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# MicroRNA-181a Regulates Apoptosis and Autophagy Process in Parkinson's Disease by Inhibiting p38 Mitogen-Activated Protein Kinase (MAPK)/c-Jun N-Terminal Kinases (JNK) Signaling Pathways

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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**Background:** microRNA (miR)-181a has been reported to be downregulated in Parkinson's disease (PD), but the regulatory mechanism of miR-181a on neuron apoptosis and autophagy is still poorly understood. We aimed to investigate the neuroprotective effects of miR-181a on PD *in vitro*.

**Material/Methods:** Human SK-N-SH neuroblastoma cells were incubated with different concentrations of 1-methyl-4-phenylpyridinium ion ($MPP^+$) to induce the PD model. The expression of miR-181a was then analyzed. After transfection with miR-181a mimic or scramble following $MPP^+$ treatment, the expression of autophagy protein markers (LC3II, LC3I, and Beclin 1) and p38 mitogen-activated protein kinase (MAPK)/c-Jun N-terminal kinases (JNK) signaling proteins (p-p38, p38, p-JNK, and JNK) and cell apoptosis were detected. Furthermore, the cells were transfected with miR-181a inhibitor and cultured in the presence or absence of p38 inhibitor SB203582 or JNK inhibitor SP600125, and the cell apoptosis was tested again.

**Results:** The expression of miR-181a was gradually decreased with the increase of $MPP^+$ concentration ($P<0.05$, $P<0.01$, or $P<0.001$). Overexpression of miR-181a significantly decreased the LC3II/LC3I ratio, Beclin 1 expression, cell apoptosis, and the expression of p-p38 and p-JNK compared to the $MPP^+$ + miR-181a scramble group (all $P<0.05$). In addition, we observed that SB203582 or SP600125 showed no effects on cell apoptosis, but the effects of miR-181a inhibitor on cell apoptosis were reversed by administration of SB203582 or SP600125 compared to the scramble group ($P<0.05$).

**Conclusions:** Our results suggest that miR-181a regulates apoptosis and autophagy in PD by inhibiting the p38 MAPK/JNK pathway.

**MeSH Keywords:** Apoptosis • Autophagy • MAP Kinase Signaling System • MicroRNAs • p38 Mitogen-Activated Protein Kinases • Parkinson Disease

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## Background

Parkinson's disease (PD) is the second most common neurodegenerative disease following Alzheimer disease (AD), which is characterized by massive degeneration and progressive loss of dopaminergic (DA) neurons in the midbrain substantia nigra pars compacta (SNpc) of the basal ganglia [1,2]. It has been reported that the prevalence of PD is approximately 0.3% of the population in developed countries [3]. Unfortunately, there are no available therapies to inhibit the degenerative process of the disease, and only symptomatic treatment exists to improve the quality of life in patients with PD due to the unclear causes [3]. Although the pathogenesis of the disease is not completely understood, aging, genetic susceptibility, mitochondrial dysfunction, inflammation, oxidative stress, and protein aggregation are responsible for PD, leading to cell apoptosis [4–6]. Recently, a growing body of evidence suggests that autophagy plays a critical role in the progression of PD [7–10]. Thus, a better understanding of cell apoptosis and autophagy involved in PD might provide an effective and efficient treatment for PD patients.

MicroRNAs (miRNAs) are small, non-coding, and highly conserved RNAs that post-transcriptionally regulate gene expression by targeting mRNAs and prompting either translation repression or RNA degradation [11]. It has been well acknowledged that miRNAs play significant roles in many human diseases, including PD [12–14]. Recent studies have suggested that a number of miRNAs such as miR-34c, miR-107, miR-133b, miR-205, and miR-433 are aberrantly expressed in PD and are involved in several cellular processes including cell replication, mature neurons' survival, neuron differentiation, neurite outgrowth, apoptosis, and autophagy [15–18]. The potential usefulness of a miRNA-based therapy in PD has been considered as a powerful tool [17]. However, the relevant mechanisms are far more complicated. Among the miRNAs, miR-181a is a conserved miRNA that is highly expressed in the brain [19] and has been reported to be increased during maturation of hippocampal neurons [20]. Besides, overexpression of miR-181 increases astrocyte death [21], and suppression of miR-181a can decrease evidence of astrocyte dysfunction [22]. In addition, a recent study revealed that the serum level of miR-181a is downregulated in PD and it could serve as a potential biomarker for the diagnosis of PD [23]. However, little information is available about the regulatory mechanism of miR-181a on DA neuron apoptosis and autophagy.

Therefore, in the present study, we aimed to investigate the regulatory mechanism of miR-181a on DA neuron apoptosis and autophagy, as well as potential signaling pathways. Human SK-N-SH neuroblastoma cells were incubated with 1-methyl-4-phenylpyridin'ium ion ($MPP^+$) to induce the PD model. The expression of miR-181a was endogenously altered, and then the effects of miR-181a on DA neuron apoptosis and autophagy were analyzed. Our study might provide new insight into novel therapeutic targets for treatment of PD.

## Material and Methods

### Cell culture

Human SK-N-SH neuroblastoma cells were obtained from the American Type Culture Collection (ATCC, Manassas, Virginia, USA). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco-BRL, Grand Island, New York, USA) containing 10% fetal bovine serum (FBS; Sigma Chemical Co., St. Louis, Missouri), penicillin 100 U/mL, and streptomycin 100 U/mL (Sigma) in an atmosphere of 5% $CO_2$ at 37°C.

