Hormone-independent assessment of human pancreatic endocrine-cell mass

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Introduction

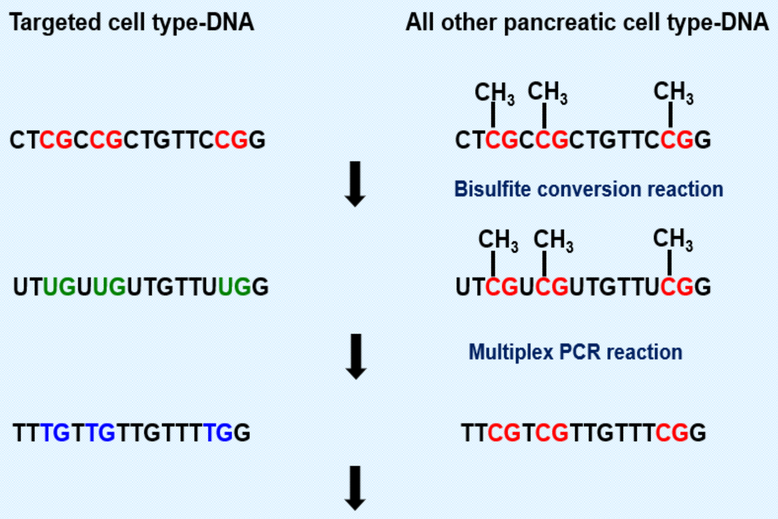
Whereas Type 1 diabetes (T1D) is characterized by autoimmune destruction of β-cells, studies on α-cell fate in T1D remain sparse. In addition, the contribution of β-cell failure in the pathogenesis of T1D is still debatable and requires further investigation; specifically, it remains unclear whether at some stages of the disease, a fraction of β-cells that have lost insulin immunoreactivity subsists in the pancreas.

Histological analyses of pancreatic tissue immunostained for insulin and glucagon remain the gold standard approach for the assessment of β and α-cell mass. However, this strategy could be flawed, would 'empty' endocrine cells with undetected levels of hormones persist in the pancreas.

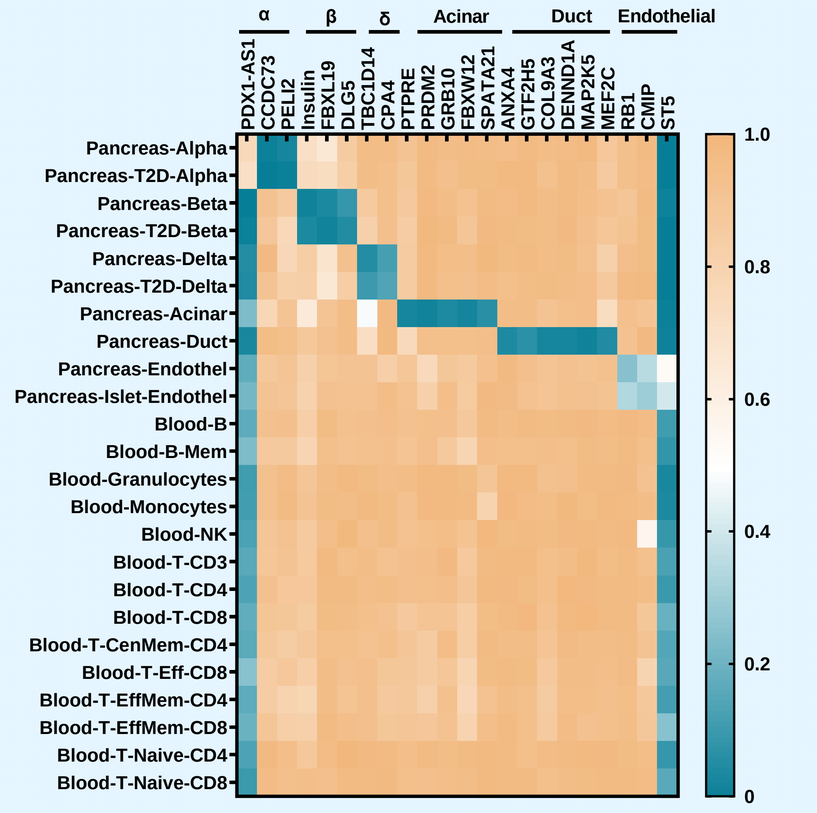
Thus, there is a pressing need for the development of a precise method to measure the fraction of α and β cells, which relies on parameters that remain stable under immune attack or metabolic stress.

