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Host and microbiota derived extracellular vesicles: Crucial players in iron homeostasis

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Iron is a double-edged sword. It is vital for all that's living, yet its deficiency or overload can be fatal. In humans, iron homeostasis is tightly regulated at both cellular and systemic levels. Extracellular vesicles (EVs), now known as major players in cellular communication, potentially play an important role in regulating iron metabolism. The gut microbiota was also recently reported to impact the iron metabolism process and indirectly participate in regulating iron homeostasis, yet there is no proof of whether or not microbiota-derived EVs interfere in this relationship. In this review, we discuss the implication of EVs on iron metabolism and homeostasis. We elaborate on the blooming role of gut microbiota in iron homeostasis while focusing on the possible EVs contribution. We conclude that EVs are extensively involved in the complex iron metabolism process; they carry ferritin and express transferrin receptors. Bone marrow-derived EVs even induce hepcidin expression in β -thalassemia. The gut microbiota, in turn, affects iron homeostasis on the level of iron absorption and possibly macrophage iron recycling, with still no proof of the interference of EVs. This review is the first step toward understanding the multiplex iron metabolism process. Targeting extracellular vesicles and gut microbiota-derived extracellular vesicles will be a huge challenge to treat many diseases related to iron metabolism alteration.

KEYWORDS

iron metabolism, extracellular vesicles, gut microbiota, ferritin, transferrin receptors, hepcidin

Abbreviations: EVs, extracellular vesicles; ROS, reactive oxygen species; DMT1, divalent metal-ion transporter 1; Dcytb, duodenal cytochrome b; TF, transferrin; FPN, ferroportin; STEAP3, six-transmembrane epithelial antigen of prostate 3; HMOX1, heme oxygenase 1; IRPs, iron regulatory proteins; IREs, iron-responsive elements; HIFs, hypoxia inducible factors; TFR 1, transferrin receptor 1; TFR 2, transferrin receptor 2; JAK/STAT, Janus kinase/signal transducers and activators of transcription; MDVs, mitochondria derived vesicles; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; DAP, 1,3-diaminopropane; SCFA, short chain fatty acids.

Introduction

Iron is a vital trace element, essential for several fundamental processes: globin synthesis and erythropoiesis (1), energy production (2), DNA synthesis and repair (3), and immune function (4). Its mode of action lies in its ability to reversibly gain or lose a single electron to participate in oxidation-reduction reactions, which also catalyze the generation of reactive oxygen species (ROS) (5). Hence, in spite of its vital role iron overload is toxic due to its ability to generate ROS and trigger cell death (6). Excess of free reactive iron leads to several types of cell death, including ferroptosis, the oxidative cell death prompted by the accumulation of iron-mediated lipid peroxidation (6).

No wonder, iron metabolism is a tightly regulated process. The regulation of iron metabolism occurs at a cellular level via transcriptional and post-transcriptional regulation of iron genes. At the systemic level, iron metabolism is regulated via the hepatic hormone hepcidin, which regulates iron absorption, plasma concentrations, and tissue distribution (7). The human microbiome, in turn, was recently identified as an effector in the iron metabolism regulation process, considering the fact that the microbiota also requires iron to maintain symbiosis. The composition of the gut microbiota is affected by iron availability, and microbiota-derived metabolites were reported to impact both iron absorption (enterocyte) and iron recycling (macrophage).

The iron metabolism process and its regulation are highly complex; up till now, there remain a lot of missing links. Ferritin, a main cargo of blood iron, and transferrin receptors, involved in the cellular membrane transportation of iron, have been identified in vesicular locations (8–10). Also, bone marrow-derived extracellular vesicles (EVs) in β thalassemia patients were reported to affect hepcidin production (11). However, no paper has yet discussed the possible role of EVs in iron homeostasis. Considering the blooming significance of extracellular EVs in cell–cell communication, delivering cargo, and modulating the physiological condition (12); we suggest that EVs might play a role in transporting iron regulators thus maintaining iron homeostasis.

Here we will discuss the critical process of iron metabolism and the systems of its regulation, while focusing on the potential interference of extracellular vesicles. We will also discuss the role of gut microbiota and its metabolites in iron homeostasis, and consider the potential involvement of microbiota-derived extracellular vesicles in this relationship.

Iron metabolism

Iron metabolism consists of iron absorption, use, storage, and transfer. Absorbable dietary iron can be in the form of heme and non-heme iron (13). Heme iron contributes to 10–15% of

the absorbed dietary iron. It is more absorbable by the body (15–35%) as compared to non-heme iron (2–20%) (14). Even though heme iron absorption surpasses that of non-heme iron, its absorption mechanisms remain ambiguous. Non-heme iron is absorbed at the level of the duodenum and proximal jejunum, by enterocytes divalent metal-ion transporter 1 (DMT1), after being reduced to ferrous iron (Fe^{2+}) via duodenal cytochrome b (Dcytb) (15, 16). If not needed inside the cell, ferrous iron, is either stored in the form of ferritin or transferred to circulating transferrin (TF) via the iron exporter ferroportin (FPN), after being oxidized to ferric iron (Fe^{3+}) by hephaestin (17). Transferrin bound iron is delivered to sites of utilization, where it binds to cell surface transferrin receptor 1 (TFR1) and endocytose into the cell (18); it then enters the cytoplasm via DMT1 in the endosomal membrane, after being reduced to (Fe^{2+}) under the action of six-transmembrane epithelial antigen of prostate 3 (STEAP3) (19). This iron can be used for metabolic functions, or stored within cytosolic ferritin- iron in the ferric form associated with hydroxide and phosphate anion (20).

