Adiponectin Stimulates Proliferation of Adult Hippocampal Neural Stem/Progenitor Cells through Activation of p38 Mitogen-activated Protein Kinase (p38MAPK)/Glycogen Synthase Kinase 3β (GSK-3β)/β-Catenin Signaling Cascade

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Background: The role of adiponectin in adult hippocampal neural stem/progenitor cell regulation is unknown.

Results: Adiponectin stimulates proliferation of hippocampal neural stem/progenitor cells in vitro and activates the p38MAPK/GSK-3β/β-catenin signaling pathway.

Conclusion: Adult hippocampal neurogenesis is positively regulated by adiponectin.

Significance: Adiponectin may alleviate neuropsychiatric disorders that involve impaired neurogenesis.

Adiponectin is the most abundant adipokine secreted from adipocytes. Accumulating evidence suggests that the physiological roles of adiponectin go beyond its metabolic effects. In the present study, we demonstrate that adiponectin receptors 1 and 2 (AdipoR1 and AdipoR2) are expressed in adult hippocampal neural stem/progenitor cells (hNSCs). Adiponectin treatment increases proliferation of cultured adult hNSCs in a dose- and time-dependent manner, whereas apoptosis and differentiation of adult hNSCs into neuronal or glial lineage were not affected. Adiponectin activates AMP-activated protein kinase and p38 mitogen-activated protein kinase (p38MAPK) signaling pathways in adult hNSCs. Pretreatment with the p38MAPK inhibitor SB203580, but not the AMP-activated protein kinase inhibitor Compound C, attenuates adiponectin-induced cell proliferation. Moreover, adiponectin induces phosphorylation of Ser-389, a key inhibitory site of glycogen synthase kinase 3β (GSK-3β), and this effect can be blocked by inhibition of p38MAPK with SB203580. Levels of total and nuclear β-catenin, the primary substrate of GSK-3β, were increased by adiponectin treatment. These results indicate that adiponectin stimulates proliferation of adult hNSCs, via acting on GSK-3β to promote nuclear accumulation of β-catenin. Thus, our studies uncover a novel role for adiponectin signaling in regulating proliferation of adult neural stem cells.

Adiponectin, the most abundant adipokine in circulation, is synthesized and secreted by adipose tissue (1, 2). It exists as either a full-length peptide or a globular form that is a product of proteolytic cleavage (3, 4). Adiponectin circulates as oligomeric complexes, including trimers, hexamers, and larger high molecular weight oligomers (5, 6). Unlike many other adipokines, plasma levels of adiponectin and mRNA expression are inversely associated with obesity in mice and humans, despite their increased adiposity (6–8). Adiponectin has insulin-sensitizing and anti-inflammatory properties. Mice deficient for adiponectin develop insulin resistance and glucose intolerance when fed a high fat diet (9–11). Conversely, adiponectin replenishment can reduce body weight, improve glucose/lipid homeostasis, and increase insulin sensitivity (12–14).

Adiponectin exerts its biological action by binding to two seven-transmembrane domain receptors termed adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2) (15, 16). They have unique distributions and affinities for the different forms of circulating adiponectin (15, 17–22). Both receptor subtypes are also expressed in the central nervous system (CNS) (15, 23, 24). Recent studies have demonstrated that adiponectin enters the brain fluid from the circulation, and the trimERIC and hexAMER complexes of adiponectin are found in the cerebrospinal fluid (23, 25–29). However, two studies failed to detect adiponectin in the cerebrospinal fluid following systemic administration of recombinant adiponectin (30, 31). The low amounts of adiponectin administered and the sensitivity of detection assays might account in part for the inconsistent results (25–27). Nonetheless, further studies are needed to define the functional role of adiponectin in the CNS.

The hippocampus is a brain region that plays a key role in memory formation and mood regulation. One unique feature of the hippocampus is that it continuously produces new neurons throughout adulthood (32, 33). Impairments in adult hippocampal neurogenesis have been implicated in the pathophysiology of mood disorders and the deficits in learning and memory (34–36). Despite significant progress that has been made in

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3 The abbreviations used are: AdipoR1 and AdipoR2, adiponectin receptor 1 and 2, respectively; GFAP, glial fibrillary acidic protein; hNSC, hippocampal neural stem/progenitor cell; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; AMPK, AMP-activated protein kinase; PARP, poly(ADP-ribose) polymerase; ANOVA, analysis of variance; GSK-3β, glycogen synthase kinase 3β.
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adult neurogenesis, molecular and cellular mechanisms that control proliferation, differentiation, and self-renewal of neural stem cells in the adult hippocampus remain unclear (37). We and others have previously reported that adipokines, such as leptin and TNF-α, regulate adult hippocampal neurogenesis, with the former increasing neurogenesis and the latter inhibiting neurogenesis (38–42). However, the role of adiponectin in adult hippocampal neurogenesis has not been identified. In the present study, we examined the expression of adiponectin receptors in adult hippocampal neural stem/progenitor cells (hNSCs) and investigated the effects of adiponectin on proliferation, apoptosis, and differentiation of adult hNSCs. Furthermore, we explored the intracellular signal transduction pathways that mediate the effect of adiponectin on adult hNSCs.

