Mol Cell Biochem (2012) 371:137–146
DOI 10.1007/s11010-012-1430-8

Interleukin-22 protects rat PC12 pheochromocytoma cells from serum deprivation-induced cell death

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Received: 13 March 2012 / Accepted: 3 August 2012 / Published online: 16 September 2012

Abstract Interleukin-22 (IL-22), an IL-10 family cytokine, mediates the crosstalk between leukocytes and epithelial cells. Previous studies reported that IL-22 expresses in mouse brain, and the rat PC12 cells are responsive to IL-22 stimulation. However, the biological roles of IL-22 in neuronal cells remain largely unknown. We show here that IL-22 activates Stat3, p38 mitogen-activated protein kinases (MAPK), and Akt pathways and inhibits Erk/MAPK pathway in naïve PC12 cells. We further demonstrate that IL-22 protects naïve PC12 cells from serum starvation-induced cell death via the Jak1/Stat3 and Akt pathways. We also show that IL-22 has no effects on naïve PC12 cell proliferation and cannot protect naïve PC12 cells from 1-methyl-4-phenylpyridinium (MPP^+)-induced cytotoxicity. However, IL-22 exerts a dose-dependent protective effect on MPP^+-induced neurodegeneration in nerve growth factor-differentiated PC12 cells. Overall, our data suggest that IL-22 might play a role in neurological processes. To our knowledge, this is the first report showing that IL-22 confers a neuroprotective function, which may provide a new therapeutic option for treatment of neurodegenerative diseases.

Keywords Interleukin-22 (IL-22) · PC12 cell · Neurodegeneration · Cell survival

Abbreviations

DA	Dopaminergic
DMEM	Dulbecco's modified Eagle's medium
FBS	Fetal bovine serum
GAP-43	Growth-associated protein 43
HS	Horse serum
IB	Immunoblot
IL-22	Interleukin-22
MAPK	Mitogen-activated protein kinases
MPP^+	1-Methyl-4-phenylpyridinium
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NGF	Nerve growth factor
PI3K	Phosphatidylinositol-3-kinases

Introduction

Interleukin-22 (IL-22), a novel member of the IL-10 family of cytokines, is produced by the cells from both innate and adaptive immune systems such as CD4 T cell subsets, NK cells, and LTi-like cells [1]. IL-22 signals through a

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receptor complex composed of the ligand-binding chain IL-22R1 and the accessory chain IL-10R2 [2, 3]. While IL-10R2 is ubiquitously expressed, the expression of IL-22R1 is detected in non-immune cells including epithelial cells, keratinocytes, or hepatocytes [4, 5]. Upon binding to its receptors, IL-22 induces Jak1 and Tyk2 phosphorylation, activates Stat3, and to a less degree activates Stat1 and Stat5 [2, 5–7]. In addition, IL-22 was also found to activate mitogen-activated protein kinases (MAPK) and Akt pathways in several kinds of cell lines including colon cancer cells SW480, HT29, and rat hepatoma cell H4IIE [6, 8, 9]. It has been shown that IL-22 plays an important role in the inflammation involved in chronic inflammatory diseases and infectious diseases [1, 10, 11]. The elevated IL-22 has been found in the patients with inflammatory bowel disease, psoriasis, and rheumatoid arthritis. The serum levels of IL-22 often correlate with disease grade [5, 8, 12, 13].

Although the expression of IL-22R1 is known to be mainly confined to epithelial cells, an early study by Dumoutier et al. [14] reported that IL-22 was found to induce activation of Stat3 and Stat5 in rat pheochromocytoma PC12 cells, suggesting IL-22R1 is expressed and might have the biological function in PC12 cells. Interestingly, Dumoutier et al. [14] also reported that constitutive IL-22 expression was also detected at a low level in the brain of 6-week-old normal mice, while Levillayer et al. [15] found that IL-22 is expressed in mouse spinal cord, indicating a role for IL-22 in neurological processes. In addition, IL-22 is found to be the candidate gene for a locus controlling mortality in a mouse experimental autoimmune encephalomyelitis (EAE) model [15] and associates with increased disease priming in a rat EAE model [15, 16]. Furthermore, a recent study observed high levels of IL-22 during the induction and peak phases that markedly decrease during recovery in an acute EAE model in a Lewis rat [17]. Interestingly, Beyeen et al. [18] reported that the soluble IL-22 binding protein, IL-22RA2, which functions as a potent antagonist of IL-22, associates with multiple sclerosis (MS) and macrophage effector mechanisms in experimental neuroinflammation. Together, these studies support a role of IL-22 in neurological processes. However, an early report by Kreymborg et al. [19] showed that IL-22 knock-out mice are fully susceptible to EAE, indicating that IL-22 is not required for the development of autoimmune encephalomyelitis. Therefore, further study is needed to illuminate the exact role of IL-22 in MS and EAE.

In this study, we demonstrated that IL-22 treatment activates Stat3 and Akt in naïve PC12 cells. We further examined IL-22-mediated effects on proliferation and neurite outgrowth in naïve PC12 cells in the presence or absence of serum or various chemical compounds. Our data indicated that IL-22 protects naïve PC12 cell from serum deprivation-induced cell death.

