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Research Report

Involvement of phosphatidylinositol 3-kinase/Akt on basic fibroblast growth factor-induced glial cell line-derived neurotrophic factor release from rat glioma cells

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ABSTRACT

Basic fibroblast growth factor (FGF-2) has a neuroprotective effect. Astrocytes support neurons by releasing neurotrophic factors including glial cell line-derived neurotrophic factor (GDNF). FGF-2 stimulates GDNF synthesis in astrocytes and the release. It has been reported that FGF-2 induces the activation of p44/p42 mitogen-activated protein (MAP) kinase, stress-activated protein kinase/c-*Jun* N-terminal kinase (SAPK/JNK) and p38 MAP kinase in C6 glioma cells, and that FGF-2 stimulates GDNF release through p44/p42 MAP kinase or SAPK/JNK, but not p38 MAP kinase. In the present study, we investigated the exact mechanism of FGF-2-induced GDNF release from C6 cells. FGF-2 induced the phosphorylation of Akt and its substrate, glycogen synthase kinase 3β (GSK3β) in addition to three MAP kinases in these cells. FGF-2-stimulated release of GDNF was suppressed by wortmannin (a phosphatidylinositol 3 (PI3)-kinase inhibitor) or LY294002 (another PI3-kinase inhibitor). The FGF-2-induced GDNF release from PI3-kinase-downregulated C6 cells was decreased compared with that in control siRNA-transfected cells. PD98059 (an inhibitor of MEK 1/2) or SP600125 (an inhibitor of SAPK/JNK), which suppressed FGF-2-induced phosphorylation of p44/p42 MAP kinase or SAPK/JNK respectively, did not affect FGF-2-induced Akt phosphorylation. Wortmannin or LY294002, which attenuated FGF-2-induced phosphorylation of Akt and GSK3β, had no effect on FGF-2-induced phosphorylation of p44/p42 MAP kinase or SAPK/JNK. These results strongly suggest that the PI3-kinase/Akt pathway plays a positive role in FGF-2-stimulated GDNF release independently of p44/p42 MAP kinase or SAPK/JNK in C6 glioma cells.

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Abbreviations: DMEM, Dulbecco's modified Eagle's medium; Egr-1, early growth response-1; ELISA, enzyme-linked immunosorbent assay; FGF, fibroblast growth factor; FGF-2, basic fibroblast growth factor; GDNF, glial cell line-derived neurotrophic factor; GSK3β, glycogen synthase kinase 3β; MAP, mitogen-activated protein; MEK, mitogen-activated extracellular signal-regulated kinase kinase; PAGE, polyacrylamide gel electrophoresis; PI3-kinase, phosphatidylinositol 3-kinase; SAPK/JNK, stress-activated protein kinase/c-*Jun* N-terminal kinase; SDS, sodium dodecyl sulfate

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1. Introduction

Fibroblast growth factors (FGFs) play an important role in cell proliferation, migration, differentiation, developmental processes, wound healing and tumor angiogenesis (Eswarakumar et al., 2005; Yun et al., 2010). FGFs are widely expressed in the CNS (Reuss and von Bohlen und Halbach, 2003). Among FGFs, basic FGF (FGF-2) is predominantly synthesized by astrocytes and has crucial roles in adult neurogenesis, neuroprotection, learning and memory (Reuss and von Bohlen und Halbach, 2003). FGF-2 expression is up-regulated at the lesion during several paradigms including ischemia in the CNS (Reuss and von Bohlen und Halbach, 2003). However, mechanism underlying FGF-2-mediated neuroprotective effects has been only partly resolved (Reuss and von Bohlen und Halbach, 2003; Wu, 2005). It has been shown that FGF-2 induces mRNA expression of glial cell line-derived neurotrophic factor (GDNF), a potent neuroprotective factor, and release of the protein from murine astrocytes, rat neurons, and rat C6 glioma cells (Lenhard et al., 2002; Shin et al., 2009; Suter-Crazzolara and Unsicker, 1996; Verity et al., 1998). FGF-2 reportedly shows neuroprotective effects through the synthesis of GDNF or the downregulation of NMDA receptor expression in rat hippocampal neurons (Lenhard et al., 2002; Mattson et al., 1993). Synthesis of neurotrophic factors, such as GDNF, brain-derived neurotrophic factor and nerve growth factor, is up-regulated in injured glial cells (Saavedra et al., 2008). GDNF plays important roles in the CNS development (Saavedra et al., 2008). Although GDNF expression is reduced in the adult brain, up-regulation of GDNF by astrocytes or microglia occurs in several injury models and shows neuroprotective effects in midbrain dopaminergic neurons, motoneurons and peripheral neurons (Saavedra et al., 2008). However, the exact mechanism behind synthesis of GDNF in the CNS is not fully clarified.

