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Resveratrol Acts Anti-Inflammatory and Neuroprotective in an Infant Rat Model of Pneumococcal Meningitis by Modulating the Hippocampal miRNome

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Abstract

Resveratrol (RSV) is anti-inflammatory and neuroprotective, cross the blood–brain barrier (BBB) and has a safe profile. Besides, RSV modulates the expression of some miRNAs related to neurological disorders. Thus, we hypothesized that RSV can be neuroprotective in pneumococcal meningitis by modulating the global microRNA expression profile (miRNome). Eleven-day old rats were intracysternally infected with *S. pneumoniae* (~ 2×10^6 c.f.u.) and were orally administered with RSV (50 mg/kg) or vehicle in pre-treatment (before infection) or post-treatment schedules (3 and 18 h p.i.). At 24 h p.i., animals were euthanized and apoptotic cells were counted in the hippocampal dentate gyrus of the right brain hemispheres. The hippocampi from left hemispheres were used for cytokines and chemokines multiplex assay and miRNome profiling with *TaqMan OpenArray Rodent MicroRNA*. Infected rats treated with RSV had lower apoptotic scores and IL-1β, CCL_2, and CCL_3 levels when compared to the infected group receiving placebo. Seven miRNAs were down regulated, and 18 were up regulated by pneumococcal acute meningitis. Thirty-seven miRNAs were down regulated, and three were up regulated (hsa-miR-15b-5p, hsa-miR-25-3p, hsa-miR-125b-5p) by the interaction between meningitis and RSV. Pathway enriched analysis revealed that meningitis and RSV modulate the expression of miRNAs targeting critical pathways related to the pathophysiology of bacterial meningitis. Nevertheless, hsa-miR-25-3p and hsa-miR-125b-5p target the transcription factor TEF-1, for which there are binding sites in *Il-1β*, *Ccl_2*, and *Ccl_3* genes. RSV is anti-inflammatory and neuroprotective in an infant rat model of pneumococcal meningitis and these positive effects involve the modulation of the hippocampal miRNome.

Keywords Meningitis · *Streptococcus pneumoniae* · Resveratrol · Neuroprotection · microRNA · miRNome

Introduction

Acute bacterial meningitis (BM) is one of the most severe infectious diseases affecting mainly children and young adults [1–3]. The most common etiological agents of BM are *Streptococcus pneumoniae* and *Neisseria meningitidis*. Despite significant advances in antimicrobial and intensive care therapies, BM is still associated with high mortality (30%) and morbidity. *S. pneumoniae* is associated with the poorest outcome among BM patients, and affects mostly children under 5 years old [4, 5]. Up to 50% of BM survivors are permanently affected by neurological sequelae [6], which are mainly due to neuron loss by necrosis in the cerebral cortex, and by apoptosis in the hippocampal dentate granule cells [7–11]. Damage to the hippocampal formation has been associated with cognitive impairments [11, 12].

The inflammatory process that drives the pathogenesis of BM is triggered by the bacteria in cerebrospinal fluid (CSF) and is characterized by the production and release of cytokines, chemokines, reactive oxygen species (ROS), reactive nitrogen species (RNS), and metalloproteinases. These inflammatory mediators increase the permeability of the blood brain barrier (BBB)

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and attract leukocytes into the central nervous system (CNS) [13–17]. In the early acute phase of BM, interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) accumulate in the CNS and act synergistically causing inflammation [18, 19]. The increased expression of chemokines, including $CXCL_8$, $CXCL_1$, CCL_2 CCL_3, and $CXCL_5$ at the site of inflammation [20–22] are crucial for the recruitment of polymorphonuclear leukocytes (PMNs) in the acute phase of the disease. IL-10 and IL-1Ra, which are anti-inflammatory cytokines, may also have their expression increased during BM [23, 24].

Resveratrol (RSV; 3,4,5-trihydroxy-trans-stilbene) is a non-flavonoid natural polyphenol abundant in red wine and grapes. It is one of the widely studied phytochemicals with known health potential due to its remarkable anti-oxidant, cardioprotective, and anti-tumoral effects [25–28]. RSV attenuates the activation of immune cells and the subsequent synthesis and release of pro-inflammatory mediators through the inhibition of transcriptional factors, such as nuclear factor-kappa B (NF-κB) and activator protein-1 (AP-1) [25]. Indeed, RSV inhibits the release of TNF-α, IL-1β, and IL-6 by lipopolysaccharide (LPS)-activated murine monocytes [29]. RSV also protects astroglial cells against glutathione depletion by modulating the heme oxygenase 1 pathway [30]. The protective effect of RSV is not limited to its anti-oxidant effect, but is in fact multifactorial targeting various signaling pathways [31, 32]. Moreover, RSV can cross the BBB and have a good safety profile, being a promisor candidate to prevent brain damage induced by BM [29].

RSV also modulates the expression of miRNAs (short non-coding RNAs that regulate the translation and/or the degradation of their target mRNAs) [33] targeting transcripts encoding oncogenes, tumor suppressor factors, or interleukins, which are relevant in the physiopathology of inflammatory and neurological diseases [34]. Unfortunately, only a few studies have addressed the effects of RSV on the differential expression of this class of non-coding RNAs [35–39], which means that no mechanistic link between a particular miRNA (or a set of miRNAs) and RSV has been identified yet. Therefore, further effort is needed to understand the molecular mechanisms underlying the RSV neuroprotective effects, shedding light on its clinical use as a therapeutic agent to minimize the BM-induced morbidity and mortality. Thus, this study assessed the potential anti-inflammatory and neuroprotective effects of RSV in the dentate gyrus of infant rats with pneumococcal acute meningitis and whether this effects involve modulation of the hippocampal global miRNA expression profile (miRNome).

Methods

Animal Model and Experimental Design

All of the experimental procedures were approved by the Ethics Committee of Care and Use of Laboratory Animals (CEUA-FIOCRUZ, protocol LW-22/13) and were conducted in accordance with the regulations described in the Committee's Guiding Principles Manual.

The experiments have been conducted using an established experimental model of pneumococcal acute meningitis in infant rats [17]. At postnatal day 11, Wistar rats (20 ± 2 g) were infected by intracisternal injection of 10 μL saline containing $\sim 2 \times 10^6$ c.f.u. / mL of *S. pneumoniae* (serotype 3, strain 38/12 MEN from the certified bacterial collection of Ezequiel Dias Foundation (FUNED) [40]). Animals in the sham-infected group were intracisternally injected with 10 μL of sterile and non-pyrogenic saline. Infected and sham-infected infant rats ($N = 70$) were randomly separated according to the therapeutic schedule tested: (1) pre-treatment ($N = 44$) with 100 μL of RSV (Sigma-Aldrich, St. Louis, MO; 50 mg/ kg) resuspended in carboxymethyl cellulose (CMC) (10 g/L), or 100 μL placebo (CMC, 10 g/ L) administered by gavage immediately before the infection; (2) post treatment ($N = 26$) with 100 μL RSV (50 mg/ kg), or 100 μL carboxymethyl cellulose (10 g/ L) administered three and 18 h post infection (p.i.). After 18 h infection, CSF (10 to 30 μL) was obtained by puncture of the cisterna magna, and 10 μL was cultured quantitatively to document meningitis [17]. After 24 h incubation at 37 °C, animals with bacterial titters in the CSF $\geq 1 \times 10^8$ c.f.u./mL were diagnosed positive to pneumococcal acute meningitis; however, in order to avoid biases due to the infection intensity, only animals with titers $\sim 1 \times 10^8$ c.f.u./mL were included in the study. All animals received antibiotic therapy (ceftriaxone at 100 mg/ kg) (EMS Sigma Pharma Ltda., São Paulo, Brazil). At the time of the infection, and at 18 and 24 h p.i., all animals were weighed and clinically scored as follows: (1) comatose, (2) do not turn upright after positioning on the back, (3) turn upright within 30 s, (4) turn within less than 5 s, and (5) for rats with normal activity [17]. Twenty-four hours p.i., the rats were euthanized by an intraperitoneal overdose of *Ketamine* (300 mg/kg) + *Xylazine* (30 mg/ kg) (Syntec, São Paulo, Brazil).

Immediately after euthanasia, the animals were perfused via the left cardiac ventricle with 7.5 mL of RNAse-free ice-cold phosphate buffered saline (PBS). The brains were removed and the two hemispheres were separated. The right hemisphere was fixed in 4% paraformaldehyde (PFA) (Sigma-Aldrich) and further processed for histological analysis. The hippocampi were removed from the left hemispheres. Samples from pre-treatment schedule were dissected in ice-cold PBS, homogenized in a cold PBS buffer and protease inhibitor cocktail (Sigma-Aldrich) to be used in cytokines and chemokines multiplex assay. Samples from post-treatment schedule were dissected and stored on RNA later (24 h at 4 °C followed by − 80 °C until use) to RNA extraction.

