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Emotional regulatory function of receptor interacting protein 140 revealed in the ventromedial hypothalamus

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ABSTRACT

Receptor-interacting protein (RIP140) is a transcription co-regulator highly expressed in macrophages to regulate inflammatory and metabolic processes. However, its implication in neurological, cognitive and emotional conditions, and the cellular systems relevant to its biological activity within the central nervous system are currently less clear. A transgenic mouse line with macrophage-specific knockdown of RIP140 was generated (MΦRIPKD mice) and brain-region specific RIP140 knockdown efficiency evaluated. Mice were subjected to a battery of tests, designed to evaluate multiple behavioral domains at naïve or following site-specific RIP140 re-expression. Gene expression analysis assessed TNF-α, IL-1β, TGF-1β, IL1-RA and neuropeptide Y (NPY) expression, and in vitro studies examined the effects of macrophage's RIP140 on astrocytes' NPY production. We found that RIP140 expression was dramatically reduced in macrophages within the ventromedial hypothalamus (VMH) and the cingulate cortex of MΦRIPKD mice. These animals exhibited increased anxiety- and depressive-like behaviors. VMH-targeted RIP140 re-expression in MΦRIPKD mice reversed its depressive- but not its anxiety-like phenotype. Analysis of specific neurochemical changes revealed reduced astrocytic-NPY expression within the hypothalamus of MΦRIPKD mice, and in vitro analysis confirmed that conditioned medium of RIP140-silnenced macrophage culture could no longer stimulate NPY production from astrocytes. The current study revealed an emotional regulatory function of macrophage-derived RIP140 in the VMH, and secondary dysregulation of NPY within hypothalamic astrocyte population, which might be associated with the observed behavioral phenotype of MΦRIPKD mice. This study highlights RIP140 as a novel target for the development of potential therapeutic and intervention strategies for emotional regulation disorders.

1. Introduction

Receptor-interacting protein 140 (RIP140), also known as nuclear receptor-interacting protein 1 (NRIP1), is recognized for its functional role as a wide-spectrum transcriptional co-regulator (Ho and Wei, 2012). A significant body of evidence demonstrates that translocation and accumulation of RIP140 in the cytoplasm promotes adipocyte dysfunctions, thus contributing to the development and progression of metabolic disease via altered regulation of glucose uptake, adiponectin secretion and lipolysis (Ho and Wei, 2012). In addition, RIP140 has been found to affect inflammatory potential in macrophages through its function as a co-activator for NF-κB to promote pro-inflammatory cytokine production following the administration of Toll like-Receptor ligands (Ho et al., 2012).

In difference from the established roles of RIP140 in modulating macrophage's activity to regulate metabolic and inflammatory processes, its involvement in human neurological, cognitive and emotional disorders and/or in animal models for such conditions has only recently been implicated. For instance, altered expression of RIP140 has been identified in Down Syndrome patients (Gardiner, 2006) and in a rodent model of aging (Ghosh and Thakur, 2009). In addition, it was suggested as a candidate gene in autism (Iurov et al., 2010) and in experience-dependent cortical plasticity (Heimel et al., 2008). Recently, direct evidence for its effects on cognitive and emotional processes was provided by Duclot et al. (2012), who demonstrated long-term memory and stress–response deficits in adult mice with constitutive, whole body inactivation of RIP140 gene expression.

In the central nervous system (CNS) of human and rodents, RIP140 has been detected in various regions [e.g., cerebral cortex, hippocampus and pituitary gland (Gardiner, 2006; Duclot et al., 2012; Zhang et al., 2007)] and cell types [neurons and immune

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cells (Duclot et al., 2012; Xue et al., 2013)]. Delineating the contributions of RIP140 expression in specific CNS targets to the rigorously regulated cognitive and emotional responses may advance our understanding of the mechanisms by which the brain translates external and internal stimuli into integrated biological responses and point the way toward selective next-generation intervention strategies aimed to promote human mental and emotional well-being. One of these targets may be the immune system. CNS-induced immune activation has been associated with various neurophysiological processes, entailing both functional neurodestructive and neuroprotective properties (Kerschensteiner et al., 2009; Hohlfeld et al., 2007; Smith et al., 2012; Minagar et al., 2002). While several studies indicate that microglia and monocyte-derived macrophages stimulation may yield protective effects via the production of neurotrophic factors including nerve growth factor, brain-derived neurotrophic factor (BDNF), and neurotrophin-4/5 (Smith et al., 2012; Minagar et al., 2002), dysregulation of macrophage/microglia-induced cytokine signaling has also been linked with the occurrence of a number of pathological conditions including schizophrenia, anxiety/depression, cognitive dysfunction and neurological diseases (London et al., 2013; Salim et al., 2012; Kohman and Rhodes, 2013). In line with the pivotal role of RIP140 in modulating the inflammatory potential of macrophages (Ho et al., 2012; Chen, 2012) we chose to explore the behavioral effects of RIP140-silencing within macrophage-lineage.