EXPERIMENTAL PROCEDURES

Cell Culture—Adult hNSCs were isolated from the hippocampus of adult Fisher rats and were positive for stem cell markers and negative for neuronal and astrocyte lineage markers (Millipore). The hNSCs were propagated in neural expansion medium (DMEM/F-12 nutrient mix, B-27 supplement, 2 mM l-glutamine, 100 units/liter penicillin, and 20 ng/ml FGF-2) on poly-l-ornithine/laminin-coated plates. For differentiation, cells were maintained in FGF-2-free neural expansion medium with the addition of 1.0 μM retinoic acid and 0.5% fetal bovine serum (FBS) to induce mixed neuronal-glial differentiation for 6 days (43). The purity of hNSCs was assessed by immunocytochemical staining with specific neuronal stem cell marker nestin. Unless indicated otherwise, cells were plated at a density of 2 × 10⁴ cells/cm² in all experiments.

Cell Proliferation Assay—The MTT assay was used to determine cell proliferation. Cells were plated in 96-well plates and grown overnight, followed by treatment with various concentrations (0–3 μg/ml) of recombinant globular adiponectin (Phoenix Pharmaceuticals Inc., Belmont, CA) or full-length adiponectin (R&D Systems, Minneapolis, MN). The MTT stock solution (5 mg/ml; Sigma-Aldrich) was added to the cells and incubated at 37 °C for 2 h. charger saturation was carefully removed and replaced with 100 μl of DMEM for 10 min. The absorbance at 590 nm was measured with a SpectraMax 340 microplate reader (Molecular Devices, Sunnyvale, CA). To determine whether inhibition of AMPK or p38MAPK affects cell proliferation, cells were pretreated with different doses of the AMPK inhibitor Compound C (0.02–2.0 μM) or adek R2 (1:1000; Millipore) were utilized. To determine the fate of differentiated stem cells, anti-Tuj1 (1:500; Covance, Princeton, NJ) and anti-GFAP antibodies (1:1000; Millipore) were used. Following incubation with primary antibodies overnight, cells were rinsed with 0.1 M PBS and incubated with secondary antibodies conjugated to Alexa Fluor 488 or Alexa Fluor 546 (1:400; Invitrogen) for 4 h. The chamber slides were then covered with ProLong Gold Antifade reagent containing 4',6-diamidino-2-phenylindole (DAPI) (Invitrogen) for nuclear counterstaining.

Cells labeled with specific neuronal or glial marker were quantified using an unbiased stereological method with Stereo Investigator (MBF Bioscience, Colchester, VT). This method determines the precise estimates of the total number of cells present in the counting area. Briefly, the counting area for each treatment condition was defined with a standard contour using a low magnification (×1.25) objective. Optical dissectors were defined using a counting frame (80 × 80 μm). Sixteen counting frames with a sampling grid dimension of 2500 × 2500 μm were randomly generated by the Stereo Investigator software. Total cell numbers positive for specific markers within each counting frame were counted using a high magnification (60 ×) oil immersion objective with a 1.4 numerical aperture. The percentage of total numbers of cells that were positive for Tuj1 or GFAP was calculated as the indicator of neuronal differentiation or glial differentiation, respectively.

Poly(ADP-ribose) Polymerase (PARP)/Apoptosis Assay—The proteolytic cleavage of PARP is an early biochemical event during apoptosis. Thus, we assessed cell apoptosis by determining PARP cleavage. Briefly, cells were seeded in 6-well plates and grown overnight, followed by treatment with various concentrations of recombinant globular adiponectin for 48 h. Western blotting was employed to detect both full-length and cleaved PARP.

Subcellular Fractionation and Nuclear Extraction—Subcellular fractionation was carried out using the CHEMICON® nuclear extraction kit (Millipore). Briefly, cells were gently scraped off the culture plate and washed with PBS, followed by resuspending the cells in 50 μl of cold cyttoplasmic lysis buffer containing 0.5 mM dithiothreitol (DTT) and protease inhibitor mixture (1:1000; Millipore). Cells were homogenized with a disposable homogenizer. Released nuclei were collected by centrifugation at 8000 × g for 20 min at 4 °C and incubated in 30 μl of ice-cold nuclear extraction buffer containing 0.5 mM DTT and protease inhibitor mixture (1:1000) with gentle agitation for 1 h at 4 °C. Samples were centrifuged at 14,000 × g for 10 min. The resulting supernatant contained the nuclear fraction, and the nuclear extraction was analyzed by Western blotting.

Western Blotting—Cells were lysed using cell lysis buffer (50 mM HEPES, pH 7.6, 150 mM NaCl, 20 mM sodium pyrophosphate, 20 mM β-glycerophosphate, 10 mM NaF, 1% Triton X-100) containing a mixture of phosphatase inhibitors (leupeptin, aprotinin, Ser/Thr phosphatase inhibitor mixture, Tyr phosphatase inhibitor mixture, phenylmethylsulfonyl fluoride).
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Seasonal Expression of AdipoR1 and AdipoR2 in cultured adult hippocampal neural stem/progenitor cells. A. Western blot demonstrating the presence of AdipoR1 (46 kDa, top) and AdipoR2 (37 kDa, bottom) in cultured adult hippocampal neural stem/progenitor cells. B. Immunocytochemical staining showing the colocalization of AdipoR1 (green, top) and AdipoR2 (green, bottom) with nestin (red), a neural stem cell marker. DAPI (blue) reveals nuclear counterstaining. Scale bar, 10 μm.

