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HEPCIDIN AND ITS ROLE IN IRON HOMEOSTASIS

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Abstract
Hepcidin, a small peptide secreted mainly by the liver, plays a central role in iron status regulation. The experiments on hepcidin seemed very promising and gave new life to understanding iron metabolism. Many authors suggest that hepcidin measurement can be used as a clinical tool for the diagnosis and management of a wide range of iron-related disorders. The current review presents data concerning hepcidin, especially its biology, mechanism of action and its role in pathomechanism of many diseases.

Key words: hepcidin, iron metabolism

1. INTRODUCTION

Hepcidin was first discovered in human blood ultrafiltrate and urine samples as a small bactericidal peptide (defensin and cathelicidin) and named liver–expressed antimicrobial peptide (LEAP–1) [1-10]. The name 'hepcidin' originates from the place of synthesis in hepatocytes (hep-) and its antimicrobial activity (-cidin) [10]. The gene encoding hepcidin (*HAMP*, 19q13) is expressed in the liver, heart, lungs, brain, spinal cord, intestine, stomach, pancreas, adipocytes, skeletal muscles, testis and macrophages [2-5, 8, 10, 11, 12]. The hepcidin genes have been also found in mice, pigs, birds and fish [3]. Hepcidin has antibacterial (*Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus* spp. group B) and antifungal activity (*Candida albicans*, *Aspergillus niger*, *Aspergillus fumigatus*) [10]. This protein is a key regulator of iron level, it decreases the iron absorption from the duodenal enterocytes, iron release from macrophages and its transport across the placenta [1, 3-8, 10, 13-16]. The main role of hepcidin in iron metabolism was confirmed on animal models and *in vitro* studies [13]. The synthesis of hepcidin in hepatocytes can be regulated by iron overload, inflammatory signals, increased erythropoiesis, hypoxia and anemia [1-9, 11, 12, 14, 16, 17].

2. THE STRUCTURE OF HEPCIDIN GENE AND PEPTIDE

The human hepcidin gene (*HAMP*) is located on chromosome 19q13.1 [18]. It is 2637 base pairs long and composed of three exons and two introns [19, 20]. It contains binding sites for such regulatory factors as HNF3β, C/EBPβ and NF-κB [19]. *HAMP* gene expression was detected mainly in the liver, but also in heart, brain, lung, prostate gland, tonsils, salivary gland and trachea [21]. *HAMP* encodes a precursor of hepcidin – preprohepcidin, which is 84 amino acids protein comprised 24 aa leader peptide at the N-terminal, a 35 aa proregion, and the C-terminal 20 or 25 aa mature peptide. Preprohepcidin is cleaved to 60 aa prohepcidin which is further amino-terminally processed and gives rise to hepcidin. There are three forms of hepcidin: 25 aa, 22 aa and 20 aa peptide. All three forms are detectable in urine, but only hepcidin-25 and hepcidin-20 are present in human serum [18, 22, 23, 24]. The structure of hepcidin-25, which is a major form of hepcidin, contains eight cysteine residues connected by disulfide bonds [21]. Analysis of hepcidin structure by NMR spectroscopy showed that this peptide forms a simple hairpin stabilized by four disulfide bonds

between the two anti-parallel strands. Unusual vicinal disulfide bridge found at the turn of the hairpin probably plays significant functional role [18, 22, 25].

3. Mechanisms of hepcidin action

Hepcidin is well known as iron-regulatory hormone. Generally, it causes a decrease in serum iron. The mechanism of hepcidin activity depends on hepcidin interactions with ferroportin. Ferroportin is the only known mammalian cellular iron exporter, which is expressed on the surface of reticulo-endothelial macrophages, hepatocytes, duodenal enterocytes and placenta cells. Hepcidin regulates posttranslationally ferroportin expression. Hepcidin binds to ferroportin and causes its internalization and degradation in endolysosomes, what in turn blocks the iron transport via ferroportin. When iron stores are adequate or high, increased hepcidin expression inhibits intestinal iron absorption, release of recycled iron from macrophages and its transport across the placenta. On the other hand, when iron stores are low, hepcidin production is suppressed. By modulating hepcidin expression, organism can control plasma iron level and maintain iron metabolism homeostasis [18, 22, 26, 27, 28].

