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Turning up the HEAT(R3) in Diamond-Blackfan anemia

Tracking no: BLD-2022-015881-CR1

Deena Iskander (Imperial College London, United Kingdom) Alan Warren (University of Cambridge, United Kingdom)

Abstract:

Conflict of interest: No COI declared

COI notes:

Preprint server: No;

Author contributions and disclosures: DI and AJW wrote the manuscript and designed the figure.

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Agreement to Share Publication-Related Data and Data Sharing Statement:

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Turning up the HEAT(R3) in Diamond-Blackfan anemia

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In this issue of *Blood*, O'Donohue et al identify biallelic mutations in *HEATR3* as the underpinning cause of Diamond-Blackfan anemia (DBA) in four unrelated pedigrees[1]. Using primary human cells, cell lines and yeast models with HEATR3 deficiency, they delineate a mechanism by which reduced HEATR3 leads to erythroid failure. The mechanism includes impaired nuclear import of ribosomal protein uL18 (encoded by *RPL5*), defects in ribosomal RNA processing and reduced production of the large (60S) ribosomal subunit, leading to p53-independent perturbation of erythroid development (**Figure 1**).

Genetic diagnosis of inherited bone marrow failure syndromes such as DBA is crucial for i) definitive diagnosis ii) screening of first-degree relatives and exclusion of asymptomatic carriers as bone marrow donors and iii) pre-implantation diagnosis. Approximately 75% of cases of DBA are caused by monoallelic loss-of function mutations in ribosomal protein (RP) genes[2]. Fewer than 1% are associated with variants in the megakaryocyte-erythroid transcription factor *GATA1*[3] or the ribosome assembly factor *TSR2*[4]; the mutations causing the remaining 20-25% of cases are unknown. In this work, O'Donohue et al elucidate the underlying pathogenetic mechanism in six individuals with DBA. Specifically, they used whole exome sequencing and confirmatory Sanger sequencing to reveal homozygous or compound heterozygous variants in *HEATR3,* with distinct missense or splice site variants detected in each of the four families. The clinical phenotypes are typical of DBA, comprised of selective erythroid hypoplasia (*n=5*) or anemia with transient but severe thrombocytopenia (*n=1*); short stature (*n=5*); facial dysmorphia and/or limb abnormalities

(*n=5*); congenital cardiac defects (*n=2*) and intellectual disability (*n=4*). One child died of osteosarcoma, one of the most common cancers arising in DBA[5].

As expected, missense variants depleted HEATR3 protein but not mRNA. To understand the mechanisms by which mutations in *HEATR3* lead to a ribosomopathy, the authors exploited the homology between one of the four identified variants affecting amino acid p.Gly584 and residue p.Gly522 of yeast Syo1, important for 60S ribosomal subunit assembly. Complementation of Syo1-null yeast cells with a DBA-related variant failed to restore growth or ribosomal subunit imbalance. By contrast, wild-type Syo1 rescued both these defects. Similarly, in lymphoblastoid cell lines (LCLs) derived from two of the DBA patients, the 60S:40S imbalance and pre-RNA processing defects were corrected by lentiviral transduction of wild-type HEATR3 but not by GFP (green fluorescent protein) control. These data suggest that the pathogenicity of the HEATR3 variants is related to efficient 60S subunit production. Syo1 facilitates nuclear translocation of a uL18-uL5 complex (**Figure 1**) through co-translational capture of uL18 and the authors show conservation of this mechanism in human cells. Further, using fluorescence microscopy of HEATR3 depleted cells (RNAi-treated HeLa cells or primary patient skin fibroblasts) compared with controls, the authors conclude that HEATR3 is important for nuclear import of uL18 but not uL5. While the reasons for this discrepancy are not established, uL5 may be recruited to the HEATR3-uL18 complex in the nucleus or through a HEATR3-independent mechanism.

Consistent with the anemia affecting these patients, O'Donohue et al show that CD34+ cells derived from patients' peripheral blood or normal CD34+ cells subjected to shRNA-mediated *HEATR3* knockdown, fail to expand in an erythroid culture assay. Additionally, they exhibit accelerated erythroid maturation and normal GATA1 expression, concordant with the recent observation of earlier acquisition of erythroid differentiation markers and preserved GATA1 expression in bone marrow erythroid progenitors harvested from patients with DBA caused by mutations in *RPL5* and *RPL11*[6]. Finally, this report demonstrates that HEATR3-depleted patient LCLs have comparable total cellular levels of uL18, consistent with the defect in uL18 subcellular localization, and express similar levels of p53 compared to control LCLs. A link between haploinsufficiency of small ribosomal subunit proteins and p53 stabilization is well established due to excess large subunit proteins which sequester HDM2 that can no longer bind to and promote proteasomal-mediated degradation of p53[7,]. The role of p53 in

DBA caused by large subunit mutations is more contentious. Although *RPL5*-deficient embryonic stem cells demonstrate p53-independent growth defects[8], in line with the findings presented here, other data show enrichment of p53 and its molecular targets in *RPL5*- or *RPL11*-mutant DBA patient cells[6]. This dichotomy implies a putative role for the bone marrow microenvironment and non-erythroid cells in modulating p53 expression. Further work is needed to discern how HEATR3 deficiency leads to erythroid failure *in vivo*.

