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Review

Molecular pathogenesis of hereditary hemochromatosis

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Summary. Hereditary hemochromatosis (HH) is an inherited iron overload disorder characterized by normal iron-driven erythropoiesis and abnormal iron metabolism, leading to excess iron deposited in parenchymal cells of liver, heart, and endocrine glands. Iron hormone, hepcidin, plays a critical role in iron homeostasis through interaction with ferroportin (FPN), a major cellular iron exporter. Hepcidin is encoded by hepcidin antimicrobial peptide (HAMP). Mutations in hepcidin and any genes that regulate the biology of hepcidin, including hemochromatosis genes (HFE), Hemojuvelin (HJV), transferring receptor 2 (TFR2) and FPN, result in hemochromatosis. The identification of hepcidin and its role will provide a better understanding for pathogenesis of HH.

Key words: Hemochromatosis, Hepcidin, HFE, HJV FPN.

Introduction

Hereditary hemochromatosis (HH) is an inherited iron overload disorder characterized by normal iron-driven erythropoiesis and abnormal iron metabolism, leading to excess iron deposition in various target organs and resulting in a spectrum of characteristic features, including hepatic cirrhosis, diabetes, cardiomyopathy, hypogonadism, arthropathy, and increased skin pigmentation (Pietrangelo, 2010a; Koperdanova and Cullis, 2015). HH is one of most common genetic diseases among Caucasians. It can be caused by any mutations that encode proteins that limit the entry of iron into the blood. These mutations include hemochromatosis gene (HFE) (Feder et al., 1996), transferring receptor 2 (TFR2) (Feder et al., 1996; Kawabata et al., 1999), Hemojuvelin (HJV) (McKie et al., 2000), Hepcidin antimicrobial peptide (HAMP) (Roetto et al., 2003; Lange et al., 2014), and ferroportin (FPN) gene (McKie et al., 2000; Montosi et al., 2001). The studies have revealed that hepcidin, a 25 amino-acid peptide, plays a critical role in the pathogenesis of hemochromatosis through interaction with FPN (encoded by SLC40A1), a major cellular iron exporter in mammals (Nemeth et al., 2006; De Domenico et al., 2007). These discoveries shed a new light on the pathogenesis of HH. This review focuses on the current understanding of the pathogenesis of HH.

Genetic classification of hereditary hemochromatosis

Based on biochemical and genetic characteristics, HH has been classified into the following four types.

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Abbreviations. BMP, Bone morphogenetic protein; FPN, Ferroportin; HAMP, Hepcidin antimicrobial peptide or Hepcidin gene; HFE, Hemochromatosis gene, or High Iron (Fe); HH, Hereditary hemochromatosis; HJV, Hemojuvelin; Matriptase 2/TMPRSS6, The serine protease 2/transmembrane serine protease 6; sHJV, Soluble form of HJV; SMAD, Sma and mothers against decapentaplegic homolog; TFR1, Transferring receptor 1; TFR2, Transferring receptor 2

HFE-related hemochromatosis (Type 1) is the most common and the first described form of the inherited disorder of iron overload. It is an autosomal recessive disorder. The most frequent mutations in HFE gene occur at C282Y and H63D loci (Fan et al., 2014; Francis and Thachil, 2015). C282Y homozygotes are observed in approximately 85-90% of patients with HH, whereas only 3-5% of HH patients have C282Y/H63D (Pietrangelo, 2010a; Li et al., 2014a).

Juvenile hemochromatosis (Type 2) is much rarer than HFE-related hemochromatosis. It is divided into two subtypes according to mutations in two different genes (hemojuvelin, Type 2A and hepcidin, Type 2B), although the clinical presentation is indistinguishable. Type 2A hemochromatosis is caused by a mutation in the haemojuvelin (HJV) gene encoding protein haemojuvelin on chromosome 1 (1q21) (Papanikolaou et al., 2004; Pelusi et al., 2014). Type 2B hemochromatosis occurs due to a mutation in the hepcidin gene (HAMP) itself on chromosome 19 (19q31) (Roetto et al., 2003).

