

capability to penetrate the challenging targets (Archakov *et al.* 2003; Watt 2006; Gozes 2007; Patel *et al.* 2007; Meade *et al.* 2009). Taken together, this study confers a precise demonstration about the broad-spectrum protective activity of ProT $\alpha$ -derived small peptide P<sub>30</sub> against ischemic damages *in vitro* and *in vivo*. Thus, it is evident that P<sub>30</sub> mimics the *in vitro* and *in vivo* neuroprotective actions of ProT $\alpha$ . The sequence homology of P<sub>30</sub> domain in ProT $\alpha$  among all species is highly conserved; furthermore, this sequence is completely equal in human, rat, and mouse. From these facts, it is speculated that P<sub>30</sub> domain may play important roles in robustness of ProT $\alpha$  against neuronal damages.

In conclusion, ProT $\alpha$ -derived peptide P<sub>30</sub> exerted its survival actions in cultured neurons against ischemic stress. P<sub>30</sub> significantly blocked the ischemia-induced functional and cellular damages in retina as well as in brain, along with inhibition of the cerebral blood vessels disruption. Therefore, detailed mechanisms underlying neuroprotection by ProT $\alpha$ -derived small peptide may provide a novel therapeutic approach for the treatment of ischemic damages in the central nervous system.

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## References

- Archakov A. I., Govorun V. M., Dubanov A. V., Ivanov Y. D., Veselovsky A. V., Lewi P. and Janssen P. (2003) Protein-protein interactions as a target for drugs in proteomics. *Proteomics* **3**, 380–391.
- Asi H. and Perlman I. (1992) Relationships between the electroretinogram a-wave, b-wave and oscillatory potentials and their application to clinical diagnosis. *Doc. Ophthalmol.* **79**, 125–139.
- Beck H. and Plate K. H. (2009) Angiogenesis after cerebral ischemia. *Acta Neuropathol.* **117**, 481–496.
- Bejot Y., Prigent-Tessier A., Cachia C., Giroud M., Mossiat C., Bertrand N., Garnier P. and Marie C. (2011) Time-dependent contribution of non neuronal cells to BDNF production after ischemic stroke in rats. *Neurochem. Int.* **58**, 102–111.
- Blanco R. E., Soto I., Duprey-Diaz M. and Blagburn J. M. (2008) Upregulation of brain-derived neurotrophic factor by application of fibroblast growth factor-2 to the cut optic nerve is important for long term survival of retinal ganglion cells. *J. Neurosci. Res.* **86**, 3382–3392.
- Buckingham B. P., Inman D. M., Lambert W., Oglesby E., Calkins D. J., Steele M. R., Vetter M. L., Marsh-Armstrong N. and Horner P. J. (2008) Progressive ganglion cell degeneration precedes neuronal loss in a mouse model of glaucoma. *J. Neurosci.* **28**, 2735–2744.
- Danielli R., Fonsatti E., Calabrò L., Giacomo A. M. and Maio M. (2012) Thymosin  $\alpha$ 1 in melanoma: from the clinical trial setting to the daily practice and beyond. *Ann. N. Y. Acad. Sci.* **1270**, 8–12.
- Dong G., Callegari E. A., Gloeckner C. J., Ueffing M. and Wang H. (2012) Prothymosin- $\alpha$  interacts with mutant huntingtin and suppresses its cytotoxicity in cell culture. *J. Biol. Chem.* **287**, 1279–1289.
