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ABSTRACTS of SFRR-INTERNATIONAL 2021 VIRTUAL MEETING

Short Oral Communications

NC1

The cellular vimentin network undergoes distinct reorganizations in response to diverse electrophiles or mutations of its single cysteine residue

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Vimentin is a type III intermediate filament protein which plays key roles in essential cellular processes such as cell migration, division, organelle positioning and homeostasis (Duarte, 2019). Moreover, vimentin behaves as a stress sensor. We previously reported that the single cysteine residue of vimentin (C328) is the target for modification by oxidants and electrophiles, such as 4-hydroxynonenal, diamide or 15-deoxy-PGJ_2. These compounds induce marked alterations in filament assembly in vitro and in vimentin network reorganization in cells. The attenuation of these effects in a C328S vimentin mutant supports the importance of this residue in the transduction of oxidative or electrophilic modifications into cytoskeletal responses (Mónico, 2019).
Here we have used vimentin-negative SW13/cl.2 cells transfected with GFP-vimentin constructs in order to explore the structure-function relationships of C328 modification. We have observed that treatment of cells with several oxidants and electrophiles, including 4-hydroxynonenal, 1,4-dinitroimidazole or H_2O_2, disrupts the organization of GFP-vimentin structures leading to diverse patterns depending on the agent used. Nevertheless, these agents can modify multiple cellular targets. To directly address whether structural modifications at C328 play a role in vimentin reorganization we have studied the behaviour of several mutants at this site. We have found that different mutations provoke the assembly of GFP-vimentin constructs into morphologically distinct arrays in two vimentin-deficient cell lines, i.e. SW13/cl.2 and MCF7 cells. Interestingly, a C328H mutant forms robust structures that are more resistant to disruption by oxidants and electrophiles than the wild type.
Altogether these observations strengthen the role of vimentin C328 as a key stress sensor and suggest that structurally different modifications at this site could result in morphologically and/or functionally distinct reorganizations of the intermediate filament network.
Duarte (2019) Nat Commun, 10:4200.
Mónico (2019) Redox Biol 23:101098.
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NC2

oxSWATH applied to the study of the alteration of intracellular and extracellular proteome of cells in response to oxidative stress

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In the present work, an exhaustive characterization of the proteome adaptions to oxidative stress was performed using the oxSWATH[1] approach. This method allows integrating the information regarding relative cysteine oxidation with the analysis of the total protein level. Thus, in a single analysis, it was possible to evaluate the alteration considering the redox status of the proteins and performed a generic differential proteomics analysis of the cells exposed to an acute stimulation with hydrogen peroxide. To completely characterize the cellular response, both the cells and the secretome were analyzed, covering the intracellular and extracellular responses, respectively. A total of 915 proteins were altered upon oxidative stress, from which, 90 were altered in both intra- and extracellular space. Moreover, a clear tendency for a remodeling of the extracellular space was observed, with near 80% of the altered proteins found altered in the secretome. The analysis of the overall redox status of the proteins reveals a tendency to have a reduced environment in the extracellular space, while an equilibrium between the reduced and oxidized proteins is achieved in the intracellular environment. Again, a higher number of secreted proteins present an alteration of their redox status upon oxidative stress when compared with the intracellular proteins (250 and 61 proteins, respectively). From those, only 4 were commonly altered between the two cellular spaces. Overall, these results indicate that there is a differential adaptation of the intracellular and extracellular proteomes, with the extracellular space being particularly affected by oxidative stress. Moreover, the potential of the oxSWATH method was confirmed in this work since a truly comprehensive evaluation of proteomics changes upon the oxidative stimulus was achieved using a single approach.
[1]Anjo, Sandra I et al. "oxSWATH: An integrative method for a comprehensive redox-centered analysis combined with a generic differential proteomics screening." Redox Biology vol. 22 (2019): 101130. doi:10.1016/j.redox.2019.101130

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NC3

H_2O_2 biosensors HyPer2, HyPer3 and GFP2-Orp1 detect rapid pH changes due to environmental CO2 fluctuations, in addition to intracellular H_2O_2, in isolated skeletal muscle fibres

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Hydrogen peroxide (H_2O_2) is one of the Reactive Oxygen Species (ROS) that seems to play an essential role in pathophysiological processes. H_2O_2 might act as a signaling molecule and modulate different crucial cellular signaling pathways, such as the glucose uptake in skeletal muscle, where H_2O_2 has been proposed to play an important role.
HyPer2, HyPer3 and GFP2-Orp1 are hydrogen peroxide biosensors. We use these biosensors to monitor intracellular H_2O_2 in single skeletal muscle fibres isolated from the flexor digitorum brevis (FDB) mouse muscle. Previously, the coding sequences of these biosensors were microinjected and electroporated in FDB.

Isolated fibres in culture that expressed one of the biosensors were settled incubation chamber coupled to the fluorescence microscope. The chamber maintains temperature (37ºC), environmental CO_2 (5%) and humidity. Different time course experimental conditions were performed where fibres were exposed to different agents (insulin, interleukin 1β, H_2O_2, DTT) and intracellular H_2O_2 flux was registered in real time using fluorescence microscopy imaging analysis. We observed that when there were environmental CO_2 (5%) fluctuations, due to initial medium stabilization or occasional interruption of CO_2 supply, the biosensors showed changes in the fluorescence emission, which were registered. The main consequence of CO_2 fluctuations is the change in the pH of medium. The main part of the biosensor structure is a fluorescent protein, YFP in de case of HyPer2 and HyPer3, and GFP2 in GFP2-Orp1. It has been reported that these fluorescent proteins are sensitive to pH and this might be a disadvantage for the biosensors. However, we believe that this pH sensitivity should be considered as an additional property of this biosensors, since they provide information in real time about the rapid changes of pH due to environmental fluctuation of CO_2 and likely other gases such as O_2 or N_2.

