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Original Article

OLR1 scavenger receptor knockdown affects mitotic gene expression but is dispensable for oxidized phospholipid- mediated stress signaling in SZ 95 sebocytes

Ionela-Mariana Nagelreiter[a,1], Masomeh Parvardeh[a,2], Marie-Sophie Narzt[a,b], Lucian Beer[c], Christopher Kremslehner[a,b], Ximena Maria Muresan[a,d], Johannes Grillari[b,e,f], Christos C. Zouboulis[g], Erwin Tschachler[a], Florian Gruber[a,b,*]

[a] Department of Dermatology, Medical University of Vienna, Währinger Gürtel 18-20, Leitstelle 7J, A-1090 Vienna, Austria
[b] Christian Doppler Laboratory for Biotechnology of Skin Aging, Austria
[c] Department of Thoracic Surgery, Medical University of Vienna, Austria
[d] Department of Life Sciences and Biotechnology University of Ferrara, Italy
[e] Department of Biotechnology, BOKU – University of Natural Resources and Life Sciences Vienna, Muthgasse 18, 1190 Vienna, Austria
[f] Austrian Cluster for Tissue Regeneration, BOKU – University of Natural Resources and Life Sciences Vienna, Muthgasse 18, 1190 Vienna, Austria
[g] Departments of Dermatology, Venereology, Allergology and Immunology, Dessau Medical Center, Theodore Fontane Medical University of Brandenburg, 06847, Dessau, Germany

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ABSTRACT

Phospholipid oxidation products (OxPL) are versatile stress signaling mediators in the skin. These lipid signaling molecules can be generated non-enzymatically or enzymatically by ultraviolet light, the major extrinsic skin aging factor. OxPL regulate cytoprotective, immunological and metabolic adaptation of the skin to oxidant stress. We here investigated whether the scavenger receptor Oxidized Low Density Lipoprotein Receptor 1 (OLR1, LOX-1) would have a function in cutaneous oxPL signaling. We found, that OLR1 is expressed in several cutaneous cell types, most prominently in cells of the sebaceous gland and in keratinocytes. We repressed OLR1 expression with siRNA in SZ95 sebocytes, exposed cells to oxidized 1-palmitoyl-2-arachidonoyl-sn-*glycero*-3-phosphocholine (PAPC) and performed transcriptomic profiling. Bioinformatic analysis revealed that OxPL exposure induced the Nrf2 antioxidant stress response and aldosterone signaling. The analysis also revealed that OLR1 is not required for the transcriptional regulation induced by oxidized PAPC but interestingly, OLR1 knockdown affected expression of CNN2, HMRR, ITGB6 and KIF20A, all genes governing cell proliferation and motility. We identify sebocytes as cutaneous cells responsive to lipid mediated redox stress which is not dependent on the scavenger receptor OLR1.

1. Introduction

Oxidative modification of cellular phospholipids was observed upon ultraviolet light exposure of the skin (Marathe et al., 2005) and cutaneous cells (Gruber et al., 2007), in skin inflammation (Rolfs et al., 2013) and in senescent cutaneous cells (Ni et al., 2016; Song et al., 2017). The oxidative modification of lipids affects their biochemical and biophysical properties, their localization within the cell and thereby stress- and inflammation signaling in skin cells. Oxidized phospholipids (OxPL) can exert biological responses via intracellular receptors or sensors like the NRF2-KEAP1 system or peroxisome proliferator-activated receptors (PPARs) (Serbulea et al., 2017). OxPL can further be exposed to the outside of the cell (Greenberg et al., 2008), can diffuse through the membranes, be endocytosed (Stemmer et al., 2012) or be secreted by exocytosis within microvesicles and exosomes (Beer et al., 2015). Such extracellular OxPL in free or protein-bound form can be recognized by components of the innate immune system and by pattern recognition receptors expressed on various cell types (Binder et al., 2016).

