-dihydrofluorescein diacetate (DCF-DA) probe, a ROS-sensitive fluorescent dye, was used to determine ROS generation according to the manufacturer's instructions. After cells were treated for 24 and 48 h with remifentanil, cells suspended in PBS were incubated with DCF-DA (10 µM) for 30 min in an incubator. Finally, the DCF fluorescence of cells was detected via a BD Accuri C6 flow cytometer.

Lactate dehydrogenase (LDH) and interleukin-6 (IL-6) measurement

After treatment with remifentanil (0.44 µg/mL) in MCF-7 cells for 24 and 48 h in 6-well plates, cell supernatant was collected to measure LDH and IL-6 levels using the Roche Cobas 6000 and e411 analyzer and related kits.

Chorioallantoic membrane (CAM) assay for angiogenesis

Fertilized chicken eggs were cultivated at 37°C and 85–90% relative humidity for the purpose of the ex ovo CAM assay. Atak-S chickens raised at the Ministry of Agriculture and Forestry's Poultry Research Institute in Ankara, Turkey, provided the fertilized chicken eggs. The cleaned eggs were incubated for 72 hours, with a 2-hour rotation, in an incubator (CIMUKA 40080 serial number-incubator). Following incubation, the embryo side of the eggs was labeled and examined under a light source. The eggs were cracked using forceps from the opposite side so that the selected region remained on top. After being placed inside the weighing container, the eggs were returned to the incubator and covered with glass lids sterilized with ethylene oxide. The eggs were incubated for an additional three days to increase the visibility of the CAM. Remifentanil (0.44 µg/mL) was applied ex ovo to the chorioallantoic membrane following incubation. Following the application, the groups were observed at 0, 24, and 48 h using a stereomicroscope (S6D; Leica Microsystems, Heerbrugg, Switzerland). Quantitative analysis of the captured images was conducted using the ImageJ program (National Institutes of Health).

Statistical analysis

GraphPad Prism 8 software was used to compare data using a one-way analysis of variance (ANOVA) test. Each experiment was conducted in two biological replicates and three technical replicates. $P<0.05$ was regarded as statistically significant after comparing the variations between means (mean±SD).

Results

Remifentanil inhibited the viability of MCF-7 breast cancer cells

To evaluate the antiproliferative effects of remifentanil, MCF-7 cells were incubated with remifentanil between 0.15625–10 µg/mL for 24 and 48 h. After exposure to remifentanil, a dose- and time-dependent decline in cell viability was observed. The results showed that remifentanil induced cytotoxicity within the range of dosages tested for 24 and 48 h (Fig. 1). As expected, remifentanil administered for 48 h at the dose of 10 µg/mL offered the highest cytotoxic effect. The IC_{50} values of remifentanil were calculated as 0.86 µg/mL for 24 h and 0.44 µg/mL for 48 h treatment. For the selection of remifentanil dosages for 24 and 48 h of treatment in the further experiments, we have taken 0.44 µg/mL, which was the IC_{50} concentration of remifentanil for 48 h in MCF-7 cells.

Remifentanil induced apoptosis in MCF-7 cells

To assess the apoptotic progression of the remifentanil-exposed cells, flow cytometry was used. The percentages of early and late apoptotic cells were significantly increased after 0.44 µg/mL remifentanil treatment for 24 h in MCF-7 cells ($p<0.05$). For 48 h treatment, the results demonstrated that remifentanil (0.44 µg/mL) promoted early and late apoptosis; however, only the proportion of late apoptosis was significantly higher in comparison to the untreated cells ($p<0.05$; Fig. 2a). The percentage of total apoptosis induced by 24 h of remifentanil treatment in MCF-7 cells (18.9%) was considerably higher than that of 48 h of treatment (14%). However, a lower percentage of MCF-7 cells undergoing necrosis after exposure to 0.44 µg/mL remifentanil for 24 h (13.3%) was observed compared to 48 h of treatment (17.3%).

Remifentanil led to nuclear fragmentation in MCF-7 cells

Nuclear condensation and apoptotic body formation are considered the core features of apoptosis in cancer cells [23]. DAPI staining was performed to observe whether remifentanil causes nuclear morphological alterations. In the untreated MCF-7 cells, there were rounded and homogeneous nuclei, while cells treated with 0.44 µg/mL remifentanil for 24 and 48 h exhibited apoptotic morphology with nuclear fragmentation and condensation. MCF-7 cells incubated with 48 h of remifentanil showed darker and more intense blue coloration compared to 24 h incubation (Fig. 2b). The results suggest that remifentanil led to cell death, and these data also correlated with the cytotoxicity results and apoptosis data obtained by flow cytometry.

