Remifentanil Protects PC-12 Cells From OGD Damage by Up-Regulating miR-124

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Research

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Abstract

The purpose of the study was to clarify the function and mechanism of Remifentanil, in PC-12 cells stimulated by oxygen-glucose deprivation (OGD). An OGD environment was constructed to induce PC-12 cells, and Remifentanil (0-2.5 μM) was used to pre-treat cells; cell viability was determined by CCK-8 assay; cell apoptosis was tested via flow cytometry; knock down of miR-124 was achieved by constructing an miR-124 inhibitor and cell transfection; miR-124 expression and the transfection efficiency were tested via quantitative real-time PCR (RT-qPCR); Western blot was used to detect the expression of Bax/Bcl-2 / cleaved-caspase 3 / cleaved-caspase 9 apoptosis protein, as well as p62/LC3-I/LC3-II and JAK2/mTOR protein. Cell viability was not affected by the low concentration of Remifentanil, but was inhibited by the *high* concentration of Remifentanil.

OGD induction reduced cell viability, enhanced apoptosis and autophagy, and activated the JAK2/mTOR pathway. The above processes were reversed by Remifentanil, alleviating the influences of OGD stimulation on PC-12 cells. Meanwhile, miR-124 was positively regulated by Remifentanil, and miR-124 silencing reversed the effects of Remifentanil on cell viability, apoptosis, autophagy and the JAK2/mTOR pathway. In conclusion, Remifentanil protected PC-12 cells from OGD damage, which was mediated by up-regulation of miR-124 and activation of the JAK2/mTOR pathway.

Introduction

Brain injury is generally considered to be a leading cause of physical disability or cognitive death [1]. Congenital brain injury (CBI), commonly known as cerebral palsy, comprises a group of permanent, non-progressive disorders caused by static lesions of the immature brain [2]. Acquired brain injury refers to changes in brain function that occur later in life through non-traumatic or traumatic origins, and which have nothing to do with congenital or degenerative diseases [3]. The symptoms of cerebral nerve injury are various, such as cerebral ischemia, blood-brain barrier injury, blood circulation disorder, elevated intracranial pressure and oedema [4]. The most common causes of cerebral nerve injury are ischemia and hypoxia, which are caused by excessive release of glutamate (Glu), and by a cellular response induced by endoplasmic reticulum stress [5]. Cerebral ischemia is caused by temporary or permanent cerebral artery occlusion. The decrease of cerebral blood flow leads to neuronal death, and eventually leads to nervous-system damage. Cerebral ischemia is also the main cause of the aggravation of brain injury and dysfunction [6]. This injury involves complex multi-factor mechanisms, including intracellular Ca2+ overload and oxidative stress damage [7]. Strategies to avoid brain injury have been designed to reduce floating thrombosis in the cerebral circulation, reduce cerebral oxygenation, and minimize the systemic inflammation in the bypass circuit. Nonetheless, the search for effective drugs to protect against brain injury remained the focus of the current research.

It had been reported that opioids play a direct or pre-emptive role in brain protection [8]. Remifentanil was a new type of ultra-short-acting phenylpiperidine opioid analgesic, which could rapidly be metabolized in blood and tissues. It has a high affinity for mu (l)-opioid receptors, but a relatively low affinity for d-and j-

opioid receptors [9]. Jeong *et al.* previously reported that Remifentanil preconditioning attenuated focal cerebral ischemic injury in rats, and this neuroprotective effect might be obtained through the activation of opioid receptors [10]. Conversely, the follow-up regulatory mechanism of Remifentanil, insofar as it may play a neuroprotective role, had not been fully studied.

MiRNA (microRNA), a member of the small non-coding RNA superfamily, is an endogenous single-stranded RNA molecule of about 18-25 nucleotides. It acts as a negative regulator of more than 60% protein-encoded gene expression through degradation or translation inhibition of target mRNA [11]. MiRNA simultaneously regulates the targets involved in the pathophysiological process of cerebral ischemia, and has been considered as a potential biomarker for the diagnosis and prognosis of cerebral ischemia [12]. MiR-124 is preferentially expressed in the cerebral cortex and cerebellum.

The expression level of miRNA is lower in neural progenitor cells, but higher in differentiated and mature neurons [13], indicating that it plays an important role in the development and differentiation of neurons. In addition, it has been reported that miR-124 protects neurons during cerebral ischemia by regulating key genes and pathological processes, including autophagy, neuroinflammation, oxidative stress, neuronal excitability and neural differentiation [14-16].

Although it was not known whether miR-124 could be used as an important link in the protective mechanism of Remifentanil cells, it was clear that miR-124 mediates the function of opioids in cells. For example, in the study of neuronal neuropathic pain, it had been found that miR-124 targeted Toll-like receptors (TLR), and signal transduction reverses the persistent sensitization effects of morphine on neurons [17]. In the context of a new type of opioid, the regulatory relationship between Remifentanil and miR-124 was worthy of further exploration.

Janus kinases (JAK) are familial non-cellular protein tyrosine kinases (PTKs), including JAK1, JAK2, JAK3 and TYK2, which selectively interact with the cytoplasm of a variety of cytokine receptors [18]. There was growing evidence that abnormal JAK2 signal transduction was associated with a variety of cellular processes, including proliferation, differentiation and apoptosis [19,20]. Chen's study demonstrated that Gap19 exerted an anti-apoptotic effect by activating the JAK2/STAT3 pathway after brain ischemia-reperfusion injury [21]. The mammalian target of rapamycin (mTOR) was a conserved serine/threonine kinase that regulated cell growth and autophagy. In previous studies, Schizandrin A protected nerve cells from oxygen-glucose deprivation/reperfusion (OGD/R) damage, by regulating the mTOR pathway to inhibit autophagy [22].

In this study, we explored the neuroprotective effect of Remifentanil treatment on OGD-induced PC-12 cell injury. It was concluded that the neuroprotective effect of Remifentanil was related to the expression of miR-124 and the activation of the JAK2/mTOR pathway.

