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OXPHOS Inhibition via LUC7L2 as a Target for SF3B1-Mutant Myelodysplastic Syndrome

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OXPHOS Inhibition via LUC7L2 as a Target for SF3B1-Mutant Myelodysplastic Syndrome

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Abstract: SF3B1 gene mutations are the most common spliceosome mutations seen in myelodysplastic syndrome (MDS) patients. Though it is well known SF3B1 mutations cause downstream changes in erythroid differentiation and the cell cycle, which leads to malignancy, metabolic changes arising from this mutation are unknown. We conducted RNA sequencing from SF3B1-mutant MDS patient samples and found several genes related to metabolism were alternatively spliced. Of these, LUC7L2 was selected as our target as previous studies show its involvement in promoting oxidative phosphorylation (OXPHOS) via various downstream mechanisms when knocked down.[1] We show that OXPHOS is increased in MOLM-13 myeloid malignant cells when LUC7L2 is inhibited. The results suggested that this gene, which is alternatively spliced and shows lower expression in SF3B1-mutant MDS, increases myeloid malignant dependence on OXPHOS.

Introduction: Myelodysplastic syndromes (MDS) are a group of blood cancers with six classifications established by the WHO. Patients typically present with cytopenias and carry a risk of progression to acute myeloid leukemia (AML). Approximately half of MDS patients harbor a spliceosome mutation, most commonly in the SF3B1 gene. SF3B1 functions as a subunit of the spliceosome factor 3b (SF3B) complex, which is considered a key U2 snRNP spliceosome component in human cells.

SF3B1 mutations are believed to be the initiating genetic lesion in SF3B1-mutant MDS. Its mutated form maintains activity which affects RNA processing of many genes, ultimately,

leading to the genesis of myelodysplastic stem cells. Downstream effects of SF3B1 mutations include changes in the cell cycle and erythroid differentiation. However, the role of SF3B1 mutations in metabolic processes is less well known. Our preliminary data shows that patient samples harboring an SF3B1 mutation exhibit alternative splicing of the gene LUC7L2. Interestingly, this gene encodes for another protein of the spliceosome complex: U1 snRNP. The gene LUC7L2 has been shown to regulate metabolic processes by promoting glycolysis while repressing oxidative phosphorylation (OXPHOS) via various downstream mechanisms.[1] We therefore hypothesize that SF3B1-mutant MDS could result in alternative splicing of the gene LUC7L2, thereby altering the metabolic dependencies of these cells.

Objective: To determine whether SF3B1-mutant MDS cells undergo a metabolic shift and more readily utilize OXPHOS for energy production as a result of altered expression of LUC7L2.

Methods: RNA sequencing was performed to compare four SF3B1 mutant MDS patient samples with non-mutant ones. LUC7L2 was found to be alternatively spliced and downregulated in SF3B1 mutant cells. Genetic inhibition of LUC7L2 was then performed in the non-mutant MOLM-13 myeloid malignant cell line using siRNA technology, and a Western blot was performed to ensure siRNA inhibition was successful. Seahorse assays were then performed to assess oxidative phosphorylation upon knockdown of LUC7L2.

Results: RNA-sequencing from SF3B1-mutant MDS patient samples compared to non-mutant ones showed alternative splicing of multiple genes involved in metabolism, including respiratory complex genes, solute carrier family, and mitochondrial membrane translocases. LUC7L2, a splicing factor known to negatively regulate OXPHOS in other tissues, was also alternatively spliced and its gene expression was decreased. Upon inhibition of LUC7L2 by siRNA technology in MOLM-13 myeloid malignant cells, we saw increased OXPHOS, confirming this

gene is tightly linked to OXPHOS regulation (Figure 1). To test whether SF3B1-mutant cells are dependent on OXPHOS for survival, we treated them with oligomycin, an ATP synthase inhibitor. Mutated cells show less viability than the wild type suggesting an increased dependence on OXPHOS (Figure 2).

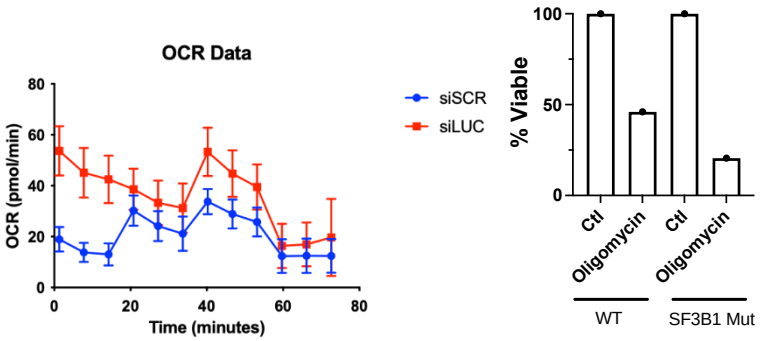

Figure 1. Seahorse assay displaying oxygen consumption rate (OCR) of two populations of MOLM-13 myeloid malignant cells.

Figure 2. Viability assay utilizing oligomycin on wild type and SF3B1-mutant cells.

Discussion: Although the role of SF3B1 mutations in metabolism is largely unknown, recent studies showing acute myeloid leukemia stem cells increased dependence on OXPHOS inspired the design of this project. As MDS can be the precursor to AML in some patients, we hypothesized that similar vulnerabilities could exist. In this study, we show SF3B1 mutations are associated with alternative splicing and downregulation of several genes involved in metabolism. Among them is LUC7L2, a gene known to negatively regulate OXPHOS. With lower expression of LUC7L2 in SF3B1-mutant MDS cells, OXPHOS becomes the main source of energy production in these cells, and it is therefore a potential therapeutic target.

References:

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