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Long Non-coding RNA BC168687 is Involved in TRPV1-mediated Diabetic Neuropathic Pain in Rats

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Abstract—Long noncoding RNAs (lncRNAs) participate in a diverse range of molecular and biological processes, and dysregulation of lncRNAs has been observed in the pathogenesis of various human diseases. We observed alterations in mechanical withdrawal thresholds (MWT) and thermal withdrawal latencies (TWL) in streptozotocin (STZ)-induced diabetic rats treated with small interfering RNA (siRNA) of lncRNA BC168687. We detected expression of transient receptor potential vanilloid type 1 (TRPV1) in rat dorsal root ganglia (DRG) by a series of molecular experiments. We determined relative levels of tumor necrosis factor (TNF)-α and interleukin (IL)-1β in rat serum by enzyme-linked immunosorbent assay (ELISA). In addition, we examined extracellular regulated protein kinases (ERK) and p38 mitogen-activated protein kinase (MAPK) signaling pathways by Western blot (WB). We showed that the MWT and TWL of diabetic rats increased significantly compared with control. Expression of TRPV1 receptors in DRG substantially decreased. Relative levels of TNF-α and IL-1β in the serum of lncRNA BC168687 siRNA-treated rats were reduced. Phosphorylation (p)-ERK and p-p38 signaling pathways in DRG were also decreased. Taken together, we concluded lncRNA BC168687 siRNA may alleviate TRPV1-mediated diabetic neuropathic pain.

Key words: diabetic neuropathic pain, long non-coding RNAs, DRG, TRPV1.

INTRODUCTION

Diabetic neuropathic pain (DNP) is a frequent complication of both type 1 and type 2 diabetes. Approximately 40–50% of diabetic patients not only have typical symptoms of hyperglycemia and dyslipidemia, but also suffer hyperalgesia, allodynia, and spontaneous pain (Mima, 2013; Didangelos et al., 2014; Gao and Zheng, 2014; Schreiber et al., 2015; Tian et al., 2016). DNP is thought to be related to primary injury or dysfunction in the peripheral nervous system (PNS) or central nervous system (CNS). DNP may involve incremental hyperalgesia to noxious stimuli, such as painful mechanical, heat, or chemical irritation (Gao and Zheng, 2014). Nevertheless, the pathogenesis of DNP remains unclear. With the increasing prevalence of DNP in diabetes, it is more important than ever to understand the etiological and regulatory mechanisms (Didangelos et al., 2014; Lu et al., 2017; Tong et al., 2017).

Transient receptor potential vanilloid type 1 (TRPV1) is a non-selective cation channel that may be a critical mediator in signal transduction of inflammatory pain (Premkumar and Abooj, 2013; Grace et al., 2014; Premkumar, 2014). TRPV1 receptors are activated by capsaicin, heat (> 43 °C) and other physical and chemical noxious stimuli. The receptor is distributed in small and medium-sized neurons in dorsal root ganglia (DRG), nodose ganglia (NG), and trigeminal ganglia (TG) (Levine and Alessandri-Haber, 2007; Spicarova and Palecek, 2008; Premkumar and Abooj, 2013). The TRPV1 channel consists of six transmembrane structures, including homo- and hetero-tetramers, with each subunit assembled as the cation channel pore (Palazzo et al., 2010). Adenosine 5′-triphosphate (ATP) activates TRPV1 by binding directly to a domain between ankyrin repeats 1–3 (Brito et al., 2014). Studies have shown that TRPV1 receptors act as pre-pain mediators in models of inflammatory pain, where they participate in generation and enhancement of pain sensitivity (Lee et al., 2005; Premkumar and Sikand, 2008; Huang et al., 2013; Brito et al., 2014; Morales-Lazaro et al., 2016). In addition,

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Abbreviations: DNP, diabetic neuropathic pain; DRG, dorsal root ganglia; FPG, fasting plasma glucose; lncRNAs, long noncoding RNAs; MAPK, mitogen-activated protein kinase; MWT, mechanical withdrawal thresholds; PBS, phosphate buffer saline; PNS, peripheral nervous system; SGCs, satellite glial cells; siRNA, small interfering RNA; STZ, streptozotocin; TNF, tumor necrosis factor; TRPV1, transient receptor potential vanilloid type 1; TWL, thermal withdrawal latencies.

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P2X receptors are known for their roles in the central and PNSs. Activated purinergic P2X receptors contribute to neuropathic pain by releasing ATP (Hanani, 2012; Huang et al., 2013; Brito et al., 2014). Thus, sensitization of TRPV1 and P2X receptors may lead to chronic inflammatory pain and peripheral neuropathy in diabetes (Premkumar and Sikand, 2008; Brederson et al., 2013; Kaneko and Szallasi, 2014; Chiba et al., 2017).

