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Research Paper

Failure to Repair Endogenous DNA Damage in β-Cells Causes Adult-Onset Diabetes in Mice

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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[1].

Age is a major risk factor for T2DM, with the majority of new diagnoses occurring in the fifth or sixth decade of life[2]. Beta-cell function decreases with age, which can be independent of peripheral insulin resistance[3]. Numerous cell-autonomous factors have been implicated in the decline in β-cell function with age, including mitochondrial dysfunction, replicative senescence, impaired autophagy, altered Ca^{2+} metabolism, and reduced expression of genes important for β-cell function[4]. 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[5]. DNA damage is one type of macromolecular damage that is causally linked to aging[5,6]. Islets from T2DM patients and mouse models of T2DM display increased levels of DNA damage[7–9]. Furthermore, diabetes is often seen in genomic instability disorders like ataxia telangiectasia[10], Werner syndrome[11], and Fanconi anemia[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*$^{d/-}$ 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[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$^{+/-}$ mice were bred as described[14] and crossed to *Ins2-cre* mice from Jackson Laboratory (Stock #003573) to generate inbred C57Bl/6J *Ins2-cre Ercc1*$^{+/-}$ breeders or *Ins1-cre* mice (Stock #026801) to generate inbred C57Bl/6J *Ins1-cre Ercc1*$^{+/-}$ breeders. They were then crossed with inbred FVB/N *Ercc1*$^{fl/+}$ mice to generate *Ins2-cre Ercc1*$^{fl/-}$ or *Ins1-cre Ercc1*$^{fl/-}$ mice on a genetically uniform C57Bl/6J:FVB/N F1 hybrid background. *Ins2-cre Ercc1*$^{fl/-}$ mice were maintained and characterized at separate institutes in The Netherlands (NL) and the USA. Mice generated in NL used an *Ercc1*^{fl} allele targeting exons 3–5, whereas mice generated in the USA used an *Ercc1*^{fl} allele targeting exons 7–10 (**Fig. S1**)[15]. *Ins1-cre Ercc1*$^{fl/-}$ mice were bred and characterized in the USA and used the *Ercc1*^{fl} allele targeting exons 7–10.

Cre recombinase activity itself has been reported to induce DNA damage, apoptosis, and toxicity[16]. *Ins2-cre Ercc1*$^{+/-}$ and *Ins1-cre Ercc1*$^{+/-}$ (Cre-positive, flox-negative), *Ercc1*$^{+/+}$ (Cre-negative, flox-negative, i.e., wild-type mice), and *Ercc1*$^{fl/-}$ (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 *ad libitum* access to water and standard chow (USA: 2020X Teklad, Envigo; NL: RM1, SDS Diets). Studies were in compliance with the U.S. HHS Guide for the Care and Use of Laboratory Animals and approved by The Scripps Research Institute IACUC, Jupiter, FL, the University of Minnesota IACUC, Minneapolis, MN, or the Ethical Committee for Animal Experiments at University Medical Center Groningen, Groningen, NL.

Metabolic studies

Glucose levels were determined using a OneTouch Ultra blood glucose meter (LifeScan, Edina, MN). Plasma insulin levels were measured by ELISA (Crystal Chem, Elk Grove Village, IL). Oral glucose tolerance tests were performed on animals fasted for 10 hours using 2 g/kg glucose administered by oral gavage. C-peptide levels were analyzed using a Milliplex Map Mouse Metabolic Hormone Magnetic Bead Panel (Millipore Sigma, St. Louis, MO) on a Luminex 200 analyzer (Thermo-Fisher).

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® *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$^+$ cells per islet was quantified on 10 islets per animal. Beta-cell proliferation was calculated using the percentage of Ki67$^+$Insulin$^+$ cells in at least 1000 β-cells per animal. H&E-stained pancreas sections were evaluated for inflammation, degeneration, necrosis, amyloid deposition, and atrophy or loss of zymogen granules within the acinar cells.

