Review

Iron regulation by hepatocytes and free radicals

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Iron is an essential metallic microelement for life. However, iron overload is toxic. The liver serves an important role as a store-house for iron in the body. About 20–25 mg of iron is required each day for hemoglobin synthesis. To maintain iron homeostasis, transferrin and transferrin receptors are primarily involved in the uptake of iron into hepatocytes, ferritin in its storage, and ferro-portin in its export. Moreover, hepcidin controls ferroportin and plays a central role in the iron metabolism. Excess "free" reactive iron produces damaging free radicals via Fenton or Harber-Weiss reactions. Produced free radicals attack cellular proteins, lipids and nucleic acid. Several detoxification system and anti-oxidant defense mechanisms exist to prevent cellular damage by free radicals. Excessive free radicals can lead to hepatocellular damage, liver fibrosis, and hepatocarcinogenesis.

Key Words: iron, hepatocyte, transferrin, hepcidin, free radicals

Iron is the most abundant metallic microelement in the body, and it performs important actions in the body; being essential in hemoglobin synthesis for new erythrocytes (oxygen transport and delivery),[1] enzymes involving electron transport, iron-sulfur proteins,[2,3] and so on. The total amount of iron in the bodies of adult human males is about 5 g, of which approximately 65% is hemoglobin iron in erythrocytes.[1] The remainder is stored in ferritin in the liver and other organs.[4,5] Some iron is also present as myoglobin iron in muscles, heme iron in enzymes involved in respiration in all cells and drug metabolism. Daily food intake contains approximately 10 mg of iron, of which 1–2 mg is absorbed by the mucosa of the upper small intestine, bound to transferrin (Tf) in blood, and transported throughout the body as diferric transferrin (Fe_2-Tf). Iron homeostasis is maintained by the loss of about 1 mg of iron per day through the sloughing of intestinal mucosa and skin. However, 20–25 mg of iron is required each day for the hemoglobinization of new erythrocytes, for which iron in senescent erythrocytes, largely processed in reticulo-endothelial cells, such as macrophages, is reused. As there appears to be no developed mechanism to evacuate accumulated iron from the body, excess iron intake can easily lead to iron overload in tissues, producing free radicals. This article provides an overview of iron metabolism in the liver, particularly in hepatocytes, and the production of free radicals caused by iron overload in the liver.

Iron in Hepatocytes

Iron absorbed from food in the upper small intestine is first transported to the liver via the portal vein. The liver not only stores iron, but also plays a crucial role in iron metabolism, such as in the production of Tf, iron carrier protein, and hepcidin known as a hormone that functions in the regulation of iron metabolism.

Mechanisms of iron transport into hepatocytes. Extra-cellular circulating iron in the plasma is present as soluble Tf-bound iron, and when there is excess iron, as non-transferrin-bound iron (NTBI), which is bound to serum proteins, such as albumin or citric acid.[6,7] Hepatocytes express both transferrin receptor (TfR) 1 and a homolog, TfR2.[8] Under neutral pH conditions, Tf binds two iron atoms with high affinity, and are circulated in the blood as Fe_2-Tf.[9] Known routes for the import of Fe_2-Tf into hepatocytes are via TfR1[10,11] and TfR2,[8] and a TfR-independent route.[12,13]
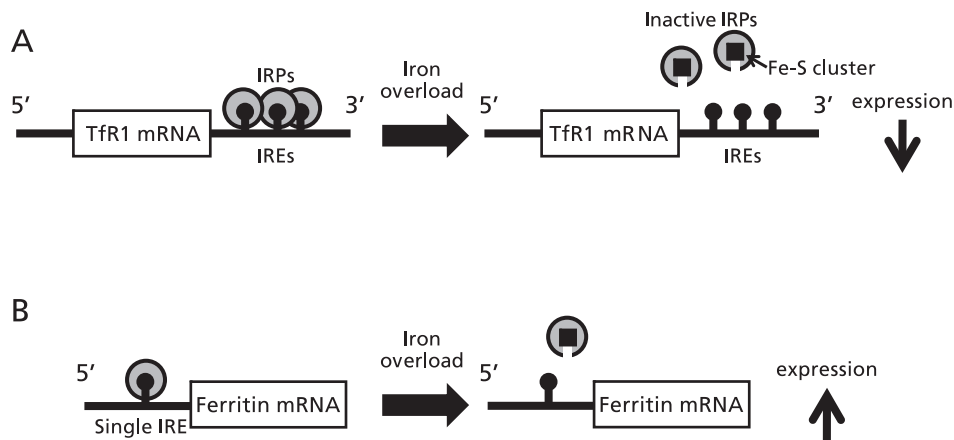
Fe_2-Tf binds with TfR1 on the cell membrane surface and forms a complex that is then taken up into hepatocytes by endocytosis.[14] Mice lacking TfR1 die during mid-gestation from reduction in erythrocyte sell size and hemoglobin content, an indication of its physiological essentiality.[15] TfR1 expression is controlled by the excess or deficiency of iron within cells, and is regulated by interactions between *trans*-acting iron regulatory proteins (IRP1 and IRP2) and multiple iron-responsive elements (IREs) present in the 3'-untranslated regions (UTRs) of TfR1 mRNA (The IRP-IRE regulatory system).[16–18] When there is insufficient iron, IRP binds with multiple IREs in the 3' UTR of TfR1 mRNA, and TfR1 protein levels increase via stabilization of the mRNA. In a state of excess iron, on the other hand, IRP is released from multiple IREs.[19] Thus, IRP acts as a sensor protein for intracellular iron and regulates the expression of IRE genes (Fig. 1A).