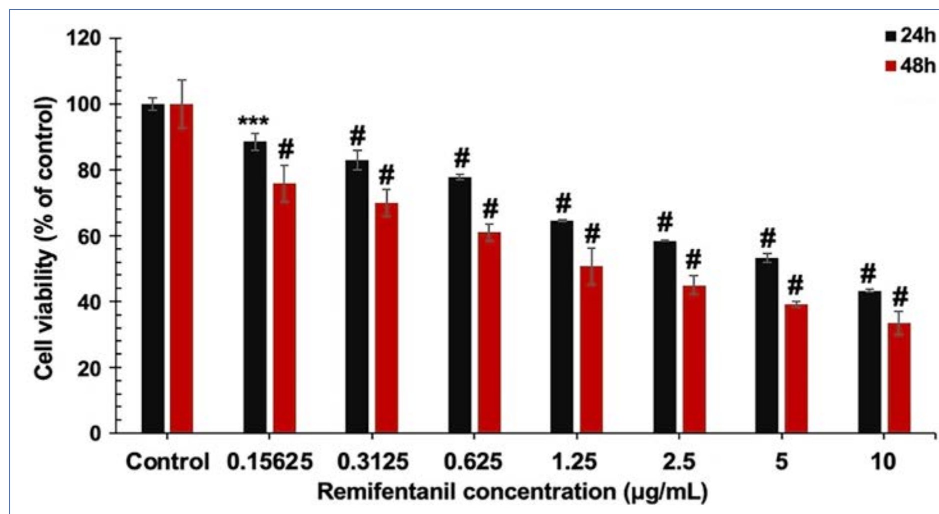

Figure 1. Determination of cell viability by MTT assay after 24 and 48 h remifentanil treatment (0, 0.15625, 0.3125, 0.625, 1.25, 2.5, 5 and 10 μg/mL).

The three separate experiments are shown as the mean±SD. ***: p<0.0005; #: p<0.0001 vs control group. MTT: 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide; SD: Standard deviation.

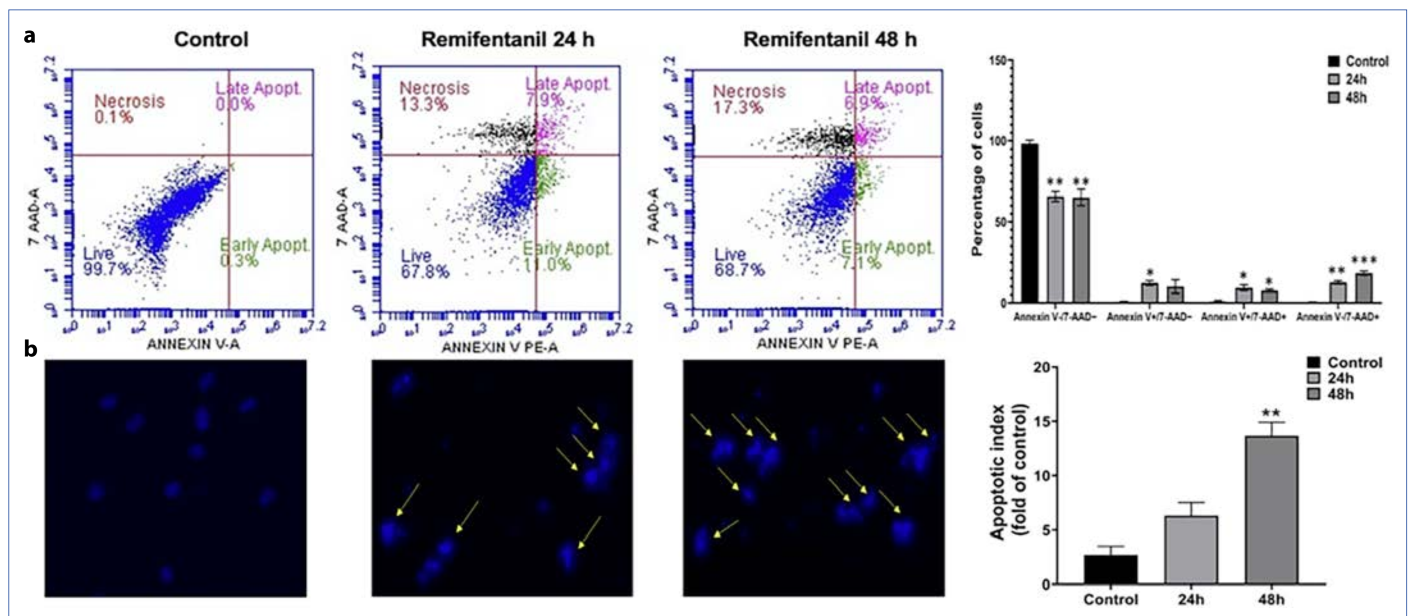

Figure 2. The effect of remifentanil (0.44 μg/mL) on the apoptosis of MCF-7 breast cancer cells after 24 and 48 h treatment. (a) Apoptotic cell death induced by 24 and 48 h remifentanil treatment via Annexin V/7-AAD double staining. The percentage of viable, early, late and necrotic cells was shown in upper right panel. (b) Fluorescence microscopy images at 20x magnification after DAPI staining of MCF-7 cells incubated with remifentanil (0.44 μg/mL) for 24 and 48 h. Apoptotic cells are depicted by the arrows. Quantitative analysis of apoptosis detected by DAPI staining is depicted in right panel.

The data are shown as the mean±SD. *: p<0.05; **: p<0.01; ***: p<0.001 vs control group. MCF-7: Michigan Cancer Foundation-7; V/7-AAD: V/7-aminoactinomycin; DAPI: Diamidino-2-phenylindole.

