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NRC-INTERACTING FACTOR DIRECTS NEURITE OUTGROWTH IN AN ACTIVITY-DEPENDENT MANNER

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Abstract—Nuclear hormone receptor coregulator-interacting factor 1 (NIF-1) is a zinc finger nuclear protein that was initially identified to enhance nuclear hormone receptor transcription via its interaction with nuclear hormone receptor coregulator (NRC). NIF-1 may regulate gene transcription either by modulating general transcriptional machinery or remodeling chromatin structure through interactions with specific protein partners. We previously reported that the cytoplasmic/nuclear localization of NIF-1 is regulated by the neuronal Cdk5 activator p35, suggesting potential neuronal functions for NIF-1. The present study reveals that NIF-1 plays critical roles in regulating neuronal morphogenesis at early stages. NIF-1 was prominently expressed in the nuclei of developing rat cortical neurons. Knockdown of NIF-1 expression attenuated both neurite outgrowth in cultured cortical neurons and retinoic acid (RA)-treated Neuro-2a neuroblastoma cells. Furthermore, activity-induced Ca^{2+} influx, which is critical for neuronal morphogenesis, stimulated the nuclear localization of NIF-1 in cortical neurons. Suppression of NIF-1 expression reduced the up-regulation of neuronal activity-dependent gene transcription. These findings collectively suggest that NIF-1 directs neuronal morphogenesis during early developmental stages through modulating activity-dependent gene transcription.

Key words: neuronal development, nuclear hormone receptor, gene transcription, neuronal morphogenesis.

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Abbreviations: AP-1, activated protein-1; CaMK, calcium/calmodulin-dependent protein kinase; C/EBPβ, CCAAT/enhancer binding protein beta; DIV, days *in vitro*; E, embryonic day; EDTA, ethylenediaminetetraacetic acid; egr-1, early growth response factor-1; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; NIF-1, NRC-interacting factor 1; NRC, nuclear hormone receptor coregulator; P, postnatal day; RA, retinoic acid; RAR, retinoic acid receptor; RXR, retinoid X receptor; siRNA, small interfering RNA.

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INTRODUCTION

The development of the nervous system requires the coordination of processes including neurogenesis, neurite outgrowth, neuronal migration, and synapse formation. To ensure proper neuronal development, these processes require precise spatiotemporal regulation of the transcription of genes involved in events such as cytoskeletal dynamics, signal transduction and protein trafficking. Nuclear hormone receptors are a family of ligand-activated transcription factors that control the expression of many of these genes (Aranda and Pascual, 2001) that are important for neuronal development (Maden, 2001; Horn and Heuer, 2010; Mandrekar-Colucci and Landreth, 2011; Srivastava et al., 2011; Frick, 2012). Nuclear hormone receptors regulate their gene targets by associating with diverse coregulators including coactivators and corepressors, which are recruited by the nuclear hormone receptors through ligand binding (McKenna and O'Malley, 2002). These coregulators mediate nuclear receptor signaling complexes by stabilizing the transcriptional machinery and altering the accessibility of transcriptional machinery to DNA through chromatin remodeling and histone modification (Kato et al., 2011). Importantly, ligand-dependent dynamic exchange between coactivators and corepressors serves as a well-regulated switch between gene activation and repression.

Nuclear hormone receptor coregulator (NRC)-interacting factor 1 (NIF-1) is ubiquitously expressed in tissues such as the skeletal muscle, thymus, placenta, and blood (Mahajan et al., 2002). Originally detected in a rat pituitary somatotrophic cell line (Mahajan et al., 2002), it contains many highly conserved domains including six zinc fingers, an N-terminal acid-rich region, an LxxLL motif, and a C-terminal leucine zipper-like motif (Mahajan et al., 2002). Human NIF-1 is a 1342 amino-acid nuclear protein that is highly conserved across mice and rats. NIF-1 indirectly interacts with type I or II nuclear hormone receptors via NRC (Mahajan et al., 2002) and subsequently potentiates the nuclear hormone receptors' transcriptional substrates such as thyroid hormone receptor and retinoic acid receptor (RAR) (Garapaty et al., 2008) through binding with components of nuclear protein complexes such as CCR4-NOT and activated protein-1 (AP-1) (Mahajan et al., 2002; Garapaty et al., 2009). These transcription factors in turn regulate the expressions of various neuronal proteins important for brain development and function (Yang et al., 2012). We previously demonstrated that NIF-1 interacts with the neuron-specific Cdk5

