Role of iron and iron-related proteins in mesenchymal stem cells: Cellular and clinical aspects

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Abstract
Mesenchymal stem cells (MSCs) are located in various tissues where these cells show niche-dependent multilineage differentiation and secrete immunomodulatory molecules to support numerous physiological processes. Due to their regenerative and reparative properties, MSCs are extremely valuable for cell-based therapy in tackling several pathological conditions including COVID-19. Iron is essential for MSC processes but iron-loading, which is common in several chronic conditions, hinders normal MSC functionality. This not only aggravates disease pathology but can also affect allogeneic and autologous MSC therapy. Thus, understanding MSCs from an iron perspective is of clinical significance. Accordingly, this review highlights the roles of iron and iron-related proteins in MSC physiology. It describes the contribution of iron and endogenous iron-related effectors like hepcidin, ferroportin, transferrin receptor, lactoferrin, lipocalin-2, bone morphogenetic proteins and hypoxia inducible factors in MSC biology. It summarises the excess-iron-induced alterations in MSC components, processes and discusses signalling pathways involving ROS, PI3K/AKT, MAPK, p53, AMPK/MFF/DRP1 and Wnt. Additionally, it evaluates the endogenous and exogenous saviours of MSCs against iron-toxicity. Lastly, it elaborates on the involvement of MSCs in the pathology of clinical conditions of iron-excess, namely, hereditary hemochromatosis, diabetes, β-thalassaemia and myelodysplastic syndromes. This unique review integrates the distinct fields of iron regulation and MSC physiology. Through an iron-perspective, it describes both mechanistic and clinical aspects of MSCs and proposes an iron-linked MSC-contribution to physiology, pathology and therapeutics. It advances the understanding of MSC biology and may aid in identifying signalling pathways, molecular targets and compounds for formulating adjunctive iron-based therapies for excess-iron conditions, and thereby inform regenerative medicine.

Abbreviations: ALP, alkaline phosphatase; AMPKα, 5'-adenosine monophosphate (AMP)-activated protein kinase; BMP, bone morphogenetic protein; BM-MSC, bone marrow mesenchymal stem cell; CDK, cyclin-dependent kinase; CXCL, C-X-C motif chemokine; DMT-1, divalent metal transporter-1; FOXO, forkhead box protein; HCC, hepatocellular carcinoma; HIF, hypoxia inducible factor; HSC, hematopoietic stem cell; KDMs, lysine-specific demethylases; LIP, labile iron pool; MAPK, mitogen activated protein kinase; MDS, myelodysplastic syndromes; MFF, mitochondrial fission factor; MSC, mesenchymal stem cell; mTOR, mechanistic target of rapamycin; NAC, N-acetyl-L-cysteine; NTBI, non transferrin bound iron; PI3K, phosphoinositide 3-kinase; PPAR-γ, peroxisome proliferator-activated receptor-γ; ROS, reactive oxygen species; RUNX-2, runt related transcription factor-2; SCF, stem cell factor; SMA, small mothers against decapentaplegic; TBI, transferrin bound iron; TIR-1, transferrin receptor-1; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor; ZIP, zrt/Irt-like protein (zinc transporter).

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INTRODUCTION

1.1 Overview of iron uptake and utilisation

Iron is crucial for numerous cellular and physiological processes. Following dietary iron uptake by the duodenal enterocytes (1–2 mg/day), iron is absorbed into the circulation, where it binds to the iron-carrier protein transferrin that transports iron to all cells of the body. Transferrin-bound iron binds to transferrin receptors on cell surfaces and iron is taken up by the cells. The imported iron is incorporated into functional enzymes/proteins such as haemoglobin (oxygen transport), myoglobin (oxygen transport), cytochromes (electron transport chain and drug metabolism), nitric oxide synthases (signal transduction) and ribonucleotide reductase (de novo synthesis of deoxyribonucleotides). Iron is exported out of the cells via the transmembrane protein ferroportin, which is present on the surfaces of hepatocytes and macrophages, the iron-storing and iron-recycling cells, respectively, as well as the enterocytes and placental cells. Excess iron is stored in the iron-storage protein ferritin, which exists both intracellularly and in circulation. As mammals do not possess the mechanisms for removal of excess iron, iron homo- eostasis is tightly regulated at cellular and systemic levels. Natural means of iron exit from the body is through menstruation and through regular sloughing of intestinal cells (Ganz, 2011; Sharp & Srai, 2007).

In iron-loaded conditions, iron levels in the plasma exceed the iron-binding capacity of transferrin, which is manifested as increased transferrin saturation. This leads to elevation in non-transferrin-bound iron (NTBI) in the circulation and the entry of NTBI into the cells via membrane metal channels causing excessive intracellular iron accrual. This can no longer be accommodated by intracellular ferritin, leading to increment in “free iron”, which is believed to collect as labile iron pool (LIP) (Tanaka et al., 2019). Excess “free iron” can accelerate the Fenton reaction and increase the generation of reactive oxygen species (ROS) to levels that are beyond the quenching capacity of cellular antioxidants, thereby dysregulating intracellular redox homeostasis. Unregulated ROS is toxic as it can directly damage proteins, lipids, membranes and DNA, and/or stimulate cell signalling pathways that alter gene/protein expression (Mehta, Farnaud, et al., 2019). Also, excess iron can directly simulate signalling pathways that may exacerbate disease pathology (Mehta et al., 2018). Excess iron deposits in the heart, liver, pancreas, bone marrow (BM), joints, endocrine glands and kidneys, and damages these organs causing heart diseases, hepatic fibrosis/cirrhosis, glucose intolerance/diabetes, arthropathy, impotence, impairment of hematopoiesis and kidney dysfunction (Lu, Zhao, Rajbhandary et al., 2013).

1.2 Mesenchymal stem cells (MSCs)

MSCs, also known as mesenchymal stromal cells/multipotent stromal cells/mesenchymal progenitor cells, are a heterogenous population found in the blood and located almost throughout the body, for example, in the perivascular spaces, BM, liver, heart, lung, gut, skin, tonsils, adipose tissue, umbilical cord, placenta, dental pulp, amniotic and synovial fluids, skeletal muscle and tendons (Figure 1) (Glenn &
There is a controversy regarding the exact origin of these cells. These nonhematopoietic, fibroblastic, immunologically naive cells are pleotropic in nature and perform two main functions. First, a subpopulation of this group plays a direct role in regeneration and tissue repair due to their ability to differentiate into various cell types. In vitro, MSCs have been shown to differentiate into cells with specialised functions such as adipocytes (fat cells), chondrocytes (cartilage cells), osteoblasts (which mature into osteocytes (bone cells)), myocytes (muscle cells) (Atashi et al., 2015; Iansante et al., 2018), functional hepatic-like cells (Lee et al., 2004) and neuron-like cells (Pittenger et al., 2019), while its differentiation into cardiomyocytes, lung cells and gut epithelial cells is debated (Glenn & Whartenby, 2014). In vivo, MSCs can self-renew and undergo microenvironment-dependent differentiation into various cell types of mesodermal lineage. For example, cell tracing experiments in mice have shown the ability of perivascular MSCs to differentiate into adipocytes, myoblasts, profibrotic fibroblasts and follicular dendritic cells (Pittenger et al., 2019). The second function of MSCs is to provide physical support in the perivascular spaces and exert paracrine effects by secreting growth factors, regulatory compounds, and extracellular vesicles/exosomes containing protein, DNA, messenger RNAs (mRNAs) and microRNAs (some of which can have immunosuppressive effects) (Crivelli et al., 2017; Glenn & Whartenby, 2014; Park et al., 2018). Through these functions, MSCs form specific niches to support several physiological processes including BM homeostasis, bone turnover, haematopoiesis, wound healing and angiogenesis (Glenn & Whartenby, 2014; B. Zhang, Wu, et al., 2015) and demonstrate antiapoptotic, antifibrotic, anti-inflammatory, proangiogenic and immunomodulatory properties (Pittenger et al., 2019). Due to their reparative characteristics and easy in vitro expansion and differentiation, MSCs make an excellent option for cell-based therapy, thereby helping to evade the challenges of whole organ transplantation in treating pathological conditions. With MSCs being one of the most sorted cell types for cell therapy, there are more than one thousand MSC-utilising clinical trials listed on the EU and NIH (US national library of Medicine) clinical trial registers.

1.3 Rationale and significance of understanding the role of iron in MSCs

Iron is essential for all cell types including the MSCs. However, excess iron has detrimental effects on all cell types and MSCs are no exception. As the MSCs have physiological and therapeutic significance, understanding MSCs from an iron perspective can provide better insights into MSC processes and functionality. For instance, the bone marrow mesenchymal stem cells (BM-MSCs) (in conjunction with hematopoietic stem cells [HSCs]) play a vital role in creating the exclusive BM niche for haematopoiesis and are located in a low oxygenic BM environment (Pittenger et al., 2019). BM-MSC’s support towards haematopoiesis is dependent on iron regulation in these cells as iron-damaged MSCs are unable to support haematopoiesis effectively. This is bound to aggravate the pathology of haematological conditions that show iron overload; for example, β-thalassaemia and myelodysplastic syndromes (MDS). Application of iron-related knowledge of BM-MSC physiology could aid in formulating adjunctive therapies to restore BM-MSC functionality in such cases. Also, it may help in developing safer therapeutic strategies that not only reduce the chemotherapeutic agent-induced damage to the BM niche but also enhance the ability of iron chelators to penetrate deeper into the BM environment and enhance BM transplantation outcomes (Crippa et al., 2019).

In addition to the aforementioned haematological diseases, iron-loading is frequently observed in chronic pathologies of the liver, brain and metabolic syndromes (Mehta, Ahmed, et al., 2019; Mehta, Farnaud, et al., 2019), where pathogenesis could be partly attributed to iron-loading. Since the MSCs are located in various tissues, it is very likely that iron-damaged MSCs contribute to the pathogenesis. For example, BM-MSC dysfunction has been described in β-thalassaemia (Crippa et al., 2019), diabetes (Fijany et al., 2019) and MDS (Poon et al., 2019), and this dysfunction could be due to iron-loading of BM-MSCs. Another example is aging, which increases the possibility of iron accumulation (Ashraf et al., 2018; Jung et al., 2008; Xu et al., 2008) while decreasing the fraction of MSCs obtainable from the BM (Pittenger et al., 2019). It is possible that these two aspects are correlated and the reduced ability to repair injured tissue in older people could be (partly or entirely) attributed to the effect of excess iron on MSC’s reparative properties. Thus, knowledge of MSC physiology from an iron perspective could help in formulating supplementary therapeutic approaches to restore lost MSC functionality in such cases. This reiterates the significance of understanding the effect of iron-loading on the MSCs, and envisages the application of this knowledge to a wide range of diseases that show iron-loading.

