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MICE DEFICIENT FOR WILD-TYPE P53-INDUCED PHOSPHATASE 1 DISPLAY ELEVATED ANXIETY- AND DEPRESSION-LIKE BEHAVIORS

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Abstract—Mood disorders are a severe health burden but molecular mechanisms underlying mood dysfunction remain poorly understood. Here, we show that wild-type p53-induced phosphatase 1 (Wip1) negatively responds to the stress-induced negative mood-related behaviors. Specifically, we show that Wip1 protein but not its mRNA level was downregulated in the hippocampus but not in the neocortex after 4 weeks of chronic unpredictable mild stress (CUMS) in mice. Moreover, the CUMS-responsive WIP1 downregulation in the hippocampus was restored by chronic treatment of fluoxetine (i.p. 20 mg/kg) along with the CUMS procedure. In addition, Wip1 knockout mice displayed decreased exploratory behaviors as well as increased anxiety-like and depression-like behaviors in mice without impaired motor activities under the non-CUMS condition. Furthermore, the Wip1 deficiency-responsive anxiety-like but not depression-like behaviors were further elevated in mice under CUMS. Although limitations like male-alone sampling and multiply behavioral testing exist, the present study suggests a potential protective function of Wip1 in mood stabilization.

Key words: Wip1, CUMS, exploratory behavior, anxiety-like behavior, depression-like behavior, fluoxetine.

INTRODUCTION

Mood disorders are a common type of severe mental illness in the world demonstrating co-occurring symptoms of anxiety and depression. A high suicide rate and a significant burden for society and family require effective treatment (Cassano and Fava, 2002). A combination of factors such as environmental, psychological, biological and genetic factors cause mood dysfunction (Ahn et al., 2009; Gillespie et al., 2009). So far, the most promising theory regarding mood dysfunction is related to the dysregulation of neurotransmitters such as serotonin (Young et al., 1994; Young and Leyton, 2002), dopamine (Diehl and Gershon, 1992; Brown and Gershon, 1993), gamma-aminobutyric acid (Petty, 1995; Krystal et al., 2001) and noradrenaline (Asnis et al., 1995; Brunello et al., 2003). Treatments through inhibition of serotonin uptake, such as selective serotonin reuptake inhibitors (SSRIs) have been widely used for depressed human patients (Vaswani et al., 2003). In the recent years, growing evidences have shown that deficiency in neurogenesis (Sablina et al., 2007; Snyder et al., 2011), increase in apoptosis (Eilat et al., 1999; Treusch et al., 2011) and increase in neuroinflammation (Raison et al., 2006; Miller et al., 2009) have also been implicated in the development of mood disorders. However, the molecular mechanisms underlying mood regulation remain poorly understood.

Wild-type p53-induced phosphatase 1 (Wip1), encoded by the protein phosphatase, Mg^{2+}/Mn^{2+}-dependent, 1D (PPM1D) gene is an oncogene initially identified in cells treated with gamma radiation (Fiscella et al., 1997). In addition, Wip1 has also shown negative responses to a variety of stresses including ultraviolet radiation (Takekawa et al., 2000), hydrogen peroxide (Oshima et al., 2007), anisomycin (Takekawa et al., 2000) and methyl methane sulfonate (Park et al., 2012). Being a serine/threonine phosphatase, Wip1 negatively

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Abbreviations: CUMS, chronic unpredictable mild stress; EOM, elevated zero maze test; FST, forced swimming test; MAPK, mitogen-activated protein kinase; OFT, open field test; PPM1D, Mg^{2+}/Mn^{2+}-dependent, 1D; SPT, sucrose preference test; SSRIs, selective serotonin reuptake inhibitors; TST, tail suspension test; Wip1, Wild-type p53-induced phosphatase 1; WT, wild-type.

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regulates mitogen-activated protein (MAP) kinase p38 by dephosphorylation of a threonine residue essential for p38 MAP kinase activation (Takekawa et al., 2000; Gururajan et al., 2014; Lu et al., 2014). Also as a stress-stimulated molecule (Sun et al., 2013; Zhou et al., 2014), the activation of p38 MAPK is associated with a number of disease-related signaling pathways such as apoptosis (Fukunaga et al., 2004; Van Laethem et al., 2004) and neuroinflammation (Herlaar and Brown, 1999; Zwerina et al., 2006), whereas selective inhibition of p38 MAPK exhibits anti-depressant and anxiolytic-related behaviors in rodents (Bruchas et al., 2007, 2011; Corsi et al., 2011, Peng et al., 2013). The positive stress-responsive p38 MAPK suggests an opposite role of Wip1 in mood regulation. Similarly, increasing evidences have shown that Wip1 may also inhibit the pro-apoptotic and pro-inflammatory pathways respectively through the deactivation of its other dephosphorylating substrates, p53 (Takekawa et al., 2000; Yu et al., 2007; Bachis et al., 2008) and NF-kappaB (Chew et al., 2009; Lowe et al., 2012; Demirtaş et al., 2014). Wip1 have also demonstrated a role in regulating neurogenesis through p53-dependent cell cycle control (Demidov et al., 2007; Zhu et al., 2009). Therefore, we hypothesize that Wip1 may play a regulatory role against stress-induced mood behaviors.

