Role of liver biopsy in disorders of iron metabolism

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Abstract

Recent advances in understanding the genetic and molecular basis of inherited disorders of iron metabolism have resulted in a new approach to the classification of hereditary iron-storage disorders; these include various forms of hereditary haemochromatosis as well as a number of other much less common genetic iron-overload disorders. As a consequence, the role of liver biopsy in assessing hepatic iron-overload disorders has changed. This review will begin by providing an overview of the molecular mechanisms involved in regulating iron homeostasis and the current approach to classifying genetic iron-overload diseases based on the identification of specific gene mutations. The pathogenesis and functional consequences of hepatic siderosis in acquired iron-overload conditions will also be considered. The histopathological assessment of hepatic iron storage will be reviewed, focusing on histological patterns of iron overload and the changing role of liver biopsy in the diagnosis and management of patients with primary and secondary hepatic iron overload.

Keywords genetic iron overload diseases; haemochromatosis; iron metabolism; liver biopsy; siderosis

Iron is an essential nutrient required for haemoglobin synthesis and various enzyme activities. However, excess iron leads to harmful effects, many of which are mediated by the induction of noxious free radical reactions. Important hepatic complications of iron overload include hepatocyte death and progressive fibrosis, leading to cirrhosis and hepatocellular carcinoma. Beginning with the landmark discovery of the *HFE* gene in 1996,[1] there have been considerable recent advances in understanding the genetic and molecular basis of inherited disorders of iron metabolism. As a consequence the role of liver biopsy in assessing hepatic iron overload has changed.

This review will begin by providing a brief summary of molecular mechanisms involved in regulating iron homeostasis. An improved understanding of this process has led to a new approach to the classification of hereditary iron-storage disorders and is also useful as a basis for considering a practical diagnostic approach to the assessment of iron overload in liver biopsies.

Normal iron uptake and homeostasis

Key factors that control iron uptake and homeostasis are the divalent metal transporter protein DMT1, ferroportin, hepcidin, transferrin receptors 1 and 2, and the HFE protein (Figure 1 and Table 1).[2–4]

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Regulation of iron absorption from the gut is the critical step in the maintenance of iron homeostasis as there are no normal mechanisms for controlling iron excretion. The liver plays a central role in regulating iron transport and homeostasis; this is principally mediated via the production of hepcidin, which has a similar function in regulating iron homeostasis to that of insulin in regulating glucose homeostasis.[3]

Dietary iron is taken up in a reduced (ferrous) form (Fe^{2+}) through the apical brush border of small intestinal enterocytes, principally in the duodenum. This process is mediated by the divalent metal transporter protein-1 (DMT1). The expression of DMT1 is mainly regulated by the intracellular concentration of iron in immature crypt epithelial cells, which in turn sense body iron requirements through the uptake of circulating transferrin-bound iron. Thus, low levels of circulating iron result in low levels of crypt epithelial iron, leading to increased DMT1 expression and vice versa.

Iron absorbed from the gut lumen can either be stored within duodenal enterocytes as ferritin and subsequently shed into the gut lumen or transported across the basolateral membrane into the circulation. The latter process is mediated by the iron transporter protein ferroportin-1, which is also expressed on other cells involved with iron transport, including macrophages and hepatocytes (Figure 1). Similar to the expression of DMT1 on the apical membrane, ferroportin expression on the basolateral membrane is regulated by the local iron concentration in duodenal crypt epithelial cells. It is also regulated by circulating levels of hepcidin (see below). Circulating iron is mainly bound to the glycoprotein transferrin.

Hepcidin is a polypeptide hormone produced by hepatocytes, whose main action is to downregulate the expression of ferroportin, by causing its internalization and intracellular degradation, thereby leading to a reduction in circulating levels of iron. Hepcidin production is upregulated by body iron excess and downregulated by low iron levels and hypoxia. Thus, in conditions where increased circulating iron is required, hepcidin production by hepatocytes decreases, leading to increased ferroportin-mediated transport of iron into the circulation. The precise signalling pathways involved in regulating hepcidin production are not fully understood, but three proteins thought to be important are transferrin receptor-2 (TFR2), haemojuvelin (HJV, also known as HFE2) and the HFE protein. Hepcidin is also released by hepatocytes in response to inflammatory signals such as pro-inflammatory cytokines. This is thought to play a protective role in response to infection with microorganisms, which also require iron for important metabolic functions. Thus, hepcidin released as part of an acute inflammatory response reduces ferroportin-mediated release of iron into plasma, thereby creating an unfavourable environment for any circulating pathogens.

The transferrin receptors TFR1 and TFR2 are also involved in mediating iron uptake by crypt epithelial cells and hepatocytes (Figure 1). This process appears to be regulated by HFE, although the precise mechanism is unknown. TFR1 is upregulated in response to low cytoplasmic iron levels, hypoxia and pro-inflammatory cytokines, whereas TFR2 is upregulated in response to high serum transferrin levels.

