A novel role for telomerase in fish

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Telomere erosion in somatic human cells occurs as a result of incomplete synthesis of linear chromosomes during cellular proliferation. Most normal cells in humans lack a mechanism to compensate for chromosome end (known as telomeres) loss, which eventually elicits a DNA damage response and the cellular arrest known as replicative senescence. The rare cell capable of overcoming the senescence block has the capacity to immortalize, and those immortal cells nearly always reactivate the enzyme telomerase to stabilize and maintain telomeres. Consistent with these observations in *in vitro* systems, the vast majority (85-90%) of human malignancies, as defined pathologically, express telomerase, making it an obvious diagnostic and therapeutic target for human cancer[9]. Germ and stem cells, as well as activated lymphocytes, express telomerase and have longer telomeres than most cancers, likely because of their high rates of turnover and their importance in tissue repair and regeneration. Inhibiting telomerase in cancer would certainly have an effect on these cells, although it is thought that, based on their longer telomeres, this effect would be minimal. The most common misconception about anti-telomerase therapy has always been that it would be used as a primary therapeutic target, rather than its more likely utility as an adjuvant therapy after tumor resection. Thus, telomerase inhibition should be utilized adjuvantly to block the recurrence of more aggressive secondary cancers and not as a primary source of cancer treatment.

Telomerase is minimally composed of a specialized reverse transcriptase, hTERT (human TElomerase Reverse Transcriptase), and its associated RNA, hTR (human Telomerase RNA). The hTERT polymerase utilizes hTR to associate with telomeres and synthesize telomeric repeats onto the single stranded 3' overhang at the very end of the telomere. This extension provides additional DNA for conventional polymerases to replicate further out on the ends of chromosomes, hence providing telomere maintenance. While there are no compounds that directly inhibit telomerase function, current indirect methods include oligonucleotides for hTR to block association with the telomere and targeting associated proteins, such as the hsp90 chaperone complex. The hypothesis is that blocking hsp90, which is required to telomerase assembly, will inhibit telomerase activity, induce telomere erosion, and elicit a senescence or apoptotic response in cancer cells. Even so, the discovery of additional pathways will provide alternative therapeutic avenues, which may accelerate the development of anti-telomerase therapy. Therefore, defining the regulation of telomerase will lead to a better understanding of enzyme function, leading to improved diagnostic and therapeutic approaches for cancer.

There have been a few reports on telomerase activity in marine animal species including lobster[7] and rainbow trout[6]. In addition, recent data generated from this laboratory indicates that telomerase is active at significant levels in both long-lived species as well as short-lived ones, suggesting an alternative function for constitutive telomerase activity[3]. One report[7] suggests that telomerase is important for longevity in marine species. However, given that a host of marine animals with short life spans (e.g. zebrafish, *Danio rerio* and Japanese medaka, *Oryzias latipes*) have telomerase and these animals have a tremendous capacity for regeneration, it is plausible to hypothesize that telomerase is critical for maintaining telomere lengths during tissue regeneration in fish. The goal of this project was to determine the role of telomerase during tissue regeneration in marine species.

Initially, the sea cucumber (*Cucumaria frondosa*) was used as a model for regeneration as they are capable of eviscerating many of their external organs as a defense mechanism, followed by complete regeneration in a short time frame[2,4]. Techniques such as mechanical force, laceration of branching tentacles, injection of high concentrations of magnesium and/or potassium, incubation in warm fresh water, and electrical shock were all unsuccessful in getting *C. frondosa* to eviscerate. Only a single cucumber expelled anything, which was considered organismal suicide (the animal did not survive) (data not shown). As such, the model system of choice was switched to zebrafish (*Danio rerio*) based on recent literature[8]. Tissue regeneration times are between 4 and 7 days when tail fins (caudal fins) were clipped from zebrafish. Collaborative efforts were made to understand the role of telomerase upregulation during caudal fin regeneration in zebrafish.

