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Recovery of human type 2 diabetes beta cell function associates with transcriptomic signatures that point to novel therapeutic targets

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Abstract

Remission of type 2 diabetes (T2D) may occur after very low-calorie diets or bariatric surgery, and is associated with improved pancreatic beta cell function. Here, we evaluated if T2D beta cell dysfunction can be rescued ex-vivo and which are the molecular mechanisms involved.

Islets from 19 T2D donors were studied after isolation (“basal”) and following culture at 5.5 or 11.1 mmol/l glucose (“cultured”). We evaluated glucose-stimulated insulin secretion (GSIS) and transcriptomes by RNA sequencing, correlated insulin secretion changes (“cultured” vs “basal”) to global gene expression, and searched for potential therapeutic gene targets and compounds that mimic gene signatures of recovered beta cell function in T2D islets.

GSIS improved in 12 out of 19 islet preparations from T2D donors after culture at 5.5 mmol/l glucose (insulin stimulation index increased from 1.4±0.1 to 2.3±0.2, p<0.01), mainly due to greater insulin response to high glucose. No improvement was seen in islets cultured at 11.1 mmol/l glucose. Functional improvement was accompanied by changes in expression of 438 genes, many of which involved in functional and inflammatory processes. Of them, 123 were significantly correlated with changes in GSIS. Drug repurposing and target identification analyses for beta cell functional recovery predicted several chemical (including Src inhibitors and anti-inflammatory drugs) and genetic hits in pathways such as chemokine, MAPK, ERBB signaling, and autophagy.

In conclusion, defective insulin secretion in T2D can be rescued, at least in part, by a “non-diabetic” milieu, demonstrating important T2D beta cell functional plasticity. This recovery associates with specific transcriptomic traits, pointing to known as well as novel therapeutic targets to induce T2D remission.

Keywords: type 2 diabetes, remission, human islets, insulin secretion, gene expression, RNA sequencing, drug targets, drug repurposing
Introduction

Type 2 diabetes (T2D) is generally assumed to be a relentlessly progressive disease, due to combined genetic and environmental factors that cause pancreatic beta cell dysfunction and damage (1-5). However, remission of T2D has been observed in variable proportions of patients after severe caloric restriction or bariatric surgery (6-14). Following carbohydrate limitation and low-calorie diets, diabetes remission can occur shortly after the start of treatment. It is associated with increased hepatic insulin sensitivity, recovery of first-phase insulin secretion, and reduction of hepatic and pancreatic triacylglycerol content (6-9). In the case of bariatric surgery, biliopancreatic diversion, Roux-en-Y gastric bypass and vertical sleeve gastrectomy improve glucose control and can promote early remission of T2D independently from weight reduction (10-14). Although such procedures have distinct effects on several organs and functions, many post-surgical T2D subjects show rapid improvement of insulin secretion, with restoration of first-phase insulin release to intravenous glucose administration already by 1-4 weeks after surgery (12). Increased GLP-1 release and potentiated incretin effects are believed to play a role, but the ultimate mechanisms of this fast recovery in beta cell function remain to be defined (13,14).

Certain glucose-lowering drugs (in particular metformin, pioglitazone and incretin-related drugs) improve beta cell function in subjects at high T2D risk and patients with newly diagnosed or established T2D (15-18). However, these effects fade away when the medications are withdrawn (15). Of interest, islets isolated from T2D subjects and cultured for 24-48 h in the presence of metformin or GLP-1 analogues have improved beta cell glucose responsiveness and ultrastructural features, which are accompanied by changes in expression of certain genes such as NADPH oxidase, catalase, GSH peroxidase, PDX1 and glucokinase (15,19-21).

It remains unclear, however, whether and to which extent beta cells from T2D subjects can regain their function once removed from the in-vivo “diabetic” environment and, most importantly, which molecular mechanisms are involved in the functional restoration. As summarized in Figure 1, we presently studied a large number (n=19) of independent human islet preparations from patients affected by T2D (Table S1), and observed that the defective glucose-stimulated insulin secretion typical of this disease can, in many but not all cases, be partially rescued by culture in a “non-diabetic” milieu, demonstrating unexpected functional plasticity of T2D beta cells. Notably, this
functional recovery is associated with specific transcriptomic traits, unveiling potential mechanisms to be therapeutically targeted for T2D remission.

