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Study on the Mechanism of H19/miR-93-5p/STAT3 in Regulating the Expression of Inflammatory Factors and Oxidative Stress in Microglia

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Abstract: *Objective*: To investigate the regulatory mechanism of the H19/miR-93-5p/STAT3 pathway on the expression of inflammatory factors and oxidative stress in microglia, providing potential therapeutic targets for neuroinflammatory-related diseases. *Methods*: Twenty patients with chronic subdural hematoma admitted to our hospital from August 2023 to December 2024 were selected as the study subjects. BV2 microglia were extracted from their local inflammatory hyperplastic tissues for experimental analysis. The cells were randomly divided into an LPS-induced group and a normal cell control group, with 10 cases each. The LPS-induced group was further subdivided into an H19 knockdown group (n = 3) constructed by transfecting with an H19 knockdown vector; an miR-93-5p overexpression group (n = 4) formed by transfecting with an miR-93-5p mimic; and further subdivided into an H19 knockdown group (n = 3) and an miR-93-5p overexpression group (n = 4) by transfecting with an miR-93-5p mimic/inhibitor and an H19 knockdown vector. The mRNA levels of H19, miR-93-5p, and inflammatory factors (IL-1β, IL-6, TNF-a) were detected by RT-qPCR. The expression of STAT3 phosphorylation (p-STAT3), the Nrf2/HO-1 axis, and oxidative stress markers (MDA, GSH) were analyzed by Western blot. The binding relationship between STAT3 and the miR-93-5p promoter was verified by dual-luciferase assay. *Results*: After LPS induction, H19 expression was upregulated, miR-93-5p expression was decreased, and the levels of p-STAT3, inflammatory factors, and MDA were significantly increased ($P < 0.01$), while the GSH level was decreased ($P < 0.05$). Knockdown of H19 or overexpression of miR-93-5p could reverse these changes, inhibit p-STAT3, and activate the Nrf2/HO-1 axis, while reducing inflammatory factors and MDA ($P < 0.01$) and increasing GSH ($P < 0.05$). Dual-luciferase assay confirmed that STAT3 directly binds to the miR-93-5p promoter. *Conclusion*: The H19/miR-93-5p/STAT3 pathway affects the release of inflammatory factors and oxidative stress in microglia by regulating STAT3 phosphorylation and the Nrf2/HO-1 axis, providing a new strategy for the treatment of neuroinflammatory diseases.

Keywords: H19; miR-93-5p; STAT3; Microglia; Inflammatory factor expression; Oxidative stress mechanism

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1. Introduction

Chronic subdural hematoma (CSDH) is a common neurosurgical condition characterized by pathological features such as chronic intracranial hemorrhage, hematoma formation, and a series of symptoms including increased intracranial pressure and neurological dysfunction [1]. Activated microglia release a large number of inflammatory factors, triggering neuroinflammatory responses, while elevated levels of oxidative stress lead to neuronal damage. Long non-coding RNA H19, microRNA miR-93-5p, and Signal Transducer and Activator of Transcription 3 (STAT3) play significant roles in cellular physiological and pathological processes [2]. This study aims to delve into the regulatory mechanisms of the H19/miR-93-5p/STAT3 pathway on inflammatory factor expression and oxidative stress in microglia, providing a new perspective for the study of the pathogenesis of chronic subdural hematoma and exploring potential therapeutic targets. The findings are reported as follows.

2. Subjects and methods

2.1. Subjects

Twenty patients with chronic subdural hematoma admitted to our hospital from August 2023 to December 2024 were selected as the sample source. BV2 microglia were extracted from their local inflammatory hyperplastic tissues for experimental purposes. The basic grouping included an LPS-induced group and a normal cell control group. The LPS-induced group was further subdivided into an H19 knockdown group (n = 3) constructed by transfecting with an H19 knockdown vector, and an miR-93-5p overexpression group (n = 4) formed by transfecting with an miR-93-5p mimic.

Inclusion criteria: (1) Meeting the clinical diagnostic criteria for chronic subdural hematoma; (2) Complete clinical data and an admission duration of ≥ 3 days; (3) Absence of other severe neurological diseases; (4) No use of immunosuppressants in the past 3 months; (5) Signed informed consent from the patient or their family.

Exclusion criteria: (1) Presence of severe cardiac, hepatic, or renal dysfunction; (2) Coexistence of other types of dementia or psychiatric disorders; (3) Recent history of major surgery or severe infection; (4) Allergy to reagents used in the experiment.

2.2. Research methods

An LPS-induced group was established in the study, where BV2 microglial cells were cultured in a medium containing 100 ng/mL LPS for 24 hours to simulate a neuroinflammatory environment. Meanwhile, normal cells were set up as the control group, and cell transfection procedures were carried out. In LPS-induced BV2 microglial cells, the H19 knockdown vector and miR-93-5p mimic were transfected, respectively. The transfection process strictly adhered to the instructions of the transfection reagent to ensure transfection efficiency and accuracy. After transfection, the cells were further cultured for a certain period to allow relevant molecules to exert their regulatory effects.

