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HHS Public Access

Author manuscript

Biol Chem. Author manuscript; available in PMC 2018 August 28.

Published in final edited form as:

Biol Chem. 2017 August 28; 398(9): 995–1007. doi:10.1515/hsz-2016-0336.

Iron Overload and Altered Iron Metabolism in Ovarian Cancer

Stephanie Rockfield[1], **Joseph Raffel**[1], **Radhe Mehta**[1], **Nabila Rehman**[1], and **Meera Nanjundan**[1,*]

[1]Department of Cell Biology, Microbiology, and Molecular Biology, University of South Florida, 4202 East Fowler Avenue, ISA2015, Tampa, Florida, 33620

Abstract

Iron is an essential element required for many processes within the cell. Dysregulation in iron homeostasis due to iron overload is detrimental. This nutrient is postulated to contribute to the initiation of cancer; however, the mechanisms by which this occurs remain unclear. Defining how iron promotes the development of ovarian cancers from precursor lesions is essential for developing novel therapeutic strategies. In this review, we discuss (1) how iron overload conditions may initiate ovarian cancer development, (2) dysregulated iron metabolism in cancers, (3) the interplay between bacteria, iron, and cancer, and (4) chemotherapeutic strategies targeting iron metabolism in cancer patients.

Keywords

Endometriosis; fallopian tube fimbrial epithelium; microbiome; ovarian surface epithelium; therapy

1. Introduction

Iron is an essential trace element that is required for maintenance of healthy mammalian cells. There exists between 3–5 grams of iron in the average adult with the large majority being associated with hemoglobin and the remainder stored within ferritin (the iron storage protein complex) in hepatocytes and macrophages (Andrews, 1999). When iron is needed, it can be released via ferritinophagy which is an autophagic process promoting iron release from ferritin (Mancias et al., 2014). Iron is needed to support important cellular processes including (1) oxygen transport via hemoglobin, (2) metabolic reactions, and (3) synthesis of DNA (Wang and Pantopoulos, 2011). Seminal discoveries identifying transferrin receptor 1 (CD71), iron regulation via iron-regulatory binding protein (IRP) binding to iron responsive elements (IRE) in specific mRNA transcripts, as well as the hepcidin (HAMP)/ferroportin (FPN1) axis are significant to our current understanding of iron regulation. Nonetheless, we have yet to fully understand iron metabolism thoroughly, particularly with respect to the biology of cancers.

*Correspondence: Meera Nanjundan, 4202 East Fowler Avenue, ISA 2015, Department of Cell Biology, Microbiology, and Molecular Biology, University of South Florida, Tampa, Florida, 33620, mnanjund@usf.edu.

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Due to the role of iron in redox reactions and the absence of mechanisms to eliminate excess iron, iron homeostasis needs to be maintained carefully by a variety of molecules involved in transportation, storage, and degradation which are discussed herein. If iron homeostasis is disrupted, iron engages in Fenton reactions (ferrous (Fe^{2+}) iron reduces hydrogen peroxide (H_2O_2) to generate hydroxyl radicals (OH•)) (Winterbourn, 1995; Wang and Pantopoulos, 2011); this event leads to the formation of reactive oxygen species (ROS) which may promote ferroptosis, a morphologically distinct form of non-apoptotic cell death (Dixon et al., 2012). Overexpression of nuclear co-activator 4 (NCOA4), a recently identified receptor involved in iron release from ferritin (Mancias et al., 2014), contributes to the ferroptotic response (Hou et al., 2016). Thus, modulation of the ferroptotic and ferritinophagic processes may be potential targets for development of novel therapeutic treatment strategies for cancer in addition to iron chelators which deplete intracellular iron essential for cancer cell survival (Lui et al., 2015).

In this review, we discuss how ovarian cancer may be initiated in response to iron overload conditions and how cancer cells may become dependent on altered iron metabolism. We present data from the Cancer Genome Atlas (TCGA) illustrating dysregulated expression of iron molecules in ovarian cancer. Finally, we discuss potential treatment strategies involving targeting of these pathways.

2. Initiation of Ovarian Cancer by Persistent Oxidative Stress Mediated by Iron Overload

Chronic iron overload can lead to a number of diseases including (1) cancer (Lagergren et al., 2016), (2) retinal degeneration (He et al., 2007), (3) neurodegenerative diseases (Berg and Youdim, 2006), (4) hyperferritinemia (Stein et al., 2010), (5) hereditary hemochromatosis (Liu et al., 2016; Powell et al., 2016), and (6) β-thalassemia (Liu et al., 2016). Ovarian cancers are characterized by elevated genomic mutations which is proposed to result from oxidative damage (i.e., DNA adducts, lipid peroxidation, and 8-OHdG) (Yamada et al., 2011). Menstrual effluent (via retrograde menstruation) is suggested to contribute to endometriosis and development of rarer types (though often more chemoresistant in relation to the more common serous ovarian carcinoma (Itamochi et al., 2008)) of ovarian cancers such as endometrioid and clear cell ovarian cancers (Defrere et al., 2006). It is indicated that the pelvic region has an elevated iron level due to retrograde menstruation which may thus promote endometrial survival and implantation at ectopic sites (Van Langendonckt et al., 2002). Iron deposits (i.e., hemosiderin) have not only been identified in endometriotic lesions but also within the fallopian tube of patients diagnosed with serous epithelial ovarian cancers (Yamaguchi et al., 2008; Seidman, 2013). In addition to ovulation, another potential contributor to ovarian cancer pathogenesis is follicular fluid (Emori and Drapkin, 2014). In particular, follicular fluid contains hormones such as estradiol (Emori and Drapkin, 2014), reactive oxygen species (ROS), and transferrin (Shigeta et al., 2016). Additionally, iron and DNA adducts are increased in follicular fluid of endometriosis patients compared to infertile controls (Singh et al., 2013; Da Broi et al., 2016). Iron and transcript levels of ferritin and transferrin receptor were increased in follicles close to an endometrioma (characterized by elevated iron levels (Yamaguchi et al.,