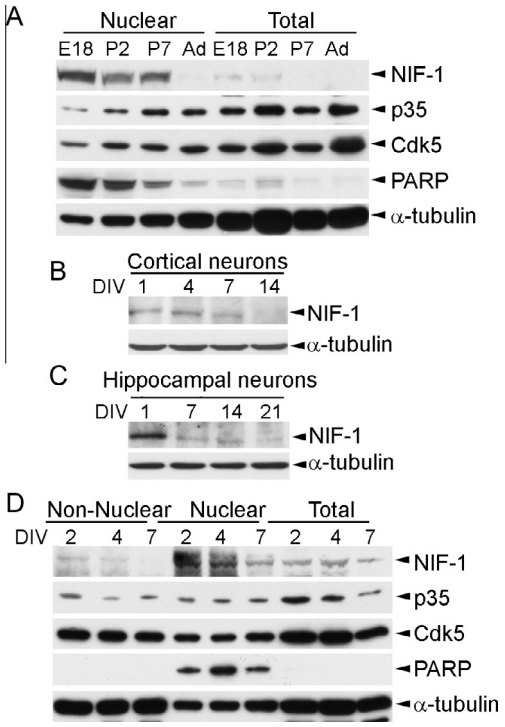

Fig. 1. NIF-1 is a nuclear protein in differentiating neurons. (A) Nuclear fractions of rat brain tissues from various developmental stages (E18, P2, P7, and adult [Ad]) were prepared and subjected to Western blot analysis. (B, C) NIF-1 protein expression in primary cultured neurons. Cortical neurons (B) and hippocampal neurons (C) were cultured for the various periods as indicated. Western blot analysis for NIF-1 and α-tubulin (as loading control). (D) NIF-1 protein was enriched in the nuclear fractions of cultured neurons. Cortical neurons were cultured for various periods, and nuclear fractions were extracted. Western blot analysis for NIF-1, p35, Cdk5, PARP (as a nuclear marker), and α-tubulin. Total: protein extracted from the same batch of neurons using RIPA.

activator p35 and that the nuclear/cytoplasmic expression of NIF-1 is controlled by p35 (Zhao et al., 2014). Given the importance of Cdk5 and p35 in neuronal morphogenesis (Nikolic et al., 1996; Rashid et al., 2001), we here sought to examine whether NIF-1 is involved in such processes.

EXPERIMENTAL PROCEDURES

siRNAs, shRNAs, and DNA constructs

A chemically modified small interfering RNA (siRNA) targeting NIF-1 was designed and synthesized by using Stealth RNA interference technology (Invitrogen, Waltham, MA, USA) according to the manufacturer's instructions. The siRNAs are as follows: NIF-1 siRNA, 5′-CCCACCAGUGCAAUCAGUGUAGCUU-3′, control siRNA, 5′-AAGUGCAUACUCAGUUCGCAGUGGG-3′. The shRNA target sequence for mouse NIF-1 is 5′-GATCTCCCAGGAACGGGCACCTCAAATTCAAGAGATTTGAGGTGCCCGTTCCTGTTTTTGGAAC-3′. The oligonucleotides were synthesized, annealed, and subcloned into the pSUPER vector.

Cell culture and transfection

Neuro-2a cells (ATCC, Manassas, VA, USA) were cultured in minimal essential medium supplemented with 10% heat-inactivated fetal bovine serum plus antibiotics. Neuro-2a cells were differentiated by treatment with 10 μM retinoic acid (RA). Primary cortical and hippocampal neuronal cultures were prepared from fetal rats at embryonic day 18 (E18). Cortices were dissected and dissociated in Dulbecco's modified Eagle medium and plated on poly-d-lysine–coated culture plates. Neurons were cultured in neurobasal medium supplemented with 2% B27 and antibiotics. The cultured cortical neurons were depolarized with 55 mM KCl (Flavell et al., 2006). All animal experiments were approved by the Animal Care Committee of the Hong Kong University of Science and Technology.

