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Telomerase, Autophagy and Acute Kidney Injury

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Abstract

In humans, aging is associated with telomere shortening and increased susceptibility to acute kidney injury. Telomerase is essential to maintain telomere length. The fourth generation mice with telomerase deletion have progressive shortening of telomeres. Those mice delayed recovery from ischemia-reperfusion injury, due to an increase in tubule cell senescence and impairment of autophagy, the latter of which may be mediated in part by increased mTOR signaling.

Keywords

Telomerase T; Telomerase C; Renal ischemia reperfusion; Autophagy; Proximal tubule; Mammalian target of rapamycin; Senescence; p16

Acute kidney injury (AKI) frequently results from tubule injury by acute ischemic or toxic exposure to the kidney, with higher morbidity and increasing morbidity and mortality seen with aging, especially in patients >65 years of age [1]. Age-related renal morphological changes, functional alterations and accompanying comorbidities may all contribute to the vulnerability of the aged population to either acute or chronic renal injury. However, intrinsic underlying predisposing molecular and genetic mechanisms related to aging per se remain incompletely studied.

Telomeres become shorter with aging, influenced by environmental factors, as well as specific genetic defects in the underlying telomere mechanisms. Telomeres become shorter each time a cell divides. Telomerase is a reverse transcriptase enzyme complex that adds DNA sequence repeats (TTAGGG) to the 3' end of DNA strands in the telomere regions at the ends of eukaryotic chromosomes. Telomerase contains 2 major components in the transcriptase ribonucleoprotein complex – the RNA-directed DNA polymerase, TerT, and the RNA template, TerC – which together prevent telomere shortening by adding telomeric DNA repeats to chromosome ends. TerC or TerT gene mutations are invariably associated with marked telomere shortening, resulting in dyskeratosis congenita and inherited bone

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marrow failure syndromes in humans and are risk factors for a range of other human telomeric syndromes, including aplastic anemia, idiopathic pulmonary fibrosis and acute myeloid leukemia. Telomerase also participates in chromosomal repair, and de novo synthesis of telomere repeats may occur at double-stranded breaks.

Telomere shortening has been described in human kidneys from aged patients, without significant association between telomere length and renal function [2]. However, shorter telomere length observed at the time of transplantation of a renal allograft was associated with decreased graft survival [3].

Compared with humans, laboratory strains of mice have much longer telomere lengths with increased telomerase activity. To study the effect of telomere shortening on pathophysiologic alterations, mice with specific genetic deletion of TerC or TerT have been generated. Absence of telomerase leads to telomere shortening progressively during successive generations of TerC- or TerT-deficient mice [4]. Genetic deletion of either TerC or TerT in mice does not lead to significant phenotypic abnormalities at an early age in the first generations, but does lead to telomere shortening by G4, with premature loss of viability and decreased lifespan associated with a number of degenerative pathologies [4]. A recent study with TerC KO mice suggested that murine kidneys with critically short telomeres were prone to acute cell death and reduced long-term regeneration [5]. The prevalence of chronic kidney disease with age also supports a potential role of telomere length and telomerase activity in its progression. Our data demonstrated delayed recovery from renal I/R in mice with deletion of either TerC or TerT (fig. 1), further suggesting an influence of telomerase on renal regeneration after injury [6].

Telomerase deficiency in mice has been shown to modulate a number of signaling pathways and genes, including the upregulation of mammalian target of rapamycin (mTOR). mTOR (especially mTORC1) is a ubiquitous kinase that regulates many different cellular processes, including possibly mediating the process of regeneration and recovery following acute injury [7]. Previous studies suggested that I/R-induced mTOR activation and inhibition by rapamycin delayed renal tubular recovery [8]. Recently, it has been reported that mTORC1-deficient mice have decreased renal recovery from injury [9], suggesting an essential role in maintaining renal tubular homeostasis. On the other hand, autophagy has been suggested to be renal protective following I/R injury, and mTOR is known to inhibit autophagy, leaving unresolved whether inhibiting mTORC1 could increase autophagic flux during AKI [10].

mTOR signaling may also have significant effects on aging [11]. Activation of this pathway may convert cellular quiescence into senescence and promote cellular and organismal aging. Inhibition of mTOR expression may increase mammalian lifespan [12].

Induction of renal ischemic injury in G4 mice with either TerT or TerC deficiency led to significantly delayed recovery compared to wild-type mice [6]. Electron microscopy demonstrated increased autophagosome formation in renal tubular epithelial cells in wild-type mice, with significant delay of autophagosome development in TerC and TerT KO mice (fig. 2). There were also delayed increases in expression of LC3 II and prolonged accumulation of P62, markers of autophagy. Therefore, ischemic kidney injury may impair

autophagy in mice with telomerase deficiency, probably triggered by endogenous and exogenous stimuli that potentially induce DNA damage (e.g., oxidative stress). Autophagy controls the quality of cellular components to prevent cell senescence [13]. Meanwhile, senescent cells increase lysosomal enzymes directed toward lipofuscin-rich lysosomes, but these enzymes lose effective autophagic degradation, leaving the lipofuscin non-degradable, which further decreases autophagy in senescent post-mitotic cells.

In addition to evidence of decreased autophagy, telomerase-deficient mice also had upregulation p16, and greater activation of the mTOR pathway following ischemic kidney injury. These results confirmed previous reports that ischemic injury or dysfunctional telomeres induces p16 [14]. p16 protein, a member of the INK4 family (p16^{INK4a}), is a cyclin-dependent kinase inhibitor that inhibits the cell cycle by blocking progression from G1 phase to S phase. It is a robust biomarker and a possible effector in mammalian renal aging. Deletion of p16 results in improved kidney regeneration and decreased capillary rarefaction after I/R [14]. The mTORC1 inhibitor, rapamycin, partially restored the autophagy response in kidneys following ischemic injury without significant effect on either upregulated p16 or renal tubule epithelial cell proliferation.

Therefore, studies to date suggest that deletion of the ability to maintain normal telomere length in mice impaired recovery from AKI, due to an increase in tubule cell senescence and impairment of autophagy, the latter of which may be mediated in part by increased mTOR signaling.

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Fig. 1.

Delayed recovery of BUN and proteinuria in TerC/TerT KO mice after ischemia-reperfusion injury. **a** I/R induced increased BUN in mice from each genetic group, but delayed recovery in TerC and TerT KO mice. $n = 8-10$, * $p < 0.05$, compared with basal level; # $p < 0.05$ TerC and TerT KO mice compared to Wt mice. **b** Delayed renal tubular restoration from I/R in TerC and TerT KO mice. Representative photos from day 0 (D0), 1, 3 and 14 were selected. Scale bar = 200 μm . **c** Tubular injury score further supports the delayed recovery from I/R in mice with telomerase deficiency. $n = 4$, * $p < 0.05$.

Fig. 2.
Representative image of I/R-induced autophagosome formation by electron microscopy.
Scale bar = 2 μ m. The areas in D3 from Wt and D14 from TerC and TerT KO mice are
composed of the enlarged autophagosomes.