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Extracellular poly(ADP-ribose) is a neurotrophic signal that upregulates glial cell line-derived neurotrophic factor (GDNF) levels *in vitro* and *in vivo*

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ABSTRACT

Synthesis of poly(ADP-ribose) (PAR) is catalyzed by PAR polymerase-1 (PARP-1) in neurons. PARP1 plays a role in various types of brain damage in neurodegenerative disorders. In neurons, overactivation of PARP-1 during oxidative stress induces robust PAR formation, which depletes nicotinamide adenine dinucleotide levels and leads to cell death. However, the role of the newly-formed PAR in neurodegenerative disorders remains elusive. We hypothesized that the effects of PAR could occur in the extracellular space after it is leaked from damaged neurons. Here we report that extracellular PAR (EC-PAR) functions as a neuroprotective molecule by inducing the synthesis of glial cell line-derived neurotrophic factor (GDNF) in astrocytes during neuronal cell death, both *in vitro* and *in vivo*. In primary rat astrocytes, exogenous treatment with EC-PAR produced GDNF but not other neurotrophic factors. The effect was concentration-dependent and did not affect cell viability in rat C6 astrocytoma cells. Topical injection of EC-PAR into rat striatum upregulated GDNF levels in activated astrocytes and improved pathogenic rotation behavior in a unilateral 6-hydroxydopamine model of Parkinson disease in rats. These findings indicate that EC-PAR acts as a neurotrophic enhancer by upregulating GDNF levels. This effect protects the remaining neurons following oxidative stress-induced brain damage, such as that seen with Parkinson disease.

1. Introduction

Oxidative and genotoxic stress generate DNA strand breaks. Under those conditions, poly(ADP-ribose) polymerases (PARPs) synthesize poly(ADP-ribose) (PAR) from nicotinamide adenine dinucleotides (NAD^+) [1,2]. PARP-1, the first identified subtype of this enzyme, is thought to produce PAR because PAR formation is markedly reduced in tissues from PARP-1 null mice [3]. In the brain, PARP-1 overactivation is induced by massive DNA damage following exposure to oxidative stress. PARP-1 consumes NAD^+ to form excessive levels of PAR [4]. According to the suicide hypothesis, NAD^+ depletion induces PARP-1-dependent cell death via necrosis [5]. The pathophysiological significance of PAR overproduction in response to oxidative stress has been well characterized in neuronal cell death-related neurodegenerative disorders [6].

Despite these extensive studies, little is known about the exact role of newly synthesized PAR. Given that massive oxidative stress causes PARP-1-dependent necrotic cell death (also known as parthanatos) [7], it is likely that PAR is released from damaged neurons through the catastrophically altered plasma membrane into the extracellular space. Therefore, we hypothesized that PAR is leaked into the extracellular space and that extracellular PAR (EC-PAR) affects functions in surrounding cells, especially activated astrocytes, which are abundant around brain lesions [8].

Abbreviations: ANOVA, one-way analysis of variance; BDNF, brain-derived neurotrophic factor; DMEM, Dulbecco's modified Eagle medium; ELISA, enzyme-linked immunosorbent assay; EDTA, ethylenediaminetetraacetic acid; EC-PAR, extracellular poly(ADP-ribose); FBS, fetal bovine serum; GDNF, glial cell line-derived neurotrophic factor; GFAP, glial fibrillary acidic protein; 6-OHDA, 6-hydroxydopamine; NAD^+, nicotinamide adenine dinucleotide; NGF, nerve growth factor; NT-3, neurotrophin-3; PAR, poly(ADP-ribose); PARP-1, poly(ADP-ribose) polymerase-1; PBS, phosphate buffered saline.

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In the present study, we aimed to identify the pathophysiological role of EC-PAR in neurodegenerative disorders *in vitro* and *in vivo* using astrocytic cell cultures and a unilateral 6-hydroxydopamine (6-OHDA) model of Parkinson disease in rats, respectively. Our findings suggest that EC-PAR increases the level of glial cell line-derived neurotrophic factor (GDNF) in astrocytes, both *in vitro* and *in vivo*. In addition, topical injection of PAR into the brain striatum improved abnormal behavior in a Parkinson disease rat model.