So, what are the clinical implications of O'Donohue et al's findings? *HEATR3* should be included in multigene panels used for diagnosing DBA, particularly in the context of consanguinity (present in three of the four families studied). More broadly, this work demonstrates the value of international collaboration in a rare disease; after elucidation of HEATR3 variants in one family, the EuroDBA consortium facilitated identification of three further families with the same genetic basis.

To date, variants in only one other gene (*TSR2*) have demonstrated autosomal recessive inheritance in DBA. TSR2 acts as a nuclear unloading chaperone for RPS26 to ensure its safe transfer to maturing pre-ribosomes[4], like HEATR3, mediating a critical role in ribosome biogenesis. Taken together, these data suggest that genetically unexplained DBA cases are likely attributable to defects in ribosome structure, assembly or function.

One caveat is that although HEATR3 is depleted in all six patients, their clinical phenotype is heterogeneous encompassing transfusion-dependence, steroid responsiveness and treatment independence. While previous studies have elucidated some genotype-phenotype associations in DBA[6,9], future work is required to reveal the genetic, epigenetic and environmental factors underpinning variable expressivity, in the hope that these could be harnessed for new therapies.

Although there was no evidence for p53 activation downstream of HEATR3 loss, notably the craniofacial defects arising in Treacher-Collins Syndrome, which resemble those observed in patients with *RPL5* or *RP11*- DBA[9] as well as the patients in this report, are caused by sensitization of neural crest cells to p53-mediated apoptosis[10]. Most DBA research has focused on elucidating the mechanisms of anemia, however delineating the relationship between ribosome defects and non-hematological manifestations- such as the congenital

anomalies, intellectual disability and cancer reported in this study- is essential for the holistic management of this complex disease and improving the quality of life of affected individuals.

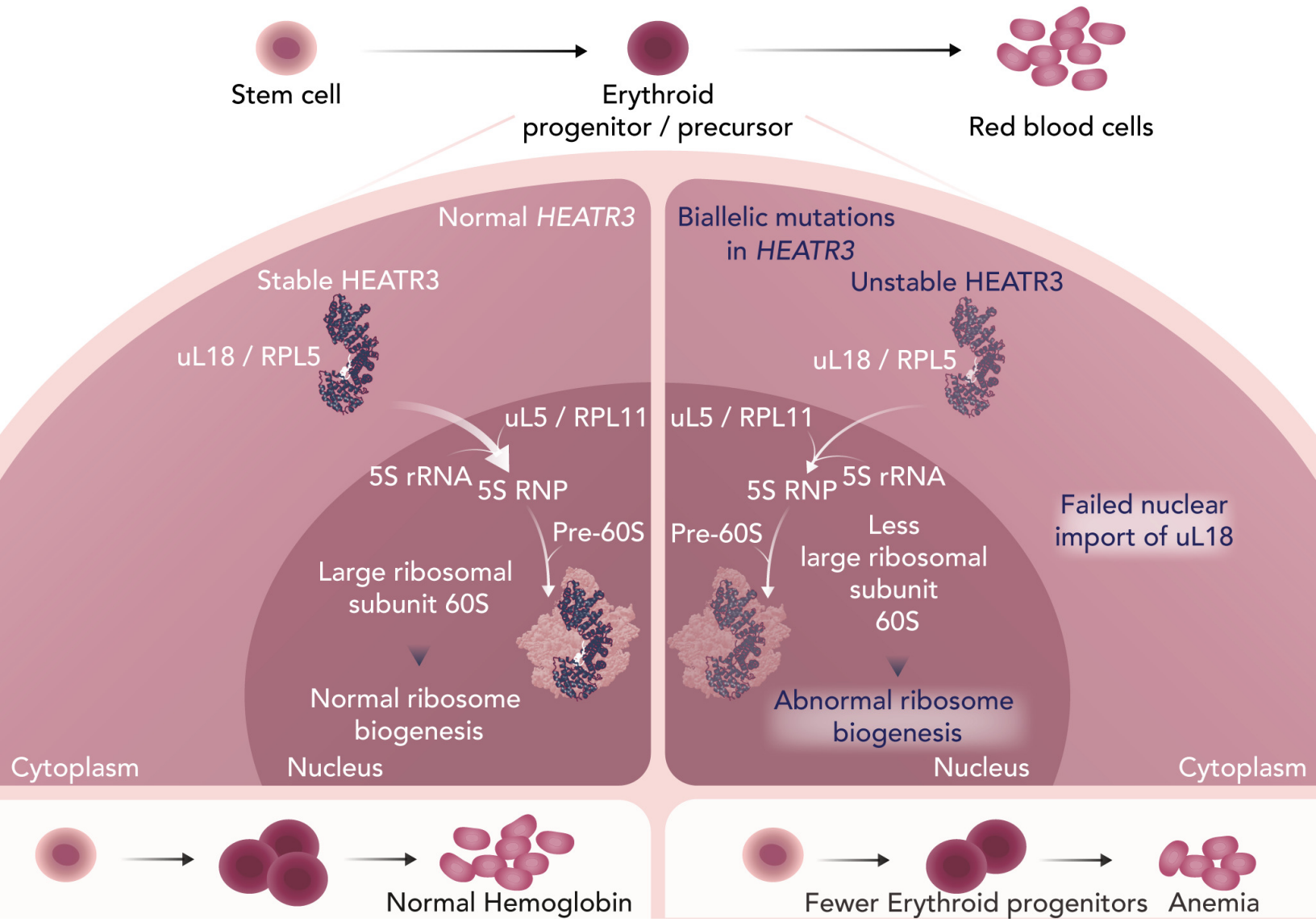
Conflict- of-interest disclosure: the authors declare no competing financial interests.