We and others have studied the cellular signaling induced by oxidized lipid mediators as messengers in aging- and senescence- associated stress of the skin. The model substance used by us and others is

* Corresponding author at: Department of Dermatology, Medical University of Vienna, Währinger Gürtel 18-20, Leitstelle 7J, A-1090 Vienna, Austria.
E-mail address: florian.gruber@meduniwien.ac.at (F. Gruber).
[1] Present address: Center for Brain Research, Department of Molecular Neurosciences, Medical University of Vienna, Austria.
[2] Present address: Hudcentrum Hagastaden, Stockholm.

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in-vitro oxidized 1-palmitoyl-2-arachidonoyl-sn-*glycero*-3-phosphocholine (Oxidized PAPC). When we mildly oxidized PAPC by exposure to UVA (yielding UVPAPC), the substance induced responses that mimic those observed upon UVA exposure in dermal fibroblasts but also in keratinocytes in humans and rodents. These responses include the so-called "antioxidant response" mediated by NRF2 (Gruber et al., 2010), the regulation of autophagy (Zhao et al., 2013), and immunomodulatory signaling (Rolfs et al., 2013;Rothe et al., 2015). How the signals by extracellular OxPL are relayed to the intracellular signaling machinery of cutaneous cells is less well known. OxPL are a major component of oxidized low density lipoprotein, and many biological effects of oxLDL can be assigned to the action of its oxidized phospholipid component (Greig et al., 2012).

Oxidized Low Density Lipoprotein Receptor 1 (OLR1) is a prime candidate for mediating oxidative stress signals in aging related disease with a lipid signaling component (D'Introno et al., 2005). OLR1 has been implicated in RhoA/Rac1 pathway signaling (Sugimoto et al., 2009), in NFkB signaling (Cominacini et al., 2000), in MAPK signaling pathways (Tanigawa et al., 2006) and is associated with pathological changes in the vasculature; reviewed in (Negre-Salvayre et al., 2017; Ogura et al., 2009)). Murphy and colleagues had shown that native phosphatidylserine (PS) but not phosphatidylcholine (PC) and phosphatidylethanolamine (PE) could bind to OLR1, however the oxidized forms of PC, which are major constituents of OxLDL, were not tested in that study (Murphy et al., 2006). Apart from a functional study in skin microvasculature (Kenney et al., 2013), neither expression nor function of OLR1 as mediator of oxidized lipid stress in the skin have been studied.

Aim of this study was to investigate whether OLR1 would be expressed in mammalian skin, and to determine whether it is functional in mediating cellular signaling in response to oxidized phospholipids. In particular, we wanted to investigate the transcriptional responses to oxidized PAPC, an accepted model for oxidized phospholipids present in oxLDL, in a cutaneous cell type that strongly expresses OLR1. Also, we wanted to study these responses in the same cell type in the absence of OLR1 expression.

2. Materials and methods

2.1. Immunohistochemistry and immunofluorescence

Tissue samples from human skin were obtained from patients undergoing routinely performed body contouring surgeries. The Ethics Committee of the Medical University of Vienna (EK2011/1149) approved the use of human tissue samples for the experiments performed in this study. The donors provided written informed consent. Murine tissue samples were isolated from tails of wild type C57/B6 mice. The skin was fixed with 7.5% formalin, paraffin embedded and microtome sections (4 mm) were done. Dewaxed sections were washed twice with phosphate-buffered saline (PBS), then incubated with 3 % H2O2/PBS for 30 min to block endogenous peroxidase activity, and finally again washed twice with PBS. Later slides were heated 20 min in steam bath in 0.01 M citrate buffer (pH 6.0) for de-masking of antigen and cooled again to room temperature. Unspecific or Fc-dependent binding of the antibodies was blocked by pre-incubation of the sections with PBS containing 10% goat serum, 2% fish gelatin and 2 % bovine serum albumin (BSA) for 30 min at room temperature. Then the slides were incubated overnight at 4 °C with anti-OLR1 antibody (ab60178, Cambridge, UK) diluted to 5 µg/ml final concentration with 2 % BSA/PBS. Isotype staining controls were prepared with rabbit IgG (Bethyl, P120-101, Montgomery Tx, USA) with the same concentration. Biotinylated goat anti rabbit IgG (Vector Labs, CA, USA) was used as secondary antibodies. Thereafter, the slides were washed again with PBS and incubated 30 min with VECTASTAIN Elite ABC Kit (Vector Labs, CA, USA). Counterstaining was performed with hematoxylin. For immunofluorescence stainings, donkey anti rabbit IgG (H + L) Alexa Fluor 488 # A11010 or Alexa Fluor 594 goat anti Guinea pig IgG (H + L) #A110762from Molecular Probes, Eugene, OR, USA were used as secondary antibodies. To detect perilipin 2 the (PLIN2), Guinea pig #ABIN113458 from antibodies-online GmbH, Aachen, Deutschland was used at a concentration of 1:2000. Bodipy 492/503# D3922 (Molecular Probes) was used at a final conentration of 25 µM. The samples were evaluated using an Olympus (Tokyo, Japan) AX 70 microscope. All image analyses were performed under the same parameter settings for a given resolution.