### Cell treatment and transfection

For cell treatment, SK-N-SH cells were seeded in plates and grown to 80-90% confluency. Thereafter, the cells were incubated with different concentrations of $MPP^+$ (0, 30, 50, 100, 200, or 400 μM), SB203582 (25 μM), or SP600125 (25 μM) for 24 h and collected for further analysis. SB203582 is a selective inhibitor of p38 mitogen-activated protein kinase (MAPK) that inactivates MAPK-activated protein kinase-K2 (MAPKAP K2) [24]. SP600125 is a selective inhibitor of c-Jun N-terminal kinase (JNK) that suppresses the phosphorylation of JNK by competitive binding to the JNK ATP-binding site [25]. For cell transfection, miR-181a mimic, inhibitor, and scramble were purchased from Sangon Biotech (Shanghai, China). Briefly, the cells ($2\times10^5$/well) were seeded in 96-well plates and then transiently transfected with miR-181a mimic, inhibitor, or scramble according to the manufacturer's instructions. Cell transfection was performed using the Lipofectamine 2000 according to the manufacture's protocol (Invitrogen, Carlsbad, California, USA).

### Cell apoptosis

Cell apoptosis was determined by flow cytometry using the Annexin V-FITC cell apoptosis kit (Abcam, Cambridge, Massachusetts, USA) according to the manufacturer's protocol. Briefly, the cells were treated with 0 or 100 μM $MPP^+$ and/or transfected with miR-181a mimic, inhibitor, or scramble for 48 h, and then the cells were cultured with fresh serum-free DMEM medium. Afterwards, the cells were washed three times with phosphate-buffered saline (PBS) buffer (pH 7.4), re-suspended in the staining buffer, and then mixed with annexin-V-FITC (5 μL) and propidium iodide (PI, 5 μL) at room temperature for 15 min. Labeled cells were analyzed by using the FACScan flow cytometry (Becton Dickinson, Franklin Lakes, New Jersey, USA). Each condition was repeated at least 3 times.





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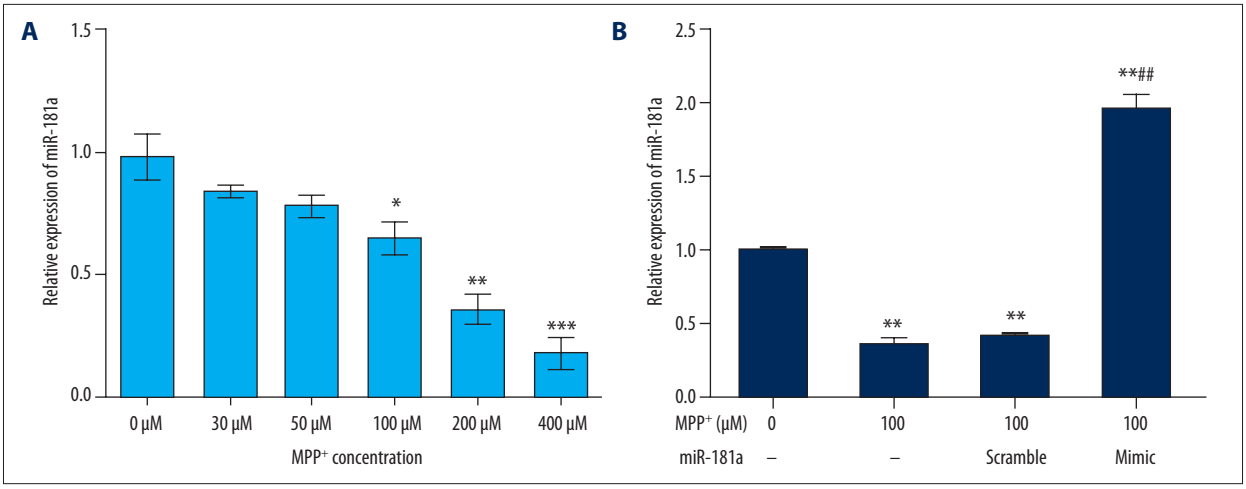


**Figure 1.** MiR-181a is downregulated in MPP+-treated SK-N-SH cells. The SK-N-SH cells were incubated with different concentrations of MPP+ (0, 30, 50, 100, 200, or 400 μM) and/or transfected with miR-181a mimic or scramble. The expression of miR-181a was then analyzed. Non-treated cells were considered as a control group. (**A**) The relative expression of miR-181a was gradually decreased with the increase of MPP+ concentration. (**B**) The relative expression of miR-181a was significantly increased by miR-181a mimic. * $P<0.05$, ** $P<0.01$, or *** $P<0.01$ compared to the control group; ## $P<0.01$ compared to the MPP+ + miR-181a scramble group. MiR – microRNA; MPP+ – 1-methyl-4-phenylpyridinium ion.

### Quantitative real time PCR (qRT-PCR) analysis

After 48 h of treatment with different concentrations of MPP+ (0, 30, 50, 100, 200, or 400 μM) and/or transfection with miR-181a mimic, inhibitor, or scramble, total mRNA was isolated from the cells using TRIzol (Invitrogen) according to the manufacturer's protocol. First-strand complementary DNA (cDNA) was produced using reverse transcriptase (iScript™ cDNA Synthesis Kit; Bio-Rad Laboratories, Hercules, CA, USA). The expression levels of mRNAs were measured by PrimeScript RT Reagent Kit (TaKaRa, Tokyo, Japan) and SYBR green-based quantitative RT-PCR (SYBR Green Master mix; Thermo Scientific, Waltham, Massachusetts, USA) based on the manufacturer's instructions. The expression levels of miRNA were measured by TaqMan miRNA assays (ABI, Forest City, California). Gene and miRNA expression was normalized by GAPDH and U6 snRNA, respectively. All primers were synthesized by Sangon Biotech. Co., Ltd. (Shanghai, China).

