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Proteomic profiling of human islets collected from frozen pancreata using laser capture microdissection

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ABSTRACT

The etiology of Type 1 Diabetes (T1D) remains elusive. Enzymatically isolated and cultured (EIC) islets cannot fully reflect the natural protein composition and disease process of in vivo islets, because of the stress from isolation procedures. In order to study islet protein composition in conditions close to the natural environment, we performed proteomic analysis of EIC islets, and laser capture microdissected (LCM) human islets and acinar tissue from fresh-frozen pancreas sections of three cadaveric donors. 1104 and 706 proteins were identified from 6 islets equivalents (IEQ) of LCM islets and acinar tissue, respectively. The proteomic profiles of LCM islets were reproducible within and among cadaveric donors. The endocrine hormones were only detected in LCM islets, whereas catalytic enzymes were significantly enriched in acinar tissue. Furthermore, high overlap (984 proteins) and similar function distribution were found between LCM and EIC islets proteomes, except that EIC islets had more acinar contaminants and stress-related signal transducer activity proteins. The comparison among LCM islets, LCM acinar tissue and EIC islets proteomes indicates that LCM combined with proteomic methods enables accurate and unbiased profiling of islet proteome from frozen pancreata. This paves the way for proteomic studies on human islets during the progression of T1D.

Significance: The etiological agent triggering autoimmunity against beta cells in Type 1 diabetes (T1D) remains obscure. The in vitro models available (enzymatically isolated and cultured islets, EIC islets) do not accurately reflect what happens in vivo due to lack of the natural environment where islets exist and the preparation-induced changes in cell physiology. The importance of this study is that we investigated the feasibility of laser capture microdissection (LCM) for the isolation of intact islets from frozen cadaveric pancreatic tissue sections. We compared the protein profile of LCM islets (9 replicates from 3 cadaveric donors) with that of both LCM acinar tissues (6 replicates from the same 3 cadaveric donor as LCM islets) and EIC islets (at least 4 replicates for each sample with the same islets equivalents) by using proteomics techniques with advanced instrumentation, nanoLC-Q Exactive HF Orbitrap mass spectrometry (nano LC-MS/MS). The results demonstrate that the LCM method is reliable in isolating islets with an intact environment. LCM-based islet proteomics is a feasible approach to obtain good proteome coverage for assessing the pathology of T1D using cadaveric pancreatic samples, even from very small sample amounts. Future applications of this LCM-based proteomic method may help us understand the pathogenesis of T1D and identify potential biomarkers for T1D diagnosis at an early stage.

1. Introduction

Pancreatic islets are composed of several types of endocrine (i.e., hormone-producing) cells, including insulin-secreting β-cells, glucagon-secreting α-cells, somatostatin-secreting δ-cells. Insulin-secreting β-cells are the major constituent of human islets, accounting for 60–87% of the total islet volume [1–3]. The autoimmune-mediated destruction of β-cells leads to the development of Type 1 Diabetes (T1D) [4,5]. Among the various risk factors proposed for this disease, genetic determinants may contribute to 40–50% risk of T1D [6]. Human leukocyte antigen (HLA) regions were reported to be the strongest genetic determinants, as a strong linkage between T1D and certain variants of the highly polymorphic HLA class II immune recognition molecules has been established [7]. Viral infection was implicated as one of the environmental factors that may trigger autoimmunity and initiate the destruction of β-cells during the development of T1D [8]. Although there is consensus on the view that T1D results from a combination of genetic and environmental factors, the pathogenetic mechanisms are not fully understood. Therefore, investigation on the

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dysfunction and death of β-cells may contribute to better understanding the pathogenic mechanism of T1D.

Enzymatically isolated and cultured (EIC) islets [9–11] have been frequently used to investigate biochemical signaling pathways that could trigger β-cell changes and death in T1D. A wealth of information can be obtained from EIC islets, and the studies focusing on those from donors with T1D are providing insights of paramount importance to the field of T1D research [12]. However, such in vitro models have some limitations: they do not fully reflect what happens in vivo due to a lack of the natural environment where islets exist and due to the changes in cell physiology induced by isolation and culture. The procedure of enzymatic isolation of pancreatic islets causes major structural changes and induces up-regulation of stress-related genes in islets [13]. The cells` phenotype, native functions, and responsiveness to stimuli can be altered during culture [14]. It is of note that mRNA levels of insulin and insulin promoter factor 1 decreased by 40% in EIC islets compared with islets in vivo [15], reflecting the inability to sustain mature beta cells in culture. Furthermore, EIC islets frequently contain a significant percentage of contaminating acinar cells and duct cells [16]. On the other hand, animal models exist, but they do not fully mimic human pancreas function, physiology, and disease due to inter-species differences [17–20]. On this note, significant differences between human and mouse islets were reported notably in the cytoarchitecture [1], immune responses, pathways controlling glucose-responsiveness, regenerative capacity, and response to therapy in diabetes [21]. In addition, obtaining fresh isolated human islet samples is challenging because of the limited material availability and because of the requirements in terms of expertise, technologies, and logistics. Networks such as the Integrated Islet Distribution Program (IIDP) <https://iidp.coh.org/>, the Alberta Islet Distribution Program (AIDP) in Canada, the Oxford Consortium for Islet Transplantation (OXCIT) in the UK, and the European Consortium for Islet Transplantation (ECIT) <http://ecit.dri-sanraffaele.org/en/index.html> provide human pancreatic EIC islets for basic research. It is noteworthy that islet isolation is frequently performed on pancreata considered not suitable for pancreas transplantation, and islet distribution for research is frequently performed when islet preparations fail to meet defined release criteria for transplantation (e.g. insufficient islet yield). Although biopsy specimens from the pancreata of living individuals can provide a wealth of information on T1D onset and recurrence [22], performing this procedure on living individuals with T1D is a challenging and risky procedure [23], that also raises important ethical concerns [24]. Alternatively, human pancreatic tissue can be collected from cadaveric individuals, from individuals at-risk for T1D, from diabetic and transplanted patients, and can be preserved frozen. Fresh-frozen pancreata can be obtained from tissue repositories such as nPOD [25,26], it is frequently available or it can be easily prepared at most pathology facilities. The preparation requires minimal manipulation of the tissue and enables the preservation of the protein content along with the natural tissue architecture. For these reasons we decided to perform laser-capture microdissection (LCM) and proteomic analysis on islets from frozen pancreas sections.

