Characterization of telomerase function in cell lines from Japanese medaka

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Normal human cells lack a mechanism to maintain the ends of their chromosomes (telomeres), due in part to the inability of conventional DNA polymerases to replicate to the very end of a linear molecule. Thus, cell division results in gradual telomere shortening, which ultimately leads to the growth-arrested state know as senescence[4]. The enzyme telomerase is capable of maintaining telomere lengths and has been shown to be associated with almost 90% of all human cancers, as well as nearly 99% of advanced malignancies, making it an obvious diagnostic and therapeutic target[6]. Most normal human cells lack expression of one of the core components of the enzyme, hTERT, which is the critical polymerase subunit. Introduction of hTERT into normal human cells reconstitutes telomerically functional telomerase, causing an extended lifespan and prevention of cellular aging[2]. Thus, understanding the regulation of telomerase at the molecular level will be critical for defining alternatives for blocking the enzyme in tumor cells (cancer) or activating it in normal cells (aging).

Our short-term objectives are to characterize telomeres and telomerase in marine animals, potentially defining new *in vitro* systems for studying aging and cancer. Long-term, we plan to utilize fish, specifically Japanese medaka and zebrafish, as *in vivo* models of vertebrate aging with specific emphasis on the role of telomeres (and telomerase) in organismal aging. Current models for the vertebrate aging include, but are not limited to, inbred strains of mice (*Mus musculus*), which have telomeres that are extraordinarily long. Telomerase is ubiquitously expressed in mice, and knocking out the telomerase gene caused minimal effects on cell growth and function through 4-5 generations[1]. This system is not ideal for studying the effects of telomerase regulation and telomere dysfunction during organismal aging as these animals take 6-7 generations for observable phenotypes to arise. Therefore, we plan to utilize fish as improved vertebrate models for organismal aging, especially considering we can follow age-related changes in these fish in a single generation.

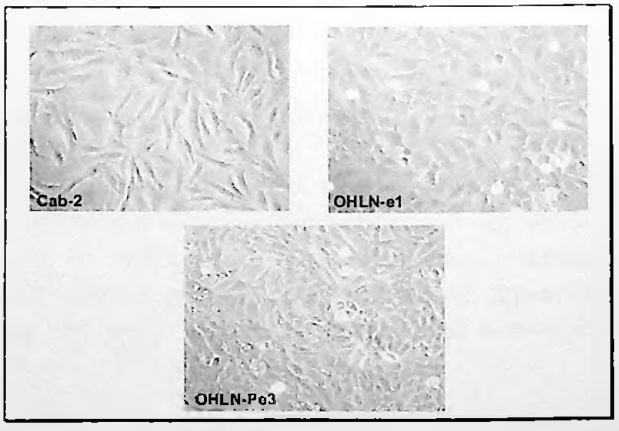

Figure 1. Morphological features of the established Japanese medaka cell lines. Cells were cultured at room temperature (22°C) in unvented T75 flasks, using a mixture of L-15 media with 10mM HEPES and 20% fetal bovine serum (Hyclone, SH30071.03). Cells were split in a 1:4 ratio when near confluent. Light microscopic images were captured (10X) in an Olympus 1X70 workstation with MagnaFIRE software. Note the expected epithelial morphology of the Pe3 and the e1 cells, while the Cab-2 cells are more fibroblastic.

To accomplish our first goal, we characterized cell lines from Japanese medaka (*Oryzias latipes*), which were developed by Dr. H. Mitani (University of Tokyo, Japan), in terms of their morphology, growth characteristics, telomeres, and telomerase activity. We show in Figure 1 the morphology of the

medaka cell lines, with the Cab-2 line of fibroblastic origin and the OHLN-e1 and OHLN-Pe3 being more epithelially derived. All grew at very similar growth rates (not shown) and have been propagated for over 100 cell divisions in our hands and likely for nearly 500 prior to our characterization (personal communication. H. Mitani, University of Tokyo). This type of continued proliferation suggests that these cells are functionally immortal.

Because human cell immortality in the classical sense involves activation of telomerase as a precursor to continued cell growth, we assessed telomerase activity levels and telomere lengths in the cell lines. Overall, each of the cell lines had extraordinarily high levels of telomerase activity (Figure 2, left panel), easily more activity than any immortal or tumor-derived human cell line tested by our laboratory (nearly 5-fold above MCF-7 breast tumor cells, not shown). Even though telomerase activity levels were excessively high, there was little effect on overall growth rate or telomere lengths; that is, telomeres were short (Figure 2, right panel), which is similar to immortal cell lines and tumor cells tested from human sources. Because of their shortened telomeres, the established cell lines from Japanese medaka should provide us with an ideal model system in which to study the regulation of telomerase and the effects of telomere dysfunction as it relates to cellular aging in the fish.

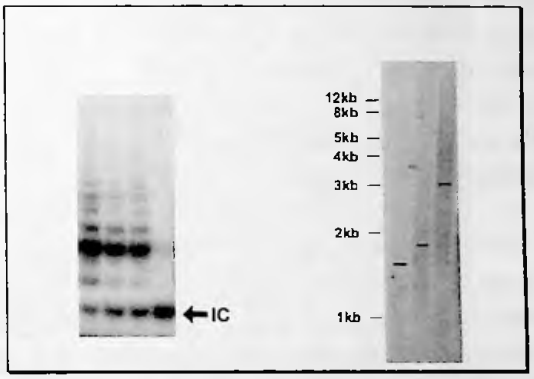

Figure 2. Medaka cell lines have telomerase activity and short telomeres. Telomerase activity (left panel) was measured using the telomeric repeat amplification protocol as recommended by the manufacturer (Clontech). The characteristic 6-bp laddering effect is indicative of telomerase activity. Lysis buffer (buffer) serves as a negative control, and IC (internal control) provides for semi-quantitation[3]. A telomere length assay (right panel) was performed on genomic DNA from all lines, and a radiolabeled telomere probe ($TTAGGG_4$) was hybridized[2]. Bars in the right panel indicate median telomere length with the migration pattern of a 1 KB ladder shown schematically.

Aging in marine animals has become an attractive area for experimentation, which initially started as a way of improving the management of fisheries[5]. Our goal is to study telomere biology and telomerase function in fish as it relates to organismal aging, where fish provide an excellent model for telomere biogenesis to replace the less telomerically optimal system of inbred strains of mice. Clearly, fish are a less expensive model for aging studies, and we have shown that they are potentially an improved telomere model, given their shorter telomere lengths, which more closely resemble those from humans when compared to mice. Ultimately, we will utilize fish as an aging model to understand the mechanisms of aging related to telomeres and telomerase function. This work was supported by a New Investigators Award and the Salisbury Cove Research Fund.

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