Neuro-2a cells were transfected using Lipofectamine PLUS reagents (Invitrogen). Cortical neurons were transfected with the Nucleofector Transfection Kit (Amaxa, Cologne, Germany).

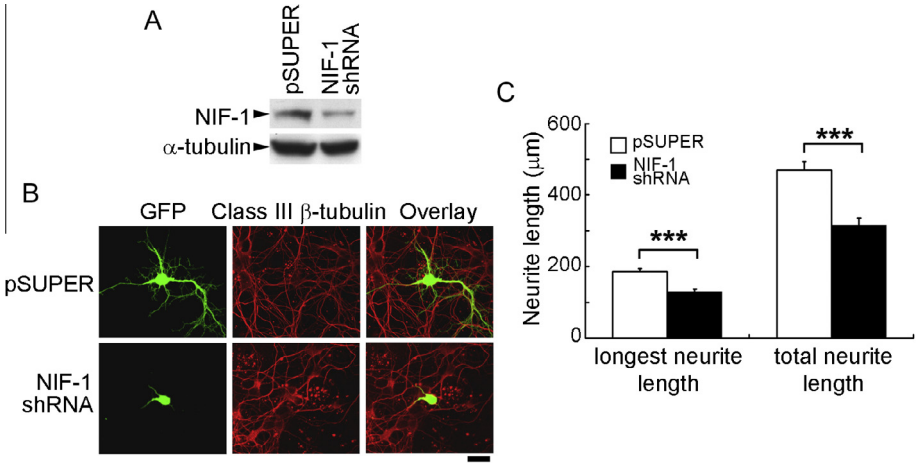

Fig. 2. NIF-1 regulates neurite outgrowth in cultured neurons. NIF-1 knockdown inhibited neurite outgrowth. (A–C) Cultured cortical neurons were co-transfected with pEGFP and pSUPER-NIF-1 shRNA. (A) Western blot analysis for NIF-1. (B) Neurons were fixed at 3 DIV post-transfection and stained with class III β-tubulin antibody. Scale bar = 10 μm. (C) Quantitative analysis of the longest and total neurite length. Results are presented as mean ± SEM ($n = 3$, $^{***}p < 0.001$, Student's t-test).

