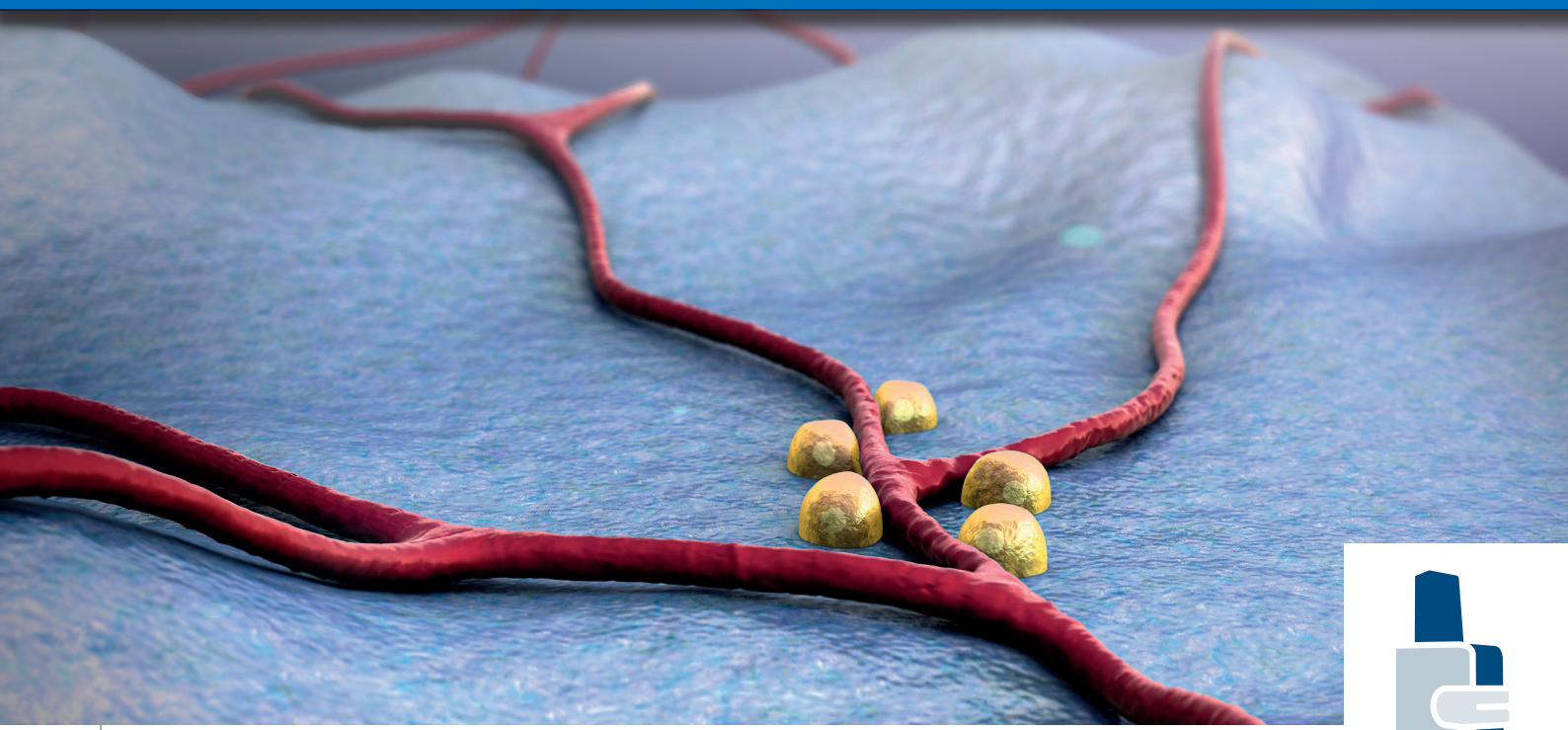

Proteomic Interrogation of Primary Insulin-secreting Pancreatic β-cells

The combination of integrated ion mobility with warp-speed MS/MS acquisition on the timsTOF Pro paves the way for deep, molecular level understanding of β-cell physiology and potential new targetable pathways for diabetes.

Abstract

In this work we leveraged the analytical performance of the timsTOF Pro to identify expression of low-level enzymes for the first time in primary insulin-secreting beta cells (β-cells). We found that these proteins are critical, functional components of key metabolic pathways which can support β-cell survival under inflammatory stress, an emerging disease etiology of diabetes. Confirmation that these factors are present and functional specifically in pancreatic β-cells points to new therapeutic avenues for human diabetes.

