The Skin Epilipidome in Stress, Aging, and Inflammation

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Lipids are highly diverse biomolecules crucial for the formation and function of cellular membranes, for metabolism, and for cellular signaling. In the mammalian skin, lipids additionally serve for the formation of the epidermal barrier and as surface lipids, together regulating permeability, physical properties, acidification and the antimicrobial defense. Recent advances in accuracy and specificity of mass spectrometry have allowed studying enzymatic and non-enzymatic modifications of lipids—the epilipidome—multiplying the known diversity of molecules in this class. As the skin is an organ that is frequently exposed to oxidative-, chemical- and thermal stress, and to injury and inflammation, it is an ideal organ to study epilipidome dynamics, their causes, and their biological consequences. Recent studies uncover loss or gain in biological function resulting from either specific modifications or the sum of the modifications of lipids. These studies suggest an important role for the epilipidome in stress responses and immune regulation in the skin. In this minireview we provide a short survey of the recent developments on causes and consequences of epilipidomic changes in the skin or in cell types that reside in the skin.

Keywords: skin, ultraviolet, inflammation, stress, oxidized phospholipid, epilipidome, aging, senescence

INTRODUCTION

The lipidome of keratinocytes (KC), the dominant cell type of the basal layer of the epidermis is made up mainly of phospholipids, cholesterol, and triacylglycerides. Differentiation of living KC into dead corneocytes, a controlled cell death process that continuously renews the epidermal barrier (1), drastically changes the KC's lipid composition several times during the process. The last living (granular) epidermal layer contains cells with lamellar bodies containing glucosylceramides, phospholipids, and sphingomyelin which are further metabolized to produce the stratum corneum (SC) lipids, a mixture of free fatty acids (FFAs), cholesterol and ceramides (2, 3). The SC lipids form the lipid matrix, a flexible connection of low water permeability between the corneocytes which remain from terminal differentiation (4) and the FFAs contribute to the required acidification of the SC (5). Part of the surface lipids derive from the sebum, a mixture of TAG, wax esters, squalene and FFA, produced by holocrine secretion of terminally differentiating cells of the sebaceous gland, a lipid producing skin appendage. Most biological consequences of epilipidomic modification take
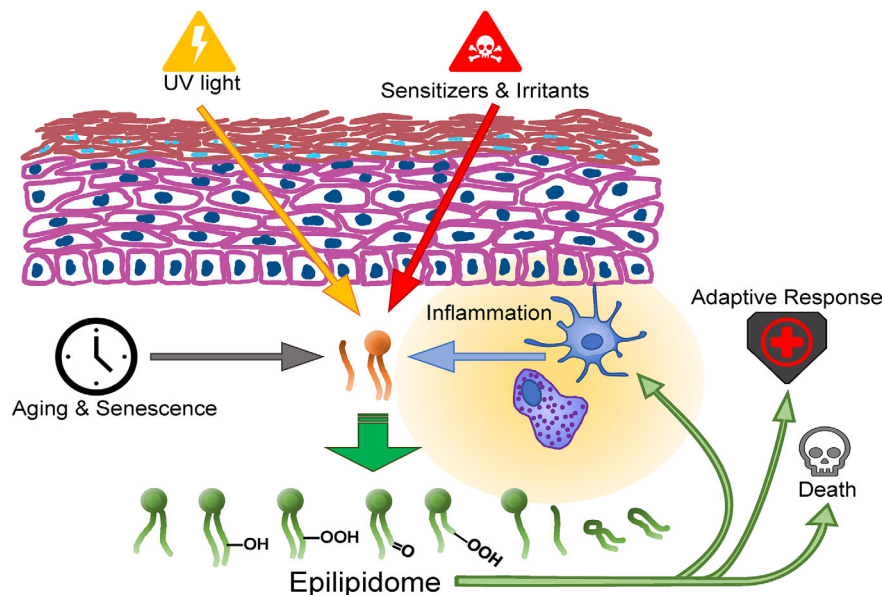
FIGURE 1 | Formation routes and action spectrum of modified lipids in the skin.

place in the living layers of the epidermis or in the dermal compartment underneath; nonetheless SC lipids are susceptible to modifications. Some of these modifications are ROS-mediated (squalene oxidation), while others depend on enzymatic cascades, as for example in the formation of the lipid envelope where hydroxyl ceramides are esterified to corneocyte proteins by specific transglutaminases.

