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ORAL ABSTRACTS

101.RED CELLS AND ERYTHROPOIESIS, EXCLUDING IRON

Mitochondrial tRNA Pseudouridylation Regulates Erythropoiesis Via the mTOR Signaling Pathway: Implications for Mlasa and Treatment Strategies

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Pseudouridine, the most abundant RNA modification found in various RNA molecules, is crucial for cellular functions. Pseudouridylation is the process of converting uridine to pseudouridine, catalyzed by pseudouridine synthases (PUSs), and abnormal pseudouridylation has been linked to human diseases, including mitochondrial myopathy, lactic acidosis, and sideroblastic anemia syndrome (MLASA). MLASA is associated with genetic mutations in the PUS1 gene, leading to progressive exercise intolerance, sideroblastic anemia, hyperlactatemia, and muscle weakness. The underlying mechanism of anemia in MLASA remains unclear.

In this study, we identified a novel homozygous frameshift deletion (c.523delC, p.P175fs*8) in the PUS1 gene in an MLASA patient with progressive anemia in our hospital. We investigated the role of pseudouridylation in erythropoiesis and its impact on MLASA using *in vitro* and *in vivo* approaches. We established an MLASA patient-derived inducible pluripotent stem cell (iPSC) line (referred to as MLASA-iPSCs) with the mutation, as well as a repaired line (MLASA-Res iPSC), and a corresponding mutant mouse model for the investigation.

During erythroid differentiation, MLASA-iPSCs showed a significant decrease in the production of erythroblasts compared to the repaired line. Also, MLASA-iPSCs exhibited a lower ratio of mitochondrial membrane potential to mitochondrial mass, decreased oxygen consumption rates, reduced ATP production, and elevated levels of ROS. Attenuated activities of NADH dehydrogenase (Complex I) and cytochrome c reductase (Complex III) were also observed in MLASA-iPSCs, indicating compromised mitochondrial functions.

Further analysis revealed a reduction in mitochondrial tRNA levels and abnormal mitochondrial translation in MLASA-iPSCs due to the specific loss of pseudouridylation. Screening of mitochondrial supplements, which could boost mitochondrial function or modulating protein synthesis, revealed that nicotinamide ribose (NR) and mitoquinone (MitoQ) improved mitochondrial function but failed to promote erythroid differentiation in MLASA-iPSCs. Interestingly, rapamycin, an mTOR inhibitor, improved erythroid differentiation in MLASA-iPSCs. Furthermore, MLASA-iPSCs showed activated mTOR signaling, increased protein synthesis and higher translation efficiency of mTOR1 targets, all of which could be rescued by rapamycin treatment, suggesting mTOR-associated translation stress.

Consistently, the corresponding PUS1 mutant mouse model exhibited anemia with blocked erythropoiesis at four weeks of age. Serial competitive transplantation experiments revealed an intrinsic impairment in the reconstitution of red blood cells. Mutant erythroblasts showed impaired oxidative phosphorylation and aberrant activation of the mTOR signaling pathway. Rapamycin treatment partially alleviated the anemia phenotype in mice.

Significantly, in the MLASA patient, sirolimus (rapamycin) administration under strict supervision and medical guidance effectively alleviated anemia symptoms and improved blood routine profile.

In summary, our findings provide insights into the critical role of mitochondrial tRNA pseudouridylation in governing erythropoiesis via mTOR signaling pathway. We propose rapamycin as potential therapeutic strategies for anemia patients experiencing protein translation pressure, such as those with MLASA.

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Fig. 1 Schematic illustration of erythropoiesis regulated by PUS1-mediated pseudouridylation.
Under physiological conditions (left), PUS1 regulates mt-tRNA abundance by catalyzing the pseudouridine synthesis of specific mt-tRNAs, thereby regulating mitochondrial translation, and ultimately affecting mitochondrial function and erythroid differentiation. In MLASA disease states (right), PUS1 deficiency causes the loss of pseudouridine modifications of specific tRNAs and thus affects the amount of PUS1 targeted tRNAs and its corresponding mitochondrial protein products, leading to mitochondrial dysfunction. The defects resulted in aberrant activation of the mTOR signaling pathway and accelerated global translation rate, ultimately leading to a blockage in erythroid differentiation, which could be relieved with rapamycin treatment.