Materials And Methods

1.1 Culture and induction of PC-12 nerve cells *in vitro*

The special nutrition incubator was ventilated with air and CO_2 (ratio 95:5), and a constant temperature of 37℃ was simultaneously set. The PC-12 cells were cultured in DMEM medium, supplemented with 10% FBS (Crumlin, UK), and 100μg/mL of penicillin-streptomycin solution (Crumlin, UK). The sterile 96-well plate was used as the incubation carrier and cultured in the aforementioned incubator. The cells of the 8^{th} passage were used as experimental materials.

The cells were treated with OGD: accordingly, PC-12 cells were inoculated at the density of 5×10^3 cells per well for one week. After the cells were twice washed with PBS, the PC-12 cells were incubated with glucose-deficient DMEM. At the same time, the incubator was filled with N_2 and CO_2 (ratio 95:5) and induced at 37 ℃ for 2 hours.

Regarding the Remifentanil induction of cells: before OGD induction, PC-12 cells were pre-treated with Remifentanil of 0, 0.5, 1.0, 1.5, 2.0 and 2.5 μM, within the DMEM medium, to detect the effect of the drug on cells, and then OGD was induced *in vitro.*

1.2 Cell transfection

The miR-124 silencing vector (miR-124 inhibitor) and the NC inhibitor were synthesized by GenePharma (GenePharma, Shanghai, China). PC-12 cells were inoculated on a 24-well plate. After the confluence of the cells reached about 50-60%, transfections were performed with the above vectors, according to the manufacturer's instructions, of lipofectamine 3000 (Invitrogen, CA, USA). The group of cells without any transfection was used as a control group. The cells were co-incubated with 0.5-5 μg/μL carrier solution in a cell incubator, at 37 ℃, for 2-3 days. When the transfection efficiency exceeded 95%, the PC-12 cells were collected.

1.3 RT-qPCR (Real-time quantitative PCR)

RT-qPCR was performed to detect the expression of mRNA. The total RNA was extracted via a Trizol method (Invitrogen, CA, USA). The cDNA was synthesized according to the manufacturer's instructions, with a PrimeScript II first-strand cDNA synthesis kit (Takara Biomedical Technology Co. Ltd, Beijing). The reaction mixture and program were prepared with the SYBR®Green Supermix (Bio-Rad Laboratories, Inc., CA, USA). The program was performed using the ABI Prism 7500 (Applied Biosystems; Thermo Fisher Scientific Inc., USA). The mRNA expression level of the target gene was standardized by reference gene β-actin, and the results were calculated by the comparative quantitative $2^{-\triangle\triangle Ct}$ method.

1.4 Determination of PC-12 cell viability

After induction by OGD and Remifentanil *in vitro,* and 48 hours after transfection, the PC-12 cells in each group were inoculated into 96-well plates and cultured for 24 hours. The viability of the PC-12 cells was detected via a Cell Counting Kit-8 cell viability assay kit (CCK-8) (Dojindo, Shanghai, China). Every 10 μL CCK-8 was mixed with 100 μL cell suspension and cultured in a CO_2 incubator for one hour. Then, the

absorbance was measured at 450nm using a Bio-Rad ELISA enzyme labelling instrument (Bio-Rad, CA, USA). Cell viability (%) = [(A treatment-A blank) / (A control-A blank)] × 100.

1.5 Determination of apoptosis by flow cytometry

Flow cytometry, Annexin V-FITC (Annexin V) and PI (propidium iodide) (BD Biosciences, NJ, USA) were used to analyse the apoptosis of the PC-12 cells. After induction by OGD and Remifentanil *in vitro,* and transfection for 48 h, the PC-12 cells (1×10^6 cells per well) in a 6-well cell-culture plate were washed twice with PBS, and then stained for 15 min in darkness, at room temperature, with Annexin V-FITC. Finally, apoptosis was analysed by flow cytometry, including early apoptosis (Annexin V positive / PI negative in the fourth quadrant).

1.6 Western blot

Total proteins were extracted from tissue and cell samples using RIPA (Beyotime, Shanghai, China), and protein density was then detected using BCA (Pierce Company, USA). After protein electrophoresis, the proteins were transferred to the polyvinylidene fluoride (PVDF) membrane. The primary antibodies (anti-Bax (ab32503) / Bcl-2 (ab32124) / c-caspase3 (ab32042) c-caspase9 (ab2324) / p62 (ab207305) / LC-3 (ab63817) / p-JAK2 (ab32101) / p-mTOR (ab131538) / JAK2 (ab39636) / mTOR (ab32028)) were used at 1/5000~ 1/10000 dilution for immune blot analysis. Then, two washes were conducted with Tween-Tris buffer (TBST). The secondary antibody was labelled with horseradish peroxidase (HRP) (anti-rabbit IgG) at 1/10000 dilution. The blots were visualized by enhanced chemiluminescence (ECL) under the ChemiDoc imaging system (Bio-Rad, USA). The strength of the strip was quantified by Image Lab strength software (Bio-Rad, Shanghai, China).

1.7 Statistical analysis

All data were analysed using SPSS 18.0 statistical software (Chicago, IL, USA), and the results were shown as mean ± standard deviation (SD). A *t*-test or analysis of variance was used to compare the measurement data of normal distribution. Thus, $p < 0.05$ denoted statistical significance, marked '*' in the figure.

Results

Remifentanil attenuated OGD-induced PC-12 cell injury

The toxicity of Remifentanil on the viability of PC-12 cells was examined by CCK-8 assay. The results showed that Remifentanil solution with a concentration of 0-2.5 μM had little effect on the viability of PC-12 cells, but the Remifentanil solution of 3.0 - 3.5 μM significantly decreased the viability of PC-12 cells in each group ($p < 0.01$and $p < 0.001$, fig. 1A). In addition, after induction by OGD, the viability of the PC-12 cells in the OGD group was significantly decreased ($p < 0.001$), while the viability of the PC-12 cells in the

OGD + Remifentanil group, which had been pre-treated with Remifentanil solution, was significantly increased ($p < 0.001$).

