Role and Metabolism of Heme-derived Iron in Cancer

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1 Introduction

Iron is an essential cofactor for many biological processes including DNA synthesis, oxygen transport, and ATP generation. Iron metabolism, recycling, and transport, is thus controlled at multiple levels. Cells undergoing rapid proliferation, such as cancer cells, are characterized by increased demand for iron and accelerated iron turnover.

Heme degradation provides the major source of iron used in iron recycling. Residential macrophages of the spleen and liver express high levels of heme oxygenase-1 (HO-1), an enzyme that degrades heme to ferrous iron (Fe^{2+}), biliverdin (BV), and carbon monoxide (CO). Tumor associated macrophages (TAM) are present in the tumor microenvironment and, dependent on their polarization phenotypes, contribute to tumor development or regression in part by regulating iron availability. “Classically activated” M1 macrophages are pro-inflammatory, express high intracellular levels of ferritin that sequesters iron, and promote tumor regression. In contrast, M2 macrophages favor tumor growth in part through upregulated HO-1-mediated iron generation and increased iron export. As HO-1 is expressed in resident macrophages and recruited monocytes in the tumor stroma, the role of iron recycling in the tumor microenvironment depends heavily on TAM activity.

In this chapter, we focus on the altered status of iron homeostasis and heme degradation in TAM and how HO-1 expression in both cancer cells and TAM may contribute to carcinogenesis.

2 Heme and Iron Recycling During Homeostasis

The average human body contains three to four grams of iron distributed throughout various organs. Hemoglobin found on circulating and developing erythrocytes binds one to two grams of total iron, while hepatocytes and residential macrophages (Kupffer cells) of the liver store an additional gram (Ganz 2007). A further significant portion of iron is present in myoglobin in muscles and in various other hemoproteins like cytochromes and nitric oxide synthase (Ganz 2007). Heme iron from hemoglobin and myoglobin derived from red meat provides the largest source of external iron and is uptaken by enterocytes via receptor-mediated endocytosis in the small intestine; nonheme iron sources include ferrous and ferric iron derived from plant products, though they are not as readily absorbed (Bastide, Pierre and Corpet 2011). Iron and heme absorption in duodenal enterocytes accounts for 1 to 2 mg of

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iron intake daily, yet the same amount of iron lost through shedding of intestinal epithelial cells and extravasated red blood cells, making physiological iron levels a constant parameter (Ganz 2007; Walsh, Kaldor, Brading and George 1955). Although there is no system for large scale removal of iron from the body, various organs do contribute to the regulation of iron levels: through reduced iron absorption by duodenal mucosa, reduced iron export by the same epithelial cells through the peptide hormone hepcidin, and sequestration of already circulating iron in the liver and spleen in ferritin and hemosiderin (Ganz 2003; Shoden, Gabrio and Finch 1953).

The relatively small exchange of iron between the body and the environment contrasts with the large amount of iron redistributed between tissues and cells. Monocytes and macrophages of the spleen, liver, lymph nodes and bone marrow form the mononuclear phagocyte system (MNP, the reticuloendothelial system), and are responsible for the degradation of hemoglobin from senescent red blood cells and generation of a systemic iron pool. The majority of recycled iron is then incorporated into nascent erythrocytes of the bone marrow (Pollycove and Mortimer 1961). Iron hemoglobin from red blood cells (RBCs) produces 25 to 30 mg of iron per day, allowing for the production of 200 billion new erythrocytes every day (Munoz, Villar and Garcia-Erce 2009).

Iron is the reactive core of a variety of enzymes necessary in all cell types. It forms the catalytic site in ribonucleotide reductases necessary for DNA synthesis, facilitates the coordination of oxygen and carbon dioxide in hemoglobin and myoglobin, and is additionally involved in ATP generation through the iron sulfur clusters of cytochromes in the mitochondrial oxidative chain (Beinert et al., 1983; Elford, Freese, Passamani and Morris, 1970; Perutz, 1979). Iron chelation has been shown to arrest human lymphocytes at the G1/S phase checkpoint due to inhibition of DNA synthesis (Lederman, Cohen, Lee, Freedman and Gelfand, 1984). Iron metabolism also has been shown to regulate cell cycle activity by affecting expression and activity of cyclins A, D, an E and cyclin dependent kinases in cancer cells. Iron chelation was shown to block cyclin dependent kinase, specifically cdks 2, 4 and 6 activities leading to hypophosphorylation of Rb and blockage of cell cycle progression(Gao and Richardson 2001; Kulp, Green and Vulliet, 1996; Lucas et al., 1995; Mitra et al., 1999; Nurtjahja-Tjendraputra, Fu, Phang and Richardson 2007). As such, iron is often the limiting factor for synthesis of proteins, DNA and cell growth.

