EUROPEAN HEMATOLOGY ASSOCIATION

Heat Shock Protein S70 (HSP70), one of the key factors in Diamond-Blackfan anemia

Lydie Da Costa[1,2,3,4]

[1]AP-HP, Hôpital Robert Debré, Service d'hématologie biologique, Paris; [2]Université Paris 7 Denis Diderot-Sorbonne Paris Cité, Paris; [3]LABEX GR-Ex, Paris; [4]INTS, UMR 1134, Paris, France

Take-home messages

- DBA is a congenital erythroblastopenia amongst the inherited bone marrow failure syndromes (IBMFS), highly heterogenous in phenotype and genotype.
- DBA is the first ribosomopathy described and p53 stabilization is involved in DBA erythroblastopenia.
- HSP70 is one of the major key factors involved in DBA pathophysiology.

Diamond-Blackfan anemia (DBA)[1, 2] has been the first ribosomopathy described[3,4] and belongs to the inherited bone marrow failure syndromes (IBMFS). DBA is characterized by a specific erythroid tropism and is associated with a lower risk of malignancies (5% of the DBA cases)[5,6] compared to Fanconi anemia, Shwachman-Diamond syndrome or dyskeratosis congenita. DBA is revealed early in infancy (median age of 2 months) with a moderate to severe, usually macrocytic aregenerative anemia and a normal platelet and white blood cell count in the vast majority of the DBA patients. The erythroblastopenia in an otherwise normal bone marrow is the main feature of the disease. The erythroid blockade has been stated between the BFU-e and the CFU-e stages.[7] DBA phenotype is however highly heterogeneous and in 50% of the DBA cases, various malformations mostly in the cephalic area and the extremities have been reported.[8,9] Steroid therapy should be initiated only after one year of age, in order to protect growth during the first year of life. More than 60% of DBA cases are steroid good responders. Corticoresistant or corticodependent more than 0.5 mg/kg/day (or even >0.3 mg/kg/day) DBA patients should enter into a regular transfusion program associated with an iron chelation after a certain amount of transfusions and based on ferritin level. However, so far bone marrow transplantation with an HLA identical intra-familial and non silent phenotype donor is the only curative treatment for DBA.[10] DBA genotype is also highly heterogeneous. A heterozygous mutation is found in more than 70% of the DBA affected patients in one of the 14 ribosomal protein genes, which have been shown to be involved in DBA, including *RPS19* (25%), *RPL5* (7%), *RPL11* (5%), *RPS24* (2,4%), *RPS26* (7%), *RPS10* (3%), *RPL35a* (3%), *RPS17* (1%), *RPS7* (<1%), *RPS28* (<1%), *RPS27a* (<1%), *RPL15*, *RPL9, RPL26*. Large deletions in these genes are reported.[11-13] The occurrence of mutations is sporadic, or *de novo*, in 55% of DBA affected patients, while in the familial cases, the inheritance is dominant. The RP gene mutation is responsible for a defect in rRNA maturation at different level depending on the RP gene.[3,14] The link between the mutation in an RP gene, the ribosome biogenesis impairment and the erythroblastopenia is still to be fully defined. However, several groups, including ours, identified p53 as one of the major proteins involved in the disease.[15,16] Indeed, it has been shown that rRNA maturation impairment leads to nucleolar or ribosomal stress which, in turn, leads to an increase in the expression level of various RP genes, with an RP binding to MDM2 (or HDM2). MDM2 is an E3 ubiquitin ligase, which binds p53 and directs it for proteasomal degradation. During the nucleolar stress, p53 is thus free and induces apoptosis and cell cycle arrest, responsible at least in part for the erythroblastopenia, the feature of the disease.[15,16] However, p53 independent pathways are now described[17] and non-RP genes have been identified in DBA, which may open new pathway(s) involved in DBA pathophysiology. Recently, two genes, namely *TSR2*[18] and *GATA1,*[19] which are not related to an RP gene have been identified in a few DBA patients. GATA1 is the major erythroid transcription factor. In GATA1 mutated DBA patients[19] and in RP mutated DBA patients,[20] the long form of GATA1 disappears, with only the short form remaining. The ribosomal defect in GATA1 mRNA translation in DBA results from this mRNA having a higher threshold for initiation of translation (highly structured 5'UTR are more poorly translated).[20] However, we recently identified another key factor that may explain the more severe DBA phenotype. Indeed, we were previously able to identify two DBA *in vitro* phenotypes resulting from the mutated RP