To test our hypothesis, we examined the expression profile of Wip1 in the neocortex and hippocampus of mice under chronic unpredictable mild stress (CUMS). We also examined the Wip1 protein expression in the hippocampus of CUMS mice under chronic treatment of fluoxetine. Finally, we measured the exploratory, anxiety-like and depression-like behaviors in Wip1 knockout mice under stress-free and stress conditions by the open field test (OFT), elevated zero maze test (EOM), tail suspension test (TST) and forced swimming test (FST).

EXPERIMENTAL PROCEDURES

Animals

C57BL/6 mouse breeders were purchased from the Vital River Laboratories (Beijing, China), and *Wip1*+/− mouse breeders (Choi et al., 2002) were provided by Dr. L. A. Donehower from the Baylor College of Medicine. All animals were bred in the animal house of the Kunming Medical University (KMU). 8∼10 weeks of C57BL/6 mice were used for experiments 1 (Wip1 expression in CUMS model) and 2 (fluoxetine treatment in CUMS model), and 8∼10 weeks of littermates of *Wip1*+/+ (wild-type (WT)) and *Wip1*−/− were used for experiment 3 (testing of mood behaviors in *Wip1*−/− mice). Animals for experiments were socially-housed (< five animals per cage) with water and food available *ad libitum* under standard housing conditions (12-h light/12-h dark cycles, 22 ± 1 °C, 52 ± 2% humidity). All procedures involving animals were conducted between 7 a.m. and 7 p.m., in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Department of Laboratory Animal Science of Kunming Medical University.

CUMS procedure

CUMS was performed as previously described (Ruan et al., 2014; Yang et al., 2014a). Briefly, as control mice were socially housed and undisturbed, mice for CUMS were singly housed and daily experienced a random stimulus of 10 for a specified duration. Stimuli were cold water swimming (13 ± 1 °C, 5 min) (A), warm water swimming (37 ± 2 °C, 5 min) (B), moist bedding (8 h) (C), cage tilt (45°, 8 h) (D), cage shaking (180 rpm, 10 min) (E), tail pinch (1 cm from the tail end, 1 min) (F), food deprivation (12 h) (G), water deprivation (12 h) (H), overnight illumination (12 h) (I) and no stress (24 h) (J). To examine the effect of CUMS on animals, OFT was weekly performed starting from the beginning, while EOM, TST as well as FST were performed at the beginning and end of the CUMS procedure.

Drug administration

To intervene the progression of mood behaviors in mice under CUMS, fluoxetine (H20110442, Lilly, Fegersheim, France), a clinical antidepressant, was used to treat the CUMS mice daily along with the CUMS procedure by intraperitoneal injection (20 mg/kg, dissolved in saline) (Chouinard, 1985; Dulawa et al., 2004; Ruan et al., 2014). Meanwhile, non-stressed mice with saline, non-stressed mice with fluoxetine and CUMS mice with saline were set as controls.

Behavioral tests

Sucrose preference test (SPT). SPT was performed as previously described (Ruan et al., 2014). Briefly, animals were trained to drink from two bottles of 1% (w/v) sucrose solution for 24 h, and one bottle of 1% sucrose solution and one bottle of tap water for the next 24 h. After another 24 h of food and water deprivation, one bottle of 1% sucrose solution and one bottle of tap water were given to the animals for 1 h, and the amount of sucrose and water consumptions were measured. The suppression of sucrose consumption indicated the depression-like behavior (Willner et al., 1987; Vollmer et al., 2013).

OFT. OFT was performed as previously described (Ruan et al., 2014). Briefly, animals were placed in an open arena and recorded by a video camera for 5 min. Travel distance, turn angle, entries in the peripheral zone, entries in the central zone and percentage of time spent in the central zone were automatically analyzed by ANY-maze video-tracking software (Stoelting, Wood Dale, IL, USA), while rearing numbers were counted manually blinding to the treatment. Travel distance and turn angle (Duffy and Ford, 1997; Spink et al., 2001; Venkitaramani et al., 2011) in the peripheral zone respectively indicated the locomotor and non-locomotor movements of the body, while travel distance and turn angle in the central zone as well as rearing indicated the exploratory behaviors. Entries in the peripheral zone and the central zone, as well as the percentage of time spent in the central zone