Supplementary Material

Supplementary Material and Methods

Telomere length measurement by quantitative fluorescence in situ hybridization (Q-FISH)

Telomere length was analyzed by quantitative FISH performed by the molecular cytogenetics core facility in MD Anderson Cancer Center. Quantitative fluorescence in situ hybridization (Q-FISH) was performed with Cy-3–labeled (CCCTAA)3 PNA probe and subsequent quantitative analysis of digital images. Briefly, slides were observed with an Axioplan microscope (Zeiss, Thornwood, NY) equipped with a charged coupled device (CCD) camera. Separate images were captured for DAPI (4,6 diamidino-2-phenylindole) and Cy-3 and subjected to telomere fluorescence measurements using TFL-Telo software. Individual telomere lengths were quantified by the level of fluorescence intensity of each telomere spot, expressed in telomere fluorescence units (TFUs).

Telomere length detection by quantitative real-time PCR

Genomic DNA was extracted from cells using QIAmp DNA blood mini kit (Qiagen) according to the manufacturer's instructions. Using quantitative real time PCR, relative telomere length (RTL) was calculated from the telomere repeat to single gene copy number ratio, ,using a modified version of the method described previously [48][49]. The single gene used was 36B4, encoding acidic ribosomal phosphoprotein P0. The assay was done on a high-throughput 96-well plate and was analyzed on a BioRad IQ5 real-time PCR detection system. Telomere and single gene copy amplifications were run separately, with a negative control (water) and a standard curve run on each plate. For each plate preparation, a master mix of SYBR Green qPCR SuperMix (Applied biosystems) was combined with either telomere or 36B4 primers. For each

20-uL reaction, 8 uL of DNA (0.4 ng/uL) was added to 10 uL of the master mix + 2 uL of primers. Telomere primer pairs were Tel 1 (5′-GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT-3′), Tel 2 (5′-TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA-3′), 36B4d (5′-CCCATTCTATCATCAACGGGTACAA-3′), and 36B4u (5′-CAGCAAGTGGGAAGGTGTAATCC-3′). The thermal cycling profile for the telomere amplification was 95°C for 10 min followed by 35 cycles of 95°C for 15 s and 56°C for 1 min. For the 36B4 amplification, the profile was 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 58°C for 1 min.

Measurement of telomerase activity

Telomerase activity of cultured cells was determined using the telomeric repeat amplification protocol (TRAP, TRAPeze telomerase detection kit; Chemicon, Billerica, MA) according to the manufacturer's instructions. In brief, T cells were suspended in CHAPS lysis buffer for 30 min on ice. The suspension was centrifuged at 12 000 g for 20 min. After determination of protein concentration, the cell extracts were incubated with TRAP buffer supplemented with dNTP mix, TS primer, TRAP primer mix and Taq polymerase (Invitrogen) in distilled water at 30ºC for 30min and PCR was performed at 94ºC for 30s, 50ºC for 30s and 72ºC for 90s for 34 cycles in a thermocycler (PTC-200, Peltier Thermal Cycler). PCR samples were run on a 12.5% (w/v) nondenaturing PAGE gel with 0.5 × TBE buffer for 2 h at 130 V. After electrophoresis, the gel was stained with SYBR® Green (Invitrogen) for 30 min and de-stained for 5 min at room temperature. The images were taken using a Kodak 2000R imaging system.

Figure S1. caAkt-transduced T cells maintained longer telomere length and increased telomerase activity in culture.

Figure S2. caAkt-T cells did not proliferate autonomously in the absence of prosurvival cytokines.

Figure S3. FoxP3 expression was reduced in caAkt-transduced CD4+CD25+ T regulatory cell.

Figure S4. Suppressive function was abrogated in caAkt transduced CD4+CD25+ T regulatory cells.

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