Musculoskeletal complications associated with pathological iron toxicity and its molecular mechanisms

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Abstract

Iron is fundamental for several biological functions, but when in excess can lead to development of toxic events. Some tissues and cells are more susceptible than others, but systemic iron levels can be controlled by treating patients with iron chelating molecules and phlebotomy. An early diagnostic can be decisive to limit the progression of musculoskeletal complications like osteoarthritis and osteoporosis because of iron toxicity. In iron-related osteoarthritis, aggravation can be associated to a small number of events that can contribute to joints articular cartilage exposure to high iron concentrations, which can promote articular degeneration with very little chance of tissue regeneration. In contrast, bone metabolism is much more dynamic than cartilage, but progressive iron accumulation and aging can be decisive factors for bone health. The iron overload associated with hereditary diseases like hemochromatosis, haemophilias, thalassemias and other hereditary anemias increase the negative impact of iron toxicity in joints and bone, as well as in life quality, even when iron levels can be controlled. The molecular mechanisms by which iron can compromise cartilage and bone have been illusive and only in the last 20 years studies have started to shed some light into the molecular mechanisms associated with iron toxicity. Ferroptosis and the regulation of intracellular iron levels is instrumental in the balance between detoxification and induced cell death. In addition, these complications are accompanied with multiple susceptibility factors that can aggravate iron toxicity and should be identified. Therefore, understanding tissues microenvironment and cell communication is fundamental to contextualize iron toxicity.

Introduction

Iron is an essential element in life and, by playing a role as metallic co-factor in several proteins and biochemical reactions, its involvement is crucial for multiple biological functions including oxygen transport and storage, respiratory chain complexes and desoxyribonucleic acid (DNA) replication [1,2]. Iron metabolism in humans is tightly regulated because there is no active mechanism of iron excretion, with losses occurring through bleeding as well as skin and enteric desquamation [3,4]. The iron metabolism balance depends on three processes, absorption, recycling and storing, and failure to regulate any of those can lead to progressive iron accumulation in tissues and organs, resulting in complications related to iron toxicity. Iron is involved in the production of oxygen reactive species (ROS) in the presence of oxygen through Fenton reactions that can promote the formation of radicals like $HO^{\cdot}$ and $(O_2^{\cdot})^{-}$ which are highly toxic, capable of promoting genome instability, proteins function impairment and lipid peroxidation [5,6].

There are several metabolic disorders that can lead to systemic and tissues iron overload including hereditary hemochromatosis [7,8], ferroportin disease [8], thalassemias, congenital dyserythropoietic anaemias , sideroblastic anaemias and myelodysplastic syndromes [9]. In addition, iron accumulation in tissues is associated with aging [10–14], contributing for the aggravation of age associated pathologies. As a consequence of the iron metabolism imbalance, an iron overload condition can be established leading to several pathological complications such as hepatic cirrhosis hepatocarcinoma, diabetes mellitus, cardiovascular disease and neuronal degeneration [7–9]. Iron overload can also lead or aggravate musculoskeletal complications like osteoporosis [15,16], osteoarthritis [15,17,18] and sarcopenia [19,20], which all have strong consequences on patients quality of life and under poor diagnostic can lead to death. Lack of a complete understanding of the molecular mechanism of iron toxicity in musculoskeletal complications prevent the development of adequate therapeutic solutions to alleviate symptoms. However, in recent years, some new evidence on the molecular mechanisms that promote the onset and acceleration of these pathogenic complications have been reported. In this article we review the present state of the art of the molecular mechanisms affected by iron toxicity associated with musculoskeletal pathologies.

Iron metabolism

Absorption

Iron is an essential element of human physiology and its homeostasis is highly regulated. The levels of iron absorption through the diet are depends on the systemic iron levels (1-2mg per day) [21]. The two main sources of iron absorbed in the intestine are haem (meat and fish) and non-haem iron (cereals, grains, and vegetables),which need different receptors, Heme carrier protein 1 (HCP1) for haem iron [22,23] and non-haem iron by divalent metal transporter 1 (DMT1), [23,24]. Non-haem iron needs to be reduced from +3 to +2 oxidation state by *cytochrome b* reductase 1 (CYBRD) before the transport by DMT1 into enterocytes [24,25]. The levels of iron absorption are regulated by the enterocytes intracellular iron levels, which can be modulated by the rate of exportation to the bloodstream by Fe^{2+} Ferroportin (FPN) [26,27]. The conversion of Fe^{2+} to Fe^{3+} by hephaestin (HEPH) allows transferrin (TF) iron loading for iron mobilization through the blood stream [26–28] (Figure 1). In addition, lactoferrin (LTF), a 77 kDa glycosylated protein, highly concentrated in human and bovine milk, similar tor TF, was shown to have an active role in non-haem iron absorption, namely in infants [29]. Enterocytes express intestinal lactoferrin receptor (also known as intelectin 1 (ITLN1)) [30] and can favor holo-LTF absorption and increase iron absorption in diet [29].