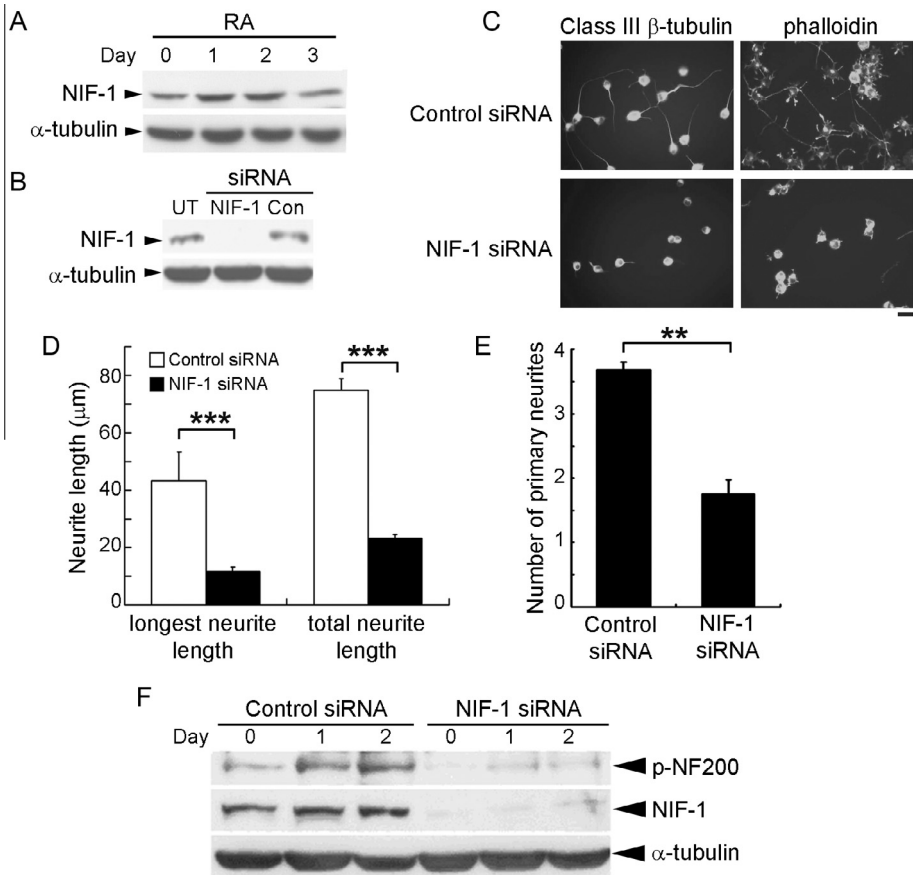

Fig. 3. NIF-1 is required for neuronal differentiation (A) NIF-1 expression was upregulated in Neuro-2a cells upon differentiation. Neuro-2a cells were differentiated by RA (10 μM), and cell lysates were collected at different periods. Western blot analysis of NIF-1 and α-tubulin expression. (B) NIF-1 protein knockdown in Neuro-2a cells using siRNA. Undifferentiated Neuro-2a cells were transfected with NIF-1 siRNA and differentiated by RA for 2 days. UT, untransfected control; Con, transfected with scramble siRNA. Western blot analysis showed the effective knockdown of endogenous NIF-1 by specific siRNA. (C–F) NIF-1 knockdown inhibited RA-induced neurite extension. Neuro-2a cells transfected with NIF-1 siRNA or control siRNA were differentiated by RA for 2 days. (C–E) NIF-1 siRNA-transfected Neuro-2a cells were stained with class III β-tubulin antibody or rhodamine-conjugated phalloidin. Scale bar = 20 μm. (C) Representative micrographs of NIF-1 siRNA-transfected Neuro-2a cells after differentiation. Quantitative analysis of (D) the longest neurite length and total neurite length, and (E) the number of primary neurites. Results are presented as mean ± SEM (**$p < 0.01$, Student's *t*-test). (F) The cell lysate of NIF-1 siRNA-transfected Neuro-2a cells after RA treatment was collected and subjected to Western blot analysis for phosphorylated neurofilament 200 and NIF-1 (α-tubulin served as an equal loading control).

Protein extraction and Western blot analysis

The nuclear fractions were prepared as described previously (Fu et al., 2004). Whole rat brains were homogenized and subsequently pelleted by low-speed centrifugation followed by a second centrifugation for 20 min at 25,000*g* to remove residual cytoplasmic material. The resultant pellet was designated as crude nuclei. The crude nuclei were resuspended in 3 mL buffer A (20 mM HEPES [pH 7.9], 25% (v/v) glycerol, 0.42 M NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM PMSF, and 0.5 mM DTT). The resultant suspension was stirred gently for 30 min and then centrifuged for 30 min at 25,000*g*. The resultant clear supernatant was dialyzed against 50 volumes of buffer B (20 mM HEPES [pH 7.9], 20% (v/v) glycerol, 0.1 M KCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM PMSF, and 0.5 mM DTT) for 5 h. The dialysate was centrifuged at 25,000*g* for 20 min, and the resultant supernatant was designated as nuclear extract. The nuclear and cytoplasmic fractions of cortical neurons were prepared according to the manufacturer's instructions (Sigma, St. Louis, MO, USA).

Antibodies used for Western blot analysis include anti-Cdk5 (C-8, 1:1000) and p35 (C-19, 1:1000) purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA), anti-α-tubulin and anti-class III -tubulin (1:5000; Sigma), anti-phosphorylated heavy-sized neurofilament (p-NF200; 1:1000; Sternberger Monoclonals Inc., Baltimore, MD, USA) and anti-PARP (1:1000; Cell Signaling Technology, Beverly, MA, USA). A custom antibody against NIF-1 was raised against the GST fusion protein of the NIF-1 C terminus (amino acids 1066–1291; 1:500). The specificity of the antibody was confirmed by pre-absorption with histidine-tagged fusion protein of the NIF-1 C-terminus (amino acids 772–1291).