A mouse line with macrophage-specific knockdown of RIP140 (MΦRIPKD), was generated on a C57BL6 background (Ho et al., 2012), and mice were subjected to a test battery which evaluated multiple behavioral domains at naïve or following site-specific RIP140 re-expression. Gene expression analysis evaluated brain-region specific RIP140 knockdown and re-expression efficiency as well as possible neurochemical correlates of RIP140 expression alternation. These included the pro-inflammatory cytokines TNF-α and IL-1β and the anti-inflammatory cytokines TGF-1β and IL1-RA, genes implicated in CNS-derived neuroinflammatory processes, depression, stress and anxiety in human and animal models [e.g., (Salim et al., 2012; Leonard and Myint, 2009)]. Because of the involvement of neuropeptide Y (NPY) in the etiology of mood, stress and resilience processes (Heilig, 2004; Hughes, 2012; Wu et al., 2011) and its known action in adaptive and innate immunity (Malva et al., 2012; Dimitrijevic and Stanojevic, 2013), we also examined the expression level of this neuromodulator agent. Finally, in vitro studies were performed to examine if RIP140 expression in macrophages could modulate NPY production from astrocytes.

2. Method

For procedural details of behavioral assays, Reverse-Transcriptase PCR, immunofluorescence, western blotting and astrocytes viability assay, see SI Appendix.

2.1. Animals and housing

MΦRIPKD male mice and WT littermates were bred in the animal facility of the University of Minnesota and used at 9–12 weeks of age. Mice were housed in a temperature controlled room (22 ± 1 °C) on a 14/10 light dark cycle (lights on/off at 0600/2000) with ad-lib food and water. Experimental procedures were conducted according to NIH guidelines and approved by the University of Minnesota Institutional Animal Care and Use Committee. For the derivation of transgenic mice that overexpress shRNA to target RIP140 in macrophage lineage, the shRNA for RIP140 was mimicked as endogenous microRNA following reported method (Rao and Wilkinson, 2006). The expression of this shRNA was driven by hCD68 promoter (Vats et al., 2006) and the expression DNA fragment was cloned into pWhere vector. Transgenic DNA fragment was excised by PacI and then injected into C57BL/6 mouse oocytes (Mouse genetics laboratory, University of Minnesota). Transgenic founder mice were genotyped by PCR using the following primer set: Forward: 5′-GAGTTCTCAGACGCTGGAAA GCC-3′ and Reverse" 5′-GTCCAATTATGTCACACCACAGAAG-3′ (for additional information, see Fig. 1A and B). F1–F3 progeny were used for this study.

2.2. Experimental design

The effect of RIP140 knockdown on the behavioral phenotype of naïve mice was evaluated using 4–11 MΦRIPKD mice and 4–7 WT littermates, subjected to a test battery designed to assess general activity, balance/motor coordination, muscle strength, sensory functioning, pain sensitivity, anxiety and depressive-like behaviors. The behavioral effect of RIP140 re-expression in MΦRIPKD and WT mice was evaluated using 9 RIP140-OE-lentivirus-injected MΦRIPKD mice, 9 GFP-control-injected MΦRIPKD mice and 8 GFP-control-injected WT mice (following the exclusion of 3 GFP-control-MΦRIPKD and one WT mice from analysis based on lentivirus-localization/expression analysis and the inability of one MΦRIPKD-RIP140-OE and one WT mice to recover from the surgical procedure). Gene expression analysis included 8–13 MΦRIPKD and 6–9 WT littermates. Experiments were performed at 2–3 partially overlapping replications and a separate cohort of mice was used for each replication.

2.3. Behavioral assessments

All behavioral assessments were performed during the light phase following habituation to the test room for 1 h on each day. General activity was assessed using the automated open field environments, motor coordination and balance using the grid walk test and the Rotarod apparatus, neuromuscular strength using the wire hang test, simple sensory functioning using the adhesive removal test and sensitivity to a painful stimulus using the hot plate test. These behavioral modalities were tested as major physical abnormalities (e.g., motor, muscular or sensory) may indicate that mice are sick or physically unable to perform the procedural aspects of more complex behavioral tasks (Crawley, 2008). The elevated plus maze (EPM), emergence test and light–dark box (LDB) were used for the assessment of anxiety-like behaviors. The forced swim test (FST) and tail suspension test (TST), common antidepressant- and depression-related behavioral-screening paradigms (Lockridge et al., 2013) were used for the assessment of depressive-like behaviors. These behavioral domains were tested based on (Duclot et al., 2012), which pointed to the possible involvement of RIP140 in stress-response and associated depressive-like behavioral phenotype. Tests were conducted either at minimal intrusion condition (*baseline phenotype*) or at a condition of behavioral stimulation, following stress-inducing assessments (*provoked phenotype*). For *baseline phenotype*, order of tests was arranged from the less to the more intrusive, a method commonly implemented in studies involving genetically altered mice (Crawley, 2008; McQuade et al., 2003) and suggested to partially overcome the effect of consecutive behavioral testing (Crawley, 2008). In this conditions, tests were also separated by 24 h (preliminary open field environments and EPM) or 2 h (grid walk, adhesive removal test, wire hang test, FST, repeated open field environments) interval. For *provoked phenotype*, tests were conducted 5 min following the termination of another, stress-inducing procedure (emergence test after repeated FST exposure, TST after hotplate exposure and LDB at the termination of the complete battery, for details, see Table S1). The behavioral battery used for the assessment of