HHS Public Access

Author manuscript

Cancer Res. Author manuscript; available in PMC 2021 June 15.

Published in final edited form as:

Cancer Res. 2020 December 15; 80(24): 5435–5448. doi:10.1158/0008-5472.CAN-20-2017.

Iron and cancer: 2020 Vision

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Abstract

New and provocative insights into the relationships between iron and cancer have been uncovered in recent years. These include delineation of connections that link cellular iron to DNA repair, genomic integrity, and oncogenic signaling as well as the discovery of ferroptosis, a novel iron-dependent form of cell death. In parallel, new molecules and pathways that regulate iron influx, intracellular iron trafficking and egress in normal cells, and their perturbations in cancer have been discovered. In addition,, insights into the unique properties of iron handling in tumor initiating cells (cancer stem cells), novel contributions of the tumor microenvironment to the uptake and regulation of iron in cancer cells, and new therapeutic modalities that leverage the iron dependence of cancer have emerged.

Introduction

The field of iron biology, particularly aspects related to cancer, has evolved substantially since we last reviewed this topic for *Cancer Research* in 2011. New connections as well as mechanistic underpinnings of previously described relationships between iron and cancer have been uncovered, and novel interventions for cancer therapy that involve iron perturbations have been proposed. Iron trafficking is now understood to be considerably more complex and nuanced than envisioned 10 years ago, involving previously unknown mechanisms of uptake and efflux as well as intracellular and intercellular redistribution of iron that impacts tumor behavior and drug response. Further, iron is now known to influence not only tumor growth, but metastatic potential. In counterpoint to these pro-tumorigenic activities, iron has also been recognized as an essential element in ferroptosis, the eponymous mechanism of cell death that may ultimately allow us to target the iron addiction of cancer cells. To adequately review these topics, we have focused on recent discoveries of molecules and pathways that link iron homeostasis and cancer. We refer the reader to additional reviews to fill out the complete picture of iron dysregulation and cancer, including population-based studies, the role of mitochondrial iron, and early experimental work linking iron and cancer(1–4).

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Conflict of interest: The authors declare no potential conflicts of interest.

Iron Uptake, Redistribution and Egress

Cellular iron uptake

Transferrin receptor 1 (TFR1)/transferrin.—A major pathway of iron intake utilizes transferrin receptor 1 (TFR1) (Figure 1). This well-studied pathway(5) involves binding of diferric transferrin (TF) to TFR1, endocytosis of the TFR1/TF-Fe complex, release of iron from TF within the acidic endosome, iron reduction by STEAP3, and subsequent exit of iron from the endosome to the cytosol via DMT1. Following release of iron, the TFR1/TF complex is recycled to the cell surface, where apo-TF dissociates from TFR1 to participate in multiple additional rounds of iron delivery. As early as the 1980's, it was shown that transferrin receptors are up-regulated in breast and other cancers(6,7), suggesting that tumor cells exhibit an enhanced demand for iron. These early findings have been corroborated by hundreds of subsequent studies, establishing TFR1 as a tumor marker (albeit not a specific marker, since non-cancer cells, especially rapidly proliferating cells, also express abundant transferrin receptors), and stimulating strategies to exploit the enhanced expression of TFR1 in anti-tumor therapy. However, TFR1 upregulation is only one of many strategies employed by tumor cells to acquire iron.

Lipocalin 2 (NGAL, *LCN2*), a member of the lipocalin superfamily, is a 25 kD secreted protein that binds bacterial siderophores or siderophore-like catechols of mammalian origin(8). Siderophores are small molecules that bind iron. Lipocalin 2 can therefore serve as an iron delivery vehicle following binding to its high affinity cell surface receptor, LCN2R (24p3R, *SLC22A17*), or megalin(9). Overexpression of *LCN2* is associated with decreased survival of breast cancer patients(10), and depletion of lipocalin 2 inhibits tumor formation in mammary(11) as well as prostate(12,13) tumor models . Consistent with a role of iron in the pro-tumorigenic effects of lipocalin 2 in breast cancer(14), tumor tissue from renal clear cell carcinoma contained approximately 10-fold more lipocalin 2-bound iron than non-tumor tissue, and mutation of amino acid residues critical to the iron-binding function of lipocalin 2 or treatment of cells with apo-lipocalin 2 abolished its pro-tumorigenic activity in experimental models(15). Strikingly, cancer cells from leptomeningeal metastases of patients with breast or lung cancer express LCN2 and its receptor, enabling them to acquire iron in this nutrient-limited microenvironment(16). Accordingly, loss of LCN2 inhibited tumor growth in the leptomeninges and conferred a survival benefit in three mouse models(16). In addition to the ability of lipocalin 2 to promote tumor growth, invasiveness, and the epithelial to mesenchymal transition (EMT) in cancer cells, the role of this secreted protein in iron trafficking in the microenvironment likely contributes substantially to its pro-tumorigenic effects(17,18).