2. Materials and methods

2.1. Materials

Unless otherwise noted, all chemicals were analytical grade. DuoSet® rat β-NGF ELISA kits were purchased from R&D systems (Minneapolis, MN). BDNF-, NT-3-, and GDNF-Emax® ImmunoAssay Systems were purchased from Promega (Madison, WI).

2.2. Preparation of PAR

PAR was purified as described previously [9] with some modifications [10,11]. Human recombinant PARP-1 (100 μg, Enzo Life Sciences, Farmingdale, NY) was incubated in reaction buffer containing 100 mM Tris-HCl (pH 8.0), 2 mM $MgCl_2$, 1 mM dithiothreitol, 1.25 μg/ml salmon sperm activated DNA, and 500 μM NAD^+ for 30 min at 30 °C. Reactions were terminated by adding 20% trichloroacetic acid and incubating for 30 min on ice. After centrifugation at 22,000×*g* for 10 min, the pellets were washed three times with diethyl ether. The pellets were hydrolyzed in a reaction buffer containing 1 M KOH and 50 mM EDTA. Hydrolyzed PAR polymers were incubated with buffer A, which contained 250 mM ammonium acetate, 6 M guanidine, and 1 mM EDTA and was adjusted to pH 9.0 using 6 N HCl. Samples were incubated with a 50% slurry of dihydroxyboryl Bio-Rex (DHBB) resin (Bio-Rad, Hercules, CA) for 12 h at 25 °C. The resin was washed three times with buffer A and once with 1 M ammonium acetate (pH 9.0). PAR was eluted using water at 37 °C, and the eluates were applied to a Detoxi-Gel Endotoxin Removing column (Thermo Fisher Scientific, Waltham, MA). To confirm the removal of endotoxins, such as lipopolysaccharides, a limulus test (Limulus HS-F Single Test Wako; Wako Pure Chemical Industries, Osaka, Japan) was performed according to the manufacturer's instructions [12]. Polymer size distribution was analyzed using 20% TBE-PAGE (90 mM Tris-borate pH 8.0, 2 mM EDTA) followed by silver staining (Thermo Fisher Scientific). The distinct polymer size distribution of PAR is shown in Fig. 1A. The concentration was calculated using the absorbance at 258 nm (E_M at 258 nm = 13,500 cm m^{-1}) [9].

2.3. Cell cultures

Primary rat astrocytes were prepared from 19- or 20-day-old embryos of Wistar rats (SLC, Shizuoka, Japan) as described previously [13]. The procedure was approved by the Animal Ethical Committee of Osaka Prefecture University. Cells were cultured to a density of 6.4 × 10^4 cells/cm^2 in 10-cm^2 dishes (Corning) using Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS; JRH Biosciences, Lenexa, KS), 100 units/ml penicillin, and 100 μg/ml streptomycin. Cells were incubated at 37 °C in a 5% CO_2 humidified incubator. Cells were treated with 10 μM PAR for 48 h in FBS-free DMEM. Rat C6 astrocytoma cells (ATCC, Manassas, VA) were cultured in DMEM supplemented with 10% FBS, 2 mM glutamine, and antibiotics–antimycotics (Invitrogen, Carlsbad, CA) under the same conditions as the primary astrocytes. The C6 cells in DMEM containing 10% FBS were plated at a density of 3.2 × 10^4 cells/cm^2 into a 96-well plate and cultured for 24 h. Then, the cells were treated with vehicle (phosphate buffered saline [PBS]) or 1, 3, 10, or 30 μM PAR in Opti-MEM medium (Invitrogen) for 48 h.

2.4. ELISA for neurotrophic factors

In primary cells, four neurotrophic proteins, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and GDNF, were extracted from cells using a sonicator (model Q-125, Qsonica LLC, Newton, CT) according to the procedure described in Refs. [14,15]. The levels of extracted neurotrophins were measured using the appropriate ELISA kit according to the manufacture's protocols. In C6 cells, the level of released GDNF in the medium was measured using the Emax® GDNF ImmunoAssay System according to previously published protocols [16]. In the rat striatum, the level of GDNF was quantified using the same procedure as primary cells [12].