Modification of The Skin Epilipidome by Ultraviolet Radiation

The best-studied oxidative modifier of skin lipids is solar radiation and wavelength bands thereof, which are used alone or in combination with photoactive chemicals as therapy for various skin diseases. The action of UV radiation (UVR) on human skin depends on wavelength and can induce acute inflammation-, immunosuppression, or cell death (6). The latter is elicited by combining UVR with photoactive drugs to specifically target cancer- or immune system cells. UVR can cause both enzymatic and non-enzymatic modification of lipids. The long-wavelength UVA (320–400 nm) oxidizes lipids in absence of enzymes (7, 8) but also shorter wavelength radiation can non-enzymatically generate oxidized lipids *via* free radical mechanisms (9). Cholesterol, phospholipids, free fatty acids, and squalene are targets for non-enzymatic lipid oxidation and yield bioactive products. Enzymatic synthesis of oxidized lipids, most prominently eicosanoids and related oxidized polyunsaturated fatty acids (PUFAs) results from UV activation of phospholipases, lipoxygenases and cyclooxygenases (10–12). Most of the work on enzymatic generation of eicosanoids [rev. in (10)] has been done on the response to

clinically relevant short wavelength UVB irradiation. This may lead to an underestimation of non-enzymatic effects to solar UV exposure which are mostly elicited by longer wavelength radiation. Similarly biasing may be that UV-regulated eicosanoids (and related FA derived mediators) are investigated mainly in their free form, while a large fraction of the modified FA may be presently attached to more complex lipids.

Previously it was observed that the UVA-photo-oxidation of PUFA esterified to phospholipids is more efficient than photo-oxidation of the same PUFA in the free form, probably due to increased UVA induced singlet oxygen generation in the PL esterified configuration of the PUFA (13). Indeed, Leung et al. found in HaCaT cells exposed to UVA little effect on n-6 PUFA and their non-enzymatic oxidation products immediately after exposure (14) but detected elevation of enzymatically modified hydroxides of docosahexaenoic acid (DHA). The authors conclude that HaCaT cells required 24 h to return to PUFA homeostasis.

In primary human dermal fibroblasts, our group identified more than 500 features corresponding in retention properties to polar and oxidized phosphatidylcholines (PCs) that were induced immediately after irradiation with UVA (15), and also in primary human keratinocytes we found significant elevation of 173 OxPC species immediately after irradiation. In both cell types, the elevated species comprised also non-enzymatic PUFA-PC oxidation products such as PC-hydroperoxides and hydroxides, di-carboxylic and carbonyl group containing PC species. In the keratinocyte investigation we found that even at the high UVA-1 fluence of 40 J/cm² the cells recover, and most lipid species return to baseline levels within 24 h, insofar as the KC appear to limit especially the amount of highly reactive carbonyl containing lipids. The restoration of phospholipid redox (or epilipidome)

homeostasis involves the antioxidant response, autophagy, the unfolded protein response and, as recent findings suggest, the transcriptional regulator NUPR1 (16). Conversely, in vitro oxidized PUFA-PC are potent inducers of autophagy and Nrf2 (17, 18). These are mechanisms and signaling pathways that can be assigned to the protective, pro-resolving spectrum of oxidized phospholipid action. At the same time these lipid extracts or *in-vitro* oxidized PAPC preparations contain phospholipids with known pro-inflammatory activity and highly reactive carbonyl compounds (19, 20). A detailed investigation of the quantities of individual lipid species and localization of the lipids, their functional groups and their adducts will be next steps for elucidating the biological net effect of epilipidomic modifications on (phospho-) lipids through oxidative stressors in the skin. Elaborate mass spectrometric methods are required for structural analysis of aldehyde adducts to proteins [rev in (21)]. Because even as antibodies to protein-lipid adducts and the dinitrophenylhydrazine method to investigate protein carbonylation are widely used, lipid oxidation products and especially malondialdehyde can show not only high diversity in the type of modification of proteins (and thereby yielding very different epitopes) (22), but also interfere with the detection of other adducts (23).