Electron microscopy (EM)

For large-scale EM studies (nanotomy), pancreas tissue (2 × 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[17], with modifications[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[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_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[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 at 55 °C. Slides were then incubated with either a scrambled nonspecific probe or a custom-designed *p16* LNA probe (5′-TCTCATGCCATTCCTTTCCTGT-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 described[13], 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 groups 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 breaks[20]. Deletion of *Ercc1* causes destabilization of the holoenzyme[21] and consequently accelerates the accumulation of spontaneous, endogenous oxidative DNA damage caused by normal metabolism[22]. Two independent *Ins2-cre Ercc1*$^{fl/-}$ 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*$^{fl/-}$ 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*$^{fl/-}$ 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*$^{fl/-}$ mice (**Fig. 1B**), consistent with prior findings in mice lacking *Ercc1*[23].

Ins2-cre Ercc1$^{fl/-}$ 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*$^{fl/-}$ mice (**Fig. 1D, Fig. S2B**). *Ins2-cre Ercc1*$^{fl/-}$ mice showed normal fasting insulin levels but significantly impaired glucose-stimulated insulin secretion compared to control mice (**Fig. 1E**). *Ins2-cre Ercc1*$^{fl/-}$ 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*$^{fl/-}$ 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*$^{fl/-}$ 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-dependent[22].

ERCC1 deficiency leads to loss of β-cell mass

The diminished secretion of insulin in *Ins2-cre Ercc1*$^{fl/-}$ 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 glucagon-expressing α-cells and diminished insulin staining (**Fig. 2B,C**). Beta-cell mass was significantly decreased in adult *Ins2-cre Ercc1*$^{fl/-}$ 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*$^{fl/-}$ 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*$^{fl/-}$ 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*$^{fl/-}$ mice (**Fig. 2I–K**), features previously reported for *Ercc1*-deficient hepatocytes and fibroblasts[24,25]. Furthermore, *Ins2-cre Ercc1*$^{fl/-}$ 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*$^{fl/-}$ islets (**Fig. 2I**), consistent with the decreased insulin content observed in *Ins2-cre Ercc1*$^{fl/-}$ 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*$^{fl/-}$ mice, multiple parameters were measured. Deletion of *Ercc1* in