Keywords: Diabetes, timsTOF Pro, proteomics, protein expression

Authors: Scott B. Ficarro, Accalia Fu, Nika N. Danial, Jarrod A. Marto
Dana-Farber Cancer Institute, Brigham and Women's Hospital, Harvard Medical School

Introduction

β-cells within islets of the pancreas integrate a myriad of endogenous and exogenous signals to properly regulate glucose levels, primarily through release of insulin. Disruptions in β-cell function and glucose homeostasis are well-established physiologic hallmarks of human diabetes. Insulin supplements can be used to maintain blood glucose, although insulin-resistance is a growing problem among diabetes patients and typically leads to severe, chronic complications which increase the clinical and economic burden associated with diabetes. More durable and less invasive disease-modifying therapies require an improved molecular-level understanding of β-cell function.

Recently, the pathogenic effects of inflammation have been recognized as a strong, confounding factor across many human diseases, including diabetes. How inflammatory signals intersect the biochemical pathways that mediate β-cell glucose metabolism and insulin secretion are unknown. Studies towards this end are complicated by the cellular heterogeneity of human islets (β-cells comprise one of many functionally distinct cell types in islets) and a lack of high-fidelity genetic or other tractable model systems for β-cells. An additional hurdle is the very low number of β-cells present in human islets, even though they supply the entire insulin demand of a healthy individual. As a result, direct interrogation of the β-cell proteome requires ultra-high sensitivity mass spectrometry platforms. Our study [1] took a combined genetic, pharmacologic, and multi-omic approach to interrogate enriched β-cells isolated from primary human islets. Importantly, the protective metabolic enzymes we identified enhance human β-cell function and survival following transplantation and reverse diabetes in mice.

This application note is focused on our use of the Bruker timsTOF Pro and PASEF® MS/MS acquisition to confirm the expression of key metabolic enzymes in primary β-cells as part of our larger effort to understand the interplay of inflammation and glucose metabolism in normal physiology and diabetes.

Methods

Primary human islets freshly isolated from deceased donors were obtained from the Alberta Islet Distribution Program (University of Alberta) and by the Integrated Islet Distribution Program (IIDP) at City of Hope (http://iidp.coh.org/) and Prodo Labs (https://prodolabs.com/human-islets-for-research/). All donor material was obtained under strict adherence to IRB guidelines.

Human islets were dispersed using Accutase (MT25058CI, Thermo Fisher Scientific) and infected with an adenovirus expressing the bright green fluorescent protein, ZsGreen (Clontech) under control of the rat insulin-1 promoter (RIP1) and a mini-CMV enhancer. Cells were collected 96 h after infection and loaded onto an Aria II Cell Sorter (BD Biosciences), to sort and isolate the live ZsGreen+ cell (β-cells) FITC-positive. The β-cell fraction was confirmed to be >92% pure by immunostaining of sorted cells with insulin, and independently, by qRT–PCR and by RNA-seq. This procedure was repeated several times using islets from n = 3 donors.

Enriched β-cells were processed for lysis, protein reduction and alkylation, trypsin digestion, and peptide clean-up. LC-MS/MS was performed by integration of our DEEP SEQ fully automated 3D fractionation platform [2] (NanoAcquity, Waters, Milford, MA) with the timsTOF Pro ion mobility mass spectrometer (Bruker Daltonics, Billerica, MA). The mass spectrometer collected ion mobility MS spectra over a mass range of m/z 100-1700 and 1/k0 of 0.6 to 1.6, and then performed 10 cycles of PASEF® MS/MS with a target intensity of 20k and a threshold of 250. Active exclusion was enabled with a release time of 0.4 minutes. MS/MS spectra were matched to peptide sequences from human proteins in the Uniprot database using PEAKS Studio 10.0 software (Bioinformatics Solutions). Search parameters specified precursor and product ion tolerances of 20 ppm and 50 mmu, respectively, as well as fixed carbamidomethylation and variable oxidation of methionine. After mapping peptide sequences to unique gene identifiers using the Pep2gene tool [3], multiplierz scripts [4] were used to provide an estimate of relative protein expression [5] based on the three most abundant peptides detected for each gene product.

