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Research perspectives

Deciphering the molecular basis of ferroportin resistance to hepcidin: Structure/function analysis of rare *SLC40A1* missense mutations found in suspected hemochromatosis type 4 patients

Mécanismes de résistance de la ferroportine à l'hepcidine : analyses structure/fonction de mutations faux-sens rares du gène SLC40A1 *identifiées chez des patients présentant une surcharge en fer typique de l'hémochromatose de type 4*

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Abstract

Genetic medicine applied to the study of hemochromatosis has identified the systemic loop controlling iron homeostasis, centered on hepcidin-ferroportin interaction. Current challenges are to dissect the molecular pathways underlying liver hepcidin synthesis in response to circulatory iron, HFE, TFR2, HJV, TMPRSS6 and BMP6 functions, and to define the major structural elements of hepcidin-ferroportin interaction. We built a first 3D model of human ferroportin structure, using the crystal structure of EmrD, a bacterial drug efflux transporter of the Major Facilitator Superfamily, as template. The model enabled study of disease-associated mutations, and guided mutagenesis experiments to determine the role of conserved residues in protein stability and iron transport. Results revealed novel amino acids that are critical for the iron export function and the hepcidin-mediated inhibition mechanism: for example, tryptophan 42, localized in the extracellular end of the ferroportin pore and involved in both biological functions. Here, we propose a strategy that is not limited to structure analysis, but integrates information from different sources, including human disease-associated mutations and functional *in vitro* assays. The first major hypothesis of this PhD thesis is that ferroportin resistance to hepcidin relies on different molecular mechanisms that are critical for ferroportin endocytosis, and include at least three fundamental steps: (i) hepcidin binding to ferroportin, (ii) structural reorganization of the N- and C-ter ferroportin lobes, and (iii) ferroportin ubiquitination.

Keywords: Iron metabolism; Hemochromatosis; Ferroportin; Hepcidin; Gain-of-function mutations

Résumé

L'étude des formes rares d'hémochromatose a contribué à une meilleure connaissance des mécanismes cellulaires et systémiques qui participent au maintien de l'homéostasie du fer et qui dépendent de l'axe hepcidine-ferroportine. Des questions fondamentales majeures restent posées sur les voies d'activation de la synthèse d'hepcidine en fonction de la quantité de fer plasmatique et de l'action à la surface des hépatocytes des protéines

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HFE, TFR2, HJV, TMPRSS6 et BMP6, ainsi que sur les bases structurales de l'interaction hepcidine-ferroportine. Nous avons construit un premier modèle tridimensionnel de la ferroportine humaine à partir de la structure cristallographiée d'EmrD, un exportateur de drogues bactérien qui appartient à la famille des transporteurs secondaires MFS (« Major Facilitator Superfamily »). Cette modélisation a permis, en lien avec l'étude fonctionnelle de mutations faux-sens identifiées chez des patients présentant des phénotypes typiques d'hémochromatose de type 4, d'identifier des acides aminés qui ont une fonction critique dans la stabilité de la ferroportine, la fonction d'export du fer, ou la régulation par l'hepcidine. Le tryptophane en position 42 s'est, par exemple, révélé important à la fois pour la prise en charge du fer et l'endocytose de la ferroportine à la suite de la fixation de l'hepcidine. Nous proposons ici une stratégie qui combine analyses structurales, corrélations génotype/phénotype et tests fonctionnels *in vitro*. Ce travail de thèse vise à révéler les différents mécanismes moléculaires qui sous-tendent le processus de dégradation de la ferroportine. Ce processus semble comprendre trois étapes essentielles : (i) fixation de l'hepcidine à la ferroportine, (ii) réorganisation structurale des lobes N et C-terminaux de la ferroportine, et (iii) ubiquitination et internalisation du transporteur.

Mots clés : Métabolisme du fer ; Hémochromatose ; Ferroportine ; Hepcidine ; Mutations « gain-de-fonction »

1. Introduction

Iron is essential to normal cell biology. As a component of heme, it is particularly important for erythropoiesis and oxygen transport. However, iron excess and "free" reactive iron is toxic. Body iron loss is insufficient, and intertwined mechanisms have evolved to maintain intracellular and systemic iron homeostasis. Ferroportin (FPN1, also referred to as SLC40A1, SLC11A1, MTP1 and IREG1) is the only known iron exporter in mammals and is considered a key coordinator of iron balance between the two compartments [1].

Ferroportin is expressed in all types of cell that handle major iron flow, including iron-recycling macrophages, absorptive enterocytes and storing hepatocytes (Fig. 1). Ferroportin is predominantly regulated by the liver-derived peptide hepcidin, which binds ferroportin on the cell surface, inducing internalization and degradation [2]. Hepcidin-ferroportin interaction is critical in both normal iron homeostasis and in common iron metabolism pathologies, including not only inherited disorders and iron overload but also non-genetic diseases and anemia [3].

1.1. Regulation of ferroportin activity

Ferroportin expression is regulated transcriptionally, post-transcriptionally, and post-translationally. At the cellular level, ferroportin synthesis is regulated via the iron-responsive element/iron regulatory proteins system. This system orchestrates the post-transcriptional regulation of several other important iron metabolism genes [4]. At the level of the organism, ferroportin is subject to post-translational downregulation via the liver-derived peptide hepcidin [2,5]. The molecular mechanism of hepcidin-mediated ferroportin downregulation is not fully understood, but includes at least three fundamental steps:

- hepcidin binding to ferroportin, a step that involves amino acid residue p.Cys326 [6];
- ferroportin ubiquitination, a step that is thought to involve several lysine residues between positions 229 and 269 [7];
- ferroportin trafficking to the multivesicular body and degradation in the late endosome/lysosome compartment [8].

1.2. Dichotomous patterns of human SLC40A1 mutation

In humans, most ferroportin mutations affect plasma membrane location and/or iron export ability. These loss-of-function mutations explain the classical reports of isolated serum ferritin elevation, relative plasma iron deficiency and preferential iron retention in cells of the reticuloendothelial system. This histological picture is commonly referred to as ferroportin disease [9].

In contrast, some *SLC40A1* mutations do not impair the ability of ferroportin to export iron but result in partial to complete resistance to hepcidin. Patients with these gain-of-function mutations (hemochromatosis type 4) usually display histological and clinical presentations similar to those of the typical HFE-related hemochromatosis. More especially, due to greater flow of iron through iron-recycling macrophages and increased iron absorption, transferrin saturation is expected to be markedly elevated in these patients [10,11].

1.3. Ferroportin structure

The structural organization of human ferroportin in the lipid bilayer is controversial. Wallace and collaborators reported the first model of the 3D structure of human ferroportin, with 12 predicted transmembrane helices built *de novo* (*i.e.*, without homology). They subsequently fitted the model to experimental data, using *Escherichia coli* glycerol-3-phosphate (GlpT) transporter as template [12]. This template belongs to the Major Facilitator Superfamily (MFS), which is the largest group of secondary active membrane transporters, transporting a range of diverse substrates across membranes.

Bonaccorsi di Pati and collaborators described a fairly similar 3D model of ferroportin, based on threading/*ab initio* modeling [13]. This is not surprising, since, independently of the algorithms used, the Italian group predicted an inward-open conformation of the ferroportin iron transporter, using GlpT as template. They used a different MFS member, *E. coli* L-fucose proton symporter (FucP), to build an outward-open conformation of ferroportin. The switch between the two conformations is believed to drive iron transport across the plasma membrane.

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