DMT1 (divalent metal transporter 1). In addition to its role in the transit of iron from the endosome to the cytosol, DMT1 serves as an apical iron transporter involved in the uptake of dietary iron by duodenal cells of the small intestine(19). Expression of DMT1 is increased in patients with colorectal carcinoma(20), and colon-selective disruption of DMT1 decreases sporadic and colitis-associated colon tumors in mice(21). The pro-tumorigenic activity of DMT1 is related to its iron transport activity: a high iron diet increased tumor burden in

mice, and this effect was attenuated following DMT1 disruption. Effects of DMT1 are linked to its ability to stimulate JAK-STAT3 signaling(21) (see Iron and Signaling section).

CD44 is a transmembrane glycoprotein and cancer stem cell marker(22). A recent publication suggests that CD44 can function to transport iron-containing hyaluronates in breast and other cancer cells, and that this pathway of iron import may be particularly important in the mesenchymal phase of the EMT transition, when epithelial cells assume the more migratory and invasive phenotype that contributes to metastatic spread(23). Iron taken up by this CD44-dependent pathway sustains EMT by supporting the catalytic activity of PHF8, an iron-dependent histone demethylase that promotes demethylation of H3K9me2, a repressive histone mark(24). The implication that alternative pathways of iron uptake, such as that mediated by CD44, may acquire increased importance in mesenchymal cells is in accord with previous work showing that CD133 (prominin-1), a mesenchymal stem cell marker, downregulates TFR1-mediated endocytosis of diferric transferrin(25).

Heme uptake.

Heme iron refers to iron coordinated within a porphyrin ring, as found in hemoglobin. Because red meat serves as the principal dietary source of heme iron in humans and intake of red meat is associated with increased cancer risk(26), a role for heme iron in malignant processes has been suggested. Pathways of heme iron uptake are upregulated in cancer, as are pathways of heme export and heme catabolism via heme oxygenase 1(27), suggesting that an increased flux of heme is important to cancer cell growth or survival(28). However, identification of critical role(s) that heme iron plays in cancer cell metabolism has remained elusive: maintenance of the function of enzymes involved in histone demethylation, microRNA processing, or a role in cataplerosis have all been proposed (28).

NTBI.

Non-transferrin-bound iron (NTBI) refers to iron present in the circulation that exceeds the binding capacity of transferrin. Although NTBI is generally not present in cancer patients, NTBI is taken up by transporters that also transport other metals (e.g. zinc, calcium) such as ZIP14, L-type and T-type calcium channels, DMT1, ZIP8, and TRPC6(29), some of which are upregulated in cancer(21) or suppressed by mutated p53(30).

Iron Redistribution.—Ferritin is an intracellular iron storage protein that can store up to 4500 atoms of iron in a non-toxic but bioavailable form(31). Ferritin is regulated post-transcriptionally by iron, but can also be regulated transcriptionally by both oxidant and oncogenic stimuli (32–36). The discovery of NCOA4 (nuclear receptor coactivator 4) as a cargo receptor for ferritin uncovered a mechanism by which iron can be dynamically reallocated among intracellular compartments(37,38). NCOA4 mediates delivery of ferritin to the lysosome for degradation and subsequent release of ferritin-bound iron to the cytosol and mitochondria. This process, termed “ferritinophagy”, is regulated by iron through HERC2, a ubiquitin ligase that targets NCOA4 for degradation under iron-replete conditions (when the iron liberated by ferritin degradation is presumably not needed)(39).

