Communication • DOI: 10.2478/s13380-014-0214-x • Translational Neuroscience • 5(2) • 2014 • 131-136

Translational Neuroscience

VERSITA

MicroRNAs EXPRESSION CHANGES IN ACUTE *STREPTOCOCCUS PNEUMONIAE* MENINGITIS

Ahmed Omran[1,2],
Mubareka Jagoo[3],
Muhammed Usman Ashhab[3],
Fang He[3],
Huimin Kong[3],
Jing Peng[3],
Fei Yin[3]*

[1]*Department of Pediatrics and Neonatology, Suez Canal University, Ismailia 41522, Egypt;*

[2]*Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, Dublin, Ireland*

[3]*Department of Pediatrics, Xiangya Hospital of Central South University, Changsha, Hunan, 410008, China;*

Abstract

Background: Despite prompt and appropriate care, *Streptococcus pneumoniae* (*S. pneumoniae*) meningitis remains a key cause of childhood morbidity and mortality. Recently, several studies suggested the possibility of microRNAs (miRNA) involvement in multiple brain and infectious diseases. This study aimed to investigate the expressional changes of brain-enriched miRNAs (124, 134, 9, and 138), and inflammation-related miRNAs (132, 181a, 221, and 222) in the cerebral cortex of acute *S. pneumoniae* meningitis in postnatal day 25 rats with and without treatment. Methodology: Quantitative polymerase chain reaction (qPCR) was used to measure the expression levels of the tested brain-enriched and inflammation-related miRNAs in the cerebral cortical tissues obtained from acute experimental meningitis rat model induced by intracranial inoculation of *S. pneumoniae* serotype 3 with and without treatment. Control rats inoculated with saline were also included. Results: Brain-enriched miRNAs are significantly downregulated in untreated animals while after treatment with antibiotic and steroid for 24 hours, miR-124 and miR-9 expressions were nearly equal to the control, while miR-134 and miR-138 were significantly upregulated. Inflammation-related miR-132 was significantly downregulated in untreated and treated animals while miRNAs (181a, 221, and 222) were significantly upregulated in untreated and treated animals. Conclusion: Acute *S. pneumoniae* meningitis leads to significant changes in the cerebral cortical expressions of some brain-enriched and inflammation-related miRNAs. These miRNAs may play a role in the pathogenesis of acute bacterial meningitis.

Keywords

• Acute meningitis • *Streptococcus pneumoniae* • microRNAs • Developing brain

Received 24 April 2014
accepted 13 May 2014

Introduction

Acute bacterial meningitis (ABM) remains a potentially debilitating infection in children resulting in high mortality as well as long-term disability in up to a third of the survivors [1]. Despite modern antibiotics and improved critical care, bacterial meningitis (BM) is still an unresolved problem in clinical medicine. Of all etiologic groups, *Streptococcus pneumoniae* (*S. pneumoniae*) has been the leading cause of ABM in young children.

Neuronal damage in meningitis is clearly multifactorial, involving bacterial toxins, cytotoxic products of immune competent cells, and indirect pathology secondary to intracranial complications. Molecular basis of bacterial meningitis development are under intense study in the hope of identifying new therapies for preventing and treating meningitis.

MicroRNAs (miRNAs) are small, non-coding RNAs that regulate post-transcriptional expression of protein-coding mRNAs, which may have key roles in the development of the central nervous system (CNS) and pathogenesis of neurological disorders in developing brains including epilepsy and hypoxic ischemic encephalopathy [2-5]. Furthermore, dysregulation of miRNAs has been implicated in the etiology of multiple acute and chronic pediatric disorders reviewed in [6-8].

miRNA expression is highly enriched in the nervous system with approximately 70% of experimentally detectable miRNAs expressed in the brain [9]. miRNAs are also involved in the regulation of several aspects of the innate, adaptive immune responses and inflammation-related disorders. Studies uncovered a subset of miRNAs which are brain-enriched including miRNAs (124, 134, 9, and 138) and inflammation-related miRNAs including (132, 181a, 221, and 222) which were extensively studied in multiple brain disease in both developing and adult brains [4,10-12], and also in infectious diseases [13-15].