Reviewing MSC functionality and signalling mechanisms under iron-loaded conditions can help identify cellular pathways and molecular targets of therapeutic significance. Based on the studies so far, some links between iron-related pathologies and MSC’s therapeutic potential can be established. For example, hepatic iron-loading increases the predisposition to hepatocellular carcinoma (Kowdley, 2004), and MSCs have been found to hinder tumour cells in hepatocellular carcinoma via specific signalling pathways (Ai et al., 2019). Similarly, neuronal iron accumulation is common in Alzheimer’s disease (Duce et al., 2010) and MSCs have shown to increase neurogenesis and neuronal differentiation via Wnt signalling in an Alzheimer’s disease model (Oh et al., 2015), probably to replace the damaged neurons.

Examining the MSCs in an iron context could be useful for improving allogeneic and autologous MSC therapy. For example, when normal MSCs and MSCs from diabetics were administered into ischaemic rat limb, normal MSCs showed better perfusion than diabetic MSCs (H. Kim, Han, et al., 2015). Here, the ineffectiveness of diabetic MSCs was attributed to their poor angiogenic ability, which is likely an effect of excess iron because iron reduces MSC angiogenic potential (Y. Zhang, Zhai, et al., 2015). Thus, knowledge of the iron-induced damage to the MSCs can help evaluate the efficacy of...
MSC therapy, while substances that have shown to rescue MSCs from the toxic effects of iron-loading (Camiolo et al., 2019; Yang et al., 2016; Yang, Yang, et al., 2017; Yao et al., 2019) could be used to treat MSCs before transplantation to restore the iron-induced MSC dysfunctions. Moreover, this knowledge could inform HSC therapy because MSCs have shown to support HSC transplantation by facilitating HSC engraftment in patients with acute graft-versus-host disease (Le Blanc et al., 2008). Successful HSC engraftment is essential in conditions like β-thalassaemia for which the only curative option is allogeneic HSC transplantation from a compatible donor (Crippa et al., 2019), often difficult to find. In current times, the understanding of MSCs from an iron perceptive is particularly useful because MSCs are now used in clinical trials to manage COVID-19 (Raza & Khan, 2020).

Collectively, viewing MSC physiology from an iron perspective can enhance our knowledge of disease pathology, aid in developing adjunctive therapeutic and management strategies for excess-iron-related conditions, and improve MSC transplantation outcomes.

Accordingly, this review examines the role of iron and related proteins in MSC biology. It addresses several aspects, namely, the damaging effects of excess iron on MSC physiology, saviours of MSCs from the detrimental effects of iron-loading, contribution of iron and endogenous iron-related proteins in normal MSC processes, and lastly, the role of MSCs in clinical conditions of excess iron.

2 | EFFECT OF EXCESS IRON ON MSC COMPONENTS AND PROCESSES

The effects of iron on MSC components and MSC processes have been summarised in Tables 1 and 2, respectively. These alterations are mediated by iron-induced changes in cell signalling pathways and/or vice versa, eventually altering MSC characteristics, processes and functionality. For example, the expansion of LIP can increase ROS production (Table 1) that can stimulate signalling pathways leading to MSC cycle arrest in G0/G1 phase, apoptosis and reduced proliferation of the MSCs (Table 2) (Y. Zhang, Zhai, et al., 2015). Likewise, excess iron in the serum of iron-loaded patients can increase cyclins A and E, inhibit intracellular cyclin-dependent kinase (CDK) inhibitor p27kip1 (Table 1), enhance mitogen-activated protein kinase (MAPK) signalling and thereby stimulate BM-MSC proliferation (Table 2) (Borriello et al., 2016).

There are some notable points about Tables 1 and 2. First, the in vitro studies cited in these tables used the NTBI ferric ammonium citrate as the iron source. Second, in most of these studies, the iron-induced alterations in MSC physiology were attributed to excess iron and/or excess-iron-induced ROS. Third, the majority of these studies were conducted on BM-MSCs. This is because BM is the most common source for MSCs. Although present as a small fraction in the BM, BM-MSCs can be easily expanded in vitro for experimental purposes (Pittenger et al., 1999). In addition, there is an important link between BM-MSCs and haematopoesis, which involves iron. In the BM, while HSCs carry out haematopoiesis, MSCs provide the niche for this process. The niche is formed by the paracrine effector molecules secreted by the BM-MSCs and the cells into which the BM-MSCs differentiate, that is, BM-stromal cells, pericytes, myofibroblasts, osteoblasts and endothelial cells; some of which also provide physical support (Fei et al., 2014). Thus, BM-MSCs control the fate of HSCs. Although low levels of ROS are required for the renewal of HSCs (Brissot et al., 2020), iron overload increases ROS levels and negatively affects haematopoiesis (Gattermann et al., 2012; Lu, Zhao, Rajbhandary, et al., 2013; Okabe et al., 2014); directly by damaging HSCs and causing apoptosis and cell cycle arrest (Chai et al., 2015; Jin et al., 2018), and indirectly by damaging the MSCs via ROS signalling (Lu et al., 2012). This hinders normal MSC contribution to haematopoiesis. Essentially, under iron-loaded conditions, while the fate of HSCs is directly affected by excess-iron-induced ROS, it is also affected by lack of sufficient support from the MSCs. This clearly explains the significance of iron regulation in the MSCs for normal haematopoiesis.

3 | EXCESS IRON INDUCES ALTERATIONS IN MSC SIGNALLING PATHWAYS

MSC survival, proliferation, differentiation, homoeostasis and functionality are mediated by myriads of signalling pathways. The activation of these pathways usually involves successive phosphorylation of several downstream mediators that ultimately modulate the expression of specific genes. Here, selected MSC pathways that show iron-induced alterations are discussed (Figure 2). These alterations may or may not encompass major ROS involvement, but these modulate MSC functionality under iron-loaded conditions and explain the excess iron and/or ROS-mediated detrimental effects on MSCs summarised in Table 2.

3.1 | Wnt signalling

Typically, Wnt signalling is initiated upon binding of extracellular Wnt proteins to the transmembrane frizzled receptors, and LRPS/6 co-receptors (canonical) or Frizzled receptors and Rho2 coreceptors (noncanonical). Canonical Wnt signalling is mediated by stabilising the cytoplasmic protein β-catenin followed by its entry into the nucleus. Here, it binds to transcription factors and activates Wnt target genes, which are involved in the regulation of cell development, stemness, fate, migration, polarity and differentiation (Lehoczky & Tabin, 2018). Accordingly, Wnt signalling regulates MSC fate, bone remodelling and homoeostasis, as evidenced by its role in the osteogenic differentiation of BM-MSCs in adult marrow (Houshyar et al., 2019; J.-A. Kim, Choi, et al., 2015). Both canonical and non-canonical Wnt pathways are involved in iron-regulated osteogenic differentiation of MSCs. For example, iron chelation increased the osteogenic differentiation of MSCs by increasing β-catenin levels (Qu et al., 2008). This demonstrated the role of iron in MSC differentiation via canonical Wnt/β-catenin signalling. Similarly, Wnt5a
| Cellular components                                      | Normal function                                                                 | Effect of iron-loading on BM-MSCs or umbilical cord MSCs, as observed in vitro, in animal or clinical studies |
|--------------------------------------------------------|--------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|
| **ROS**                                                | • Regulated levels of ROS facilitate redox signalling, cell proliferation, apoptosis, senescence and normal cellular metabolism (Liou & Storz, 2010) | • Increased (Crippa et al., 2019; Lu, Zhao, Chai, et al., 2013; Lu, Zhao, Rajbhandary, et al. 2013; Lu, Zhao, Sajin, et al., 2013; Shen et al., 2018; Yang, Li, et al., 2017; Yang, Yang, et al., 2017; Yao et al., 2019; Yuan et al., 2019; Y. Zhang, Zhai, et al., 2015; Zheng et al., 2018) |
|                                                       | • During MSC differentiation, the major sources of ROS production include the NADPH oxidase isoform NOX4 and mitochondrial complexes I and III. These influence the transscription machinery required for MSC differentiation including the Wnt, Hedgehog and FOXO signalling pathways and thus tightly regulate the adiopgenic and osteogenic differentiation ratio of BM-MSCs (Atashi et al., 2015) | • Increased in MSCs from iron-loaded patients with myelodysplastic syndrome (Zheng et al., 2018) |
| **Intracellular iron and LIP**                         | • Intracellular iron is the total iron present inside the cells that could be bound to enzymes/proteins and participate in cellular activities | • No elevation (Borriello et al., 2016) |
|                                                       | • LIP is that fraction of intracellular iron in which iron is loosely bound to complexes | • Elevation in NOX4 protein levels (Yuan et al., 2019) |
|                                                       | • LIP is a collection of redox-active iron, and therefore, the main determinant of cellular oxidative stress | • Increased iron levels observed in mice BM-MSCs (Y. Zhang, Zhai, et al., 2015) |
| **Ferritin**                                           | • Iron-regulated iron-storage protein | • Iron-induced upregulation of ferritin expression (Balogh et al., 2016) |
|                                                       | • Made up of light and heavy chains | • Accumulated in human BM-MSCs (Borriello et al., 2016) |
|                                                       | • Can act as an antioxidant molecule as well as a pro-oxidant molecule | • Expression of light and heavy chains was lower in BM-MSCs of β-thalassaemia patients than healthy subjects (Crippa et al., 2019) |
|                                                       | • Exists intracellularly and in the circulation | • Treatment with exogenous ferritin caused anti-osteogenic effect (Balogh et al., 2016) |
| **RUNX-2**                                             | • Key transcription factor for osteogenic differentiation (osteoblastic differentiation marker) (Y. Zhang, Zhai, et al., 2015) | • Expression reduced/inhibited (Balogh et al., 2016; Yang, Yang, et al., 2017; Yao et al., 2019; Yuan et al., 2019; Y. Zhang, Zhai, et al., 2015) |
|                                                       | • Regulates transcription of bone-related proteins including osteocalcin, alkaline phosphatase, osteopontin and collagen-1-α1 (Balogh et al., 2016) | • Expression reduced in BM-MSCs of patients with low-risk myelodysplastic syndromes (Fei et al., 2014) |
| **Osteocalcin (bone γ-carboxyglutamate protein, encoded by BGLAP gene)** | • A versatile hormone secreted solely by osteoblasts (in the bone) | • Insufficient activation in BM-MSCs from β-thalassaemia patients (Crippa et al., 2019) |
|                                                       | • Osteoblastic differentiation marker; target of RUNX-2 | • Expression reduced (Balogh et al., 2016; Borriello et al., 2016; Yang, Yang, et al., 2017; Yuan et al., 2019; Y. Zhang, Zhai, et al., 2015) |
|                                                       | • Affects bone mineralisation and bone density | • Expression decreased in BM-MSCs of patients with low-risk myelodysplastic syndromes (Fei et al., 2014) |
|                                                       | • Controls other physiological processes including glucose homoeostasis, male fertility and brain development in an endocrine manner (Moser & van der Eerden, 2019) | • Ureupregulation induced by the iron-binding protein lactoferrin (Li et al., 2019) |
| **Alkaline phosphatase**                               | • Enzyme present in high levels in bone and liver | • Activity reduced (Balogh et al., 2016; Fei et al., 2014) |
|                                                       | • Osteoblast-specific gene, target of RUNX-2 | • Expression decreased (Yang, Yang, et al., 2017; Y. Zhang, Zhai, et al., 2015) |
|                                                       | • Marker of osteogenic differentiation (pro-osteoblastic) in MSCs (Hanna et al., 2018) | • Expression decreased in BM-MSCs of patients with low-risk myelodysplastic syndromes (Fei et al., 2014) |
|                                                       | | • Expression and activity increased slightly (Borriello et al., 2016) |
TABLE 1 (Continued)

