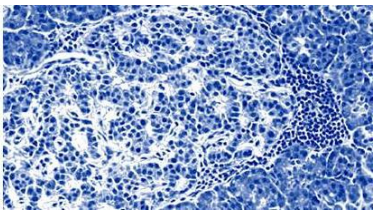
nPOD
Network for Pancreatic Organ Donors with Diabetes



Protein Modifications that Alter both Autoimmunity and Beta Cell Metabolism

AUTHORS

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PURPOSE

Our laboratory and others have identified novel properties of self proteins, namely posttranslational protein modifications (PTMs) that may initiate autoimmune responses in human Type 1 diabetes. In particular, a growing number of self proteins acquire PTMs within cells and become targets of B and T cell autoimmunity, leading to inflammation and pathology in the pancreas. Examples of important modifications to self proteins include citrullination, oxidation, deamidation reactions, and isoaspartyl modification, all responses of self proteins within cells that undergo cytokine or reactive oxygen-mediated stress. As importantly, these PTMs within cells may alter the biological properties of proteins within beta cells. This study examines PTMs of beta cell proteins that not only trigger autoimmunity but also alter metabolism and are sentinels of beta cell health or demise.

METHODS

The work primarily utilized human islets treated with inflammatory cytokines or reactive oxygen species (H_2O_2) followed by deep proteomic profiling by LC tandem mass spectroscopy. A variety of PTMs were identified that were unique to inflammatory conditions, including citrullination and carbonyl modification. Candidate proteins identified with PTMs were assayed utilizing T1D serum and peripheral T cells from early onset and established disease for autoreactivity. While more than 10 candidate proteins with PTMs were identified, we followed up on two specific beta cell proteins, glucokinase (GK) and Prolyl-4-hydroxylase (P4Hb). Binding motifs to HLA DR0401 were identified and utilized to investigate T cell autoimmunity to these proteins as well as the presence of autoantibodies. Finally, studies of beta cell metabolism were performed that included both glucose sensing pathways and insulin release, both in the context of glucokinase and P4Hb modifications.

SUMMARY OF RESULTS

Initial studies of new-onset T1DM patient serum (obtained from Drs. Cate Speake and Dr. Kevan Herold) indicate that 75% of the patients have IgG autoantibodies to P4Hb. As we first observed with mice, Abs to P4Hb precede the onset of anti-insulin Abs in human T1D. That is, anti-insulin Abs are never found without pre-existing Ab responses to P4Hb, linking these autoimmune responses, possibly due to the role P4Hb in insulin biosynthesis. We also identified specific citrullinated sites of human glucokinase. Similarly, T1D patients

develop both autoantibody and CD4 T cell responses to citrulline-GK. Finally, inflammation that leads to PTM of both GK and P4Hb alter glucose sensing and insulin release, respectively.

CONCLUSIONS

The present study has identified novel PTMs within two beta cell proteins, glucokinase and proly-4-hydroxylase beta (P4Hb) to which T1D patients have both B and T cell autoimmunity. P4Hb is a critical macromolecule for the accurate folding of insulin. Oxidation and carbonylation of P4Hb may lead to the misfolding of insulin, making insulin itself immunogenic, or even lead to proteotoxicity and/or the death of beta cells. We also identified specific sites of citrulline modification of glucokinase. Our studies indicate that citrulline modified GK alters glucose sensing by beta cells and reduces insulin release. Understanding how self-proteins are specifically modified in T1D can lead to therapeutic strategies to selectively target enzymatic pathways and reduce PTMs in beta cells. Moreover, these autoantigens have immediate diagnostic value as candidate biomarkers of disease and reflect beta cell health. Restoration of beta cell functions, via pharmaceutical correction of the aberrant modifications, may be capable of delaying or preventing disease in individuals at high-risk for T1D.