Different responses to DNA damage determine ageing differences between organs

Maria Vougioukalaki1 | Joris Demmers1 | Wilbert P. Vermeij2 | Marjolein Baar3 | Serena Bruens1 | Aristea Magaraki4 | Ewart Kuijk5 | Myrthe Jager6 | Sarra Merzouk1 | Renata M.C. Brandt1 | Janneke Kouwenberg1 | Ruben van Boxtel2 | Edwin Cuppen5,7 | Joris Pothof1 | Jan H. J. Hoeijmakers1,2,8

1Department Molecular Genetics, Erasmus University Medical Center Rotterdam, Rotterdam, The Netherlands
2Princess Máxima Center for Pediatric Oncology, Oncoide Institute, Utrecht, The Netherlands
3Center for Molecular Medicine, University Medical Center Utrecht, Utrecht, The Netherlands
4Department of Developmental Biology, Oncoide Institute, Rotterdam, The Netherlands
5Division Biomedical Genetics, Center for Molecular Medicine and Cancer Genomics Netherlands, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands
6Department of Genetics, Center for Molecular Medicine, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands
7Hartwig Medical Foundation, Amsterdam, Netherlands
8Institute for Genome Stability in Ageing and Disease, Cologne Excellence Cluster for Cellular Stress Responses in Aging-Associated Diseases (CECAD), University Hospital of Cologne, Cologne, Germany

Correspondence
Jan Hoeijmakers, Department Molecular Genetics, Erasmus University Medical Center Rotterdam, Dr. Molewaterplein 40, 3015 GD, Rotterdam, The Netherlands.
Email: j.hoeijmakers@erasmusmc.nl

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Abstract
Organs age differently, causing wide heterogeneity in multimorbidity, but underlying mechanisms are largely elusive. To investigate the basis of organ-specific ageing, we utilized progeroid repair-deficient Ercc1Δ−/− mouse mutants and systematically compared at the tissue, stem cell and organoid level two organs representing ageing extremes. Ercc1Δ−/− intestine shows hardly any accelerated ageing. Nevertheless, we found apoptosis and reduced numbers of intestinal stem cells (ISCs), but cell loss appears compensated by over-proliferation. ISCs retain their organoid-forming capacity, but organoids perform poorly in culture, compared with WT. Conversely, liver ages dramatically, even causing early death in Ercc1-KO mice. Apoptosis, p21, polyplodization and proliferation of various (stem) cells were prominently elevated in Ercc1Δ−/− liver and stem cell populations were either largely unaffected (Sox9+), or expanding (Lgr5+), but were functionally exhausted in organoid formation and development in vitro. Paradoxically, while intestine displays less ageing, repair in WT ISCs appears inferior to liver as shown by enhanced sensitivity to various DNA-damaging agents, and lower lesion removal. Our findings reveal organ-specific anti-ageing strategies. Intestine, with short lifespan limiting time for damage accumulation and repair,

Abbreviations: DDR, DNA damage response; GG-NER, Global genome nucleotide excision repair; HR, Homologous recombination; HSC, Hematopoietic stem cell; ICS, Intestinal stem cell; LCS, Liver stem cell; NHEJ, Non-homologous end-joining; SC, Stem cell; SI, Small intestine; TCR, Transcription-coupled repair; TLS, Translesion synthesis; XLR, Cross-link repair.

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1 | INTRODUCTION

Accumulation of DNA damage is recognized as a principal cause of systemic ageing (Hoeijmakers, 2009; Niedernhofer et al., 2018; Schumacher et al., 2021). DNA lesions interfere with DNA function and activate an intricate DNA damage response (DDR), which triggers repair and decides on cell fate including cell cycle arrest (senescence), mutagenesis, premature differentiation or cell death. Since DNA is at the top of the informational hierarchy, genetic erosion has very diverse, lasting consequences, impairing cell and tissue functioning causing pathology and cancer (Marteijn et al., 2014; Vermeij, Dollé, et al., 2016; Vermeij, Hoeijmakers, & Pothof, 2016). However, how DNA damage shapes ageing and how this relates to organ/tissue-specific ageing trajectories is largely unknown.

Adult stem cells (SC) gradually lose functionality with age (McNeely, 2020). Mouse models mimicking DNA-repair-deficient human syndromes, revealed numerous features of ageing pathology (Marteijn et al., 2014), including functional impairment of hematopoietic stem cells (HSCs) (Cho et al., 2013; Navarro et al., 2006; Nijnik et al., 2007; Prasher et al., 2005; Rossi et al., 2007; Walter et al., 2015). Also, damage likely compromises HSCs and progenitor functionality in wt mice and humans (Beerman et al., 2014; Flach et al., 2014; Rube et al., 2011). In telomerase-deficient mice, intestinal stem cells (ISCs) and progenitors undergo apoptosis or cell cycle arrest (Begus-Nahrmann et al., 2009; Sperka et al., 2011). Hair follicle stem cells prematurely differentiate in a mouse model of the human progeroid DNA repair syndrome trichothiodystrophy and upon exogenous damage, similarly to naturally aged mice (Matsumura et al., 2016). Muscle stem cells decline in number and function in Ercc1-repair-deficient progeroid mutants, as in aged mice (Alyodawi et al., 2019; Lavasani et al., 2012).