Histological Analysis

To assess the RSV adjunctive therapy effect on preventing hippocampal damage caused by BM, the brains were analyzed

histomorphological as previously described [11, 17] ($N = 10$ per group in pre-treatment; $N = 3$ per group in post treatment, except the infected group treated with RSV, for which $N = 4$). Briefly, the right hemisphere was fixed in 4% PFA, embedded in paraffin, and then sliced to 5 μm thickness with a microtome (Leica CM1850, Wetzlar, Germany). The coronal histological sections were Nissl stained with Cresyl violet. Sections were analyzed under optical microscopy with a 40× objective.

Neurons of the lower blade of dentate gyrus with morphological changes characteristic for apoptosis (shrunken cytoplasm, condensed, fragmented nuclei, and/or apoptotic bodies) were counted in six sections for each rat. An average score per animal was calculated from all sections, applying the following scoring system: 0–5 cells = 0; 6–20 cells = 1; and > 20 cells = 2 [9].

Inflammatory Biomarkers Quantification by Luminex Assay

The following chemokine/cytokines were assayed with Milliplex MAP rat kit (Millipore, Billerica, MA; Cat #RECYTMAG-65 K-7): CCL_2, CCL_3, IL-1β, IL-6, IL-10, TNF-α, and IFN-γ according to the manufacturer's instructions ($N = 6$ animals per group). Plates were read on a Luminex 200 (Luminex Corporation, Austin, TX). Data for 100 beads per cytokine were collected for each standard and sample dilution, and the results were expressed as picograms of chemokine/cytokines per milligram of protein. Total protein concentrations were determined using the BCA quantification method (Sigma-Aldrich).

Total RNA Extraction Enriched with miRNA

Total RNA enriched with miRNA was obtained from post-treatment hippocampus samples using a combination of Trizol™ reagent (Invitrogen, Carlsbad, CA) and chloroform (Merck, Kenilworth, NJ) and were purified using the miRNeasy Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol. Total RNA was treated with RNase-Free DNase Set (Qiagen). Total RNA was quantified using the Qubit 2.0 Fluorometer (Thermo Fisher, Waltham, MA) and RNA integrity was analyzed by Agilent RNA 6000 Nano (Agilent Technologies, Waldbronn, Germany).

miRNA Expression Profiling

The TaqMan OpenArray Rodent MicroRNA panel (Thermo Fisher, Catalog #4470188) was used to evaluate the expression of 750 validated rodent miRNAs in samples from 12 rats, three in each experimental group: infected + RSV; infected + placebo; sham-infected + RSV; and sham-infected + placebo. Real-time PCR reactions were conducted on the QuantStudio 12 K Flex Real-Time PCR System (Thermo Fisher).

Each sample was divided and processed in parallel using Megaplex Primer Pools A and B and the MicroRNA Reverse Transcription kit (Thermo Fisher), according to the manufacturer's protocol. One hundred nanogram in 3 μL of total RNA extracted from each sample was used. The resulting cDNA was amplified prior to the real-time PCR reaction using the primer pools Megaplex PreAmp Pools A and B and the TaqMan PreAmp Master Mix solution (Thermo Fisher). The amplified cDNA was added to the TaqMan OpenArray Real-Time PCR Master Mix (Thermo Fisher) and distributed on a 384 wells plate. Then, samples were loaded onto the slides with the aid of the AccuFill system (Thermo Fisher). The sealed slide was transferred to the QuantStudio™ 12 K Flex Real-Time PCR System where real-time PCR reactions occurred. Raw data were analyzed with the Applied Biosystems analysis software, v. 1.0. Global normalization was used to determine the amount of each miRNA in a total RNA sample, using a 1.5 fold change threshold. Statistical significance was established at $P \leq 0.01$ (Student t test) [41]. Results were represented as a heat map of the global normalized Z-scores of miRNAs expression data using GenePattern [42].

Pathways Enrichment Analysis

MicroRNAs with altered expression in the hippocampus in response to BM, to RSV, or to the interaction between these two factors were analyzed using the DNA Intelligent Analysis (DIANA) miRPath v3.0 program with *H. sapiens* miRNA orthologues (http://snf-515788.vm.okeanos.grnet.gr/) [43]. The miRPath was parameterized with the options: pathways union, FDR correction, conservative stats, P value 0.05 and Fisher's exact test. The databases chosen were TarBase, which consider only miRNA-mRNA interactions that have been previously experimentally validated, and Kyoto Encyclopedia of Genes and Genomes (KEGG), which maps miRNA targets to pathways. Five groups were queried separately: (1) miRNAs down regulated, and (2) up regulated by BM; (3) miRNAs down regulated, and (4) up regulated by interaction (BM + RSV); and (5) miRNAs down regulated by RSV (no miRNA was up regulated by RSV in sham-infected animals).

Additionally, targets of differentially expressed miRNAs were retrieved from mirTarBase (http://mirtarbase.mbc.nctu.edu.tw/) [44], and Motifmap (http://motifmap.ics.uci.edu/) [45, 46] was used to disclose the transcription factors (TFs) with binding sites on the regulatory regions of a selection of these targets, which have been previously implicated in the host response to pneumococcal acute BM.

Statistical Analysis

The statistical analysis was performed using GraphPad Prism (version 5.0) (GraphPad Software Inc., Irvine, CA). The

Shapiro–Wilk test was used to verify the normal distribution of data. When applicable, outliers were excluded before statistical analysis using the standard interquartile range (IQR) criteria (values above 75% and below 25%). The data are reported as the mean ± SD. Differences between groups were evaluated using a two-way ANOVA followed by the Bonferroni test. P values less than 0.05 were considered to be statistically significant.

Results

Clinical Parameters of BM

After 18 h infection, all animals infected with *S. pneumoniae* had acute meningitis, as evidenced by positive bacterial titters in the CSF ($\sim 1 \times 10^8$ c.f.u./mL) regardless of the therapeutic schedule (placebo or RSV in pre- or post-treatment). The RSV dose used in this study is below the minimum inhibitory concentration previously reported to the pneumococci strain 38/12 MEN [40]. Accordingly, the bacterial titters assessed in the CSF at 18 h p.i. were not affected by RSV. With this strategy, we aimed to exclude the RSV antimicrobial effect to test its neuroprotective and anti-inflammatory effects.

The body weight variation and the activity score were significantly decreased by BM, and the adjunctive treatment with RSV did not affect these parameters (data not shown). It is worth mentioning that, at 3 h p.i, all infected animals had reduced activity score in our model. Moreover, Barichello et al. [47] have reported inflammatory mediators to peak at 6 h p.i. (the first time point assessed after 0 h p.i.) in the hippocampi of adult rats with pneumococcal acute meningitis. Thus, it is reasonable to assume that dramatic changes in the hippocampal content of inflammatory mediators are already in place at 3 h p.i.

RSV-Treated Infected Rats Had Significantly Lower Apoptotic Cell Score

Figure 1a–c shows the apoptosis in the lower blade of the dentate gyrus granular layer. Infection with *S. pneumoniae* caused extensive apoptosis ($P < 0.001$), while adjunctive pre-treatment with RSV reduced in 40% the apoptotic score ($P < 0.05$) (Fig. 1d). The adjunctive post-treatment reduced in 65% the apoptotic score (Fig. 1e). All infected animals had brain vascular dilation and congestion.

RSV Reduces Hippocampal Protein Levels of IL-1β, CCL_2, and CCL_3 in Counteracting the Effect of BM

Figure 2 summarizes the effect of pre-treatment with adjunctive RSV on inflammatory biomarkers induced by pneumococcal acute BM in the hippocampus. IL-1β, CCL_2, and CCL_3 levels were increased by BM ($P < 0.001$); the interaction between infection and RSV treatment decreased these levels ($P < 0.05$) at 57, 33, and 58% as compared to the infected placebo-treated group. BM increased IL-6, IL-10, TNF-α, and IFN-γ levels ($P < 0.01$), but RSV did not influence these parameters (Table 1).

