Title and abstract for the presentation for Dansk Selskab for Flowcytometri's årsmöte 28. mars.

Purification of functional human and rat pancreatic alpha cells using the BD InFlux cell sorter

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Pancreatic alpha cells contribute to glucose homeostasis by the regulated secretion of glucagon, which increases glycogenolysis and hepatic gluconeogenesis in response to hypoglycemia. Alterations of glucagon secretion are observed in diabetic patients and exacerbate the disease. The restricted availability of purified primary alpha cells has limited our understanding of their function in health and disease. We have now established convenient protocols for the purification of viable alpha cells from rat and human pancreatic islets by FACS (in our case BD InFlux), using intrinsic cellular properties. Islets were isolated from the pancreata of Wistar rats or deceased human organ donors. Dispersed islet cells were separated by FACS based on light scatter and autofluorescence. Purity of sorted cells was evaluated by immunocytochemistry using hormone specific antibodies (using the BD Pathway 855 High-Content Bioimager). Relative hormone expression was further determined by quantitative RT-PCR. Viability was determined by Annexin V and propidium iodide staining and function was assessed by monitoring cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_i$) using Fura-2/AM (using the BD Pathway 855 High-Content Bioimager). We developed species-specific FACS gating strategies that resulted in populations consisting mainly of alpha cells (96.6 ± 1.4 %, n = 3 for rat; 95.4 ± 1.7 %, n = 4 for human, mean ± SEM). These cell fractions showed ~ 5-fold and ~ 4-fold enrichment (rat and human, respectively) of glucagon mRNA expression compared to total ungated islet cells. Most of the sorted cells were viable and functional, as they responded with an increase in $[Ca^{2+}]_i$ upon stimulation with L-arginine (10 mM). The majority of the sorted human alpha cells responded also to stimulation with kainate (100 μM), whereas this response was infrequent in rat alpha cells. Using the same sample preparation, but a different gating strategy, we were also able to sort rat and human populations enriched in beta cells. In conclusion, we have simplified and optimized a method for the purification of rat alpha cells, as well as established a novel approach to separate human alpha cells using neither antibodies nor dyes possibly interfering with cellular functions.