Here, we compared intestine and liver, and their stem cells, organoids and damage responses in the Ercc1-deficient mouse model of the XFE human progeroid syndrome (Niedernhofer et al., 2006). ERCC1 is in a complex with XPF involved in damage excision in multiple DNA repair systems: global genome nucleotide excision repair (GG-NER) and transcription-coupled repair (TCR), which remove helix-distorting and transcription-stalling lesions, (deficient in the rare human genetic disorders xeroderma pigmentosum and Cockayne syndrome, respectively (de Laat et al., 1999; Marteijn et al., 2014)), as well as interstrand cross-link repair (defective in Fanconi’s anemia) and single-strand annealing repair of persistent double strand breaks (Ahmad et al., 2008; Gillet & Scharer, 2006; Kuraoka et al., 2000; Niedernhofer et al., 2004). Thus, Ercc1ΔErcc1Δ mice carrying one hypomorphic truncation allele and one null allele, harbour defects in four repair systems and combine multiple human genome instability disorders. They have largely normal embryonal development, but show after birth numerous progressive progeroid symptoms (Dolle et al., 2011), strikingly similar to natural ageing, which limit lifespan to 4–6 months (Vermeij et al., 2016b). Using this model, we analysed at tissue, SCs and organoids levels liver and small intestine, which vastly differ in ageing features in order to comprehend why organs and tissues age differently.

![Figure 1](https://example.com/figure1.png)

**Figure 1** Aging-related phenotypic features of small intestinal and liver from progeroid Ercc1ΔErcc1Δ mice. (a) Intestinal tissue from 15-week-old Ercc1ΔErcc1Δ and control mice stained with haematoxylin and eosin. Bars 200 μm. (b, c) Intestinal length (b, n = 2) and perimeter (c, n = 4) from 15-week-old mice of indicated genotypes. (d) Jejunal crypt density of 15-week-old wt and mutant mice. Crypts were counted on paraffin-embedded 4μm slices of intestinal tissue. The number of crypts of progeroid Ercc1ΔErcc1Δ mice is not significantly reduced in spite of the overall cachexia and decreased organ size, p = 0.7328 (n = 3). (e) Cell density in jejunal crypts of 15-week-old wt and Ercc1ΔErcc1Δ mice. Cells were counted on DAPI stained intestinal tissue slices as in (d), p = 0.5415 (n = 3 mice). (f) Immunofluorescent images of small intestine crypt and villi from sections stained for apoptosis (TUNEL), counterstained with DAPI. Bars 50 μm. (g, h) Apoptosis index in crypts (g) and villi (h), (n = 3 mice). (i) Liver tissue from 15-week-old Ercc1ΔErcc1Δ and wt mice assessed for apoptosis (TUNEL). Red arrows: TUNEL+ cells; black cut-out depicts a TUNEL+ large hepatocyte. Bars 100 μm. (j–m) Apoptosis index in the liver, parenchymal, non-parenchymal (l), and biliary cell (m) population of 15-week-old wt and mutant mice (n = at least 3 mice for WT, n = 5 mice for mutant groups). Quantification of TUNEL+ nuclei was performed on DAB-stained liver sections. Data: mean ± SEM. *p < 0.05, **p < 0.01

**KEYWORDS**

adult stem cells, DNA damage response, ERCC1, genome maintenance, liver, nucleotide excision repair, organoids, small intestine
(a) Hematoxylin/Eosin

(b) (c) Intestinal crypts Villi

(d) (e) % TUNEL+ crypts

(f) TUNEL/DAPI

(g) (h) % TUNEL+ villi

(i) % TUNEL+ ile cells

(j) (k) TUNEL+ hepatocytes per field

(l) TUNEL+ non par/mal per field

(m) % TUNEL+ ile cells
2 | RESULTS

2.1 | Contrasting ageing physiology and checkpoints of small intestine and liver from Ercc1Δ− mice

While liver suffers dramatic ageing pathology in Ercc1Δ−/− mutants and in full Ercc1-KO is lifespan limiting, small intestine (SI) seems unaffected (Dolle et al., 2011; Gregg et al., 2012; Wang et al., 2012). To compare these extremes in ageing, we choose the age of 15 weeks, when Ercc1Δ−/− mice manifest numerous ageing symptoms, without being moribund (Dolle et al., 2011). Histochemical evaluation of Ercc1Δ−/− SI revealed no overt abnormalities (Figure 1a), aside from a smaller intestinal tract and perimeter (Figure 1b,c) in line with the reported cachexia of the mutant (Dolle et al., 2011) and smaller overall size of Ercc1Δ−/− mice. The number and density of crypts appeared similar to WT (Figure 1d,e) and the ability of goblet cells to secrete mucus seems unchanged, suggesting normal intestinal function (Figure 1A). However, apoptosis—an outcome linked with ageing-related homeostatic deregulation (Muradian & Schachtschabel, 2001) in naturally aged crypts (Martin et al., 1998) appeared significantly elevated with cells dying at the bottom and higher-up in both Ercc1Δ−/− villi and crypts. Hence, apoptosis is not restricted to a specific cellular compartment or stage of differentiation (Figure 1f–h), consistent with a stochastic origin and congruent with accelerated ageing.

Contrary to SI, Ercc1Δ−/− liver is known to suffer from severe ageing pathology (Dolle et al., 2011; Gregg et al., 2012; Vermeij et al., 2016b; Weeda et al., 1997), confirmed for the Lgr5EGFP-Ercc1Δ−/− model used here in Figure S1B–E. TUNEL immunostaining revealed apoptosis to be clearly enhanced in Ercc1Δ−/− liver (Figure 1i–j). Close morphological inspection revealed that nearly all cell types are affected (Figure 1k–m). In fact, the increase of TUNEL + hepatocytes was relatively modest, although, notably, apoptosis included also polykaryons, that is, the equivalent of many diploid hepatocytes. Moreover, hepatocytes expressing cyclin-dependent kinase inhibitor p21 were ~40-fold increased (Figure S2A–B) and a ~4-fold increase in biliary cells was found (Figure S2C), consistent with the systemic nature of the repair defect and wide-spread premature ageing features.

p21 expression in mice carrying (liver-specific) DNA repair deficiency is correlated with senescence (Ogrodnik et al., 2017). Recently, Yousefzadeh and coworkers (Yousefzadeh et al., 2020) convincingly demonstrated extensive senescence in 10 organs of Ercc1Δ−/− mice, including liver, starting at the age of 12 weeks, progressively increasing with time. We examined liver tissue of 15-week-old Ercc1Δ−/− mice, but unexpectedly, despite multiple trials and positive controls (see below), did not detect significant loss of LaminB1 and nuclear HMGB1 immunosignals nor significantly increased IL-6 expression that would suggest overt senescence, at this age in our Ercc1Δ−/− mouse line (Figure S3A–F). Further investigation is warranted to find out whether differences in, for example housing conditions, play a role in the age of onset of senescence in liver, including food, for which Ercc1Δ−/− mutants are extremely sensitive regarding accelerated ageing (Vermeij, Dollé, et al., 2016) and which likely influences antioxidant buffering and DNA damage load (Milanese et al., 2019).

