

MICRORNA-132 DYSREGULATION IN *TOXOPLASMA GONDII* INFECTION HAS IMPLICATIONS FOR DOPAMINE SIGNALING PATHWAY

J. XIAO,^{a,*} Y. LI,^{a,b} E. PRANDOVSKY,^a
S. S. KARUPPAGOUNDER,^{c,d,h} C. C. TALBOT, Jr.^e
V. L. DAWSON,^{c,d,f,g,h,i} T. M. DAWSON^{c,d,g,h} AND
R. H. YOLKEN^a

^a Stanley Division of Developmental Neurovirology, Department of Pediatrics, Johns Hopkins School of Medicine, Baltimore, MD 21287, USA

^b Department of Psychiatry, Renmin Hospital, Wuhan University, Wuhan, PR China

^c Neuroregeneration and Stem Cell Programs, Institute for Cell Engineering, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

^d Department of Neurology, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

^e Institute for Basic Biomedical Sciences, Johns Hopkins School of Medicine, Baltimore, MD 21287, USA

^f Department of Physiology, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

^g Solomon H. Snyder Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

^h Adrienne Helis Malvin Medical Research Foundation, New Orleans, LA 70130-2685, USA

ⁱ Diana Helis Henry Medical Research Foundation, New Orleans, LA 70130-2685, USA

Abstract—Congenital toxoplasmosis and toxoplasmic encephalitis can be associated with severe neuropsychiatric symptoms. However, which host cell processes are regulated and how *Toxoplasma gondii* affects these changes remain unclear. MicroRNAs (miRNAs) are small noncoding RNA sequences critical to neurodevelopment and adult neuronal processes by coordinating the activity of multiple genes within biological networks. We examined the expression of over 1000 miRNAs in human neuroepithelioma cells in response to infection with *Toxoplasma*. MiR-132, a cyclic AMP-responsive element binding (CREB)-regulated miRNA, was the only miRNA that was substantially upregulated by

all three prototype *Toxoplasma* strains. The increased expression of miR-132 was also documented in mice following infection with *Toxoplasma*. To identify cellular pathways regulated by miR-132, we performed target prediction followed by pathway enrichment analysis in the transcriptome of *Toxoplasma*-infected mice. This led us to identify 20 genes and dopamine receptor signaling was their strongest associated pathway. We then examined myriad aspects of the dopamine pathway in the striatum of *Toxoplasma*-infected mice 5 days after infection. Here we report decreased expression of D1-like dopamine receptors (DRD1, DRD5), metabolizing enzyme (MAOA) and intracellular proteins associated with the transduction of dopamine-mediated signaling (DARPP-32 phosphorylation at Thr34 and Ser97). Increased concentrations of dopamine and its metabolites, serotonin (5-HT) and 5-hydroxyindoleacetic acid were documented by HPLC analysis; however, the metabolism of dopamine was decreased and 5-HT metabolism was unchanged. Our data show that miR-132 is upregulated following infection with *Toxoplasma* and is associated with changes in dopamine receptor signaling. Our findings provide a possible mechanism for how the parasite contributes to the neuropathology of infection. © 2014 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: *Toxoplasma gondii*, miR-132, dopamine receptor pathway, alteration in expression, mouse striatum.

INTRODUCTION

Toxoplasma gondii is an obligate intracellular pathogen within the phylum Apicomplexa. *Toxoplasma* is capable of infecting and replicating within virtually any nucleated mammalian or avian cell. Moreover, *Toxoplasma* is one of the few pathogens that regularly cross the placenta. Brain and eye lesions are the most common consequences of *in utero* infection. While infection of healthy adults is usually relatively mild, the tropism of *Toxoplasma* for brain tissue has been linked with specific behavioral changes in humans and in animals (Vyas and Sapolsky, 2010; Webster et al., 2013). In immunocompromised patients, severe neurological disease such as toxoplasmic encephalitis can occur due to either acute infection or reactivation of chronic infection. Taken together, these lines of evidence document that *Toxoplasma* infection has specific effects on the brain. However, which host cell processes are regulated and how the parasite effects these changes remain unclear.

*Corresponding author. Tel: +1-410-502-6825; fax: +1-410-955-3723. E-mail address: jxiao4@jhmi.edu (J. Xiao).

Abbreviations: 3-MT, 3-methoxytyramine; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; BDNF, brain derived neurotrophic factor; COMT, catechol-O-methyltransferase; DOPAC, 3, 4-dihydroxyphenylacetic acid; DRD1, dopamine receptor D1; DRD5, dopamine receptor D5; DARPP-32, dopamine- and cAMP-regulated neuronal phosphoprotein of molecular mass of 32 kDa; dpi, days post infection; HPLC, high-performance liquid chromatography; HVA, homovanillic acid; IPA, Ingenuity Pathway Analysis; LPS, lipopolysaccharides; MAOA, monoamine oxidase A; MAOB, monoamine oxidase B; miRNA, MicroRNA; PBS, Phosphate-buffered saline; PKA, protein kinase A; PP1, protein phosphatase 1; QC, quality control; qPCR, quantitative real-time PCR; TLR, Toll-like receptor.

