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Review

An overview of molecular basis of iron metabolism regulation and the associated pathologies

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ABSTRACT

Iron is essential for several vital biological processes. Its deficiency or overload drives to the development of several pathologies. To maintain iron homeostasis, the organism controls the dietary iron absorption by enterocytes, its recycling by macrophages and storage in hepatocytes. These processes are mainly controlled by hepcidin, a liver-derived hormone which synthesis is regulated by iron levels, inflammation, infection, anemia and erythropoiesis. Besides the systemic regulation of iron metabolism mediated by hepcidin, cellular regulatory processes also occur. Cells are able to regulate themselves the expression of the iron metabolism-related genes through different post-transcriptional mechanisms, such as the alternative splicing, microRNAs, the IRP/IRE system and the proteolytic cleavage. Whenever those mechanisms are disturbed, due to genetic or environmental factors, iron homeostasis is disrupted and iron related pathologies may arise.

1. Introduction

The central role of iron in cells and organisms is widely known, as many branches of essential metabolisms, encompassing a full range of cellular processes, energy production, biosynthesis, replication and locomotion, require iron in order to occur.

Despite being so important, it has toxic properties when presented on its free form. Its regular ability to mediate electron transfer, changing between the +2 and +3 oxidation states, may elicit the production of reactive oxygen species responsible for cellular and tissue damage. To avoid those negative effects, iron is usually found coupled with proteins. In serum, it is mainly associated with transferrin, while within the cells it is driven by chaperones or stored within ferritin.

In humans, erythropoiesis is the biological process with the highest demand for iron atoms because of its requirement to heme synthesis and subsequent incorporation into hemoglobin molecules. Circulating erythrocytes consist mainly of hemoglobin containing four heme groups that temporarily binds to oxygen molecules in the lungs and release them throughout the body. When senescent, erythrocytes are phagocytized by macrophages and iron becomes available to be reutilized. Consequently, an organism needs to absorb from diet only the amount of iron strictly necessary to overcome the nonspecific body iron losses. The control of dietary iron absorption by enterocytes and its release from macrophages and from storing hepatocytes are the main examples of mechanisms through which iron homeostasis is maintained. Commonly, these processes are regulated by hepcidin, a liver-derived hormone (Fig. 1). Hepcidin gene (*HAMP*) transcription is up-regulated by high iron levels, infection and inflammation, while anemia and erythropoiesis inhibit its expression. Hepcidin acts by binding to the cell surface ferroportin-1 (Fpn1), the only known iron exporter, inducing its internalization and degradation. As a consequence, iron release from enterocytes, macrophages and hepatocytes is prevented. Besides the systemic regulation of iron homeostasis provided by hepcidin, cells enclose both general and specific mechanisms to regulate themselves the expression levels of iron metabolism-related genes. For instance, the iron regulatory protein (IRP)/iron responsive element (IRE) system controls both mRNA stability and translation of transcripts coding for proteins involved in iron uptake, export, transport and

Abbreviations: β2M, beta 2-microglobulin; aa, amino acid; ACD, anemia of chronic diseases; BMP, bone morphogenetic protein; BMPR, bone morphogenetic protein receptor; CD, cluster of differentiation; Cp, ceruloplasmin; DcytB, duodenal cytochrome b; Dmt1, proton-coupled divalent metal transporter 1; Epo, erythropoietin; ER, endoplasmic reticulum; Fe^{2+}, ferrous iron; Fe^{3+}, ferric iron; Fpn1, ferroportin-1; Ft, ferritin; Ft-H, ferritin heavy chain; Ft-L, ferritin light chain; GDF-15, growth differentiation factor 15; Gpi, glycosylphosphatidylinositol; *HAMP*, hepcidin gene; HCP1, heme-carrier protein 1; Heph, hephaestin; HH, hereditary hemochromatosis; HIF, hypoxia inducible factor; Hjv, hemojuvelin; HO1, heme oxygenase 1; HRE, hypoxia-response element; IDA, iron deficiency anemia; IL, interleukin; IRE, iron responsive element; IRIDA, iron-refractory iron deficiency anemia; IRP, iron regulatory protein; ISC, iron–sulfur cluster; JH, juvenile hemochromatosis; MT2, matriptase-2; Neo, neogenin; NTBI, non-transferrin bound iron; PC, proprotein convertase; ROS, reactive oxygen species; sCp, serum ceruloplasmin; sFt, serum ferritin; sHFE, soluble HFE; sHJV, soluble hemojuvelin; Stat, signal transducer and activator of transcription; Steap, six transmembrane epithelial antigen of the prostate; sTFR1, soluble transferrin receptor 1; TBI, transferrin-bound iron; Tf, transferrin; TfR, transferrin receptor; TfSat, transferrin saturation; TWSG-1, twisted gastrulation 1; UPR, unfolded protein response; UTR, untranslated region

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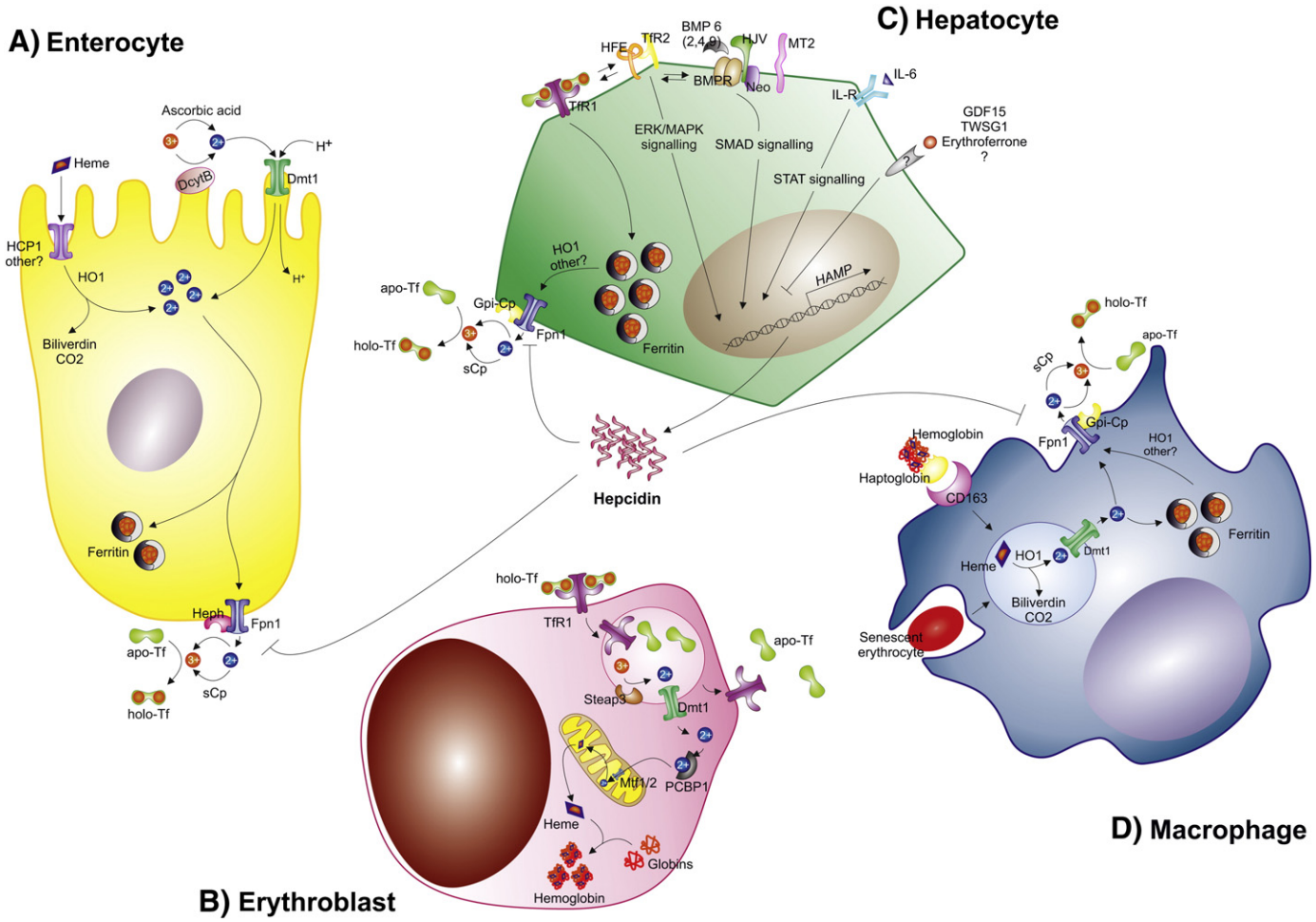