Merely 1–2 mg of dietary iron are absorbed in the gut daily; most iron is recycled upon phagocytosis of erythrocytes by macrophages (21). Senescent/damaged erythrocytes are phagocytosed by macrophages. Macrophages recover iron from heme via heme oxygenase 1 (HMOX1) for utilization, conservation or recycling depending on the body needs (22).

Iron homeostasis

The iron metabolism process is highly critical, considering the danger of excess free reactive iron. It is supposed to be tightly regulated. Iron homeostasis is essential to maintain normal physiology. To achieve homeostasis, iron regulation takes place at both cellular and systemic levels. At the cellular level, iron homeostasis involves mechanisms that balance iron uptake with intracellular iron storage and utilization (23). Systemic iron homeostasis embodies the mechanisms that synchronize dietary iron absorption and iron concentration in plasma and the extracellular milieu (24).

Iron homeostasis at the cellular level

At the cellular level, iron metabolism regulation is based on transcriptional and post-transcriptional regulation of iron genes. Post transcriptional regulation is facilitated by the binding of iron regulatory proteins (IRPs) to iron-responsive elements (IREs) of their mRNA untranslated regions (25). In case of iron-deficiency, IRP1 and IRP2 bind to the IREs in TFR1 mRNA and stabilize it, thus increasing iron uptake. They also bind to the 5'-UTR of the mRNAs that encode ferroportin and ferritin to suppress their translation, thus blocking iron export and storage (26). Conversely in iron-replete cells, iron

can bind IRPs and induce their conformational change. Notably, IRP1 assembles an aconitase-type 4Fe-4s and this assembly alters its conformation (27, 28). The latter conformational changes weaken the IRPs IRE-binding ability, thus leading to (a) destabilization and degradation of TFR1 mRNA and (b) facilitated translation of the target 5'-UTR mRNA encoding ferroportin and ferritin (29). Micro RNAs (miRNAs) also count as significant posttranscriptional regulators of gene expression. They can regulate cellular iron homeostasis by influencing iron absorption, transport, storage, and utilization. For example, miR-Let-7d was reported to target the DMT1-non-IRE isoform (30) and miR-320 was found to post-transcriptionally control TfR1 expression (31). The mRNA encoding FPN in turn was shown to be targeted by miR-485-3p (32). Furthermore, the storage of iron as ferritin is downregulated by miR-200b (33).

Transcriptional regulation is dependent on hypoxia inducible factors (HIFs). Hypoxia inducible factor -2 α (HIF-2 α) plays an important physiological role in transcriptional regulation of iron homeostasis. HIF-2 α regulates iron absorption notably by activating the expression of DcytB and DMT1 proteins during iron deficiency or ineffective erythropoiesis to increase iron uptake (34, 35). In fact, HIF-2 α mRNA 5'-UTR contains an IRE that binds both IRP1 and IRP2 (36, 37) for iron-dependent regulation of the transcript. By regulating HIF-2 α mRNA, IRP1 amends the erythropoietic response to hypoxia. This IRP1-HIF-2 α axis synchronizes both iron and oxygen sensing with erythropoiesis and iron absorption (38). Furthermore, HIF regulates hepcidin, the orchestrator of systemic iron homeostasis, via erythropoietin-induced erythropoiesis. HIF can suppress the hepcidin gene *Hamp1* indirectly through erythropoietin-induced erythropoiesis (39, 40).

Iron homeostasis at the systemic level

Systemic iron homeostasis is regulated via hepcidin, a hormone that is primarily secreted by hepatocytes. Iron and inflammatory cytokines induce hepcidin expression, while iron deficiency, erythropoiesis, and anemia/hypoxia downregulate it (17). High circulating iron levels upregulate hepcidin expression by hepatocytes, through the BMP/SMAD pathway (41). The binding of transferrin to transferrin receptor 2 (TFR2), to which it has less affinity than TFR 1, in case of high plasma iron concentration, has been anticipated to affect hepcidin expression. The binding of diferric transferrin to TFR2 induces overexpression of hepcidin in hepatocytes and reduced erythropoietin responsiveness in erythroid cells (42), where TFR2 binds erythropoietin receptors (43). Inflammatory cytokines upregulate hepcidin gene expression through the Janus kinase/signal transducers and activators of transcription (JAK/STAT); for example, IL-6, increases hepcidin expression via activating the IL-6R-JAK2-STAT3 pathway (44). When

activated, hepcidin combines FPN1, internalizes it, and degrades it in the lysosome (45), thereby reducing iron absorption by duodenal cells and iron recycling by macrophages.