Materials and methods

Antibodies and reagents

Antibodies to active Erk1/2, Jnk, and total Erk1/2 were from Promega, and antibody to α-synuclein and β-actin were from Sigma. Antibodies to p38 MAP kinase, Stat3, Stat5, and their phosphorylated forms were from Cell Signaling Technology. Antibody to growth-associated protein 43 (GAP-43) was purchased from Millipore. Recombinant murine IL-22 (mIL-22) and nerve growth factor (NGF) were from Peprotech. Inhibitors of phosphatidylinositol-3-kinases [PI3K]/Akt (Wortmannin), Jak inhibitor 1, and Stat3 inhibitor III (WP1066) were purchased from Calbiochem. Another PI3K/Akt inhibitor LY294002 was obtained from Cell Signaling Technology. 1-Methyl-4-phenylpyridinium (MPP^+) was from Sigma.

Cell culture and treatments

Rat naïve PC12 pheochromocytoma cells were purchased from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China). Naïve PC12 cells were grown in poly-L-Lysine-coated dishes in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % horse serum (HS), 5 % fetal bovine serum (FBS), 1 % penicillin/streptomycin, and 2 mM L-glutamine. The culture medium was changed every 3 days and cells were subcultured once a week.

To induce PC12 cell differentiation by NGF, naïve PC12 cells were differentiated with 50 ng/ml NGF, which was added to the culture medium with low serum (0.5 % FBS and 1 % HS). Fifty percent of the culture medium was replaced with a fresh one every 2 days. Cells were differentiated for 9 days as described previously [20]. For treatments with MPP^+, NGF-differentiated or naïve PC12 cells were incubated for 24 h with IL-22 (20, 50, 100, 200, 500 ng/ml) or NGF (50 ng/ml), which served as a positive control. Cells were then treated with MPP^+ (5 mM) in the DMEM containing 1 % HS and 0.5 % FBS for another 24 h.

Measurement of neurite outgrowth

Naïve PC12 cells were plated at a density of 4×10^4 cells in poly-L-Lysine-coated six-well plates. At 24 h after incubation, the medium was changed to low serum (0.5 % FBS and 1 % HS), cells were then treated with IL-22 (10, 20, 50, 100, 200 ng/ml) or NGF (50 ng/ml). Fifty percent of the culture medium was replaced every 2 days. Five days later, cells were examined by counting neurite-positive cells using an inverted microscope. Neurite-positive

cells were defined as cells exhibiting a process greater than 1.5-cell body diameter and possessing a terminal growth cone. Such cells were counted in 5 microscopic fields, among which 100 cells were examined.

Cell viability and proliferation assay

Naïve PC12 cells were plated at a density of 1×10^4 cells in poly-L-Lysine-coated 96-well plates. For serum-free experiments, naïve PC12 cells were washed three times with DMEM. Cells were mock-treated or pretreated with various indicated inhibitors for 1 h and then incubated with IL-22 (100 ng/ml) for 2 days. Cell viability and proliferation were measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described previously [21]. Experiments were performed in triplicate.

Immunoblot analysis

Immunoblot (IB) was performed as described previously [22]. All IB experiments were carried out in duplicate.

Statistical analysis

Data were summarized as mean ± SD. Statistical analysis was first performed for all groups by a one-way analysis of variance (ANOVA) to determine statistically significant variance between the groups for each endpoint assessed. Dunnett's LSD tests were used to determine the significant difference among multiple groups by means of the SPSS 11.0 software (SPSS Inc., Chicago, IL, USA). The statistical significances were achieved when $P < 0.05$ ($^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$, and $^{\#}P < 0.001$).

Results

IL-22 signaling in naïve PC12 cells and the effect of IL-22 on serum starvation-induced PC12 cells

PC12 cells were reported to respond to IL-22 [14]. In our preliminary experiment, IL-22R1 mRNA was detectable in naïve PC12 cells by RT-PCR analysis (data not shown). We then investigated the signaling pathways mediated by IL-22 in naïve PC12 cells. Figure 1 shows the kinetics of the activation of Stat3, MAPK, and Akt pathways. Within