We have developed an experimental platform based on cell type-specific DNA methylation signatures to measure the fraction of α and β cells (and additional cell-types) in human pancreatic islets and tissues. We have used this method to measure α-cell area in pancreatic sections of T1D and T2D donors and found increased α-cell fraction in both pathologies. We have also measured β-cell DNA per insulin-stained area on pancreatic sections to test for the presence of 'empty' β-cells in the pancreas of T1D (and T2D) donors. This assay has revealed the presence of insulin-depleted cells that retained an intact β-cell methylation signature in a fraction of T1D donors.

Next-Generation-Sequencing Strategy for the Quantification of Endocrine Cell-Type fractions

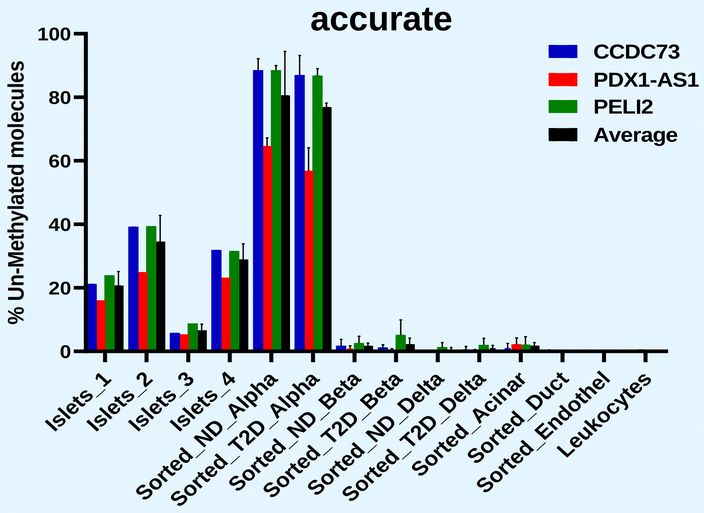


Quantification of main islet cell-types within the Pancreas

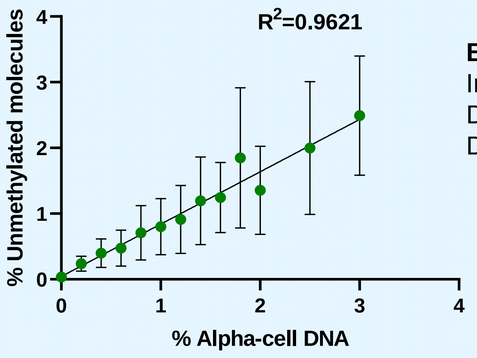


Specifically-unmethylated loci (columns) were selected for the accurate measurement of alpha, beta, delta, acinar, ductal and pancreatic endothelial cells.