Highly proliferative cells, such as cancer cells, have high iron requirements. Early studies on iron metabolism in cancer showed that addition of iron to culture media of hepatoma cells enhanced cell growth via increased iron retention, and this effect was reversed with addition of the iron chelator, desferroxamine (Hann, Stahlhut and Hann, 1990). Similar studies on iron deprivation have been performed in neuroblastoma (Blatt and Stitely, 1987), leukemia (Foa, Maiolo, Lombardi, Villa and Polli, 1986; Kim et al., 2011), breast cancer (Reddel, Hedley and Sutherland, 1985) and hepatocellular carcinoma (Ba et al., 2011) cell lines showing iron deprivation to block cancer growth. Iron deprivation was shown to have minimal or no effect on normal non-transformed cells growth. Moreover, other iron depleting compounds such as deferasirox, tachpyridine, and thiosemicarbazones were also effective in suppressing cancer cell growth in vivo and in vitro(Kim et al., 2011; Lui et al., 2013; Torti et al., 1998; Turner et al., 2005)

In addition to iron chelation, mechanisms reducing iron uptake — through transferrin — and enhancing iron export — through ferroportin — were also shown to be effective approaches
for inhibition of cancer growth. Blockage of transferrin receptors with monoclonal antibody blocked growth of human melanoma xenografts (Trowbridge and Domingo, 1981). Similarly, overexpression of iron exporter ferroportin or alteration of ferroportin expression may also be viable options for cancer therapy (Pinnix et al., 2010; Sow et al., 2009).

The multiple processes in which iron participates are due to its flexible coordination chemistry and redox reactivity allowing oxygen binding and transfer of electrons with ease (Aisen, Enns and Wessling-Resnick 2001). However, the same high energy properties of iron makes it cytotoxic as free iron reacts in Fenton chemistry to generate hydroxyl radicals and reactive oxygen species that damage cellular macromolecules indiscriminately (Graf, Mahoney, Bryant and Eaton, 1984). The most significantly damaged molecules include DNA (double stranded breaks, base shifts, base modifications) and lipids (oxidation) (Chevion, 1988; Lloyd and Phillips, 1999; Mello Filho, Hoffmann and Meneghini, 1984; Meneghini, 1997; Morel et al., 1990). Dietary analyses have linked high fats and heme-iron intake to colon cancer and ovarian cancers, likely due to the generation of lipid peroxyl radicals that have DNA damaging capacities (Sawa et al., 1998; Yamaguchi et al., 2008). Transgenic mice fed a high iron diet were also shown to have increased accumulation of lipid peroxidation products and DNA oxidative damage correlating with increased risk of developing hepatocellular carcinoma (Furutani et al., 2006). Iron is thus capable of accelerating tumor growth as both a limited resource and through the generation of ROS and DNA mutations.

As mentioned previously, various organs participate in regulating iron homeostasis but there is no efficient mechanism for large-scale removal of iron from the body (Ganz 2007; Kohgo, Ikuta, Ohtake, Torimoto and Kato 2008). Iron overloading can occur through abnormalities due to genetic disorders, repeat blood transfusions, chronic inflammation and dietary choices. Iron that exceeds export and sequestering capacities circulates as non-transferrin bound iron which then accumulates in the liver, heart, and pancreas (Andrews, 1999; Brissot, Ropert, Le Lan and Loreal 2012; Cabantchik, Breuer, Zanninelli and Ciacciulli 2005). Such free iron causes organ dysfunction through enhanced ROS production and can lead to liver failure, cirrhosis, arrhythmias, heart failure, and diabetes (Brissot et al., 2012; Hirano et al., 2001). Diseases characterized by high iron accumulation like hereditary hemochromatosis, beta-thalassemia and end stage liver disease have been associated with increased risk of hepatocellular carcinoma (Kowdley 2004; Pietrangelo 2010). Similarly to cancer cells, invading pathogens also depend on iron for their exponential growth, resulting in iron withholding by the MNP system as a form of primary immune defense. Continued iron sequestration in these instances however, results in chronic anemia with symptoms of decreased survival of mature red blood cells and poor maturation of erythroid precursors (Rivera et al., 2005). These data suggest that iron is a critical regulator of cell proliferation and therefore imbalance in iron recycling, storage or uptake may result in pro-proliferative disease and/or malignancy.

