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Red Cell membrane disorders

Role of Lu/BCAM glycoproteins in red cell diseases

Les protéines Lu/BCAM dans les maladies du globule rouge

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Abstract

Lu/BCAM glycoproteins (gps) are the unique erythroid receptors of laminin α5 chain, a major component of the extracellular matrix. They interact with the membrane skeleton by binding directly to spectrin *via* the Lu/BCAM RK573-574 motif. Lu/BCAM gps are involved in abnormal sickle red blood cell (RBC) adhesion to components of the vascular wall. This adhesion is activated by the phosphorylation of the Lu/BCAM long isoform Lu in a protein kinase A-dependent manner. A similar high adhesion to laminin was also observed with RBCs from Hereditary Spherocytosis (HS) patients suffering from haemolytic anaemia subsequent to spectrin deficiencies. We investigated the molecular mechanisms responsible for the Lu/BCAM-mediated abnormal RBC adhesion to laminin in sickle cell disease (SCD) and HS. We showed that SCD patients treated with hydroxycarbamide (HC) had a diminished RBC adhesion to laminin that was associated with reduced levels of the PKA upstream effector cAMP and a severe decrease in Lu isoform phosphorylation. On the other hand, we showed that increased Lu/BCAM-mediated HS RBC adhesion to laminin was independent of Lu/BCAM phosphorylation. A cellular model expressing the RK573-574AA Lu/BCAM mutant, which is unable to bind to spectrin, showed increased Lu/BCAM detergent extractability and enhanced cell adhesion to laminin. Similar results were obtained with HS RBCs, strongly suggesting that their increased adhesion could result from alteration of the Lu/BCAM-spectrin interaction following the severe spectrin deficiency.

Keywords: Lu/BCAM; Adhesion; Laminin; Spectrin; Red blood cells; Sickle cell disease; Hereditary spherocytosis

Résumé

Les glycoprotéines (gp) Lu/BCAM sont les récepteurs érythroïdes uniques de la chaîne α5 de la laminine, composant majeur de la matrice extracellulaire. Elles interagissent également avec le squelette membranaire en liant directement la spectrine par leur motif cytoplasmique RK573-574. Les gp Lu/BCAM sont impliquées dans l'adhérence anormale des globules rouges (GR) drépanocytaires à la paroi vasculaire. Cette adhérence est stimulée par la phosphorylation de leur isoforme longue Lu après activation de la voie protéine kinase A (PKA). Une adhérence anormale à la laminine est également observée pour les GR de patients atteints de sphérocytose héréditaire (SH) qui présentent une anémie hémolytique suite à une déficience sévère en spectrine. Nous avons étudié les mécanismes moléculaires à l'origine de cette adhérence anormale des GR drépanocytaires et sphérocytaires. Nous avons montré que les GR de patients drépanocytaires traités par l'hydoxycarbamide (HC) avaient une adhérence diminuée à la laminine associée à une baisse des taux d'AMPc, effecteur de la voie PKA, et à une forte inhibition de la phosphorylation de l'isoforme Lu. À l'opposé, l'adhérence augmentée des GR SH était indépendante de la phosphorylation de Lu/BCAM. Un modèle cellulaire exprimant le mutant Lu RK573-574AA, ne liant plus la spectrine, a montré que la mutation augmentait la solubilisation de Lu/BCAM par le Triton-X100 ainsi que l'adhérence cellulaire à la laminine. Des résultats similaires ont été obtenus avec des GR SH, suggérant fortement que l'augmentation de leur adhérence pourrait résulter de l'altération de l'ancrage de Lu/BCAM au squelette membranaire suite au défaut en spectrine érythroïde.

Mots clés : Lu/BCAM ; Adhérence ; Laminine ; Spectrine ; Globules rouges ; Drépanocytose ; Sphérocytose héréditaire

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1. Introduction

Lutheran (Lu) blood group antigens and Basal Cell Adhesion Molecule (BCAM) tumor-associated antigen are carried by Lu/BCAM (CD239) glycoproteins (gps) of the immunoglobulin superfamily. Lu/BCAM are transmembrane proteins represented by two isoforms of 628 (Lu) and 588 [Lu(v13)] amino acids which differ by the length of their cytoplasmic domain (Fig. 1) [1]. They show a broad expression in human tissues, including epithelial and endothelial cells. Lu/BCAM gps are the unique erythroid receptors of laminin α5 chain, a major component of the extracellular matrix (ECM); they bind to laminin through aspartic acid 343 (Fig. 1) [2–4].

Lu/BCAM gps are involved in abnormal adhesion of RBCs from sickle cell disease (SCD) patients to components of the vascular wall. Two interactions have been described: (i) erythroid Lu/BCAM-mediated adhesion of sickle (SS) RBCs to laminin and (ii) interaction of integrin α4β1 on SS reticulocytes with endothelial Lu/BCAM [2,3,5].