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2008)) affecting oocyte retrieval (Sanchez et al., 2014) although another study did not identify any differences in iron content or ferritin levels (Benaglia et al., 2015). Exposure of fallopian epithelium to follicular fluid leads to a small increase in cell proliferation along with IL-8 cytokine levels (Bahar-Shany et al., 2014). High ROS containing follicular fluid was capable of inducing early onset B-cell lymphoma in mammary fat pads in mice lacking p53 (Huang et al., 2015).

The detailed molecular mechanism underlying initiation of ovarian cancers remains unclear (Figure 1). Since iron induces ROS via its participation in Fenton reactions (Winterbourn, 1995; Yamada et al., 2011), iron overload may initiate carcinogenesis by increasing oxidative stress levels and thus mutagenesis (Vercellini et al., 2011). Precursor cells postulated to contribute to specific subtypes of ovarian cancers (i.e., endometriotic cells, fimbrial secretory epithelial cells, and ovarian epithelial cells) may be exposed to a sublethal dose of oxidative stress and thus may have the potential to undergo tumorigenesis due to persistent anti-oxidant defenses (Kobayashi, 2016). Ferritin, nuclear factor (erythroid-derived 2)-like 2 (NRF2), as well as heme oxygenase 1 (HO-1) amongst others (i.e., NAD(P)H:Quinone Oxidoreductase 1 (NQO1), glutathione S-Transferase Pi 1 (GSTP1), and glutathione-dependent peroxidases (GPX)) contribute to such anti-oxidant defenses and may therefore participate in the tumorigenesis process along with persistent DNA damaging events (Iwabuchi et al., 2015; Kobayashi, 2016). This would enable a proliferative environment for survival advantage (Iwabuchi et al., 2015). Indeed, for example, the incidence of hepatocellular carcinoma (HCC) is elevated 200-fold in patients with hereditary hemochromatosis; the resulting increase in ROS coinciding with unbalanced anti-oxidant defenses is suggested to promote tumorigenesis (Marrogi et al., 2001). In endometriotic cysts, iron was measured to be ~100mM and was associated with lipid peroxide, 8-OHdG positivity, and evidence of iron deposits (via Prussian blue staining) (Yamaguchi et al., 2008). Iron deposits have also been identified in the fallopian tube (Seidman, 2013). Fimbrial secretory epithelial cells treated with increasing doses of iron elicited increased cellular proliferation along with changes in p53, MAPK, AKT, and c-Myc proteins, as well as increased ROS species (0.05–100mM) (Lattuada et al., 2015). Furthermore, vitamin D3 could oppose the oxidative stress-induced events mediated by iron in these fimbrial cells (Uberti et al., 2016). In support of the transition from precursor cells to cancer, it is notable that a clear cell ovarian cancer gene signature was induced upon iron treatment in immortalized ovarian surface epithelial cells; this was regulated partially by DNA methylation (Yamaguchi et al., 2010).

ROS damages not only DNA but also proteins and lipids; products of lipid peroxidation can themselves damage DNA (Luczaj and Skrzydlewska, 2003). DNA damaging events could lead to (1) its repair unless the damage is extensive leading to cell death or (2) survival of mutant cells if other changes are present leading to an accumulation of mutations (Tak et al., 2000). ROS cause numerous types of DNA damage such as strand breakages, formation of apurinic/apyrimidinic sites, base modifications (8-OHdG), thymidine glycols, and ring-opened based products (Toyokuni, 1996). There are likely “specific” sites that are susceptible to such DNA damage (Tanaka et al., 1999). These can be mutagenic resulting in Ras activation and p53 inactivation (Du et al., 1994). Indeed, K-Ras and the MAPK signaling cascade have been shown to be hyperactivated in response to oxidative stress

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(Yamada et al., 2011). Furthermore, PTEN can be inactivated directly by hydrogen peroxide via oxidation which was accompanied by elevated PIP_3 levels and AKT activation (Leslie et al., 2003). Mutations in K-Ras (a codon 12 G to T transversion) can be induced by nickel (similar chemical and physical properties to iron (Valko et al., 2006)) in renal sarcomas and mutations in p53 (G to T transversion) can be induced by iron in renal cancers (Toyokuni, 1996). Further, K-Ras was identified to be mutated (GGT to GCT mutation at codon 12) at 8 weeks in one rat out of 26 exposed to cigarette smoke for a 1 hour time twice a day (8, 12, or 20 weeks) (Maehira et al., 1999). Oxidative stress can also lead to marked increases in c-Myc (a known mediator of cell proliferation) transcripts (Toyokuni, 1996; Li and Spector, 1997; Elouil et al., 2005). Altogether, these events may contribute to iron-induced persistent oxidative stress events, thereby leading to precursor lesions transitioning to various subtypes of ovarian cancer (Yamada et al., 2011).