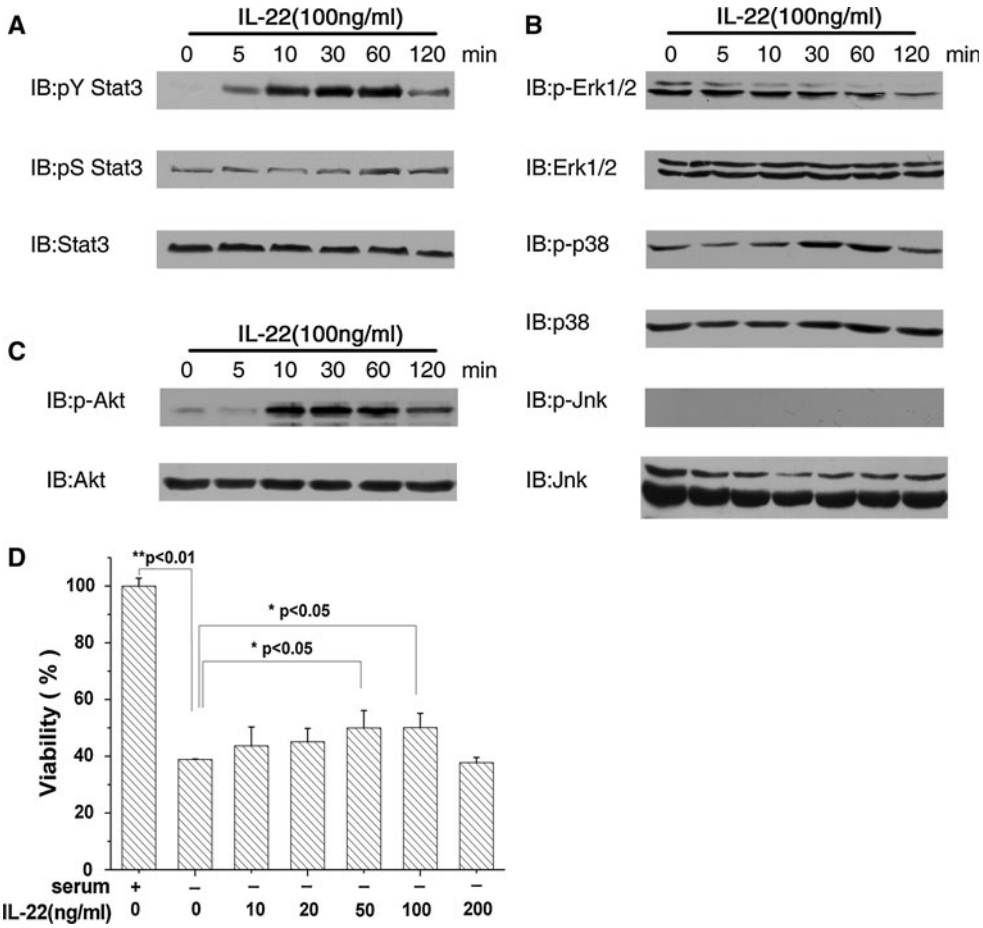

Fig. 1 Activation of Stat3, p38 MAPK, and Akt pathways in IL-22-stimulated PC12 cells and IL-22-mediated protective effect on serum starved-PC12 cells. Near-confluent naïve PC12 cells in 6 cm dishes were starved for 18 h and then stimulated with IL-22 (100 ng/ml) for the indicated times. Total lysates were analyzed by IB assay with antibodies against the phosphorylated forms of Stat3 (Tyr 705), Stat3 (Ser 727) (**a**), Erk1/2, p38, and Jnk (**b**), and Akt (**c**). The membranes were then stripped and reprobed with antibodies against total Stat3, Erk1/2, p38, Jnk, and Akt. All IB experiments were performed twice. **d** Naïve PC12 cells were incubated in serum-free medium and various concentrations of IL-22 were added to the culture. Cells grown in DMEM supplemented with complete serum were used as control. After 2 days incubation, viable cells were counted by means of MTT assay. Values are mean ± SD ($n = 3$). $^{**}P < 0.01$ versus serum (+) group. $^*P < 0.05$ versus serum (−) group

5 min, mIL-22 induced tyrosine phosphorylation of Stat3 (Fig. 1a). The phosphorylation was markedly increased at 10 min after stimulation and reached the maximal phosphorylation level at 30–60 min (Fig. 1a). In addition, serine phosphorylation of Stat3 was observed in PC12 cells from 60 to 120 min after IL-22 stimulation. During the observation, total Stat3 levels remained unchanged (Fig. 1a). However, no activation of Stat1 and Stat5 was observed in IL-22-stimulated PC12 cells (data not shown). We next analyzed the ability of IL-22 to activate the MAPK and Akt pathways. As shown in Fig. 1b, increased phosphorylation of p38 MAPK was detected during 30–60 min after IL-22 stimulation, whereas no significant activation of Jnk was observed. Surprisingly, compared to basal levels of phosphorylation of Erk1/2 in unstimulated PC12 cells, phosphorylation of Erk1/2 significantly decreased in response to IL-22 stimulation. Further examination of Akt phosphorylation revealed that 10 min of IL-22 stimulation led to the phosphorylation of Akt at Serine 473 with a peak at 30 min, which decreased afterward during 120 min of stimulation (Fig. 1c). Taken together, the data indicate that IL-22 activates Stat3, p38 MAPK, and Akt pathways and inhibits Erk/MAPK pathway in naïve PC12 cells.

It has been reported that serum deprivation for 48 h results cell death in naïve PC12 cells [23, 24]. To examine the effect of IL-22 on PC12 cell death by serum deprivation, naïve PC12 cells in DMEM deprived of serum were treated with various concentrations of IL-22 or mock-treated for 48 h. As shown in Fig. 1d, upon serum deprivation, approximately 60–70 % of the inoculated cells died. IL-22 at concentrations of 50 and 100 ng/ml exerted a significantly protective effect on naïve PC12 cell death.

IL-22-mediated protective effect against serum deprivation through the Jak1/Stat3 and Akt pathways

