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Review

Renal phenotype of young and old telomerase-deficient mice

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ABSTRACT

Telomere shortening in the kidney explains the impaired regenerative capacity, but may not drive the ageing phenotype itself. We investigated kidneys from young and old *Terc*$^{+/+}$ and *Terc*$^{-/-}$ mice of early (G1) and late (G4, G5) generations.

Functional parameters declined and age-related morphological changes increased in late generation *Terc*$^{-/-}$ mice and with further age. Podocyte loss was only seen in old G4 *Terc*$^{-/-}$. Whereas p21$^{CIP1/WAF1}$ was highest in old G1 and G4 *Terc*$^{-/-}$, telomere shortening and p16^{INK4a} expression, also significantly associated with later generation young *Terc*$^{-/-}$, were not further induced in old *Terc*$^{-/-}$ mice. Both, young and old late generation *Terc*$^{-/-}$, showed increased pro-inflammatory cytokine levels.

Young late generation *Terc*$^{-/-}$ animals show mild functional and histological abnormalities, the presence of cellular senescence explains their kidneys' limited regenerative capacity. While these aspects resemble the situation seen in aged human kidneys, the lack of telomere shortening and p16^{INK4a} induction in older *Terc*$^{-/-}$ animals differs from observations in old human kidneys and may result from clearance of senescent cells. This animal model is well suited to investigate the mechanisms of impaired renal regeneration in aged human kidney, but may not fully explain the natural course of the human renal ageing phenotype.

1. Introduction

Aged human kidneys show an inadequate ability to respond to injury and an impaired regeneration when exposed to stress. With age increased vascular resistance, reduced renal plasma flow and increased filtration fraction are observed resulting in a measurable decline in renal function in a large subgroup of subjects (Lindeman and Goldman, 1986). Acute kidney injury is significantly more common in the elderly (Schmitt et al., 2008) and old individuals are more likely to develop end-stage renal failure than young (Saran et al., 2015). Moreover, donor age is a strong predictor of graft survival and old donor kidneys are more likely to fail, when they experience rejection (de Fijter et al., 2001). Morphologic changes of renal ageing include cortical thinning (Wang et al., 2014), interstitial fibrosis (IF), tubular atrophy (TA) and glomerulosclerosis (Melk et al., 2004). This renal ageing phenotype is linked with the occur-

Abbreviations: FISH, fluorescence in situ hybridisation; G-CSF, granulocyte-colony stimulating factor; GFR, glomerular filtration rate; HPF, high power field; IF, interstitial fibrosis; IFN, interferon; MT, Masson Trichrome; PAS, periodic acid–Schiff; PDGF, platelet-derived growth factor; SIPS, stress induced premature senescence; STASIS, stress and aberrant signaling-induced senescence; TA, tubular atrophy; Terc, telomerase RNA component; *Terc*$^{-/-}$, telomerase RNA component-deficient; TFI, telomere fluroescence intensity; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.

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rence of certain senescence markers, such as telomere shortening and increased $p16^{INK4a}$ expression (Melk et al., 2003, 2000, 2004; Wills and Schnellmann, 2011).

Cellular senescence, a state of permanent irreversible growth arrest, is reached via two different pathways. One is referred to as stress and aberrant signaling-induced senescence (*STASIS*) or stress induced premature senescence (*SIPS*) and is caused by different cellular stresses like DNA damage, oxidative stress, Ras induction and epigenetic alterations (Wright and Shay, 2000). $P16^{INK4a}$, a cell cycle regulator and tumor suppressor, is a key player in *STASIS* that is detectable in humans and rodents (Serrano et al., 1996; Weinberg, 1995). The other that is focussed on in this publication, is called replicative senescence and is triggered by telomere shortening (Collado et al., 2007). Telomere length is a crucial determinant for human lifespan and the regenerative capacity of organs exposed to extrinsic stresses (Blasco, 2007). Telomeres are specialised nucleoprotein structures at the end of eukaryotic chromosomes (Blackburn, 1991) consisting of repetitive DNA elements that protect the DNA ends from degradation, recombination and end fusions. Due to the end replication problem, telomeres become progressively shorter with every round of cell division (Blasco, 2005). Reaching a critically short length, they behave as double-stranded DNA breaks that activate the p53 tumor suppressor protein and result in telomere-initiated replicative senescence or apoptosis (de Lange, 2005). Replicative senescence is mediated via the $p53/p21^{CIP1/WAF1}$ pathway and specifically mediates the pro-ageing effect of short telomeres (Choudhury et al., 2007).

Telomere maintenance involves a ribonucleoprotein with reverse transcriptase activity, called telomerase (Blackburn, 1992). Telomerase synthesises telomeric repeats *de novo* and adds them to chromosome ends using an associated RNA molecule (Telomerase RNA component, *Terc*) as a template (Chan and Blackburn, 2002). Telomerase is active during human embryonic development and downregulated in the kidney at gestational week 17 (Ulaner et al., 1998). Somatic cells in most human tissues, including the kidney, lack detectable telomerase activity, whereas cells from tissues with a high tissue turnover, e.g. germline cells, express high levels of telomerase activity (Blasco, 2007; Kim et al., 1994).

