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MiR-206 is involved in neuroprotective effects of Dexmedetomidine in H_2O_2-induced SK-N-SH cells by targeting ANXA1

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Abstract

This study focused on exploring the neuroprotective role of Dexmedetomidine (DEX) and miR-206 in H_2O_2-induced SK-N-SH cells. First, we dectected the cell viability, apotosis, oxidative stress and miR-206 expression in H_2O_2-treated SK-N-SH cells. Next, we examined above content in H_2O_2-induced SK-N-SH cells though DEX treatment. Besides, the level of cell viability, apotosis, oxidative stress were evaluated in H_2O_2-treated SK-N-SH cells transfected with miR-206 mimics and miR-206 inhibitor. Moreover, the target gene of miR-206 were predicted and verifed by binformatics tools and luciferase reporter assay. These data indicated that H_2O_2 evoked the apotosis, oxidative stress and inhibition of miR-206 expression in SK-N-SH cells in a dose manner. Besides, DEX attenuated H_2O_2-induced oxidative damage, apotosis and promoted miR-206 expression in SK-N-SH cells. Moreover, overexpression of miR-206 augmented the cell viablity as well as suppressed apotosis and oxidative stress in H_2O_2-induced SK-N-SH cells, while down-expression of miR-206 showed opppsite effects. Further, Annexin A1 (ANXA1) was verified as a directly target of miR-206, and over-expression of ANXA1 could slack the neuroprotective effect of miR-206 in H_2O_2-induced SK-N-SH cells. DEX exerted neuroprotective effects on H_2O_2-treated SK-N-SH cells in vitro by negatively reguating ANXA1 expression via activation of miR-206.

Keywords: dexmedetomidine; miR-206; annexin A1; hypoxic–ischaemic brain damage.

Practical Application: Neuroprotective effects of dexmedetomidine on H2O2-treated SK-N-SH cells.

1 Introduction

It is widely recognized that, hypoxic–ischaemic brain damage (HIBD) is a fatal neurological injury in newborn infants and the patients with stroke or cardiac arrest, which has become a major public health issues worldwide in past decades, leading to a high alarming cognitive impairment and mortality (Disdier & Stonestreet, 2020). The pathophysiological process of HIBD is very complicated, including a great diversity of factors and signaling pathways, including calcium overload, oxygen free radicals, inflammation, and apoptosis, nevertheless, there is no hypothesis which can perfect description of the development and progression of this disease (Cardinali, 2019). Although plentiful basic and clinical researches of HIBD have been implemented, there is still no effective strategy for HIBD patients (Sisa et al., 2019). Thus, it is critically urgently to investigate the unearthing the underlying mechanism behind HIBD.

Dexmedetomidine (DEX), a novel sedative-analgesic agent, is regarded as a highly specific and selective a2-adrenoceptor agonist which has sedative, analgesic, and anxiolytic properties, and it is commonly used in clinical practice as an anesthetic (Peng et al., 2019). In recent years, increasing studies indicated that DEX can provide neuroprotective effects against HIBD in multiple ways, such as inhibition of apoptosis, blocking the release of inflammatory cytokines and relieving oxidative stress, which also is able to improve the cognitive function under the condition of hypoxia-ischemia (Degos et al., 2013; Ren et al., 2016; Akpınar et al., 2016). However, the exact underlying mechanisms of DEX protective effect on neurocytes from ischemic-hypoxia injury remain to be deeply explored.

MicroRNAs (miRNAs) is group of non-coding single chain small-molecule RNAs molecule with a length of about 18-25 nucleotides, that function in post-transcriptional regulation of targeting gene expression (Kobayashi et al., 2012). A growing body of evidence affirmed that miRNAs serve pivotal roles in multitudinous pathological an processes in hypoxia-ischemia damage including oxidative stress response, autophagy apoptosis and cytotoxic reaction (Ponnusamy & Yip, 2019; Di et al., 2014). MiR-206 is located on the human chromosome 6p12.2, which is originally thought essential for growth and rebuilding of skeletal muscle (Ma et al., 2015). With the deepening of researches, some studies have shown that miR-206 is also closely involved in regulating ischemia-reperfusion injury (Kong et al., 2019), oxidative stress damage (Ciesla et al., 2016) and neuroprotection (Zhao et al., 2019). Nevertheless, what roles of miR206 act as in the neuronal ischemic hypoxia injury remains to be delved. Additionally, as far as we know, the correlation between miR206 and the neuroprotective effect of DEX has not yet been reported.

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In present study, we focused on the molecular mechanism of H_2O_2-induced cytotoxicity, apoptosis and oxidative stress damage, and the associated roles of DEX and miR-206 of neuroprotective effect on SK-N-SH cells. We identified that DEX protected SK-N-SH cells from cytotoxicity, apoptosis and oxidative damage through regulation of miR-199a and subsequent down-regulated ANXA1 expression.