In conclusion, in this and other studies (Dolle et al., 2011; Weeda et al., 1997; Yousefzadeh et al., 2020), Ercc1Δ−/− liver displays numerous accelerated ageing features, in hepatocytes and biliary cholangiocytes, in sharp contrast to SI, although elevated apoptosis in villi and the regenerative and amplifying compartments of crypts suggests altered homeostatic regulation in this organ as well.

2.2 | Tissue-specific regenerative responses parallel homeostatic deregulation and pathology in Ercc1Δ−/− small intestine and liver

Previous research provided evidence for limited regenerative proficiency of Ercc1Δ−/− liver following partial heptectomy (Gregg et al., 2012), but which cells are implicated and whether SI is affected as well is unknown. Ki67 immunostaining indicated that the proliferative index of Ercc1Δ−/− intestinal crypts did not differ from controls (Figure 2a,b). In contrast, Ercc1Δ−/− liver showed a prominent upregulation of Ki67+ cells in nearly all cell populations (Figure 2c–g). Thus, in progeroid Ercc1Δ−/− mice SI displays normal regeneration rates, in spite of increased apoptotic events. However, compared with the very high cell turn-over, cell loss is relatively small and compensatory over-proliferation would likely go undetected. In contrast, in the liver, which normally has low turn-over, homeostasis is severely affected, as for other NER-deficient mouse mutants (Barnhoorn et al., 2014). Hence, DNA repair deficiency triggers cell loss in many cell types in liver, but also enhanced compensatory cell division.

2.3 | Distinct responses of Ercc1Δ−/− SC types to unrepaired damage

To further investigate the fate of SCs, we focused on Lgr5+ stem cells responsible for steady-state intestinal homeostasis but also

FIGURE 2 Steady-state regenerative potential of intestine and liver of progeroid Ercc1Δ−/− mice. (a) Immunofluorescence of intestinal tissue sections from 15-week-old wt and mutant mice stained for proliferation marker Ki67, nuclei counterstained with DAPI. Insets here and in other panels are digitally magnified images. Bars 10 μm (5 μm for insets). (b) Steady-state proliferative index of small intestine from 15-week-old Ercc1Δ−/− (n = 3) and control mice (n = 2). (c) Immunohistochemical staining for proliferation marker Ki67 of liver from 15-week-old Lgr5EGFP-Ercc1Δ−/− and wt mice. Nuclei counterstained with haematoxylin. Bars 250 μm. (d) Proliferative index of Lgr5EGFP-Ercc1Δ−/− liver. Quantitation of Ki67+ cells per total cells in a field as represented in (c) from 15-week-old mutant and wt mice. More than 5 fields were quantified from n = 7 mice per genotype. (e–g) Proliferative index of various cell populations (identified on morphology and location) in progeroid Lgr5EGFP-Ercc1Δ−/− and wt liver. Note that nearly all cell populations in Ercc1Δ−/− liver show increased proliferation. Over 5 fields were quantified from at least 4 mice per genotype. Data: mean ± SEM, **p < 0.01, ***p < 0.001
WT
Ercc1Δ/−

% Ki67+ cells per total cells

% Ki67+ hep/tes per field

% Ki67+ non par/mal cells per field

intestine

liver tissue

liver

Intestine

WT
Ercc1Δ/−

% of Ki67+ cells per crypt

p=0.5415

0
20
40
60
80

WT
Ercc1Δ/−

liver tissue

liver

liver

WT
Ercc1Δ/−

0
2
4
6
8

WT
Ercc1Δ/−

0
1
2
3
4

WT
Ercc1Δ/−

0
10
20
30

WT
Ercc1Δ/−

0
10
20
30

liver

liver

liver

% Ki67+ biliary cells per field

Ki67+ hepatocytes per field

Ki67+ non-partial cells per field

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damage-induced liver regeneration (Barker et al., 2007; Huch et al., 2013). We crossed Lgr5EGFP-ires-CreERT2 (Barker et al., 2007) and Ercc1Δ− mice and monitored EGFP-expressing (EGFP+) cells in 15-week-old Lgr5EGFP Ercc1Δ− SI and liver. Flow cytometry revealed a strikingly reduced number of high EGFP-expressing (EGFPhi) ISCs in progeroid mice (Figure 3a,b). Immunohistology and flow cytometry disclosed also erosion of the total EGFP+ population in mutant intestine (Figure 3c). Unaltered ratios of EGFPlow to EGFP hi cells (Figure 3d) in crypts and seemingly unaffected intestinal functionality (Figure S1A) suggest that ISCs in progeroid mice are eliminated randomly through apoptosis (as shown in Figure 1g,h), rather than premature differentiation. To examine how Ercc1Δ− intestine maintains homeostasis, given the increased apoptosis and reduced numbers of SCs, we analysed SI sections for co-occurrence of Ki67 and EGFP expression and found that mutant crypts bear a higher percentage of Ki67+ EGFP hi ISCs and progenitors indicating that they are more in cycle than crypts of Lgr5EGFP control mice (Figure 3e,f).

A liver cell type, attributed with SC properties, is the Sox9+ cholangiocyte (Gilgenkrantz & Collin de l’Hortet, 2018). Close examination of bile ducts revealed unaltered Sox9+ cell pools (Figure 3h), but increased bile cell nuclear size (Figure S1c,d) and a more disorganized structure of the ducts in Ercc1Δ− liver (Figure 3g). In addition, the number of cells per bile duct was significantly reduced (Figure 3i), in line with the evidence for apoptosis of this cell population in mutant liver (Figure 1m). Apparently, cell loss is not adequately compensated by increased cell division leading to aberrant duct morphology and possibly function.

