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PEP-1-PEA15 suppresses inflammatory responses by regulation of MAPK in macrophages and animal models

Ji In Yong[a,1], Dae Won Kim[b,1], Min Jea Shin[a,1], Hyo Sang Jo[a], Jung Hwan Park[a], Su Bin Cho[a], Chi Hern Lee[a], Hyeon Ji Yeo[a], Eun Ji Yeo[a], Yeon Joo Choi[a], Ji An Kim[a], Jung Soon Hwang[a], Duk-Soo Kim[c], Hyun Ah Kim[d], Yong-Jun Cho[e], Keun Wook Lee[a], Kyu Hyung Han[a], Jinseu Park[a], Won Sik Eum[a,*], Soo Young Choi[a,*]

[a] Department of Biomedical Science and Research Institute of Bioscience and Biotechnology, Hallym University, Chuncheon 24252, Republic of Korea
[b] Department of Biochemistry and Molecular Biology, Research Institute of Oral Sciences, College of Dentistry, Gangneung-Wonju National University, Gangneung 25457, Republic of Korea
[c] Department of Anatomy, College of Medicine, Soonchunhyang University, Cheonan-Si 31538, Republic of Korea
[d] Division of Rheumatology, Department of Internal Medicine, Hallym University Sacred Heart Hospital, Kyunggi 14068, Republic of Korea
[e] Department of Neurosurgery, Hallym University Medical Center, Chuncheon 24253, Republic of Korea

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ABSTRACT

Phosphoprotein enriched in astrocytes 15 (PEA15) plays a multi-functional role in neuronal cell survival, however the effects of PEA15 against inflammation have not been investigated yet. To examine the effects of PEP-1-PEA15 protein against lipopolysaccharide (LPS)-induced inflammatory responses in Raw 264.7 cells and in a 12-O-tetradecanoylphobol 13-acetate (TPA)-induced mouse model, we constructed and purified PEP-1-PEA15 protein, which can transduce into cells or tissues. PEP-1-PEA15 inhibited LPS-induced damage in cells including that caused by reactive oxygen species (ROS) production and DNA fragmentation. PEP-1-PEA15 also significantly suppressed activation of mitogen activated protein kinases (MAPKs), pro-inflammatory mediator proteins and various cytokines. In a TPA-induced mouse ear edema model, PEP-1-PEA15 significantly reduced ear weight and thickness as well as MAPK activation as well as the expression levels of COX-2, iNOS, IL-6, IL-1β, and TNF-α. These results demonstrated that PEP-1-PEA15 showed anti-inflammatory effect in cells and animal model suggesting that this fusion protein protects cells or skin tissues from inflammatory response.

1. Introduction

Inflammation plays a crucial role in protecting cells or tissues against foreign invasion and tissue injury. Prolonged exposure to inflammation sources such as lipopolysaccharide (LPS)-induced chronic inflammation leads to cellular or tissues damage (Ferrero-Miliani et al., 2007; Serhan et al., 2008). LPS is a well described endotoxin which induces an inflammatory response by macrophage activation (Mayeux, 1997). During inflammatory response, macrophages produce inflammatory mediators including cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and reactive oxygen species (ROS) as well as pro-inflammatory cytokines including interleukin (IL)-6, IL-1β, and tumor necrosis factor-α (TNF-α) (Burmester et al., 1997; Duffield, 2003; Fujihara et al., 2003). Many studies have demonstrated that excessive production of pro-inflammatory mediators and cytokines has been implicated in various diseases such as cancer, asthma, Parkinson's disease, Alzheimer's disease, and rheumatoid arthritis (Hale and Lightman, 2006; Ferrero-Miliani et al., 2007; Glezer et al., 2007; Wang et al., 2010). Inflammatory response is highly associated with signaling pathways through the activation of transcription factors including nuclear factor κB (NF-κB) and activation protein-1 (AP-1). These transcription factors are closely involved in the regulation of inflammatory response. Mitogen-activated protein kinases (MAPKs) such as extracellular signal-regulated kinase (ERK), p38, and c-Jun N-terminal kinase (JNK) are activated during inflammatory response and the subsequently activated MAPKs lead to the activation of transcription factors (Rahman and MacNee, 1998; Buchanan et al., 2011; Khan et al., 2011; Su et al., 2011). Therefore, inhibition of inflammatory responses or regulation of transcription factors and MAPKs activation are considered to be important targets for the treatment of diseases caused by

* Corresponding authors.
E-mail addresses: wseum@hallym.ac.kr (W.S. Eum), sychoi@hallym.ac.kr (S.Y. Choi).
[1] These authors contributed equally to this work.