### Western blotting

After 48 h of treatment with 0 or 100 μM MPP+ and/or transfection with miR-181a mimic, inhibitor, or scramble, protein was extracted from the cells and the protein concentration was determined by using Bio-Rad protein assay reagent (Bio-Rad). The protein samples (30 μg per lane) were separated on a 10–12% sodium dodecyl sulfate (SDS)-polyacrylamide gel, blotted onto polyvinylidene difluoride membranes (GE Healthcare, Little Chalfont, England), and blocked in 5% non-fat dried milk in Tris-buffered saline with Tween (TBST) for 2 h. Then the membranes were probed with the following primary antibodies overnight at 4°C: anti-LC3 A/B (ab128025, Abcam), anti-Beclin-1 (ab55878, Abcam), anti-phospho-p38 (anti-p-p38; #4511, Cell Signaling Technology Inc., Beverly, Massachusetts, USA), anti-p38 (#8690, Cell Signaling Technology), anti-phospho-JNK (anti-p-JNK; #4668, Cell Signaling Technology), or anti-JNK (#9252, Cell Signaling Technology). The membranes were then incubated with the appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies. The immunoreactive protein bands were analyzed using enhanced chemiluminescence (Pierce, Rockford, Illinois, USA). GAPDH was used as the internal control.

### Statistical analysis

Data are expressed as mean ± standard deviation (SD). Student's t test was performed to analyze the significant difference between two groups. Statistical differences between gene expression were assessed using a one-way variance analysis (ANOVA), followed by Tukey's multiple comparison test. For measurements of protein expression, post hoc testing was employed when there was a significant difference. Statistical analyses were performed using Graph prism 6.0 software (GraphPad Prism, San Diego, California, USA). $P<0.05$ was considered as statistically significant.

## Results

### MiR-181a was downregulated in MPP+-treated SK-N-SH cells

To investigate the functional role of miR-181a in PD, we first evaluated the expression levels of miR-181a in an *in vitro* MPP+





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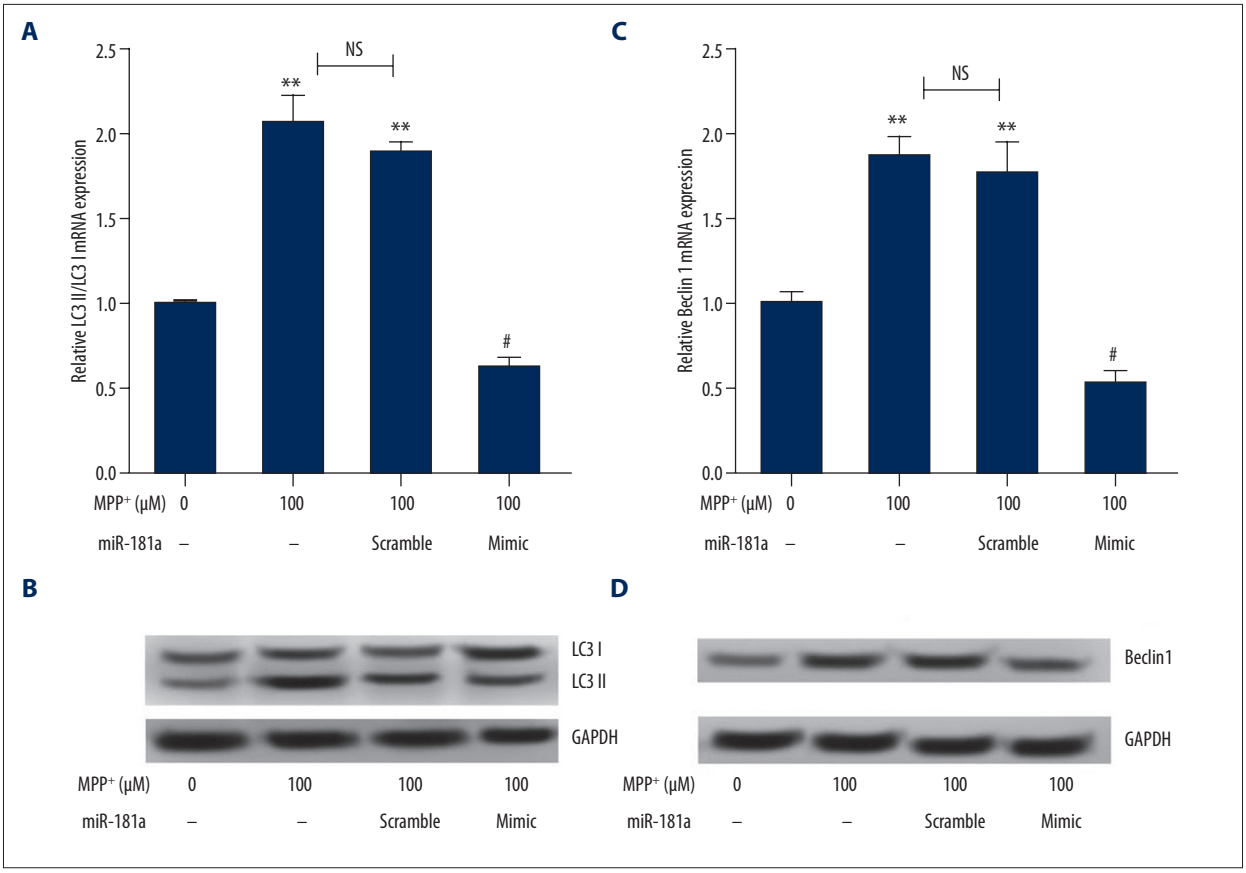


