powerful oxidant HOCl. This mediates pathogen killing, but misplaced generation is linked with extracellular matrix damage (via MPO binding) and human diseases. Chlorination of Tyr residues gives 3-chloroTyr, a specific biomarker of MPO damage, but this is challenging to detect due to its low yield. In this study, a new optimised protocol has been developed to detect and quantify modifications. Systematic evaluation of previous protocols has identified factors that limit detection. Alternative methods allow the detection of a large number of modified residues induced by pathologically-relevant HOCl levels. High sequence coverage and reproducibility can be achieved for large, disulfide-rich proteins without reduction and alkylation. Using label-free quantification at a peptide level, in shotgun MS of single proteins, chlorination can be detected down to a relative site occupancy of ~0.15%. This method is effective for complex samples, and minimizes artefactual oxidation, facilitating quantification of modifications to readily-oxidized residues such as Met, His, Cys, and Trp.

E-mail address: adelinar@bmb.sdu.dk

http://dx.doi.org/10.1016/j.freeradbiomed.2018.04.187

P-41

Myeloperoxidase-derived chlorination and oxidation human plasma fibronectin

Tina Nybo [1], Huan Cai [1], Christine Y. Chuang [1], Adelina Rogowska-Wrzesinska [2], Michael J. Davies [1]

[1] *Department of Biomedical Sciences, University of Copenhagen, Copenhagen N, Denmark*
[2] *Department of Biochemistry and Molecular Biology and VILLUM Center for Bioanalytical Sciences, University of Southern Denmark, Odense M, Denmark*

Immune system cells are a major source of oxidants. Leukocyte activation generates $O_2^{\bullet-}$ and H_2O_2, and results in myeloperoxidase (MPO) release. MPO uses H_2O_2 and Cl^- to generate the powerful oxidant HOCl. This mediates pathogen killing, but misplaced formation is linked with extracellular matrix damage (via MPO binding) and human disease. In this study, we have quantified chlorination (3-chloroTyr) and oxidation of human extracellular matrix fibronectin (FN) induced by HOCl and an enzymatic MPO system, and its consequences for human coronary artery smooth muscle cell adhesion, proliferation, migration and gene expression. 3-ChloroTyr and oxidation are detected at multiple sites on FN, including within functionally-important heparin-, cell- and collagen-binding domains, in a dose-dependent manner. Damage is localized to specific residues and occurs to markedly different extents. Heparin-binding and cell adhesion to HOCl-modified FN is decreased in a dose-dependent manner. Cell proliferation on HOCl-damaged FN is modulated, and migration attenuated. Expression of matrix proteins and matrix metalloproteinases in cells exposed to modified FN is significantly altered. These data indicate that FN modification by HOCl and MPO markedly affects matrix properties and cell behaviour.

E-mail address: adelinar@bmb.sdu.dk

http://dx.doi.org/10.1016/j.freeradbiomed.2018.04.188

P-42

Identification of the channel responsible for the ozone stress induced chloride current in human lung epithelial cells

Rita Canella [1,2], Mascia Benedusi [1,2], Marta Martini [1,2], Carlotta Cavicchio [1,2], Franco Cervellati [1,2], Giuseppe Valacchi [1,2]

[1] *Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy*
[2] *NC State University, Plants for Human Health Institute, Animal Science Dept., Kannapolis, NC, USA*

In our previous work we investigated the effects of O_3, one of the most noxious pollutants to which respiratory tract is the most exposed organ, on Cl currents in human cultured lung epithelial cells (A549 line) and demonstrated that O_3 exposure significantly affects Cl current, inducing a large outward rectifier component.

Among the different types of chloride channels present on the cell membrane, Cl-C2 and ORCC (Outward Rectifier Chloride Channel) were the mainly involved, based on the experimental conditions. Functional experiments with specific channel blockers failed to uniquely identify the channel responsible for this stress induced behavior.

In this study we show how silencing of the ANO6 gene (functional part of the ORCC channel) is able to completely eliminate the outward rectifier component of the current. Surprisingly, it was also observed that even under control conditions, a large part of the chloride current of the cell is sustained by the activity of this channel, usually considered inactive in the absence of oxidative stress or voltage stimulation.

This work provides new explanations on the mechanisms of control of water-salt balance in lung epithelial cells at rest and subjected to oxidative stress.

E-mail address: cnr@unife.it

http://dx.doi.org/10.1016/j.freeradbiomed.2018.04.189

P-43

Genome-wide sequencing reveals small nucleolar RNAs downregulated in cerebral cavernous malformations

Souvik Kar [1], Kiran Kumar Bali [2,3], Arpita Baisantry [4], Robert Geffers [5], Christian Hartmann [6], Amir Samii [1], Helmut Bertalanffy [1]

[1] *International Neuroscience Institute, Hannover, Germany*
[2] *Pharmacology Institute, Medical Faculty Heidelberg, Heidelberg University, Heidelberg, Germany*
[3] *Molecular Medicine Partnership Unit with European Molecular Biology Laboratory, Heidelberg, Germany*
[4] *Department of Kidney, Liver and Metabolic Diseases, Children's Hospital, Hannover Medical School, Hannover, Germany*
[5] *Genome Analytics Research Group, Helmholtz Centre for Infection Research, Braunschweig, Germany*
[6] *Department of Neuropathology and Neurosurgery, Hannover Medical School, Hannover, Germany*

Cerebral cavernous malformations (CCM) are vascular malformations associated with abnormally dilated blood vessels and leaky capillaries that