gene.[16] The respective DBA patients, affected by an *RPS19* mutation, exhibited a decrease in erythroid proliferation, but a normal erythroid differentiation and no apoptosis, while DBA patients carrying a mutation in RPL5 or RPL11 exhibited a dramatic decrease in erythroid proliferation, a delayed erythroid differentiation, and significant erythroid cell apoptosis.[16] In all these DBA patients, independently of the RP gene mutated, we found a cell cycle arrest in G_0/G1. Seeking to decipher the significance of these dual phenotypes, we were able to identify the protein involved in these discrepancies. Strikingly, the Heat Shock Protein S70 (HSP70) was expressed at a normal level in RPS19 mutated DBA patients and in shRNA-RPS19 infected $CD34^+$ from cord blood, while absent or largely decreased in RPL5 or RPL11 mutated DBA patients or in the shRNA-RPL5 or -RPL11 infected erythroid cells (manuscript in revision). The decrease in HSP70 is due to an enhanced proteasomal degradation of polyubiquitinylated HSP70. Overexpression of wild type HSP70 is able to restore the erythroid defect caused by DBA, in particular the erythroid proliferation and differentiation defect in the severe DBA phenotype, confirming the specific role of HSP70 in DBA pathophysiology. Furthermore, overexpression of wild-type HSP70 reduced p53 stabilization. In a parallel study, we were able to show a disequilibrium between the heme and globin synthesis. We observed a normal or slightly decreased total heme content, but an excess of free heme, in DBA affected patients in association with both, transcriptional and translational, defect in globin protein expression level. GATA1 targets, namely HRI, ALAS2 and globin genes, were indeed downregulated. Wild-type HSP70 overexpression was able to increase GATA1 and its targets, restoring globin chain expression levels. HSP70 should thus be considered as one of the key factors of DBA pathophysiology (Figure 1). New therapeutic developments involving the HSP70 nuclear re-localization and its GATA1 chaperon function may be considered as an innovative treatment for DBA patients, who faced until now in the vast majority of cases, only iterative transfusions or long-term steroid therapeutics options.

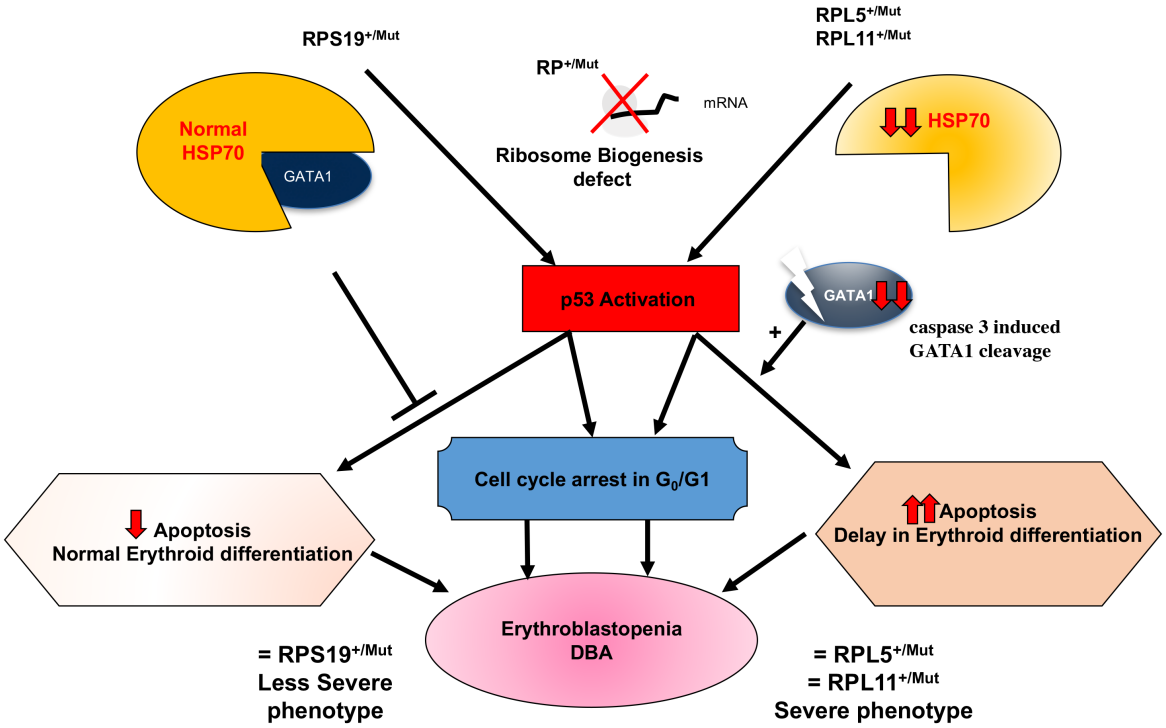

Figure 1. HSP70 as a major key factor in DBA pathophysiology.

