Cytokine-induced phosphorylation dynamics reveals molecular mechanisms underlying human β-cell stress

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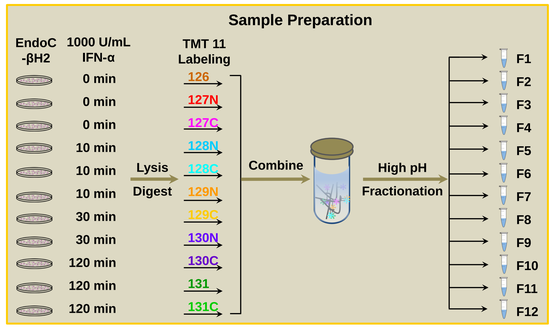
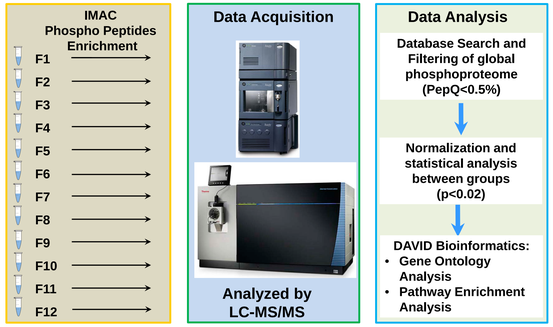
Overview

- Cytokines, including type 1 interferons (IFN1), 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.[1]
- 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.





Coverage of phosphoproteome

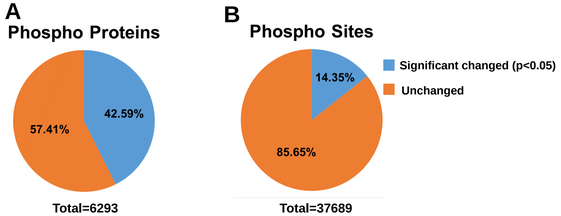
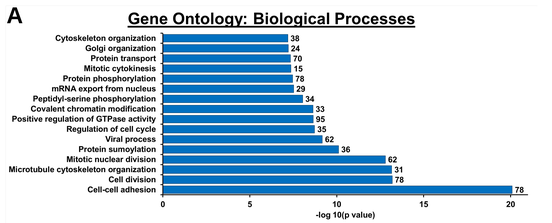


Figure 1. Total number and percentage of identified phosphoproteins (A) and phosphor sites (B) with significant changes (p<0.05) after IFNα treatment.

Gene Ontology Analysis



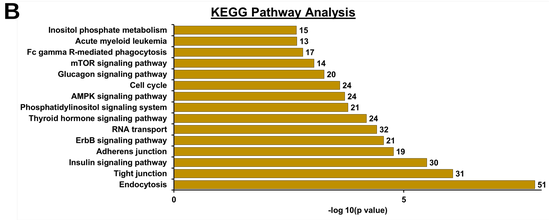


Figure 2. Gene Ontology Analysis was performed on proteins that displayed significant changes (p<0.02) between different time points using the DAVID bioinformatics database. Shown above are Biological Processes (A) and KEGG Pathway Analysis (B).

Upregulated phosphorylation dynamics

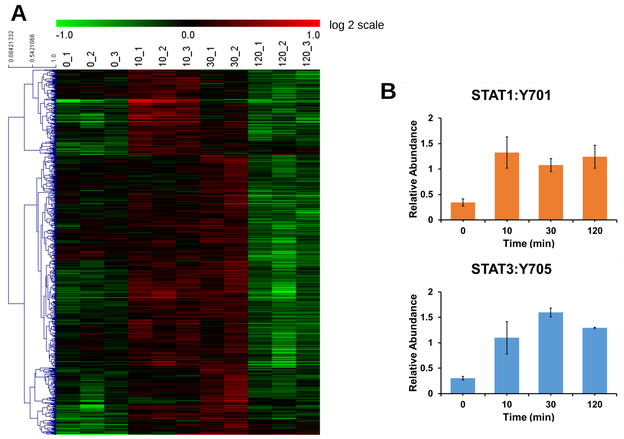


Figure 3: Upregulated phosphorylation dynamics after IFNα treatment using ANOVA analysis. (A) Heat map of upregulated phosphorylation dynamics as a function of time (0, 10,30,120 min) following IFNα treatment. Each time point has two or three biological replicates. Signal intensities were log 2 transformed and normalized based on median value. For better visualization, the response of each phosphorylation site at each time point was scaled to the average signal intensity of each cell line passage. (B) Phosphorylation dynamics of representative early and late response sites on key proteins. Error bars represent standard error of the replicates at each time point.

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 EndoC-βH2 cell line samples.
- 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 include endocytosis, tight junction, insulin, ErbB, phosphoinositide, AMPK, glucagon, and mTOR signaling pathways.
- K-Means clustering was applied to identify early and late response sites that are significantly upregulated after IFN1 treatment.
- Significant upregulation of STAT1[2] Y701 and STAT3[3] Y705 indicates macrophage activation induced by IFNα via a STAT-dependent pathway.
- This technique will be applied to study more biological replicates of IFNα treated EndoC-bH2 cell line samples and human islet samples. We expect to identify differently regulated and novel pathways as well as key phosphorylation sites.

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