Iron recycling

Splenic and hepatic macrophages are the main players of iron recycling, they scavenge senescent erythrocytes and for haem iron [31,32], and its contribution to iron mobilization pool is higher than dietary iron absorption [33]. Macrophages can promote the release of iron from haem complex in haemoglobin through heme oxygenase-1 (HMOX1) activity [34,35]. HMOX1 catalyzes the reaction that degrades the heme group, producing $Fe^{2+,}$ biliverdin and CO. The Fe^{2+} released from hemoglobin can be stored in ferritins or mobilized to be exported by FPN [35]. HMOX1 can be viewed as the key player in iron metabolism, contributing to recycle iron from senescent erythrocytes The recycled iron will be stored in macrophages ferritins or exported by FPN Fe^{2+} transporter, followed by oxidation of Fe^{2+} to Fe^{3+} by ceruloplasmin (CP) or HEPH, depending on the tissue, thus allowing to complete the loading process of TF [28,32,34,36] (Figure 1).

Iron mobilization and storage

The systemic transport of iron is made through the bloodstream mainly by transferrin [37,38] but complemented by serum ferritin [39–41] and non-transferrin bound iron (NTBI) [42,43] . Transferrin loading occurs with iron exported from tissues, in general by FPN, and this is especially relevant for the mobilization of iron from enterocytes and macrophages to storing tissues like liver and spleen (Figure 1). Transferrin (TF) delivers iron to cells by interacting with Transferrin-receptor 1 (TFRC) and Transferrin-receptor 2 (TFR2) on cells surface [44]. The liver, being the main iron storing organ in the body, has an important role in iron storing and homeostasis. Hepatocytes are the main source of the hepcidin hormone (HAMP), fundamental in the regulation of systemic iron concentrations [2,45]. HAMP is expressed in response to increased levels of iron in the blood stream and its release will act on FPN transporters [46] (Figure 1). HAMP interaction with FPN expressing cells will promote its degradation, leading to a decrease of iron export to the blood stream, promoting a net effect that will contribute to decrease the systemic iron concentrations, dietary absorption, and macrophage iron release [46,47] (Figure 1). The expression of *HAMP* is regulated by bone morphogenetic proteins (BMP's)/ son of mothers against decapentaplegic homologues (SMAD's) and extracellular signal regulating kinase (ERK)/mitogen activated protein kinase (MAPK) [48–50]. This pathway is reinforced by the action of hemojuvelin (HJV), which interact with bone morphogenetic proteins receptors (BMPRs) and TFR2 [48,51] to promote SMADs pathway signalling increase, [52,53]. The main regulator of HAMP is BMP6 which in the combination of HJV can promote upregulation of *HAMP* expression (Figure 1) [54]. The finetuning of iron homeostasis is done by the homeostatic iron regulator (HFE) which is especially expressed by hepatocytes and involved in the regulation of TF interaction with TFRC by competing with TF for TFRC binding, thus promoting intracellular iron entry inhibition [55] (Figure 1). In addition, HFE and HJV interacts with TFR2 [44,51,56] and BMPRs in a non-competitive way, to reinforce the signal pathway for *HAMP* expression [51,57,58] (Figure 1). The mobilization of iron through NTBI is made through oxidized iron complexed with small molecules of citrate. Hepatocytes and T-lymphocytes are the major cells involved in NTBI intake [43,59]. The absorption of NTBI by the cells is made mainly by DMT1 and by solute carrier family 39 (metal ion transporter) members 8 (SLC39A8) and 14 (SLC39A14) transporters [60,61].