Conversely, the following apoptosis assay showed that the apoptosis rate of PC-12 was significantly increased in the OGD group ($p < 0.001$), while the apoptosis rate of the PC-12 cells decreased in the OGD + Remifentanil group ($p < 0.001$). Further, the expressions of pro-apoptotic proteins of Bax, cleaved-caspase3 and cleaved-caspase9 were significantly increased in the PC-12 cells of the OGD group by Western blot assay ($p < 0.001$), while the expression of anti-apoptotic proteins of Bcl-2 was significantly decreased ($p < 0.001$), and the expression of related proteins evinced the opposite pattern in the PC-12 cells of the OGD + Remifentanil group ($p < 0.001$ or $p < 0.001$). The above results showed that low concentrations of Remifentanil did not affect the viability of PC-12 cells, but a high concentration of drugs had a negative effect on their viability. Inhibition of cell viability and apoptosis could be induced by OGD, but Remifentanil alleviated the damage caused by OGD to the PC-12 cells, thus exerting a cytoprotective effect.

Remifentanil attenuated autophagy of PC-12 cells induced by OGD

Next, the effects of OGD induction and Remifentanil treatment were determined regarding the autophagy of PC-12 cells. As shown in figure 2, the expression of p62 autophagy-related proteins decreased significantly in PC-12 cells ($p < 0.001$), while the ratio of LC3-I/LC3-II increased significantly ($p < 0.001$) after induction by OGD. At the same time, compared with OGD treatment, the expression of p62 protein increased significantly in the PC-12 cells in the OGD + Remifentanil group ($p < 0.001$), while the ratio of LC3-I/LC3-II decreased accordingly ($p < 0.05$). Remifentanil reduced the promoting effect of OGD on the autophagy of PC-12 cells.

Remifentanil promoted the expression of miR-124

We sought to explore further the molecular mechanism of Remifentanil in protecting PC-12 cells from OGD damage. Thus, after PC-12 cells were exposed to OGD, and pre-treated with Remifentanil solution, the expression level of miR-124 was detected by RT-PCR. The results, illustrated in figure 3, showed that the mRNA expression of miR-124 in the PC-12 cells of the OGD group was significantly lower than that of the untreated group ($p < 0.001$), while the expression level of miR-124 in the PC-12 cells of the OGD + Remifentanil group was significantly higher than that of the OGD group ($p < 0.001$). These data suggested that miR-124 was a factor related to OGD damage in PC-12 cells. The expression of miR-124 was inhibited by OGD induction in PC-12 cells, while Remifentanil played a cytoprotective role by promoting the expression of miR-124.

Remifentanil attenuated OGD-induced apoptosis and autophagy, by up-regulating the expression of miR-124

In order to verify our inference and clarify the function of miR-124 in the cytoprotective mechanism of Remifentanil, an miR-124 silencing vector and miR-124 inhibitor were synthesized and constructed, and

were then used to knockdown miR-124 in PC-12 cells. After transfection, the expression level of miR-124 in the miR-124 inhibitor group was significantly decreased, compared with the NC inhibitor group ($p < 0.001$, figure 4A). Furthermore, the viability of the CCK-8 cells in the OGD + Remifentanil + NC inhibitor group was significantly lower than that in the OGD + Remifentanil + NC inhibitor group ($p < 0.05$, figure 4B). This showed that the inhibition of miR-124 expression reduces the promoting effect of Remifentanil on the viability of PC-12 cells induced by OGD. At the same time, the apoptosis rate in the OGD + Remifentanil + miR-124 inhibitor group was significantly higher than that in the OGD + Remifentanil + NC inhibitor group ($p < 0.05$, figure 4C). Similarly, the protein levels of Bax, c-caspase3 and c-caspase9 in the OGD + Remifentanil + miR-124 inhibitor group were higher than those in the OGD + Remifentanil + NC inhibitor group (all $p < 0.001$), but the protein expression level of Bcl-2 was in exact contrast. These data suggested that miR-124 silencing reverses the mitigation effect of Remifentanil on OGD-induced apoptosis in PC-12 cells. In addition, the results of Western blot showed that the miR-124 inhibitor significantly decreased the expression of p62 autophagy-related proteins, compared with NC inhibitor transfection ($p < 0.05$).

Nonetheless, compared with the OGD + Remifentanil + NC inhibitor group, the ratio of LC3-I/LC3-II in the PC-12 cells in the OGD + Remifentanil + miR-124 inhibitor group was significantly increased ($p < 0.001$). Our results suggested that the inhibitory effect of Remifentanil on the autophagy of PC-12 cells against OGD damage was reversed by the silencing of miR-124. To sum up, the low expression of miR-124 undermined the protective effect of Remifentanil on PC-12 cells, and Remifentanil alleviated the damage induced by OGD in PC-12 cells by up-regulating the expression of miR-124.

Remifentanil activated the JAK2/mTOR signal pathway by up-regulating the expression of miR-124

The JAK2/mTOR pathway affected many different cellular processes by regulating downstream genes. In our study, we detected the expression of JAK2/mTOR-related proteins in PC-12 cells, serving to clear the interaction between miR-124 and the JAK2/mTOR pathway. According to the data, compared with the control group, the phosphorylation of JAK2/mTOR protein in PC-12 cells induced by OGD was significantly decreased in terms of protein level ($p < 0.001$), while the phosphorylation level of JAK2/mTOR protein in the OGD + Remifentanil group was significantly increased ($p < 0.001$). Nevertheless, after down-regulation of miR-124, the changes in these proteins induced by Remifentanil were significantly reversed ($p < 0.001$). The results showed that OGD induces a decrease in the activity of the JAK2/mTOR signalling pathway, while Remifentanil facilitates the activation of this pathway, and the low expression of miR-124 reverses the activation of Remifentanil on the JAK2/mTOR pathway. In short, Remifentanil activated the JAK2/mTOR signal pathway by up-regulating the expression of miR-124, in OGD-induced PC-12 cells.