In light of being brain-enriched and their growing roles in the inflammation-related disorders, we hypothesize that these brain-enriched miRNAs (124, 134, 9, and 138) and inflammation-related miRNAs (132, 181a, 221, and 222) expression levels could change in the cerebral cortex of acute *S. pneumoniae* induced meningitis in the developing brains with and without treatment and may have a role in the disease pathogenesis. In this study, we monitored for the first time, changes in the expressions of these 8 miRNAs in the cerebral cortex of acute *S. pneumoniae* induced meningitis in developing brains.

Experimental procedures

Animal model preparation

We started our experiment with 30 Sprague Dawley (SD) at postnatal day 25 (PN25) from the Experimental Animal Center of Xiangya Medical College, Central South University. The animals were housed in a room with controlled temperature 20 ± 2° C and humidity (50 – 60%) and were kept on an alternating 12h light dark cycle. Animals had free access to food and water. The 30 rats were randomly divided into

* *E-mail: yf_2323@yahoo.com*

three groups: control group C (n = 10) and experimental group (E = 20), which sub-divided into two sub-groups, meningitis without treatment group (n =10), and meningitis with treatment (antibiotic and dexamethasone) group (n = 10). All procedures were approved by the Institutional Animal Care and Use Committee of Central South University.

Bacterial strain

S. pneumoniae serotype 3 was commercially bought from American Type Culture Collection (ATCC, Manassas, VA, USA). The bacteria was diluted in a dose of 0.2 mg/ml then cultured on solid blood agar medium. The blood agar was prepared according to the manufacturer's instructions. On the solid blood agar, glistening colonies were formed after 18 hours, measuring about 1 mm each. *S. pneumoniae* strains were grown on Trypticase soy agar (TSA II, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) supplemented with 5% sheep's blood at 37°C in 5% CO_2 in liquid media. The growth was observed at 6, 12 and 18 hours. The bacteria were grown on a serial of 4 subsequent cultures on liquid blood agar. With initial growth beginning in optimal conditions, autolysis usually begins within 18-24 hours, with colonies collapsing in the centers. They were examined after 15 hours for solid residues in the tubes. The red blood cells were lysed and the cultures compared to the 0.5 - 5 McFarland bacterial comparator.

Animal model

On PN 25, the experimental and control rats were given intraperitoneal chloral hydrate (10%, 5 ml/kg) to induce anesthesia and placed on stereotaxic frame. A central incision was made in the scalp and 10 µl cerebrospinal fluid (CSF) was obtained by puncture of the cisterna magna. For the experimental group (n = 20) the bacteria was inoculated in the intracranial route in a dose of 10^3 colony forming unit (CFU), of *S. pneumoniae* in 10 µl of 0.9% sterile saline using the microsyringe with a 26 mm gauge needle. After 1 hour, the rats were observed for signs of meningism including piloerection, irritability, hunched position, and decreased spontaneous activity. The experimental group was randomly subdivided into two subgroups, meningitis without treatment (n = 10), this group was observed for 24 hours without receiving any treatment (mortality = 40%) and meningitis with treatment group (n = 10) 16 hours after bacterial inoculation this group received a single injection of subcutaneous ceftriaxone (100 mg/kg) and dexamethasone in a dose of 0.25mg/kg, and killed 24 hours after the bacterial inoculation (mortality = 20%). Meningitis was documented 16 hours after bacterial induction by a quantitative culture of 5 µl of CSF obtained by puncture of the cisterna magna [16]. In the control group (n = 10), 10 µl of CSF was obtained but instead of the bacteria, normal saline was injected (mortality = 10%).

Rat tissue preparation for RNA isolation

The rats were sacrificed under deep anesthesia by an intraperitoneal injection of chloral hydrate (10%, 5 ml/kg) 24 hours after bacterial inoculation in the untreated group and 24 hours after ceftriaxone and dexamethasone in the treated group. Control rats were also included. After decapitation, the cerebral cortex was removed quickly using RNase-free instruments. All materials were frozen on dry ice and stored at -80°C until use.

RNA isolation

For RNA isolation, frozen material was homogenized in 1 ml Trizol Reagent (Invitrogen, Carlsbad, CA, USA) for each 50 mg of hippocampal tissue. After adding 0.2 ml of chloroform, the aqueous phase was isolated using Phase Lock tubes (Eppendorf, Hamburg, Germany). RNA was precipitated with 0.5 ml isopropyl alcohol, washed twice with 75% ethanol, and dissolved in nuclease-free water. The concentration and purity of RNA were determined at 260/280 nm using a nanodrop spectrophotometer (Ocean Optics, Dunedin, FL, USA).

