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Mini-review

Iron homeostasis in breast cancer

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ABSTRACT

Iron is an essential element and a critical component of molecules involved in energy production, cell cycle and intermediate metabolism. However, the same characteristic chemistry that makes it so biologically versatile may lead to iron-associated toxicity as a consequence of increased oxidative stress. The fact that free iron accumulates with age and generates ROS led to the hypothesis that it could be involved in the etiogenesis of several chronic diseases. Iron has been consistently linked to carcinogenesis, either through persistent failure in the redox balance or due to its critical role in cellular proliferation. Several reports have given evidence that alterations in the import, export and storage of cellular iron may contribute to breast cancer development, behavior and recurrence. In this review, we summarize the basic mechanisms of systemic and cellular iron regulation and highlight the findings that link their deregulation with breast cancer. To conclude, progresses in iron chelation therapy in breast cancer, as a tool to fight chemotherapy resistance, are also reviewed.

1. Preface

Iron is an essential trace element and the most abundant transition metal in the human body. Due to its ability to accept and donate electrons, while conversion amid ferric (Fe^{3+}) and ferrous (Fe^{2+}) oxidation states, iron is a critical component of sensor, transporter and storing molecules and enzymes involved in energy production and intermediate metabolism [1,2]. Iron is also vital for the cell division process once the enzyme responsible for the synthesis of deoxyribonucleotides, ribonucleotide reductase (RR), is iron dependent [3]. Iron presence is imperative for the R2 RR function, and a limitation of iron availability for R2 leads to loss of RR activity [4,5] resulting in G1/S phase arrest. Iron availability was also shown to regulate other proteins implicated in cell cycle modulation and DNA damage sensing, such as Mdm2, GADD45 and p21/WAF1 [6–9]. Moreover, iron is a functional component of heme and iron-sulfur cluster-containing proteins synthesized in mitochondria [10]. Iron is, thus, fundamental for cell survival, growth and differentiation [11].

On the other side, iron associated toxicity may occur, as a consequence of its strong Lewis acidity and multiple valence [12]. This characteristic aspect of iron chemistry contributes to the formation of hazardous molecules through Fenton and Haber–Weiss reactions. These are classical iron-catalyzed redox reactions that result in the production of hydroxyl radicals and anions in the presence of hydrogen peroxide [13–15]. Consequently, an excess in the cell's labile iron can result in increased oxidative stress and damage in the DNA, lipids and proteins [16–19].

The cell's constant need for iron is challenging in a way that the organism must acquire enough iron for all biological processes where it is needed, while avoiding free iron toxicity [20,21]. Although almost all organisms possess the adequate mechanisms to regulate iron acquisition and maintain its homeostasis, there has been growing body of evidence linking a deregulation of iron homeostasis and a number of diseases, such as cancer, inflammatory and neurodegenerative diseases [22].

Breast cancer is the most common type of cancer in women worldwide [23] and despite recent advances many tumors become chemo-resistant, requiring new strategies for disease control. Recently, several groups have attempted to link a deregulation of iron's metabolism with breast cancer progression, aggressiveness and recurrence.

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2. Introduction

2.1. Systemic iron homeostasis and regulation

Given the fact that there is no physiologically regulated pathway for iron elimination its regulation is achieved through absorption, utilization, storage and export [24].

Generally, iron is obtained through the diet, which is composed by inorganic non-heme iron (±10%), mostly in vegetables, and heme iron (±90%) in meat. Erythropoiesis iron source comes from recycling of senescent macrophages from the reticuloendothelial system [1].

Iron reaches blood circulation through the apical and basolateral membranes of the enterocyte [25]. Inorganic non-heme iron is imported through DMT1 (divalent metal transporter 1), after reduction of its Fe^{3+} form, most likely, by the reductase DcytB (Duodenal cytochrome B), whose expression is induced by iron deficiency and is localized in the apical membrane of intestinal enterocytes [26]. Nevertheless, DcytB does not seems to be the only ferrireductase at the apical membrane of enterocytes, since DCYTB (Cybrd1−/−) knockout mice do not develop iron malabsorption [27,28]. DMT1 belongs to the Nramp family of transmembrane-segment proteins [29–31] and was recognized as essential through defects in iron absorption and assimilation by erythroid precursor cells in mice with microcytic anemia (mk) and Belgrade (b) rats, who share a unique spontaneous mutation in DMT1 (G185R) [30,32]. DMT1 is regulated at the transcriptional level and at its subcellular localization by iron availability [29,30,33]. Heme iron is putatively absorbed by HCP1 (heme carrier protein 1) [34,35]. Although this membranar protein is associated with the greatest heme iron absorption, its mechanism remains unknown because the transporter appears to carry mostly folate [25]. Some heme iron might follow a different trail and bypass enterocytes through Bcrp/Abcg2 and/or Feline Leukemia Virus C Receptor (FLVCR) [34,36,37]. Iron inside the enterocyte is then either stored inside ferritin or transported across the basolateral membrane to be exported into circulation, by the only iron exporter identified to date, ferroportin 1 (FPN1). The iron stored inside enterocyte's ferritin is never used due to enterocyte sloughing into the gut lumen [20]. FPN1 is most likely the only iron exporter, given that gene targeted inactivation of FPN1 is embryonic lethal in Slc40a1−/− mice [38]. Iron export at the basolateral membrane also depends on hephaestin ferroxidase activity, for Fe^{3+} incorporation into transferrin (Tf), and on the multicopper oxidase ceruloplasmin (Cp) [39–42]. The peptide regulatory hormone hepcidin was shown to play a critical role not only in iron absorption, but ultimately, in iron homeostasis through its ability to down-modulate the cellular expression of FPN1 [43–46]. The bioactive hepcidin is a 25 amino acid peptide derived from an 84 amino acid prepropeptide by furin cleavage [47]. Hepcidin is mainly produced by hepatocytes and circulates in blood bound to α2-macroglobulin [48] and its expression is regulated by iron stores, hypoxia, inflammation and rate of erythropoiesis [20,49,50].

