Clinica Chimica Acta 434 (2014) 34–40

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Invited critical review

Iron homeostasis and anemia markers in early breast cancer Iron and breast cancer

Pierre-Jean Lamy [a,*], Anna Durigova [b], William Jacot [c]

[a] Laboratoire de biologie spécialisée et d'oncogénétique, Institut régional du Cancer, rue de la croix verte, 34298 Montpellier, France
[b] Service d'oncologie médicale, Hôpitaux Universitaires de Genève, Rue Gabrielle-Perret-Gentil 4, CH-1205 Genève, Switzerland
[c] Service d'oncologie médicale, Institut régional du Cancer, rue de la croix verte, 34298 Montpellier, France

ARTICLE INFO

Article history:
Received 19 February 2014
Received in revised form 7 April 2014
Accepted 8 April 2014
Available online 24 April 2014

Keywords:
Iron
Breast cancer
Ferritin
Transferrin
Ferroportin
Hepcidin

ABSTRACT

Iron plays a fundamental role in cell life and its concentration in living organisms is precisely regulated. Different molecules for iron storage and transport are used to maintain its intracellular homeostasis which is often altered in cancer cells. Specifically, recent studies have demonstrated that in breast cancer cells, the expression/activity of several iron-related proteins, such as ferritin, hepcidin and ferroportin, is deregulated and that these alterations may have a prognostic impact in patients with breast cancer. Moreover, molecules that regulate iron metabolism could become therapeutic targets. This review focuses on recent findings on iron metabolism particularly in breast cancer and on the development of new biomarkers that may be used in the clinical routine for the diagnosis, prognosis and management of cancer-associated anemia as well as for monitoring personalized treatments.

Contents

1. Introduction

Iron plays a key and fundamental role in eukaryotic cells. It is found in many heme proteins, such as hemoglobin and myoglobin. It is also involved in cell respiration through the iron-containing cytochrome proteins and in redox reactions catalyzed by ribonucleotide reductases and xanthine oxidases, which are iron-dependent enzymes that play a role in DNA damage. It is also present in cyclo-oxygenases and lipoxygenases that are enzymes implicated in inflammation. Iron is also involved in the function of catalases and peroxidases that protect cells against the formation of free radicals. Iron concentration must be finely regulated because any excess of free iron is rapidly toxic and its deficit induces anemia-related hypoxia. In these last years, our

* Corresponding author at: Laboratoire de Biologie Spécialisée, ICM, rue de la croix verte, 34298 Montpellier cedex 05 France. Tel.: +33 467613140; fax: +33 476632873.
E-mail addresses: pierre-jean.lamy@icm.unicancer.fr (P.-J. Lamy), Anna.DurigovaAbdouh@hcuge.ch (A. Durigova), william.jacot@icm.unicancer.fr (W. Jacot).

http://dx.doi.org/10.1016/j.cca.2014.04.011
0009-8981/

understanding of iron metabolism in humans has considerably improved thanks to the identification of many molecules involved in its storage and transport in the blood circulation and of the mechanisms that regulate its intra and extra-cellular concentration. Moreover, several studies have described the many alterations of iron metabolism occurring in tumor cells and in patients with cancer. More recently, the role of iron deregulation in breast cancer development/progression has been confirmed by basic and clinical studies [1]. In this review, we will summarize our current knowledge on the iron alterations in cancer, leading to the so-called anemia of inflammation. Then, we will focus specifically on the deregulations of iron metabolism in breast cancer, the prognostic value of new iron-related markers and the therapeutic potential of targeting iron metabolism.

2. The regulation of iron metabolism

The human body contains 3 to 5 g of iron that is almost all recycled during its metabolism. Only 2 mg of iron are adsorbed as heme (organic iron, mainly from meat) each day. Heme proteins from the diet are digested and heme is liberated and adsorbed by enterocytes via the heme carrier protein (HCP) discovered in 2005. Non-heme (inorganic, from cereals, fruits and vegetables) iron is first reduced by the gastric acid environment and is transported in the form of ferrous iron (Fe^{2+}) across the apical membrane of enterocytes to the cytoplasm by the dimetal transporter 1 (DMT1) that also reduces iron. The enterocyte iron absorption capacity is modulated by signals coming from other cells that require iron (particularly erythroid precursors) or that store iron (hepatocytes, macrophages). In case of iron excess, DMT1 expression is down-regulated and this could explain the reduction in iron intestinal absorption in this situation [2]. Conversely, iron intestinal absorption is higher in case of iron deficiency or increased erythropoiesis. This regulation is very complex and involves also inflammatory and hypoxic signals, particularly through the iron-regulatory hormone hepcidin [3].

Before its excretion from enterocytes to the blood circulation, cytoplasmic Fe^{2+} is oxidized to Fe^{3+} by the ferroxidase hephaestin. Intracellular Fe^{3+} is then excreted by ferroportin at the level of the latero-basal portion of enterocytes. Ferroportin allows iron binding to transferrin (also called siderophilin, iron-regulated transporter 1 or SLC40A1 [3]) that is required for Fe^{3+} export from enterocytes to plasma and its transport to the cells, particularly erythroblasts. Finally, iron that is not transferred to plasma is eliminated with the enterocytes in the feces.

