Beta Cell Stress triggers formation of Hybrid Insulin Peptide through granular pH modulation

Jason Groegler, Mylinh Dang, Thomas Delong

University of Colorado Anschutz, United States of America; jason.groegler@cuanschutz.edu

Background: The Eisenbarth model of type 1 diabetes (T1D) describes a linear decline in pancreatic beta cells but fails to explain the variable disease progression in at-risk individuals. Hybrid insulin peptides (HIPs) have emerged as potential disease triggers. HIPs form when proinsulin peptides link to other beta cell protein fragments, creating peptides not expressed in the thymus. This allows HIP-reactive T cells to escape negative selection, potentially contributing to beta cell destruction in T1D.

Aim: We investigated HIP formation by cathepsin D (CatD), a protease that creates disease-relevant HIPs in beta cell insulin granules. While HIPs are consistently detectable in murine islets, their detection in human islets is sporadic. We hypothesized this difference is due to varying pH optima for CatD activity between species. Murine CatD forms HIPs within pH 5.0-6.0, while human CatD only forms HIPs at pH 5.0 or less. We proposed that treating human islets with small molecules that lower insulin granule pH would activate HIP formation.

Methods: Human islets were treated with varying glucose concentrations (0-481 mg/dL) or C381 (0-400 μM), a compound that boosts vATPase activity and lowers lysosomal and insulin granule pH. Samples were then lysed, fractionated, and analyzed by LC-MS/MS.

Results: We detected a novel HIP (GAGSLQPL-TPIESHQV) in islets treated with 285 mg/dL glucose or 100 μM C381. The peptide's identity was confirmed by comparison to a synthetic HIP, with a Pearson correlation coefficient of 0.987 and a retention time deviation of 0.02 min.

Conclusions: Our findings suggest that HIP formation occurs under specific stress conditions, such as elevated blood glucose, which can decrease granular pH. These HIPs may then become targets for the adaptive immune system, mediating beta cell destruction in T1D. The diverse environmental exposures throughout a human's lifetime could explain the highly variable disease progression observed. This research may provide a modification to the Eisenbarth model and offer new insights into T1D progression. Future studies will explore how stressors affect immune targeting of HIP-containing beta cells and whether this leads to gradual beta cell depletion.