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Genetic disorders of the red cell membranes

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Abstract The red cell membrane is comprised of a lipid bilayer studded with transmembrane proteins, and laminated by a protein network, the membrane skeleton, at the surface of the inner monolayer. The erythrocyte owes its mechanical properties to the membrane skeleton. Hereditary spherocytosis, hereditary elliptocytosis or poikilocytosis, Southeast Asian ovalocytosis are hereditary hemolytic anemias, due to mutations in the genes encoding ankyrin, the anion exchanger, spectrin, protein 4.1 or protein 4.2, which are main proteins of the membrane. Recent advances in the field have led to fundamental questions.

Key words: Red cell; Membrane; Protein; Hereditary hemolytic anemia; Genomic mutation

1. Introduction

The red cell membrane is comprised of a lipid bilayer studded by numerous transmembrane proteins, many of which are involved in exchange processes. The phospholipids have an assymetric distribution. Glycan moieties, covalently linked to transmembrane proteins and sphingolipids, and glycosylphosphatidylinositol-anchored proteins (GPI-proteins), protrude on the outer side. The inner monolayer of the surface is laminated by a protein assembly, the red cell (or membrane) skeleton. Over several years, many mutations have been found in abnormal red cell membrane proteins. Blood group antigens and/or GPI-proteins will be not to delt with here.

2. The red cell membrane and its skeleton

The red cell must achieve remarkable mechanical performances, that is, endure the turbulences prevailing in large vessels and negociate their passing through capillaries. These properties are ensured by the red cell skeleton in connection with some transmembrane proteins. Fig. 1 provides a cross-section of the membrane and its skeleton. Table 1 gives the most salient data pertaining to the main proteins and their genes. A number of significant features may be pinpointed [1].

1. All the proteins envisaged are widely distributed among cell-types through a spectrum of isoforms. Isoforms diversity derives mainly from gene multiplication (spectrin chains, ankyrin, anion exchanger) and/or alternative splicing of the transcript from a single gene (protein 4.1, protein 4.2).
2. Ankyrin contains three functional domains : 89 kDa (binding site for band 3), 63 kDa (binding site for spectrin β-chain) and 55.5kDa ('regulatory domain') [2]. The 89 kDa domain is comprised of 24 homologous segments of 33 amino acids. This recurrent motif is found in a variety of proteins, such as NF-κD, a transcription factor precursor [3]. The multiprotein complex held together by ankyrin connects the skeleton to transmembrane proteins. The primary absence of one protein is likely to disrupt the whole complex, at least in part, and yield a reduction or the absence of other proteins.
3. Basically, the anion exchanger (AE1), also designated band 3, has a cytoplasmic domain (amino acids 1to 403) and a membrane domain comprising 14 membrane-spanning segments (TM1 to 14; amino acids 404–882) [4]. The cytoplasmic domain binds the N-terminal, 89 kDa domain of ankyrin. It also binds protein 4.2 and many other proteins. Band 3 exists as immobile tetramers, bound to ankyrin and spectrin, and mobile dimers.
4. Protein 4.2 has homologies with transglutaminases [5]. It binds to the cytoplasmic domain of the anion exchanger. Its N-terminal glycyl residue is acylated by myristic acid.
5. Each α- and β-spectrin chains are comprised of 106 amino acids homologous segments (α1 to α22, and β1 to β17, respectively) [6,7]. In space, the conformation units are described by two models [8,9], that both imply three α-helical segments. The phasing of conformational units is shifted downstream by 26amino acids with respect to that of homologous segments [10]. An α- and a β-chain of spectrin initiate dimerization at two complementary nucleation sites, one on the α-chain (α18 to α21), the other on the β-chain (β1 to β4) [11]. Then, the two chains complete dimerization by laying alongside in an antiparallel fashion. Two dimers self-associate head to head through complementary sites located near the N- and C-termini of the α- and β-chains, respectively.
6. The transcript of protein 4.1 is the subject of complex splicing patterns that are cell and stage-specific [12,13]. For example, in the early erythroid precursors, the 5′-portion (17 nt) of exon 2, containing the main initiation codon, is conserved whereas exon 16 (63 nt) is skipped. The situation reverses at a latter stage, though non-synchronically. Then, a downstream ATG (exon 4) ensures entirely the initiation of translation. Exon 16, along with exon 17, complete the binding site of protein 4.1 for the spectrin-actin complex.
7. p55 and glycophorin C anchor protein 4.1 to the bilayer. p55 is palmitoylated, has a SH3 domain and a guanylate kinase activity[14]. Its homologue in *Drosophila* acts as a tumor suppressor. Glycophorin C interacts with protein 4.1 and GPC through its cytoplasmic segment [15].

3. Hereditary spherocytosis

Hereditary spherocytosis (HS) is the most common (approx. 1/2000 kindreds) of congenital hemolytic anemia [1]. Icterus, anemia, splenomegaly and biliary stones are the main signs. The whole spectrum of gravity may be covered, from no clinical expression at all to death in utero (hydrops fetalis). HS occurs in nearly all ethnic groups, but appears more seldom among

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black people. The inheritance pattern is dominant or recessive. Yet, compounded modes of transmission are often recorded.