RSV and BM Modulate the miRNA Profile in Hippocampus

Of the 750 miRNAs represented in TaqMan OpenArray Rodent MicroRNA panel, seven were down regulated, and 18 were up regulated by BM (when comparing infected + placebo vs. sham-infected + placebo) (Fig. 3). A different pattern was observed as a result of the interaction between pneumococcal acute BM and adjunctive therapy with RSV, where thirty-seven miRNAs were down regulated, and three were up regulated (when comparing infected + RSV vs. infected + placebo). Interestingly, hsa-miR-186-5p, hsa-miR-708-5p, mmu-miR-1193-3p, hsa-miR-101-3p, rno-miR-134-5p, and rno-miR-381 and were up regulated by BM and down regulated in RSV-treated infected animals. Six miRNAs were down regulated by RSV in the absence of infection, and none were up regulated in this condition (when comparing sham-infected + RSV vs. sham-infected + placebo).

BM and RSV Modulate the Expression of miRNAs Targeting Crucial Signaling Pathways Related to the Pathophysiology of the Disease

The lists of miRNAs up or down regulated in the hippocampus during pneumococcal acute BM, or in response to RSV in sick or healthy rats were used as inputs to DIANA mirPath v3.0 for pathway enrichment analyses. MirPath identified the potential KEGG pathways that are targeted by the miRNAs in the queries, and the results are displayed in Table 2. The ensemble of miRNAs down regulated in the hippocampi of infected animals receiving placebo was associated with 23 signaling pathways, while those miRNAs with increased expression were associated with nine pathways. Adjunctive RSV administered to animals with BM down regulated miRNAs associated with 15 signaling pathways, and up regulated miRNAs associated with 36 signaling pathways. Adjunctive RSV administered to sham-infected animals down regulated miRNAs associated with three signaling pathways. Among the pathways likely to be modulated by changes in the miRNome (the full spectrum of miRNAs expressed in a specific genome in a given condition) due to pneumococcal acute BM, RSV or the interaction between these two factors, some are closely related to pathophysiological hallmarks of BM. For instance, pneumococcal acute BM down regulated miRNAs targeting components of FOXO and Thyroid hormone signaling pathways (Fig. 4; Online resources 1 and 2, respectively, and Online resource 3). Pneumococcal

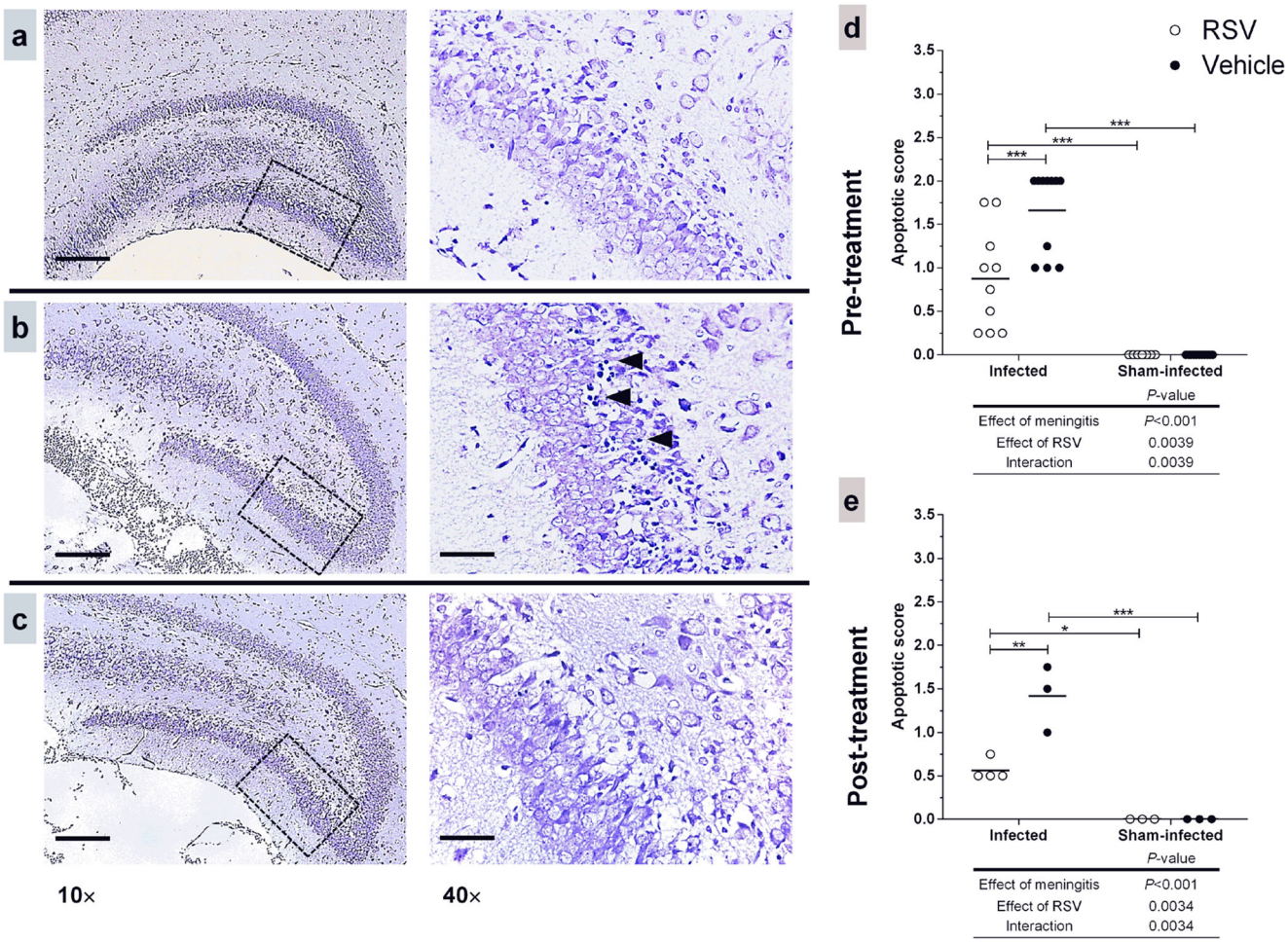

Fig. 1 Effect of adjunctive pre- and post-treatment with RSV on neuron apoptosis in the dentate gyrus of infant rats with pneumococcal meningitis. Histological sections of hippocampus Nissl stained with Cresyl violet in **a** sham-infected rats treated with placebo, **b** infected rats treated with placebo, and **c** infected rats treated with RSV. **d** Apoptotic scores in adjunctive pre-treatment and **e** post-treatment with RSV. Left panels (**a**, **b**, **c**)-Barr = 200 μm; right panels (**a**, **b**, **c**)-Barr = 50 μm. Dashed square represents the amplified region. Black arrows show apoptosis. Horizontal bars represent means. The effects of the BM and the RSV treatment were compared using a two-way ANOVA (Bonferroni test). $*P < 0.05$; $**P < 0.01$; $***P < 0.001$

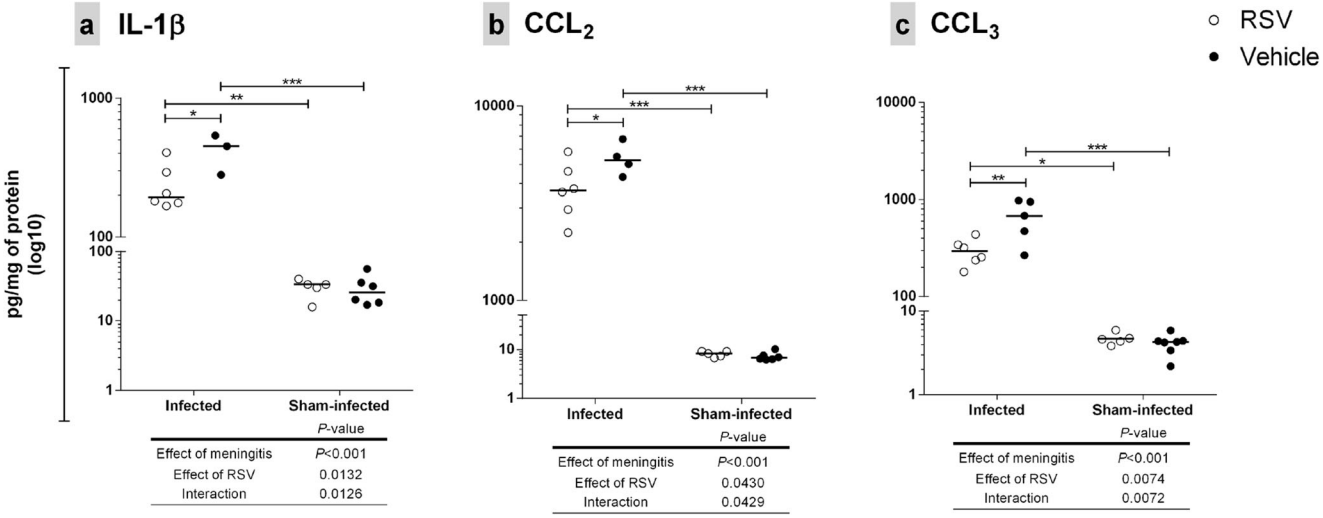

Fig. 2 Effect of adjunctive pre-treatment with RSV on inflammatory biomarkers induced by pneumococcal meningitis in the hippocampus of infant rats. Cytokine (**a**) and chemokines (**b** and **c**) reduced by pre-treatment with RSV. Horizontal bars represent the means. The effects of BM and RSV were compared using a two-way ANOVA (Bonferroni test). $*P < 0.05$; $**P < 0.01$; $***P < 0.001$