Lu/BCAM-mediated SS RBC adhesion to laminin is stimulated by the physiological stress mediator epinephrine in a protein kinase A (PKA) dependent manner and is associated with a PKA-dependent phosphorylation of the Lu/BCAM long isoform Lu [6,7]. Likewise, epinephrine also activates integrin α4β1-mediated SS reticulocyte adhesion to Lu/BCAM on resting endothelial cells [5].

Lu/BCAM gps are associated with the erythroid membrane cell skeleton [8]. The Lu/BCAM cytoplasmic domain interacts with the spectrin-actin-protein 4.1 complex by binding directly to spectrin α4 repeat *via* the Lu/BCAM RK573-574 motif (Fig. 1) [9,10]. Hereditary spherocytosis (HS) patients, suffering from haemolytic anaemia subsequent to spectrin deficiencies, exhibit high Lu/BCAM-mediated RBC adhesion to laminin [11].

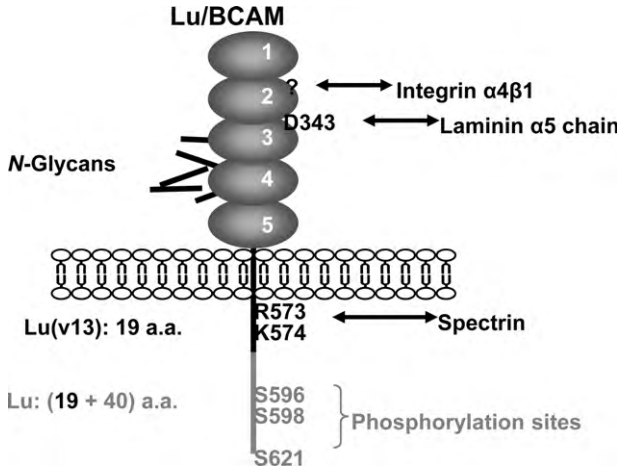

Fig. 1. Lu/BCAM proteins. Lu/BCAM are type I transmembrane glycoproteins, members of the immunoglobulin superfamily. Lu/BCAM isoforms, Lu and Lu(v13), share the same extracellular and transmembrane domains but differ by the length of their cytoplasmic domain: 19 a.a. for Lu(v13) and 59 a.a. for Lu. The 40 extra amino acids of Lu isoform comprise three phosphorylated serines. Lu/BCAM isoforms interact with laminin through D343 of their domain 2-domain 3 interdomain region and with spectrin through their RK573-574 juxtamembrane cytoplasmic motif. Their integrin α4β1 interaction site is still unknown.

This review addresses the molecular mechanisms responsible for the Lu/BCAM-mediated abnormal RBC adhesion to laminin in SCD and HS. It also reports recent advances in the comprehension of hydroxycarbamide (HC) mechanism of action in SCD.

2. Lu/BCAM in sickle cell disease

2.1. Introduction

SCD is a monogenic red blood cell disorder resulting from a Glu→Val substitution in the sixth codon of β hemoglobin (Hb). This abnormal Hb, named HbS, polymerizes under deoxygenated conditions leading to less deformable sickle RBC formation.

Vaso-occlusion crises (VOC) are the main acute complication of SCD. Growing evidence supports the hypothesis that VOC could be initiated by abnormal RBC adhesion to the vascular wall through interactions between erythroid adhesion molecules and proteins of the vascular wall [12–14]. Such interactions could play a critical role in HbS polymerization and VOC development by prolonging RBC transit time in capillaries.

HC is the only drug now available having demonstrated benefit for SCD patients, with fewer VOC and lower mortality and morbidity [15–18]. It was commonly thought that HC acts as an anti-sickling agent by increasing fetal hemoglobin (HbF) levels, leading to significantly less hemoglobin S polymerization. However, HC was often associated with clinical improvement before HbF rose significantly, suggesting that it could also act through other mechanisms [16].

Several interactions have been described between SS RBCs and the endothelial vascular wall involving erythroid integrin α4β1, Landsteiner–Wiener/intercellular adhesion molecule-4 (LW/ICAM-4) and Lu/BCAM gps as well as the ECM proteins, fibronectin, TSP and laminin. HC diminishes SS RBC adhesion to endothelial cells and the ECM proteins [19–21]. These decreases are consistent with less CD36, $\alpha_4\beta_1$ and LW/ICAM-4 expression on the surfaces of SS reticulocytes and erythrocytes [22]. However, Odièvre et al. showed that RBC expression of Lu/BCAM was significantly elevated in HC-treated children [22]. Consequently, SS RBC adhesion to laminin could not be directly linked to the cell-surface Lu/BCAM expression level.

2.2. Effects of hydroxycarbamide on Lu/BCAM-mediated SS RBC adhesion

SS RBC adhesion to laminin was investigated under HC treatment. Because of wide interindividual variability in SCD, SS RBC samples were obtained from 13 prospectively followed patients before starting HC and at regular intervals during treatment: 15 days, 2, 4 and >6 months after starting HC. Adhesion assays under flow conditions showed that adhesion was significantly lower after 6 months of HC ($P = .013$), and dramatically diminished for the five patients with the highest adhesion levels before starting HC (Fig. 2A). In contrast with this diminished adhesion, flow cytometry analysis showed