Original Article
LncRNA MALAT1 affects the biological behavior of Parkinson’s disease model cells by downregulating miR-181a

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Abstract: To explore the value of lncRNA MALAT1 in Parkinson’s disease (PD) and its effects on PD model cells through miR-181a. A total of 64 PD patients admitted to The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, from August 2017 to August 2019 were enrolled as a research group (res group), and 70 healthy individuals during the same period were enrolled as a control group (con group). The expression of MALAT1 in the peripheral blood of participants in the two groups was determined. In addition, human neuroblastoma cells (SH-SY5Y) and normal human brain glial cells (HEB) were purchased, and MPP+ was used to construct PD model cells. The biological behavior of SH-SY5Y cells transfected with MALAT1 or miR-181a was detected, and the relationship between MALAT1 and miR-181a was verified by a dual luciferase reporter (DLR) assay. MALAT1 was overexpressed in the peripheral blood of the PD patients and in Parkinson’s model cells, and miR-181a had lower expression in PD model cells (all P<0.05). After being transfected with MALAT1 inhibition sequence and miR-181a mimic sequence, the PD model cells showed weakened proliferation and invasion abilities and an increased apoptosis rate (all P<0.05). Additionally, the DLR assay revealed that the luciferase activity of MALAT1-WT was inhibited by miR-181a-mimics (P<0.05), and the co-transfection experiment of si-MALAT1 and miR-181a-mimics revealed that the influence of miR-181a-mimics on cell viability was reversed by si-MALAT1. MALAT1 was highly expressed in PD, and it promotes the proliferation and invasion of PD model cells and inhibits their apoptosis by targeting the inhibition of miR-181a, which may be the key to the diagnosis and treatment of PD in the future.

Keywords: MALAT1, miR-181a, Parkinson’s disease, proliferation, apoptosis

Introduction

Parkinson’s disease (PD), also known as Parkinson’s paralysis agitans, is a common neurological disease among the middle-aged and elderly [1]. At present, its incidence accounts for 1% among all diseases in people above 60 years old, and some studies show that its incidence has been continuously rising in recent years [2, 3]. The pathogenesis of PD is still unclear yet, and some studies have pointed out that PD is related to aging, genetic susceptibility, and environmental toxins [4]. PD not only induces neuronal degradation and dysfunction, but also brings about complications in daily skills requiring clinical attention [5]; including nerve injury and disorder, digestive system abnormalities, and respiratory tract infections, which seriously endangers the life and health of patients [6]. Currently, conservative treatment is recommended for PD patients in clinical practice, but the treatment cycle is extremely long and the possibility of cure it is relatively low [7]. Therefore, it is particularly important to fully understand the pathogenesis of PD and find a novel and effective treatment.

With the continued investigation of research into long-chain noncoding RNAs (lncRNAs) they have gradually become a hot research topic in the treatment of nervous system diseases [8, 9]. They have been confirmed to be involved in the occurrence of many diseases and have extremely strong regulatory effects on biologi-
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Table 1. Primer sequences

<table>
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<th>F (5′-3′)</th>
<th>R (5′-3′)</th>
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<tr>
<td>MALAT1</td>
<td>GAATTGCGTCTATTA-AAGGCTAGGT</td>
<td>GTTCATC-CTACCACCTCCACAATTAA</td>
</tr>
<tr>
<td>β-actin</td>
<td>GACCTGACT-GAATCTCCTGATGAA</td>
<td>GT-AAGCCTGACTCTGCCAATAG</td>
</tr>
<tr>
<td>miR-181a</td>
<td>GAAAAGCAGACATTGAAGCCTAC</td>
<td>CCATCCTTACATCTCTGCTGG</td>
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<tr>
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Intracellular cell behaviors [10, 11]. Among them, IncRNA MALAT1 is closely related to cerebral vessels and involved in the biological behavior of human brain microvascular endothelial cells [12, 13], but its relationship with PD is still under investigation. At present, the pathogenesis of PD is still under investigation, and there is also a lack of relevant research on the molecular regulation of cell behavior affecting PD patients. We suspect that MALAT1 is also closely related to the occurrence of PD. In order to verify this, we explored the effects of MALAT1 on PD models to verify the relationship between MALAT1 and PD. It is expected that the occurrence and development process of PD can be identified by detecting MALAT1 in future clinic practice, and targeted therapy from a molecular perspective may be better than the current conventional methods for treating PD; which is of great significance for clinical diagnosis and treatment of PD in the future.