**Figure 2.** Overexpression of miR-181a inhibits autophagy. The mRNA and protein expressions of LC3II, LC3I, and Beclin 1 were detected after administration with MPP+ and/or transfection with miR-181a mimic or scramble. Non-treated cells were considered as a control group. (**A, B**) The mRNA and protein expressions of the LC3II/LC3I ratio were statistically decreased by MPP+ + miR-181a mimic. (**C, D**) The mRNA and protein expressions of Beclin 1 were significantly reduced by MPP+ + miR-181a mimic. ** *P*<0.01 compared to the control group; # *P*<0.05 compared to the MPP+ + miR-181a scramble group. MiR – microRNA; MPP+ – 1-methyl-4-phenylpyridinium ion; NS, no significance.

model of PD. SK-N-SH cells were maintained in different concentrations of MPP+ (0, 30, 50, 100, 200, or 400 μM), and then the expression levels of miR-181a in SK-N-SH cells were assessed by qRT-PCR. As shown in Figure 1A, we observed that the relative expression of miR-181a was gradually decreased with the increase of MPP+ concentration. However, significance was reached by 100 (*P*<0.05), 200 (*P*<0.01), and 400 μM (*P*<0.001) MPP+. The results suggested that miR-181a was downregulated in PD. Then we transfected miR-181a mimic or scramble into MPP+-treated (100 μM) SK-N-SH cells. The untreated cells were regarded as a control group. As expected, the relative levels of miR-181a were significantly decreased by MPP+ and MPP+ + miR-181a scramble compared to the control group (both *P*<0.05), while the relative levels were statistically increased by miR-181a mimic compared to the control group (*P*<0.01) or 100 μM MPP+-treated group (*P*<0.01) (Figure 1B), indicating that all the vectors were successfully transfected into SK-N-SH cells.

### Overexpression of miR-181a inhibited autophagy

It is well known that autophagy is tightly linked to PD. To explore the effects of miR-181a overexpression on autophagy, the expressions of autophagy protein markers (LC3II, LC3I, and Beclin 1) were detected after transfection with miR-181a mimic by Western blotting. As indicated in Figure 2A, 2B, a significant increase in LC3II/LC3I ratio expressions was found in the MPP+ and MPP+ + miR-181a scramble group compared to the control group (both *P*<0.01). However, there was no significant difference between the MPP+ group and MPP+ + miR-181a scramble group. Interestingly, we found that the LC3II/LC3I ratio was statistically reduced by overexpression of miR-181a compared to the MPP+ + miR-181a scramble group (*P*<0.05). In addition, the expression of Beclin 1 revealed results similar to those of the LC3II/LC3I ratio (Figure 2C, 2D). These results demonstrated that overexpression of miR-181a could significantly inhibit autophagy in PD.





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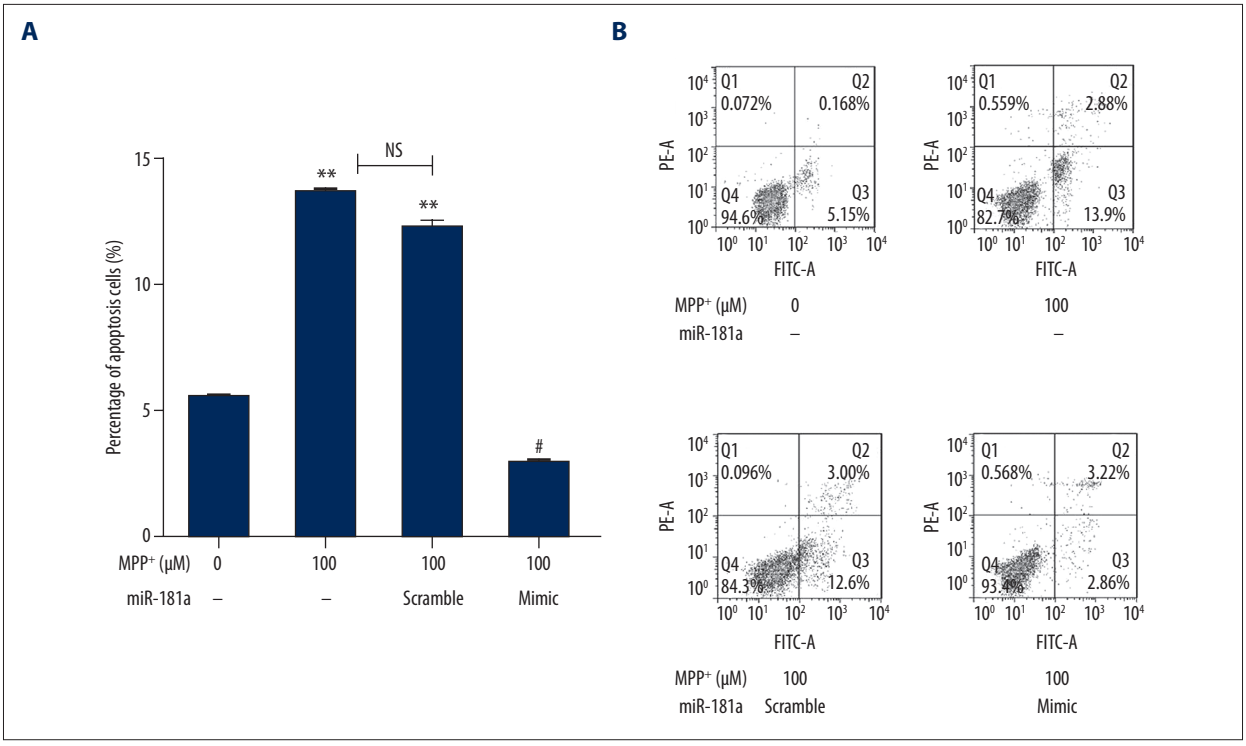


**Figure 3.** Overexpression of miR-181a reduces neuron apoptosis. The percentages of cell apoptosis were analyzed after administration with MPP+ and/or transfection with miR-181a mimic or scramble. Non-treated cells were considered as a control group. (**A, B**) The percentages of cell apoptosis were distinctly decreased by MPP+ + miR-181a mimic. ** *P*<0.01 compared to the control group; # *P*<0.05 compared to the MPP+ + miR-181a scramble group. MiR – microRNA; MPP+ – 1-methyl-4-phenylpyridinium ion; NS – no significance.

