The Oxidized Proteome of Peripheral Blood Mononuclear Cells: A Valuable Repository for Clinical Proteomics

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Overview

Purpose: PBMCs and its specific cell subsets have allowed for a very broad collection of applications such as in vitro cell-based assays to study the immune response to a given stimuli, or to monitor in vivo or ex vivo changes before and after a drug treatment. More importantly, they are an easily accessible cellular part of the blood, and they are the only component of the blood that could have a gene expression activity. Access to complete atlas of the gene expression and posttranslational modifications in PBMCs will permit more sophisticated studies such as the selection of potential biomarkers that could be used for many purposes ranging from diagnostic, prognosis or even help selecting the appropriate therapy for a patient.

Results: This study compiles the most extensive proteome map of PBMCs. We have demonstrated that the combination of the results yielded the identification of over 8000 proteins. In addition, over 5000 proteins were accurately quantified and over 7000 oxidation events were identified. Remarkably, our data also suggest that H2O2 might play a role modulating signaling pathways by reacting with specific protein targets. Overall, this study not only adds significant value in the mechanistic understanding of redox signaling, but it also creates a valuable protein repository that could lead to the development of new therapeutic strategies.

Introduction

Peripheral blood mononuclear cells (PBMCs) are a popular model system to study the physiological and metabolic activity of cells within the body. PBMCs have enabled a very broad collection of biomedical applications. Monitoring gene expression and posttranslational modifications are very promising areas in biomarker discovery and translational research. In this study, we have aimed to have the most extensive proteome map of PBMCs and monitor the in vitro effect of reactive peroxide at low concentration under different exposition times. Over 8000 proteins were mapped, more than 5000 proteins were accurately quantified and over 7000 oxidation events were identified. These observations represent the largest proteome profiling dataset for PBMCs to date, and create useful warehouse in the clinical blood proteomics field.

Methods

Sample Preparation
PBMCs from a healthy male individual were purchased from AllCells. 1ml cells aliquots were in vitro treated with 5 mM H2O2 for 0, 2, 10, 30 and 80 min. Cell lysis, protein precipitation and digestion were performed using the Mass Spec Sample Prep Kit for Cultured Cells (Pierce, Rockford IL.).

Liquid Chromatography and Mass Spectrometry Analyses
Peptide digestes were then analyzed by LC-MS/MS analysis on a Thermo ScientificTM EASY-nLC™1000 system, coupled to a Thermo ScientificTM Q Exactive™ Plus mass spectrometer over a 2-hour gradient.

Data Analysis
Database search and oxidation site localization were performed using SEQUEST and phosphor RS. These tools were used as nodes within Thermo Scientific™ Protein Discoverer™ Software (v 2.0.0). Inferno was then used for further statistical analysis and ProteinCenter was used to extract biological context and set comparisons with publicly available datasets.