- Dvorianchikova G., Barakat D. J., Hernandez E., Shestopalov V. I. and Ivanov D. (2010) Liposome-delivered ATP effectively protects the retina against ischemia-reperfusion injury. *Mol. Vis.* **16**, 2882–2890.
- Feigin V. L. (2005) Stroke epidemiology in the developing world. *Lancet* **365**, 2160–2161.
- Flynn R. W., MacWalter R. S. and Doney A. S. (2008) The cost of cerebral ischaemia. *Neuropharmacology* **55**, 250–256.
- Fornage M. (2009) Genetics of stroke. *Curr. Atheroscler. Rep.* **11**, 167–174.
- Fujita R. and Ueda H. (2003) Protein kinase C-mediated cell death mode switch induced by high glucose. *Cell Death Differ.* **10**, 1336–1347.
- Fujita R. and Ueda H. (2007) Prothymosin-alpha1 prevents necrosis and apoptosis following stroke. *Cell Death Differ.* **14**, 1839–1842.
- Fujita R., Ueda M., Fujiwara K. and Ueda H. (2009) Prothymosin-alpha plays a defensive role in retinal ischemia through necrosis and apoptosis inhibition. *Cell Death Differ.* **16**, 349–358.
- Garaci E., Favalli C., Pica F. *et al.* (2007) Thymosin alpha 1: from bench to bedside. *Ann. N. Y. Acad. Sci.* **1112**, 225–234.
- Goldstein A. L. and Goldstein A. L. (2009) From lab to bedside: emerging clinical applications of thymosin alpha 1. *Expert Opin. Biol. Ther.* **9**, 593–608.
- Gozes I. (2007) Activity-dependent neuroprotective protein: from gene to drug candidate. *Pharmacol. Ther.* **114**, 146–154.
- Halder S. K., Matsunaga H. and Ueda H. (2012) Neuron-specific non-classical release of prothymosin alpha, a novel neuroprotective DAMPs. *J. Neurochem.* **123**, 262–275.
- Hofmeijer J. and van Putten M. J. (2012) Ischemic cerebral damage: an appraisal of synaptic failure. *Stroke* **43**, 607–615.
- Iadecola C. and Anrather J. (2011) The immunology of stroke: from mechanisms to translation. *Nat. Med.* **17**, 796–808.
- Jiang X., Kim H. E., Shu H. *et al.* (2003) Distinctive roles of PHAP proteins and prothymosin-alpha in a death regulatory pathway. *Science* **299**, 223–226.
- Karapetian R. N., Evstafieva A. G., Abaeva I. S. *et al.* (2005) Nuclear oncoprotein prothymosin alpha is a partner of Keap1: implications for expression of oxidative stress-protecting genes. *Mol. Cell. Biol.* **25**, 1089–1099.
- Korada S., Zheng W., Basilico C., Schwartz M. L. and Vaccarino F. M. (2002) Fibroblast growth factor 2 is necessary for the growth of glutamate projection neurons in the anterior neocortex. *J. Neurosci.* **22**, 863–875.
- Krysl D., Deykun K., Lambert L., Pokorny J. and Mares J. (2012) Perifocal and remote blood-brain barrier disruption in cortical photothrombotic ischemic lesion and its modulation by the choice of anesthesia. *J. Physiol. Pharmacol.* **63**, 127–132.
- Madinier A., Bertrand N., Mossiat C., Prigent-Tessier A., Beley A., Marie C. and Garnier P. (2009) Microglial involvement in neuroplastic changes following focal brain ischemia in rats. *PLoS ONE* **4**, e8101.
- Maiese K., Li F. and Chong Z. Z. (2004) Erythropoietin in the brain: can the promise to protect be fulfilled?. *Trends Pharmacol. Sci.* **25**, 577–583.
- Matsunaga H. and Ueda H. (2010) Stress-induced non-vesicular release of prothymosin-alpha initiated by an interaction with S100A13,