Keywords: Hydrogen peroxide; CO2; biosensors; skeletal muscle fibres

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NC4

PRDX2- and PRDX3-rsGFP2 fusions: response to relevant oxidants and redox sensors during hypoxia and reoxygenation inside living cells

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Peroxiredoxins (Prx) are thiol-dependent peroxidases playing key roles in antioxidant defence and redox signalling and are preferential intracellular targets for peroxides. Genetically encoded probes based on Prx and redox-sensitive GPF2 (rsGFP2) fusions allow to detect elevated H_2O_2 levels in stimulated or stressed cells. We have developed bovine aortic endothelial cells (BAECs) stably expressing a hsPrx2-rsGFP2 protein and evaluated the response to exogenous addition of H_2O_2. The oxidation levels of the probe (OxD) were dependent on peroxide concentration but did not reach maximum levels, probably due to partial overoxidation of Prx, as detected by western blot. Interestingly, the endogenous and rsGFP fusion forms of Prx2 showed distinct sensitivity to overoxidation. We also measured the real-time oxidation of hsPrx2-rsGFP2 sensor when the cells were exposed to a continuous flux of H_2O_2, peroxynitrite and drugs known to induce ferroptosis via accumulation of lipid peroxides (RSL3 and erastin). Our group is advocated to the study of redox responses to hypoxia and reoxygenation. We observed a time dependent oxidation of the peroxide probe 2',7'-dichlorofluorescein (*DCF*) during reoxygenation after 2 hours of hypoxia, which was accompanied by a reversible oxidation of endogenous Prx1 and 2 to disulphide-bond dimers. Accordingly, oxidation of hsPrx2-rsGFP2 was detected under the same conditions, making this probe an excellent tool for our studies in the living cell. A hsPrx3-rsGFP2 containing the Prx3 mitochondrial target was developed. Confocal microscopy studies confirmed the localization of the probe in the mitochondria. We aim to use both probes to study the redox response of the cell to hypoxia and reoxygenation in both cellular compartments.

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NC5

Performance evaluation tests of an auto-fluorescence observation system for non-invasive biological measurements

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Conventional oxidative stress markers, such as lipid-derivers free radicals have been studied to cause damages to cell membranes, proteins and other biomolecules. Decompositions of lipid hydroperoxides are known to release excited triplet states of biomolecules composed with carbonyl groups. The previous study suggested that ultra-weak photon emissions of the carbonyl groups composed with various wave lengths. On the other hand, more detailed investigations on in vivo redox status are needed to elucidate the mechanisms contributing to damage caused by stress. Recently, glycative stress related to accumulation of advanced glycation products (AGEs) might be important to monitor in the redox status because AGEs have been used as biomarkers for non-invasive measurement techniques. However, a skin condition marked by an overgrowth of layers of horny skin and distance from skin surface to high moisture stage layer might affect measuring auto-fluorescence in vivo. To elucidate mechanisms of photon emissions from human fingers including fluorescent oxidation products, we executed performance tests of an auto-fluorescence observation system. Our findings provide that auto-fluorescence intensities of human fingers changed during COVID-19 related crisis. The auto-fluorescence observation system for non-invasive biological measurements might be a lifestyle habit improvement support device.

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NC6

Redox control of the transcriptional circadian rhythmicity by SOD2

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Endogenous control of circadian rhythmicity is mainly governed by clock genes at the genomic level. Furthermore, both clock genes and redox regulation also modulate cell metabolism, thus showing a closed and strong interconnection between both regulatory pathways.

Mitochondrial superoxide dismutase (SOD2/MnSOD) is a key antioxidant and redox-regulating enzyme, depurating superoxide anion mainly generated in the electron transport chain. The increase in the activity of this enzyme has been postulated as a major event during the antioxidant defense response; on the other hand, loss of SOD2 function constitutes a significant oxidative stress factor. With this premise, the work presented here used two transgenic murine models for SOD2 characterized by either a reduced function in hemizygous ($SOD2^{+/-}$) and by a SOD2 overexpressing mice ($SOD2^{+/++}$) in order to study the role of oxidative stress and redox regulation on the physiological circadian transcriptional rhythmicity.

Interestingly, both transgenic models, $SOD2^{+/-}$ and $SOD2^{+/++}$, displayed a similar transcriptional profile which differed to WT, sharing some transcriptional changes regarding cytokeratin, calcium binding, and kallikrein serine proteases. Both genotypes presented a significant loss of rhythmic metabolic transcripts, when compared to WT, resulting in a lower number of rhythmic transcripts. However, the changes in circadian rhythmic transcripts in heterozygous $SOD2^{+/-}$ mice were greater than those observed in $SOD2^{+/++}$ overexpressing mice. Therefore, a change in the expression pattern of ARNTL/BMAL1, the transcriptional decrease in NADH dehydrogenase or the pro-inflammatory transcript profile were among the major features observed in $SOD2^{+/-}$ mice.

The study points that deregulation of SOD2 expression accounts for important changes in the metabolic transcriptional machinery, especially in a situation of oxidative stress, in which clock genes and the metabolic activity appear to be highly compromised.

Keywords: Superoxide dismutase 2, Circadian rhythm, Metabolism, ARNTL, Electron transport chain.

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NC7

Lipid peroxidation as measured by chromatographic determination of malondialdehyde. Human plasma reference values in health and disease

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