CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

Andre L. Cornman

Project Number

S1706

Project Title

Rapamycin Treatment Decreases the Secretion of Senescent Murine Cells with Wild-Type and Inactive p53

Abstract

Objectives/Goals

Cellular senescence is a tumor suppressor mechanism which functions by permanently arresting cell cycle, while keeping cells metabolically active. Senescence may be triggered by a number of factors, including dysfunctional telomeres, DNA damage, chromatin perturbation, and oncogenic stimuli. Recently, senescent cells have been found to secrete 40-80 factors which are mainly composed of growth factors, proteases, chemokines and cytokines. The purpose of this research is to test the effects of blocking the mTOR pathway by treating senescent murine fibroblasts with Rapamycin on the senescence-associated secretory phenotype (SASP). We tested Rapamycin both on wild-type murine cells, as well cells carrying an inactive form of p53.

Methods/Materials

Control (wild-type) and p53 mutated primary mouse embryonic fibroblasts (MEFs) were cultured in media. The cells were irradiated using X-ray (10 Gy) or not for controls. After irradiation, the cells were treated with either 12.5 uM Rapamycin (RAPA) or vehicle (dimethyl sulfoxide, DMSO). Induction of senescence was measured using Beta-galactosidase. qRT-PCR reactions for p16, IL-6 and MMP-3 were performed to measure their RNA expression. Western blot was performed to measure p16 protein expression. Supernatant was collected and analyzed for IL-6 secretion using an ELISA assay.

Results

We found that p16, IL-6, and MMP-3 expression increased dramatically with senescent cells. Rapamycin treatment effectively reduces the secretion of SASP factors, such as IL-6 and MMP-3, in murine senescent cells. Interestingly, we found that inactive p53 increases SASP factors and that Rapamycin restrains the induction.

Conclusions/Discussion

The results supported our hypothesis that Rapamycin can effectively reduce the SASP in senescent murine cells, both wild-type and with inactive p53. These results show that Rapamycin could be used to reduce pro-inflammatory and paracrine activities of the SASP, which may drive age-related phenotypes and pathologies, including cancer. Also, conventional anticancer therapies, including chemotherapy and radiation therapy, have been shown to induce senescence. By reducing the SASP, we can improve prognosis and long term outcome of the therapy.

To improve the experiment, we are planning to test other factors such as Cxcl1, or repeat the experiment with human cells.

Summary Statement

This project tests the effectiveness of Rapamycin treatment on reducing secretion of factors caused by cellular senescence, both in wild-type and p53 mutated cells.

Help Received

Used lab equipment at the Buck Institute for Research on Aging under the supervision of Dr. Demaria