A Single c-Jun N-terminal Kinase Isoform (JNK3-p54) Is an Effector in Both Neuronal Differentiation and Cell Death*

Received for publication, July 23, 2002, and in revised form, October 16, 2002
Published, JBC Papers in Press, October 24, 2002, DOI 10.1074/jbc.M207391200

Vicki Waetzig and Thomas Herdegen‡
From the Institute of Pharmacology, Kiel University Medical Center, D-24105 Kiel, Germany

The c-Jun N-terminal kinases (JNKs) mediate degeneration and apoptosis in the brain. Particularly, JNK3 is considered to be a degenerative enzyme with c-Jun as a relevant substrate. The contribution of individual JNK isoforms, however, to pathological as well as to physiological processes remains to be defined. To analyze the effects of a single JNK isoform on neuronal cell death and differentiation, we transfected PC12 cells, which normally express only JNK1 and JNK2, with JNK3-p54. Transfected JNK3 significantly enhanced cell death after UV irradiation (0.5–6 J/cm²) and paclitaxel/taxol treatment (1–10 μM). In contrast, in the context of nerve growth factor-induced (10 or 50 ng/ml) differentiation of PC12 cells, JNK3 expression significantly increased the number and length of neurites. This functional dichotomy of JNK3 was mirrored by differential activation and induction of nuclear JNK substrates; although activating transcription factor-2 phosphorylation was enhanced by death signaling in response to UV and taxol, c-Jun protein expression and N-terminal phosphorylation were increased during nerve growth factor-induced differentiation. The absence of significant JNK activation or target phosphorylation in response to H₂O₂ (60 μM) further supports the hypothesis that JNK isoforms are not merely injury- or stress-specific kinases but also have context-specific physiological functions.

In recent years, members of the c-Jun N-terminal kinase (JNK) family of mitogen-activated protein kinases have emerged as important players in neurodegenerative disorders, such as Alzheimer’s (1) and Parkinson’s disease (2), as well as in cellular stress responses (Ref. 3; reviewed in Ref. 4).

The JNK family consists of the ubiquitously expressed JNK1 and JNK2 and of JNK3, which is primarily expressed in the heart, brain, and testis (5, 6). Differential splicing and exon usage yield a total of 10 different isoforms. After activation, JNKs phosphorylate various substrates in the nucleus, e.g. c-Jun or activating transcription factor-2 (ATF-2), and in the cytoplasm, e.g. Bcl-2, neurofilaments or tau (6, 7).

JNKs were first described as stress kinases, and it is their involvement in the response to cellular stress that has been studied extensively (6, 8, 9). Especially JNK3 has mostly been associated with apoptosis in pathological contexts and, because of its defined expression, predominantly with neuronal apoptosis. For example, disruption of the mouse JNK3 gene caused resistance to the excitotoxic glutamate receptor agonist kainic acid, with reduction in seizure activity and apoptosis of hippocampal neurons (10). JNK3 is likely to contribute to ischemic death in perinatal and adult rats (11, 12). In cortical and cerebellar neurons, JNK3 holds a central role in apoptosis caused by UV irradiation, β-amyloid, growth factor withdrawal, and sodium arsenite (13–16) or at least a partial role in cell death following DNA damage (17). Finally, a crucial function has been attributed to JNK3 in the induction of p75-mediated apoptosis in oligodendrocytes (18).

Thus, the majority of reports indicate a pro-apoptotic role for JNKs. Their function, however, is clearly not restricted to apoptosis. Already at the beginning of JNK research, a dichotomous role for activated JNKs and their phosphorylated substrate c-Jun was suggested in neuronal injury connecting cell death with the competence for regeneration (19–21). Numerous reports attribute JNKs functions in neurite outgrowth in vitro (22–26), in the neuronal response to growth factors (27, 28), and in regeneration in vivo (21, 29). The wide range of JNK actions is further emphasized by the observation that within a single cell, different subpools of JNKs have distinct functions (3). In summary, JNKs have an extensive potential to perform different actions. However, the contribution of individual isoforms is still obscure.

