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PERSPECTIVE

Perspective on Diamond–Blackfan anemia: lessons from a rare congenital bone marrow failure syndrome

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Congenital and inherited bone marrow failure syndromes are rare and not exactly public health hazards. However, they have important lessons to teach about hematology in particular and cell biology in general. First, significant advances in scientific knowledge and understanding of pathogenesis can be obtained from studying rare diseases in children. Second, studies on rare syndromes provide insights into the normal physiology at both the cellular and global levels. Finally, as in the case of del(5q) myelodysplastic syndrome (where RPS14 was found to be mutated), discoveries from studying rare diseases such as Diamond–Blackfan anemia (DBA) can have important implications for our understanding of other congenital, inherited and acquired diseases in children and adults. In this perspective we briefly review progress and milestones in DBA research over the past two decades.[1]

DBA was first described in 1936 by Hugh Josephs but was ultimately named after Louis Diamond and Kenneth Blackfan who described the hypoplastic anemia syndrome in 1938.[2] Initially, DBA was thought to be an immune-mediated disease that led to corticosteroid therapy that has become the standard of care. However, after 50 years, it is still unclear how precisely corticosteroids improve erythropoiesis in children with DBA. An elevated eADA has been noted since the 1980s in the majority (>75%) of patients with DBA.[3–5] Why adenosine deaminase should be elevated and what role it might have in DBA again remains uncertain. In 1997, a mutation was noted on chromosome 19 in a child with DBA,[6,7] and in 1999 was determined to be within the gene encoding *ribosomal protein S19* (*RSP19*). Mutations in *RSP19* were subsequently detected in ~25% of children with DBA.[8] Studies of ribosomal protein encoding genes were then extended. We now know that ~70% of individuals with DBA have ribosomal protein (RP) mutations. DBA was the first disease to be linked to impaired ribosome function and is the founding member of a group of disorders now known as ribosomopathies.[9] Ribosomal insufficiency associated with small ribosomal subunits leads to increased ratio of 28S/18S ribosomal RNA, whereas mutations in large ribosomal subunit results in decreased ratio of 28S/18S ribosomal RNA and nucleolar stress.[10,11] Ebert and co-workers[12,13] also identified RPS14 insufficiency in individuals with myelodysplastic syndrome and anemia, suggesting ribosome insufficiency contributes to acquired and congenital bone marrow failure syndromes. Exactly how RP insufficiency results in anemia and bone marrow failure is another unknown fact that is under study.

Progress in DBA research has come from the development of *in vitro* and *in vivo* model systems. The discovery of Yamanaka factors enabled scientists to generate pluripotent stem cells from children with DBA that could then be differentiated into erythroid progenitor cells.[14,15]

Mice with ribosomal mutations are smaller, have dark skin and decreased numbers of erythrocytes.[16] p53 protein accumulates in epidermal melanocytes and induces C-Kit ligand expression in these mice. Zebrafish embryos injected with morpholinos recapitulate the congenital abnormalities found in children with DBA such as small size and severe anemia.[17,18] Like humans, red blood cell adenosine deaminase levels are increased in these mouse and zebrafish models. p53 is upregulated in the zebrafish embryos resulting in apoptosis. Another important *in vivo* model recently described is the inducible RPS19 knockdown mouse that has an anemia phenotype that will be a model in which to test potential therapies.[19]

Genome sequencing of RNA and DNA has contributed to important advances in DBA research. These studies identified novel RP mutations in children with DBA, highlighting the importance of banking clinical samples.[1] DNA sequencing information has led to identification of certain phenotype–genotype correlations, such as *RPL5/RPL11* mutations and craniofacial abnormalities.[1] *GATA1* mutations are also found in some children with DBA.[20,21] The inability of GATA1 to normally regulate erythropoiesis in DBA may result from failure to interact with erythroid-specific promoter elements.[22] The DBA Mutation Database and the DBA Registry will undoubtedly help identify new mutations in the future.[23]

Going forward, improved genomic sequencing will likely uncover new genes and pathways critical to the pathogenesis of DBA. RNA-sequencing approaches have identified aberrantly regulated genes in bone marrow progenitors of children with DBA.[24,25] Such studies raise important issues regarding the complexity of differential gene expression in diverse cell populations, culture conditions and bioinformatics data analyses.

Additional insights into DBA come from studies of iron metabolism. For example, an imbalance in heme/globin ratio seems to contribute to impaired erythropoiesis in DBA and myelodysplastic syndrome.[26] These data suggested that drugs that suppress heme synthesis might benefit individuals with these disorders. More recently, the use of inducible pluripotent stem cells from individuals with DBA were used to perform an unbiased chemical screen, resulting in the identification of an inducer of autophagy that stimulated erythropoiesis and upregulated globin genes.[27] As technology develops the hope is that new drugs will be identified to help individuals with DBA.

Finally, individuals with DBA have an increased risk of developing malignancies, suggesting the need to study

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predisposing factors that may have broader implications.[28–31] Recent analysis from the DBA Registry of North America (DBAR) reported 702 subjects with 12 376 individual-years of follow-up. The incidence of cancer was significantly increased with a 4.75 observed-to-expected ratio for all combined cancers. Significant observed-to-expected ratios were 352 for myelodysplastic syndrome, 45 for colony carcinoma, 42 for osteogenic sarcoma and 29 for acute myelogenous leukemia.[32] The overall cancer risk appears to be approaching those observed in dyskeratosis congenital but lower than in Fanconi anemia. Interestingly, the risk of developing specifically gastrointestinal carcinoma and osteogenic sarcoma is higher in DBA. Studies have not found any genotype–phenotype correlation in terms of cancer risk.[32]

SUMMARY AND FUTURE DIRECTIONS

Despite substantial advances in our understanding of the molecular aspects of DBA, many questions remain. We do not understand why a germline mutation of RPS19 should affect specific cells, in particular, erythroid progenitors. Future directions will likely focus on the role of inflammation and the bone marrow cytokine milieu that could also contribute to aberrant erythropoiesis.[33] The role of ribosome insufficiency and specific signaling pathways resulting in ineffective erythropoiesis are not well understood. The noncoding RNAs and epigenetic regulation of gene expression in DBA is not well studied.[34] Also unstudied is the role of the ubiquitin–proteasome system and its regulation of erythropoiesis.[35,36]

In summary, therapy of children with DBA has not advanced for decades. Corticosteroids, red blood cell transfusions and hematopoietic cell transplants are the only currently effective therapies. Each is associated with significant morbidity and mortality. We hope future research will uncover new, effective therapies and will improve the quality of life of DBA.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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