Cell Reports
Report

Increased Mammalian Lifespan and a Segmental and Tissue-Specific Slowing of Aging after Genetic Reduction of mTOR Expression

J. Julie Wu,[1] Jie Liu,[1] Edmund B. Chen,[1] Jennifer J. Wang,[1] Liu Cao,[5] Nisha Narayan,[1] Marie M. Fergusson,[1] Ilsa I. Rovira,[1] Michele Allen,[2] Danielle A. Springer,[2] Cory U. Lago,[1] Shuling Zhang,[3] Wendy DuBois,[3] Theresa Ward,[6] Rafael deCabo,[6] Oksana Gavrilova,[4] Beverly Mock,[3] and Toren Finkel[1,*]

[1]Center for Molecular Medicine, National Heart, Lung and Blood Institute
[2]Murine Phenotyping Core, National Heart, Lung and Blood Institute
[3]Laboratory of Cancer Biology and Genetics, National Cancer Institute
[4]Mouse Metabolism Core, National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health, Bethesda, MD 20892, USA
[5]Key Laboratory of Medical Cell Biology, China Medical University, Shenyang 110001, China
[6]Translational Gerontology Branch, National Institute of Aging, National Institutes of Health, Baltimore, MD 21224, USA
*Correspondence: finkelt@nih.gov
http://dx.doi.org/10.1016/j.celrep.2013.07.030

SUMMARY

We analyzed aging parameters using a mechanistic target of rapamycin (mTOR) hypomorphic mouse model. Mice with two hypomorphic ($mTOR^{\Delta/\Delta}$) alleles are viable but express mTOR at approximately 25% of wild-type levels. These animals demonstrate reduced mTORC1 and mTORC2 activity and exhibit an approximately 20% increase in median survival. While $mTOR^{\Delta/\Delta}$ mice are smaller than wild-type mice, these animals do not demonstrate any alterations in normalized food intake, glucose homeostasis, or metabolic rate. Consistent with their increased lifespan, $mTOR^{\Delta/\Delta}$ mice exhibited a reduction in a number of aging tissue biomarkers. Functional assessment suggested that, as $mTOR^{\Delta/\Delta}$ mice age, they exhibit a marked functional preservation in many, but not all, organ systems. Thus, in a mammalian model, while reducing mTOR expression markedly increases overall lifespan, it affects the age-dependent decline in tissue and organ function in a segmental fashion.

INTRODUCTION

Inhibiting target of rapamycin (TOR) activity appears to extend lifespan in various model systems, including yeast, worms, and flies (Bjedov et al., 2010; Kaeberlein et al., 2005; Kapahi et al., 2004; Medvedik et al., 2007; Vellai et al., 2003). Moreover, deletion of the TOR1 gene in yeast results in an increase in replicative lifespan that cannot be further extended by nutrient restriction (Kaeberlein et al., 2005). Evidence also suggests that mechanistic TOR (mTOR) plays a role in regulating mammalian lifespan. Treatment of mice beginning at 20 months of age with rapamycin, a pharmacological inhibitor of mTOR, results in an extension of lifespan that averages 9% for males and 13% for females (Harrison et al., 2009). When rapamycin was initiated at 9 months of age, median survival was increased to 10% for males and 18% for females (Miller et al., 2011). Similarly, deletion of ribosomal S6 protein kinase 1 (S6K1), a downstream effector of mTOR, extends the median lifespan of female $S6K1^{-/-}$ mice by approximately 19% (Selman et al., 2009). Very recently, an additional genetic model consisting of mice heterozygous for deletion of both mTOR and mLST8 (mammalian lethal with Sec13 protein 8) also demonstrated lifespan extension, again only evident in female mice (Lamming et al., 2012).

In mammals, mTOR exists in two distinct complexes, termed mTORC1 and mTORC2. Each of these mTOR complexes has distinct protein components, although both share the catalytic mTOR subunit, as well as mLST8 (Dazert and Hall, 2011; Laplante and Sabatini, 2012; Zoncu et al., 2011). Agents such as rapamycin are known to acutely inhibit mTORC1, although chronic treatment can also affect the activity of mTORC2 (Lamming et al., 2012; Sarbassov et al., 2006). How reducing mTOR activity extends lifespan remains incompletely understood. In addition, whether manipulations of pathways that regulate mammalian lifespan will slow aging and age-related pathologies in a uniform or segmental fashion remains largely unexplored. Here, using a genetic model of reduced mTOR expression, we provide evidence that reducing mTOR activity produces a marked increase in overall lifespan while also regulating an important, but not universal, subset of tissue-specific, age-dependent parameters.

RESULTS

Reduced mTOR Expression Increases Survival

To assess the role of mTOR in mammalian aging, we used a model of hypomorphic mTOR expression that has been recently