4. Factors responsible for the regulation of hepcidin synthesis

Hepcidin synthesis is modulated by different stimuli, which are divided into positive and negative regulators. The molecular mechanisms of regulation of hepcidin expression are not completely understood.

Positive regulators of hepcidin production

Inflammation

Hepcidin is not only iron-regulatory hormone but also type II acute-phase reactant. It means that its synthesis can be induced by inflammatory cytokine IL-6. Some studies show that inflammation and infection rapidly decrease serum iron levels, dietary iron absorption and iron release from RE macrophages [29, 30]. IL-6 acts via its receptor and causes phosphorylation of signal transducer and activator of transcription 3 (STAT 3), dimerization of phospho-STAT3 and its translocation to nucleus, where it interacts with hepcidin promoter. What is important, STAT3 activation requires the presence of SMAD4 to affect the *HAMP* gene expression [6, 31]. This data confirms that hepcidin could be the pathogenic mediator of anemia of chronic diseases (ACD).

Increase in iron stores

Under normal conditions *HAMP* gene expression is regulated by BMP/SMAD and STAT3 pathways. Bone morphogenetic proteins (BMPs), activated by elevated circulating iron level, bind and form complex with type I and II cell serine/threonine kinase BMP receptors in hepatocytes, that results in phosphorylation of SMAD proteins receptors (R-SMADs). Phospho-R-SMADs form complex with SMAD4, which translocates into the nucleus and activates the transcription of *HAMP* gene [31, 32]. Membrane isoform of hemojuvelin (m-HJV) as a BMP co-receptor also takes part in positive regulation of *hepcidin* expression via BMP/SMAD pathway. Another hepatocyte iron sensors activating hepcidin synthesis are hemochromatosis protein (HFE) and transferrin receptor 2 (TfR2). Further studies are needed to define precisely their role in regulation of iron status [31, 32, 33].

Negative regulators of hepcidin production

Hypoxia, anemia, increased erythropoiesis

It has been confirmed that anemia and hypoxia belong to regulators of hepcidin expression. Experiments on mice have demonstrated that anemia induced by phenylhydrazine or phlebotomies triggered a considerable decrease in hepcidin mRNA [34]. Hypoxia and anemia regulate the erythrocytes production through erythropoietin (Epo) synthesis. Some authors claim that Epo is a hormone down-modulating hepcidin mRNA expression. In their opinion the effect of Epo is mediated via Epo receptor signaling and regulation of C/EBPα. Moreover, observations on thallasemia patients reported growth differentiation factor–15 (GDF-15) as a possible factor causing hepcidin downregulation [35, 36]. Obviously erythropoietic activity is a potential hepcidin synthesis suppressor, but specific erythropoiesis-associated mediator that negatively regulates hepcidin production is still unknown.

It is highly likely that in hypoxic conditions the hypoxia inducible factor/von Hippel-Lindau (HIF/vHL) pathway can inhibit hepcidin expression in hepatocytes [31, 33]. It is also suggested that hypoxia activates furin to increase amount of soluble hemojuvelin (s-HJV) by proteolytic cleavage of membrane hemojuvelin (m-HJV) [31].

DECREASE IN IRON STORES

In plasma hemojuvelin exists in soluble form (s-HJV), which in response to low serum iron level binds to BMPs and inhibits BMP/SMAD signaling [31, 33]. Very intriguing is the role of matriptase-2 (transmembrane serine protease 6, TMPRSS6) in hepcidin production. Some authors indicate that matriptase-2 is a negative regulator of hepcidin expression because it is cleaving membrane hemojuvelin [37].

5. HEPCIDIN IN THE PATHOGENESIS OF IRON DISORDERS AND OTHER DISEASES

The studies on hepcidin provide essential information about the etiology and pathomechanisms of iron metabolism disorders and other diseases.

