Molecular Immunology 67 (2015) 248–255

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Lipoteichoic acid isolated from *Lactobacillus plantarum* down-regulates UV-induced MMP-1 expression and up-regulates type I procollagen through the inhibition of reactive oxygen species generation

Yi-Fan Hong[a,c,1], Hea young Lee[b,1], Bong Jun Jung[a], Soojin Jang[e], Dae Kyun Chung[a,c,d,**], Hangeun Kim[d,*]

[a] *Graduate School of Biotechnology and Institute of Life Science and Resources, Kyung Hee University, Yongin 446-701, Republic of Korea*
[b] *Cancer Research Institute, Seoul National University, College of Medicine, Yongon-dong, Chongno-gu, Seoul 110-744, Republic of Korea*
[c] *Skin Biotechnology Center, Gyeonggi Biocenter, Suwon, Gyeonggi-do 443-766, Republic of Korea*
[d] *RNA Inc., #308 College of Life Science, Kyung Hee University, Yongin 449-701, Republic of Korea*
[e] *Institute Pasteur Korea, Sampyeong-dong, Seongnam-si, Gyeonggi-do 463-400, Republic of Korea*

ARTICLE INFO

Article history:
Received 15 January 2015
Received in revised form 30 April 2015
Accepted 22 May 2015
Available online 6 June 2015

Keywords:
Matrix metalloproteinases-1
Lipoteichoic acid
Reactive oxygen species
Photo-aging
Lactobacillus plantarum
Human dermal fibroblasts

ABSTRACT

Background: Ultraviolet (UV) irradiation from the sun is the primary environmental factor that causes human skin aging. UV irradiation induces the expressions of matrix metalloproteinases (MMPs) and extracellular matrix degrading enzymes. Among the members of MMP family, MMP-1 is an interstitial collagenase that degrades the collagen triple helix. We investigated the effect of *Lactobacillus plantarum*, well known as useful microorganism, on UV-induced-MMP-1 expression in human dermal fibroblasts.
Methods: Human dermal fibroblasts (HDF) was pre-stimulated with lipoteichoic acid isolated from *L. plantarum* followed by UV irradiation. Secreted protein level of MMP-1 was evaluated by Western blot analysis. The phosphorylation of mitogen-activated protein kinases (MAPKs) and nuclear factor-kappa B (NF-κB) from the cell lysates was also examined by western blotting. Electrophoretic mobility-shift assay (EMSA) was used to detect the activated transcription factor, AP-1 and NF-κB. The detection of type 1 procollagen was carried with Procollagen type 1 C-peptide (PIP) EIA kit. The generation of reactive oxygen species (ROS) by LTA and UV irradiation was examined by Griess reagent assay and fluorescence microscope.
Results: We found that lipoteichoic acid (LTA), a cell-wall component of Gram-positive bacteria, isolated from *L. plantarum*, inhibited MMP-1 expression. Pretreatment with LTA from *L. plantarum* (pLTA) reduced MMP-1 expression in a dose-dependent manner and inhibited activation of extracellular signal-regulated kinases (ERK) and c-Jun N-terminal kinases (JNK). It also led to the inhibition of DNA binding activity of activator protein-1 (AP-1) and of nuclear factor kappa-light-chain-enhancer of activated B cell (NF-κB). Furthermore, LTA promoted type 1 procollagen synthesis and reduced the generation of ROS induced by UV irradiation.
Conclusion: Our study demonstrates that pLTA inhibits degradation of collagen and promotes its synthesis and that pLTA contributes to a decrease in ROS production. Therefore, pLTA from *L. plantarum* has potential abilities to prevent and treat skin photo-aging.

Abbreviations: UV, ultraviolet; MMP, matrix metalloproteinase; LTA, lipoteichoic acid; pLTA, *Lactobacillus plantarum* lipoteichoic acid; ERK, extracellular signal-regulated kinases; JNK, c-Jun N-terminal kinases; AP-1, activator protein-1; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cell; ROS, reactive oxygen species.

