Tomas Ganz: Disclosure of Interests

- Intrinsic LifeSciences (founder, stockholder, officer)
- Merganser Biotech (consultant, stockholder)
- Keryx (consultant)
- Xenon Pharma (stockholder, former consultant)
Outline

• Iron homeostasis: basic science and lessons from genetic disorders
• Iron overload disorders
• Iron deficiency anemia and anemia of inflammation
• Application to CKD
Iron

- A trace element essential for oxygen transport, oxygen storage and catalysis of redox reactions (energy production, nucleoside synthesis, intermediary metabolism…)
- Bioavailable iron is scarce in most environments
- Living organisms conserve and recycle iron
- Disorders of iron homeostasis are common
  - Hereditary hemochromatosis
  - Iron-loading anemias (e.g. β-thalassemia)
  - Iron deficiency anemia
  - Anemia of inflammation
  - Anemia of chronic kidney disease
Human iron economy

- Plasma iron maintained normally at 10-30 µM
- Chronically >30 µM leads to iron deposition in tissues, injury, organ damage
- Chronically <10 µM causes cellular dysfunction, anemia
- In humans, iron flow to hemoglobin synthesis is highly dependent on recycling by macrophages
Iron homeostasis

- **Extracellular:** The organism regulates its dietary iron absorption, concentration of iron in extracellular fluid and iron storage
- **Intracellular:** Each cell regulates its iron uptake and subcellular distribution
Organismal iron homeostasis

- Response to hemorrhage, iron deficiency:
  - dietary absorption of iron increased
  - stored iron released from macrophages and hepatocytes

- Response to iron overload:
  - absorption of dietary iron decreased

- Response to infection or inflammation:
  - iron release from macrophages and hepatocytes decreased
  - dietary iron absorption decreased
  - hypoferremia
Key organs in iron homeostasis

- Liver
- Spleen
- Duodenum
- Bone marrow

1 ml of PRBC = 1 mg Fe
Ferritin

- Cytoplasmic Fe storage protein, 24 L/H subunits
- Serum form is 24 L>>H subunits, Fe poor
- In subjects without inflammation or maldistribution of Fe, serum ferritin reflects systemic Fe stores
- Inflammation causes increased serum ferritin
  - Very high serum ferritin in macrophage activation syndromes
- Fe in macrophages causes more ferritin secretion than Fe in hepatocytes
  - Ferritin very high in ferroportin disease, caused by mutations in ferroportin, where Fe accumulates in macrophages (vs hereditary hemochromatosis)
Transferrin

- Abundant plasma protein, 2x Fe$^{3+}$ carrier
- Normally ~1/3 saturated with iron
- Essential for delivery of iron to hemoglobin synthesis
- Transferrin receptor 1 mediates cellular iron uptake
- Transferrin receptor 2 involved in hepcidin regulation
Hepcidin

- Iron-regulating peptide hormone
- Made in the liver
- Binds to the cellular iron exporter ferroportin
- Induces the endocytosis and proteolysis of ferroportin
Regulation of intestinal iron absorption

Low hepcidin

High hepcidin

Dietary iron uptake

Food

Dietary iron uptake

Duodenal enterocytes

Ferritin

Fpn

Fe

Iron released to transferrin

Hepcidin

Controversies Conference on Iron Management in CKD | March 27-30, 2014 | San Francisco, California, USA
Regulation of erythrocyte iron recycling

Low hepcidin  |  High hepcidin

Erythrocyte uptake

Iron released to plasma transferrin

Fpn

Fe

Erythrocyte uptake

Macrophages

Fpn

Ferritin

Hepcidin

Controversies Conference on Iron Management in CKD  |  March 27-30, 2014  |  San Francisco, California, USA
How hepcidin regulates iron

Liver
- Hepcidin
- Fpn

Duodenum
- Hepcidin
- Fpn

Spleen
- Hepcidin
- Fpn

Bone marrow and other sites of iron usage

Plasma Fe-Tf
Signals regulating hepcidin
Consequences of hepcidin dysregulation

Hepcidin deficiency = iron overload
- Hereditary hemochromatosis
- Iron-loading anemias

Hepcidin excess = iron-restriction, anemia
- Anemia of inflammation
- Anemia of CKD
- IRIDA
Hereditary hemochromatosis

- Loss of splenic iron stores
- Cardiomyopathy
- Pancreatic endocrine failure (diabetes)
- Skin pigmentation
- Arthritis of small joints
- Arthritis of large joints with chondrocalcinosis
- Hypogonadism
- Cirrhosis with hepatocellular carcinoma
- Anterior pituitary failure

*Molecular Medicine Today*
Hepcidin deficiency causes most forms of hereditary hemochromatosis.
Non-transferrin bound iron (NTBI)

- Fe bound to citrate, acetate, albumin
- Seen when transferrin is (nearly) saturated with Fe
- NTBI is selectively taken up by the liver and other parenchymal tissue and causes injury
- Labile plasma iron (LPI) is the redox-active portion of NTBI
- LPI is detectable when Tsat >75%

Le Lan et al. Blood 2005
Hereditary hemochromatosis

HFE, TF, HJV

Liver

Spleen

Duodenum

Bone marrow

Plasma Fe-Tf

RBC

Hepcidin deficiency, absolute or relative

Hepcidin resistance

Hepcidin resistance

Hepcidin
Biomarkers of systemic iron overload associated with potential tissue injury