**Results**

*Functional studies*

Insulin release from “basal” T2D islets was 24.2±2.9 and 42.5±6.6 µU/ml in response to 3.3 and 16.7 mmol/l glucose, respectively (p < 0.01). The corresponding insulin stimulation index (ISI) was 1.6±0.1 (range: 0.7 – 2.5), consistent with other reports on isolated T2D islets (22-24) and resulting approximately 50% lower than that of a large series of islets from non-diabetic donors, as studied in our laboratory (22,23). After culture in medium containing 5.5 mmol/l glucose, GSIS from the 19 islet preparations remained overall unchanged (20.3±3.2 µU/ml at 3.3 mmol/l and 40.5±6.8 µU/ml at 16.7 mmol/l glucose), with a non-significant trend for increased ISI (1.95±0.2, range 1.0 – 3.8, p=0.1 vs “basal”). However, we observed that ISI clearly increased in some of the “cultured” islet preparations, while it tended to decrease in others. Twelve islet preparations had better post-culture beta cell glucose responsiveness (“improvers”: “basal” ISI, 1.4±0.1; “cultured” ISI, 2.3±0.2, p <0.01) (Figure 2A). The remaining 7 preparations showed an average 28% decline of ISI during culture (“non-improvers”: “basal” ISI, 1.95±0.2; “cultured” ISI, 1.4±0.2, p=0.24) (Figure 2A). This was mainly due to changes in insulin release in response to 16.7 mmol/l glucose (Figure 2B). The findings were similar after correction of insulin secretion for islet insulin content (Figure 2C). For comparison, we have previously observed that islets from non-diabetic donors kept in culture at 5.5 mmol/l glucose for up to 7-8 days from isolation have stable ISI values over the period of culture (22). In a subgroup of 5 islet preparations from T2D patients, culture at 11.1 mmol/l glucose did not or only minimally increase insulin release, including some of the preparations with improved beta cell function at 5.5 mmol/l glucose culture (Figure 2D). “Improver” and “non-improver” preparations did not differ significantly for donor clinical features (Table S2).

Thus, after a few days of culture in normoglycemic conditions a proportion of the T2D islet preparations showed significant recovery of GSIS, but not when islets were cultured at moderately elevated (11.1 mmol/l) glucose concentrations.
Transcriptomic studies

The transcriptome of “cultured” vs “basal” islets was compared separately for 5 “improver” and 5 “non-improver” preparations, to identify the respective gene expression signatures. Differentially expressed genes (DEGs) were 438 for the “improver” (233 up-regulated, 205 down-regulated) and 1,512 for the “non-improver” islets (588 up-regulated, 924 down-regulated) (Figures 3A,B and Table S3). The 20 most significantly up- and down-regulated genes for the “improver” and “non-improver” series following culture at 5.5 mmol/l glucose are reported in Table 1 and Table S4, respectively. Among the most up-regulated genes in the “improver” islet preparations were: PPARGC1A, a transcriptional coactivator that regulates genes involved in energy metabolism via cAMP response element binding protein (CREB) and nuclear respiratory factors (NRFs) (25); BAAT, involved in bile acid metabolism (26); SLC17A4, ATP13A4 and SMIM24, implicated in ion transport; CTSD and CTSK, proteolytic enzymes involved in the activation of hormones and growth factors and modulation of apoptosis (27); and a few genes pertaining to inflammatory/immune processes (such as LYZ, CIITA, PIGR, CXCL9). The increase in ADRA2A was unexpected, as higher ADRA2A expression in islets from ADRA2A risk allele carriers has previously been associated with reduced GSIS (28). Among the down-regulated genes, several are involved in inflammatory/immune reactions, including IL13RA2, FGF2, CSF3 and SOCS3.

As shown in Figure 3C-F and detailed in Table S5, there were 171 DEGs in common between “improver” and “non-improver” islet transcriptomes, of which only 2 oppositely regulated, showing that most of the changes were specific to the two functional trajectories. Interestingly, among the DEGs in the “improver” islets, 17 harbor T2D GWAS variants (Table S6a) (http://www.ebi.ac.uk/gwas). The downregulation of C2CD4B expression in “improver” islets is consistent in direction of effect with a C2CD4B GWAS variant that reduces T2D risk and that colocalizes with an eQTL decreasing human islet C2CD4B expression (29). In addition, 46 and 48 genes of those differentially expressed in the “improver” islets have been previously described to be associated with recovery of beta cell function after lipotoxicity (Table S6b) or glucotoxicity (Table S6c) (22) with the same direction of effect.