Assessment of brain-enriched miRNAs (124, 134, 9, and 138) and inflammation related miRNAs (132, 181a, 221, and 222) relative expressions

All the miRNAs primers were purchased from GeneCopoeia Company (USA). cDNA synthesis was performed using the One Step PrimeScript® miRNA cDNA Synthesis Kit (TAKARA, Dalian, China), which includes three mixes (2×miRNA Reaction Buffer Mix, miRNA PrimeScript® RT Enzyme Mix and 0.1% BSA). A 10 µl reaction contained 5 µl 2×miRNA Reaction Buffer Mix, 1 µl miRNA PrimeScript® RT Enzyme Mix and 1 µl 0.1% BSA, 100 pg RNA, and DEPC-treated water up to 10 µl. The tube was incubated at 37°C for 60 min, the reaction was terminated at 85°C for 5 sec, and then the reaction was held at 4°C.

Quantitative polymerase chain reaction (qPCR) reaction was performed using the SYBRR® Premix Ex Taq™ II (TAKARA, Dalian, China) kit. qPCR was performed in triplicates. The 10µl PCR reaction contained the following: 5 µl SYBRR® Premix Ex Taq™ II, 0.4 µl Uni-miR qPCR Primer, 2 µl miR Specific Primer (GeneCopoeia, USA), 1 µl cDNA, and 1.6 µl diethylpyrocarbonate (DEPC)-treated water. The PCR reactions were incubated at 50°C for 2 min (uracyl-DNA glycosylase incubation) and 95°C for 30 sec, followed by 40 cycles of 95°C for 5 sec and 60°C for 30 sec, followed by melting curve analysis from 65.0 to 95.0 °C (increment 0.5°C, 0:05). The relative expression level for each of the 8 miRNAs was calculated using the comparative CT method. The expression of the U6 small nucleolar RNA gene was used as the internal control.

Statistical analysis

All of the data are expressed as means ± standard deviation. A Student's t test was performed to determine significant differences between two groups. One-way analysis of variance was utilized to determine significant differences among multiple groups. $P < 0.05$ was considered to be statistically significant.

Results

Relative expression pattern of brain-enriched miRNAs (124, 134, 9, and 138)

qPCR results showed significant downregulation of miR-124 expression in the cerebral cortical tissue in the acute stage of *S. pneumoniae* meningitis group with mean 0.110 ± 0.020 compared to control group mean of 0.450 ± 0.050, while after treatment with (ceftriaxone and dexamethasone) its expression matched

the control with mean 0.450 ± 0.050 (Figure 1a). miR-134 expression showed significant downregulation in the meningitis group with mean 0.100 ± 0.0 compared to the control mean 0.300±0.050, while after treatment it become significantly upregulated with mean 0.700 ± 0.050 (Figure 1b). miR-9 expression was also downregulated in the meningitis group with mean 0.150 ± 0.050 compared to the control mean 0.400 ± 0.050, while after treatment its expression matched the control with mean 0.450 ± 0.050 (Figure 1c). miR-138 expression was downregulated in the meningitis group with mean 0.200 ± 0.050 compared to the control mean, while after treatment its expression become significantly upregulated with mean 0.900 ± 0.100 (Figure 1d).

In rat tissues, miRNAs (124, 134, 9, and 138) expressions were normalized to that of the rat U6B small nuclear RNA gene (rnu6b) ($P < 0.05$).

Relative expression pattern of inflammation-related microRNAs (132, 181a, 221, and 222)

qPCR results showed significant downregulation of miR-132 expression in the cerebral cortical tissue in the acute stage of *S. pneumoniae* meningitis group with mean 0.250 ± 0.050 and also in the treatment group with mean 0.500 ± 0.050 compared to the control mean of 0.800 ± 0.100 (Figure 2a).