* Corresponding author. Tel.: +82 31 201 2487; fax: +82 31 202 3461.
** Corresponding author at: Graduate School of Biotechnology and Institute of Life Science and Resources, Kyung Hee University, Yongin 446-701, Republic of Korea. Tel.: +82 31 201 2465; fax: +82 31 202 8333.
E-mail addresses: dkchung@khu.ac.kr (D.K. Chung), giam6009@gmail.com (H. Kim).
[1] Both authors contributed equally to this work.

http://dx.doi.org/10.1016/j.molimm.2015.05.019
0161-5890/

1. Introduction

Human skin is often directly exposed to the environment, especially to sun. Therefore, skin undergoes not only chronological aging, but also photo-aging (Fisher et al., 2002). Both are cutaneous and cause laxity, wrinkles and a decrease in elasticity. However, their molecular mechanisms and morphological changes are distinct (Helfrich et al., 2008; Rittie and Fisher, 2002). Photo-aging is also called extrinsic aging and is caused by external factors such as ultraviolet (UV) radiation. UV exposure can cause pigmented and wrinkled skin. UV irradiation induces the expression of MMPs and triggers cleavage of cell-surface receptors and protein kinase signal transduction pathways and activation of transcription factors (Fisher et al., 2002). MMPs degrade all kinds of extracellular matrix proteins and damage connective tissues (Kahari and Saarialho-Kere, 1997) and induce the release of cytokines and chemokines and cleave cell surface receptors (Van and Libert, 2007). They also play a role in tissue remodeling and disease processes such as arthritis (Burrage et al., 2006). MMP-1, interstitial collagenase, is a member of the MMP family and is responsible for the degradation of collagen fibers (Moon et al., 2004). An increase in MMP-1 expression causes skin connective tissue damage. Collagen is the richest protein of the dermal connective tissue and is broken down by MMP-1. Among the 29 types of collagen, type 1 collagen is one of the most abundant proteins in the human body (Di Lullo et al., 2002). UV irradiation impairs synthesis of new type 1 collagen. Degradation and damage of collagen is the major feature of photo-aging (Brennan et al., 2003).

Mitogen-activated protein kinase (MAPK) pathways are activated in response to extracellular stimuli including UV irradiation (Fisher et al., 1998), leading to the activation of activator protein 1 (AP-1) (Fisher and Voorhees, 1998; Kim et al., 2005; Whitmarsh and Davis, 1996). AP-1 is a transcription factor that regulates MMP-1 gene expression in response to various stimuli (Karin, 1995; Wenk et al., 1999). Down-regulation of type 1 collagen and procollagen is mediated by UV-induced AP-1 (Fisher et al., 2002). NF-κB activation occurs in response to various stimuli such as stress, free radicals and ultraviolet irradiation. UV-induced NF-κB activation leads to the induction of various genes including MMP-1 and the production of MMP-1 in skin fibroblasts (Brenneisen et al., 2002; Tanaka et al., 2005). Reactive oxygen species (ROS) are free radicals and are naturally produced during cellular metabolism. The production of ROS is significantly increased by environmental stresses including UV irradiation, leading to the induction of MMPs (Bond et al., 1999). ROS causes oxidative damage and are a major contributor to aging (Muller et al., 2007).

Lipoteichoic acid (LTA) is a major cell-wall component of Gram-positive bacteria and has antigenic properties that stimulate specific immune responses. LTA is known to increase the activity of MMP-9, play an active role in otitis media, inhibit melanogenesis in B16F10 mouse melanoma cells, protect UV-induced carcinogenesis and increases skin mast cell antimicrobial activity against vaccinia viruses (Park et al., 2012; Wang et al., 2012; Weill et al., 2013; Kim et al., 2015). In addition, tolerance using LTA has been used to introduce to protect against pro-inflammation and other diseases (Kim et al., 2008b; Ellingsen et al., 2002). Thus, to identify the role of LTA (pLTA) isolated from *Lactobacillus plantarum* on UV-mediated photo-aging, MMP-1 expression and ROS generation by pLTA was examined.