2.2. Cellular iron homeostasis and regulation

Once in circulation, iron is incorporated into Tf. Tf function is to transport iron in the bloodstream and deliver it in iron requiring organs, while keeping it nonreactive in the circulation and extravascular fluids [11]. Cellular iron uptake is primarily regulated by the presence of the ubiquitous membrane protein transferrin receptor 1 (TfR1). Tf binding to TfR1 forms a complex that induces receptor mediated endocytosis and internalization of the clathrin-coated endosome [51–54]. A proton pump mediated decrease in the pH occurs (pH ~ 5.5) facilitating iron release due to conformational changes in the Tf–TfR1 complex [55,56]. Fe^{3+} is then reduced to Fe^{2+} by the endosomal ferrireductase six-transmembrane epithelial antigen of the prostate 3 (Steap3) [57] and DMT1 transports it to the cytosol [29,30,32,58]. A TfR1 homolog, TfR2, is also capable of holo-transferrin binding and internalization [59]. However, this does not appear to be its primary function since TfR2 knockout mice are capable of iron storage and accumulation [60]. It is thought that the HFE gene product, a MHC class I-type protein, in conjunction with β2-microglobulin associates with either TfR1 or TfR2 and senses transferrin saturation, to consequently regulate hepcidin expression [61–63].

In cytoplasm, iron enters the 'labile iron pool' (LIP), and from there it can be used in the production of iron–sulfur (Fe–S) containing proteins or it can be stored in ferritin [64,65]. Ferritin is an ubiquitous heteropolymer consisting of 24 heavy (H) and light (L) subunits that are encoded by different genes. These chains assemble into a shell-like structure capable of storing up to 4500 Fe^{3+} in form of ferric oxy-hydroxide phosphate [64,66,67]. Ferritin is essential for health, as shown by the embryonic lethality of H-ferritin knockout [68]. Iron entrance to ferritin appears to be aided by poly(rC)-binding protein 1 (PCBP1) [69]. H-ferritin has ferroxidase activity, necessary for the incorporation of iron into holo-ferritin, and L-ferritin functions as a nucleation centre. Iron deposits may also be found inside haemosiderin, an iron storage-complex composed by ferritin degradation products [70].

In order to finely control iron uptake, storage and export, sensing of cellular iron content is achieved by a post-transcriptional mechanism operated by the iron-regulated RNA binding proteins 1 and 2 (IRP1 and IRP2) that interact with conserved iron responsive elements (IREs) in the untranslated regions of central components in the iron homeostasis system in vertebrates [71–77]. In iron-depleted cells, IRPs are capable of high affinity binding to their target IREs. The interaction between IRPs and the five IRE copies in TfR1 3′-UTR mRNA stabilizes it, by inhibiting nuclease digestion, and favors its translation [71]. Conversely, IRP binding to the IRE in the 5′-UTR of Ft mRNA leads to a steric blockade in the translation initiation complex, and therefore to decreased protein abundance, in order to enhance metal availability [78]. In conditions of high cellular iron content, IRP1 and IRP2 are inactivated and cannot bind IREs. This leads to TfR1 mRNA degradation and Ft mRNA translation. This reaction inhibits further iron uptake by transferrin, while promoting storage of excess cellular iron, capable of mediating chemical reactions involving the production of reactive oxygen species (reviewed in [20,70,73,79]). The recognition of the existence of putative IREs in other mRNAs has increased the complexity of the IRE-IRP system, while unraveling new functions beyond the regulation of iron uptake and storage [75,80]. These include IREs in 5′-UTR of ferroportin [81,82], aminolevulinic acid synthase (ALAS2) [83], mitochondrial aconitase (ACO2) [84], succinate dehydrogenase [85], glycolyate oxidase (GOX) [86] and hypoxia-inducible factor 2α (HIF2α) [87,88] mRNAs, and in the 3′-UTR of DMT1 [29], cell division cycle 14 homolog A (CDC14A) [89], hydroxyacid oxidase 1 (HAO1) [90] and CDC42 binding protein kinase α/MRCKa (CDC42BPA) [91] mRNAs. The presence of IREs in several mRNAs, identified in a transcriptome-wide approach, suggests that IRP control extends well beyond cellular iron regulation [92,93].

3. Cancer cell iron metabolism

Deregulation of the molecular mechanisms of iron absorption, storage, use and removal can result in disease [75,94,95]. The observation that iron tends to accumulate in the elderly and that free iron in excess is prone to chemical reactions involved in the generation of ROS, led to the hypothesis that iron could be involved