Materials and methods

Patient data

A total of 64 patients with PD admitted to The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, from August 2017 to August 2019 were enrolled as a research group (res group), and 70 healthy individuals during the same period were enrolled as a control group (con group). Fasting venous blood (4 mL) was extracted from each participant for prospective analysis. The inclusion criteria of the patients: Patients diagnosed with PD in our hospital, patients with complete case data, patients willing to cooperate with and participate in this study, and those who signed informed consent forms or whose immediate relatives signed them. The exclusion criteria of the patients: Patients who received disease-related treatment 6 months prior to admission, patients with other congenital diseases or autoimmune defects, patients with liver or renal insufficiency due to organ failure, patients allergic to drugs, and those transferred to our hospital. Th-

Cellular data

Human neuroblastoma cells (SH-SY5Y) and normal human brain glial cells (HEB) were provided by the American Type Cell Culture, with batch numbers BNCC338056 and BNCC338123, respectively, and the cells were incubated in Dulbecco’s modified eagle medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin in a 5% CO₂ incubator at 37°C.

Cell transfection

SH-SY5Y and HEB cells in a logarithmic growth phase under normal culturing conditions were assigned to a model group and a normal group, respectively. Cells in the model group were treated using 1 mmol/L MMP-1 for 24 h according to the methods in the study by Xicoy et al. [14], and then MALAT1 inhibitor (si-MALAT1), negative control (NC), miR-181a mimics (miR-181a-mimics), and negative control (miR-NC) were transfected into the cells using ExFect 2000 Transfection Reagent, separately.

PCR assay

Total RNA was extracted from the upper serum or cell lysate collected after centrifugation, and the concentration, purity, and integrity of the total RNA were determined using an ultraviolet spectrophotometer and agarose gel electrophoresis. Subsequently, the total RNA was reversely transcribed to collect cDNA for PCR amplification. The primer sequence is shown in Table 1. The qPCR amplification system consisted of 20 μL total volume containing 1μL cDNA 1 μL, 0.4 μL upstream and downstream primers, 10 μL 2×TransTaq® Tip Green qPCR SuperMix, 0.4 μL Passive Reference Dye (50X), and ddH₂O added to adjust the volume. The amplification conditions: Pre-denaturation at 94°C for 30 s, followed by 40 cycles of denaturation at 94°C for 5 s, and annealing and exten-

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sion at 60°C for 30 s. In addition, data of this experiment was analyzed using 2-ΔΔct, with β-actin as an internal reference for MALAT1 and U6 for miR-181a.

CCK-8 assay

After 24 hours of transfection, cells were harvested, and transferred to a 96 well plate with 4*10^6 cells per well, and then incubated for 24 h, 48 h, 72 h, and 96 h, separately. At each culturing time point, 10 μL CCK solution was added to the cells, and cultured in 90 μL basal medium (DMEM) at 37°C for an additional 2 hours. Finally, the optical density of cells in each group at measured at 450 nm was determined using a microplate reader.

Flow cytometry

The transfected cells were digested with trypsin, and then washed twice, and mixed with 100 μL binding buffer to prepare a 1*10^6 cells/mL suspension. The suspension was mixed with AnnexinV-FITC and PI in order, cultured in the dark at room temperature for 5 min, and finally detected using the FC500MCL flow cytometer system. The experiment was repeated three times, and the data were averaged as the results.

Transwell assay

The transfected cells were transferred to a 6-well plate, and washed with PBS three times, and then transferred to the upper compartment, followed by addition of 200 μL DMEM in the upper compartment and addition of 500 mL DMEM containing 20% FBS in the lower compartment. The plate was incubated at 37°C for 48 h, and the substrates and cells that did not penetrate the membrane surface in the upper compartment were wiped off. The plate was cleaned with PBS three times, cells were immobilized with paraformaldehyde for 10 min, washed again with double distilled water three times, and dyed with 0.5% crystal violet after being air dried, and then detected with regards to cell invasion with a microscope.