FGFs mediate their cellular responses by binding to and activating a family of four receptor tyrosine kinases designated as the high-affinity FGF-receptors (Eswarakumar et al., 2005; Reuss and von Bohlen und Halbach, 2003). It is generally known that FGFs stimulate the activation of the mitogen-activated protein (MAP) kinase superfamily, protein kinase C pathway or phosphatidylinositol 3 (PI3)-kinase/Akt pathway in the cells (Dailey et al., 2005; Eswarakumar et al., 2005). The MAP kinase superfamily includes p44/p42 MAP kinase, stress-activated protein kinase/c-*Jun* N-terminal kinase (SAPK/JNK) and p38 MAP kinase (Kyriakis and Avruch, 2001). In C6 glioma cells, it has been reported that FGF-2 induces the activation of p44/p42 MAP kinase, SAPK/JNK and p38 MAP kinase (Shin et al., 2009). It has been shown that FGF-2 stimulates early growth response-1 (Egr-1) expression via p44/p42 MAP kinase or SAPK/JNK but not p38 MAP kinase, which promotes transcriptional activation of the GDNF gene in C6 cells (Shin et al., 2009). On the other hand, it is generally recognized that the PI3-kinase/Akt pathway relates to the regulation of cell growth, proliferation, migration, glucose metabolism, protein synthesis and apoptosis (Brazil and Hemmings, 2001; Brazil et al., 2004). In the CNS, the PI3-kinase/Akt pathway has important functions in modulation of synapse activity, neuroprotection and neurodegeneration (Brazil et al., 2004; Burke, 2007; Zhao et al., 2006). It has been shown that growth factors including GDNF block neural apoptosis after transient ischemia through Akt activation in rat (Zhao et al., 2006). Concerning about astrocytes, it has been reported that the activation of PI3-kinase/Akt pathway suppresses apoptosis of rat cortical astrocytes and shows cell survival after hypoxia (Gao et al., 2005). FGF-2 is generally known to activate the PI3-kinase/Akt pathway in a variety type of cells (Dailey et al., 2005; Eswarakumar et al., 2005). In addition, FGF-2 reportedly shows neuroprotective effects against glutamate through GDNF synthesis in rat neurons (Lenhard et al., 2002). It has recently been shown that heme oxygenase-1 induces GDNF expression through Akt activation in rat glial cells (Hung et al., 2010). However, the role of PI3-kinase/Akt pathway in FGF-2-induced GDNF release from astrocytes remains to be elucidated.

Herein, we investigated whether the PI3-kinase/Akt pathway is involved in FGF-2-induced GDNF release from C6 glioma cells and the relationship with the MAP kinase superfamily.

2. Results

2.1. *Effects of wortmannin or LY294002 on FGF-2-induced GDNF release*

It is known that FGFs induce PI3-kinase activation in various types of cells (Dailey et al., 2005; Eswarakumar et al., 2005). The activated PI3-kinase converts the plasma membrane lipid PI-4,5-bisphosphate to PI-3,4,5-trisphosphate. Accumulation of this lipid leads to recruitment of Akt from cytosol to the plasma membrane, subsequently activated by phosphorylation on Thr308 and Ser473 residues. Akt phosphorylates a variety of substrates including glycogen synthase kinase 3β (GSK3β) (Brazil et al., 2004; Cantley, 2002; Dailey et al., 2005; Katoh and Katoh, 2006). First, we showed that FGF-2 markedly stimulated phosphorylation Akt at Thr308 and Ser473 residues and GSK3β in a time dependent manner in C6 glioma cells. FGF-2-induced phosphorylation of Akt and GSK3β reached its peak at 10 min after the stimulation and continued up to 90 min (Fig. 1).

In order to investigate whether the PI3-kinase/Akt pathway is involved in FGF-2-induced GDNF release from C6 glioma cells, we examined the effects of PI3-kinase inhibitors on FGF-2-induced GDNF release. Wortmannin, a PI3-kinase inhibitor (Arcaro and Wymann, 1993), significantly suppressed the FGF-2-induced GDNF release in addition to the basal levels of GDNF (Fig. 2A). Wortmannin remarkably attenuated FGF-2-induced Akt phosphorylation at Thr308 and Ser473 residues and GSK3β phosphorylation (Fig. 2B). The viability of cells stimulated by FGF-2 after 36 h with pretreatment of 7 μM wortmannin or 20 μM LY294002 was above 98% compared to that of cells without pretreatment by trypan blue staining.

LY294002, another PI3-kinase inhibitor (Vlahos et al., 1994), also significantly reduced the FGF-2-induced GDNF release (Fig. 3A). LY294002 truly suppressed FGF-2-induced Akt phosphorylation at Thr308 and Ser473 residues and GSK3β phosphorylation (Fig. 3B). Therefore, it is suggested that the PI3-kinase/Akt pathway is involved in FGF-2-induced GDNF release from C6 cells.