Title: *RPS26*-deficiency in Diamond-Blackfan Anemia syndrome represents a distinct subset of disease

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Introduction: Diamond-Blackfan Anaemia Syndrome (DBAS) is a rare inherited bone marrow failure syndrome, characterized by hypoplastic anaemia, congenital anomalies, and an increased cancer risk. It is mostly caused by loss-of-function mutations in ribosomal protein (RP) genes, disrupting ribosome biogenesis. The mechanisms underlying DBAS anaemia are still unclear, and research is limited due to the scarcity of patient samples and disease models. We recently developed an induced pluripotent stem cell (iPSC) platform for generating hematopoietic organoids, offering a valuable tool for studying erythropoiesis.

The RPS26 gene is frequently mutated in DBAS patients. It has been reported that those patients have a worse response to treatment with glucocorticoids compared to patients with other molecular subtypes. Notably, RPS26-DBAS is the only molecular subgroup in which no DBAS-associated cancers have been reported so far, suggesting possible protective mechanisms.

Methods: DBAS patients, including one carrying the RPS26 c.95-98 dup. (p. Asp. 33 Glu fs*6) mutation, were selected from the Dutch Registry (DBAN). PBMC-derived erythroblasts were reprogrammed to iPSC using Cytotune-Sendai-IPS2.0-kit. Here we study three iPSC lines derived from this patient of which one was genetically corrected using CRISPR/Cas9-mediated homology directed repair. We analyzed embryoid body formation, hematopoietic organoid development, and hematopoietic cell production.

Results: We have collected clinical data from our DBAN registry study, and from international registry reports in the literature. In the RPS26 group, more patients were treated with chronic blood transfusions or allogeneic hematopoietic stem cell transplantation, suggesting that patients with RPS26 defects indeed respond less to glucocorticoids. In our RPS26-DBAS iPSC lines, harboring the c.95-98 duplication, embryoid bodies often disaggregated after a few days, in contrast with those from other DBAS or healthy iPSC lines, suggesting a specific role for RPS26 in embryoid body formation. Intriguingly, even though the patient carrying this mutation displays a very mild DBAS phenotype, the iPSC lines failed to produce functional hematopoietic organoids. CRISPR Cas9 mediated genetic correction restored embryoid body formation, suggesting a RPS26-specfic role. We are also introducing RPS26 haploinsufficiency into a healthy donor iPSC line to further study its effects on embryoid body and hematopoietic organoid formation.

Conclusion: Studies in RPS26-DBAS patients and RPS26-deficient iPSC lines suggest a specific subtype of disease, illustrated by compromised embryoid body formation, and impaired formation of functional hematopoietic organoids. This may be explained by altered translation by RPS26-deficient ribosomes, which can be resolved by genetic correction. Further research will demonstrate how RPS26 mutations affect cell viability and hematopoietic organoid formation in iPSC models, and the specific role of RPS26 in erythropoiesis, and other hematological and non-hematological manifestations in DBAS.