Remifentanil arrested the MCF-7 cell cycle in the G1 phase

To better clarify the mechanism of remifentanil-induced cytotoxicity, the distribution of cell cycle phases was determined in MCF-7 cells after incubation with 0.44 μg/mL remifentanil for 24 and 48 h. As presented in Figure 3, the cell population after 24 and 48 h of remifentanil treatment was highly accumulated in the G1 phase (28.5% and 37.6%, respectively) compared to the untreated cells (19.5%), which was accompanied by a concomitant decrease in the G2 population. Following 24 and 48 h of treatment with remifentanil, the percentage of MCF-7 cells in the G2 phase was 70.2% and 60.6%, respectively, while 75.9% of the control cells were found to be in the G2 phase (Fig. 3a, b). Moreover, there were no alterations in the S phase among groups. The data suggest that

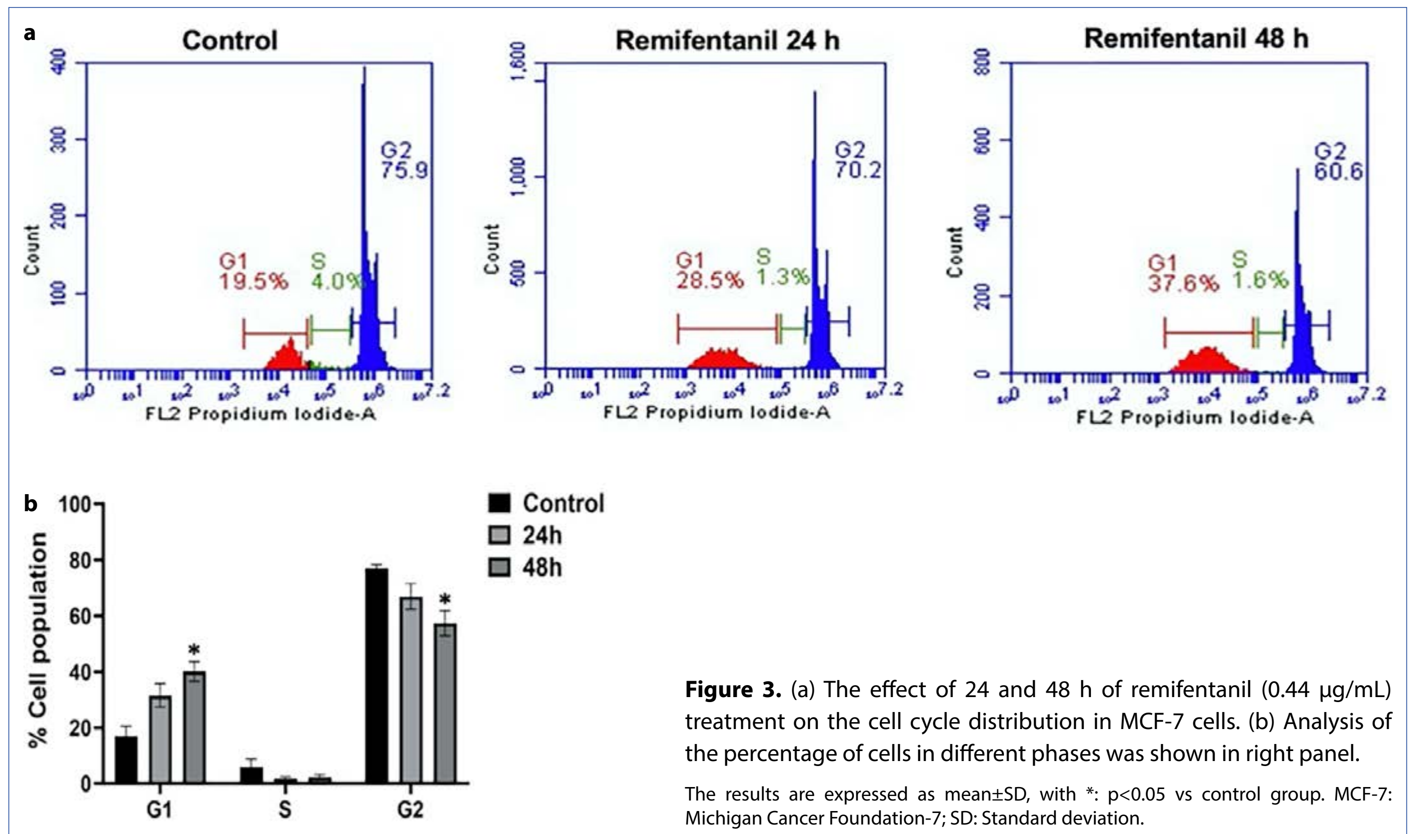

Figure 3. (a) The effect of 24 and 48 h of remifentanil (0.44 µg/mL) treatment on the cell cycle distribution in MCF-7 cells. (b) Analysis of the percentage of cells in different phases was shown in right panel.

The results are expressed as mean±SD, with *: p<0.05 vs control group. MCF-7: Michigan Cancer Foundation-7; SD: Standard deviation.

remifentanil induces apoptosis by causing cell cycle arrest in the G1 phase in MCF-7 cells.

Remifentanil promoted ROS overproduction in MCF-7 cells

In the present study, we evaluated whether remifentanil influences the generation of ROS in MCF-7 cells. Remifentanil exposure caused a rise in ROS production to 42.5% after 24 h and 47.2% after 48 h of 0.44 µg/mL remifentanil treatment compared to the control group (13.7%) (p<0.01, Fig. 4a, b).

Remifentanil increased lactate dehydrogenase (LDH) release and repressed interleukin (IL)-6 levels in MCF-7 cell culture media

Remarkable upregulation of LDH release in the supernatant of MCF-7 cells was observed after 48 h of treatment with 0.44 µg/mL remifentanil (p<0.01, Fig. 5a). The IL-6 levels for remifentanil-treated MCF-7 cells at a dose of 0.44 µg/mL diminished in a time-dependent manner (p<0.05 and p<0.01 for 24 and 48 h, respectively; Fig. 5b).

