Cytokine-induced phosphorylation dynamics reveals molecular mechanisms underlying human β-cell stress
Lian Yi1, Britney N. Newby2, Adam C. Swensen1, Therese Rw Clau51, Marina A. Gritensko1, Ronald J. Moore1, Mark W Wallet2, Rohit N. Kulkarni3, Richard D. Smith1, Clayton E. Mathews2 and Wei-Jun Qian1

1Pacific Northwest National Laboratory, Richland, WA; 2University of Florida Diabetes Institute, Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, FL; 3Joslin Diabetes Center, Harvard Medical School, Boston, MA

Overview

• Cytokines, including type 1 interferons (IFN), released by islet-infiltrating immune cells play a crucial role in the pathogenesis of type 1 diabetes where they impair β cell function, enhance immune-surveillance, and augment CD8+ cytolytic T cell (CTL) mediated β cell killing.
• Protein phosphorylation is essential in orchestrating pancreatic β-cell function, including beta-cell proliferation, apoptosis, and insulin resistance of peripheral tissues.
• Mass spectrometry has enabled the study of cellular signaling on a system-wide scale, through the quantification of protein phosphorylation.
• Here, we report a quantitative study of phosphorylation dynamics induced by cytokine treatment in human beta-cell line using isobaric labeling combined with immobilized metal ion affinity enrichment and mass spectrometry.

Method

• Experimental workflow consisted of sample treatment, process, data acquisitions and analysis.

Sample Preparation

| Time Post Treatment (min) | TMT 11 Labeling | TMT 12 Labeling | ESI-T 1 Labeling | ESI-T 2 Labeling | Digestion | Trypsin | In-gel Digestion | Interleukin-2 | Interleukin-10 | Glucose | Lipid | Fasting | Fasting + Lipid | Endocell | Endocell | Sedox | Sedox | Sedox | Sedox | Sedox | Sedox | Sedox | Sedox
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Gene Ontology: Biological Processes

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Gene Ontology Analysis

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Conclusions

• This is the first large-scale quantification of phosphorylation dynamics in a human beta cell line under autoimmune stress.
• With a deep-profiling phosphoproteomics workflow, we identified and quantified ~38,000 distinct phosphorylation sites on >6,200 proteins from 300 µg of cytokine treated EndoB-H2 beta cell lines.
• More than 5,400 phosphorylation sites were identified as significant changed sites between different time points using ANOVA analysis.
• These proteins are involved in viral process, regulation of cell cycle, and mitotic cytokinesis. Interestingly, the top pathways observed included endocytosis, tight junction, insulin, ErbB, phosphoinositide, AMPK, glucon, and mTOR signaling pathways.
• Means clustering was applied to identify early and late response sites that are significantly upregulated after IFN treatment.
• Significant upregulation of STAT1 Y701 and STAT3 Y705 indicates macrophase activation induced by IFNs via a STAT-dependent pathway.
• This technique will be applied to study more biological replicates of IFNα treated EndoB-H2 cell line samples and human islet samples. We expect to identify differently regulated and novel pathways as well as key phosphorylation sites.

Acknowledgements

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References