Table 1 Effect of BM on inflammatory biomarkers induced by pneumococcal meningitis in the hippocampus of infant rats

	Groups				*P* values		
	Sh-Sal	Sh-RSV	Infect-Sal	Infect-RSV	Effect of BM	Effect of RSV	Interaction
IL-6	43.39 ± 5.80	60.25 ± 18.50	1575 ± 257.9*	1323 ± 220.3*	< 0.001	0.4886	0.4709
TNF-α	0.23 ± 0.05	0.41 ± 0.11	4.13 ± 1.29*	2.75 ± 0.59*	< 0.001	0.6856	0.3817
IL-10	7.98 ± 0.89	13.56 ± 5.06	42.30 ± 5.77*	38.20 ± 3.8*	< 0.001	0.6854	0.2490
IFN-γ	8.40 ± 1.02	14.72 ± 4.09	36.6 ± 13.45	44.05 ± 9.22*	0.0010	0.2707	0.7236

Data are expressed as means ± S.D. Statistical differences were determined using a two-way ANOVA to examine the effects of BM and RSV post-treatment, followed by Bonferroni post hoc analyses. * denotes statistically significant differences at Bonferroni test when compared with the respective sham-infected control (Sh-Sal or Sh-RSV). Sh-Sal, sham-infected–placebo; Sh-RSV–sham-infected–resveratrol; Infect-Sal, infected–placebo; Infect-RSV, infected–resveratrol

acute BM up regulated miRNAs targeting components of the extracellular matrix (ECM)-receptor interaction pathway (Fig. 5; Online resource 4B and 5), while RSV in health subjects down regulated miRNAs targeting components of this same pathway (Online resource 4A and 5). Interestingly, RSV administered to infected animals down regulated eight miRNAs, one of them was up regulated by pneumococcal acute BM (hsa-miR-708-5p) (Fig. 5; Online resource 4C and 5). Pneumococcal acute BM also down regulated miRNAs targeting components of the TGF-beta and p53 signaling pathways (Fig. 6; Online resource 6A and 7, respectively, and Online resource 8A and 9, respectively), whereas the adjunctive RSV administered to infected animals up regulated miRNAs targeting components of the TGF-beta and p53 signaling pathways (Fig. 6; Online resource 6B and 7, respectively, and Online resource 8B and 9, respectively). The relationships between these pathways and the pathophysiology of BM are explored in the "Discussion" section.

Interplay of miRNAs, Transcription Factors and Target Genes

Target genes that have been previously implicated in the host response to pneumococcal acute BM had their transcription factors with binding sites on the regulatory regions disclosed using Motifmap. We have used the human h19 multiz46way placental database, with default parameters (FDR: 0.50; NLOD: 0.65, *Z*-score: 1.00) and distance from transcription start site (TSS) upstream and downstream = 1000 bp to disclose the transcription factors with binding sites on the regulatory regions of *Il-1β* (NM_000576), Ccl_2 (NM_002982), and Ccl_3 (NM_002983). *Il-1β* retrieved 19 entries; Ccl_2 retrieved 43 entries; and Ccl_3 retrieved 26 entries (Online resource 10). They showed in common the transcriptional enhancer factor (TEF)-1, which is a target of hsa-miR-25-3p and hsa-miR-125b-5p, both up regulated by RSV in infected rats. TEF-1 binding site is located 270 bp downstream in *Il-1β*, − 928 and − 379 bp upstream of Ccl_2, and − 734 and − 318 bp upstream of Ccl_3.

Discussion

The main finding with this study was to prove the anti-inflammatory and neuroprotective effects of adjunctive RSV treatment in an infant rat model of pneumococcal acute meningitis, shedding light on its mechanism of action. We provided evidences that RSV, as an adjunctive drug in pneumococcal

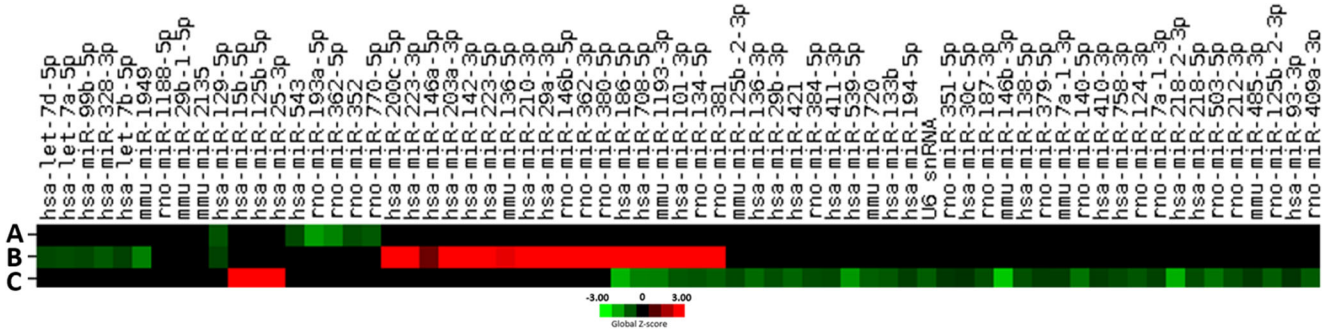

Fig. 3 Hierarchical clustering of miRNAs differentially expressed in the hippocampus in response to BM and RSV. MiRNAs were grouped by hierarchical clustering based on the correlations between their expression profiles. Global normalized *Z*-scores values were represented in the heat map. This analysis was performed using GenePattern. The color range in the heat map represents the fold change of differentially expressed miRNAs (FC > 1.5; $P < 0.01$). A) Sham-infected + RSV vs. Sham-infected + placebo; B) Infected + placebo vs. Sham-infected + placebo; C) Infected + RSV vs. Infected + placebo

Table 2 Results from Diana mirPath v.3 prediction of KEGG pathways influenced by miRNAs in BM, RSV, and the interaction (BM + RSV)

KEGG pathway	miRNAs DOWN regulated by BM			miRNAs UP regulated by BM			miRNAs DOWN regulated by BM + RSV			miRNAs UP regulated by BM + RSV			miRNAs DOWN regulated by RSV		
	P value	#genes	#miRNAs	*P* value	#genes	#miRNAs	*P* value	#genes	#miRNAs	*P* value	#genes	#miRNAs	*P* value	#genes	#miRNAs
Adherens junction (hsa04520)	7.60e-08	38	3/ 7	0.0019	31	3/ 18	4.04e-06	57	9/ 37	6.23e-07	33	3/ 3			
Bacterial invasion of epithelial cells (hsa05100)										0.0159	13	1/ 3			
Bladder câncer (hsa05219)	0.0122	18	2/ 7							0.0386	11	1/ 3			
Cell cycle (hsa04110)	2.55e-15	64	3/ 7				0.0083	62	4/ 37	1.01e-05	48	2/ 3			
Chronic myeloid leucemia (hsa05220)	9.61e-05	32	3/ 7							0.0003	18	1/ 3			
Colorectal câncer (hsa05210)	0.0113	21	2/ 7				0.0119	38	4/ 37	0.0232	12	1/ 3			
ECM-receptor interaction (hsa04512)				0	25	3/ 18	0	37	9/ 37				0.0389	2	1\ 6
Endocytosis (hsa04144)	0.0025	75	3/ 7												
Endometrial câncer (hsa05213)										0.0040	12	1/ 3			
Epstein-Barr virus infection (hsa05169)										0.0190	50	1/ 3			
Fatty acid biosynthesis (hsa00061)				0	3	3/ 18	0	4	6/ 37	0	4	2/ 3			
Fatty acid degradation (hsa00071)										0.0031	9	1/ 3			
Fatty acid elongation (hsa00062)							0.01927	8	3/ 37	0.0014	6	1/ 3			
Fatty acid metabolism (hsa01212)							5.86e-08	22	6/ 37	0	16	2/ 3			
Focal adhesion (hsa04510)							0.0305	53	3/ 37						
FoxO signaling pathway (hsa04068)	0.0061	50	2/ 7												
Glioma (hsa05214)	8.57e-06	30	4/ 7												
Hepatitis B (hsa05161)	3.36e-08	61	3/ 7							0.0002	46	2/ 3			
Hippo signaling pathway (hsa04390)	1.42e-09	61	4/ 7	0.0067	42	4/ 18	1.40e-07	74	8/ 37	2.98e-09	52	3/ 3			
Lysine degradation (hsa00310)	2.37e-09	19	3/ 7	9.41e-05	21	4/ 18	0.0024	22	4/ 37	6.17e-07	21	3/ 3			
Melanoma (hsa05218)	0.0039	23	2/ 7							0.0055	13	1/ 3			