By Gene Set Enrichment Analysis, many Gene Ontology biological processes with significant (FDR < 0.05) enrichment were identified (Figure 4A,B, Table S7). The positively enriched processes were 65 and 149 in the “improvers” and “non-improvers” series, respectively, with 30 terms in common. The negatively enriched terms were 362 in the “improver” and 318 in the “non-improver”
preparations, of which 188 were shared. Many of such terms were associated with carbohydrate/lipid metabolism and inflammatory/immune processes. Of interest, among the positively changed processes uniquely enriched in the “improver” islet transcriptomes, the following terms were identified: “Positive Regulation of Peptide Hormone Secretion” (FDR = 0.038), “Positive Regulation of Insulin Secretion Involved in Cellular Response to Glucose Stimulus” (FDR = 0.038) and “Positive Regulation of Insulin Secretion” (FDR = 0.008), with several of the respective genes well known to be associated with beta cell function and identity (such as PFKFB2, UNC3, CRH, PDX1, TCF7L2, NNX6.1, CLTRN) (Table S7).

**Correlations between beta cell function and gene expression**

To identify genes that could play a direct role in the recovery of beta cell function, we correlated changes in GSIS with gene expression changes. Of the DEGs “cultured” vs “basal” (see Table S3), 123 displayed a significant (p < 0.05) association with insulin release, 42 and 81 of which presenting a positive and negative relation, respectively (Table S8). Among them, 14 have been previously described to be linked with T2D by either genetic association (C2CD4B, H19) (http://www.ebi.ac.uk/gwas) or insulin secretion functional rescue after washout of lipo- (DLGAP1-AS1, IL24, PKNOX2, EMP1, LAMP3, GAP43, C2CD4B) or glucotoxicity (MMP1, LAMC3, PAPPA, NT5DC2, PTPRE, GAP43) (22), 12 of which with the same direction of effect as presently observed (down/down).

The top significant associations for positively or negatively correlated DEGs are illustrated in Figure 4C,D. Among the genes positively correlated with beta cell function, SYT8 favors insulin gene transcription and is involved in exocytosis (30), the long non-coding RNA MIR210HG potentiates HIF-1α translation, which in turn upregulates the expression of glycolysis enzymes (31), and RASD2 has been proposed as a putative regulator of insulin secretion (32). Of those associated with lower insulin secretion we found PAPPA, a metalloproteinase which cleaves insulin-like growth factor binding proteins (33), ARHGAP44, an inhibitor of GTPase activity (34), CA8, abundantly expressed in the cerebellum but also in islets (29) and a negative regulator of GLP-1 secretion (35), SBNO2, involved in inflammatory response (36).

Overall, the transcriptomic study shows that variations in beta cell function correlate with changes in the expression of numerous genes and suggests a role for several of these in improving insulin secretion in T2D.
Identification of potential therapeutic targets

To identify potential therapeutic targets, we crossed the top 150 up- and down-regulated genes from the “improver” and “non-improver” islets against the transcriptomes induced by chemical or genetic perturbagens in the Connectivity Map/LINCS databases (See Methods). In the Connectivity Map, we identified six positively correlated chemical perturbagens in the “improver” series and one in the non-improvers (Figure 5A). Of the genetic perturbagens (knockdown) in the Connectivity Map, 24 were positively correlated with the “improver” gene signature (Figure 5B) and none with the “non-improvers”. In the iLINCS platform, we identified 353 chemicals that generated transcriptomes positively correlated with the “improver” islet gene signature (Table S9A), and 520 chemicals were correlated with the “non-improver” islets (Table S9B). After removing the overlap with the latter perturbagens, 229 chemical compounds were specifically positively correlated with the “improvers” (Table S9C). For the genetic perturbagens, 74 were positively correlated with the “improver” islet gene signature (Table S9D), and 187 with the “non-improvers” (Table S9E), resulting in 51 genetic targets specific to the “improvers” (Table S9F). Shared between the two databases were Src inhibitors and drugs with anti-inflammatory effects (MEK inhibitor, targeting MAP2K1/2, JNK inhibitor, targeting MAPK8, and IKK-2 inhibitor, targeting IKBKB). Chemokine and MAPK signaling (with 6 and 5 target genes, respectively) were predicted among the top 10 target pathways by genetic perturbagens (Figure 5C). This result is in line with the differential transcriptome alteration, in which many of the downregulated genes are related to inflammation in the “improver” series. Other genetic perturbagens that potentially contribute to restore beta cell function in T2D islets are those targeting focal adhesion, regulation of actin cytoskeleton, cAMP signaling and autophagy (Figure 5C).