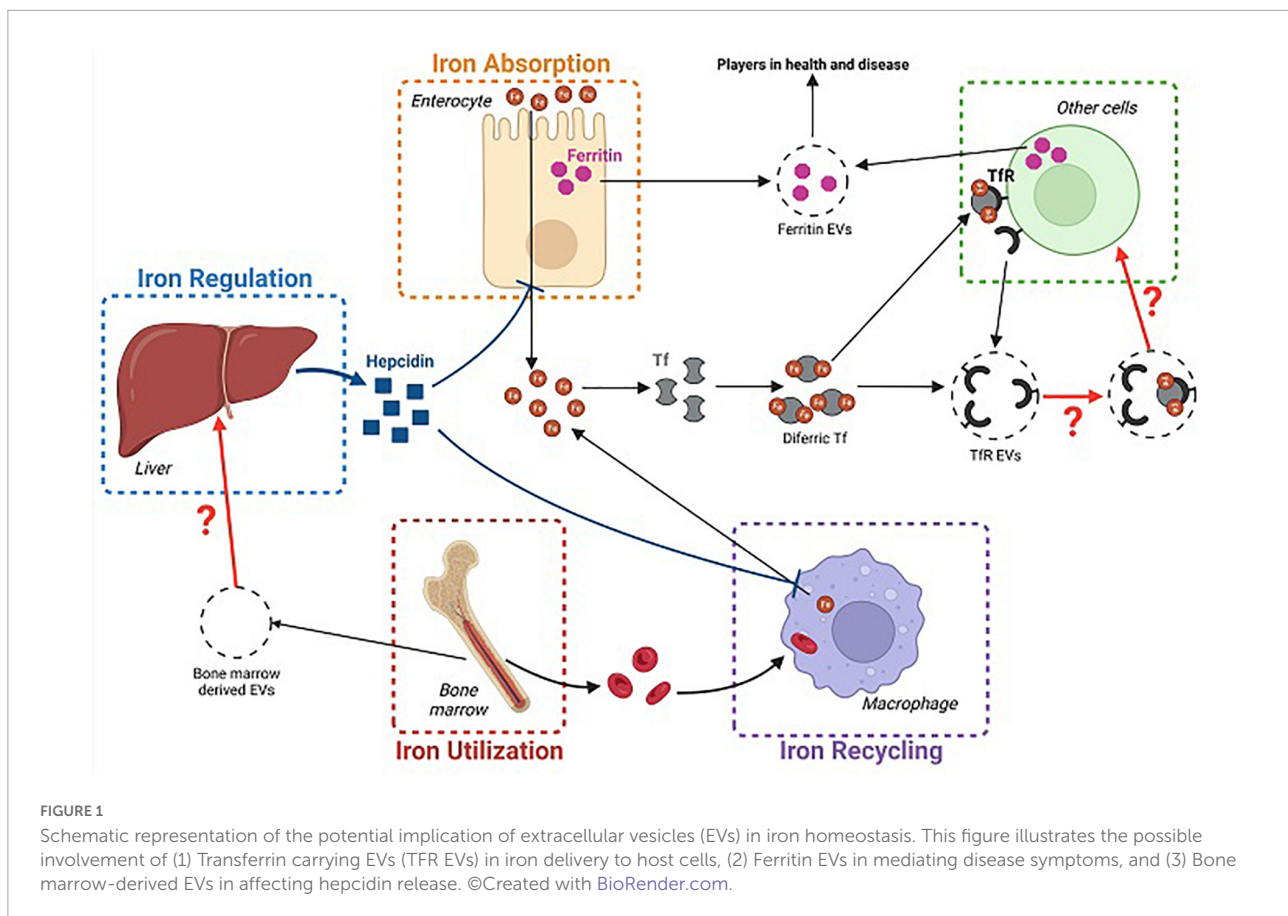
Extracellular vesicles: Key players in iron homeostasis?

Extracellular vesicles are membrane-bound vesicles secreted by cells into the extracellular space. They constitute microvesicles, exosomes, and apoptotic bodies- released by dying cells; they vary in size with microvesicles being the smallest (less than 100 nm–1 μ m), followed by exosomes and apoptotic bodies, respectively (46). They function by facilitating the intercellular exchange of proteins, lipids, and genetic material, thus facilitating intercellular signaling/communication (47). While it is known that iron metabolism requires a lot of cell communication, the exact role of EVs in either iron metabolism or the maintenance of iron homeostasis remains to be elucidated. The putative role of EVs in iron homeostasis is summarized in [Figure 1](#).