### Overexpression of miR-181a reduced neuron apoptosis

Next, we analyzed the effects of miR-181a overexpression on neuron apoptosis by flow cytometry using the Annexin V-FITC cell apoptosis kit. The results showed that the percentages of cell apoptosis were markedly increased by MPP+ and MPP+ + miR-181a scramble compared to the control group (both *P*<0.01). No significant differences were observed between the two groups. However, the percentages of cell apoptosis were distinctly decreased by overexpression of miR-181a compared to the MPP+ + miR-181a scramble group (*P*<0.05) (Figure 3A, 3B). The results indicated that overexpression of miR-181a could significantly prevent neuron apoptosis in PD.

### Overexpression of miR-181a inhibited p38 MAPK/JNK signal activation

Activation of p38MAPK/JNK has been reported to be associated with PD. Therefore, we analyzed the effects of miR-181a overexpression on p38 MAPK and JNK phosphorylation by qRT-PCR and Western blotting. As demonstrated in Figure 4A, the results revealed that both the relative mRNA expression levels of p-p38 and p-JNK were significantly elevated by MPP+ and MPP+ + miR-181a scramble group compared to the control group (*P*<0.05). Also, we found that there was no distinct difference in levels of p-p38 and p-JNK between the MPP+ group and MPP+ + miR-181a scramble group. Besides, the levels of p-38 and JNK were not dramatically changed. However, the levels of p-p38 and p-JNK were remarkably lowered by overexpression of miR-181a compared to the MPP+ + miR-181a scramble group (*P*<0.05). The protein expression of p-p38 and p-JNK showed similar results (Figure 4B). These results demonstrated that overexpression of miR-181a could activate p38 MAPK/JNK pathways.

### Suppression of miR-181a promoted p38 MAPK/JNK signal activation

To further confirm whether miR-181a regulates neuron apoptosis by modulating p38 MAPK/JNK pathways, we administered the inhibitor of p38 MAPK (SB203582) and the inhibitor of JNK (SP600125), and then analyzed the effects of miR-181a suppression on neuron apoptosis. As expected, the expression of p-p38 was markedly decreased by its inhibitor SB203582 but was elevated by MPP+ and miR-181a inhibitor compared to the control group. It was noteworthy that the effects of SB203582 on the expression of p-p38 were eliminated by miR-181a inhibitor, indicating that miR-181a inhibitor could activate the p38 MAPK





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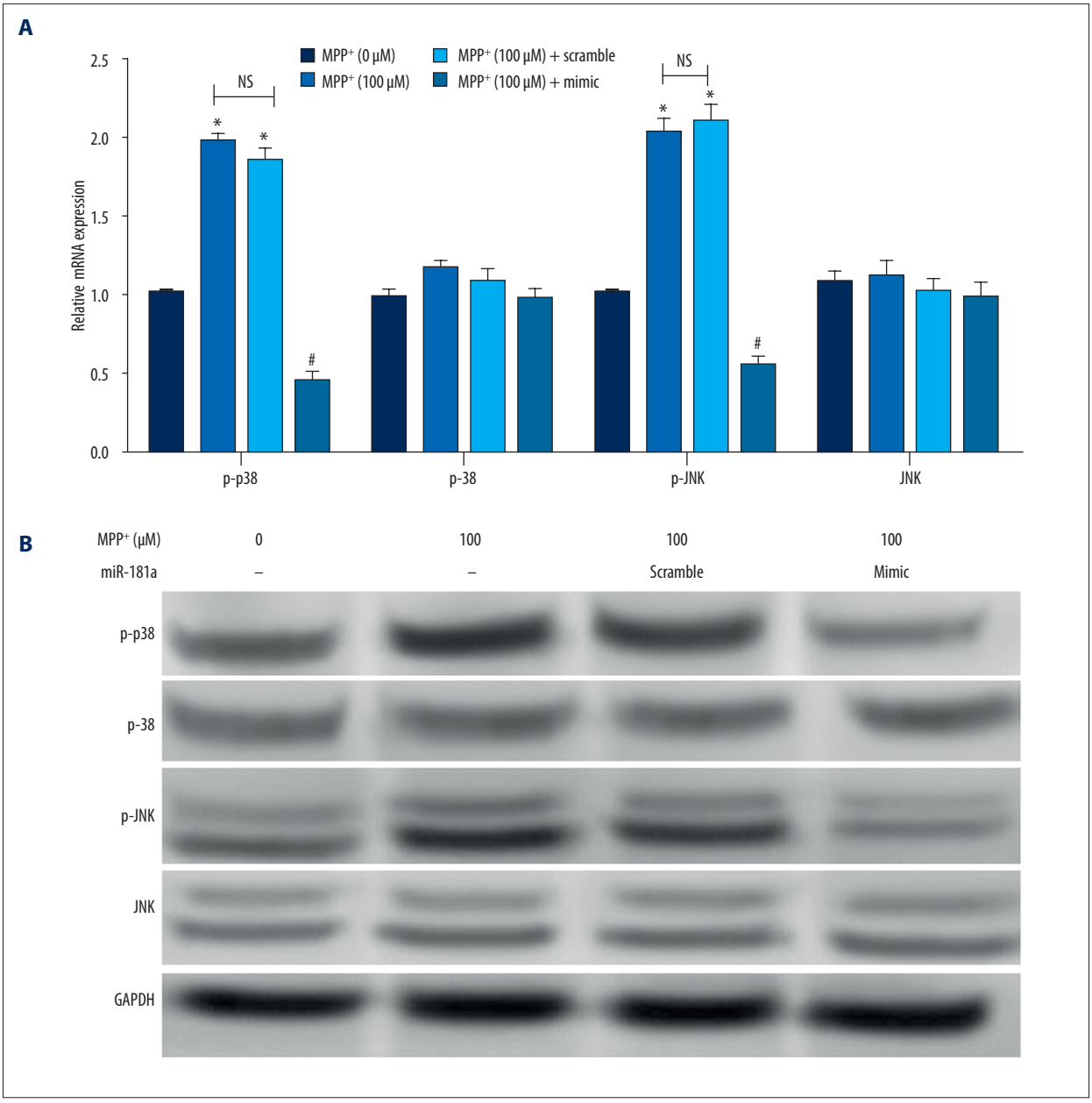