Laboratory mice have very long telomeres (40–60 kb) compared to humans (5–15 kb) and express telomerase. Telomere shortening and chromosomal instability is only seen after successive intercrossings of Telomerase-deficient ($Terc^{-/-}$) mice (Wright and Shay, 2000; Lee et al., 1998). Late generation $Terc^{-/-}$ mice with critically short telomeres display a typical phenotype of premature ageing (Rudolph et al., 1999; Herrera et al., 1999), which includes decreased survival, reduced fertility, premature hair greying, alopecia and ulcerative skin lesions (Rudolph et al., 1999). Moreover, they show decreased body weight, small intestine atrophy, splenic atrophy, abnormal hematology and reduced proliferative capacity of B and T cells after mitogenic stimulation (Herrera et al., 1999). Due to neural tube closure defects embryonic lethality is increased (Lee et al., 1998; Herrera et al., 1999). Late generation $Terc^{-/-}$ mice show defects in homeostasis of proliferative organs (Lee et al., 1998) and a reduced capacity to respond to stresses such as wound healing and hematopoietic ablation (Rudolph et al., 1999). Similarly, liver regeneration after acute and chronic stresses is diminished and associated with an increased number of senescent cells (Satyanarayana et al., 2003). We and others have previously shown that also renal regeneration after ischemic injury is decreased in late generation $Terc^{-/-}$ mice (Westhoff et al., 2010; Cheng et al., 2015).

Renal ageing in humans goes along with a marked decrease in the kidney's regenerative capacity that can be explained by the increase in senescent cells. It is unclear, however, whether telomere shortening drives the ageing phenotype itself. We therefore investigated the renal phenotype of young and old $Terc^{+/+}$ and $Terc^{-/-}$ mice that have not been exposed to any stresses such as ischemia–reperfusion injury.

2. Materials and methods

2.1. Animals

All procedures performed on animals were done in accordance with institutional guidelines for animal research and approved by the local government authorities. Animals were kept in pathogen-free conditions and had free access to standard chow and water.

2.2. Generation and genotyping of $Terc^{-/-}$ and $Terc^{+/-}$ littermates

$Terc^{+/-}$ mice in the C57BL/6 background were purchased from Jackson Laboratories (Lee et al., 1998). G1 $Terc^{-/-}$ and $Terc^{+/+}$ control animals were derived from heterozygous intercrosses according to previously published breeding strategies (Rudolph et al., 1999). Mating of G1 $Terc^{-/-}$ to each other generated G2 $Terc^{-/-}$ animals. Following this mating scheme, $Terc^{-/-}$ animals up to the 5th generation were bred. G4 and G5 $Terc^{-/-}$ mice displayed the typical phenotype including decreased survival, reduced fertility, premature hair greying, alopecia and ulcerative skin lesions (Rudolph et al., 1999; Herrera et al., 1999).

For our study we compared young (3–8 months old) female mice ($Terc^{+/+}$, $n = 10$; mean 5.3 ± 0.4 months; G1 $Terc^{-/-}$, $n = 11$, mean 4.5 ± 0.3 months; G4 $Terc^{-/-}$, $n = 11$, mean 4.9 ± 0.3 months; G5 $Terc^{-/-}$, $n = 7$, mean 6.0 ± 0 months) with old (14–25 months old) mice ($Terc^{+/+}$, $n = 7$, mean 18.2 ± 0.7 months; G1 $Terc^{-/-}$, $n = 11$, mean 19.0 ± 0.8 months; G4 $Terc^{-/-}$, $n = 5$, mean 14.3 ± 0.3 months; no old G5 $Terc^{-/-}$ were investigated as they did not survive sufficiently long).

2.3. Creatinine clearance determination

All mice were kept in metabolic cages for the last 24 h before being sacrificed. Creatinine clearances in old animals were only measured in $Terc^{+/+}$ and G1 $Terc^{-/-}$ animals as old G4 $Terc^{-/-}$ animals did not survive the necessary metabolic cages. Twenty-four-hour urine was collected, urine volume was recorded and aliquots were stored at −80 °C for subsequent analysis. Blood was obtained by retrobulbar puncture under deep anaesthesia right before the mice were sacrificed. Plasma and urinary creatinine were determined using an enzymatic method that has been validated in rodents (Keppler et al. 2007). Creatinine clearance (ml/min) was derived from the following formula: Urinary creatinine × urine volume × 1440 min^{-1} × plasma $creatinine^{-1}$.

2.4. Body and relative kidney weight in $Terc^{-/-}$ mice

Mice were weighed and killed by cervical dislocation under deep anaesthesia. Organs were immediately retrieved and sections were divided for snap freezing (histology and RT-PCR) and paraffin histology.

2.5. Renal histology

Paraffin-embedded tissue sections were cut at 3 μm sections with a Leica RM 2165 microtome (Leica Instruments, Nussloch, Germany) and stained with periodic acid–Schiff (PAS) or Masson Trichrome (MT). High power field (HPF) pictures of the whole cortex (>20) and corticomedullary junction (>10) were taken of each mouse kidney using a Leica DM LB2 digitising microscope and a