Heme degradation is mediated by the heme-oxygenase family of enzymes. Although there are two isoforms of the heme oxygenases, HO-1 is the predominant form associated with iron recycling and hemoglobin turnover and is highly expressed in the specialized cells of the MNP system—specifically the macrophages of the spleen and liver. HO-1 is generally considered to be a cytoprotective molecule as it degrades proinflammatory heme and generates biologically active products biliverdin, carbon monoxide gas, and free iron (Maines, 1988; Tenhunen, Marver and Schmid, 1968). Through its importance in macrophages, HO-1 is also a critical mediator of iron recycling. A case study of a 6-year-old boy suffering from HO-1 defi-
ciency reported persistent erythrocyte fragmentation, increased serum iron and heme levels, and iron deposition in distal tissues (Kawashima, Oda, Yachie, Koizumi and Nakanishi 2002; Yachie et al., 1999). Similar MNP system-related abnormalities were made for HO-1 knockout (Hmox -/-) mice which are characterized by high oxidative stress, low survival in utero, and exaggerated response to tissue injury (Poss and Tonegawa, 1997). Hmox -/- mice, in addition to dysfunctional and mostly absent residential splenic and liver macrophages, showed tissue inflammation due to intravascular erythrocyte hemolysis and iron overload in the kidneys, suggesting difficulties with iron trafficking (Kovtunovych, Eckhaus, Ghosh, Ollivierre-Wilson and Rouault 2010). Furthermore, bone marrow progenitor cells isolated from HO-1 knockout mice were unable to differentiate fully even under favorable macrophage growth conditions, suggesting the importance of HO-1 in the maturation of myeloid cells (Wegiel et al., 2014).

**Figure 1: The heme degradation pathway.** Residential macrophages of the liver and spleen express high levels of heme oxygenase-1 (HO-1) enzyme. HO-1 degrades heme from hemoglobin released from senescent erythrocytes to biliverdin (BV), carbon monoxide (CO), and Fe2+. Biliverdin is further converted to bilirubin (BR) by biliverdin reductase (BVR) while CO is a freely diffusible gas product. Fe2+ iron is additionally sequestered by ferritin into the intracellular labile iron pool to prevent Fenton chemistry generation of reactive oxygen species. All of the products of the heme degradation pathway have been shown to be cytoprotective.

As mentioned previously, the energy potential and flexibility of heme to be incorporated into a variety of hemoproteins is due to the coordination chemistry possible with the iron center of the heme porphyrin. Biological iron is rarely found unaccompanied, likely due to its insolubility and reactivity. Generation of intracellular iron in the heme degradation pathway leads to induction of heavy chain of ferritin, a sequestration molecule considered to possess protective properties. Ferritins have been shown to not only play a role in iron storage and detoxification but also mediate cytoprotective effects in several models of the diseases mimicking and mediating in part the effects observed with induction of HO-1 (Harrison and Arosio, 1996). Overexpression of ferritin was shown to inhibit tumor human H1299 xenografts growth (Nie, Chen, Sheftel, Pantopoulos and Ponka 2006). Deficiency in light chain of ferritin in human leads to reduced cellular iron availability, diminished levels of cytosolic catalase superoxide dismutase 1 (SOD1) protein levels, enhanced reactive oxygen species (ROS) production and higher levels of oxidized proteins (Cozzi et al., 2013). Expression of ferritin is induced by acute inflammatory cytokines such as IFNγ, IL-6, and TNF-α that increase iron sequestration in macrophages (Feelders et al., 1998).
3 Iron Degradation in Macrophages of the Mononuclear Phagocyte (MNP) System

Iron regulation by macrophages of the mononuclear phagocyte system (MNP) is facilitated through two pathways: uptake of circulating iron complexes or uptake of heme/hemoglobin. Circulating heme complexes include transferrin (Tf)-bound iron (Fe$_2$-Tf) at physiological iron concentrations, and non-transferrin-bound iron (NTBI) under iron overload conditions (Trinder, Fox, Vautier and Olynyk 2002). NTBI is biologically more toxic than Tf-bound iron. Among the NTBI fractions, labile plasma iron (LPI) is the most toxic because unlike Tf-bound iron, the cellular uptake of NTBI is not dependent on the presence of transferrin receptors (TfR), and therefore the resulting iron is diffusely distributed throughout the organs (Breuer, Hershko and Cabantchik 2000; Cabantchik et al., 2005).

