Hepcidin and iron disorders: new biology and clinical applications

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Disclosures

Co-founder and consultant for:

- Intrinsic LifeSciences (hepcidin diagnostics)
- Merganser Biotech (hepcidin agonists)
- Silarus Therapeutics (erythroferrone-targeted therapeutics)
Iron homeostasis

Total body iron: ~4 grams

Liver
(1000 mg)

(Storage)

Spleen (recycling)

Bone marrow

RBC
2400 mg

Duodenum
(absorption)

Iron loss (1-2 mg/d)
(not regulated)

Plasma
Fe-Tf
3-4 mg

0-5 mg/d
1-2 mg/d
20 mg/d
20 mg/d

• Chronically increased iron leads to iron deposition in tissues, organ damage
• Chronically decreased iron causes cellular dysfunction, anemia
How is iron homeostasis regulated and how do iron disorders develop?
Hepcidin—the iron-regulatory peptide hormone

- Made in the liver as 84 aa preprohepcidin
- Cleaved to 25 aa bioactive hepcidin by furin
- Short half-life in circulation, cleared by the kidneys

Jordan et al. JBC 2009
Ferroportin – the iron exporter

- The only cellular iron exporter known, supplies iron to plasma
- The receptor for hepcidin
Hepcidin causes ferroportin endocytosis and degradation

- Hepcidin binds to ferroportin
- Ferroportin is ubiquitinated, endocytosed and targeted to lysosomes for degradation

control  + hepcidin

HEK293 cells expressing Fpn-GFP

Nemeth et al. Science 2004
Regulation of intestinal iron absorption

Dietary iron uptake

Food

Dietary iron uptake

Low hepcidin

High hepcidin

Fpn

Fe

Dietary iron uptake

Duodenal enterocytes

Ferritin

Fpn

Hepcidin
Regulation of erythrocyte iron recycling

Erythrocyte uptake

Iron release into plasma

Low hepcidin

High hepcidin

Erythrocyte uptake

Macrophages

Fpn

Fe

Ferritin

Hepcidin
How is hepcidin regulated?

- **Liver**
- **Spleen**
- **Bone marrow**
- **Duodenum**
- **Plasma Fe-Tf**

**Arrows and Signals:**
- Inflammation
- Iron signal
- Erythropoietic signal
Hepcidin regulation by iron
Hepcidin response to oral iron

Hepcidin is appropriately low in iron deficiency

NL = healthy subjects
ID = iron deficiency defined as ferritin <10 ng/ml
Dotted line = lower limit of detectability

Ganz et al, Blood 2008
Current model of hepcidin regulation by iron

Intracellular iron

BMP6

Fe-Tf

HFE

TfR2

TfR1

BMPR

HJV

Fe-Tf

BMP6

Smad4

R-Smad

P

R-Smad

hepc

TFs
When hepcidin regulation by iron goes wrong...
Mutations causing hereditary hemochromatosis

- BMP6
- HJV
- Fe-Tf
- Tfr2
- HFE
- Tfr1

Intracellular iron

Tmprss6

hepatocyte

TFs

Smad4

R-Smad

FS

R-Smad P

hpec

hepc

Fe-TF

BMPR

R-Smad P

R-Smad

BMPR

HJV

Fe-Tf
Molecular basis of hereditary hemochromatosis

- **Hepcidin deficiency**
  - mutations in hepcidin or its regulators (HFE, TfR2 and HJV)

- **Resistance to hepcidin** (rare but instructive)
  - mutations in the hepcidin receptor ferroportin
Hereditary hemochromatosis

Hepcidin deficiency, absolute or relative

Plasma Fe-Tf

NTBI

RBC

Bone marrow

Duodenum

Liver

Spleen
Hepcidin regulation by erythropoiesis
Hepcidin is suppressed by erythropoietic activity

- Stimulated erythropoiesis in mice by phlebotomy (0.5 ml) or EPO injection (200 U)

Hepcidin suppression depends on functional marrow (Pak 2006)
Erythroferronone (ERFE): an erythroid hepcidin suppressor