To elucidate the mechanism underlying the pro-survival effect of IL-22, we investigated the signaling pathways responsible for the effect. As revealed by our results in this study, IL-22 induces the activation of Stat3 and Akt. Therefore, naïve PC12 cells were treated with specific inhibitors of their respective pathways: Jak inhibitor 1, an inhibitor of Jak1 kinase activity; WP1066, an inhibitor of Stat3 phosphorylation; LY294002 and Wortmannin, both inhibitors of Akt activation. The effective concentration of these inhibitors was selected by dose–response assays in order to prevent cytotoxicity (data not shown). These inhibitors at different concentrations were then added to the naïve PC12 cell cultures 1 h before the addition of IL-22 (100 ng/ml). As shown in Fig. 2a and b, IL-22-increased survival of PC12 cells was significantly inhibited by Jak inhibitor 1 (100 nM) and WP1066 (6 μM). IL-22-triggered tyrosine phosphorylation of Stat3 was blocked by the two inhibitors, suggesting that the Jak1/Stat3 pathway contributes to IL-22-mediated PC12 cells' survival. Similar results were also obtained in cells treated with the PI3K inhibitor Wortmannin (500 nM), while activation of Akt upon IL-22 stimulation was inhibited in the presence of Wortmannin (Fig. 2c). Treatment with another PI3K inhibitor, LY294002, resulted in markedly decreased survival of PC12 cells in a dose-dependent manner (Fig. 2d). In addition, LY294002 inhibited IL-22-mediated PC12 cells' survival, while activation of Akt was totally blocked (Fig. 2d). Taken together, the results indicated that the PI3K/Akt pathway is also involved in IL-22-mediated naïve PC12 cell survival.

Effect of IL-22 on cell proliferation and neurite outgrowth in naïve PC12 cells

IL-22 was shown to induce proliferation of a variety of cells such as keratinocytes and SW480 cells [8, 9, 25]. To investigate the IL-22-mediated effect on PC12 cell proliferation, naïve PC12 cells were cultured in the low-serum medium (0.5 % FBS and 1 % HS) for 24 h and then treated with mIL-22 for the indicated time points. NGF treatment was used as a positive control. As shown in Fig. 3a, IL-22 treatment did not affect PC12 cell proliferation, while 24 and 72 h treatments of NGF induced significant cell proliferation (***$P < 0.005$; $^{\#}P < 0.001$). Furthermore, NGF-triggered cells' proliferation was not altered by treatment with IL-22. These results indicate that IL-22 has no effect on naïve PC12 cell proliferation in the presence or absence of NGF.

To quantify neurite outgrowth in PC12 cell, the number of naïve PC12 cells exhibiting neurites was measured during a 5-day period of exposure to IL-22 or NGF, or both of them. NGF treatment (50 ng/ml) was used as a positive control. Larger cell bodies and elaboration of an extensive network of neurites were seen in NGF-treated PC12 cells (data not shown). As shown in Fig. 3b, neurite outgrowth was not observed in naïve PC12 cells treated with IL-22 of various concentrations (10–200 ng/ml), whereas NGF induced significant neurite outgrowth in naïve PC12 cells. A number of neurotypical proteins have been shown to be associated with naïve PC12 cells' differentiation and neurite outgrowth, including GAP-43 [26, 27]. GAP-43 protein levels were extremely low in naïve PC12 cells on day 0 and IL-22 treatment did not alter the expression of GAP-43 (Fig. 3c). Consistent with previous results [28], increased expression of GAP-43 was detected upon NGF treatment.

We further examined whether IL-22 affected NGF-induced neurite outgrowth; naïve PC12 cells were co-treated with 50 ng/ml NGF and IL-22 at various

