DNA methylation status correlates with adult β-cell regeneration capacity

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The role of DNA methylation in β-cell neogenesis is poorly understood. We report that during the process of induced cell reprogramming, methylation content of the Ngn3 and Sox11 genes are diminished. These findings emphasise DNA methylation is a barrier in β-cell regeneration in adulthood, a well described pathophysiological phenomenon of major significance in explaining β-cell deficiency in diabetes in the adult pancreas.

npj Regenerative Medicine (2021)6:7 ; https://doi.org/10.1038/s41536-021-00119-1

The development of diabetes involves pathogenetic processes that either destroy the β-cells of the pancreas or result in resistance to insulin action. Type 1 diabetes (T1D) is an autoimmune disease that selectively destroys insulin-producing β-cells in the pancreas. Even though symptoms usually do not appear before 80% of the β-cell mass has been destroyed, absolute destruction of these cells leads to the dependence on exogenous insulin administration for survival. In patients with Type 2 diabetes (T2D), insulin is either produced in insufficient quantities so the response to insulin is weak or it is produced in normal amounts, but the target organs become insulin resistant.

Two solutions aimed at replacing the damaged β-cell mass in diabetic patients exist, such as whole pancreas or islets transplantation. Although efficient, these therapies face the shortage of organ donors together with the associated side-effects of immunosuppressive drugs. Consequently, current research focuses on the replacement of the lost β-cell in diabetic patients using several approaches and cell sources. However, critical to exploiting the potential of these regenerative approaches, is understanding how tissue and cellular processes are controlled during development.

In the pancreas, endocrine cell allocation and maintenance of the different endocrine cell lineages are controlled by transcription factors that precisely regulate glucose homeostasis. During development, this transcriptional hierarchy itself is in part regulated by epigenetic modifications. The master gene involved in endocrine fate determination is Neurogenin3 (Ngn3).1,2 Ngn3 is required for the development of all the endocrine cells (α-, β-, δ-, PP- and ε-cells), that are all associated with the secretion of specific endocrine hormones. Moreover, during pancreas morphogenesis, Ngn3 induces the delamination of progenitors from the ductal epithelium through an epithelial-to-mesenchymal transition (EMT) process3. EMT is a key developmental program by which cells located within an epithelial layer acquire the ability to spread and migrate to a distant site to form new structures mediated by Sox114. Ngn3-expressing progenitors, subsequently migrate and emerge from the ductal epithelium and aggregate to eventually form the islets of Langerhans.

Arx and Pax4 are key transcription factors for the specification towards the a-/PP- and β-/δ- cell fates, respectively2. Indeed, Pax4 is critical for β-cell determination and is exclusively expressed in β-cells in the adult pancreas, whereas Arx plays a key role in the determination of the α/PP-cell lineage and is restricted to mature glucagon-expressing cells where it is involved in maintaining their identity. In fact, Arx and Pax4 display antagonistic activities with respect to the allocation of the endocrine precursors through an inhibitory cross-regulatory circuit that controls the transcriptional state of these two genes5.

A potential source of β-cells was previously demonstrated with the discovery of α-cell plasticity and the ability of α-cell to convert into insulin-producing cells. This is dependent on the ectopic expression of Pax4 in adult or embryonic α-cells for conversion into β-like cells.6,7 Conversely, the loss of Arx in glucagon-expressing cells triggers their conversion into functional insulin-producing cells.8-10 Equally important was the finding that the α- to β-like cell conversion observed in these models induces the re-expression of Ngn3 in ductal cells and their differentiation into endocrine cells by reawakening EMT.

In this study, we assessed DNA methylation in order to gain a better understanding how this epigenetic mark impacts gene expression during cell reprogramming. We show that in two transgenic mouse models of α-to-β-cell conversion by way of directed transcription factor reprogramming, Ngn3 and Sox11 genes undergo drastic reductions in DNA methylation content which is consistent with re-expression at the mRNA level. Our in vivo studies propose the Ngn3 and Sox11 genes are demethylated during adult β-cell regeneration.