References

1. O'Donohue M-F, Da Costa L, Lezzerini M, et al. HEATR3 variants impair nuclear import of uL18 (RPL5) and drive Diamond-Blackfan anemia. Blood 2021; in press.
2. Ulirsch JC, Verboon JM, Kazerounian S, et al. The Genetic Landscape of Diamond-Blackfan Anemia. *The American Journal of Human Genetics*. 2018;103(6):930–947.
3. Sankaran VG, Ghazvinian R, Do R, et al. Exome sequencing identifies GATA1 mutations resulting in Diamond-Blackfan anemia. *Journal of Clinical Investigation*. 2012;122(7):2439–2443.
4. Gripp KW, Curry C, Olney AH, et al. Diamond-Blackfan anemia with mandibulofacial dystostosis is heterogeneous, including the novel DBA genes TSR2 and RPS28. *Am J Med Genet A*. 2014;164A(9):2240–2249.
5. Vlachos A, Rosenberg PS, Atsidaftos E, et al. Increased risk of colon cancer and osteogenic sarcoma in Diamond-Blackfan anemia. *Blood*. 2018;132(20):2205–2208.
6. Iskander D, Wang G, Heuston EF, et al. Single-cell profiling of human bone marrow progenitors reveals mechanisms of failing erythropoiesis in Diamond-Blackfan anemia. *Science Translational Medicine*. 2021;13(610).
7. Fumagalli S, di Cara A, Neb-Gulati A, et al. Absence of nucleolar disruption after impairment of 40S ribosome biogenesis reveals an rpL11-translationdependent mechanism of p53 induction. *Nature Cell Biology*. 2009;11(4):501–508.
8. Singh SA, Goldberg TA, Henson AL, et al. p53-Independent Cell Cycle and Erythroid Differentiation Defects in Murine Embryonic Stem Cells Haploinsufficient for Diamond Blackfan Anemia-Proteins: RPS19 versus RPL5. *PLoS ONE*. 2014;9(2):e89098--10.
9. Gazda HT, Sheen MR, Vlachos A, et al. Ribosomal Protein L5 and L11 Mutations Are Associated with Cleft Palate and Abnormal Thumbs in Diamond-Blackfan Anemia Patients. *American Journal of Human Genetics*. 2008;83(6):769–780.

10. Calo E, Gu B, Bowen ME, et al. Tissue-selective effects of nucleolar stress and rDNA damage in developmental disorders. *Nature*. 2018;554(7690):112–117.

Figure 1. In normal bone marrow erythroid progenitors (depicted in left panel), HEATR3 acts as a shuttling factor that imports ribosomal protein uL18 from the cytoplasm to the nucleus. There, it associates with uL5 and the 5S rRNA to form the 5S RNP complex which is incorporated with maturing large ribosomal subunits to form the central protuberance. Intact ribosome biogenesis is a prerequisite for erythroid progenitor proliferation and differentiation to red blood cells. Biallelic *HEATR3* variants destabilize the HEATR3 protein, reducing nuclear uL18 (right panel). HEATR3 variants cause strong pre-RNA processing defects, reduced 60S ribosomal subunits and failure of erythropoiesis, presenting clinically as anemia. Professional illustration by Somersault18:24.


Stem cell
Erythroid progenitor / precursor
Red blood cells
Normal *HEATR3*
Stable HEATR3
uL18 / RPL5
uL5 / RPL11
5S rRNA
5S RNP
Pre-60S
Large ribosomal subunit 60S
Normal ribosome biogenesis
Cytoplasm
Nucleus
Biallelic mutations in *HEATR3*
Unstable HEATR3
uL18 / RPL5
uL5 / RPL11
5S RNP
5S rRNA
Pre-60S
Less large ribosomal subunit 60S
Failed nuclear import of uL18
Abnormal ribosome biogenesis
Nucleus
Cytoplasm
Normal Hemoglobin
Fewer Erythroid progenitors
Anemia