Quantification of neurite length

To study the effect of NIF-1 on neurite outgrowth, Neuro-2a cells were transfected with NIF-1 siRNA or control siRNA. Two days after RA-induced differentiation, the cells were fixed and incubated with rhodamine-conjugated phalloidin (Invitrogen, 1:3000) for 1 h to stain F-actin or class III β-tubulin antibody as a neuron-specific marker.

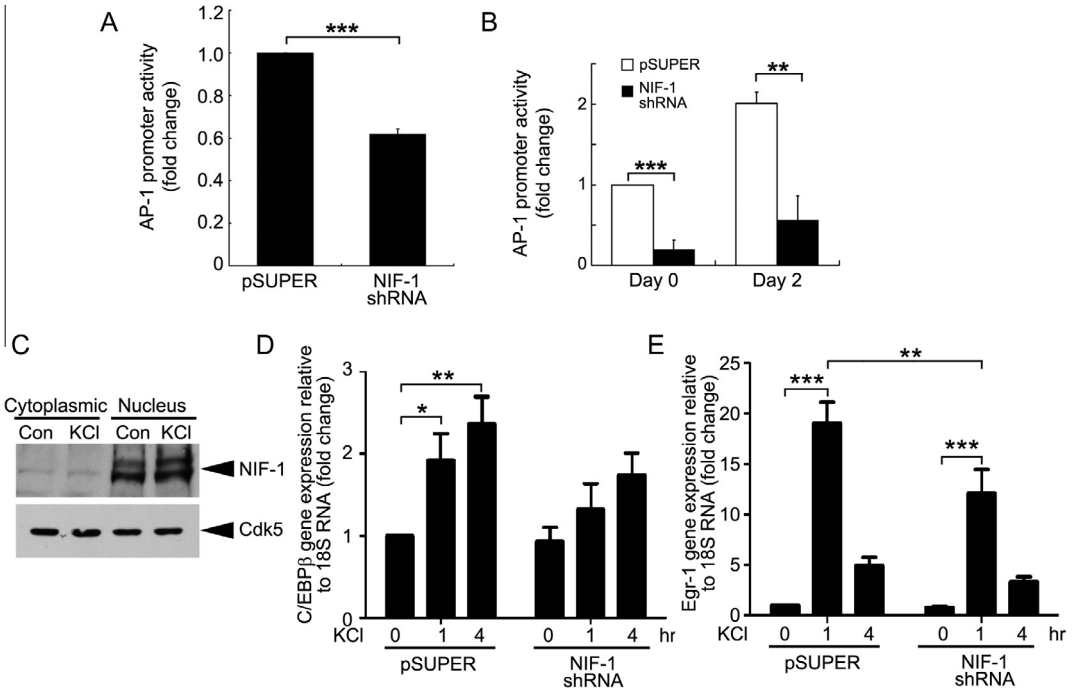

Fig. 4. NIF-1 regulates AP1-mediated gene transcription in neurons (A) NIF-1 knockdown reduced AP-1-mediated transcriptional activity in neurons. Cultured cortical neurons were co-transfected with pAP-1-Luc reporter construct and pSUPER-NIF-1 shRNA. Cell lysate was harvested, and luciferase activity was measured. Results are presented as the fold change of luciferase activity of pSUPER-NIF-1 shRNA-transfected cells versus that of cells transfected with pSUPER. (B) NIF-1 knockdown inhibited the AP1-mediated transcriptional activity induced by RA. Neuro-2a cells were co-transfected with an AP-1 promoter-reporter construct (pAP-1-Luc) together with pSUPER-NIF-1 shRNA followed by RA treatment for 2 days. Luciferase activity was measured and is presented as the fold change of AP-1 promoter activity relative to that of pSUPER-transfected cells. Data are presented as mean ± SD (***$p < 0.001$, **$p < 0.01$, Student's *t*-test). (C) Depolarization induced NIF-1 nuclear translocation in cortical neurons. Cultured cortical neurons were treated with 55 mM KCl for 5 min, and the nuclear protein was extracted. Western blot analysis for NIF-1 and Cdk5. (D, E) NIF-1 knockdown in cortical neurons attenuated the depolarization-induced gene expression of C/EBPβ (D) and egr-1 (E). Total RNA was collected from cortical neurons, which had been transfected with pSUPER NIF-1 shRNA, after treatment with 55 mM KCl for 1–4 h. C/EBPβ mRNA level was measured by reverse-transcription and quantitative PCR and normalized to 18S as control. Results are presented as the mean ± SEM of 3–4 independent experiments (*$p < 0.05$, **$p < 0.005$, a two-way ANOVA followed by Newman–Keuls test).