Classification of hepatic iron-storage diseases

Iron-overload disorders can be subdivided into hereditary and acquired forms.

Main actions and factors regulating the expression of the major iron transporter proteins

Transporter protein	Localization	Action	Regulatory factors
DMT-1	Duodenal enterocytes (apical membrane)	Iron uptake from gut lumen	Stimulated by low iron concentration in enterocyte cytoplasm
Ferroportin-1	Duodenal enterocytes (basolateral membrane) Macrophages (including Kupffer cells), Hepatocytes	Iron transport from cell cytoplasm into plasma	Stimulated by high cytoplasmic iron concentration. Inhibited by hepcidin
TFR1	Duodenal enterocyte (basolateral membrane), hepatocytes	Iron transport from plasma into cell cytoplasm	Stimulated by low cytoplasmic iron levels, hypoxia and pro-inflammatory cytokines Also regulated by HFE (mechanism unknown)
TFR2	Duodenal enterocyte (basolateral membrane), hepatocytes	Iron transport from plasma into cell cytoplasm. Also regulates hepcidin production by hepatocytes	Stimulated by high transferrin levels. Also regulated by HFE (mechanism unknown)

Table 1

Genetic iron-overload disorders

Recent advances in elucidating the molecular genetics of iron homeostasis have led to an improved understanding of the pathophysiology of genetic iron-overload disorders, which can now be classified on the basis of specific gene mutations (Table 2).[4]

Haemochromatosis

The commonest form of genetic haemochromatosis (type 1) is due to mutations in the *HFE* gene located on chromosome 6. This accounts for more than 90% of hereditary iron-overload syndromes, mostly presents in middle-aged men and is almost

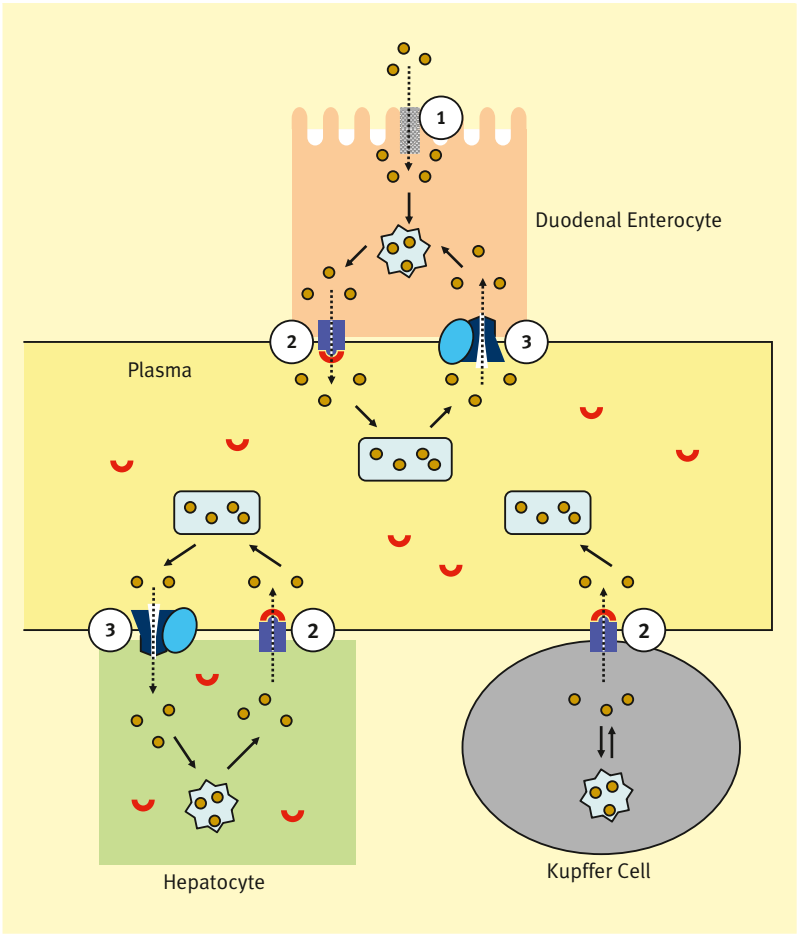

Figure 1 Main pathways of iron transport: (1) DMT-1 mediated uptake of ferrous iron from the gut lumen into the duodenal enterocyte; (2) Ferroportin-1 mediated transfer of iron (mainly present as ferritin) from the cytoplasm of duodenal enterocytes, hepatocytes and macrophages into plasma; (3) Transferrin receptors 1 and 2 mediate uptake of circulating iron (mainly present as transferrin) into the cytoplasm of duodenal enterocytes and hepatocytes. Key to Symbols: Iron, Ferritin, Transferrin, Divalent metal transporter 1 (DMT-1), Ferroportin-1, Transferrin receptor (1 and 2), HFE, Hepcidin.