2.5. Quantitative real-time PCR

Quantitative real-time PCR was performed as described previously [13]. The targeted primers for GDNF and β-actin mRNA (internal control) are as followed: GDNF forward, 5′-ACGAAA CCAAGGAGGAACTGA-3′, GDNF reverse, 5′-TTTGTCGTACATTGTCTC GGC-3′, β-actin forward, 5′-AACCGCGAGAAGATGACCCAGTCATGT TT-3′, and β-actin reverse 5′-AGCAGCCGTGGCCATCTCTTGCTCG AAGTC-3’.

2.6. Animal procedures

All animal experiments were approved by the Animal Ethical Committee of Osaka Prefecture University and were performed according to the animal ethical guidelines of Osaka Prefecture University. Male Wistar rats (SLC Japan, Shizuoka, Japan) weighing 200–250 g were provided standard laboratory chow and tap water ad libitum and housed under a constant 12-h light/12-h dark cycle. The Parkinson disease model was induced using a unilateral injection of 6-OHDA into the right striatum as described previously [17] with some modifications. Rats were fixed to a stereotaxic apparatus (Narishige, Tokyo, Japan) and anesthetized using 1.0–1.5% isoflurane (MSD Animal Health, Tokyo, Japan) in air delivered through a facial mask. Injection of 6-OHDA (20 μg/rat; 4 μl of 5 μg/ml in 0.9% saline containing 0.01% ascorbic acid; Sigma-Aldrich, St. Louis, MO) or vehicle into the right striatum was performed at a rate of 1 μl/min using a 28-gauge Hamilton syringe (coordinates: 1.0 mm anterior to bregma, 3.0 mm lateral to midline, and 5.0 mm ventral to the skull surface).

The successful formation of a lesion in the right striatum was confirmed by recording the number of rotation behaviors evoked when the rat was treated with methamphetamine (2 mg/kg, intraperitoneal, Dainippon Sumitomo Pharm., Osaka, Japan) at 7 days after injection of 6-OHDA (designated as pre). The rotation test (n = 21) was performed as described previously [18] with some modifications. Rats were equally divided into three groups based on a rotation criterion of 50–250 turns within 30 min of methamphetamine treatment. A complete turn was defined as a 360° turn to the right. One day after the pre test, rats were anesthetized and injected with EC-PAR (240 or 720 pmol/rat; 4 μl of 60 or 180 μM in PBS) or vehicle in the right striatum. The amount injected was determined based on *in vitro* data. The injected concentrations of 60 or 180 μM EC-PAR are estimated to be 10 or 30 μM in the striatum assuming an average striatum volume of 30 μl (in-house data). All injection coordinates and infusion rates were the same as those for the injection of 6-OHDA. To evaluate the dose-dependent

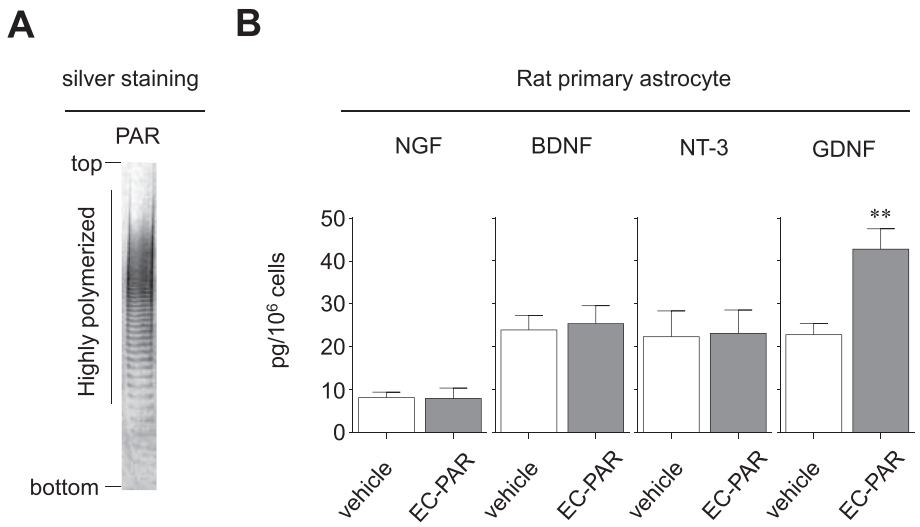

Fig. 1. The effect of extracellular PAR (EC-PAR) on neurotrophic protein levels in rat primary astrocytes. (A) Purified PAR is visualized using silver staining. This sample was used for all EC-PAR experiments. (B) The levels of four neurotrophic proteins were measured using an ELISA. EC-PAR only increased GDNF protein levels. Data represent means ± SD (N = 4, $^{**}p < 0.01$).