Iron overload causes

The mechanisms of iron overload establishment are different depending on the metabolic origin of the hereditary disorder. For example, hemochromatosis is associated with mutations in genes encoding iron metabolism players like *HFE* (most common disease), *HAMP* and *TFRC* [7]. These mutations lead to hepcidin deficiency that can promote progressive systemic iron overload [7,62]. Ferroportin disease (mutations on *FPN* gene) can also promote systemic iron increases but does not decrease hepcidin expression [7]. The iron accumulation in some hereditary anaemias [63,64] and haemophilia [65,66] is related with the systematic blood transfusions and in some anaemias like thalassemias, hepcidin suppression secondary to ineffective erythropoiesis increases iron absorption [63,67]. In general, anaemia-associated iron overload occurs within tissues of several organs and not at the systemic level because iron metabolism homeostasis is regulated by hepcidin.

Iron overload contributes to the onset and progression of osteoarthritis

Osteoarthritis is one of the most frequent joint diseases, with significant implications in patient morbidity levels. It is characterized by structural and molecular modifications in articular cartilage, subchondral bone, synovial membrane, muscle and ligaments, contributing to compromise the affected joint function [68,69]. The onset and progression of osteoarthritis depends on several factors like mechanical overload, aging, genetic background, obesity, and metabolic syndromes [70]. Iron overload has been established as a risk factor for the development of osteoarthritis [14,15,71–74], however the molecular mechanisms associated with the impact of iron overload on patients' joints and respective tissues has only recently been addressed using several approaches and biological models [66,75–78].

Recently, it was shown that excess iron exposure of synovial tissue and articular cartilage can contribute for osteoarthritis-like phenotypes [74,76,77]. Systemic iron overload alone is thought not to be enough to produce osteoarthritis [75]. Indeed, additional susceptibility factors appear to be required, like mechanical stress associated with microbleeding events, in order to expose synovial membrane and articular cartilage to high iron concentrations from blood (Figure 2). Through *in vitro* studies it was shown

that chondrocytes exposed to iron loads reproduce an osteoarthritis-like phenotype [75,79]. Exposure of mouse primary chondrocytes to 50µM of iron citrate, equivalent to systemic iron overload concentrations in *Hfe*-KO mouse models [80], led to changes in chondrocyte iron metabolism and an increase in intracellular iron levels [75]. The exposure of both *wt* and *Hfe*-KO cells to this iron citrate concentration promoted significant increases in expression of metalloproteases such as matrix metalloprotease 3 (MMP3) and 13 (MMP13) and aggrecanases like disintegrin and metalloproteinase with thrombospondin motifs like 5 (ADAMTS5), which were correlated with significant decreases in extracellular matrix (ECM) production by the chondrocytes (Figure 2) [75]. These results agreed with reported *in vivo* studies, which showed that an increase in levels of iron deposits in synovial tissue and articular cartilage was accompanied by an increase in metalloproteases expression and ECM degradation (Figure 2) [74,76–78,81]. In line with these results, recent reports have shown that excess iron can lead to chondrocyte death by ferroptosis and acceleration of osteoarthritis phenotype [78,82]. Iron overload can promote the upregulation of endothelial PAS domain-containing protein 1 (*EPAS1*) [75], also known as *HIF-2a*, probably as a result of hypoxia promoted by high levels of intracellular iron in chondrocytes, and induce the expression of MMPs and ADAMT5, both targets of EPAS1 [75,83]. In addition, primary chondrocytes culture exposed to iron showed an upregulation of chondrocyte hypertrophy markers like osteocalcin, also known as bone gamma-carboxyglutamate protein 2 (*Bglap2*), and collagen 1a1 (*Col1a1*), and downregulation of SRY-Box Transcription Factor 9 *(Sox9)*[75], suggesting an acceleration of articular cartilage hypertrophy. *In vivo*, the presence of osteophyte formation and increase subchondral bone sclerosis were observed as consequences of articular cartilage degradation associated with iron overloads [76–78]. In metabolic diseases like hereditary anaemias, the iron overload responsible to trigger arthropathies in the joints likely results from outcomes of consecutive blood transfusions and suppression of hepcidin as a result of defective erythropoiesis [63,67]. In the case of haemophilia, the iron overload associated with blood transfusions can be aggravated by hemarthrosis in the joints due to defective expression of coagulation factors [66,79,84].