Table 2 (continued)

KEGG pathway	miRNAs DOWN regulated by BM			miRNAs UP regulated by BM			miRNAs DOWN regulated by BM + RSV			miRNAs UP regulated by BM + RSV			miRNAs DOWN regulated by RSV		
	P value	#genes	#miRNAs	*P* value	#genes	#miRNAs	*P* value	#genes	#miRNAs	*P* value	#genes	#miRNAs	*P* value	#genes	#miRNAs
N-Glycan biosynthesis (hsa00510)													0.0273	1	1\ 6
Oocyte meiosis (hsa04114)	9.58e-06	45	3/ 7							0.0049	30	1/ 3			
Other types of O-glycan biosynthesis (hsa00514)										0.0004	6	1/ 3			
p53 signaling pathway (hsa04115)	0.0028	29	2/ 7							0.0014	25	2/ 3			
Pathways in câncer (hsa05224)	2.21e-06	128	3/ 7				3.08e-05	191	6/ 37	0.0014	96	2/ 3			
Prion diseases (hsa05020)				4.53e-05	4	1/ 18	0.0378	4	1/ 37	0	3	1/ 3			
Prostate câncer (hsa05215)	0.0002	43	4/ 7							5.73e-05	42	3/ 3			
Protein processing in endoplasmic reticulum (hsa04141)	0.0135	60	2/ 7	0.0412	60	3/ 18	0.0199	87	6/ 37	1.34e-05	75	3/ 3			
Proteoglycans in câncer (hsa05205)	1.33e-07	74	3/ 7	0.0003	53	2/ 18	6.38e-12	113	8/ 37	1.55e-08	75	3/ 3			
Regulation of actin cytoskeleton (hsa04810)										0.0034	49	2/ 3			
RNA transport (hsa03013)										0.0272	28	1/ 3			
Small cell lung cancer (hsa05222)	0.0271	33	2/ 7												
Steroid biosynthesis (hsa00100)													3.19e-05	1	2\ 6
TGF-beta signaling pathway (hsa04350)	9.16e-05	34	2/ 7							0.0109	22	1/ 3			
Thyroid hormone signaling pathway (hsa04919)	5.16e-06	49	3/ 7												
Ubiquitin mediated proteolysis (hsa04120)	0.0397	51	2/ 7												
Viral carcinogenesis (hsa05203)	2.40e-12	77	4/ 7	6.63e-07	85	4/ 18	6.80e-08	93	6/ 37	4.47e-12	76	3/ 3			

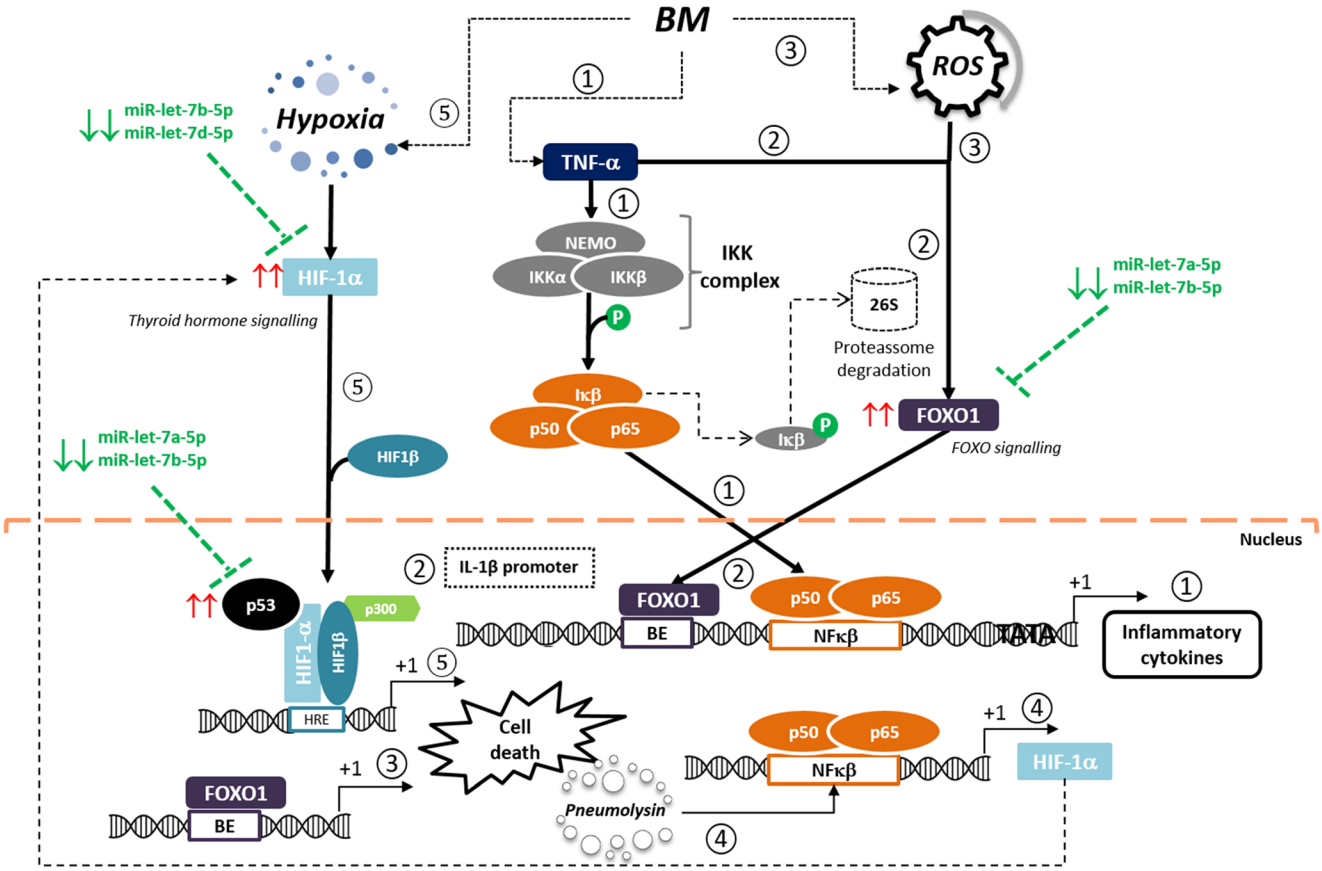

Fig. 4 BM down regulates miRNAs targeting components of FOXO and Thyroid hormone signaling pathway. ① BM increases TNF-α level, which can trigger the recruitment and activation of the IKK complex (including IKKα and IKKβ catalytic subunits and two molecules of the regulatory non-enzymatic scaffold protein NEMO). The IKK complex activates the NF-κβ dimmers (p50 and p65), phosphorylating Iκβ subunit and leading to its degradation by the proteasome. Then, NF-κβ translocate to the nucleus to activate target genes [48]. ② TNF-α also activates FOXO1, inhibiting its AKT-mediated phosphorylation and enhancing FOXO1 translocation to the nucleus, where it binds to promoters of pro-inflammatory genes, such as CCL_2 and IL-6, enhancing their expression [49]. In addition, FOXO1 plays a cooperative role in inflammatory signaling through NF-κB, being both FOXO1 and NF-κB necessary to induce IL-1 transcription [50]. ③ FOXO1 plays a protective role against oxidative stress in normal conditions. However, in extreme conditions such as BM, FOXO1 promotes cell death [51]. ④ Bacterial products signals through NF-kB to increase *Hif1α* transcription. ⑤ Bacterial infection typically results in a hypoxic condition, leading to HIF-1α accumulation and nuclear translocation, heterodimerization with HIF-1β, and recruitment of p300/CBP, a member of the HIF complex that acts as a transcriptional coactivator of target genes. The HIF transcriptional complex binds to hypoxia-responsive elements (HREs) to control the expression of target genes. P53 is also activated in hypoxic conditions and is stabilized during the interaction with the HIF-1 protein, activating genes that cause cell death [52, 53]. BM, bacterial meningitis; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-α; NFκβ, nuclear factor kappa beta; FOXO1, forkhead box 1; BE, binding elements; HIF-1, hypoxia inducible factor; HRE, hypoxia-response elements; p300: histone acetyltransferase (also known as p300 HAT). Green letters/ symbols; down regulated miRNA or genes. Red letters/ symbols; up regulated miRNA or genes. For more details, see online resources 1 and 2