Ferritinophagy and the consequent release of iron supports heme synthesis during erythropoesis(40), and also has important consequences in non-hematopoietic cells, including cancer cells. For example, interference with ferritin degradation by knockdown of NCOA4 inhibits ferroptosis, a death pathway dependent on iron, and overexpression of NCOA4 sensitizes pancreatic cancer cells to ferroptosis(41). Intriguingly, an oncogenic NCOA4-RET fusion protein has been identified in a small fraction of thyroid, breast and other cancers(42). Although NCOA4-RET is not predicted to retain the ferritin binding domain of the intact NCOA4 protein, cells expressing this fusion protein are sensitive to sorafinib(43), a multikinase inhibitor that induces ferroptosis. The effect of NCOA4-RET on iron metabolism may merit investigation.

Mitochondria are essential to intracellular iron metabolism, serving both as a site of FeS cluster biogenesis and heme synthesis. Mitochondrial iron accumulation has also been linked to cancer. For example, the NEET proteins mitoNEET (*CISD1*) and NAF-1 (*CISD2*) are Fe-S cluster-containing proteins located in the mitochondrial outer membrane that modulate mitochondrial iron homeostasis and oxidative stress; upregulation of NEET proteins have been implicated in breast and other cancers(44). PINK1 and PRKN, proteins that play a role in mitophagy, suppress *KRAS*-driven pancreatic tumor growth by inhibiting the mitochondrial iron importers mitoferrin-1 (*SLC25A37*) and mitoferrin-1 (S*LC25A28*) and attenuating mitochondrial iron accumulation(45,46). Inhibition of PINK1 and PRKN results in mitochondrial iron overload, chronic inflammation, and tumorigenesis in a mouse model of spontaneous pancreatic cancer. The iron chelator deferiprone reduced these pancreatic tumors, suggesting that mitochondrial iron homeostasis may constitute a therapeutic target.

Intracellular iron regulation.—IRP1 and IRP2 are a post-transcriptional regulators of intracellular iron metabolism that bind to IREs located in mRNAs encoding several critical proteins of iron metabolism (reviewed in (47)). Binding of IRP1 or IRP2 represses ferritin and the iron efflux pump ferroportin, and stabilizes TFR1, thus acting to increase intracellular iron. IRP2 is regulated by FBXL5, a ubiquitin ligase that degrades IRP2 under iron replete conditions. Inappropriate activation of IRP2 has been linked to cancer(48–50), perhaps in part by activating MDM2 (51) or suppressing TAp63(52). Dysregulation of FBXL5 is also associated with poor prognosis in human hepatocellular carcinoma (53). Further, in the first genetically engineered model of liver cancer promoted by iron overload (53), tissue-specific FBXL5 ablation in mouse liver was shown to result in iron overload, oxidative stress and inflammation, and promote tumor formation. Unexpectedly, the anti-cancer drug cisplatin may also act in part by disrupting IRP2-mediated iron regulation: recent work showed that cisplatin binds directly to cys512 and cys516 in human IRP2, disrupting IRP2 function and depleting the LIP(54). Accordingly, combining cisplatin with the iron chelator desferoxamine (DFO) led to enhanced iron depletion and reduced tumor growth in a mouse xenograft model of colon adenocarcinoma(54).

Iron efflux.—In 2010, our laboratory observed that ferroportin (FPN), a cellular iron efflux pump, is downregulated in breast cancer(55). We subsequently reported similar results in prostate(56) and ovarian(57) cancer. Overexpression of ferroportin reduced intracellular iron

and slowed growth of cancer cells *in vitro* and *in vivo*. Ferroportin expression predicted outcome for women with breast cancer(55,57), implicating intracellular iron as a major driver of cancer cell growth, and suggesting that perturbation of this efflux pathway is sufficient to drive the biological behavior of cancers. Evaluation of additional iron regulatory genes revealed that gene combinations that minimized intracellular iron content conferred favorable prognosis regardless of whether they reduced iron import [anti-import TFR1(Low)/HFE(High)]; or increased iron export [SLC40A1 (ferroportin)(High)/ HAMP(Low)] (58). Others have confirmed and expanded these results in other malignancies, including lung cancer(59), multiple myeloma(60) adrenocortical carcinoma(61), and a subset of patients with AML(62). Highlighting the crucial importance of iron to malignant processes, an iron-regulatory gene signature (IRGS) composed of 16 “iron” genes strongly predicted clinical outcome in women with breast cancer(58).