Ferric iron Fe$_3^+$ bound to transferrin (Fe$_2$-Tf) is the primary form of physiological iron found circulating in the blood. Transport of iron between organs and uptake of iron is facilitated by Tf and transferrin receptor (TfR1). The Fe$_2$-Tf complex binds to transferrin receptor 1 (TfR1) on the cell surface and is endocytosed. Once internalized, the endosome is acidified, uncoupling ferric iron from transferrin (Dautry-Varsat, Ciechanover and Lodish, 1983). STEAP3 reductase catalyzes the conversion of ferric (Fe$_3^+$) iron to ferrous (Fe$_2^+$) iron which binds to the divalent metal transporter 1 (DMT1; also known as natural resistance associated macrophage protein (NRAMP2)) (Ohgami et al., 2005). DMT1 then transports the ferrous ion across the endosomal compartment to the cytoplasm (Fleming et al., 1997; Gunshin et al., 1997). In the cytoplasm, ferrous iron is either directly utilized by mitochondria for synthesis of heme and iron-sulfur complexes or stored by ferritin into the intracellular labile iron pool (Napier, Ponka and Richardson 2005). Apo-transferrin bound to its receptor is recycled to the cell surface where they uncouple at neutral pH, and both participate in additional cycles of iron transport and intake (Figure 2).

Ferroportin is thus far the only iron export channel discovered in mammalian cells (Donovan et al., 2005). Iron for delivery to systemic tissues is first exported by ferroportin, oxidized to ferric iron by multicopper ferroxidase ceruloplasmin and then scavenged by transferrin which maintains Fe$_3^+$ in a redox inert state for delivery (Harris, Durley, Man and Gitlin, 1999). Iron export is negatively regulated by hepcidin, a circulating peptide hormone that binds to ferroportin, leading to its internalization and degradation (Nemeth et al., 2004). Hepcidin is produced by the liver in the state of high iron intake and under inflammatory conditions, resulting in reduced iron absorption by duodenal enterocytes and iron retention in macrophages (Feelders et al., 1998).

Iron transport is controlled at the post-transcriptional level via two iron regulatory proteins (IRP1 and IRP2) and iron responsive elements (IREs). IREs are found within both the 5’ and 3’ UTRs of various target mRNAs encoding proteins involved in iron trafficking: ferritin and ferroportin mRNAs contain IREs in their 5’ UTRs, and transferrin receptor and DMT1 contain IREs in their 3’ UTR (Hubert and Hentze 2002; Torti and Torti 2013). Binding of IRPs to IREs occurs only in conditions of low iron and serves to increase levels of intracellular iron. Binding of IRPs to 5’ UTRs blocks mRNA translation, reducing expression of proteins involved in iron storage and efflux, while binding of IRPs to 3’ UTRs inhibits mRNA degradation, stabilizing expression of iron importers. In conditions of high intracellular iron IRP1 acquires an iron sulfur cluster and is converted to a cytoplasmic aconitase, IRP2 is degraded,
Figure 2: Scheme of iron recycling in macrophages. Iron is derived from two primary pathways: intake of transferrin bound iron and degradation of hemoglobin (Hb). Circulating iron is bound to transferrin. Macrophages uptake diferric-bound transferrin through endocytosis via the transferrin receptor (TfR). Acidification of the endosome releases iron from transferrin, which is then reduced by Steap3 metalloreductase before transport to the cytosol via divalent metal transporter (DMT1). Additionally, hemolysis of RBCs released hemoglobin, which could be bound by circulating haptoglobin (Hb:Hpt). Macrophage specific CD163 scavange both hemoglobin and hemoglobin:haptoglobin complexes and allows for endocytic heme degradation. Acidification of the endosomal compartment uncouples heme from its globin component. Heme is then transported to the cytosol by heme transporter heme carrier protein 1 (HCP-1). Heme degradation through ER-anchored heme oxygenase-1 (HO-1) produces biliverdin (BV), which is then reduced to bilirubin (BR) by biliverdin reductase (BVR), carbon monoxide (CO), and Fe^{2+} iron. Heavy and light chains of ferritin sequester free iron generated from both heme degradation and iron import into the labile iron pool until utilization by mitochondria or export. Release of iron from macrophages is facilitated through ferroportin followed by oxidation by membrane bound ceruloplasmin and is finally bound to transporter transferrin.
and ferritin and ferroportin mRNA is translated (Thomson, Rogers and Leedman, 1999). In addition to iron levels, IREs are sensitive to factors such as nitric oxide, reactive oxygen species, and hypoxia (Pantopoulos, Gray and Hentze, 1995; Pantopoulos et al., 1997; Tacchini, Recalcati, Bernelli-Zazzera and Cairo, 1997) suggesting their function during oxidative stress or injury associated with ischemia-reperfusion.