Ferritin extracellular vesicles and their fate

Iron is stored in the cells in the form of ferritin, as mentioned before. The synthesis and degradation of ferritin are both orchestrated by cellular iron status. Under low iron levels, ferritin synthesis decreases via translational repression (48), and ferritin lysosomal degradation, mediated by the selective autophagy receptor NCOA4, increases. The degradation of the ferritin to free the iron stored is a selective macro-autophagy mechanism called ferritinophagy and is mediated by NCOA4 (49). On the other hand, high iron levels decrease the entry of ferritin into the lysosome (50). Ferritin can be found in the cytoplasm (51), nucleus (52), and mitochondria (53). However, it has also been observed in vesicular locations.

Some researches succeeded to report the presence of ferritin in human urinary exosomes, via a large-scale proteomic analysis (9). However, there is a gap in our knowledge underlying ferritin intracellular trafficking and secretion. It was thought to be secreted through the Endoplasmic Reticulum–Golgi route (54), but Cohen et al. showed that ferritin is secreted primarily by macrophages through lysosomal pathway, by secretory lysosomes (55). Truman-Rosentsvit et al. provided evidence on the secretion of ferritin via the multivesicular body–exosome pathway (56). Yanatori et al. studied the mechanism of secretion of ferritin in extracellular vesicles (57). They discovered that CD63, which plays a role in EVs secretion (58), is post-transcriptionally regulated by iron via the IRE-IRP system, the system that induces ferritin under iron upsurge (59). Thus, increased levels of iron induce CD63 expression. Upon loading



iron, intracellular ferritin is transported via nuclear receptor coactivator 4 (NCOA4)/ferritin vesicles to $CD63^+$ EVs that are secreted (57).

Exosomes play a role in iron homeostasis after all, but the destination of ferritin carried by the exosomes remains to be explored. Is it transferred to hepatocytes, the major sites for iron storage? Can those exosomes fuse with the free mitochondria and deliver ferritin to them? Can the ferritin stored in mitochondria be released via mitochondria-derived vesicles (MDVs) too? Vasam et al. had reported that MDVs revealed high levels of mitochondrial iron-sulfur clusters biogenesis proteins, that are responsible for the biogenesis of iron-containing cofactors, and iron-binding capable proteins. They suggested that MDVs can serve as a potential source of biomarkers for mitochondrial stress (60).

The serum ferritin level mirrors the body's iron stock; it is considered a hematologic index for iron-associated diseases (61). Whether this serum ferritin is contained in EVs or possibly released from ferritin carrying EVs is ambiguous. Excessively elevated levels of both ferritin (62, 63) and EVs (64) have long been noted in the circulation of iron overload β -thalassemia patients. Recently, Atipimonpat et al. reported the presence of high levels of ferritin-bearing exosomes in the plasma of β -thalassemia patients (65). They explained that those high levels

of circulating red blood cells and activated platelets derived EVs, especially ferritin-carrying exosomes, can speed up the proliferation of H9C2 cardiac cells leading to cell hyperplasia, progression of cardiac hypertrophy, and eventually heart failure. What remains to be clarified is whether iron overload can induce or increase the ferritin-loaded exosomes and if ferritin-loaded exosomes can contribute to disease symptoms.

Strzyz in turn reported ferritin exosomes to induce ferroptosis resistance (66). In this regard, exosomes provide a route for ejecting iron out of ferroptotic cells, thus protecting them from ferroptosis. Accordingly, Mukherjee et al. reported that disrupting the EV release or ferritin heavy chain expression in oligodendrocytes resulted in neuronal loss and oxidative damage in mice (67). In case of cancer, carcinoma cells may use this iron export pathway involving multivesicular body/exosome trafficking of iron out of the cell to avoid ferroptotic death (68). Interestingly, a study conducted by Ito et al. showed that macrophages that engulf asbestos produce ferroptosis-dependent extracellular vesicles that contain ferritin and transport it to mesothelial cells, thereby contributing to mesothelial carcinogenesis by loading ferritin (69). Alterations in EV levels during therapy have been reported in Glioblastoma patients; interestingly the EV protein signature showed common iron metabolism proteins and disappeared

post-surgical resection (70). We can speculate that ferritin extracellular vesicles can halt ferroptosis in health and in disease. They show potential in becoming biomarkers for disease diagnosis, notably cancer.

Transferrin receptor carrying extracellular vesicles

Transferrin receptor expression was also detected in exosomes. The presence of Glycerinaldehyde-3-phosphate dehydrogenase (GAPDH), which has been characterized as a transferrin receptor (71, 72), in exosomes from different cell lines was confirmed by Malhotra et al. along with its ability to bind transferrin (10). They also reported that iron preloaded exosomes delivered more iron into various cells, thus raising an interesting chance of exosomes playing a role in the delivery of iron and iron homeostasis. Interestingly a recent study conducted by Dar et al. demonstrated that GAPDH also induces clustering of EVs *in vitro* and *in vivo* (73). Further, Mattera et al. identified the expression of TFR1 in extracellular vesicles derived from human and mouse plasma, rat oligodendrogloma cells, mouse neuroblastoma cells, and rat astrocytes (8). However, no research has been conducted on the presence of TFR2, that induces hepcidin expression upon binding diferric transferrin, in hepatocytes and erythroblasts derived EVs.