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inflammation.

Phosphoprotein enriched in astrocytes 15 (PEA15) protein is a small phosphoprotein (15 kDa) abundantly expressed in brain astrocytes as well as lung and eye. It is known to have multiple cellular functions related to cell proliferation, survival, and apoptosis. PEA15 protein has an N-terminal death effector domain and phosphorylated serine residue in the C-terminal tail (Danziger et al., 1995; Renault et al., 2003; Krueger et al., 2005). Renganathan et al. showed that phosphorylation of PEA15 protein plays opposing roles between cell survival and apoptosis (Renganathan et al., 2005). PEA15 is also known to play both anti- and pro-tumorigenic functions in cancers. In ovarian cancer patients, high expression of PEA15 protein led to increased patient survival depending on the expression levels whereas high PEA15 protein expression in glioblastoma cells contributed to increased proliferation of cancer cells (Xiao et al., 2002; Bartholomeusz et al., 2008). In addition, overexpression of PEA15 protein in skeletal muscle cells ameliorated insulin-stimulated diabetes in diabetes patients (Condorelli et al., 1998). Furthermore, it has been reported that PEA15 protein has a protective effect against ischemic injury and Parkinson's disease (Koh, 2011; Ahn et al., 2014). Even though this protein is involved in various diseases, its effect on inflammation remains unclear. Therefore, we fused the protein transduction domains (PTDs) PEP-1 to the PEA15 protein to determine whether or not this protein has anti-inflammatory effects.

The lipid bilayer and its low permeability limits the transduction of proteins into cells. To overcome these obstacles, PTDs has been used to transduce the target proteins into cells (Ramsey and Flynn, 2015). Since PTDs such as PEP-1 or Tat peptide are known to be capable of delivery of proteins *via* transduction into cells or tissues, PTDs have been widely used as therapeutic agents for applications in protein therapy in the treatment of various diseases (Choi et al., 2010; Sakurazawa et al., 2012; Liu et al., 2013; Jeong et al., 2014; Kim et al., 2014a; Shin et al., 2014; Kim et al., 2015a, 2015b; Zhang et al., 2015). In this study, we investigated the anti-inflammatory effects of PEP-1-PEA15 in Raw 264.7 cells and in an animal model.

2. Materials and methods

2.1. Materials and cell culture

Histidine, COX-2, and iNOS antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). JNK, p-JNK, p38, p-p38, and beta-actin antibodies were purchased from Cell Signaling (Danvers, MA, USA). LPS and TPA were purchased from Sigma-Aldrich (St. Louis, MO, USA). Male 4–6-week-old ICR mice were obtained from the Hallym University Experimental Animal Center. Unless otherwise stated, all other chemicals and reagents were of the highest analytical quality grade accessible.

Raw 264.7 murine macrophage cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 20 mM HEPES/NaOH (pH 7.4), 5 mM $NaHCO_3$, 10% fetal bovine serum (FBS), and antibiotics (100 μg/ml streptomycin and 100 U/ml penicillin) at 37 °C under humidified conditions of 95% air and 5% CO_2.

2.2. Transduction of PEP-1-PEA15 proteins

PEP-1-PEA15 protein was purified as described previously (Ahn et al., 2014). Purified PEP-1-PEA15 and control PEA15 proteins were treated with Detoxi-Gel™, an endotoxin removing gel (Pierce, Rockford, IL, USA) according to manufacturer's instructions. After endotoxins were removed, proteins (< 0.03 EU/ml) were confirmed using a Limulus amoebocyte lysate assay (BioWhitaker, Walkersville, MD, USA). The purified protein concentration was then determined using the Bradford assay (Bradford, 1976).