The extracted proteins were denatured, separated by SDS-PAGE, and transferred to a nitrocellulose membrane. The membrane was blocked in a blocking buffer (0.01 M Tris-buffered saline with 1% dry milk and 0.1% Tween 20), followed by incubation with specific primary antibodies overnight at 4 °C. The primary antibodies included anti-AdipoR1 (1:1000), anti-AdipoR2 (1:1000), anti-PARP (1:5000; Neomarker, Fremont, CA), anti-p38MAPK, anti-phosphorylated p38MAPK*Thr-180/Tyr-182*, anti-AMPK, anti-phosphorylated AMPK*Thr172*, anti-GSK-3β, and anti-histone 3 (1:1000; Cell Signaling Technology Inc., Danvers, MA), anti-β-actin (1:3000; Cell Signaling Technology Inc.), anti-phosphorylated-GSK-3β*Ser-389* (1:500; Millipore), and anti-β-catenin (1:1000; Abcam, Cambridge, UK). Horseradish peroxidase-conjugated anti-mouse or anti-rabbit immunoglobulin G was used as secondary antibodies (1:5000; Pierce, Rockford, IL). Signal detection was performed using enhanced chemiluminescence (Thermo Scientific, Rockford, IL). Quantification of Western blotting was performed using ImageJ software with normalization to total protein levels.

Statistical Analysis—All results were expressed as mean ± S.E. Statistical analysis was performed by two-tailed Student’s t test or ANOVAs, followed by Bonferroni/Dunn post hoc comparisons. p < 0.05 was considered statistically significant.

RESULTS

Expression of AdipoR1 and AdipoR2 in Adult Hippocampal Neural Stem/Progenitor Cells—To determine whether adiponectin receptors are expressed in hNSCs, we first examined the expression of AdipoR1 and AdipoR2 using immunoblotting with antibodies specific for each receptor subtype. Western blot assay indicated that the AdipoR1 antibody recognized a 46-kDa protein, and the AdipoR2 antibody recognized a 37-kDa protein (Fig. 1A), corresponding to AdipoR1 and AdipoR2 receptor proteins, respectively. Immunocytochemical staining with the antibodies against AdipoR1 and AdipoR2 confirmed the expression of AdipoR1 and AdipoR2 in individual hNSC (Fig. 1B). Both AdipoR1 and AdipoR2 were found in cells positive for nestin, a marker for neural progenitor cells (44) (Fig. 1B).

Effect of Adiponectin on Proliferation of Adult Hippocampal Neural Stem/Progenitor Cells—The purity of adult hNSC culture was assessed by determining the expression of the specific neural stem cell marker nestin using immunocytochemical staining. We found that more than 90% of cultured cells were positive for nestin (Fig. 2A). To determine the effects of adiponectin on proliferation of cultured hNSCs, a dose-response relationship between globular adiponectin concentrations and cell proliferation was determined using the MTT assay at 48 h after adiponectin treatment. ANOVA analysis revealed a significant effect of treatment (F(3,16) = 18.822, p < 0.0001). Post hoc analysis indicated that globular adiponectin significantly increased total cell number at concentrations of 0.03–3 μg/ml (Fig. 2B). Moreover, we determined the effects of different doses of full-length adiponectin on proliferation of hNSCs. ANOVA analysis also revealed a significant effect of treatment (F(4,10) = 4.214, p < 0.05). Post hoc analysis revealed that full-length adiponectin significantly increased total cell number at concentrations of 0.03–3 μg/ml (Fig. 2C). These results indicated that both globular and full-length forms of adiponectin are effective in promoting proliferation of hNSCs.

Next, the time course of the effects of adiponectin on proliferation of hNSCs was determined at various times (24, 48, and 72 h) after treatment with globular adiponectin (3 μg/ml). ANOVA revealed significant effects of treatment (F(1,16) = 33.469, p < 0.0001), time (F(3,16) = 695.854, p < 0.0001), and treatment × time (F(3,16) = 10.346, p < 0.01). Post hoc analysis indicated that globular adiponectin significantly increased cell proliferation at 48 h (p < 0.05) and 72 h (p < 0.05) post-treatment (Fig. 2D).
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**FIGURE 3. Effect of adiponectin on PARP cleavage in cultured adult hippocampal neural stem/progenitor cells.** Adult hippocampal neural stem/progenitor cells were incubated with various doses of globular adiponectin (gAd) for 48 h. Cleavage of PARP precursor protein was determined by Western blot analysis. A, representative immunoblots of full-length and cleaved PARP. B, quantitative data showing the effect of adiponectin on cleaved PARP. C, quantitative data showing the effect of adiponectin on full-length PARP. Data are expressed as mean ± S.E. (error bars), n = 4 per group.

**Effect of Adiponectin on Apoptosis of Adult Hippocampal Neural Stem/Progenitor Cells**—Adiponectin has been reported to induce apoptosis in several cancer cell lines (45). We therefore investigated whether adiponectin affects apoptosis in hNSCs. Using the PARP cleavage assay, we showed that treatments with different doses of globular adiponectin for 48 h had no significant effect on cleaved PARP (F(3,8) = 0.238, p = 0.868) (Fig. 3) or full-length PARP levels (F(3,8) = 0.086, p = 0.966) (Fig. 3). These data indicate that apoptosis of hNSCs is not affected by adiponectin treatment.

**Effect of Adiponectin on Differentiation of Adult Hippocampal Neural Stem/Progenitor Cells**—To determine the effect of adiponectin on differentiation of hNSCs into neuronal or glial lineage, cells were treated with 3 μg/ml globular adiponectin in differentiation medium with 1 μM retinoic acid and 0.5% FBS for 6 days. Double-labeling immunocytochemical staining was conducted with anti-Tuj1 to assess cell differentiation into neuronal lineage and with anti-GFAP to assess cell differentiation into glial lineage. Retinoic acid-induced differentiation of hNSCs into either neurons (p = 0.563) or glia (p = 0.08) was not significantly affected by globular adiponectin treatment (data not shown).