HFE-hemochromatosis is characterized by HFE loss of function. The most common mutation, associated with a single nucleotide variant (SNP) in *HFE* gene (rs1800562), results into a substitution of one adenine (A) for a cytosine (C) in homozygosity (NG_008720.2:g.10633G4A; NM_000410.3:c.845G4A), leading to the substitution of a cysteine for a tyrosine in position 282 of its amino acid sequence

(NP_000401.1:p.Cys282Tyr) [7,85,86]. This mutation is particularly common in Caucasians where 1 in 200–300 individuals are found to be homozygous [7,85]. In HFE-related hemochromatosis, besides the impact that systemic iron overload may have on articular cartilage, loss of function of HFE can also be a susceptibility factor for intracellular iron accumulation. Accordingly, in *Hfe*-KO mice primary chondrocytes, the osteoarthritis-like phenotype was aggravated when compared with *wt* cells [75]. Indeed, HFE loss of function was found to promote a significant increase in intracellular iron levels when compared with *wt* cells subject to the same iron loads. The mechanism by which this susceptibility is established is related with Hfe interaction with TFRC, which under normal conditions limits iron import to the cells, while in *Hfe*-KO chondrocytes TFRC-mediated iron import was not regulated by HFE. A downregulation of *Tfrc* upon iron load in *wt* chondrocytes was observed as previously reported [44], however in *Hfe*-KO chondrocytes subject to iron load, no significant downregulation was found, leading to significant increases in intracellular iron and upregulation of *Fth1* and *Fpn* [75]. In addition, compared to *wt*, *Hfe*-KO chondrocytes showed significant differences in MMP3 expression, both at the transcript and protein levels, suggesting an aggravation of the osteoarthritis phenotype. These results were in agreement with what was observed *in vivo* for *Hfe*-KO mice subject to knee destabilization, which showed an aggravation of osteoarthritis phenotype when compared with *wt* mice [75,76], confirming the susceptibility associated with HFE loss of function. Altogether, results from those studies suggest an explanation for why HFE-related hemochromatosis patients present significant increases in the incidence of osteoarthritis when compared with idiopathic osteoarthritis [15,17,18,87–90] and that can be correlated with increases in systemic iron concentrations, serum ferritin and HFE loss of function. Epidemiological results and analysis of articular cartilage of *Hfe*-KO mice showed that HFE loss of function alone should not be enough to promote osteoarthritis [15,75]. Available data indicates that joint lesions in combination with the pC282Y mutation should increase the probability of osteoarthritis onset and progression [76], but additional susceptibility factors need to be identified [91]. Non-HFE-related hemochromatosis and ferroportin diseases are characterized by the appearance of a much earlier systemic iron overload in patients [7]. Implications on the onset and progression of iron related osteoarthritis should be similar to HFE-hemochromatosis, but with different susceptibility mechanisms associated with their mutations. However, its impact is unclear and remains poorly studied, possibly because they are much less frequent than HFE-related hemochromatosis [7].

Iron overload associated with bone loss acceleration

Bone remodelling is a physiological process characterized by cycles of bone formation and resorption. The cycles of bone matrix deposition are mediated by osteoblasts and osteocytes (mesenchymal lineage) and mineral matrix degradation by osteoclasts (hematopoietic lineage) [92,93]. Bone formation is favoured during growth and in young adulthood until bone mineral mass pics between 25 to 30 years of age [94]. From 30 year old onward, bone mineral density starts do decrease, especially in women after menopause [94]. Several factors have been reported as susceptibility factors for bone loss acceleration and onset. Gender, aging, unbalanced diet, low vitamin K availability, diabetes, hyperthyroidism and excess glucocorticoids [94–96]. Associated with aging, iron accumulation in bone tissue has been reported and shown to be associated with bone loss phenotype [13,97]. Accordingly, bone loss is a frequent consequence of pathologies like hemochromatosis, thalassemia, haemophilia and other hereditary anaemias capable of producing iron overload phenotype [16,64,98–101].

At the molecular level, the iron toxicity mechanisms associated with bone loss have only been addressed, with some detail, in the last 20 years. Several studies favour an increase in bone resorption [101–103], others suggest an osteoblast differentiation inhibition [104–106], but all mention a significant toxic impact of iron on bone mineralization process, with significant increase in osteoblast death and consequently low mineralization capability [101,102,107–110].

The crosstalk between bone and iron metabolisms is becoming more evident and recent results unveil how iron metabolism can interfere with the balance between bone resorption and formation. In particular, hepcidin with an active role in iron and calcium transport [111–113] and ferroxidase activity of ferritins involved in decreasing mineralization. TFR2 associated with upregulation of BMPs/SMADs pathways, which favours osteoblast differentiation and TRFC associated with intracellular iron incorporation in osteoblasts are also examples of that [50,100,101,106,114–118].