The dietary intake of fatty acids affects the systemic and cutaneous composition of systemic free fatty acids and the composition of phospholipids to which these fatty acids are dynamically esterified. It also affects the potential enzymatic and non-enzymatic oxidation products that will form after UV exposure. Supplementation with eicosapentaenoic acid (EPA) and a subsequent UV exposure led to a shift in the UVA induced eicosanoids that were recovered from skin suction blisters from arachidonic acid metabolites (prostaglandin E2 and 12-HETE) towards EPA metabolites (prostaglandin E3 and 12-hydroxy-eicosapentaenoic acid, respectively) which have less pro-inflammatory activity (24). When administering docosahexaenoic acid (DHA) to cultured fibroblasts, we observed an elevation of DHA-containing phospholipids which were highly susceptible to photo-oxidation. Only in Nrf2 deficient cells this increased oxidation susceptibility led to increased expression of inflammation markers. Therefore, both the type of UV-induced lipid signaling mediator and the cell's capability to limit peroxidation may determine the epilipidomic effect on UV mediated inflammation regulation. UV not only can enzymatically generate immunomodulatory platelet activating factor (PAF), but PAF-like lipids can also result from free radical action on phospholipids. PAF and PAF-like lipids relay both acute inflammatory and delayed immunosuppressive UV effects, and potentially elicit systemic signals by releasing microvesicles from KC (25).

The effects of UV exposure are not restricted to cellular lipids. Also the sebum is susceptible to modification. Hydroperoxides of squalene generated by UV exposure have been identified *in vitro* and *in vivo* (26, 27), and as squalene is a major component of the epidermal surface lipids, its peroxidation products including also reactive aldehydes (28) were proposed as sensors conveying metabolic and inflammatory responses to UV radiation (29). One study even suggested that corneocyte dust containing high levels of oxidized squalene may be a relevant environmental irritant (30). The full spectrum of immunomodulatory actions of (UV-) oxidized squalene and other sebaceous lipids is discussed in (31), where the epidermal NLRP3 inflammasome is suggested as the cellular component that senses and relays inflammatory signaling.

An amplification of photo-damage is elicited by photosensitizers in photo(dynamic) therapy. Porphyrins and their derivatives have hydrophobic properties that locate them to membranes of target cells, allowing to kill those with light through photosensitized ROS generation. At the same time, this treatment leads to massive oxidation of (phospho) lipids (32), and it remains to be elucidated whether oxidized lipids interfere with- or contribute to the therapeutic efficacy. Lipotoxicity upon oxidative stress is mainly exerted by aldehydolipids and was reviewed in (33). In the skin context, the OxPL POVPC was toxic in melanocytes in the micromolar range (32), at which (34) we detected this lipid after exposure to physiologic fluences of UVA in other cell types (15).

