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MICROCYTOSIS REVISITED: LESSON FROM MURINE MODELS

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Microcytosis and hypochromia were once simply observed by the appearance of small and pale red cells on a peripheral blood smear under a light microscope and are, at present, easily identified by reduced red cell indices, especially MCV and MCH, with automated cell counters. Microcytic hypochromic red cells are present in several common and rare anaemias (Table 1), due to either heme or globin deficiency, but a unifying model to explain the phenomenon in molecular terms has been lacking.

The issue has recently been clarified in mice, demonstrating that the main actor in microcytosis is a kinase regulated by heme (heme-regulated inhibitor kinase or Hri)(1). When heme synthesis is deficient Hri is activated and inhibits protein synthesis by blocking a factor (the eukaryotic initiation factor 2 or eIFa2), which is essential to initiate protein translation (Figure 1). That Hri activation occurs in heme deficiency, is suggested by the study of Hri knock out mice (Hri -/-). These animals are phenotypically normal in basal conditions except for an increased globin production in their reticulocytes (2). When Hri -/- mice are kept on an iron-poor diet and develop iron deficiency, they have *normocytic normochromic* red cells and a severe hemolytic anaemia with splenomegaly. Unlike normal mice they are unable to develop microcytosis under conditions of iron deficiency. This result indicates that Hri is responsible for microcytosis associated with iron deficiency anaemia in mice. Hri is also protective against apoptosis of the more mature erythroblasts in the iron deficient bone marrow (2).

Since iron deficiency causes heme deficiency the problem remained whether Hri activation was caused by iron or by heme deficiency. That was the second option formally proved by the model of erythropoietic protoporphyria (EPP), an inherited disorder of heme synthesis due to deficiency of ferrochelatase (fech), the last enzyme of heme synthetic pathway, that inserts iron into protoporphyrin IX (PPIX). Fech -/- mice develop a disorder similar to the human one with microcytic anaemia, photosensitivity and liver disease, due to skin and liver PPIX accumulation respectively (3). The Authors demonstrate that Hri is activated in this condition, proving that heme and not iron is responsible for Hri activation. The double knock out mice (Fech -/- Hri -/-) were smaller, had more severe, but normocytic anaemia and more severe jaundice and liver disease than Fech -/- with normal Hri. They also died shortly after UV exposure. Fech -/- mice with Hri haploinsufficiency (Hri -/+) had an intermediate phenotype, still more severe than Fech -/- Hri +/+ (3). The greater PPIX accumulation in both models with partial or total inactivation of Hri suggests that Hri is able to limit the synthesis of the enzymes of the heme pathway, thus reducing toxic PPIX accumulation in EPP. Altogether these results indicate a protective role for Hri *in vivo* in low heme conditions and suggest that Hri is a modifier of EPP, an hypothesis that should be verified in patients.

Hri was shown to play an important role also in murine models of β thalassaemia ($Hbb^{th3/+}$)(4). Crossing Hri-/- with $Hbb^{th3/+}$, a model of thalassaemia intermedia did not produce viable animals, but in the Hri haploinsufficiency ($Hbb^{th3/+}$ and Hri -/+) anaemia, as well as splenomegaly, cardiomegaly and iron overload, were all more severe than in simple β thalassaemic mice (3). This points to a role for Hri in conditions of defective globin synthesis. Since heme deficiency is apparently not present in this condition, another mechanism has to be postulated.

The molecular mechanism of HRI activity
Heme binds to several hemoproteins through the heme regulatory motif. Hri protein has binding sites for heme in the N-terminal portion of the chain. After heme binding Hri becomes inactive and does not interfere with protein synthesis. When binding does not occur, Hri is activated by multiple autophosphorylation. Phosphorylated Hri (Hri-P) phosphorylates the β subunit of the initiation factor 2, EIFβ2 with a consequent block of protein synthesis. In red cell precursors this block results mainly in globin synthesis suppression (Figure 1). In this way Hri is a sensor of heme in the cells and a feedback inhibitor of globin synthesis, a mechanism that ensures the balance between globin and heme production. In thalassaemic cells where heme synthesis is apparently not impaired and Hri is activated by the oxidative stress that occurs because of degradation of large amounts of free □chains. Indeed Hri, as demonstrated for other kinases of the same family (5) is involved in "stress response" (such as heat or osmotic shock, oxidation...).

Perspective
All these results have been obtained in murine models, but it may be predicted that the protein will have a similar role in humans, since the strong similarities of iron and heme metabolism in humans and mice. We may predict that Hri is activated not only in iron deficiency and thalassaemia, but in all microcytic anaemias, as congenital sideroblastic anaemias due to heme deficiency or the recently identified DMT1 defects in which heme synthesis is impaired because of defective iron utilization.

