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Volume 12

Washington University
Undergraduate Research Digest

Spring 2017

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Recommended Citation

Hogan, Reuben Aaron, "Induction of IDH2 R140Q Mutation in Stem Cells with Doxycycline Increases Rate of Cell Death" (2017). *Volume 12*. 76.

https://openscholarship.wustl.edu/wuurd_vol12/76

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INDUCTION OF IDH2 R140Q MUTATION IN STEM CELLS WITH DOXYCYCLINE INCREASES RATE OF CELL DEATH

Reuben Aaron Hogan

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Mutations in isocitrate dehydrogenase 1 or 2 (*IDH1* or *IDH2*) occur in a variety of human cancers. IDH enzymes normally convert isocitrate to alpha-ketoglutarate (α KG) in the citric acid cycle. However, the cancer-associated mutations occur at specific positions in each gene, including position 140 of *IDH2*, and lead to the production of a novel molecule, R-2-hydroxyglutarate (2HG). 2HG has been shown to result in DNA and histone hypermethylation of leukemia samples in comparison to normal cells. To understand how this specifically blocks cellular differentiation and allows increased proliferation, we set up an experimental model within stem cells.

H9 cells were infected using a lentivirus which delivered DNA containing the IDH2 R140Q mutation, the GFP gene, and a promoter region that binds a receptor for doxycycline (DOX). When cells are treated with DOX, the receptors diffuse from the promoter, allowing transcriptional machinery to take over. The cell then produces mutant IDH2; however, induction of IDH2 R140Q kills stem cells instead of causing increased proliferation. Our hypothesis is that there is a differential gene expression in stem cells from leukemic cells that must be controlled for to use the model.

To test this hypothesis, we generated both an H9 IDH2 R140Q line and a H9 Vector line. Both were treated with DOX for at least 2 days and were brought up with untreated samples as controls. GFP expression was measured by flow cytometry as an indicator of IDH2 R140Q positivity. Cell counts were taken for all conditions (H9 IDH2 and H9 Vector \pm DOX) at different time points to determine the rate of cell death. RNA sequencing was done for all conditions to determine what genes were differentially expressed, and analysis was done to compare the expression between samples and with leukemia tumor samples as well.