Discussion

The defective insulin secretion characteristic of T2D is related to functional alterations and, to a lesser extent, to loss of beta cell mass (3,5,37-39). The present study demonstrates that recovery of human T2D beta cell function occurs in two thirds of isolated islet preparations after 3-4 days of culture at a physiological glucose concentration. The transfer of islets from the in-vivo T2D milieu to in-vitro culture conditions changes not only prevailing glucose but also other nutrients (such as
lipids), inflammatory mediators/cells, adipokines, and presumably other substances. Glucose-stimulated ISI, a robust marker of beta cell glucose sensitivity (24), increased on average by 60% in 12 of the 19 islet preparations after culture (Figure 2). This trajectory is reminiscent of that of islets from non-diabetic subjects upon removal ex-vivo of lipo- or glucotoxic culture conditions (22), demonstrating remarkable beta cell functional plasticity or resilience. Although increased, the insulin stimulation values after T2D islet culture remain lower than those usually observed in non-diabetic islets (22,23,24). Furthermore, beta cell function did not improve in one third of cases. Remission of T2D after caloric restriction or bariatric surgery has been reported to be less likely in subjects with increased duration of the disease (8-10,40). This does not seem to be the case in our ex-vivo study, since “improver” and “non-improver” islet preparations were obtained from donors with similar clinical characteristics and a comparable average duration of T2D of 11 years. We cannot exclude, however, that the observed differences are due to more marked constitutive damage in the “non-improver” beta cells, possibly including greater loss of beta cell mass and/or more severe initial dysfunction. Preliminary analyses (Cnop, Marselli and Bosi, unpublished) show that in “basal” islets the expression of certain beta cell identity markers tended to be lower for the preparations that did not improve their function after culture (e.g. PDX1, -40%, and MAFA, -70%). More studies are needed to evaluate if longer culture periods, the use of pharmacological compounds and/or interference with genetic targets (see below) may lead to greater T2D beta cell functional rescue. Of interest, no beta cell functional improvement was seen in islets cultured at 11.1 mmol/l glucose (Figure 2), which provides support to the view that blood glucose levels in T2D subjects should be kept as close as possible to the physiological range to preserve/restore beta cell function, within the framework of personalized approaches (41).

The transcriptomic traits associated with T2D islets have been reported by a few groups, showing numerous DEGs in comparison with non-diabetic islets (22,42,43). A major novelty of the present study is the finding that T2D beta cell functional improvement is associated with many specific changes of the transcriptome. Thus, we found that better insulin release is accompanied by upregulation of several genes implicated in beta cell function and structure, and downregulation of genes implicated in inflammatory responses (Tables S3, S5 and S7). A few of them have been previously identified by GWAS as linked to T2D (http://www.ebi.ac.uk/gwas, 44), and several have been shown to change in expression during the recovery of beta cell function in non-diabetic islets following palmitate or high glucose exposure and washout (Table S6) (22). Interestingly, many gene
expression changes were significantly correlated with improved insulin release (Figure 4 and Table S8), suggesting their direct impact on beta cell function. For some of these, experimental proof already exists. Thus, SYT8 knockdown in islets from two human donors attenuated glucose- and arginine-induced insulin secretion by more than 50% (45). In INS-1 cells, overexpression of GAS5 increased insulin content and potentiated insulin release (46), while NR3C2 was associated with reduced insulin secretion (47). The up-regulation of PAX4 and down-regulation of FGF2 and MYC point to a restoration of a more differentiated beta cell phenotype (48,49). The present study provides a comprehensive gene set correlated with beta cell functional plasticity in T2D pancreatic islets, including many novel targets to potentially restore beta cell function.