Fig. 1. Systemic iron metabolism regulation. The maintenance of iron homeostasis is a complex process that encompasses the regulation of (A) dietary iron by the duodenum enterocyte, (B) usage by erythroblasts, (C) storage by hepatocytes and (D) recycling by splenic macrophages. After being reduced by ascorbic acid and Dcytb at the apical membrane of enterocytes, dietary iron is absorbed by Dmt1 and driven to the basolateral membrane of these cells where it is exported by Fpn1 to circulation in association with transferrin (holo-Tf). Erythrocytes, which are the cells that require the major amounts iron, capture holo-Tf through the membrane-associated TfR1. After being endocytosed, iron is used by the mitochondria in the synthesis of heme, which will be incorporated in the hemoglobin, functioning as a transporter of oxygen. Whenever the organism absorbs more iron than the required, it is stored within ferritin, mostly at the hepatocytes. The most common source of iron are the macrophages, as they phagocyte the senescent erythrocytes, releasing iron from heme through the HO1, rendering it available to be re-utilized by the cells. In the control of all of these processes we find hepcidin, a circulatory protein synthesized in the liver accordingly to iron levels. Hepcidin acts by binding to membrane-associated Fpn1, inducing its internalization and degradation and, consequently, preventing dietary iron absorption and release from storing hepatocytes and recycling macrophages.

storage. Whenever these mechanisms are perturbed, due to genetic or environmental factors, iron overload or iron deficiency pathologies may arise. This manuscript will provide a general view of the iron metabolism regulatory mechanisms required to maintain homeostasis and the causes and consequences of their deregulation.

2. Inorganic and organic iron

2.1. The origin of life

The ability to complex with organic ligands is one of the reasons why iron is a co-factor of several enzymes. In nature, it can be found in eight oxidation states, ranging from −2 to +6 [1]. This redox property makes iron useful for electron transfer reactions. Recently, it has been hypothesized that, iron could have a crucial role on the origin of life. It is supported that it was an essential element for the development of the primordial membrane bioenergetics [2]. The process of serpentinization at alkaline thermal vents generates natural proton gradients similar to the ones used by modern cells [3]. Briefly, at high pressures and temperatures, iron-containing minerals, such as olivine, react with water to form serpentine and high concentrations of magnetite and H_2. The thin mineral walls would form osmotic barriers separating the warm H_2-rich alkaline fluids from the cold, Fe^{2+}-rich oxidized ocean. There, it would have been gathered the conditions for the formation of primordial natural proton gradients and, consequently, of the energy required for the reduction of CO_2, synthesis of complex organic compounds and the development of the first proto-cells.

2.2. Iron and evolution

Iron is an element required by almost all species within the six kingdoms of life. Before oxygenic photosynthesis, where O_2 and H_2O started to cycle between photosynthesis and respiration, redox cycles took advantage of other elements in order to maintain microbial metabolism [4]. Some bacteria and archea retain the ability to extract energy from sources that are inaccessible to other organisms. For example, instead of acquiring electrons from water, some photosynthetic bacteria oxidize Fe^{2+} to promote CO_2 fixation (anoxygenic photosynthesis) while others transfer electron from organic carbon to Fe^{3+} (heterotrophic respiration) or obtain energy by oxidizing Fe^{2+} and reducing O_2 or NO_3 (lithotrophic respiration) [5].

Throughout evolution, and due to the increase of oxygen tension, redox properties of iron made it extremely useful, so that its usage in eukaryotes is focused on oxygen metabolism, both as an oxygen carrier or

an electron carrier. There are three major classes of iron-containing proteins: iron–sulfur cluster-containing proteins (ISCs), heme-containing proteins and iron-containing enzymes. ISCs catalyze chemical reactions or are components of electron transport complexes [6]. Heme-containing proteins play functions related to oxygen transport or metabolism [7]. Finally, iron-containing enzymes play roles in a vast range of reactions [8].

While Fe^{2+} is soluble in anoxic conditions, it becomes insoluble in the presence of oxygen, being rapidly oxidized to Fe^{3+}, a form that can be solubilized by acidification [9]. There are two mechanisms that allow the organisms to acquire iron. One involves the acidification of the environment in order to solubilize Fe^{3+} and to promote its reduction to Fe^{2+} which will be transported across the plasma membrane. The second approach is the synthesis and secretion of siderophores (small organic molecules with affinity for Fe^{3+}) [10].

3. Iron metabolism in humans

The most common forms in the human body are ferrous (Fe^{2+}) and ferric (Fe^{3+}) iron. The one-electron transfer between these oxidation states is easily achieved in a way that regular reducing agents perform the reduction of aqueous Fe^{3+} to Fe^{2+}, while dioxygen (O_2) promotes the reverse reaction [9]. Under physiological O_2 concentrations the most stable form of iron is Fe^{3+}. The reduction of O_2 by Fe^{2+} results in the formation of superoxide radicals [11]. The hydroxyl radical, one of the most potent oxidants found in the organism, is responsible for the attack to proteins, nucleic acids and carbohydrates, for triggering the propagation of lipid peroxidation and, ultimately, cell apoptosis [12]. Therefore, the organism has developed mechanisms to prevent the toxicity of free iron. These mechanisms comprise the dietary iron absorption by the duodenum, transport in the circulation, cellular uptake and consumption, recycling by macrophages and storage in the liver [13–16] (Fig. 1).

3.1. Absorption

Dietary iron is classified as heme or non-heme iron [17]. Heme iron is highly abundant in meat, as a component of the hemoproteins, hemoglobin and myoglobin. The low pH of the stomach coupled to proteolytic enzymes is responsible for heme release from hemoproteins. The mechanism responsible for heme uptake is not yet well understood, however it is known to occur by receptor-mediated endocytosis (Fig. 1A). The heme-carrier protein 1 (HCP1), together with the proton coupled folate transporter (PCFT), has been identified as the most probable receptor involved in this process. However, it has low-affinity to heme and is more involved in folate absorption [18]. Dietary ferritin is also absorbed by the enterocytes through an adaptor-related 2 protein complex (AP2)-dependent endocytosis mechanism [19]. Once in the enterocyte, heme is broken by heme oxygenase 1 (HO1) and iron is released in its ferric state.

Non-heme iron is mostly presented to the organism on its oxidized form (Fe^{3+}). In order to be absorbed by the enterocyte, it is reduced to Fe^{2+} by the low pH of the stomach together with ascorbic acid [17]. Differentiated enterocytes express on their surface the proteins required for dietary iron absorption. At the apical membrane, facing the gut lumen, there are expressed proteins that facilitate iron reduction, such as duodenal cytochrome b (DcytB) and, most probably, six transmembrane epithelial antigen of the prostate 2 (Steap2) [20,21], as well as, the proton-coupled divalent metal transporter 1 (Dmt1), an iron importer (Fig. 1A). Dmt1 is responsible for the absorption of the ionic forms of iron, cobalt, zinc, cadmium and others. It is a transmembrane protein that takes advantage of the proton gradient existing between the gut lumen and the enterocyte cytoplasm, to perform the symport of Fe^{2+} coupled with H^+ [22].