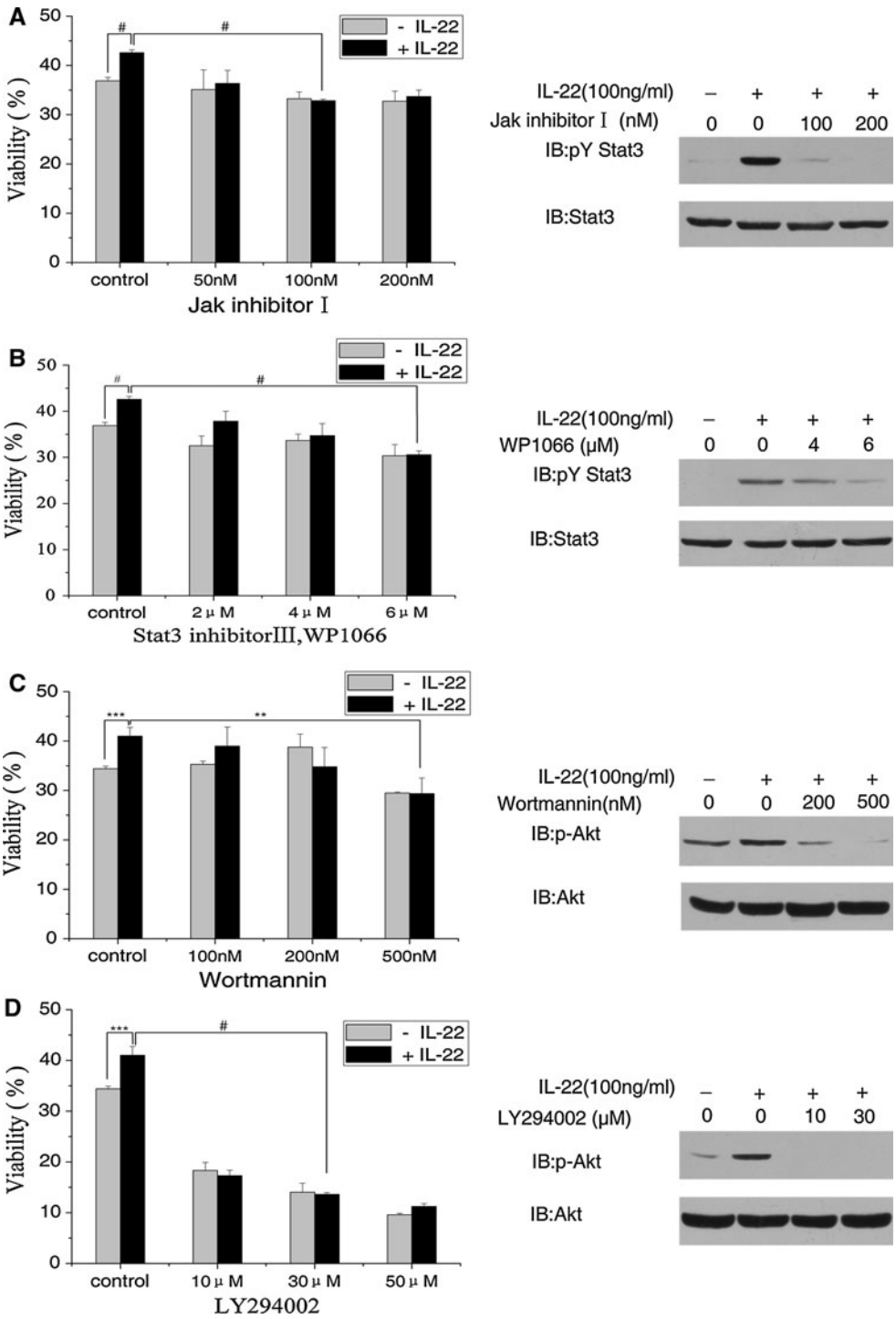

Fig. 2 Role of Stat3 and PI3K pathways in IL-22-exerted protective effect on PC12 cells. **a–d** Naïve PC12 cells were incubated in serum-free medium and mock-treated or treated with specific inhibitors for 1 h. 100 ng/ml IL-22 was then added to the medium for 2 days. Cell viability and activation of a specific pathway were determined by MTT assay and IB, respectively. **a** Jak1 inhibitor I (50, 100, 200 nM), **b** WP1066 (2, 4, 6 μM), **c** Wortmannin (100, 200, 500 nM), **d** LY294002 (10, 30, 50 μM). Values are mean ± SD ($n = 3$, each). $^{\#}P < 0.001$; $^{***}P < 0.005$; $^{*}P < 0.05$. IB experiments were performed twice

concentrations. As illustrated in Fig. 3d, co-incubation of NGF and IL-22 at concentrations from 10 to 100 ng/ml decreased the effect of NGF on neurite outgrowth, and 100 ng/ml IL-22 induced a significant inhibitory effect on NGF-induced neurite outgrowth ($^{*}P < 0.05$), suggesting that NGF-induced expression of GAP-43 was partially attenuated by IL-22 (Fig. 3e). Collectively, these data suggest that IL-22 might down-regulate NGF-primed effects on neurite outgrowth in naïve PC12 cells.

Effect of IL-22 on MPP^{+}-induced cytotoxicity in naïve PC12 cells and neurodegeneration in NGF-differentiated PC12 cells

Parkinson's disease (PD) is characterized by the progressive degeneration of dopaminergic (DA) neurons with the presence of α-synuclein inclusions, termed Lewy bodies. Many kinds of neurotoxins can be used to induce the model of PD such as paraquat, rotenone, 6-OHDA