**Effect of Adiponectin on AMPK and p38MAPK Phosphorylation in Adult Hippocampal Neural Stem/Progenitor Cells**—Adiponectin exerts its action in peripheral tissues or cell lines by activating AMPK and p38MAPK signaling pathways (15). To determine whether these signaling pathways are stimulated by globular adiponectin in hNSCs, phosphorylation of AMPK and p38MAPK was assessed at different times following treatment with globular adiponectin (3 μg/ml). ANOVA revealed significant effects of treatment on phosphorylation of Thr-172 of AMPK (F(2,9) = 13.53, p < 0.005) and phosphorylation of Thr-180/Tyr-182 of p38MAPK (F(2,6) = 8.18, p < 0.05). Post hoc analysis indicated that significant increases in phosphorylation of both AMPK and p38MAPK occurred at 15 and 30 min post-treatment (Fig. 5A). These data indicate that both AMPK and p38MAPK signaling pathways can be activated by adiponectin in hNSCs.

**Effect of Inhibition of AMPK and p38MAPK on Adiponectin-induced Proliferation of Adult Hippocampal Neural Stem/Progenitor Cells**—To determine whether activation of the AMPK signaling pathway mediates adiponectin-induced proliferation of hNSCs, the cells were pretreated with different doses of the AMPK inhibitor Compound C (0.02–2.0 μM) for 2 h prior to a 48-h incubation with 3 μg/ml globular adiponectin. Cell proliferation was determined using the MTT assay. ANOVA revealed a significant effect of treatment (F(7,43) = 7.820, p < 0.0001). Inhibition of AMPK by Compound C alone showed no significant effect on cell proliferation under basal conditions, although there was a tendency to increase at the highest concentration of Compound C (2.0 μM) (Fig. 5B). Adiponectin-induced cell proliferation was not altered by pretreatment with varying doses of Compound C (Fig. 5B).

To determine whether activation of the p38MAPK signaling pathway mediates adiponectin-induced proliferation of hNSCs, the cells were pretreated with different doses of the p38MAPK inhibitor SB203580 (1–10 μM) for 2 h prior to a 48-h incubation with 3 μg/ml globular adiponectin. ANOVA revealed a significant main effect of treatment (F(7,39) = 10.472, p < 0.0001). Inhibition of p38MAPK by pretreatment with SB203580, at a dose of 3.0 μM, significantly attenuated adiponectin-induced cell proliferation without affecting basal proliferation of adult hNSCs (Fig. 5C). Interestingly, SB203580 at a dose of 10 μM caused a significant decrease in cell viability, suggesting a role of p38MAPK in maintaining the neural stem cell population. Taken together, these results suggest that
stimulatory effect of adiponectin on proliferation of hNSCs is dependent on activation of the p38MAPK signaling pathway.

Effect of Adiponectin on GSK-3β/β-Catenin in Adult Hippocampal Neural Stem/Progenitor Cells—Adaptation of p38MAPK has been shown to inhibit GSK-3β activity via phosphorylating Ser-389 in thymocytes and brain tissue homogenates (46). GSK-3β plays a crucial role in adult neurogenesis (47–49). Thus, we determined the effect of adiponectin on Ser-389 phosphorylation of GSK-3β. We found that Ser-389 phosphorylation was significantly stimulated at 15 and 30 min after treatment with globular adiponectin ($F(2,6) = 6.822, p < 0.05$) (Fig. 6A).

To determine whether adiponectin-induced phosphorylation of GSK-3β on Ser-389 is associated with activation of p38MAPK, hNSCs were pretreated with a 3.0 μM concentration of the p38MAPK inhibitor SB203580 2 h prior to 30-min adiponectin treatment. As shown in Fig. 6B, SB203580 was sufficient to block phosphorylation of p38MAPK induced by adiponectin. Inhibition of p38MAPK by SB203580 also attenuated adiponectin-induced phosphorylation of Ser-389 of GSK-3β. These results suggest that adiponectin-induced Ser-389 phosphorylation of GSK-3β requires activation of p38MAPK signaling.

GSK-3β activity is a determinant of β-catenin stabilization and its accumulation in the nucleus, which is required for neurogenesis (50, 51). To determine the effects of adiponectin treatment on β-catenin, hNSCs were treated with different doses of globular adiponectin (0, 0.3, and 3 μg/ml) for 48 h, and levels of β-catenin in whole cell and nuclear fractions were determined by Western blotting. ANOVA revealed a significant effect of treatment on total intracellular β-catenin ($F(2,6) = 4.758, p < 0.05$) and nuclear β-catenin ($F(2,8) = 7.864, p < 0.05$). Post hoc analysis indicated that globular adiponectin at a dose of 3 μg/ml significantly increased total intracellular β-catenin levels (Fig. 6C). Nuclear levels of β-catenin were significantly increased by globular adiponectin at concentrations of 0.3 and 3 μg/ml (Fig. 6C).
**DISCUSSION**

In the present study, we demonstrate that adiponectin increases proliferation of adult hNSCs, accompanied by activation of AMPK and p38MAPK signaling pathways. Adiponectin-induced cell proliferation was attenuated by inhibition of p38MAPK but not by inhibition of AMPK. Furthermore, our results indicate that adiponectin stimulates phosphorylation of GSK-3β on Ser-389, a key inhibitory site (46), and results in nuclear accumulation of the GSK-3β substrate β-catenin. These results support a novel function and signaling mechanism of adiponectin in adult hippocampal neural stem/progenitor cell regulation.