THE SKIN EPILIPIDOME IN INFLAMMATION

The two major chronic inflammatory skin diseases associated with impaired barrier function, psoriasis and atopic dermatitis (AD), affect composition and ordering of the epidermal barrier lipids and composition of basal epidermal, dermal, and systemic lipids [reviewed in (10, 35, 36)]. Metabolites attributable to the epilipidome are regulated and likely contribute to the disease, but functional data are yet limited. 9- and 13-hydroxyoctadecadienoic acids (9- and 13-HODE) were significantly elevated in plasma samples from psoriatic patients, as was 7-hydroxycholesterol. In skin biopsies from the same patients the free and esterified levels of 8- and 12-hydroxy-eicosatetraenoic acids (8- and 12 HETE) and 9- and 13-HODE were accordingly elevated, but also eicosanoids with known anti-inflammatory properties (37). First data where resolvin D1 was applied on patient KC and reduced interleukin synthesis by these cells indicate that small pro-resolving mediators of the epilipidome that are topically applied or generated *in situ* could be useful for the treatment of psoriasis (38). At the same time the pro-inflammatory components of the epilipidome likely contribute to the inflammation. Interestingly, a phospholipase that is transferred *via* exosomes to Langerhans cells seems to process psoriasis specific antigens (39). Thus, clear spatial localization of lipid metabolites, *e.g.* with high resolution mass spectrometric imaging and detailed functional studies are needed to fully understand the contribution of the epilipidome in psoriasis.

In the sera of juvenile AD patients, leukotriene B4 (LTB4), thromboxane 2 (TXB2), prostaglandins, HETE and HODE were found elevated, and lipidomic analysis could distinguish between clinically relevant subgroups of patients with high *versus* low immunoglobulin E levels (40). Among the distinguishing markers lysophosphatidyl-ethanolamine (18:2), thromboxane b 2 (TXB2), and 11-, 12-dihydroxyeicosatrienoic acid (DHET) can

be attributed to the epilipidome. TXB2 and 11, 12-DHET were found elevated in skin tissue lipid samples in a comparable study (41), that came to the conclusion that the ratio of pro-inflammatory to pro-resolution mediators was increased in the patients, especially PPARalpha agonistic oxidized lipids. These, especially 12-HETE mediate inflammation and disturb differentiation in AD organotypic skin models (42). Further research will elucidate the contribution of non-enzymatically formed isoforms or mimetics to the downstream signaling of these enzymatically generated mediators in skin inflammation. Agonism or signaling *via* prostaglandin receptors, PPARs, and pattern recognition receptors (PRR) through ROS mediated changes to lipids in other context has been reported (43–45).

MODIFICATIONS OF THE SKIN EPILIPIDOME BY EXPOSURE TO AGING, CHEMICAL IRRITANTS, DRUGS, AND OTHER STRESSORS

Highly reactive lipid oxidation products and their adducts to other macromolecules accumulate in the skin that prematurely aged due to sun exposure (46, 47). However, also chronologic aging of the skin at the cellular level and senescence of cells are similarly associated with lipoxidizing redox events, for example ROS accumulation in mitochondrial dysfunction and in senescence related chronic inflammation (48). The skin's cellular composition as well as the synthetic and metabolic fidelity changes during the mammalian lifespan, and these changes leave traces in the skin's lipidome and epilipidome. Those epilipidomic changes introduce a novel, autonomous layer of signaling for complex exposure–response relationships (49) in cellular stress, aging, and inflammation. Recently, elevated leukotriene generation was identified as a feature of senescent fibroblasts that promotes lung fibrosis (50), and we found compatible changes in the oxidized phospholipidome of senescent dermal fibroblasts (51).

The skin is exposed to temperature fluctuations, which likely affects the dynamics of enzymatic- and ROS-mediated epilipidomic modifications. One study monitored barrier lipids of acne and control patients over the course of a year, together with trans-epidermal water loss (TEWL) measurements and assessment of acne severity. The authors found that in acne-affected skin the ceramide species Cer[NH] and Cer[AH] were significantly reduced. This effect was greatest in winter and correlated with the highest TEWL measurements. Ceramide species with 18-carbon species of 6-hydroxysphingosine appeared to be most significantly reduced, an example of the diverse consequences that oxidative modification of lipids has in epidermal barrier function (52). Compatible with the latter finding, a (redox-) lipidomic study (53) on SC lipids from volunteers receiving glucocorticosteroids (GC) identified that the barrier damage, which is a side effect of GC therapy, was associated with reduction of ceramides with an esterified omega-hydroxy acyl chain. Furthermore, anti-cancer chemotherapy can affect the skin