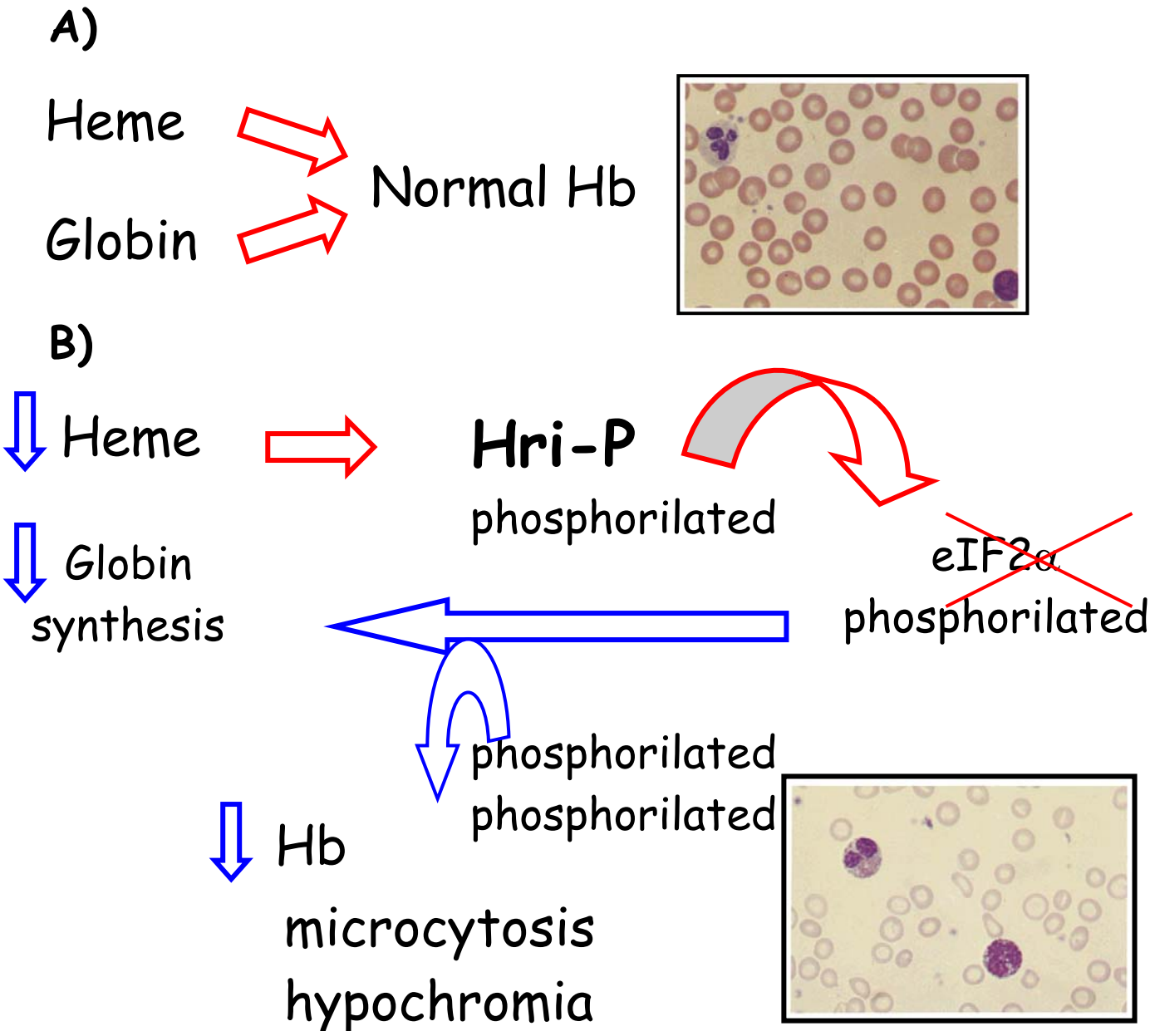
Based on its activation by oxidative stress it is reasonable to predict that Hri has a role in β thalassaemia, characterized by microcytosis without anaemia, in sickle cell anaemia and even in other intrinsic non microcytic defects of the red cells. In this way the Hri protective role overtakes the adaptive mechanism played in microcytosis. Red cells are containers full of proteins in the form of haemoglobin and cannot tolerated further protein production, that would result in inclusion body formation and red cell destruction. Hri could also be protective against ineffective erythropoiesis and, as such, could have a role in acquired anaemias e.g. myelodysplasia (1).

Finally Hri is expressed mainly in red cell precursors and its function seems to be limited to erythropoiesis. However, we cannot exclude a role in cells with high heme content. The role of this interesting protein has just started to be unravelled and in the future its study could reveal other surprises.

Table 1. Classification of microcytic anaemia

Reduction of body iron stores	Iron deficiency anaemia
Defective iron transport	Hypotransferrinemia
Defective iron utilization	Defects of DMT1
Defective iron recycling	ACD (rarely microcytic, only when longstanding) Aceruloplasminemia
Disorders of heme synthesis	Sideroblastic anaemias Erythropoietic protophorphyria (some cases)
Disorders of globin synthesis	Thalassaemia syndromes (a and bthalassaemia) Other microcytic hemoglobinopathies

Figure 1. Schematic effect of Hri on globin synthesis based on murine results.
A: normal conditions. B: heme deficiency. Red arrows indicate positive effect, blue negative effects.



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