Transferrin receptor 2(TFR2) hemochromatosis (Type 3) is an autosomal recessive disorder caused by mutations of the TFR 2 gene located on the long arm of chromosome 7 (7q22) (Camaschella et al., 2000; Benyamin et al., 2014). Type 3 HH can be considered as an ''intermediate'' syndrome between juvenile and HFE related hemochromatosis.

Type 4 hemochromatosis (also referred to as Ferroportin Disease) is an autosomal dominant disease caused by the mutation of the Ferroportin gene (SLC40A1) located on chromosome 2 (Montosi et al., 2001; Callebaut et al., 2014; Sharma et al., 2014), which is the second most common inherited iron overload syndrome following HFE-related hemochromatosis (Abboud and Haile, 2000; Muehlenberg et al., 2014).

Mutation of several genes which lead to hemochromatosis and genic polymorphism indicate that a better understanding of characteristics of HH is needed.

Molecular pathogenesis

In 1935, Joseph Sheldon (Sheldon, 1935) first described the inherited nature of hemochromatosis and the association with abnormal iron metabolism. Over the next few decades, studies of iron traffic provided more information about iron regulation. The discovery of hepecidin during the last decade has triggered a virtual explosion of studies on iron metabolism, which sheds new light on the pathogenesis of hemochromatosis (Pietrangelo, 2010b; Gutschow et al., 2015).

Hepcidin: a key regulator of iron homeostasis

Hepcidin was first isolated in 2000 from human blood ultrafiltrate (Krause et al., 2000; Park et al., 2001; Pigeon et al., 2001). Active mature hepcidin is a 25 amino-acid peptide with four disulfide bonds. Its C-terminus has weak antibacterial and antifungal activity. The protein itself is classified, along with the thionins and the defensins, as a member of the cationic cysteine rich antimicrobial peptide family. Hepcidin is mainly synthesized in the liver by hepatocytes, but by other cells such as macrophages and adipocytes as well at a much lower level (Bekri et al., 2006; Peyssonnaux et al., 2006; Praschberger et al., 2014). Due to its small size (2.7 kD), hepcidin is cleared by kidney through entering the glomerular membrane, and then it is taken up and degraded in the proximal tubule (Ganz and Nemeth, 2012; Mascitelli and Goldstein, 2014).

The first evidence that hepcidin plays a critical role in iron homeostasis was observed by Pigeon et al. (2001). Meanwhile, HAMP knock-out mouse suffered from severe iron overloading was confirmed by genetically modified (Nicolas et al., 2001), whereas serious anemia was caused by over expression of hepcidin (Viatte et al., 2006; Roy et al., 2007). By now, it is clear that the central control site of iron traffic is the liver, and its effector is hepcidin. Of particular significance, except for FPN disease, all forms of HH are characterized by a low level of hepcidin (Preza et al., 2011; Ramos et al., 2012). These results indicate that iron hormone hepcidin is the central regulator of systemic iron homeostasis.

Physiologic regulation of hepcidin

Similar to other hormones, hepcidin expression by the liver is feedback-regulated by iron, whose concentration is controlled by hepcidin. In humans, administration of iron in healthy volunteers increased hepcidin levels, which were observed within 24 hours (Nemeth et al., 2004). Rather, iron depletion caused decreased level of hepcidin (Theurl et al., 2009). Iron overload is a potent stimulus for increasing hepcidin synthesis in mice. Thus, expression of hepcidin is regulated clearly by iron levels (Hentze et al., 2010).