epilipidome, shown in a murine melanoma model, where chemotherapy generated, probably due to ROS generation, PAF-receptor agonistic lipids which negatively affected anti-tumor immunity (54). In murine epidermis exposed to the carcinogenic chemical irritant 12-O-tetradecanoylphorbol 13-acetate (TPA), we found strong epilipidome modification. Phospholipid hydroperoxides were elevated three days after the last treatment, and we found that peroxiredoxin 6 is an important regulator of epidermal lipid (per) oxidation *in vivo* (55). Cigarette smoke (CS) is a lifestyle-related environmental stress for the skin, and exposure of KC to CS increases the formation of carbonyl (4-hydroxy-2-nonenal; 4-HNE) adducts which likely result in part from lipid oxidation (56), and the immunosuppressive PAF-like lipids (57). A novel therapeutic option for dermatological wound- and inflammation management is the directed application of beams of cold atmospheric plasma (CAP) which contains highly dynamic matter, to tissue (58). One consequence when this treatment is applied to surface lipids is a massive change in the skin epilipidome (59), and it remains to be investigated whether epilipidomic changes contribute to the efficacy of the treatment which appears to involve activation of the antioxidant response (60).

Whereas most of the studies discussed so far have investigated the modification of fatty acid residues, Maciel and colleagues reported that the radical generating 2,20-azobis(2-amidinopropane) dihydrochloride (AAPH) modifies the headgroup of phosphatidylserines in cultured keratinocytes, adding an additional layer of complexity and novel potential biological consequences to the epilipidome (61). Beyond the oxygen-mediated modifications to lipids, the complexity of the epilipidome can be increased by sulfonation of lipids (62) nitration and nitroxidation of phospholipids, observed *in vivo* in diabetes models and under metabolic stress [Rev. in (63)] and several nitro- and nitroso modifications of unsaturated PC and PS have been characterized (64). Nitro fatty acids were also found in dermal fibroblasts upon virus infection and impaired interferon gamma signaling (65) by modulating the palmitoylation of the adaptor molecule stimulator of IFN genes (STING) which led to inhibition of interferon release, and the authors suggested the pharmacological potential of these lipids in diseases caused by abnormally high STING activity.

DISCUSSION AND OUTLOOK— CONNECTION OF THE EPILIPIDOME WITH OTHER NON-CANONICAL REGULATORS AND LOCALIZATION OF EPILIPIDOMIC MODIFICATIONS WITHIN THE SKIN

Although the importance of the epilipidome for the regulation of cellular processes is clearly evidenced (66), little is known about its interaction with other non-canonical regulators of cell fate ("epi-omics"), such as the epigenome, epitranscriptome, epiproteome or epimetabolome. As all of these "epi-omics" are influenced by oxidative stress, it is well conceivable that oxidized lipids further

exacerbate the effects of the original redox stressor. For example, 4-HNE is formed by lipid peroxidation and is highly reactive towards cysteine, lysine and histidine residues. Thereby, protein adducts are formed which do not only impinge on the epiproteome (67), but also on the epigenome through covalent modification of histones. Histones are common advanced lipoxidation endproducts (ALEs), and some of them are associated with human disorders, such as systemic lupus erythematosus or Alzheimer's disease (68). ALE formation impairs the interaction of histones with DNA and consequently leads to increased vulnerability of exposed DNA stretches to oxidative stress (69, 70). Similarly, chromatin reader, writer and eraser enzymes might be covalently modified by oxidized lipids and thereby their function might be altered. Besides histone acetylation, the epigenome is shaped by methyltransferases, adding methyl groups to bases of DNA. The metabolite S-adenosyl-methionine (SAM) might represent an important link between the different layers of "epi-omics", because it acts as the universal methyl group donor for most DNA, RNA, lipid, and protein methylation reactions. Phospholipid methylation is the major consumer of SAM and SAM availability in cells is limited. Thus, changes in the methylation of phospholipids strongly reflect on methylation reactions of other substrates. Ye and colleagues provided evidence for this phenomenon by demonstrating that loss of phospholipid methylation causes hypermethylation of histones as well as of the major phosphatase PP2A (71). In contrast to DNA methylation, chemical modifications of different RNA species came into focus only recently (72), and might be subject to similar redox- and metabolism-based connections with the epilipidome (73–75). Moreover, RNA modifications were already implicated in the interaction of specific RNA molecules with lipid bilayers (76). N6-adenosine methylation of ribosomal RNA (rRNA) by METL-5 represents an interesting example for a complex crosstalk between the different layers of "epi-omics" in *Caenorhabditis elegans*. Methylation of A_{1717} on 18S rRNA enhances selective ribosomal binding and translation of CYP-29A3 mRNA. This enzyme is required for oxidation of eicosapentaenoic acid to eicosanoids and modulates heat stress resistance (77). Oxidized lipids might also directly influence selective protein synthesis through oxidation of ribosomal proteins (78). Since the synthesis of post-translational protein modifications, such as glycosylations, is tightly synchronized with translation, the epiproteome might be regulated by the epilipidome as well.