Once the Fe_2-Tf/TfR1 complex is endocytosed, the pH of endosomes is lowered to 5–6 by a proton pump ATPase.[20] The Fe_2-Tf bond is broken within acidic endosomes, and Fe^{3+} (ferric iron) is released within the endosome.[21] The free Fe^{3+} in endosomes then becomes Fe^{2+} (ferrous iron) by enzymes of the six-transmembrane epithelial antigen of the prostate (STEAP) family,[22,23] and sub-sequently transported to the cytoplasm by divalent metal trans-porter 1 (DMT1, also known as Nramp2 or DCT1), a member of the NRAMP (natural resistance-associated macrophage protein) family of metal iron transporters.[24,25] Much of the iron transported into the cytoplasm is employed in reactions for essential physio-logical reactions, such as mitochondrial adenosine triphosphate (ATP) generation, TCA cycle, and DNA damage repair.[26] Mean-while, unused iron is stored in the iron-binding protein, ferritin. After dissociating from iron, apo-Tf and TfR1 return to the cell membrane surface, and are reused for further iron binding and uptake (Fig. 2 A–F).[21]

TfR2 was discovered in 1999 as a molecule highly homologous with the TfR1 gene,[8] and it was later shown that mutations in human TfR2 gene induced iron overload syndrome,[27] consistent with its important role in iron metabolism. TfR2 protein is distributed on the basolateral membrane of hepatocytes, and like the TfR1 protein, binds specifically to Tf.[28] IRE is not present in TfR2 mRNA; thus, its mRNA expression is almost completely unaffected by iron levels.[29] Nevertheless, when there is excess iron, Fe_2-Tf stabilizes the TfR2 protein, resulting in increased TfR2 protein levels.[30,31]

There are also reports of a route for the import of iron into hepatocytes that is not mediated by TfRs (Fig. 2G),[12,32] and the

