Pyruvate kinase deficiency

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Over the past few years the inherited disorders of erythrocyte metabolism have been the object of intensive research which has resulted in a better understanding of their molecular basis. However, curative therapy for red blood cell (RBC) enzyme defects still remains undeveloped.

Among glycolytic defects causing chronic non-spherocytic hemolytic anemia, red cell pyruvate kinase (PK) deficiency is the most common, its prevalence being 1:20.000 in the general white population.[1]

The PK-catalysed reaction is the second ATP-generating step of the glycolytic pathway and is of particular importance in energy production, yielding nearly 50% of the total ATP. Four PK isoenzymes (M1, M2, L, and R), encoded by two separate genes (*PK-M* and *PK-LR*) and expressed in a tissue-specific manner, are present in humans.[2] The *PK-LR* gene, located on chromosome 1 (1q21),[3] codes for both L-PK (expressed in liver, renal cortex, and small intestine) and R-PK (restricted to erythrocytes) through the use of alternate promoters.[4] Although abnormalities in the *PK-LR* gene may result in alterations of both erythrocyte and liver enzyme, clinical symptoms are confined to RBC, the hepatic deficiency usually being compensated by the persistent enzyme synthesis in hepatocytes.[5]

PK deficiency is transmitted as an autosomal recessive trait, clinical symptoms occurring in homozygous and compound heterozygous patients. The degree of hemolysis is variable, ranging from mild or fully compensated forms to life-threatening neonatal anemia necessitating exchange transfusions and subsequent continuous transfusion support;[6] hydrops fetalis and death in the neonatal period have also been reported in rare cases.[7-12]

Among the 180 different mutations so far identified in the *PK-LR* gene,[13-16] mostly missense, 1529A is the most common in the USA, and Northern and Central Europe, 1456T is prevalent in Southern Europe, and 1468T in Asia.[6] Molecular studies indicate that a severe syndrome is commonly associated with disruptive mutations and with missense mutations more or less directly involving the active site or protein stability.[13] Among severe missense mutations, 994A (Gly332Ser) is one of the most common in Caucasians and may be associated in the homozygous state with intrauterine death.[17] Similarly, the rare patients reported in the literature with homozygous *null* mutations (i.e. mutations resulting in the absence of a functional protein product) displayed intrauterine growth retardation, severe anemia at birth with need of exchange blood transfusion, transfusion dependence and, in rare cases, intrauterine death or death in the first days of life.[11,12,18-21] The production and characterization of the recombinant mutant proteins of human R-PK have recently enabled the effects of amino acid replacements on the enzyme molecular properties to be determined and helped to correlate genotype to clinical phenotype.[12,22-23] However, although there is in general correlation between the nature and location of the replaced amino acid and the type of molecular perturbation, caution is needed in predicting the consequence of a mutation simply considering the target residue *per se*: in fact, the clinical manifestations of red cell enzyme defects are not merely dependent on the molecular properties of the mutant protein but rather reflect the complex interactions of additional factors, including genetic background, concomitant functional polymorphisms of other enzymes, post-translational or epigenetic modifications, ineffective erythropoiesis and differences in splenic function.

In spite of a variety of drugs and chemicals administered to improve *in vivo* activity,[24-26] no specific therapy for PK deficiency is available, and the treatment of this disease is, therefore, based on supportive measures. Red cell transfusions may be required in severely anemic cases, particularly in the first years of life. Splenectomy usually results in an increase of 1-3 g/dL in hemoglobin and reduces or even eliminates transfusion requirement in most transfusion-dependent cases.[25] Iron chelation may be required since iron overload is rather common in PK deficiency, even in non-transfused patients.[27-28] Bone marrow transplantation has been successfully performed in one severely affected child.[29]

A gene repair strategy may be a feasible goal for enzyme defects mainly confined to hematopoietic cells. The feasibility of gene therapy in PK deficiency was first demonstrated by Tani *et al.*,[30] who introduced the human liver-type PK (L-PK) cDNA into mouse and human leukemic cell lines, and into mouse bone marrow cells using a retroviral vector. They demonstrated the prolonged expression of human L-PK mRNA in both the peripheral blood and hematopoietic organs after bone marrow transplantation.