Discussion

In our study, it was found that the viability of PC-12 cells decreased significantly, while the degree of apoptosis and autophagy *increased* significantly, when PC-12 cells were treated with OGD. After the use

of Remifentanil, the OGD damage of cells was alleviated, which was manifested by the recovery of cell viability and the decrease of apoptosis and autophagy. The expression of apoptosis and autophagy-related proteins also changed accordingly. The following mechanism analysis showed that miR-124 was positively regulated by Remifentanil, and the cytoprotective effect of Remifentanil was reversed by knocking down miR-124.

In the context of neurosurgery operations, patients often experience hypoxic and ischemic brain injury. Cerebral ischemia leads to neuronal death and eventually leads to neurological dysfunction. The ischemia-reperfusion (I/R) process of the brain puts the brain tissue in a state of hypoxia and ischemia, thus activating various cell-death processes, such as necrosis, apoptosis or autophagy-related cell death [23-25]. Apoptosis, a form of cell death, plays a vital role in normal development and tissue homeostasis. By contrast, inappropriate or excessive apoptosis is associated with many types of neurodegenerative disease, including cerebral ischemia [26]. In addition, autophagy is an evolutionarily conservative process of massive degradation and recycling of cytoplasmic proteins and organelles [27]. As a degradation / recirculation system, autophagy appears in the pathological changes of many organs and plays an important role in cerebral ischemia [28-29]. Our results showed that the neuroprotective effect of Remifentanil on PC-12 cells was indeed achieved through apoptosis and autophagy, which was consistent with the above studies.

Opioid agonists evinced neuroprotective effects in relieving hypoxia injury. Remifentanil was a new type of opioid drug, which had the following characteristics: quick action, fast clearance rate and short blood-brain balance time. Previous studies had shown that Remifentanil plays a role in the cardio-protection of ischemic cardiac injury through opioid receptors [29]. In addition, recent reports had found that Remifentanil preconditioning had a neuroprotective effect against cerebral ischemic injury. For example, Remifentanil exerted a neuroprotective effect on global cerebral ischemia-reperfusion injury, by improving spatial learning and memory in rats. It was related to the inhibition of neuronal apoptosis in the hippocampal CA1 region, and the regulation of signal-pathway and apoptosis-related genes. Remifentanil treatment enhanced the expression of the anti-apoptotic gene Bcl-2 and inhibited the expression of the pro-apoptotic gene Bax in the hippocampal CA1 region [30]. Animal experiments showed that Remifentanil preconditioning significantly reduced the infarct size and neurological damage after focal cerebral ischemia in rats. Remifentanil had an anti-apoptotic effect in vitro, but had no effect on necrotizing death. Conversely, it significantly decreased the activity of caspase-3 and the level of cleaved caspase-3, inhibited the expression of Bax protein and the activity of caspase-9 in the cortex, and maintained the integrity of mitochondria. Mechanism studies had found that this involved opioid and N-methyl-D-aspartate (NMDA) receptors, as well as mitochondrial-dependent apoptosis pathways [31].

These results suggested that Remifentanil had the potential effect of reducing hypoxia injury and protecting the survival of nerve cells. In a manner similar to our experimental results, OGD significantly decreased the viability of PC-12 cells, and deepened the degree of apoptosis and autophagy. Nonetheless, after pre-treatment with Remifentanil, this situation was reversed, which fact was reflected in the obvious enhancement of cell viability, the decrease in the number of apoptotic cells and the expression of

intracellular cleaved caspase-3 and Bax proteins. Meanwhile, there was an increase of p62 expression and a decrease in the LC-I/LC-II ratio, which meant that the process of apoptosis and autophagy was weakened. This was consistent with the conclusions obtained by our predecessors.

MiRNAs is one of the main types of non-coding RNA derived from most genes, and it plays an important role in mRNAs transcription and protein synthesis [32]. MiR-124 is the key regulator of microglia in the central nervous system [33]. It has been reported that up-regulation of miR-124 may regulate apoptosis and autophagy in patients with Parkinson's disease (PD), thereby reducing the loss of dopaminergic neurons [14]. More importantly, miR-124 also played a dual role by regulating the apoptosis of cerebral ischemic cells [34]. Sun et al. reported that high levels of miR-124 in ischemic penumbra can enhance the expression of anti-apoptosis proteins Bcl-2 and Bcl-xl, while the up-regulated expression of miR-124 protects neurons from ischemic apoptosis [35].

All these studies suggest that miR-124 is an important factor involved in the process of anoxic injury or brain injury of nerve cells, and that high levels of miR-124 contribute to the survival of nerve cells. In this study, Remifentanil inhibited the damaging effect of OGD on PC-12 cells, while increasing the level of intracellular miR-124. Further studies have shown that the low expression of miR-124 weakens the protective effect of Remifentanil on PC-12 cells induced by OGD. This was mainly reflected in the enhancement of apoptosis and autophagy, and the decrease of cell viability. Therefore, we believe that Remifentanil reduced OGD damage by up-regulating miR-124, and miR-124 was an important participant in the mechanism of Remifentanil cytoprotective function. This was also the first time that the pharmacological effects of miR-124 and Remifentanil were directly discussed.

Earlier studies had confirmed that I/R injury in brain tissue promoted the phosphorylation of JAK2 and its downstream signal transducers, which exerted an anti-apoptotic effect and promoted cell viability [36]. In addition, mTOR activity was regulated by a variety of intracellular and extracellular factors; in turn, mTOR affected the rates of translation, transcription, protein degradation, signal transduction, metabolism and cytoskeleton dynamics [36]. Meanwhile, mTOR played a key role in the OGD damage of cells. Conversely, circZNF292 targeted BNIP3 by activating the mTOR signalling pathway, thereby alleviating OGD-induced damage in H9c2 cells [37].