Cells were then washed 3 times with phosphate-buffered saline and mounted with ProLong Gold antifade reagent (Invitrogen) for microscopy. To examine the effect of NIF-1 on primary cultured cortical neurons, pSUPER and pSUPER-NIF-1 shRNA with the pEGFP construct were co-transfected into cortical neurons. Cells were fixed at 3 days *in vitro* (DIV) and stained with class III β-tubulin antibody. The morphology of the neurons was visualized by GFP fluorescence using confocal microscopy (FV1000, Olympus, Tokyo, Japan). Lengths of neurites were measured using MetaMorph version 5.0r1 software. At least 50 cells from randomly selected fields in each condition were counted per trial ($n \geqslant 3$ trials).

Reporter assay and qPCR analysis

Subconfluent Neuro-2a cells and primary cortical neurons were transfected with pSUPER or pSUPER-NIF-1 with pAP-1-Luc. Twenty-four hours after transfection, Neuro-2a cells were induced to differentiate by RA. Two days after the addition of RA, Neuro-2a cells and cortical neurons were lysed in luciferase lysis buffer (Roche, Basel,Switzerland). Cell extracts were assayed for luciferase activity using luciferase buffer (Roche). Transfection efficiencies were normalized according to protein concentration.

Total RNA was extracted using the QIAGEN RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Synthesis of cDNA was performed as described previously (Ng et al., 2006). Quantitative real-time PCR was performed on an ABI 7500 Fast Real-Time System (Invitrogen).

Statistical analysis

All data are expressed as arithmetic mean ± SEM. Comparisons between groups were performed using Student's *t*-test and a two-way ANOVA.

RESULTS

NIF-1 is prominently expressed in neurons during early development

To determine the functional role of NIF-1 in neurons, we first investigated its temporal expression pattern in the rat brain and cultured neurons. NIF-1 (~200 kDa) was predominantly expressed in the nuclear fraction of rat whole-brain preparations during early development (E18 to postnatal day (P) 7), but its presence was dramatically downregulated by adulthood (Fig. 1A). Interestingly, NIF-1 was prominently enriched in the nuclei of early developing neurons and downregulated

upon development (Fig. 1B–D), whereas it was barely detectable in the non-nuclear fractions (Fig. 1D) at the later stage. In contrast, p35 and Cdk5 proteins were detected in both the nuclear and non-nuclear fractions of cultured neurons (Fig. 1D). Together, these findings demonstrate that NIF-1 is a nuclear protein, suggesting it has functional roles in the regulation of gene transcription in neuronal morphogenesis.

NIF-1 is required for neurite outgrowth

Because the expression pattern of NIF-1 suggests the protein is involved in neuronal morphogenesis, we subsequently investigated the effect of loss-of-function on this process. To this end, cultured cortical neurons were co-transfected with shRNA NIF-1 to attenuate NIF-1 expression or an empty pSUPER construct as a control together with a pEGFP construct to visualize the transfected cells. The reduction of NIF-1 protein expression by pSUPER NIF-1 shRNA in cortical neurons was confirmed by Western blot analysis (Fig. 2A). After 2 days, fixed cells were analyzed immunocytochemically for class III β-tubulin, a neuron-specific cell marker (Lee et al., 1990) (Fig. 2). NIF-1 knockdown significantly inhibited neurite extension in cortical neurons (Fig. 2B, C).