The novel gold standard methods for redox- and other epilipidomic investigations are typically based on high resolution mass spectrometry (HRMS), often in combination with chromatographic separation and require intensive bioinformatic post-processing. These methods and their application on the lipidome, redoxlipidome and especially the skin are the topic of recent reviews that are suggested to the reader (35, 66, 79–85). The emerging technology of mass spectrometry-based imaging (MSI) has the unique feature to reveal the distribution of analytes within a tissue allowing the detection, localization and identification of multiple lipid species in an area of interest. Ionization techniques like secondary ion mass spectrometry (SIMS) (86), matrix assisted-laser desorption/ionization (MALDI) or desorption electrospray (DESI) (87) are the methods of choice allowing sensitive measurements. One tissue section can be used for consecutive measurements in positive and negative ion modes depending on the lipid class under investigation (88). However, low concentrations and ion suppression effects can lead to low ion intensities making lipid identification difficult. However, low signal intensities in respect to concentration levels of lipid peroxidation products or method-inherent ion suppression effects makes lipid identification by tandem MS often infeasible and HRMS (*i.e.* Fourier Transform Ion Cyclotron or Orbitrap) is indispensable. The novelty of MSI in the context of skin research is reflected by the limited number of publications available. Few papers focusing on sample preparation (89), few studies are available giving a general overview of lipid changes in skin during wound healing (90), in reconstructed skin equivalents (91) studying lipid profiles over time and in *ex vivo* human skin samples (92). Worth mentioning is research on the effect of topically applied compounds on lipid changes in the skin (93, 94). Despite the promising future of MS imaging, limitations have to be considered and challenges have to be met. One limitation is the rather low spatial resolution achieved with most instruments. (Nano)DESI provides spatial resolutions of approximately 40 to 100 μm, and conventional MALDI measurements can be carried out at pixel sizes down to 10 μm, still larger than most mammalian cells. As a result, each pixel represents the average lipid profile of maybe multiple cells and not of individual cells within the tissue. Reducing the spot size to a single cell level is therefore one of the most important endeavors in MSI research and instrument development (95). SIMS on the one hand has the potential to measure at a few nm spot size (approximately 30 nm), easily reaching cellular levels. However, SIMS is not a soft ionization technique, fragmenting lipid species and providing only lipid class information by head group analysis but not the full molecular information one is usually striving for. MALDI on the other hand is allowing the detection of intact lipid species at rather low resolution, being therefore the most often used method so far. But MALDI shows different ionization efficiencies for different lipid classes, making a comprehensive analysis for the entire lipidome a challenge, choosing the appropriate matrix is key (96). In summary, combining a multimodal approach at high spatial and mass resolution information on the skin's epilipidome with immunohistological features of individual cells, their activation- and differentiation state, their metabolic configuration and their (epi-) transcriptome will be an important task in the imminent future that will help elucidate the contribution of the epilipidome to skin biology (**Figure 1**).

AUTHOR CONTRIBUTIONS

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