Macrophages have the capacity to not only endocytose senescent erythrocytes but also scavenge hemoglobin and free heme released from hemolysis of red blood cells. Endocytosis of free heme and hemoglobin by the monocytes/macrophage is mediated by scavenger receptor CD163. CD163 is currently the only known pathway for intake of free circulating hemoglobin and hemoglobin:haptoglobin complexes (Kristiansen et al., 2001; Møller, Peterslund, Graversen and Møestroep 2002; Schær et al., 2006). Following ingestion of the receptor:ligand complexes, increased acidification of the endosomal compartment uncouples both hemoglobin:CD163 and subsequently heme from its globin component as well. Free heme is exported to the cytoplasm by the heme transporters HCP-1 and then degraded by ER-bound heme oxygenase-1 (HO-1) while globins are digested in the maturing lysosome, and the receptor is eventually recycled back to the cell surface (Schaer, Vallelian, Imhof, Schoedon and Schaer 2008). Schaer et al showed colocalization of iron transporter DMT1 within the late endosome/lysosome suggesting heme breakdown and release of iron occurring in the lysosome (Schaer et al., 2008). Although he attributed this to nonenzymatic heme degradation due to the various peroxides or at low pH in the presence of proteases, Gagnon et al provided evidence that phagosomal membrane is derived from the endoplasmic reticulum, suggesting ER associated HO-1 could catalyse heme degradation and iron recycling within the phagolysosome as well (Gagnon et al., 2002).

4 Cancer and Reprogramming of Iron Homeostasis: Role of Tumor-associated Macrophages (TAM)

Because iron is key in the regulation of cell growth, cancer cells reprogram their iron metabolism and transport to facilitate enhanced iron acquisition. Studies on the role of iron in tumorigenesis have shown that iron accumulates in the tumor stroma (Alkhateeb, Han and Connor 2013). Tumor stroma consists of a mixture of cell types including endothelial cells, fibroblasts, and various other cells such as lymphocytes, myeloid cells and smooth muscle cells. Accumulation of iron in the stroma suggests that iron-dependent effects may be mediated by cells in the tumor microenvironment rather than tumor cells themselves.

Among the cells in tumor stroma, macrophages have the highest iron storage capabilities. Increased infiltration of ferritin-rich CD68 positive macrophages was detected in more advanced breast cancer tumors characterized by high histological grade (Alkhateeb et al., 2013). Tumor associated macrophages (TAM) and infiltrating monocytes and polarized macrophages express different profiles of iron receptors, iron transporters and exporters. TAM in the tumor stroma is present in close proximity to cancer cells and therefore can facilitate their growth and metastasis. There are two distinct polarization statuses of tumor-associated macrophages: “classically activated” M1s and “alternatively activated” M2s (Figure 3). M1 macrophages are generated in vitro by stimulation with lipopolysaccharide (LPS) and IFNγ while M2 macrophages are typically polarized under Th2 stimulatory conditions including IL4 and
IL13 (Mantovani and Locati 2013). The M1 phenotype is proinflammatory and is characterized by the release of inflammatory cytokines, reactive nitrogen intermediates, reactive oxygen species, and anti-tumoral activities (Mantovani, Sozzani, Locati, Allavena and Sica 2002). In contrast, M2 macrophages are involved in tissue remodeling and repair with immunosuppressive and anti-inflammatory functions that promote tumor growth (Mantovani et al., 2002; Sica and Mantovani 2012). M2 macrophages show low IL12 expression, high IL10 expression, high scavenging potential, and ability to support angiogenesis, tissue remodeling and repair (Biswas, Sica and Lewis 2008). It is prudent to mention now that while dividing the macrophage population under the properties mentioned above is tempting, such classifications are general observed characteristics, and that macrophages display remarkable plasticity with some expressing none or all M1/M2 markers, and these even subject to change depending on the local environment. Recently, vascular cell adhesion molecule 1 (VCAM-1) positive and CD11b low population with intermittent expression of mannose receptor (MMR, CD206) was shown to influence mammary tumor growth in transgenic model (Franklin et al., 2014).