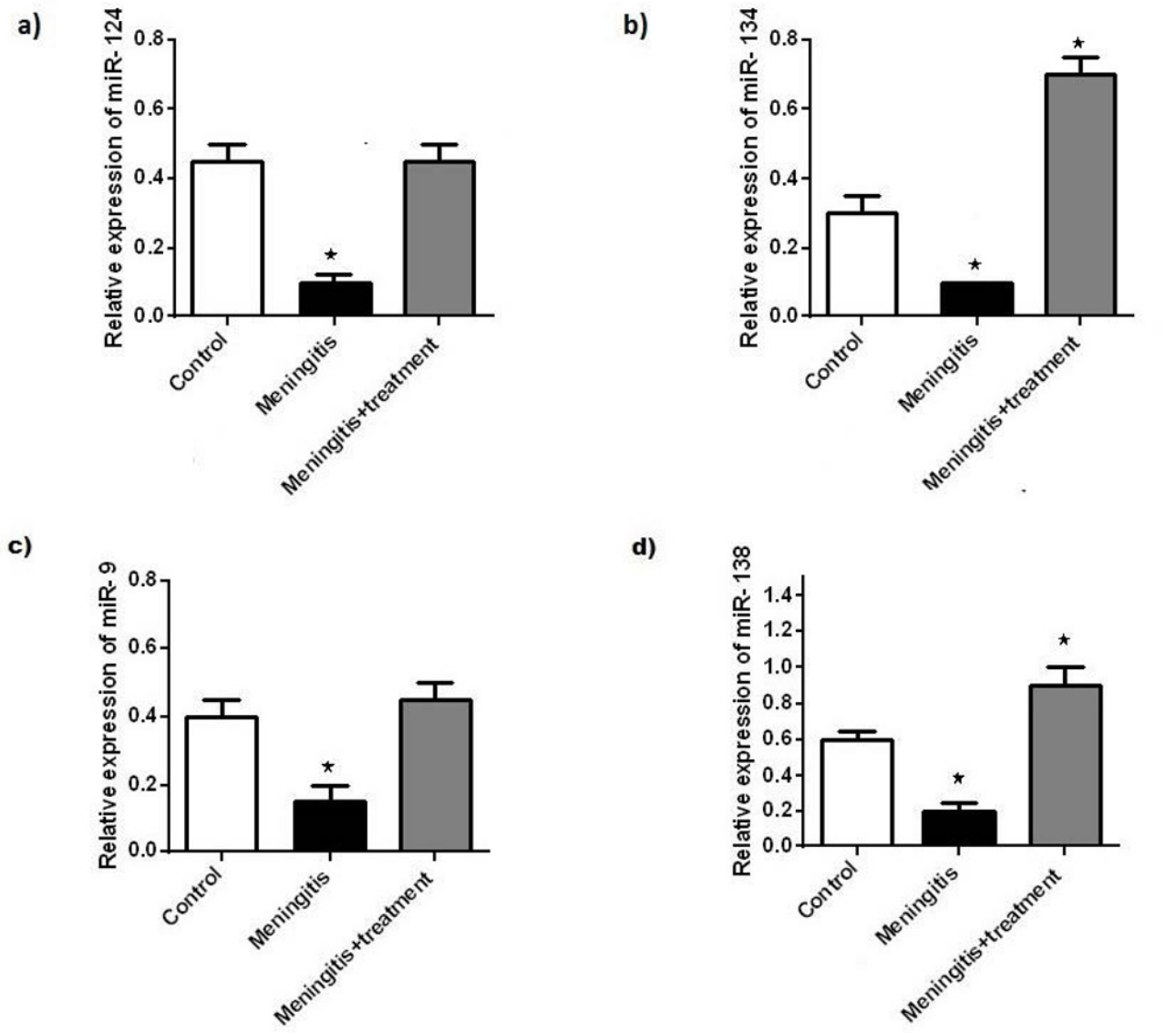

Figure 1. Relative expressions of brain-enriched microRNAs (124, 134, 9, and 138) in the cerebral cortex of acute *S. pneumoniae* meningitis model with and without treatment. a. miR-124 was significantly downregulated in the meningitis group, while it matched the control with treatment. b. miR-134 was significantly downregulated in the meningitis group, while it became significantly upregulated in the treatment group. c. miR-9 was significantly downregulated in the meningitis group, while it matched the control with treatment. d. miR-138 was significantly downregulated in the meningitis group, while it became significantly upregulared in the treatment group. N = 6 per group. The asterisk denotes $P < 0.05$.