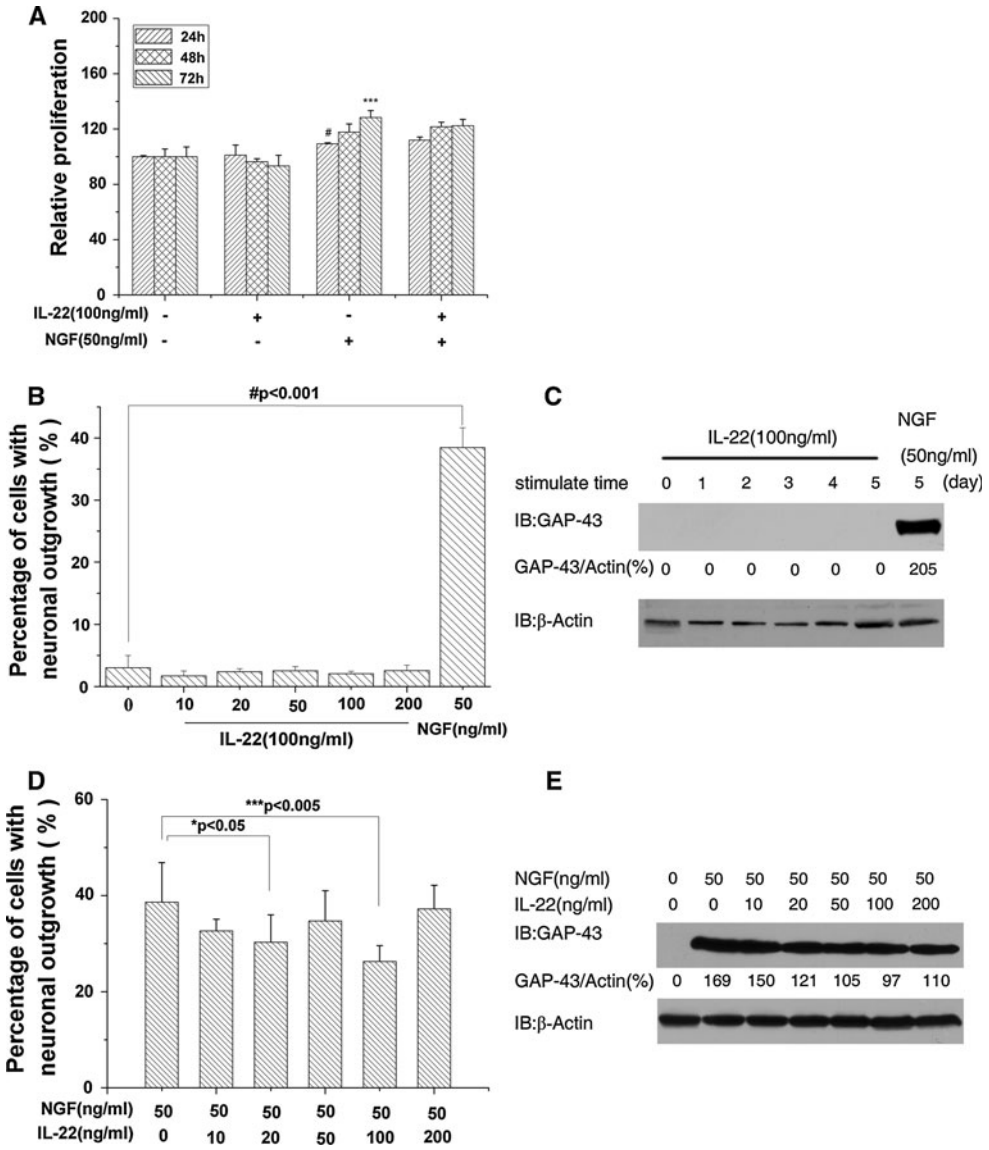

Fig. 3 IL-22-induced effects on PC12 cell proliferation and neurite outgrowth. **a** Naïve PC12 cells were seeded onto 96-well plates at a density of 10,000 cells per well and were grown for 1 day. After starvation in low-serum (0.5 % FBS and 1 % HS) medium overnight, the cells were stimulated with IL-22 (100 ng/ml) or NGF (50 ng/ml), which was set as positive control, or stimulated with both IL-22 (100 ng/ml) and NGF (50 ng/ml) or with cytokine-free medium (negative control) for 24, 48, and 72 h. The cell proliferation rate was determined by MTT assay. Values are mean ± SD ($n = 3$, each). $^{\#}P < 0.001$; $^{***}P < 0.005$ versus unstimulated control. **b** and **d** For quantification of neurite outgrowth, naïve PC12 cells were plated at a density of 4×10^4 cells in poly-L-Lysine-coated six-well plates. At 24 h after incubation, the medium was changed to low serum (0.5 % FBS and 1 % HS), and PC12 cells were treated with IL-22 (10, 20, 50, 100, 200 ng/ml) and/or NGF (50 ng/ml) alone. Fifty percent of the culture medium was replaced every 2 days, including fresh stimulator. Five days later, cells were examined by counting neurite-positive cells using an inverted microscope. Values are mean ± SD ($n = 3$, each). $^{\#}P < 0.001$; $^{***}P < 0.005$; $^{*}P < 0.05$. **c** and **e** To determine the expression of GAP-43, **c** PC12 cells were stimulated with IL-22 (100 ng/ml) or NGF (50 ng/ml) for the indicated days or **e** stimulated with IL-22 (10, 20, 50, 100, 200 ng/ml) and NGF (50 ng/ml) for 5 days. Cell lysates were subjected to SDS-PAGE and IB assay was performed. IB experiments were performed twice

(6-hydroxydopamine), and MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). The most widely used were 6-OHDA and MPTP [29]. Both neurotoxins caused a significant loss of nigral tyrosine hydroxylase-immunostained cells and striatal dopamine depletion, but 6-OHDA caused more widespread and intense cell loss, more intense body weight loss, and more mortality than MPTP [29, 30]. The main advantage of the MPTP was that MPTP-lesioned rats are a good model of early phase PD because these animals presented less loss of body weight, resulting in lower animal suffering, morbidity, and mortality compared to the 6-OHDA rat model [29, 31]. MPP^+, the active metabolite of MPTP, has been extensively used in various mammalian species to produce an experimental model of PD [32].