In the cytoplasm iron is driven to the basolateral membrane of the enterocyte, or stored in ferritin. Here, the 'mucosal block' mediated by ferritin plays a crucial role on the effective absorption of iron. For instance, it was shown that, in mice, when ferritin levels are upregulated by the absence of the IRE/IRP system, iron is mostly stored at the enterocyte instead of being delivered to the circulation, independently of the iron status of the organism [23].

The export of iron from the enterocyte to the circulation is a crucial step for the entrance of iron in the body. The enterocytes express on their basolateral membrane the protein Fpn1, the only known mammalian iron exporter [24]. Fpn1 transports Fe^{2+} to the extracellular side of the basolateral membrane, where it is oxidized by the ferroxidases, hephaestin (Heph) and ceruloplasmin (Cp) in order to be associated with the circulatory transferrin (Tf) [25,26] (Fig. 1A).

3.2. Transport and cellular uptake

Following absorption by the enterocyte, iron must be delivered to cells for either general or specific functions (Fig. 1B). The stability of transferrin-bound iron (TBI) is dependent on several factors, such as the oxidation state of iron, the protein conformation and the environmental pH. Tf is found in the plasma in three states: apo-transferrin (apo-Tf), when no iron is bound; monoferric transferrin (bounded to a single iron atom); and diferric transferrin, also known as holo-transferrin (holo-Tf; bounded to two iron atoms) [27]. The concomitant existence of these three states of Tf in a healthy individual allows the response to acute increases of iron absorption, preventing the toxic effects of excess of iron in the organism.

The cellular uptake of TBI is mainly mediated by the transferrin receptor 1 (TfR1), located at the cell membrane [28]. Upon cell surface binding, the TBI–TfR1 complex undergoes a clathrin-dependent endocytosis. The endosome pH decreases by the entry of H^+ mediated by an ATP-dependent proton pump, and Fe^{3+} is released, while apo-Tf remains bound to TfR1. In the endosome, free iron is reduced by Steap 3 and transported to the cytoplasm by Dmt1 [22,29]. Meanwhile, the Tf–TfR1 complex is driven to the cell surface where apo-Tf is released to the plasma (Fig. 1B).

Non-transferrin bound iron (NTBI) species are also frequently found in the plasma [30]. It has been suggested that the main form of NTBI is Fe^{3+} bound to citrate. However, other iron transporters, such as acetate, have been detected [31]. The origin and the mechanisms involved in the cellular uptake of plasma NTBI remain unclear. It is known that it has a high-affinity to hepatocytes and its uptake seems to be mediated by an endocytosis-independent mechanism. The most probable NTBI transporter that was so far identified is the zinc transporter Zrt–Irt-like protein 14 (Zip 14) [32].

3.3. Consumption and storage

The organism has a high requirement for iron, being its majority used by the erythroblasts for hemoglobin synthesis. About 70% of the body iron pool is found as heme iron in erythrocytes [33]. Mitochondrion is the major human organelle responsible for maintaining the cellular iron homeostasis. After being released in the endosomes, iron is driven to this organelle. Iron may be delivered to mitochondria both by a cytosolic iron chaperon, the poly (rC) binding protein 1 (PCBP1) [34], or directly from the endosome through a 'kiss-and-run' mechanism. The latter was observed in the developing erythrocytes, which have higher demands of iron for hemoglobin synthesis [35]. The transport through the inner membrane is mediated by mitoferrins 1 and 2 [36]. Once in the mitochondria, iron is used in the biosynthesis of heme and of ISCs, which are incorporated into several proteins involved in electron transfer.

Iron storage prevents the presence of free iron whenever body iron levels increase. It also ensures its immediate availability during iron deficiency. The major protein responsible for iron storage is ferritin (Ft) [37] (Fig. 1C). It exists as a 24-subunit multimer of heavy (Ft-H) and light (Ft-L) polypeptide chains. When synthesized, these chains self-

assemble, forming a spherical capsular structure that is able to store up to 4500 iron atoms on its core. Cytosolic Fe^{2+} is driven to Ft by the PCBP chaperones, being oxidized by the Ft-H subunits, while Ft-L subunit promotes its uptake into the core [34]. Ft is localized in cell cytosol, nucleus and mitochondria, but is also present in the serum (sFt). Mitochondria express their own Ft-H-like chains, and it seems that mitochondrial Ft (mFt) is able to store iron even more efficiently than the cytosolic one [38]. The mechanisms used for iron release from Ft are thought to be driven both by lysosome- and proteasome-mediated degradation mechanisms [39,40]. It has been shown that the nuclear receptor coactivator 4 (NCOA4) acts as a cargo receptor that binds and delivers ferritin to ferritinophagy in lysosomes, thus regulating iron bioavailability [41]. In agreement, *NCOA4* expression is up-regulated during erythropoiesis, a process with high demands for iron [42]. Also, the importance of NCOA4 in iron metabolism has been highlighted in *Ncoa4*$^{-/-}$ mice, which developed a severe iron overload in splenic macrophages, important effectors of iron recycling from senescent erythrocytes [43].

3.4. Recycling

One of the main functions of splenic and hepatic macrophages is to scavenge the senescent erythrocytes in order to release iron from the hemoglobin, rendering it available for another hemoglobin cycle [44] (Fig. 1D). Recycling macrophages contribute to the major pool of plasma iron, exceeding the contribution of dietary iron absorption. There are several alterations that are recognized by macrophages as erythrocyte markers of aging [45–48]. These include modifications on the erythrocyte solute carrier family 4 (anion exchanger) member 1 (SLC4A1), the presence of membrane phosphatidylserine, and the decrease of membrane flexibility, sialic acid and in the cluster of differentiation 47 (CD47) antigen. A combination of these modifications trigger erythrocyte phagocytosis by the macrophage.

Once in the phagolysosome, the erythrocyte is subjected to reactive oxygen species (ROS) and hydrolytic enzymes that promote the release of heme to the vacuolar fluid. Then, HO1 together with O_2 cleaves heme to iron, carbon monoxide and biliverdin [49]. Macrophages also capture the hemoglobin released to the serum by ruptured erythrocytes. Plasma hemoglobin complexes with haptoglobin, which is recognized by the macrophage cell surface receptor CD163 [50] (Fig. 1D).

The transport of iron within the macrophage involves (i) the movement of iron across the phagosome membrane by the transporters Dmt1 and the natural resistance-associated macrophage protein (Nramp1) [51]; (ii) the transport through the cytoplasm mediated by the PCBP chaperones [34]; and (iii) the delivery of Fe^{2+} to the Fpn1 for cellular export, followed by reduction by the glycosylphosphatidylinositol-linked (Gpi)-Cp and consequent binding to serum Tf [52].

4. Iron metabolism regulation

The human organism has several mechanisms through which iron levels are kept in homeostasis. Controlled iron absorption, recycling and storage represent part of these mechanisms. Since there are no active ways of excreting iron, the human body needs to absorb approximately 1 mg of dietary iron every day. This process overcomes the non-specific iron losses through bleeding, sweat and sloughing of epithelial cells. However, changes in the absorption, storage and cellular iron release may occur. Whenever more iron is required, the organism increases its duodenal absorption and release from macrophages and from storing cells. On the other hand, when facing conditions of iron overload the absorption is inhibited and storage increased, in order to prevent the toxic effects of free iron excess [53,54]. All the steps required for keeping iron homeostasis are strictly regulated at both systemic and cellular levels.

