Human SHRNA Library Screening to Dissect Pathways Involved In Telomerase Actions

Hirotoshi Hoshiyama, Ph.D.
The University of Texas Southwestern Medical Center at Dallas, 2011
Graduate School of Biomedical Sciences
Supervising Professors: Jerry W. Shay, Ph.D. and Woodring E. Wright, M.D., Ph.D.

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The minimal components of human telomerase are the human telomerase reverse transcriptase (hTERT) and the human telomerase template RNA (hTR). Although it is known that both components are minimally sufficient to reconstitute telomerase activity, the factors involved in any of the multiple steps of telomerase action such as telomerase assembly, telomerase recruitment to telomeres, and telomere extension/regulation are not well understood. There are a large numbers of proteins that have associations with telomerase, yet the functional roles of those in telomere maintenance and telomerase regulation are not well understood. Identifying novel proteins and pathways involved in any of these important telomerase-associated functions will be useful for identifying new targets for the development of novel inhibitors that block telomerase function in cancer cells. Therefore, my goal has been to develop methods to dissect these molecular pathways and identify functional factors involved in any step of telomerase actions. To accomplish this, I designed a selective screening system by exploiting lentiviral shRNA libraries and tetracycline inducible-hTERT cell lines that is hTR deficient but expressing mutant hTR. Thus, the overall strategy of the screening system may be considered a “synthetic rescue screen”. In brief this screen was set up to rescue cells from apoptotic death due to mutant sequence incorporation at telomeres by reducing the gene expressions with lentiviral shRNA libraries. This allows us to look a set of genes involved in pathways involved in functional aspects of telomerase actions, not based on structural association with telomerase.

During the work, I have established multiple lines of inducible-hTERT cells to use in selective screening systems. I have also developed a method for rapid construction of high-complexity custom shRNA libraries for targeted screening and re-evaluate hundreds of primary candidate genes to identify smaller numbers of secondary candidate genes by removing false positives. In order to analyze the pooled shRNA screening result, I have developed a method for quantitative identification of half-hairpins from a pooled shRNA library based on the pGIPZ vector. Introducing multiplexing codes and refining sample preparation schemes resulted in the predicted ability to detect two-fold enrichments followed by massive parallel sequencing. Development of those methods allowed me to identify several candidate proteins, which may be involved in telomerase actions.