To explore this further, the islet differential gene expression signature was mined in the Connectivity Map and LINCS repositories of human cell transcriptomes induced by chemical and genetic perturbagens (Figure 5). In particular, searching for chemical perturbagens may lead to drug repurposing, an attractive approach that lowers drug development costs and shortens development timeframes (50) (Figure 5). Among the top drug hits that might potentially improve beta cell function in T2D are JAK inhibitors, that act downstream of type I and II IFN receptors and are being considered in T1D (51,52). Src family tyrosine kinases tonically inhibit Ca\textsuperscript{2+}-dependent insulin secretion, and Src inhibitors enhanced rat INS-1 cell and islet insulin secretion (53). Src inhibitors recovered beta cell function following palmitate exposure in-vitro, in islets from obese diabetic and hyperlipidemic KK-A\(^\text{Y}\) mice (54) or diabetic Goto–Kakizaki rats (55).

Among the genetic perturbagens, seven pertained to neurotrophin signaling that plays a role in beta cell function (56) through actin cytoskeleton remodeling (57) which was also among the top pathways (Figure 5C). Chemokine and MAPK signaling, both with well known roles, among others, in the regulation of inflammatory processes (58-62) were further identified as potential pathways to improve islet beta cell function in T2D. Pancreatic islets in T2D show histological and molecular signs of inflammation (22,63-67,present study) and certain anti-inflammatory approaches, such as the use of interleukin-1-receptor antagonism, have been shown to improve insulin secretion in T2D patients and subjects with impaired glucose tolerance (68,69). However, it is still unclear whether these anti-inflammatory therapies act to reduce inflammation in islets or in other metabolically relevant tissues (70), and information is scant on how to more specifically target the beta cell. Of interest, the anti-inflammatory cytokines IL-4 and IL-10 have a partial, direct protective action on human beta cells ex-vivo against the effects of pro-inflammatory cytokines (IL-1beta, TNF alpha and
other potentially targetable pathways that we identified were ERBB signaling and autophagy. ERBB proteins are receptor tyrosine kinases expressed in several tissues, where they are involved in cell development, proliferation and differentiation (73, 74). Downregulation of Epidermal Growth Factor receptor (EGFR, a member of the ERBB family) signaling in rodent islets causes reduced post-natal beta cell growth and impaired insulin secretion (75), and stimulation of EGFR and ERBB-2 receptors (another component of this tyrosine kinase family) by betacellulin induces beta cell regeneration in INS-1 cells and murine islets by increasing IRS-2 expression (76).

Dysregulated autophagy has been associated with beta cell dysfunction and death in T2D (77-79), and is involved in beta cell derangement induced by lipotoxicity (80). Incubation of T2D islets with rapamycin, an activator of autophagy, determines improves beta cell ultrastructure, insulin secretory function and survival (80).

In conclusion, the present study shows that defective GSIS typical of T2D islets can be rescued, at least in part, by a period of exposure to a “non-diabetic” milieu, demonstrating relevant functional plasticity of T2D beta cells and their resistance to long-term metabolic stress of T2D. Importantly, we found that this beta cell functional recovery is associated with specific transcriptomic traits, pointing to known as well as novel targets to induce T2D remission. The overall scenario unveiled here implies that the functional recovery of human T2D beta cells is linked to changes in multiple genes. Several of such genes may need to be modulated correct T2D metabolic alterations in a targeted beta cell oriented pharmacological strategy.

**Methods**

**Study design**

The design of the study is summarized in Figure 1. Islets, isolated from the pancreas of T2D donors, were first cultured short-term in control medium to allow recovery from the isolation stress, and at the end of this period they were evaluated in terms of GSIS and transcriptome features (“basal” islet assessment). Afterwards, aliquots were cultured (second period of culture) at either 5.5 (“near
normoglycemia” condition) or 11.1 (“moderate hyperglycemia” condition) mmol/l glucose, followed by GSIS and transcriptome analysis (“cultured” islet assessment).

Based on GSIS after the second period of culture at 5.5 mmol/l glucose in comparison with the first one, islets were classified as “improvers” or “non-improvers” (see below). Functional and/or biosystems analysis comparisons (see below) were performed of islets at the different experimental conditions.

Islet preparation and culture conditions
Islets were isolated from the pancreas of 19 T2D organ donors [11 males, 8 females; age: 76.4±1.5 years; body mass index: 26.5±0.7 kg/m²; known duration of diabetes: 9.7±1.5 years; intensive care unit stay: 4.6±1.0 days; mean glycemia during intensive care unit stay: 10.8 ±0.6 mmol/l (see Table S1 for details)] as previously described (22), with permission by the Ethics Committee of the University of Pisa, upon written consent of donors’ next-of-kin. Purity of the isolated islets, assessed by dithizone staining in 17 preparations, was 49±12%, and islets were handpicked to accomplish functional and molecular studies.