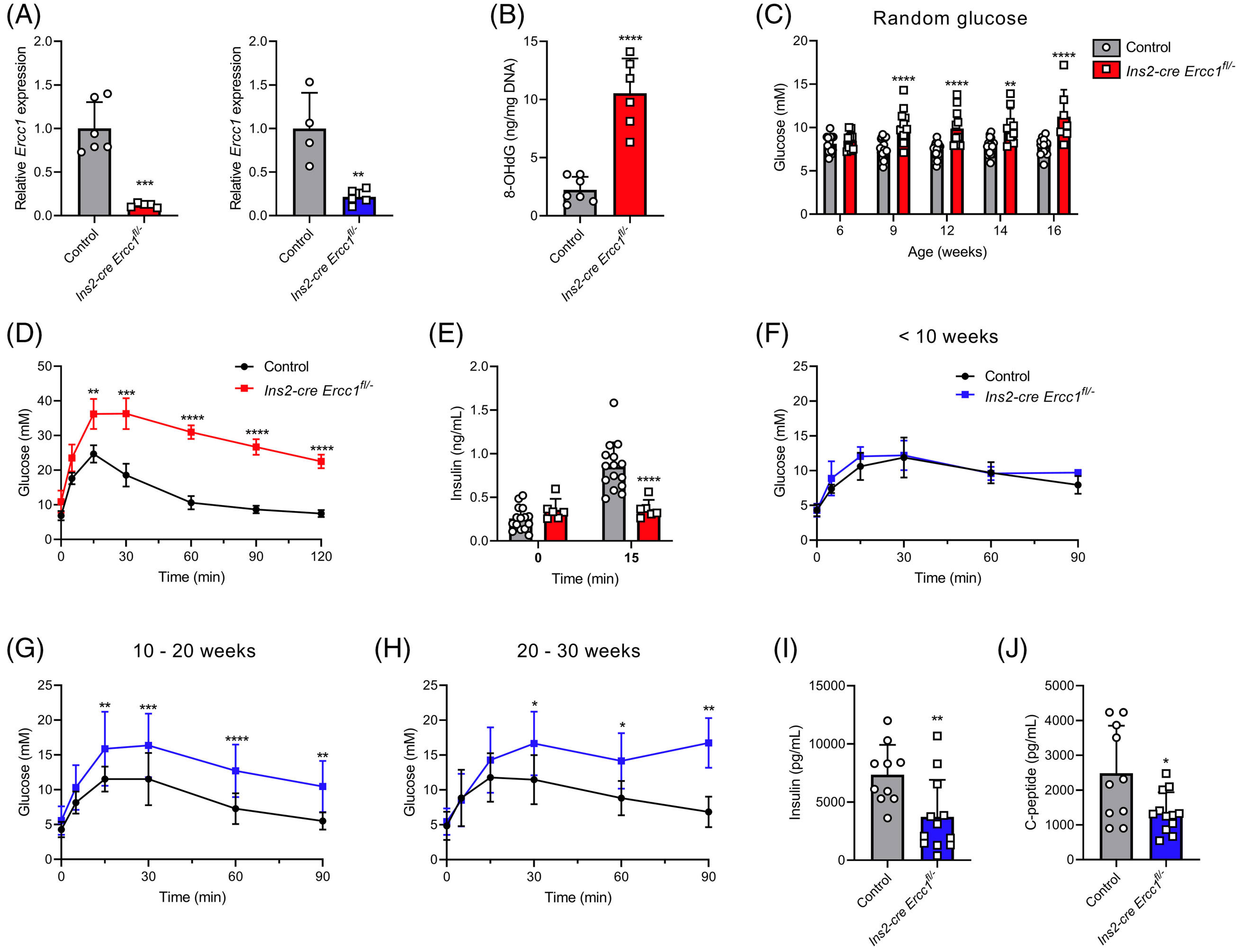

Figure 1. Deletion of *Ercc1* in β-cells causes glucose intolerance. **(A)** *Ercc1* expression was measured by qPCR using mRNA prepared from pancreatic islets isolated from 3- to 4-month-old male *Ins2-cre Ercc1*$^{fl/-}$ mice and their littermate controls (in red, NL mice, n = 4–5 mice per group, and in blue, USA mice, n = 4–5 mice per group). **(B)** Measurement of 8-hydroxy-2′-deoxyguanosine (8-OHdG), a marker of oxidative DNA damage, in pancreatic islets of 9–12-month-old male *Ins2-cre Ercc1*$^{fl/-}$ mice and their littermate controls (n = 6–7; NL). **(C)** Blood glucose levels of non-fasted male *Ins2-cre Ercc1*$^{fl/-}$ mice and age-matched controls at multiple ages (n = 7–23; NL). **(D)** Oral glucose tolerance test on 3-month-old male *Ins2-cre Ercc1*$^{fl/-}$ mice and age-matched controls (n = 6–14; NL). **(E)** Plasma insulin levels in 3-month-old male mice before and 15 min after an oral glucose bolus (n = 6–15; NL). Oral glucose tolerance test on **(F)** <10-week-, **(G)** 10–20-week-, and **(H)** 20–30-week-old *Ins2-cre Ercc1*$^{fl/-}$ mice and age-matched controls (both sexes) (n = 3–20; USA). **(I)** Serum insulin and **(J)** C-peptide levels in non-fasted 6–8-month-old mutant and littermate control mice (both sexes) (n = 10–12; USA). Data represent mean ± SD, unpaired Student's *t*-test, or repeated measurement two-way ANOVA. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$.

β-cells resulted in increased expression of the senescence marker *p16*^{Ink4a} (Fig. 3A). In addition, islets isolated from *Ins2-cre Ercc1*$^{fl/-}$ mice showed increased expression of *p21*^{Cip1} 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 Ercc1*$^{fl/-}$ 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 Ercc1*$^{fl/-}$ islets (Fig. 3D). Other genes involved in glucose metabolism or insulin processing were either marginally affected (*Glut2*) or remained unaffected in *Ins2-cre Ercc1*$^{fl/-}$ 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 Ercc1*$^{fl/-}$ 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 Ercc1*$^{fl/-}$ 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 Ercc1*$^{fl/-}$ mice. Basal OCR was equivalent between islets from *Ins2-cre Ercc1*$^{fl/-}$ 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 Ercc1*$^{fl/-}$ 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 Ercc1*$^{fl/-}$ 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