To further investigate the impact of Ercc1 deficiency on liver stem cells (LSCs), we quantified Lgr5EGFP+ cells on paraffin-embedded liver sections. EGFP+ mutant SCs appeared more abundant and display intensified GFP fluorescence than in WT (Figure 3j,k). A prominent characteristic of Ercc1Δ− liver is the abundance of large polyploid nuclei (McWhir et al., 1993; Weeda et al., 1997). We found that a significant fraction of Ercc1Δ− Lgr5EGFP+ cells has enlarged nuclear size. But despite some EGFP+ cells in mutant mice looking polyploid (Figure S4), overall, nuclei of EGFP+ cells seem smaller than the rest of the (GFP+) population in both genotypes (Figure S4B). We conclude that, in contrast to intestine, the number of Lgr5+ cells in Ercc1Δ− liver is increased. A fraction of LSCs suffers from polyploidy most likely as a consequence of accumulated DNA damage suggesting limited functional potential.

2.4 | Functional exhaustion of liver but not intestinal SC populations of progeroid mice

To assess functional consequences of Ercc1 deficiency on SCs, we examined the ability of mutant and WT ISCs to expand into organoids. After seeding an equivalent number of crypts, organoid-forming capacity of ISCs seems similar for both genotypes (Figure 4a). However, mutant differentiated organoids, appeared smaller than WT with lower numbers of organoid-budding crypts (Figure 4b–d). Culturing crypts in 3% O2 did not significantly improve Ercc1Δ− (or WT) organoid development (Figure S5A) ruling out that it was caused by increased oxidative stress due to culturing in atmospheric oxygen, as previously noted in Ercc1Δ− primary mouse embryonic fibroblasts (Fuhrmann-Stroissnigg et al., 2017; Niedernhofer et al., 2006; Tilstra et al., 2012). Therefore, the reduced number of ISCs in isolated crypts in Ercc1Δ− intestine (Figure 3b,c) might explain the difference in organoid size.

To probe Ercc1Δ− LSC self-renewal potential, we used ex vivo organoid cultures (Huch et al., 2013). After seeding equal numbers of isolated liver cells, bile cells from mutant liver yielded far fewer organoids than controls (Figure 4e). To examine the possible effect of a difference in starting amount of bile cells (Figure 2f), from which organoids spawn, we plated secondary cultures from the organoids. This showed that organoid-forming ability of Ercc1Δ− LSCs is impaired (Figure 4f). Moreover, mutant organoids are dramatically smaller (Figure 4g). Both phenotypes were not rescued using low (3%) oxygen culture conditions (Figure S5B).

To conclude, whereas organoid-forming capacity seems unaltered, Ercc1Δ− ISCs show reduced growth and formation of budding crypts. Mutant LSCs, however, display strongly diminished ability to...
FIGURE 4 Ex vivo culture of intestinal and liver SCs from Ercc1Δ/− mice. (a) Organoids grown from intestinal crypt cell suspensions, derived from 15-week-old Ercc1Δ/− and wt mice, after 9 days in culture, \( p \) = not significant (\( n = 5 \) mice per genotype). (b) In vitro cultures of freshly isolated crypts from 15-week-old mice of the indicated genotypes. Images after 9 days in culture. (c) Average organoid size of the indicated genotypes after 9 days in culture (\( n = 3 \) independent cultures derived from different mice per group). (d) Average number of crypts budded in organoids of the indicated genotypes after 9 days in culture (at least two cultures for each mouse and 3 mice per genotype). (e) Number of organoids grown after plating bile cell containing liver cell suspensions, derived from 15-week-old Ercc1Δ/− mice and wt controls (at least two cultures for each mouse and 3 mice per genotype). Organoids were counted at Day 7 of culture. (f) Number of liver organoids grown in secondary cultures, after plating single liver stem cells derived from a primary organoid culture (at least three cultures were measured for each mouse and 3 mice per genotype). Organoids were counted at Day 7 of culture. (g) In vitro cultures of liver organoids grown from bile cell suspensions derived from livers of 15-week-old mice of the indicated genotypes. Images were taken after 7 days in culture. Data: mean ± SEM. *\( p < 0.05 \), **\( p < 0.01 \).
form organoids, and drastically compromised development, suggesting SC exhaustion.

2.5 | Diverse functional outcomes of DNA damage in Ercc1Δ−/− intestinal and liver SCs

Ercc1Δ−/− SI organoids show Ki67+ cell content similar to WT (Figure 5a), indicating that proliferative capacity in vitro is largely unaffected, consistent with the in vivo findings. TUNEL and cleaved Caspase-3 staining revealed that Ercc1Δ−/− SI organoid crypts contain cells undergoing apoptosis (Figure 5b), also recapitulating the mouse tissue phenotype. Interestingly, mutant crypts were found positive for senescence-associated β-galactosidase (SA-β-Gal), indicating the features of senescence (Figure 5c).

In contrast, Ercc1Δ−/− liver organoids display considerably less Ki67+ cells (Figure 5d) and a significant fraction of SCs was apoptotic or positive for senescence (Figure 5e,f), the latter deviating from our findings in 15-week-old liver (Figure S3A–F), suggesting that culture conditions are more stressful than in situ and reach the threshold for senescence. LSCs show moderately compromised proliferation (as assessed by EdU incorporation Figure 5d and S6A,B) and γH2AX foci indicative of DNA breaks (Figure S6C). We found little overlap of γH2AX signal with replicating cells (Figure S6D), consistent with the notion that Ercc1 defects also include repair systems such as TCR (amplified by GG-NER deficiency) linked with transcription, affecting all cell cycle stages.