During the last decades, the rat pheochromocytoma cell line PC12 has been established as a model system for neuronal apoptosis as well as for neuronal differentiation and neurite outgrowth in response to NGF. Therefore, PC12 cells are the system of choice for investigating the potential of JNKs in the context of neuronal apoptosis and differentiation. Importantly, PC12 cells only express JNK1 and JNK2 (30, 31), and these qualities render PC12 cells into a well defined cell culture system for the analysis of transfected JNK3.

In the present study, we have investigated the capacity of a single JNK3 isoform for the propagation of cell death and differentiation. We demonstrate that the expression of JNK3 promotes cell death after UV irradiation and application of taxol but not after H₂O₂. On the other hand, however, transfected JNK3 significantly increases NGF-induced neurite outgrowth. Concomitant with the shift of function is the switch of JNK3 substrates, changing from ATF-2 in the apoptotic context to c-Jun during neuronal differentiation. Our results not only shed new light on the role of ATF-2 in the context of cell death but also define a model case for the dichotomous role of JNKs in coupling neuronal cell death and regeneration (32).

EXPERIMENTAL PROCEDURES

Materials—The following materials were used: anti-active JNK (Promega), anti-c-Jun (Santa Cruz), anti-mouse IgG (Amersham Bio-
RESULTS

Characterization of Transfected Cells—After the selection of stable clones, vector- and JNK3-transfected PC12 cells were monitored with regard to cell morphology, growth characteristics, and the expression status of different mitogen-activated protein kinases and their substrates. No morphological changes were detected; under normal growth conditions, both untransfected and transfected PC12 cells had a round or polygonal shape and did not extend processes (Fig. 1A). Apart from their similar appearance, all of the cells displayed an almost identical growth rate with a doubling time of ∼72 h. When screened for the expression of different mitogen-activated protein kinases and their substrates, the transfection with pEGFP or JNK3-EGFP did not cause any changes in the amount of JNK1, JNK2, p38α, and ATF-2 in wild type (lane wt), vector-transfected (lane ve), and JNK3-transfected (lane JNK3) PC12 cells showed no difference in protein expression. Bars, 50 μm.

Activation of JNKs by Cellular Stress—To investigate the effect of different stress stimuli on the phosphorylation of all JNK isoforms, PC12 cells were stimulated with UV irradiation (0.5, 2, or 6 J/cm²; 312 nm), H2O2 (60 μM), or paclitaxel/taxol (1 μM, 5 μM, or 10 μM) and incubated for 90 min before protein extraction. JNK activities were analyzed by Western blotting with antibodies directed against dual phosphorylated JNK isoforms, Western blots of total JNK, JNK1, JNK2, ERK1/2, p38α, and ATF-2 in wild type (lane wt), vector-transfected (lane ve), and JNK3-transfected (lane JNK3) PC12 cells showed no difference in protein expression. Bars, 50 μm.

Characterization of JNK3-Transfected PC12 Cells. A, cell morphology of wild type (wt) and JNK3-transfected PC12 cells. Both were kept under the same culture conditions and displayed an indis-tinguishably similar phenotype. B, Western blots of total JNK, JNK1, JNK2, ERK1/2, p38α, and ATF-2 in wild type (lane wt), vector-transfected (lane ve), and JNK3-transfected (lane JNK3) PC12 cells showed no difference in protein expression. Bars, 50 μm.

Activation of JNK3 by Stress—JNK3 is a stress-activated kinase that is known to play a role in neuronal differentiation and death. To investigate whether JNK3 is activated by stress stimuli, PC12 cells were exposed to various stressors, including UV irradiation, H2O2, and paclitaxel/taxol. Western blots of total JNK, JNK1, JNK2, p38α, and ATF-2 in wild type (lane wt), vector-transfected (lane ve), and JNK3-transfected (lane JNK3) PC12 cells showed no difference in protein expression. Bars, 50 μm.

Activation of JNK3 by Stress—JNK3 is a stress-activated kinase that is known to play a role in neuronal differentiation and death. To investigate whether JNK3 is activated by stress stimuli, PC12 cells were exposed to various stressors, including UV irradiation, H2O2, and paclitaxel/taxol. Western blots of total JNK, JNK1, JNK2, p38α, and ATF-2 in wild type (lane wt), vector-transfected (lane ve), and JNK3-transfected (lane JNK3) PC12 cells showed no difference in protein expression. Bars, 50 μm.