2. Materials and methods

2.1. Cell culture

Human dermal fibroblasts (HDF) were kindly provided by Dr. Y. S. Son (Kyung Hee University, Korea). NDFs were grown in FBM with FGM-2 (Lonza, Basel, Switzerland) in humidified incubator of 5% CO_2 at 37 °C.

2.2. Preparation and treatment of LTA

Highly purified LTA was isolated from *L. plantarum* by *n*-butanol extraction. The purity of the purified LTA was determined by measuring the protein and endotoxin contents through the conventional silver staining after polyacrylamide gel electrophoresis and through the *Limulus amebocyte lysate* (LAL) assay (BioWhittaker, Walkersville, MD), respectively. DNA or RNA contamination was assessed by measuring UV absorption at 260 and 280 nm, and no nucleic acids were detected. To treat the cells, LTA was diluted with non-pyrogenic water at the indicated concentrations.

2.3. UV irradiation

Vilber Lourmat Bio-sun lamps (Marine, France) were used as a UV source. The emission spectrum was between 275 and 380 nm (peak spectrum, 312 nm) and UV strength was measured by UV meter (Waldmann, Germany). Before UV irradiation, the cells were washed with Dulbecco's phosphate-buffered saline (DPBS) and medium was replaced by DPBS. After UV irradiation, the cells were washed with DPBS and cultured for 24 h in the serum-free media.

2.4. Western blot analysis

For detection of MMP-1, cultured supernatants were harvested and for detection of phospho-ERK1/ERK2 and phospho-JNK, cells were lysed with RIPA buffer containing proteinase inhibitors. Insoluble debris was removed by centrifugation at 12,000 rpm for 10 min. For detection of NF-κB, cells were lysed using nuclear extraction kit (Marligen Biosciences, Rockville, MD) and nuclear protein was extracted. Protein concentration was quantified using Bradford assay reagent (Bio-Rad, Hercules, CA). Equal amounts of protein were resolved on SDS–polyacrylamide gels (10–15%) and then electrophoretically transferred onto nitrocellulose membrane (100 V, 2 h). Membranes were subsequently blocked with 5% skim milk in TBS-T (20 mM Tris–HCl, pH 7.6, 137 mM NaCl, and 0.05% Tween 20) and incubated with the indicated antibodies. Commercially available anti-JNK, anti-phospho-JNK, anti-ERK, anti-phospho-ERK, anti-phospho-c-Jun, anti-phospho-p65, and anti-β-actin were purchased from Cell Signaling Technology (Boston, MA). Blotting proteins were visualized by enhanced chemiluminescence (ECL) reagents (GE Healthcare).

2.5. Electrophoretic mobility shift assay (EMSA)

Fibroblasts were pretreated with the indicated concentrations of pLTA for 20 h, washed with DPBS and irradiated with 75 mJ/cm^2 of UV in DPBS. To prepare nuclear fractions, cells were washed with DPBS and nucleus was extracted using nuclear extraction kit (Marligen Biosciences, Rockville, MD). The cytoplasmic extracts were removed by centrifugation and nuclear pellet were washed and solubilized with extraction buffer. AP-1 and NF-κB consensus oligonucleotides (Jany et al., 1995; Husain et al., 2007) were labeled with 10 μCi/ml [γ-^{32}P] ATP by T4 polynucleotide kinase (Fermentas, Burlington, ON) at 37 °C for 30 min. The sequence of the AP-1 and NF-κB oligonucleotide is as follows: AP-1 sense 5′-CGCTTGATGAGTCAGCCGGAA-3′, AP-1 antisense 5′-TTCCGGCTGACTCATCAAGCG-3′, NF-κB sense 5′-AGTTGAGGGGACTTTCCCAGGC-3′, NF-κB antisense 5′-GCCTGGGAAAGTCCCCTCAACT-3′. Because unincorporated labeled nucleotides cause non-specific binding, purification was performed to remove them using PROBER Probe DNA purifying kit (Intron Biotechnology, Korea). After determination of nuclear