Acquired and hereditary red cell anomalies - Section 3

References

1. Diamond LK, Wang WC, Alter BP. Congenital hypoplastic anemia. Adv Pediatr 1976;22:349-78.
2. Lipton JM, Ellis SR. Diamond-Blackfan anemia: diagnosis, treatment, and molecular pathogenesis. Hematol Oncol Clin North Am 2009;23:261-82.

*3. Leger-Silvestre I, Caffrey JM, Dawaliby R, et al. Specific role for yeast homologs of the Diamond Blackfan anemia-associated Rps19 protein in ribosome synthesis. J Bio Chem 2005;280:38177-85.

First article showing that haploinsufficiency in RPS19 is responsible for an impairment of rRNA maturation. DBA was ever since considered as the first ribosomopathy.

4. Liu JM, Ellis SR. Ribosomes and marrow failure: coincidental association or molecular paradigm? Blood 2006;107:4583-8.
5. Vlachos A, Rosenberg PS, Atsidaftos E, et al. Incidence of neoplasia in Diamond Blackfan anemia: a report from the Diamond Blackfan Anemia Registry. Blood 2012;119:3815-9.
6. Alter BP, Giri N, Savage SA, et al. Malignancies and survival patterns in the National Cancer Institute inheited bone marrow failure syndromes cohort study. Br J Haematol 2010;150:179-88.
7. Ohene-Abuakwa Y, Orfali KA, Marius C, Ball SE. Two-phase culture in Diamond Blackfan anemia: localization of erythroid defect. Blood 2005;105:838-46.
8. Vlachos A, Ball S, Dahl N, et al. Diagnosing and treating Diamond Blackfan anaemia: results of an international clinical consensus conference. Br J Haematol 2008;142:859-76.
9. Willig TN, Niemeyer C, Leblanc T, et al. Diamond Blackfan anemia: clinical and epidemiological studies with identification of new long-term prognosis factors from the analysis of a registry of 234 patients. Pediatr Res 1999;46:553-61.
10. Dietz A, Savage SA, Vlachos A, et al. Late effects screening guidelines after hematopoietic cell transplantation (HCT) for inherited bone marrow failure syndromes (IBMFS): Consensus statement from the second pediatric blood and marrow transplant consortium international conference on late effects after pediatric HCT. Biol Blood Marrow Transplant 2017.
11. Farrar JE, Vlachos A, Atsidaftos E, et al. Ribosomal protein gene deletions in Diamond-Blackfan anemia. Blood.2011;118:6943-51.

*12. Kuramitsu M, Sato-Otsubo A, Morio T, et al. Extensive gene deletions in Japanese patients with Diamond-Blackfan anemia. Blood 2012;119: 2376-84.

First study (ASH2011 poster) showing deleterious mutations in RP genes involved in DBA (almost 20% of DBA affected patients).

13. Quarello P, Garelli E, Brusco A, et al. High frequency of ribosomal protein gene deletions in Italian Diamond-Blackfan anemia patients detected by multiplex ligation-dependent probe amplification assay. Haematologica 2012;97:1813-7.
14. Choesmel V, Fribourg S, Aguissa-Toure AH, et al. Mutation of ribosomal protein RPS24 in Diamond-Blackfan anemia results in a ribosome biogenesis disorder. Hum Mol Genet 2008;17:1253-63.
15. Dutt S, Narla A, Lin K, et al. Haploinsufficiency for ribosomal protein genes causes selective activation of p53 in human erythroid progenitor cells. Blood 2011;117:2567-76.

*16. Moniz H, Gastou M, Leblanc T, et al. Primary hematopoietic cells from DBA patients with mutations in RPL11 and RPS19 genes exhibit distinct erythroid phenotype in vitro. Cell Death Dis 2012;3:e356.

Article showing that p53 is stabilized in DBA affected patients and there are two in vitro phenotype depending on the RP mutated gene, RPS19+/Mut and non RPS19+/Mut.

*17. Horos R, Ijspeert H, Pospisilova D, et al. Ribosomal deficiencies in Diamond-Blackfan anemia impair translation of transcripts essential for differentiation of murine and human erythroblasts. Blood 2012;119:262-72.

First article showing a defect in transcript translation in DBA namely Bag1, Csde1 and p53 independent pathways.

18. 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:2240-9.
19. Sankaran VG, Ghazvinian R, Do R, et al. Exome sequencing identifies GATA1 mutations resulting in Diamond-Blackfan anemia. J Clin Invest 2012;122:2439-43.

*20. Ludwig LS, Gazda HT, Eng JC, et al. Altered translation of GATA1 in Diamond-Blackfan anemia. Nat Med 2014;20:748-53.

Important article showing the specific defect in GATA1 translation after haploinsufficiency in different ribosomal proteins involved in DBA.