2 Materials and methods

2.1 Cell culture and H_2O_2 treatment

Human SK-N-SH cells were obtained from the Chinese Academy of Sciences cell bank in Shanghai, China. This study was approved by the Ethics Committee of Affiliated Hospital of Xuzhou Medical University. The resuscitated cells were cultured in Dulbecco's modified Eagle Media (DMEM, Invitrogen ,USA) containing 10% fetal bovine serum (FBS, Gibco USA). The cells lines were cultured at 37 °C in saturated humidity containing 5% CO_2. Furthermore, a total of 20 μm DEX (Hengrui Medicine Co., Ltd, China) was dissolved in DMEM medium.

For H_2O_2 treatment, the cultured SK-N-SH cells were respectively treated with H_2O_2 (Sigma-Aldrich, USA) at different concentrations (0, 12.5, 50, 100 or 200 μm) for 6 h. The qRT-PCR and western bolt were performed to detect expression level of miR-206 and Annexin A1 (ANXA1).

2.2 Cell tranfection

The ANXA1 over-expressing vector (pcDNA3.1- ANXA1) and its negative control (pcDNA3.1), miR-206 mimics, miR-206 inhibitors and negative controls were all purchased from GenePharma (Shanghai, China). The cells were transfected with Lipofectamine 3000 (Invitrogen, USA) according to the instructions. After 48 h of transfection, the cells were harvested for further analysis.

2.3 Real time quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from SK-N-SH cells by using TRIzol reagent (Takara, Japan) according to the manufacturer's recommendations. Then, the purified RNA was used to transcribed into cDNA using a PrimerScript RT Reagent kit (TaKaRa, Japan), and miRNA was reversely transcribed by using the Prime-Script miRNA cDNA Synthesis Kit (TaKaRa, Japan) in ABI Prism 5700 Sequence Detection System (Applied Biosystems, USA). according to the manufacturer's recommendations. The PCR reaction amplification conditions were 95 °C for 30 s, 54 °C for 1 min and 72 °C for 1 min for 35 cycles followed by 72 °C for 10 min. GAPDH and U6 was severally used as an internal reference for mRNA and miRNA. The relative expression of miR-206 and ANXA1 was quantified using $2^{-\Delta\Delta Ct}$ method. The primers sequences for miR-206 and ANXA1 were listed in Table 1.

2.4 Cell Counting Kit-8 (CCK-8) assay

The cells after trasfection were cultured in 96-well plates at a density of 5 x 10^4 cell/well. Next, CCK-8 solution (Beyotime, China) was added to the maintenance cell medium and incubated for 2 h at 37 °C. The absorbance values were determined at 450 nm using the E Thermo Scientific Microplate Reader (BioTek, USA).

Table 1. List of primers used for real-time polymerase chain reaction.

Name of genes	Sequence (5'-3')
miR-206	Fwd: CGTCAGAAGGAATGATGCACAG
	Rev: ACCTGCGTAGGTAGTTTCATGT
ANXA1	Fwd: ATGGCAATGGTATCAGAA
	Rev: GTTTCCTCCACAAAGACG
U6	Fwd: CACTG GGTGC GGCAG GT
	Rev: TCATC ACCGA TCGA TACGA TGA
GAPDH	Fwd: CACTCACGGCAAATTCAACGGCA
	Rev: GACTCCACGACATACTCAGCAC

2.5 Detection content of Malondialdehyde (MDA), Lactate Dehydrogenase (LDH) and Reactive Oxygen Species (ROS) level

The MDA, LDH and ROS level were explored in H_2O_2 induced SK-N-SH cells for evaluating oxidative stress. The concentration of MDA and LDH was measured with MDA assay kit (Beyotime, China) and LDH Assay Kit (Beyotime, China) respectively following manufacturers' contract.

For ROS level, the cells were firstly incubated with diluted Dihydroethidium (Beyotime, China) based on manufacturers' instructions. Then, the levels of ROS were measured by flow cytometry analysis (BD Biosciences) in the FL2 channel.

2.6 Flow Cytometry (FCM) apoptosis assay

In brief, the collected cells were resuspended and diluted in a concentration of 1×10^6 per milliliter with PBS, and then stained with PI-conjugated anti-Annexin V antibodies under darkness for 20 min at room temperature. Next, apoptosis were carried out by FCM assay (BD biosciences, USA) and assessed with FACScan system (BD biosciences, USA).

2.7 Western blot

The total proteins extracted from cells using RIPA lysis buffer (Beyotime, China). The protein concentrations were measured using a Protein Assay Reagent (Roche Molecular Diagnostics, USA) in line with manufacturer's explanatory memorandum.

Equal amount of protein was separated with 10% sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred onto a polyvinylidene fluoride (PVDF) membrane (Sigma, USA) blocking with 5% non-fat milk for 2 h at room temperature. In rapid sequence, the membranes were incubated with primary antibodies anti-ANXA1 and anti-GAPDH (1:1000, Abcam, UK) overnight at 4 °C, and then incubated with the appropriate horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. Finally, the blots were visualized by using enhanced chemi-luminescence (ECL) detection regent (Pierce, Rockford, IL, USA).

