THE 4.1. (–) HEREDITARY ELLIPTOCYTOSIS

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SUMMARY

The 4.1(–) hereditary elliptocytosis is a variety of hereditary elliptocytosis that results from the partial or total absence of skeletal protein 4.1. The heterozygous state, referred to as the 4.1(–) trait, displays to following features: absence of clinical symptoms, dominant transmission, existence of numerous, long and smooth elliptocytes, apparent reduction of band 4.1 by about 30%. The homozygous state yields a pronounced hemolytic anemia and causes some elliptocytes to bud. No band 4.1 is detectable. In addition, sialoglycoproteins β and γ are sharply reduced and do no appear in the Triton-shells, indicating an interaction with protein 4.1. 4.1(–) Hereditary elliptocytosis allows to relate a cellular abnormality to a molecular change. It provides a model for a better understanding of red cell shape and deformability.

INTRODUCTION

The genetic defects of the red blood cell can be distributed into three classes, depending whether they involve hemoglobin, cytosolic enzymes or membrane proteins. It is interesting that the first abnormal hemoglobin was recognized in 1949 and that the first enzyme deficiency was described in 1956. Although the chemical characterization of numerous alterations of the membrane, especially those concerning the blood group molecules, has started long ago, it can be said that it is not until 1980 that a primary change concerning a membrane protein was discovered.[1-3]. It involves this variety of hereditary elliptocytosis, that will been referred to as the 4.1(–) hereditary elliptocytosis (HE) and that results from a partial or total absence of protein 4.1, a major component of the membrane skeleton. We shall describe this novel abnormality at the heterozygous state which has been termed the 4.1(–) trait by Alloisio et al.[4] Through homozygous 4.1(–) HE, we will also describe the insight that the defect of protein 4.1 provides, concerning some new interections between the membrane skeleton and transmembrane proteins. Finally, we will discuss how one can approach more generally the molecular basis of red cell deformability.

The membrane skeleton comprises several major proteins

The molecular basis of red cell shape and deformability is mostly accounted for by a submembrane protein network, referred to as the membrane skeleton. This molecular assembly forms a continous strucutre beneath the cell membrane and is formed basically by four proteins, e.g. spectrin, protein 4.1, actin and protein 4.9.

Spectrin is a heterodimer $\alpha\beta$. The molecular weights of α and β chains are 240 and 220 kDa, respectively. The structure of spectrin may be approached by electron microscopy,[5] since it is a 100 nm-long, noodlelike molecule, or by limited proteolysis that yields characteristic fragments.[6] Part of the sequence of the α-chain has been determined.[7] It appears that the polypeptide chain contains repeated, homologous though not identical segments of 106 aminoacids, each of them being organized in the form of triple helices.[8] Within the dimer, α and β chains are placed side to side in an antiparallel fashion. Remarkably, spectrin must not be specific of the red cell, but belongs to a family of related proteins. Fodrin in the brain,[9] or polypeptide TW 260/240 from the intestinal brush border [10] are other members of this family.

Protein 4.1 splits into two components, polypeptides 4.1a and 4.1b (80 kDa and 78 kDa, respectively) that are sequence-related phosphopolypeptides.[11] The two polypeptides seem to be the products of a single gene. When the red cell ages, polypeptide 4.1b apparently generates polypeptide 4.1a throught an unknown post-translational mechanism. Again, protein 4.1 is not specific of the erythrocyte and similar proteins have been detected in other tissues.[12-15] Erythrocyte actin (45 kDa) belongs to the family of β-actins. It forms but short filaments comprising no more than 15-20 monomers.[16] This behaviour would be the results of protein 4.9, that would act as an actin bundling protein.[17]

The skeletal proteins interact with each other

Skeletal proteins interact in two sites: the spectrin self-association site and the spectrin-actin-protein 4.1 interaction site. Two spectrin heterodimers can self-associate, conforming to an association-dissociation equilibrium. The most common polymer delt with *in vitro* is the heterotetramer $\alpha_2\,\beta_2$.[18, 19] However, higher order oligomers have been obtained *in vitro* with high concentrations of the dimer [20] and the possibility has been presented that not only the tetramer, but also higher order oligomers occur *in situ*. The self-association sites involves one end of α and β chains. In the α-chain, the site is located on a tryptic peptide (80 kDa) that contains the amino terminus.[6]. In the β-chain, it appears on a 6 kDa tryptic peptide, containing the carboxy terminus as well as four phosphorylation sites.[21]