‘Regulated in development and DNA damage responses 1’ (aka REDD1) regulated hypoxia-induced neuronal oxidative stress damage, by mediating the mTOR autophagy signal. The down regulation of REDD1 also interfered with the increase in apoptosis caused by OGD/R, as well as the overexpression of Bax and caspase-3. In addition, blocking the mTOR pathway attenuated the protective effects of the REDD1 inhibitor on OGD-induced neuronal injury and oxidative stress [38].

More importantly, the activity of JAK2/mTOR was regulated by miR-124. For example, miR-124 inhibited apoptosis by activating a series of JAK2 pathways, and by promoting the expression of its downstream anti-apoptotic protein Bcl-2, thus protecting CIRI. In LPS-stimulated microglia-BV-2, acetylcholine (Ach) activated the JAK2 pathway deactivated by LPS, while the levels of miR-124 and downstream target C/EBP α were significantly increased, indicating the regulatory relationship between miR-124 and the

JAK2 pathway [39]. Huang *et al.* confirmed that the inhibitory effect of miR-124-3p on neuronal inflammation was achieved by regulating the activity of mTOR signal transduction. MiR-124-3p affected the activity of the mTOR pathway by targeting PDE4B [40]. MiR-124 acted as a growth inhibitory miRNA, and played an important role in inhibiting tumorigenesis by targeting mTOR [41]. Similarly, hypoxia led to the inactivation of the JAK2/mTOR pathway in OGD-stimulated PC-12 cells, and Remifentanil pre-treatment relieved this inhibitory effect. Further mechanism studies showed that miR-124 silencing partially eliminated the function of Remifentanil, and reduced the activity of the signal pathway. MiR-124 mediated the activation of the JAK2/mTOR pathway by Remifentanil.

In conclusion, Remifentanil activated the JAK2/mTOR pathway by up-regulating miR-124 in PC-12 cells, which played a protective role in the response of these PC-12 cells to OGD stimulation.

Abbreviations

OGD: oxygen-glucose deprivation; RT-qPCR: quantitative real-time PCR; CBI: Congenital brain injury; Glu: glutamate (Glu); TLR: Toll-like receptors; JAK: Janus kinases; PTKs: protein tyrosine kinases; mTOR: mammalian target of rapamycin; PD: Parkinson's disease.

Declarations

Authors' contributions

Conceptualization, Hongyan Zhang and Suyan Xu. Methodology, Fabin Chi and Guofeng Li. Validation, Yujie Zhou and Chenghai Li. Data curation, Hongyan Zhang. Writing, original draft preparation: Hongyan Zhang. Writing, review & editing, Hongyan Zhang and Aifeng Wang. All authors read and approved

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that there are no conflicts of interest.