2.8 Luciferase reporter assay

The wild type (WT) of AXNA1 3'-untranslated region (3'-UTR) including binding sites of miR-206 was amplified by PCR from human genomic DNA and cloned in the pmirGLO vector (Invitrogen, USA). The luciferase plasmids AXNA1-WT and AXNA1-MUT were co-transfected with miR-206 mimics

into cells. After 48h of transfection, the luciferase activity was measured using the dualluciferase reporter system (Promega, USA) following by manufacturer's instructions.

2.9 Statistical analysis

Statistical analysis was carried out by using SPSS 22.0 software (IBM, USA). All data, except for special instructions, were expressed by mean + standard deviation. or Student's t test or One-way ANOVA with Dunnett's post-hoc test were used for comparison between groups. All experiments were performed at least 3 times. P value less than 0.05 ($p < 0.05$) was considered statistical significant.

3 Result

3.1 Effect of H_2O_2 on cellular viability, oxidative stress, apoptosis and miR-206 expression of SK-N-SH cells

In order to explore the effect of H_2O_2 on SK-N-SH cells, we firstly treated the cells with different concentration of H_2O_2 (0, 12.5, 50, 100, 200 μm) for 6 h. After that, the CCK-8 assay showed that H_2O_2 could observably suppress the cell viability by a concentration gradient (Figure 1A), analogously, H_2O_2 was able to promote cell apoptosis in a dose-dependent dose (Figure 1B). As described in Figure 1C, D, the levels of cytotoxic markers, containing LDH leakage and MDA content were notably increased in H2O2induced SK-N-SH cells in a dose-dependent dose. In the meantime, the ROS assay indicated that ROS level was dramatically elevated by H_2O_2, that means the higher concentrations of H_2O_2 might cause more severe oxidative stress injury (Figure 1E). Astoundingly, our results found that the expression level of miR-206 in H_2O_2-simulated SK-N-SH cells was gradually rise with increase of H_2O_2 concentration (Figure 1F). Therefore, our findings remaindered that miR-206 might be involved in H_2O_2-evoked oxidative stress injury in SK-N-SH cells.

3.2 DEX attenuated H_2O_2-induced oxidative damage and apoptosis, and miR-206 expression suppression

To investigate the effect of DEX on H_2O_2-induced oxidative damage and apoptosis of SK-N-SH cells, we separated experiment groups as follow: negative control group, H_2O_2 group (100 μm) and H_2O_2 (100 μm) + DEX (20 μm) group. As illustrated in Figure 2A, B, DEX was capable of alleviating H_2O_2-evoked cytotoxic reaction and apoptosis in SK-N-SH cells. Similarly, our results showed that LDH leakage and MDA content were also declined in H_2O_2 + DEX group when compared with H_2O_2 group (Figure 2C, D). Moreover, the increased ROS level caused by H_2O_2 was partly reversed by DEX in SK-N-SH cells (Figure 2E). Furthermore, our finding elucidated that the expression level of miR-206 was significantly increased by DEX+H_2O_2 treatment in comparison with H_2O_2 alone (Figure 2F). That meant, miR-206 might take part in neuroprotective effect of DEX in H_2O_2-treated SK-N-SH cells. Finally, we verified the transfection efficiency of miR-206 mimics and miR-206 inhibitor (Figure 2G).