Extracellular vesicle-mediated hepcidin modulation

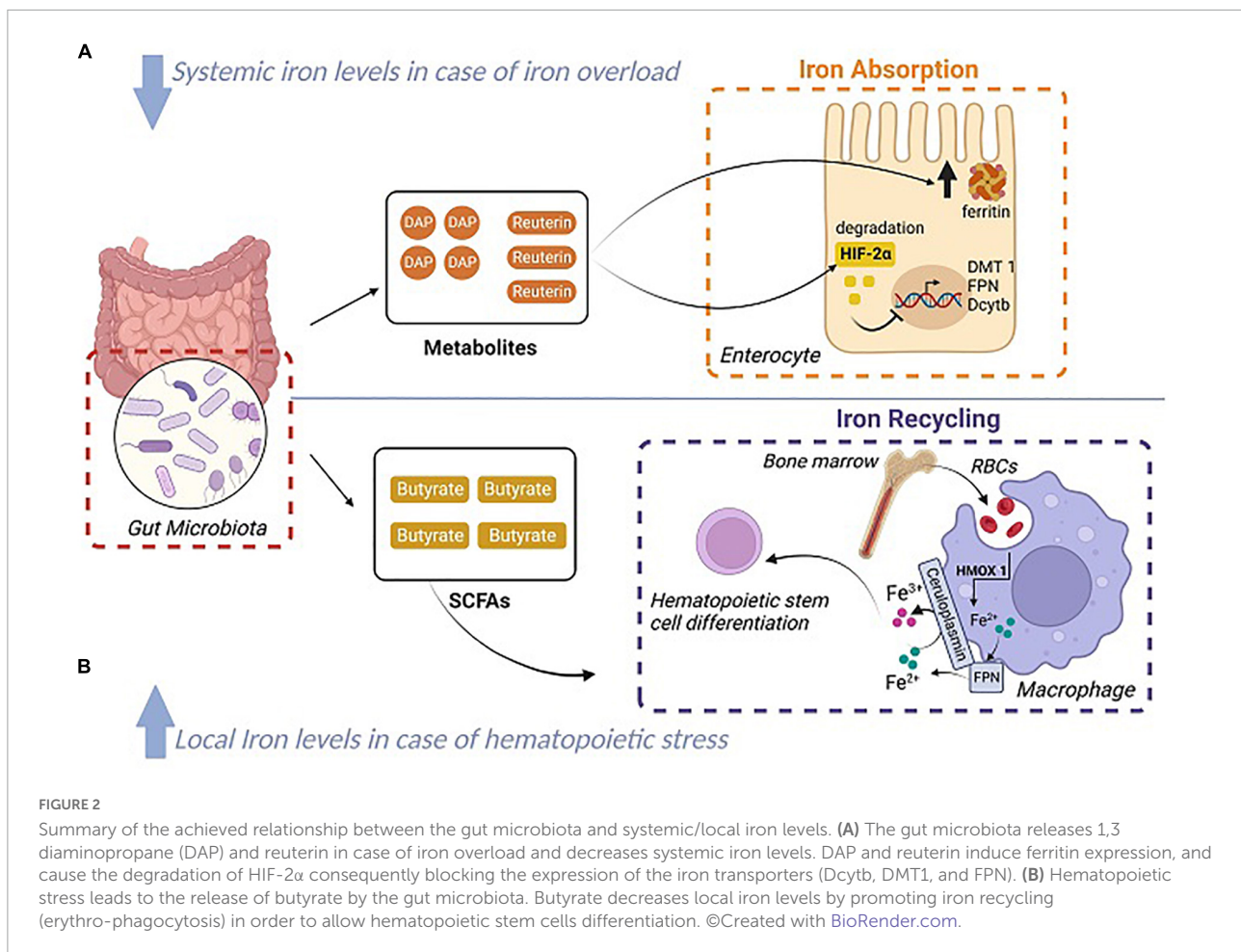
Hepcidin, the key iron regulator, is produced primarily by hepatocytes neighboring the portal veins and Kupffer cells (7), macrophages (74), adipocytes (75), and dendritic cells (76). Also, several studies have reported local synthesis of hepcidin by multiple other tissues, notably in disease, like the and the lungs (77), kidney (78), stomach (79), adipose tissue (75), brain (80), heart (81), and even the skin (82). The organ that produces the most hepcidin after the liver is the heart (83). As mentioned before, under active erythropoiesis, hepcidin production is somewhat inhibited via erythroferrone, an erythroid factor produced by the erythroblasts, that suppresses the BMP/SMAD pathway in the liver (84). β -Thalassemia patients are known to have ineffective erythropoiesis and iron overload. This ineffective erythropoiesis suppresses hepcidin leading to iron overload (85). Ruiz Martinez et al. hypothesized that bone-marrow derived exosomes modulate hepcidin expression and regulate iron metabolism (11); they investigated the link between exosomes and hepcidin regulation in β -thalassemia. They were able to demonstrate that those exosomes boost hepcidin expression by increasing SMAD1/5/8 signaling. Increased hepcidin, in