miR-181a expression showed also significant upregulation in the meningitis group with mean 0.650 ± 0.050 and also in the treatment group with mean 0.600 ± 0.100 compared to the control mean 0.200 ± 0.0 (Figure 2b). miR-221 expression showed significant upregulation in the meningitis group with mean 0.500 ± 0.100 and also in treatment group with mean 0.800 ± 0.050 compared to the control mean 0.200 ± 0.050 (Figure 2c). miR-222 expression showed significant upregulation in the meningitis group with mean 0.617±0.028 and also in the treatment group with mean 0.517 ± 0.028 compared to the control mean 0.183 ± 0.028 (Figure 2d).

In rat tissues, miRNAs (132, 181a, 221, and 222) expressions were normalized to that of the rat U6B small nuclear RNA gene (rnu6b) ($P < 0.05$).

Discussion

Despite the significant advances in treatment, bacterial meningitis remains one of the most detrimental infectious diseases of the CNS with high mortality and morbidity. *S. pneumoniae* is one of the major causative pathogens of the most severe form of BM, in terms of mortality and morbidity [17]. A better understanding for the ABM molecular basis in the developing brains may result in strategies for the treatment of this infection.

In addition to well-established miRNA functions in a wide range of pathological and physiological process, it is becoming clear that miRNAs also play crucial roles during microbial

