Analyzing the Effect of HADH Overexpression on Response to Immune Checkpoint Blockade

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Introduction

Immune checkpoint blockade (ICB), which targets the inhibitory immune checkpoints on the T cells, has recently revolutionized cancer treatment. ICB works by introducing antibody, such as anti-PD1 and anti-CTLA, that can bind to the inhibitory molecules on CD8+ T cells in cancer patients. Upon binding to the targets such as PD1 and CTLA4, the inhibitory signals on the T cells are removed, which reinvigorates the T cells anti-tumor functions. ICB has been shown to induce favorable and long-lasting anti-tumor response in many cancer types including bladder cancer. However, only around 30% of bladder cancer patients respond to ICB, highlighting the urgent need to understand the resistance mechanisms to ICB and improve its efficacy to benefit more cancer patients.

Hypothesis

To systematically investigate the resistance mechanisms to ICB and explore the potential targets that can improve ICB efficacy, Dr. Sharma’s lab has previously performed an in vivo CRISPR knockout screen and identified a list of 50 genes which can modulate a tumor’s response to ICB. One of the genes is HADH, which encodes 3-hydroxyacyl-CoA dehydrogenase and catalyzes the third step in beta oxidation. During lipid beta oxidation, NADH is produced and used by mitochondria oxidative phosphorylation (OXPHOS) to provide ATP for the tumor cells. Enhanced OXPHOS has been shown to promote lipid oxidation, which can sensitize tumor cells to a type of cell death called ferroptosis. Based on our preliminary data that HADH knockout conferred tumor cell resistance to ICB, we hypothesize that HADH expression affects response to ICB by affecting beta oxidation, production of ROS, lipid peroxidation, and CD8 T cell induced ferroptosis.

Results

Figure 2. In vivo CRISPR knockout screen identified 50 genes as potential targets for improving ICB. Cells with HADH knockout were enriched under anti-PD1 selective pressure, suggesting that knocking out HADH can confer cancer cell resistance to ICB.

Figure 3. Project hypothesis. HADH activity affects response to ICB by affecting beta oxidation, synthesis of ROS, lipid peroxidation, and response to ferroptosis.

Materials and Methods

Generating Cells with HADH

Cells transfected with HADH

Figure 4. An HADH⁰ plasmid was transfected into MB49 cells, a mouse urothelial carcinoma cell line. Overexpression of the gene was analyzed by conducting qPCR and a Western blot.

Figure 5. Western blot analysis of HADH expression shows successful transfection of HADH overexpression plasmid into MB49 cells.

HADH

bActin

Figure 6. qPCR analysis of HADH overexpression shows that the HADH overexpression cell line had a 16-fold change in transcription level compared to wild type cells.

Conclusions

We successfully established a mouse bladder cancer cell line with HADH overexpression. We expect to find that overexpressing HADH can delay tumor growth and improve animal survival when combined with anti-PD1 treatment.

Future Directions

More experiments can be done in vitro to analyze ferroptosis, levels of NAD, and levels of ROS to better understand how overexpression of HADH affects each process or type of molecule.

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References