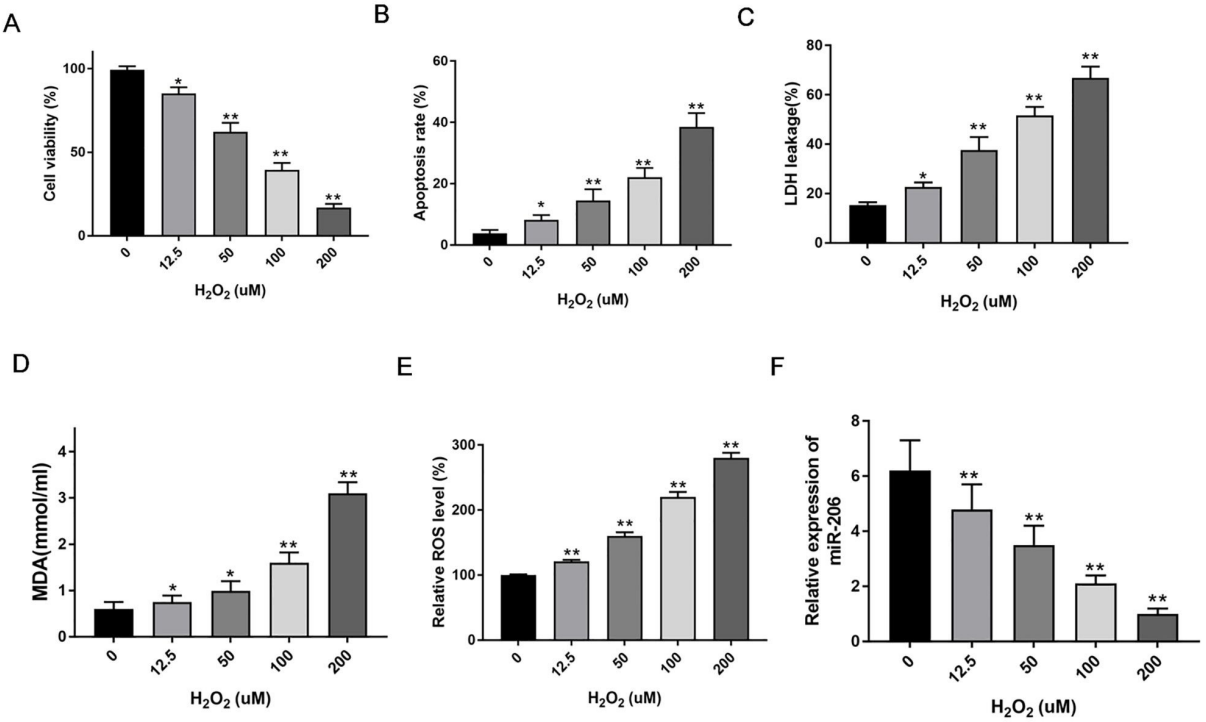

Figure 1. Effect of H_2O_2 on cellular viability, oxidative stress, apoptosis and miR-206 expression of SK-N-SH cells. (A) The CCK-8 assay was used for detecting cell viability of H_2O_2-simulated SK-N-SH cells; (B) Flow cytometry analysis was carried out to explore the apoptosis of H_2O_2-simulated SK-N-SH cells; (C) LDH leakage; (D) MDA release; (E) ROS production of H_2O_2-treated SK-N-SH cells; (F) the expression level of miR-206 in H_2O_2-treated SK-N-SH cells; *P < 0.05; **P < 0.01 vs. the control group; LDH = Lactate dehydrogenase; MDA = Malondialdehyde; ROS = Reactive Oxygen Species.

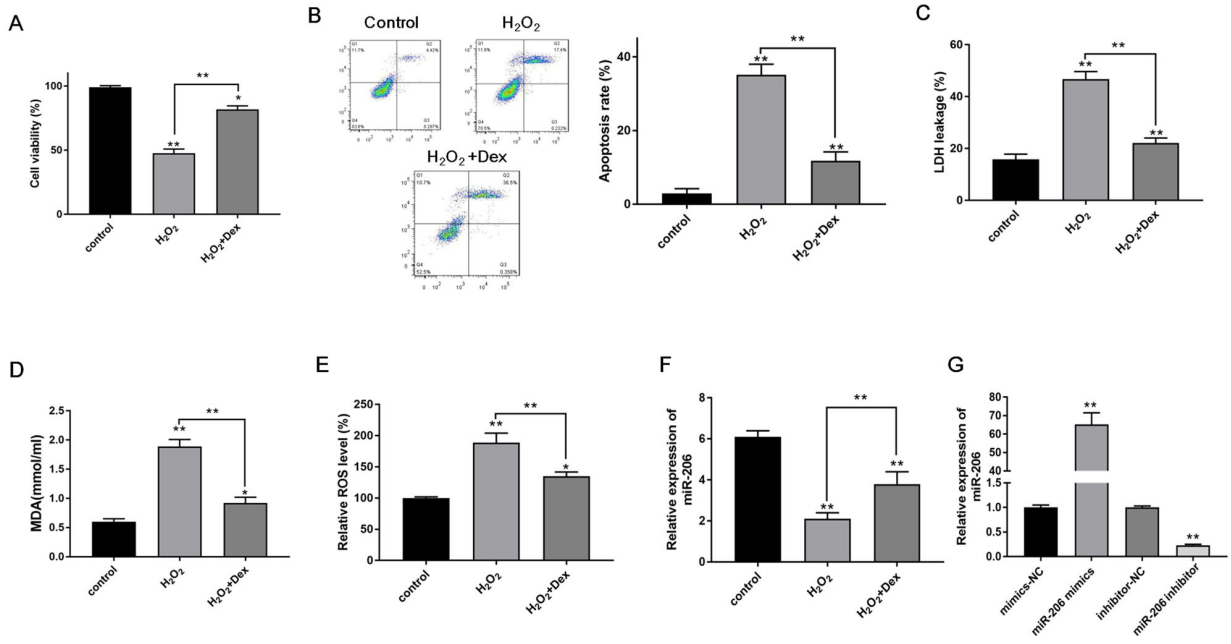

Figure 2. DEX attenuated H_2O_2-induced oxidative damage and apoptosis, and miR-206 expression suppression. (A) The cell viability of H_2O_2 and DEX co-treated SK-N-SH cells by using CCK-8 assay; (B) The apoptosis rate of H_2O_2 and DEX co-treated SK-N-SH cells by using flow cytometry analysis; (C) LDH leakage; (D) MDA release; (E) ROS production of H_2O_2 and DEX co-treated SK-N-SH cells; (F) The expression level of miR-206 in H2O2 and/or DEX co-treated SK-N-SH cells by qPCR; (G) The qRT-PCR was used for identify the transfection efficiency of miR-206 mimics and miR-206 inhibitor; *P < 0.05; **P < 0.01 vs. the control group; LDH = Lactate Dehydrogenase; MDA = Malondialdehyde; ROS = Reactive Oxygen Species.