Activation of JNK3 by Stress—JNK3 is a stress-activated kinase that is known to play a role in neuronal differentiation and death. To investigate whether JNK3 is activated by stress stimuli, PC12 cells were exposed to various stressors, including UV irradiation, H2O2, and paclitaxel/taxol. Western blots of total JNK, JNK1, JNK2, p38α, and ATF-2 in wild type (lane wt), vector-transfected (lane ve), and JNK3-transfected (lane JNK3) PC12 cells showed no difference in protein expression. Bars, 50 μm.

Activation of JNK3 by Stress—JNK3 is a stress-activated kinase that is known to play a role in neuronal differentiation and death. To investigate whether JNK3 is activated by stress stimuli, PC12 cells were exposed to various stressors, including UV irradiation, H2O2, and paclitaxel/taxol. Western blots of total JNK, JNK1, JNK2, p38α, and ATF-2 in wild type (lane wt), vector-transfected (lane ve), and JNK3-transfected (lane JNK3) PC12 cells showed no difference in protein expression. Bars, 50 μm.

Activation of JNK3 by Stress—JNK3 is a stress-activated kinase that is known to play a role in neuronal differentiation and death. To investigate whether JNK3 is activated by stress stimuli, PC12 cells were exposed to various stressors, including UV irradiation, H2O2, and paclitaxel/taxol. Western blots of total JNK, JNK1, JNK2, p38α, and ATF-2 in wild type (lane wt), vector-transfected (lane ve), and JNK3-transfected (lane JNK3) PC12 cells showed no difference in protein expression. Bars, 50 μm.

Activation of JNK3 by Stress—JNK3 is a stress-activated kinase that is known to play a role in neuronal differentiation and death. To investigate whether JNK3 is activated by stress stimuli, PC12 cells were exposed to various stressors, including UV irradiation, H2O2, and paclitaxel/taxol. Western blots of total JNK, JNK1, JNK2, p38α, and ATF-2 in wild type (lane wt), vector-transfected (lane ve), and JNK3-transfected (lane JNK3) PC12 cells showed no difference in protein expression. Bars, 50 μm.

Activation of JNK3 by Stress—JNK3 is a stress-activated kinase that is known to play a role in neuronal differentiation and death. To investigate whether JNK3 is activated by stress stimuli, PC12 cells were exposed to various stressors, including UV irradiation, H2O2, and paclitaxel/taxol. Western blots of total JNK, JNK1, JNK2, p38α, and ATF-2 in wild type (lane wt), vector-transfected (lane ve), and JNK3-transfected (lane JNK3) PC12 cells showed no difference in protein expression. Bars, 50 μm.

Activation of JNK3 by Stress—JNK3 is a stress-activated kinase that is known to play a role in neuronal differentiation and death. To investigate whether JNK3 is activated by stress stimuli, PC12 cells were exposed to various stressors, including UV irradiation, H2O2, and paclitaxel/taxol. Western blots of total JNK, JNK1, JNK2, p38α, and ATF-2 in wild type (lane wt), vector-transfected (lane ve), and JNK3-transfected (lane JNK3) PC12 cells showed no difference in protein expression. Bars, 50 μm.

Activation of JNK3 by Stress—JNK3 is a stress-activated kinase that is known to play a role in neuronal differentiation and death. To investigate whether JNK3 is activated by stress stimuli, PC12 cells were exposed to various stressors, including UV irradiation, H2O2, and paclitaxel/taxol. Western blots of total JNK, JNK1, JNK2, p38α, and ATF-2 in wild type (lane wt), vector-transfected (lane ve), and JNK3-transfected (lane JNK3) PC12 cells showed no difference in protein expression. Bars, 50 μm.

Activation of JNK3 by Stress—JNK3 is a stress-activated kinase that is known to play a role in neuronal differentiation and death. To investigate whether JNK3 is activated by stress stimuli, PC12 cells were exposed to various stressors, including UV irradiation, H2O2, and paclitaxel/taxol. Western blots of total JNK, JNK1, JNK2, p38α, and ATF-2 in wild type (lane wt), vector-transfected (lane ve), and JNK3-transfected (lane JNK3) PC12 cells showed no difference in protein expression. Bars, 50 μm.