4.1. Systemic regulation

The major known regulator of iron homeostasis is hepcidin, a 25 amino-acid (aa) peptide hormone mainly expressed by the liver [55]. Its expression is regulated by several physiological conditions, such as systemic iron levels, hypoxia, anemia, erythropoiesis, infection and inflammation [56–58] (Figs. 1C and 2).

Hepcidin is expressed from the *HAMP* gene located at the long arm of chromosome 19 (19q13.1). It is firstly synthesized as a biologically inactive 84 aa pre-proprotein, comprised of a signal peptide, a pro-region and the fully-active 25 aa sequence [59]. Its maturation occurs within the cell and it is performed by proteolytic cleavage mediated by the prohormone convertase furin [60]. Hepcidin acts by triggering Fpn1 internalization and, consequent degradation [61] (Fig. 1). It was proposed that hepcidin binding to Fpn1 induces a rapid ubiquitination of the latter, responsible for its internalization [62].

Therefore hepcidin regulates systemic iron metabolism by reducing Fpn1 levels and consequently, dietary iron uptake and release from macrophages, hepatocytes and other cell types. The elimination mechanism of serum hepcidin has been poorly studied. Hepcidin is found in urine as a 20–25 aa peptide. It is still controversial, whether hepcidin degradation is a consequence or the cause of kidney filtration [63,64].

Hepcidin is mainly expressed in the liver, although its expression has been also detected in macrophages, pancreatic beta cells, kidney, adipocytes and lung [63,65–68]. While iron overload, infection and inflammation up-regulate *HAMP* expression [56,69,70], iron deficiency, hypoxia, anemia and erythropoiesis act on the opposite way [57,58] (Fig. 2).

4.1.1. Iron levels

The regulation of *HAMP* expression by iron levels is a process through which the human body avoids both the toxic outcomes of iron overload and the negative physiological consequences of its deficiency. Several proteins located at the surface of hepatocytes are considered to be 'iron sensors'. These are hemojuvelin (Hjv), HFE, TfR1, and TfR2 (Fig. 2A).

One of the pathways involved in *HAMP* expression regulation by iron is the Hjv-bone morphogenetic protein (BMP) axis [71]. Hjv is a Gpi-linked membrane protein that is considered to be the major regulator of *HAMP* expression in the liver. Hjv acts at the cell surface as a co-receptor of BMPs, a subfamily of cytokines belonging to the transforming growth factor β superfamily [72]. Despite BMPs playing an important role in human development, it is long known their role in iron homeostasis regulation. The Hjv–BMP signaling pathway is triggered upon BMPs binding to Hjv and BMP receptor (BMPR) complexes. The activated receptors induce the phosphorylation of cytosolic SMADs 1, 5 and 8 [71,73,74]. The phosphorylated SMADs form complexes with SMAD4, which are translocated to the nucleus where they bind to the BMP responsive elements (BMPREs) present at *HAMP* promoter, inducing its transcription [75,76]. The BMPs that have been identified as activators of *HAMP* expression are BMPs 2, 4, 5, 6, 7, and 9 [71]. However, only the knockout in mice of *BMP6* drives to an iron loading phenotype similar to the ones observed for the knockouts of *HJV* and *HAMP*, revealing its high importance in iron metabolism regulation [77,78]. The mechanisms regulating BMP expression seems to involve iron but are far to be known. However, it was shown that BMP6 mRNA levels correlate positively with both body iron and hepcidin levels [79]. The expression of BMP6 occurs majorly in the liver. However, while hepcidin is synthesized by the hepatocytes, BMP6 expression in response to iron occurs preferentially at non-parenchymal liver cells, such as sinusoidal endothelial cells, hepatic stellate cells and Kupffer cells [80]. So, whenever iron levels increase in the body, BMP6 is produced by these cells, secreted to the interstitial environment, where it binds to the Hjv in hepatocyte, triggering hepcidin expression.

Although the Hjv–hepcidin axis represents the most important mechanism for the up-regulation of *HAMP* expression during iron overload, there are other few mechanisms that can restrain its way of action

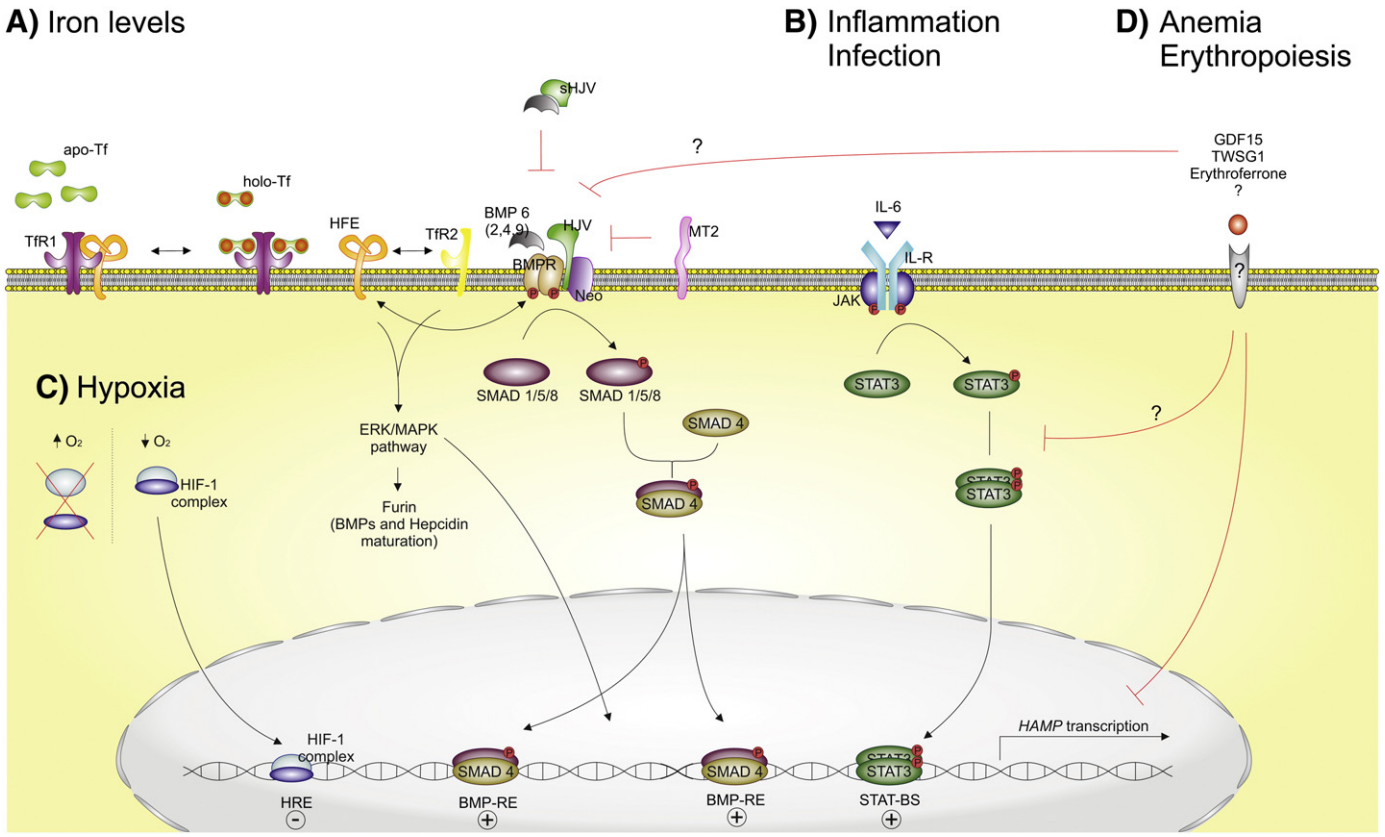

Fig. 2. Mechanisms of *HAMP* gene expression regulation in the hepatocyte. (A) The increase of iron levels and (B) inflammation and infection up-regulate the expression of *HAMP* gene, while (C) hypoxia and (D) anemia and erythropoiesis repress its expression. The mechanisms involved in the up-regulation comprise the recognition of circulatory proteins, as holo-Tf, BMPs and ILs, by membrane-associated proteins. This recognition trigger ERK/MPK, SMAD and JAK-STAT signaling pathways that culminate in the activation of *HAMP* gene expression. On the other hand, both soluble and membrane proteins, such as sHJV and MT2, are known to repress the SMAD signaling pathway by arresting BMPs or degrading mHJV, respectively. In hypoxic conditions, the HIF-1 protein complex is stabilized, being able to bind to *HAMP* promoter and repressing hepcidin transcription. Also, during anemia and expanded erythropoiesis circulatory proteins are produced (GDF15, TWSG1 and erythroferrone), which will act through yet unknown membrane receptors to repress *HAMP* expression, possibly by inhibiting SMAD and JAK-STAT signaling pathways.

