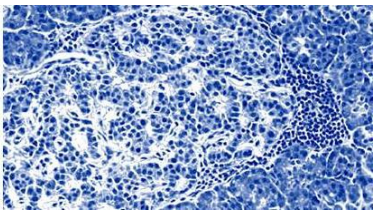
nPOD
Network for Pancreatic Organ Donors with Diabetes



In Situ Post-Translational Modifications in Islets as Potential Neoantigens in Type 1 Diabetes

AUTHORS

Adam C. Swensen[1], Xi Wang[1], Tai-Tu Lin[1], Paul D. Piehowski[1], Ying Zhu[1], Jing Chen[3], Mark A Atkinson[2], Martha Campbell-Thompson[3], David A Ostrov[2], Clayton E Mathews[2], and Wei-Jun Qian[1]

[1]Biological Sciences Division and Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, Washington 99352, [2]Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, FL 32610

PURPOSE

Increasing lines of evidence support that post-translational modification (PTM) of proteins represent a key mechanism of producing potential neoantigens and neoepitopes that are unique to β-cells, which may play a prominent role in triggering T1D. Thus, the discovery and characterization of in-situ islet PTMs as potential neoantigens could lead to important insights into the immuno-pathogenesis and provide new avenues of prevention and intervention. However, there is still little evidence of in situ PTMs in islets of pre-T1D or T1D patients. Herein, we applied mass spectrometry-proteomics to characterize in situ PTMs in islets obtained by laser microdissection from both presymptomatic autoantibody positive (AAb+) cases and T1D cases.

METHODS

Human islet sections from non-diabetic controls, multiple AAb+ cases, and T1D subjects from the Network for Pancreatic Organ donors with Diabetes (nPOD) program were isolated by laser microdissection (LMD), with islet sections subjected to global proteome profiling by nanoscale proteomics technologies. Direct identification of in situ PTMs (e.g., phosphorylation, deamidation, citrullination, oxidation) was identified by advanced informatics tools. The potential impact of modifications on HLA binding, TCR binding or both was determined by generation of atomic models of HLA-A*02:02 complexed with specific sequence motif based on the crystal structure of the most closely related crystal structure. Molecular graphic images were generated with PyMol.

SUMMARY OF RESULTS

We have applied nanoscale proteomics to profile PTMs in laser microdissected human islet sections from pre-symptomatic AAb+ subjects and age/sex matched controls (n=6) as well as one T1D donor. Our initial analyses resulted in the identification of a relatively large set of in situ PTMs including phosphorylation and several other PTMs reported to be involved in autoantigen formation will including deamidation, citrullination, and cysteine oxidation products: sulfinic (SO_2H) and sulfonic acid (SO_3H). Specifically, with <5% FDR, we identified ~350 unique phosphopeptides; ~170 peptides with sulfonic and sulfinic acid modifications, and ~570 peptides with deamidation and citrullination. To enable accurate identification of error-prone PTMs such as deamidation, we have developed a novel dual-search delta score strategy by comparing the same MS/MS spectra with and without deamidation in searching parameters and true and false positives can be clearly differentiated by the delta scores between the two independent searches. In total, we observed ~55% non-enzymatic asparagine (N) deamidation, 38% glutamine (Q) deamidation, and 7% arginine (R) citrullination.

Interestingly, many PTMs were observed on known autoantigens with insulin and proinsulin being modified by several types of PTMs, suggesting modified insulin as a key source of neoepitope. A further highlight is that the observed deamidated sequence from proinsulin were recently reported to be preferentially bound to HLA-DQ8. Another example highlights a SO_2H-modified insulin B-chain peptide, where the modification was predicted to impact T-cell recognition based on molecular modeling.

CONCLUSIONS

Proteomic profiling of in-situ PTMs in LMD islets from AAb+ and T1D subjects provides a valuable resource of candidate neoantigens and neoepitopes. Such resource will provide a knowledgebase to identify and confirm novel functional in situ neoepitope from human patients.