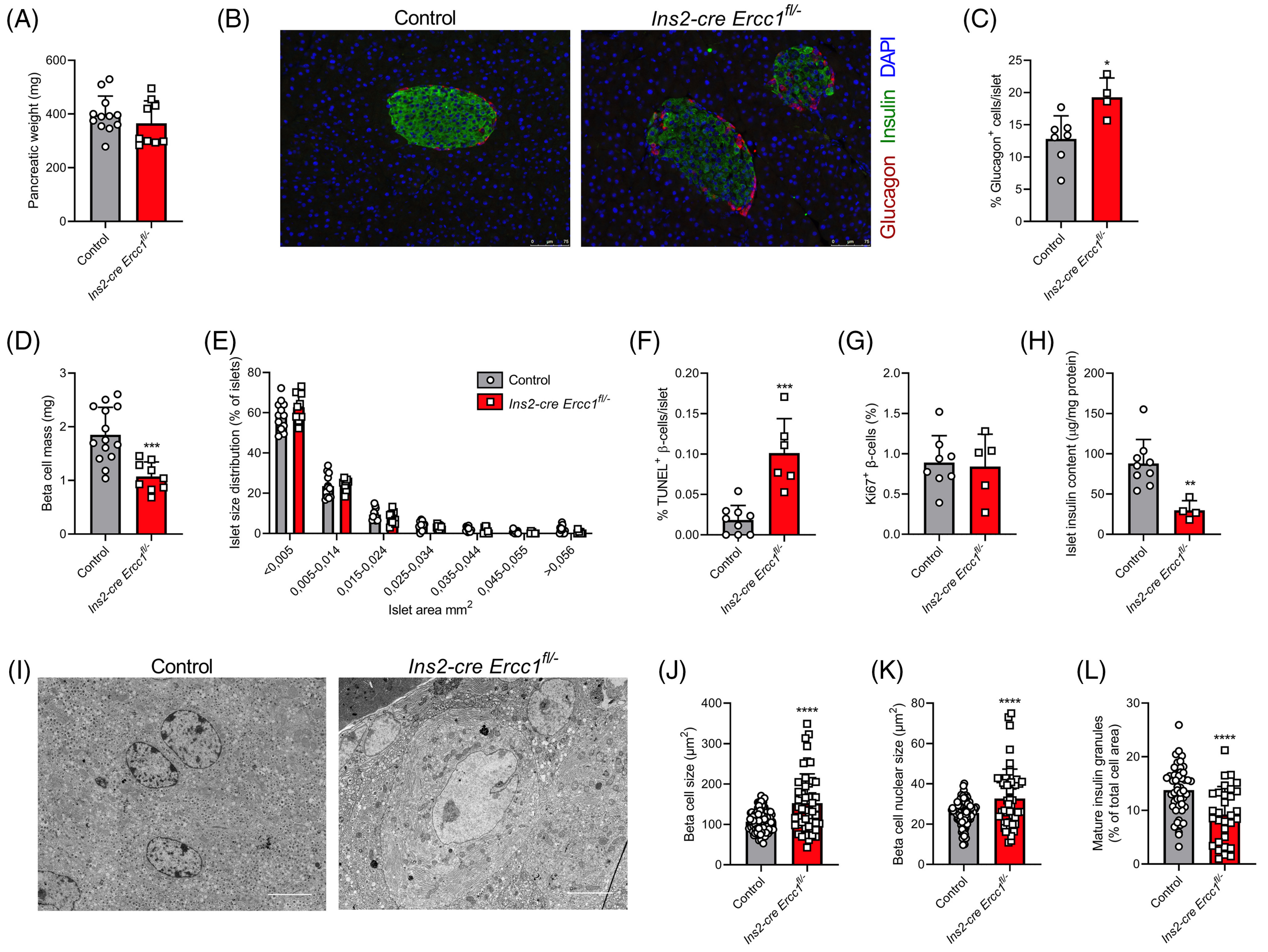

Figure 2. Reduced β-cell mass and altered β-cell morphology in *Ins2-cre Ercc1*$^{fl/-}$ mice. **(A)** Average weight of pancreatic tissue isolated from male *Ins2-cre Ercc1*$^{fl/-}$ mice and age-matched controls (n = 9–12). **(B)** Representative fluorescence images of islet morphology after immunostaining 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)** Islet 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)** Islet 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).

by *Ins2-cre Ercc1*$^{fl/-}$ islets possibly arises as a consequence of reduced mitochondrial function, as previously reported in *Ercc1* deficiency[26], 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[27]. *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