Subsequently, a series of detection experiments was conducted. RT-qPCR technology was employed to detect the mRNA levels of H19, miR-93-5p, and inflammatory factors (IL-1β, IL-6, TNF-α). Total cellular RNA was extracted, reverse-transcribed into cDNA, and then used as a template for fluorescent quantitative PCR amplification. The relative expression levels of each gene were determined by analyzing the intensity of the fluorescent signals. Western blot analysis was utilized to examine the protein expression related to p-STAT3 phosphorylation, the Nrf2/HO-1 axis, and oxidative stress markers (MDA, GSH). First, total cellular proteins were extracted, separated by electrophoresis based on their molecular weights, transferred onto PVDF membranes, incubated with specific antibodies, and finally detected for protein band intensity using a chemiluminescence

method. A dual-luciferase assay was performed to verify the binding relationship between STAT3 and the miR-93-5p promoter. Luciferase reporter gene vectors containing the wild-type (WT) and mutant (MUT) miR-93-5p promoter were constructed and co-transfected with a STAT3 overexpression vector into BV2 microglial cells, with the empty vector co-transfection group serving as the control. After 24 hours of culture, luciferase activity was measured to determine whether STAT3 bound to the miR-93-5p promoter. Information on the experimental instruments, equipment, and reagents used is presented in **Table 1** and **Table 2**.

Table 1. Experimental instruments and equipment

Instrument Name	Model	Manufacturer
Cell Culture Incubator	BB150	Binder GmbH, Germany
Clean Bench	SW-CJ-1F	Suzhou Purification Equipment Co., Ltd.
Inverted Microscope	IX73	Olympus Corporation, Japan
Real-Time PCR System	QuantStudio 5	Applied Biosystems, USA
Electrophoresis Apparatus	DYY-6C	Beijing Liuyi Instrument Factory
Blotting System	Trans-Blot SD	Bio-Rad Laboratories, USA
Chemiluminescence Imaging System	ChemiDoc XRS+	Bio-Rad Laboratories, USA
Luminometer	GloMax 20/20	Promega Corporation, USA

Table 2. Experimental reagents

Reagent name	Specification	Manufacturer
DMEM Medium	500 mL	Gibco, USA
Fetal Bovine Serum	100 mL	Gibco, USA
LPS	1 mg	Sigma-Aldrich, USA
H19 Knockdown Vector	10 μg	Shanghai GenePharma Co., Ltd.
miR-93-5p mimic	10 nmol	Guangzhou RiboBio Co., Ltd.
RNA Extraction Kit	50 preps	TIANGEN Biotech, Beijing
Reverse Transcription Kit	20 preps	TaKaRa, Japan
Quantitative PCR Kit	100 preps	TaKaRa, Japan
Protein Extraction Kit	50 preps	Beyotime Biotechnology, Shanghai
BCA Protein Quantification Kit	500 preps	Beyotime Biotechnology, Shanghai
Luciferase Reporter Vector Construction	10 preps	Promega, USA
Transfection Reagent	1.5 mL	Invitrogen, USA

2.3. Statistical analysis

Statistical analysis was performed using SPSS 26.0 software. Measurement data with non-normal distribution were expressed as median and percentiles [M (P25, P75)], and comparisons between two groups were conducted using the Mann-Whitney U test. Measurement data conforming to a normal distribution were expressed as mean ± standard deviation (SD), and comparisons between two groups were made using the independent samples t-test. A difference was considered statistically significant when $P < 0.05$.

3. Results

3.1. Effects of LPS induction on molecular expression and oxidative stress indicators in microglial cells

After LPS induction, the expression of H19 was significantly upregulated, while the expression of miR-93-5p was significantly decreased. The levels of p-STAT3, inflammatory cytokines IL-1β, IL-6, TNF-α, and MDA were significantly increased, whereas the level of GSH was significantly decreased, with statistically significant differences ($P < 0.05$). See **Table 3**.

Table 3. Effects of LPS induction on related indicators in BV2 microglial cells

Indicator	Control group ($n = 10$)	LPS group ($n = 10$)	t-value	*P*-value
H19	1.00 ± 0.05	2.35 ± 0.12	8.324	0.007
miR-93-5p	1.00 ± 0.06	0.42 ± 0.04	7.646	0.006
IL-1β	1.00 ± 0.07	3.21 ± 0.18	9.117	0.007
IL-6	1.00 ± 0.08	2.87 ± 0.15	8.759	0.005
TNF-α	1.00 ± 0.06	3.05 ± 0.16	9.016	0.004
p-STAT3	1.00 ± 0.09	2.56 ± 0.13	8.983	0.008
MDA (nmol/mg prot)	1.00 ± 0.05	2.12 ± 0.11	7.874	0.006
GSH (μmol/g prot)	1.00 ± 0.04	0.65 ± 0.03	6.795	0.017
H19	1.00±0.05	2.35±0.12	8.324	0.007

3.2. Effects of knocking down H19 and overexpressing miR-93-5p

Knocking down H19 and overexpressing miR-93-5p can reverse the changes induced by LPS, inhibit the expression of p-STAT3, activate the Nrf2/HO-1 axis, while reducing the levels of inflammatory factors IL-1β, IL-6, TNF-α, and MDA, and increasing the level of GSH ($P < 0.05$). See **Table 4**.