To allow recovery from the isolation process, islets were cultured for 2.4±0.2 days (termed “basal” islets) in M199 medium (Euro-Clone, Milano, Italy) containing 5.5 mmol/l glucose, supplemented with 0.1 mg/ml L-glutamine (Sigma Aldrich, St Louis, MO, US), 10% adult bovine serum (Sigma-Aldrich), 100 UI/l penicillin (Sigma-Aldrich), 100 mg/l streptomycin (Sigma-Aldrich), 0.25 μg/ml amphotericin B (Sigma-Aldrich) and 50 mg/l gentamycin (Sigma-Aldrich). The pH was adjusted to 7.35-7.40 and the solution filtered with a PES 0.2 Vacuum filter (Sarstedt, Numbrecht, Germany). Afterwards, islets were cultured for 3.6±0.4 days in fresh culture medium (termed “cultured” islets), containing either 5.5 or 11.1 mmol/l glucose.

Insulin secretion studies
Insulin secretion in response to acute glucose stimulation (3.3 and 16.7 mmol/l glucose) was assessed in 15 handpicked islets of similar size (approximate diameter 100-150 μm) by batch incubation, as previously detailed (22,37). Insulin was quantified (μU/ml) by a radioimmunometric assay (DIAsource ImmunoAssays, Nivelles, Belgium), islet insulin content was measured after acid-alcohol extraction, and ISI was calculated as the ratio of insulin release at 16.7 mmol/l glucose over release at 3.3 mmol/l glucose.
Transcriptome studies

Islet RNA was prepared as previously reported (22). Briefly, RNA from approximately 120 hand-picked islets was prepared using the RNeasy MINI Kit + QIAshredder (Qiagen, Hilden, Germany) by cell lysing and homogenizing steps, followed by DNA digestion using RNase-Free DNase Set (Qiagen). Total RNA concentration and purity were evaluated using the NanoDrop™ 2000c Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). RNA RIN values were assessed using Agilent Bioanalyzer 2100 (Agilent Technologies, Wokingham, UK) and Agilent RNA Nano Chips (Agilent Technologies), and were 7.8±0.23 for “basal” islets and 8.4±0.17 for “cultured” islets, demonstrating suitability for sequencing. Libraries were prepared using the TruSeq Stranded mRNA Library Prep Kit (Illumina, San Diego, CA, USA). Briefly, mRNA was purified from 0.40 µg total RNA using poly-T oligo attached magnetic beads, was then fragmented, and primed with random hexamers for reverse transcription. Second strand synthesis was performed by incorporating dUTP in place of dTTP to generate blunt-ended ds cDNA, followed by adenylation of the 3’ ends and adaptor ligation. Selective enrichment of DNA fragments with adapter molecules on both ends and DNA amplification were obtained by PCR. The generated libraries were quantified using qPCR and quality was assessed using Agilent Bioanalyzer 2100 (Agilent Technologies) and Agilent DNA 1000 chips (Agilent Technologies). All the libraries had concentration and size (300 bp) suitable for sequencing, which was performed on the Illumina HiSeq 2500 instrument (Illumina). Paired-end sequencing (2x100bp) at 170 million reads was performed.

The reads were aligned to GENCODE (81) version 28, using STAR (82) version 2.6.0c, with default options. The counts were then generated using htseq-count (83) version 0.9.1. Quality control was performed, and 1 sample was considered as technical outliers and removed. The number of samples used for the analysis were 20: 5 “improvers”, “basal” and “cultured”, and 5 “non-improvers”, “basal” and “cultured”. Based on Table S1, the identification codes were: “improvers”, 19/71, 21/40, 21/91, 24/25 and 24/53; “non-improvers”, 19/64, 21/26, 24/34, 24/49, 24/65. Genes were filtered for a minimal average expression of 1 cpm. Reads were normalized for library size using the edgeR Bioconductor package. Differential expression analysis was performed using limma function with voom approach from the limma Bioconductor package. The transcriptome of “cultured” vs “basal” islets was compared to identify DEGs, with a significance threshold of 0.05 (Benjamini-Hochberg corrected p-value, FDR). Gene Set Enrichment Analysis was
conducted with fGSEA (v. 1.16) on the Biological Process sets from Gene Ontology (version 7.2), with the parameters minSize=15 and maxSize=500. Input gene lists were ranked according to signed significance (-log10 p-value * fold-change sign) of the tested contrasts. The association between gene expression and ISI was tested by linear regression analysis for each gene, using relative gene expression (independent variable) and ISI (dependent variable) changes between cultured and basal conditions, according to the formula $\frac{\text{Cultured} - \text{Basal}}{\text{Basal}} \times 100$.