Bone marrow becomes a significant iron accumulating tissue along with liver and spleen, upon iron overload conditions [63,67,101,107]. Iron starts first to accumulate in bone osteoid, affecting terminal differentiation of osteoblasts associated with a significant downregulation of osteocalcin, and progressing towards mineral matrix incorporation [102,107,108]. Results reported suggest that exposure of osteoblasts and osteocytes to

iron can promote increase intracellular iron levels that in turn can lead to expression of pro-inflammatory molecules (Figure 3) [107].

When iron levels become toxic, changes in iron metabolism of osteoblasts and osteocytes become evident, with significant increases in FTH (inhibitor of mineralization)[106,119] and TFRC (active role in iron incorporation in osteoblasts) [101,107,109,112], suggesting the establishment of ferroptosis, a mechanism of programmed cell death associated with iron (Figure 3) [120,121]. The decrease in osteoblast and osteocyte numbers leads to inhibition of bone formation, as observed by downregulation of mineralization markers like osteocalcin, lactoferrin and alkaline phosphatase and upregulation of acid phosphatase 5, tartrate resistant (*Acp5*), associated with increase osteoclast activity (Figure 3) [103,107,108]. Osteoblasts and osteocytes iron toxicity is the establishment of an inflammatory microenvironment in bone tissue [107,109,110,122,123], which has been associated with bone loss [124–127]. Osteocytes death in association with iron overload was showed to promote osteoclastogenesis by favouring Receptor activator of nuclear factor kappa-B ligand (RANKL) [128]. This microenvironment is characterized by increased levels of oxidative stress related associated with high levels of iron in bone and can promote osteoblasts and osteocytes death [6,129], which can favour osteoclastogenesis and osteoclast recruitment, thus leading to bone metabolism uncoupling [107,128]. This mechanism favours bone resorption in opposition to bone formation leading to significant bone mineral loss (Figure 3). This phenotype was observed in *Hfe*-KO mice subject to iron rich diets, which revealed significant decreases in bone volume, trabecular number, and osteoblasts on bone surface area, with depression of bone formation markers and upregulation of osteoclast activity [107]. These results revealed the susceptibility of HFE loss of function in the establishment of iron overload and osteoblast iron incorporation when compared with a fully functional iron metabolism regulation in C57BL/6 *wt* mice. These data confirmed the association between iron overload and osteoporosis establishment reported previously [15,97,100,130–132].

In C57BL/6 mice subject to an iron rich diet for 3 months, transient iron overload can lead to full depression of bone metabolism, both bone formation (downregulation osteocalcin and lactoferrin) and resorption (downregulation of *Acp5*) [107]. It has been showed that hepatic hemosiderosis can promote hypoparathyroidism in thalassemia patients [133,134]. This was also confirmed in C57BL/6 subject to iron enriched diet, showing downregulation of *Pthr* [107], with implication on sclerostin (*Sost)* expression.

Decrease levels of PTHR activation promotes a significant downregulation of *SOST*, which can favour upregulation of WNT signalling pathway [135], because SOST binds to LDL receptor related protein 4, 5 and 6 (LRP4, LRP5 and LRP6) receptors, promoting WNT signalling inhibition [136]. SOST downregulation have been associated with increase bone density [137] and may contribute to explain why iron rich diet favoured bone mineralization transiently [107,138], with progressive incorporation of iron in mineral matrix. In addition, at the molecular level, no significant differences were seen in *Fth1*, *Tfrc* and *Tnf*, markers for ferropoptosis induction mechanism in osteoblasts and osteocytes [5,107,120], which contribute to block osteoclast recruitment. These results suggest a mechanism which could mask the toxic effect of iron transiently if just looking at bone mineral content, because bone tissue of C57BL/6 *wt* mice revealed a depressed osteoblast differentiation process, with upregulation of catalase (*Cat)* and *Il6*, which eventually will lead to bone loss phenotype [107].

Conclusions

The fact that there is not an active mechanism for iron excretion evidence the relevance for the iron metabolism regulation and iron detoxification mechanisms. Imbalance of iron metabolism can contribute to abnormal iron deposits in several tissues, and even with systemic iron levels under control, through time, iron accumulation can lead to musculoskeletal complications associated with pathological iron toxicity. They are a common denominator in hereditary pathologies like hemochromatosis, haemophilia and anaemias, contributing to decrease patient´s life quality and possibly increase life-threatening situations when diagnostic errors occur. In addition, iron accumulation associated with aging contributes to aggravate the onset of osteoporosis and osteoarthritis. The key denominator seems to be the threshold for cells to deal with iron toxicity before starting to promote chronic inflammatory microenvironment and eventually cell death by ferropoptosis. The identification of susceptibility factors that can contribute to the acceleration of musculoskeletal pathologic phenotypes in association with iron overload can contribute to develop early diagnostic tools and improve patients' quality of life.