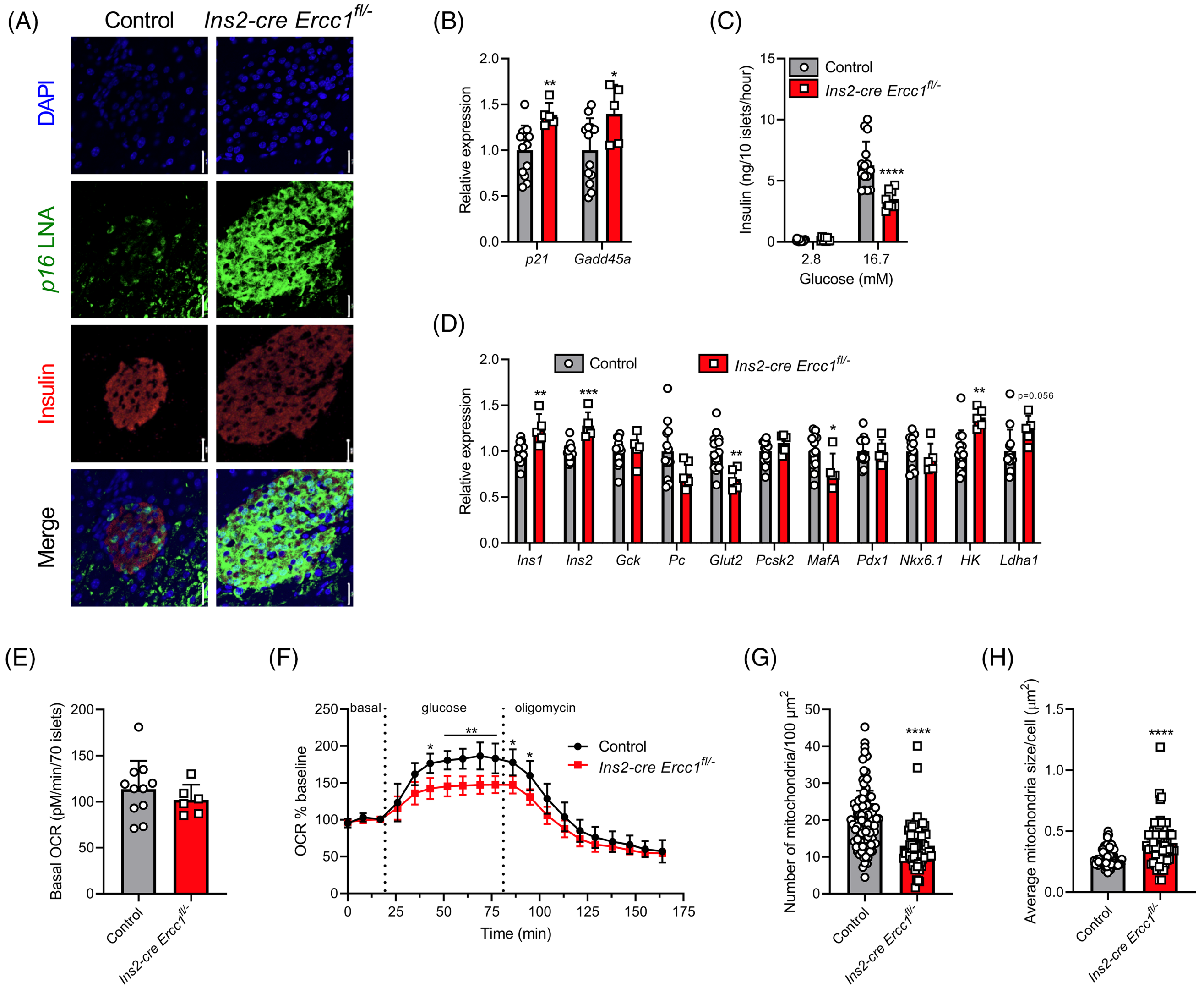

Figure 3. Loss of β-cell *Ercc1* causes senescence and impaired glucose-stimulated insulin secretion and mitochondrial function. **(A)** Representative image of *p16*^{Ink4a} expression with insulin co-staining in pancreatic islets detected by fluorescence *in situ* hybridization (male mice, USA). Scale bars: 20 μm. **(B)** Relative expression levels of *p21*^{Cip1} and *Gadd45a* in isolated islets from *Ins2-cre Ercc1*$^{fl/-}$ mice and age-matched controls (n = 5–13). **(C)** Glucose-stimulated insulin secretion of isolated islets from *Ins2-cre Ercc1*$^{fl/-}$ mice and age-matched controls (n = 8–15). **(D)** Relative expression of genes involved in glucose metabolism, insulin production, and β-cell identity in islets isolated from *Ins2-cre Ercc1*$^{fl/-}$ mice and age-matched controls (n = 5–13). **(E)** Seahorse analysis was performed to measure oxygen consumption rate (OCR) in β-cells isolated from *Ins2-cre Ercc1*$^{fl/-}$ and control mice (n = 6–11). Basal OCR and **(F)** stimulated OCR. **(G)** Mitochondria density and **(H)** mitochondria size in β-cells of *Ins2-cre Ercc1*$^{fl/-}$ and age-matched control mice measured from EM images obtained for **Figure 2**. (40–60 β-cells per group originating from n = 3 mice per group). Data represent mean ± SD, unpaired Student's *t*-test, or repeated measurement two-way ANOVA. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$. Data in panels (B)–(H) were collected from 3- to 4-month-old male mice at 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[6], 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[28]. Interestingly, endocrine pancreatic cells show a higher somatic mutation frequency relative to brain tissue[28]. Pancreatic β-cells are under high metabolic demand, with a relatively high rate of ATP-dependent processes such as protein synthesis and

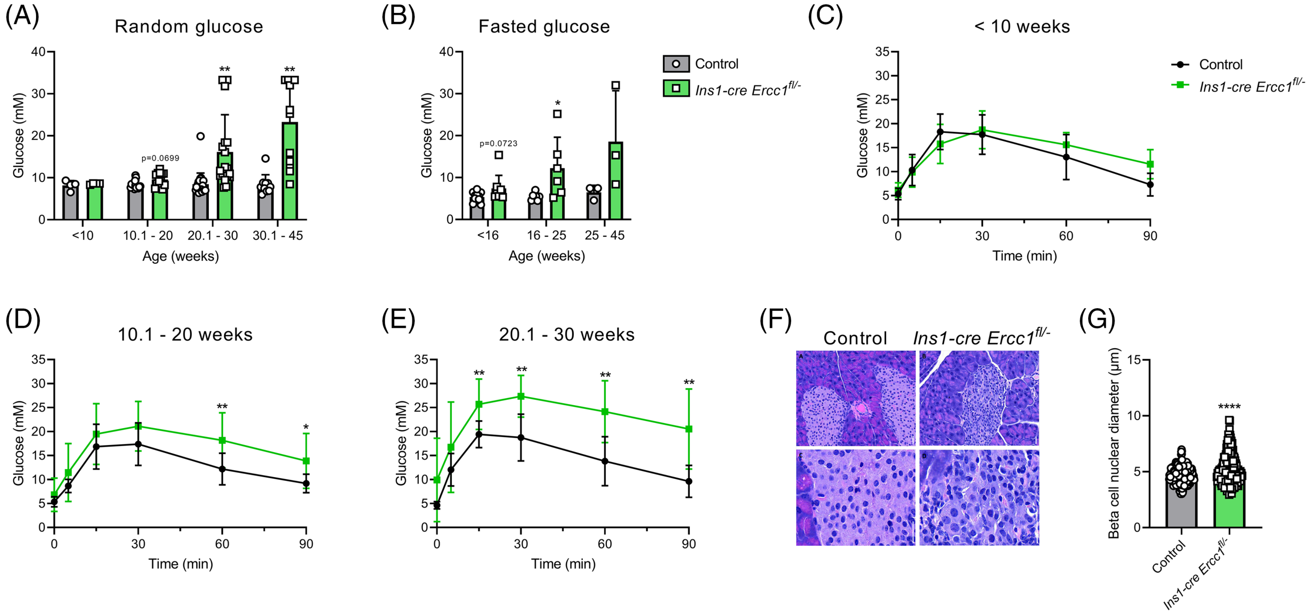

Figure 4. ***Ins1-cre Ercc1$^{fl/-}$* mice develop adult-onset hyperglycemia. (A)** Blood glucose levels of non-fasted *Ins1-cre Ercc1$^{fl/-}$* mice and age-matched controls at multiple ages (both sexes, n = 4–24; USA). **(B)** Blood glucose levels of fasted male *Ins1-cre Ercc1$^{fl/-}$* mice by age (n = 3–11; USA). **(C–E)** Oral glucose tolerance test on *Ins1-cre Ercc1$^{fl/-}$* 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 Ercc1$^{fl/-}$* 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. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$.