- 50 kD glycoprotein (C1q-TNFα family), highly expressed in Epo-stimulated erythroblasts
- Hepatocytes treated with recombinant Erfe

Predicted structure of Erfe

Hepcidin mRNA at 15 h

EC50 = 7ng/ml (~100pM)
Erfe-/- mice do not suppress hepcidin in response to bleeding or EPO

Phlebotomy (0.5 ml)

ERFE deficiency
Lack of hepcidin suppression
Delayed recovery from anemia

Kautz et al., Nat Genet 2014
The role of ERFE in iron regulation

- **ANEMIA HYPOXIA**
- **KIDNEYS**
- **DUODENUM**
- **LIVER**
- **BONE MARROW**

**ERFE**

**ERYTHROBLASTS** (Jak2/Stat5)

**IRON**

**HEPCIDIN**

**EPO**
When hepcidin regulation by erythropoiesis goes wrong...
Anemias with ineffective erythropoiesis (iron-loading anemias)

- β-thalassemia, congenital diserythropoietic anemia, X-linked sideroblastic anemia
- Ineffective erythropoiesis $\rightarrow$ erythroid expansion $\rightarrow$ hepcidin suppression
- Iron overload even without transfusions
- Iron overload is the major cause of morbidity and mortality
Increased ERFΕ expression in a mouse model of β-thalassemia

- th3/+ mice (anemic and have high Epo)

### Erfe mRNA

<table>
<thead>
<tr>
<th></th>
<th>Bone marrow</th>
<th>Spleen</th>
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<tbody>
<tr>
<td>WT</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>th3/+</td>
<td>16 (***</td>
<td>16 (***</td>
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### Hepcidin mRNA

<table>
<thead>
<tr>
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<th>Serum hepcidin (ng/mL)</th>
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<tbody>
<tr>
<td>WT</td>
<td>100</td>
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<tr>
<td>th3/+</td>
<td>50 (***</td>
</tr>
<tr>
<td>thfe-/-</td>
<td>200 (***</td>
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*Significance levels: *** p < 0.001*
Iron-loading anemias

Liver
- Hepcidin deficiency

Duodenum
- Erythroid factor (Erythroferrone, ?)

Plasma Fe-Tf
- Hepcidin

Spleen
- RBC

Bone marrow
Hepcidin regulation by inflammation
Hepcidin regulation by inflammation

- Hepcidin expression is potently and rapidly induced by multiple cytokines
Hepcidin role in innate immunity?

- Hypothesis: hepcidin limits iron availability to pathogens

[Diagram showing liver, hepcidin, iron, and infection signal (PAMPs)]
Hepcidin and siderophilic infections

- Siderophilic ("iron-loving") bacteria, e.g. *Vibrio vulnificus*
- Pathogenicity increased by iron
- Hereditary hemochromatosis patients are highly susceptible to siderophilic infection
- *Vibrio* infection leads to fulminant sepsis (>50% mortality) and necrotizing skin infection

Is hepcidin a mediator of the host defense against *V. vulnificus*?
Hepcidin mediates host defense against *Vibrio vulnificus*

**Survival: wild-type vs hepcidin KO mice**

Induction of hepcidin by infection $\rightarrow$ acute decrease in plasma iron $\rightarrow$ protective against infection with *V. vulnificus*

*Arezes et al. Cell Host Microbe 2015*
The downside of the inflammatory regulation of hepcidin
Hepcidin excess in human diseases

• Anemia of inflammation
  – Rheumatoid arthritis
  – Inflammatory bowel disease
  – Infections
  – Critical care, burns
  – Juvenile rheumatoid arthritis
  – Obesity
  – Anemia of aging

• Anemia of cancer
  – Multiple myeloma
  – Ovarian cancer
  – Renal cell carcinoma
  – Hodgkin's lymphoma
  – Hepatic adenomas

Hepcidin excess due to decreased clearance

- Chronic kidney disease, uninflamed patients
- Inverse correlation between GFR and serum hepcidin

Zaritsky et al, CJASN 2009
Genetic disease of hepcidin excess: Iron-refractory iron deficiency anemia (IRIDA)

