Age is the greatest risk factor for the development of type 2 diabetes mellitus (T2DM). Age-related decline in organ function is attributed to the accumulation of stochastic damage, including damage to the nuclear genome. Islets of T2DM patients display increased levels of DNA damage. However, whether this is a cause or consequence of the disease has not been elucidated. Here, we asked if spontaneous, endogenous DNA damage in β-cells can drive β-cell dysfunction and diabetes via deletion of Ercc1, a key DNA repair gene, in β-cells. Mice harboring Ercc1-deficient β-cells developed adult-onset diabetes, as demonstrated by increased random and fasted blood glucose levels, impaired glucose tolerance, and reduced insulin secretion. The inability to repair endogenous DNA damage led to an increase in oxidative DNA damage and apoptosis in β-cells and a significant loss of β-cell mass. Using electron microscopy, we identified β-cells in clear distress that showed an increased cell size, enlarged nuclear size, reduced number of mature insulin granules, and decreased number of mitochondria. Some β-cells were more affected than others, consistent with the stochastic nature of spontaneous DNA damage. Ercc1-deficiency in β-cells also resulted in loss of β-cell function as glucose-stimulated insulin secretion and mitochondrial function were impaired in islets isolated from mice harboring Ercc1-deficient β-cells. These data reveal that unrepaired endogenous DNA damage is sufficient to drive β-cell dysfunction and provide a mechanism by which age increases the risk of T2DM.

Introduction

Type 2 diabetes mellitus (T2DM) is a progressive metabolic disease characterized by chronic hyperglycemia. Worldwide, approximately 10% of the adult population is affected by T2DM, and the incidence is expected to rise as a result of increased life expectancy and obesity. T2DM is a major health problem as it increases the risk of comorbidities such as cardiovascular disease, nephropathy, neuropathy, and retinopathy. Hyperglycemia has long been considered to be primarily the consequence of insulin resistance in peripheral tissues, resulting in increased secretion of insulin by pancreatic β-cells, followed by β-cell exhaustion and, ultimately, β-cell failure. Beta-cell dysfunction is an invariant finding among individuals with T2DM, and reduced β-cell function is an important step in the progression to T2DM.

Age is a major risk factor for T2DM, with the majority of new diagnoses occurring in the fifth or sixth decade of life. Beta-cell function decreases with age, which can be independent of peripheral insulin resistance. Numerous cell-autonomous factors have been implicated in the decline in β-cell function with age, including mitochondrial dysfunction, replicative senescence, impaired autophagy, altered Ca2+ metabolism, and reduced expression of genes important for β-cell function. This lack of precise mechanism underlying age-related loss of β-cell function impedes the design of rational strategies for disease prevention.

One of the key mechanisms that drives aging is the accumulation of macromolecular and organelle damage. DNA damage is one type of macromolecular damage that is causally linked to aging. Islets from T2DM patients and mouse models of T2DM display increased levels of DNA damage. Furthermore,
diabetes is often seen in genomic instability disorders like ataxia telangiectasia\textsuperscript{10}, Werner syndrome\textsuperscript{11}, and Fanconi anemia\textsuperscript{12}, suggesting the involvement of DNA damage in T2DM development. Although the accumulation of DNA damage has been found in T2DM individuals, it is not known whether this is a cause or consequence of T2DM. Previously, we found that progeroid Ercc1\textsuperscript{−/−} mice, which have a systemic deficiency in the key DNA repair gene Ercc1, showed a reduced β-cell area and diminished glucose-stimulated insulin secretion\textsuperscript{13}. Here, we deleted Ercc1 specifically in β-cells to ask if endogenous DNA damage in β-cells is sufficient to induce β-cell dysfunction through a cell-autonomous mechanism.