3.3 MiR-206 was involved in H_2O_2 inducedcytotoxicity, apoptosis and oxidative damage

To identify how miR206 participated in DEXmediated neuroprotective effect on SK-N-SH cells against H_2O_2-evoked cytotoxicity, apoptosis and oxidative damage, the cells were transfected with miR206 mimics or miR206 inhibitor or corresponding controls in a H_2O_2 treatment (100 μm). Our results showed that over-expression of miR206 could enhance cell viability, whereas down-expression of miR206 displayed an opposite effect (Figure 3A). Likewise, flow cytometry assays indicated that miR206 mimics was able to inhibit H_2O_2- triggered apoptosis, where miR2233p inhibitor could aggravate this effect (Figure 3B). As shown in Figure 3C, E, over-expression of miR206 markedly suppressed the LDH leakage, MDA release and ROS production, in contrast, down-expression of miR206 could sharpen the oxidative damage caused by H_2O_2 treatment. Therefore, our findings suggest miR-206 was implicated in H_2O_2 inducedcytotoxicity, apoptosis and oxidative damage in SK-N-SH cells.

3.4 ANXA1 is a target gene of MiR-206

To detect the correlation between ANXA1 and miR-206, we primarily measured the expression level of ANXA1 in H_2O_2-induced SK-N-SH cells. By using RT-PCR and western blot, we found that the mRNA and protein level of ANXA1 were dramatically increased in H_2O_2-treated SK-N-SH cells in a dose-dependent dose (Figure 4A, B). Contemporarily, our results indicated that DEX could repressed the facilitation effect on mRNA and protein expression of ANXA1 by H_2O_2 (Figure 4C, D). Bioinformatics software prediction implied that miR-206 had a binding site with ANXA1 3'-UTR (Figure 4E). Next, the dual luciferase reporter assay was implemented to explore the relationship between miR-206 and ANXA1. As demonstrated in Figure 4F, luciferase activity of ANXA1 3'-UTR WT was down-regulated in cells transfected with miR-206 mimics, where its luciferase activity was up-regulated in cells transfected with miR-206 inhibitor, meanwhile the uciferase activity of ANXA1 3'-UTR MUT showed no noticeable change both in miR-206 mimics group and miR-206 inhibitor group. Moreover, the mRNA and protein level of ANXA1 was significant suppressed by over-expression of miR-206 in H_2O_2-treated SK-N-SH cells, whereas down-expression of miR-206 showed an opposite phenomenon (Figure 4G, H). Conclusively, these results suggest that ANXA1 was a direct target of miR-206.

3.5 MiR-206 mediated the H_2O_2 tiggeredcytotoxicity, apoptosis and oxidative damage by targeting ANXA1

To investigate whether miR-206 mediated H_2O_2 tiggeredcytotoxicity, apoptosis and oxidative damage though regulating ANXA1 expression, we detected the transfection efficiency of pcDNA3.1- ANXA1 at first (Figure 5A). Whereafter, our results showed up-regulated ANXA1 was able to abrogate the growth promotion effect caused by miR-206 in H_2O_2-treated SK-N-SH cells (Figure 5B). In like manner, up-regulated ANXA1 prominently attenuated apoptosis inhibition in H_2O_2-treated SK-N-SH cells transfected miR-206 mimics (Figure 5C). As