- High transferrin saturation
- Increased non-transferrin bound iron (NTBI)*
- Increased labile plasma iron (LPI)*
- Ferritin > 1000 only in the absence of:
  - inflammation
  - hepatitis
  - ferroportin disease
  - hyperferritinemia with cataracts syndrome
Erythroid factor(s) regulate hepcidin to assure adequate iron for erythropoiesis

Erythropoietic stimulation (hemorrhage, EPO)

Erythropoiesis

LIVER

Hepcidin suppression

Increased iron availability

Pak et al., Blood, 2006
The effect of erythropoietin on iron homeostasis

- Five male volunteers
- Erythropoietin 5000 u at 9am on day 2
- Serum hepcidin drop 9-24h later
- The effect lasts at least 5 days
- Very slow change in iron parameters

Ashby et al., Haematologica 2010
Erythropherrone in iron regulation

- Anemia
- Hypoxia
- Kidneys
- Duodenum
- Liver
- Bone marrow

\[ \uparrow \text{EPO} \quad \downarrow \text{Hepcidin} \quad \uparrow \text{Iron} \quad \uparrow \text{ERFE} \]

- EPOR
- Jak2/Stat5
- EPO-stimulated erythroblasts

- Anemia
- Hypoxia
- Epo-stimulated erythroblasts
- Iron
- Hepcidin

- Kidneys
- Duodenum
- Liver
- Bone marrow
Ineffective erythropoiesis

- Failure of red cell precursors to mature into functional RBCs
- Expansion of immature precursors
- E.g.: β-thalassemia (Cooley’s anemia)
- Causes increased Fe absorption
- Probably caused by a hepcidin-suppressive signal generated by increased RBC precursors
Iron-loading anemias

- Liver
  - Hepcidin deficiency
- Spleen
- Duodenum
- Bone marrow
- Erythroferrone
- Plasma Fe-Tf
- RBC

Erythroid factor (Erythroferrone)?
# Iron-restricted anemias

<table>
<thead>
<tr>
<th>Prototype</th>
<th>Fe deficiency</th>
<th>Inflammation</th>
<th>“Pure” hepcidin excess</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic bleeding</td>
<td>Rheumatologic diseases</td>
<td>IRIDA (Tmprss6 mutations)</td>
<td></td>
</tr>
<tr>
<td>Serum Fe</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Tsat</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Macrophage Fe</td>
<td>No</td>
<td>Yes</td>
<td>Yes (Fe tx)</td>
</tr>
<tr>
<td>Ferritin</td>
<td>Low</td>
<td>High</td>
<td>Mostly normal</td>
</tr>
<tr>
<td>Hepcidin</td>
<td>Low to absent</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>MCV</td>
<td>Low to very low</td>
<td>Normal</td>
<td>Very low</td>
</tr>
<tr>
<td>Response to “usual” oral Fe</td>
<td>Good</td>
<td>Poor</td>
<td>Poor</td>
</tr>
</tbody>
</table>
Iron deficiency

- Normal absorption 1-2 mg Fe/day
- Normal stores in adult men ~1 g
- It takes more than 2 yrs of iron-free diet to deplete stores
- Women have lower stores so depletion is faster
- Bleeding: each 1 ml of RBC = 1 mg of Fe
- Iron deficiency = blood loss, in the US and other developed countries
- Iron malabsorption is rare: Celiac disease, Helicobacter pylori, autoimmune atrophic gastritis
Anemia of inflammation

- Suppression of erythropoiesis
  - Switch to leukocytes and platelets?
- Iron restriction
  - IL-6 → hepcidin → hypoferremia
- Destruction of erythrocytes
  - Macrophage activation

Attributed to A. Einstein: “Make things as simple as possible, but not simpler”
Iron-restricted anemias

- Inflammation

- Liver
  - mutTMPRSS2
  - hepcidin

- Spleen
  - Plasma
    - Fe-Tf
      - RBC

- Bone marrow

- Kidneys

- Duodenum

- Adenoma

- hepcidin clearance
Pathogenesis of anemia of CKD

• EPO deficiency
• Inflammation leading to EPO resistance  
  – Iron restriction due to hepcidin  
  – Suppression of erythropoiesis
• Other effects of inflammation  
  – Shortened erythrocyte lifespan
• True iron deficiency from blood loss and decreased iron absorption from chronic inflammation
Serum hepcidin is high in CKD

Zaritsky et al. CJASN. 2010; 5: 1010-1014.
Hepcidin is cleared by HD

Polyflux Revaclear dialyzer (Gambro) with a dialysate flow (Qd) of 800 ml/min for an average of 3.2 ± 0.2 and 3.0 ± 0.4 hours in pediatric and adult patients, respectively (NS).

The average blood flow (Qb) was 320 ± 52 and 375 ± 32 ml/min in pediatric and adult patients, respectively Hepcidin clearance by HD was 141 +/- 40 and 128 +/- 44 ml/min in pediatric and adult patients, respectively (NS).

Za faire et al. CJASN 2010 June; 5: 1010-1014.
Hepcidin recovers rapidly after HD

Iron restriction and deficiency in CKD

- Elevated hepcidin causes:
  - malabsorption of iron
  - sequestration of iron in macrophages
- Hemodialysis causes blood loss
- Iron deficiency and iron restriction contribute to erythropoietin resistance
- Erythropoietin dose-dependently suppresses hepcidin but causes side effects, risks and costs
- IV iron reduces erythropoietin resistance but:
  - may increase infections
  - questions about long term safety