Activation of JNK3 by Stress—JNK3 is a stress-activated kinase that is known to play a role in neuronal differentiation and death. To investigate whether JNK3 is activated by stress stimuli, PC12 cells were exposed to various stressors, including UV irradiation, H2O2, and paclitaxel/taxol. Western blots of total JNK, JNK1, JNK2, p38α, and ATF-2 in wild type (lane wt), vector-transfected (lane ve), and JNK3-transfected (lane JNK3) PC12 cells showed no difference in protein expression. Bars, 50 μm.
both wild type (wt) JNK1/2 and EGFP-tagged JNK3 (83 kDa) were dose-dependently activated (i.e., dual phosphorylated; p-JNK1/2 and p-JNK3-EGFP, respectively) by UV irradiation (312 nm) as compared with untreated control cells (lane C). Stimulation with hydrogen peroxide (60 µM) caused only a weak activation of JNKs. B, taxol (1, 5 or 10 nM) evoked a dose-dependent response, but differences in JNK activation between high and low doses of taxol were less pronounced than with UV (A). With all stress stimuli, the p46 and p54 splice variants of JNK1/2 were equally activated. No changes in protein expression of JNK1/2 or JNK3-EGFP were observed. The data are representative of four independent experiments.

In response to all stress stimuli and doses used, the survival of PC12 cells was significantly reduced as compared with untreated controls (not indicated). With higher doses of UV irradiation (2.0 and 6.0 J/cm²), a significantly larger proportion of JNK3-transfected cells died compared with wild type and vector-transfected cells (60 and 11%) and vector-transfected cells (66 and 11%) (p < 0.02; indicated by two asterisks). In contrast, there was no increase in cell death of JNK3-transfected cells after treatment with H₂O₂ (Fig. 3). This finding shows that the expression of JNK3 and the subsequently increased supply of JNKs did not automatically integrate JNKs in the realization of cell death to all stimuli but rather emphasizes the selectivity and specificity of JNK functions. To control the cell death rates observed with trypan blue staining, lactate dehydrogenase release assays were conducted that confirmed the results obtained so far (data not shown).

**JNK3 Enhances the Phosphorylation of ATF-2—**The two transcription factors c-Jun and ATF-2 are commonly associated with the JNK stress response. Therefore, we studied the effects of JNK3 activation on the phosphorylation of c-Jun and ATF-2 in PC12 cells following UV irradiation (0.5, 2, and 6 J/cm²; 312 nm), H₂O₂ (60 µM), and taxol (5 µM). The nuclear proteins were extracted after an incubation time of 90 min.

ATF-2 phosphorylation was significantly increased in response to UV treatment with H₂O₂ (Fig. 4A). In contrast, there was no increase in cell death of JNK3-transfected cells after treatment with H₂O₂ (Fig. 3). This finding shows that the expression of JNK3 and the subsequently increased supply of JNKs did not automatically integrate JNKs in the realization of cell death to all stimuli but rather emphasizes the selectivity and specificity of JNK functions. To control the cell death rates observed with trypan blue staining, lactate dehydrogenase release assays were conducted that confirmed the results obtained so far (data not shown).

In response to the high UV doses and to taxol treatment in JNK3-transfected cells as compared with wild type PC12 cells was only observed after taxol (TX) stimulation. This increase in phosphorylated c-Jun was, however, partly due to an increased induction of c-Jun protein expression in the JNK3-transfected cells. The data shown represent five separate experiments.
NF-H was also significantly higher in JNK3-transfected cells. Induction of c-Jun protein expression and phosphorylation of c-Jun \( \text{p-c-Jun} \) of JNK1 and JNK2. Induction of c-Jun protein expression and phosphorylation levels were similar in wild type and transfected cells. The induction/phosphorylation of NF-H was also significantly higher in JNK3-transfected cells.

induced cell death (see above), JNK3 transfection did not alter ATP-2 phosphorylation in response to \( \text{H}_2\text{O}_2 \) (Fig. 4, A and B).

Following UV irradiation and taxol stimulation, we detected a similar increase in c-Jun protein expression and N-terminal phosphorylation in the transfected and untransfected PC12 cells (Fig. 4C). Normalization of phospho-c-Jun and c-Jun levels showed that the overall increase in phosphorylated c-Jun was partly due to the enhanced expression of the c-Jun protein. Similar to the minor effect on JNK phosphorylation, \( \text{H}_2\text{O}_2 \) did not enhance c-Jun phosphorylation or protein expression, whereas taxol evoked the strongest c-Jun induction and phosphorylation in JNK3-transfected cells (Fig. 4C).