Flow cytometry for polyploidy showed a trend for more >4n cells in Ercc1Δ−/− organoids, however, increased DNA content did not reach statistical significance (Figure S6E). Therefore, we measured the perimeter of EdU+ nuclei 48 hours after a short pulse, analysing exclusively cells that have undergone replication. Indeed, a significant fraction of Ercc1Δ−/− LSC nuclei seems to be larger and some very large, indicating that they further progress in polyploidization in

![Figure 5](image-url)
vitro (Figure S6F,G). Staining for albumin, a marker of differentiated hepatocytes, showed that Ercc1Δ+ LSCs in culture do not spontaneously undergo differentiation (data not shown). Collectively, we reveal diverse DNA damage responses in Ercc1Δ- SI and liver SCs in organoid cultures, affecting stemness and regenerative capacity.

2.6 | Comparison of functional DNA repair capacity of intestinal and liver stem cells

Since un repaired DNA damage is the most logical culprit for the SC phenotypes of Ercc1Δ- mice, we wished to examine SC responses to different classes of DNA-damaging agents. We chose UV that causes base-pair-disrupting photoproducts mainly repaired by GG-NER; I lludin S, which induces lesions that block transcription and are repaired by TCR (Jaspers et al., 2002); and interstrand cross-links inflicted by cisplatin, which require cross-link repair and mostly affect replication. Ercc1 mutants are deficient in all of these pathways (Martijn et al., 2014; Niedernhofer et al., 2006). We assessed the ability of cultured repair-proficient ISCs and LSCs to survive and expand into organoids, following increasing doses of the above genotoxins. Surprisingly, although the intestine compared with liver shows less (premature) ageing in WT and when Ercc1 is mutated, ISCs appeared consistently more sensitive to all genotoxins than LSCs (Figure 6a-d). ISCs were previously shown to be more sensitive to γ-irradiation (Barker, 2014). Cytochrome C release following treatment with illudin S and cisplatin illustrates the hypersensitivity of ISCs to apoptotic cell death compared to LSCs (Figure 6e,f). Apparently, after equal DNA damage ISCs opt for cell death and LSCs prioritize survival. We wondered whether this differential genotoxic sensitivity correlates with differential repair capacity. Previously, we noted that core NER genes are higher expressed in LSCs than ISCs (Jager et al., 2019). However, expression levels do not always correlate with repair efficiency (Naipal et al., 2015). We examined 6,4-photoproduct resolution after treatment of SCs with UVB, reflecting mainly GG-NER and to a lesser extent TCR activities. As shown by the remaining 6,4-photoproduct, immunosignals LSCs appear superior in repairing these lesions (Figure 6g). Inefficient repair likely enhances the propensity of ISCs to undergo apoptosis. Taken together, despite the virtual absence of accelerated ageing features in intestine, ISCs appear inferior in major DNA repair systems when assayed in parallel with LSCs.

3 | DISCUSSION

At tissue level, we found no overt features of accelerated ageing in Ercc1Δ- SI. Since Ercc1Δ- mutants include cross-link repair and GG-NER defects, which are highly relevant for replication, this is unexpected as intestine is the most proliferative organ. Cell death affects many tissues of naturally aged mice (Pollack et al., 2002), including SI (Martín et al., 1998; Nalapareddy et al., 2017; Steegenga et al., 2017). Interestingly, Ercc1Δ- crypts, transient amplifying zone and villi, reveal increased cell death in all stages of intestinal development, consistent with underlying stochastic damage events. Overall regenerative potential of the crypts is maintained, in line with the plasticity reported for ISC populations (Ritsma et al., 2014; Tetteh et al., 2016; Tian et al., 2011) and the presence of slow-cycling, reserve ISCs (Barriga et al., 2017). Although we cannot exclude alterations in cell cycle rates, elevated spontaneous apoptosis had no detectable effect on tissue proliferation. Possibly, this went undetected amidst the high cell renewal in intestine. In sharp contrast, Ercc1Δ- liver displays dramatic ageing pathology (Gregg et al., 2012; Vermeij, Dollé, et al., 2016; Weeda et al., 1997). Here, we show that apoptosis is prominent in nearly all liver cell types and p21 elevated in a fraction of cells. The intercellular heterogeneity in p21 levels parallels p53 in Ercc1Δ- liver (McWhir et al., 1993), consistent with stochastic DNA damage. The p21 response likely reflects resistance to apoptosis—explaining the modest increase in hepatocyte death, prior to reaching moribund stages, where senescence and apoptosis are strongly raised (Gregg et al., 2012; Yousefzadeh et al., 2021). The elevated proliferative index, persistent damage and different DDR, likely explain the increased mutations in LSCs compared with ISCs (Jager et al., 2019).

At the SC level, Ercc1Δ- ISCs are diminished in number, possibly through apoptosis. However, remaining ISCs appear functionally normal. Nevertheless, the number of quiescent Lgr5- cells is reduced in Ercc1Δ- crypts, which can be either due to apoptosis and/or cell cycle entry to restore regenerative capacity. In contrast, Ercc1Δ- liver displays increased levels of Lgr5+ cells, but these cells show evidence of impaired functionality: some are polyploid—indicative of genomic stress. The bile cell population appears compromised. Elevated apoptosis and p21 upregulation point to loss of stemness properties and reduced regenerative potential.