Perspectives

- To avoid the onset and progression of osteoarthritis and osteoporosis as consequence of iron overload it is necessary to promote an early diagnostic for the potential establishment of iron overload conditions.
- Identification of susceptibility factors that can contribute for an aggravation of bone and cartilage degradation can diminish the impact that iron overload associated diseases can have on patients' quality of life.
- Ferroptosis seems to be part of the mechanisms associated to tissue degradation upon iron overload therefore, besides the treatments to decrease this metal levels. Prevention with antioxidant supplementations in diets should contribute to decrease the susceptibility for iron toxicity.

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Declaration of interest

The authors have no conflict of interest do disclose.

Author Contributions

Márcio Simão and M. Leonor Cancela conceived and wrote the review.

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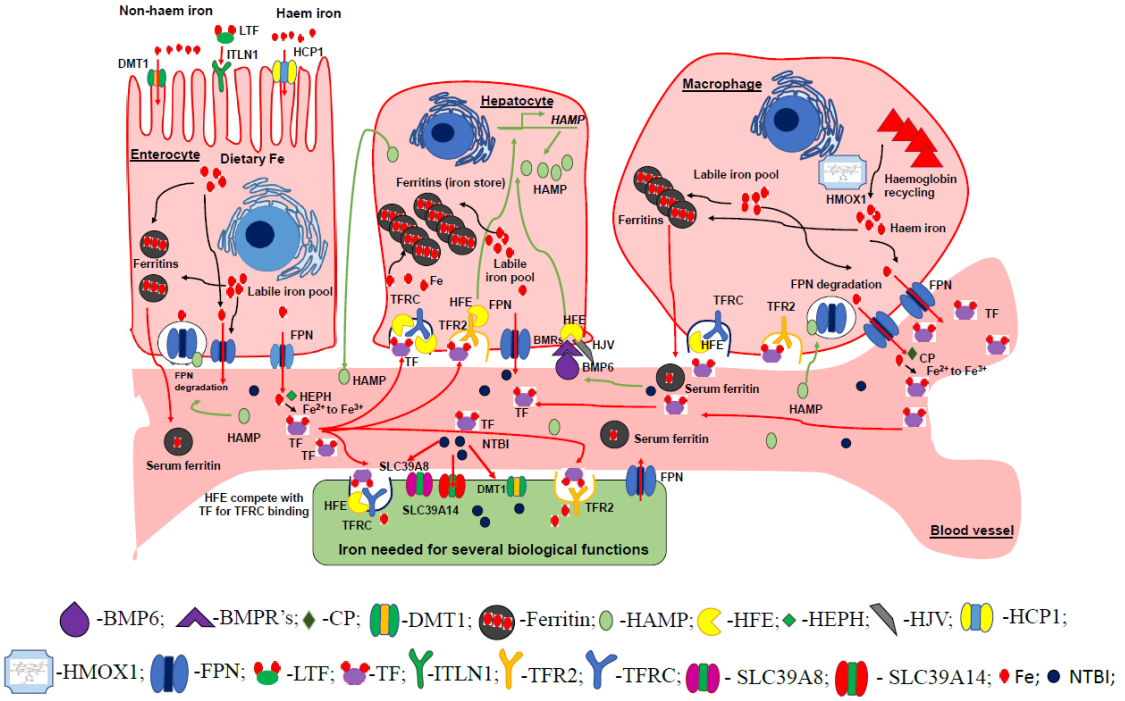