Iron is essential for nearly all microbes. Excess iron in the bloodstream and/or tissues can promote microbial proliferation, viability and pathogenicity. Hepcidin secretion is a part of the innate immune response, so its production and blood concentrations will increase during infections and systemic inflammatory diseases. Molecular pathogenesis involved in inflammatory cytokines, especially interleukin-6 (IL-6) (Ganz, 2006; Siddique and Kowdley, 2012), which activates hepcidin transcription by binding to its receptor (activating JAK2 signaling and STAT3 phosphorylation) (Xia et al., 2008; Corradini et al., 2011a). An integrity of the bone morphogenetic proteins (BMP)-SMAD pathway is required in order to fully activate hepcidin. (Vecchi et al., 2009). Hepcidin transcription also appears to be activated by proinflammatory cytokines and lipopolysaccharide (LPS) by endoplasmic reticulum stress through C-AMP responsive element binding protein H (CREBH) (Vecchi et al., 2009).

Erythropoiesis can inhibit hepcidin synthesis (Ganz,

2011), but the mechanism of this regulatory process is unclear. One possibility is the decreasing levels of plasma iron during active erythropoiesis because erythropoietic precursors are the main consumers of plasma iron. Anemia induces circulating regulatory factors released by the bone marrow, which act on the liver to regulate the synthesis of hepcidin. These circulating regulatory factors are being investigated. Several candidates have recently been described, for example, the growth differentiation factor 15 (GDF-15) (Tanno et al., 2007, 2009).

Anemia leads to tissue hypoxia, which also inhibits hepcidin synthesis. Several putative mediators such as hypoxic inducible factor (Peyssonnaux et al., 2007) and erythropoietin (Pinto et al., 2008) have been proposed.

In a word, hepcidin senses a variety of physiologic stimuli to maintain plasma iron levels within a physiologic range.

Hepcidin–ferroportin axis

The iron found in the plasma compartment comes from dietary iron and reticuloendothelial macrophages. The dietary iron is uptaken by enterocytes, either stored intracellularly as ferritin or oxidized to Fe3+ by the multicopper oxidase hephaestin expressed on the basolateral surface of the intestine then released into plasma by the iron transport protein FPN (Abboud and Haile, 2000). Macrophages degrade senescent or damaged red blood cells and their hemoglobin iron is returned to plasma, transferred by FPN, and they produce new red cells in the bone marrow or for other purposes. FPN is a multi-domain transmembrane protein encoded by the SLC40A1 genes (Abboud and Haile, 2000), which are expressed in reticuloendothelial macrophages, hepatocytes and placental syncytiotrophoblasts. FPN is the main cellular iron exporter regulating the iron metabolism in duodenal enterocytes. The binding of hepcidin with FPN is the main axis for the regulation of iron metabolism. The molecular mechanisms have been verified *in vitro* and *in vivo* studies. After the binding of the two factors, a conformational change occurred, and then both FPN and hepcidin internalized, dephosphorylated, ubiquitinated, and ultimately degraded in the late endosome/lysosome compartment (Nemeth et al., 2006; De Domenico et al. 2007). Those processes resulted in reduced iron export from duodenal enterocytes and macrophages.

Type 2B hemochromatosis arises from alterations in hepcidin gene HAMP while other types of hemochromatosis (except for type 4 HH) are caused by mutations in genes, including HFE, TFR 2, and HJV, which regulate hepcidin synthesis. These changes of genes result in hepcidin insufficiency that hamper the interaction of hepcidin with FPN and lead to abnormally high iron levels. Type 4 haemochromatosis arises from rare mutations in FPN gene leading to a loss of FPN or hepcidin resistance by interfering with hepcidin binding or hepcidin-mediated internalization (Fernandes et al., 2009; Sham et al., 2009; Pietrangelo, 2010c).

Molecular regulation of hepcidin of HH related genes

The molecular mechanisms of type 2 HH caused by mutation in HAMP and HJV is fully convinced that characterized by low expression of hepcidin and thus resulted in iron overloading. Otherwise HH suffer from mutation in HFE and TFR2 was not clarified up to now.