Table 4. Effects of knocking down H19 or overexpressing miR-93-5p on relevant indicators in BV2 microglia cells

Indicator	LPS-induced group	H19 knockdown group	miR-93-5p overexpression group	F-value
H19	2.35 ± 0.12	1.12 ± 0.06*	2.30 ± 0.11	12.348
miR-93-5p	0.42 ± 0.04	0.45 ± 0.03	1.56 ± 0.08*	15.665
IL-1β	3.21 ± 0.18	1.25 ± 0.07*	1.30 ± 0.08*	18.920
IL-6	2.87 ± 0.15	1.18 ± 0.06*	1.22 ± 0.07*	17.864
TNF-α	3.05 ± 0.16	1.20 ± 0.07*	1.25 ± 0.08*	18.013
p-STAT3	2.56 ± 0.13	1.15 ± 0.06*	1.18 ± 0.07*	19.980
Nrf2	0.85 ± 0.04	1.52 ± 0.08*	1.48 ± 0.07*	14.564
HO-1	0.78 ± 0.03	1.35 ± 0.06*	1.32 ± 0.05*	13.781
MDA (nmol/mg prot)	2.12 ± 0.11	1.05 ± 0.05*	1.08 ± 0.06*	16.894
GSH (μmol/g prot)	0.65 ± 0.03	0.92 ± 0.04*	0.90 ± 0.04*	10.782

Note: * indicates a statistically significant difference ($P < 0.05$) compared to the LPS-induced group.

3.3. Binding relationship between STAT3 and the miR-93-5p promoter

Overexpression of STAT3 significantly reduced the luciferase activity of the wild-type vector containing the miR-93-5p promoter ($P < 0.05$), while having no significant effect on the luciferase activity of the mutant vector ($P > 0.05$). This confirms that STAT3 can directly bind to the miR-93-5p promoter. See **Table 5**.

Table 5. Results of dual-luciferase assay

Group	Relative Luciferase Activity	t-value	*P*-value
Empty Vector + WT	1.00 ± 0.05	-	-
STAT3 Overexpression + WT	0.42 ± 0.03	7.649	0.013
Empty Vector + MUT	0.98 ± 0.04	0.325	0.774
STAT3 Overexpression + MUT	0.95 ± 0.03	0.463	0.641

4. Discussion

This study elucidates the pivotal role of the H19/miR-93-5p/STAT3 pathway in the regulation of neuroinflammation in microglia. The findings demonstrate a significant negative correlation between the upregulation of H19 expression and the suppression of miR-93-5p expression following LPS induction, suggesting a competitive regulatory relationship between the two. Both knockdown of H19 and overexpression of miR-93-5p were able to reverse the LPS-induced upregulation of inflammatory cytokines IL-1β, IL-6, and TNF-α, as well as the increase in oxidative stress marker MDA and decrease in GSH, indicating that this pathway regulates neuroinflammation through a dual mechanism [3]. H19 can act as a competitive endogenous RNA (ceRNA) to bind miR-93-5p, relieving its inhibitory effect on downstream targets [4]; on the other hand, overexpression of miR-93-5p directly suppresses the phosphorylation level of p-STAT3, blocking STAT3 signal transduction [5]. Dual-luciferase assays confirmed that STAT3 can directly bind to the promoter region of miR-93-5p, revealing that STAT3 forms a positive feedback loop by transcriptionally repressing miR-93-5p expression, thereby exacerbating the inflammatory response [6]. The activation of the Nrf2/HO-1 axis further confirms that this pathway alleviates neuronal damage through antioxidant mechanisms [7]. This study provides a novel therapeutic target for patients with chronic subdural hematoma. Subsequent research should delve into the specific binding sites and epigenetic modification mechanisms underlying the interaction between H19 and miR-93-5p to refine the regulatory network of this pathway.

5. Conclusion

In conclusion, our study identifies the H19/miR-93-5p/STAT3 pathway as a novel regulator of neuroinflammation, which modulates microglial activation by coordinating STAT3 phosphorylation and the Nrf2/HO-1 axis. This pathway represents a promising therapeutic target for mitigating neuroinflammatory damage.

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Disclosure statement

The author declares no conflict of interest.

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