"FunDNAmethyl" Mechanism for Developmental Restriction of a β-Cell Subpopulation

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β-Cell heterogeneity is a phenomenon that has been recognized and studied for decades from different perspectives: function (1), telomere length (2), cellular age (3), chromatin structure (4), methylation status (5), cyclical or compensatory changes to endoplasmic reticulum (ER) stress and unfolded protein response (6,7), pathophysiologic states like senescence (8), and pliability, such as states of transdifferentiation and dedifferentiation (9). Many studies of β-cell subpopulations have focused on what characterizes their functional profile based on insulin secretion (10,11). Determinants of heterogeneity in β-cell function have been examined, such as glucose sensitivity (12) and the biosynthetic pathways (13). All of these characteristics have been used to describe β-cell subpopulations that vary throughout stages of development, age, and pathology, but defining common mechanisms that determine this heterogeneity has remained difficult.

Recent single-cell and sorted-cell sequencing studies have uncovered new markers of β-cell heterogeneity. Four subtypes of human β-cells were defined by cell surface markers that are proportionally altered in type 2 diabetes (T2D) (14), whereas other surface markers have been linked to the individual age of the cells (3). Additionally, two populations of proliferating (*Fltp*$^{+}$) and mature (*Fltp*$^{-}$) mouse β-cells have been identified (15). Finally, specialized hub cells, identified as 1–10% of β-cells with more active mitochondria and less insulin, were recently reported to synchronize β-cell oscillations (16). The pathophysiological relevance of β-cell heterogeneity has been explored in seminal publications where researchers performed single-cell sequencing on human islets from people with and without T2D (17–19). While successful in defining a robust set of β-cell signature genes and identifying hundreds of differentially expressed candidate genes in T2D β-cells versus nondiabetic β-cells, the small number of donors and modest number of cells per donor profiled in each study has hindered the ability to define robust and reproducible human β-cell subpopulations to date.

While there have been clear advances in identifying and describing markers of putative β-cell subpopulations, open questions remain about the developmental time point(s) at which they are generated or specified, the correlation between markers of β-cell heterogeneity, their dynamics (stable versus cyclical), and, more importantly, the molecular mechanism(s) that establishes and maintains these (meta)stable differences. Previous DNA methylation analyses suggested that changes in mature islets were driven by age-dependent demethylation of regulatory elements and altered chromatin accessibility or structure, with consequent changes in β-cell function (5).

In this issue of *Diabetes*, Parveen et al. (20) define an epigenetic mechanism that contributes to pancreatic β-cell heterogeneity based on the analysis of a developmentally regulated tyrosine hydroxylase (TH^{+}) subpopulation, which they thoroughly characterize at transcriptomic, molecular, and metabolic levels. Strong and convincing data indicate that developmental restriction of this subpopulation is determined by de novo DNA methylation of the TH promoter during embryonic β-cell differentiation (Fig. 1). Conditional ablation of the de novo DNA methyltransferase Dnmt3a in PDX1^{+} pancreatic progenitors or NGN3^{+} endocrine progenitors leads to increased TH^{+} β-cells, suggesting that this subpopulation emerges from progenitors in which the DNA is demethylated at the *Th* gene promoter. Consequently, when Dnmt3a is deleted from mature β-cells, the TH^{+} subpopulation is unchanged. In adulthood, restriction of the TH^{+} subpopulation is maintained by Dnmt1, but it dynamically reemerges in response to metabolic challenges such as high-fat diet (Fig. 1).

The proportion of TH^{+} β-cells, which resolves to between 1% and 3% after birth, is similar to the frequency of senescent cells in young mice (3,8). However, the TH^{+}