Results

We performed biochemical studies demonstrating that different activators of glucokinase, which regulates the initial step in glucose metabolism, resulted in divergent cell survival scenarios, depending on the presence of inflammation-associated cytokines [1]. Using unbiased metabolomic analysis performed on primary human islets, we observed that the balance between cell survival and cell death correlated with the shunt of arginine to production of either protective (urea) or damaging (nitric oxide) nitrogen metabolites. How inflammatory cytokines interact with glucose metabolism to favor or oppose β-cell survival is unknown, in part because cellular heterogeneity of human islets makes it difficult to deconvolute the functional contribution of β-cells independently from the bulk tissue.

We reasoned that interrogating protein expression directly in β-cells would provide important molecular insight for the intrinsic pathways by which these cells balance urea versus nitric oxide production. Towards this end we undertook the challenging task of enriching β-cells from bulk islets obtained from deceased human donors (Figure 1 A). We collected approximately 30,000 viable β-cells from each of three individual donor islets. To maximize the depth of proteome coverage we integrated our fully automated DEEP SEQ peptide fractionation platform [2] with the timsTOF Pro mass spectrometer (Figure 1 B). Using this approach, we detected more than 5000 proteins from the combined samples based on peptides that map uniquely into the genome [3, 4]. These data supported relative quantification [5] of more than 3000 proteins, including several enzymes that regulate the metabolic axis responsible for balancing the output of the urea cycle (Fig. 2, Left). To our knowledge, systematic proteomic analysis of enriched β-cells has not been previously reported. Interestingly we found good concordance between protein and gene expression data for these enzymes (Fig. 2, Left). These data provided important guidance for an intensive series of follow-up functional assays in primary human islets and mouse models [1]. Collectively our data suggested that in the face of inflammatory cytokines, pyruvate carboxylase (PC, confirmed in β-cells by our timsTOF Pro data) integrates glucokinase (GK) signaling to shunt arginine metabolism to ureagenesis, thereby suppressing generation of deleterious nitric oxide. These observations nominate the GK-PC axis as an important pro-survival pathway for β-cells which are actively metabolizing glucose under conditions of inflammatory stress. Our study motivates future efforts to credential the link between glucose and arginine metabolism as a new therapeutic target for diabetes.

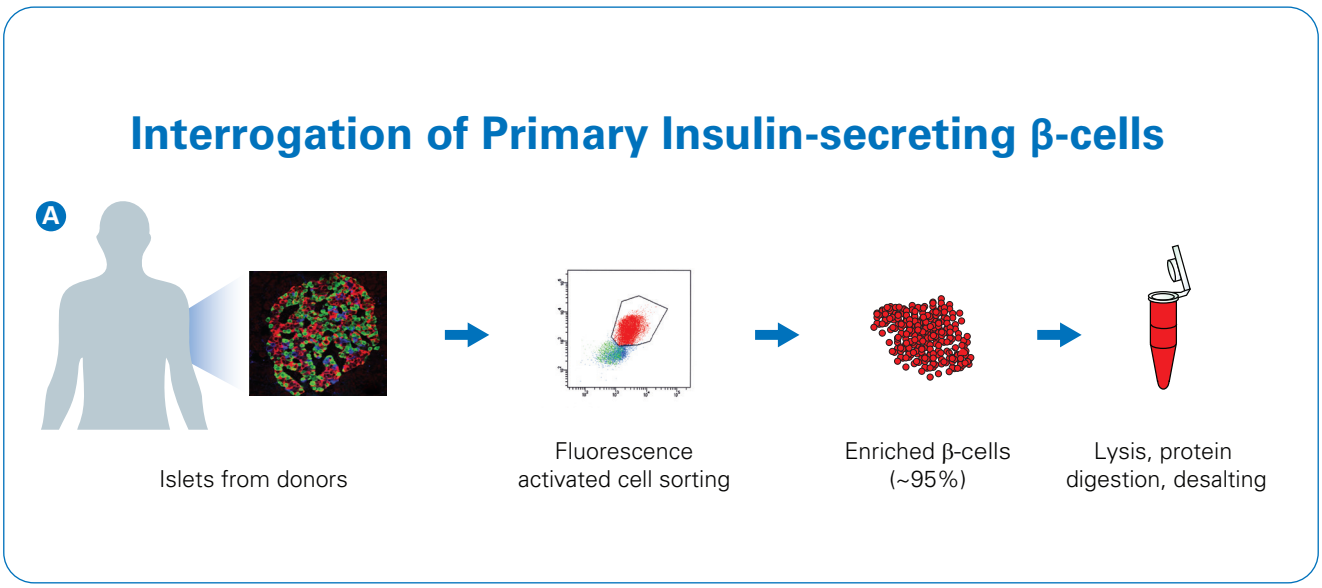

*Figure 1Ⓐ: **Workflow for biochemical enrichment and proteomic analysis of primary β-cells.***
Human islets, comprising multiple cell types, obtained from deceased donors are processed to facilitate enrichment of insulin-secreting β-cells (red), followed by lysis, cysteine alkylation, digestion with trypsin, and peptide de-salting.