It is well-established that estrogen is associated with an increased risk of tumorigenesis in ovarian cancers (Liehr and Jones, 2001; Mungenast and Thalhammer, 2014; Jeon et al., 2016; Kyriakidis and Papaioannidou, 2016) as well as endometrial and breast cancers (Brown and Hankinson, 2015). Strikingly, there are links between estrogen and iron which stems from a hamster kidney tumor model in which the animals were implanted with estrogen and provided either normal chow or a diet with elevated iron levels which doubled tumor incidence (Wyllie and Liehr, 1998). There are also notable changes in intracellular levels of iron upon estrogen treatment in breast cancer cells (Liehr and Jones, 2001). Specifically, metabolic estrogen components generates ROS which promotes iron release from ferritin in the Fe^{+2} form (Liehr and Jones, 2001) and furthermore, there is evidence that estrogen metabolites can themselves form DNA adducts (Cavalieri and Rogan, 2016). Since c-Myc can control gene expression of molecules involved in iron metabolism (i.e., ferritin heavy chain (FTH1) and IRP-2), it is interesting that estrogen can modulate expression of c-Myc (Liehr and Jones, 2001). Balance of intracellular iron is tightly controlled by the HAMP/FPN1 axis (Hou et al., 2012) and both mediators are regulated via estrogen response elements (ERE) within their promoter regions (Hou et al., 2012; Qian et al., 2015). Estrogen also can increase transferrin mRNA by binding to specific elements within the promoter region of transferrin via an atypical (non-consensus) ERE sequence (Vyhlidal et al., 2002).

3. Dysregulation of Iron Metabolism in Cancer

Iron mediates its effects via complex mechanisms of regulation including transcriptional and post-transcriptional means (i.e., via IRP) (Liu et al., 2005) and changes in iron metabolism are a property of tumors (Miller et al., 2011). The pathways of iron metabolism that are altered in cancer include (1) iron uptake/export processes, (2) storage, and (3) regulation (Zhang and Zhang, 2015). Indeed, iron is critical for a number of cellular processes including heme biosynthesis and the production of iron-sulphur clusters, which regulate a large array of enzymatic activities required for sustaining increased cellular proliferation and metabolic functions (Rouault, 2015). The number of molecules identified to be involved in iron metabolism has increased exponentially in the last decade (Hower et al., 2009). Indeed, a network of pathways integrating these molecules has been developed for 107 reactions/ transport processes (using CellDesigner version 3.5.2) for various cell types including intestine, liver (important in uptake and storage), as well as reticulocytes and macrophages

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(utilization and recycling) (Hower et al., 2009). To comprehend the complexity of iron regulation, global gene profiling approaches such as Affymetrix oligonucleotide arrays have been used to define patterns of genes that are altered (Liu et al., 2005). For example, an Iron Regulatory Gene Signature (IRGS) has been utilized to identify high risk cancer patients (Miller et al., 2011). This signature is comprised of 16 iron regulatory genes including CYBRD1 (cytochrome b reductase 1), STEAP1 (six transmembrane epithelial antigen of the prostate 1), STEAP2 (six transmembrane epithelial antigen of the prostate 2), HFE (hemochromatosis), SCARA5 (scavenger receptor class A, member 5 (putative)), LTF (lactotransferrin), TFRC (transferrin receptor 1 (CD71)), SLC40A1 (solute carrier family 40 (iron-regulated transporter), member 1), ISCU (iron-sulfur cluster scaffold homolog), SFXN1 (sideroflexin 1), EPAS1 (endothelial PAS domain protein 1), SLC25A37 (solute carrier family 25, member 37), ABCG2 (ATP-binding cassette, subfamily G (WHITE), member 2), SFXN5 (sideroflexin 5), HIF1AN (hypoxia inducible factor 1, alpha subunit inhibitor), and ALAD (aminolevulinate dehydrogenase) (Miller et al., 2011). This signature was demonstrated to predict breast cancer recurrence as well as subclassify molecular subtypes of breast cancer, which may aid in determination of patient outcomes (Miller et al., 2011).