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Fig. 1. The IRP-IRE regulatory system. A: Regulation of TfR1; at the high level of iron, the IRPs binding with multiple IREs in the 3' UTRs of TfR1 mRNA promotes TfR1 mRNA's translation via mRNA stabilization. B: Regulation of ferritin; the IRP binding with the single IRE in the 5' UTRs of ferritin mRNA blocks the translation of ferritin message. In iron overload, the IRP contained the Fe-S cluster cannot bind with the IRE in the 5' UTRs of ferritin mRNA, resulting in the increased translation of ferritin message.
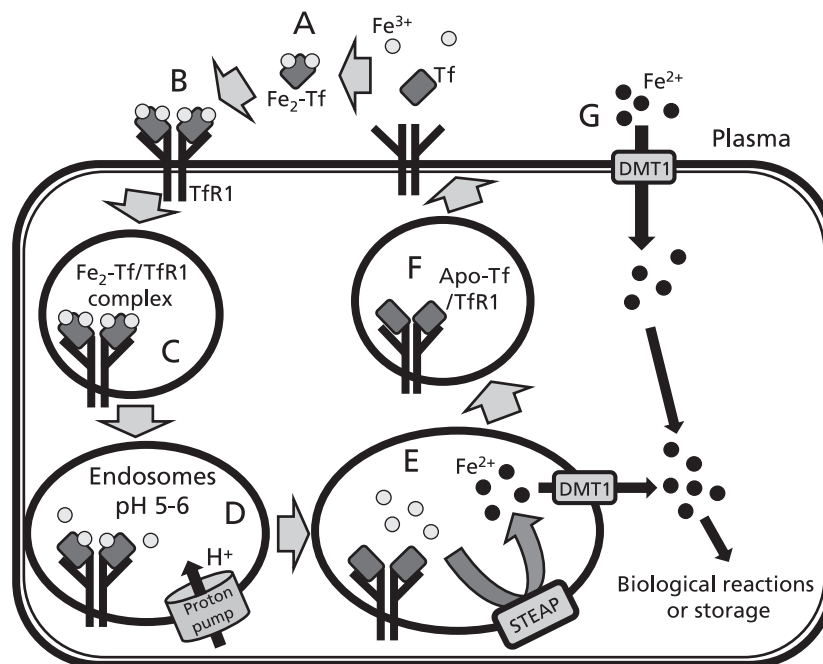
Fig. 2. Iron metabolism in hepatocytes. A–F: TfR-dependent route (the Tf cycle). A: Two ferric irons (Fe^{3+}) in the plasma bind to Tf with high affinity. B: Fe_2-Tf binds to TfR1 on the surface of hepatocytes. C: Fe_2-Tf/TfR1 complex is taken up into hepatocytes by endocytosis. D: The pH of endosomes is lowered to 5–6 by an action of proton pumps, and then Fe^{3+} is released at this acidic pH. E: Released Fe^{3+} becomes ferrous iron (Fe^{2+}) by the enzymes of STEAP family, and then Fe^{2+} moves across the endsomal membrane through the DMT1 transporter. F: Apo-Tf still binds to TfR1 at the lower pH of endosomes. Apo-Tf/TfR1 is returned to the surface of the hepatocyte for further iron binding and uptake. G: TfR-independent route. At the high level of iron in plasma, NTBI is reduced to Fe^{2+} by ferric reductase. Fe^{2+} is rapidly transported into the hepatocytes through DMT1.

involvement of DMT1[13] and the zinc transporter ZIP14 (Zrt-Irt-like protein 14)[33] molecules in the uptake of NTBI. In especially cardiomyocytes, it has been reported that L-type voltage-dependent calcium channels involved in the uptake of NTBI under iron overload condition.[34]

Iron storage in hepatocytes. Hepatocytes have a large capacity for iron storage. After iron's entering hepatocytes, the portion that is not used is stored in the cores of ferritin shells, in order to prevent subsequent toxicity.[35] When hepatocytes become heavily iron overload, an insoluble hemosiderin is derived from iron-rich ferritin.[5] Ferritin is an iron-storage protein comprising heavy (H) and light (L) subunits.[36] Fe^{2+} is oxidized to Fe^{3+} by ferroxidase in the H subunit, and then Fe^{3+} is stored in spaces within ferritin.[37] The synthesis of ferritin, like the TfR protein, is also regulated by IRP protein binding in the mRNA IRE region, and when the concentration of iron in cells increases, the amount of ferritin protein increases.[38,39] As some of the ferritin in the cell cytoplasm is secreted into extracellular medium, it can be measured in clinical tests to gauge the amount of iron stored in the body under non-inflammation conditions.

Regulation of iron export from hepatocytes. There is only 4 mg of iron in blood, and stored iron must be sent quickly

to the blood in order to maintain serum iron concentrations. Some kinds of cells, hepatocytes, intestinal epithelial cells and macrophages, have mechanisms to export iron. Iron is exported from cells by the ferroportin membrane protein (also known as IREG1 or MTP1),[40] and it is reported that in mice or Zebrafish lacking ferroportin, the supply of iron to the blood is almost entirely lost.[41] Iron sent into the blood is again transferred to Tf and is used in erythrocyte production in bone marrow. The expression of ferroportin is also regulated by the IRP-IRE regulatory system and hepcidin,[42] which is currently thought to play a central role in the iron metabolism.[43–46]