High levels of iron released within the tumor microenvironment provide a source of iron for cancer proliferation. Since M2 macrophages exhibit a gene expression profile con-
sistent with iron efflux—an increase in ferroportin and decreased ferritin accumulation—M2 macrophages may represent a mechanism behind promotion of cancer growth (Corna et al., 2010; Recalcati et al., 2010). Measurements of iron release into the culture medium showed that after 4 hours, M2 macrophages released approximately four times more iron as M1 (Recalcati et al., 2010). Supernatants from M2 macrophage cultures were shown to promote tumor cell proliferation, an effect inhibited by iron chelation, suggesting that M2 macrophages may promote tumor growth at least partly by supplementing tumor cells with iron (Corna et al., 2010; Recalcati et al., 2010). In a model of urethane-induced lung carcinogenesis, cancer growth was associated with a phenotypic switch from M1 (early initiation) to M2 (late promotion and progression). However, depletion of alveolar macrophages during both early and late stages impaired tumor growth suggesting a tumor-promoting role for M1 macrophages as well, though dependent on the tumor progression stage (Zaynagetdinov et al., 2011).

Although ferritin is most commonly found as an intracellular iron storage protein, ferritin has also been found in circulation. Increased levels of serum ferritin correlated with poor clinical outcome in breast cancer patients (Alkhateeb et al., 2013). Macrophages, but not breast cancer cells, were able to actively secrete L ferritin in vitro in an nonclassical secretion pathway following N-glycosylation and ER/golgi processing, an effect enhanced in response to pro-inflammatory cytokines (Cohen et al., 2010). Macrophages are known to extensively infiltrate breast tumors and are often rich in ferritin (Simson and Spicer, 1972). Staining of ferritin in tissue stroma showed high density of ferritin rich CD68+ TAM in the stroma of invasive ductal carcinomas (Alkhateeb et al., 2013). Ferritin was shown to increase proliferation of T47D and MCF7 epithelial breast cancer cell lines however with little intracellular iron level changes (Alkhateeb et al., 2013). Release of ferritin by M2 macrophages within the stroma in breast tumors may represent a mechanism by which ferritin stimulates tumor growth and progression. The role of iron in cancer progression is summarized in Table 1.

Tumors regulate their own iron metabolism in ways to favor net iron influx and retention. TfR1 is highly expressed in breast cancer (Hogemann-Savellano et al., 2003; Shindelman et al., 1981), leukemia (Sutherland et al., 1981), lymphoma (Habeshaw et al., 1983), prostate cancer (Keer et al., 1990), and lung cancer (Kukulj et al., 2010). Analysis of squamous cell carcinomas showed that expression of TfR1 is a marker of poor prognosis and outcome. Small interfering RNA against TfR used in culture resulted in reduced proliferation and cell cycle arrest (Chan et al., 2014; Schaar, Medina, Moore, Strair and Ting 2009). High TfR1 expression has additionally been associated with poor response to tamoxifen treatment and short breast cancer-specific survival (Habashy et al., 2010). Heme transporter heme carrier protein 1 (HCP-1) was shown to be highly expressed in Caco-2 and HeLa cancer cell lines and moderately expressed in Hep2G and HEK293 cells suggesting the higher demands for heme uptake by cancer cells (Latunde-Dada, Takeuchi, Simpson and McKie 2006). A study on small lung cancer cell and a variety of human lung cancer tumors additionally showed upregulated heme transporters HCP-1 and heme responsive gene 1 (HRG-1) expression in combination with enhanced ALAS1 expression (the rate limiting enzyme involved in heme synthesis) and increased production of hemoproteins like cytochromes and cytoglobins (Hooda et al., 2013). These data, in addition to evidence regarding altered localization of HO-1 and potential altered function suggest perhaps cancer cells in part utilize heme and iron independent of HO-1 involvement. Though one might expect intracellular ferritin to diminish with accelerated iron-containing macromolecule synthesis, intracellular iron has been shown to be upregulated
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<td>† Iron importer DMT1(Brookes <em>et al.</em>, 2006).</td>
<td>† H ferritin following continual heme treatment in Caco-2 and BT-20 tumor cells (Cermak <em>et al.</em>, 1993).</td>
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<td>† STEAP family (1,2,3) metallo-reductases (Ohgami <em>et al.</em>, 2005; Torti and Torti 2011)</td>
<td>≫ Intracellular iron levels following ferritin stimulation in MC7 and T47D breast cancer cell lines (Alkhateeb <em>et al.</em>, 2013).</td>
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<td>† Ribonucleotide reductase correlated with increased growth rate (Elford <em>et al.</em>, 1970).</td>
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<td><strong>Utilization</strong></td>
<td>↓ TfR1 (Corna <em>et al.</em>, 2010).</td>
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<td>↓ HCP-1 expression following stimulation with TLR1-9 agonists (Schaer <em>et al.</em>, 2008).</td>
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<td>↓ STEAP3 (Corna <em>et al.</em>, 2010).</td>
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<td>↑ NRAMP1(Corna <em>et al.</em>, 2010)</td>
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<td><strong>Transport</strong></td>
<td>↓ Ferroportin in colorectal carcinoma (Brookes <em>et al.</em>, 2006), breast cancer cell lines(Jiang, Elliott and Head 2010) and breast cancer patients(Miller <em>et al.</em>, 2011; Pinnix <em>et al.</em>, 2010)</td>
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<td>↓ Ferroportin following treatment with TLR1-9 agonists(Schaer <em>et al.</em>, 2008)</td>
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*Table 1*: Altered Iron Homeostasis in Cancer Cells and Macrophages of the Tumor Microenvironment.
in a variety of cancers (Alkhateeb et al., 2013). Treatment with hemin and FeSO$_4$ was shown to induce H-ferritin in breast cancer cells but not colon cancer cells, allowing for protection against subsequent oxidants; Cermak et al hypothesized this as due to the differences between basal ferritin amounts and that while acute exposure of tumors to heme-derived iron allowed for oxidant-mediated sensitivity and lysis, chronic exposure to oxidants may result in resistance to oxidant stressors, especially due to increased intracellular iron storage capacity (Cermak et al., 1993). These two observations suggest that cancer cells have a high rate of iron import and utilization with little export.