**Figure 4.** Overexpression of miR-181a inhibits p38 MAPK/JNK signal activation. The mRNA and protein expressions of p-p38, p38, p-JNK, and JNK were determined after administration with MPP+ and/or transfection with miR-181a mimic or scramble. Non-treated cells were considered as a control group. (**A, B**) The mRNA and protein expressions of p-p38 and p-JNK were dramatically decreased by MPP+ + miR-181a mimic, and no significant difference was found in levels of p38 and JNK. * $P<0.05$ compared to the control group; # $P<0.05$ compared to the MPP+ + miR-181a scramble group. MiR – microRNA; MPP+ – 1-methyl-4-phenylpyridinium ion; NS – no significance.

signaling pathway (Figure 5A). Furthermore, we observed that SB203582 had no significant effect on cell apoptosis. The percentages of cell apoptosis were significantly increased by MPP+ and miR-181a inhibitor compared to the control group ($P<0.05$), but the effects of miR-181a inhibitor on apoptosis were reversed by administration with SB203582 compared to the scramble group ($P<0.05$) (Figure 5B, 5C), demonstrating that miR-181a inhibitor promotes cell apoptosis by activating the p38 MAPK signaling pathway. Meanwhile, the results showed that the JNK signaling pathway could also be activated by miR-181a inhibitor (Figure 5D). The effects of miR-181a inhibitor on cell apoptosis were also reversed by administration with SP600125 (Figure 5E, 5F). These results suggested that suppression of miR-181a could promote the cell apoptosis by activation of p38 MAPK/JNK pathways.





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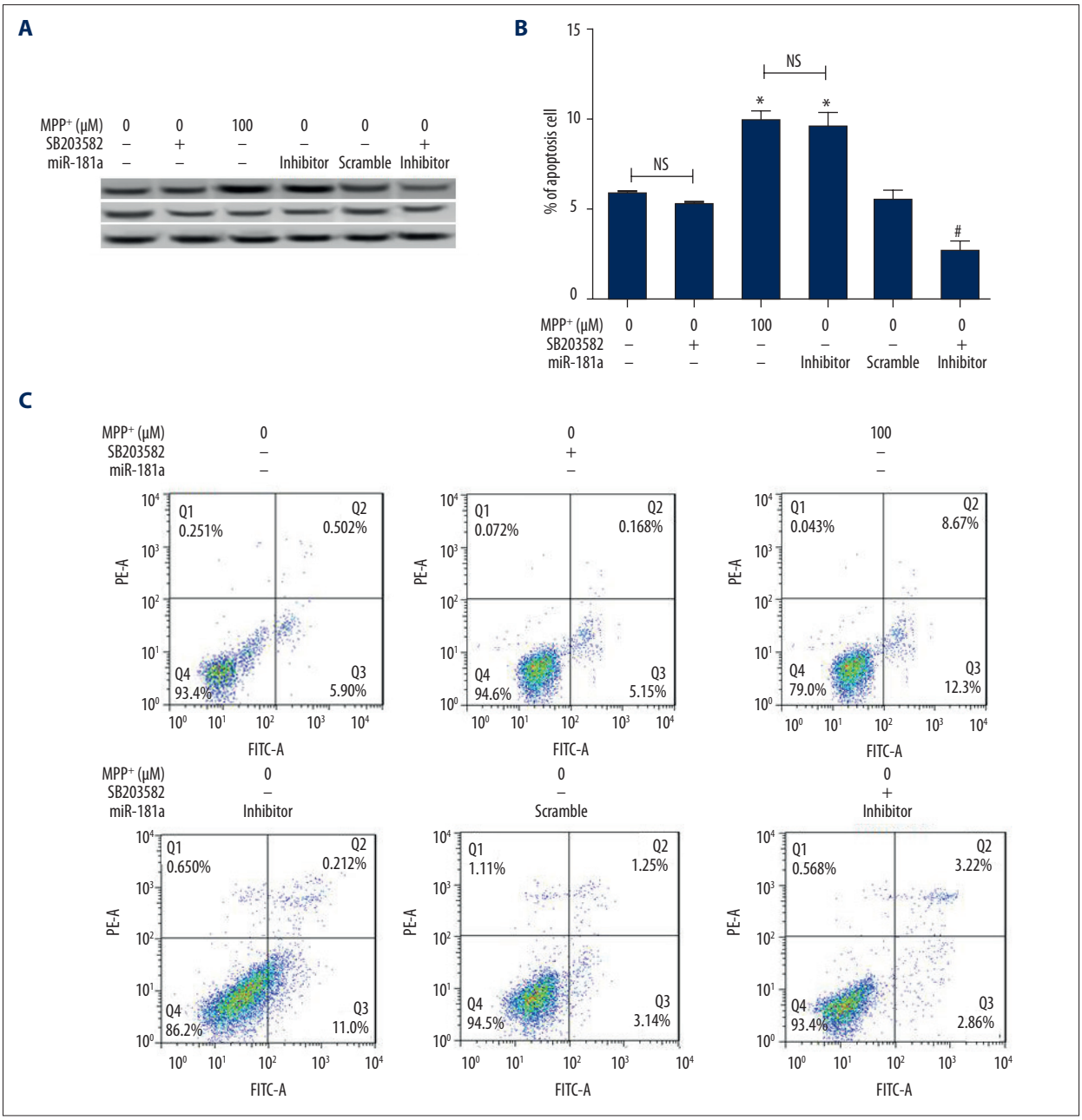
## Discussion