Activation of JNK3 and JNK Targets in Response to NGF—JNKs have been associated with neurite outgrowth in vitro (25) and regeneration in vivo (21, 29). To examine whether JNK3 takes part in the process of neuronal differentiation in PC12 cells, we treated serum-starved PC12 cells with different doses of NGF (10 and 50 ng/ml) for 3 and 5 days. At these time points, JNK activities were analyzed in whole cell extracts. JNK1 and JNK2 were phosphorylated to a similar extent in JNK3-transfected cells (1.3- and 2.3-fold) and in wild type cells (1.5- and 2-fold), but JNK3 showed an enhanced response (2.5- and 3-fold) to NGF treatment (Fig. 5). JNK protein levels did not change (Fig. 5).

Because transfection of PC12 cells with JNK3 led to an altered phosphorylation pattern of ATF-2 in response to apoptotic stress stimuli (see above), we investigated the expression and phosphorylation levels of c-Jun and ATF-2 in nuclear proteins on days 3 and 5 of the differentiation process. Importantly, transfection with JNK3 increased the induction and phosphorylation of c-Jun (Fig. 5), whereas ATF-2 phosphorylation was equal in untransfected and transfected cells (Fig. 5).

To further characterize the differentiation processes induced by NGF and the involvement of JNK3, we determined the expression and phosphorylation of the heavy unit of the neurofilament protein (NF-H), which is a JNK substrate as well as a feature of neuronal differentiation in PC12 cells. For this purpose, we used an antibody that detected both the phosphorylated and the unphosphorylated form. Transfection of JNK3 substantially increased the expression and/or phosphorylation of NF-H as compared with wild type or vector-transfected cells (Fig. 5).

FIG. 5. JNK3-transfected PC12 cells displayed a stronger induction and phosphorylation of JNK targets following NGF treatment (10 ng/ml). Both wild type JNK1/2 (wt) and transfected JNK3 were activated \( (p-\text{JNK1/2} \) and \( p-\text{JNK3-EGFP} \), respectively) after 3 and 5 days in medium supplemented with NGF. The activation of JNK3 in response to NGF was higher than the combined activity levels of JNK1 and JNK2. Induction of c-Jun protein expression and phosphorylation of c-Jun \( (p-\text{c-Jun}) \) were significantly stronger in JNK3-transfected PC12 cells, whereas ATF-2 phosphorylation levels were similar in wild type and transfected cells. The induction/phosphorylation of NF-H was also significantly higher in JNK3-transfected cells.

JNK3 Promotes Formation and Elongation of Neurites in PC12 Cells in Response to NGF—In JNK3-transfected cells, treatment with 10 ng/ml NGF significantly accelerated the morphological changes linked to the neuronal phenotype as compared with wild type and vector-transfected cells (Fig. 6A). Thereby, transfected JNK3 was present both in the nucleus and the cytoplasm (Fig. 6B); even though the cytoplasm contained substantially more JNK3 (Fig. 6C), the presence of JNK3 in the nucleus was repeatedly established by microscopic analysis. This confirms the finding that JNK3 transfection affected both cytoplasmic and nuclear JNK substrates (Fig. 5).

Analysis of the differentiation status after 3 and 5 days of NGF application revealed an increase in both the number of neurites \( (p < 0.000001; \) Fig. 7A) and the length of neurites (Fig. 7B) as compared with wild type and vector-transfected cells. The enhanced length of the neurites became apparent on day 5 with a higher percentage of neurites longer than 40 \( \mu \)m in JNK3 transfected cells (57%) compared with wild type (32%) or vector-transfected cells (35%). A similar effect was observed with 50 ng/ml NGF (data not shown).