effects of EC-PAR on pathogenic rotation behavior induced by 6-OHDA lesions, the rotation test was conducted 7 days after EC-PAR injection (designated as post) using the same protocol as that used for the pre rotation test.

2.7. Immunofluorescent staining in rat brain

To identify cells expressing GDNF in astrocytes of rat brain striatum, frozen coronal sections (10 μm) collected from the striatal and cortical region (+1 mm) through the bregma were incubated at 4 °C overnight with goat polyclonal anti-GDNF antibody (1:200, R&D systems) and rabbit polyclonal anti-glial fibrillary acidic protein (GFAP, 1:500, Dako, Glostrup, Denmark), which is a marker of astrocytes [19]. After three washes, sections were incubated with Alexa 488-conjugated anti-rabbit IgG antibody (1:5000, Invitrogen) and Alexa 568-conjugated anti-goat IgG antibody (1:5000, Invitrogen) for 1 h. Square images (5 × 5 mm) were collected using confocal microscopy (C1si-TE2000-E; Nikon). Raw images were initially grayed and converted to binary using the Scion image software package (version 4.0.3., Scion cooperation, Fredrick, MD). The numbers of binary pixels in the images were measured automatically [20].

2.8. Statistical analysis

All data are represented as the mean ± SD of independent experiments, and N are indicated in each Figure legend. For statistical analysis, two-group and multiple-group comparisons were analyzed using the unpaired Student's *t*-test or one-way analysis of variance (ANOVA) followed by Dunnett's multiple test, respectively. The results obtained from the rotation test (the numbers of turns at pre versus post for each rat) were analyzed using the paired Student's *t*-test.

3. Results and discussion

To determine the effects of EC-PAR on the levels of neurotrophic proteins, we treated rat primary astrocytes with purified PAR. EC-PAR significantly increased the level of GDNF but not that of NGF, BDNF, or NT-3 (Fig. 1B). Because the EC-PAR was verified to be endotoxin free, the effect of EC-PAR on the GDNF level is based purely on its potency.

We next examined if EC-PAR affects the synthesis and release of GDNF. In this test, we used rat C6 astrocytoma cells because they have characteristically high levels of GDNF synthesis and release [16]. EC-PAR increased GDNF release (Fig. 2A) and mRNA synthesis (Fig. 2B) in a concentration-dependent manner without affecting cell viability (supplementary Fig. 1). Further, treatments with ADP-ribose, nicotinamide, or NAD^+ did not upregulate GDNF levels (data not shown). Thus, the polymerized structure of EC-PAR could is indispensable for GDNF expression in astrocytes *in vitro*.

To examine the effect of EC-PAR on GDNF expression *in vivo*, we locally injected purified PAR into rat brain striatum (Fig. 3A). The striatum regions were selected because that is the most appropriate area to determine the *in vivo* effectiveness of GDNF [21]. Injection of EC-PAR into right striatum increased the levels of GDNF in GFAP-positive cells (i.e., astrocytes) (Fig. 3B), and the relative number of GDNF/GFAP double-positive cells increased approximately two-fold (Fig. 3C, left panel). By contrast, there were almost no cells that were GDNF-positive and GFAP-negative (Fig. 3C, right panel). Further, the GDNF protein levels were increased significantly following injection of EC-PAR in the striatum (Fig. 3D). These results clearly indicate that EC-PAR upregulated GDNF levels in astrocytes *in vivo*.

