ISCHEMIA/REPERFUSION INCREASES THE OXIDIZED STATE OF SPECIFIC CYS RESIDUES IN PROTEINS FROM MITOCHONDRIAL SUBPOPULATIONS FROM RAT MIOCARDIUM THAT ARE PROTECTED BY ISCHEMIA PRECONDITIONING

M. Moreno [(1)], P. Martínez-Acedo [(1)], E. Miró-Casas [(2)], E. Núñez [(1)], D. García-Dorado [(2)], J. Vázquez [(1)].

[(1)] Centro de Biología Molecular Severo Ochoa, [(2)] Servicio de Cardiología, Hospital Universitari Vall d'Hebron, Barcelona.

When heart is subjected to ischemia, mitochondria generate an excess of partially reduced oxygen species and reperfusion can exacerbate the damage. Understanding the causes of reperfusion injury and providing ways of preventing it is a challenge clinical importance. However, the alterations in the mitochondrial proteome in these situations have not been analyzed yet, and information about mitochondrial redox proteomics is scarce.

In this study we used GELSILOX, a stable-isotope labelling technique newly developed in our laboratory, to perform at the same time a quantitative analysis and the determination of exact Cys sites that change their oxidation state in proteomes from two different mitochondrial populations, subsarcolemmal (SSM) and intermyofibrillar (IFM), obtained from rat hearts subjected to ischemia preconditioning (IP) and/or ischemia-reperfusion (IR). These subpopulations were chosen because, in contrast to SSM, IFM are more resistant of IR and cannot be preconditioned.

From a total of more than 350 quantified Cys sites, we were able to detect an increase on the oxidized state in 29 specific Cys in SSM submitted to IR when compared to their normoxic pair, whereas no oxidation increases were detected in IFM. Most of the oxidized sites correspond to subunits of the Complex I of the electron transport chain and to active or metal binding sites of proteins with potentially important biological relevance. On other hand, the oxidation levels in SSM subjected to IP before to IR were lower than those detected in SSM subjected to IR.

This study is the first that combines a comparative differential expression proteomics analysis in SSM and IFM mitochondria in response to preconditioning and ischemia/reperfusion with the characterization of a record number of changes in the redox state of specific Cys sites. Our results may contribute to a better understanding of the molecular events underlying the heart damage produced by ischemia reperfusion.