DISCUSSION

The present study demonstrates that individual JNK isoforms, e.g. JNK3-p54, can exert substantial but contrasting effects in neurons. Transfection of JNK3 into PC12 cells, which normally only express the JNK1 and JNK2 isoforms, significantly enhanced cell death in response to UV irradiation and taxol. In the context of NGF-induced differentiation of PC12 cells, however, transfected JNK3 evoked a substantial increase...
in the number and length of neurites compared with untransfected cells. This functional dichotomy of JNK3 was mirrored by a differential activation and induction of nuclear JNK substrates, i.e. enhanced phosphorylation of ATF-2 following death signaling in response to UV or taxol and increased expression and phosphorylation of c-Jun during NGF-induced differentiation. The absence of significant JNK activation or target phosphorylation in response to the stress stimulus H2O2 supports our hypothesis (21, 32) that JNKs, including JNK3, are not merely injury- or stress-specific kinases but also have relevant physiological functions depending on the cellular context and the switch of nuclear effector substrates.

**JNK3 as a Mediator in Neuronal Cell Death**

**UV Irradiation**—We have extended the knowledge about intracellular alterations in mammalian cells triggered by UV irradiation (33) by novel insights into the function of JNKs. UV irradiation activated all of the JNK isoforms and induced expression and N-terminal phosphorylation of c-Jun. In this context, transfected JNK3 not only increased cell death but enhanced the phosphorylation of ATF-2, whereas c-Jun expression and activation was not affected by the increased pool of JNK molecules.

UV irradiation is a particularly strong stimulus for both the activation of JNKs, including JNK3, and the expression/phosphorylation of c-Jun (30, 34, 35). The fact that transfected JNK3 had no impact on c-Jun expression and phosphorylation suggests that c-Jun activation following UV is mediated via the Ras/ERK pathway (36, 37) rather than by the characteristic phospho-c-Jun autoregulatory loop (38, 39). It remains to be elucidated whether the function of the Ras/ERK pathway is confined to the induction of c-Jun or includes a JNK-independent N-terminal phosphorylation of c-Jun (40).

It is the ATF-2 transcription factor that serves as the predominant nuclear receptor for JNKs in response to UV irradiation (41, 42). By its high transactivating potential, ATF-2 can enhance or even replace the action of c-Jun (43–46). Thus, despite the general view that JNKs exert their degenerative-apoptotic effects via N-terminal phosphorylation of c-Jun, the previous reports and the present results demonstrate that the increased phosphorylation of ATF-2 is linked to the enhanced potential of JNK3 to trigger UV-evoked cell death, which sheds new light on stimulus-specific neurodegenerative actions of ATF-2 downstream of JNKs. In this context, ATF-2 might cooperate with c-Jun to induce death effectors such as Fas ligand (15, 45, 46) and should not be overlooked as an important JNK target in neuronal cell death. We could exclude the contribution of p38 to the enhanced ATF-2 phosphorylation in JNK3-transfected cells, because p38 was activated to a similar extent in wild type and transfected PC12 cells in response to UV.

**Taxol**—The chemotherapeutic agent taxol suppresses mitotic spindle assembly and chromosome movement, which leads to cell cycle arrest at the G2/M phase (47) and dysfunction of the cytoskeleton. Similar to UV irradiation, treatment of PC12 cells with taxol activated all JNK isoforms and c-Jun. Transfection with JNK3 selectively increased the phosphorylation of ATF-2, but not of c-Jun, and in consequence significantly enhanced cell death. As reported recently, taxol is a particularly potent activator of JNKs and their nuclear substrates c-Jun and ATF-2 (47–50). Most authors agree that JNKs propagate cell death in response to microtubule inhibiting agents (51–53), whereas single observations indicate a protective role for JNKs in this context (54). In cortical neurons, taxol has been shown to promote cell death by activating the nuclear JNK pool and c-Jun; unfortunately, the phosphorylation of ATF-2 was not investigated (51).

**H2O2**—In our study, H2O2 (60 μM) had only moderate effects on both cell death and the activation of JNKs and their targets. So far, the JNK activation by H2O2 had not specifically been addressed in PC12 cells, but JNK activation has been described following reactive oxygen species and redox reactions (55). Transfected JNK3 did not alter the death rate or the phosphorylation levels of c-Jun and ATF-2. These observations convincingly demonstrate that the increase in JNK molecules by JNK3 transfection per se is not sufficient to influence cell survival or activation of nuclear substrates and that the action of JNKs in PC12 cells is highly stimulus-specific.

**JNK3 Promotes Neuronal Differentiation**

In response to NGF, PC12 cells differentiate into neuron-like cells with formation and elongation of neurites. We could demonstrate that this differentiation went along with the activation of all JNK isoforms as well as an increased expression and phosphorylation of c-Jun. All of these effects, including the formation of neurites, were enhanced by transfection with JNK3.