At its other end, spectrin interacts with actin and protein 4.1 in the form of ternary complex. Under certain experimental circunstances, spectrin causes actin to polymerize.[22] The possibility exists, therefore, of a direct binding between spectrin and actin. On the other hand, tetrameric spectrin binds with high affinity to two molecules of protein 4.1 (per spectrin dimer).[23] Binding of protein 4.1 on spectrin strenthens the attachment of actin to spectrin [24] and, in the same time, protein 4.1 causes spectrin-actin gels to become thixiotropic.[25]

Skeletal proteins interact with transmembrane proteins

Beside internal interactions, skeletal proteins are connected with transmembrane proteins. In this manner, the skeleton is attached to the membrane and, in turn, it stabilizes the latter, reducing its tendency to vesiculate. The connections involve spectrin and protein 4.1.

Spectrin binds with high affinity to band 2.1, or ankyrin (215 kDa).[26] The binding side occurs in spectrin β-chain, 20 nm from its head, e.g. its carboxy terminus. Ankyrin binds itself to transmembrane protein 3 through its 43 kDa cytosolic fragment.[26] There is a large excess of band 3 with respect to ankyrin and there would be only one molecule of ankrin per spectrin tetramer. Although evidence is indirect, protein 4.1 would bind to transmembrane proteins including glycosphorin A,[27] also termed sialoglycoprotein α, and to sialoglycoprotein β, as will be discussed below.

Finally, it is likely that skeletal proteins have direct contacts with the membrane phospholipids. The spectrin-actin complex [28] and protein 4.1 [29, 30] interact with synthetic phosphatidylserine vesicles, this class of phospholipids being prevalent at the inner surface of the red cell.

4.1(—) Hereditary elliptocytosis results from the absence of protein 4.1

Hereditary elliptocytosis (HE) comprises a variety of conditions defined on the basis of a common morphological feature of the red cell. Even this feature may display some variability, depending on the elliptocyte percentage, the degree of elongation and the tendency to bud of the red cells. Most variable, however, are the clinical and genetic backgrounds of HE. Some cases are clinically silent while others result in a dramatic hemolytic anemia. Some are transmitted on the dominant mode, whereas others appear recessive, at least morphologically. It has been a challenging problem to find out the molecular basis of the red cell abnormal shape. 4.1(—) HE represents the first example of a response to this question,[1-3] although other molecular changes have been described soon afterwards. It was first recognized by Féo et al.[1] and Tchernia et al.,[2] who reported a homozygous case, and by Alloisio et al.,[3] who observed a person combining the 4.1(—) gene, i.e. the gene responsable for 4.1(—) HE, and a gene encoding a shortened variant of protein 4.1, since then referred to as protein 4.1 Presles.[31, 32]

Homozygous 4.1(—) HE was described in three Algerian children from the same sibship who were the products of a consanguineous marriage.[1, 2] The clinical picture, though somewhat variable, was severe and early splenectomy was necessary in one child. All the red cells were elliptocytes, displaying some tendency to bud. Upon electrophoresis, the total absence of band 4.1 was the most striking feature.

In the course of a 3-year screening of HE, Alloisio et al. described clear-cut picture which was referred to as the 4.1(—) trait.[4] The condition is clinically silent. In the only case in which isotopic studies could be carried out, however, the red cell half life appeared shortened. The genetic transmission was invariably dominant. There was a 100% elliptocytosis, and elliptocytes appeared smooth and well elongated. The recognition of a partial absence of protein 4.1 was based on a careful densitometric determination of the electrophoretic profiles. This form of HE would represent roughly one half of the cases of mild HE, at least among whites and North-Africans. It has not been described so far in Blacks, in whom a distinct form of HE, termed type I HE and due to a defect of the head of spectrin α-chain, seems to be prevalent.[33-35]

