



Editorials

Lipoxidation targets: From basic mechanisms to pathophysiology



Proteins are structurally and functionally diverse species that serve multiple functions in the cell and in the extracellular medium. A key factor in the generation of this diversity is their capacity of undergoing posttranslational modifications. In an ample sense, this implies all the chemical modifications of proteins occurring after translation, or sometimes co-translationally, and, aside from enzymatic modifications, it includes a plethora of non-enzymatic modifications by endogenous or exogenous reactive species. These species can be of varied nature and derive from sugars, amino acids, oxygen and nitrogen species, nucleotides and lipids, among others. The development of lipidomics is unveiling the complexity and diversity of the “lipoxidome” present in biological systems, in health and disease, with hundreds of reactive lipid species, which arise from the peroxidation or dehydration of lipids [1]. These molecules can form adducts with other biomolecules, including sugars, nucleic acids and proteins and this is a key factor in their biological effects. Protein modification by addition of these electrophilic lipids is called lipoxidation [2]. This process occurs under (patho)physiological conditions and can contribute to signaling processes or to adaptive or defense responses, as well as a mechanism of instauration or perpetuation of disease in inflammation, neurodegeneration or cancer, among others [3–6] (see Fig. 1).

The Special Issue on “Lipoxidation targets: from basic mechanisms to pathophysiology” aims to travel the route from a better understanding of the reactive oxidized lipid species generated in pathophysiology to their involvement in the formation of new structurally and functionally diverse protein species and their role in health and disease. This encompasses their contribution to defense mechanisms as well as their pathogenic role, their use as biomarkers and the strategies to either circumvent their action or use them as therapeutic tools. Both comprehensive reviews and original articles have been gathered in order to offer a broad perspective of the topic as well as cutting edge knowledge and current challenges in this field.

The article by Jové et al. [7], unravels the complexity of the brain lipidome with a region-specific perspective and sets the focus on the involvement of lipid oxidation and lipoxidative damage in human brain aging. In their view, a restricted set of lipoxidized proteins emerge as important players in this process, with mitochondrial ATP synthase playing a pivotal role. Circulating lipoxidized proteins can serve both as vehicles of danger signals and as biomarkers for disease processes. Among them, lipoproteins play key roles in metabolic and cardiovascular homeostasis. Afonso and Spickett [8] provide a comprehensive overview of the analysis of lipoxidized lipoproteins, their role as biomarkers in disease and their pathophysiological implications and mechanisms of action, which will constitute a valuable reference in this field. The advances in the understanding of the implications of electrophilic lipids and their metabolites, their generation and their implications in cardiovascular disease are thoroughly reviewed in the

article by Gianazza et al. [9], which also provides a clinical perspective and details the clinical trials and therapeutic approaches to prevent or ameliorate lipoxidative damage.

As other oxidative modifications, protein lipoxidation can constitute a double edged sword by either contributing to disease or becoming the basis for novel therapeutic strategies [10]. In their article, Lee et al. [11], show for the first time that the electrophilic prostaglandin 15-keto-PGE₂ directly modifies and suppresses STAT3 signaling, a pathway constitutively activated in many cancer types, which contributes to tumor progression. These effects associate with an inhibition of proliferation of breast cancer cells in culture, as well as of the growth of tumor xenografts. Therefore, this study provides an interesting example of how cancer cell growth can be perturbed by lipoxidation and in particular by electrophilic prostaglandins. Nevertheless, tumoral cells also exploit antioxidant defenses triggered by lipoxidation in order to acquire increased resistance against stress or chemotherapy [12]. As reviewed by Martín-Sierra et al., lipoxidation can exert either anti-carcinogenic or pro-carcinogenic effects and have implications on tumorigenesis that do not only depend on the interactions of electrophilic lipids with cancer cells, but also with immune cells, altering the immune response in cancer [13]. Connections between lipoxidation and pathways involved in cellular defense or toxicity are analyzed in the reviews addressing stress signaling [14] and cell death by ferroptosis [15], as well as in the research article assessing the interplay of lipoxidation and cellular degradation and signaling pathways [16]. Lipoxidation of critical protein sensors can activate various adaptive signaling pathways including heat shock response, Nrf2 and stress kinase signaling, which can lead to increase resistance to stress [14]. Nrf2 activity also plays a key role in the cellular defense against ferroptosis, a recently characterized mode of cell death caused by the accumulation of lipid peroxides [15] and that has become a novel target for therapeutic discovery. Overcoming of the antioxidant defense mechanisms will lead to protein damage and, in many cases, accumulation of damaged proteins and impairment of the cellular degradative pathways [17]. Importantly, impairment of the degradative machinery coupled with increased lipoxidative damage occurs in brain pathology associated with Down syndrome, as shown in the article by Di Domenico et al. [18], with a particular focus on the lipoxidation of protein phosphatase 2A, an event that may have broad consequences on the phosphorylation status of many proteins. Under these conditions there is an abnormal increase in mTOR signaling. Experimental therapeutic approaches administering rapamycin to bring down mTOR signaling to its normal activity could help reducing the levels of key lipoxidized proteins and exert beneficial effects on cognitive functions in their model. The impact of the activity of the autophagy-lysosomal pathway on the fate of carbonylated biomolecules is explored by Coliva et al. in a cellular model of nitrosative stress [16]. They show that mild