α-cell methylation markers are specific and accurate



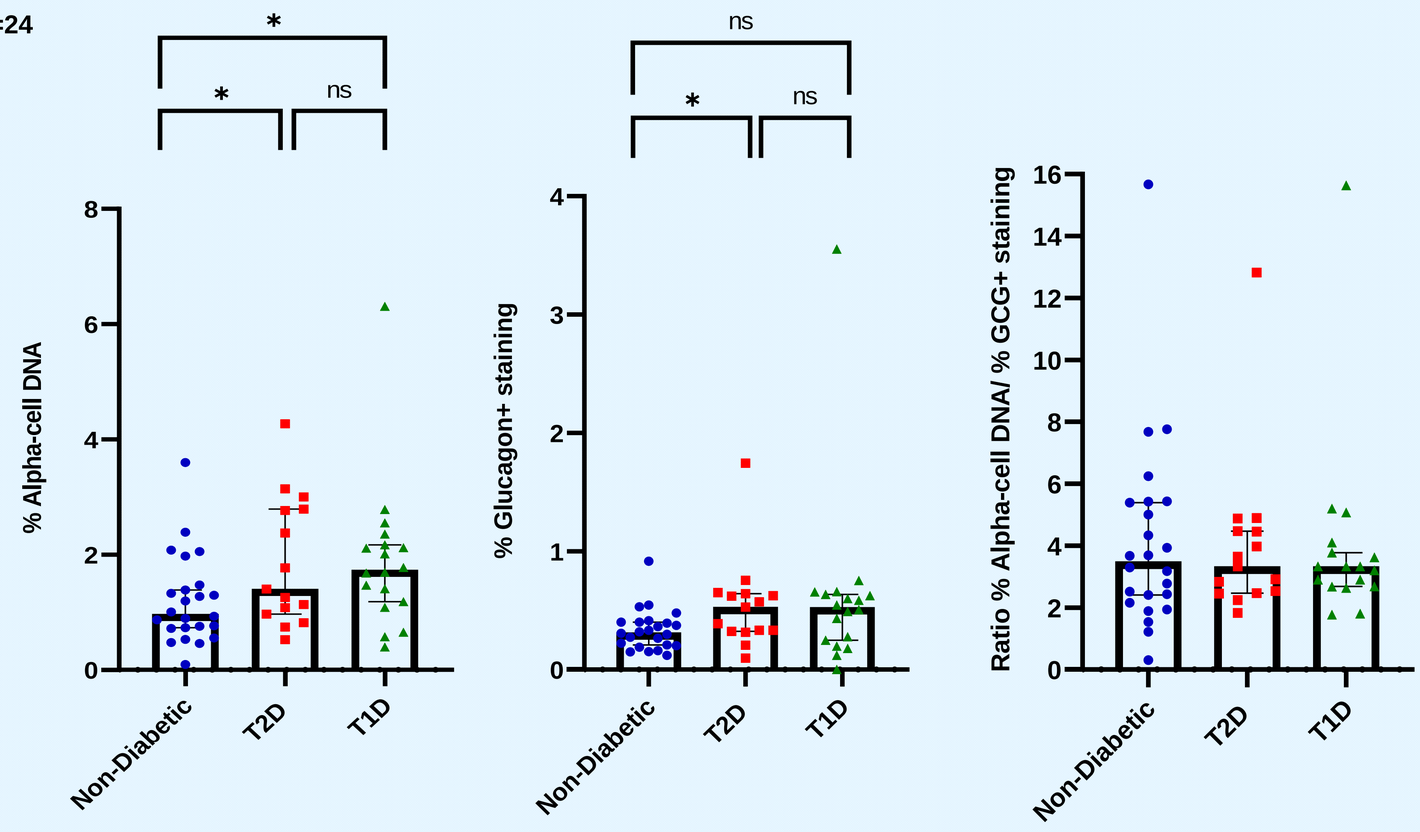
A. Assay specificity The methylation status of three α-cell markers was determined in DNA from islets and sorted pancreatic cells



B. Assay accuracy
Increasing amounts of α-cell DNA spiked in ductal cell DNA was quantified

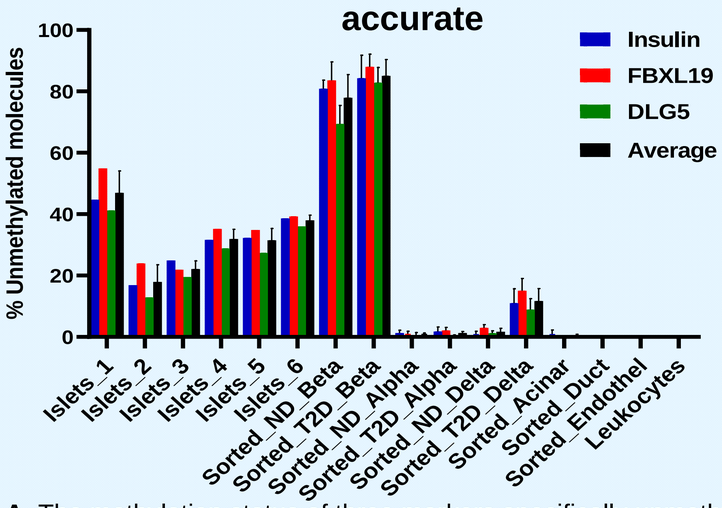
α-cell DNA and Glucagon staining show higher α-cell fraction in T2D and in T1D

Non-Diabetic n=24
T2D n=15
T1D n=18

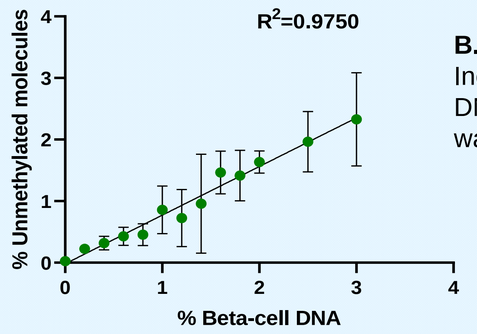


Quantification of the percentage of α-cell DNA and Glucagon⁺ cells in serial **paraffin-embedded pancreatic sections** from non-diabetic, T2D and T1D donors. Each dot in each graph represents a different donor