At the organoid level, Ercc1Δ- ISCs show –WT ability to form organoids, which are smaller and contain less crypts, suggesting reduced ability to grow and differentiate. Although proliferative potential is marginally decreased, apoptosis and senescence in these crypts are elevated, in line with time-dependent stochastic DNA damage causing the organoid’s limited net growth under culture conditions. In comparison, LSCs appear to be functionally even more compromised in all regards: they form fewer organoids, which are also much smaller and contain fewer proliferative cells, suggesting proliferative exhaustion. Apoptosis, senescence, polyploidy and irregularly shaped nuclei are also increased in liver organoids, accounting for their poor growth. Increased yH2AX foci, mainly in non-replicating cells provides evidence for DNA damage likely related to transcription (rather replication) stress, a genome-wide ageing phenomenon, first discovered in Ercc1Δ- and XpgΔ- liver and subsequently also in natural ageing (Vermeij, Dollé, et al., 2016), which is associated with functional decline, cell cycle arrest, senescence and cell death. This novel ageing feature occurs primarily in organs with low cell renewal (since DNA replication dilutes damage) and affects expression preferentially of large genes, consistent with accumulating random DNA lesions compromising transcription in a gene-length-dependent manner (Vermeij, Dollé, et al., 2016). The
origin of the endogenous DNA damage is unclear, but in our in vitro studies appears largely independent from O$_2$ levels (see Figure S5b), fitting our observation (Milanese et al., 2019) that TCR-defects trigger a potent anti-oxidant defence (Garinis et al., 2009; Niedernhofer et al., 2006; van der Pluijm et al., 2007).

Directly comparing the DDR and repair properties of WT ISCs and LSCs, we show that ISCs are more sensitive to diverse genotoxins attesting different repair systems. Individual ISC types have distinct damage sensitivities (Shivdasani, 2014). Quiescent Lgr5$^+$ cells appear more resistant, while fast-dividing ISCs have a low apoptotic threshold, as shown for ionizing radiation (Barker, 2014; Barriga et al., 2017). Consistent with low expression of NER genes (Jager et al., 2019), we find ISCs to be less efficient in repair of UV-lesions and more sensitive to UV, illudin S and cisplatin damages eliminated by GG-NER, TCR and cross-link repair, respectively, supporting the idea that the entire repair machinery is functionally inferior in ISCs.

As liver exhibits much more ageing features than intestine, one might expect genome maintenance in ISCs to be superior. However, in a direct comparison, repair in LSCs appears superior. This explains why Ercc1 (and all combined TCR/GG-NER) mutants display much less ageing in intestine than liver (and, as evident from human and mouse TCR/GG-NER mutants, also e.g., neurons and kidney). Apparently, organs utilize different anti-ageing strategies, involving DDR mechanisms. This raises the question—why do ISCs not invest maximally in DNA repair? One reason may be their tight replicative and differentiation schedule imposed by the rapid renewal of intestinal epithelium, which may not permit spending much time and energy for repair. Intestinal epithelial cells have only a lifespan of only 3–5 days (Gehart & Clevers, 2015) with limited time for DNA

**FIGURE 6** Differential responses of repair-proficient liver and intestinal stem cells to exogenous damage. (a) Schematic of experimental set-up for organoid formation assay following DNA damage induction. For each genotoxic agent, cultures from 2 wt mice were used and for each mouse, organoids grown in triplicates were quantified. (b-d) Clonogenic survival of wt liver and intestinal stem cells upon UVC-exposure (b); 2 h treatment with the indicated concentrations of illudin S, which is only removed by transcription-coupled repair (c) and the DNA-crosslinking agent cisplatin (d). Organoids from single stem cell containing cultures were counted 5 days after treatment ($n=2$ mice per group). (e, f) Number of stem cells that have lost cytochrome c at various time points following treatment with cisplatin (e) or illudin S (f). Data: means and SD of replicate samples from 2 mice in total. (g) Quantitation of 6,4-photoproducts’ immunosignal in organoid stem cells at the indicated time points following UVB irradiation. Plotted is the percentage of remaining fluorescence relative to the 5 min time point from at least 500 nuclei of separate organoids of each mouse (3 mice per time point). p-values were calculated using Student’s t test. Data: mean ± SEM unless otherwise specified. *p < 0.01, **p < 0.001, ***p < 0.0001
damage to accumulate, rendering repair less critical, and hence, prefer they opt for apoptosis. The high proliferation rate of ISC and the reserve capacity probably can easily compensate for the loss of a relatively small fraction of cells.

The above strategy may be less suitable when cell turnover is slower with more time for lesions to accrue, increasing dependence on repair. This matches with the severe functional and numerical SC exhaustion in Ercc1 mutant mice for the hematopoietic system (Cho et al., 2013; Prasher et al., 2005; Rossi et al., 2007), in which SCs have an estimated average turn-over of ~2 months. Presumably, exhaustion of HSCs in the Ercc1 mutant is largely caused by its replication-associated cross-link repair defect, as in Fanconi’s anaemia (FA), in line with the notion that defects in the ERCC1 partner XPF can cause FA (Bogliolo et al., 2013; Kuraoka et al., 2000).

Presumably, the investments in high intestinal tissue turn-over cannot be afforded by many other organs and cell turn-over is incompatible with the primary function of, for example most post-mitotic neurons. Moreover, the intestine resides in a very hostile environment, including the microbiome, with all metabolites passing through its epithelium rendering the organ with the highest exposure to exogenous compounds, explaining its 3–5 day cell turn-over. Although liver, as main detoxification organ, is also exposed to numerous toxics, we assume that its superior repair and high damage tolerance enable hepatocytes to live longer. Since cells in liver replicate only occasionally,

**FIGURE 7** Tentative model for organ-specific anti-ageing strategies. Remaining cellular lifespan largely determines which DNA damage response strategy is preferred by organs/tissues to counteract ageing. For intestine, cell death is preferred, as cells have to function only for 3–5 days and cell loss can be compensated by increased (stem) cell proliferation. Obviously, this is very energy-demanding (daily a human body produces 200 gram intestinal epithelium) and unaffordable for many organs. Other tissues with continuous but slower cell renewal such as the hematopoietic system (average cell turn-over ~2 months) rely mostly on replication-related repair (such as NHEJ/HR and XLR) and apoptosis (Hoeijmakers, 2001). Skin, as an organ with high UV exposure and also intermediate cell renewal combines GG-NER and TCR with apoptosis and premature differentiation of damaged stem cells (Kim et al., 2020). Finally, tissues with slow (e.g., liver, on average ~1 year) or no cell turn-over (e.g., the central nervous system, life time) depend on constitutive (cell cycle independent) DNA repair systems, most notably TCR to permit long-term unperturbed use of the transcribed compartment of the genome, needed for sustained proper cellular functioning. Global genome repair systems (base and nucleotide excision repair) are probably important for all organs and tissues for preventing mutagenesis and permit survival. TLS allows replication bypass of lesions to rescue stalled replication and cellular proliferative capacity, however, at the expense of elevated mutagenesis and cancer risk. Cellular (replicative) senescence opposes cell death in most organs. This model explains the segmental nature of repair-deficient progeroid syndromes, in which inherited deficiencies in different repair systems are associated with a different subset of organs and tissues displaying accelerated ageing.