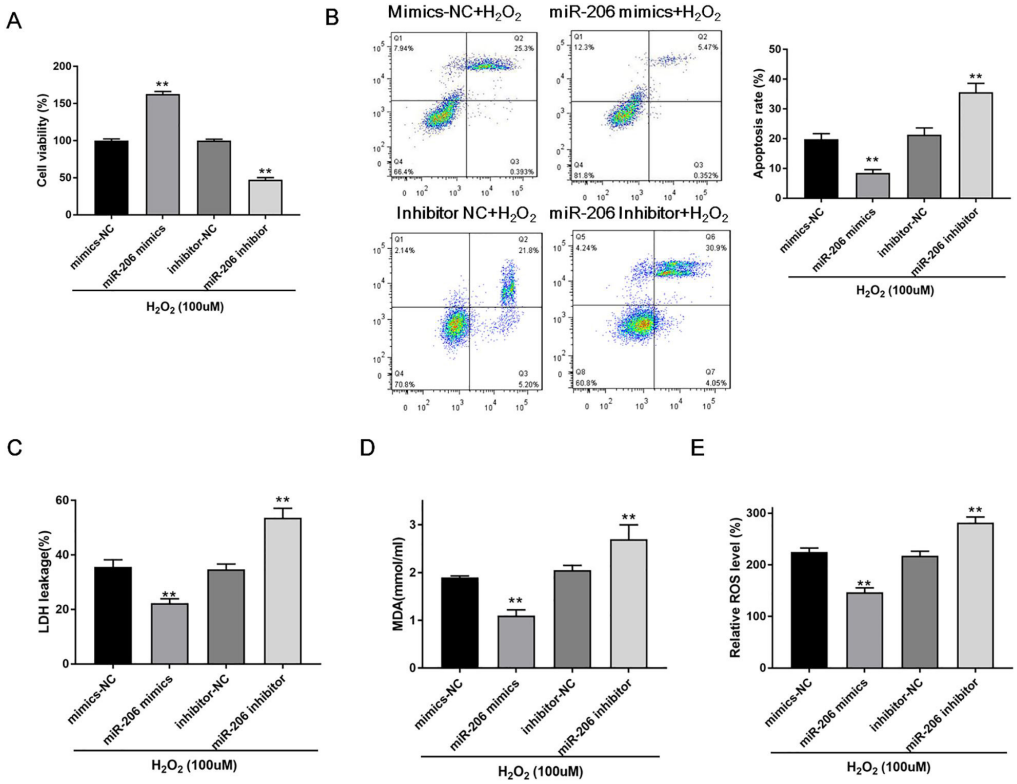

Figure 3. MiR-206 was involved in H_2O_2-induced cytotoxicity, apoptosis and oxidative damage. (A) The cell viability of H_2O_2-treated SK-N-SH cells transfected with miR-206 mimics or miR-206 inhibitor by using CCK-8 assay; (B) The apoptosis rate of H_2O_2-treated SK-N-SH cells transfected with miR-206 mimics or miR-206 inhibitor by using flow cytometry analysis; (C) LDH leakage; (D) MDA release; (E) ROS production of H_2O_2-treated SK-N-SH cells transfected with miR-206 mimics or miR-206 inhibitor; *P < 0.05; **P < 0.01 vs. the control group; LDH = lactate dehydrogenase; MDA = malondialdehyde; ROS = reactive oxygen species.

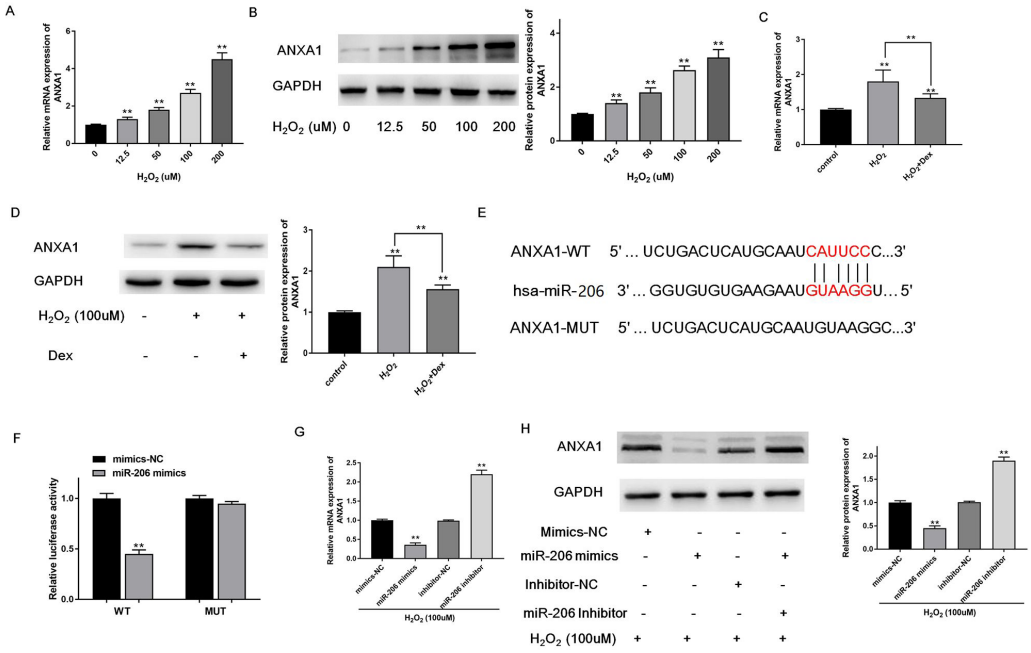

Figure 4. ANXA1 is a target gene of miR-206. The mRNA (A) and protein (B) expression level of ANXA1 in H_2O_2-treated SK-N-SH cells; The mRNA (C) and protein (D) expression level of ANXA1 in H_2O_2 and DEX co-treated SK-N-SH cells; (E) The prediction of putative alignment of miR-206 on ANXA1 3'-UTR; (F) The luciferase activity of ANXA1 WT 3'-UTR in cells transfected with miR-206 mimics or miR-206 inhibitor; The mRNA (G) and protein (H) expression level of ANXA1 in H_2O_2-treated SK-N-SH cells trasfeced with miR-206 mimics or miR-206 inhibitor; *P < 0.05; **P < 0.01 vs. the control group.