From the presently available data, protein 4.1 seems to be encoded by a single locus, the 4.1(—) locus. We assume that 4.1(—) HE results from a thalassemia-like mutation occuring in this locus. A distinct case of mild, dominant HE has been reported by Garbarz et al.,[36] resulting from a shortened variant of protein 4.1. In contrast, no change at all existed in the carriers of another truncated protein 4.1, protein 4.1 Presles.[31, 32]

The absence of protein 4.1 results in a change of the level of sialoglycoprotein β

Two interesting observations have been made by Morrison and his group. Sialoglycoprotein β, as named after Anstee et al.,[37] was found to be extracted with the Triton-shells, e.g. the skeletal proteins precipitated by the detergent Triton X-100.[38] This strongly suggested, and indeed virtually proved, that there was a linkage between the membrane skeleton and sialoglicoprotein β. Soon afterwards, they observed the absence of sialoglycoprotein β in the Triton-shells of an elliptocytic child lacking protein 4.1.[39] Sialoglycoprotein β was also reported to be completely missing in native ghosts. In the absence of clinical and genetic data, it was not certain whether this child displayed 4.1(—) HE, or any other condition, such as those recently described by Anstee et al.[40, 41]

We examined sialoglycoprotein β in homozygous and heterozygous 4.1(—) HE, using the labelling of sialic acid residues.[42] In homozygous 4.1(—) HE, sialoglycoprotein β was sharply reduced in the ghosts and entirely absent from the Triton-shells. In the 4.1(—) trait, there was presumably a small decrease of sialoglycoprotein β, but the latter appeared in normal amount in the Triton-shells. These results further support the view of a specific interaction of protein 4.1 and sialoglycoprotein β, notwithstanding other constacts between protein 4.1 and the membrane. They fit with the fact that protein 4.1 largely saturates sialoglycoprotein β.[43] In the 4.1(—) trait, the absence of one haploid set of protein 4.1 has a limited effect on sialoglycoprotein β, because the remaining haploid set is sufficient to support most of the contact. Interestingly, abnormalities of sialoglycoprotein γ and other sialoglycoproteins accompany the absence of protein 4.1 in homozygous 4.1(—) HE. These findings indicate that there might be a very complicated nexus between protein 4.1 and sialoglycoprotein β and γ, as well sialoglycoprotein α,[27] although this nexus would be most diferent from that formed by the spectrin-ankyrin-band 3 complex.

Toward a molecular understanding of red cell shape and deformability

We have briefly presented the current representation of the membrane skeleton. We have also shown the information that a spontaneous mutation can provide concerning the linkage between the molecular composition of the skeleton and the cell architecture. Beside the lack of protein 4.1, a number of other remarkable genetic defects of the membrane skeleton have been reported since 1981. In addition, in numerous genetic alterations, involving hemoglobin and red cell enzymes, hemolysis ultimately results from some acquired membrane damage. One frequent event that seems to result from the increased oxidant stress associated with highly unstable hemoglobins, major forms of thalassemia or some enzyme defects, is the proteolytic cleavage of numerous proteins, including spectrin and sialoglycoproteins. The red cell deformability becomes dramatically reduced.

One may ask the question as to whether external stress may also alter red cell shape and deformability. The answer is yes. In Laennec's cirrhosis, a disturbance of lipid metabolism ultimately modifies the red cell membrane lipid composition and leads the erythrocytes to become acanthocytes, which are less deformable.[44] In diabetes mellitus [45] and, to a lesser extent, in chronic arterial disease, a reduction of red cell deformability has been recorded.[46-48] This observation was made, in general, using the filtration of blood or of washed red cells through nucleopore membranes. In neither diabetes mellitus nor arterial disease has one been able to detect so far any clear-cut molecular abnormality. However, it seems conceivable that increased levels of blood glucose may generate, through non enzymatic overglycosylation, subtle changes of membrane proteins, just as they yield higher proportions of glycosylated hemoglobin. Repeated mechanical stress may also modify red cell calcium permeability.[49] A large field of investigations is open concerning the acquired changes of the red cell in conditions that do not concern primarily this cell but that secondarily yield a reduction of its deformability.

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