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

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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 UV -oxidized 1-palmitoyl-2-arachidonoyl-sn-phosphatidylcholine (UV-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.

Results

The scavenger receptor OLR1 is expressed in human and murine skin, most prominently in sebaceous glands.

Fig.1 OLR1 in human and mouse skin.

(A-E) Sections of sun-protected human abdominal skin (A) show OLR1 immunoreactivity in epidermis (B), sebaceous glands (C), isolated dermal cells and in vascular endothelial cells (D). Isotype control staining in (E). (F-I) Sections of murine back skin (F) show OLR1 immunoreactivity in epidermis (G) and in the sebaceous glands of the pilosebaceous unit (H). Isotype control staining in (I) Scale bar is 50 μm.

OLR1 expression is inducible in cultured keratinocytes and fibroblasts by redox stress, in SZ95 sebocytes high expression but no stress regulation.

Fig.2 OLR regulation (A-C) Primary human keratinocytes (A), fibroblasts (B) and SZ95 sebocytes (C) were cultured and either stressed with UV-PAPC (25 μg/ml) UVA (40 J/cm²), hydrogen peroxide (H₂O₂; 100 μM) or left untreated. Seven hours after the stress the expression of OLR1 mRNA was quantified by qPCR and normalized to expression of beta-2-microglobulin as housekeeping gene

OLR1 knockdown in SZ95 sebocytes increases promitotic gene expression.

Fig.3 OLR1 knockdown – transcriptomic analysis

SZ95 sebocytes were transfected with either scramble siRNA or OLR1 siRNA (A,B) shows the number of transcripts significantly up or downregulated, in red transcripts regulated more than two-fold (B). Bioinformatic analysis with IPA software identified genes for the molecular functions “M-phase” and “Chromosome segregation” to be significantly enriched. Network view (C) shows OLR1 knockdown regulated genes (red is up-, green is downregulated) and the predicted effect on activation (orange) or inhibition (blue) on the pathway drawn in the center.

UV- Oxidized phospholipids induce the Nrf2/ antioxidant stress response and aldosterone signaling in SZ95 sebocytes independent of OLR1.

Fig.4 – UV- Oxidized PAPC (UVPAPC) signaling in sham transfected and OLR1 knockdown SZ95 sebocytes.

Scramble siRNA transfected and OLR1 siRNA-transfected SZ95 sebocytes were treated for 7 hours with UVPAPC (25 μg/ml) or left untreated, and microarray transcriptome analysis was performed. Transcripts with a mean two-fold up- or downregulation in either scramble siRNA transfected (black bars) or OLR1 siRNA transfected cells (grey bars) are shown in (A). IPA analysis of regulated canonical pathways in scramble and OLR1 siRNA transfected cells, sorted by ascending p-value of predicted activation (B). Regulation of genes in the NRF2 (C) and aldosterone (D) signaling pathways; mean -fold regulation is displayed as expression heat maps in scramble and OLR1 si transfected samples.

Conclusion

Our work identifies oxidized phospholipids as inducers of the NRF2 stress response which is novel in the sebocyte field and may have importance for the sebaceous gland function. Further aldosterone signaling through oxidized lipids warrants future research on how the major lipid metabolizing cells deal with lipid oxidation which generates both, reactive noxae and essential signaling compounds. While lipid signaling was independent of OLR1, this scavenger receptor which is highly expressed in sebaceous glands, appears to regulate sebocyte mitosis and cytoskeletal rearrangement.