Bone marrow is the main iron consumer. Iron enters in the erythroid precursors via the transferrin type 1 receptors (TfR) that bind to transferrin and are internalized via endocytic vacuoles. The acid pH of this environment allows the liberation of Fe^{3+} from transferrin. Once liberated, Fe^{3+} is not reactive and soluble Fe^{3+} is reduced by the membrane cytochrome b reductase and is internalized in the cytoplasm by the DMT1 transporter. It then forms a labile pool of iron that can bind to different iron-dependent proteins, which have important roles particularly in mitochondria. Ferritin, a storage molecule, will take care of any iron surplus in the cell. A single ferritin molecule can store more than 4000 iron atoms and the normal ferritin blood level ranges between 50 and 350 ng/ml in men and 30 and 120 ng/ml in women. It is composed of two structurally similar, but functionally distinct subunits: the L-subunit (19 kDa) and the H-subunit (21 kDa). The H-subunit has ferroxidase activity, whereas the L-subunit contributes to the stable storage of iron in the ferritin core [4] Iron can be exported from the cells to the blood circulation by using the same mechanism described for enterocytes, but with ceruloplasmin as ferroxidase [5].

Iron homeostasis is regulated only through its absorption and recycling by macrophages. Serum iron is mainly controlled by hepcidin, a protein produced by the liver [6] that negatively regulates the intestinal absorption of iron and its intracellular export from macrophages and hepatocytes. Increased hepcidin production has been reported in a group of patients with large hepatic carcinomas, leading to severe iron-deficiency anemia [7], and also in anemia of inflammation in response to excess iron, leading to iron sequestration. Hepcidin role in iron homeostasis is comparable to that of insulin in the regulation of glycemia. The administration of synthetic hepcidin in mice leads to sideropenia within one hour of injection [8]. Complete absence of hepcidin leads to juvenile hemochromatosis, a severe type of iron overload in which iron is still absorbed despite excessive iron stores. Conversely, excessive production of hepcidin leads to iron deficiency and to anemia caused by the intestine's inability to absorb iron despite normal or increased iron supply. The severity of these two pathological conditions suggests that there is no compensatory mechanism for the effects of hepcidin. Therefore, hepcidin role in iron homeostasis seems to be crucial [3]. Hepcidin acts by binding to an extra-cellular loop of ferroportin, leading to the phosphorylation of ferroportin and its internalization in enterocytes and destruction by the proteasome [9]. Hepcidin expression is negatively regulated by increased erythropoiesis [10] or hypoxia. Conversely, it is stimulated by an increase in the circulating level of iron and by inflammation. In the presence of inflammation, hepcidin expression is induced by lipopolysaccharides (LPS) and interleukin 6 (IL-6), which activates hepcidin synthesis through the signal transducer and activator of transcription 3 (STAT3) pathway [10]. This mechanism allows linking together immune response, iron homeostasis and anemia of inflammation in cancer. Indeed, hepcidin was initially identified as an anti-microbial protein and it seems that its role in iron homeostasis is the result of the evolution of the body defense systems in order to decrease the amount of iron available to pathogens or cancer cells. High Iron Fe (HFE), which is encoded by the *hemochromatosis* gene is also involved in the regulation of iron absorption. Defects in this gene cause the main form of hereditary hemochromatosis. Hepcidin deficiency has been reported in patients with this disease or with other forms of hereditary hemochromatosis [11]. In the case of juvenile hemochromatosis of type 2AB, iron overload is linked directly to a mutation of the gene encoding hepcidin (*HAMP*) on chromosome 19.

The regulation of iron metabolism in non-erythroblastic cells is based on the post-transcriptional control of ferritin and TfR synthesis by *iron regulatory proteins1 and 2* the action of which is modulated directly by the labile iron pool levels (the iron not bound to ferritin and directly available) [5].

3. Iron metabolism and anemia in cancer

Perturbations, at least indirect, of iron metabolism in cancer have been suspected for a long time. Indeed, anemia is frequently reported in patients with cancer [12]. Cancer is often associated with chronic inflammation that can lead initially to normochromic, normocytic, non-regenerative anemia with reduced transferrin saturation coefficient (TSC) linked to a decrease in plasma iron levels [13]. The cause of this sideropenia is not a deficit in iron supply because the body iron stores are not reduced. It is rather the results of a very strong inflammatory stimulus associated with an important secretion of pro-inflammatory cytokines (such as IL-1a, IL-6, interferon gamma, TGF beta and TNF alpha) by cancer cells and macrophages infiltrating the cancer tissue. Pro-inflammatory cytokines directly inhibit the differentiation and proliferation of erythroid progenitors [14] by causing their apoptosis or by producing free radicals that alter EPO binding capacity to its receptors. For instance, TNF alpha promotes apoptosis of erythroid cells, and interferon gamma seems to be the most powerful inhibitor of erythropoiesis. Pro-inflammatory cytokines also inhibit the synthesis of erythropoietin (EPO) and lead to resistance to EPO therapy [15]. They also affect directly iron homeostasis by inducing iron sequestration, thus explaining the possible progression of the anemia towards a hypochromic and microcytic form (although less severe than in anemia caused by a true iron deficit). Specifically, interferon gamma and TNF alpha induce DMT1 and TfR expression, leading to an increase of iron uptake by macrophages that may also increase concomitantly their tumor-suppression activity. IL-1 and IL-6 as well as TNF alpha also promote ferritin