A Deadly Combination: Complete Knockout of Neutral Ceramidase Effectively Kills Lung Cancer While Protecting the Kidneys from Cisplatin Chemotherapy

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Abstract

According to the CDC, lung cancer is the third most diagnosed cancer in the world. Kentucky has one of the highest lung cancer rates with the lowest survival and early diagnosis rates in the nation. Further research is needed to better understand the mechanisms driving lung cancer's high mortality rate. One potential novel therapeutic target is neutral ceramidase (NC). The Siskind lab has shown loss of NC in mice is protective to the kidneys from chemotherapy-induced toxicity. NC is encoded by ASAH2 and metabolizes the sphingolipid ceramide. Sphingolipids are involved in a variety of cell functions such as cell growth, differentiation, and apoptosis. Effects of NC knockdown in cancer are unknown. This study investigated the role of NC knockout and knockdown in human lung adenocarcinoma cell lines (A549, H1437). These cell lines, stably expressing CAS9, were transfected with gRNAs or siRNAs to knockout or knockdown ASAH2. Cell viability was determined following knockout or knockdown via AlamarBlue and was compared with positive (KIF11 and RPS11) and negative (non-targeting) controls. Additionally, in an attempt to confirm the knockout or knockdown of ASAH2, activity assays for NC were performed in the surviving cells. Our preliminary results show knockout of ASAH2 in A549 is lethal when compared to the positive controls, but in H1437 had no effect. However, knockdown with siRNA had opposite effects for A549 with a significant increase in growth, but again had no effect in H1437 cells. Results at this stage are not conclusive as to the role of NC in lung cancer cell viability because we could not achieve effective decrease in its enzyme activity. Further studies are needed with a system that allows for inducible knockout or knockdown to more completely understand the role of ASAH2 in lung cancer.

Background

- Lung cancer is one of the leading causes of cancer-related deaths.
- The Siskind lab has shown loss of NC in mice is protective to the kidneys from chemotherapy-induced toxicity.
- NC is encoded by ASAH2 and metabolizes ceramide, which is a sphingolipid, to a fatty acid and a sphingosine.
- Sphingolipids are involved in a variety of cell functions such as cell growth, differentiation, and apoptosis.

Hypothesis

- We hypothesize that complete knockout or knockdown of neutral ceramidase effectively kills lung cancer while still protecting the kidneys from injury that is caused by cisplatin chemotherapy.

Methods

- Human cancerous cell lines A549 and H1437 were cultured in RPMI-1640 +10% FBS and pen/strep. Cells grew in 37ºC at 5% CO_2.
- Cells stably expressing CAS9 were transfected with gRNAs and siRNAs to knockout or knockdown expression of the target gene (ASAH2) along with positive (KIF11 and RPS11) and negative (non-targeting) transfection controls using DharmaFECT 1 transfection reagent.
- Once transfected, cells continued to grow at 37ºC at 5% CO_2.
- Cells were grown for 7-days post transfection before measuring cell number using AlamarBlue
  - Neutral ceramidase activity was measured in vitro using NBD labeled C12-ceramide as substrate and TLC to separate substrate and product.

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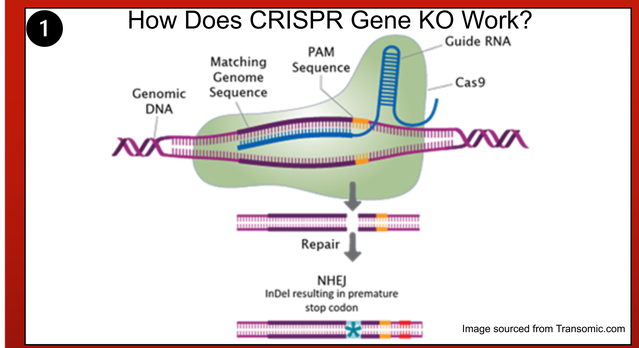

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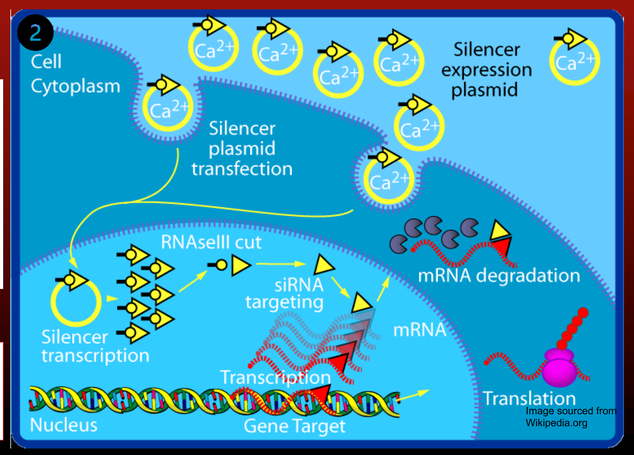

