Brain, Behavior, and Immunity 46 (2015) 270–279

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Behavioral stress reduces RIP140 expression in astrocyte and increases brain lipid accumulation

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ARTICLE INFO

Article history:
Received 21 October 2014
Received in revised form 2 February 2015
Accepted 9 February 2015
Available online 16 February 2015

Keywords:
RIP140
Astrocyte
Cholesterol
Stress

ABSTRACT

Receptor-interacting protein 140 (RIP140) is highly expressed in the brain, and acts in neurons and microglia to affect emotional responses. The present study reveals an additional function of RIP140 in the brain, which is to regulate brain lipid homeostasis via its action in astrocytes. We found forced swim stress (FSS) significantly reduces the expression level of RIP140 and elevates cholesterol content in the brain. Mechanistically, FSS elevates endoplasmic reticulum stress, which suppresses RIP140 expression by increasing microRNA 33 (miR33) that targets RIP140 mRNA's 3′-untranslated region. Consequentially, cholesterol biosynthesis and export are dramatically increased in astrocyte, the major source of brain cholesterol. These results demonstrate that RIP140 plays an important role in maintaining brain cholesterol homeostasis through, partially, regulating cholesterol metabolism in, and mobilization from, astrocyte. Altering RIP140 levels can disrupt brain cholesterol homeostasis, which may contribute to behavioral stress-induced neurological disorders.

1. Introduction

Stressful experiences can contribute to mood changes and psychological and neurological disorders. For instance, chronic stress leads to hippocampal structure remodeling and cognitive impairment in rodents (McEwen et al., 2012). While alteration in adrenal steroid and neurotransmitter release can cause brain structural changes and behavioral disorders (McEwen, 2012), the molecular mechanism underlying stress-induced neurological disorder is poorly understood. Recent studies indicated that, chronic stress such as social defeats can disrupt lipid metabolism by increasing transcriptional activity of genes involved in cholesterol synthesis (Chuang et al., 2010); as a result, the level of circulating cholesterol is significantly elevated minutes after exposure to psychological stressor (Muldoon et al., 1992). Brain contains the highest level of cholesterol, and almost all the brain cholesterol is formed by *de novo* synthesis due to efficient blockade in the uptake of circulating cholesterol by the blood–brain barrier (Wang and Eckel, 2014). A disruption in brain cholesterol level has also been related to cognitive impairment (Martin et al., 2010). However, whether and how brain cholesterol homeostasis may be directly affected by stress is unclear.

Receptor-interacting protein 140 (RIP140), a transcriptional co-regulator for numerous transcription factors and a signal transduction regulator, is recently implicated in the regulation of cognitive function, emotional response and neuron health via its activities in microglia and neurons (Feng et al., 2014; Flaisher-Grinberg et al., 2014). RIP140 is first known to co-regulate the activities of many nuclear receptors/transcription factors that regulate lipid metabolism. For instance, in adipose tissues, RIP140 regulates the storage of lipids by inhibiting the expression of genes involved in fatty acid oxidation (Leonardsson et al., 2004; Ho et al., 2011). In hepatocytes and macrophages, RIP140 is involved in both positive and negative actions of liver X receptor (LXR)-regulated lipid and glucose metabolism (Herzog et al., 2007). In microphages, cholesterol overload activates microRNA 33 (miR33), a regulator of hepatocyte cholesterol homeostasis which decreases RIP140 mRNA level (Ping-Chih Ho et al., 2011). RIP140 is highly expressed in the brain (Lee et al., 1998) and has been detected in various cell types including neurons, astrocytes and microglia. Whole body RIP140 knockout mice are lean and exhibit memory deficits and increased

Abbreviations: ABCA, ATP-binding cassette transporter; ABCG1, ATP-binding cassette sub-family G; ApoE, Apolipoprotein E; CYP46, cholesterol 24S-hydroxylase; CYP51, Sterol 14 alpha-demethylase; ER, endoplasmic reticulum; FSS, forced swim stress; H_2O_2, Hydrogen peroxide; HMGCR, 3-hydroxy-3-methyl-glutaryl-CoA reductase; LDLR, low density lipoprotein receptor; LPR, lipoprotein receptor; LXR, liver X receptor; miR33, microRNA 33; RIP140, Receptor-interacting protein 140; SREBP, Sterol regulatory element-binding proteins.

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http://dx.doi.org/10.1016/j.bbi.2015.02.008
0889-1591/

stress responses, in addition to numerous defects particularly in reproduction (Duclot et al., 2012). Macrophage specific RIP140 knockdown mice have decreased microglia RIP140 level in ventromedial hypothalamus and the cingulate cortex, and exhibit increased anxiety- and depressive-like behaviors (Flaisher-Grinberg et al., 2014). More recently, we found that loss of RIP140 in hippocampal neurons can result in increased vulnerability to ER stress-induced death (Feng et al., 2014). Whether RIP140 plays a role in another prominent cell type of the brain, astrocyte, is still unclear.

In the current study, we aim to determine if RIP140 expression in the brain responds to stress, and whether this response is causally related to brain cholesterol metabolism. Forced swim stress (FSS) was used to generate the behaviorally stressed animal model. RIP140 level and cholesterol metabolic genes expression, as well as cholesterol content, in the brain and primary cultured astrocyte were examined. The experiments results show that a stressor like FSS can decrease RIP140 level in the brain, and simultaneously increase brain cholesterol level. Further, RIP140 negatively regulates cholesterol biosynthesis in, and cholesterol exportation from, astrocyte. Thus, a reduction in RIP140 expression such as in astrocytes, which can be caused by FSS, can result in brain cholesterol accumulation. This may contribute to certain stress-induced pathological outcomes in the brain including cognitive impairment.