LCM employs a high-energy laser source to separate the desired cells from the remaining tissue section, enabling isolation and downstream analysis [27]. This technique allowed the study of islet- and β-cell-specific genes [16,28,29] and proteins [30]. LCM enables the extraction of samples from an environment which is well conserved and close to the natural condition, to better investigate cell physiology [31], cell biology [32], cell transcriptome [13], and proteome [30]. Islets obtained with this method are expected to reflect closely the in vivo molecular composition and pathology of in situ islets. The exploration of the proteome signature of LCM islets with an unbiased method may provide information on the changes in protein composition occurring in dysfunctional islets, even with limited sample amounts, and may thus facilitate understanding of the pathogenesis of T1D.

In this study, we aimed at characterizing the proteome of a limited amount of LCM islets from frozen tissue blocks. In order to confirm the fidelity of the proteome of the LCM material and the accuracy of the microdissection, we compared the proteome of LCM islets from 3 cadaveric donors, each with 3 technique replicates, and we compared the protein profile of LCM islets with that of EIC islets and that of LCM pancreatic acinar tissue. We applied a mass spectrometry-based label-free proteomics approach with advanced instrumentation, nanoLC-Q Exactive HF Orbitrap mass spectrometry (QE-HF MS). With this study we aimed at establishing the feasibility of using LCM to isolate limited amounts of islets from frozen pancreatic tissue sections for proteomics analysis. This study opens the path to future endeavors aimed at investigating the pathophysiology of T1D.

2. Materials and methods

2.1. Pancreatic tissue sourcing

Research pancreata from three non-diabetic deceased heart-beating donors were obtained from Organ Procurement Organizations, in accordance with federal guidelines for organ donation. Organs were processed at the cGMP facility of the Diabetes Research Institute, University of Miami, under Institutional Review Board-issued waiver that states that islet cells isolation from donors does not constitute human subject research. Organ recovery and transport met transplant-grade criteria. Tissue processing procedures were conducted by the Diabetes Research Institute's (DRI) cGMP Cell Processing Facility personnel and Pathology core staff. Islet cell isolation was performed following the method reported in a previous study [33].

Briefly, the pancreatic ducts of Wirsung and Santorini were stapled at the pancreas/duodenum interface and the pancreas was separated from the duodenum and blunt-cut at the neck. A tissue block was resected in the region of the neck for immediate embedding in TissueTek OCT and freezing on dry ice. Pancreatic duct in the head and tail sections of the pancreas was cannulated with 18G angiocatheters, and 350 mL of 1.4 mg/mL of Liberase C/T GMP Grade (Roche Diagnostics GmbH, Cat # 05339880001) solution was introduced through the pancreatic duct. The enzymatic solution was injected while keeping the pancreas cold. Following organ distention, the tissue was cut into 7–9 pieces, and loaded in the 500 mL digestion chamber containing a stainless steel screen. Digestion was allowed to proceed at 37 °C until it was judged complete, according to the number of free islets and the amount of acinar tissues observed in the digestion samples collected every minute during the digestion process. Gradient centrifugation on a COBE cell processor 2991 enabled separation of islets from pancreatic acinar and ductal tissue. Following purification and washing, isolated islet cells were cultured overnight in Supplemented CMRL-1066 culture medium (Mediatech, Cat # 99-603-CV), at 22 °C, with 5% CO_2. Preparation of EIC islets for proteomic analysis was started following 12 hour culture.

In addition, all work reported here was approved by the University of North Carolina at Greensboro.

2.2. Solvents and chemicals

Common solvents were purchased from Sigma-Aldrich (St. Louis, MO). Tissue-Tek O.C.T. compound (Sakura 4583, Cat # 25608–930) and 100% ethanol (Decon Labs, Cat # 71006–012) were purchased from VWR. Toluidine blue O (Cat # 198161-25G) was purchased from Sigma-Aldrich. Anti-insulin antibody clone K36aC10 (Cat # I2018, 1:1000) was purchased from Sigma-Aldrich. PPS Silent Surfactant was purchased from Expedeon (San Diego, California, Cat # 21011). Histostain Plus Broadspectrum AEC kit was purchased from Invitrogen (Cat # 85–8943).