Long noncoding RNAs (LncRNAs) are non-protein-coding RNA transcripts longer than 200 nucleotides that participate in various biological processes and diseases (Ponting et al., 2009; Schmitz et al., 2016; Wu et al., 2017). Evolutionarily conserved in mammals, lncRNAs mediate various regulatory functions in gene expression networks, including regulating transcriptional states, modifying nuclear architecture, splicing, and mRNA translation (Ponting et al., 2009; Schmitz et al., 2016; Wu et al., 2017). Dysfunction of lncRNAs may participate in the pathogenesis of several human diseases (Ponting et al., 2009; Schmitz et al., 2016). Inhibitors of TRPV1 receptors in terminals of primary sensory neurons effectively attenuate inflammation and neuropathic pain in several animal models (Bolcskei et al., 2005; Sousa-Valente et al., 2014; Morales-Lazaro et al., 2016; Berta et al., 2017; Leo et al., 2017). In the present study, the expression of TRPV1 receptors was down-regulated in the DRG of DNP rats as a result of treatment with lncRNA BC168687 small interfering RNA (siRNA). This suggested that lncRNA BC168687 siRNA alleviated the generation of chronic pain mediated by TRPV1.

EXPERIMENTAL PROCEDURES

Animal model and groups

Healthy male Sprague–Dawley rats (weight 200–250 g, 24 rats in total) were obtained from the Medical Animal Experimental Center of Nanchang University. Rats were housed in clean standard metabolic cages on a 12-h light/dark cycle with free access to food and water in the environment of 40–70% humidity and 20–25 °C. The treatment of rats followed the regulations of the Care and Use of Animals Ethics Committee. A high-sugar and high-fat diet (general feed 66.5%, cholesterol 2.5%, sodium cholate 1%, lard 10%, sucrose 20%) was provided for 4 weeks. Impairment of pancreatic β-cell function was induced by intraperitoneal injection of streptozotocin (STZ) (35 mg/kg). At 72 h after injection, we collected blood from the tail vein, and measured fasting plasma glucose (FPG) and mechanical withdrawal thresholds (MWT). When FPG > 16.7 mmol/L and MWT < 15 g, the animals were considered as STZ-induced diabetic rats (Gao and Zheng, 2014). The animals were then separated into a healthy rat group (Control), a DNP model group (DNP), a DNP injected with BC168687 siRNA group (DNP + BCsi), and a DNP injected with negative control siRNA group (DNP + NCsi). All rats participated in each and every procedure with six for each group. In order to screen effective duplexes-siRNA of lncRNA BC168687, three sequences were synthesized by NOVOBIO Company (Shanghai, China) (Table 1). BC168687-rat-2400 was obtained by targeted-siRNA by cell transfection: 25-μl transfection complexes consisting of siRNA were transfected into rats by intrathecal injection using Entranster™-*in vivo* transfection regents (18668-11-1, Engreen Biosystem Co, Ltd., Beijing, China). Control and DNP groups were injected with equivalent volumes of saline. MWT and thermal withdrawal latencies (TWL) were measured on the 2nd, 4th, 6th and 8th days after injection. After measurement of MWT and TWL at the 8th day, 10% chloral hydrate was used to euthanize the rats, and DRG were isolated from each group.

Mechanical withdrawal threshold (MWT)

MWT was measured with an Electrical Mechanical Analgesia Tester (EMAT) (BME-410C, XiHuaYi, Beijing, China). The threshold of painful sensitivity was determined by mechanical-pressure stimuli. Rats were set free in a transparent plastic chamber (20 × 15 × 20 cm), with the bottom made of stainless steel mesh (1 × 1 cm grid). The environment was maintained quiet, and temperature was maintained at 20–25 °C. At the beginning of the trials, all rats were allowed to adapt to the new environment. The EMAT was applied to detect withdrawal responses to mechanical stimulation. We used filaments with stochastic bending force to stimulate mid-plantar glabrous skin. We selected six peak values and averaged them to represent MWT.

Table 1. The siRNAs of lncRNA BC168687 and relative primer duplexes

Gene name	Gene sequences
BC168687 siRNA-rat-159	Sense 5′-GAGAUUAUUAAGGUGUACUTT-3′
	Antisense 5′-AGUACACCUUAAUAAUCUCTT-3′
BC168687 siRNA-rat-1172	Sense 5′-GACGGUUGAUACUGACUCUTT-3′
	Antisense 5′-AGAGUCAGUAUCAACCGUCTT-3′
BC168687 siRNA-rat-2400	Sense 5′-GUUGGAUCCUUCUCAAUCATT-3′
	Antisense 5′-UGAUUGAGAAGGAUCCAACTT-3′
Negative Control siRNA	Sense 5′-UUCUCCGAACGUGUCACGUTT-3′
	Antisense 5′-ACGUGACACGUUCGGAGAATT-3′
TRPV1 151 bp	Forward primer 5′-CTGCCTACTATCGGCCTGTG-3′
	Reverse primer 5′-GGTCGCCTCTGCAGGAAATA-3′
Actin 111 bp	Forward primer 5′-CCTAAGGCCAACCGTGAAAAGA-3′
	Reverse primer 5′-GGTACGACCAGAGGCATACA-3′

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