Remifentanil restrained angiogenesis in MCF-7 cells

The CAM model was used to evaluate the effect of remifentanil on angiogenesis *in vivo* [24]. The effect of 24 and 48 h of remifentanil (0.44 µg/mL) on the inhibition of angiogenesis was demonstrated by a decreased percentage of vascular proliferation (p<0.01; Fig. 6a, b). The ability of remifentanil to repress angiogenesis might contribute to its antitumor effect in the MCF-7 cell line.

Discussion

Remifentanil, a fentanyl derivative, is an ultra-short-acting, highly effective opioid analgesic agent with its predictable pharmacokinetic profile [8]. It is especially valuable in anesthesia due to its rapid action and metabolism, making it more potent than morphine [9]. In clinical practice, the dosing of remifentanil varies depending on the context of its use [25]. For the induction of anesthesia, a loading remifentanil dose of 1.0 microgram per kilogram (mcg/kg) is administered over 30 to 60 seconds, followed by maintenance of anesthesia typically ranging from 0.1 to 1.0 mcg/kg/minute based on the patient's response and surgical conditions [25]. In the intensive care unit, for sedation, remifentanil is used at a much lower continuous infusion rate, usually between 0.01 and 0.05 mcg/kg/minute [25]. Its pharmacokinetic profile prevents accumulation, even with prolonged use, making it ideal for long surgeries [8]. The drawbacks of using remifentanil in intensive care, such as the potential for hyperalgesia and withdrawal symptoms upon discontinuation, can be effectively controlled [26]. These concerns should not prevent its utilization, as they can be addressed by providing analgesics or opiates after remifentanil infusion and before dose tapering [26]. High doses of remifentanil may pose a risk that necessitates careful monitoring and management to address potential complications [9].

Research in the literature has increasingly supported the anticancer effects of fentanyl and its derivatives [22, 27, 28]. However, studies focusing on the apoptotic effects of

Figure 4. (a) The effect of 24 and 48 h of remifentanil (0.44 μg/mL) treatment on MCF-7 cell ROS production by flow cytometric analysis. (b) Mean fluorescence intensity was illustrated in the lower left panel.

The results are expressed as mean±SD, with **: $p<0.01$ vs control group. MCF-7: Michigan Cancer Foundation-7; ROS: Reactive oxygen species; SD: Standard deviation.

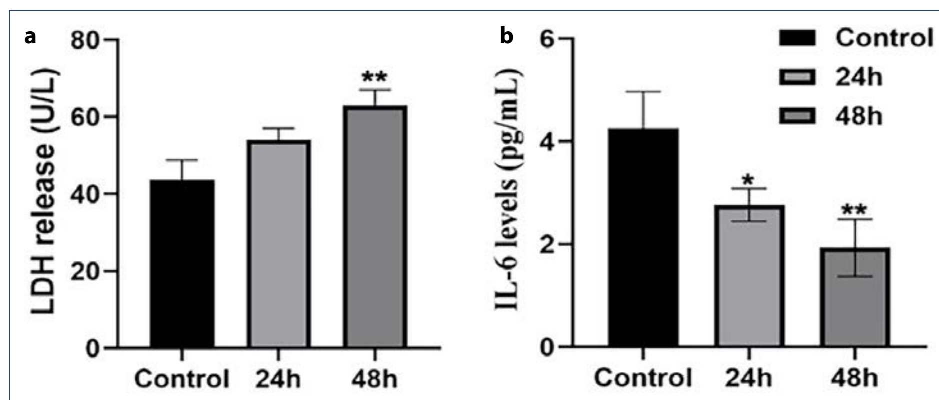

Figure 5. Levels of (a) LDH and (b) IL-6 in MCF-7 cell culture media after 24 and 48 h exposure of remifentanil (0.44 μg/mL).

The results are expressed as mean±SD, with *: $p<0.05$; **: $p<0.01$ vs control group. LDH: Lactate dehydrogenase; IL-6: Interleukin-6.

remifentanil in different cancer cells are limited in number [29–31]. The beneficial effects of remifentanil in normal cell lines at nanomolar levels have been previously reported [32, 33]. These data may demonstrate the high safety and selectivity profile of remifentanil due to its decreased cytotoxicity to normal cells and antitumor effects on various cancer cell lines [29–31], even though the applied concentrations of remifentanil vary. Another opioid agent, morphine, also showed higher cytotoxicity against the human MCF-7 breast cancer cell line than normal human cells [34] and significantly increased the proliferation of MDA-MB-231 breast cancer cells at low concentrations but demonstrated cytotoxic effects at higher concentrations [35].

In addition, a growing body of evidence has indicated good anti-inflammatory and antioxidant activities of remifentanil in various animal disease models [15–17]. Based on the confirmed relationship between inflammation, oxidative stress, and the carcinogenesis process, we have therefore proposed that remifentanil can exert anticancer properties via anti-inflammatory and antioxidant mechanisms of action.