Western blot assay

Total protein was extracted using the RIPA lysis method, and the protein concentration was determined using the BCA method, and adjusted to 4 μg/μL. Afterwards, the total protein was isolated using 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred to a polyvinylidene fluoride (PVDF) membrane, after which the membrane was dyed with Ponceau’s stain, soaked in phosphate buffer saline with Tween (PBST) for 5 min for washing, sealed with 5% skim milk for 2 h, and then incubated with Bax, Bcl-2, and β-Actin primary antibody (1:1000), and sealed at 4°C overnight. Afterwards, the membrane was washed to remove the primary antibody, incubated with horseradish peroxidase-labeled goat anti-rabbit secondary antibody (1:5000), cultured at 37°C for 1 h, and washed with PBS three times, 5 min each time. Finally, the membrane was developed in the dark, and visualized with electrochemiluminescence (ECL) and developed. The protein bands were scanned, and the gray value was quantified using Quantity One software.

Dual luciferase reporter (DLR) assay

Complementary DNA fragments containing wild-type (MALAT1-WT) or mutant-type MALAT1 (MALAT1-mut) fragments were subcloned downstream of the luciferase genes in psi-CHECK2 luciferase reporter vectors, and miR-181a mimics were co-transfected with MALAT1-WT or MALAT1-mut reporter vectors using transfection reagents (Invitogen, United States). After 48 hours of transfection, a DLR Assay Kit (Promega, United States) was applied to determine the firefly luciferase activity and renin-luciferase activity in cell lysates.

Statistical analysis

The data were analyzed statistically using SPSS 24.0, in which quantitative data were expressed as rate, and compared between groups using the chi-square test. In addition, quantitative data were expressed as the mean ± standard deviation, and comparison between groups was carried out using the t test. Data at different time points was compared using the repeated measures analysis of variance and Bonferroni post hoc test. P<0.050 indicates a significant difference.

Results

Comparison of MALAT1 expression between the res group and the con group

The MALAT1 expression in the peripheral blood of the res group was higher than that of the con group (P<0.050). Figure 1.
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Effects of MALAT1 on SH-SY5Y cells

The MALAT1 expression in the cells from the model group was higher than that in the cells from the normal group (P<0.050). However, after transfection of MALAT1, cells in the si-MALAT1 group showed significantly weaker proliferation and invasion abilities, lower Bcl-2 protein levels, stronger apoptosis, and higher Bax protein level than those in the NC group (all P<0.050). Figure 2.

Effects of miR-181a on SH-SY5Y cells

The miR-181a expression in cells from the model group was lower than that in cells from the normal group (P<0.050). However, after transfection of miR-181a, cells in the miR-181a-mimics group showed significantly weaker proliferation and invasion abilities, lower Bcl-2 protein level, stronger apoptosis, and higher Bax protein levels than those in the miR-NC group (all P<0.050). Figure 3.

Relationship between MALAT1 and miR-181a

It was found through online target gene prediction websites that MALAT1 and miR-181a had the same binding locus, and the DLR assay for the relationship between the two revealed that the luciferase activity of MALAT1-WT was strongly inhibited by miR-181a-mimics (P<0.050). Moreover, after transfection of si-MALAT1, the expression of miR-181a in SH-SY5Y cells increased significantly (P<0.001). Figure 4.

Effects of co-transfection of MALAT1 and miR-181a on SH-SY5Y cells

si-MALAT1 and miR-181a-mimics were transfected into SH-SY5Y cells (Group A), and si-MALAT1 alone was transfected into SH-SY5Y cells (Group B). Based on the detection of the biological behavior of cells, it was found that there was no difference in cell activity between Group A and the NC group (P>0.050), while cell proliferation and invasion activity and Bcl-2 protein level in Group B were all lower than those in the rest groups (all P<0.050), while apoptosis rate and Bax protein level in Group B were higher than those in the rest groups (both P<0.050). Figure 5.

Discussion

At present, the incidence of PD is increasing daily, bringing increasing challenges to the clinical practice [15], so it is a major focus of clinical research to fully understand the pathogenesis of PD and to carry out targeted therapy from a molecular point of view. In this study, we have explored MALAT1 in PD and detected its mechanism in cells, which is of great value for future clinical diagnosis and treatment of PD.