Circulating adiponectin exists either as the full-length form, having a higher affinity for AdipoR2, or in a cleaved globular form, having a relatively higher affinity for AdipoR1 (15). In the present study, we showed that both AdipoR1 and AdipoR2 were expressed by hNSCs. The proliferation rate of hNSCs was increased by treating the cells with either the globular or full-length form of adiponectin. Globular adiponectin appeared to have a greater potency than full-length adiponectin, which may reflect the relative contributions of AdipoR1 and AdipoR2 to adult hNSC proliferation. Such a relative potency pattern of globular versus full-length forms of adiponectin was also noted in hematopoietic stem cells (52). In contrast to the proproliferative effects on neural and hematopoietic stem cells, adiponectin has been shown to have antiproliferative effects in several types of cancer cells, including breast carcinoma cells, colon cancer cells, and endometrial carcinoma cells (53–56). Interestingly, it was reported that full-length adiponectin produced an inhibitory effect, whereas globular adiponectin had no effect on cancer cell proliferation (56). Furthermore, it has been suggested that the antimitogenic actions of adiponectin are mediated via sequestering growth factors instead of directly interacting with AdipoRs (56, 75). These studies imply that distinct mechanisms may underlie the effects of adiponectin on proliferation of different types of stem cells.

Through the stimulation of AdipoR1 and AdipoR2, adiponectin has been shown to activate AMPK and p38MAPK signaling pathways in various cell lines and tissues (16). In this study, we found that these two signaling pathways were also activated by adiponectin in adult hNSCs, as indicated by stimulation of phosphorylation of AMPK at Thr-172 and p38MAPK at Thr-180/Tyr-182. Although inhibition of AMPK by Compound C had no effect on adiponectin-induced proliferation of hNSCs, inhibition of p38MAPK by SB203580, at a dose that did not affect basal proliferation of hNSCs, significantly blocked adiponectin-induced cell proliferation. This finding suggests that activation of p38MAPK signaling is required for mediating the proproliferative effect of adiponectin in hNSCs. Notably, the p38MAPK inhibitor SB203580 at high concentrations decreased the total number of hNSCs, indicating that basal p38MAPK activity contributes to maintaining adult hNSC population.

The p38MAPK belongs to the MAPK family, along with stress-activated kinases, c-Jun NH2 terminal kinases (JNKs), and extracellular signal-regulated protein kinase (ERK). The ERK cascade can be activated by growth factors and transmit signals to promote cell proliferation (57). Recent studies have reported that ERK1/2 activation is involved in regulating proliferation of adult hNSCs (58–60). JNK signaling has been shown to mediate FGF2-stimulated proliferation of embryonic neural stem cells (61). JNK signaling is also involved in differentiation and apoptosis of neural stem cells (62–64). In contrast, p38MAPK signaling in neural stem cell regulation has been less characterized. The classic p38MAPK signaling pathway is generally activated in response to cellular stress and leads to cell cycle arrest and apoptosis (65, 66). In this study, we demonstrate that activation of p38MAPK mediates adiponectin-induced proliferation of adult hNSCs. One study has reported that activation of the p38MAPK pathway by gp120 leads to suppression of proliferation of adult hNSCs (67). Although the mechanisms for activation of p38MAPK leading to contrasting effects on adult hNSC proliferation remain to be defined, several studies have indicated that sustained activation of the MAPK family members can lead to a cellular response different from that induced by transient activation (68, 69). It is noted that gp120 treatment causes sustained phosphorylation of p38MAPK in hNSCs (67). By contrast, phosphorylation of p38MAPK induced by adiponectin in hNSCs declined after 30 min (data not shown). Transient p38MAPK activation has been implicated in mediating stimulatory effects on cancer cell proliferation, whereas prolonged activation of p38MAPK results in decreased proliferation of cancer cells (70). Thus, it is possible that different durations of p38MAPK activation evoked by different stimuli in adult hNSCs may recruit distinct downstream cascades, thereby leading to stimulation or suppression of cell proliferation.

A recent study has shown that activation of p38MAPK is linked to the Wnt/GSK-3β/β-catenin signaling pathway (46), a crucial pathway in the regulation of neurogenesis (50). GSK-3β is inactivated by phosphorylation of serine, and its activity is increased by phosphorylation of tyrosine (71). It has been demonstrated that p38MAPK directly inactivates GSK-3β by phosphorylating Ser-389 in the C terminus of GSK-3β in the brain and thymocytes, leading to an accumulation of intracellular β-catenin (46). In the present study, we found that adiponectin induces phosphorylation of Ser-389 of GSK-3β in adult hNSCs, and this effect can be attenuated by pretreatment with the p38MAPK inhibitor SB203580. This suggests that adiponectin induces Ser-389 phosphorylation of GSK-3β via stimulating the p38MAPK signaling pathway. Ser-389 phosphorylation induced by adiponectin would decrease GSK-3β activity, which in turn results in reduced degradation and increased intracellular level and nuclear accumulation of β-catenin, where it activates the transcription of the target genes, including those promoting proliferation (72–74). The signaling intermediates between activation of AdipoR, p38MAPK, and Ser-389 phosphorylation of GSK-3β in neural stem cell regulation, however, require further study.