**Methods**

**Animals**

Ercc1\textsuperscript{+/−} mice were bred as described\textsuperscript{14} and crossed to Ins2-cre mice from Jackson Laboratory (Stock #003573) to generate inbred C57Bl/6J Ins2-cre Ercc1\textsuperscript{+/−} breeders or Ins2-cre mice (Stock #026801) to generate inbred C57Bl/6J Ins2-cre Ercc1\textsuperscript{−/−} breeders. They were then crossed with inbred FVB/N Ercc1\textsuperscript{+/−} mice to generate Ins2-cre Ercc1\textsuperscript{+/−} or Ins2-cre Ercc1\textsuperscript{−/−} mice on a genetically uniform C57Bl/6J:FVB/N F1 hybrid background. Ins2-cre Ercc1\textsuperscript{−/−} mice were maintained and characterized at separate institutes in The Netherlands (NL) and the USA. Mice generated in NL used an Ercc1\textsuperscript{−/−} allele targeting exons 3–5, whereas mice generated in the USA used an Ercc1\textsuperscript{−/−} allele targeting exons 7–10 (Fig. S1)\textsuperscript{15}. Ins2-cre Ercc1\textsuperscript{−/−} mice were bred and characterized in the USA and used the Ercc1\textsuperscript{−/−} allele targeting exons 7–10.

Cre recombinase activity itself has been reported to induce DNA damage, apoptosis, and toxicity\textsuperscript{16}. Ins2-cre Ercc1\textsuperscript{+/−} and Ins1-cre Ercc1\textsuperscript{+/−} (Cre-positive, flox-negative), Ercc1\textsuperscript{+/−} (Cre-negative, flox-negative, i.e., wild-type mice), and Ercc1\textsuperscript{−/−} (Cre-negative, flox-positive) mice were used as controls (all in a C57Bl/6J:FVB/N F1 hybrid background). Animals were housed in a light- and temperature-controlled facility with access to water and standard chow (USA: 2020X Teklad, negative, Fl), characterized in the USA and used the

**Electron microscopy (EM)**

For large-scale EM studies (nanotomy), pancreas tissue (2 x 2 mm) isolated from 3- to 4-month-old mice (n = 3 per genotype) was fixed in 2% glutaraldehyde and 0.5% paraformaldehyde in 0.1M cacodylate buffer. Agarose-embedded islets were sectioned (50 μm), postfixed with osmium, embedded in Epon epoxy resin, ultra-thin sectioned (80 nm), and contrasted as described\textsuperscript{17}, with modifications\textsuperscript{18}. Nanotomy data were obtained using a Supra 55 (Zeiss, White Plains, NY) in scanning transmission EM (STEM) mode at 29KV. Beta-cell size, nuclear size, mitochondrial number, and mitochondrial area were quantified on 60–100 β-cells per group using Fiji. Mature insulin granule area was quantified on 30–50 β-cells per group. Nanotomy data are available at full resolution via http://www.nanotomy.org.

**Islet studies**

Islets were isolated from pancreata as described\textsuperscript{13} and snap frozen for subsequent RNA, DNA, or protein isolation. For insulin content measurement, islets were lyzed in NP40 buffer, protein content was measured by a DC protein assay (Bio-Rad, Hercules, CA), and insulin was measured by ELISA (Mouse-Insulin Ultra-Sensitive ELISA, Alpco, Salem, New Hampshire). Pancreatic islet levels of 8-oxo-guanine (Abcam) were measured by ELISA using a Varioskan plate reader (Thermo-Fisher). For insulin secretion assays, islets were cultured overnight in RPMI containing 10% FBS and 1% penicillin/streptomycin. Similar-sized islets were handpicked and incubated in Krebs-Ringer bicarbonate (pH 7.5) containing 0.1% bovine serum albumin (BSA) and 2.8 mM glucose for 2 hours, after which 10 islets/well were plated in 1.67 mM glucose or 16.7 mM glucose for 1 hour. After incubation, medium was removed, and insulin was measured by ELISA (Mouse-Insulin Ultra-Sensitive ELISA, Alpco). Mitochondrial respiration was measured by a Seahorse XF24 Extracellular Flux Analyzer (Agilent, Santa Clara, CA). After overnight culture, 70 islets/well were preincubated in Seahorse XF assay medium containing 3 mM glucose and 1% FBS for 2 hours at 37 °C without CO\textsubscript{2} then transferred to the XF flux analyzer for respiration measurement. Glucose (20 mM) was used to stimulate oxygen consumption and oligomycin (5 μM) to inhibit ATP synthesis. The data shown are an average of ≥2 replicates from 4 to 6 mice/group.