| Cellular components | Normal function | Effect of iron-loading on BM-MSCs or umbilical cord MSCs, as observed in vitro, in animal or clinical studies |
|---------------------|-----------------|-------------------------------------------------------------------------------------------------------------------|
| Osteopontin         | Secreted extracellular matrix protein that has diverse biological functions including bone remodelling, immune functions and a role in cancers and inflammatory diseases | • Expression inhibited (Yao et al., 2019)  
• Expression decreased in BM-MSCs of patients with low-risk myelodysplastic syndromes (Fei et al., 2014)  
• Expression stimulated by the iron-binding protein lactoferrin (Li et al., 2019) |
| Active β-catenin    | Cell-cell adhesion, gene transcription and intracellular signal transducer in the Wnt signalling pathway | • Expression inhibited (Yao et al., 2019) |
| Adipogenic genes    | Peroxisome proliferator-activated receptor gamma (PPARγ), adipin and adipocyte protein-2 mediate adipogenesis and are adipogenic differentiation markers  
• Fatty acid-binding protein 4 (Fabp4) is an adipocyte specific protein | • Expressions increased (Y. Zhang, Zhai, et al., 2015)  
• Expression of PPARγ increased (Yang, Yang, et al., 2017)  
• No effect on Fabp4 expression; no influence on adipogenic differentiation of BM-MSCs (Balogh et al., 2016) |
| Chondrogenic marker | Aggrecan is the main component of extracellular matrix of cartilaginous tissues | • No effect on aggrecan expression; no influence on chondrogenic differentiation of BM-MSCs (Balogh et al., 2016) |
| Cyclin-dependent kinase inhibitor (CDKI)1B [p27 (Kip1)] | Regulates (inhibits) cell cycle progression at G1 phase by binding to and preventing the activation of cyclin E-CDK2 or cyclin A-CDK2 or cyclin D-CDK4. Thus, acts as a tumour suppressor (Chu et al., 2008) | • Expression reduced (Borriello et al., 2016) |
| Cyclins A and E      | Group of proteins that bind to and activate the CDKs for regulating cell cycle progression. The two together phosphorylate target proteins that trigger a specific event within the cycle | • Expressions increased (Borriello et al., 2016) |
| • Caspase-3  
• BCL-2 protein  
• BAX protein | Caspases facilitate cell apoptosis. Caspase-3 is activated upon cleavage, which mediates classical apoptotic pathways (Walters et al., 2009)  
• BAX protein pierces the mitochondrial membrane and induces apoptosis (Westphal et al., 2011)  
• BCL-2 inhibits apoptosis, cleaved by caspases during apoptosis | • Increased levels of cleaved caspase-3 (Yang, Li, et al., 2017; Yao et al., 2019; Yuan et al., 2019).  
• Promoted BAX protein expressions (Yang, Li, et al., 2017; Yao et al., 2019)  
• Inhibited Bcl-2 protein expression (Yang, Li, et al., 2017; Yao et al., 2019) |
| Mitochondrial proteins | FIS1: mitochondrial fission protein  
• Mitofusion 2 (MFN2): mitochondrial fusion protein  
• DRP1: mediates mitochondrial fission (Fonseca et al., 2019)  
• Cytochrome C: synthesised in cytosol and translocated to mitochondria, where it associates with haem and functions in the respiratory chain (Garrido et al., 2006) | • Inhibition of FIS1, MFN2 and DRP1 (Yao et al., 2019)  
• Inhibition of translocation of Cytochrome C from cytoplasm to mitochondria (Yao et al., 2019) |
| Cytokine profile    | Immunomodulatory (proinflammatory or anti-inflammatory) proteins that act as disease markers | • Altered (Tanaka et al., 2019) |
| CXCL-12             | Activates or induces migration of hematopoietic progenitor cells, stem cells and endothelial cells; vital for haematopoiesis, embryogenesis and angiogenesis (Janssens et al., 2018) | • Expression reduced in the bone marrow (Y. Zhang, Zhai, et al., 2015) |
gene, the noncanonical ligand that promotes osteoblast differentiation (Maeda et al., 2012) was induced by iron chelation (deferoxamine) (via activation of the PI3K and nuclear factor of activated T cell pathways) and thereby promoted the osteogenic differentiation of BM-stromal cells (Baschant et al., 2016). The necessity of Wnt5a in the chelator-mediated pro-osteogenic effects was further confirmed when the osteogenic effect was abolished in Wnt5a−/− cells (Baschant et al., 2016). Collectively, this indicated a role of iron in MSC differentiation via noncanonical Wnt signalling.

Wnts function as messengers shuttling between stromal cells and cancer cells, and thereby enhance proliferation and invasion of cancer cells. Although increased haem iron intake is associated with increased risk of gastric cancer (Forseca-Nunes et al., 2014), in MSCs, activation of a branch of Wnt signalling (Wnt/Ror2) enhances gastric cancer cell proliferation (Zhao et al., 2018). Although the effect of excess iron on Wnt/Ror2 in the MSCs is yet to be fully elucidated, it could be extrapolated that under iron-loaded conditions, MSCs may promote cancer cell proliferation and accelerate cancer progression via Wnt signalling. Also, Wnt/β-catenin signalling can enhance ROS production, induce aging in MSCs via p53 and p21 (Zhang et al., 2013) and thereby hamper the reparative functions of MSCs. This further adds to the recognised role of Wnt in cancer development and represents Wnt signalling as a target for cancer therapy.

3.2 | ROS signalling

Iron treatment increases ROS production in the MSCs (Table 1), which positively correlates with LIP (Lu, Zhao, Rajbhandary, et al., 2013; Y. Zhang, Zhai, et al., 2015). ROS is not only a by-product of cell metabolism, but also a participant and modulator of many pathways. Hence, excess-iron-induced elevation in ROS affects several cellular functions and involves multiple pathways. For example, iron-loading in the MSCs leads to unregulated ROS levels, which increase MSC apoptosis and decrease viability (Balogh et al., 2016; Zheng et al., 2018). ROS also affect MSC proliferation and differentiation (Atashi et al., 2015). Regulated levels of ROS mediate osteogenic differentiation of MSCs (Atashi et al., 2015) while excess-iron-induced ROS elevation is anti-osteogenic. Normally, the osteogenic differentiation of MSCs into osteoblasts occurs via stimulation of the Wnt and Hedgehog pathways in the MSCs, but excess ROS inhibit these pathways and decrease bone formation (Atashi et al., 2015). Iron-induced inhibition of MSC osteogenic differentiation has been observed consistently in various studies (Balogh et al., 2016; Fei et al., 2014; Yang, Yang, et al., 2017). Unlike this, there is variability in the data on the effect of excess iron on MSC adipogenic differentiation (Figure 3). Stimulation of osteogenesis is believed to inhibit adipogenesis and vice versa (Su et al., 2015), and increased iron levels increase ROS, which favours adipogenesis (Atashi et al., 2015). Thus, it would be expected that iron-loading in MSCs would always favour adipogenic differentiation over osteogenic differentiation. Although some studies reported favoured adipogenic inclination of BM-MSCs under iron loaded conditions (Yang, Yang, et al., 2017; Y. Zhang, Zhai, et al., 2015), Balogh et al. observed no effect of iron-loading on adipogenic and chondrogenic differentiation of BM-MSCs (Balogh et al., 2016). Moreover, in BM-MSCs from β-thalassaemia patients, iron-loading appeared to simultaneously impair both osteogenesis and adipogenesis (Crippa et al., 2019).

While understanding the reasons for such differences, an aspect to consider is the mechanism via which ROS mediates MSC differentiation. Increased oxidative stress/ROS can phosphorylate the forkhead box (FOXO) protein family of transcription factors and this phosphorylated FOXO is known to attenuate the transcription of osteogenic genes and promote adipogenic differentiation (Atashi et al., 2015). Thus, the observed differences in MSC adipogenesis could be partly due to differences in FOXO functionality. In addition, the inability to re-establish the osteogenic potential of iron-treated BM-MSCs by neutralising excess ROS by treatment with the antioxidant N-acetyl-L-cysteine (NAC) (Balogh et al., 2016) suggests that ROS is not the only inhibitor of osteogenic differentiation and other factors may play a role. Nonetheless, excess-iron-induced alteration or exaggeration of ROS signalling can change MSC functionality (Table 2). For example, a study showed that activation of the

### Table 1 (Continued)

| Cellular components | Normal function | Effect of iron-loading on BM-MSCs or umbilical cord MSCs, as observed in vitro, in animal or clinical studies |
|---------------------|-----------------|-----------------------------------------------------------------------------------------------------------|
| Stem cell factor    | A cytokine that can act as a transmembrane protein as well as a soluble protein and plays a role in haematopoiesis. | Expression reduced in the bone marrow (Y. Zhang, Zhai, et al., 2015) |
| Vascular endothelial growth factor | Plays an important role in haematopoiesis, angiogenesis, bone formation, wound healing and development (Duffy et al., 2013) | Expression reduced in the bone marrow (Y. Zhang, Zhai, et al., 2015) |

Abbreviations: BAX, BCL-2-associated X; BCL-2, B-cell lymphoma-2; BM-MSC, bone marrow mesenchymal stem cell; CXCL-12, C-X-C motif chemokine 12; MSC, mesenchymal stem cell; LIP, labile iron pool; ROS, reactive oxygen species; RUNX-2, runt-related transcription factor 2.
The pathway in the MSCs may be hampered by excess ROS alters Wnt signalling in MSCs, the reparative function of this induced injury to lung epithelium in mice (Cai et al., 2015). As excess Wnt/β-catenin pathway in MSCs can repair lipopolysaccharide-induced injury to lung epithelium in mice (Cai et al., 2015). As excess ROS alters Wnt signalling in MSCs, the reparative function of this induced injury to lung epithelium in mice (Cai et al., 2015).