acute BM, acts by modulating the global expression pattern of miRNAs in the hippocampus. Besides, we presented the first snapshot of the hippocampal miRNome during the acute phase of pneumococcal BM. In brief, we found that (a) pneumococcal acute BM up regulated 18 miRNAs, and six among them (hsa-miR-186-5p, hsa-miR-708-5p, mmu-miR-1193-3p, hsa-miR-101-3p, rno-miR-134-5p, and rno-miR-381) were down regulated by RSV; however, apart from miR-708-5p, this subset of miRNAs are poorly annotated for targets with experimental evidence; (b) pneumococcal acute BM down regulated miRNAs targeting components of FOXO and Thyroid hormone signaling pathways related to apoptosis and inflammation (FOXO1 and HIF-1α); (c) RSV counteracted some of the deleterious effects of pneumococcal acute BM by down regulating miRNAs over-expressed in the disease, such as miR-708-5p, which targets components of ECM-receptor interaction signaling pathway. Thus, RSV may contribute to preserve or restore the BBB integrity; (d) RSV up regulated miR-25-3p and miR-15-b, which target TP53 and SMAD2, respectively. These two molecules are components of p53 and TGF-beta signaling pathways. By modulating these pathways, RSV may inhibit apoptosis and attenuate hippocampal damage; (e) RSV also up regulated miRNAs (miR-25-3p and miR-125b-5p) that have among

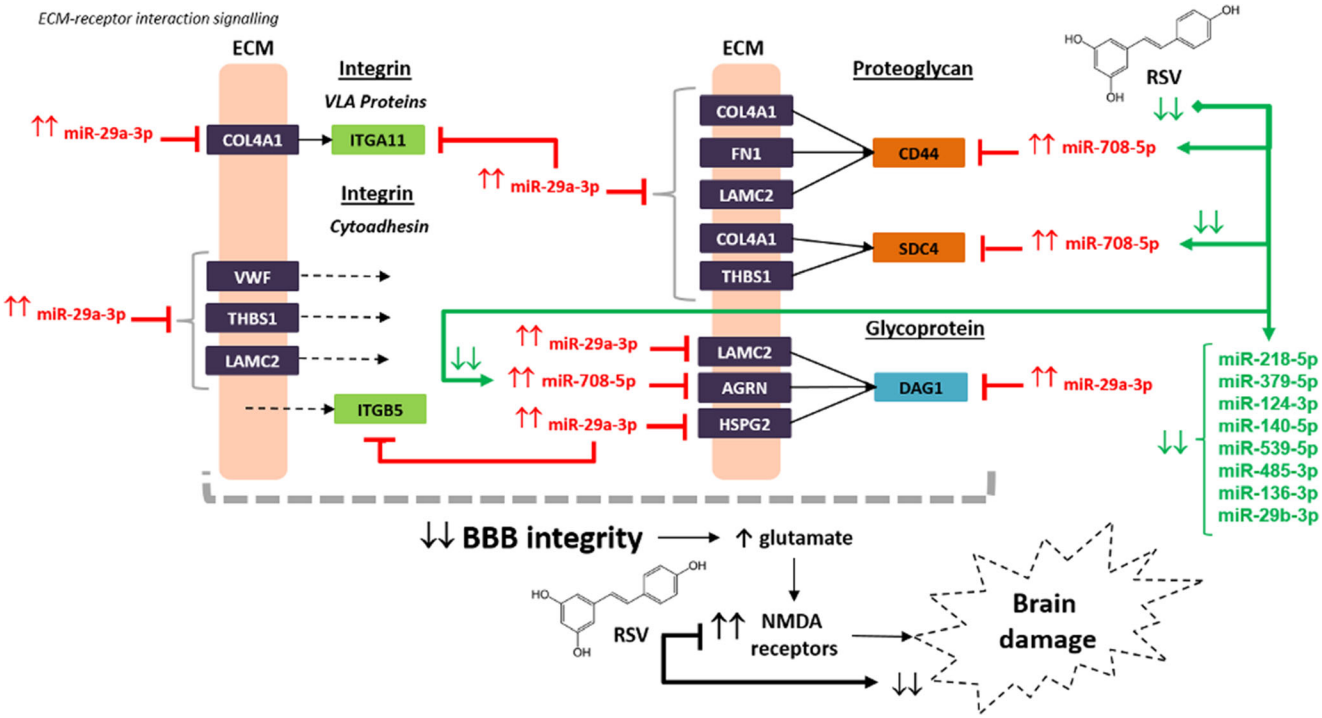

Fig. 5 RSV down regulates miRNAs targeting components of ECM-receptor interaction signaling pathway, counteracting the effect of BM. BM up regulated miRNAs targeting integrins, ECM components, proteoglycans and glycoproteins, which may contribute to impair BBB's tight junctions and integrity [54]. The increase in the BBB permeability results in the accumulation of glutamate in the CNS [55], which stimulates excessively the NMDA receptors and contributes to brain damage associated to BM [12, 13]. RSV administered to infected animals down regulated miRNAs targeting components of ECM-receptor interaction signaling pathway, probably aiding to preserve or restore de BBB integrity. In addition, RSV has inhibitory effect on ionotropic glutamate receptors [56], including NMDA receptors, which may account to attenuate neuron death by excitotoxicity. RSV, resveratrol; ECM, extracellular matrix; CNS, central nervous system; BBB, blood brain barrier; COL4A1, collagen type IV alpha 1 chain; VWF, Von Willebrand factor; THBS1, thrombospondin 1; LAMC2, laminin subunit C2; ITGA11, integrin subunit alpha 11; ITGB5, integrin subunit beta 5; FN1, fibronectin 1; AGRN, agrin; HSPG2, heparan sulfate proteoglycan 2; CD44, cell-surface glycoprotein involved in cell–cell interactions; SDC4, syndecan 4; DAG1, dystroglycan 1; NMDA receptor, N-methyl-D-aspartate receptor. Green letters/ symbols; down regulated miRNA or genes. Red letters/ symbols; up regulated miRNA or genes. For more details, see online resource 4

their targets the transcription factor TEF-1, which interacts with binding sites on the regulatory regions *of Il-1β*, Ccl_2, and Ccl_3.

Pneumococcal acute meningitis modulated the global expression pattern of miRNAs in the hippocampus. To our knowledge, this is the first study to implicate the miRNome as a key regulator of pneumococcal acute BM pathophysiology. BM is likely to indirectly up regulate genes in FOXO (targets of down regulated miRNAs) (Fig. 4; Online resources 1 and 3). FOXO is a class of transcription factors with crucial roles in cell proliferation, differentiation, autophagy, apoptosis, metabolism, inflammation, and stress resistance [50]. FOXO1 is targeted by two miRNAs down regulated by pneumococcal acute BM (let-7a-5p, and let-7b-5p), and this could account to its over expression during pneumococcal acute BM. FOXO1 is strongly activated by TNF-α [64], a potent pro-inflammatory and pro-apoptotic mediator that accumulates in the CNS and causes inflammation in the early acute phase of BM [18, 19]. Therefore, the increase in TNF-α levels activates FOXO1, which, in its turn enhances the expression of other pro-inflammatory cytokines [51]. Also, FOXO1 plays a cooperative role in inflammatory signaling via NF-κB, being both FOXO1 and NF-κB necessary to induce Il-1 transcription [50]. It is noteworthy that FOXO1 plays an important role in cell protection against oxidative stress. Under normal conditions, FOXO1 induces the expression of antioxidant genes to attenuate apoptosis [65]. However, FOXO1 seems to promote cell death when oxidative stress is more extreme, having a damaging rather than a protective role [51]. Pneumococcal acute BM is also likely to up regulate the Thyroid hormone signaling pathway since three miRNAs down regulated by the disease (let-7a-5p, let-7b-5p, and let-7d-5p) had as target the hypoxia inducible factor (HIF)-1 (Fig. 4; Online resources 2 and 3). HIF-1 is a heterodimeric transcription factor comprising a constitutively expressed β-subunit and an oxygen-regulated α-subunit. It is a central regulator of the adaptation process of hypoxia during infectious diseases and inflammatory conditions. For instance, HIF-1α is critical to myeloid cell-mediated inflammation, and phagocytes bactericidal capacity [66]. Accordingly, HIF-1α