Hepcidin is synthesized in hepatocytes, secreted from hepatocytes, and excreted through the kidneys.[43,47] Hepcidin in its mature form is a 25-amino acid-peptide, hepcidin-25.[47] Hepcidin-25 binds with ferroportin and both are broken down in lysosomes by endocytosis.[46,48] Hepcidin-25 expression decreases and ferroportin iron export is promoted, when the body requires more serum iron, such as iron deficiency, hypoxia, and pregnancy.[47,49–50] However, in iron deficiency anemia in states of chronic inflammation, the supply of iron to the blood is inhibited by the reduced ferroportin function. It is thought that the inflammatory cytokine, interleukin-6 (IL-6), induces the expression of hepcidin-25 in hepatocytes, resulting in decreased ferroportin.[49,51] And when serum iron is not needed, hepcidin-25 expression increases and ferroportin is broken down, restricting the supply of iron.[47,49]

Iron Overload and Free Radicals

In healthy individuals, almost all of the iron in circulating blood is bound to Tf, while those in hepatocytes is bound to ferritin and isolated.[35] Ferritin has the capacity to hold 2,000–5,000 iron ions per a molecule, and as mentioned above, the amount of ferritin protein expressed increases with elevated intracellular levels of iron by the IRP-IRE regulatory system (Fig. 1B).[39] Ferritin can therefore handle even high amounts of iron imported into hepatocytes.[52] However, during iron overload, ferritin that is bound with iron undergoes denaturation in lysosomes to become hemosiderin, and Fe^{2+} is released from NTBI that is not bound to ferritin. When this occurs, Fe^{2+} reacts with hydrogen peroxide (H_2O_2) to generate hydroxyl radical (·OH) which have the highest toxicity among reactive oxygen species (ROS), via the Fenton reaction or Harber-Weiss reaction. And Fe^{2+} also reacts with lipid peroxides to generate lipid radicals. These radicals oxidize various cell components; including lipid membranes, proteins, and DNA, causing apoptosis or other cell damage.[53] The solubility of iron ions in the cytoplasm is very low; thus, they are assumed to be bound with other molecules, such as adenosine diphosphate (ADP), rather than be present as single ions. This notion is supported by the fact that administration of the iron chelating agent deferoxamine almost completely suppresses the cell damage from free radicals.[54,55] The risk of hepatocellular carcinoma (HCC) from hereditary hemochromatosis (HH) is about 200 times higher than that in healthy individuals. Interestingly, excess deposits of iron are also seen in hepatic tissues in chronic hepatitis C patients. It has been reported that chronic hepatitis C patients and hepatitis C virus (HCV) transgenic mice have lower levels of hepcidin, consistent with iron accumulation in the liver.[56–58] In such chronic hepatitis C patients, 8-hydroxydeoxy-guanosine (8-OHdG), an indicator of DNA damage by ROS, is significantly higher.[59] On the other hand, the rate of HCC from chronic hepatitis C patients treated with phlebotomy is significantly lower.[60] Moreover, it has been reported that the supplementation of exogenous antioxidants (vitamins A and E) reduced 8-OHdG levels in dietary iron overloaded rats.[61] These evidences *in vivo* and *in vitro* studies suggest that iron overload in the cytoplasm contributes, through the production of ROS, to both cellular damage and hepatocarcinogenesis.

Conclusions

Various molecules are involved in iron transport, storage, and export in hepatocytes, and because iron intake tends to be insufficient, despite being essential for life activity, systems for the reuse of iron have evolved. However, in states of iron overload from excess iron intake or chronic inflammation, harmful ROS are produced, and these may be able to cause hepatocellular damage and hepatocarcinogenesis.

Acknowledgment

Abbreviations

Tf	transferrin
Fe2-Tf	diferric transferrin
NTBI	non-transferrin-bound iron
TfR	transferrin receptor
IRP	iron regulatory proteins
IRE	iron-responsive element
UTRs	untranslated regions
STEAP	six-transmembrane epithelial antigen of the prostate
DMT1	divalent metal transporter 1
NRAMP	natural resistance-associated macrophage protein
ATP	adenosine triphosphate
ZIP	Zrt-Irt-like protein
IL	interleukin
H2O2	hydrogen peroxide
ROS	reactive oxygen species
ADP	adenosine diphosphate
HCC	hepatocellular carcinoma
HH	hereditary hemochromatosis
HCV	hepatitis C virus
8-OHdG	8-hydroxydeoxy-guanosine

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