The main goal of this study was to determine whether the reactivation potential of Ngn3 and Sox11 by way of direct lineage conversion is dependent on DNA methylation. We made use of two transgenic models generated previously by our group7,8 in which α-cells are continuously regenerated and converted into functional β-like cells through Pax4 overexpression (PaxOE) or Arx

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deletion (ArxKO) (Fig. 1a). Both these models conclusively established the ductal and α-cell ontogeny of these transdifferentiated β-cells by direct lineage tracing experiments. We showed that Ngn3 re-expression is a feature of pancreatic progenitors in the duct, and Sox11 is a hallmark of EMT (Figs. 1b, c). Indeed, using immunofluorescence, we confirmed the detection of numerous insulin-producing cells co-expressing Ngn3 in islets. Sox11 was also assessed because DNA demethylation is thought to be an early regulatory event required for epithelial gene reactivation. Both Ngn3 and Sox11 were undetected in adult WT mice (data not shown).
DNA methylation-dependent reprogramming of islet cells derived from Arx knockout and overexpression of Pax4 animal models. 

Fig. 1  DNA methylation-dependent reprogramming of islet cells derived from Arx knockout and overexpression of Pax4 animal models. 

Transgene therapy: the POU transcription factor family, (wt) to transgenic mouse models PaxOE and ArxKO, using development genes in transgenic mouse models, PaxOE and ArxKO. Data show DNA methylation (fold-change) for Sox11 and Ngn3 and its re-expression in the mouse pancreata. The expression of Ngn3 was analysed by immunohistochemistry in WT/Dox- controls and Dox-treated Pax4OE and ArxKO pancreata. Ngn3 labelling was absent in controls, while strongly re-expressed in induced animals (b, c). Ngn3 is re-expressed in the ductal lining and epithelium (b and i) as well as in the islets (b, c) of transgenic mice while being absent in controls. 

Workflow of DNA methylation capture and analysis of transgenic mice islets using methyl-domain-binding (MBD) capture and downstream qPCR (MBD-qPCR) were used for the assessment of the reprogramming (re) amplons Ngn3 and Sox11. The reprogramming amplons Ngn3 and Sox11 were designed from the mouse genome assembly (mm10) using UCSC browser. Cpg Islands ( CGI) are shown in green for Ngn3 and Sox11. Oct4 does not have a CGI and served as a control for DNA methylation. The positions of the transcription start sites (TSS) are also shown relative to the 1 kb scale. Chromosome positions are shown Ngn (chr10), Sox11 (chr11) and Oct4 (chr17). DNA methylation analysis of islet development genes in transgenic mouse models, PaxOE and ArxKO. Data show DNA methylation (fold-change) for Ngn3 and Sox11. A member of the POU transcription factor family, Oct4, central to the machinery governing pluripotency served as an endogenous control and remains stable for DNA methylation. Error bars are defined as standard error of the mean (s.e.m) with significance calculated by comparing wild-type (wt) to transgenic mouse models PaxOE and ArxKO, (*P < 0.05, **P < 0.01, ***P < 0.001). g mRNA expression of genes associated with islet lineage reprogramming. Data shows gene expression (fold-change) normalised to housekeeping gene (H3F3A). SEM error bars with significance calculated by comparing wild-type (wt) to transgenic mouse models PaxOE and ArxKO, (*P < 0.05). h mRNA expression of ten eleven translocation (Tet) enzymes in transgenic mice islets. Error bars are defined as standard error of the mean (s.e.m) with significance calculated comparing wild-type (wt) to transgenic mouse models PaxOE and ArxKO, (*P < 0.05).

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greatly assist in resolving a major obstacle in regenerating β-cells in adulthood thereby restoring the β-cell mass in pathophysiological conditions such as T1 and T2D.