Figure 2 summarizes the major mediators in iron metabolism. Briefly, in enterocytes, duedonal cytochrome b (DCYTB) reduces Fe^{+3} to Fe^{+2}, which can then be imported into the cell by the divalent metal transporter 1 (DMT1) (Canonne-Hergaux et al., 1999; McKie et al., 2001). The ferric Fe^{+3} can also bind to transferrin (holo-transferrin); the iron-bound transferrin then binds to CD71 on the plasma membrane and enters cells via endocytosis (Byrne et al., 2010; Wang and Pantopoulos, 2011). Iron is then released from transferrin within the endosomal compartment and reduced to Fe^{+2} by six-transmembrane epithelial antigen of prostate 3 (STEAP3) (Zhang et al., 2012), allowing its transport to the cytosol via DMT1 (Anderson et al., 2013). Fe^{+2} becomes part of the labile iron pool (LIP) which may then be used for generating iron-dependent protein complexes, iron-sulfur clusters, heme, or used in metabolic pathways as an enzymatic cofactor (Wang and Pantopoulos, 2011). If in excess, iron may then be stored within the ferritin complex or exported by FPN1 (Donovan et al., 2000; Shi et al., 2008). Elevated serum iron levels promote production of the 84 amino acid protein hormone HAMP in the liver which initiates endocytosis of FPN1 on the surface of hepatocytes, macrophages, enterocytes, and placental cells (Pigeon et al., 2001; Anderson et al., 2002). FPN1 is the only known exporter channel for intracellular iron in the Fe^{+2} form (Ward and Kaplan, 2012). Ferroportin disease is characterized by mutations in FPN1 leading to hyperferritinemia and macrophage iron loading (Mayr et al., 2010) which may be due to altered trafficking of FPN1 to the cell surface (rate of synthesis, rate of internalization, and/or rate of degradation) (Ward and Kaplan, 2012). FPN1 levels can also be modulated via mechanisms distinct from HAMP such as post-transcriptionally by the IRP/IRE system (Ross et al., 2012; Miseta et al., 2015) as well as transcriptionally by (1) hypoxia, (2) transition metals and heme, as well as (3) inflammation (Ward and Kaplan, 2012). The presence of HAMP reduces circulating iron levels leading to intracellular sequestration of iron (Miseta et al., 2015). HAMP/FPN1 axis is critical to body iron homeostasis; HAMP is upregulated by a variety of mechanisms involving transferrin receptor-2, hemochromatosis (HFE), bone morphogenic protein 6 (BMP6), and hemojuvelin (iron sensing extracellular

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system) (Miseta et al., 2015). Erythropoietin negatively influences HAMP gene expression (Miseta et al., 2015). Furthermore, IL-6 (which is upregulated in cancers and promotes cellular proliferation in human breast carcinoma (Sansone et al., 2007)) signals through the JAK/STAT signaling cascade and upregulates HAMP expression (Miseta et al., 2015). As indicated earlier, estrogen, vitamin D, and other hormones also influence HAMP expression (Miseta et al., 2015). Finally, iron regulatory proteins (IRPs) regulate translation of iron transport proteins (such as FTH1 and CD71), through their interactions with 3′-UTR or 5′-UTR iron responsive elements (Weiss et al., 1997).

A new mediator in iron metabolism, namely NCOA4 (previously known as androgen-associated protein 70 (ARA70) (Ligr et al., 2010)), was identified via a quantitative proteomics approach and demonstrated to be involved in the process of ferritinophagy (ferritin breakdown in lysosomes) (Dowdle et al., 2014; Mancias et al., 2014; Mancias et al., 2015). NCOA4 interacts with both the heavy and light chain of ferritin (Dowdle et al., 2014). Reduction of NCOA4 protein hindered co-localization of FTH1 with LC3B/LAMP1; furthermore, NCOA4 reduces IRP-2 and CD71 protein (Mancias et al., 2014). Functions independent of ferritinophagy include NCOA4's ability to regulate DNA replication via binding to MCM7; further, deficiency of NCOA4 activates DNA replication origin activation via CMG helicase regulation (Bellelli et al., 2014). Additionally, NCOA4 mediates DNA damage/replication stress events (i.e., fork stalling, inhibition of fork speed, and premature senescence) (Bellelli et al., 2014). Using a NCOA4 knockout mouse model, the ferritinophagy receptor was demonstrated to be involved in promoting iron balance (Bellelli et al., 2016). In addition to interacting with the androgen receptor, NCOA4 can bind to the estrogen receptor (ERα) (Lanzino et al., 2005). While these studies refer to the full-length form (NCOA4α/ARA70α), an alternative splice variant (NCOA4β/ARA70β) was identified lacking an internal sequence of 985bp (Alen et al., 1999). The two NCOA4 isoforms elicit distinct roles in breast and prostate cancer cells; in particular, NCOA4α elicits tumor suppressive activity whereas NCOA4β elicits oncogenic activity (Peng et al., 2008; Ligr et al., 2010; Wu et al., 2011). It is presently unclear how these two isoforms mediate distinct functional activities; however, based on functional domains that have so far been identified, the AhR and FTH binding domains are lacking in the shorter variant (Kollara and Brown, 2006; Mancias et al., 2015). Aside from one report demonstrating that NCOA4/ARA70 mRNA expression was increased in human ovarian cancers compared to normal ovarian surface epithelium via *in situ* hybridization, further investigations are needed to fully comprehend the role of NCOA4 (as a ferrinophagic mediator and a nuclear co-activator) in the biology of ovarian cancers (Shaw et al., 2001).