(Fig. 2A). Hjv is a target of the furin family of proprotein convertases [81,82]. Its cleavage product is secreted from the cells as a soluble form (sHjv). The subcellular location where furin-mediated cleavage takes place is controversial since, in the literature, two positions were reported, in the endoplasmic reticulum (ER) and in the trans-golgi network [81,83]. When released to the extracellular environment, sHjv is thought to act as an antagonist of *HAMP* expression by high-jacking the BMPs, preventing them to bind to the membrane Hjv and to trigger the signaling pathway [71,83].

Besides furin-mediated cleavage, Hjv is also a target to cleavage by matriptase-2 (MT2), a protein predominantly expressed in the liver and an hepcidin expression suppressor [84]. *In vitro* studies suggest that MT2 binds to the membrane Hjv cleaving it in a ladder of fragments that are subsequently released to the medium [85].

The pathway through which Hjv triggers the expression of *HAMP* is well characterized, however there are some aspects regarding Hjv membrane stabilization that are not yet disclosed. For instance, in the cell surface Hjv interacts with neogenin (Neo) [86]. The role of this protein seems to be bidirectional. Neo is either required for HJV-mediated hepcidin expression, as well as for sHjv production and Hjv cleavage by MT2, inhibiting hepcidin production [87,88].

Another pathway responsible for the regulation of hepcidin expression dependent on iron levels involves the membrane proteins HFE and TFR2 (Fig. 2A). Under iron homeostatic conditions, HFE is partially localized at the hepatocyte cell surface bound to TfR1 [89]. Whenever plasma iron levels increase, the iron loaded transferrin (Fe_2-Tf) gains high affinity for the same receptor. Since the TfR1 binding sites for HFE and Fe_2-Tf overlap, and as the TfR1 affinity for Fe_2-Tf is higher than for HFE, it dissociates from this receptor and becomes available to associate with TfR2 [69,90]. The formed Fe_2-Tf–TfR2–HFE complex triggers the ERK1/2 signaling pathway and ultimately *HAMP* expression [91]. The cytosolic proteins involved in this pathway have not been determined. Moreover, it has been reported that, in mice, the knockouts of *HFE* and *TFR2*, alone or together, have slightly different outcomes regarding *HAMP* expression [92]. Therefore, it is accepted that both HFE and TfR2 may trigger other signaling pathways independent of each other. Whether HFE and TfR2 interact and form a complex containing Hjv at the lipid rafts within cell membrane also remains controversial [93,94]. Nevertheless, it was recently shown that HFE interacts with the BMP receptor ALK3, promoting its stabilization at cell surface by inhibiting its ubiquitination and proteasomal degradation, though regulating the expression of hepcidin [95].

4.1.2. Infection and inflammation

Similar to other organisms, pathogens require iron for survival and proliferation. Whenever the human body is infected, the pathogen takes advantage of the host's iron pool, for its own needs. The hypoferremia commonly associated with the infection is one of the several innate and adaptive responses of the immune system [70]. By lowering the iron levels, the organism tries to arrest the proliferation of iron-dependent pathogens avoiding a fast septicemia outcome and allowing an effective elimination of the infection.

The induction of the hypoferremia during inflammation/infection is mediated by hepcidin [96]. For instance, the injection of hepcidin in mice induces a rapid decrease in serum iron levels [97]. As circulatory hepcidin levels increase, Fpn is internalized and degraded prompting

the organism to absorb lower amounts of iron from the diet and to arrest the body iron pool in macrophages and storing cells. During infection, pathogens are recognized as foreign elements by several cell types, such as macrophages. This recognition triggers the expression and secretion of the pro-inflammatory cytokines interleukin (IL)-6, -22 and type-1 interferon (INF) [70,96,98,99] (Fig. 2B). This cytokines are recognized by surface Toll-like receptors expressed by hepatocytes and leukocytes, triggering the Jak-signal transducer and activating the transcription 3 (Stat3) pathway. The translocation of factor Stat3 to the nucleus and its binding to the Stat3 binding sites in the *HAMP* promoter trigger the expression of this gene [100]. Therefore, extracellular pathogens are deprived of iron.

4.1.3. Hypoxia

Erythropoiesis is the process through which red blood cells are produced and, consequently, the oxygen transporter hemoglobin. During hypoxia, erythropoiesis increases in order to overcome the low body oxygen levels.

The hypoxia inducing factor (HIF) system is recognized as being the main modulator of both iron metabolism and erythropoiesis during hypoxia [101] (Fig. 2C). HIFs are transcription factors that bind to the hypoxia-response elements (HREs), controlling gene transcription [102]. HIF protein levels are regulated by oxygen. Under normoxia, all HIFα-subunits are targeted for proteasome degradation [103]. Under hypoxia, the stabilized HIFs regulate the expression of *TF*, *TFR1*, and *CP*, as well as of *HMOX*, *SLC11A2*, *CYBRD1* and *EPO* (the genes responsible for the expression of HO1, Dmt1, DcytB and erythropoietin, respectively) [104–107]. While HIF-2 has been shown to increase the expression of *SLC11A2* and *CYBRD1*, though enhancing intestinal iron uptake, HIF-1 has been suggested to play a role in the inhibition of *HAMP* expression in the liver, through a HRE-mediated mechanism [57]. However, it remains controversial the way how HIF-1 acts on *HAMP* expression. Despite the promoter of hepcidin gene presenting consensus sequences for hypoxia responsive elements, both the overexpression and knockdown of HIF-1α do not affect the expression of hepcidin [108,109]. On the other hand, some data supports an indirect regulation of hepcidin expression by HIF-1. For instance, HIF-1 upregulates the expression of furin and consequently an indirect downregulation of *HAMP* expression [81]. Also, it has been recently shown that the platelet derived growth factor BB (PDGF-BB), which expression is upregulated by HIF-1 during hypoxia, is responsible for the repression of hepcidin expression mediated by the downregulation of both CREB and CREB-H transcription factors [110,111].