response to exosomes, will possibly influence several signaling pathways by an autocrine mechanism. Exosomes compensated for suppressed hepcidin in the exosome-depleted serum of β -thalassemic samples. Proteomic analysis of β -thalassemic patients' bone marrow derived exosomes can help us better understand their role in hepcidin regulation. On the other hand, EVs derived from the plasma of β -thalassemic patients showed dysregulation of certain miRNAs involved in oxidative stress, erythropoiesis, and apoptosis- in particular overexpressed miR-144-3p. They were reported to induce apoptosis in endothelial, pancreatic, and hepatic cells, possibly contributing to the organ damage in β -thalassemia (86). Thus, studying EVs derived from β -thalassemic patients not only can help to understand the implication of EVs in iron metabolism regulation, but they can also help to comprehend the health complication associated with β -thalassemia. They can also serve as biomarkers for β -thalassemia severity.

Gut microbiota: Modulation of iron homeostasis

Almost all living organisms require iron in order to survive. A deficiency or excess in iron is dangerous hence iron homeostasis is firmly regulated. Iron acquisition takes place at the level of the small intestine (87). The small intestine is colonized by symbiotic microorganisms, called the "gut microbiota," that share a mutually beneficial relationship with the host (88, 89). The gut microbiota relies on the host for nutrients and survival, while it plays an indirect role in regulating complex endocrine networks. Gut microbiota compete with the host to acquire iron, for survival. Studies have reported how iron deficiency/repletion in rats (90) or genetic modification of iron metabolism in mice (91) affect the gut microbiota composition and metabolic activity. The fecal microbiota has even been proposed as a non-invasive biomarker for tissue iron accumulation prediction in intestine epithelial cells and liver (92).

The gut microbiota must in turn also impact the iron absorption process and play an indirect role in regulating iron homeostasis. Most studies, however, focus on the strategies used by microbiota to acquire iron, studies on the possible role of microbiota in regulating iron homeostasis are scarce. Deschemin et al. were the first to investigate the impact of microbiota on host iron sensing (93). They demonstrated that gut microbes induce a specific iron-related protein signature and revealed a new feature of the microbiota – intestinal epithelium crosstalk. Further, Das et al., in their turn, then studied the impact of gut microbiota on iron intestinal absorption, and discussed its unforeseen role in regulating host iron homeostasis (94). They identified that the host iron-sensing mechanism is connected to the gut microbiome and regulated by it. They reported that, intestinal iron deficiency leads to the positive



selection of *Lactobacillus* species that produce reuterin and 1,3 diaminopropane (DAP). Those microbiota metabolites (a) suppress iron absorption, by inhibiting the transcription factor HIF-2 α that targets the expression of key iron transporters (34, 35, 95), and (b) induce ferritin expression to eventually lead to its degradation by the host in due to the iron overload caused - in order to maintain homeostasis (34); thereby indirectly preventing tissue iron accumulation (94).

From another perspective, Zhang et al. also reported that microbiota derived short chain fatty acids (SCFAs), specifically butyrate, can lead to iron distribution, for fueling hematopoietic regeneration, by promoting emergency erythro-phagocytosis by bone marrow macrophages (96). However, it is important to note that microbiota depletion resulted in only reduced local iron levels without affecting systemic iron homeostasis (96). Yet, future studies should further explore a possible involvement of a microbiota-macrophage-iron axis in iron homeostasis. All the current knowledge on the relationship between gut microbiota and host iron levels is summarized in **Figure 2**.

In reality, we are still in the infancy of understanding the metabolic crosstalk between gut microbiota and the host intestinal epithelium- in terms of iron homeostasis. A lot of

studies are required to identify the role of microbiota-derived metabolites in context of iron homeostasis, and to determine whether microbiota commensals can secrete hormones that directly regulate or impact iron metabolism. Additionally, it is important to study the potential of targeting gut microbiota therapeutically via prebiotics, probiotics, or fecal microbiota transplant for iron-related diseases.

Gut microbiota and hepcidin production: An intriguing question

As explained before, hepcidin is the central iron regulator, it is overexpressed in response to iron overload and inflammation (97); it blocks iron export (98) and degrades the iron exporter ferroportin (45), thereby reducing iron absorption by duodenal cells and iron recycling macrophages. The microbiota produces a wide range of metabolite-derived humoral agents including SCFA, secondary bile acids, and neurotransmitters all of which play important body functions (99). As mentioned above, *Lactobacillus* derived metabolites were lately reported to play an indirect role in regulating iron homeostasis.