Disclosures No relevant conflicts of interest to declare.

OffLabel Disclosure: RAPAMUNE (sirolimus) is an mTOR inhibitor that is used off-label for the treatment of patients with mitochondrial myopathy, lactic acidosis, and sideroblastic anemia (MLASA) to alleviate anemia in this study.

Fig. 1

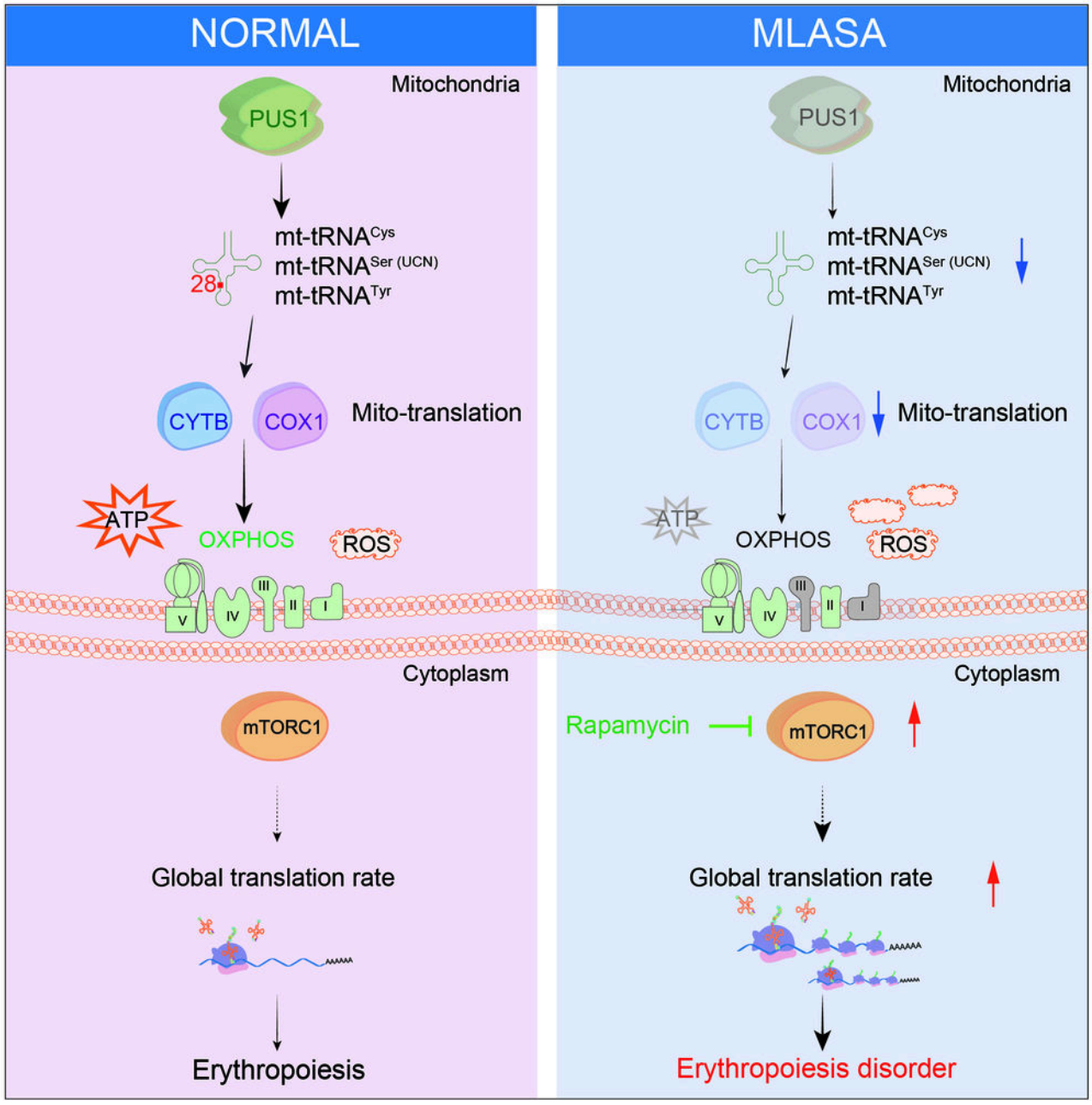

Figure 1

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