- Iron deficiency despite adequate dietary iron content, only partially corrected by parenteral iron
- Mutations in the membrane protease TMPRSS6 (hepcidin suppressor)
Iron-restricted anemias

Inflammation (IL-6) → Liver

Liver → TMPRSS6 mutations

Liver → Plasma Fe-Tf

Plasma Fe-Tf → Spleen

Spleen → RBC

Kidneys → Duodenum

Duodenum → Plasma Fe-Tf

Plasma Fe-Tf → Bone marrow

Bone marrow

Liver → TMPRSS6 mutations

Liver → Plasma Fe-Tf

Plasma Fe-Tf → Spleen

Spleen → RBC

Kidneys → Duodenum

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Bone marrow

Kidneys

Duodenum

Liver

Spleen

RBC

Bone marrow

Iron-restricted anemias
Hepcidin clinical applications
Hepcidin diagnostic potential

• Anemias:
  – Diagnosis of IRIDA
  – Differential diagnosis of anemias (AI vs IDA)
  – Prediction of efficacy of oral iron therapy
  – Monitoring of anemia therapy (for AI, CKD, IDA)

• Iron overload:
  – Screening assay for hereditary hemochromatosis
  – Stratification of hereditary hemochromatosis
  – Monitoring of treatment in iron overload states

• Inflammation: clinical monitoring of inflammatory states

• Predict risk for acute renal failure after surgery

• Companion diagnostic for pharmacological studies
Current state of hepcidin assays

• Research use only

• Types:
  – Immunoassays (competitive ELISA, sandwich ELISA, RIA)
  – Mass spectrometry assays (LC-MS/MS, MALDI-TOF MS, Q-TOF LC/MS, SELDI-TOF MS)

• Harmonization needed (absolute levels vary widely between assays)
Hepcidin-targeted therapeutics
## Hepcidin-targeted therapeutics

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<tr>
<th>Therapeutic approach</th>
<th>Mode of action</th>
<th>Agents</th>
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<tr>
<td><strong>Hepcidin agonists</strong></td>
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<td></td>
<td>Hepcidin mimics</td>
<td>Minihepcidins [47]</td>
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<td></td>
<td>Stimulators of hepcidin production</td>
<td>Gene silencing of TMPRSS6 [50,51]</td>
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<td>BMP pathway agonists [52]</td>
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<tr>
<td><strong>Hepcidin antagonists</strong></td>
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<tr>
<td></td>
<td>Suppressors of hepcidin production</td>
<td>BMP pathway inhibitors [54,56,74]</td>
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<td>Anti-inflammatory agents [60–62]</td>
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<td>Erythropoiesis-stimulating agents [65]</td>
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</table>
|                          |                                                      | Gene silencing of hepcidin and its regulators [66]  
|                          | Hepcidin peptide neutralizing binders               | Anti-hepcidin antibodies [67]  
|                          |                                                      | Anticalins [68]                                                        |
|                          |                                                      | Spiegelmers [69]                                                       |
|                          | Agents interfering with hepcidin-ferroportin        | Anti-ferroportin antibodies [71]                                       |
|                          | interaction                                         | Thiol modifiers [72]                                                   |
Clinical trials!

- **Anti-hepcidin antibodies (Lilly)**
  - Phase 1, anemia of cancer, just completed

- **Ferroportin Antibody (Lilly)**
  - Phase 1, ESRD on hemodialysis

- **Soluble HJV-Fc (Ferrumax)**
  - Phase 2, ESRD on hemodialysis (terminated due to low number of patients); Phase 0 in CKD, exploratory

- **Spiegelmers (Noxxon), RNA-based hepcidin binder**
  - Anemia of cancer (Phase 2, completed); ESA-hypo responsive anemia in dialysis patients (Phase 1/2)
Summary

• Hepcidin-ferroportin interaction controls systemic iron homeostasis
• Hepcidin dysregulation causes common iron disorders
  – Hepcidin deficiency causes iron overload
  – Hepcidin excess contributes to iron-restricted anemias
• Hepcidin assays will help the diagnosis and clinical monitoring of iron disorders
• The hepcidin-ferroportin pathway can be targeted for drug leads, a number of potential therapeutics under development