P16 in situ hybridization

The detection of p16 mRNA was performed as described\textsuperscript{19}. Pancreas sections were deparaffinized, rehydrated, and boiled in sodium citrate buffer. Slides were prehybridized in a 4X saline-sodium citrate (SSC) solution containing 3% BSA

Histology, immunostaining, and histopathological analysis

Formalin-fixed pancreatic tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). For immunofluorescence, primary antibodies against insulin (Abcam, Cambridge, MA, 1:500), glucagon (Dako, Glostrup, Denmark, 1:500), and Ki67 (Abcam, 1:500), and secondary antibodies conjugated to FITC or Cy3 (Thermo-Fisher, Waltham, MA, 1:250) were used. Apoptotic cells were detected using the Apoptag\textsuperscript{8} In Situ Apoptosis Detection Kit (Roche, Indianapolis, IN). For β-cell area measurements, the percentage of insulin-positive area was determined using ImageScope (Aperio, Sausalito, CA) from eight evenly spaced sections per pancreas. Beta-cell mass was calculated by multiplying the percentage of insulin-positive area by pancreas weight. The percentage of glucagon\textsuperscript{+} cells per islet was quantified on 10 islets per animal. Beta-cell proliferation was calculated using the percentage of Ki67\textsuperscript{+} insulin\textsuperscript{+} cells in at least 1000 β-cells per animal. H&E-stained sections were evaluated for inflammation, degeneration, necrosis, amyloid deposition, and atrophy or loss of zymogen granules within the acinar cells.
at 55 °C. Slides were then incubated with either a scrambled nonspecific probe or a custom-designed p16 LNA probe (5′-TCTGATGACACCTTCTCCCT-3′, Exiqon, Woburn, MA, USA) diluted in hybridization buffer containing 10% dextran sulfate in 4X SSC. Slides were hybridized at 55 °C for 1 hour and then submitted to a series of five washes of decreasing stringency. Tissues were stained with an anti-insulin antibody before being imaged using confocal scanning laser microscopy.

RNA isolation and quantitative PCR (qPCR)

Tissues were harvested and snap frozen in liquid nitrogen. Tissues were homogenized using the FastPrep-24 homogenizer (MP Biomedicals, Irvine, MD) or by vortexing (islets), and total RNA was isolated by Trizol extraction according to the manufacturer’s specifications (Thermo Fisher). RNA was reverse transcribed, and qPCR was performed as described13, using primers in Table S1. Gene expression levels were normalized to Gapdh.

Statistical analysis

Graphpad Prism 9.1.0 was used for statistical analysis. Data are presented as mean ± standard deviation. Differences between group were calculated by unpaired Student’s t-test with a P value of 0.05 is considered significant. Multiple comparisons were tested using a one-way analysis of variance (ANOVA) followed by a Dunnett’s multiple comparisons test. A repeated measurement two-way ANOVA, followed by Bonferroni post hoc tests, was used to evaluate glucose tolerance, body weight, and oxygen consumption rate (OCR) in time.

Results

Adult onset of hyperglycemia in mice harboring Ercc1-deficient β-cells

To examine the effect of increased DNA damage on β-cell function, the key DNA repair gene Ercc1 was deleted in the β-cells of mice. Ercc1 encodes one subunit of ERCC1-XPF, a structure-specific endonuclease required for nucleotide excision repair (NER) of bulky DNA adducts, as well as repair of interstrand crosslinks and some DNA double-strand breaks50. Deletion of Ercc1 causes destabilization of the holoenzyme21 and consequently accelerates the accumulation of spontaneous, endogenous oxidative DNA damage caused by normal metabolism22. Two independent Ins2-cre Ercc1+/− mouse lines were generated by crossing mice carrying a floxed allele of Ercc1 and mice carrying a knockout allele. The floxed allele of Ercc1 differed between the mouse lines, which were maintained and characterized at separate institutes in NL and USA (Fig. S1). Ins2-cre Ercc1+/− mice developed normally and were born with Mendelian frequency (Table S2). The Cre recombinase efficiently excised Ercc1 in pancreatic β-cells, as shown by decreased Ercc1 mRNA in islets isolated from Ins2-cre Ercc1+/− mouse pancreata compared to littermate controls in both mouse lines (Fig. 1A, red bars NL, blue bars USA). Oxidative DNA damage was significantly elevated in pancreatic β-cells from Ins2-cre Ercc1+/− mice (Fig. 1B), consistent with prior findings in mice lacking Ercc123.

