Cisplatin-induced cytotoxicity is blocked by brain-derived neurotrophic factor activation of TrkB signal transduction path in neuroblastoma

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Abstract

We evaluated the ability of brain-derived neurotrophic factor (BDNF) to decrease the chemosensitivity of neuroblastoma cells to cisplatin. Two cell lines, one derived from SH-SY5Y (SY5Y-TB8) and the other from SK-N-AS (AS-TB8), transfected with a TrkB plasmid were generated, and used to assess the effects of activation of the TrkB signal transduction path on cisplatin (Cis) induced apoptosis. BDNF treatment of each of the TrkB expressing cells blocked cisplatin-induced cell death. BDNF’s ability to rescue the cells from cisplatin-induced cell death was inhibited by treatment with the Trk tyrosine kinase inhibitor, K252a, and the phosphatidylinositol 3'-kinase (PI)-3-kinase inhibitor, LY294002. This indicates that the activation of the TrkB path through PI-3-kinase is required for BDNF’s survival-promoting effects.

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1. Introduction

Neuroblastoma (NB) is a pediatric solid tumor derived from neural crest precursor cells [1]. It has been shown that favorable-prognosis NB tumors express relatively high levels of TrkA, whereas cell lines and tumors from patients with poor prognosis are

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insults [14–17]. Thus for normal neurons BDNF, usually via activation of TrkB, protects cells from injury.

We hypothesize that activation of TrkB would protect NB cells from chemotherapy. In NB cells expressing a transfected TrkB or endogenous TrkB induced after retinoid treatment, we have demonstrated that BDNF could partially protect from vinblastine-induced cell death [8]. To investigate the signaling system by which BDNF protects NB cells from chemotherapy, we developed TrkB expressing SY5Y cell lines [18] and TrkB-expressing AS cell lines. Recently we found that activation of the TrkB via the propidium iodide (PI)-3 kinase path BDNF/TrkB rescues NB cells from etoposide-induced cytotoxicity [10]. Similarly, in this study we find that BDNF stimulation of TrkB rescues NB cells from apoptosis induced by cisplatin. Additionally, we find that the BDNF-induced rescue of cells from cisplatin can be blocked by inhibiting Trk tyrosine kinase activity, and selectively targeting the PI-3-kinase path.

2. Materials and methods

2.1. Construction of vectors and establishment of stable transfectants

A 3.1 kb fragment of rat TrkB spanning a full coding region was subcloned into the pBSTR1 vector [18] and transfected into the human NB cell lines SH-SY5Y and SK-N-AS using lipofectAMINE (Life Technologies, Inc.). Stable transfectants (SH-SY5Y-TB8 (SY5Y-TB8); SK-N-AS-TB8 (AS-TB8)) were obtained after selection with puromycin (0.5 μg/ml) [18].

2.2. Cell culture

Cells were cultured in RPMI 1640 containing 10% fetal bovine serum, 2 mM glutamine and antibiotics as previously described [18]. To maintain selection pressure the AS-TB8 and SY5Y-TB8 cells were cultured in puromycin (0.5 μg/ml).

2.3. Reagents

Recombinant human BDNF (100 ng/μl) (PeproTech, Inc.) stocks were prepared as directed and stored at −20 °C. Cisplatin was obtained from Sigma, and 1 mg/ml stocks prepared according to the company’s specification, and stored at −20 °C. The pharmacological inhibitors, LY294002 (1 mM) and K252a (1 mM) were obtained from Sigma, and reconstituted according to manufacturer’s specification.

2.4. Immunoblotting/immunoprecipitation

For protein analysis, cells were washed in phosphate-buffered saline (PBS), centrifuged and cell pellets kept in −70 °C until analysis and prepared as described previously [6]. The cells were then lysed in Nonidet P-40 (NP-40) lysis buffer containing 20 mM Tris pH 7.4, 150 mM NaCl, 1% NP-40, 10% glycerol, 1 mM phenylmethylsulphonyl fluoride, 10 μg/ml aprotinin, 1 μg/ml leupeptin, and 500 μM sodium orthovanadate in ice-cold PBS, and immunoprecipitation was performed as previously described [10]. Akt phosphorylation changes were analyzed by Western blotting analysis of 50 μg of protein loaded onto 12% Tris–Glycine bis-acrylamide gels, and probed with rabbit monoclonal antibodies to phosphoAkt (Cell Signaling). Total Akt protein levels were determined by Western blotting analysis of 50 μg of protein with rabbit monoclonal antibodies to Akt. Signals were detected using enhanced chemiluminescence reagents (Pierce).

2.5. Cell survival analysis

NB cells (1 × 10⁴) per well were plated into 96-well plates. After 16–24 h, the cells were treated with control media or BDNF (100 ng/ml) for 15 min. The next day, the indicated concentrations of cisplatin were added for 2 h. The culture supernatant was then removed, and fresh media added for the remainder of the culture period. Cell number was assessed 48 h later using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as previously described [10]. Each value represents six replicates, and each experiment was repeated 2–3 times.
3. Results

3.1. Characterization of TrkB-expressing NB cell lines

In order to evaluate the effect of TrkB activation on cisplatin chemosensitivity, we utilized TrkB-transfected cell lines from SK-N-AS (AS-TB8) and SH-SY5Y (SY5Y-TB8) cell lines for evaluation. Upon treatment by BDNF (100 ng/ml) for 5 min, autophosphorylation of TrkB can be detected in each of the cell lines (Fig. 1A). In addition, TrkB phosphorylation is abrogated by pre-treatment for 30 min with the selective Trk kinase inhibitor, K252a (500 nM), indicating intact TrkB signal transduction in these cells (Fig. 1A). Activation of the TrkB path leads to activation of several downstream signaling paths, one of which is the PI-3-kinase pathway. A key target of the PI-3-kinase pathway is phosphorylation of Akt (PO4Akt). Following BDNF activation, PO4Akt is detected in both the AS-TB8 and SY5Y-TB8 cell lines (Fig. 1B). Activation of PO4Akt can be abrogated by pre-treatment with the selective PI-3-kinase inhibitor, LY294002 (50 μM) (Fig. 1B).