SNPs in the germline for cellular transport genes was found to be associated with epithelial ovarian cancer risk (Chornokur et al., 2015). Specifically, one gene that was associated with serous epithelial ovarian cancers was the Hephaestin (HEPH) gene which is involved in catalyzing redox-active iron to its non-redox active form for transport from the enterocytes to the blood (Chornokur et al., 2015). Furthermore, we analyzed The Cancer Genome Atlas (TCGA) via cBIOPORTAL (Cerami et al., 2012; Gao et al., 2013) for alterations in iron pathway-mediating genes in ovarian cancers and this is presented in Table 1. Several studies have presented evidence implicating transferrin in ovarian cancer biology. For example, OVCAR-3 responded to transferrin with increased proliferation (Martinez et al., 2000).

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Transferrin was found to antagonize the cell death response induced by FTH1 and c-Myc in the N.1 human ovarian carcinoma cell line (Fassl et al., 2003). Treatment of fallopian epithelial cells with transferrin increased phosphorylation of H2AX in a CD71 and ROS-dependent manner (Shigeta et al., 2016). DNA double strand breaks were also observed in response to transferrin treatment in murine fallopian tubes (*ex vivo*) (Shigeta et al., 2016). Finally, there exists clinical data implicating transferrin as a biomarker to classify ovarian cancers (Ahmed et al., 2005; Kozak et al., 2005; Hogdall et al., 2011; Macuks et al., 2012; Moore et al., 2012). In addition, the transferrin receptor 2 appears to play a role in mediating iron homeostasis; it is highly expressed in ovarian cancer cell lines and inversely associated with CD71 expression (Calzolari et al., 2007). Elevated redox active iron, elevated CD71, and lowered FPN1 levels were present in ectopic endometrial stromal cells resulting in increased intracellular labile iron pool compared to normal eutopic endometria (Mori et al., 2015). The human hemochromatosis protein has two prevalent mutations at H63D and C282Y that are associated with iron overload diseases such as type I hemochromatosis (Gannon et al., 2011). Interestingly, the C282Y mutation was more frequent in patients with epithelial ovarian cancer and associated with a poor patient outcome (Gannon et al., 2011). Contradictory findings are reported for the iron storage protein, ferritin. Reduced FTH1 mRNA was identified in ovarian cancer specimens and associated with a poor patient outcome (Lobello et al., 2016) whereas its expression was elevated in metastatic ovarian cancer specimens relative to primary tumors (Tripathi and Chatterjee, 1996). However, upon FTH1 knockdown, SKOV3 ovarian cancer cells were more proliferative and migratory (Lobello et al., 2016) and furthermore, FTH1 was induced by c-Myc expression leading to increased cell survival (Fassl et al., 2003).

4. Bacteria, Iron, and Cancer

It is hypothesized that changes in the microbiome of the pelvic region may be associated with the development of gynecological cancers including ovarian cancers (Chase et al., 2015). It is thus interesting that the microbiome of the gut is altered with increased dietary iron (Ng, 2016). This metal is critical for promoting pathogenic bacteria virulence, growth, and colonization (Ng, 2016). Evidence suggests that the protective microorganisms (*Bifidobacterium longum* and *Lactabacillus acidophilus)* which reduce oxidative stress in the gut become overwhelmed by pathogenic bacteria (Ng, 2016). Furthermore, utilization of antibiotics or breeding microorganism-free mice are associated with reduced colon cancer (Ng, 2016). Mutations in genes involved in iron metabolism (i.e., IRP-2) promotes a protective microbiome (Ng, 2016). In addition, heme may damage the colonic surface epithelium leading to crypt cell hyperproliferation which fills-in the denuded area; heme mediates these effects by altering the levels of *Bacteroidetes* and *Firmicutes* in the colonic environment (N et al., 2012). Lipid peroxidation mediated by heme was reduced in rats treated with antibiotics, which was accompanied by decreased crypt cell proliferation (Martin et al., 2015). Siderophores enable bacteria to solubilize iron from their environment to promote their cellular proliferation (Ellermann and Arthur, 2016). For example, uropathogenic *E. coli* (UPEC) bacteria depend on iron for growth and the ferritinophagy pathway was recently demonstrated to contribute to its increased survival (Bauckman and Mysorekar, 2016). It is suggested that epigenetic changes (i.e., methylation of CDKN2A) in

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the host uroepithelial cells due to UPEC infection may lead to bladder cancer (Tolg et al., 2011).