Finally, we evaluated how EC-PAR-induced GDNF expression in the striatum affects methamphetamine-evoked rotation behavior in a unilateral 6-OHDA model of Parkinson disease in rats. The study design is shown in Fig. 4A. Injection of EC-PAR into the striatum attenuated abnormal rotation behaviors in a dose-dependent manner (Fig. 4B). Additionally, treatment with EC-PAR did not influence the body weight of the rats (supplementary Fig. 2). These results suggest that EC-PAR improves pathological motor function in the Parkinson disease rat model by increasing GDNF levels in the striatum.

In this study, we found that EC-PAR has potent effects on astrocytes by the increasing the expression of GDNF both *in vitro* and *in vivo*. These results indicate that in neurodegenerative disorders, such as Parkinson disease, the PAR released into the extracellular space after parthanatos potently upregulates GDNF expression in activated astrocytes. Thus, EC-PAR could act as a rescue molecule that protects the remaining neurons from undergoing parthanatos [7]. Interestingly, several studies have found anti-PAR antibodies in

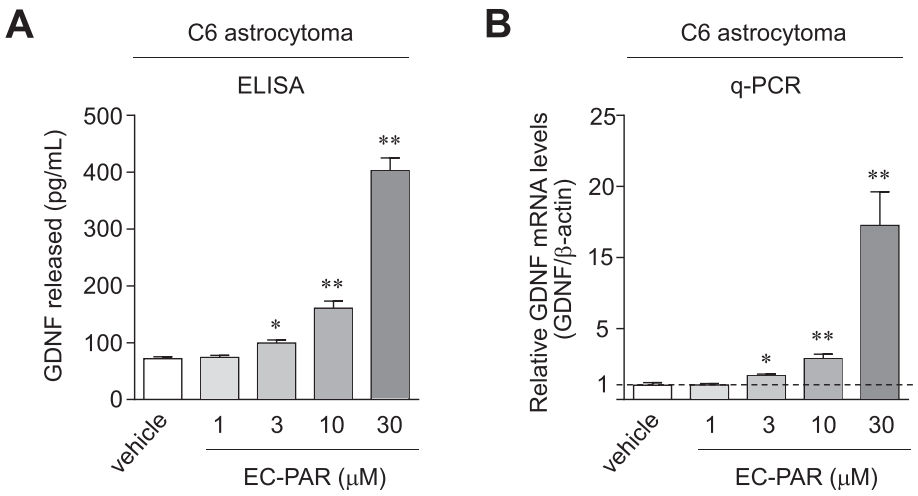

Fig. 2. Characterization of EC-PAR-induced GDNF expression in rat C6 astrocytoma. C6 cells were incubated with 1, 3, 10, or 30 μM EC-PAR at 37 °C for 48 h. (A) The release of GDNF into the medium was measured using an ELISA. (B) The expression of GDNF mRNA was measured using quantitative PCR (q-PCR). Data represent means ± SD (N = 4, $^{*}p < 0.05$, $^{**}p < 0.01$, relative to vehicle).