In summary, we provide the first evidence that adiponectin promotes proliferation of adult hNSCs in vitro via a mechanism through activating the p38MAPK/GSK-3β/β-catenin signaling cascade (Fig. 7). It will be important in future studies to investigate the involvement and functionality of adiponectin and its signaling pathways in regulating adult hNSCs in vivo. Impaired
hippocampal neurogenesis has been implicated in a range of neurological and psychiatric disorders, and our results suggest that adiponectin may serve as a therapeutic agent or target for these disorders.

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REFERENCES

1. Scherer, P. E., Williams, S., Fogliano, M., Baldini, G., and Lodish, H. F. (1995) J. Biol. Chem. 270, 26746–26749
2. Maeda, K., Okubo, K., Shimomura, I., Funahashi, T., Matsuzawa, Y., and Matsubara, K. (1996) Biochem. Biophys. Res. Commun. 221, 286–289
3. Fruebis, J., Tsao, T. S., Javorsch, S., Ebbets-Reed, D., Erickson, M. R., Yen, F. T., Bihain, B. E., and Lodish, H. F. (2001) Proc. Natl. Acad. Sci. U.S.A. 98, 2005–2010
4. Waki, H., Yamauchi, T., Kamon, J., Kita, S., Ito, Y., Hada, Y., Uchida, S., Tsuchida, A., Takekawa, S., and Kadowaki, T. (2005) Endocrinology 146, 790–796
5. Tsao, T. S., Tomas, E., Murrey, H. E., Hug, C., Lee, D. H., Ruderman, N. B., Heuser, J. E., and Lodish, H. F. (2003) J. Biol. Chem. 278, 50810–50817
6. Waki, H., Yamauchi, T., Kamon, J., Kita, S., Ito, Y., Hada, Y., Uchida, S., Tsuchida, A., Takekawa, S., and Kadowaki, T. (2003) J. Biol. Chem. 278, 40352–40363
7. Cnop, M., Havel, P. J., Utzschneider, K. M., Carr, D. B., Sinha, M. K., Boyko, E. J., Retzlaff, B. M., Knopp, R. H., Brunzell, J. D., and Kahn, S. E. (2003) Diabetes 46, 459–469
8. Lara-Castro, C., Luo, N., Wallace, P., Klein, R. L., and Garvey, W. T. (2006) Diabetes 55, 249–259
9. Nawrocki, A. R., Rajala, M. W., Tomas, E., Pajvani, U. B., Saha, A. K., Trumbauer, M. E., Pang, Z., Chen, A. S., Ruderman, N. B., Chen, H., Rossetti, L., and Scherer, P. E. (2006) J. Biol. Chem. 281, 2654–2660
10. Maeda, N., Shimomura, I., Kishida, K., Nishizawa, H., Matsuda, M., Nagaretani, H., Furuyama, N., Kondo, H., Takahashi, M., Arita, Y., Komuro, R., Ouchi, N., Kihara, S., Tochino, Y., Okutomi, K., Horie, M., Takada, S., Aoyama, T., Funahashi, T., and Matsuzawa, Y. (2002) Nat. Med. 8, 731–737
11. Kubota, N., Terauchi, Y., Yamauchi, T., Kubota, T., Moroi, M., Matsui, I., Eto, K., Yamashita, T., Kamon, J., Satoh, H., Yano, W., Foguel, P., Nagai, R., Kimura, S., Kadowaki, T., and Noda, T. (2002) J. Biol. Chem. 277, 25863–25866
12. Yamauchi, T., Kamon, J., Waki, H., Terauchi, Y., Kubota, N., Hara, K., Mori, Y., Ide, T., Murakami, K., Tsuboyama-Kasaoka, N., Ezaki, O., Akanuma, Y., Gavrilova, O., Vinson, C., Reitman, M. L., Kagechika, H., Shudo, K., Yoda, M., Nakano, Y., Toke, K., Nagai, R., Kimura, S., Tomita, M., Foguel, P., and Kadowaki, T. (2001) Nat. Med. 7, 941–946
13. Combs, T. P., Pajvani, U. B., Berg, A. H., Lin, Y., Jelicks, L. A., Lapiante, M., Nawrocki, A. R., Rajala, M. W., Parlow, A. F., Cheesemore, L., Ding, Y. Y., Russell, R. G., Lindemann, D., Hartley, A., Baker, G. R., Obici, S., Deshaies, Y., Ludgate, M., Rossetti, L., and Scherer, P. E. (2004) Endocrinology 145, 367–383
14. Yamauchi, T., Kamon, J., Waki, H., Imai, Y., Shimozawa, N., Hioki, K., Uchida, S., Ito, Y., Takakuwa, K., Matsu, T., Takata, M., Eto, K., Terauchi, Y., Komeda, K., Tsunoda, M., Murakami, K., Ohnishi, Y., Naitoh, T., Yamamura, K., Ueyama, Y., Foguel, P., Kimura, S., Nagai, R., and Kadowaki, T. (2003) J. Biol. Chem. 278, 2461–2468
15. Yamauchi, T., Kamon, J., Ito, Y., Tsushima, A., Yokomizo, T., Kita, S., Sugiyama, T., Miyagishi, M., Hara, K., Tsunoda, M., Murakami, K., Ohketa, T., Uchida, S., Takekaway, K., Waki, H., Tso, N. H., Shibata, Y., Terauchi, Y., Foguel, P., Toke, K., Koyasu, S., Taira, K., Kitamura, T., Shimizu, T., Nagai, R., and Kadowaki, T. (2003) Nature 423, 762–769