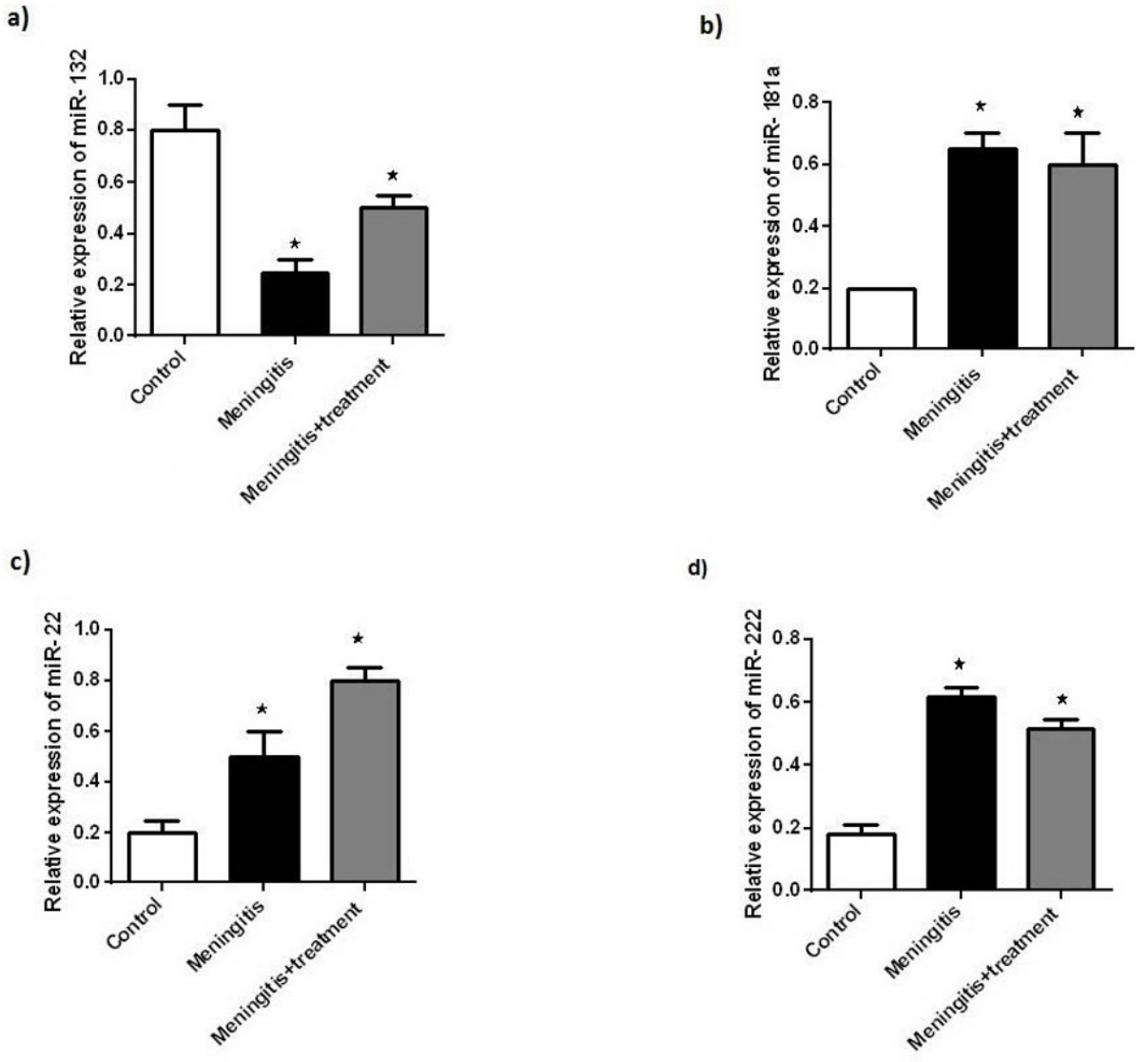

Figure 2. Relative expressions of inflammation-related microRNAs (132, 181a, 221, and 222) in the cerebral cortex of acute *S. pneumoniae* meningitis model with and without treatment. a. miR-132 was significantly downregulated in both meningitis groups with and without treatment. b, c, d. miR-181a, miR-221, and miR-222 were significantly upregulated in both meningitis groups with and without treatment. n= 6/group. The asterisk denotes $P < 0.05$.

infections. Furthermore, the role of miRNAs in regulating the host response to bacterial infection is increasingly recognized [18]. When compared with viral and parasitic infections, the changes in miRNA expression in response to bacterial pathogens have been less explored, especially in BM.

In this experiment, we focused for the first time on the expressional changes of brain-enriched miRNAs (124, 134, 9, and 138) and inflammation-related miRNAs (132, 181a, 221, and 222) in the cerebral cortical tissues of acute *S. pneumoniae* meningitis with and without treatment in the PN25 rats.

Our results revealed that the most abundant brain-specific miR-124 and miR-9 which play a major role in brain development nearly takes the same pattern of expression to be significantly downregulated in untreated animals, while with treatment there expression nearly return to the level of control. Recently, Ma et al. (2014) suggested that miR-124 is a potential target for preventive and therapeutic intervention against pulmonary tuberculosis through modulation of the Toll-like receptor (TLR) signalling cascade [19]. Glucocorticoid treatment induced expression of miR-124 in T-cells [20]. This could explain the expression change of miR-124 with treatment in our model. miR-9 was also found to be dysregulated in progressive human immunodeficiency virus (HIV) infection [21]. Modulation of miR-124 and miR-9 expression in models of meningitis in the developing brains may explore important roles for these miRNAs in pathogenesis and response to treatment.

miR-134 and miR-138 shows almost the same pattern of expression to be downregulated without treatment and then upregulated with treatment. Wippel *et al.* outlined for the first time the occurrence of synaptic pathology in human *S. pneumoniae* meningitis autopsy cases and in an animal model [22]. The observed long-term cognitive decline in survivors of pneumococcal meningitis may relate to synaptic damage and loss. miR-134 was the first miRNA shown to be involved in synapse formation [23]. miR-134 silencing reduces spine density in hippocampal pyramidal neurons in vivo and protecting from epileptic seizure [11]. miR-138 is a neuron-specific miRNA, which dynamically regulates neural development by controlling the shape and size of dendrites and thereby influences long-term memory [24]. miR-138 was also found to be downregulated in the seizure-related stages of the mesial temporal lobe epilepsy (MTLE) model in the developing brains [10]. miR-134 and miR-138 may be good candidates to study the long term cognitive abnormalities and epilepsy development which complicate bacterial meningitis. Neuronal activity *in vitro* and *in vivo* can trigger increased degradation of miRNAs [25], this may explain the down-regulation of the four brain-enriched miRNAs in untreated animals.

miR-132 that is known to be TLR-responsive and potentiates anti-inflammatory signaling [26], was the only miRNA in our experiment which downregulated in the treated and untreated animals. Recently, Yu *et al.* suggested a role for miR-132 in CNS inflammation induced by *Angiostrongylus cantonensis* [14].