### TABLE 2

| Cellular processes/activities | Effect of iron-loading on BM-MSCs or umbilical cord MSCs, as observed in vitro, in animal or clinical studies |
|-----------------------------|---------------------------------------------------------------------------------------------------|
| **Cell viability**          | • Reduced (Yang, Li, et al., 2017; Yang, Yang, et al., 2017; Y. Zhang, Zhai, et al., 2015; Zheng et al., 2018)  
• Reduced in MSCs from iron-loaded patients with myelodysplastic syndrome (Zheng et al., 2018) |
| **Cell apoptosis/necrosis/autophagy** | • Increased (Lu, Zhao, Chai, et al., 2013; Lu, Zhao, Rajbhandary, et al., 2013; Lu, Zhao, Sajin, et al., 2013; Yang, Li, et al., 2017; Yao et al., 2019; Yuan et al., 2019; Y. Zhang, Zhai, et al., 2015; Zheng et al., 2018)  
• No apoptotic signals (Borriello et al., 2016)  
• Increased in MSCs from iron-loaded patients with myelodysplastic syndrome (Zheng et al., 2018) |
| **Cell cycle**              | • Arrested (Lu, Zhao, Rajbhandary, et al., https://doi.org/10.1002/jcp.303832013)  
• Changed the G0/G1 ratio in vitro (Yuan et al., 2019)  
• High percent of cells in the S phase indicating high rate of cell division (Borriello et al., 2016) |
| **Cell proliferation**      | • Disturbed (Lu, Zhao, Chai, et al., 2013; Lu, Zhao, Rajbhandary, et al., 2013; Y. Zhang, Zhai, et al., 2015), decreased (Lu, Zhao, Sajin, et al., 2013)  
• Increased in a concentration dependent manner (Borriello et al., 2016)  
• Reduced MSC proportion in mice bone marrow cells (Yuan et al., 2019)  
• Slower in BM-MSCs from β-thalassaemia patients (Crippa et al., 2019)  
• Hampered proliferation and differentiation of BM-MSCs in hereditary hemochromatosis and β-thalassaemia patients receiving chronic blood transfusion (Danjou et al., 2013; Suzuki et al., 2008) |
| **Mitochondrial physiology**| • Fragmentation induced via ROS dependent process (Zheng et al., 2018)  
• Altered mitochondrial morphology and function; depolarisation of mitochondrial membrane potential (Yang, Yang, et al., 2017; Yao et al., 2019)  
• Inhibition of mitochondrial fusion/fission (Yao et al., 2019)  
• Increased mitochondrial fragmentation in MSCs from iron-loaded patients with myelodysplastic syndrome (Zheng et al., 2018) |
| **Osteogenic commitment and differentiation of BM-MSCs into osteoblasts** | • Inhibited (Yang, Yang, et al., 2017); iron showed anti-osteogenic effect (Balogh et al., 2016)  
• Impaired in BM-MSCs of patients with low-risk myelodysplastic syndromes (Fei et al., 2014)  
• Limited in BM-MSCs from β-thalassaemia patients (Crippa et al., 2019) |
| **Osteogenic: adipogenic differentiation ratio** | • Disturbed in BM-MSCs; increased adipogenic differentiation (lipid accumulation increased in iron-loaded lipoblasts) (Yang, Yang, et al., 2017; Y. Zhang, Zhai, et al., 2015)  
• No effect on adipogenic and chondrogenic differentiation of BM-MSCs (Balogh et al., 2016)  
• Disturbed adipocyte differentiation of BM-MSCs from β-thalassaemia patients (Crippa et al., 2019) |
| **Extracellular matrix mineralisation (of mature osteoblasts)** | • Attenuated in a dose dependent manner in BM-MSCs (Balogh et al., 2016)  
• Inhibited in BM-MSCs (Borriello et al., 2016; Yao et al., 2019; Y. Zhang, Zhai, et al., 2015) |
| **Hematopoietic supporting functions** | • Inhibited in BM-mononuclear cells from iron-loaded patients and in iron-loaded umbilical cord blood MSCs (Lu, Zhao, Chai, et al., 2013; Lu, Zhao, Rajbhandary and et al., 2013)  
• Decreased in BM-MSCs of β-thalassaemia patients (Crippa et al., 2019)  
• Decreased in BM-MSCs of patients with low-risk myelodysplastic syndromes (Fei et al., 2014)  
• Haematopoiesis is suppressed in iron-loaded patients (Lu, Zhao, Rajbhandary, et al., 2013) and in mouse model of iron-overload (Okabe et al., 2014). Suppression is mainly due to injury to BM-MSCs, that is, impaired quality and quantity of BM-MSCs (Crippa et al., 2019; Yuan et al., 2019; Y. Zhang, Zhai, et al., 2015) |

Abbreviations: BM-MSC, bone marrow mesenchymal stem cell; MSC, mesenchymal stem cell; ROS, reactive oxygen species.

### 3.3 PI3K/AKT pathways

Phosphoinositide 3-kinases (PI3Ks) are intracellular phosphorylating enzymes that transduce signals to downstream signalling molecules.
Activation of PI3K/AKT pathway involves mechanistic target of rapamycin complex 2 (mTOR-C2) mediated phosphorylation (activation) of protein kinase B (AKT), a signal transducer, which phosphorylates multiple downstream effectors including mTOR-C1 and FOXO3 proteins. Phosphorylation of mTOR-C1 mediates PI3K/AKT/mTOR signalling, which promotes anabolic metabolism, cell survival and growth (Hoxhaj & Manning, 2020; Saxton & Sabatini, 2017). Iron-loading in rat BM-MSCs inhibited the phosphorylation of PI3K, AKT and mTOR, thereby inactivating the PI3K/AKT/mTOR pathway (Yao et al., 2019). Thus, the iron-induced MSC apoptosis and death (Table 2) could be partly attributed to the inactivation of this pathway.

FOXO3 proteins are transcription factors that activate target genes to mediate apoptosis and cell cycle arrest, offer protection against oxidative stress, and maintain the HSC pool (Miyamoto et al., 2007). Activation of PI3K/AKT/FOXO signalling involves downregulation of FOXO, which prevents/reduces FOXO-dependent transcription and results in increased ROS levels and enhanced sensitivity to oxidative stress (Dolado & Nebreda, 2008; Farhan et al., 2017; Hoxhaj & Manning, 2020). In mice BM-MSCs, high doses of iron-dextran activated PI3K/AKT/FOXO signalling, where PI3Ks were elevated and FOXO3 expression was reduced (Y. Zhang, Zhai, et al., 2015); a scenario that can continuously increase intracellular ROS (Y. Zhang, Zhai, et al., 2015) and explain the iron-induced detrimental effects on MSCs (Table 2). The effects of iron on the subbranches of PI3K/AKT signalling in MSCs have been captured in Figure 4. As PI3K/AKT signalling is commonly activated in human cancers (Hoxhaj & Manning, 2020) and high iron intake increases the risk for some cancers (Torti & Torti, 2013), the iron-induced activation of PI3K/AKT/FOXO pathway together with inactivation of PI3K/AKT/mTOR pathway in the MSCs could have major implications in iron loaded patients and in their increased predisposition to cancers, such as in case of hereditary hemochromatosis (Kowdley, 2004).

### 3.4 MAPK pathways

The MAPK cascades encompass major signal transduction pathways that maintain cell survival, growth, differentiation, cell cycle and...
apoptosis in response to extracellular stimuli. These cascades involve signalling via four main subgroups: extracellular signal-related kinases (ERK1/2), c-Jun N-terminal or stress-activated protein kinases (JNK/SAPK), ERK/big MAP kinase 1, and the p38 group of MAP kinases, with a phosphatase-mediated cross-talk between these MAPK cascades (Junttila et al., 2008; Zarubin & Han, 2005).

Iron overload in rat BM-MSCs increased the phosphorylation of ERK1/2 and JNK (Yao et al., 2019) and increased ERK levels in mice BM-MSCs (Yang, Yang, et al., 2017). As increased intracellular ROS activate the ERKs, JNKs or p38-MAPKs (Son et al., 2011), this excess iron-induced activation of MAPK/ERK and MAPK/JNK pathways in rat BM-MSCs could be mediated via iron-induced ROS. However, in human BM-MSCs, although iron increased the phosphorylation of nuclear ERK1/2, activation of this pathway occurred without apoptotic signals and ROS elevation (Borriello et al., 2016). Thus, apart from ROS, there may be other mediators that activate these pathways under excess iron conditions. It is also possible that iron may directly stimulate these pathways, as indicated in the case of TGF-β signalling in the hepatic stellate cells (Mehta et al., 2018).

Under physiological and cancerous conditions, the MAPK/ERK1/2 and MAPK/JNK pathways additionally regulate the Warburg effect, that is, metabolic (glucose) reprogramming of tumour cells, which allows continuous activation of signalling pathways leading to increased cell proliferation and evasion of apoptosis. Activation of MAPK/ERK1/2 promotes the Warburg effect while activation of JNK1 suppresses this effect and promotes apoptosis (Papa et al., 2019). This suggests that while the iron-induced activation of ERK1/2 in rat BM-MSCs (Yao et al., 2019) could be to initiate cancerous effects, the simultaneous increase in phosphorylation and activation of JNK (Yao et al., 2019) could be a counter protective response to maintain cell sanity and prevent tumorigenesis under iron-loaded conditions. Induction of such opposing effects are possible because the JNK family includes distinct proteins JNK1, JNK2 and JNK3, whose functions can differ or be antagonistic depending on the cell type in cancer (Papa et al., 2019).

Excess iron can increase ROS levels, which can stimulate p38-MAPK signalling (Son et al., 2011). Activation of this pathway includes phosphorylation of the protein p38 and promotion of cell death (Zarubin & Han, 2005). In mice BM-MSCs, iron treatment increased the levels of p38 and phospho-p38 (Shen et al., 2018; Yang, Yang, et al., 2017). Also, in human BM-mononuclear cells (a heterogenous group of cells that include the BM-MSCs), iron increased the levels of phospho-p38 and p38-MAPK, collectively suggesting iron-induced stimulation of this pathway (Lu, Zhao, Rajbhandary, et al., 2013), which could be one of the causes of iron-induced MSC death (Table 2). However, in iron-treated human umbilical cord MSCs, total p38-MAPK expression remained unaltered (Lu, Zhao, Rajbhandary, et al., 2013) and in iron-treated rat BM-MSCs, phosphorylation of p38 remained unaltered (Yao et al., 2019). Further clarification is needed on the iron activation of the p38-MAPK pathway in MSCs. Nonetheless, activation of p38-MAPK is associated with apoptosis (Zarubin & Han, 2005) and this explains the iron-induced apoptosis of MSCs (Table 2). Also, p38 controls the cell cycle via p21, a cyclin dependent kinase inhibitor that mediates cell cycle arrest, and which is a substrate of p38 (Zarubin & Han, 2005). Hence, the iron-induced MSC cycle arrest (Table 2) could be partly attributed to the iron-induced increment in p21 levels, as observed in mice BM-MSCs (Yang, Yang, et al., 2017).