16. Kadowaki, T., and Yamauchi, T. (2005) Endocr. Rev. 26, 439–451
17. Staiger, H., Kaltenbach, S., Staiger, K., Stefan, N., Fritzsche, A., Gruiguis, A., Peterfi, C., Weisser, M., Machicaco, F., Stumvoll, M., and Haring, H. U. (2004) Diabetes 53, 2195–2201
18. Neumeier, M., Weigert, J., Schaffler, A., Weiss, T., Kirchner, S., Laberer, A. M., and Buechler, C. (2005) Biochem. Biophys. Res. Commun. 334, 924–929
19. Palanivel, R., Xing, X., Kohn, A., Schölmerich, J., and Buechler, C. (2007) Diabetes 56, 999–1009
20. Blüher, M., Fasshauer, M., Kralisch, S., Schönh, M. R., K and Paschke, R. (2005) Biochem. Biophys. Res. Commun. 329, 1127–1132
21. Berg, H. S., Nygstad, D., Spahr, A., Monjo, M., Thremen, L., Drevon, C. A., Syversen, U., and Reseland, J. E. (2004) Bone 35, 842–849
22. Kharroubi, I., Rasschaert, J., Eizirik, D. L., and Cnop, M. (2003) Biochem. Biophys. Res. Commun. 312, 1118–1122
23. Qi, Y., Takahashi, N., Hileman, S. M., Patel, H. R., Ahima, R., Kumar, S., and Scherer, P. E. (2007) J. Clin. Invest. 119, 1541–1544
24. Kadowaki, T., and Yamauchi, T. (2005) J. Clin. Invest. 114, 391–401
25. Neumeier, M., Weigert, J., Schaffler, A., Weiss, T., Kirchner, S., Laberer, A. M., and Buechler, C. (2005) Biochem. Biophys. Res. Commun. 334, 924–929
26. Pali, R., Xing, X., Kohn, A., Schölmerich, J., and Buechler, C. (2007) Diabetes 56, 999–1009
27. Kubota, N., Yano, W., Kubota, T., Yamauchi, T., Itoh, S., Kumagai, H., Kozono, H., Takamotono, I., Okamoto, S., Suzuki, T., Satoh, H., Tsuchida, A., Moroi, M., Sugiyama, T., Noda, T., Ebinuma, H., Ueta, Y., Kondo, T., Araki, E., Ezaki, O., Nagai, R., Toke, K., Terauchi, Y., Ueki, K., Minokoshi, Y., and Kadowaki, T. (2007) Cell Metab. 6, 55–68
28. Neumeier, M., Weigert, J., Buechler, C. (2003) Am. J. Physiol. Endocrinol. Metab. 292, E965–E969
29. Kusminski, C. M., McTernan, P. G., Schraw, T., Kos, K., O’Hare, J. P., Ahima, R., Kumar, S., and Scherer, P. E. (2007) Diabetesologia 50, 634–642
30. Kos, K., Harte, A. L., da Silva, N. F., Tonchev, A., Chaldakov, G., James, S., Sneed, D. R., Hoggart, B., O’Hare, J. P., McTernan, P. G., and Kumar, S. (2007) J. Clin. Endocrinol. Metab. 92, 1129–1136
31. Ebinuma, H., Miida, T., Yamauchi, T., Hada, Y., Hara, K., Kubota, N., and Kadowaki, T. (2007) Clin. Chem. 53, 1541–1544
32. Pan, W., Tu, H., and Kastin, A. J. (2006) Peptides 27, 911–916
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31. Spranger, J., Verma, S., Göhring, I., Bobbert, T., Seifert, J., Sindler, A. L., Pfeiffer, A., Hileman, S. M., Tschöp, M., and Banks, W. A. (2006) Diabetes 55, 141–147
32. Ming, G. L., and Song, H. (2005) Annu. Rev. Neurosci. 28, 223–250
33. Gage, F. H. (2000) Science 287, 1433–1438
34. Zhao, C., Deng, W., and Gage, F. H. (2008) Cell 132, 645–660
35. Kempermann, G., Krebs, J., and Fabel, K. (2008) Curr. Opin. Psychiatry 21, 290–295
36. Snyder, J. S., Soumier, A., Brewer, M., Pickel, J., and Cameron, H. A. (2011) Nature 476, 458–461
37. Ming, G. L., and Song, H. (2011) Neuron 70, 687–702
38. Garza, J. C., Guo, M., Zhang, W., and Lu, X. Y. (2008) J. Biol. Chem. 283, 18238–18247
39. Cacci, E., Claasen, J. H., and Kokaia, Z. (2005) J. Neurosci. Res. 80, 789–797
40. Keohane, A., Ryan, S., Maloney, E., Sullivan, A. M., and Nolan, V. J. (2010) Mol. Cell Neurosci. 43, 127–135
41. Iosif, R. E., Ekdahl, C. T., Ahlenius, H., Pronk, C. J., Bonde, S., Kokaia, Z., Jacobsen, S. E., and Lindvall, O. (2006) J. Neurosci. 26, 9703–9712
42. Ben-Hur, T., Ben-Menachem, O., Furer, V., Einstein, O., Mizrahi-Kol, R., and Grigoriadis, N. (2003) Mol. Cell Neurosci. 24, 623–631