Ins2-cre Ercc1+/− mice (NL) showed significantly higher random and fasted blood glucose levels compared to littermate controls (Fig. 1C, Fig. S2A). However, this increase did not occur until adulthood, indicating a degenerative process in the islets rather than a developmental problem. In line with this, oral glucose tolerance was impaired in 3–5-month-old male and female Ins2-cre Ercc1+/− mice (Fig. 1D, Fig. S2B). Ins2-cre Ercc1+/− mice showed normal fasting insulin levels but significantly impaired glucose-stimulated insulin secretion compared to control mice (Fig. 1E). Ins2-cre Ercc1+/− mice (USA) showed a similar glucose intolerance in both sexes as evidenced by hyperglycemia in 3–4-month-old mice (Fig. 1F–H, Fig. S2C) as well as decreased serum insulin (Fig. 1I) and C-peptide (Fig. 1J) in a non-fasted state. In both lines, Ins2-cre Ercc1+/− mice showed an increased body weight at 4 months of age compared to littermate controls (Fig. S3, USA and NL). Notably, the response to glucose challenge was unaffected in mice-expressing Ins2-cre in the absence of a floxed allele of Ercc1, as were random and fasted serum glucose levels and insulin secretion (Fig. S4, NL and USA). Moreover, glucose homeostasis did not differ between age-matched wild-type and heterozygous animals (Fig. S4). Hence, littermates of Ins2-cre Ercc1+/− mice were analyzed together as controls. These data indicate that spontaneous, endogenous DNA damage in β-cells, if not repaired, is sufficient to drive impaired glucose homeostasis, illustrating a cell-autonomous mechanism of β-cell dysfunction that in wild-type mammals would be age-dependent22.

ERCC1 deficiency leads to loss of β-cell mass

The diminished secretion of insulin in Ins2-cre Ercc1+/− mice (Fig. 1E,I) could be mediated by a reduction in β-cell mass, impairment of β-cell function, or a combination of both. To determine if the reduced insulin secretion resulted from a reduction in islet mass, pancreatic weight was measured. Mutant and control mice showed no difference in pancreatic weight at 3–4 months of age, a time point when glucose homeostasis was impaired (Fig. 2A). In addition, loss of Ercc1 in β-cells did not induce major changes in islet architecture in the small islets of 4-month-old mice. However, larger islets showed an increase in glucagonexpressing α-cells and diminished insulin staining (Fig. 2B,C). Beta-cell mass was significantly decreased in adult Ins2-cre Ercc1+/− mice (Fig. 2D). The distribution of islet size did not differ between mutant and control mice at 3–4 months of age (Fig. 2E). However, there were significantly more apoptotic TUNEL-positive β-cells in the islets of Ins2-cre Ercc1+/− mice (Fig. 2F), while the fraction of proliferating Ki67-positive β-cells was not altered (Fig. 2G). Islet insulin content was reduced in islets isolated from Ins2-cre Ercc1+/− mice compared to age-matched littermate controls (Fig. 2H), consistent with decreased insulin staining and a patchy β-cell appearance within islets (Fig. 2B).

Visualization of the anatomy of islets using large-scale EM revealed the presence of significantly enlarged β-cells and enlarged irregular nuclei in islets from Ins2-cre Ercc1+/− mice (Fig. 2I–K), features previously reported for Ercc1-deficient hepatocytes and fibroblasts24,25. Furthermore, Ins2-cre Ercc1+/− islets contained a larger population of β-cells with a significantly reduced number of mature insulin vesicles (Fig. 2L). In addition, degranulated β-cells with a high number of empty vesicles were observed more frequently in Ins2-cre Ercc1+/− islets (Fig. 2I), consistent with the decreased insulin content observed in Ins2-cre Ercc1+/− islets (Fig. 2H) and impaired insulin secretion in response to a glucose challenge (Fig. 1E,I). Together, these data indicate that deletion of Ercc1 affects β-cell mass and number as well as the insulin content of the remaining β-cells.