5. Targeting the Iron Pathway in Cancer and Future Perspectives

Since there is no mechanism to eliminate excess iron from the body, iron levels need to be tightly controlled; if dysregulated, disease conditions develop (Blanchette et al., 2016). Thus, modulating any of the processes involved in iron metabolism could be feasible targeting strategies for cancer treatment (Zhang and Zhang, 2015). There is evidence that phlebotomy may have potential as a cancer treatment strategy (Zacharski et al., 2008; Assi and Baz, 2014; Chifman et al., 2014; Nirei et al., 2015; Distante et al., 2016; Inati et al., 2017). Since HAMP levels are critical in regulating body iron levels, therapeutic strategies such as antagonists (via altering function (binding properties to FPN1) or modulation of its levels (via regulatory mechanisms)) to target this molecule would be worthwhile (Blanchette et al., 2016). Curcumin, a primary component of turmeric associated with anti-inflammatory properties (Funk et al., 2006; Jiao et al., 2009), is an iron-chelating agent that can also inhibit HAMP production thereby altering body iron levels (Jiao et al., 2009). Additionally, a variety of iron chelating agents exists, some of which have been approved for clinical use to treat iron overload disorders though not yet in use for treatment of cancer (Lui et al., 2015). For example, deferrioxamine (DFO) is the accepted chelator for use in treatment of β-thalassemia (Lui et al., 2015). However, it has a short half-life in plasma and thus requires long period of infusion (8–12 hours) resulting in poor patient compliance (Lui et al., 2015). Therefore, oral chelators would be superior in this regard; indeed, deferasirox (EXJADE) has not only improved tolerance for such iron overload disorders but also elicits higher anti-proliferative activity (Lui et al., 2015). Thiosemicarbazone chelators (3-AP, PIH analogs, DpT analogs) not only target iron metabolism but also innumerable signaling pathways; therefore, they appear to have more potency and thus may be beneficial in their use as a cancer treatment strategy although not yet in use for this purpose (Lui et al., 2015) (Heath et al., 2013). Curcumin can also modulate IRP activity leading to changes in FTH1 and CD71 expression (Jiao et al., 2006; Hatcher et al., 2008). Since CD71 is markedly elevated in cancers (Daniels et al., 2006), it can be targeted with monoclonal antibodies which diminish cellular proliferation leading to an increased cell death response *in vitro* (Daniels et al., 2006). Currently used as efficient targeting agents, there is evidence of the therapeutic benefit of transferrin-conjugates with therapeutic compounds such as doxorubicin, cisplatin, and tirapazamine thus far in mouse xenograft models although these have been unsuccessful in clinical trials (Tortorella and Karagiannis, 2014).

We previously reported that iron treatment in a series of gynecological cell lines resulted in cell death initiated by the MAPK pathway (and dependent on Ras mutational status) leading to increased autophagosome and lysosomes (Bauckman et al., 2013); these changes were accompanied by increased ROS and mitochondrial damage (Bauckman et al., 2015). The cell death induced by FAC appeared to be distinct from the recently described ferroptosis; this is a cell death pathway distinct from apoptosis, necrosis, and autophagy though with features similar to glutamate-induced death (Dixon et al., 2012). Cells that are rapidly dividing are seemingly addicted to higher levels of iron and thus may be more responsive to changes in ROS levels resulting in cell death such as ferroptosis (Tarangelo and Dixon,

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2016). Interestingly, C′ (Cornell) dots (poly(ethylene glycol)-coated silica nanoparticles) can eliminate cancer cells in a growth restricted medium similar to the environmental conditions found *in vivo* for a tumor (Tarangelo and Dixon, 2016). These dots bind to extracellular iron and following their entry into cells, they increase intracellular redox active iron and ROS leading to a ferroptotic mechanism of cell death (Tarangelo and Dixon, 2016). The use of C′ dots in *in vivo* mouse xenograft models led to regression of tumors (Tarangelo and Dixon, 2016). Via a screening approach, Ferrostatin-1 was identified to be a specific inhibitor for ferroptosis which involves inhibition of cysteine uptake via the cysteine/glutamate antiporter system (xc-) which decreases the anti-oxidant response (Dixon et al., 2012; Skouta et al., 2014). Further, Sorafenib induces cell death by chelation of intracellular iron, targeting of the xc- system, and increasing ROS; these events could be blocked with ferrostatin-1 (Louandre et al., 2013; Dixon et al., 2014). Strengthening of the ferroptotic response was promoted by Rb deficiency and p53 which transcriptionally upregulates SLC7A11 (a cysteine/glutamate antiporter component) to reduce cysteine uptake (Jiang et al., 2015; Louandre et al., 2015). Pancreatic ductal adenocarcinomas (PDAC), which are generally insensitive to apoptosis, were sensitized to Artesunate (ART) via induction of ferroptosis, specifically in cells with constitutive K-Ras activity (Eling et al., 2015). In this regard, it is suggested that profiling of patient tissues for ferroptotic genes may identify responsiveness to ferroptosis-inducing agents (Eling et al., 2015). ART also hindered growth of ovarian cancer cell lines and tumors in a mouse xenograft model via induction of ROS leading to DNA damage and a ferroptotic response (Greenshields et al., 2016). The ferroptotic response is associated with a reduction in glutathione (GSH) levels via GPX enzymes (Yang et al., 2014). Negative regulators of the ferroptotic response include (1) HSPB1 (Heat Shock Protein Family B Member 1) in an HSF-dependent manner (Sun et al., 2015) (2) CISD1 (CDGSH iron sulfur domain 1) which is an iron-containing outer mitochondrial membrane protein implicating mitochondrial lipid damage (via peroxidation) (Yuan et al., 2016), (3) NRF2 via activation of the p62-Keap1 signaling pathway (key regulators of the antioxidant response) (Sun et al., 2016) in which its activation leads to increased HO-1, FTH1, as well as NQO1 transcripts (Sun et al., 2016), (4) reduction of autophagy markers (ATG5 or ATG7) thereby decreasing autophagic flux turnover (Hou et al., 2016), and (5) NCOA4 depletion (Hou et al., 2016).