The activation of JNKs after NGF treatment in PC12 cells has already been described (23, 25, 56), but so far, its meaning is completely unknown. Our finding of the differentiation-triggering role of the JNK3 isoform, however, strikingly counteracts the widely accepted role of JNK3 as a pro-degenerative effector molecule (10, 18). It deserves major interest that the promotion of differentiation by JNK3 involves the induction and phosphorylation of c-Jun. In both PC12 cells and oligodendrocytes, NGF treatment results in JNK3 activation with p75 and Rac as upstream intermediates (18, 25, 28, 35). In striking contrast to the differentiation of PC12 cells, oligodendrocytes undergo apoptosis in response to NGF, suggesting that the change in JNK3 signaling is mediated either by elements downstream of Rac or by modulation of the p75-tyrosine receptor kinase A interaction.
The enhanced induction/phosphorylation of NF-H in JNK3-transfected cells further supports the neurite outgrowth-promoting role of JNK3. NF-H expression and phosphorylation underlies the elongation as well as the stabilization of neurites and facilitates its own transport. Therefore, by inducing increased expression/phosphorylation of NF-H, JNK3 can further advance sprouting.

The substrate switch between ATF-2 and c-Jun not only represents a novel facet of the functional JNK repertoire but can also be considered to be the first direct evidence of the bipartite action of JNKs. JNK isoforms can be recruited by several scaffold proteins such as JNK-interacting protein (59), bZIP transcriptional-regenerating potential of phosphorylated c-Jun in vitro, and scaffold alterations in the JNK3 signalosome might account for the substrate switch, but these mechanisms remain to be elucidated.

The activation of c-Jun by JNK3 potentiates the differentiating-regenerating potential of phosphorylated c-Jun in vitro, which also holds a particular position in the cell body response in the brain following nerve fiber damage (21, 63, 64). Our findings support the importance of JNKs and phosphorylated c-Jun for neuronal differentiation (23, 25, 65, 66). In detail, we have directly proven the contribution of the JNK3 isoform to the enhanced induction/phosphorylation of NF-H in JNK3- and scaffold alterations in the JNK3 signalosome might account for the substrate switch, but these mechanisms remain to be elucidated.

In the present study, we have shown that a single splice form of JNK3 (JNK-p54) has the potential to exert pivotal effects in a defined cell culture system such as PC12 cells. On the one hand, it enhances cell death caused by UV irradiation and taxol treatment, and on the other hand, transfected JNK3 increases the number and length of neurites in PC12 cells. The underlying substrate switch of JNK3 sheds new light on the role of ATF-2 in neurodegeneration. The functional versatility of JNK3 in physiological and pathological situations requires a substantial revision of the general notion of JNK3 as a neuronal stress kinase.

Acknowledgment—We thank Aninka Dorst for expert technical assistance.