3.5 | p53 signalling

p53 is a tumour suppressor protein and a transcription factor that is recognised as the guardian of the human genome. Upon activation, p53 exerts several protective effects including cell cycle arrest,
apoptosis, DNA-repair and regulates ROS levels and cell metabolism (Budanov, 2014). Iron-loading significantly increased p53 levels in human BM-monomonuclear cells (Lu, Zhao, Rajbhandary, et al., 2013), in human umbilical cord MSCs (Lu, Zhao, Rajbhandary, et al., 2013), and in mice BM-MSCs (Yang, Yang, et al., 2017); likely a defensive response against the toxic effects of excess iron. This elevation in p53 levels may be due to iron-induced or ROS-induced stimulation of the p38-MAPK pathway (Lu, Zhao, Rajbhandary, et al., 2013), which involves p38-mediated phosphorylation and activation of p53 (Zarubin & Han, 2005). Elevation in p53 may also be due to iron/ROS-induced DNA breaks, which may stimulate the ataxia-telangiectasia-mutated (ATM)-Chk2-p53-p21 pathway. In this pathway, DNA damage induces phosphorylation (activation) of p53 and its stabilisation by ATM and ataxia telangiectasia and Rad3-related protein (ATR), which acts on p21. Thus, the iron-induced increment in p53 levels may have contributed to the cycle arrest of iron-loaded BM-monomonuclear cells and umbilical cord MSCs, and led to apoptosis of iron-treated MSCs (Lu, Zhao, Rajbhandary, et al., 2013) (Table 2); probably a defence mechanism against the toxic effects of excess-iron-induced ROS.

3.6 | AMPK/MFF/DRP1 pathway

5′-Adenosine monophosphate (AMP)-activated protein kinase (AMPK) is considered as the "guardian of cell metabolism and mitochondrial homeostasis" (Herzig & Shaw, 2018). It acts as an energy and redox sensor. When the ratio of AMP:ATP is high (i.e., ATP levels are low), AMPK is activated via phosphorylation, which leads to phosphorylation of the downstream signalling molecule mitochondrial fission factor (MFF). MFF is the mitochondrial outer membrane receptor for DRP1 (the GTPase dynamin-related protein 1). MFF recruits DRP1 from the cytosol and promotes DRP1 binding to MFF to induce mitochondrial degradation (mitophagy) (Toyama et al., 2016; Trewin et al., 2018). In iron-treated MSCs, increased ROS levels and low electron transport chain activity reduced ATP concentrations, which phosphorylated and thereby activated AMPK. This activation increased MFF phosphorylation and increased total DRP1 protein levels, thereby mediating mitochondrial fragmentation via activation of AMPK/MFF/DRP1 pathway (Zheng et al., 2018). The effect of iron on this pathway was further confirmed when AMPK−/− iron-loaded MSCs showed comparatively lower iron-induced cell death, increased cell viability and decreased mitochondrial fission. Similarly, MFF−/− iron-loaded MSCs showed reduced AMPK-induced mitochondrial fragmentation (Zheng et al., 2018). Together, the data demonstrated the role of iron in MSC mitochondrial physiology.

4 | Saviours of MSCS AGAINST THE DETRIMENTAL EFFECTS OF IRON-LOADING

4.1 | Endogenous proteins

Under physiological conditions, endogenous antioxidants scavenge the excess free radicals and thereby regulate ROS levels to prevent damage. Additionally, the iron-binding proteins (e.g. systemic proteins like transferrin and lactoferrin, and intracellular proteins such as cytochromes and iron regulatory proteins) bind to iron, which reduces the probability of free iron accelerating the Fenton reaction, and thus prevent oxidative damage.

Moreover, specific endogenous proteins/peptides protect various cells/tissues including the MSCs from the detrimental effects of iron-loading. For example, the liver-secreted iron-hormone hepcidin maintains systemic iron homoeostasis in the body. Essentially, plasma iron elevation increases hepcidin secretion from the hepatocytes. Secreted circulatory hepcidin binds to and degrades the iron-exporter ferroportin present on the surfaces of various cell types. This prevents iron entry into the circulation not only from the denal enterocytes but also from the iron-storing hepatocytes and macrophages (Ganz, 2011). Ferritin is another protein that protects from the toxic effects of excess iron by sequestering reactive iron and storing it in a redox inactive form. Under basal conditions, both light and heavy chains of ferritin are highly expressed in MSCs, indicating an increased capacity of MSCs to store iron under iron-loaded conditions (Crippa et al., 2019) and presenting MSCs as local iron-regulators. Haem oxygenase-1 is a haem-degrading enzyme and a protector against oxidative stress. It was upregulated during the early stages of transfusion-induced iron-loading in mice (Yu et al., 2018), and this protected the iron-loaded BM-MSCs from oxidative stress and reduced iron-induced apoptosis. The rescue effect was executed by haem oxygenase-1-induced decrement in ROS levels and increment in interleukin 10 (IL-10) secretion via the ERK pathway (Yu et al., 2018). More recently, a new dermal cell subpopulation was identified that expressed the ATP-binding cassette subfamily B member 5 (ABCB5) transporter. When dermal MSCs expressing ABCB5 were administered in an iron-overloaded mouse that modelled nonhealing human venous leg ulcers, these cells reduced the macrophage-induced inflammation and accelerated wound healing. This benefit was because ABCB5+ dermal MSCs secreted IL-1-receptor antagonist, which reduced the inflammation and shifted the macrophage mode from proinflammatory to anti-inflammatory. This promised a cure for chronic nonhealing wounds in iron-loaded conditions (Vander Beken et al., 2019).

4.2 | Exogenous iron-chelators and antioxidants

Some exogenously administered compounds such as iron chelators and antioxidants showed to offer protection to the iron-loaded MSCs and reverse the excess-iron-induced negative effects. For example, in mice, the iron chelator deferasirox reduced iron deposition and LIP, and partially attenuated the iron-induced injury to BM-MSCs by rescuing cell viability and proliferation. Moreover, it restored the iron-induced reduction in C-X-C motif chemokine 12 (CXCL-12), stem cell factor, and vascular endothelial growth factor (VEGF) in the BM (Y. Zhang, Zhai, et al., 2015); molecules that participate in haematopoiesis and development. CXCL-12 can additionally induce cell migration and is vital for angiogenesis.
(Janssens et al., 2018), while VEGF is involved in bone formation and wound healing (Duffy et al., 2013). Iron-induced reductions in the levels of these molecules in MSCs followed by restoration of levels upon iron chelation jointly highlight the significance of iron levels in the MSCs and indicates the role of iron in the processes these molecules mediate. In addition, deferasirox inhibited PI3K/AKT/FOXO signalling in BM-MSCs (Y. Zhang, Zhai, et al., 2015); the pathway, which, if activated, has the potential to enhance oxidative stress. Predictably, in iron-loaded mice, deferasirox showed protective effects on BM haematopoiesis against iron-loading (Shen et al., 2017) and there are ample clinical studies that show positive effects of iron chelation on haematopoiesis.

Deferoxamine, another iron chelator, showed similar remedial effects by rescuing the iron-induced suppression of BMP-2treated BM-MSCs and in iron-loaded BM-MSCs and in iron-loaded mice. It decreased the levels of NOX4, an activator of ROS, and repressed the iron-induced bone loss in mice (Yuan et al., 2019). In iron-treated umbilical cord MSCs, deferoxamine or the antioxidant NAC reduced apoptosis, decreased ROS levels and inhibited p38-MAPK signalling by reducing p-p38 MAPK expression, thereby attenuating the iron-induced injury (Lu, Zhao, Rajbhandary, et al., 2013). Also, NAC more effectively reduced the iron-induced increment in p53 levels (Lu, Zhao, Rajbhandary, et al., 2013), indicating that the detrimental effects of iron on p53-related signalling may be predominantly mediated via ROS in excess iron conditions. Moreover, in iron-loaded BM-MSCs from patients with MDS, iron chelation and NAC attenuated the iron-induced AMPK phosphorylation, and the overall activity of the AMPK/MFF/DRP1 pathway, thereby reducing excess-iron-induced mitochondrial fragmentation (Zheng et al., 2018).

Excess iron hampers MSC osteogenic differentiation (Atashi et al., 2015), whereas bone morphogenetic protein 2 (BMP-2) promotes MSC osteogenic differentiation. In BMP-2 treated MSCs, deferoxamine enhanced the osteogenic differentiation ability of BMP-2, as evidenced by increased alkaline phosphatase (ALP) activity and calcium deposition in MSCs. This was accompanied by enhanced phosphorylation of glycogen synthase kinase-3β and increment in β-catenin levels; reiterating the involvement of the Wnt/β-catenin pathway in the iron-regulated osteogenic differentiation of MSCs (Qu et al., 2008). In an instance, when superparamagnetic iron oxide nanoparticles (commonly used for labelling and in vivo tracing of administrated MSCs) increased the MSC iron content as desired but diminished the osteogenic differentiation of human MSCs, deferoxamine eliminated this iron-induced antiosteogenic effect and reiterates the role of free iron in preventing MSC osteogenic differentiation (Chen et al., 2010). In iron loaded BM-MSCs of β-thalassaemia patients, deferoxamine relieved the iron-induced repression of the genes CXCL-12, VEGF-A, angiopoietin-1 (ANGPT1) and IL-6 (Crippa et al., 2019), whose products form the BM niche and enhance MSC functionality. This demonstrates that iron regulation in the MSCs is essential for maintaining BM homoeostasis.

### 4.3 Herbs

Usage of herbal ingredients in enhancing osteogenesis in MSCs has been discussed previously (Udalamaththa et al., 2016). Icariin, a flavonoid glucoside isolated from *Herba Epimedi* protected rat BM-MSCs in vitro from the iron-induced ROS generation, apoptosis and inhibition of osteogenic differentiation. In addition, it rescued BM-MSC proliferation and prevented the iron-induced depolarisation of mitochondrial membrane potential, which is an indicator of mitochondrial dysfunction and leads to mitophagy. Icariin exerted these protective effects by (a) modulating mitochondrial fusion and fission, (b) enhancing the mRNA and protein expressions of RUNX-2, osteopontin and active β-catenin (components involved in osteogenic differentiation), (c) activating the PI3K/AKT/mTOR pathway and (d) inhibiting the ERK1/2 and JNK pathways (Yao et al., 2019). Another example is Astragalus polysaccharide, an active ingredient of *Astragalus membranaceus* that is often used in Chinese medicine. In mice BM-MSCs, it prevented mitochondrial ROS accumulation, decreased the iron-induced apoptosis and senescence, and attenuated the downregulation of the pluripotent genes SOX-2, OCT-4 and NANOG, thus reducing excess-iron-induced BM-MSC dysfunction (Yang et al., 2016).