Ovarian cancer remains one of the deadliest gynecological cancers in women in the United States. Regrettably, the disease is typically only identified once the cancer has already progressed to advanced stages. Although considerable knowledge has thus far been obtained regarding iron metabolism, further work is direly needed to clarify the mechanisms how this essential nutrient leads to ovarian cancers from precursor lesions. This will be essential for developing improved therapeutic strategies for these ovarian cancer patients.

Acknowledgments

This work was supported by funding from the National Cancer Institute to MN (R21 CA178468-01). We apologize to those in this area of research whose work we have not cited.

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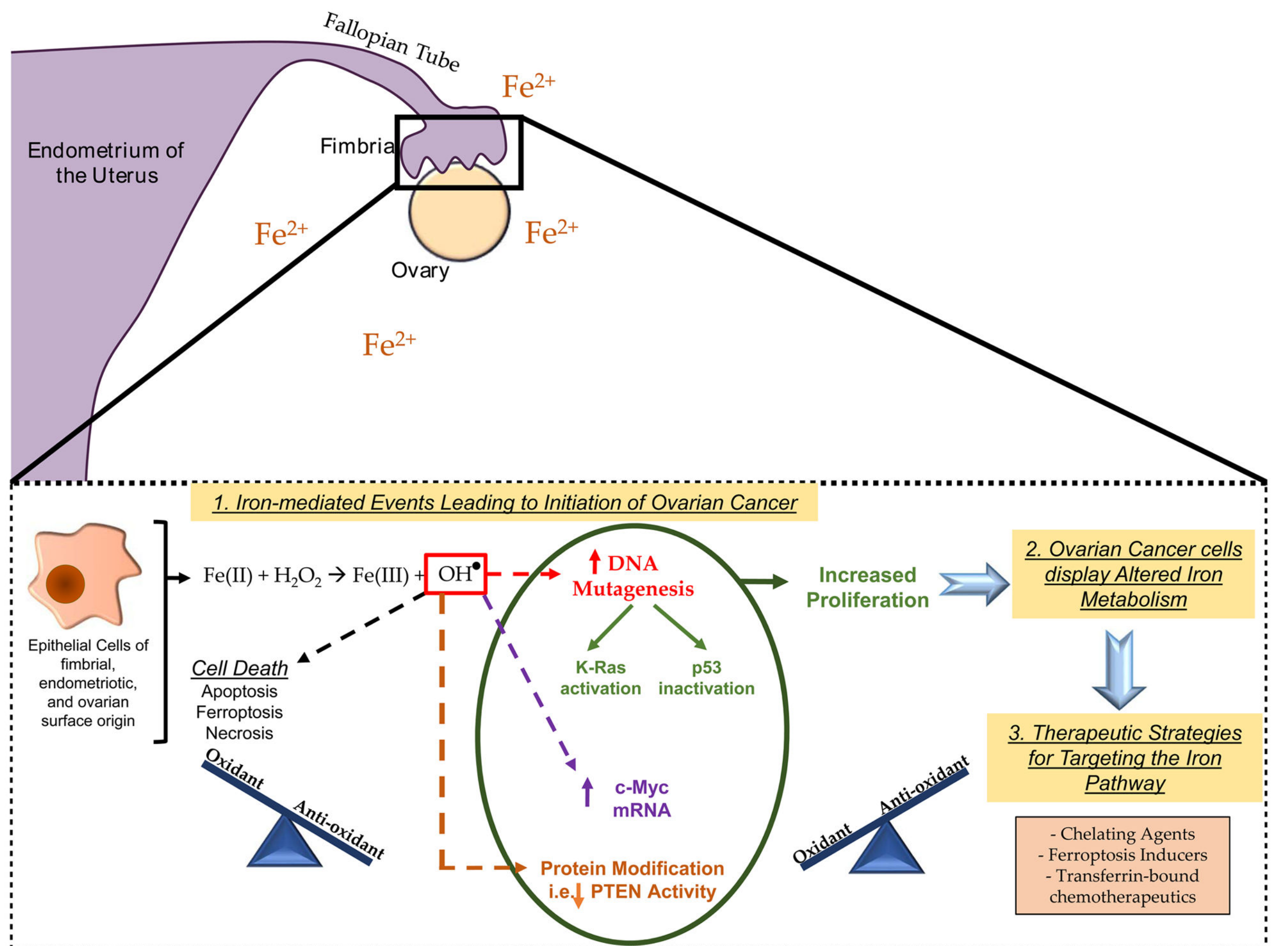

Figure 1. Model of the Contribution of Iron to Ovarian Cancer Development

Iron is elevated in the peritoneal cavity of women with endometriosis, in endometriotic cysts, and in fallopian tube fimbriae. Iron engages in Fenton reactions resulting in ROS such as hydroxyl radicals. An excess of ROS can promote cell death such as ferroptosis. However, sublethal levels of ROS may lead to increased proliferative capacity (if balanced with an increase in anti-oxidant response) thereby promoting tumorigenesis. ROS can lead to increased c-Myc transcripts, directly reduce PTEN activity, and cause DNA mutations leading to inactivation of p53 and activation of K-Ras. Tumor cells are characterized by increased iron dependence. Therefore, therapeutic strategies targeting components of the iron pathway may prove useful in treating ovarian cancer patients.