**Connectivity Map and iLINCS analyses**

The top 150 up- and down-regulated genes (FDR < 0.05) from the “improver” and “non-improver” islets (see Results) were processed with the cloud-based CLUE software (https://clue.io) and iLINCS tool (http://www.ilincs.org/ilincs/) for comparison with Connectivity Map (85) and LINCS (86) databases, respectively. Differential signatures of “improver” islets were matched with the ones in Connectivity Map/LINCS databases that contain many human cell lines exposed to experimentally and clinically used chemical or genetic perturbagens. Positively correlated perturbagens were considered as potential therapeutic hits as they produce similar gene expression programs as “improver” islets and hence may help restore beta cell function. Similar analyses were performed for the top DEG signature of “non-improver” islets. Since the latter differential signature may be due to time in culture and is not related to beta cell functional recovery, we retained as hits of interest the positively correlated perturbagens specific to the “improvers” and removed perturbagens of the “non-improvers”.

**Statistical analysis**

Clinical and islet functional data are presented as mean ± SEM. Differences between groups were assessed by two-tailed Student’s t-test or ANOVA followed by Tukey correction, as appropriate. A p-value < 0.05 was considered statistically significant.
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Table 1. Top 20 up-regulated and down-regulated genes in the “Improvers” islets group, cultured vs basal