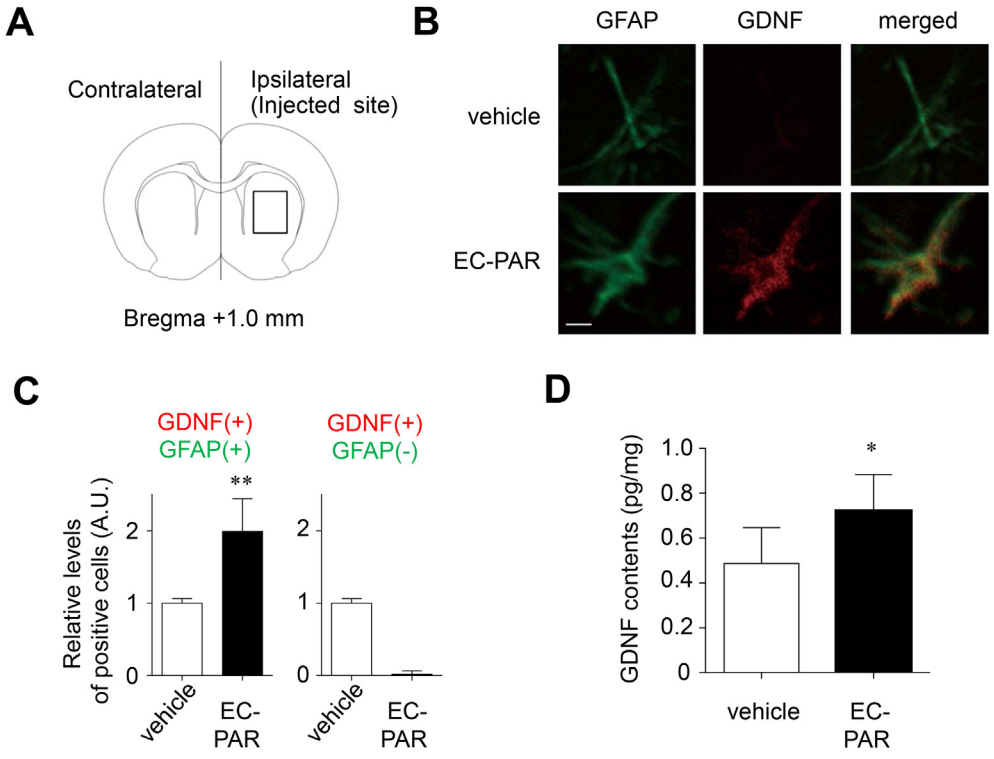

Fig. 3. The effect of EC-PAR on GDNF levels in rat striatum. (A) Squares represent the brain regions analyzed in the following experiments. (B) Immunofluorescence analysis of GFAP (green) and GDNF (red) in the striatum following an intrastriatal injection of 720 pmol/rat EC-PAR. Scale bar = 10 μm. (C) Semi-quantification of GDNF/GFAP double-positive signals (left panel) and GDNF-positive and GFAP-negative signals (right panel) are shown for vehicle- and EC-PAR-injected striatum. (D) The effect of EC-PAR on GDNF levels in the striatum was measured using an ELISA. Data represent means ± SD (N = 4, $^{*}p < 0.05$, $^{**}p < 0.01$).

the serum of patients with Alzheimer disease [22]; autoimmune diseases, such as systemic lupus erythematosus [23]; and systemic autoimmune rheumatic disease [24]. These data support our hypothesis that PAR is released in the extracellular space, possibly through catastrophically damaged cells, following oxidative stress.

In the immune system, EC-PAR is a pro-inflammatory signal for macrophages [25], and the T-cell surface molecule, mono-ADP-ribosyltransferase 2, systematically generates EC-PAR for this purpose [26]. To our knowledge, this is the first report of the effects of EC-PAR in the central nervous system. Here, we showed that EC-PAR upregulates GDNF levels in astrocytes. GDNF is one of the most promising agents for a curative therapy for Parkinson disease [27]. The results of this study indicated that EC-PAR could potentially be used to increase GDNF levels in patients with Parkinson disease. Moreover, EC-PAR could play a cytoprotective role in parthanatos through upregulation of rescue agents, such as GDNF. Further

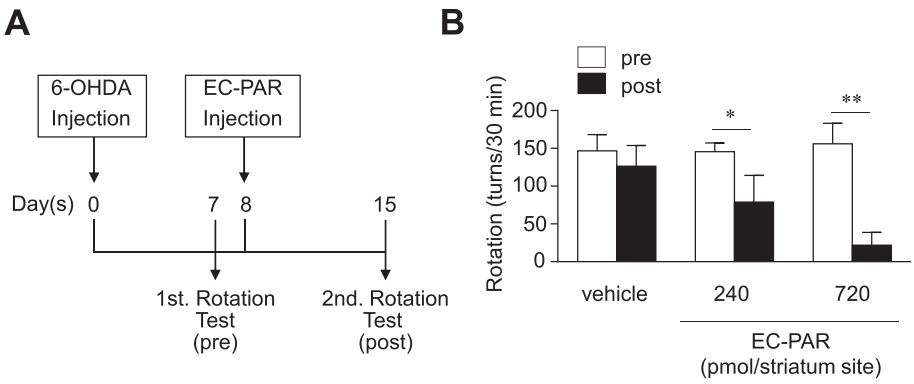

Fig. 4. Dose-dependent effect of EC-PAR on 6-OHDA Parkinson disease rat model. (A) An overview of the study design and striatum injection schedule. (B) Effects of intrastriatal injection of EC-PAR (240 or 720 pmol/rat) or vehicle on methamphetamine-induced rotation behaviors. Data represent means ± SD (N = 7, $^{*}p < 0.05$, $^{**}p < 0.01$).