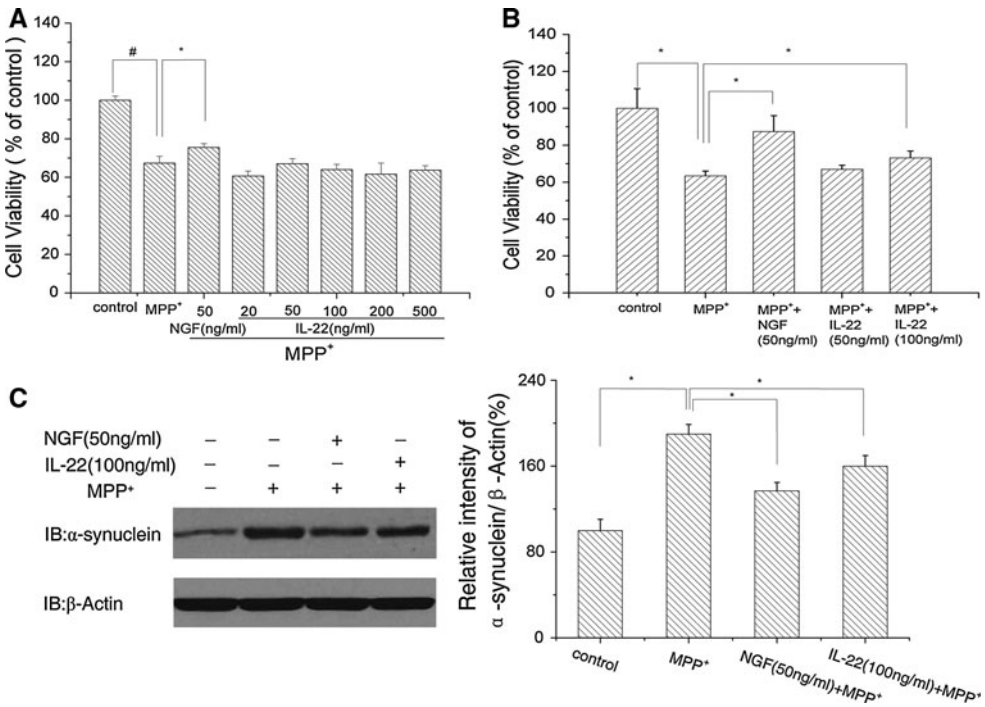

Fig. 4 IL-22-exerted protective effect on NGF-differentiated PC12 cells from MPP^+. The naïve (**a**) or NGF-differentiated (**b**) PC12 cells were treated with MPP^+ (5 mM) as described in experimental procedures. Cell viability was measured by MTT assay. **c** NGF-differentiated PC12 cells were pre-treated with 100 ng/ml IL-22 or 50 ng/ml NGF for 24 h, then incubated with MPP^+ (5 mM) for another 24 h. α-Synuclein expression was determined by IB analysis. β-Actin was used as an internal standard and the ANOVA showed significant differences among the groups. IB experiments were performed twice. Values are mean ± SD ($n = 3$, each). $^{\#}P < 0.001$; $^{*}P < 0.05$

MPP^+ induced cell death and/or apoptosis in PC12 cells [33]. To investigate whether IL-22 could protect naïve PC12 cells against MPP^+-induced cytotoxicity, naïve PC12 cells in DMEM containing 1 % FBS in order to avoid the effect of serum deprivation were treated with NGF (50 ng/ml) or IL-22 at a variety of concentrations or mock-treated for 24 h and then incubated with MPP^+ (5 mM) for another 24 h. MPP^+ at the concentration of 5 mM was used in previous studies to induce 30 % cell death [34]. As illustrated in Fig. 4a, IL-22 at the concentrations of 50–500 ng/ml had no protective effect on MPP^+-induced cytotoxicity. Consistent with previous reports, 50 ng/ml NGF treatment displayed significant protective effects against MPP^+-induced cytotoxicity [35].

PC12 cells are DA neuron-like cells and have physiological properties of DA neurons [36]. MPP^+ provokes the selective degeneration of DA neurons [37]. NGF-differentiated PC12 cells are more sensitive to MPP^+ than naïve cells [38], which serves the basis for the classic method of MPP^+-induced PC12 cells. Thus, we adopted this method to establish an in vitro PD model to induce neurodegeneration in NGF-differentiated PC12 cells. To investigate whether IL-22 affects MPP^+-induced neurodegeneration, NGF-differentiated PC12 cells cultured in DMEM containing 1 % FBS were incubated with NGF (50 ng/ml) or IL-22 (50 and 100 ng/ml) or mock-treated for 24 h and then incubated with MPP^+ (5 mM) for another 24 h. In line with a previous report, NGF treatment showed a significant protective effect on MPP^+-induced neurodegeneration [35]. Figure 4b shows that 100 ng/ml IL-22 treatment significantly reduced MPP^+-induced neurodegeneration ($^{*}P < 0.05$), whereas 50 ng/ml IL-22 exhibited no protective effect, indicating a dose-dependent protective effect by IL-22 on MPP^+-induced neurodegeneration of NGF-differentiated PC12 cells.

One of the hallmarks of PD is the presence of α-synuclein inclusions, termed Lewy bodies, in the DA neurons of the substantial nigra [39, 40]. Upon stimulation with MPP^+, the monomeric α-synuclein (19 kDa) was easily oligomerized to form into oligomers (57 kDa) [39, 40]. It has been reported that MPP^+ can increase the expression of oligomeric α-synuclein (57 kDa) in PC12 cells [41]. To further confirm the protective role of IL-22 in MPP^+-induced neurodegeneration of NGF-differentiated PC12 cells, the expression of α-synuclein was detected by IB analysis. NGF-differentiated PC12 cells were pre-treated with 100 ng/ml IL-22 or 50 ng/ml NGF for 24 h, then 5 mM MPP^+ was added and the incubation continued for the other 24 h. As shown in Fig. 4c, both IL-22 and NGF significantly inhibited the augmentation of oligomeric α-synuclein expression in MPP^+-treated differentiated PC12 cells. Together, these data indicated that IL-22 can protect the NGF-differentiated PC12 cells against neurodegeneration induced by MPP^+.

Discussion

To date, studies on IL-22 focused on its role in mucosal immunity in target cells of the digestive, skin, and respiratory organ systems [1, 10]. Recently, several studies showed that IL-22 and its receptors are implicated in the

pathogenesis of MS and EAE [15–18]. In the present study, we show for the first time that IL-22 protects rat naïve PC12 pheochromocytoma cells from serum deprivation and MPP^+-induced neurodegeneration in the NGF-differentiated PC12 cells. Since constitutive IL-22 expression was detected in the brain of 6-week-old normal mice and in mouse spinal cord [15, 42], our study suggests that IL-22 might act as an important messenger to mediate the communication between the central nervous system (CNS) and the immune system.