1 Not including mismatch repair, which is a replication error correction system, important for preventing mutations and cancer, particularly in highly proliferative tissues such as intestine. GG-NER, global genome nucleotide excision repair; NHEJ/HR, Non-Homologous End-Joining/Homologous recombination repair two pathways for double strand break repair; TCR, transcription-coupled repair; TLS, translesion synthesis; XLR, cross-link repair
they must rely on replication-independent DNA repair pathways. Particularly, their transcribed compartment is vital for sustained functionality, explaining why TCR mutants display a segmental progeroid phenotype strongly biased towards post-mitotic organs and tissues, such as the neuronal system, liver, kidney, fat tissue and skeleton, but less bone marrow and intestine (Lans et al., 2019). A combined TCR/GG-NER deficiency as in Ercc1 mutants dramatically augments these segmental ageing features, as all GG-NER lesions also hamper replication (Andressoo et al., 2006; de Boer et al., 2002).

3.1 A model for the relationship between DNA damage responses and tissue-specific ageing trajectories

Based on the central role of DNA damage for ageing, we propose a tentative model (Figure 7), in which different DNA repair and damage response systems linked with replication and transcription (cell cycle arrest, senescence, cell death and mutagenesis) are differentially employed as anti-ageing/cancer strategies. Very frequent replicating cells with a short lifespan, as in intestinal epithelium opt primarily for apoptosis and cell replacement. On the other extreme, liver (and by inference from the phenotypes of mouse and human progeroid mutants with defects in the same repair pathways also neurons, kidney, and skeleton with mostly post-mitotic cells with a long lifespan invest in cell survival by damage sensing and repair primarily linked with transcription and the transcribed compartment (i.e., the most important functional part), involving TCR with the help of global genome repair systems (GG-NER and base excision repair). They also may have high damage tolerance and consequent senescence levels. Tissues with an intermediate stem cell turn-over and lifespan, such as the hematopoietic system, invoke both apoptosis and replication-linked DDR such as cross-link and double-strand break repair by non-homologous-endjoining and homologous recombination repair. Global genome and replication-linked repair and response mechanisms are critical for preventing mutagenesis and cancer. Such a model provides an explanation for the segmental ageing phenotypes of progeroid syndromes in man and mouse mutants in which deficiencies in different DNA repair systems are associated with a different subset of accelerated ageing symptoms. Natural variation in genome maintenance mechanisms, metabolism and exogenous exposure likely contributes to the different ageing trajectories between organs and inter-individual differences in normal ageing. Finally, we show that organoid cultures from progeroid mice faithfully recapitulate various ageing features and may be useful tools for studying regenerative interventions.

4 | METHODS

4.1 Mice

All animals were housed in the Erasmus Medical experimental animal center and handled in accordance with the recommendations and regulations of the animal ethical committee (DEC-consult) and national/EU legislation. Ercc1Δ/Δ mice were bred in a hybrid F1 genetic background (FVB/N: C57BL/6J) and genotyped as previously described (Vermeij, Dollé, et al., 2016). The Lgr5EGFP-IRES-CRE2 transgenic mouse line has been previously described (Barker et al., 2007). Lgr5EGFP-IRES-CRE2 Ercc1Δ/Δ mice were generated by first crossing male Ercc1Δ/+ mice with female Lgr5EGFP-IRES-CRE2, all in a pure C57BL/6J background. Female Lgr5EGFP-IRES-CRE2 Ercc1Δ/+ C57BL/6J progeny were subsequently crossed with male Ercc1Δ/Δ mice, in a pure FVB background and the desired Lgr5EGFP-IRES-CRE2 Ercc1Δ/Δ offspring were obtained in a 50/50 F1 C57BL6J/FVB hybrid background. Ercc1Δ/+ and Ercc1Δ/Δ mice have been previously described (Weeda et al., 1997).

4.2 Immunohistochemistry and immunocytochemistry, microscopy, FACS and flow cytometric analysis

Immunohistochemistry and immunocytochemistry of intestinal and liver tissues were done according to established procedures and detailed in supplementary materials, including the specific reagents (e.g., antibodies) used. Stainings of paraffin-embedded SI and liver tissue sections and liver organoids for senescence markers were performed as described (Baar et al., 2017). Procedures for microscopy, FACS and flow cytometric analysis and equipment are specified in Supplementary materials.

4.3 Apoptosis

TUNEL staining of tissue sections and organoid cultures was performed using an in situ cell death kit (Roche) or the Apoptag Plus Peroxidase in situ apoptosis detection kit (Millipore). Apoptosis of ISCs and LSCs after treatment with illudin S and cisplatin was assessed by staining for cytochrome c of single ISC and LSC suspensions—derived from undifferentiated organoid cultures—embedded in Matrigel using standard immunofluorescence protocol for cells (Baar et al., 2017).