studies investigating the distinct pathophysiological significance of EC-PAR in other tissues are needed to obtain a clear picture of poly(ADP-ribosyl)ation during parthanatos.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2017.01.129

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2017.01.129

References

[1] G. de Murcia, J. Menissier de Murcia, Poly(ADP-ribose) polymerase: a molecular nick-sensor, Trends Biochem. Sci. 19 (1994) 172–176.
[2] O. Hayaishi, K. Ueda, Poly(ADP-ribose) and ADP-ribosylation of proteins, Annu. Rev. Biochem. 46 (1977) 95–116.
[3] J.C. Ame, V. Schreiber, V. Fraulob, P. Dolle, G. de Murcia, C.P. Niedergang, A bidirectional promoter connects the poly(ADP-ribose) polymerase 2 (PARP-2) gene to the gene for RNase P RNA. structure and expression of the mouse PARP-2 gene, J. Biol. Chem. 276 (2001) 11092–11099.
[4] M.J. Eliasson, K. Sampei, A.S. Mandir, P.D. Hurn, R.J. Traystman, J. Bao, A. Pieper, Z.Q. Wang, T.M. Dawson, S.H. Snyder, V.L. Dawson, Poly(ADP-ribose) polymerase gene disruption renders mice resistant to cerebral ischemia, Nat. Med. 3 (1997) 1089–1095.
[5] H.C. Ha, S.H. Snyder, Poly(ADP-ribose) polymerase is a mediator of necrotic cell death by ATP depletion, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 13978–13982.
[6] A. Chiarugi, Poly(ADP-ribose) polymerase: killer or conspirator? The 'suicide hypothesis' revisited, Trends Pharmacol. Sci. 23 (2002) 122–129.
[7] K.K. David, S.A. Andrabi, T.M. Dawson, V.L. Dawson, Parthanatos, a messenger of death, Front. Biosci. (Landmark Ed.) 14 (2009) 1116–1128.
[8] P. Teismann, J.B. Schulz, Cellular pathology of Parkinson's disease: astrocytes, microglia and inflammation, Cell Tissue Res. 318 (2004) 149–161.
[9] G.M. Shah, D. Poirier, C. Duchaine, G. Brochu, S. Desnoyers, J. Lagueux, A. Verreault, J.C. Hoflack, J.B. Kirkland, G.G. Poirier, Methods for biochemical study of poly(ADP-ribose) metabolism in vitro and in vivo, Anal. Biochem. 227 (1995) 1–13.
[10] S.A. Andrabi, H.C. Kang, J.F. Haince, Y.I. Lee, J. Zhang, Z. Chi, A.B. West, R.C. Koehler, G.G. Poirier, T.M. Dawson, V.L. Dawson, Iduna protects the brain from glutamate excitotoxicity and stroke by interfering with poly(ADP-ribose) polymer-induced cell death, Nat. Med. 17 (2011) 692–699.
[11] H. Nakajima, H. Nagaso, N. Kakui, M. Ishikawa, T. Hiranuma, S. Hoshiko, Critical role of the automodification of poly(ADP-ribose) polymerase-1 in nuclear factor-kappaB-dependent gene expression in primary cultured mouse glial cells, J. Biol. Chem. 279 (2004) 42774–42786.
[12] A. Motoyoshi, H. Nakajima, K. Takano, M. Moriyama, Y. Kannan, Y. Nakamura, Effects of Amphotericin B on the expression of neurotoxic and neurotrophic factors in cultured microglia, Neurochem. Int. 52 (2008) 1290–1296.
[13] A. Motoyoshi-Yamashiro, M. Tamura, M. Moriyama, K. Takano, K. Kawabe, H. Nakajima, R. Katoh-Semba, T. Furuichi, Y. Nakamura, Activation of cultured astrocytes by amphotericin B: stimulation of NO and cytokines production and changes in neurotrophic factors production, Neurochem. Int. 63 (2013) 93–100.