### 4.4 Melatonin

Melatonin is a pineal-gland-secreted naturally occurring hormone that performs numerous physiological functions including the regulation of sleep patterns and acting as an endogenous antioxidant (Reiter et al., 2016). When added exogenously to iron-loaded mice BM-MSCs, it rescued osteogenic differentiation. It reversed the iron-induced reduction in the expressions of the osteoblast-specific genes alkaline phosphatase (ALPL), collagen-1 (COL-1), BMP-2 and BMP-4, and increased the expressions of osteogenic transcription factors RUNX-2 and BGLAP (ostecalcin). In addition, melatonin partially prevented iron-induced adipogenic differentiation by inhibiting the expressions of the adipogenic genes PPARγ and C/EBPα (Yang, Yang, et al., 2017). This may have additionally favoured BM-MSC osteogenic differentiation because inhibition of adipogenic regulators can promote osteogenic differentiation (Atashi et al., 2015; Su et al., 2015). The results were replicated in in vivo studies (Yang, Yang, et al., 2017). Melatonin also rescued cell proliferation, attenuated premature senescence and reduced depolarisation of mitochondrial membrane potential in iron-loaded BM-MSCs. Mechanistically, it prevented the upregulation of p53, ERK and p38 proteins, thereby blocking p53/ERK/p38 activation (Yang, Yang, et al., 2017). In another study involving mice BM-MSCs, melatonin inhibited iron-induced ROS accumulation (Yang, Li, et al., 2017), likely by modulating the expressions of genes involved in antioxidant and ROS generation (Hu & Li, 2019) and prevented iron-induced apoptosis by altering the expressions of apoptosis-related proteins BCL-2, BAX and cleaved caspase-3. The functional properties of
melatonin could be exploited to enhance MSC therapy. Before MSC transplantation, during the in vitro proliferation and differentiation stage, MSCs are placed in adverse environments where excessive ROS production can substantially reduce their therapeutic potential. Pretreatment of BM-MSCs with melatonin has shown to prevent MSC death (Yang, Li, et al., 2017). Thus, addition of optimised concentrations of melatonin to the growth medium could regulate ROS levels and prevent the loss of MSC reparative potential.

### 4.5 α-Lipoic acid

α-Lipoic acid is a naturally occurring fatty acid present in prokaryotic and eukaryotic cell membranes, and the cytosol. It is an antioxidant and a metal chelator that is used for ameliorating various chronic conditions including diabetes mellitus, hypertension and Alzheimer’s disease. α-lipoic acid treatment reduced the iron-induced detrimental effects in a human MSC line. It decreased iron accumulation, ROS elevation, loss of mitochondrial membrane potential and autophagy (systematic degradation and recycling of cellular parts). Also, α-lipoic acid increased intracellular glutathione levels to enhance cellular defence against ROS and regulate intracellular redox state (Camilo et al., 2019).

Identification of such proteins/hormones/compounds and investigation on how these rescue MSCs from iron-induced toxicity can be very useful in formulating supplementary iron-related therapies for iron-loaded conditions.

### 5 IRON AND IRON-RELATED ENDOGENOUS EFFECTORS OF MSCs

MSCs express the mediators of iron regulation, namely, transferrin receptor-1 (TfR-1), ferroportin, hepcidin, ferritin, divalent metal transporter 1 (DMT-1), Zrt-/Irt-like protein 14 (ZIP14) and ZIP8, the zinc transporters (Crippa et al., 2019; Esfandiyari et al., 2019). Their ability to uptake iron through the iron-uptake channels and store it as ferritin shows their direct contribution in managing excess iron, like the macrophages. However, prolonged iron (and ROS) exposure appears to damage the iron-sensing and iron-storing machinery in the MSCs. Although iron and iron-related proteins have a canonical role in mediating normal MSC physiology, these also have specific roles in MSC biology, as described in this section.

#### 5.1 Iron

At the physiological level, ascorbate promotes intestinal iron absorption (Sharp & Srai, 2007). At cellular level, iron and ascorbate are important for determining cell fate specification. Histone methylation/demethylation is an important epigenetic event that modulates the expression of genes by turning them off and on. Several MSC genes whose products contribute to the development of BM niche and provide hematopoietic supportive functions are subjected to H3K36 methylation, which in turn is regulated by lysine-specific demethylases (KDMs) that are responsive to iron (Crippa et al., 2019). This suggests that via modulation of KDMs, iron may play a crucial role in determining the MSC’s function of supporting haematopoiesis. In vitro, a combination of iron and ascorbate regulated histone methylation in human skeletal MSCs, where the ascorbate-regulated histone demethylase KDM4B was found to be crucial and sufficient to promote the specification of MSCs from mesoderm progenitors. This ascorbate-induced promotion of MSC specification was iron-dependent but redox-independent, which clearly shows the significance of iron in MSC specification. Also, the ascorbate-iron combination promoted long-term MSC self-renewal and increased the osteochondrogenic potential (Liu et al., 2020). This approach could be utilised to extend the lifespan of MSCs in vitro during pretransplantation procedures and to increase the probability of repairing cartilage injuries posttransplantation.

#### 5.2 Hepcidin

MSCs can secrete the iron hormone hepcidin (Esfandiyari et al., 2019). Since excess iron is a risk factor for osteoporosis (Weinberg, 2006), it is possible that the MSC-secreted hepcidin reduce local tissue iron levels and thereby act as an endogenous protector against the development of osteoporosis (Zhang et al., 2018). Hepcidin may also execute its anti-bone-loss function by modulating BM-MSC differentiation. Hepcidin treatment has been shown to enhance osteogenic differentiation and mineralisation, and increase the levels of ALP and osteocalcin in rat BM-MSCs. Simultaneous increments in the mRNA expressions of BMP-2 and SMADs 1, 5 and 8 indicate that hepcidin-induced osteoblastic differentiation is mediated via the BMP2/SMAD pathway (H. Lu, Lian, et al., 2015). As BM-MSC osteogenic differentiation involves p38-MAPK-SMAD signalling too (Kim et al., 2013), the effect of hepcidin treatment on the components of MAPK signalling were examined. Data showed that hepcidin upregulated phospho-p38 levels, suggesting that hepcidin-induced osteogenic differentiation may additionally involve the p38-MAPK pathway (M. Lu, Xia, et al., 2015). Together, this supports the role of MSC-derived hepcidin in MSC differentiation into osteoblasts and bone metabolism.

#### 5.3 Transferrin receptor, ferroportin and iron transporters

Iron entry in the MSCs occurs via similar routes to other cell types, that is, via transferrin-dependent and transferrin-independent mechanisms (Borriello et al., 2016). Transferrin-dependent mechanism involves iron entry into the cells via the cell-surface protein TfR-1. Cellular iron ejection is mediated via the transmembrane protein ferroportin. In response to alterations in cellular iron status, their mRNA transcripts are subjected to regulatory mechanisms to
produce these proteins at optimal levels so that intracellular iron homoeostasis is maintained. For example, a normal regulatory response of proliferating nonerythroid cells to increased intracellular iron is Tfr-1 mRNA degradation (to prevent further iron uptake) and ferroportin mRNA upregulation (to remove excess intracellular iron) (Muckenthaler et al., 2008). Such responses were observed in iron-treated MSCs from healthy subjects (Crippa et al., 2019) demonstrating that not only the iron entry but the regulation of iron import-export in healthy MSCs is similar to other cell types. However, BM-MSCs from β-thalassaemia patients showed lower levels of Tfr-1 and ferroportin expression than BM-MSCs from healthy subjects. Moreover, upon iron treatment, the iron-loaded BM-MSCs diverted from the canonical regulation and showed upregulation of Tfr-1, which was attributed to excess ROS production. Like Tfr-1, ferroportin regulation contrasted the norm and showed an inability to induce its expression rapidly in iron-treated BM-MSCs from β-thalassaemia patients (Crippa et al., 2019). These “atypical” responses of Tfr-1 and ferroportin clearly reflect impaired iron-sensing in BM-MSCs under iron-loaded conditions, as generally observed in transfusion-dependent β-thalassaemia patients. Thus, the atypical responses of Tfr-1 and ferroportin contribute to dysregulating intracellular iron homoeostasis in BM-MSCs of iron-loaded patients. The mechanisms by which these iron-injured BM-MSCs divert from canonical regulation and acquire an iron-gaining and retaining phenotype should be investigated.

Notably, there are additional aspects to Tfr-1 responses, and it is yet to be confirmed which of the following aspects are applicable to MSCs. First, Tfr-1 response to intracellular iron concentration (direct or inverse association) is cell-type specific and is modulated by cell proliferation, differentiation, antigens and mitogens (Schäfer et al., 2007). For example, in proliferating nonerythroid cells, Tfr-1 expression is negatively regulated by intracellular iron (Chan et al., 1994), whereas in cultured human monocytes-macrophages, iron treatment upregulates Tfr-1 expression (Testa et al., 1989). Also, in proliferating nonerythroid cells, Tfr-1 numbers are positively correlated with proliferation, while in haemoglobin-synthesising cells, Tfr-1 numbers elevate during differentiation, so these negatively correlate with proliferation (Chan et al., 1994), in light of the fact that usually, increased proliferation is linked with reduced differentiation.

With regard to transferrin-independent mechanisms of MSC iron uptake, levels of the NTBI transporters ZIP14, ZIP8 and DMT-1 in BM-MSCs from healthy and β-thalassaemia patients were similar, unlike the case with Tfr-1. However, like Tfr-1, these NTBI transporters were induced at higher levels in iron-treated BM-MSCs from β-thalassaemia patients compared to BM-MSCs from healthy subjects (Crippa et al., 2019). Collectively, this suggests that in β-thalassaemia, the MSCs not only lose their iron-sensing ability (partly due to the impaired iron-sensing mechanism mediated via Tfr-1 and ferroportin transcripts) but also acquire unregulated iron levels via two routes; transferrin receptor-dependent and independent mechanisms; the latter mediated via the aforementioned iron transporters that allow NTBI uptake from plasma (Knutson, 2019). As such, β-thalassaemia patients show high levels of NTBI in the serum (al-Refaie et al., 1992).