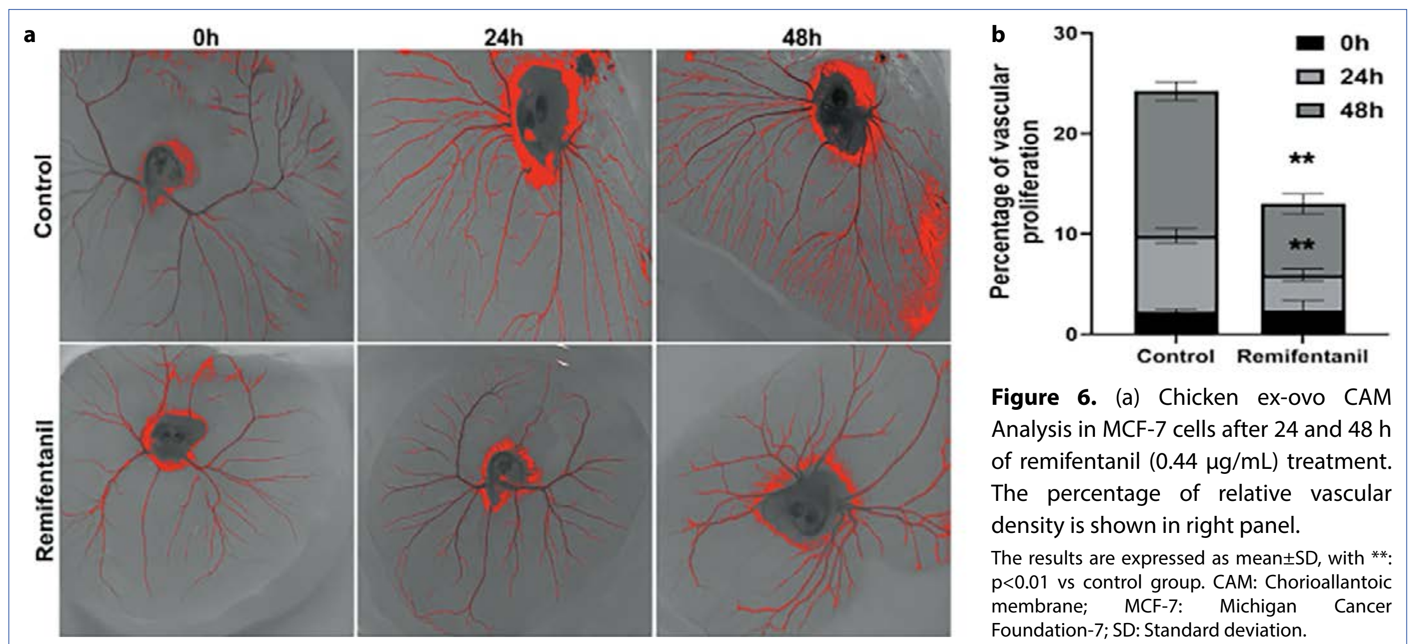

Figure 6. (a) Chicken ex-ovo CAM Analysis in MCF-7 cells after 24 and 48 h of remifentanil (0.44 μg/mL) treatment. The percentage of relative vascular density is shown in right panel.

The results are expressed as mean±SD, with **: p<0.01 vs control group. CAM: Chorioallantoic membrane; MCF-7: Michigan Cancer Foundation-7; SD: Standard deviation.

In this study, remifentanil significantly diminished cell proliferation in a time-dependent manner and also, as expected, promoted apoptosis. In addition, apoptotic nuclear morphology, as evidenced by fragmented nuclei, was observed after remifentanil treatment according to DAPI staining. Supporting this, a previous study indicated that remifentanil inhibited cell viability and triggered apoptotic cell death in C6 glioma cells [29]. Another fentanyl derivative, sufentanil, has been shown to lead to apoptosis and a decrease in the growth of MDA-MB-231 breast cancer cells [28]. Moreover, the arrest of cell cycle progression is thought to be an efficient approach for the elimination of cancer cells via apoptosis [36].

Our findings indicate that remifentanil treatment leads to an arrest of MCF-7 cells in the G1 phase, accompanied by a diminished cell population in the G2 phase. This suggests that remifentanil may promote apoptosis by halting cell cycle progression at the G1 phase. Cancer cells usually develop a defective G1/S checkpoint, whereas the G2/M checkpoint is often intact and crucial for the survival of cancer cells [37]. Thus, remifentanil, as a potential anticancer agent, could lead to G1 arrest rather than arrest in the S or G2/M phases. In addition, it is known that ROS-induced DNA damage activates the DNA damage response (DDR) [38]. Based on this, the oxidative stress induced by elevated ROS generation after remifentanil treatment could activate DDR and subsequently lead to cell cycle arrest. Thus, the remifentanil-induced mechanisms involved in DDR require further investigation.

Moreover, as supporting evidence, Yang et al. [39] demonstrated that the upregulation of ROS levels plays a key role in apoptosis through inhibiting cell proliferation, activating pro-apoptotic protein expression, and triggering cell cycle arrest in breast cancer cells. It can be suggested that the inhibition of cell proliferation and cell cycle arrest induced by remifentanil treatment might be mediated by ROS.

ROS accumulation has been found to promote cancer cell death via irreversible oxidative damage to cellular structures, which is the mechanism of many chemotherapeutic drugs [40]. We observed that exposure to remifentanil for 24 and 48 h caused elevated levels of ROS in MCF-7 cells. In a study by Yao et al. [41], fentanyl was shown to induce ROS generation in A549 lung cancer cells. The apoptosis-inducing effects of remifentanil might be attributed to increased production of ROS.

The main enzyme, lactate dehydrogenase (LDH), catalyzes the aerobic glycolysis of pyruvate in cancer cells to produce ATP [42]. It has been shown that increased LDH release into the culture supernatants of cancer cells is related to irreversible cell death due to cell membrane damage and disruption of membrane integrity [42]. The higher amount of LDH in the culture supernatant is a supportive marker for the cytotoxic potential of remifentanil in MCF-7 cells, as supported by similar *in vitro* results with other cytotoxic agents [43].