As NIF-1 is necessary for proper neurite extension in developing cortical neurons, we next sought to determine the molecular pathways responsible for this particular effect. RA and RAR represent one pathway known to be critical for various neuronal developmental processes throughout the central nervous system, including neurite outgrowth (Maden et al., 1998; Maden, 2001). Given that NIF-1 was prominently expressed in developing brains (Fig. 1) and has been shown to potentiate the ligand-dependent transcriptional activity of nuclear hormone receptors including RAR (Mahajan et al., 2002), we hypothesized that NIF-1 is involved in RA-regulated neurite outgrowth. Treating Neuro-2a cells with RA triggers neuronal differentiation (Morton and Buss, 1992). NIF-1 expression was detected in undifferentiated Neuro-2a cells, and its level increased following 2 days of RA treatment (Fig. 3A). We next determined the effect of NIF-1 deficiency on the RA-induced differentiation of Neuro-2a cells. Neuro-2a cells were transfected with an siRNA targeting NIF-1 expression, and the cells were subsequently subjected to RA-induced differentiation (Fig. 3B–F). Both the longest neurite length and total neurite length were significantly reduced in NIF-1 knockdown cells upon RA treatment (Fig. 3C, D). Moreover, NIF-1 knockdown reduced the number of primary neurites (Fig. 3E). NIF-1 depletion in Neuro-2a cells did not affect the expressions of cell cycle proteins upon RA treatment (data not shown), suggesting that NIF-1 does not affect cell cycle exit but instead regulates neuronal morphology during the process of RA-induced neuronal differentiation (Fig. 3F). While RA treatment increased neurofilament-200 phosphorylation in Neuro-2a cells, which is concordant with development, NIF-1 knockdown completely inhibited this specific modification (Fig. 3F). Thus, the present findings collectively demonstrate that NIF-1 regulates neuronal morphogenesis in both developing cortical neurons as well as in the RA-induction of Neuro-2a cells *in vitro*.

NIF-1 regulates AP-1–mediated transcription

Having identified the RA-induction of neurite outgrowth as a mechanism reliant upon NIF-1, we subsequently determined which downstream signaling molecules are affected by NIF-1 interaction with RA/RAR. RA-induced neurite outgrowth can be mediated by enhanced activation of mitogen-activated protein kinase (MAPK) (Encinas et al., 1999; Singh et al., 2003), which in turn leads to the activation of transcription factors such as Elk-1 and AP-1 (Hill and Treisman, 1995; Cavanaugh et al., 2001). As NIF-1 enhances the activation of AP-1 transcription factors including c-Fos and c-Jun (Mahajan et al., 2002), we hypothesized that its depletion affects AP-1-mediated transcriptional activity in developing cortical neurons. Accordingly, luciferase assay in cultured neurons showed AP-1 promoter activity was reduced upon NIF-1 shRNA-mediated knockdown (Fig. 4A). Consistent with this finding, the RA treatment of Neuro-2a cells led to an increase in AP-1 promoter activity, whereas NIF-1 knockdown inhibited this elevation (Fig. 4B). These findings collectively suggest that NIF-1 regulates neuronal morphogenesis by facilitating AP-1-mediated transcriptional activation.

Neuronal activity regulates neurite outgrowth in cortical neurons by activating the transcription of various genes (Li et al., 2004). Given the role of NIF-1 in gene transcription during neuronal morphogenesis, we asked whether the effects of NIF-1 are activity dependent. To this end, we depolarized cultured primary neurons by treatment with KCl, which led to an increase in NIF-1 nuclear localization (Fig. 4C). This suggests that NIF-1 may undergo nucleocytoplasmic shuttling in an activity-dependent manner, which may in turn regulate gene transcription in neurons. Next, we identified specific transcription factors that are possibly affected by the nuclear localization of NIF-1 after depolarization. The CCAAT/enhancer binding protein beta (C/EBPβ) and early growth response factor-1 (egr-1) are transcription factors that play critical roles in regulating the neuronal gene expression during neuronal morphogenesis (Levkovitz et al., 2001; Halterman et al., 2009; MacGillavry et al., 2011). KCl-induced depolarization increased both C/EBPβ and egr-1 transcription in control neurons, whereas NIF-1 knockdown inhibited this activity-dependent increase of C/EBPβ and egr-1 mRNA (Fig. 4D, E). This finding suggests NIF-1 plays a specific role in the regulation of activity-dependent transcriptional activation in neuronal morphogenesis.

