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TITLE: Isolation of Factors that Disrupt Critical Protein/Protein Interactions within the Telomerase Holoenzyme for Use in Breast Cancer Therapies

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Telomerase activity is required to maintain telomere integrity on chromosomes of proliferating cells and thus is critically involved in regulating cellular replicative lifespan. Telomerase is repressed in most adult somatic cells, and activation of telomerase activity is an early event associated with tumor progression. Expression of telomerase is sufficient to greatly prolong proliferative lifespan of human cells in culture. Because telomerase activity is not required to maintain viability of post-mitotic somatic cells, but is required to maintain the proliferative capacity of tumor cells, telomerase is an ideal target for anti-cancer therapies. Here we have produced mammalian expression vectors containing PolIII-driven short-hairpin siRNA precursors targeting hTERT mRNA. We show that these vectors dramatically repress telomerase activity when delivered to telomerase positive immortal human tumor cells, resulting in dramatic telomere shortening and a limited replicative life-span in culture.

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Introduction:

The goal of our grant is to apply our knowledge of the functional assembly of telomerase to design novel inhibitors of telomerase activity as a mechanism of limiting the proliferative potential of breast tumor cells in vivo. The telomerase holoenzyme is a multi-subunit complex consisting of a reverse transcriptase catalytic core (hTERT) and an RNA template component (hTR) together with other known and unknown accessory factors. It has recently been demonstrated that small interfering RNA duplexes (siRNAs) can effectively and specifically mediate degradation of mRNAs in human cells. We have assessed the efficacy of siRNA mediated gene silencing in normal and tumor-derived human mammary epithelial cells. We find specific silencing of gene products can be achieved at high efficiency in these cells. Therefore, we are developing a system for delivery of siRNAs targeting hTERT and hTR.

Body:

Our original approach to inhibition of telomerase was through isolation of peptide aptamers that disrupt telomerase assembly by disrupting association of hTERT with p23. As described in last year's progress report, we successfully isolated a pool of peptide aptamers that bind hTERT such that they block association with p23 (task 1). Unfortunately, we have had great difficulty introducing these peptides into cells at sufficient concentrations to compete with native p23 (a relatively abundant protein) (task 2). While there are a number of strategies that can be employed to find a solution to this problem, we have elected to alter our approach to take advantage of recent technological developments in the field of RNAi. This modification of our work plan was approved following last year's progress report.

This new approach has been very successful, and as described below, accomplishes the goals of the original proposal.

Our progress with this approach is as follows: 1) Synthetic siRNA duplexes were synthesized targeting unique 21 nucleotide sequences in the hTERT mRNA and hTR. These were introduced into telomerase positive breast cancer derived cell lines to identify siRNAs that effectively reduced telomerase activity. Anti-hTERT siRNAs with the capacity to dramatically and specifically inhibit hTERT expression were identified. No siRNAs designed to target hTR showed any efficacy in vivo. 2) Replication defective retroviral vectors were constructed that express short hairpin RNAs from polIII promoters corresponding to the effective synthetic siRNAs targeting hTERT. Viral particles were produced and used to infect breast cancer cell lines. 3) 12 cell lines were cloned following infection. Of these, three lines showed reduced TRAP activity (Fig. 1). Clone C8 failed to expand further- presumably due to replicative senescence. Clones C2 and C5 were expanded for a total of 15 population doublings, then telomere length was assessed using standard TRF assay (Fig. 1). Both cell lines displayed dramatically shortened telomeres relative to the parentals and parallel clonal control lines. The telomerase impaired clones failed to proliferate beyond approximately 10 additional population doublings.

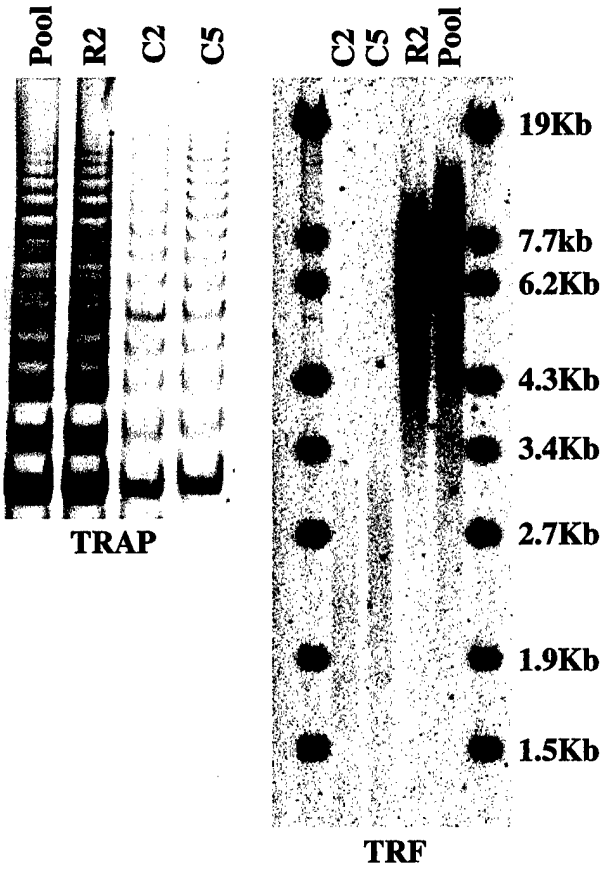

Figure 1. anti-hTERT shRNA inhibits telomerase activity and telomere elongation in human tumor cells. Two stable clones expressing anti-hTERT shRNA (C2, C5), a control clone (expressing anti-RalB siRNA) and a pooled population of parental cells are shown. Left panel: TRAP activity from equal cell equivalents. Right panel: telomere restriction fragment analysis.

Current focus: 1) We are constructing adenoviral vectors based on the successful retroviral vectors described in 2 above. The reason for this is that we have discovered that polIII-mediated expression of shRNAs is far more effective when using episomal vectors than when integrated into the genome. 2) We are evaluating the efficacy of systemic delivery of synthetic siRNA-related compounds targeting telomerase in mouse xenograft model systems.

Key Research accomplishments:

-islolation of a panel of peptide aptamers that competitively association with the p23 binding domain of hTERT
-validation of siRNA technology in human mammary epithelial cells.
-generation of retroviral expression vectors for stable expression of anti-hTERT shRNA.
-validation of anti-hTERT shRNA as a mechanism to limit replicative lifespan of human tumor cells.

Reportable outcomes: These observations are currently being prepared for publication. The anti-hTERT shRNA retroviral vectors will be made freely available to the research community.

Conclusions:

The reagents that are being developed will be useful in examining the biological consequences of inhibiting telomerase activity in human breast cancer cells. These reagents will have potential clinical applications to breast cancer therapy and prevention.