2. Materials and methods

2.1. Porsolt forced swim stress (FSS) and behavioral tests

Male C57BL/6 mice (8–9 weeks old), from Charles River Laboratories, were maintained and experimental procedures were conducted according to NIH guidelines and approved by the University of Minnesota Institutional Animal Care and Use Committee (Protocol No. 1306A30679). All behavioral assessments were performed as previously described (Flaisher-Grinberg et al., 2012, 2014). The general activity was assessed by the automated open field environments. Motor coordination and balance were determined by the grid walk test and the rotarod apparatus to indicate the physical abnormalities (Crawley, 2008).

Repeated exposure to a modified Porsolt forced swim test (FST) was used to induce stress (FSS). Mice were placed in a transparent cylindrical container (30 cm tall, 20 cm in diameter), filled to a depth of 20 cm with tap water at 30 °C, to swim for 15 min each trial. Between each trial, the water was replaced and the mice were towel dried and returned to their home cage placed on heat pad to keep the mice warm. The trial was repeated for 14 days (1 trial per day). At the day 15, all mice were subjected to the stress-like behavior tested with automated open field test and emergence test. In the automated open field test, the time spent in the central area of open field, number of enters into the central area of open field, and as well as the distance traveled in the central were recorded. In the emergence test, the time spent out of the shelter, latency to exit shelter and number of out of the shelter were recorded.

The stress-like behaviors of the normal control ($n = 9$) and FSS ($n = 12$) groups were assessed according to the five recordings (Rec. 1: time in the central area; Rec. 2: number enters into the central area; Rec. 3: time spent out of the shelter; Rec. 4: latency to exit shelter; Rec. 5: number of out of the shelter). For each recording, the average of the control group was defined as "standard", indicating normal behaviors with regard to this particular recording. Each recording of a stressed mouse was converted into a percentage change, compared to the standard, for that particular recording. An overall percentage change (indicated as "Average") was determined by averaging the five recordings for each animal. The stressed animals were subdivided into stress-responsive (S-R) and stress-unresponsive (S-UR) groups based on the average percentage change: stressed animal displaying >30% difference in its average percentage change was regarded as stress responsive; stressed animal displaying <10% difference in its average percentage change was regarded as stress unresponsive. Stressed animals ($n = 2$) with an average percentage change of 10–30% were excluded from further experiments.

2.2. Cell culture

Primary astrocyte from day 1 mice pups was performed as previously described (Feng et al., 2011). Briefly, whole cortex were isolated from neonatal mice and digested in 0.25% trypsin at 37 °C. The dissociated cells were cultured in poly-D-lysine (5 μg/mL) coated flasks in DMEM/F12 (10% FBS) at 37 °C under 5% CO_2. Neurons and microglia were removed by shaking the flasks in the culture box for 15 h at 250 rpm, and the 3-week old cells were used for the experiments. Cultures consisting of more than 95% astrocytes were determined by glial fibrillary acidic protein (GFAP) immunocytochemical staining.

Mouse hippocampal neuron cells (HT22 cells, from Salk Institute) were maintained with DMEM supplemented with 10% FBS and differentiated in neurobasal medium with N2 supplement for 3 days before treatment.

2.3. siRNAs and transfection

Scrambled RNA and siRNAs for RIP140 (5′-CGGCGTTGACATCAAAGAA-3′ and 5′-GCTTCTTTCTTTAATCTAA-3′, SI02698759, Qiagen) were transfected into astrocytes or HT22 cells to knockdown RIP140 using HiPeFect transfect reagent (301707, QIAGEN) according to the manufactural instruction. siRNA for miR33 (SI02655450, Qiagen) was transfected into astrocytes to knockdown miR33 by HiPeFect transfect reagent.

2.4. Gene expression assay by quantitative real-time PCR (qPCR)

Total RNA was isolated from astrocytes and HT22 cells or the different brain region by TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA samples' cDNA was synthesized using Omniscript RT kit (QIAGEN), and qPCR was performed with SYBR-Green QPCR reagent (Agilent, Santa Clara, CA, USA) and detected by the Mx3005P QPCR system (Agilent). QPCR primer sequences were presented in Supplemental Table 1.

The miR33 expression level was detected using real-time PCR with miR33 specific primer (MS00032697, Qiagen).

2.5. Immunofluorescence (IF) assay

Animals were anesthetized and transcardially perfused with 0.9% saline followed by 4% paraformaldehyde. Brain samples were post fixed with 4% paraformaldehyde overnight and equilibrated in 20% and 30% sucrose. Coronal sections of 15 μm were prepared with a sliding microtome. IF was performed using as primary antibody rabbit anti-RIP140 and Bip or goat anti-GFAP IgG (Abcam, ab42126, ab21685, ab7260) and as secondary antibody FITC, Cy3 or Cy5 conjugated monkey anti-rabbit IgG (Santa Cruz). Nuclei were stained by DAPI (Sigma–Aldrich). Images were acquired with an Olympus FluoView 1000 IX2 upright confocal microscope. The fluorescence intensity presenting RIP140 or Bip level was calculated with Zen 2011(ZEISS) software.

2.6. Cholesterol assay

Cellular and brain tissue cholesterol content were detected with Amplex® Red Cholesterol Assay Kit (Invitrogen, A12216) according