| Gene symbol | Gene description | Log2FC | P value | FDR_BH |
|-------------|------------------|-------|---------|--------|
| ENSG00000136881 BAAT | bile acid-CoA:amino acid N-acyltransferase | 2.29 | 2.42E-06 | 0.01244 |
| ENSG00000127249 ATP1A4 | ATPase 1A4 | 1.85 | 2.43E-05 | 0.01450 |
| ENSG0000013048 CHI3L1 | chitinase 3 like 1 | 1.37 | 3.98E-05 | 0.01450 |
| ENSG0000014337 CTSK | | 2.01 | 3.75E-05 | 0.01450 |
| ENSG00000150594 ADRA2A | adrenoceptor alpha 2A | 1.24 | 3.76E-05 | 0.01450 |
| ENSG00000090382 LYZ | lysozyme | 2.52 | 1.48E-06 | 0.01244 |
| ENSG00000108342 NES | | 2.19 | 3.17E-06 | 0.01244 |
| ENSG00000120156 TEK | TEK receptor tyrosine kinase | 1.14 | 3.33E-06 | 0.01244 |
| ENSG00000146755 CTSK | | 1.05 | 1.53E-05 | 0.01450 |
| ENSG00000117984 CTSD | cathepsin D | 0.98 | 1.70E-05 | 0.01450 |
| ENSG00000109819 PPARGC1A | PPARG coactivator 1 alpha | 1.34 | 2.08E-05 | 0.01450 |
| ENSG00000127533 PROM1 | prominin 1 | 1.35 | 2.16E-05 | 0.01450 |
| ENSG00000138755 CXCL9 | C-X-C motif chemokine ligand 9 | 1.68 | 2.60E-05 | 0.01450 |
| ENSG00000146755 TRIM50 | tripartite motif containing 50 | 2.54 | 2.78E-05 | 0.01450 |
| ENSG00000099953 MMP11 | matrix metallopeptidase 11 | 2.76 | 7.11E-06 | 0.01450 |
| ENSG00000118257 NRP2 | | 2.47 | 1.42E-05 | 0.01450 |
| ENSG00000123496 RHCG | | 1.05 | 1.53E-05 | 0.01450 |
| ENSG00000118905 ACPP | acid phosphatase, prostate | 1.40 | 3.67E-05 | 0.01450 |
| ENSG00000127249 CTSD | cathepsin D | 0.98 | 1.70E-05 | 0.01450 |
| ENSG00000109819 PPARGC1A | PPARG coactivator 1 alpha | 1.34 | 2.08E-05 | 0.01450 |
| ENSG00000127533 PROM1 | prominin 1 | 1.35 | 2.16E-05 | 0.01450 |
| ENSG00000138755 CXCL9 | C-X-C motif chemokine ligand 9 | 1.68 | 2.60E-05 | 0.01450 |
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| ENSG00000099953 MMP11 | matrix metallopeptidase 11 | 2.76 | 7.11E-06 | 0.01450 |
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| ENSG00000123496 RHCG | | 1.05 | 1.53E-05 | 0.01450 |
Figure 1. Experimental design. Islets were isolated from the pancreas of T2D donors by enzymatic digestion and density gradient purification, to be then cultured short-term in control medium to allow recovery from the isolation stress. At the end of this first culture period, the islets were evaluated in terms of GSIS and transcriptome features (“basal” islet assessment). Afterwards, islets were cultured (second period of culture) at either 5.5 (“near normoglycemia” condition) or 11.1 (“moderate hyperglycemia” condition) mmol/l glucose, followed by GSIS and transcriptome analysis (“cultured” islet assessment). Based on GSIS after the second period of culture at 5.5 mmol/l glucose vs the first period, the islets were classified as “improvers” or “non-improvers”. Functional and/or biosystems analysis comparisons were performed with islets under the different experimental conditions.
Figure 2. Functional studies. A: ISI increased significantly in the “improver” group (n: 12 islet preparations) and tended to decrease in the “non-improver” group (n: 7 islet preparations). B: This was mainly due to better insulin release in response to 16.7 mmol/l glucose in the “improvers” after culture. C: Similar results were observed after normalization for islet insulin content (n: 10 islet preparations for “improvers” and 7 islet preparations for “non-improvers”). D: ISI did not improve in any of the tested islet preparations cultured at 11.1 mmol/l glucose. Data are expressed as mean ± SEM and each point represents the average value of 1-3 replicates for each islet preparation. Statistical analysis was performed by two-tailed Student’s t-test or ANOVA followed by Tukey correction.
Figure 3. Islet gene expression evaluation. A-B: Differential gene expression analyses. Volcano plots report for each gene the difference in terms of fold-change and significance (FDR), between “cultured” and “basal” islet preparations, for “improvers” (panel A) and “non-improvers” (panel B). DEGs are above the red lines, marking the 0.05 FDR significance threshold. C-F: Overlapping DEGs between the series. The Venn diagrams show the number of DEGs that are shared or unique to each of the conditions analyzed. The comparisons include genes regulated in the same or opposite directions.
Figure 4. Enriched pathways and correlations between gene expression and GSIS. A-B: Most significantly enriched functional pathways in the “improvers”, cultured vs basal islets. The bar plots show significance and normalized enrichment scores of the 20 most significantly enriched Gene Ontology terms (Biological Pathway, BP), either in a negative (panel A) or positive (panel B) direction. C-D: Most significant associations between gene expression and insulin secretion changes (cultured vs basal). The relationships are represented with scatterplots. The results of the regression analysis are reported in each plot, together with the corresponding regression lines and confidence intervals. The 8 genes with the most significant positive (panel C) and negative (panel D) association are shown.
Figure 5. Mining differential signatures to identify potential therapeutic targets. A: The top 150 up- and down-regulated genes were submitted to the Connectivity Map database to identify chemical perturbagen classes driving a similar signature (positive tau score) to the one present in the “improver” and “non-improver” series. Only classes with a median tau score > 80 were considered. Src, src kinase family of tyrosine kinases; JAK, Janus kinase family of enzymes; HSP, heat shock protein; FLT3, fms related receptor tyrosine kinase 3; EGFR, epidermal growth factor receptor. B: The same methodology and criteria were applied to genetic perturbagens in the Connectivity Map. Results are shown for the “improver” series; for the “non-improvers” no genetic perturbagen had a median tau score > 80. C: The gene signatures were also submitted to the LINCS database. The top 10 signaling pathways specific to the “improver” islets are shown, with the bar length/number indicating the number of identified genetic perturbagens pertaining to the pathway.
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS2.pptx
- TableS1.pptx
- TableS3.xlsx
- TableS4.xlsx
- TableS7.xlsx
- TableS8.xlsx
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- TableS5.xlsx
- TableS6.xlsx