DISCUSSION

Nuclear hormones including thyroid hormone, retinoid, and estrogen are essential for proper brain development, as their signaling pathways act on various processes such as neuronal migration, morphogenesis, differentiation and synaptogenesis (Maden, 2001; Miglio et al., 2009; Horn and Heuer, 2010; Puttagunta et al., 2011; Srivastava et al., 2011; Maggio et al., 2012).

However, the precise signaling and regulatory mechanisms of these hormones are poorly understood. The nuclear protein NIF-1 was initially identified according to its association with NRC and regulation of the transcriptional activity of nuclear receptors (Mahajan et al., 2002, 2004). NIF-1 is one component of a nuclear hormone receptor transcriptional coactivator complex and functions in histone modification to regulate gene transcription, consequently affecting the signal transduction of such hormones (Garapaty et al., 2009). The present study demonstrates that NIF-1 regulates neuronal morphogenesis by facilitating neurite outgrowth. Furthermore, NIF-1 acts in RA-induced neurite outgrowth by regulating the AP-1 promoter, C/EBPβ and egr-1 transcription factor expression.

Since the identification of the first nuclear hormone receptor coactivator, SRC-1, (Onate et al., 1995), several hundred coregulators have been identified. Of these, only a few have been identified to participate in neuronal morphogenesis, including CBP/p300 (Gaub et al., 2010), and PHF8 (Asensio-Juan et al., 2012) as well as the corepressor, NCOR2 (Foley et al., 2011). The present study identified NIF-1 as a nuclear hormone receptor cofactor that regulates the RA-induced transcriptional activity of AP-1 to affect receptor hormone-mediated neuronal morphogenesis. The activation of AP-1 transcription factors, including homo- or heterodimers of c-Fos and c-Jun basic leucine zipper transcription factors, is sufficient to induce the gene transcription required for neuronal morphogenesis (Robinson et al., 1998; Dragunow et al., 2000; Gil et al., 2004). Therefore, NIF-1 may be a critical regulator of neuronal morphogenesis.

The neuronal activity-dependent regulation of gene expression is important for shaping brain development and plasticity, which are based on tightly controlled and coordinated processes including epigenetic regulation (West et al., 2002). Neuronal activity stimulates calcium influx, and elevated intracellular calcium activates several downstream signaling molecules for regulating gene transcription (West et al., 2002). Neuronal activity-dependent gene expression may involve the transcriptional activities of nuclear receptor signaling complexes. Previous studies demonstrate that calcium/calmodulin-dependent protein kinase II beta (βCaMKII) can be associated with retinoid X receptor (RXR) (Lin et al., 2012) and that CaMKIV regulates the transcriptional activity of the vitamin D receptor signaling pathway (Ellison et al., 2005). The present study demonstrates that NIF-1 is central to neuronal activity-induced neurite outgrowth. The nuclear translocation of NIF-1 was stimulated by neuronal activity, which suggests NIF-1 may be calcium responsive and associate with nuclear receptor complexes such as RAR/RXR to regulate gene transcription. In addition, NIF-1 modulated the transcriptional activities of neuronal activity-mediated C/EBPβ and egr-1 gene expression in neuronal cells. C/EBPβ and egr-1 are transcriptional factors that play important roles in neuronal morphogenesis (Levkovitz et al., 2001; Halterman et al., 2009; MacGillavry et al., 2011). The transcriptional activities of C/EBPβ can be regulated by neuronal activity and the signaling of nuclear hormone receptors such as RAR and estradiol receptor beta (Worley et al., 1991; Maharjan et al., 2005; Sun et al., 2007; Ohoka et al., 2009). Our observations of the regulation of C/EBPβ gene expression by NIF-1 underscore the importance of NIF-1-mediated gene regulation in neuronal morphogenesis.

The present findings corroborate the notion that NIF-1 associates with nuclear hormone receptor signaling complex transcriptional hierarchies and mediates complex gene expression regulation during neurite outgrowth and that this process can be controlled by neuronal activity. Although NIF-1 is indispensable for neuronal morphogenesis, the precise mechanism underlying NIF-1-dependent transcriptional activation in neuronal development remains to be elucidated. To further understand the molecular role of nuclear hormone receptor complexes in regulating neuronal development, additional studies must examine whether NIF-1 regulates these transcriptional hierarchies by stabilizing general transcriptional machinery or chromatin remodeling during neuronal development.

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