4.4 In vitro small intestinal organoid culture

Intestinal organoid formation from crypts was performed as described (Sato et al., 2009). A total of 1000 crypts was mixed with 50 μl of Matrigel and plated in 24-well plates. Intestinal organoids were grown in ENR medium (Advanced DMEM/F12, B27 and N2 supplement (Invitrogen), 50 ngμl⁻¹ epidermal growth factor, 100 ngml⁻¹ Noggin (Peprotech), 500 ngml⁻¹ R-spondin-1, 1.25 mM N-acetylcystein) supplemented with Wnt3α-conditioned medium34 when appropriate to keep organoids in undifferentiated state. For organoid growth from single cell crypt suspensions, crypts were incubated with TrypLE (Invitrogen) and passed through a 40 μm cell strainer. Resulting cell suspensions were mixed with Matrigel and plated as described with
supplementation of growth medium with 10μM Rock inhibitor Y27632 (Sigma). The medium was refreshed every two days. Organoids were passaged every 10 days.

4.5 | In vitro liver organoid culture

Growth of organoid cultures from liver bile duct enriched cell suspensions was performed as described (Broutier et al., 2016; Huch et al., 2013, 2015). A total of 100,000 cells was mixed with 50 μl of Matrigel and plated on 24-well plates. Culture medium was refreshed every two days, and organoids were passaged every 8 to 10 days. Growth of liver organoids from single cells was performed with fluorescence-activated cell sorting of cell suspensions derived from TrypLE-digested primary cultures.

4.6 | EdU incorporation assay

In intestinal and liver organoid culture medium, EdU was added for 2 h at a final concentration of 10 μM, cultures were washed with PBS, replenished and at the appropriate time points, fixed with 4% paraformaldehyde in PBS for 15 min at room temperature. Edu immunofluorescence was performed using Click-IT® EdU Alexa Fluor® Imaging Kit (Invitrogen), according to manufacturers’ instructions.

4.7 | Genotoxic sensitivity assay

Organoids were dissociated into single cell suspension using TrypLE reagent and passed through a 40 μm cell strainer. Following cell counting, 10,000 single ISCs and 500 LSCs were resuspended in 20 μl Matrigel and plated in a 48-well plate. Cisplatin and illudin S were diluted in HBSS at appropriate concentrations and added to each well. Two hours after treatment medium was removed and replaced with appropriate stem cell culture medium supplemented with 10μM Rock inhibitor Y27632 (Sigma). For ISC cultures, Gsk3 inhibitor CHIR-9921 (Axon MedChem) was added to the culture medium at a final concentration of 10 μM.

For UV sensitivity assessment, suspensions of single stem cells were plated in 12-well plates at different densities and centrifuged at 600 relative centrifugal force for 20 min. At 32°C, medium was carefully removed, and the cells at the well bottom were exposed to the indicated doses of UV (254nm, TUV Lamp Philips). Cells were collected in culture medium, centrifuged at 1500 rpm, resuspended in Matrigel and plated in triplicates in 48-well plates. Organoids were counted 5 days after plating.

4.8 | Assessment of DNA lesion resolution in SCs

Organoids were grown in 8-well tissue culture slides, exposed to UVB (200J/m², Philips 40W/12RS UVB lamp) and at selected time points fixed (2% formalin, 20 min. at RT), washed with PBS and 0.1 M glycine, permeabilized with 0.5% Triton-X-100 in PBS for 20 min. RT and treated with 2N HCL for 30 min to denature DNA. After extensive washes with PBS and incubation with blocking solution (1% BSA in PBS), 150μl of anti-6,4-PP antibody (COSMO-BIO, Cat#CAC-NM-DND-002) mix (1:500 in blocking solution) was added to each well, and the slides were transferred to a 37°C incubator for 3 h. Organoids were washed 3 times with PBST (PBS and 0.05% Triton-X-100) and incubated with secondary antibody (Alexa Fluor 555) diluted 1:100 in PBS for 2 h at 37°C. Nuclei were counterstained with Hoechst for 30min. Subsequently, Prolong Diamond mounting medium (Thermo Fisher) was added and slides were incubated overnight in a freezer. Images were captured with a confocal microscope. Fluorescence measurements and analysis were performed using image J software.

4.9 | Statistics

Experimental sample size was not strictly chosen based on utilization of statistical methods prior to initiation of the study. Experiments were not standardly performed and analysed in a randomized, blinded fashion. Statistical analysis was performed with GraphPad Prism software. Student’s t test and nonparametric Mann-Whitney test were used to calculate P values. For multiple comparisons, two-way ANOVA was implemented for evaluation of statistical significance (GraphPad PRISM).

For all other procedures, see Supplementary Materials.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

M.V. designed experimental work, performed experiments, analysed data and wrote the manuscript. J.D. J.D. designed, performed and analysed experiments. M.B. performed senescence stainings. S.B. performed flow cytometry, A.M. Ki67, and GFP immunostainings and critically commented on experiments. E.K., R.v.B. and M.J. grown organoid cultures and provided expertise, reagents and protocols. S.M. commented on experiments, data analysis and participated in cell viability experiments. R.M.C.B. helped with the
mouse experiments. W.P.V. provided data on p21 and apoptosis and commented on study design and manuscript. J.K. performed immunostainings. R.v.B. and E.C. were involved in the conceptual design. J.P. and J.H.J.H. conceived and supervised the study and wrote the manuscript.

**DATA AVAILABILITY STATEMENT**

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

**ORCID**

Maria Vougioukalaki https://orcid.org/0000-0001-9868-820X
Joris Demmers https://orcid.org/0000-0002-3962-0480
Wilbert P. Vermeij https://orcid.org/0000-0002-9690-1385
Ewart Kuĳk https://orcid.org/0000-0002-1385-6516
Myrthe Jager https://orcid.org/0000-0002-1406-1715
Renata M.C. Brandt https://orcid.org/0000-0003-0514-5623
Janneke Kouwenberg https://orcid.org/0000-0003-2595-3951
Ruben van Boxtel https://orcid.org/0000-0003-1285-2836
Edwin Cuppen https://orcid.org/0000-0002-0400-9542
Joris Potthof https://orcid.org/0000-0003-3536-3162
Jan H. J. Hoeijmakers https://orcid.org/0000-0003-3526-7795

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