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FORUM EDITORIAL

Protein Carbonylation

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PROTEIN CARBONYLATION is a type of protein oxidation that can be promoted by reactive oxygen species. It usually refers to a process that forms reactive ketones or aldehydes that can be reacted by 2,4-dinitrophenylhydrazine (DNPH) to form hydrazones. Direct oxidation of side chains of lysine, arginine, proline, and threonine residues, among other amino acids, in the "primary protein carbonylation" reaction produces DNPH detectable protein products (11, 15, 23). DNPH derivatizable protein products can also be formed in the "secondary protein carbonylation" reaction via the addition of aldehydes such as those generated from lipid peroxidation processes (14, 21). Oxidative decomposition of polyunsaturated fatty acids initiates chain reactions that lead to the formation of a variety of carbonyl species (three to nine carbons in length), the most reactive and cytotoxic being α,β-unsaturated aldehydes (4-hydroxy-*trans*-2-nonenal and acrolein), di-aldehydes (malondialdehyde and glyoxal), and keto-aldehydes (4-oxo-*trans*-2-nonenal). Although the biology of oxidative protein modifications is complex and remains incompletely defined, protein carbonylation and chemistry of the reactions that give rise to carbonyl groups have been well characterized (24).

The development of the antibody against DNPH-derivatized proteins revolutionalized the studies of carbonylated proteins by allowing for the use of immunological techniques (16, 17, 22). More recently, these methods contributed to a rapid progress in proteomic analyses of carbonylated proteins using two-dimensional gel electrophoresis, followed by immunoblotting and mass spectrometry. This redox proteomics approach allowed for the identification of carbonylated proteins in various diseases in humans, animals models, and cell models, and has provided important information to biologists by describing the effects of modifications by carbonyl species on protein function, as well as the consequences of such modifications at the cellular level.

Butterfield and co-workers developed this proteomics approach to identify specifically oxidized proteins in Alzheimer's disease by detecting carbonylated proteins (7, 10, 12). In this issue, Sultana *et al*. (25) used a redox proteomics approach to identify specifically carbonylated proteins in the inferior parietal lobule from human subjects with mild cognitive impairment and early stage Alzheimer's disease, providing insights to the mechanism of the progression of this disease.

Hussain, Barreiro and co-workers have championed the understanding of carbonylated proteins in skeletal muscle dysfunctions during various disease processes such as chronic obstructive pulmonary disease and sepsis (5, 6). In this issue, Barreiro and Hussain (4) review their studies on carbonylated proteins in skeletal muscle dysfunction. Further, Barreiro and co-workers report their data on carbonylated proteins in skeletal and cardiac muscle of cachectic rats (18).

Burcham and co-workers have previously shown that cell exposure to acrolein results in the reaction with cysteine groups, forming protein carbonyls (8). In this issue, Burcham *et al*. (9) demonstrate that intermediate filament proteins are targets of acrolein-induced protein carbonylation in A549 lung epithelial cells, providing evidence for the involvement of carbonylation of these proteins during smoke-induced lung injury.

In addition to the identification of proteins that are carbonylated in various disease models, advancement in mass spectrometry technology has allowed for sophisticated mechanistic studies of carbonylated proteins in oxidative stress conditions. In this issue, Dalle–Donne and co-workers report the identification of amino acids within the human serum albumin molecule that are carbonylated in response to cigarette smoke extract exposure (13).

In addition to the well-established roles of protein carbonylation in oxidative stress, this oxidation process may also play roles in cell signal transduction as described by Suzuki and co-workers (26, 27). This suggests that cellular regulatory mechanisms of protein carbonylation may be complex, which might include means to promote and eliminate protein carbonyls. While enzymatic reversal of the protein–carbonyl modifications has not yet been detected, an enzymatic reversal mechanism for protein–methionine sulfoxide modifications exits and may play a role in the regulation of protein carbonylation, as described by Moskovitz and co-workers (19).

These pathophysiologic roles of protein carbonylation in oxidation stress and oxidant signaling suggest that compounds, which regulate carbonyl content, may have clinical value. An alternative strategy to the antioxidant intervention based on compounds acting as free radical scavengers is to detoxify oxidative-derived carbonyl reaction products. Trapping of lipid-derived reactive carbonyl species (identified as

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the chemical intermediates between hyperglycemia, hyperlipidemia, and their complications) seems to be very promising, and represents a new therapeutic target on which the efforts of the medicinal chemists should focus in the near future. Promising results have been obtained in preclinical studies, where compounds belonging to different chemical classes, but sharing a carbonyl quenching mechanism, were found to be effective in the prevention/treatment of carbonyl-associated diseases such as diabetes and metabolic distress syndrome (2, 3). Hence, the generation of drugs sharing both antioxidant and carbonyl scavenging properties represents a new therapeutic challenge in the treatment of carbonyl stress-associated diseases (20). In this regard, Carini and co-workers report in this issue that edaravone is not only a reactive oxygen species scavenger, but also a reactive carbonyl scavenger (1).

Protein carbonylation is a well-used marker for oxidative stress. Recent progress in redox proteomics and mass spectrometry identified carbonylated proteins during various disease states. Protein carbonylation may also mediate redox signaling processes, which may result in pathogenesis. Thus, the development of agents, which can control cellular protein carbonylation status, should contribute to the development of therapeutic strategies against various diseases.

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Abbreviation Used

DNPH = 2,4-dinitrophenylhydrazine