2.2. Cell culture

The immortalized human SZ95 sebaceous gland cell line (Zouboulis et al., 1999) was cultured in Sebomed basal medium (Merck, Biochrom, Berlin, Germany) supplemented with 10 µg/ml gentamicin, 50 ng/ml human epidermal growth factor (EGF) and 10% fetal bovine serum. Cells were maintained at 37 °C at 5% CO2 and the medium was replaced every 2 days. Neonatal human epidermal keratinocytes (KCs) derived from foreskin were obtained from Clonetics (San Diego, CA). Keratinocytes were cultured in keratinocyte growth medium up to the fifth passage. Human neonatal skin fibroblasts (FB) were obtained from Cascade Biologics (Portland, OR) and grown in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% fetal calf serum and penicillin/streptomycin (1000 units/ml; Invitrogen) to sub-confluence.

2.3. OLR1/scramble siRNA knockdown

For the OLR1-knockdown cells were cultivated in 12 well plates (3000cells/cm2) to reach a 40% confluence. Cells were transfected with 20 nM equimolar mixture of OLR1HSS107426 and OLR1HSS107427 stealth RNA (Invitrogen, Life Tec, Carlsbad, CA) and Lipofectamine®2000 transfection reagent (Thermo Fisher Scientific) in OptiMem (Gibco) and sebocytes medium without serum. As control, cells were transfected with 20 nM Stealth siRNA Negative Control, Med GC (Invitrogen Life Tec, Carlsbad, CA). After 12 h 10% FBS was added.

2.4. Stress treatment

24 h after transfection SZ95 sebocytes were exposed to UVPAPC (25 µg/ml), UVA (40 J/cm^2) or 100 µM H2O2. UVPAPC was produced by irradiation of PAPC (1-palmitoyl-2-arachidonoyl-sn-*glycero*-3-phosphorylcholine; Avanti, Alabaster, AL) with 80 J/cm2 of UVA-1 (Gruber et al., 2007). UVA irradiation was carried out using a Sellamed 3000 UVA-1 therapy lamp (Sellas, Ennepetal, Germany), filtered for the emission at 340–400 nm at a distance of 20 cm for 10 min to reach a total fluence of 40J/cm2. During the irradiation cells were kept in phosphate-buffered saline on a cooling plate at 25 °C. "Sham" cells were treated identically but without irradiation. After 7 h the RNA was isolated using the RNeasy 96 system (Qiagen, Redwood City, CA). 350 ng of total RNA was reverse-transcribed with an iScript cDNA Synthesis Kit (Biorad, Hercules, CA). Mice were maintained according to the animal welfare guidelines of the Medical University of Vienna, Austria. The in vivo treatment had been authorized under austrian law by the protocols TVA 66.009/0123-II/10b/2010 and 0090-WF/II/3b/2014.

2.5. Relative quantitative real time PCR (qPCR)

qPCR was performed using LightCycler 480 and the LightCycler 480 SYBR Green I Master (Roche, Basel, Switzerland). Expression of target genes was normalized to the expression of β-2 microglobulin. Relative quantification was performed according to the model of (Pfaffl, 2001). The significance of differences in relative expression between two biological triplicate groups was determined by one-way ANOVA. Statistical significance was considered at $p < 0.05$. Primer sequences are given in Supplementary Data Table 1.