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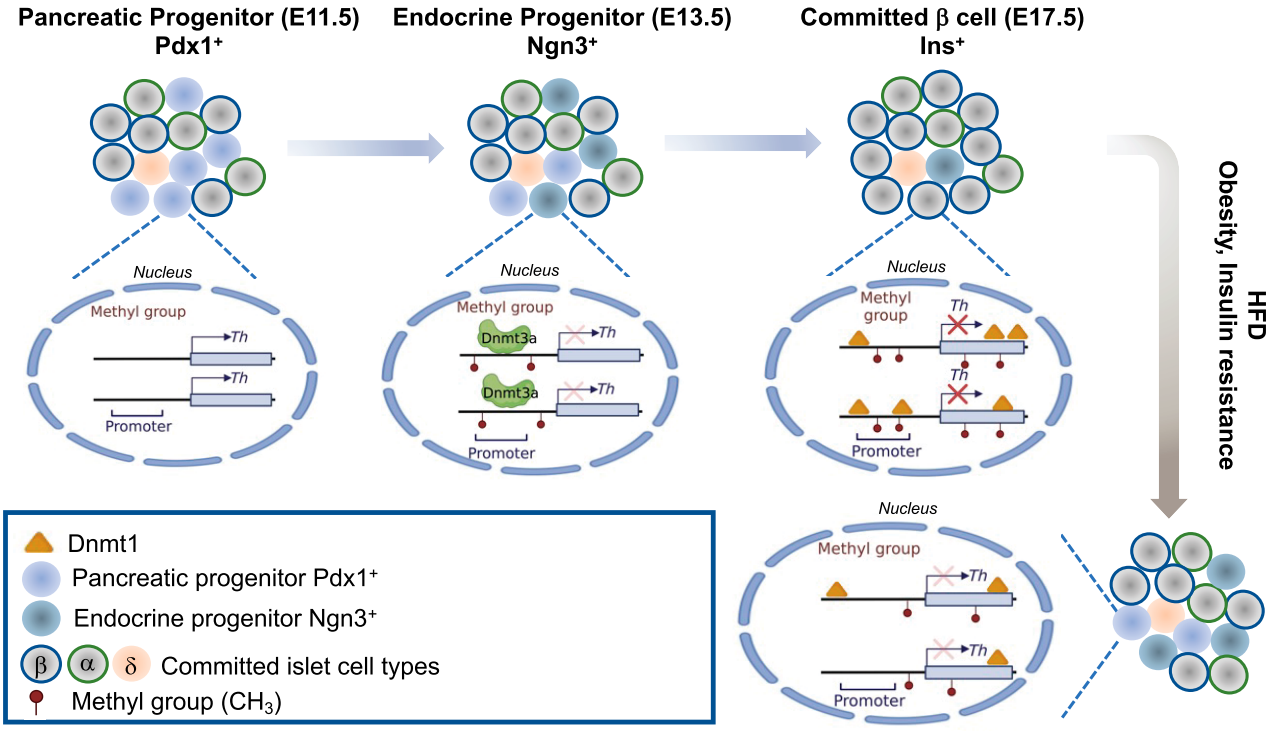

Figure 1—De novo DNA methylation in PDX1$^+$ pancreatic progenitors or NGN3$^+$ endocrine progenitors restricts Th$^+$ β-cells. This population becomes restricted to 1–3% of β-cells in the postnatal period but can be dynamically restored in response to high-fat diet (HFD) through demethylation. BioRender.com was used to create part of the figure. E, embryonic day.

population does not express markers of senescence, is not proinflammatory, and can proliferate, in stark contrast to senescent cells, which, by definition, are unable to divide. Instead, TH$^+$ cells express ER stress and unfolded protein response markers, which could be compatible with Fltp$^+$ or ER and oxidative stress subpopulations that were previously described (6,7,15). Further studies will be important to corroborate or refute this possibility.

This study establishes de novo and maintenance of promoter DNA methylation as a mechanism contributing to developmental specification of a β-cell subpopulation. Given the universal nature of this epigenetic modification genome-wide, it will be important to determine its role in regulating other key gene targets that are developmentally controlled. Additionally, this work provides new insights into the developmental regulatory dynamics of the TH$^+$ subpopulation, which initially appears during the developing or neonatal period in the mouse, peaks during a postnatal critical window, and becomes restricted in adults. TH$^+$ cells are also present in the human pancreas, supporting their physiological importance and pointing to DNA methylation as a preserved developmental mechanism across species. This concept may be particularly important for the directed differentiation of progenitors into functional β-cells. Given the developmental restriction of the TH$^+$ subpopulation, it should serve as a useful marker to study models such as transgenic mice, and these markers can be used to evaluate the relevance of DNA methylation in determining other key parameters of β-cell heterogeneity. For example, the functional characteristics of TH$^+$ cells, and their physiological role within the islets and the organism, remain to be determined.

The results of this study naturally raise new questions. Studies could focus on understanding the relationship between the TH$^+$ subpopulation and previously described β-cell subpopulations in mice and humans (calcium currents, hub cells, age markers, insulin secretion, and telomere length, among others). The presence of TH$^+$ β-cells in the human pancreas raises questions about their pathophysiological relevance and the role they may play in type 1 diabetes and the different subtypes of T2D (21).

Another interesting area for investigation arising from this study is the mechanism(s) that determines DNA methylation and chromatin structure, which impact the transcriptomic cellular identity of β-cells. To resolve this question, it will be important to understand whether these are universal regulators of methylation states that lead to the identification of other key β-cells that are developmentally controlled or restricted by this mechanism. However, due to the low frequency and exceeding rarity of the TH$^+$ population in mice and humans, respectively, it remains unclear if or how the DNA methylation at work in this subpopulation represents a robust and generalizable mechanism contributing to the general determination, maintenance, or restriction of other β-cell subpopulations and other islet cell types.