IL-6, as one of the inflammatory cytokines, exhibits abnormal expression in many cancer cells, with a critical role in tumor progression [44]. In the current study, remifentanil reduced the production of IL-6 in MCF-7 cells. Similarly, remifentanil restricts the release of IL-6 in LPS-induced BV2 microglia cells and an acute lung injury rat model [17, 45]. ROS levels are also critical to inducing IL-6 gene expression [46], and IL-6 can also promote the production of ROS [47]. Depending on the tissue microenvironment and concentration, IL-6 may exhibit either pro- or anti-inflammatory actions [48]. Interestingly, in the present study, the downregulation of IL-6 levels coincided with increased ROS levels in MCF-7 cells exposed to remifentanil, which could be related to the induction of ROS-mediated signaling pathways associated with IL-6 or the alterations in transcriptional activation of the IL-6 gene through ROS production.

Furthermore, tumor progression is profoundly affected by angiogenesis, and the inhibition of angiogenesis might block

the growth of cancer cells [49]. The inhibition of angiogenesis, along with decreased vascular proliferation, was observed after remifentanil treatment according to the CAM assay. The inhibitory effect of remifentanil on IL-6 cytokine production and angiogenesis in MCF-7 cancer cells is possibly related to its ability to induce apoptotic cell death.

Evidence suggests that anesthetic and pain management techniques can significantly impact underlying biological processes, potentially altering the risk of cancer recurrence for better or worse [50]. In a recent study, the combination of remifentanil and dexmedetomidine showed improvement in analgesia following surgery while lowering immunosuppression by regulating T lymphocyte subsets [51]. Despite concerns about remifentanil-induced hyperalgesia [26], it remains a preferred choice due to its various benefits [52]. While our data point to potential benefits for cancer patients, further research is essential. Comprehensive *in vitro* and *in vivo* experiments, along with clinical trials, are needed to fully understand and confirm the anticancer effects of remifentanil. These studies should involve combinations with other agents and various cancer cell lines to fully explore the potential of remifentanil in cancer therapy. Since most current evidence on the effects of anesthetic and analgesic agents on cancer cells comes from experimental models, there is a need for more retrospective and prospective studies to establish a relationship between anesthesia and improved cancer outcomes.

The current study contains a number of limitations. Firstly, only the MCF-7 cell line was used in this *in vitro* study. Animal models and various kinds of cancer cell lines could be used to determine and validate the *in vivo* and comprehensive *in vitro* anticancer impacts of remifentanil in further studies. In our study, we used 0.44 µg/mL remifentanil for experiments conducted for 24 and 48 h. The beneficial effect of remifentanil on healthy cell lines at the nanomolar level [32,33] and its antitumor effects on different cancer cell lines at the micromolar level have been reported in studies [29,31]. Due to the application of higher remifentanil concentrations in cancer cell lines compared to normal cells, remifentanil treatment at the micromolar level could lead to potential side effects. More experimental data are needed to investigate the effects of remifentanil at two different concentrations ($2\times IC_{50}$, IC_{50}) to explore dose-dependent toxicity in noncancerous cells in future studies.

It should also be considered that cancer patients frequently need to take other analgesics and antitumor medications simultaneously [41]. Further studies should also be conducted to determine the molecular mechanisms by which remifentanil impacts cancer cell sensitivity to common chemotherapy. Moreover, by presenting more reliable data regarding the toxicity, stability, and negative effects of remifentanil, future research applying various animal models and clinical trials will generate stronger results to clarify the potential of remifentanil for clinical application.

Conclusion

This study, for the first time, delineated that remifentanil showed significant cytotoxic and apoptotic effects in MCF-7 cells, which could be associated with G1 cell cycle arrest, elevated ROS production, and inhibition of IL-6 release and angiogenesis *in vitro*. Any off-target effects or interactions with standard cancer therapies require thorough investigation. This preliminary study warrants more experimental *in vitro* and *in vivo* evidence from future research to clearly support the great potential of remifentanil as an anticancer agent against breast cancer.

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References

1. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. CA Cancer J Clin 2023;73(1):17–48. [CrossRef]
2. Wang Y, Minden A. Current molecular combination therapies used for the treatment of breast cancer. Int J Mol Sci 2022;23(19):11046. [CrossRef]
3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71(3):209–49. [CrossRef]
4. Pfeiffer RM, Webb-Vargas Y, Wheeler W, Gail MH. Proportion of U.S. trends in breast cancer incidence attributable to long-term changes in risk factor distributions. Cancer Epidemiol Biomarkers Prev 2018;27(10):1214–22. [CrossRef]
5. Subik K, Lee JF, Baxter L, Strzepek T, Costello D, Crowley P, et al. The expression patterns of ER, PR, HER2, CK5/6, EGFR, Ki-67 and AR by immunohistochemical analysis in breast cancer cell lines. Breast Cancer Auckl 2010;4:35–41. [CrossRef]
6. Duff S, Connolly C, Buggy DJ. Adrenergic, inflammatory, and immune function in the setting of oncological surgery: their effects on cancer progression and the role of the anesthetic technique in their modulation. Int Anesthesiol Clin 2016;54(4):48–57. [CrossRef]
7. Ziesenitz VC, Vaughns JD, Koch G, Mikus G, van den Anker JN. Pharmacokinetics of fentanyl and its derivatives in children: A comprehensive review. Clin Pharmacokinet 2018;57(2):125–49. [CrossRef]