ERCC1 deficiency leads to loss of β-cell function

To further characterize changes in β-cells in Ins2-cre Ercc1+/− mice, multiple parameters were measured. Deletion of Ercc1 in

AgingBio, 1, 1–10, October 23, 2023
β-cells resulted in increased expression of the senescence marker p16Ink4a (Fig. 3A). In addition, islets isolated from Ins2-cre Ercc1fl−/− mice showed increased expression of p21Cip1 and Gadd45a (Fig. 3B), consistent with the notion that loss of Ercc1 results in the accumulation of endogenous DNA damage and thereby senescence.23

Insulin secretion in islets isolated from Ins2-cre Ercc1fl−/− mice was significantly reduced compared to control islets (Fig. 3C). This blunted response was not due to decreased insulin gene expression, as Insulin mRNA levels were modestly but significantly increased in Ins2-cre Ercc1fl−/− islets (Fig. 3D). Other genes involved in glucose metabolism or insulin processing were either marginally affected (Glut2) or remained unaffected in Ins2-cre Ercc1fl−/− islets relative to control animals (Fig. 3D). Gene expression of genes involved in β-cell identity, such as transcription factors Pdx1 or Nkx6.1, was unchanged; however, expression of Mafa was decreased in Ins2-cre Ercc1fl−/− islets relative to control animals (Fig. 3D). Expression of HK and Ldha1, genes normally repressed in mature β-cells, was modestly increased in Ins2-cre Ercc1fl−/− islets (Fig. 3D).

As insulin secretion is also controlled by the oxidative metabolism of glucose in mitochondria, we examined mitochondrial function in islets isolated from Ins2-cre Ercc1fl−/− mice. Basal OCR was equivalent between islets from Ins2-cre Ercc1fl−/− mice and controls (Fig. 3E). Upon stimulation with 20 mM glucose, control islets responded robustly, with a 1.8-fold increase in their OCR. In contrast, Ins2-cre Ercc1fl−/− islets showed reduced glucose-stimulated OCR (1.4-fold) under the same conditions (Fig. 3F). Inhibition of ATP synthesis by oligomycin, as measured by OCR, was reduced in β-cells from both Ins2-cre Ercc1fl−/− mice and controls, indicating that uncoupled respiration is similar in both groups. EM (Fig. 2I) revealed that loss of Ercc1 in β-cells resulted in decreased mitochondrial density and increased mitochondria size (Fig. 3G,H). Therefore, impaired insulin secretion...
by *Ins2-cre Ercc1*fl/*−* islets possibly arises as a consequence of reduced mitochondrial function, as previously reported in *Ercc1* deficiency\(^2^6\), in combination with reduced insulin content. Collectively, these data indicate that repair of spontaneous DNA damage in β-cells is critical to maintain β-cell mass and function.

**Ins1-cre Ercc1*fl/*− mice develop adult-onset hyperglycemia**

The insulin II promoter (*Ins2*) was previously reported to also drive Cre expression in the hypothalamus\(^2^7\). *Ercc1* mRNA levels, however, were not reduced in hypothalamic tissues from *Ins2-cre Ercc1*fl/*−* mice compared to controls (Fig. S5). In addition, expression of p21\(^{Cip1}\) or Gadd45a, measures of genotoxic stress, was not changed (Fig. S5). Although our results do not indicate *Ercc1* deletion in the hypothalamus, we aimed to verify our findings in a β-cell-specific *Ercc1* knock-out model without expression of Cre recombinase in neuronal cells: the *Ins1-cre Ercc1*fl/*−* mouse model. Similar to *Ins2-cre Ercc1*fl/*−* mice, *Ins1-cre Ercc1*fl/*−* mice showed significantly elevated random and fasted blood glucose levels as well as impaired glucose tolerance after an oral glucose bolus compared to control mice in both sexes (Fig. 4A–E, Fig. S2D). Again, perturbations in glycemic control did not occur until adulthood (Fig. 4A–E), supporting a degenerative process in the islets rather than a developmental defect in both models. Histological analysis of pancreatic sections revealed that islets of 10-month-old *Ins1-cre Ercc1*fl/*−* mice showed similar pathological changes to *Ins2-cre Ercc1*fl/*−* mice, including enlarged