METHODS

Mice and animal procedures
Animal care and experimental procedures were conducted according to the French ethical guidelines. Animal protocols were reviewed and approved by an institutional ethics committee (Ciepal-Azur) at the University of Nice, and all colonies were maintained following European animal research guidelines. This project received approval from ethics committee (NCE/2011-22, University of Nice). Wild-type (WT) 129/sv mice were obtained from Charles River Laboratories and from Taconic. The animal research guidelines. This project received approval from ethics committee (Ciepal-Azur) at the University of Nice, and all colonies were maintained following European ethical regulations. Animal protocols were reviewed and approved by an institutional ethics committee (Ciepal-Azur) at the University of Nice, and all colonies were maintained following European animal research guidelines. This project received approval from ethics committee (NCE/2011-22, University of Nice).

Islet isolation and DNA methylation analysis
Methyl-CpG-binding domain capture was used to investigate DNA methylation in the ArxKO knockout and barrier for islet transition and reprogramming in the pancreas. αPax4 overexpression in adult α-cells (orange) induce their trans-differentiation and conversion into β-like cells (blue). This leads to a shortage in glucagon, which is responsible for the mobilisation of ductal precursor cells (green), these re-expressing the pro-endocrine gene Ngn3 (green), prior to undergoing an EMT and concomitant differentiation into endocrine cells. Such a continuous cycle of conversion/regeneration results in insulin- cell hyperplasia. b Methylation writing (DNMT’s) and erasing (Tet’s) enzymes are implicated in the regulation of Arx and Pax4, respectively. c Re-expression of Ngn3 is inversely associated with loss of DNA methylation in the ArxKO knockout and Pax4 misexpression animal models. This model of DNA demethylation-mediated reprogramming or dmrE closely corresponds with Tet expression and the loss of gene methylation content and enhanced activity of the mesenchymal marker Sox11.

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were the following: mouse monoclonal anti-insulin (1/500; Sigma; catalogue #2018, mouse anti-Ngn3 (1/10,000; Millipore; catalogue #AB5684), rat monoclonal anti-somatostatin (1/250; Sigma; catalogue #AB354). Pictures were processed using ZEISS Axiomager Z1.

Reporting summary
Further information on research design is available in the Nature Research Reporting Summary linked to this article.

DATA AVAILABILITY
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Received: 15 November 2020; Accepted: 14 January 2021; Published online: 12 February 2021

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ACKNOWLEDGEMENTS
This work was supported in part by the NSFC and NHMRC International Joint Program (81561128017 and 1113188). Department of Health, Australian Government. Assam El-Osta is a National Health and Medical Research Council (NHMRC) Senior Research Fellow (1154650, 0526681). P.C. is supported by the JDRF (2-SRA-2017-416-S-B, 2-SRA-2017-417-S-B), the Agence Nationale pour la Recherche (ANR-16-CE18-0005-01, ANR-17-CE14-0034), MSD-Avenir, and French Government (National Research Agency, ANR) through the “Investments for the Future” programs LABEX SIGNALIFE ANR-11-LABX-0028-01 and IDEX UCAJedi ANR-15-IDEX-01. A.E.O. dedicates this article to his late grandmother.

AUTHOR CONTRIBUTIONS
I.K., P.C., K.A.H., S.M., H.K. and J.O. performed the experiments and analysed the data. I.K., K.A.H., P.C. and A.E.O. interpreted the data. I.K., K.A.H., M.C., P.C. and A.E.O. wrote the article. All authors were involved in revising the manuscript for intellectual content. A.E.O. revised the manuscript according to feedback from the reviewers and supervised I.K., K.A.H., S.M., H.K. and J.O. on the project. All authors approved the final version to be published and agreed to be accountable for all aspects of the work. I.K. and K.A.H. are considered co-first authors.

COMPETING INTERESTS
The authors declare no competing interests.

ADDITIONAL INFORMATION
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41536-021-00119-1.
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Title: DNA methylation status correlates with adult -cell regeneration capacity.

Date: 2021-02-12

Citation: Khurana, I., Al-Hasani, K., Maxwell, S., K N, H., Okabe, J., Cooper, M. E., Collombat, P. & El-Osta, A. (2021). DNA methylation status correlates with adult -cell regeneration capacity.. NPJ Regen Med, 6 (1), pp.7-. https://doi.org/10.1038/s41536-021-00119-1.

Persistent Link: http://hdl.handle.net/11343/272983

File Description: Published version

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