In the present study, we found that the expression of miR-181a was significantly downregulated in the PD model. Overexpression of miR-181a inhibited the expression of autophagy protein markers (LC3II/LC3I ratio and Beclin 1) and reduced the percentages of cell apoptosis. Further, the results demonstrated that overexpression of miR-181a inhibited the activation of p38MAPK/JNK pathways, while suppression of miR-181a promoted the activation of p38MAPK/JNK pathways. Additionally, suppression of miR-181a statistically induced cell apoptosis, but the effects were prominently alleviated by application of the inhibitor of p38 MAPK (SB203582) and the inhibitor of JNK (SP600125).

Growing evidence suggests that miRNAs play a significant role in the regulation of a variety of basic biological and pathological processes in many brain diseases, including PD. Several miRNAs have been identified to be abnormally expressed in the brain tissues of patients or animals with PD and are responsible for the development of DA neurons [12,26–28]. Thus, many miRNAs are regarded as novel biomarkers for molecular diagnosis and potential therapy targets. Among the miRNAs, miR-181a is a member of miR-181 family, which shows





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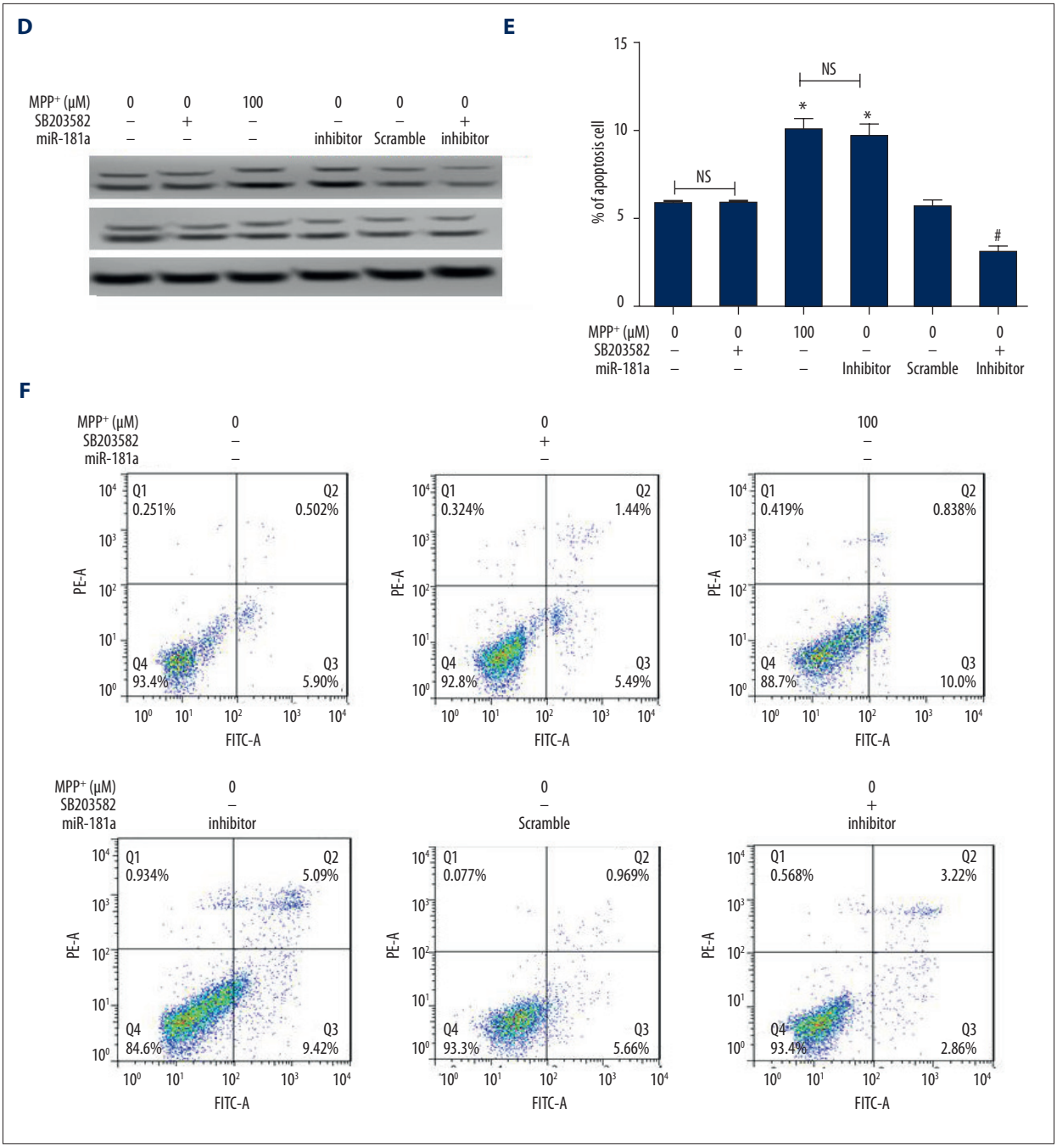


