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Molecular characterization of mammalian secretory pathway

The mammalian secretory pathway is critical forsecretion of hormones and enzymes and disruption of its homeostasis leads to human diseases including diabetes and pancreatitis. Applying quantitative proteomics in specialized pancreatic secretory cells, we aim to understand the regulation of the secretory pathway under different metabolic conditions. A molecular model of pancreatic zymogen granule (ZG) is critical for understanding its functions. After extensive characterizations of the components and topology of ZG membranes,here we report the determination of the absolute quantities of ZG proteinsusing GeLC-MRM and AQUA peptides. To determine thecopy numbers/ZG, atomic force microscopy was utilized to estimate the numbers and sizes of isolated ZGs. Pancreatic beta cell has well-developed endoplasmic reticulum (ER) for massive production of proinsulin. Perturbation of ER homeostasis contributes to diabetes. To characterize the molecular machinery for proinsulin biogenesis and ER homeostasis, MIN6 cell was used to purify rough ERby two different purification schemes, differential ultracentrifugation and step sucrose gradient. A total of 1696 proteins were identified in three experiments, among which 1279 proteins found in at least two experiments. This represents a comprehensive coverage of all known and many novel players in beta cell ER. To identify beta cell-specific ER proteins, tissue specific gene expression and Blast analysis were performed between our data and published ER proteomic data. Furthermore, targeted proteomics approach has been developed to validate mouse islets enriched proteins. iTRAQ-based proteomics analysis is also used to quantify the perturbation of beta cell ER homeostasis under different diabetes-causing conditions.

Biography

Xuequn Chen is currently an assistant professor in the School of Medicine at Wayne State University. He holds a PhD from the University of Michigan (2003) and did his postdoctoral work with Dr. Phil Andrews in the National Proteomics Center at Michigan. He has been conducting quantitative subsellular proteomics research in the mammalian secretory pathway and published his works in Molecular & Cellular Proteomics, Journal of Proteome Research, Proteomics and Journal of Proteomics

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