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[29].

As β-cells are long-lived, terminally differentiated cells[30], 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[31,32]. DNA repair capacity is reported to decline with chronological age[6]. NER, which requires ERCC1, is reduced in fibroblasts from aged humans compared to young[33], and in hepatocytes from old rats relative to young animals[34]. Base excision repair, which is critical for the repair of spontaneous, endogenous DNA lesions, is decreased in the tissues of old mice and rats compared to young animals[35,36], and in human fibroblasts and leukocytes from older individuals compared to young[37]. In mice with a transgene that reports nonhomologous end-joining (NHEJ) capacity, aging reduces NHEJ repair in a tissue-specific manner[38]. As DNA repair is energetically costly, DNA repair is attenuated in postmitotic cells such as neurons, which may also occur in cells reaching their replicative lifespan[39].

Increased DNA damage has been reported in β-cells of T2DM patients and in mouse models of T2DM[7–9]. *In vitro* studies suggest that elevated glucose levels can increase genomic instability by inhibiting DNA repair pathways[40]. In addition, obesity and diabetes are both associated with chronic low-grade inflammation. Pro-inflammatory cytokines can induce DNA damage, ultimately leading to cytokine-induced β-cell death[41] or inflammation-derived β-cell dysfunction[3], 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[42]. Furthermore, mice with deficient double-strand break repair and impaired p53 signaling develop severe diabetes[43]. This supports the role of persistent DNA damage in playing a causal role in β-cell dysfunction and loss.

Beta-cell mass was reduced in adult *Ins2-cre Ercc1$^{fl/-}$* mice, and using EM, we noticed variable levels of distressed β-cells in the islets of *Ins2-cre Ercc1$^{fl/-}$* mice. This finding is in line with the stochastic nature of spontaneous DNA damage and the heterogeneity of β-cells within an islet[44]. 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[45,46]. 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[46]. 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 *Ercc1$^{d/-}$* mice[13]. 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[41,47]. Both cell fates are detected in the islets of *Ins2-cre Ercc1$^{fl/-}$* mice (Figs. 2F, 3A,B). Previously, we demonstrated that loss of *Ercc1* resulted in oxidative DNA damage and consequently senescence[23]. Furthermore, we found evidence of increased senescence in the pancreas of *Ercc1$^{d/-}$* and naturally aged wild-type mice[48]. 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[49]. The exocrine 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* $Ercc1^{fl/-}$ mice could be due to increased stochastic damage in those cells or potentially to the paracrine effects of senescent β-cells on healthy β-cells[50,51], as previously described *in vitro* in human fibroblasts and in $Ercc1^{d/-}$ mouse skin[52]. 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 function[23,53]. 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 secretion[54], and by influencing mitochondrial homeostasis[55]. Mitochondrial morphology is altered in *Ins2-cre* $Ercc1^{fl/-}$ islets compared to controls, suggesting a possible involvement of the p53-Parkin interaction[55]. 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 tissues[56]. Inhibition of the DREAM-complex via inhibition of the upstream kinase DYRK1A induces the expression of DNA repair genes and resistance to DNA damage[56]. Interestingly, DYRK1A inhibitors are also being evaluated as human β-cell regenerative drugs due to their capacity to stimulate human β-cell proliferation[57]. 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 response[58].

In conclusion, the analysis of *Ins1*- and *Ins2-cre* $Ercc1^{fl/-}$ 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* $Ercc1^{fl/-}$ 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.

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Author Contributions

M.J.Y., A.P.H.G., A.C.P., R.R.F., S.J.M., B.J.L.E., J.W.J., P.D.R., L.J.N., and J.K.K. designed the experiments. M.J.Y., A.P.H.G., A.C.P., R.R.F., T.S., L.A., A.J., S.J.M., R.D.O., E.A.W., L.V.G., D.H.W., S.H., T.A.R., and C.E.T. performed the experiments. M.J.Y., A.P.H.G., A.C.P., S.J.M., L.V.G., M.K., R.R.F., and N.F.L. analyzed the data. M.J.Y., A.H.G., A.C.P., J.W.J., P.D.R., L.J.N., and J.K.K. wrote this article. All authors reviewed the article and approved the final version.

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/10.59368/agingbio.20230015.

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