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AUTHOR'S VIEW

The metabolic reprogramming and vulnerability of *SF3B1* mutations

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ABSTRACT

Mutations in the splicing factor 3b subunit 1 (*SF3B1*) gene create a neomorphic protein that disrupts RNA splicing, but the downstream consequences of this missplicing are unclear. Our recent study of isogenic human cells demonstrated that *SF3B1*^{MUT} induces reprogramming of energy metabolism and a targetable vulnerability to deprivation of the nonessential amino acid serine.

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Initially found in >70% of myelodysplastic syndromes (MDS), spliceosome mutations have now been described in many hematologic and solid malignancies.[1–3] The splicing factor 3b subunit 1 (*SF3B1*) gene is the most widely mutated, occurring in MDS, acute myeloid leukemia (AML), chronic lymphocytic leukemia, melanoma, breast carcinoma, pancreas adenocarcinoma, and many other cancers. *SF3B1* is a member of the U2 small nuclear ribonucleoprotein (U2snRNP) complex, essential for branch point sequence recognition in pre-mRNA. Mutant *SF3B1* is a neomorphic protein that disrupts the usage of thousands of splice junctions, leading to altered expression of hundreds of genes, affecting dozens of cellular pathways.[1,3] Given the variety of affected processes, it has been a challenge to understand what missplicing events are physiologically impactful in *SF3B1*^{MUT} cells.

To understand consequences of *SF3B1*^{MUT} missplicing, we recently characterized the transcriptome and proteome of *SF3B1*^{MUT} human isogenic cells.[4] This analysis showed enrichment of metabolic genes in *SF3B1*^{MUT} cells, including a decrease in mitochondrial complex III of the electron transport chain (ETC), essential for cellular respiration. This was mediated through missplicing and downregulation of its assembly factor, ubiquinol-cytochrome c reductase complex assembly factor 1 (*UQCC1*), as re-expression of this protein was able to rescue complex III levels. *SF3B1*^{MUT} also decreased cellular respiration, reduced citric acid cycle metabolites, and misspliced and downregulated other metabolic enzymes of the mitochondria – dihydrolipoamide S-succinyltransferase (*DLST*) and methylmalonyl-CoA mutase (*MUT*) (Figure 1). This reprogramming of mitochondrial metabolism bears particular relevance to the form of MDS in which *SF3B1* is most frequently (>85% of cases) mutated: MDS with ring sideroblasts (MDS-RS).[5] This disease is characterized by dysplastic erythroblasts (the "sideroblasts") with iron-overloaded mitochondria (the "rings"). Interestingly, some forms of congenital sideroblastic anemia (CSA) are characterized by mutations in ETC genes, causally implicating impaired cellular respiration in ring sideroblast formation.[6] In this context, our results suggest that ETC disruption by *SF3B1*^{MUT} may contribute to the sideroblastic anemia of MDS-RS. Consistent with this, data from 40 years ago showed that in MDS-RS patients, granulocytes (*SF3B1*^{MUT} in >85% of cases) had reduced ETC activity when compared to healthy controls, while ETC activity of lymphocytes (unmutated in MDS-RS) was similar between groups.[7] Hsu et al also recently reported a decrease in metabolically active mitochondria in induced pluripotent stem cells derived from *SF3B1*^{MUT} MDS.[8] It will be interesting for future studies to determine how missplicing events, such as in *UQCC1, DLST* or *ABCB7* (the latter downregulated by *SF3B1*^{MUT} and also a cause of CSA), drive impairment of cellular respiration and ring sideroblast formation in *SF3B1*^{MUT} MDS.[5]

Our study also identified another metabolic gene that was heavily misspliced and downregulated by SF3B1^{MUT}: phosphoglycerate dehydrogenase (*PHGDH*), the gatekeeper enzyme controlling synthesis of the nonessential amino acid serine. We found that *SF3B1*^{MUT} cells had lower baseline serine synthesis, as well as a reduced ability to increase its relative synthesis when deprived of exogenous serine. Accordingly, *SF3B1*^{MUT} cells exhibited greatly decreased growth without exogenous serine, as compared to their wild type counterparts. This we observed in several contexts, including untransformed and transformed breast epithelial cell knockins, leukemia cell knockins, and AML cell lines from patients with naturally acquired *SF3B1*^{MUT} (the latter cells simply died in the absence of exogenous serine). Overexpression of *PHGDH* – or supplementation with its reaction product, phosphohydroxypyruvate (PHP) – was able to rescue growth without exogenous serine, supporting deficient serine synthesis as a mechanism for this vulnerability. Since Maddocks et al showed in mice that a serine-free diet will drop serine levels by 60%, exert no clear toxicity, and can produce anticancer activity,[9] we performed this intervention on two different naturally *SF3B1*^{MUT} AML cell lines. These experiments showed that *SF3B1*^{MUT} cancers in mice fed a serine-free diet grew significantly slower than that of mice given a serine-replete diet, demonstrating that this vulnerability could be exploited in vivo.