The secretion of hepcidin by myeloid cells occurs via the toll-like receptor 4-hepcidin pathway as a host response to bacterial pathogens (100). Layoun and Santos showed that bacterial cell wall lipopolysaccharide induced hepcidin expression in macrophages (101). Microbiota dysbiosis in the inflamed intestine of humans was found to induce the release of hepcidin by conventional dendritic cells, for tissue repair (76). However, one intriguing question that remains to be answered is whether the microbiota can modulate iron homeostasis, directly, by producing hepcidin.

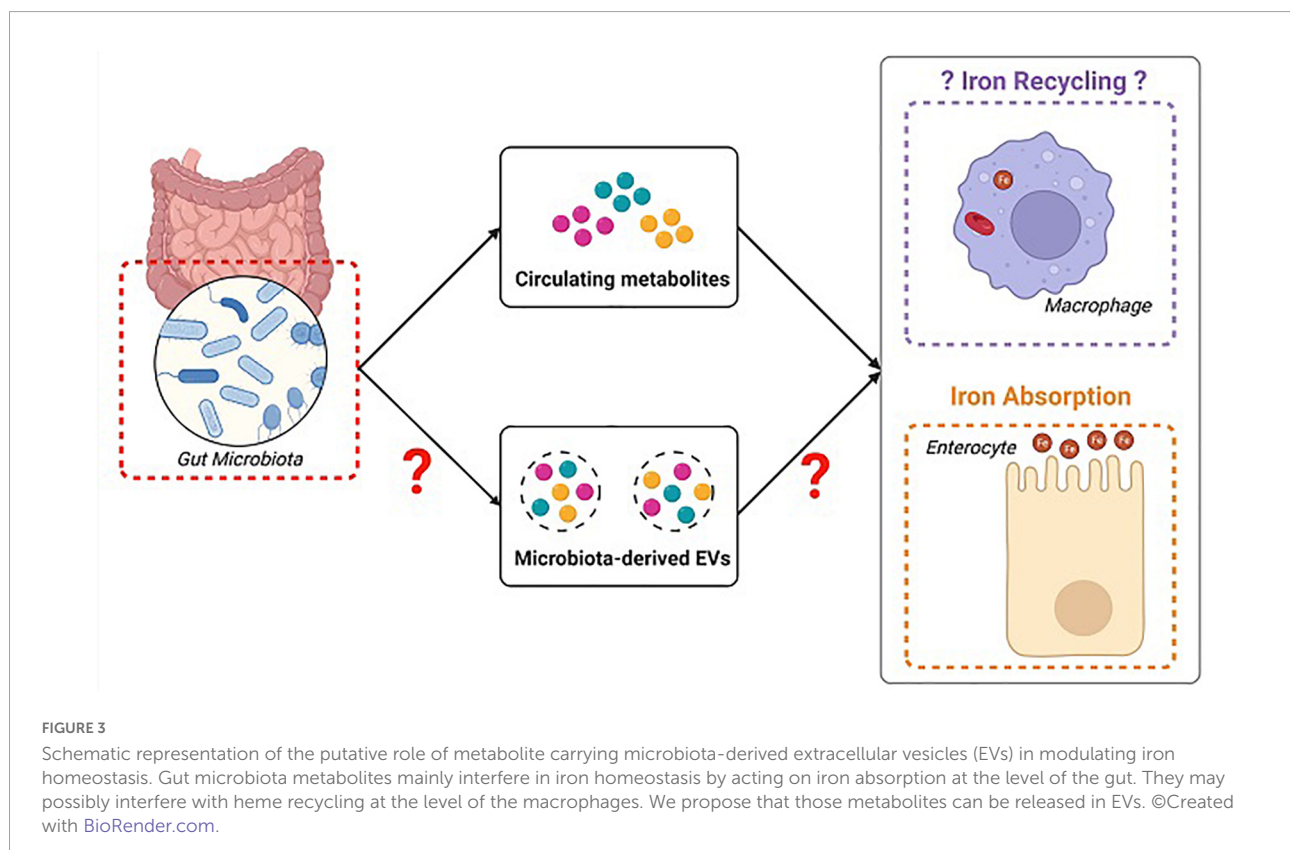
Gut microbiota derived extracellular vesicles: Players in iron homeostasis?

Iron is essential for the majority of microorganisms. Some bacteria have evolved efficient strategies to acquire iron from the host. The microbiota acquires iron from the host by using host iron compounds (e.g., heme, transferrin, lactoferrin, ferritin), producing high-affinity iron chelators called siderophores, and/or uptaking ferrous iron (102). Gram-negative bacteria derived outer membrane vesicles (103) and gram-positive bacteria derived extracellular vesicles (104) are well reported to play significant roles in bacterial survival, material exchange, cell-to-cell communication, and pathophysiology. EVs of some pathogenic bacteria are known to help them acquire iron from

the host (105). Additionally, bacteria-derived EVs (*Diaetzia sp.*) were found to allow homologous bacterial species to share iron (106).