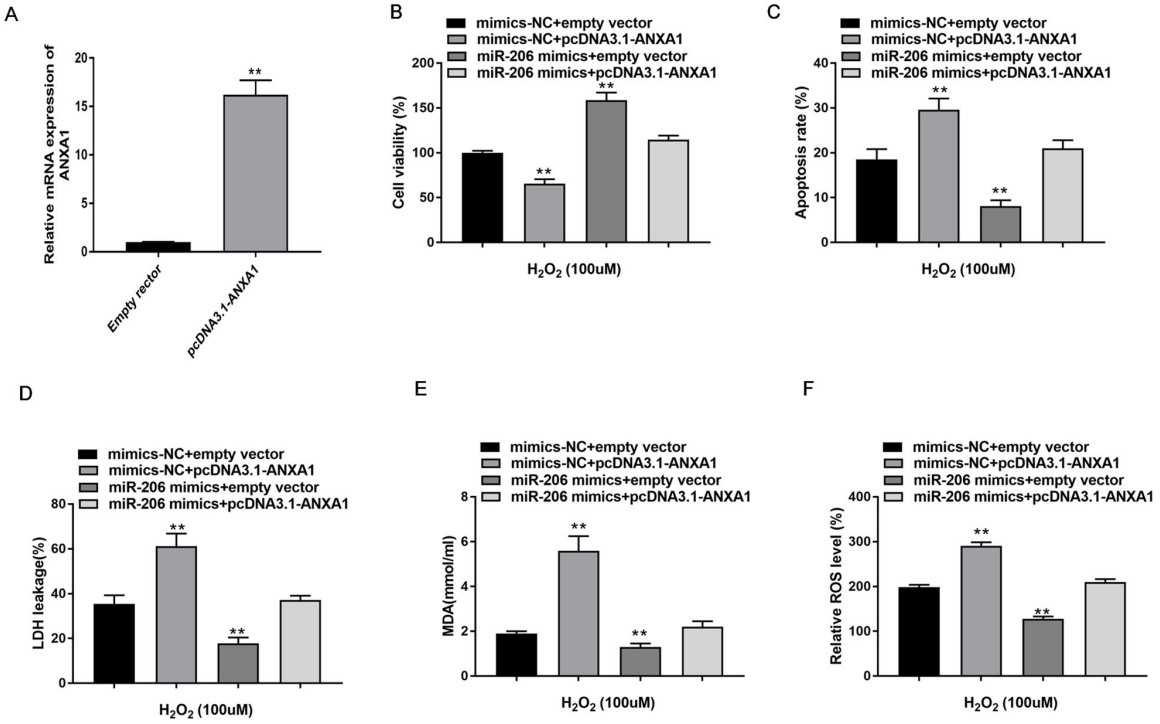

Figure 5. MiR-206 mediated the H_2O_2 tiggeredcytotoxicity, apoptosis and oxidative damage by targeting ANXA1. (A) The qRT-PCR was used for identify the transfection efficiency of pcDNA3,1- ANXA1; (B) The cell viability of H_2O_2-treated SK-N-SH cells co-transfected with miR-206 mimics and/or pcDNA3,1- ANXA1; (C) The apoptosis rate of H_2O_2-treated SK-N-SH cells co-transfected with miR-206 mimics and/or pcDNA3,1- ANXA1; (D) LDH leakage; (E) MDA release and (F) ROS production of H_2O_2 induced SK-N-SH cells co-transfected with miR-206 mimics and/or pcDNA3,1- ANXA1. *P < 0.05, **P < 0.01 vs. the control group; LDH = Lactate Dehydrogenase; MDA = Malondialdehyde; ROS = reactive oxygen species.

shown in Figure 5D-F, LDH leakage, MDA release and ROS production were all partly reversed in H_2O_2-treated SK-N-SH cells co-transfected with pcDNA3.1-ANXA1 and miR-206 mimics, when compared with that transfected with miR-206 mimics alone. To sum up, our above findings considered that DEX exerted neuroprotective effects through up-regulation of miR206 and subsequent down-regulation of ANXA1.

4 Discussion

HIBD, attributes to a series changes of ischemia and hypoxia in brain, which is a primary component of acute deaths and chronic nervous system damage around the world (Han et al., 2018). Recently studies supported that, the ischemic-hypoxic-insult itself and subsequent reoxygenation chain reaction, give rise to a large amount of reactive nitrogen and oxygen species generation, leading to multifarious neurodegenerative processes containing oxidative stress damage, overwhelming releasing of pro-inflammatory cytokines, and even neuronal apoptosis (Blanco et al., 2017; Zhao et al., 2016). In general, after a hypoxic-ischemic attack, several oxygen free radicals and relative pro-inflammatory mediators were generated by activated resident immune cells in the brain, which therewith evoke extreme damages biological macromolecules of neurocytes (Zhao et al., 2016). In present study, we used H_2O_2-treated SK-N-SH cells to simulate the condition that nervous tissue attacked by local ROS after hypoxia-ischemia. From our results, it was showed that H_2O_2 could distinctly evoked oxidative stress injury, cytotoxicity and apoptosis in SK-N-SH cells.