8. Feldman PL. Insights into the chemical discovery of remifentanil. Anesthesiology 2020;132(5):1229–34. [CrossRef]
9. Santonocito C, Noto A, Crimi C, Sanfilippo F. Remifentanil-induced postoperative hyperalgesia: Current perspectives on mechanisms and therapeutic strategies. Local Reg Anesth 2018;11:15–23. [CrossRef]
10. Komatsu R, Turan AM, Orhan-Sungur M, McGuire J, Radke OC, Apfel CC. Remifentanil for general anaesthesia: A systematic review. Anaesthesia 2007;62(12):1266–80. [CrossRef]
11. Reade MC, Finfer S. Sedation and delirium in the intensive care unit. N Engl J Med 2014;370(5):444–54. [CrossRef]
12. Barr J, Fraser GL, Puntillo K, Ely EW, Gélinas C, Dasta JF, et al. Clinical practice guidelines for the management of pain, agitation, and delirium in adult patients in the intensive care unit. Crit Care Med 2013;41(1):263–306. [CrossRef]
13. Droge W. Free radicals in the physiological control of cell function. Physiol Rev 2002;82(1):47–95. [CrossRef]
14. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: How are they linked? Free Radic Biol Med 2010;49(11):1603–16. [CrossRef]
15. Zhou Q, Song J, Wang Y, Lin T. Remifentanil attenuates cardiac dysfunction, lipid peroxidation and immune disorder in rats with isoproterenol-induced myocardial injury via JNK/NF-KB p65 inhibition. Ann Transl Med 2020;8(8):551. [CrossRef]
16. Shen J, Zhan Y, He Q, Deng Q, Li K, Wen S, et al. Remifentanil Promotes PDIA3 expression by activating p38MAPK to Inhibit Intestinal ischemia/reperfusion-induced oxidative and endoplasmic reticulum stress. Front Cell Dev Biol 2022;10:818513. [CrossRef]
17. Zhang Y, Du Z, Zhou Q, Wang Y, Li J. Remifentanil attenuates lipopolysaccharide-induced acute lung injury by downregulating the NF-kappaB signaling pathway. Inflammation 2014;37(5):1654–60. [CrossRef]
18. Ma Y, Ren Z, Ma S, Yan W, He M, Wang D, et al. Morphine enhances renal cell carcinoma aggressiveness through promotes survivin level. Ren Fail 2017;39(1):258–64. [CrossRef]
19. Harimaya Y, Koizumi K, Andoh T, Nojima H, Kuraishi Y, Saiki I. Potential ability of morphine to inhibit the adhesion, invasion and metastasis of metastatic colon 26-L5 carcinoma cells. Cancer Lett 2002;187(1–2):121–7. [CrossRef]
20. Dai H, Feng J, Nan Z, Wei L, Lin F, Jin R, et al. Morphine may act via DDX49 to inhibit hepatocellular carcinoma cell growth. Aging Albany NY 2021;13(9):12766–79. [CrossRef]
21. Qin Y, Chen J, Li L, Liao CJ, Liang YB, Guan EJ, et al. Exogenous morphine inhibits human gastric cancer MGC- 803 cell growth by cell cycle arrest and apoptosis induction. Asian Pac J Cancer Prev 2012;13(4):1377–82. [CrossRef]
22. Li C, Qin Y, Zhong Y, Qin Y, Wei Y, Li L, et al. Fentanyl inhibits the progression of gastric cancer through the suppression of MMP-9 via the PI3K/Akt signaling pathway. Ann Transl Med 2020;8(4):118. [CrossRef]
23. Hacker G. The morphology of apoptosis. Cell Tissue Res 2000;301(1):5–17. [CrossRef]
24. Mitrevska K, Merlos Rodrigo MA, Cernei N, Michalkova H, Splichal Z, Hynek D, et al. Chick chorioallantoic membrane (CAM) assay for the evaluation of the antitumor and antimetastatic activity of platinum-based drugs in association with the impact on the amino acid metabolism. Mater Today Bio 2023;19:100570. [CrossRef]
25. Battershill AJ, Keating GM. Remifentanil : A review of its analgesic and sedative use in the intensive care unit. Drugs 2006;66(3):365–85. [CrossRef]
26. Joly V, Richebe P, Guignard B, Fletcher D, Maurette P, Sessler DI, et al. Remifentanil-induced postoperative hyperalgesia and its prevention with small-dose ketamine. Anesthesiology 2005;103(1):147–55. [CrossRef]
27. Wu W, Wei N, Jiang CN, Cui S, Yuan J. Effects of sufentanil on human gastric cancer cell line SGC-7901 *in vitro*. Cent Eur J Immunol 2014;39(3):299–305. [CrossRef]
28. Li M, Gu K, Kong Q, Wang G, Gu J. Sufentanil inhibits the metastasis and immune response of breast cancer via mediating the NF-kappaB pathway. Immunopharmacol Immunotoxicol 2023;45(6):663–71. [CrossRef]
29. Xu J, Xu P, Li Z, Xiao L, Yang Z. The role of glycogen synthase kinase-3beta in glioma cell apoptosis induced by remifentanil. Cell Mol Biol Lett 2013;18(4):494–506. [CrossRef]
30. Chang J, Zhang W. Remifentanil modulates the TLR4-mediated MMP-9/TIMP1 balance and NF-kappaB/STAT3 signaling in LPS-induced A549 cells. Exp Ther Med 2023;25(2):79. [CrossRef]
31. Liang W, Ke J. Remifentanil reduces the proliferation, migration and invasion of HCC cells via lncRNA NBR2/miR-650/TIMP3 axis. Int J Exp Pathol 2022;103(2):44–53. [CrossRef]
32. Kwon JY, Park BS, Kim YH, Kim YD, Kim CH, Yoon JY, et al. Remifentanil protects human keratinocytes against hypoxia-reoxygenation injury through activation of autophagy. PLoS One 2015;10(1):e0116982. [CrossRef]
33. Yoon JY, Park CG, Park BS, Kim EJ, Byeon GJ, Yoon JU. Effects of remifentanil preconditioning attenuating oxidative stress in human dermal fibroblast. Tissue Eng Regen Med 2017;14(2):133–41. [CrossRef]
34. Hatsukari I, Hitosugi N, Ohno R, Hashimoto K, Nakamura S, Satoh K, et al. Induction of apoptosis by morphine in human tumor cell lines *in vitro*. Anticancer Res 2007;27(2):857–64.
35. Sezer G, Caner A, Onal MG, Cumaoglu A. Morphine counteracts the effects of paclitaxel in triple-negative breast cancer cells. Indian J Med Res 2022;156(1):70–6. [CrossRef]
36. Otto T, Sicinski P. Cell cycle proteins as promising targets in cancer therapy. Nat Rev Cancer 2017;17(2):93–115. [CrossRef]
37. Yan Y, Hein AL, Greer PM, Wang Z, Kolb RH, Batra SK, et al. A novel function of HER2/Neu in the activation of G2/M checkpoint in response to gamma-irradiation. Oncogene 2015;34(17):2215–26. [CrossRef]
38. Kuczler MD, Olseen AM, Pienta KJ, Amend SR. ROS-induced cell cycle arrest as a mechanism of resistance in polyaneuploid cancer cells (PACCs). Prog Biophys Mol Biol 2021;165:3–7. [CrossRef]
39. Yang B, Zhu W, Zheng Z, Chai R, Ji S, Ren G, et al. Fluctuation of ROS regulates proliferation and mediates inhibition of migration by reducing the interaction between DLC1 and CAV-1 in breast cancer cells. *In Vitro* Cell Dev Biol Anim 2017;53(4):354–62. [CrossRef]