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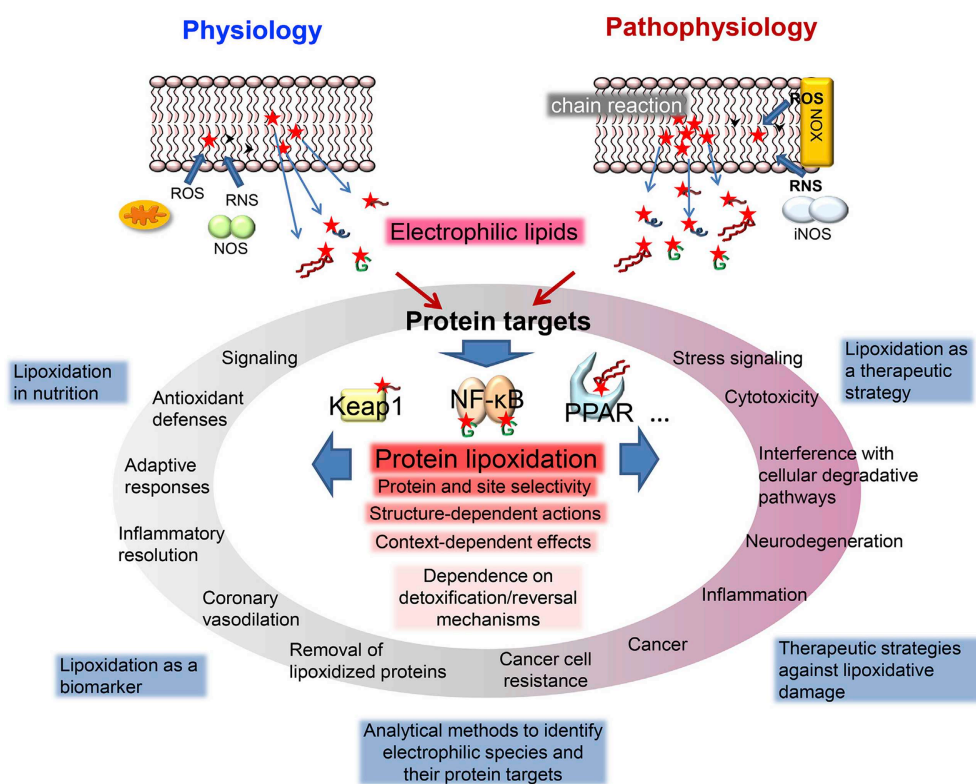


Fig. 1. Protein lipoxidation in health and disease. The generation of electrophilic lipids, increased in pathophysiological conditions due to enzymatic or non-enzymatic lipid (per)oxidation, triggers the formation of covalent adducts with proteins (lipoxidation). Some of the key lipoxidation targets and the characteristics of the lipoxidation process are depicted in the central oval. Functional consequences in physiology (left) or pathophysiology (right) are outlined in the shaded oval. Blue boxes depict strategies for analysis, modulation or therapeutic intervention. ROS, reactive oxygen species; RNS, reactive nitrogen species; iNOS, inducible nitric oxide synthase; NOX, NADPH oxidases.

nitrosative stress in combination with inhibition of the autophagy-lysosomal pathway leads to accumulation of carbonylated molecules in droplets or vesicles, as well as to interfering with the regulation of cellular pathways, with a selective down-regulation of insulin signaling.

