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Research Report

Involvement of NFκB signaling in mediating the effects of GRK5 on neural stem cells

*Yun Zhang, Guang-li Shen, Li-juan Shangguan, Yang Yu, Mao-lin He**

Department of Neurology, Beijing Shijitan Hospital, Capital Medical University, 10 TieYi Road, Haidian District, Beijing 100038, PR China

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ABSTRACT

Nuclear factor κB (NFκB) signaling plays ubiquitous roles in inflammation, immune response and neurogenesis. G protein-coupled receptor kinase 5 (GRK5) can protect neurons from degeneration. GRK5 also mediates tumor necrosis factor-α (TNFα)-induced NFκB signaling through the phosphorylation of IκBα. Here, we show that NFκB signaling is involved in neural stem cell (NSC) differentiation. The IκBα/p65 pathway was activated by phorbol myristate acetate (PMA), a stimulator of protein kinase C (PKC). Once the NFκB was activated, the initial stage of neural differentiation was induced, with an increased level of GRK5 in NSCs. This finding was reversed in response to the NFκB inhibitor N-acetyl cysteine (NAC). To evaluate the effect of GRK5-NFκB signaling crosstalk on NSC neurogenesis and apoptosis, GRK5 was knocked down by siRNAs in cell culture. SiRNAs against GRK5 not only impaired neural differentiation and axogenesis, but also induced apoptosis of NSC. GRK5 knockdown affected the transcription of NFκB, phosphorylation of the liver kinase B1 (LKB1) and the activity of caspase 3, thereby modulated neurogenesis and apoptosis. Taken together, our findings reveal a novel function of GRK5 in neurogenesis and provide insight into the molecular mechanisms underlying neurodevelopmental disorders and neurodegenerative diseases.

1. Introduction

Abnormal neurogenesis contributes to a variety of disorders, including neurodevelopmental defects, stroke and neurodegenerative diseases (Lazarov and Marr, 2013). During adult neurogenesis, NSCs follow a differentiation pattern that is similar to embryonic neurogenesis (Gonzalez-Perez et al., 2012; Encinas et al., 2013; Furutachi et al., 2013). Although many extrinsic factors and intrinsic proteins have been identified in the regulation of neurogenesis, the underlying signaling pathways and molecular mechanisms remain poorly understood (Govindarajan and Kempermann, 2014).

G protein-coupled receptor (GPCR) kinases (GRKs) regulate GPCR signaling by inducing receptor desensitization. Among

Abbreviations: AD, Alzheimer's disease; GPCR, G protein-coupled receptor; GRK, G-protein receptor kinase; IL-6, interleukin-6; IL-1β, interleukin-1β; NAC, N-acetyl cysteine; NFκB, nuclear factor κB; NPCs, neural progenitor cells; NSCs, neuron stem cells; PKA, protein kinase A; PMA, phorbol myristate acetate; TNFα, tumor necrosis factor-α

*Corresponding author.
E-mail address: maolinh09@sina.com (M.-l. He).

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the GRK family members, GRK5 is ubiquitously expressed in mammalian tissues. GRK5 can regulate GPCR signaling via 7-transmembrane receptors, several of which have been implicated in neurodegenerative diseases (Liu et al., 2010; Suo and Li, 2010). Recent studies have demonstrated that GRK5 expression is decreased in Alzheimer's disease (AD) (Suo et al., 2004; Suo and Li, 2010). Furthermore, GRK5 deficiency alone can enhance axonal defects and promote apoptosis during aging, leading to dementia (Suo et al., 2007). Interestingly, GRK5 has been shown to interact with and affect NFκB signaling. NFκB proteins are a family of ubiquitously expressed transcription factors that regulate cellular proliferation, apoptosis and cell cycle progression (Imielski et al., 2012). GRK5 can mediate the phosphorylation of IκBα in Raw264.7 macrophages (Patial et al., 2009). As IκBα is one of the key factors in NFκB activation (Brasier, 2006; Imielski et al., 2012), TNFα-induced NFκB signaling may be mediated by GRK5 (Patial et al., 2009). In contrast, recent studies have demonstrated that NFκB plays a critical role in the regulation of GRK5 transcription in myocytes (Crampton and O'Keeffe, 2013). It is inferred that a reciprocal link may present between these two systems.

Although some aspects of GRK5-dependent signaling have been well established (Suo et al., 2004; Suo and Li, 2010), its function in neurogenesis and apoptosis remains unclear. Based on previous studies, we hypothesized that GRK5 could enhance NFκB transcriptional activity and consequently promote neural differentiation and inhibit apoptosis of NSCs. In this study, we treated primary NSCs obtained from E14.5 rat embryos brain with specific GRK5 siRNA or activators of the NFκB pathway. We investigated whether GRK5 affects IκBα cellular levels and NFκB transcriptional activity. Moreover, we assessed the effects of GRK5 on NFκB-dependent phenotypes *in vitro*, such as NSCs proliferation and differentiation, axogenesis and apoptosis. Our observations provide new insight into the functional role of GRK5.

