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DBA syndrome erythroid progenitors display abnormal metabolomic profile

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INTRODUCTION

- Diamond-Blackfan anemia syndrome (**DBAS**) is a rare, hereditary bone marrow failure syndrome, commonly caused by **haploinsufficiency** of genes encoding for ribosomal proteins (RPs).-This results in **reduced proliferation** and **defective differentiation** of CD34⁺ erythroid progenitors from DBA patients.
- In DBAS, ***RPS19*** and ***RPL5*** are among the **most frequently affected RP genes**. We generated **two conditional mouse models of *Rps19* and *Rpl5*** haploinsufficiency under a Vav-iCre promoter. These models revealed distinct, RP-specific, disease mechanisms.
- Mouse ***Rpl5* haploinsufficient embryos** displayed **an increase of the HSPC** compartment as a compensation for the severe anemia arising from ferroptosis-driven cell death at the erythroid progenitor stage.

AIM

To understand how *RPL5* haploinsufficiency impacts the erythroid progenitor's metabolome.

METHODS

- **CD34⁺ cells isolation** from peripheral blood of *RPL5* haploinsufficient DBAS patients (without steroid treatment) and healthy controls.
- In vitro **differentiation** of isolated CD34⁺ cells for XX days, leading to differentiation into erythroid progenitors
- **Isolation** of **cKit⁺ fetal liver cells** from *Rpl5* haploinsufficient murine embryos (Vav-iCre⁺ Rpl5$^{lox/+}$) as well as from littermate controls (Vav-iCre⁻ Rpl5$^{lox/+}$).
- **Metabolomic analysis** of cultured human erythroid progenitors and of isolated murine cKit⁺ with **High-Throughput Metabolomics** using Isocratic and Gradient Mass Spectrometry Methods

RESULTS

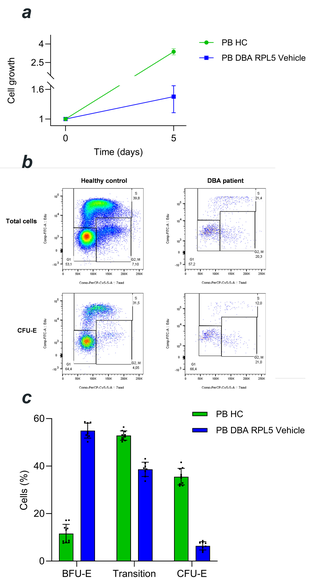

***Figure 1. P*rimary progenitor cell culture from RPL5 DBA patients showed impaired proliferation and differentiation**
a. Cell growth of DBA RPL5 erythroid precursor cells compared to healthy control.
b. Gating strategy used to analyze sub-populations in DBA RPL5 erythroid precursor cells and healthy control ones.
c. Quantification of different sub-populations (BFU-E, Transition and CFU-E) in DBA erythroid precursor samples compared to healthy control ones.

RPL5 haploinsufficient DBA cells are characterized by increased fatty acids and acylcarnitine's, reduction in redox metabolites levels and nucleotides associated with alterations of glucose metabolites

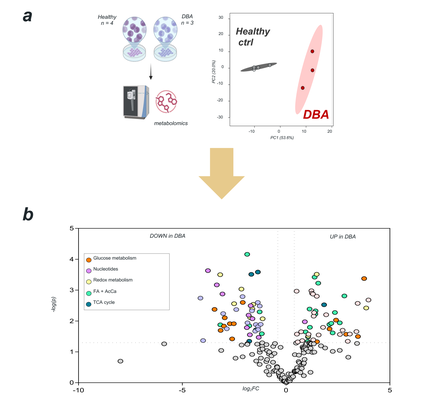

Figure 2. Metabolic profiles of erythroid precursors from subjects with RPL5 haploinsufficiency Diamond-Blackfan anemia (DBA, n=3) versus healthy controls (n=4).
a. Left panel. Experimental overview of erythroid precursor analysis by mass spectrometry-based metabolomics. **Right panel.** Principal component analysis (PCA) of metabolomics data from healthy control erythroid precursor cells versus DBA precursor cells at baseline.
b. Volcano plot indicating pathway-level up- and down-regulation of metabolites. FA = free fatty acid, AcCa = acylcarnitine, TCA = tricarboxylic acid.