TLR4 related miR-146a was also significantly downregulated in acute *Escherichia coli* meningitis of immature rat models [27]. It will be interesting to determine if miR-132 downregulation plays an anti-inflammatory effect in the meningitis animal model.

Other inflammation-related miRNAs (181a, 221, and 222) were upregulated in the treated and untreated animals. In our previous work we found significant upregulation in these miRNAs expressions in the astrocytes stimulated with lipopolysaccharides (LPS) [28] and also in microglia exposed to oxygen glucose deprivation (OGD) [29]. Further experiments will need to prove if these miRNAs have a pro-inflammatory function or not in this animal model.

In conclusion, our study shows that *S. pneumoniae* meningitis with and without treatment leads to significant changes in the expression of some brain-enriched and inflammation-related miRNAs. Modulation in the expression of these miRNAs may be a novel molecular target for *S. pneumoniae* bacterial meningitis treatment in developing brains.

Acknowledgments

This work was kindly supported by the National Natural Science Foundation of China (NO.81301031). All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design AO, MJ, FH, and JP. Acquisition of data: AO, HK and MUA. Analysis and interpretation of data: AO, MUA and JP. Writing the draft of the manuscript: AO and MJ. Critical revision of the manuscript for important intellectual content: AO, MUA, and JP. Statistical analysis: MUA and HK. Administrative, technical, and material support: AO and FY. Study supervision: FY. None of the authors have any conflict of interest to disclose.

References

[1] Saez-Llorens X., McCracken G. H. Jr., Bacterial meningitis in children, Lancet, 2003, 361, 2139-2148

[2] Omran A., Peng J., Zhang C., Xiang Q. L., Xue J., Gan N., et al., Interleukin-1β and microRNA-146a in an immature rat model and children with mesial temporal lobe epilepsy, Epilepsia, 2012, 53, 1215-1224

[3] Ashhab M. U., Omran A., Kong H., Gan N., He F., Peng J., et al., Expressions of tumor necrosis factor alpha and microRNA-155 in immature rat model of status epilepticus and children with mesial temporal lobe epilepsy, J. Mol. Neurosci., 2013, 51, 950-958

[4] Peng J., Omran A., Ashhab M. U., Kong H., Gan N., He F., et al., Expression patterns of miR-124, miR-134, miR-132, and miR-21 in an immature rat model and children with mesial temporal lobe epilepsy, J. Mol. Neurosci., 2013, 50, 291-297

[5] Qu Y., Wu J., Chen D., Zhao F., Liu J., Yang C., et al., MiR-139-5p inhibits HGTD-P and regulates neuronal apoptosis induced by

hypoxia-ischemia in neonatal rats, Neurobiol. Dis., 2014, 63, 184-193

[6] Omran A., Elimam D., Shalaby S., Peng J., Yin F., MicroRNAs: a light into the "black box" of neuropediatric diseases?, Neuromolecular Med., 2012, 14, 244-261

[7] Omran A., Elimam D., Webster K., Shehadeh L., Yin F., MicroRNAs: a new piece in the paediatric cardiovascular disease puzzle, Cardiol. Young, 2013, 23, 642-655

[8] Omran A., Elimam D., Yin F., MicroRNAs: new insights into chronic childhood diseases, Biomed. Res. Int., 2013, 291826

[9] Fineberg S. K., Kosik K. S., Davidson B. L., MicroRNAs potentiate neural development, Neuron, 2009, 64, 303-309

[10] Ashhab M. U., Omran A., Gan N., Kong H., Peng J., Yin F., microRNAs (9, 138, 181A, 221, and 222) and mesial temporal lobe epilepsy in developing brains, Transl. Neurosci., 2013, 4, 357-362

[11] Jimenez-Mateos E. M., Engel T., Merino-Serrais P., McKiernan R. C., Tanaka K., Mouri G., et al., Silencing microRNA-134 produces neuroprotective and prolonged seizure-suppressive effects, Nat. Med., 2012, 18, 1087-1094

[12] Nudelman A. S., DiRocco D. P., Lambert T. J., Garelick M. G., Le J., Nathanson N. M., et al., Neuronal activity rapidly induces transcription of the CREB-regulated microRNA-132, in vivo, Hippocampus, 2010, 20, 492-498