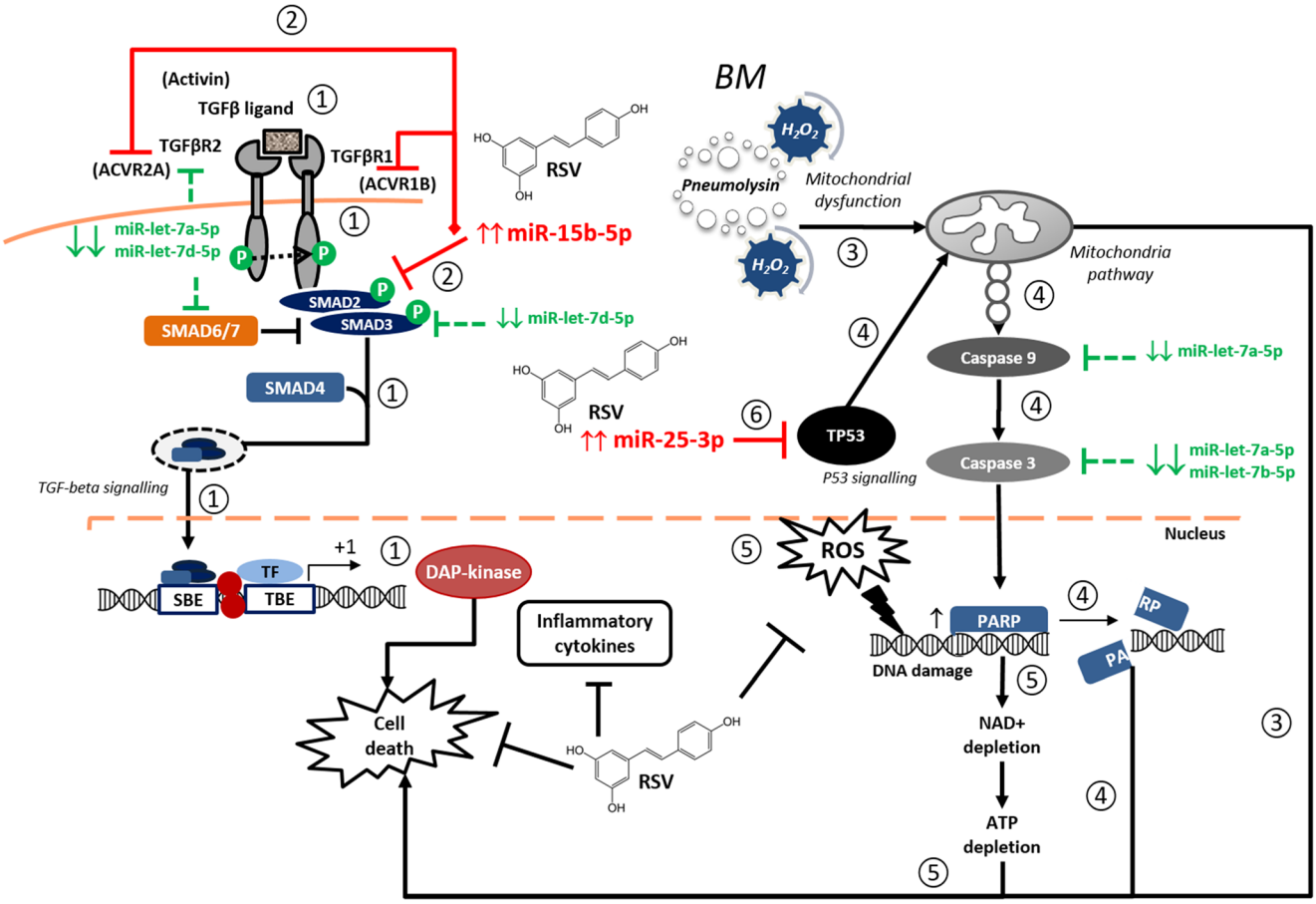

Fig. 6 In the context of BM, adjunctive RSV up regulated miRNAs targeting components of TGF-beta and P53 signaling pathways. ① TGF-beta signaling is triggered by the binding of TGFβ ligands (such as activin A) to type II TGFβ receptors (such as ACVR2A). The type II receptor recruits, phosphorylates, and activates the type I TGFβ receptor (such as ACVR1B). Then, they phosphorylate SMAD2 and SMAD3 that subsequently bind to SMAD4. The SMAD complex is translocated to the nucleus, where it binds to SRE and interact with transcription factors that drives the gene expression of inflammatory cytokines and DAP-kinase [57]. ② The adjunctive RSV administered to infected animals up regulated the miR-15b-5p that has as target the type I and type II receptors ACVR1B and ACVR2A, and SMAD2, impairing the signal transduction through TGF-beta signaling, attenuating the inflammatory processes and apoptosis in the brain. ③ Pneumolysin and oxygen peroxide induce mitochondrial dysfunction, ultimately leading to caspase-independent cell death during the early phase of BM. ④ TP53 activates the mitochondrial pathway, which results in the caspase-dependent apoptosis. In the nucleus, caspase-3 cleaves the DNA repair enzyme PARP-1 preventing its recruitment to sites of DNA damage and causing apoptosis [58, 59]. ⑤ In addition, ROS generated during BM induces DNA damage [60], and then PARP-1 is activated for repairing activity. PARP-1 consumes large amounts of energy, which results in depletion of NAD^+ and ATP reserves, leading to neuronal death [61–63]. ⑥RSV administered to infected animals up regulated miR-25-3p, which targets TP53. BM: bacterial meningitis; ROS: reactive oxygen species; RSV: resveratrol; TGFβ: transforming growth factor-beta; TGFβR2: type II TGFβ receptors; TGFβR1: type I TGFβ receptors; SRE: SMAD responsive elements; TF: transcription factor; TBE: TF bind elements; PARP: poly (ADP-ribose) polymerase; TP53: tumor protein 53. Green letters/ symbols: down regulated miRNA or genes. Red letters/ symbols: up regulated miRNA or genes. For more details, see Online resources 6 and 7

knockout mice show an impairment of myeloid cell aggregation, motility, invasiveness, and bacterial killing capacity [67]. Additionally, HIF-1α has been identified as a key regulator of the inflammatory transcription factor NF-κB [68]. NF-κB activation and PMN transmigration across the BBB are hallmarks of BM [12]. In addition, HIF-1 plays a role in hypoxia-induced apoptosis. Although the exact mechanism remains unclear, data from previous studies suggest that the tumor suppressor p53 is activated in hypoxic conditions. P53 is stabilized during the interaction with the HIF-1 protein and activates cell death genes [52, 53].

Some miRNA up regulated by pneumococcal acute BM are poorly annotated in TarBase, despite relevant information available in the literature. For example, Jimenez-Mateos et al. [69] have reported that silencing miR-134 leads to neuroprotective and prolonged seizure-suppressive effects, which is in line with its up regulation by pneumococcal acute BM in our infant rat model. Regarding to miR-381, its over expression was related to promotion of neural stem cells proliferation, induction of their differentiation to neurons, and inhibition of their differentiation to astrocytes [70]. Indeed, increased neurogenesis occurs after experimental pneumococcal acute

meningitis [71]. This may account to limit the extent hippocampal damage in pneumococcal acute BM [72]. Functional data about miR-186, miR-101, and miR-1193 are still scarce in the literature.