5.4 | Lactoferrin

Lactoferrin is an iron-binding glycoprotein found in body secretions such as saliva, tears, serum, colostrum and milk. It exhibits antimicrobial, anti-inflammatory and immunomodulatory activities and has an anabolic effect on the bone (Cornish et al., 2004). Lactoferrin induced proliferation and osteogenic differentiation of mouse MSCs C3H10T1/2, as evidenced by elevations in osteoblastic markers osteocalcin, osteopontin, collagen-2α1 and fibroblast growth factor-2 (Li et al., 2019). The osteogenic differentiation occurred via stimulation of the canonical transforming growth factor β (TGF-β) signalling pathway (Li et al., 2019). This pathway involves ligand-mediated activation of TGF-β-receptor(RI/II, followed by phosphorylation (activation) of TGFβ-RI and then of SMAD 2/3, which subsequently binds to SMAD-4. The complex of SMADs translocates into the nucleus and stimulate TGF-β target genes for cell growth and differentiation (Mehta, Farnaud, et al., 2019). Lactoferrin-treated cells increased the levels of TGF-β receptors I and II and activated SMAD-2 (Li et al., 2019). Such lactoferrin-induced stimulation of the canonical TGF-β pathway in the MSCs is similar to that induced by holotransferrin in murine hepatic stellate cells; an event which has been proposed to enhance liver fibrosis in iron-loaded conditions (Mehta et al., 2018). These examples collectively demonstrate that iron-induced activation of signalling pathways is independent of cell type or purpose; physiological or pathological.

In the MSCs, lactoferrin activated p38 and thereby the p38-MAPK signalling, which was dependent on TGF-β/Smad-2 signalling (Li et al., 2019). As the MAPK-pathway induces osteogenic differentiation in MSCs and bone formation, it is not surprising that in postmenopausal women, lactoferrin reduced bone resorption markers and was thus beneficial for bone turnover (Bharadwaj et al., 2009). As the MAPK-pathway reflects one of the noncanonical TGF-β signalling routes, the effect of lactoferrin on p38-MAPK signalling in the MSCs indicates that like canonical TGF-β signalling, noncanonical TGF-β signalling networks may also be affected by iron.

5.5 | Lipocalin-2

Lipocalin-2 is a small iron-binding cytokine of innate immunity that is released by various cell types/tissues under physiological and pathological conditions. It exhibits iron regulatory and transporting ability. By scavenging iron, it not only reduces iron availability for pathogens and provides protection against bacterial infections but also protects from oxidative stress (Xiao et al., 2017).

MSCs can secrete lipocalin-2 and upregulate its secretion following lipopolysaccharide stimulation (Gupta et al., 2012). Lipocalin-2 treatment to human BM-MSCs increased the production of osteoblasts via upregulation of RUNX-2, increased osteocalcin
levels, and promoted fibroblast formation (M. Lu, Xia, et al., 2015). Also, lipocalin-2 treatment primed BM-MSCs for osteogenesis and chondrogenesis (Tsai & Li, 2017) and promoted the generation of ROS, which is known to modulate BM-MSC differentiation (M. Lu, Xia, et al., 2015). However, adipogenesis was impaired via decrement in PPARγ expression (M. Lu, Xia, et al., 2015). This was expected because inhibition of adipogenic differentiation is usually accompanied by promotion of osteogenic differentiation of BM-MSCs (Su et al., 2015). Notably, this MSC response to lipocalin-2 contrasts the MSC response to iron, where iron diminishes osteogenic differentiation and in some instances, promotes adipogenic differentiation in MSCs (Table 1) (Atashi et al., 2015); rightly so because lipocalin-2 is an iron scavenger and is expected to trigger a response opposite to that induced by NTBI (Tables 1 and 2). Alongside, lipocalin-2 has shown to elevate the expressions of TGF-β, VEGF and BMP-2. (growth factors involved in regulating marrow environment) and increase the osteoblast products osteoprotegerin and collagen type-1. Collectively, this indicates a role of lipocalin-2 in BM-MSC differentiation and remodelling of the BM environment (M. Lu, Xia, et al., 2015).

In addition to understanding the physiological role of lipocalin-2 in MSC biology, it is important to study MSC responses to lipocalin-2 treatment because lipocalin-2 overexpression in MSCs is being explored as a means of protecting and strengthening the MSCs to tackle the adverse environments during their in vitro expansion for transplantation (Halabian et al., 2015).

5.6 | Bone morphogenetic proteins

BMPs are multifunctional circulatory growth factors that are essential for a variety of developmental and physiological processes. BMP-2 and BMP-6 secreted by the liver endothelial cells play important roles in inducing hepcidin in hepatocytes via the BMP/SMAD-1/5/8-SMAD4 pathway (Canali et al., 2017, p. 6). Although BMP-2 is thought to mediate basal hepcidin induction in the liver, BMP-6 regulates (induces) hepatic hepcidin in response to increased tissue iron and thereby plays a central role in iron homeostasis (Corradini et al., 2011; Silvestri et al., 2019). Since MSCs can produce hepcidin (Esfandiary et al., 2019), it is likely that the MSCs may utilise this pathway for hepcidin production. BMPs also mediate osteogenic and chondrogenic differentiation of MSCs via the BMP/SMAD pathway and aid in bone formation. Although BMP-6 is produced by BM-MSCs before differentiation into osteoblasts (Vukicevic & Grgurevic, 2009), BMP-2 promotes osteoblastic differentiation of MSCs and is essential for bone formation (Beederman et al., 2013). Thus, the usage of BMP-2 has been explored to enhance MSC therapy. When human BM-MSCs were exposed to pulsed electromagnetic fields (clinically used for bone fracture healing) in combination with BMP-2, the osteogenic activity of BMP-2 was enhanced via activation of SMAD1/5/8 and p38-MAPK signalling (Martini et al., 2020). This demonstrates the significance of BMP-2 in MSC biology. Presuming the MSCs utilise the BMP- SMAD1/5/8 pathway for hepcidin induction like the hepatocytes, activation of the BMP-SMAD1/5/8 pathway in the MSCs may have a dual role; first, secretion of hepcidin to provide local protection against the toxic effects of excess iron, and second, simultaneous osteoblastic differentiation of MSCs for bone formation. Thus, this pathway may be common for iron regulation and osteogenic differentiation in the MSCs.

5.7 | Hypoxia-inducible factors (HIFs)

HIFs are transcription factors that sense oxygen concentration (hypoxia) and induce the expression of target genes to modulate several processes including iron metabolism and MSC proliferation. The context of hypoxia and the role of HIFs in MSCs is important because BM-MSCs and placental MSCs reside and function in a low oxygen microenvironment, probably to keep ROS concentration low and protect against DNA damage, while favouring haematopoiesis in the BM (Wessling-Resnick, 2017). Typically, hypoxia suppresses hepcidin expression via HIFs (Mastrogiannaki et al., 2012) resulting in increased intestinal iron uptake and release of iron from the iron-storing cells. HIFs not only function in iron regulation but also impact MSC biology. HIF-2α suppresses hepcidin expression via erythropoietin induction (erythropoiesis) (Mastrogiannaki et al., 2012). It also promotes the proliferation of human placenta-derived MSCs under hypoxic conditions via the MAPK/ERK pathway, as evidenced via increased proliferation-related transcription factors and binding of HIF-2α to MAPK3 (ERK1) promoter (Zhu et al., 2016). Similarly, while HIF-1α suppresses hepcidin, it plays a central role in cartilage formation during development. HIF-1α also plays a role in the differentiation of MSCs and chondroprogenitors into cells that can produce cartilage-like ECM and maintain the articular phenotype of chondrocytes following differentiation. The differential regulation of chondrogenesis in human BM-MSCs by the inhibitors of HIF-1α hydroxylase is believed to have implications in developing cartilage repair therapies (Taheem et al., 2018).

This overview of the MSC effectors clearly shows a link between iron regulation and MSC functionality and suggests that the mechanisms involved in these processes may be mediated via common signalling pathways and/or factors.

6 | CLINICAL CONDITIONS OF IRON EXCESS AND MSCS

Iron overload can occur due to mutations in the iron-regulatory genes, as characterised in hereditary hemochromatosis (Pietrangelo, 2016) or iron can be accumulated as seen in African iron overload (dietary iron loading), neurodegenerative disorders, chronic liver diseases (alcoholic and nonalcoholic liver disease), menopause and in conditions which require repeated blood transfusions such as β-thalassaemia, aplastic anaemia, BM failure, MDS, sickle cell anaemia or other acquired and inherited refractory anaemias (Jeney, 2017; Mehta et al., 2016; Mehta, Ahmed, et al., 2019; Mehta,
Farnaud, et al., 2019; Wang et al., 2016). Notably, some of these conditions are not independent of each other but are interconnected, often one leading to another (Figure 5). For instance, excess iron is a risk factor for cancers (Fonseca-Nunes et al., 2014; Torti & Torti, 2013). Hereditary hemochromatosis patients are at risk of developing hepatocellular carcinoma, and also show increased risk for diabetes and metabolic syndrome (Simcox & McClain, 2013; Wessling-Resnick, 2017). Inevitably, hereditary hemochromatosis patients and β-thalassaemia major patients can develop diabetes (Barnard & Tzoulis, 2013; Barton & Acton, 2017). This section discusses the contribution of MSCs in some of these iron loading pathologies and envisages a role of MSCs in promoting interconnections between different pathologies in excess iron states.

### 6.1 Hereditary hemochromatosis

Mutations in the genes that either encode hepcidin or modulators of hepcidin (HFE, HJV, TFR-2) or ferroportin lead to iron deposition in the body; a group of conditions called hereditary hemochromatosis (Pietrangelo, 2010). Approximately 40%–80% and 30% of hereditary hemochromatosis patients suffer from osteopenia and osteoporosis, respectively, involving a disbalance between bone resorption and bone formation (Jeney, 2017). Thus, in the context of MSCs in hereditary hemochromatosis, the most studied topic has been the impact of excess iron on BM-MSC differentiation and bone health. The ability of BM-MSCs to differentiate into osteoblasts is crucial for bone remodelling and healing (Jeney, 2017). Several secreted differentiation factors like TGF-β, BMPs, Wnt proteins and the Indian HedgeHog protein can activate respective signalling cascades to stimulate osteogenic differentiation of BM-MSCs. All these pathways in the BM-MSCs converge on the main osteogenic transcription factor RUNX-2, which mediates the transcription of the main bone-specific proteins osteocalcin, osteopontin collagen I-α1, and ALP (Jeney, 2017). Iron (and ferritin) selectively inhibits the osteogenic differentiation of BM-MSCs by preventing the upregulation of RUNX-2 and its target genes osteocalcin and ALP (Balogh et al., 2016). Such an effect of iron and ferritin is also seen in osteoblasts, where the iron-induced inhibition of osteoblast activity is mediated via ferritin’s ferroxidase activity. Here, ferritin downregulates the osteoblast-specific markers ALP, osteocalcin and CBF-α1 and inhibits calcification (Zarjou et al., 2010). This makes elevated iron a risk factor for osteoporosis in hereditary hemochromatosis (Guggenbuhl et al., 2005; Jeney, 2017; Valenti et al., 2009). Also, iron-loading causes apoptosis of BM-MSCs (Yuan et al., 2019) and affects osteoblasts negatively, which reduces bone formation (Tsay et al., 2010). The increased risk for osteoporosis is linked with low osteogenic potential of the circulating MSCs, even though MSC numbers may be increased (Jeney, 2017). This explains bone loss and low bone mineral density in hereditary hemochromatosis patients (Jandl et al., 2020).