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**Figure 2. Reduced β-cell mass and altered β-cell morphology in *Ins2-cre Ercc1*fl/*−* mice.** (A) Average weight of pancreatic tissue isolated from male *Ins2-cre Ercc1*fl/*−* mice and age-matched controls (n = 9–12). (B) Representative fluorescence images of islet morphology after immunostaining for insulin (green) and glucagon (red). Scale bars: 75 μm. (C) Quantification of the percentage of glucagon+ cells per islet on fluorescence images (n = 4–7). (D) Beta-cell mass of *Ins2-cre Ercc1*fl/*−* male mice and age-matched controls (n = 9–14). (E) Ilet size distribution of islets from *Ins2-cre Ercc1*fl/*−* male mice and age-matched controls (n = 9–12). (F) Detection of β-cell apoptosis by TUNEL assay and percentage of TUNEL+ β-cells per islet were calculated (n = 6–9). (G) The proliferation of β-cells was determined by quantification of the percentage of Ki67+ insulin+ cells in the same mice as panel (F). (H) Ilet insulin content was measured by ELISA in samples isolated from *Ins2-cre Ercc1*fl/*−* mice and age-matched controls (n = 4–9). (I) Representative electron microscopy (EM) images illustrating ultrastructural changes in islets of *Ins2-cre Ercc1*fl/*−* mice compared to genetic control animals. Scale bars: 5 μm. (J) Beta-cell size and (K) nuclear size measured in EM images from *Ins2-cre Ercc1*fl/*−* mice and genetic control mice (40–60 β-cells per group originating from n = 3 mice per genotype). (L) Area of mature insulin granules measured in EM images of β-cells from *Ins2-cre Ercc1*fl/*−* and genetic control mice (20–30 β-cells per group originating from n = 3 mice per genotype). Data represent mean ± SD, unpaired Student’s t-test, or repeated measurement two-way ANOVA. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001. All data were collected from 3- to 4-month-old male mice at the University Medical Center Groningen (UMCG) (NL).
β-cells and enlarged irregular nuclei (Fig. 4F,G). Collectively, these findings show that deletion of Ercc1 only in β-cells can drive adult-onset hyperglycemia.

Discussion

Here, we demonstrate that loss of the DNA repair gene Ercc1 in β-cells of mice results in the development of adult-onset diabetes due to loss of β-cell mass and β-cell function. This phenotype is observed in mice with two distinct floxed alleles of Ercc1 and expression of the Cre recombinase driven by either the insulin I or insulin II promoter. These results strongly suggest that failure to repair spontaneously occurring endogenous DNA damage in β-cells is sufficient to drive β-cell dysfunction and T2DM. Notably, the same spontaneous DNA lesions accumulate with aging in wild-type mice and humans that have a normal level of DNA repair, raising the possibility that endogenous DNA damage is a physiologically relevant cause of β-cell loss and dysfunction.

In support of this, single-cell analysis of human endocrine pancreas from healthy donors revealed that aging is accompanied by a gradual accumulation of DNA mutations. Pancreatic β-cells display an enrichment of a specific mutational signature, p16 expression, and oxidative damage with increasing chronological age. Interestingly, endocrine pancreatic cells show a higher somatic mutation frequency relative to brain tissue. Pancreatic β-cells are under high metabolic demand, with a relatively high rate of ATP-dependent processes such as protein synthesis and
secretion. Furthermore, insulin secretion is coupled to nutrient oxidation by the generation of ATP and the production of secondary messengers such as mitochondria-derived reactive oxygen species. Therefore, β-cells express relatively low levels of antioxidant enzymes.

As β-cells are long-lived, terminally differentiated cells, DNA repair is predicted to be crucial to maintain their metabolic function. Indeed, we show that failure to repair DNA damage in β-cells due to the loss of Ercc1 results in increased levels of oxidative DNA lesions that were previously associated with T2DM. DNA repair capacity is reported to decline with chronological age. NER, which requires ERCC1, is reduced in DNA repair capacity is predicted to be crucial to maintain their metabolic function.