Figure 4: Scheme of iron import and utilization by cancer cells. Cancer cells maintain high level of total iron import through increased expression of transferrin receptors and minimal ferroportin expression. Low ferritin measurements and high aminolevulinic acid synthase (ALAS1) activity in cancer cells indicate low sequestration and high iron utilization by mitochondria. Iron is essential for macromolecule synthesis such as various hemoproteins, iron-sulfur (Fe-S) containing proteins, and ribonucleotide reductases that synthesize deoxyribonucleotides that are utilized by cancer cells for their abnormal and accelerated growth. Free iron further contributes to carcinogenesis by Fenton chemistry through catalysis of mitochondrial and lipid mediated ROS formation that causes DNA mutations. Nuclear translocation of HO-1 has also been reported for various cancers, though its function in the nucleus is currently unknown.
The role of HO-1 in cancer has been either linked to more advanced disease in the presence of dysfunctional HO-1 in the nucleus in prostate cancer (Sacca et al., 2007; Wegiel et al., 2013) or to the better prognosis if present in the cytoplasm in breast cancer (Lin, Shen, Hou, Yang and Chen 2008). Recent data suggests an alternative utilization of HO-1 protective properties in assisting in the progression of malignant cells (Jozkowicz, Was and Dulak 2007). The protective properties of HO-1 can also be considered as a hallmarks of carcinogenesis, and elevated HO-1 expression have been reported in various tumor tissues. In renal cell carcinoma, elevated HO-1 in tumor tissues was suggested to contribute to neoplastic development by reducing oxidative damage caused by cytochrome P450 metabolites (Goodman, Choudhury, da Silva, Schwartzman and Abraham, 1997). Overexpression of HO-1 accelerated tumor growth through the promotion of angiogenesis in prostate cancer (Birrane, Li, Yang, Tachado and Seng, 2013). HO-1 is also implicated as BCR-ABL-mediated antiapoptotic survival signal in chronic myeloid leukemia (Tibullo et al., 2013). Importantly, HO-1 expression is also potently increased in response to chemotherapy, radiation, and photodynamic therapy (Jozkowicz et al., 2007).