### 6.2 Diabetes

Diabetes is characterised by prolonged elevation of blood sugar levels. A strong correlation between high iron levels and diabetes is well-established (Swaminathan et al., 2007). Contextually, two

![Figure 5](image-url)
factors increase the risk for type 2 diabetes; high iron stores, that is, increased ferritin levels, and a low transferrin receptor: ferritin ratio. Inevitably, type 2 diabetes is frequently associated with high ferritin levels. Insulin resistance in these patients can be ameliorated by iron chelation (Lecube et al., 2004). In addition, decreased iron-binding antioxidant capacity (Van Campenhout et al., 2006) and the prevalence of NTBI in type 2 diabetic patients indicates the contribution of NTBI in diabetes (Lee et al., 2006). Together, this reiterates the significance of iron in the development of diabetes. On the other hand, physiological stress in diabetes causes functional defects in the MSCs. Here, MSCs show elevated oxidative stress, reduced proliferation and differentiation, increased apoptosis and altered cytokine profile (Fijany et al., 2019). Thus, it is likely that iron-damaged MSCs may partly contribute to diabetes pathology.

Normally, MSCs promote vasculogenesis, that is, de novo formation of blood vessels. However, in diabetes, MSCs show altered support towards vasculogenesis and angiogenesis (growth of newly formed blood vessels) by expressing substantially low levels of VEGF, a potent angiogenic factor, and αβ-crystallin, a chaperone for VEGF (Fijany et al., 2019). Simultaneously, iron-induced reduction of VEGF in the MSCs has been reported (Table 1). Therefore, altered vasculogenesis and angiogenesis in diabetes could be partly or fully attributed to the effects of excess iron on MSCs. In addition, hyperglycaemia decreases RUNX-2 expression, which in turn affects osteopontin, osteocalcin and osteoprotegerin (all osteogenic factors), and thereby reduces the osteogenic differentiation potential of MSCs (Fijany et al., 2019). Although this attenuation of MSC’s osteogenic potential in diabetes could be due to the excess-iron induced effects on MSCs (Table 1), it could also be due to the reduced expression of BMP-2 (upstream activator of RUNX-2 expression) in high glucose states. Here, involvement of BMP-2, the mediator of basal iron regulation via basal hepcidin induction, and the restoration of RUNX-2 expression by addition of exogenous BMP-2 jointly reiterate the link between iron and glucose regulation. Furthermore, it presents BMP-2 as an important target for repairing impaired osteogenic differentiation potential of MSCs in diabetic states (Fijany et al., 2019). Although MSC osteogenic differentiation is hampered in diabetes, adipogenic differentiation is favoured, which leads to disturbance in osteoblast:adipocyte ratio. This greater ability of MSC adipogenic differentiation (Fijany et al., 2019) could be partly attributed to the combination of elevated levels of ROS and iron in diabetes. Importantly, autologous MSC therapy in diabetes would be challenging because such chronic conditions diminish the functional and reparative ability of the MSCs (van de Vyver, 2017). This could be partly or largely because of the excess-iron-induced damage to the MSCs in pathological states.

6.3 | Myelodysplastic syndromes

MDS are a group of blood disorders characterised by ineffective haematopoiesis in the BM leading to insufficient numbers and abnormality of blood cells. Here, an abnormal BM environment is accompanied by altered functions of BM-MSCs. Iron-loading in these patients begins when ineffective erythropoiesis represses hepcidin production in the liver leading to uncontrolled intestinal iron uptake and uncontrolled release of iron from splenic macrophages (Brissot et al., 2020; Gattermann, 2018). Blood transfusion is one of the treatment options for MDS which adds to the existing iron load and becomes the main cause of iron overload in these patients. The consequent increase in ROS production and iron-induced toxicity (Angelucci et al., 2017) is bound to affect the BM-MSCs. Accordingly, iron-loaded MDS patients show diminished BM-MSC quantity, proliferative ability and differentiation potential. In these patients, iron-loading promotes mitochondrial fragmentation in the MSCs (Zheng et al., 2018), and downregulation of VEGF-A, CXCL-12 and TGF-β1 along with concomitant high expression of capsase-3 and β-catenin in the MSCs (Fu et al., 2019).

The BM-MSCs in lower-risk MDS patients insufficiently support the stromal environment. Here, the osteogenic differentiation of BM-MSCs is impaired, as evidenced by reduced expressions of the key osteogenic genes Osterix and RUNX-2 in the undifferentiated BM-MSCs (Fei et al., 2014). Although some iron-induced damage to the MSCs, for example, ROS elevation and apoptosis can be reversed with iron chelation, increased genetic mutations together with decreased expression of p-AKT may promote transformation to acute myeloid leukaemia in iron-loaded MDS patients (Fu et al., 2019). Generally, iron-loading can aggravate BM failure by negatively affecting both haematopoietic stem cells and BM-MSCs. Like in the case of other transfusion-dependent conditions, iron chelation improves haematopoiesis and survival in MDS patients to some extent, but detailed data on this is lacking (Gattermann, 2018). Recently, human MDS-MSCs were shown to exhibit epigenetic, transcriptomic and phenotypic abnormalities and while their significance in disease progression is recognised, their role in the prognosis and therapeutics of MDS is envisioned (Poon et al., 2019).

6.4 | β-Thalassaemia

β-Thalassaemia is an inherited disorder characterised by ineffective erythropoiesis and anaemia due to mutations in the β-globin gene, which results in the reduction or absence of β-globin chains of haemoglobin. Thalassaemia major patients receive regular blood transfusions to maintain haemoglobin levels, which causes iron-loading (high iron and ferritin) despite the concomitant use of iron chelators (Jeney, 2017). Iron-loading is aggravated due to ineffective-erythropoiesis-induced hepcidin dysregulation (Crippa et al., 2019). This predisposes the patients to multiple end-organ complications including a risk for osteoporosis (Tsay et al., 2010; Wong et al., 2016). Thus, more than 60% adult patients in this group have low bone mineral density (Jeney, 2017). An iron impact on bone health implies an impact on BM-MSCs in these patients.

BM-MSCs from β-thalassaemia patients showed insufficiently activated RUNX-2 osteogenic gene, limited osteogenic potential, and impaired differentiation into adipocytes; the latter occurred possibly due to lack of sufficient multipotent progenitors that would additionally form the adipose tissue (Crippa et al., 2019). Along with altered clonogenicity capacity, these cells showed reduced proliferation and reduced ability to
attract the HSCs. The reason for impoverished BM-MSC quality and reduced frequency of primitive MSCs (CD271 and CD146) in these patients was attributed to elevated ROS (Crippa et al., 2019), which is one of the direct results of iron-loading. Other altered characteristics of these MSCs such as diminished antioxidative response and reduced expression of the genes whose products contribute to the formation of the bone-marrow niche (Crippa et al., 2019) could be partly attributed to elevated iron levels in these patients (Tables 1 and 2). The latter group includes reduced expressions of the following in BM-MSCs of β-thalassaemia patients (Crippa et al., 2019): CXCL-12 and KITLG that are essential for the engraftment, retention, survival and proliferation of HSCs (Zhang et al., 2016), CDH-2, encoding the cell adhesion molecule N-cadherin, which plays a role in regulating and retaining HSCs in the BM (Araki et al., 2012), FGF-2 and IL-6 that help stem cells self-renew and maintain stemness of BM-MSCs, respectively (Coutu & Galipeau, 2011; Pricola et al., 2009), and VEGF-A and ANGPT-1 that regulate HSC quiescence and engraftment during autologous HSC transplantation (Nowicki et al., 2017). In cases of iron overload, the resultant unregulated ROS levels can promote stem cell activation and therefore the HSCs are likely to exit quiescence (Chaudhari et al., 2014). Moreover, it postulated that chronic iron exposure might alter the activities of the iron-dependent proteins that modulate chromatin exposure, alter histone methylation patterns, and cause epigenetic remodelling of MSCs in β-thalassaemia patients leading to altered functionality of the BM-MSCs (Crippa et al., 2019), a scenario similar to MDS patients (Poon et al., 2019).

7 | SUMMARY

MSCs demonstrate reparative and regenerative properties in vivo and are extremely valuable for cell-based therapy. Iron and iron-related proteins play an essential role in MSC physiology. The latter include hepcidin, ferroportin, transferrin receptor, lactoferrin, lipocalin-2, BMPs and HIFs. Excess iron causes detrimental effects on the MSCs and alter their functionality, differentiation potential, haematopoiesis supportive functions, epigenetics and the signalling pathways of ROS, PI3K/AKT, MAPK, p38, AMPK/MFF/DRP1 and Wnt. Endogenous proteins like hepcidin, ferritin and haem oxygenase-1 can protect the MSCs from iron-loading or its effects. In addition, exogenous iron chelators like deferasirox and deferoxamine, herbs like Herba Epimedi and A. membranaceus, and naturally occurring compounds like melatonin and α-lipoic acid can reduce or reverse some of the detrimental effects of iron-loading on the MSCs. MSCs contribute to the disease pathology of iron-loading conditions like hereditary hemochromatosis, diabetes, β-thalassaemia and MDS (Figure 6).
The review summarises the iron-mediated processes in the MSCs and helps understand MSC contribution to physiology and pathology under normal and iron-loaded conditions, respectively. This knowledge can help identify signalling pathways, molecular targets and endogenous/exogenous compounds that could be explored to formulate adjunctive iron-based therapy to ameliorate numerous conditions of iron excess. Also, it can inform MSC therapeutics, as it recognises the challenges of autologous MSC therapy in iron-loaded conditions.

ACKNOWLEDGEMENTS
This article was made open access with the financial support of King’s College London.

CONFLICT OF INTERESTS
The authors declare that there are no conflict of interests.

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How to cite this article: Mehta, K. J. (2021). Role of iron and iron-related proteins in mesenchymal stem cells: Cellular and clinical aspects. *Journal of Cellular Physiology*, 1–24. https://doi.org/10.1002/jcp.30383