- and its blockade by caspase-3 cleavage. *Cell Death Differ.* **17**, 1760–1772.
- Meade A. J., Meloni B. P., Mastaglia F. L. and Knuckey N. W. (2009) The application of cell penetrating peptides for the delivery of neuroprotective peptides/proteins in experimental cerebral ischaemia studies. *J. Exp. Stroke Transl. Med.* **2**, 22–40.
- Mosoian A., Teixeira A., Burns C. S., Sander L. E., Gusella L. G., He C., Blander J. M., Klotman P. and Klotman M. E. (2010) Prothymosin- $\alpha$  inhibits HIV-1 via Toll-like receptor 4-mediated type I interferon induction. *Proc. Natl Acad. Sci. USA* **107**, 10178–10183.
- Nagai N., Van Hoef B. and Lijnen H. R. (2007) Plasminogen activator inhibitor-1 contributes to the deleterious effect of obesity on the outcome of thrombotic ischemic stroke in mice. *J. Thromb. Haemost.* **5**, 1726–1731.
- Neroev V. V., Zueva M. V. and Kalamkarov G. R. (2010) Molecular mechanisms of retinal ischemia. *Vestn. oftalmol.* **126**, 59–64.
- Osborne N. N., Casson R. J., Wood J. P., Chidlow G., Graham M. and Melena J. (2004) Retinal ischemia: mechanisms of damage and potential therapeutic strategies. *Prog. Retin. Eye Res.* **23**, 91–147.
- Paolucci S., Antonucci G., Grasso M. G., Bragoni M., Coiro P., Angelis D. D., Morelli D., Venturiero V., Troisi E. and Pratesi L. (2003) Functional outcome of ischemic and hemorrhagic stroke patients after inpatient rehabilitation: A matched comparison. *Stroke* **34**, 2861–2865.
- Patel L. N., Zaro J. L. and Shen W. C. (2007) Cell penetrating peptides: intracellular pathways and pharmaceutical perspectives. *Pharm. Res.* **24**, 1977–1992.
- Paul R., Zhang Z. G., Eliceiri B. P., Jiang Q., Boccia A. D., Zhang R. L., Chopp M. and Cheresch D. A. (2001) Src deficiency or blockade of Src activity in mice provides cerebral protection following stroke. *Nat. Med.* **7**, 222–227.
- Pierluigi B., D'Angelo C., Fallarino F., Moretti S., Zelante T., Bozza S., De Luca A., Bistoni F., Garaci E. and Romani L. (2010) Thymosin alpha1: the regulator of regulators?. *Ann. N. Y. Acad. Sci.* **1194**, 1–5.
- Prasad S. S., Kojic L., Wen Y. H., Chen Z., Xiong W., Jia W. and Cynader M. S. (2010) Retinal gene expression after central retinal artery ligation: effects of ischemia and reperfusion. *Invest. Ophthalmol. Vis. Sci.* **51**, 6207–6219.
- Rhee K. D., Ruiz A., Duncan J. L., Hauswirth W. W., Lavail M. M., Bok D. and Yang X. J. (2007) Molecular and cellular alterations induced by sustained expression of ciliary neurotrophic factor in a mouse model of retinitis pigmentosa. *Invest. Ophthalmol. Vis. Sci.* **48**, 1389–1400.
- Sherry D. M., Mitchell R., Standifer K. M. and du Plessis B. (2006) Distribution of plasma membrane-associated syntaxins 1 through 4 indicates distinct trafficking functions in the synaptic layers of the mouse retina. *BMC Neurosci.* **7**, 54.
- Sims N. R. and Muyderman H. (2010) Mitochondria, oxidative metabolism and cell death in stroke. *Biochim. Biophys. Acta* **1802**, 80–91.
- Siren A. L., Fratelli M., Brines M. *et al.* (2001) Erythropoietin prevents neuronal apoptosis after cerebral ischemia and metabolic stress. *Proc. Natl Acad. Sci. USA* **98**, 4044–4049.
- Skopeliti M., Kratzer U., Altenberend F., Panayotou G., Kalbacher H., Stevanovic S., Voelter W. and Tsitsilonis O. E. (2007) Proteomic exploitation on prothymosin alpha-induced mononuclear cell activation. *Proteomics* **7**, 1814–1824.
- Skopeliti M., Iconomidou V. A., Derhovanessian E., Pawelec G., Voelter W., Kalbacher H., Hamdrakas S. J. and Tsitsilonis O. E. (2009) Prothymosin alpha immunoactive carboxyl-terminal peptide TTKKQKTDEDD stimulates lymphocyte reactions, induces dendritic cell maturation and adopts a beta-sheet conformation in a sequence-specific manner. *Mol. Immunol.* **46**, 784–792.
- Tanaka Y., Koizumi C., Marumo T., Omura T. and Yoshida S. (2007) Serum S100B is a useful surrogate marker for long-term outcomes in photochemically-induced thrombotic stroke rat models. *Life Sci.* **81**, 657–663.
- Ueda H. (2008) Prothymosin alpha plays a key role in cell death mode-switch, a new concept for neuroprotective mechanisms in stroke. *Naunyn Schmiedebergs Arch. Pharmacol.* **377**, 315–323.
- Ueda H. (2009) Prothymosin alpha and cell death mode switch, a novel target for the prevention of cerebral ischemia-induced damage. *Pharmacol. Ther.* **123**, 323–333.
- Ueda H. and Fujita R. (2004) Cell death mode switch from necrosis to apoptosis in brain. *Biol. Pharm. Bull.* **27**, 950–955.
- Ueda H., Fujita R., Yoshida A., Matsunaga H. and Ueda M. (2007) Identification of prothymosin-alpha1, the necrosis-apoptosis switch molecule in cortical neuronal cultures. *J. Cell Biol.* **176**, 853–862.
- Ueda H., Matsunaga H., Uchida H. and Ueda M. (2010) Prothymosin alpha as robustness molecule against ischemic stress to brain and retina. *Ann. N. Y. Acad. Sci.* **1194**, 20–26.
- Ueda H., Matsunaga H. and Halder S. K. (2012) Prothymosin  $\alpha$  plays multifunctional cell robustness roles in genomic, epigenetic, and nongenomic mechanisms. *Ann. N. Y. Acad. Sci.* **1269**, 34–43.
- Watt P. M. (2006) Screening for peptide drugs from the natural repertoire of biodiverse protein folds. *Nat. Biotechnol.* **24**, 177–183.
- White B. C., Sullivan J. M., DeGracia D. J., O'Neil B. J., Neumar R. W., Grossman L. I., Rafols J. A. and Krause G. S. (2000) Brain ischemia and reperfusion: molecular mechanisms of neuronal injury. *J. Neurol. Sci.* **179**, 1–33.
- Witmer M. T., Pavan P. R., Fouraker B. D. and Levy-Clarke G. A. (2011) Acute retinal necrosis associated optic neuropathy. *Acta Ophthalmol.* **89**, 599–607.
- Yin K. J., Deng Z., Huang H., Hamblin M., Xie C., Zhang J. and Chen Y. E. (2010) miR-497 regulates neuronal death in mouse brain after transient focal cerebral ischemia. *Neurobiol. Dis.* **38**, 17–26.

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