**Figure 5.** Suppression of miR-181a promotes p38 MAPK/JNK signal activation. The inhibitor of p38 MAPK (SB203582) and the inhibitor of JNK (SP600125) were added to the cells, and then the effects of miR-181a suppression on neuron apoptosis were analyzed. Non-treated cells were considered as a control group. (**A**) The protein expression of p-p38 was inhibited by SB203582 but promoted by MPP+ and miR-181a inhibitor, and the effects of miR-181a inhibitor on p-p38 expression were reversed by SB203582. (**B, C**) The percentages of cell apoptosis were significantly increased by MPP+ and miR-181a inhibitor, but the effects of miR-181a inhibitor on apoptosis were reversed by SB203582. (**D**) The protein expression of p-JNK was decreased by SP600125 but increased by MPP+ and miR-181a inhibitor, and the effects of miR-181a inhibitor on p-JNK expression were reversed by SP600125. * *P*<0.05 compared to the control group; # *P*<0.05 compared to the scramble group. MiR – microRNA; MPP+ – 1-methyl-4-phenylpyridinium ion; NS – no significance.





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a robust enrichment in the brain [19,29]. It has been reported that miR-181a is increased during maturation of hippocampal neurons and in the infarct core, but decreased in the penumbra after focal ischemia [20,30]. MiR-181a has been established to regulate the functions of synapsis, and miR-181a expression could be induced by DA signaling in primary neurons [20]. Besides, suppression of miR-181a reduced astrocyte dysfunction and increased cornu ammonis (CA1) neuronal survival [22]. Recently, it has been demonstrated that miR-181a is involved in PD [23,31]. However, the clear mechanism for the effects of miR-181a on PD remains to be identified. In the present study, we focused on the functional role of miR-181a in DA neuron apoptosis and autophagy.

Apoptosis and autophagy are basic physiologic processes that are responsible for the maintenance of cellular homeostasis [32]. They are independent processes but inversely related. Emerging evidence supports the view that neuron apoptosis and autophagy play critical roles in the development of PD [8–10,33,34]. MiRNAs have been reported to be involved in PD by regulating the cell apoptosis and autophagy. For example, miR-124 could regulate apoptosis and autophagy processes in the 1-methyl-4-pheny-1, 2, 3, 6-tetrahydropyridine (MPTP) model of PD by targeting Bim [35] and by regulating the adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK)/mammalian target of rapamycin (mTOR) pathway [36]. To confirm the functional role of miR-181a in apoptosis and autophagy, we used $MPP^+$, a frequently used neurotoxin [37], to induce cellular models of PD. SK-N-SH neuroblastoma cells were incubated with $MPP^+$, and then the expression of miR-181a was analyzed. Consistent with Ding et al. [23], the expression of miR-181a in $MPP^+$ treated cells was significantly downregulated compared to the non-treated cells. Subsequently, the effects of overexpression of miR-181a on cell apoptosis and autophagy were determined. As expected, $MPP^+$ caused higher percentages of apoptotic cells, but the effects were alleviated by overexpression of miR-181a, demonstrating the inhibitory effects of miR-181a overexpression on cell apoptosis.

Our results were in line with those of a previous study, in which the results showed that apoptosis was inhibited by overexpression of miR-181a in osteosarcoma cells [38]. In the scenario of autophagy, the expression of LC3II, LC3I, and Beclin 1 was measured after application of $MPP^+$ and transfected with miR-181a mimic. LC3II is the cleaved form of LC3I, and the ratio of LC3II/LC3I demonstrates the degree of autophagosome formation and is also a marker widely used to indicate autophagy [39]. An increase of the LC3II/LC3I ratio reflects active autophagy. Beclin 1 is an essential autophagy-related protein that has been linked to apoptosis and autophagy [40]. In spite of many studies supporting the protective role of autophagy in PD, extreme autophagy activation is related to neuronal loss [41]. Therefore, reduction of autophagy may be of benefit in PD. Our results revealed that the ratio of LC3II/LC3I and the expression of Beclin 1 were statistically downregulated by overexpression of miR-181a compared to the scramble group, indicating the autophagy inhibition was generated bymiR-181a upregulation.

p38 MAPK and JNK are two important components of MAPK cascades, which play significant roles in regulation of diversiform cellular activities such as cell survival, cell proliferation, differentiation, and apoptosis [42]. Recent studies have highlighted that p38 MAPK and JNK are activated by neurotoxicants, stress, and/or inflammation and may be responsible for PD, and that blocking of the p38 MAPK and JNK pathways may help for the development of therapeutic approaches that could benefit PD patients [43,44]. Interestingly, our results showed that miR-181a upregulation caused lower levels of p-p38 and p-JNK. The results implied that overexpression of miR-181a inactivated the p38 MAPK and JNK pathways. The results were partly similar to those of Song et al. [45], in which miR-181a achieved its functionalities by regulating the p38 MAPK pathway. The present study also showed, for the first time to the best of our knowledge, that miR-181a regulated the JNK pathway. To further confirm that the effects of miR-181a on cell apoptosis might be via the p38 MAPK and JNK pathways, we administered the inhibitors of the two pathways, SB203582 and SP600125, and then analyzed the suppression of miR-181a on cell apoptosis. The results revealed that suppression of miR-181a activated the p38 MAPK and JNK pathways, which reversely verified that overexpression of miR-181a inactivated the two pathways. Moreover, we observed that suppression of miR-181a significantly increased the percentages of cell apoptosis; however, these effects were statistically relieved by application of SB203582 and SP600125.

## Conclusions

In conclusion, our results suggest that miR-181a is downregulated in $MPP^+$-intoxicated SK-N-SH neuroblastoma cells. Overexpression of miR-181a inhibits autophagy and reduces apoptosis. The effects of miR-181a on autophagy and apoptosis might be achieved by regulation of the p38 MAPK and JNK pathways.

### Conflicts of interest

The authors have no conflicts of interest to declare.





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