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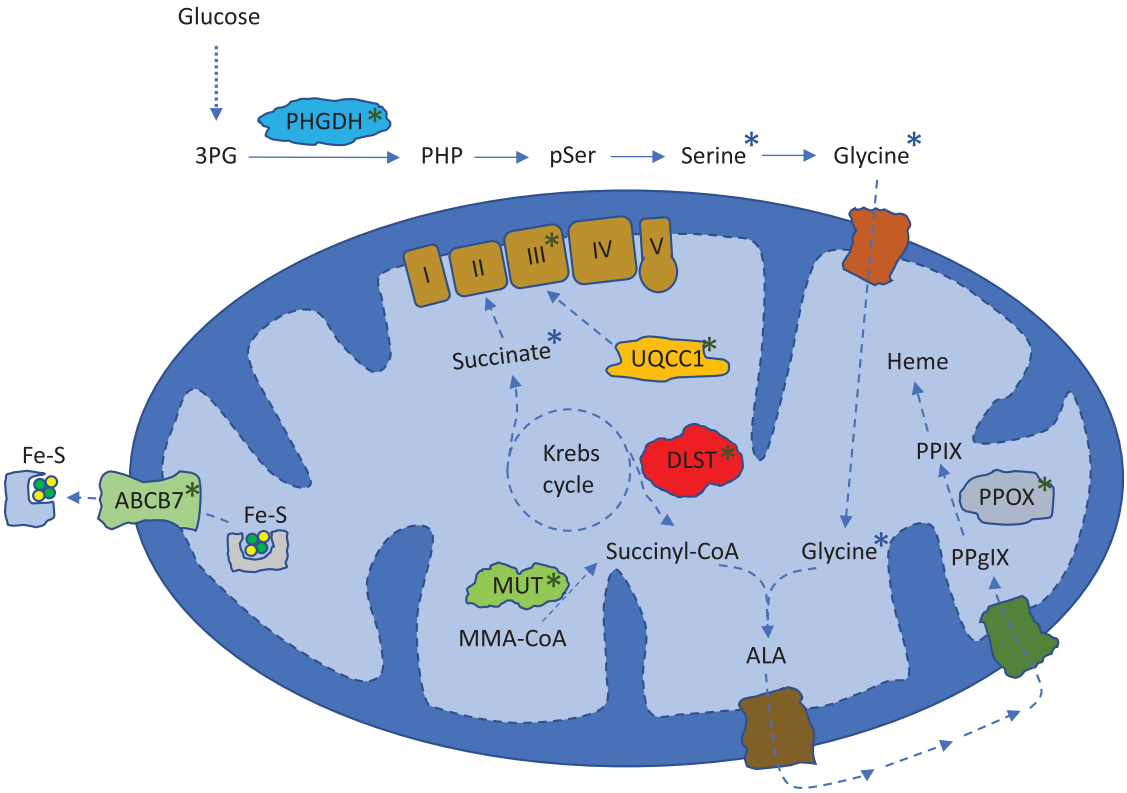

Figure 1. Metabolic pathways affected by mutant SF3B1. Blue asterisks indicate metabolites that were found to be downregulated by SF3B1^{MUT}. Green asterisks show metabolic proteins found to be misspliced and downregulated by SF3B1^{MUT}. 3PG = 3-phosphoglycerate. PHP = phosphohydroxypyruvate. pSer = phosphoserine. I, II, III, IV, V = mitochondrial complexes. Fe-S = iron-sulfur complex. MMA = methylmalonyl. ALA = aminolevulinic acid. PPgIX = protoporphyrinogen IX. PPIX = protoporphyrin IX. PHGDH = phosphoglycerate dehydrogenase. UQCC1 = ubiquinol-cytochrome c reductase complex assembly factor 1. DLST = dihydrolipoamide S-succinyltransferase. MUT = methylmalonyl-CoA mutase. ABCB7 = ATP binding cassette subfamily B member 7. PPOX = protoporphyrinogen oxidase.

Our findings offer serine deprivation as a novel treatment strategy for *SF3B1*^{MUT} cancers. Modulating serine availability in humans might be approached in several ways, including dietary serine restriction (dietary restriction of individual amino acids is commonplace in management of inborn errors of metabolism), development of a therapeutic serine-catabolizing enzyme (asparaginase is a backbone of acute lymphoblastic leukemia treatment), inhibition of serine transport (pharmacologic inhibition of cystine transport is under clinical investigation), and other modalities.[10] Further insight into the mechanisms of serine auxotrophy in *SF3B1*^{MUT} cells may also help such efforts. Though our data causally implicates decreased serine synthesis through *PHGDH* downregulation, other mechanisms may contribute, such as decreased cellular respiration, given that human cells increase their reliance on oxidative phosphorylation as a compensatory adaptation to serine starvation.[9] Finally, it is worth noting that it remains largely unclear how spliceosome mutations confer a clonal advantage to cancer cells, so it is possible that metabolic reprogramming by *SF3B1*^{MUT} has advantageous, and not just detrimental, effects. Future research is certainly needed to better understand the mechanisms and consequences of metabolic rewiring by *SF3B1* mutations. Nonetheless, our study provides a novel and therapeutically relevant connection between two previously unacquainted processes in molecular and cellular oncology: mutant spliceosomes and metabolic reprogramming.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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