4.1.4. Anemia and erythropoiesis

Ineffective erythropoiesis is responsible for a hypoxic condition observed during anemia. Erythropoietin (Epo) is a glycoprotein hormone mainly synthesized in the adult kidney [112]. It prevents the apoptosis of the colony-forming unit-erythroid cells and erythroblasts that have not begun the synthesis of hemoglobin [101]. Hypoxia is the primary known physiological stimulus that controls Epo production. According to hypoxia severity, Epo serum levels may raise up to 100-fold of the levels found during normoxia. It was shown that, in mice, HIF-2 enhances Epo expression in the liver during hypoxic conditions [113]. Since hypoxia is the main regulator of Epo and consequently erythropoiesis, it was believed for a long time that the down-regulation of hepcidin expression observed during anemia was a consequence from hypoxia rather than from erythropoiesis itself. However, it was reported that the administration of erythropoietin to healthy individuals lowered the levels of circulatory hepcidin, while no immediate changes of iron levels were detected [114]. This outcome led to the search of a hepcidin-suppression factor that could be expressed by the erythroid precursors in response to Epo. Two BMP family members, the growth differentiation factor 15 (GDF-15) and the twisted gastrulation 1 (TWSG-1) are up-regulated in pathologies that present an expanded but ineffective erythropoiesis, as β-thalassemia and congenital dyserythropoietic anemias [115–117] (Fig. 2D). GDF-15 was shown to regulate hepcidin expression in hepatoma cells but its specific role in the regulation of iron homeostasis or in the development of pathological iron-related conditions remains elusive. Recently, it was discovered a new hormone, erythroferrone, produced by the erythroblasts in response to erythropoietin during the erythropoiesis process, which is responsible for the repression of hepcidin expression (Fig. 2D). However, its action is independent on the BMP–SMAD pathway, as well as, on TfR2 [118]. The transcription factor Atoh8 is a possible mediator of this process as it induces hepcidin expression in HEK293 cells, as well as, its expression is downregulated in conditions associated with high erythropoietic activity [119].

4.2. Cellular regulation

While hepcidin plays a crucial role in the systemic regulation of iron metabolism, cells present other mechanisms that specifically regulate the expression of iron metabolism-related genes. These include the modulation of general cellular mechanisms that give rise to alternative transcript variants (such as alternative transcription initiation, polyadenylation and splicing) or of more specific systems that control the stability of the mRNAs and proteins.

4.2.1. Transcriptional initiation

Gene transcription initiation is a complex mechanism through which RNA polymerases recognize the DNA sites from where the RNA synthesis should be started. So far, *DMT1*, *SLC40A1* (gene that expresses Fpn1) and *TFR2* are the only iron metabolism-related genes described as having more than one transcription initiation site associated with alternative promoters [120,121].

DMT1 transcription is regulated by two alternative promoters that give rise to two 5′ DMT1 protein variants which differ in the N-terminal sequence (the variant originated by the transcript with the upstream 5′ exon has an additional 29 N-terminal residues). The expression of this upstream 5′ exon variant seems to occur preferentially in the kidney and duodenum [122]. The transcription of *SLC40A1* originates two mRNAs, *FPN1A* and *FPN1B*, with different 5′-regions but coding for the same protein [120]. However, only the *FPN1A* mRNA contains an IRE motif, being regulated by iron levels. *FPN1B* is only expressed in the duodenum and in the erythroid precursors. On the other hand, the transcripts that are produced by the alternative *TFR2* transcription sites, give rise to two slightly different proteins [121]. The *TFR2α* transcript originates the full-length membrane protein involved in the regulation of hepcidin expression, while the *TFR2β* transcription is initiated within the *TFR2* DNA sequence corresponding to the intron 3 of *TFR2α*. The protein originated by *TFR2β* mRNA is similar to the full-length TFR2 however it lacks the intracellular and transmembrane domains. The knockout of the *TFR2β* in mice showed that it plays a role in the transcriptional control of *SLC40A1* in the spleen and, therefore, in the control of the iron efflux from this organ [123].

4.2.2. Post-transcriptional regulation

It is estimated that almost all human genes suffer post-transcriptional regulation [124] through mechanisms such as, the alternative polyadenylation, the alternative splicing, and also all the regulatory mechanisms that affect the RNA stability, translation and protein processing. For instance, iron metabolism post-transcriptional regulation is mediated by both specific systems that regulate mRNA stability and its translation, such as the IRP/IRE system, as well as by general processes, such as the microRNAs and protein proteolytic cleavage.

4.2.2.1. Alternative polyadenylation. Poly(A) tails are found in the 3′-terminal region of almost all eukaryotic fully-maturated mRNAs [125]. The size of a poly(A) tail influences mRNA stability, translation and transport. Alternative polyadenylation occurs whenever the polyadenylation factors fail to recognize the main poly(A) consensus sequence, binding

to other less specific sequences. The IRP-2 has three poly(A) signals responsible for the existence of the 6.4, 4.0 and 3.7 kb mRNAs [126]. The 3.7 kb transcript is preferentially expressed in iron-depleted cells, possibly as a response to the high needs of IRP-2 protein during iron deficiency, since this RNA is more stable and efficiently translated. Also, *HFE* has, at least, four active poly(A) signals [127,128]. The most upstream signal is responsible for the generation of an mRNA that is resistant to the nonsense-mediated mRNA decay (NMD) mechanism, possibly being recognized more efficiently in cells that require increased amounts of the HFE protein [128].

4.2.2.2. Alternative splicing. Splicing is probably the post-transcriptional mechanism that brings more variability to gene expression [129]. It is responsible for the production of different proteins from the same gene. The *HFE* gene produces several alternative transcripts [127,130]. From those, the ones corresponding to the inclusion of the total or partial HFE intron 4 give rise to a soluble form of the HFE protein (sHFE), which regulates the expression of hephastin in the duodenum through a clathrin-independent, dynamin-mediated and RhoA-regulated endocytosis mechanism. So, sHFE acts, most likely, as a regulator of dietary iron absorption in the duodenum [131]. On the other hand, *SLC11A2* alternative splicing gives rise mainly to two variants, the *DMT1A* and *DMT1B*, which are the result of the alternative usage of the 3′-exons of the gene. This way the *DMT1A* variant presents an IRE and produces a protein that is preferentially expressed in the epithelium. On the other hand, *DMT1B* does not have the IRE, thus it does not respond to iron, and it is expressed mainly by blood cells [132]. Also, *CP* is a target for post-transcriptional processing by alternative splicing giving rise to two main Cp isoforms, the sCp and the Gpi-Cp. These are the result of alternate usage of exons within the 3′ region of *CP* [133].

4.2.2.3. The IRP/IRE system. In general, mechanisms of post-transcriptional regulation play an important role in the maintenance of cellular iron metabolism. In fact, cells have developed a specific mechanism to control the translation and the stability of iron metabolism-related mRNAs. The IRP/IRE system was developed by the cell to maintain the cellular iron content through the regulation of its uptake, storage and export [134]. The IRP1 and IRP2 proteins are the main regulators of cellular iron in humans [135,136]. They bind to the IREs present at the 5′ and 3′-untranslated regions (UTRs) of mature mRNAs that code for proteins responsible for iron uptake (Dmt1 and TfR1), storage (Ft-H and Ft-L) and export (Fpn1) [134]. IREs are 28–30 nucleotide sequences with a highly conserved secondary structure. During iron deficiency, IRP1 binds to IRE through a cavity on its structure, while under iron overload that same cavity is occupied by the 4Fe–4S cluster and unavailable to bind to the IRE [137,138]. The activity of IRP2 is regulated by iron in a different way. An increase of cellular iron promotes IRP2 ubiquitination and consequent proteosomal degradation, therefore preventing its binding to the IRE [139]. This process is mediated by E3 ubiquitin ligase complex containing the F box and leucine-rich repeat protein 5 (FBXL5). FBXL5 stabilizes after the binding of iron and oxygen to its hemerythrin domain, promoting IRP2 ubiquitination [140]. Also, the knockout of FBXL5 gene induces a massive iron overload that is fatal to the embryos. This phenotype is only prevented by the concomitant knockout of the IRP2 gene [141].

When cells are deprived from iron, IRPs bind to the IREs located at the 5′UTRs of *FTH* and *FTL* and *SLC40A1*, preventing ribosome assembly and further translation [142,143]. On the other hand, they also bind to the IREs located at the 3′UTR of *TFR1* and *SLC11A2* increasing their stability and consequently their translation [144,145]. This way, iron uptake is up-regulated while iron storage and export are inhibited. When iron levels increase, IRPs are not able to bind to the IREs, limiting cellular iron acquisition.