HJV

Patients with HJV mutations as well as HJV-/- mice show lowered hepcidin levels (Niederkofler et al., 2005; Li et al., 2014b) and a massive iron deposition in the liver and other tissues, suggesting that HJV is involved in the regulation process of hepcidin expression, in which BMP/SMAD signaling pathway plays the central role (Babitt and Lin, 2011). Members of the transforming growth factor beta (TGF- beta) superfamily (Corradini et al., 2009a), Bone morphogenetic proteins (BMPs) combine with type I and type II cell serine/threonine kinase receptors (BMPRs) leads to SMAD1/5/8 proteins phosphorylation. Phosphorylated SMAD1/5/8 binds to the common mediator SMAD4 to form a complex. The activated complex translocates into the nucleus and induces the expression of target genes, including HAMP (Fig. 1) (Mleczko-Sanecka et al., 2010). It has now emerged that HJV acting as a BMP coreceptor plays a significant role in controlling the timing, location, and specific downstream effects of BMP signaling (Papanikolaou et al., 2004; Pelusi et al., 2014). A protein of 426 amino acids encoded by HJV contains a C-terminal glycosylphosphatidylinositol anchor, so it can function in either a soluble or cell-associated form (Pietrangelo, 2011). A soluble form of HJV (sHJV) inhibits the activation of the BMP–receptor complex (whose activation results in the expression of hepcidin) through competing with cell-associated counterpart for BMP binding. sHJV release was progressively inhibited by increasing iron concentrations, which might be a mechanism of iron regulation of hepcidin expression *in vivo* (Fig. 1) (Pietrangelo, 2011).

Expression of hepcidin can be stimulated by various BMPs, especially BMP6. Andriopoulos et al. have shown that BMP6-/- mice exhibit a phenotype resembling hemochromatosis, with a low level of hepcidin and tissue iron overload. They demonstrated that BMP6 acts by interacting with sHJV to increase hepcidin expression in mice (Andriopoulos et al., 2009; Meynard et al., 2009). These data indicate that BMP6 (as a ligand of HJV) is an endogenous regulator of hepcidin expression and iron metabolism *in vivo*.

Mleczko-Sanecka et al. (2010) have shown that SMAD7 is coregulated with hepcidin by BMPs in primary hepatocytes from mice. Its overexpression completely abolishes BMP-mediated activation of hepcidin. SMAD7 was known as inhibitory SMADs,

which was able to down regulate hepcidin synthesis (Mleczko-Sanecka et al., 2010) (Fig. 1).

Neogenin, a member of the deleted in colorectal cancer (DCC) family of tumor suppressor molecules, appears to be able to interact with HJV to regulate expression of hepcidin (Kuns-Hashimoto et al., 2008; Yang et al., 2008) (Fig. 1). Nevertheless, its role in modulation of hepcidin expression and iron traffic is still controversial (Lee et al., 2010). Conflicting reports exist regarding the function of Neogenin in modulation of HJV activity and BMP/SMAD signaling pathways. The livers of Neogenin-mutant mice have reduced BMP signaling (Lee et al., 2010), however, inhibition of HJV shedding by Neogenin would increase BMP signaling.

Recent studies have suggested that the serine protease 2/transmembrane serine protease 6 (matriptase 2/TMPRSS6) (Maxson et al., 2010) appears to act by cleaving HJV, which facilitates release of soluble HJV to down-regulate hepcidin expression (Maxson et al., 2010) (Fig. 1). Guo et al. (2013) showed that reduction of TMPRSS6 expression using antisense technology dramatically decreased serum iron in HFE-/- mice (Guo et al., 2013). In humans, TMPRSS6 mutations fail to turn off hepcidin signaling, leading to iron-refractory iron deficiency anemia (Finberg et al., 2008; Bardou-Jacquet et al., 2013).

In summary, SMAD7 and TMPRSS6/matriptase 2 down-regulate hepcidin expression; BMPs, especially BMP6 up-regulate hepcidin expression, while the function of Neogenin is still controversial.