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Figures

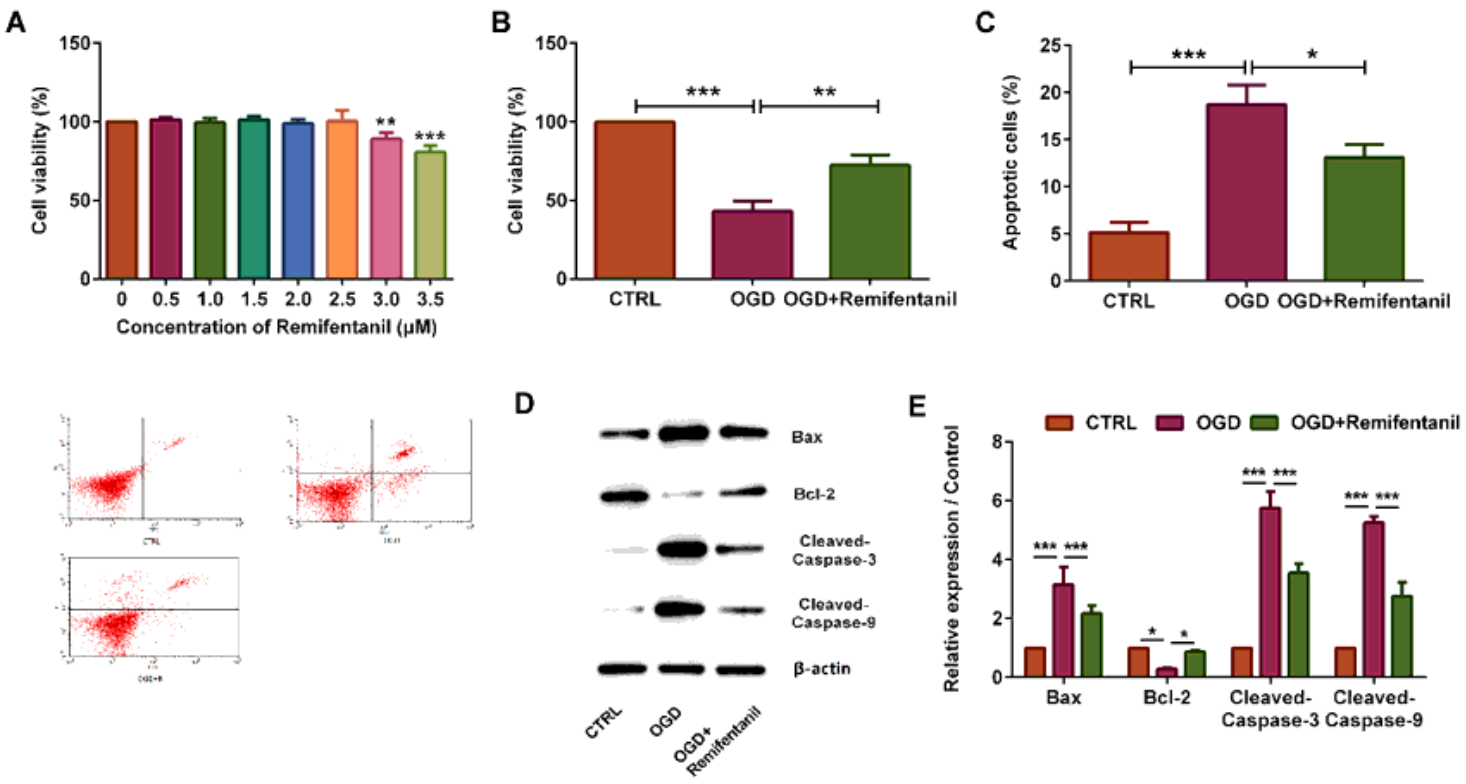

Figure 1

Remifentanil attenuated OGD-induced PC-12 cell injury. A. Low concentrations of Remifentanil had no toxicity in terms of cell viability, while high concentrations of Remifentanil inhibited viability; B. Remifentanil increased the viability of PC-12 cells induced by OGD; C-E. Remifentanil reduced the apoptosis of PC-12 cells induced by OGD. * denotes $p < 0.05$, ** denotes $p < 0.01$ and *** denotes $p < 0.001$.

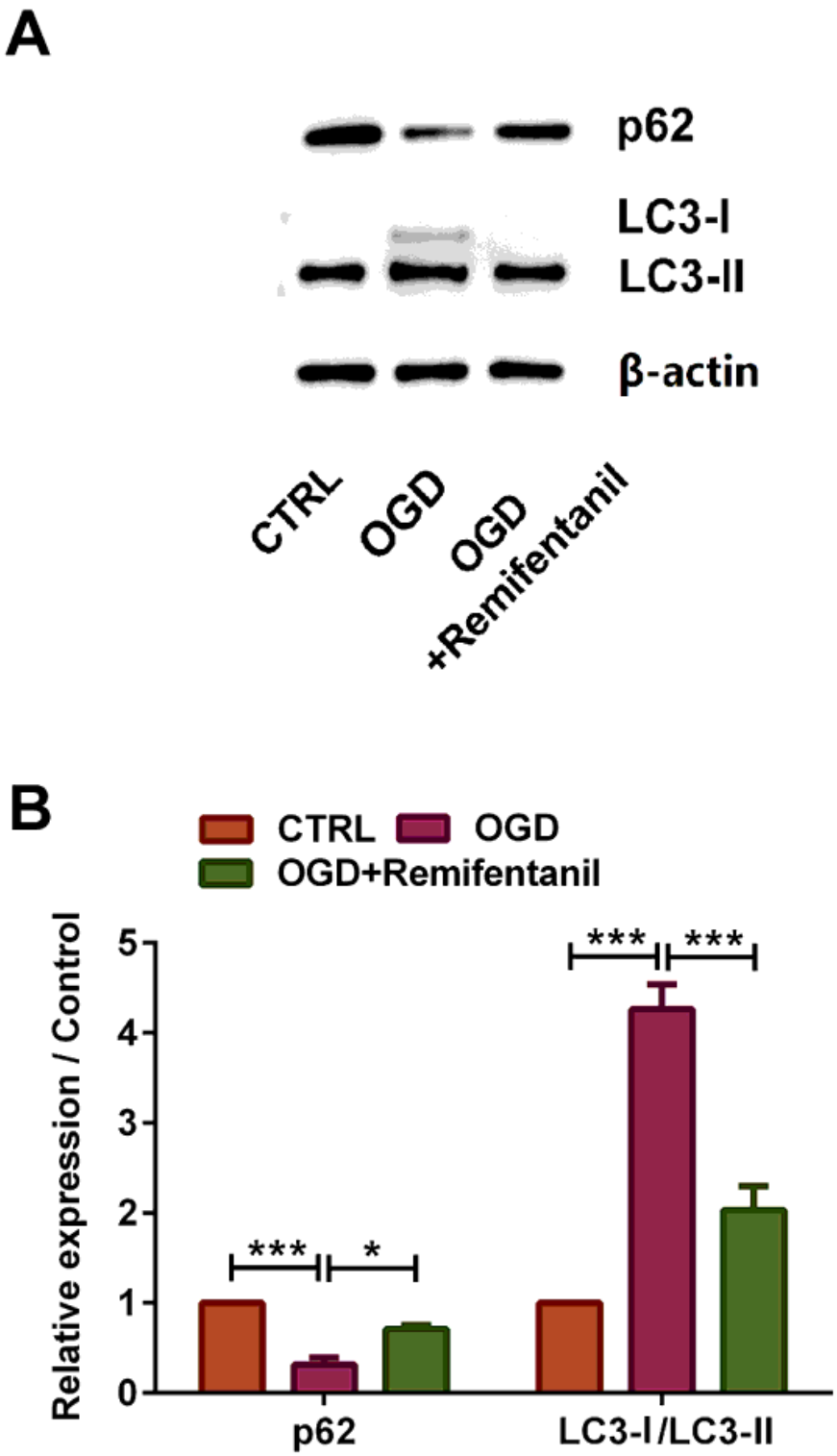

Figure 2

Remifentanil alleviated autophagy of PC-12 cells induced by OGD. A-B. Autophagy of PC-12 cells was attenuated by Remifentanil. * denotes $p < 0.05$, ** denotes $p < 0.01$ and *** denotes $p < 0.001$.