Hemochromatosis (HH), the most common form of genetic iron overload, is divided into two groups: iron overload associated with defective or suppressed hepcidin gene and ferroportin disorders [12, 31]. The first group of diseases is caused by mutation in four genes: *HFE-1*, *HJV*, *TfR-2* and *HAMP*. Patients with defect of these genes have low hepcidin mRNA level in comparison with normal subjects [6, 10, 12, 13, 16, 31]. The mutation in *HAMP* causes rare form of juvenile hemochromatosis (JH) type 2B and leads to downregulation of hepcidin expression [10, 13, 31]. Moreover, juvenile hemochromatosis is associated with lower hepcidin level than in adult forms of hemochromatosis [31].

Hepcidin, as an acute phase protein is the key mediator of anemia observed in inflammatory disorders known as anemia of chronic diseases (ACD) [3, 7, 8, 10, 16]. Pathogenesis of ACD is associated with decreased iron absorption and impaired mobilization of iron stores [8]. The individuals with the anemia of inflammation, characterized by disturbance of iron absorption, hypoferremia and hyperferritinemia, have higher hepcidin levels than healthy subjects [9, 10, 15, 38]. The higher concentration of serum hepcidin in patients with ACD than in the healthy people can be explained by the IL-6 increase [1, 2]. Interestingly, the relationship between the IL-6 and hepcidin level was observed in patients with acute inflammatory reaction and in healthy volunteers after lipopolysaccharide injection [1, 10]. The increased level of serum hepcidin is observed in many chronic inflammatory diseases such as: chronic kidney diseases, thallassemia, glucose-6-phosphate dehydrogenase deficiency, sickle cell disease (SCD), coronary artery disease (CAD) and myelodysplasia [7, 9, 10, 14]. The recent studies have shown elevated hepcidin level during radiotherapy for prostate cancer in individuals with acute proctitis and after hematopoietic stem cell transplantation (SCT) [1, 8].

The overexpression of hepcidin has been shown in clinical studies on dysmetabolic iron overload syndrome (DIOS) [11, 12]. In patients with DIOS the iron absorption is significantly decreased than in controls with normal iron status [12]. In other liver diseases like obesity related to non-hereditary mild iron overloading hepatic disease (NHIOD), alcoholic liver disorders and hepatitis C virus infection (HCV) enterocytes are resistant to circulating hepcidin, while macrophages are more sensitive [11]. Serum hepcidin correlates positively with hepatic hepcidin mRNA level and ferritin level in chronic hepatitis C (CHC) [15]. Hepcidin may be a prognostic and monitoring test of iron overload in patients with NHIOD and HCV. Normalization of hepcidin concentration may be also an indicator of HCV eradication [11].

6. HEPCIDIN AS A POTENTIAL DIAGNOSTIC AND THERAPEUTIC TOOL

The discovery of hepcidin in 2000 by Krause et al. [21] and Park et al. [20] not only opened the way to understand the iron metabolism but also helped to elucidate the pathomechanisms of many diseases. The studies on hepcidin raise the question of the use of hepcidin as a diagnostic and therapeutic tool in many diseases. Hepcidin measurement can be helpful test distinguishing anemia of chronic diseases (ACD) from iron deficiency anemia (IDA), as it is known that hepcidin production is induced by inflammation (ACD) and reduced in iron deficiency states (IDA) [29, 39]. One of the greatest promises for the practical application of hepcidin assay is the utilization of hepcidin in diagnosis and monitoring of hemochromatosis. What is more, the possible therapeutic value of hepcidin is investigated. The development of synthetic hepcidin should be useful in the treatment of hemochromatosis and other iron-loading conditions [40].

In 2008 Ganz et al. [41] performed successful validation of a competitive enzyme-linked immunoassay (C-ELISA) for human hepcidin. This simple and robust assay can be used in detecting physiologic and pathologic changes in serum or urine hepcidin levels. It is probable that this test will be widely available for use in clinical chemistry laboratories in the near future.

However, a lot remains to be uncovered on the biology and function of hepcidin. Its signaling pathways are as yet to be delineated. Further studies are needed to define precisely the hepcidin role in iron metabolism homeostasis and its utility in the diagnosis and treatment of iron disorders.

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