Figure 1-**Iron metabolism**. Description of iron metabolism regulatory mechanism and associated tissues and molecules. Upon systemic iron overload, a main response will lead to BMP6 upregulation and consequently will increase the expression of HAMP by increasing BMP's/SMADs pathway. Iron overload will also increase TF saturation, which will implicate not only the entry of elevated levels of iron to cells through TFRC affinity but, will also increase TFR2 affinity to TF. This will lead not only to intracellular iron absorption, but also have implications on the upregulation of HAMP expression. High levels of HAMP increase the degradation of FPN, leading to decreased levels TF saturation. As a consequence, lower concentrations of iron will be transported and locally, dietary absorption by the enterocytes will be inhibited and haem iron recycled by macrophages will not be exported Hepatocytes, enterocytes and macrophages will increase the levels of intracellular iron accumulation by cytosolic ferritins. The red arrows presented in figure represent iron absorption to cells or blood stream. The green arrows represent a response to decrease systemic iron levels. The black arrows represent intracellular iron mobilization. Molecules represented in figure: Bone morphogenetic protein 6 (BMP6); Bone morphogenetic protein receptors (BMPR's); Ceruloplasmin (CP); Divalent metal transporter 1 (DMT1); FT-Ferritin; sFT-serum ferritin; Hepcidin (HAMP), Human hemochromatosis protein (HFE); Hephaestin (HEPH); Hemojuvelin

(HJV); Haem carrier protein 1 (HCP1); heme oxygenase 1 (HMOX1); Ferroportin (FPN); Lactoferrin (LTF); intestinal lactoferrin receptor (also known as intelectin 1 (ITLN1), Transferrin (TF); Transferrin receptor 1 (TFRC); Transferrin receptor 2 (TFR2), solute carrier family 39 member 8 (SLC39A8) and solute carrier family 39 member 14 (SLC39A14);. In addition, two different representations for iron were presented, elemental iron (Fe) and Non-transferrin-bound serum iron (NTBI).