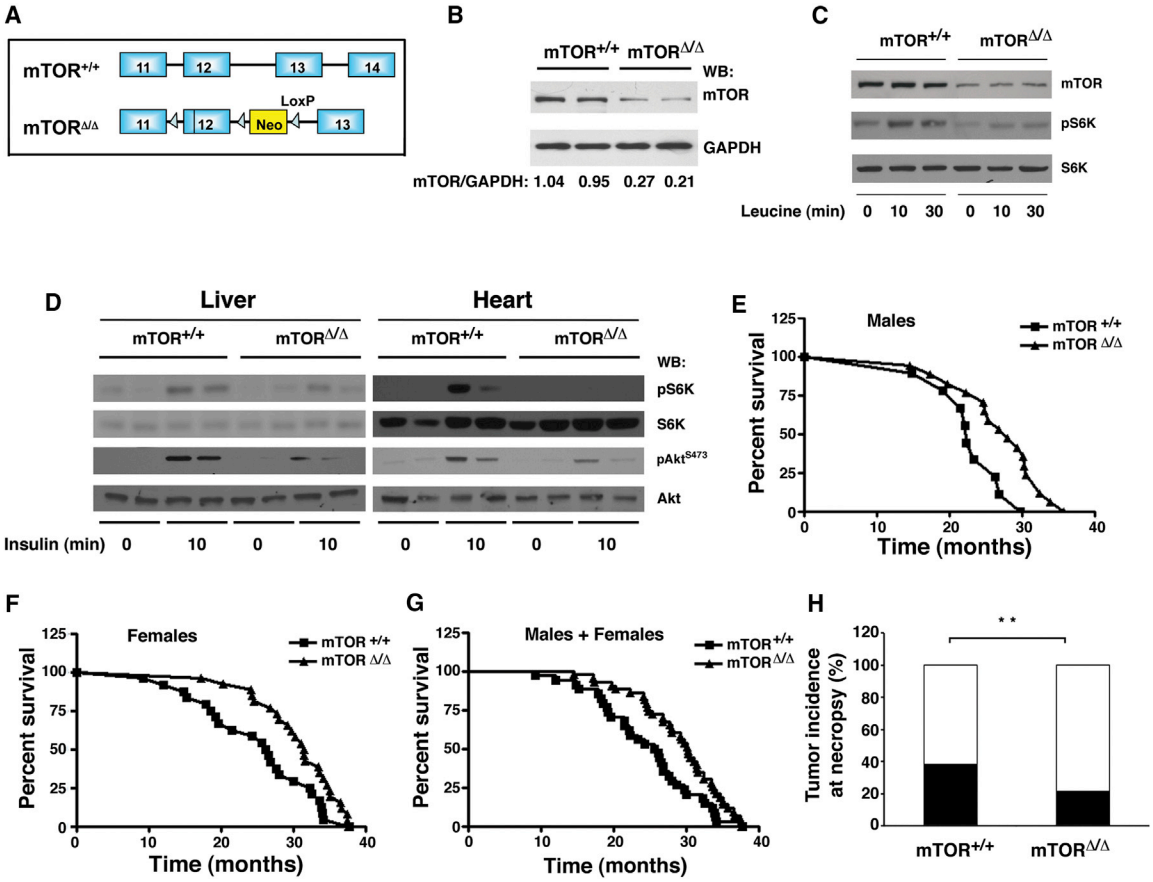

Figure 1. A Mouse Model of Reduced mTOR Expression Extends Life Span

(A) Genomic Organization of the WT Allele (+) and the Hypomorphic mTOR Allele (Δ).

(B) Representative mTOR protein expression in the liver of two WT ($mTOR^{+/+}$) and two $mTOR^{\Delta/\Delta}$ mice. GAPDH is used as a loading control, and the normalized expression (WT = 1) of mTOR to GAPDH is shown for each mouse.

(C) Leucine-stimulated S6 Kinase phosphorylation (pS6K) in primary mouse embryonic fibroblasts isolated from WT or $mTOR^{\Delta/\Delta}$ mice.

(D) Insulin-stimulated mTOR activity in pairs of WT or $mTOR^{\Delta/\Delta}$ mice.

(E) Survival of a cohort of male WT and $mTOR^{\Delta/\Delta}$ mice.

(F) Survival of female members of the cohort.

(G) Survival of the overall cohort.

(H) Incidence of malignant tumors found at necropsy denoted by shaded portion of each bar. While the overall incidence of cancer was different between the two genotypes, the spectrum of tumors observed was similar. **$p < 0.001$, Fisher's exact test.

See also Figure S1.

described (Zhang et al., 2011). This model results from a floxed neomycin cassette inserted between exons 12 and 13 of the mTOR locus that results in the partial disruption of mTOR transcription (Figure 1A). While complete disruption of Raptor, Rictor, mLST8, or mTOR is embryonically lethal (Gangloff et al., 2004; Guertin et al., 2006; Murakami et al., 2004), $mTOR^{\Delta/\Delta}$ mice were viable in a mixed 129/C57BL/6 background. Analysis of tissues derived from $mTOR^{\Delta/\Delta}$ mice revealed that the level of mTOR protein was reduced to approximately 25% of wild-type (WT) levels (Figures 1B and S1A). Mouse embryonic fibroblasts (MEFs) derived from $mTOR^{\Delta/\Delta}$ mice also exhibited reduced mTOR expression, with no apparent alteration in the expression of associated proteins such as Raptor and Rictor (Figures 1C and S1B). When MEFs derived from $mTOR^{\Delta/\Delta}$ mice were analyzed, levels of TORC1 and TORC2 complexes appeared to be reduced to a similar degree (Figure S1B). As expected, $mTOR^{\Delta/\Delta}$ MEFs had reduced activation of S6 kinase following leucine addition (Figure 1C), although the overall level of protein translation was not altered (Figure S1C). We noted that $mTOR^{\Delta/\Delta}$ mice also exhibited a decrease in mTOR signaling in vivo. In particular, the activation of S6 kinase following insulin administration was markedly attenuated in $mTOR^{\Delta/\Delta}$ mice (Figure 1D). Similarly, the mTORC2 dependent serine 473 phosphorylation of Akt was also reduced in these mice.

We next asked whether this reduction in mTOR activity was sufficient to provide an extension in lifespan. Median survival of the $mTOR^{\Delta/\Delta}$ male mice was significantly higher than observed in $mTOR^{+/+}$ (WT) male mice (Figure 1E; median survival for WT, 22.9 months [n = 10]; for $mTOR^{\Delta/\Delta}$, 28.0 months [n = 17]; 22% extension, $p = 0.02$ by log rank [Mantel-Cox] test). Similarly,