Given its non-enzymatic nature, protein lipoxidation could be considered a random process. However, protein lipoxidation displays selectivity determined by the nature of the targets, the electrophilic lipid (s) involved in the modification and by other context factors [19]. The latter include the status of antioxidant defenses of the cell and the occurrence of other modifications or of protein-protein interactions [20]. Therefore, not all proteins are lipoxidized in cells to the same extent and even within proteins there are residues especially prone to modification that are considered “hot spots” and likely act as sensors for electrophilic stress [21]. The single cysteine residue of the cytoskeletal protein vimentin appears to be one of these sensors. In their article, Mónico et al., show that lipoxidation of soluble vimentin alters the morphology of the subsequently polymerized filaments in a cysteine-dependent manner [22]. As preformed filaments are more resistant to lipoxidation-induced disruption, dynamic assembly-disassembly would be necessary for the functional outcome. Moreover, the morphological alterations of filaments display different patterns depending on the lipoxidative agent. This and other evidence confirm the existence of structure-function relationships of lipoxidation. For instance, lipoxidation by small or large electrophilic moieties can induce opposite effects on the activity of certain targets [23]. Therefore, the identification and characterization of novel electrophilic lipids involved in lipoxidation or of new types of adducts, is critical to fully understand the consequences of lipoxidation. Importantly, Yoshitake et al., describe a novel alkanolic acid-type histidine adduct which occurs in hemoglobin and depends on the presence of heme iron [24]. Thus, this adduct, likely occurring in the proximity of the heme group, could have important functional consequences. The discovery of new adducts and/or protein or signaling targets will open the way for the dissection of novel mechanisms of action. In particular, lipoxidation can hamper but also favor protein-protein interactions by altering the charges of amino acid residues. This is the case of the modification of albumin by reactive aldehydes, which,

by reducing the basicity of the adducted residues allow the interaction of neighboring negative residues with positively-charged residues in RAGE (receptor for advanced glycation end-products) thus explaining how a lipoxidized protein is converted in a ligand for this receptor, facilitating damage through the induction of pro-inflammatory and oxidative responses [25]. Moreover, the interplay of lipoxidation with other posttranslational modifications, such as phosphorylation, as suggested in Ref. [22], unveils additional regulatory possibilities.

Protein lipoxidation is thus emerging as a critical and versatile protein modification in pathophysiology. Given the broad structural variety of the lipid moieties involved, understanding the structural and functional complexity of lipoxidation requires high precision methodology. In this issue, the reviews by or Afonso and Spickett [8], Melo et al. [26], and Gianazza et al. [9], provide a comprehensive overview of the available methods for the detection of lipoxidation adducts. There has been an increasing interest in the biological effects of protein lipoxidation, but their elucidation has been hampered to some extent by the limited availability of methods to determine the sites and exact nature of such modification. More recently, the readiness of a wide range of antibodies towards lipoxidation products, as well as advances in analytical techniques such as liquid chromatography tandem mass spectrometry (LC-MS/MS), provided an increase in the knowledge in this type of posttranslational modification. Afonso et al., review the latest developments for MS detection of lipoxidation adducts on apolipoproteins [8], Melo et al., reviewed the MS-based approaches available for the identification of lipoxidation adducts with nitrated lipids, including some recently detected lipid species, that are gaining momentum as important endogenous signaling molecules in health and disease [26]. In the last decade it has been proposed that the nitro lipid-protein adducts with nitro fatty acids and nitrophospholipids can trigger a series of downstream signaling events associated with anti-inflammatory, anti-hypertensive, and cytoprotective effects [26,27]. Furthermore, Patinen et al. [14], call attention on recently developed approaches for the mechanistic study of lipoxidation in cells. Nevertheless, advances in the clinical setting will be highly dependent on the fast development of label free high resolution MS approaches for the analysis of ex vivo

samples. In addition, novel affinity strategies like that reported by Mol et al. [25], are being developed, allowing the enrichment of adducts from clinical samples and enabling, in combination with MS, the detection of minute amounts of adducts.

Expanding the boundaries of lipoxidation signaling, J.D. Alché recapitulates the importance of lipoxidation in plants. Lipoxidation is involved in the response of plants to both biotic and abiotic stress. Moreover, the importance of lipoxidation in foodstuffs is considered, given its potential beneficial or detrimental roles for human health [28]. Although a number of lipoxidation targets have been identified in plants, the wealth of knowledge in other experimental systems, from yeast to mammals, paves the way for elucidating novel roles and mechanisms of action of lipoxidation in plants.

Concluding remarks

Protein lipoxidation is emerging as a multi-faceted protein modification with critical roles in health and disease, eliciting structure-dependent functional changes in proteins in a selective manner, and becoming both a potential therapeutic target and tool. Interest in this type of modification, as well as in the process of lipid oxidation that underlies it, is greatly on the increase and important advances are being made in the area, as illustrated by the articles contained in this Special Issue. Although much is yet to be learnt about protein lipoxidation, the development of high precision MS approaches combined with detailed functional studies will help elucidate its significance in a context-specific manner and exploit its potential both as a biomarker and as an element for the modulation of disease. Recent developments in methodology for analysing oxidation and nitroxidation of lipids, as well for identifying protein lipoxidation, are reported in a companion Special Issue in Free Radical Biology and Medicine entitled "Redox Lipidomics and Adductomics - advanced analytical strategies to study oxidized lipids and lipid-protein adducts". Together these two Special Issues offer a comprehensive resource both for a general readership and for specialists in this field.

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