While HO-1 normally carries out catabolism of heme on the endoplasmic reticulum, membrane-bound caveaole, or in mitochondria, recently the truncated isoform has been described in the nucleus of cancer cells. While HO-1 has also been located in endothelial cell calveolae to modulate carbon monoxide (CO) and nitric oxide (NO) production (Kim, Wang, Galbiati, Ryter and Choi, 2004) and in mitochondria to protect against mitochondrial oxidative stress (Bindu et al., 2011), nuclear translocation is atypical in that the HO-1 enzyme is both modified—nuclear translocation is dependent on proteolytic cleavage of a c-terminal tail—and has abrogated enzymatic activity (Lin et al., 2007; Wegiel et al., 2013). Nuclear HO-1 showed protective and progressive effects in prostate cancer (Sacca et al., 2007), head and neck squamous cell carcinoma ( Gandini et al., 2012), and oral epithelial dysplasia (Lee et al., 2008). Nuclear localization has also been correlated to higher VEGF production (Birrane et al., 2013) and resistance to chemotherapy (Tibullo et al., 2013). Interestingly, unlike BVR, HO-1 does not have DNA binding motifs and thus cannot act as a transcription factor, although many have speculated on the role of HO-1 mediated chromatin modifications and transactivation of transcription factors including AP-1 or NFκB (Lin et al., 2007). The specifics of how nuclear translocation of HO-1 affects tumor growth have yet to be elucidated.

5 Heme Degradation-derived Iron in Tissue Homeostasis and Cancer

Information about the role and expression of HO-1 in TAMs is limited. HO-1 was identified as a key gene out of 54 genes associated with iron recycling that distinguished the polarization status between M1 and M2 macrophages. (Recalcati et al., 2010) During the early stages of cancer, myeloid cells are recruited from the blood into the tissues and in the presence of pathogen or sterile inflammation differentiate and are polarized towards M1 “classically activated” macrophages that activate an immune response, promote inflammation, sequester iron, promote cancer cell regression, and inhibit development of tumours (Sica, Schioppa, Mantovani and Allavena, 2006). However, in the later stages of cancer progression, recruited and resident macrophages in the tumor microenvironment become polarized towards an “al-
ternatively activated” M2 phenotype with anti-inflammatory, immunosuppressive, pro-angiogenic, and favour iron intake and iron export characteristics. Since HO-1 is expressed in both M1 and M2 macrophages, being more pronounced in M2 macrophages, we speculate that HO-1 may have a significant role in shaping the tumor microenvironment. The switch between M1 and M2 macrophages is important for responses against the tumor as well as tumor maintenance.

![Diagram](image)

**Figure 5: Hypothetical scheme on the role of macrophages in tumor initiation and progression.** Transformation of cells leads to early recruitment of myeloid cells from the bone marrow and their differentiation to macrophages as a host response to the carcinogenesis. Pro-inflammatory mediators from M1 polarized macrophages released to the tumor microenvironment accelerate early progression of tumor. Cancer development is strongly associated with inflammation. Once the tumor expands, generation of an immune-suppressive niche is associated with reprogramming of macrophages in response to mediators released from cancer cells towards a tumor-supportive M2 phenotype.

In metastatic melanoma, HO-1 expression was primarily detected in CD163-positive TAM, typically associated with M2 polarization (Sierra-Filardi et al., 2010). Administration of cobalt protoporphyrin, a potent inducer of HO-1, resulted in increased IL-10 release from M2 macrophages, suggesting that HO-1 is important for the anti-inflammatory activities of MCSF M2 polarized macrophages (Sierra-Filardi et al., 2010). M1-polarizing cytokines (GM-CSF, IFNγ) inhibited, while IL-4 enhanced, M-CSF-driven HO-1 expression (Sierra-Filardi et al., 2010). MCSF-stimulated macrophages showed high levels of HO-1 and their functional status depends on the generation of carbon monoxide (Wegiel et al., 2014). Furthermore, binding of hemoglobin-haptoglobin to CD163 cells elicited IL10 secretion and resulted in HO-1 induction (Philippidis et al., 2004). Surprisingly, stable overexpression of HO-1 cells resulted in lower ferritin concentrations while the labile iron was not shown to increase significantly (Li et al., 2012).

6 Concluding Remarks

In summary, iron metabolism, recycling and storage are critical determinants of cancer development and progression. All above processes are controlled by macrophages and therefore
TAM with altered iron homeostasis may contribute to cancer growth and promotion. The heme degradation pathway and HO-1 are important regulators of iron recycling and utilization and as such dictate cancer growth and metastases. Cancer cells are characterized by increased iron intake and use, and M2 macrophages are uniquely positioned in the tumor microenvironment to facilitate enhanced heme degradation and iron export that contributes to cancer progression to more advanced disease.

References