In this issue of the journal, Kanno and co-workers report a genetic rescue study of PK deficiency using human R-PK transgenic mice by means of a gene-addition strategy.[31] The mouse model used in the protocol (Pk-1slc) shows moderate hemolytic anemia and marked splenomegaly and is homozygous for the missense mutation Gly338Asp of the murine *PK-LR* gene,

which affect a residue located near the substrate-binding site.[32,33] Two transgenic lines were obtained, one with low (hRPK_lo) and the other with high (hRPK_hi) expression of the transgene. hRPK_lo mice showed RBC PK activity at the same level of littermates, mean hemoglobin levels of 13.0 g/dL and overt reticulocytosis; contrariwise, hRPK_hi mice had PK activity double that of controls and hemolytic anemia was fully recovered, with hemoglobin levels of about 15.1 g/dL and almost normal reticulocyte counts. Even with a high level of high expression of the transgene, splenomegaly was still present. Moreover, the authors observed a negative correlation between RBC PK activity and the number of apoptotic erythroid progenitors in the spleen, providing direct evidence that the metabolic alteration of PK deficiency affects RBC maturation, resulting in ineffective erythropoiesis. Since the occurrence of dyserythropoiesis has been occasionally reported in some PK-deficient patients, but never explained,[6] this is an interesting finding, and contributes to our knowledge of the consequences of the metabolic impairment in PK-deficient erythroid cells.

Taken together, these results indicate that overexpression of the wild-type *PK* gene can lead to the successful recovery of hemolytic anemia in homozygous PK-deficient mice, and ameliorate erythroid apoptosis; further studies are needed to identify the most appropriate enhancer/promoter system to obtain timely erythroid lineage-specific expression and therapeutic levels of gene expression.

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The spectrum of molecular aberrations in myelodysplastic syndromes: in the shadow of acute myeloid leukemia

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How are the genetic profiles of acute myeloid leukemia (AML) and the myelodysplastic syndromes (MDS) similar, and how do these two disorders differ? These are complex questions – and difficult ones to answer intelligently right now, chiefly because so little is currently understood about the molecular etiology of MDS. Furthermore, despite several features in common, both AML and MDS are phenotypically and genetically heterogeneous, making it hard to tease out unifying threads binding their molecular pathobiology. Yet some recent progress has been made, including insights from a large mutation screening study published in this issue of Haematologica. A few common themes are beginning to emerge, and the near future holds great promise (Figure 1).

Molecular basis of AML

There is widespread support for the concept that the development of AML requires at least two somatic gene alterations: one mutation to augment the rate of cellular proliferation or enhance cell survival (a *class I mutation*, usually constitutively activating a tyrosine kinase or a RAS family member), and another mutation impairing normal cellular differentiation (a *class II mutation*, usually deregulating a hematopoietic transcription factor or a transcriptional co-activator, such as homeobox family members or the components of core binding factor).[1] The repertoire of known genetic changes capable of filling one of those two pathologic demands continues to expand. Recurrent chromosomal translocations, gene rearrangements (including amplifications and deletions), and point mutations are all now well recognized as important contributors to the myeloid neoplastic phenotype.[2]

Recently described mutations in the nuclear-cytoplasmic shuttling factor nucleophosmin (NPM1) lay claim to the title of the overall most frequent AML-associated genetic lesion, common balanced translocations notwithstanding.[3] Truncating NPM1 mutations result in protein mislocalization and are remarkably prevalent in AML, being detectable in 45-50% of cases of *de novo* disease with a normal karyotype and in 5-10% of patients with abnormal cytogenetics.[3,4] More generally, the likelihood of finding a given mutation such as NPM1 depends on the karyotype, the clinicopathological subtype of AML, and whether or not the patient has a history of therapy with DNA-damaging agents (*therapy-related AML*, t-AML) or was diagnosed with another clonal myeloid disorder prior to developing AML (*secondary AML*, s-AML).

NPM1 mutations are followed in frequency by the former *record holder*: activating mutations of the *FLT3* tyrosine kinase, which often co-exist with NPM1 mutations and likely co-operate in leukemogenesis.[5] Internal tandem duplications of the juxtamembrane domain of *FLT3* can be found in ~20-25% of patients with *de novo* AML, and, like NPM1 mutations, are more common in people with a normal karyotype, while FLT3 D835 activating loop point mutations are detectable in another 5-10% of cases.[6]

Over the last two decades, careful work by dozens of research groups has defined the prognostic implications of recurrent AML-associated karyotypes (not considered further here), and also uncovered a number of other important acquired mutations in AML (e.g., TP53, NRAS, KRAS, KIT, PTPN11, CEBPA, CSF1R (C-FMS), NF1, and BRAF point mutations, and MLL partial tandem duplications).[1] However, each of those individual