In sham-infected animals, RSV down regulated miR-193a-5p that targets COL1A1 and COL1A2. This down regulation may result in an increase of these two genes that encode the α1 and α2 subunits of collagen 1. Thus, our result suggests that RSV may stimulate the collagen 1 production in the hippocampus of healthy animals. Collagenases [matrix metalloproteinases (MMP)-8 and − 13] are overexpressed in the CNS during BM [73]. They disrupt the BBB, facilitate leukocyte pleocytosis, and ultimately lead to vasculitis, brain edema, and ischemia [12, 74, 75]. Pharmacological inhibition of MMPs ameliorates the outcome of infant rats with pneumococcal acute meningitis [76]. In the present study, pneumococcal acute BM was likely to down regulate genes in ECM-receptor interaction pathway (targets of up regulated miRNAs) (Fig. 5; Online resource 4B and 5). In this pathway, ECM components, integrins, and proteoglycans are targeted by mir-29a-3p, miR-708-5p. Integrins are a class of adhesion mechanoreceptors that functionally links the ECM and the cytoskeleton [77]. Agrin (AGRN), an heparan sulfate proteoglycan, which is a target of the miRNA miR-708-5p, initiates a link of the dystroglycan (DAG)/dystrophin-glycoprotein complex to the astrocyte actin cytoskeleton, contributing to BBB integrity and function [78]. Moreover, integrin-mediated signaling events in neurons regulate glutamate receptor (NMDA) activity [79]. Thus, inhibition of the ECM-receptor signaling pathway via the up regulation of miRNAs targeting its components could impair the BBB integrity and the homeostasis of excitatory amino acids. Indeed, during BM, increased BBB permeability allows the accumulation of glutamate in the CNS [55], causing excessive stimulation of glutamate receptors and excitotoxicity. The excessive activation of NMDA receptors contributes to neuronal cell death in BM [12]. As opposite, adjunctive RSV administered to infected animals down regulated hsa-miR-708-5p (Fig. 5; online resource 4C and 5). Other miRNAs inhibited by RSV during BM (miR-218-5p, miR-379-5p, miR-124-3p, miR-140-5p, miR-539-5p, miR-485-3p, miR-136-3p, miR-29b-3p) also target ECM components, integrins, and proteoglycans. Summarizing, in pneumococcal acute BM, adjunctive therapy with RSV down regulates miRNAs targeting components of ECM-receptor interaction pathway, enhancing by this way the expression of genes responsible for maintaining the BBB integrity. Additional support to the hypothesis that RSV favors the BBB integrity and the homeostasis of excitatory amino acids can be found in the works of Gao et al. [56], who demonstrated that RSV inhibits MMP-9 expression in a mice model of cerebral ischemia, and Chang and Wang [80], who reported that RSV inhibits NMDA receptors post-synaptically and glutamate release pre-synaptically.

Pneumococcal acute BM is likely to up regulate genes in TGF-beta signaling pathways (targets of down regulated miRNAs), while adjunctive RSV administered to infected animals up regulated miRNAs targeting components of this pathway (Fig. 6; Online resources 6 and 7). Transforming growth factor-beta (TGF-beta) is involved in a number of biological processes (i.e., proliferation, differentiation, migration, and apoptosis) [81], playing an essential role in tissue homeostasis and immune functions. TGF-beta signaling is triggered by the binding of TGFβ ligands, such as activin, bone morphogenetic proteins (BMPs), Nodal and TGFβs, to type II TGFβ receptors (TGFβR2). Then, TGFβR2 recruits, phosphorylates, and activates the type I TGFβ receptor (TGFβR1), which phosphorylates SMAD2 and SMAD3 that subsequently bind to SMAD4, forming SMAD complexes. It is noteworthy that different kinds of type I and type II receptors were described, being type I essentially for signaling, whereas type II are required for binding ligands and for regulation of type I receptors expression. The nuclear translocation of SMAD complexes allows their interactions with TFs to activate the expression of TGFβ responsive genes [57]. SMAD6 and SMAD7 can also modulate this pathway, binding to the activated receptors and avoiding further propagation of TGF-beta signaling [82, 83]. In pneumococcal acute meningitis mice, deletion of TGFβR2 on leukocytes increased neutrophils recruitment to the infection site to enhancement bacterial clearance [84], suggesting that TGF-beta signaling suppresses host defense against bacterial infection in the CNS [85, 86]. One of the outputs of TGF-beta signaling pathway is the induction of apoptosis, which depends on the different SMAD targets. It has been described that the SMAD target death-associated protein kinase (DAP-kinase) is an effector of TGF-β-dependent apoptosis, acting immediately downstream from SMADs and upstream of mitochondrial pro-apoptotic events [87]. RSV administered to infected animals up regulated miR-15b-5p that targets SMAD2, ACVR1B, and ACVR2A; the last two are types I and II TGFβ receptors, respectively. ACVR2A and ACVR1B are activated after activin binding, which is up regulated in inflammatory conditions [88]. In a rabbit model of BM, activin levels positively correlated with the number of apoptotic neurons in the dentate gyrus and also modulated the release of several pro-inflammatory cytokines [89]. Thus, the up regulation of miR-15b-5p induced by RSV during pneumococcal acute BM suggests an inhibitory mechanism, which may attenuate the inflammatory process and apoptosis. Moreover, SMAD2 inhibition by miR-15b-5p may hamper the downstream activation of the DAP-kinase [87], preventing apoptosis and leading to neuroprotection. It is noteworthy that, although modulating different miRNAs, RSV down regulated SMADs in human SW480 colon cancer cells [36]. Thus, miR-15b-5p, by regulating SMADs, the main effectors of the canonical TGF-beta signaling pathway, seems to play a central role in

the mode of action of RSV as a neuroprotective drug to pneumococcal acute BM.

Pneumococcal acute BM is likely to up regulate genes in p53 signaling pathway (targets of down regulated miRNAs), while adjunctive RSV administered to infected animals up regulated miRNAs targeting components of the same pathway (Fig. 6; Online resources 8 and 9). Briefly, p53 (also known as tumor protein - TP53) is a tumor suppressor that induces apoptosis [90]. The p53 signaling pathway regulates caspase-3, which is centrally involved in brain cell apoptosis during BM [91, 92]. It has been established that BM-associated neuronal apoptosis, particularly in the hippocampus occurs in two distinguished steps in vivo, wherein bacterial components stimulate different pathways in the host: (1) pneumolysin (a pneumococcal toxin) and hydrogen peroxide lead to mitochondrial dysfunction, that induces caspase-independent cell death in the early phase of BM; (2) afterwards, pneumococcal cell-wall components are released, which fosters the inflammation and caspase-dependent events maintain the apoptosis [93]. Another characteristic event of apoptosis is the proteolytic cleavage of the nuclear enzyme poly (ADP-ribose) polymerase 1 (PARP-1) involved in DNA repair, DNA stability, and transcriptional regulation. Caspase-3 (and caspase-7) cleaves PARP-1 between Asp214 and Gly215 [58]. This cleavage results in the separation of the two zinc-finger DNA-binding motifs from the catalytic domain, preventing the recruitment of the enzyme to sites of DNA damage and causing apoptosis [59]. In addition, ROS generated during the inflammatory process induces DNA cleavage [60], which activates PARP-1. Excessive PARP activation depletes NAD^+ and ATP cellular stores, leading to neuronal death by necrosis or apoptosis [61–63]. Therefore, BM-induced down regulation of miRNAs targeting components of p53 signaling pathway is likely to contribute to apoptosis in the post-mitotic neurons and progenitor cells of the dentate gyrus. Adjunctive RSV administered to infected animals up regulated hsa-25-3p, which targets TP53. By this mode of action, RSV may prevent hippocampal apoptosis in BM. In line with this findings, RSV inhibited neuronal apoptosis and p53 expression by hippocampal granular cells in a murine model of chronic fatigue [94]. It also blocked the activation of NF-kB signaling in the brain, preventing cognitive impairments in rats exposed to ethanol during the postnatal period [95].

Although pathway enrichment analysis and direct search for targets of miRNAs up regulated by RSV in infected rats did not disclose IL-1-β, CCL_2, and CCL_3 (up regulated by BM and down regulated by the interaction between BM and RSV), there are in silico evidences that the transcription factor TEF-1, targeted by miR-25-3p and miR-125b-5p, has specific binding sites in the regulatory regions of these inflammatory mediators [45, 46].

Sheu et al. [96] have previously reported that adjunctive RSV attenuates inflammation and hippocampal apoptosis in adult rats with meningitis caused by *Klebsiella pneumoniae*. It is difficult to compare their results with the present study, since they have tested the effect of RSV in adult rats infected with *K. pneumoniae*, an etiological agent of BM in newborns, and, unfortunately, did not inform by which via RSV was administered to their animals. Our infant rat model of pneumococcal acute meningitis is a well-established method that reproduces the most relevant pathophysiological aspects of BM in humans [11, 17]. However, further studies are necessary to assess the effect of adjunctive RSV on other pathophysiological aspects of pneumococcal meningitis, such as the subarachnoid inflammation, brain vasculitis, and cortical necrosis.

Altogether, our results support the hypothesis that RSV's mode of action underlying its neuroprotective and anti-inflammatory effects in the infant rat model of pneumococcal meningitis involves the modulation of the hippocampal miRNome. In this BM model, RSV regulated the expression of miRNAs targeting components of critical pathways related to maintenance of BBB integrity (ECM-receptor interaction pathway) and apoptosis (TGF-beta and p53 signaling pathways). RSV also up regulated miRNAs targeting the transcription factor TEF-1, that, in its turn, controls the expression of genes coding for *Il-1-β*, Ccl_2, and Ccl_3. Moreover, we provide herein the first snapshot of the hippocampal miRNome during the acute phase of pneumococcal meningitis.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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