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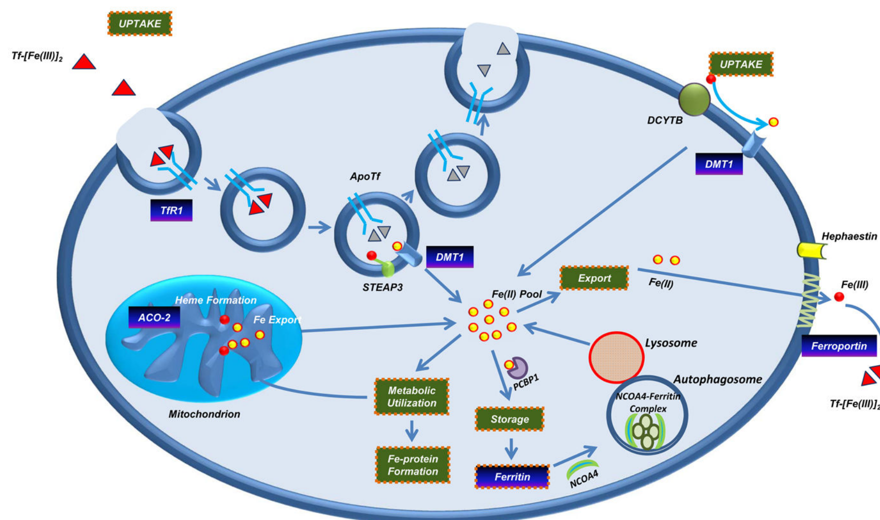

Figure 2. The Iron Regulatory Pathway

Iron bound transferrin is endocytosed into the cell via CD71. Iron is then reduced by STEAP3 from the Fe^{+3} to Fe^{+2} state, which then is shuttled out of the endosome via DMT1. Holo-transferrin and CD71 are recycled back to the cell surface. Non-transferrin bound iron may also be reduced to Fe^{+2} by DCYTB and transported into the cell by DMT1. Intracellular redox active iron may then be used for metabolic processes, formation of iron-sulphur clusters, or shuttled into the mitochondria for heme synthesis. If intracellular iron is in excess, it can be stored (via the chaperone protein PCBP1) in the ferritin storage complex. When needed, iron can then be released from the ferritin complex through the process of ferritinophagy, mediated by NCOA4. Iron may also be exported from the cell by FPN1, following oxidation to Fe^{+3} by HEPH.

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Table 1

Summary of alterations in the iron metabolic pathway in ovarian cancers obtained from the TCGA via cBIOPORTAL. Information on the chromosomal location of the genes listed was obtained from Genecard (http://www.genecards.org/).

Gene	Symbol	GCID	Chromosome Location	Genomic Location (base pairs)	Mutations	Deletions	Amplifications
Aconitase 1; Iron-Responsive Element Binding Protein 1	ACO1; IRP1	GC09P032374	9p21.1	Start: 32,384,603 bp End: 32,454,769 bp	2/311 (0.6%)	1/311 (0.3%)	5/311 (1.6%)
Ferritin Heavy Chain	FTH1	GC11M061959	11q12.3	Start: 61,959,718 bp End: 61,967,660 bp	1/311 (0.3%)	0/311 (0%)	6/311 (1.9%)
Ferritin Light Chain	FTL	GC19P048965	19q13.33	Start: 48,965,301 bp End: 48,966,879 bp	0/311 (0%)	3/311 (1%)	2/311 (0.6%)
Hepcidin Antimicrobial Peptide	HAMP	GC19P035281	19q13.12	Start: 35,280,716 bp End: 35,285,143 bp	0/311 (0%)	0/311 (0%)	30/311 (9.6%)
Iron-Responsive Element Binding Protein 2	IREB2; IRP2	GC15P078437	15q25.1	Start: 78,437,431 bp End: 78,501,456 bp	0/311 (0%)	1/311 (0.3%)	5/311 (1.6%)
Nuclear Receptor Coactivator4	NCOA4	GC10M046005	10q11.22	Start: 46,005,088 bp End: 46,030,714 bp	0/311 (0%)	0/311 (0%)	3/311 (1%)
Transferrin	TF	GC03P133663	3q22.1	Start: 133,661,998 bp End: 133,779,006 bp	1/311 (0.3%)	0/311 (0%)	23/311 (7.4%)
Transferrin Receptor	TFRC; CD71	GC03M196027	3q29	Start: 196,027,183 bp End: 196,082,189 bp	1/311 (0.3%)	1/311 (0.3%)	76/311 (24.4%)
Solute Carrier Family 11 (Proton-Coupled Divalent Metal Ion Transporter), Member 2	SLC11A2; DMT1	GC12M050979	12q13.12	Start: 50,979,401 bp End: 51,028,566 bp	0/311 (0%)	1/311 (0.3%)	2/311 (0.6%)
Solute Carrier Family 40 (Iron-Regulated Transporter), Member 1	SLC40A1; FPN1	GC02M189560	2q32.2	Start: 189,560,579 bp End: 189,583,758 bp	1/311 (0.3%)	0/311 (0%)	25/311 (8%)