HFE and TFR2

The hepcidin regulatory signaling pathways might be regulated by HFE, which can interact with TFR1. TFR1 is a type II transmembrane glycoprotein that mediates uptake of transferrin-bound iron. Expression of TFR1 is low in the liver and is affected by intracellular levels of iron. HFE binds to beta 2-microglobulin to enable it to be transported to the cell surface and

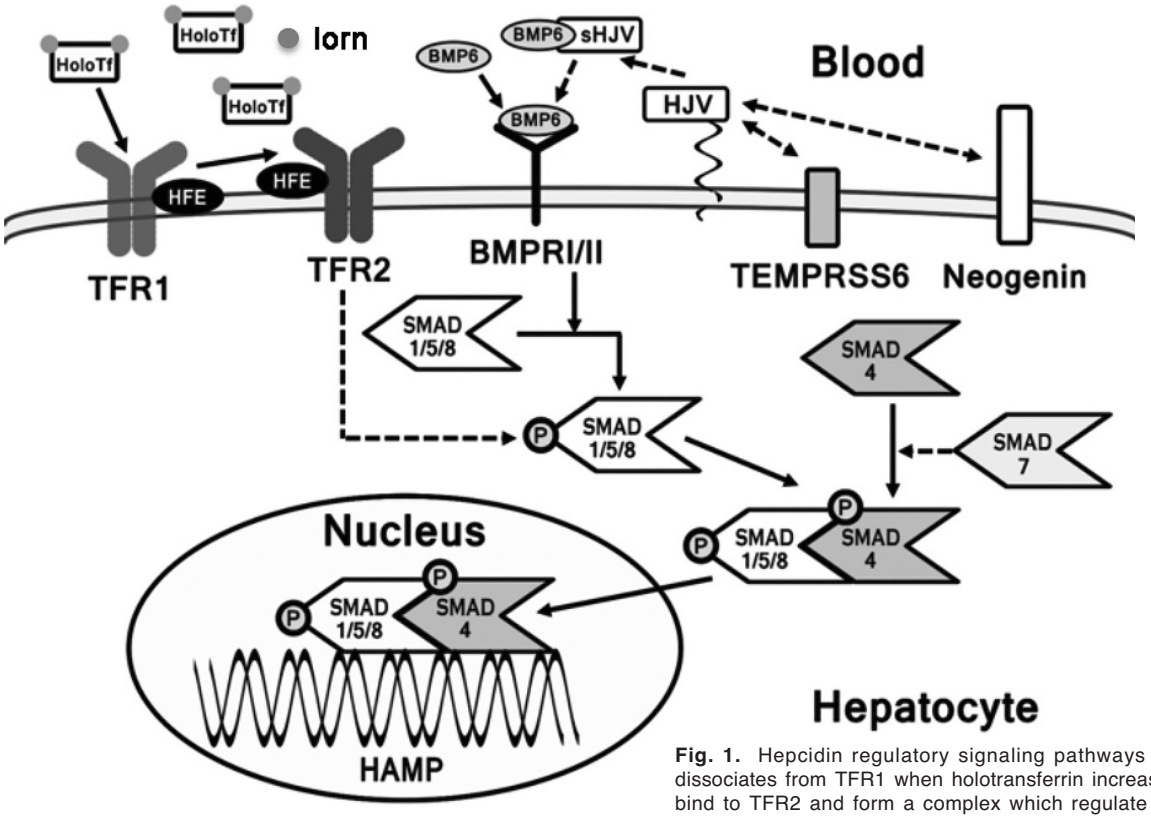

Fig. 1. Hepcidin regulatory signaling pathways in hepatocyte. HFE dissociates from TFR1 when holotransferrin increases, this makes HFE bind to TFR2 and form a complex which regulate hepcidin expression though p38MAPK/ERK1/2 and BMP-SMAD signaling pathways. BMP6 combine with type I and type II cell serine/threonine kinase receptors (BMPRs) leads to SMAD1/5/8 proteins phosphorylation. Phosphorylated SMAD1/5/8 bind to SMAD4 forming a complex whose activated form translocate into the nucleus and induce the expression of target genes, such as HAMP. A soluble form of HJV (sHJV) down regulate hepcidin expression through inhibiting the activation of the BMP–receptor complex. hepcidin expression increases by interaction between BMP6 and sHJV. The expression of hepcidin was likely to accommodate by interreaction of Neogenin and HJV by cleaving HJV.TMPRSS6 appears to facilitate the release of soluble HJV to down-regulate hepcidin expression. SMAD7 was known as inhibitory SMADs, are able to negatively regulate hepcidin synthesis. [Notes: HFE: Hemochromatosis gene, or High Iron (Fe); TFR: Transferring receptor; Holo Tf: holotransferrin; HJV: Hemojuvelin; HAMP: Hepcidin gene; BMP: Bone morphogenetic protein; SMAD: Sma and mothers against decapentaplegic homolog; sHJV: Soluble form of HJV: matriptase 2/TMPRSS6: The serine protease 2/transmembrane serine protease]

endosomal membranes, where it interacts with TFR1. The C282Y mutation in HFE disrupts a disulfide bond that is necessary for HFE binding to beta2-microglobulin. TFR2 is a liver-specific homologue of TFR1 and its expression is not affected by intracellular levels of iron. It is still not well demonstrated that whether TFR2 participate in upregulation of hepcidin all alone or in form of complex with HFE (Schmidt et al., 2008). Because the binding site for HFE on TFR1 overlaps with the binding site for transferrin, holotransferrin competes with HFE for binding to TFR1. One model proposes that HFE dissociates from TFR1 (Schmidt et al., 2008) when holotransferrin increases, and this result makes HFE avaliable to bind to TFR2 and form a complex (Gao et al., 2009; Ali-Rahmani et al., 2014) (Fig. 1). The complex might signal the high iron content of the blood to an iron sensor and upregulate hepcidin expression in response to the increased circulating holotransferrin, but the precise molecular mechanism is still unclear.