It is therefore unlikely that persistent DNA damage in playing a causal role in β-cell dysfunction and loss. Beta-cell mass was reduced in adult Ins2-cre Ercc1fl/mice, and using EM, we noticed variable levels of distressed β-cells in the islets of Ins2-cre Ercc1fl/mice. This finding is in line with the stochastic nature of spontaneous DNA damage and the heterogeneity of β-cells within an islet. Others have shown that mice with a β-cell deficiency in the transcription factor Yin Yang 1 (YY1) developed severe β-cell loss and diabetes. YY1 regulates key biological processes, including cell identity, cell cycle control, DNA damage recognition, and DNA repair. Interestingly, YY1 is downregulated in β-cells of prediabetic and diabetic db/db mice and of human donors with T2DM. YY1-deficient mice already displayed a diabetic phenotype at 2–3 weeks of postnatal life, indicating a role for YY1 in β-cell development. In contrast, loss of Ercc1 did not result in an early life phenotype, as evident from normal glucose homeostasis in young β-cell-specific Ercc1-deficient mice and normal β-cell area in young Ercc1fl/mice. It is therefore unlikely that Ercc1 plays a key role during the differentiation or maturation of β-cells.

Persistent DNA damage in β-cells is reported to result in apoptosis and senescence. Both cell fates are detected in the islets of Ins2-cre Ercc1fl/mice (Figs. 2F, 3A,B). Previously, we demonstrated that loss of Ercc1 resulted in oxidative DNA damage and consequently senescence. Furthermore, we found evidence of increased senescence in the pancreas of Ercc1fl/mice and naturally aged wild-type mice. Systemic loss of Ercc1 has further been reported to drive pancreatic pathology such as fibrosis, loss-of-tissue architecture, and chronic pancreatitis through a mechanism likely involving cellular senescence. The exocrine

Figure 4. Ins1-cre Ercc1fl/mice develop adult-onset hyperglycemia. (A) Blood glucose levels of non-fasted Ins1-cre Ercc1fl/mice and age-matched controls at multiple ages (both sexes, n = 4–24; USA). (B) Blood glucose levels of fasted male Ins1-cre Ercc1fl/mice by age (n = 3–11; USA). (C–E) Oral glucose tolerance test on Ins1-cre Ercc1fl/mice and littermate controls at multiple ages (both sexes, n = 5–17; USA). (F) Representative images of hematoxylin and eosin (H&E)-stained pancreatic sections from Ins1-cre Ercc1fl/mice and controls at 10 months of age (USA). Scale bars: 100 μm. (G) Beta-cell nuclear size measured in H&E-stained images from panel (F). 200–300 β-cells were measured originating from n = 2–3 mice per genotype (USA). Data represent mean ± SD, unpaired Student’s t-test, or repeated measurement two-way ANOVA. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.

beta-cell dysfunction3, which can accelerate T2DM. Retrospective studies with childhood cancer survivors show a dose-response relation between radiation exposure to the pancreas and the subsequent risk of diabetes. Furthermore, mice with deficient double-strand break repair and impaired p53 signaling develop severe diabetes. This supports the role of persistent DNA damage in playing a causal role in β-cell dysfunction and loss.
pancreas of β-cell-specific Ercc1 mutant mice did not show an overt phenotype, suggesting that pancreatitis and pancreatic fibrosis may be largely driven by the exocrine pancreas or peripheral factors rather than by the consequences of β-cell dysfunction driven through DNA damage. Increased β-cell apoptosis in Ins2-cre Ercc1fl/fl mice could be due to increased stochastic damage in those cells or potentially to the paracrine effects of senescent β-cells on healthy β-cells26–31, previously described as in vitro in human fibroblasts and in Ercc1−/− mouse skin32. Dedifferentiation of mature β-cells might play an additional role in the β-cell dysfunction in our model. Due to the stochastic nature of DNA damage, future analysis on a cellular level, by single-cell RNA sequencing, would provide further insight into the molecular mechanisms by which failure to repair endogenous DNA damage results in β-cell dysfunction.