Microbiota-derived EVs are involved in inter-kingdom communication with host cells in the gut (107, 108). They were reported to deliver to host cells effector molecules that modulate host signaling pathways and cell processes (108). The probable effects of gut microbiota-derived EVs on metabolic diseases such as obesity and diabetes have been reviewed (109). Knowing that the gut microbiota plays an indirect role in regulating the host's iron homeostasis, no research has yet addressed the potential role of microbiota-derived EVs in the regulation of iron homeostasis.

Bacteria can store iron in the form of bacterial ferritin, heme-containing bacterioferritin, and DNA binding dodecameric ferritin (110). The existence of bacterial ferritin, the prototype of ferritin that possesses a classical ferritin H-chain with ferroxidase activity (111), in microbiota-derived EVs might suggest their interference in host iron homeostasis. In fact, Zakharzhevskaya et al. were able to identify non-heme ferritin with special oxidoreductase activity in toxigenic *Bacteroides fragilis* (112). Further research is required to test for the presence of bacterial ferritin in microbiota-derived EVs and unravel the possibility of the association of bacterial ferritin-carrying vesicles with host iron homeostasis.



The presence of iron modulating microbial metabolites in microbiota-derived EVs should also be addressed. The potential implication of metabolite-carrying microbiota-derived EVs in the microbiota host iron regulatory mechanisms is depicted in **Figure 3**. As reported above, the gut microbiota plays a role in regulating the host's iron homeostasis via *Lactobacillus* derived metabolites reuterin and DAP (94). Moreover, proteomic analysis of *L. reuteri* derived EVs revealed the presence of different functional and structural proteins signifying the possible involvement of *L. reuteri* EVs in metabolism, transport, and signaling (113). Further studies ought to specifically test for the presence of the bacteriocins reuterin and DAP in *L. reuteri*-derived EVs, taking into consideration that species like *Lactobacillus acidophilus* can carry bacteriocin peptides via EVs in order to deliver them to opportunistic pathogens (114).

The presence of SCFAs specifically butyrate in EVs should also be tested to assess the prospective involvement of bacteria-derived EVs in modulating the iron recycling process. *Streptococcus pneumoniae* derived EVs for example were enriched in short chain saturated fatty acids (115).

Thus, the characterization of bioactive molecules and different types of cargo of gut microbiota- and probiotic-derived EVs is required. Once this is achieved, we can begin to explore the potential role of microbiota-derived EVs in regulating the host iron homeostasis. However, it is important to note that there are current limitations to characterize those EVs and their cargo due to the similarity between bacterial and mammalian EVs (107).

Conclusion

Maintaining iron homeostasis is a challenge reflected by the complexity of iron metabolism. EVs seem to be indispensable in mediating this process. They appear to be key players in iron homeostasis, ferritin is released via exosomes under high iron levels, transferrin receptors are also expressed in exosomes. The question of the destination of ferritin carrying EVs has yet to be answered. Is ferritin transferred via EVs to be stored in hepatocytes? Do they serve in delivering ferritin to free mitochondria? Do mitochondria export iron via MDVs? On another note, EVs should be tested for any ability to deliver iron to distal sites especially to the brain knowing that they can cross the blood brain barrier (116).

Extracellular vesicles are involved in iron regulation in health and disease. Bone marrow-derived EVs can regulate iron homeostasis by compensating for low hepcidin in β -thalassemic patients. However, plasma-derived EVs were also reported to contribute to organ dysfunction and complications of β -thalassemia. A better understanding of EVs composition and cargo might help identify new mechanisms underlying iron-overload diseases such as β -thalassemia or even treat them. Ferritin-containing EVs were reported to suppress ferroptosis in health, but also promote cell survival and proliferation in cancer.

This opens doors for better diagnostics and therapeutic options in the future; what if cancer can be diagnosed by identifying ferritin-containing EVs? What if it can be treated by targeting those EVs and modulating iron levels?

Achieving iron homeostasis is also linked to the gut microbiota, the endocrine organ that keeps surprising us with its importance day by day. Gut microbiota metabolites can participate in achieving iron homeostasis by acting on intestinal iron absorption, and can impact local iron levels by affecting bone marrow macrophage erythro-phagocytosis. Knowing that the gut microbiota secretes a wide range of metabolite-derived humoral agents, can it regulate iron metabolism by directly releasing hepcidin? We are still in the infancy of understanding the many roles of gut microbiota in achieving/maintaining iron homeostasis. Later studies should address the effect of gut dysbiosis on iron homeostasis. They should also investigate where microbiota-derived EVs stand in this gut microbiota - iron homeostasis relationship. The future of medicine lies in the microbiota (117). Microbiota derived EVs might be key in future therapies for iron related diseases. Targeting host and gut microbiota-derived EVs will be a huge challenge to prevent and treat many diseases related to the alteration of iron homeostasis and metabolism.

Author contributions

ME: conception. ME, MF, CPo, CPe, and YD: writing, interpretation, and critical revision of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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