[13] Qi Y., Zhu Z., Shi Z., Ge Y., Zhao K., Zhou M., et al., Dysregulated microRNA expression in serum of non-vaccinated children with varicella, Viruses, 2014, 6, 1823-1836

[14] Yu L., Liao Q., Chen X., Xu L., Zeng X., Lv Z., et al., Dynamic expression of miR-132, miR-212, and miR-146 in the brain of different hosts infected with Angiostrongylus cantonensis, Parasitol. Res., 2014, 113, 91-99

[15] Liu Y., Zhao J. J., Wang C. M., Li M. Y., Han P., Wang L., et al., Altered expression profiles of microRNAs in a stable hepatitis B virus-expressing cell line, Chin. Med. J. (Engl.), 2009, 122, 10-14

[16] Barichello T., Silva G. Z., Generoso J. S., Savi G. D., Michelon C. M., Feier G., et al., Time-dependent behavioral recovery after pneumococcal meningitis in rats, J. Neural Transm., 2010, 117, 819-826

[17] Swartz M. N., Bacterial meningitis—a view of the past 90 years, N. Engl. J. Med., 2004, 351, 1826-1828

[18] Staedel C., Darfeuille F., MicroRNAs and bacterial infection, Cell. Microbiol., 2013, 15, 1496-1507

[19] Ma C., Li Y., Zeng J., Wu X., Liu X., Wang Y., Mycobacterium bovis BCG triggered MyD88 induces miR-124 feedback negatively regulates immune response in alveolar epithelial cells, PLoS One, 2014, 9, e92419

[20] Ledderose C., Möhnle P., Limbeck E., Schütz S., Weis F., Rink J., et al., Corticosteroid resistance in sepsis is influenced by microRNA-124-induced downregulation of glucocorticoid receptor-α, Crit. Care Med., 2012, 40, 2745-2753

[21] Seddiki N., Phetsouphanh C., Swaminathan S., Xu Y., Rao S., Li J., et al., The microRNA-9/B-lymphocyte-induced maturation protein-1/IL-2 axis is differentially regulated in progressive HIV infection, Eur. J. Immunol., 2013, 43, 510-520

[22] Wippel C., Maurer J., Förtsch C., Hupp S., Bohl A., Ma J., et al., Bacterial cytolysin during meningitis disrupts the regulation of glutamate in the brain, leading to synaptic damage, PLoS Pathog., 2013, 9, e1003380

[23] Schratt G. M., Tuebing F., Nigh E. A., Kane C. G., Sabatini M. E., Kiebler M., et al., A brain-specific microRNA regulates dendritic spine development, Nature, 2006, 439, 283-289

[24] Siegel G., Obernosterer G., Fiore R., Oehmen M., Bicker S., Christensen M., et al., A functional screen implicates microRNA-138-dependent regulation of the depalmitoylation enzyme APT1 in dendritic spine morphogenesis, Nat. Cell Biol., 2009, 11, 705-716

[25] Krol J., Busskamp V., Markiewicz I., Stadler M. B., Ribi S., Richter J., et al., Characterizing light-regulated retinal microRNAs reveals rapid turnover as a common property of neuronal microRNAs, Cell, 2010, 141, 618-631

[26] Shaked I., Meerson A., Wolf Y., Avni R., Greenberg D., Gilboa-Geffen A., et al., MicroRNA-132 potentiates cholinergic anti-inflammatory signaling by targeting acetylcholinesterase, Immunity, 2009, 31, 965-973

[27] Omran A, Peng J, Zheng C, Xue J, Xiang QL, Yin F., The expression of interleukin-1β and miRNA-146a in the cerebral cortex of acute Escherichia coli meningitis immature rat model, Afr. J. Infect. Dis., 2012, 6, 41-47

[28] Omran A., Ashhab M. U., Gan N., Kong H., Peng J., Yin F., Effects of MRP8, LPS, and lenalidomide on the expressions of TNF-α, brain-enriched, and inflammation-related microRNAs in the primary astrocyte culture, ScientificWorldJournal, 2013, 2013, 208309

[29] Kong H., Omran A., Ashhab M. U., Gan N., Peng J., He F., et al., Changes in microglial inflammation-related and brain-enriched microRNAs expressions in response to in vitro oxygen-glucose deprivation, Neurochem. Res., 2014, 39, 233-243