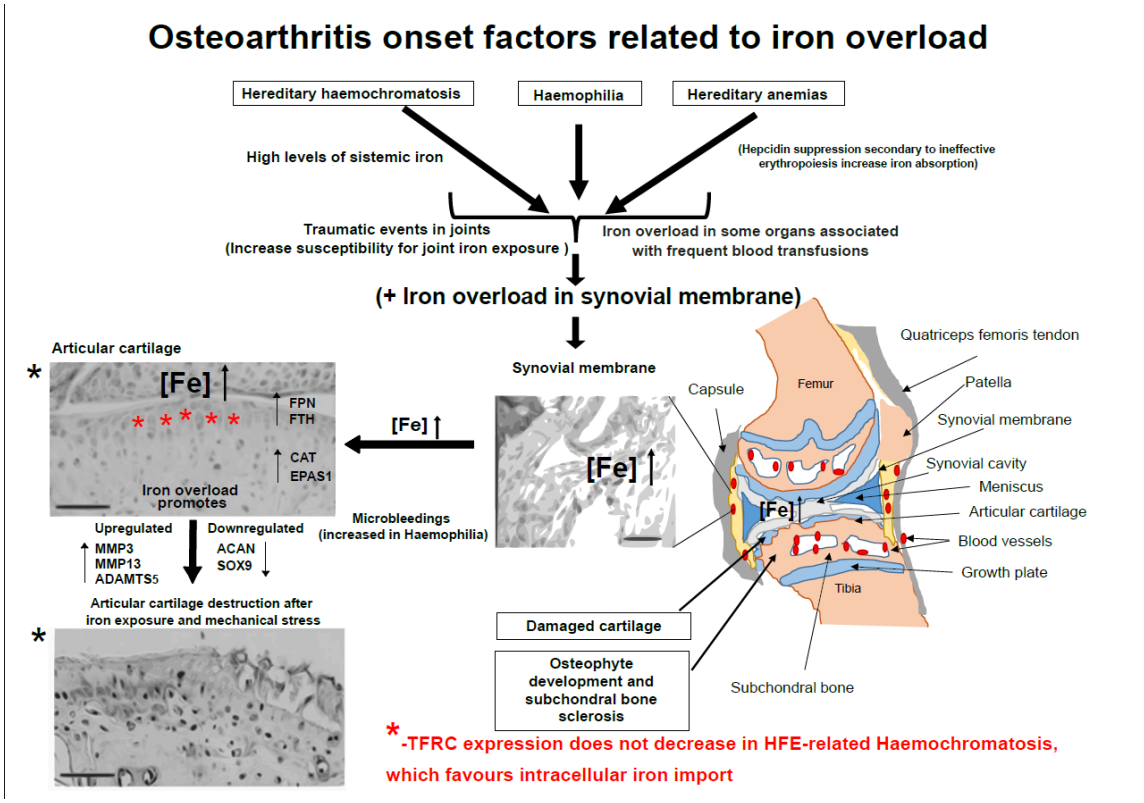

Figure 2-**Osteoarthritis onset factors related to iron overload**. Hereditary diseases like hemochromatosis, haemophilias, thalassemias and other hereditary anemias can promote generalized iron overload. Arthropaties are complications commonly associated with excess iron affecting articular cartilage associated with additional susceptibility factors like traumatic events and synovial tissue iron exposure. Articular cartilage exposure to intracellular accumulation of iron at toxic levels leads to extracellular matrix degeneration, upregulation of metalloproteinases and acceleration of hypertrophy. Articular cartilage promotes a hypoxic microenvironment, which can favour ROS production by iron and eventually cell death by ferroptosis. (*)- Mouse articular cartilage before and after destabilization and iron exposure. These images are adapted from Simão et al (2019) results [75]. (*)-TFRC expression does not decrease in HFE-related

Haemochromatosis, which favours intracellular iron import Molecules represented in figure: Aggrecan (ACAN); ADAM metallopeptidase with thrombospondin type 1 motif 5 (ADMTS5); Catalase (CAT); Endothelial PAS domain protein 1 (EPAS1); Ferritin H (FTH); Ferroportin (FPN); Matrix metallopeptidase 3 (MMP3); Matrix metallopeptidase 13 (MMP13); SRY-Box Transcription Factor 9 (SOX9); Transferrin receptor 1 (TFRC); Intracellular iron levels ([Fe]). ( )-Iron deposits in articular cartilage.

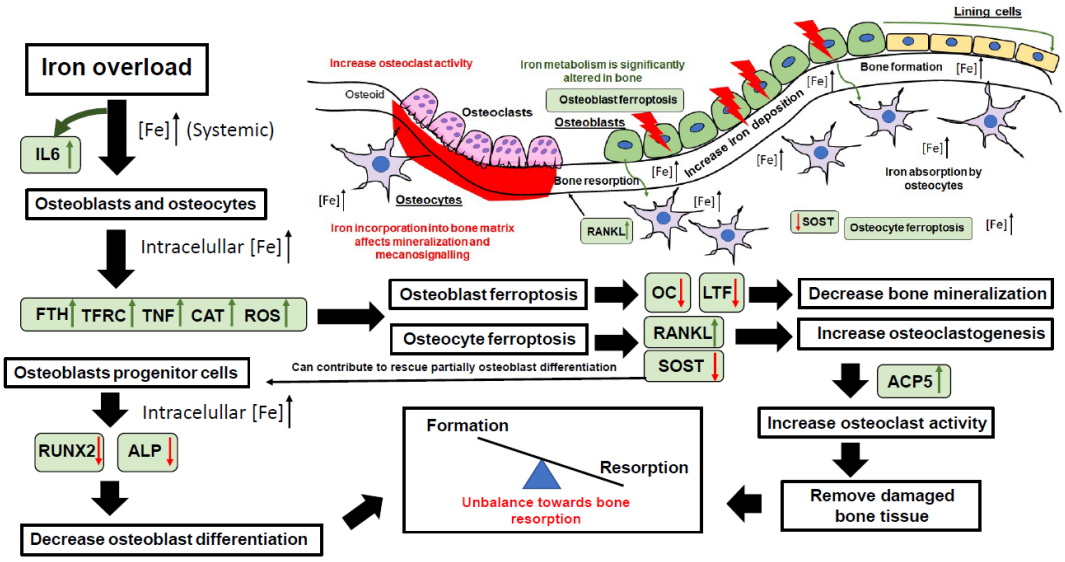

Figure 3-**Molecular mechanisms contributing to bone loss within a microenvironment under iron overload**. The excess iron in bone marrow and at the systemic levels is progressively incorporated into osteoid and mineralized matrix, leading to depression of osteoblast and osteocyte terminal differentiation and consequently decreasing mineralization. The excess iron promotes cell death and creates a pro-inflammatory microenvironment that favours osteoclastogenesis and osteoclast recruitment, which leads to increase bone resorption, and consequently bone loss. Green arrows increase expression. Red arrows represent decrease expression. Molecules represented in figure: Acid phosphatase 5, tartrate resistant (ACP5); Alkaline phosphatase (ALP); Catalase (CAT); Ferritin H (FTH); Interleukin 6 (IL6); Lactoferrin (LTF); Osteocalcin (OC); Receptor activator of nuclear factor kappa-B ligand (RANKL); Reactive oxygen species (ROS); RUNX family transcription factor 2 (RUNX2); Transferrin receptor (TFRC); Tumor necrosis factor (TNF); Sclerostin (SOST); Physiological iron concentrations ([Fe]).