3.1. Ankyrin

70% of HS cases stems from ankyrin mutations. Twenty percent of these mutations would be de novo. Most of HSmutations are 'private', being restricted to one or a few families. A number of mutations lead to the absence of one haploid set of ankyrin [16] or the truncation of the latter molecule [17]. These forms display a dominant inheritance pattern. The decrease of ankyrin yields a secondary reduction in protein 4.2 and, inconstantly, in spectrin. Other ankyrin mutations are simple amino-acid substitutions [1]. Two such mutations in *trans* to one another are required to produce a clinical picture, that is often severe. The *nb/nb* mouse (*nb*: normoblastosis) discloses HS and a delayed neurological syndrome associated with degenerating Purkinje cells [18], both stemming from an ANK1 gene mutation.

3.2. Anion exchanger

25% of HS cases are associated with a 20–40% reduction of the anion exchanger (AE1). The responsible mutations are either stop codons that suppress the synthesis of one AE1 haploid set, or mutations that prevent AE1 from being conveyed and/or inserted into the membrane [19–21]. The clinical picture is of intermediate severity but often requires splenectomy at last. Basically, the inheritance pattern is dominant. Some AE1 mutations hit specifically the binding site for protein 4.2 [22]. While these mutations remain symptomless in the heterozygous state, they produce a severe picture in the homozygous state. The main biochemical feature, then, is the lack of protein 4.2 whereas the amount of AE1 is normal (or nearly so). A deletion of 9 nt [23,24], involving the very junction of the AE1 cytoplasmic and membrane domains, is the cause of Southeast Asian ovalocytosis in the heterozygous state (the homozygous state must be lethal).

3.3. Protein 4.2

Protein 4.2 total deficiency, arising now from mutations in protein 4.2 gene itself, yields a somewhat atypical form of HS (scarce spherocytes, nearly normal osmotic resistance) in the homozygous state [25–27]. It has a recessive inheritance pattern. Except for two cases, the mutations are single amino acid substitutions and it is not always understood why they result in the lack of protein 4.2. One allele, allele NIPPON, appears in a recurrent fashion in Japan [25].

3.4. Spectrin

A few mutations of spectrin β-chain have been found to be responsible for HS in the heterozygous state in man [28–30] and one nonsense mutation does so in the *ja/ja* mouse[31]. The mutation may occur all over the β-chain molecule, except in the β17 homologous segment. The mouse displays an array of spectrin α-gene mutations liable to cause HS.

4. Hereditary elliptocytosis

Hereditary elliptocytosis (HE) is the second most common hereditary hemolytic anemia [1]. The clinical features are not very different from those of HS, yet they are usually milder. Diagnosis relies on the presence of elliptic red cells on smears. The severe forms achieve hereditary poikilocytosis (HP): on smears, elliptocytes are replaced by cells having all sorts of shapes and sizes (poikilocytosis). HE is quite evenly distributed in ethnic groups, but are more common among black people. The inheritance pattern is dominant or recessive, however many compounded patterns are also observed.

4.1. Spectrin α- and β-chains

The HE mutations are clustered in the self-association sites of the α-chain (α1 homologous segment) and of the β-chain (β17 homologous segment) [32,33]. In addition, some mutations are more remote from the self-association site in the α-chain, occurring down to homologous segment α8. β-Chain mutations often lead to truncation the C-terminal segment. At all events, the self-association process is disturbed. Most often, mutations hit helix 3 of homologous segments, however changes altering helix 2 and even helix 1 (β-chain) have been reported.

Remarkably, any α-allele responsible for HE (α^{HE} alleles)

Table 1
Main features of the considered proteins and their genes. Some proteins are only mentioned in Fig. 1, not in the text.

	Amino acids (calculated MW)	Monomers per cell	Gene symbol and chromosomal location	Gene size (kb) and exon number	Size of mRNA (kb)	Disease
Spectrin α-chain	2429 (281)	242 000	SPTA 1; 1q22–q23	80; 52	8	HE
Spectrin β-chain	2137 (246)	242 000	SPTB; 14q23–q24.2	>100; 36	7.5	HE, HS
Ankyrin	1880 (206)	124 000	ANK1; 8p11.2	>120; 42	6.8–7.2	HS
Adducin α-chain	737 (81)	30 000	ADDA; 4p16.1	–	–	–
Adducin β-chain	726 (80)	30 000	ADDB; –	–	–	–
Anion exchanger	911 (102)	1 200 000	EPB3; 17q12–q21	17; 20	4.7	HS
Protein 4.1	588 (66)	~200 000	EL1; 1p33–p34.2	>250; 23	5.6	HE
Protein 4.2	691 (77)	~250 000	ELP42; 15q15–q21	20; 13	2.4	HS[a]
Dematin	383 (43)	~140 000[b]	– –	–	–	–
	– (52)[c]		–; 8p21.1	– –	–	–
p55	466 (53)	~80 000	MPP1; Xq28	~30; –	2	–
β-Actin	375 (42)	700 000[d]	ACTB; 7pter-q22	>4; 6	–	–
Tropomodulin	359 (41)	~30 000	TMOD; 9q22	–	–	–
Tropomyosin	239 (22)	~ 70 000[e]	TPM3; 1q31	– –	–	–
Glycophorin C	128 (14)[f]	~200 000	GYPC; 2q14–q21	14; 4	1.4	HE
Glycophorin D	107 (11)[f]					

These data are largely from reference 1; a: atypical form of HS; b: dematin occurs as trimers; c: determined MW; d: β-actin occurs as oligomers (~14 monomers); e: tropomyosin occurs as dimers; f: the contribution of the glycan moieties is not included in the MW; glycophorin D is an isoform of glycophorin C, the translation being initiated at Met22 of glycophorin C.