40. Arfin S, Jha NK, Jha SK, Kesari KK, Ruokolainen J, Roychoudhury S, et al. Oxidative stress in cancer cell metabolism. Antioxidants Basel 2021;10(5):642. [CrossRef]
41. Yao J, Ma C, Gao W, Liang J, Liu C, Yang H, et al. Fentanyl induces autophagy via activation of the ROS/MAPK pathway and reduces the sensitivity of cisplatin in lung cancer cells. Oncol Rep 2016;36(6):3363–70. [CrossRef]
42. Yao H, Yang F, Li Y. Natural products targeting human lactate dehydrogenases for cancer therapy: A mini review. Front Chem 2022;10:1013670. [CrossRef]
43. Gabr SA, Elsaed WM, Eladl MA, El-Sherbiny M, Ebrahim HA, Asseri SM, et al. Curcumin modulates oxidative stress, fibrosis, and apoptosis in drug-resistant cancer cell lines. Life Basel 2022;12(9):1427. [CrossRef]
44. Hodge DR, Hurt EM, Farrar WL. The role of IL-6 and STAT3 in inflammation and cancer. Eur J Cancer 2005;41(16):2502–12. [CrossRef]
45. Huang Y, Cai Q, Liu H, Wang Y, Ma W. Remifentanil inhibits the inflammatory response of BV2 microglia and protects PC12 cells from damage caused by microglia activation. Bioengineered 2022;13(5):13944–55. [CrossRef]
46. Henriquez-Olguin C, Altamirano F, Valladares D, Lopez JR, Allen PD, Jaimovich E. Altered ROS production, NF-kappaB activation and interleukin-6 gene expression induced by electrical stimulation in dystrophic mdx skeletal muscle cells. Biochim Biophys Acta 2015;1852(7):1410–9. [CrossRef]
47. Wu Y, Antony S, Meitzler JL, Doroshow JH. Molecular mechanisms underlying chronic inflammation-associated cancers. Cancer Lett 2014;345(2):164–73. [CrossRef]
48. Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochim Biophys Acta 2011;1813(5):878–88. [CrossRef]
49. Rajabi M, Mousa SA. The role of angiogenesis in cancer treatment. Biomedicines 2017;5(2):34. [CrossRef]
50. Wall T, Sherwin A, Ma D, Buggy DJ. Influence of perioperative anaesthetic and analgesic interventions on oncological outcomes: A narrative review. Br J Anaesth 2019;123(2):135–50. [CrossRef]
51. Zhang Y, Jiang W, Luo X. Remifentanil combined with dexmedetomidine on the analgesic effect of breast cancer patients undergoing modified radical mastectomy and the influence of perioperative T lymphocyte subsets. Front Surg 2022;9:1016690. [CrossRef]
52. Yi S, Cao H, Zheng W, Wang Y, Li P, Wang S, et al. Targeting the opioid remifentanil: Protective effects and molecular mechanisms against organ ischemia-reperfusion injury. Biomed Pharmacother 2023;167:115472. [CrossRef]