Islets deficient in β-cell Ercc1 display decreased glucose-induced insulin secretion associated with decreased mitochondrial function. Aging, loss of Ercc1, and the accumulation of nuclear DNA damage are all reported to impact various mitochondrial properties, including morphology, density, and respiratory function23,53. In β-cells, activation of p53, a key player in DNA damage response pathways, reduced mitochondrial function by repressing the anaplerotic enzyme pyruvate carboxylase (PC), which plays an important role in coupling mitochondrial metabolism to insulin secretion34, and by influencing mitochondrial homeostasis35. Mitochondrial morphology is altered in Ins2-cre Ercc1−/− islets compared to controls, suggesting a possible involvement of the p53-Parkin interaction35. In addition, mice harboring Ercc1-deficient β-cells show a significant loss of β-cell mass, leading to more pressure on the remaining β-cells to compensate, potentially causing exhaustion and failure of these β-cells. Both mechanisms could independently or simultaneously contribute to β-cell dysfunction in mice harboring Ercc1-deficient β-cells.

From a therapeutic point, enhancing DNA damage repair in β-cells might be beneficial to treat diabetes. Recently, the DREAM-complex, which governs the induction and maintenance of quiescence, was identified as a negative regulator of DNA repair in somatic tissues26. Inhibition of the DREAM-complex via inhibition of the upstream kinase DYRK1A induces the expression of DNA repair genes and resistance to DNA damage36. Interestingly, DYRK1A inhibitors are also being evaluated as human β-cell regenerative drugs due to their capacity to stimulate human β-cell proliferation37. Although it is unknown whether stimulation of DNA repair contributes to the beneficial effects of DYRK1A inhibitors, proliferation of adult human β-cells is limited and, at least in vitro, inhibited by activation of the DNA damage response38.

In conclusion, the analysis of Ins1- and Ins2-cre Ercc1fl/fl mice, models with DNA repair deficiency in β-cells, reveals that failure to repair spontaneous, endogenous DNA damage in β-cells is sufficient to drive β-cell dysfunction. Our results reveal a new mechanism that contributes to the initiation or exacerbation of T2DM. In addition, the Ins1- and Ins2-cre Ercc1fl/fl mice represent novel, physiologically relevant, and rapid models of spontaneous damage in which therapeutic strategies to prevent or delay the onset of T2DM can be investigated.

Acknowledgments

The authors would like to thank Ben N. G. Giepmans and Jeroen Kuipers (Department of Cell Biology, University Medical Center Groningen) for electron microscopy (EM) acquisition, training, and advice. Part of the work has been performed in the UMCG Microscopy and Imaging Center (UMIC), sponsored by ZonMW grant 91111.006 (Zeiss Supra55 ATLAS). The authors acknowledge Prof. Jan H. J. Hoeijmakers for helpful discussion as well as Mariah Witt and Dr. Akilavalli Narasimhan for careful editing. The authors are grateful for the help of the TSRI Animal Resource Center, Histology Core, and Metabolic Core, as well as the UMN Resources for Research Animal Resources.

Funding

This work was supported by NIH grants P01AG043376, R01AG063543, R56AG059676, R56AG059675, P01AG062412, and U19AG056278 to P.D.R. and L.J.N. and the Glenn Foundation (L.J.N.). M.J.Y. was supported by the Irene Diamond Fund/AFAR Postdoctoral Transition Award. A.P.H.G. was supported by the Mexican National Council of Science and Technology (CONACyT). This work was supported by grants from the Dutch Diabetes Research Foundation (Diabetes II Breakthrough project 459001005 to J.K.K.) and Stichting De Cock-Hadders (to A.C.P.). This project was cofinanced by the Ministry of Economic Affairs and Climate Policy by means of the public–private partnership (PPP) allowance made available by Health—Holland, Top Sector Life Sciences & Health to stimulate PPPs.

Author Contributions


Declaration of Interests

The authors declare no competing interests.

Data Availability

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

Supplementary Materials

Supplemental information can be found online at https://doi.org/10.5936/agingbio.20230015.

Accepted September 20, 2023
Published October 23, 2023

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