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Project Number
38062

Project Title
Using CRISPR-Cas9 to Elucidate How p53 Functional Status Modulates Telomerase Inhibition Treatment Efficacy

Abstract

Objectives/Goals
Over the past decade, many mechanisms of telomerase inhibition have been developed to treat cancer. Yet certain barriers prevent telomerase inhibition from becoming a robust treatment strategy for all cancers, of which dysfunctional p53 is simultaneously most critical and controversial. We address controversy from prior publications by investigating how p53 functional status modulates telomerase inhibition treatment efficacy, with the purpose of elucidating the contexts in which treatment efficacy can be maximized.

Methods/Materials
We used CRISPR-Cas9 to induce genetic knockdown of TERT in A549 lung adenocarcinoma. Separately, we also used BIBR1532, a telomerase inhibitor, to eliminate telomerase function. Telomerase-inhibited A549 was next treated with PFT (p53 inhibitor), or RITA (p53 activator). Cell viability and replicative senescence were subsequently quantified using the MTT and SA Beta-Galactosidase Assays, respectively.

Results
TERT-KD A549 exhibited 34.3% viability reduction. However, TERT-KD A549 treated with PFT exhibited 113.2% viability increase. Finally, TERT-KD A549 treated with RITA exhibited 118.6% viability increase. This final piece of data was initially unexpected; however, we explain this viability increase by hypothesizing that an uptick in induction of senescence occurred. We are currently in progress of gathering data to confer support for this conjecture. Results are also pending for treatment with BIBR1532, although we predict findings to be similar to that encountered with TERT-KD.

Conclusions/Discussion
Although telomerase inhibition is capable of inducing cellular apoptosis in the absence of p53, maintaining low levels of p53 function can greatly enhance this cytotoxic effect. In addition, while activation of p53 in telomerase-inhibited cells leads superficially to an increase in viability, this is likely countered simultaneously by increased induction of replicative senescence. Telomerase inhibition is currently an area of active research for its use in sensitizing cancer to other treatment protocols (ex. chemotherapy, radiation), with promising results. The world#s first telomerase inhibitor drug is currently in clinical trials. Developing a better understanding of the context in which telomerase inhibition treatment can be maximized in efficacy today, will improve our ability to treat cancer tomorrow.

Summary Statement
We sought to understand how manipulating the functional status of p53 can make telomerase inhibition treatment more effective at curing cancer.

Help Received
Dr. Mi Shi from Applied Stem Cell taught us how to conduct the CRISPR-Cas9 procedure.