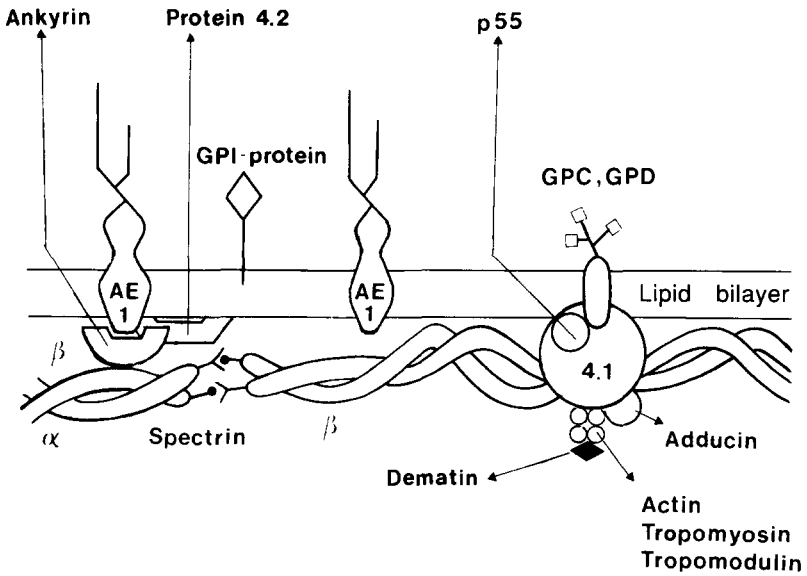

Fig. 1. Section of the erythrocyte membrane and skeleton. Only relevant proteins and some other major proteins are shown. AE1 : anion exchanger. GPC and GPD : glycophorins C and D, respectively. GPI-protein : glycosylphosphatidylinositol-anchored protein. Horizontal interactions: $\alpha\beta$-dimers self-associate head-to-head to form $\alpha_2\beta_2$ heterotetramers; each of the extremities of the tetramers interacts with actin and protein 4.1 to form the junctional complex. This complex extends to a larger complex including adducin (α- and β-chains), dematin, tropomyosin, tropomodulin (Table 1) and still other proteins. Vertical interactions: β-spectrin interacts with ankyrin that, in turn, interacts with the cytoplasmic domain of AE1. Protein 4.1, p55 and GPC (GPD) combine into a distinct complex.

yields two possible levels of clinical expression, schematically mild or severe. It has been shown that the aggravating factor is carried by the α-allele in *trans*. Unless it is merely another α^{HE} mutation, it is more often a low expression allele, allele α^{LELY} (LELY: Low Expression LYon) [34,35]. Allele α^{LELY} carries two mutations: α1857 Leu→Val, a functionnally neutral polymorphism, and a C to T substitution at position −12 of intron 45. Remarkably, exon46 (18 nt), a normally constitutive exon, is skipped in 50% of α^{LELY} transcripts. Alpha-chains stemming from α^{LELY} allele compete defavourably with α^{HE}chains, that all contain, in principle, the 6 amino acids encoded by exon 46 and thereby are preferentially recruited by the β-chains. Hence a prevalence of $\alpha^{HE}\beta^{dimers}$ that will turn out to poorly self-associate subsequently. Alpha^{LELY} alleles appear with a uniform frequency (20–30%) in all ethnic groups investigated so far (Caucasians, African Blacks, Japanese and Chinese) [36].

4.2. Protein 4.1

4.1(−) HE may be associated with a reduction (heterozygous state) or the absence (homozygous state) of protein 4.1. 4.1(−) alleles account for approximately 30% of HE cases among Caucasians. Heterozygous 4.1(−) HE is clinically silent. It is only manifested by a 20–30% decrease of protein 4.1. Exceptional homozygous 4.1(−) HE yields a severe clinical presentation and the total absence of protein 4.1. Due to the complexity of protein 4.1 gene and of the splicing of its transcript, only two mutations have been elucidated at the gene level so far [37,38]. In the homozygous state, the absence of protein 4.1 yields a sharp decrease of glycophorin C [39] and abolishes the presence of protein p55 [40].

5. Conclusions

Elucidating the mutations responsible for genetic disorders of the red cell membrane have delineated by default many important features of normal gene expression and protein function. It opened new ways in fundamental research, and received important applications in diagnosis and treatment.

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