RPL5 haploinsufficient human DBA cells display alterations of glucose downstream metabolites characterized by lower levels of ATP, pentose phosphate pathway intermediates, Krebs cycle metabolites and higher pyruvate and lactate content and decrease GSH system

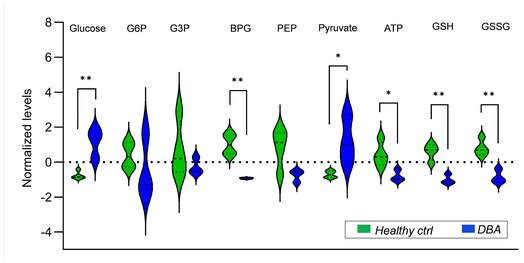

Figure 3. Relative levels at baseline of key energy and redox metabolites including glycolysis, ATP, and glutathione metabolism. Statistics are based on unpaired t-test. * p<0.05, ** p<0.01. G6P = glucose 6-phosphate, G3P = glyceraldehyde 3-phosphate, BPG = bisphosphoglycerate, GSH = reduced glutathione, GSSG = glutathione disulfide. Statistics are based on two-way ANOVA with multiple comparisons within genotypes. ** p<0.01.

RPL5 haploinsufficient DBA cells display increased pyruvate content and up-regulation of both PKR and PKM gene expression, associated with reduced ATP level when compared to health

Figure 4. Left to right panel. Intracellular pyruvate levels- PKLR and PKM gene expression- ATP level in RPL5 haploinsufficiency vs healthy cells. Statistics are based on two-way ANOVA with multiple comparisons within genotypes. * p<0.05. H: Intracellular ATP levels. Statistics are based on two-way ANOVA with multiple comparisons within genotypes

RPL5 haploinsufficient mouse *ex vivo* fetal liver derived erythroid cells display alterations of pentose phosphate pathway intermediates, Krebs cycle metabolites similarly to the human counterpart associated with persistent up-regulation of PKM2 expression

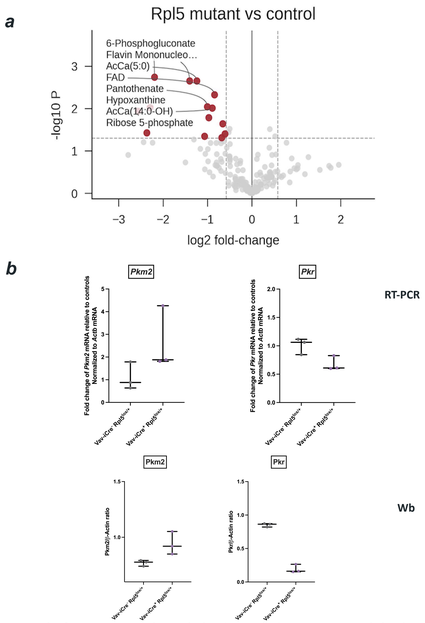

Figure 5.a. Results from untargeted metabolomics analysis, comparing *Rpl5* haploinsufficient cKit⁺ fetal liver cells with those of littermate controls**. b.** Gene **(upper panel)** and protein **(lower panel) expression of PK isoforms in *Rpl5* haploinsufficient cKit+ fetal liver cells.** Data are presented as median with range.

CONCLUSIONS

- In ***RPL5* haploinsufficient cells** from DBAS patients, our metabolomic data indicate increased **pro-oxidant environment** associated with **deep perturbation** of **cellular energy** when compared to healthy controls.
- *RPL5* haploinsufficient cells from DBAS patients display **up-regulation of both PKR and PKM gene expression and increased pyruvate content , associated with reduced ATP level when compared to healthy cells**
- In our *Rpl5* haploinsufficient mouse model, the oxidative stress observed is **paired with distinct metabolic disturbances in pathways related to oxidative stress response and mitochondrial metabolism.**
- Our *Rpl5* mouse model confirmed the abnormal expression of both PKM2 and PKR when compared to healthy cells, reflecting our observations in the human DBA cells.
- In *RPL5* haploinsufficient cells, the persistent increased expression of PKM2 suggests a possible **non-glycolytic function** of **PKM2**, which might **play a role in the failure of erythroid progenitors to progress to the late progenitor/early precursors stage.**

REFERENCES

Tang et al. *RPS19 and RPL5 haploinsufficient models reveal divergent ribosomal subunit controls of fetal hematopoiesis*. Nat Commun. 2026 Apr 8. Epub ahead of print.

Siciliano et al. *Mitapivat metabolically reprograms human β-thalassemic erythroblasts, increasing their responsiveness to oxidation*. Blood Adv. 2025 Jun 10;9(11):2818-2830.

De Wilde et al. *Activation of pyruvate kinase by mitapivat potentially rescues ineffective erythropoiesis in models of diamond blackfan anemia*. Blood 2025; 146 (Supplement 1): 1121.

Prosser et al. *Stem Cell Model of Novel RPL30 Variant in Diamond Blackfan Anemia with Downregulated GATA1-HSP70 in Early Erythroid Progenitors*. Blood 2024; 144 (Supplement 1): 2710.

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