[14] R. Katoh-Semba, M. Tsuzuki, N. Miyazaki, A. Yoshida, H. Nakajima, C. Nakagawa, S. Kitajima, M. Matsuda, Distribution and immunohistochemical localization of GDNF protein in selected neural and non-neural tissues of rats during development and changes in unilateral 6-hydroxydopamine lesions, Neurosci. Res. 59 (2007) 277–287.
[15] T. Mele, D.M. Juric, Metrifonate, like acetylcholine, up-regulates neurotrophic activity of cultured rat astrocytes, Pharmacol. Rep. 66 (2014) 618–623.
[16] K. Hisaoka, A. Nishida, T. Koda, M. Miyata, H. Zensho, S. Morinobu, M. Ohta, S. Yamawaki, Antidepressant drug treatments induce glial cell line-derived neurotrophic factor (GDNF) synthesis and release in rat C6 glioblastoma cells, J. Neurochem. 79 (2001) 25–34.
[17] M. Itakura, T. Kohda, T. Kubo, Y. Semi, Y.T. Azuma, H. Nakajima, S. Kozaki, T. Takeuchi, Botulinum neurotoxin A subtype 2 reduces pathological behaviors more effectively than subtype 1 in a rat Parkinson's disease model, Biochem. Biophys. Res. Commun. 447 (2014) 311–314.
[18] M. Itakura, T. Kohda, T. Kubo, Y. Semi, K. Nishiyama, Y.T. Azuma, H. Nakajima, S. Kozaki, T. Takeuchi, Botulinum neurotoxin type A subtype 2 confers greater safety than subtype 1 in a rat Parkinson's disease model, J. Vet. Med. Sci. 76 (2014) 1189–1193.
[19] H. Nakajima, T. Kubo, Y. Semi, M. Itakura, M. Kuwamura, T. Izawa, Y.T. Azuma, T. Takeuchi, A rapid, targeted, neuron-selective, in vivo knockdown following a single intracerebroventricular injection of a novel chemically modified siRNA in the adult rat brain, J. Biotechnol. 157 (2012) 326–333.
[20] H. Nakajima, W. Amano, T. Kubo, A. Fukuhara, H. Ihara, Y.T. Azuma, H. Tajima, T. Inui, A. Sawa, T. Takeuchi, Glyceraldehyde-3-phosphate dehydrogenase aggregate formation participates in oxidative stress-induced cell death, J. Biol. Chem. 284 (2009) 34331–34341.
[21] M. Aoi, I. Date, S. Tomita, T. Ohmoto, Single administration of GDNF into the striatum induced protection and repair of the nigrostriatal dopaminergic system in the intrastriatal 6-hydroxydopamine injection model of hemiparkinsonism, Restor. Neurol. Neurosci. 17 (2000) 31–38.
[22] Y. Kanai, H. Akatsu, H. Iizuka, C. Morimoto, Could serum antibody to poly(ADP-ribose) and/or histone H1 be marker for senile dementia of Alzheimer type? Ann. N. Y. Acad. Sci. 1109 (2007) 338–344.
[23] Y. Kanai, T. Sugimura, Comparative studies on antibodies to poly(ADP-ribose) in rabbits and patients with systemic lupus erythematosus, Immunology 43 (1981) 101–110.
[24] S. Garcia, C. Conde, The role of poly(ADP-ribose) polymerase-1 in rheumatoid arthritis, Mediat. Inflamm. 2015 (2015) 837250.
[25] K.A. Krukenberg, S. Kim, E.S. Tan, Z. Maliga, T.J. Mitchison, Extracellular poly(ADP-ribose) is a pro-inflammatory signal for macrophages, Chem. Biol. 22 (2015) 446–452.
[26] A.R. Morrison, J. Moss, L.A. Stevens, J.E. Evans, C. Farrell, E. Merithew, D.G. Lambright, D.L. Greiner, J.P. Mordes, A.A. Rossini, R. Bortell, ART2, a T cell surface mono-ADP-ribosyltransferase, generates extracellular poly(ADP-ribose), J. Biol. Chem. 281 (2006) 33363–33372.
[27] A. Saavedra, G. Baltazar, E.P. Duarte, Driving GDNF expression: the green and the red traffic lights, Prog. Neurobiol. 86 (2008) 186–215.