4.2.2.4. MicroRNAs. The non-coding RNAs have assumed an extraordinary importance in the last few years when it became clear that they have critical functions in physiological processes (such as cell differentiation and development) but also in pathological processes. MicroRNAs are non-coding RNAs with an average length of 22 nucleotides which function is being antisense regulators of other RNAs [146]. Originally, they are expressed within the nucleus as longer RNAs (pri-microRNAs) with a stable hairpin structure and are subsequently processed by Drosha into ~70 nucleotide microRNA precursors (pre-microRNAs) that are translocated to the cytoplasm and processed by Dicer into double-stranded ~22 nucleotide microRNAs. Then, one of the microRNA strands is assembled to the RNA-induced silencing complex (RISC), followed by binding to the target mRNA and consequent translation inhibition and/or degradation [147].

In recent times, it has been clear that microRNAs are implicated in iron metabolism regulation, as well as, in the development of iron-related disorders. Most of microRNAs involved in those processes were reviewed by Davis and Clarke [148] but, more recently, few others were reported (Table 1). The latter group consists of microRNAs-130, -199a, -221, -200a and -223. MicroRNA-130 was found upregulated in the liver of iron-deficient mice. It targets the BMPR Alk2 and, consequently, represses hepcidin expression [149]. MicroRNA-199a targets the 3′-UTRs of HIF-1α and HIF-2α. Under hypoxia, the microRNA-199a expression is decreased, which consequently promotes increased HIF levels. It was demonstrated a regulatory relationship between microRNA-199a and HIF with implications in ovarian cancer metastasis [150]. Moreover, microRNA-221 was shown to regulate *TFR2* expression in an *in vitro* model of Parkinson disease [151]. Finally, both microRNA-200a and microRNA-223 expressions are inversely correlated with the expression of IRP1 in mice with induced nonalcoholic fatty liver disease [152].

Interestingly, iron has been shown to be a crucial element during the biogenesis of microRNAs. For instance, the activity of DGCR8 (a co-factor for Drosha) is affected *in vitro* by the absence of heme-iron and, consequently, it perturbs the processing of pri-microRNAs to pre-microRNAs [153]. Moreover, it was also found that the free cytosolic iron prevents pre-microRNA processing to mature microRNA by 'sequestering' PCBP2, a co-factor for Dicer protein [154].

4.2.2.5. Proteolytic cleavage. The production of many secreted proteins is dependent on the proteolysis of a precursor protein at specific internal sites by a conserved family of proprotein convertases (PCs) [155]. The shedding of TfR1 from cellular membrane is mediated by PC7. This is a ubiquitously expressed convertase which is located within the *trans*-golgi network and the cell surface [156]. The function of the formed soluble TfR1 (sTfR1) remains unclear. However, its levels in plasma are useful to differentiate between anemia of inflammation and iron deficiency anemia. PC7 mRNA and protein levels are regulated by iron status [156]. As described before, membrane Hjv may also be cleaved giving rise to sHjv, an antagonist of the membrane Hjv by sequestering

Table 1
Iron metabolism genes which expression is regulated by microRNAs.

Target	MicroRNA	Reference
HFE; *HJV*	miR-122	[183]
TFR1	miR-320, miR-210	[184,185]
ISCU1/2	miR-210	[185]
Lactoferrin	miR-214	[186]
Lactoferrin receptor	miR-584	[187]
DMT1-non IRE	miR-let-7	[188]
BACH1	miR-let-7, miR-98, miR-196	[189,190]
FTH	miR-200b	[191]
SLC40A1	miR-485-3p	[192]
ALK2	miR-130	[149]
HIF-1α, *HIF-2α*	mir-199a	[150]
TFR2	miR-221	[151]
IRP1	miR-200a, miR-223	[152]

BMPs in the plasma and ultimately preventing the activation of *HAMP* expression [71,81–83]. The cleavage of Hjv is mediated by the PC-like protein, furin.

Hepcidin is initially expressed as pre-proprotein [59]. This protein precursor is presented as an 84 aa peptide that contains a N-terminal 24 aa sequence that targets the nascent protein for the ER. To be matured, hepcidin undergoes two cleavages to remove firstly the signal peptide sequence and then the pro-region. Since prohepcidin precursor has a signal peptide for the ER it is believed that the first cleavage occurs in this compartment through an enterokinase-like protein, forming the prohepcidin. As prohepcidin keeps to be processed, it may be translocated to the *trans*-golgi network where it is cleaved on its pentarginyl motif by furin [60,157]. This way it is formed the bioactive 25 aa hepcidin, that is secreted to the circulation.

5. Iron metabolism disorders

Despite the existence of a highly organized and regulated network that keeps the body iron homeostasis, several factors either at genetic, physiological or environmental levels may drive to the development of iron overload or deficiency conditions (Table 2). During iron overload it is observed a deposition of this metal in the liver, heart, as well as in other tissues [158]. When the iron overload is triggered by an increase in dietary absorption, iron accumulates in parenchymal cells of the liver, forming a gradient of deposition decreasing from the periportal hepatocytes to the centrilobular hepatocytes. If the origin of iron overload is associated with an uncontrolled release from macrophages, iron accumulates at the mesenchymal level [159]. Here, it is observed a punctual deposition of iron in the hepatocytes that surround the macrophages.

The iron overload disorders are classified as genetic or acquired. Within the genetic disorders we can find the four types of hemochromatosis, aceruloplasminemia, atransferrinemia and others [159]. The acquired iron loading diseases are not directly originated by mutations in iron metabolism genes. They give rise to a secondary iron overload.

The deficiency of iron in the organism results in the development of several types of anemia. Iron deficiency may also be classified as acquired or genetic. The most common examples of iron deficiency pathologies are the iron deficiency anemia (IDA), anemia of chronic diseases (ACD) and the iron-refractory iron-deficiency anemia (IRIDA) [160–162].

5.1. Iron overload disorders

Hereditary hemochromatosis (HH) is the most frequent iron overload disease characterized by an excessive parenchymal iron deposition in vital organs such as the liver, heart and pancreas. The several hereditary forms conduce to the same pathological mechanism of entry of iron to the plasma in amounts higher than the required to sustain the iron-depending processes, such as the erythropoiesis. The severity of the different forms of HH is variable, however they present common features. The uncontrolled plasma iron ingress results primarily in an increased Tf saturation and then in parenquimal iron deposition at several organs, what may drive to cirrhosis, hypogonadism, cardiomyopathy, arthropathy, diabetes and hepatocellular carcinomas [163].

The molecular mechanism that is usually affected in HH is the iron sensing-hepcidin axis. In this disease, hepcidin levels are abnormally low despite the high iron status presented by the body [164–168]. According to the clinical outcomes of the disease and the genes affected, HH is divided in four types: (i) *HFE*-related HH, (ii) juvenile hemochromatosis, (iii) *TFR2*-associated hemochromatosis and (iv) ferroportin disease.

The *HFE*-related HH, also known as classic HH or HH-type I is the mildest form of the disease, being characterized by a gradual deposition of iron in organs along life with major clinical manifestations in males with ages higher than 40–50 years old and post-menopausal women [163]. It is characterized by elevated Tf saturation (TfSat) and sFt levels. Clinically it is responsible for fatigue, dark skin and hepatomegaly symptoms. It represents approximately 90% of the total cases of hemochromatosis [169]. The most frequent mutation associated with *HFE*-related HH is the p.C282Y [164]. It is most frequent in individuals of north-European descent, with an allelic representation of approximately 6%. It is estimated that 1:200 to 1:300 Caucasians are homozygous for this mutation [170]. The majority of the individuals with hemochromatosis are actually homozygotes for p.C282Y. Biologically, the cysteine–tyrosine substitution disrupts the formation of the disulfide bond that physiologically occurs between C225 and C282 residues, and that is essential for HFE association with beta2-microglobulin (β_2M) [171]. Therefore the p.C282Y mutated HFE protein is unable to bind to β_2M and to be transported to the cell surface where it would interact with TfR1 and TfR2 in order to regulate hepcidin expression. The retention of the C282Y mutated HFE in the ER is known to trigger the unfolded protein response (UPR) [172]. It has been shown that the efficiency of this process may modulate the clinical outcomes of

Table 2
Most common features of iron metabolism disorders.