Studies have been shown that HFE-/- mice, TFR2-/- mice and combined HFE-/- and TFR2-/- double knockout mice have lower phospho-ERK1/2 levels (Wallace et al., 2009; Stickel et al., 2014), and exhibit impaired BMP-SMAD signaling (Poli et al., 2010; Corradini et al., 2011b), suggesting that the p38MAPK/ERK1/2 and BMP-SMAD signaling pathways may also be involved in HFE and TFR2-mediated regulation of hepcidin expression (Fig. 1).

Studies offer attractive clues linking HFE loss to blunted BMP6 signaling. Elena Corradini et al. (2010) showed that levels of hepatic phosphorylated SMAD 1/5/8 protein (an intracellular mediator of BMP6 signaling) in HFE knockout mice, were inappropriately low relative to high BMP6 mRNA levels in response to the body iron burden compared with wild-type (WT) mice, BMP6 induction of hepcidin expression is impaired in HFE knockout mice (Corradini et al., 2009b). Transgenic hepatic HFE overexpression in the mouse led to increased BMP6 signaling, hepcidin excess and iron deficiency anemia (Corradini et al., 2010; Schmidt et al., 2010). Together, these data suggest that HFE might be essential for an optimal response to BMP6.

Mice with combined HFE-/- and TFR2-/- display a more severe iron overload phenotype compared with either single HFE-/- or TFR2-/- mice (Wallace et al., 2009; Corradini et al., 2011b), i.e., HFE and TFR2 are not only independent but also complementary in regulating hepcidin expression and iron homeostasis.

The identification of hepcidin and its pathway above mentioned not only elucidate the pathogenesis of HH, but also provide novel diagnostic and therapeutic approaches based on conventional scenarios.

Conclusions

Hemochromatosis is a common hereditary disorder caused by reduced hepatic hepcidin secretion, which is characterized by abnormal iron homeostasis. The molecular pathogenesis of mutations in HJV and HAMP resulting in decreased hepcidin expression are now clear, but the detailed pathogensis of HFE and TFR2 in regulation of iron homeostasis is still incompletely understood. Understanding of the exact role of HFE, TFR2 and modifier genes such as Neogenin, TMPRSS6, and SMAD/7, may shed new light on the pathogenesis of hemochromatosis.

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