β-cell methylation markers are specific and accurate

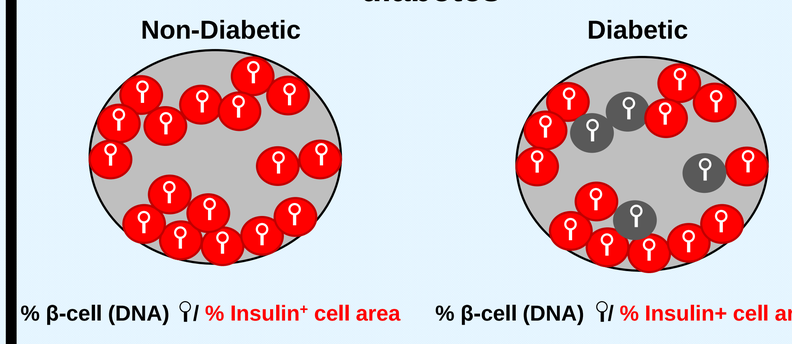


A. The methylation status of three markers specifically unmethylated in β-cells was determined in DNA from islets and sorted pancreatic cells



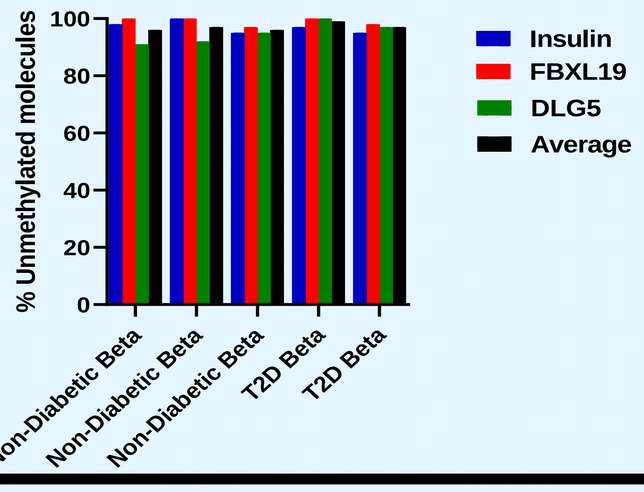
B. Assay accuracy
Increasing amounts of β-cell DNA spiked in 293T-cell DNA was quantified

Testing for the presence of 'empty' β-cells in diabetes



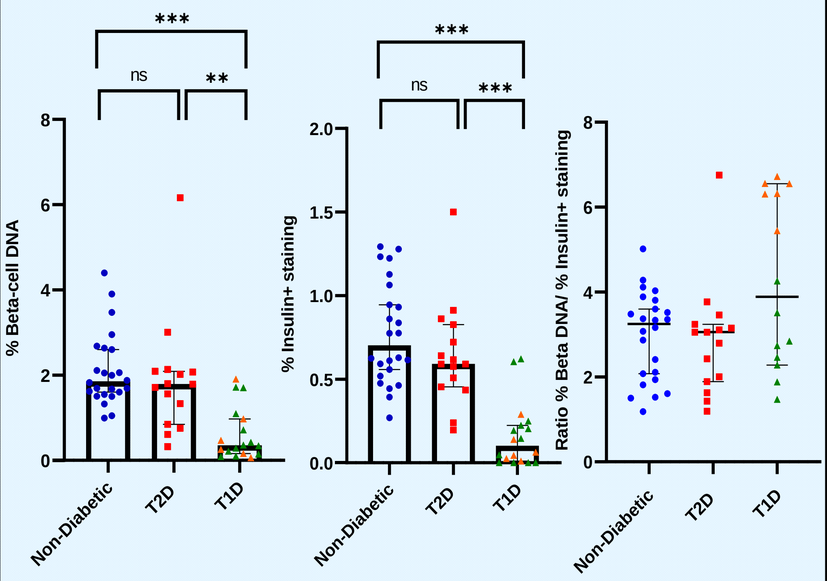
The presence of 'empty' β-cells in diabetes will result in increased **% β-cell DNA / % Insulin⁺ cells** ratio

The β-cell methylation signature is preserved in T2D



'Empty' β-cells are detected in a fraction of T1D donors

Non-Diabetic n=24
T2D n=15
T1D n=18



The percentage of β-cell DNA and Insulin⁺ cells was quantified in two serial pancreatic sections from non-diabetic, T2D and T1D donors. The increased % β-cell DNA/ % Insulin⁺ cells ratio in the pancreas of some T1D donors (orange triangles) supports the possibility that the pancreas of patients with T1D contains a significant number of insulin-depleted beta-cells.
Each dot in each graph represents a different donor.

Summary

- A DNA methylation-based assay for the accurate quantification of alpha and beta-cells in human pancreatic islets and tissues.
- A significant increase in the alpha-cell fraction in diabetic donors compared to non-diabetic.
- Preliminary data indicate that the pancreas of some T1D donors contains 'empty' β-cells.