Previous studies have indicated that *Toxoplasma* infection affects the levels of certain neurotransmitters (e.g. monoamines) and their metabolites in both the acute and chronic phases of infection (Stibbs, 1985; Gatkowska et al., 2013). Moreover, a study on rats has demonstrated that treatment with the dopamine antagonist haloperidol during the tachyzoite replicative stage diminishes the behavioral effects of *Toxoplasma* infection (Webster et al., 2006). In infected mice, dopamine uptake inhibitor GBR12909 modifies behavioral responses associated with latent toxoplasmosis (Skallová et al., 2006). It thus has been speculated that the dopaminergic system may be involved in the neurological effects of infection. Indeed, *Toxoplasma* harbors two genes encoding tyrosine hydroxylase catalyzing the rate-limiting step in dopamine biosynthesis (Gaskell et al., 2009), and an increase in dopamine level during infection of neural cells *in vitro* has been observed (Prandovszky et al., 2011).

Dopamine is a catecholamine neurotransmitter that controls a diverse range of physiological processes. Dopamine exerts its effects by acting on two primary receptor subtypes: D1-like (DRD1 and DRD5) and D2-like (DRD2, DRD3, and DRD4) receptors. Activation of D1-like receptors leads to the activation of adenylyl cyclase and increase in cyclic adenosine monophosphate (cAMP) and Ca^{2+} levels, whereas activation of D2-like receptors leads to a decrease in adenylyl cyclase and cAMP levels. DARPP-32 (dopamine and cyclic AMP-regulated 32-kDa phosphoprotein) was identified as a major target for dopamine-activated adenylyl cyclase in the striatum. Two phosphorylation sites, threonine-34 (Thr34) and threonine-75 (Thr75), make DARPP-32 a bifunctional signal transduction molecule that controls the activities of protein phosphatase 1 (PP1) and protein kinase A (PKA), and thereby controls the phosphorylation state and activity of many downstream physiological effectors (Nairn et al., 2004; Svenningsson et al., 2004). Disturbances of dopaminergic signaling have been implicated in many pathological conditions including Parkinson's disease, schizophrenia, attention-deficit/hyperactivity disorder and addiction. Not surprisingly, dopaminergic signaling in the central nervous system (CNS) is highly regulated and subject to precise temporal control (Kotowski et al., 2011).

MicroRNAs (miRNAs) comprise a class of small noncoding RNAs (~20–23 nt) that regulate gene expression. Dysregulation of a single miRNA can be sufficient to alter the gene-expression profile and developmental trajectory of cells (Lim et al., 2005; Friedman et al., 2009). Approximately 70% of known miRNAs are expressed in the nervous system, often with a high degree of spatial and temporal specificity (Krichevsky et al., 2003). MiR-132 is a cyclic AMP-responsive element binding (CREB)-regulated miRNA and is enriched in neuronal cells (Cheng et al., 2007). MiR-132 function has been suggested within both the nervous and the immune systems with the majority of function in a neuronal context. Dysregulation of miR-132 is associated with several neurological disorders, such

as schizophrenia, Alzheimer, Parkinson's disease and tauopathies (Miller et al., 2012; Wanet et al., 2012), suggesting a broader impact of this miRNA on diseases of brain development. A role for miRNAs in *Toxoplasma* infections was first documented in human fibroblasts, where the transcription of the miR-17/92 loci was specifically increased by two- to three-fold (Zeiner et al., 2010).

We first performed a comprehensive genomewide miRNA expression profiling of neural cells infected by *Toxoplasma*, with the objective of understanding the pathogenetic role of miRNA dysregulation during infection. This led us to identify the increased expression of miR-132 as a common effect of infection with three canonical *Toxoplasma* strains. In our further investigation on cellular pathways regulated by miR-132, we were surprised to find the dopamine receptor pathway had the strongest association. Therefore, we examined myriad aspects of the dopamine system in mice with acute *Toxoplasma* infection to evaluate the balance of dopaminergic neurotransmission. In addition to examining changes in the dopamine metabolism in the striatal regions of the mouse brain, we have evaluated the expression of genes that regulate dopaminergic pathways, such as dopamine receptors and some of the intracellular proteins associated with the transduction of dopamine-mediated signaling. Our findings support the hypothesis that abnormal dopamine signaling may account for numerous data suggesting some neuropsychiatric symptoms (e.g. mental impairment such as learning, motor disabilities and personality changes) observed in congenital toxoplasmosis and toxoplasmic encephalitis.

EXPERIMENTAL PROCEDURES

Infection of human neuroepithelioma cells

MiRNA expression profiles were measured in RNA samples obtained from SK-N-MC cells (ATCC, HTB-10) infected or mock infected with three major clonal *Toxoplasma* strains: (RH-2F (type I), PRU (type II) or CTG (type III)). SK-N-MC is a human neuroepithelioma cell line expressing neuronal characteristics (Barnes et al., 1981). Cell culture of parasite strains and infection of SK-N-MC cells were conducted as previously described (Xiao et al., 2011) except that cells were infected at multiplicity of infection 5. Infections and mock-infected controls (no tachyzoites) were performed for each strain on three separate occasions in order to have biological replicates. RNA was extracted 20 h following infection using the miRNeasy kit (QIAGEN, Valencia, CA, USA).

MiRNA profiling and analysis

MiRNA expression was profiled using Affymetrix miRNA 2.0 arrays containing 1, 105 human mature miRNA and carried out at the Johns Hopkins Deep Sequencing & Microarray Core Facility. Briefly, RNA samples were prepared following the Affymetrix FlashTag™ Biotin HSR RNA Labeling kit protocol (Affymetrix Inc., Santa

Download English Version:

<https://daneshyari.com/en/article/4337704>

Download Persian Version:

<https://daneshyari.com/article/4337704>

[Daneshyari.com](https://daneshyari.com)