Proving the Synthetic Lethal Interaction between ARID1A and EZH2 in Hepatocellular Carcinoma

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Abstract— **Liver cancer, of which the most common form is hepatocellular carcinoma (HCC), is one of the leading causes of cancer deaths worldwide. Owing to its limited treatment options, HCC has a poor prognosis. Current research is focused on targeted therapies, an example of which is synthetic lethality. Synthetic lethality describes a relationship between two genes in which the mutation or deletion of one gene is not lethal to the cell, while the alteration of both genes is lethal. A previous study established the synthetic lethal interaction between ARID1A and EZH2 in ovarian cancer. Since ARID1A is frequently mutated in HCC, this project sought to determine if ARID1A and EZH2 had a synthetic lethal interaction in HCC as well. To address that aim, EZH2 activity was inhibited through GSK126, a small molecule inhibitor, in both ARID1A-mutant and ARID1A wild type HCC cell lines. Ultimately, there was no clear sensitivity to EZH2 inhibition in ARID1A-mutant HCC cell lines, indicating that no such relationship exists in HCC.**

I. Introduction

Liver cancer is the second most lethal cancer worldwide, with the United States' five-year survival rate at 8.9%. In the past decade, there have been over 667,000 new cases diagnosed each year, 83% of which are hepatocellular carcinoma (HCC) [1]. Targeted therapies (using tumor-specific traits for selective targeting) represent a strategy that could combat both HCC's limited treatment options and its low survival rate. A potential treatment option is the exploitation of the concept of synthetic lethality, which is based on the interaction between two genes that contribute to similar essential processes. After the mutation or deletion of one of these genes, the cell is viable; however, mutations in both of these genes are lethal to the cell [2]. Targeting genes that are synthetic lethal pairs may be beneficial, as only cancer cells with certain genetic alterations would be killed.

A previous study established the synthetic lethal interaction between the genes ARID1A and EZH2 in ovarian cancer [3]. Nevertheless, it cannot be assumed that the same is true for all cancers. It is hypothesized that inhibiting EZH2 in ARID1A-mutant HCC cell lines will cause cell death, demonstrating the synthetic lethal interaction between the two genes. This could lead to better treatments for HCC, as the cancer cells now lacking ARID1A and EZH2 activity would die without significantly harming healthy cells.

II. Methods

The following human HCC cell lines were used in this study: Huh7, SNU423, SNU398, and SNU475 (obtained from the JCRB and ATCC). Huh7 and SNU423 are ARID1A-mutant, while SNU398 and SNU475 are ARID1A wild type. The cell lines were treated for 72 hours with 10 concentrations between 0 and 20 μM of GSK126, an EZH2-activty inhibitor, before an MTS assay was performed. An MTS assay is a colorimetric assay that tests cell viability. To check how the drug affects cell growth, a colony formation assay was performed after cells were treated with 4 concentrations of GSK126 for 14 days. Lastly, a western blot confirmed that GSK126 was successfully working.

III. Results and Conclusions

The most sensitive cell line to EZH2 inhibition, especially at high doses, was SNU398, despite its ARID1A wild type status, while SNU423, an ARID1A-mutant cell line, was the most resistant. Moreover, GSK126 was not selective among different cell lines. There was no significant difference in the number of live cells for all cell lines at low doses (between 0 and 0.5 μM, t-test p-values>0.05), while there was a significant difference in the number of cells for all cell lines at high doses (between 0 and 5 μM, t-test p-values<0.05).

This result indicates there is no convincing evidence for the synthetic lethal interaction between ARID1A and EZH2 in HCC. There is not a clear sensitivity to GSK126 treatment in ARID1A-mutant cells lines, nor is there a clear resistance in ARID1A wild type cell lines, as predicted. Based off these data, EZH2 inhibition in ARID1A-mutated HCC is not an effective treatment option. While this study was unable to establish a new treatment option, it ruled out an unsuccessful treatment strategy, limiting the number of additional experiments that must be done to identify a synthetic lethal target in HCC.

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