Differential CpG methylation at *Nnat* in the early establishment of beta cell heterogeneity

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Introduction

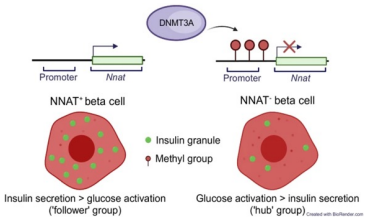

Figure 1. Neuronatin (Nnat) is an imprinted gene in humans and mice, critical for glucose-stimulated insulin secretion in vivo [1]. Its expression is regulated by CpG methylation. Pancreatic beta cells are functionally heterogeneous, with distinct subpopulations that coordinate islet-wide Ca2+ dynamics.

This paper aims to determine whether Nnat marks a specific subpopulation of functional beta cells and to explore how CpG methylation and other epigenetic pathways contribute to their formation and maintenance.

METHODS

Single cell RNA-seq islet data were downloaded from Gene Expression Omnibus database and filtered according to the respective published work and hypervariability of gene expression was assessed by variance analysis to avoid artefacts resulting from technical dropouts. Extraction of islet clusters identified two distinct subpopulations of beta cells where cells with Nnat expression <3 was dedicated as Nnat- and >3 as Nnat+. Mice with Nnat-driven enhanced GFP under the control of the Nnat enhancer/promoter regions were purchased from the Mutant Mouse Resource and Research Centre while mice with deletion of the DNMT3A from the pancreatic progenitor stage were obtained from The Jackson Laboratory. From the mice, pancreatic islets expressing Nnat-eGFP were isolated and Ca2+ imaging of whole islets was performed.

RESULTS

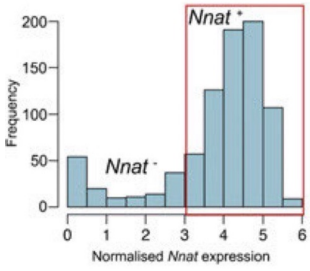

Figure 2. Distribution of Nnat expression among beta cell clusters, along with the expression range used in defining Nnat+ beta cells (highlighted by the red rectangle). 2 beta cell populations with distinct levels of Nnat expression were identified indicating the heterogenous expression of Nnat across the islet [2,3]

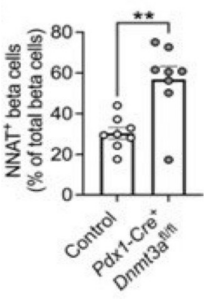

Figure 3. Quantification of *Nnat*+ beta cells, expressed as *Nnat*/INS co-positive cells as a percentage of total INS-positive cells. Scale bar, 50μm (*n*=8 mice per genotype, unpaired Student's *t* test, ***p*<0.01). Nuclei are visualised with DAPI

Nnat staining in control mice at P6 demonstrated expression in a subpopulation of beta cells, whereas deletion of DNMT3A resulted in a loss of this heterogenous expression across the islet. Nnat mRNA was differentially expressed in a discrete beta cell population in a DNMT3A-dependent manner.

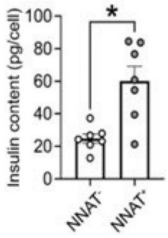

Figure 4. Insulin content assessed in *Nnat*+and *Nnat*- beta cells (*p<0.05, n=7m FACS-purified populations from individual mouse islet preparations, Wilcoxon matched-pairs signed rank test). FACS-sorted primary Nnat+ beta cells had a significantly higher insulin content than Nnat- cells.

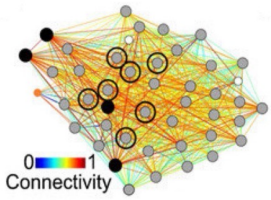

Figure 5. Representative Cartesian map of beta cells with colour-coded lines connecting cells according to the strength of coactivation (colour-coded *R* values from 0 to 1, blue to red).

Beta cells are represented by differently coloured nodes depending on their coactivity with the other beta cells, where black nodes indicate coactivity with ≥80% of the remaining beta cells, while grey, white and orange nodes represent coactivity with ≥60%, ≥40% and <40%, respectively. Nodes circled with a solid black line indicate *Nnat*+ cells.

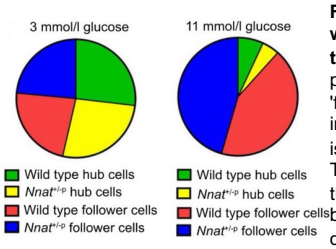

Figure 6. The wild-type 'hub' cells were significantly more connected than *Nnat*-deficient 'hub' cells. The proportion of highly-connected 'hubs' to 'followers' were significantly decreased in both wild type and *Nnat* deficient islets under high glucose (p<0.001). There were no significant differences in the proportion of 'hub'/follower ells between the wild type and *Nnat* deficient islets under high glucose conditions (p=0.36).

DISCUSSION

CpG methylation at the Nnat promoter plays a crucial role in early beta cell maturation by restricting its expression to a specialized subpopulation of beta cells. This process establishes functional heterogeneity within beta cells, essential for distinct roles in insulin production and glucose metabolism [1,4]. NNAT+ beta cells are enriched for pathways related to translation initiation and oxidative phosphorylation, emphasizing their specialization in insulin synthesis rather than glucose sensing.

The loss or reduction of NNAT+ beta cells correlates with impaired glucose-stimulated insulin secretion, a hallmark of Type 2 diabetes. Altered CpG methylation at the Nnat locus may contribute to the disruption of beta cell hierarchy and functionality in diabetic states. These findings highlight the therapeutic potential of targeting CpG methylation and other epigenetic modifications to preserve or restore beta cell function [5]. Such strategies could provide novel approaches for beta cell replacement, regeneration, or modulation in diabetes treatment.

CONCLUSION

CpG methylation plays a crucial role in early beta cell developmental maturation, restricting Nnat expression in specific beta cells during early maturation. This leads to reduced glucose-stimulated insulin secretion, contributing to the development of Type 2 diabetes [6]. Retention of Nnat+ beta cells in adulthood is also indicative of its distinct functional role in the mature islets, contributing to a heterogenous population of beta cells [6]. Chemical modification of the epigenome through CpG methylation may provide an attractive therapeutic angle for both beta cell replacement or regeneration and modulation of beta cell function and cell-cell connectivity in type 2 diabetes.

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