Considerable evidence illustrated that DEX developed neuroprotective roles in different mechanisms, such as anti-inflammatory and anti-apoptotic effects, inhibiting vasoconstriction, reducing oxidative stress and enhancing immunity (Dong et al., 2017; Yoshikawa et al., 2018). For instance, Guo et al. (2020) found that DEX could improve the cognitive degeneration and the iron disorder by suppressing neuroinflammation and oxidative stress in vivo models (Guo et al., 2020). Likewise, Chen et al. (2019) indicated that DEX protected against diabetic hyperglycemia-exacerbated cerebral ischemia reperfusion damages by remission of oxidative stress, inflammation and apoptosis via NFAT5/SIRT1/Nrf2 signaling pathway dependent (Chen et al., 2019). Irrefutably, DEX was always considered as a vital defender to the pathophysiology of cerebral oxidative stress injury and is known to prevent neuronal damage after ischemia and hypoxia condition. In our study, we found that DEX was able to significantly enhance cell viability, reduce apoptosis, lower cellular MDA, LDH and ROS levels in H_2O_2 –treated SK-N-SH cells, which acted a protective role in neurocytes the after oxidative stress injury.

Currently, a number of studies noticed that miRNAs were associated with neuronal survival and control of the accumulation of toxic proteins associated with neurodegeneration, for example, Mu et al. (2020) implied that miR-532-5p promoted cell viability,

decreased cell apoptosis in OGD-treated N2a cells by suppressing PTEN expression and activating the PI3K/Akt signaling pathway, regarded as a novel therapeutic target for ischemic stroke; Fan et al. (2020) denounced that miR-140-5p was involved in isoflurane-aggravated neurotoxicity via negatively regulating of SNX12 expression , suggesting a novel target for neuroprotection in diabetes under isoflurane treatment (Fan et al., 2020).

Initially, miR-206 was recognized as a myogenic miRNA since miR-206 is highly expressed in skeletal muscle tissue and actively supports myogenesis when expressed at the right moment, with development of researches, it was reconsidered as a tumor suppressor, inflammatory modulator and functional biomarker for a variety of diseases and disorders (Ma et al., 2015). In the area of neuroprotection, Zhao et al. (2019) firstly found that hucMSCs-derived miR-206-knockdown exosomes prevented early brain injury from apoptosis via BDNF/TrkB/CREB signaling in subarachnoid hemorrhage (SAH) rat models (Zhao et al., 2019). Inconsequently, Valsecchi et al. (2020) claimed that over-expression of miR-206 exerted an neuroprotective effect on mouse model of spinal muscular atrophy (SMA) via reduction of the predicted target NCX2 which is one of the main regulators of intracellular [Ca2+] and [Na+]. In our research, we firstly found that expression level of miR-206 in H_2O_2-simulated SK-N-SH cells was gradually rise with increase of H_2O_2 concentration, and over-expression of miR-206 could ameliorate H_2O_2-inducedcytotoxicity, apoptosis and oxidative damage, whereas down-expression of miR-206 showed opposite effects.

Previous studies have reported the interaction between DEX and miRNAs in neuroprotection against ischemic hypoxia injury, for examples, Han et al. (2018) showed clearly that miR-140-5p enhanced the cerebral protective effects of DEX against HIBD in neonatal rats by directly binding to WNT1 through the negative regulation of the Wnt/β-catenin signal pathway (Han et al., 2018); Wang et al. (2019) noticed that DEX could carry out its neuroprotective effects though modulating the relationship between miR2233p and TIAL1; Zhou et al. (2018) suggest that miR-129-5p was able to improve neuroprotective role of DEX in HIBI by targeting COL3A1 via Wnt/β-catenin signaling pathway in neonatal rats (Fan et al., 2020). To determine whether miR-206 was implicated in neuroprotection of DEX, we detected the expression of miR-206 in H_2O_2 and/or DEX treated SK-N-SH cells. Excitingly, our results disclosed that miR-206 expression was significantly increased by DEX and H_2O_2 treatment in comparison with H_2O_2 treatment alone, implicating miR-206 might take part in neuroprotective effect of DEX in H2O2-treated SK-N-SH cells.

Accordingly, we predicted the miR-206 had a binding site with ANXA1 3'-UTR by using bioinformatics softwares. ANXA1, also known as lipocortin-1 or calpactin II, is a 38 kDa protein from 2 annexin family of calcium and phospholipid-binding proteins, which widely engaged in progression of multiple disorders such as muscular disease, diabetes and microvascular diseases (Purvis et al., 2019). Most interestingly, our findings firstly showed that the expression level of ANXA1 was positively related with H_2O_2 concentration in SK-N-SH cells, besides, we proved that ANXA1 was a directly target gene of miR-206. Hence, we suspected the interaction beween miR-206 and ANXA1 might be impicited in H2O2 induced oxidative stress injury in SK-N-SH cells. No surprisely, our results interpreted overexpression of ANXA1 rescued the inhibition of apoptosis, LDH leakage, MDA release and ROS production, and promotion of cell viability tiggered by miR-206 in H_2O_2-treated SK-N-SH cells, suggesting DEX exerted neuroprotective effects through up-regulation of miR206 and subsequent down-regulation of ANXA1.

5 Conclusion

Collectively, the present study suggested that DEX exerted neuroprotective effects on H_2O_2-treated SK-N-SH cells in vitro by negatively reguating ANXA1 expression via activation of miR206, which might provide a novel therapy for HIBD.

Conflict of interest

The authors have no conflicts of interest to declare.

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