Results

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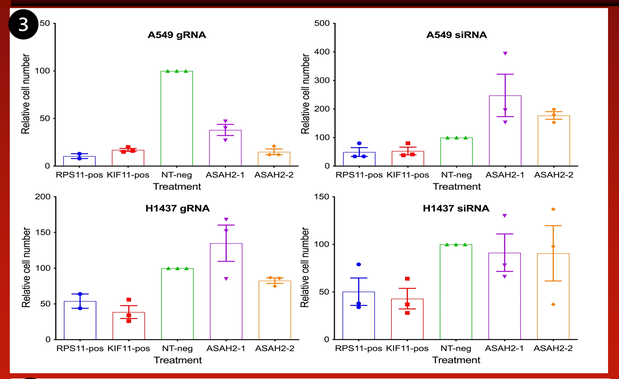

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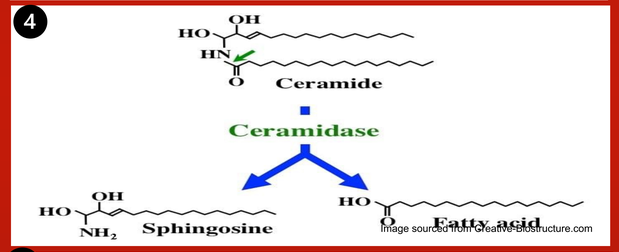

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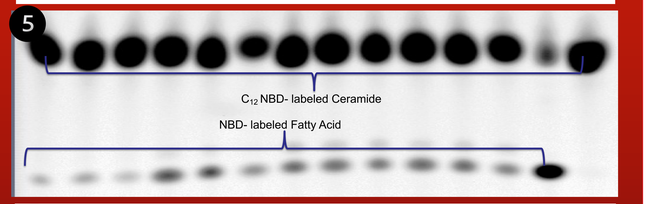

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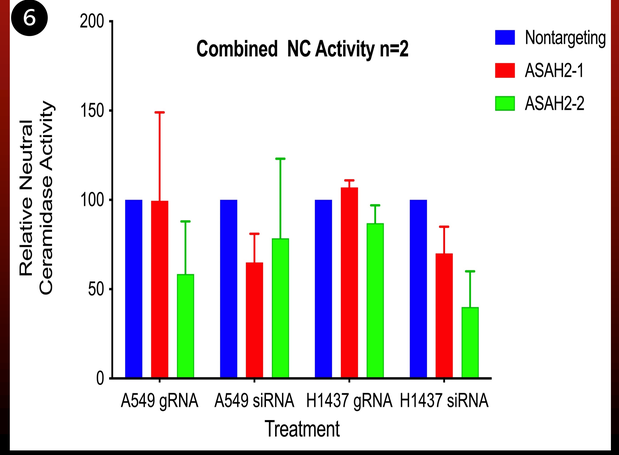

- Figure 1 depicts how CRISPR gene knockout works.
- Figure 2 shows how SiRNA can knockout mRNA as well as protein expression.
- Figure 3 is a bar graph with combined results from two separate AlamarBlue assays to measure relative cell number.
- Figure 4 is a schematic diagram of the ceramidase hydrolysis process.
- Figure 5 is a TLC plate used to separate the reaction components of the NC experiment. Very top is NBD-labeled ceramide substrate and the middle is NBD-labeled fatty acid product.
- Figure 6 shows a bar graph with combined results from two separate NC activity assays.

Conclusion and Future Directions

- Knockout of neutral ceramidase appears to be lethal in some lung cancer cell lines (A549) but not others (H1437).
- Knockdown of neutral ceramidase appears to lead to significant increases in growth within A549 cells but no effect on H1437.
- Further studies are needed to more completely understand the role of ASAH2 in lung cancer.
- Exploration of other cell lines such as non-cancerous human cells and cancerous/non-cancerous mice cells will be used and have same tests conducted.

Acknowledgments

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