2. Results

2.1. PMA increases GRK5 mRNA levels and protein levels in NSCs

The NFκB proteins are a family of transcription factors that consist of five members: p65 (RelA), RelB, c-Rel, NFκB1 (p50 and its precursor 105) and NFκB2 (p52 and its precursor p100). The p65–p50 heterodimer is transcriptional activator of the canonical NFκB pathway. While the p50–p50 homodimer can repress the expression of their target genes (Yamamoto and Gaynor, 2004). PMA is known to activate several PKC isoforms, which can regulate the activation of NFκB in several tissue types (Churchill et al., 2008). Using RT-PCR, we determined the effects of PMA on mRNA expression of GRK5 in NSCs. As shown in Fig. 1A, 20 nM of PMA induced the expression of p65 and p50 within 1 h, and this effect was maintained for over 24 h. Importantly, GRK5 level increased after NSCs were treated with PMA (Fig. 1A). Moreover, Western blot analysis showed enhanced p65, p50 and GRK5 expression at 24 h post-PMA treatment (Fig. 1B and C, $P<0.01$). These results suggest that PMA can increase the expression of NFκB and GRK5.

2.2. Alterations in NFκB expression affect GRK5 protein levels and neural differentiation of NSCs/NPCs (neural progenitor cells)

Moreover, we examined the effect of PMA and an NFκB inhibitor (NAC) on GRK5 protein levels and the differentiation of NSCs. We cultured NSCs in suspension culture in the presence of PMA or NAC alone or in combination for 24 h prior to differentiation. There were four groups in this study: control (without PMA nor NAC treatment), PMA(20 nM) treatment, NAC(5 mM) treatment, and PMA+NAC treatment (where NSCs were maintained in the presence of PMA+NAC) (Islam and Koch, 2012).

Western blot was used to determine the effect of PMA±-NAC on the protein levels of NFκB and GRK5 in NSCs. PMA increased GRK5, p65 and p50 protein levels (Fig. 2A and B, $P<0.01$). And NAC partially attenuated PMA-induced increase in GRK5, p65 and p50 levels (Fig. 2A and B, $P<0.01$). We deduced that inhibition of NFκB with NAC cannot entirely block the stimulation of PMA on this pathway. Decreased expression of p50 and p65 when NFκB was inhibited by NAC

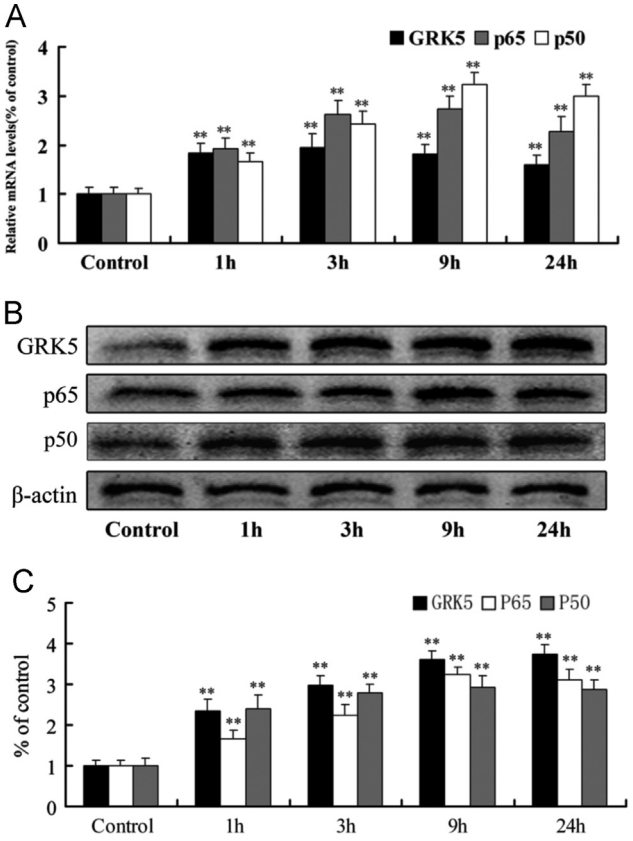

Fig. 1 – Effects of PMA on the expression of GRK5 and NFκB in cultured NSCs. NSCs were incubated with 20 nM PMA for different times. (A) GRK5, p65 and p50 mRNA levels were quantified using real-time PCR. The units were arbitrary with the control, and expressed as means±SD ($N=6$). (B) GRK5, p65 and p50 NFκB proteins were analyzed using Western blot with β-actin as a loading control. (C) Semi-quantitative analyses for Western blot in panel (B). **, $P<0.01$ *versus* control; Student's *t*-test ($N=6$).