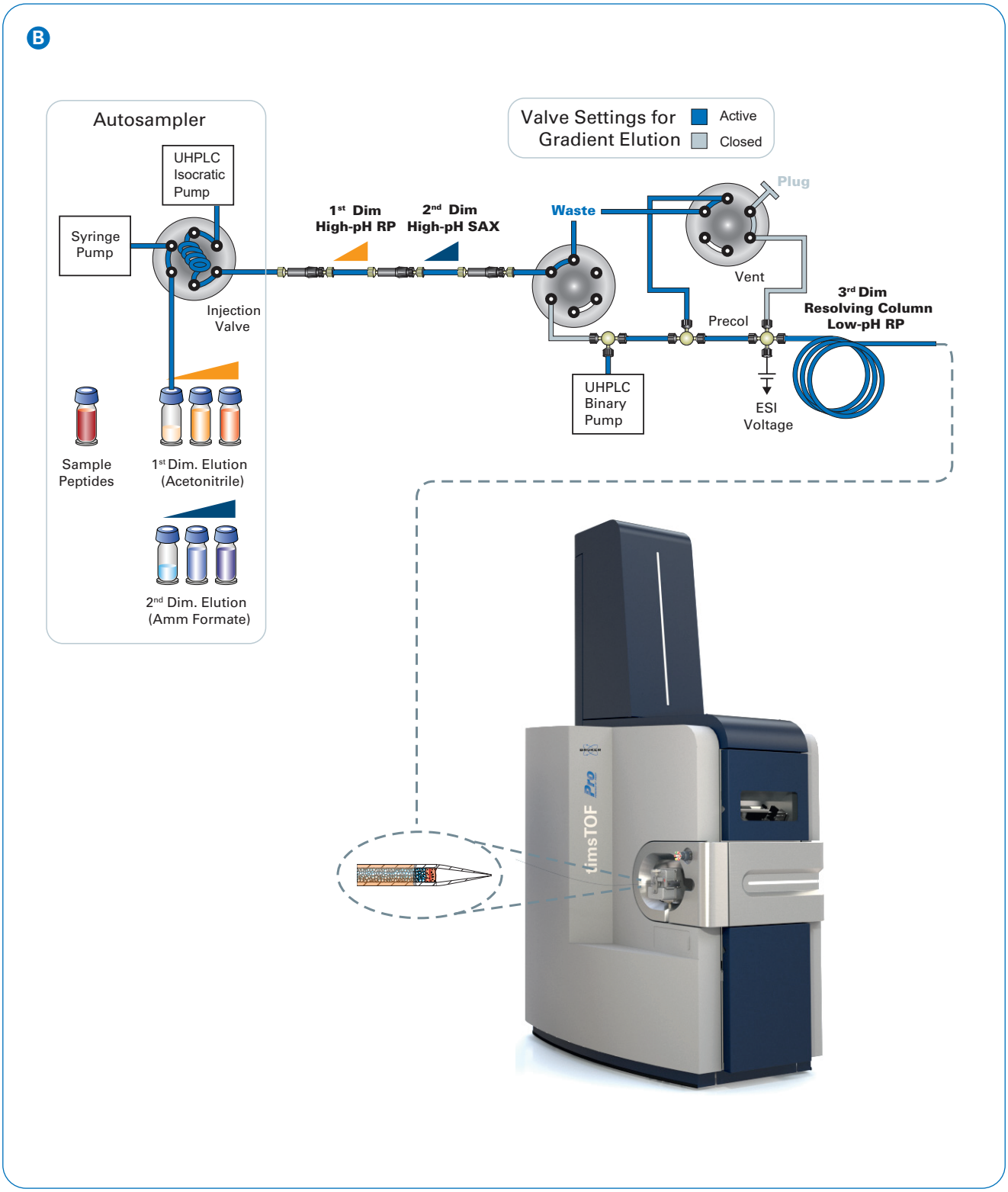

Figure 1Ⓑ: ***Workflow for biochemical enrichment and proteomic analysis of primary β-cells.***
Fully automated, online three-dimension fractionation (1st dim., high-pH reversed phase; 2nd dim., high-pH strong anion exchange; 3rd dim., low-pH reversed phase) [2, 6, 7] provides high peptide yield and in-depth proteome coverage on limiting numbers of enriched β-cells. Sample peptides from β-cells are injected in pH=10 ammonium format loading buffer, and then fractionated via discrete eluents for the 1st dimension (acetonitrile/formic acid) and 2nd dimension (ammonium formate), respectively. The final, low-pH reversed phase separation is performed in a 30 µm × 50 cm self-packed fused silica column with integrated emitter [8] interfaced directly with the Bruker timsTOF Pro instrument.