REFERENCES

1. Savage, M. J., Lin, Y. G., Caillella, J. R., Flood, D. G., and Scott, R. W. (2002) J. Neurosci. 22, 3376–3385
2. Saporto, M. S., Thomas, B. A., and Scott, R. W. (2000) J. Neurosci. 20, 7602–7613
3. Herdegen, T., and Waetzig, V. (2001) J. Neurosci. 21, 1297–1304
4. Herdegen, T., and Waetzig, V. (2001) Oncogene 20, 2424–2437
5. Ip, Y. T., and Davis, R. J. (1998) Curr. Opin. Cell Biol. 10, 205–219
6. Widmann, C., Gibson, S., Jarpe, M. B., and Johnson, G. L. (1999) Physiol. Rev. 78, 143–180
7. Giasson, B. I., and Mushynski, W. E. (1996) J. Biol. Chem. 271, 30404–30409
8. Davis, R. J. (1994) Biochem. Soc. Symp. 192, 1208–1209
9. Bruckner, S. R., Tammaro, S. P., Kuan, C. Y., Flavell, R. A., Rakic, P., and Estus, S. (2001) J. Neurosci. 21, 286–303
10. Morishima, Y., Godth, Y., Zieg, J., Barrett, T., Takanu, H., Flavell, R., Davis, R. J., Hirai, S., and Greenberg, M. E. (2001) J. Biol. Chem. 276, 5551–5760
11. Li, S. B., Zhang, J., Takahashi, S., Ma, W., Jaffe, H., Kulkarni, A. B., and Pant, H. C. (2002) EMBO J. 21, 1432–1443
12. Shackleford, D. A., and Yeh, R. Y. (2001) Brain Res. Mol. Brain Res. 94, 178–192
13. Naughton, U., and Xia, Z. (2000) J. Neurosci. 20, 6442–6451
14. Coffer, T. E., Smidens, G., Rangl, V., Cao, J., Brecht, S., Herdegen, T., and Courtney, M. J. (2002) J. Neurosci. 22, 4353–4354
15. Herdegen, T., Blume, A., Burkach, T., Pfasch, M., Winter, C., Schmidt, W. H., Hech, T. F., Zimmermann, M., and Gass, P. (1997) Neuroscience 119, 191–212
16. Herdegen, T., Blume, A., Pfasch, M., Winter, C., and Kainz, M. (1997) J. Neurosci. 17, 5124–5135
17. Heasley, L. E., Storey, B., Fager, G. R., Butterfield, L., Zamarrpia, J., Blumberg, D., and Masse, R. A. (1996) Mol. Cell. Biol. 16, 648–656
18. Kobayashi, M., Nagata, S., Kita, Y., Nakatsu, N., Ibata, S., Ikeda, K., Kuroda, S., U., M., Iba, H., Konishi, H., Kikkawa, U., Saotob, I., and Fukui, Y. (1997) J. Biol. Chem. 272, 10689–10692
19. Yan, R., Yoshizawa, H., and Osada, H. (1997) J. Biol. Chem. 272, 18261–18266
20. Zita, Y., Kimura, K., Kobayashi, M., Ibata, S., Kikuchi, K., Kuroda, S., M., Iba, H., Konishi, H., and Fukui, Y. (1999) J. Cell Sci. 111, 907–915
21. Giasson, B. I., Brecht, S., Henderson, L. L., Valin, P. D., and Mushynski, W. E. (1999) J. Neurochem. 72, 1081–1087
22. Cano, E., and Mahadevan, L. C. (1996) Trends Biochem. Sci. 20, 117–122
23. M., A., and Akai, F. X., Abo, A., and Karin, M. (1995) Cell 195, 1147–1157
24. Kenney, A. M., and Kocsis, J. D. (1998) J. Neurosci. 18, 1318–1328
25. Butterfield, L., Zentrich, E., Beekman, A., and Heasley, L. E. (1999) Biochem. J. 338, 681–686
26. Widmann, C., Gibson, S., Jarpe, M. B., and Johnson, G. L. (1999) Biochem. J. 324, 681–686
27. Cano, E., and Mahadevan, L. C. (1996) Trends Biochem. Sci. 20, 117–122
28. Minde, A., Lin, A., Smeal, T., Minde, A., and Karin, M. (1998) Genes Dev. 11, 2072–2105
29. Herdegen, T., Claret, F. X., Martin-Villalba, A., Winter, C., Kallunki, T., and Kainz, M. (1997) J. Neurosci. 17, 5124–5135
30. Herdegen, T., Blume, A., Burkach, T., Pfasch, M., Winter, C., and Kainz, M. (1997) J. Neurosci. 17, 5124–5135
31. Herdegen, T., Blume, A., Pfasch, M., Winter, C., and Kainz, M. (1997) J. Neurosci. 17, 5124–5135
32. Herdegen, T., Blume, A., Pfasch, M., Winter, C., and Kainz, M. (1997) J. Neurosci. 17, 5124–5135
33. Herdegen, T., Blume, A., Pfasch, M., Winter, C., and Kainz, M. (1997) J. Neurosci. 17, 5124–5135
A Single c-Jun N-terminal Kinase Isoform (JNK3-p54) Is an Effector in Both Neuronal Differentiation and Cell Death
Vicki Waetzig and Thomas Herdegen

J. Biol. Chem. 2003, 278:567-572.
doi: 10.1074/jbc.M207391200 originally published online October 24, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M207391200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 66 references, 40 of which can be accessed free at http://www.jbc.org/content/278/1/567.full.html#ref-list-1