Increasing Healthspan by Rapid and Transient Telomere Extension

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Telomeres are composed of repetitive TTAGGG sequences located at the ends of chromosomes and are essential for the preservation of genome integrity. Telomere shortening can result in genomic instability and the induction of a DNA damage response that may result in either senescence or apoptosis. Telomeres are extended by the enzyme telomerase, which comprises a protein component telomerase reverse transcriptase (TERT) and an RNA component (TR). Mutations in TERT or TR are found in a spectrum of diseases, the telomeropathies, including dyskeratosis congenita (50-60% of patients) and familial idiopathic pulmonary fibrosis (8-15% of patients). More broadly, as we have shown, short telomeres are implicated in other genetic diseases including Duchenne Muscular Dystrophy (1), and in diseases of aging including cancer and heart disease. Telomere extension has been proposed as a means to improve cell culture and tissue engineering, and to treat disease. However, telomere extension by non-viral, non-integrating methods remains inefficient. Here we report that delivery of modified mRNA encoding telomerase reverse transcriptase (TERT) to diverse human cell types increases telomerase activity transiently (24-48 h) and rapidly extends telomeres. Successive transfections over a four-day period extended telomeres up to 0.9 kb in a cell type-specific manner conferring up to 28 ± 1.5 additional population doublings. Notably, unlike immortalized cells, all treated cell populations eventually stopped increasing in number and exhibited senescence markers to the same extent as untreated cells. To deliver TERT mRNA in vivo we have made progress using exosomes. Exosomes are advantageous as they are 40-100 nm vesicles that transport mRNA, microRNA, and proteins naturally between cells in a targeted manner, are non-immunogenic, and release their contents directly into the cytoplasm of target cells. We are developing biomimetic synthetic exosomes that have the advantages of natural exosomes but avoid the endogenous targeting moieties and cargo that may have unwanted effects or sterically hinder loading of therapeutic molecules including modified mRNA. These technologies for rapidly extending telomeres and increasing cell proliferative capacity without risk of insertional mutagenesis should have broad utility in disease modeling, drug screening, and regenerative medicine.

(1) Sacco, A. *et al.* Short Telomeres and Stem Cell Exhaustion Model Duchenne Muscular Dystrophy in mdx/mTR Mice. *Cell* **143,** 1059–1071 (2010)
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