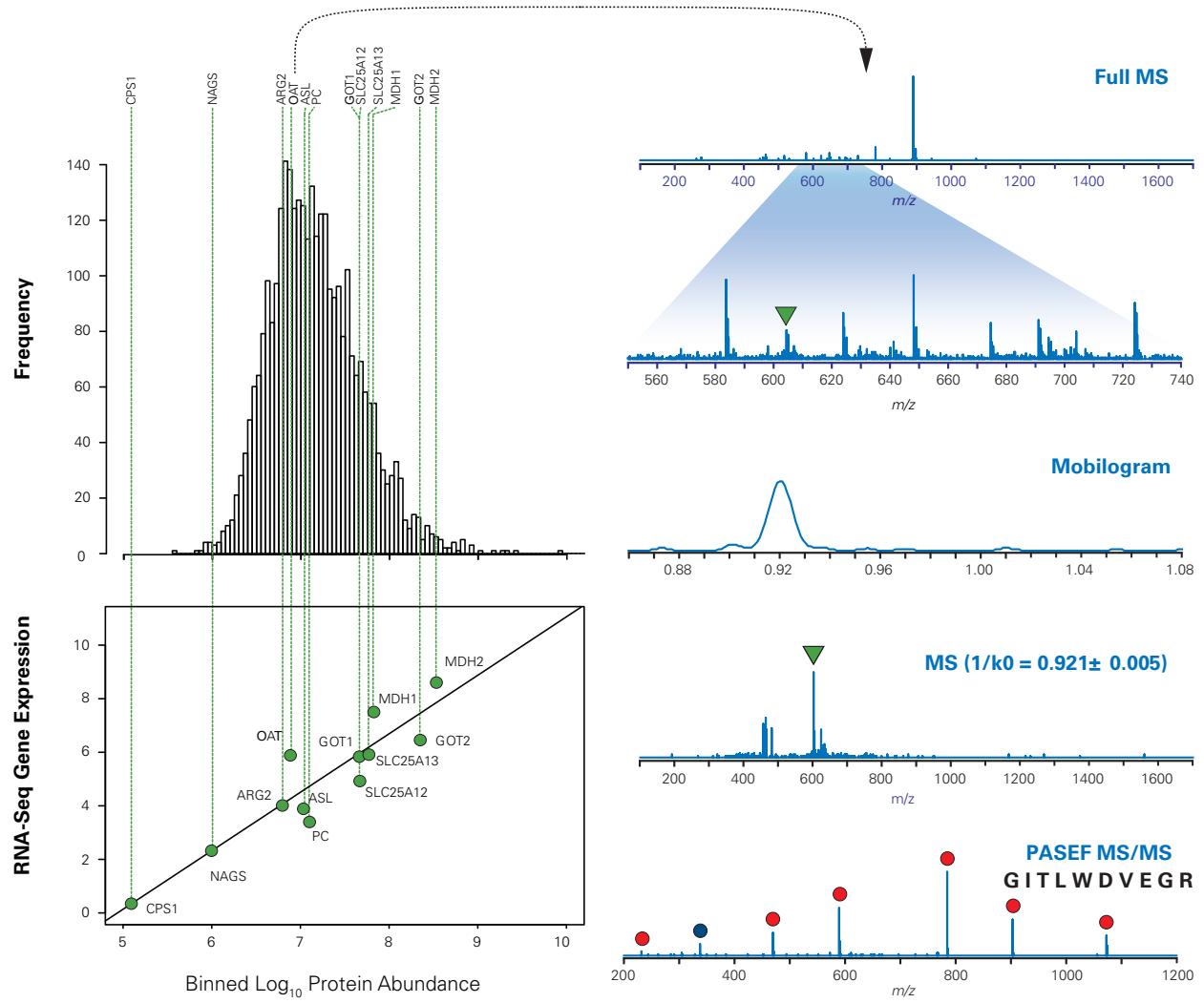

Figure 2. timsTOF Pro PASEF® MS/MS analysis of the proteome of primary β-cells. (Left, Top) Histogram distribution of proteins identified in primary β-cells by at least 3 unique peptides, displayed by estimated relative abundance [5]. Enzymes and other proteins related to the urea cycle as well as argininosuccinate and aspartate metabolism are highlighted with green dashed lines. (Left, Bottom) The concordance with gene expression by RNA-seq for this subset of functionally relevant gene-products is illustrated by the scatterplot. (Right) Scan sequence for a tryptic peptide detected from ornithine aminotransferase (OAT), showing full-scan MS spectrum, with zoom-in illustrating low signal-to-noise ratio for peptide precursor (green triangle). Integrated ion mobility on the timsTOF Pro provides gas phase enrichment of the peptide precursor and rich fragment ion spectrum via PASEF® MS/MS analysis. Red and blue circles indicate c-terminal y-type and n-terminal b-type ions, respectively.

Conclusion

- Integrated ion mobility combined with PASEF® on the timsTOF Pro facilitates deep proteome analysis in purified human β-cells; the analytical performance of the timsTOF Pro paves the way for systematic interrogation of β-cell proteome to support diabetes research.
- timsTOF Pro data provides evidence to further confirm functional role of key enzymes which may shield pancreatic insulin-secreting β-cells from the harmful effects of chronic inflammation.
- Together, data from our study highlights exciting new targetable pathways for diabetes therapy.

Learn More

You are looking for further Information?
Check out the link or scan the QR code for more details.

www.bruker.com/timstofpro

References