To assess the transduction of PEP-1-PEA15 protein in accordance with protein concentrations, Raw 264.7 cells were grown on a 60 mm dish plate for 1 h and treated with different concentrations of PEP-1-PEA15 or control PEA15 protein (0.2–1 μM) for 1 h. Also, cells were treated with PEP-1-PEA15 or control PEA15 proteins (1 μM) for various times (10–60 min). Then, the cells were treated with trypsin-EDTA, washed twice with phosphate-buffered saline (PBS), and harvested to perform Western blot analysis. Transduced PEP-1-PEA15 protein was detected using an anti-histidine antibody.

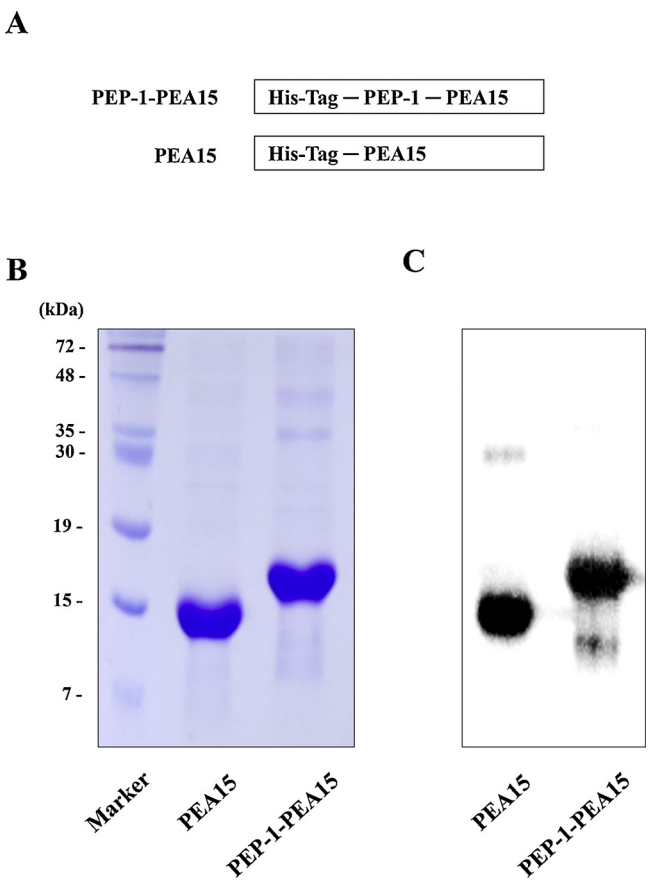

Fig. 1. Purification of PEP-1-PEA15 protein. Diagram of the expressed control PEA15 and PEP-1-PEA15 proteins. Each consists of a His tag consisting of six histidine residues (A). After induction by IPTG, purified control PEA15 and PEP-1-PEA15 proteins were analyzed by 15% SDS-PAGE (B) and Western blot analysis was performed with an anti-histidine antibody (C).

2.3. Western blot analysis

Western blot analysis was performed as described previously (Kim et al., 2015a; Seo et al., 2015). Briefly, sample proteins were separated by 15% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane. Western blotting was performed with the primary antibodies at 4 °C overnight and the protein bands were detected using an ECL kit according to the manufacturer's instructions (Amersham, Franklin Lakes, NJ, USA). The bands were quantified by Image J software (NIH, Bethesda, MD, USA).

2.4. Fluorescence microscopy analysis

Fluorescence microscopy analysis was performed as described previously (Choi et al., 2015; Hu et al., 2015; Kim et al., 2015a). Raw 264.7 cells were grown on coverslips and incubated with control PEA15 or PEP-1-PEA15 protein (1 μM) for 1 h at 37 °C. The cells were triple washed with PBS, fixed with 4% paraformaldehyde at room temperature for 5 min and then permeabilized and blocked with PBS buffer containing 3% bovine serum albumin (BSA) and 0.1% Triton X-100