According to the results of this study, MALAT1 was highly expressed in the peripheral blood of PD patients, suggesting that MALAT1 may participate in the development and progression of PD. In addition, according to previous references, we found that MALAT1 also showed an increasing trend in gastric cancer and osteosarcoma [16, 17], which can testify the accuracy of this study. MALAT1 can promote the progression of lung cancer and the angiogenesis of thyroid carcinoma [18, 19], but the influencing mechanism of MALAT1 on PD remains unclear. Neuroblastoma is a common malignant childhood solid tumor of migrating neuroectodermal cells derived from the neural crest, with unique biological characteristics of spontaneous regression and reversion [20]. As human neuroblastoma cells, SH-SY5Y have many characteristics of dopaminergic neurons, including expression of the activity of dopamine-β-hydroxylase, tyrosine hydroxylase, and dopamine turnover, which can simulate dopaminergic neurons. Currently, they have been widely
used as a dopaminergic neuron model cell for Parkinson’s research [21, 22]. Therefore, we purchased SH-SY5Y cells and obtained a PD model with MPP+, and then quantified MALAT1 in them, finding that MALAT1 was highly expressed in SH-SY5Y cells, which also supports the results of our above experiments. What’s more, we transfected MALAT1 inhibitor sequences into SH-SY5Y cells, finding that it strengthened cell proliferation, invasion, and apoptosis, suggesting that highly expressed MALAT1 plays a role in promoting the development of PD in model cells. The results are also consistent with the regulatory effects of MALAT1 on cell behavior in previous studies [23, 24], which supports our study. Moreover, we also found that miR-181a was closely related to PD according to one study by Liu Y et al. [25], and that MALAT1 had a potential relationship with miR-181a [26]. Therefore, we speculated that there may be a certain regulatory relationship between the two in PD. In order to confirm this, we detected the expression of miR-181a in cells. It turned out that miR-181a showed a low expression in PD model cells, and transfection of miR-181a mimic sequence weakened cell proliferation and invasion, and intensified cell apoptosis, which confirmed our above conjecture. MiR-181a and MALAT1 have opposite relationships in regulating cell biological behavior. With the aim of further confirming the relationship between miR-181a and MALAT1, we also measured the expression of apoptosis-related proteins such as Bax and Bcl-2. We found that the expression of Bax was significantly increased in the malat1-overexpressed group, while the expression of Bcl-2 was significantly decreased. This indicates that high expression of MALAT1 promotes cell apoptosis, which is consistent with previous studies. Additionally, we observed that the expression of Bax and Bcl-2 in the miR-181a-overexpressed group showed the opposite trend compared to the MALAT1-overexpressed group, further confirming the regulatory role of miR-181a in cell apoptosis.
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Figure 3. Effect of miR-181a on SH-SY5Y cells. A. Comparison of miR-181a expression between the model group and the con group. The miR-181a expression in the model group was significantly lower than that in the normal group, *** indicates P<0.001. B. Cell proliferation. C. Cell invasion. D. Cell apoptosis. E. Expression of apoptosis-related proteins. * indicates P<0.050 vs. the miR-181a-mimics group.

Figure 4. Relationship between MALAT1 and miR-181a. A. Binding locus between MALAT1 and miR-181a based on online predication websites. B. DLR assay results. * indicates P<0.050 vs. the luciferase activity of MALAT1-WT after transfection of miR-181a-mimics. C. Expression of miR-181a in SH-SY5Y cells transfected with MALAT1. * indicates P<0.050 vs. the si-MALAT1 group.
MALAT1, we predicted a binding locus of miR-181a and MALAT1 based on online databases, finding that they had the same binding locus. We also carried out a DLR assay, finding that the luciferase activity of MALAT1-WT was inhibited by miR-181a-mimics, and transfection of si-MALAT1 upregulated miR-181a, which confirms that there is a targeted relationship between the two. Moreover, it was also found that the viability of cells co-transfected with MALAT1 and miR-181a was not different from cells in the NC group, implying that the effects of miR-181a-mimics on cells were reversed by si-MALAT1. Therefore, we come to the conclusion that MALAT1 affects the biological behavior of PD model cells by targeted inhibition of miR-181a.

This study has investigated the influence and mechanisms of MALAT1 on PD, but there are still some deficiencies due to limited experimental conditions. For example, the signaling pathway through which MALAT1 affects PD model cells by targeting miR-181a is still unclear, which requires further experimental exploration. In addition, whether MALAT1 can be used as a therapeutic target for PD also requires confirmation. Moreover, due to the short experimental period, we cannot evaluate the long-term prognosis of PD patients affected by MALAT1. In the future, we will conduct further in-depth experimental analysis on the above deficiencies as soon as possible to obtain more complete experimental results for clinical reference.

To sum up, MALAT1 was highly expressed in the peripheral blood of PD patients, and it promotes the proliferation and invasion of PD model cells and inhibits their apoptosis by tar-
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geted inhibition of miR-181a, which may be a key to the diagnosis and treatment of PD in the future.

Disclosure of conflict of interest

None.

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References

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