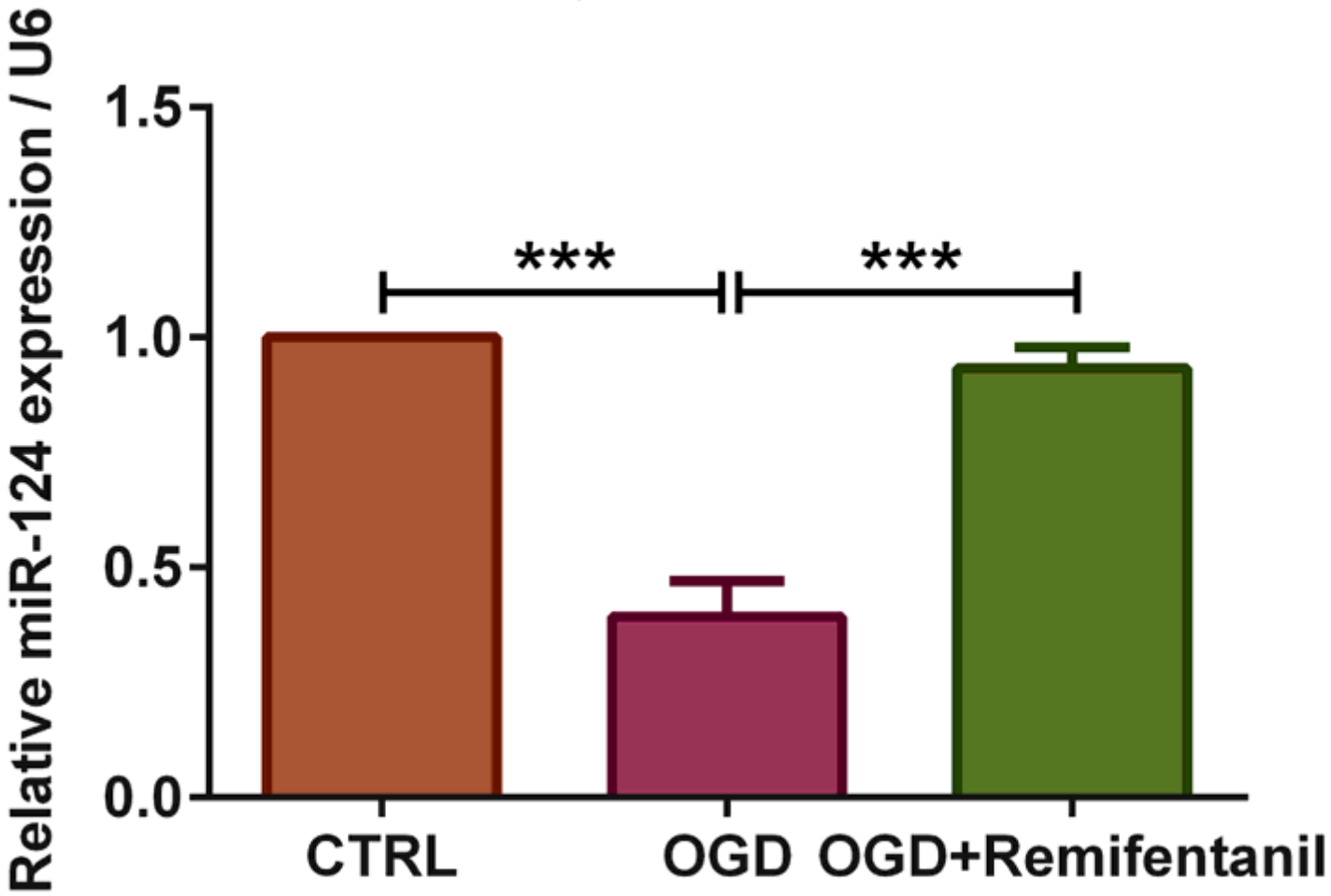

Figure 3

The expression of miR-124 induced by OGD is regulated by Remifentanil in PC-12 cells. * denotes $p < 0.05$, ** denotes $p < 0.01$ and *** denotes $p < 0.001$.