IL-22 stimulation primarily results in activation of Stat3 signaling pathways. We observed both tyrosine and serine phosphorylation of Stat3 in IL-22-stimulated naïve PC12 cells, indicating a functional Stat3 pathway in naïve PC12 cells induced by IL-22. However, activation of Stat1 and Stat5 was not detected in naïve PC12 cell upon exposure to IL-22, whereas IL-22 was shown to activate Stat1 and/or Stat5 in cell lines such as TK-10 and HT29 [2, 8], suggesting a cell type-dependent response to IL-22. In addition, IL-22 selectively activates some signals in MAPK pathways in a cell type-dependent manner. For instance, it activated all three (Erk1/2, Jnk, and p38) major MAPK pathways in H4IIE rat hepatoma cells and intestinal subepithelial myofibroblasts [6, 12], Erk1/2 and Jnk pathways in human keratinocytes and intestinal epithelial cells [8, 43], or Erk1/2 and p38 in synovial fibroblasts from rheumatoid arthritis patients [13]. We also observed activation of Erk1/2 in IL-22-stimulated human colorectal cancer SW480 cells and IL-22-induced activation of the three major MAPK pathways (Erk1/2, Jnk, and p38) in IL-22R1-expressing HEK293 cells [9]. On the other hand, it inhibited phosphorylation of Erk1/2 in EMT6 murine breast cancer cells [44]. In this study, we observed that IL-22 activated p38 MAPK pathway, inhibited phosphorylation of Erk/MAPK, but did not affect the Jnk phosphorylation in naïve PC12 cells. This observation is generally consistent with the fact that the effect of IL-22 on the MAPK pathways is cell type dependent.

Induction of cell growth by IL-22 is observed in some cell types. We show that IL-22 treatment has no effect on naïve PC12 cell proliferation and neurite outgrowth. Previous reports indicated that IL-22 induces the proliferation of several cell lines including keratinocytes, SW480, and IL-22R1-transfected BaF3 and inhibits keratinocyte differentiation [9, 45–47]. However, IL-22 was shown to reduce EMT6 cell proliferation in both in vitro and in vivo [44]. Together, our data and others' further support the notion that IL-22 exerts specific effects in different cell types. Interestingly, although treatment with IL-22 alone does not affect the neurite outgrowth of naïve PC12 cells, NGF-induced neurite outgrowth was attenuated by IL-22 as demonstrated by a decreased number of cells with neurite outgrowth and decreased GAP-43 expression.

MPTP is one of the well-characterized neurotoxins responsible for the sudden onset of Parkinsonism-like symptoms in humans, monkeys, as well as mice [48–50]. We demonstrate that IL-22 reduces MPP^+-induced neurodegeneration in NGF-differentiated PC12 cells in a dose-dependent manner, but it cannot protect naïve PC12 cells against MPP^+-induced cytotoxicity. The data suggested a role of IL-22 in preventive and/or complementary therapies for several human neurodegenerative diseases. It is well established that pro-inflammatory cytokines such as IL-1, IL-6, and tumor necrosis factor, which are expressed and released from resident cells of the CNS and other immune cells, play a critical role during the onset and progress of neurodegenerative diseases [51, 52]. Taken together, our data provide a new therapeutic option for neurodegenerative diseases.

The key finding of this study is that IL-22 at concentrations of 50–100 ng/ml significantly increases naïve PC12 cell viability in the absence of serum ($^*P < 0.05$), suggesting IL-22 can protect naïve PC12 cells from serum deprivation. The protective effect of IL-22 on PC12 cell survival was significantly inhibited by the Jak1/Stat3 pathway inhibitors Jak inhibitor 1 and WP1066, or the PI3K/Akt pathway inhibitors LY294002 and Wortmannin, suggesting both Jak1/Stat3 pathway and PI3K/Akt pathway contribute to the protective effect of IL-22 on naïve PC12 cell viability in the absence of serum. A similar mechanism has been reported for IL-6-induced protecting of PC12 cells from serum deprivation [23]. Therefore, common signaling pathways might be utilized by different cytokines to exert their protective effects in PC12 cells. Interestingly, in contrast to IL-22, IL-17, a cytokine also produced by Th17 cell, enhances neuronal injury induced by oxygen-glucose deprivation [53]. The opposite biological effects exerted by IL-17 and IL-22 were also observed in the pathogenesis of psoriasis. Wolk et al. [54] reported that IL-22 is one of the key mediators of the epidermal alterations in psoriasis, while IL-17 is not. Considering different cytokines such as IL-22 and IL-17 might exist in same sites in the nervous system, it will be intriguing to investigate how these cytokines interplay with each other and how they are tightly regulated in particular settings.

Collectively, we demonstrate here that IL-22 protects naïve PC12 cells from serum deprivation and this effect might be mediated by the Jak1/Stat3 and PI3K/Akt pathways. Moreover, we show that IL-22 reduces MPP^+-induced neurodegeneration in NGF-differentiated PC12 cells. Therefore, our data suggest that IL-22 might be a new link between the CNS and immune cells with an

implication in the therapeutic avenue for neurodegenerative diseases.

Acknowledgments This work was supported by the National Science Foundation of China (30870792 and 81030025).

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