3.2. BDNF rescues NB cells from cisplatin-induced apoptosis

BDNF pre-treatment of retinoic-acid treated NB cells has been demonstrated to rescue cells from vinblastine-induced cytotoxicity [8]. Recently we showed that BDNF via activating the TrkB path through PI-3-kinase also protects cells from etoposide cytotoxicity [10]. Cisplatin is an important chemotherapeutic agent in the treatment of NB. In this study, we investigate the ability of BDNF to protect two different TrkB-expressing cells from cisplatin-induced cytotoxicity. BDNF does not have a significant effect on the proliferation of control cells [7]. In order to determine the effect of cisplatin on the viability of NB cells, we treated the cells with different concentrations of cisplatin (0–100 μg/ml) for 2 h, and then incubated the cells with untreated media. After 48 h, relative cell number was assessed by using an MTT assay (Fig. 2A,B). Treatment of the AS-TB8 and SY5Y-TB8 cells with chemotherapy results in a dose-responsive cell death as measured by MTT (Fig. 2A,B). Pre-treatment of these cells with BDNF (100 ng/ml) for 15 min prior to addition of cisplatin shows a significant protection from cisplatin-induced cytotoxicity at the indicated drug doses (Fig. 2A,B). Both AS and SY5Y cells treated with BDNF required almost 5-fold more Cisplatin to reach the IC50.

3.3. Inhibition of the Trk tyrosine kinase and PI-3-kinase pathways

In order to show the dependence of the BDNF rescue of cisplatin on TrkB, we pre-treated the cells with the selective Trk kinase inhibitor, K252a (500 nM). K252a was incubated for 30 min prior to addition of BDNF (100 ng/ml). Following BDNF treatment, the cells were treated with either 5 μg/ml cisplatin (AS-TB8) or 10 μg/ml (SY5Y-TB8). After 2 h, cells were washed and cultured for 48 h with media alone. Pre-treatment with K252a results in complete abrogation of BDNF rescue of both the AS-TB8 and SY5Y-TB8 cell lines from cisplatin-induced cytotoxicity (Fig. 3A,
B), demonstrating that rescue is dependent on TrkB activation. Nerve growth factor (NGF), which binds the p75 receptor, but not TrkB fails to rescue cells from cisplatin under these conditions (data not shown).

To determine the role of the PI3-kinase pathway in the BDNF-mediated rescue from cisplatin, we used the PI-3-kinase inhibitor, LY294002. AS-TB8 and SY5Y-TB8 cells were pre-treated with LY294002 (50 μM) and culture as above. LY294002 abrogated the BDNF rescue of cisplatin-induced cytotoxicity, indicating that the PI-3-kinase pathway is primarily responsible for BDNF rescue. Incubation with pharmacologic inhibitors of the mitogen-activated protein kinase (MAPK) pathway (PD98059) and the PLCγ pathway (U73122) failed to block BDNF rescue from cisplatin induced cell death (data not shown).

4. Discussion

Neurotrophins have the ability to protect normal neurons from both chemical and physical injuries [19]. We have hypothesized that the expression of BDNF and TrkB by neuroectodermal tumors may contribute to chemoresistance [8]. Several studies have shown that neurotrophins, particularly BDNF, can promote the survival [2,4,5] and chemoresistance of neuroblastoma cells, however the mechanism of this chemoprotection has yet to be elucidated. In this study, we demonstrate that BDNF is capable of rescuing NB cells from cisplatin-induced cell death. In addition, we have determined that the BDNF rescue can be inhibited by pre-treatment with either a Trk kinase or PI-3-kinase inhibitor.

We have found that BDNF blocks cisplatin, as well
as etoposide, doxorubicin and vinblastine [10], through activation of TrkB. This is evident in that the selective Trk tyrosine kinase inhibitor, K252a, is able to prevent rescue by BDNF. Further evidence lies in the fact that both parental cell lines are unable to be rescued by BDNF treatment (data not shown). One NB model has shown that NGF can mediate protection from neocarzinostatin-induced apoptosis by a p75 dependent mechanism [20]. However the role of p75 signaling can be confusing in that it has been known to have both pro- and anti-apoptotic signaling capacities, and the ability of p75 to rescue is dependent on the levels of Trk receptors present [21].

Using a pharmacologic approach, we have also shown that inhibition of the PI-3 kinase path is able to prevent BDNF rescue. In neurons, various signaling paths have been implicated in rescue from ischemic injury. For example, in neonatal brains BDNF rescue from hypoxic-ischemic injury is dependent on activation of the MAPK pathway [22]. In oxidative stress-induced [23] and axotomy-induced [24] cell death, cerebellar granule neurons and retinal ganglion cells, respectively, are rescued by activation of both the PI-3 kinase and MAPK pathways.

Our studies would indicate that chemotherapy in combination with a PI-3-kinase inhibitor could rescue from BDNF induced chemoresistance. We believe the implication of this study for treatment of aggressive and chemoresistant NB tumors is important. CEP751, a K252a derivative with greater selectivity for the Trk kinase, has already been shown to inhibit growth in TrkB-expressing neuroblastoma xenografts in nude mice [25]. Pharmacologic targeting of the PI-3 kinase path in NB represents an alternative strategy with potentially fewer or different toxicities when combined with chemotherapy to treat NB tumors.

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References