[1] Fu A, Alvarez-Perez J, Avizonis D, Kin T, Ficarro SB, Choi DW, Karakose E, Badur MG, Evans L, Rosselot C, Bridon G, Bird GH, Seo HS, Dhe-Paganon S, Kamphorst JJ, Stewart AF, Shapiro J, Marto JA Walensky LD, Jones RG, Garcia-Ocana A, Danial NN. Glucose-dependent partitioning to urea cycle spares β-cells from inflammation. Nature Metabolism 2020;2:432-446.

[2] Zhou F, Lu Y, Ficarro SB, Adelmant G, Jiang W, Luckey CJ, Marto JA. DEEP SEQ mass spectrometry: a platform for genome-scale proteome quantification. Nat Comm 2013;4:2171.

[3] Askenazi M, Marto JA, Linial M. The complete peptide dictionary – a meta-proteomics resource. Proteomics 2010;10:4306-10.

[4] Alexander WM, Ficarro SB, Adelmant G, Marto JA. Multiplierz v2.0: a python-based ecosystem for shared access and analysis of native mass spectrometry data. Proteomics 2017;17:1700091.

[5] Silva, J. C., Gorenstein, M. V., Li, G.-Z., Vissers, J. P. C. & Geromanos, S. J. Absolute quantification of proteins by LCMSE. Mol Cell Proteom 2006;5:144–156.

[6] Zhou F, Sikorski TW, Ficarro SB, Webber JT, Marto JA. An online nanoflow rp-sax-rp lc-ms/ms platform for efficient and in-depth proteome sequence analysis of complex organisms. Anal Chem 2011;83:6996-7005.

[7] Ficarro SB, Zhang Y, Alfonso MC, Adelmant GO, Garg B, Webber JT, Luckey CJ, Marto JA. Online nanoflow mult-dimensional fractionation strategies for high efficiency phosphopeptide analysis. Mol Cell Proteomics 2011;10:M111.011064, 1-19.

[8] Ficarro SB, Zhang Y, Lu Y, Moghimi AR, Askenazi M, Hyatt E, Smith ED, Boyer L, Schlaeger TM, Luckey CJ, Marto JA. Improved electrospray ionization efficiency compensates for diminished chromatographic resolution and enables proteomics analysis of tyrosine signaling in embryonic stem cells. Anal Chem 2009;81:3440-7.

For Research Use Only. Not for Use in Clinical Diagnostic Procedures.

Bruker Daltonics GmbH & Co. KG

Bremen · Germany
Phone +49 (0)421-2205-0

Bruker Scientific LLC

Billerica, MA · USA
Phone +1 (978) 663-3660

ms.sales.bdal@bruker.com – www.bruker.com