43. Hsieh, J., Aimone, J. B., Kaspar, B. K., Kwabana, T., Nakamichi, K., and Gage, F. H. (2004) J. Cell Biol. 164, 111–122
44. Lendahl, U., Zimmerman, L. B., and McKay, R. D. (1990) Cell 60, 585–595
45. Kelesidis, I., Kelesidis, T., and Mantzoros, C. S. (2006) Br. J. Cancer 94, 1221–1225
46. Thornton, T. M., Pedraza-Alva, G., Deng, B., Wood, C. D., Aronshtam, A., Clements, J. L., Sabio, G., Davis, R. J., Matthews, D. E., Doble, B., and Rincon, M. (2008) Science 320, 667–670
47. Kim, W. Y., Wang, X., Yu, P., Hu, J., Gu, W., Xu, X. M., and Lu, P. (2007) Mol. Cell Neurosci. 36, 343–354
48. Kim, S. J., Son, T. G., Kim, K., Park, H. R., Mattson, M. P., and Lee, J. (2007) Neurochem. Res. 32, 1399–1406
49. Hoeck, J. D., Jandke, A., Blake, S. M., Xie, Z., Spencer-Dene, B., Brandner, S., and Behrens, A. (2010) Nat. Neurosci. 13, 1365–1372
50. Bulavin, D. V., Amundson, S. A., and Fornace, A. J. (2002) Curr. Opin. Genet. Dev. 12, 92–97
51. Mikhailov, A., Shinhara, M., and Rieder, C. L. (2004) J. Cell Biol. 166, 517–526
52. Okamoto, S., Kage, Y. J., Brechtel, C. W., Siviglia, E., Russo, C., Clemente, A., Harrop, A., McKercher, S., Kaul, M., and Lipton, S. A. (2007) Cell Stem Cell 1, 230–236
53. Liu, J. F., Issad, T., Chevet, E., Ledoux, D., Courty, J., Caruelle, J. P., Barriot, D., Crépin, M., and Bertin, B. (1998) Eur. J. Biochem. 258, 271–276
54. Chen, Y. R., Meyer, C. F., and Tan, T. H. (1996) J. Neurosci. Res. 43, 523–527
55. Mikhailov, A., Shinhara, M., and Rieder, C. L. (2004) J. Cell Biol. 166, 517–526
56. Wang, Y., Lam, J. B., Lam, K. S., Liu, J., Lam, M. C., Hoo, R. L., Wu, D., Cooper, G. J., and Xu, A. (2006) Cancer Res. 66, 11462–11470
57. Seger, R., and Krebs, E. G. (1995) FASEB J. 9, 726–735
58. Ma, D. K., Ponnusamy, K., Song, M. R., Ming, G. L., and Song, H. (2009) Mol. Brain 2, 16
59. Tien, H. P., Huang, B. S., Zhao, J., Hu, X. H., Guo, J., and Li, L. X. (2009) BMC Neurosci. 10, 139
60. Gomez-Nicola, D., Valle-Argea, B., Pallas-Bazarra, N., and Nieto-Sampedro, M. (2008) Mol. Biol. Cell 22, 1960–1970
61. Sanalkumar, R., Indulekha, C. L., Divya, T. S., Divya, M. S., Anto, R. J., Vinod, B., Vidyaneel, S., Jagatha, B., Venugopal, S., and James, J. (2010) J. Neurochem. 113, 807–818
62. Wang, X., Fu, S., Wang, Y., Yu, P., Hu, J., Gu, W., Xu, X. M., and Lu, P. (2007) Mol. Cell Neurosci. 36, 343–354
63. Kim, S. J., Son, T. G., Kim, K., Park, H. R., Mattson, M. P., and Lee, J. (2007) J. Cell Biol. 171, 1019–1030
64. Hoeck, J. D., Jandke, A., Blake, S. M., Xie, Z., Spencer-Dene, B., Brandner, S., and Behrens, A. (2010) Nat. Neurosci. 13, 1365–1372
65. Bulavin, D. V., Amundson, S. A., and Fornace, A. J. (2002) Curr. Opin. Genet. Dev. 12, 92–97
66. Mikhailov, A., Shinhara, M., and Rieder, C. L. (2004) J. Cell Biol. 166, 517–526
67. Okamoto, S., Kage, Y. J., Brechtel, C. W., Siviglia, E., Russo, C., Clemente, A., Harrop, A., McKercher, S., Kaul, M., and Lipton, S. A. (2007) Cell Stem Cell 1, 230–236
68. Liu, J. F., Issad, T., Chevet, E., Ledoux, D., Courty, J., Caruelle, J. P., Barriot, D., Crépin, M., and Bertin, B. (1998) Eur. J. Biochem. 258, 271–276
69. Chen, Y. R., Meyer, C. F., and Tan, T. H. (1996) J. Biol. Chem. 271, 631–634
70. Kim, C. S., Kim, J. M., Nam, S. Y., Yang, K. H., Jeong, M., Kim, H. S., Lim, Y. K., Kim, C. S., Jin, Y. W., and Kim, J. (2007) J. Radiat Res. 48, 407–415
71. Doble, B. W., and Woodgett, J. R. (2003) J. Cell Sci. 116, 1175–1186
72. Susse, A. (2008) Cell Res. 18, 523–527
73. He, T. C., Sparks, A. B., Rago, C., Hermeking, H., Zawel, L., da Costa, L. T., Nusse, R., and Morin, P. J. Vogelstein, B., and Kinzler, K. W. (1998) Science 281, 1509–1512
74. Tetsu, O., and McCormick, F. (1999) Nature 398, 422–426
75. Wang, Y., Lam, K. S., Xu, J. Y., Lu, G., Xu, L. Y., Cooper, G. J., and Xu, A. (2005) J. Biol. Chem. 280, 18341–18347