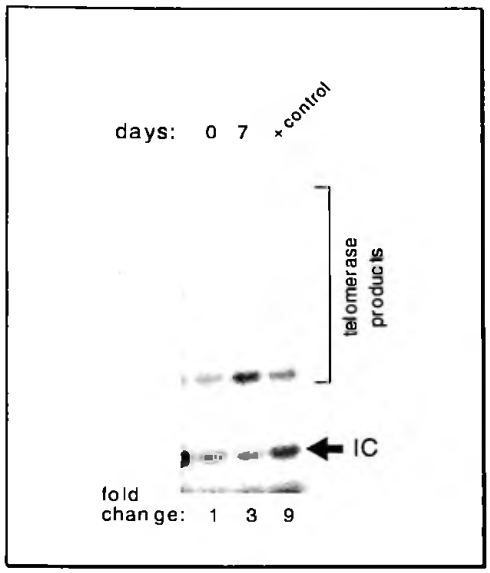

Figure 1. Increased telomerase activity during tissue regeneration in zebrafish (*Danio rerio*). Zebrafish caudal fins were clipped at day 0 and reclipped after a 7-day regeneration time. 6 individual fish were utilized and tested for telomerase activity using the TRAP assay. Shown is a representative telomerase gel with the characteristic 6-bp ladder indicative of telomerase activity and the 36-bp internal control (IC) used for quantitation. The IC is used to normalize sample to sample variation, where a ratio of the telomerase ladder to IC is calculated and referred to "fold change" when day 0 is set to 1. 1 μg of total cellular protein was used, and a reproducible increase in telomerase activity is observed at the 7d time point (compared to 0 day).

Caudal fins were clipped and allowed to regenerate for 7 days. Fish fins were cut using a sterile scalpel and subsequent tissue was divided into 2 tubes: 1 for DNA and 1 for protein. Individual fish were separated into 2 tanks (3 per tank) and marked by clipping either the dorsal, pelvic, or anal fins. Fin samples were extracted by standard lysis conditions (1% NP-40 in a buffered solution) and mechanical homogenization, followed by determination of protein concentration using a standard Lowry-based assay (BioRad). The telomerase activity assay (TRAP-telomeric repeat amplification protocol) was utilized to assess the upregulation of telomerase during tissue regeneration. The TRAPeze® telomerase detection kit was used for all telomerase measurements, as recommended by the manufacturer (Intergen, Inc., Gaithersburg, MD)[5]. After a 7-day regeneration time, telomerase activity was tested and shown to be upregulated during the regeneration process (Figure 1), indicating the importance for maintaining telomere length and integrity during tissue regeneration in the caudal fins of zebrafish.

Given that previous data generated from this laboratory has shown that a number of vertebrate and invertebrate marine species have telomerase activity[3], additional model systems (invertebrate organisms) will be used for defining the role of telomeres and telomerase expression during regeneration in starfish and sea cucumber. Clarifying their roles during tissue regeneration will be critical for understanding their importance in tissue engineering and overall organismal aging. Related to the vertebrate species, Japanese medaka (*Oryzias latipes*), which has high levels of telomerase, has telomere lengths similar to normal human cells without telomerase[3], which differs from inbred strains

of mice with telomerase activity and inordinately long telomeres. Because telomeres shortening is a causal factor during aging and current vertebrate models for aging and age-related disease (rodents) have 50-80 kb telomeres[1], understanding organismal aging as it relates to overall telomere shortening will be necessary to define telomere effects in age-related disorders. Thus, for the medaka system, because they have telomere lengths resembling normal human cells[3], knocking out telomerase will provide a model for studying aging and age-related abnormalities associated with telomere erosion and dysfunction within a single generation of fish, as well as the role of telomeres and telomerase in metal-induced carcinogenesis and pharmacology/toxicology studies. The role of telomere shortening and dysfunction during organismal aging will provide important clues to the regulation of certain diseases and disorders associated with aging, including cancer. These long-term goals would not be possible without the funding provided by the Department of Pathology at Virginia Commonwealth University, New Investigator Award from the NIEHS Center for Membrane Toxicity Studies at MDIBL (P30 ESO3828-18), and the Salisbury Cove Research Fund.

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