In summary, Parveen et al. (20) provide an important proof-of-principle study that demonstrates a role for de novo DNA methylation in refining islet β-cell expression of one target gene (*Th*) (Fig. 1). This is a model that can be used to make further analyses of broader changes with potentially important implications to understand more complex mechanisms behind β-cell heterogeneity.

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References

1. Salomon D, Meda P. Heterogeneity and contact-dependent regulation of hormone secretion by individual B cells. Exp Cell Res 1986;162:507–520
2. Peng SW, Zhu LY, Chen M, et al. Heterogeneity in mitotic activity and telomere length implies an important role of young islets in the maintenance of islet mass in the adult pancreas. Endocrinology 2009;150:3058–3066
3. Aguayo-Mazzucato C, van Haaren M, Mruk M, et al. β Cell aging markers have heterogeneous distribution and are induced by insulin resistance. Cell Metab 2017;25:898–910.e5
4. Arda HE, Li L, Tsai J, et al. Age-dependent pancreatic gene regulation reveals mechanisms governing human β cell function. Cell Metab 2016;23:909–920
5. Avrahami D, Li C, Zhang J, et al. Aging-dependent demethylation of regulatory elements correlates with chromatin state and improved β cell function. Cell Metab 2015;22:619–632
6. Xin Y, Dominguez Gutierrez G, Okamoto H, et al. Pseudotime ordering of single human β-cells reveals states of insulin production and unfolded protein response. Diabetes 2018;67:1783–1794
7. Sharma RB, Landa-Galván HV, Alonso LC. Living dangerously: protective and harmful ER stress responses in pancreatic β-cells. Diabetes 2021;70:2431–2443
8. Aguayo-Mazzucato C, Andle J, Lee TB Jr, et al. Acceleration of β cell aging determines diabetes and senolysis improves disease outcomes. Cell Metab 2019;30:129–142.e4
9. Talchai C, Xuan S, Lin HV, Sussel L, Accili D. Pancreatic β cell dedifferentiation as a mechanism of diabetic β cell failure. Cell 2012;150:1223–1234
10. Pipeleers D, Kiekens R, Linz Z, et al. Physiologic relevance of heterogeneity in the pancreatic beta-cell population. Diabetologia 1994;37(Suppl 2):S57–S64
11. Hiriart M, Ramirez-Medeles MC. Functional subpopulations of individual pancreatic B-cells in culture. Endocrinology 1991;128:3193–3198
12. Van Schravendijk CF, Kiekens R, Pipeleers DG. Pancreatic beta cell heterogeneity in glucose-induced insulin secretion. J Biol Chem 1992;267:21344–21348
13. Kiekens R, In 't Veld P, Mahler T, Schuit F, Van De Winkel M, Pipeleers D. Differences in glucose recognition by individual rat pancreatic B cells are associated with intercellular differences in glucose-induced biosynthetic activity. J Clin Invest 1992;89:117–125
14. Dorrell C, Schug J Canaday PS, et al. Human islets contain 4 distinct subtypes of beta cells. Nat Commun 2016;7:11756
15. Bader E, Migliorini A, Gegg M, et al. Identification of proliferative and mature β-cells in the islets of Langerhans. Nature 2016;535:430–434
16. Johnston NR, Mitchell RK, Haythorne E, et al. Beta cell hubs dictate pancreatic islet responses to glucose. Cell Metab 2016;24:389–401
17. Lawlor N, George J, Bolisetty M, et al. Single-cell transcriptomes identify human islet cell signatures and reveal cell-type-specific expression changes in type 2 diabetes. Genome Res 2017;27:208–222
18. Segerstolpe Å, Palasantza A, Eliasson P, et al. Single-cell transcriptome profiling of human pancreatic islets in health and type 2 diabetes. Cell Metab 2016;24:593–607
19. Xin Y, Kim J, Okamoto H, et al. RNA sequencing of single human islet cells reveals type 2 diabetes genes. Cell Metab 2016;24:608–615
20. Parveen N, Wang JK, Bhattacharya S, et al. DNA methylation–dependent restriction of tyrosine hydroxylase contributes to pancreatic β-cell heterogeneity. Diabetes 2023;72:575–589
21. Ahlqvist E, Prasad RB, Groop L. Subtypes of type 2 diabetes determined from clinical parameters. Diabetes 2020;69:2086–2093