Pathology		Affected gene(s)	Mode of transmission	Age of clinical onset (years)	Clinical and biochemical features
Hereditary hemochromatosis (HH)	Type I	*HFE*	Recessive	40–50	High sFt, high TfSat fatigue, tissue iron deposition, hepatomegaly.
	Type II (juvenile HH)	*HAMP* *HJV*	Recessive	15–20	High sFt, high TfSat cardiomyopathies and reproductive defects.
	Type III	*TFR2*	Recessive	30–40	High sFt, high TfSat fatigue, tissue iron deposition.
	Type IV (ferroportin disease)	*SLC40A1*	Dominant	40–50	Ferroportin disease, form A — high sFt, normal or low TfSat, iron accumulation within splenic and hepatic macrophages.
				10–80	Ferroportin disease, form B — high sFt, high TfSat, tissue iron accumulation, preferentially within hepatocytes, cardiomyopathies, and reproductive defects.
Hyperferritinemia–cataract syndrome		*FTL*	Dominant	Lifespan	High sFt, normal TfSat, cataracts.
Aceruloplasminemia		*CP*	Recessive	Lifespan	Retinal degeneration, ataxia, dementia, diabetes, parenchymal iron accumulation.
Atransferrinemia		*TF*	Recessive	Lifespan	Microcytic and hypochromic anemia, tissue iron overload, increased sFt, low Tf.
Iron deficiency anemia (IDA)		n.a.	n.a.	Lifespan	Microcytic and hypochromic anemia, fatigue, immune dysfunction, neurocognitive impairment.
Anemia of chronic diseases (ACD)		n.a.	n.a.	Lifespan	Microcytic and hypochromic anemia, fatigue, immune dysfunction, neurocognitive impairment.
Iron-refractory iron-deficiency anemia (IRIDA)		*TMPRSS6*	Recessive	New borns	Microcytic and hypochromic anemia, unresponsive to treatment with oral and intravenous iron

hemochromatosis. C282Y patients expressing higher amounts of calreticulin (a protein involved in UPR) present a lower phenotypic presentation of the disease [173].

Another *HFE* variant has also been associated with *HFE*-related HH, the p.H63D [164]. This variant has a higher prevalence in the general population than the p.C282Y (the average allelic frequency of H63D is 14%); however, only when presented in a compound heterozygous state with p.C282Y is considered as a risk factor for the development of HH [174].

The other forms of HH present worst clinical outcomes than the ones driven by the *HFE*-related HH. The Juvenile hemochromatosis (JH), also known as HH-type II is a rare disease of iron overload metabolism. It occurs mainly due to mutations in the *HJV* and *HAMP* genes [165,166]. The age of onset of the disease is at 15–20 years old and the clinical outcomes of the disease are cardiomyopathies and reproductive defects. It is characterized by a severe iron overload driven by the full saturation of Tf, high sFt levels and iron deposition [163].

The HH-type III is similar to the HFE-related HH. However, it is originated by mutations in the *TFR2* gene. It leads to increased TfSat and sFt at the average age of 30–40 years old [163,168]. Finally, the HH-type IV, also known as ferroportin disease, is an iron overload disease with a autosomal dominant transmission, associated with mutations in the *SLC40A1* gene. Two forms are considered. In form A (also known as classic) the ferroportin loss of function mutants are unable to export iron from cells leading to cellular (especially macrophage) iron accumulation with decreased availability of iron for serum transferrin, which is reflected in low transferrin saturation [163]. On the other hand, in ferroportin disease form B mutations are responsible for a gain of function with full iron export capacity but insensitivity to down-regulation by hepcidin, so iron is constitutively absorbed at the enterocyte and released by cells, resulting in increased TfSat and parenchymal iron overload [167].

In addition to the above described types of hereditary hemochromatosis other iron overload disorders may occur. One is caused by mutations in *FTL* which may drive to increase of ferritin levels in young individuals and consequently the development of the hyperferritinemia–cataract syndrome [175]. Another rare iron metabolism-related disease is aceruloplasminemia which arises as a consequence of mutations in the *CP* gene. Due to the importance of the ceruloplasmin protein in the brain, the main symptoms of aceruloplasminemia are brain iron deposition, retinal degeneration, ataxia and dementia [176]. On the other hand, atransferrinemia is a microcytic and hypochromic anemia associated with tissue iron overload, increased sFt and low levels of Tf due to mutations in transferrin gene [177]. While the hyperferritinemia–cataract syndrome has a dominant pattern of transmission, aceruloplasminemia and atransferrinemia are recessive disorders.

5.2. Iron deficiency pathologies

Iron deficiency anemia (IDA) is the most common nutritional deficiency worldwide. It occurs more frequently in determined groups of individuals, such as the premenopausal woman, children, hospitalized individuals that require frequent diagnostic blood sampling and blood loosing patients [178]. Also, it may arise as a consequence of an iron-poor diet. The most harmful outcome of IDA is a microcytic and hypochromic anemia [160] that, in its worst degrees is manifested by fatigue, impairment of thermoregulation, immune dysfunction and neurocognitive damage that can lead to psychomotor and cognitive abnormalities in children [179,180].

Anemia of chronic diseases (ACD) is a condition that arises from individual presenting a permanent state of inflammation as a consequence of a chronic disease [161]. During inflammation, macrophages produce high levels of IL-6, the pro-inflammatory cytokine that triggers the expression of hepcidin and, consequently, lowers dietary iron absorption and stored iron release [70], leading to anemia development.

Despite the acquired conditions of iron deficiency, there are also genetic determinants responsible for the etiology of anemia. The iron-refractory iron-deficiency anemia (IRIDA) is one of these examples [181]. It is characterized by congenital microcytic and hypochromic anemia, low mean corpuscular erythrocyte volume, low TfSat, unresponsiveness to oral iron administration, abnormal iron utilization after parenteral iron administration and a recessive pattern of transmission. IRIDA is detected in early life and is developed due to mutations in the MT2 producing gene (*TMPRSS6*) [182]. The spectrum of *TMPRSS6* mutations associated with IRIDA is wide. Since matriptase-2 is a negative regulator of hepcidin expression by the cleavage of membrane Hjv, levels of hepcidin in IRIDA patients are extremely high and consequently the absorption of dietary iron by the duodenum is impaired [85].

6. Conclusions

In conclusion, iron is an essential element for a vast range of biological processes, however, it does also have toxic properties when on its free state. In this manuscript we have reviewed the molecular mechanisms responsible for the maintenance of iron homeostasis in the organism. We have also described the most common pathologies that may arise from the dysregulation of those mechanisms. However, the field of iron metabolism regulation is far from being understood, therefore more research is needed to deepen the knowledge in this field. While hepcidin has been assumed, for long years, as being the primordial regulator of iron homeostasis, we cannot neglect the vital importance of other mechanisms involved in iron metabolism regulation. In future, those mechanisms might be considered for the design of new effective therapeutic strategies able to greatly reduce iron-related disorders.

Conflict of interest

The authors declare no conflict of interest.

Transparency documents

The Transparency documents associated with this article can be found, in online version.

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