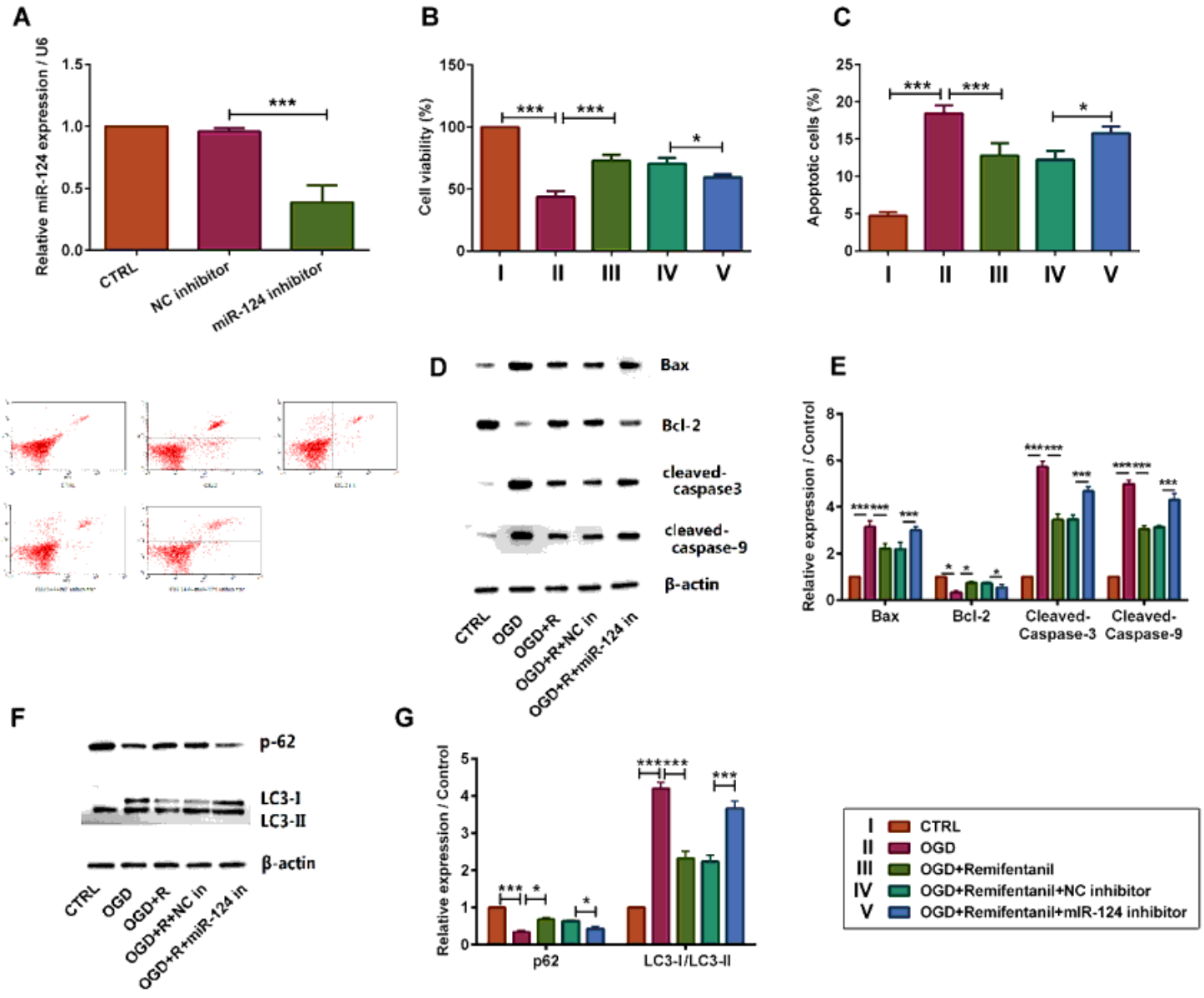

Figure 4

Remifentanil attenuated OGD-induced apoptosis and autophagy by up-regulating the expression of miR-124. A. miR-124 inhibitor down-regulated the expression of miR-124; B. Cell viability was inhibited by miR-124 inhibitor; C-E. miR-124 inhibitor promoted apoptosis; F-G. Cell autophagy was promoted by miR-124 inhibitor. * denotes $p < 0.05$, ** denotes $p < 0.01$ and *** denotes $p < 0.001$.

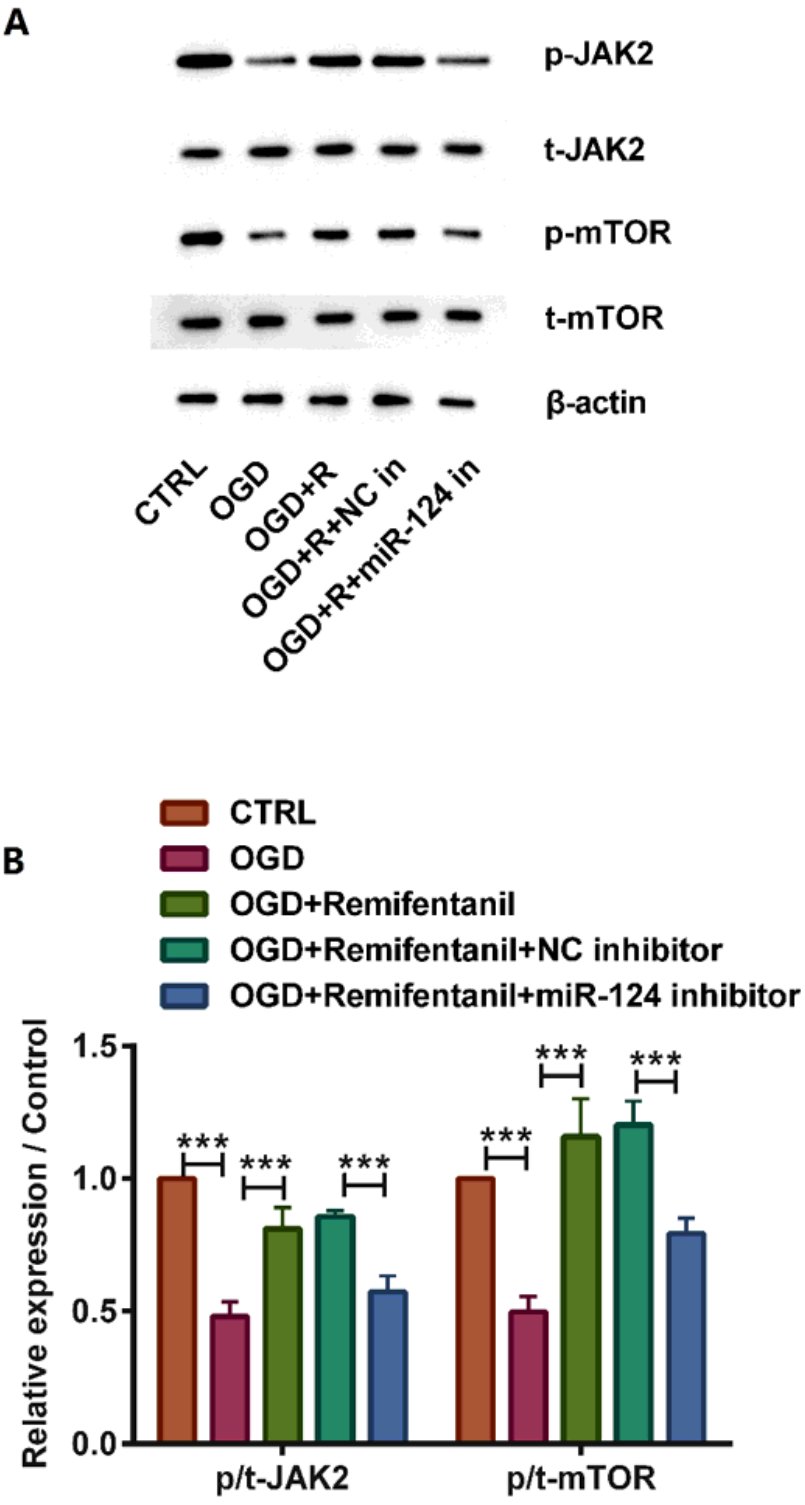

Figure 5

Remifentanil activated the JAK2/mTOR signal pathway by up-regulating expression of miR-124. A-B. The phosphorylation level of JAK2/mTOR protein was decreased by OGD, while increased by Remifentanil, and inhibited by miR-124 inhibitor. * denotes $p < 0.05$, ** denotes $p < 0.01$ and *** denotes $p < 0.001$.