An additional mechanism of enhanced iron retention through downregulation of iron efflux was recently described(63). G9a, a H3K9 methyltransferase, is overexpressed in breast cancer and correlated with poor patient survival. Hephaestin, a ferroxidase involved in basolateral intestinal iron transport that facilitates iron egress from cells, was identified as an important target that is repressed by G9a. Blockade of iron efflux through silencing of hephaestin in cancer cells may thus represent an additional mechanism of iron retention.

Cells may also control intracellular iron by eliminating ferritin and its associated iron by secretion into the bloodstream (64,65). Additionally, breast cancer cells can activate a MVB-mediated pathway of ferritin secretion following detachment from the cell surface; this pathway is dependent on prominin-2(66). Ferritin released through this or other pathways may subsequently be taken by binding to TFR1 or other receptors(67–71).

Iron Addiction

Observations of enhanced iron uptake, redistribution and retention in cancer cells suggest that cancers are addicted to iron. What metabolic needs underpin this addiction? The answers are evolving, complex, and multifactorial. At a minimum, iron is a critical element in the proliferation, cell cycle control and genomic integrity of cancer cells.

Cellular proliferation:

Iron is essential for the catalytic function of ribonucleotide reductase, the enzyme that converts ribonucleotides to deoxyribonucleotides, the rate-limiting step in DNA synthesis and an obligate step in cell replication. Ribonucleotide reductase depends on a tyrosyl radical for ribonucleotide reduction that in turn requires a di ferric center coordinated with oxygen (Fe^{3+}-O-Fe^{3+}) for activity. Ribonucleotide reductase is highly regulated, and an imbalance in dNTP pools leads to increased DNA mutation rates, genome instability and replication anomalies. Iron chelators are a major class of ribonucleotide reductase inhibitors.

Cell cycle entry and progression:

Iron regulatory proteins (IRP1 and IRP2) are master regulators of intracellular iron. Both IRP2 knockdown (which results in iron depletion) and iron chelation induce the cell cycle regulators p15, p21 and p27, leading to cell cycle arrest with accumulation of cells in

G0/G1(50). Mechanisms of p21 induction are still incompletely elucidated: in melanoma cells, SP1 and its interacting partners ER-α and c-Jun were implicated(72), whereas in prostate cancer cells, p53 and KLF6 appear to play a major role in iron-dependent regulation of p21(73). Through these effects on the cell cycle, iron directly impacts cell proliferation.

Genomic integrity:

Iron, in the form of iron-sulfur clusters, is required for the activity of virtually all replicative DNA polymerases as well as proteins involved in genome maintenance and repair(74). For example, iron-sulfur clusters are critical to the function of helicases such as XPD and FancJ, glycosylases involved in DNA repair, the primases Chir1 and DNA2 that are involved in DNA replication, RTEL1 that is involved in homologous recombination, and others(74). These pathways are used to initiate and maintain a highly replicative state.

For these and other reasons (for example, iron dysregulation also contributes to the activation of metastatic pathways [see Iron and Metastases section]), at least some cancer cells develop a dependence on iron that exceeds that of their non-malignant counterparts, a phenomenon we have termed “iron addiction”. As a result, these cancer cells are more sensitive to the anti-proliferative effects of a range of chemically distinct iron chelators, including desferoxamine, tachpyridine, the di-2-pyridylketone class of thiosemicarbazones (e.g. Dp44mT and DpC), than non-cancer cells(75,76). Similarly, forced expression of ferroportin or treatment with anti-hepcidin antibodies, which reduce intracellular iron by enhancing its efflux , preferentially reduces the rate of proliferation of breast, prostate and ovarian cancer cells(56,57,73) .

Iron and Metastases

With a few notable exceptions, most patients who die from cancer do so as the result of distant metastases rather than the growth of the primary tumor. Recent evidence has begun to link cancer cell iron to the ability of cancer cells to metastasize, although mechanisms are still uncertain. The observation that expression of ferroportin and TFR1 predict survival of breast cancer patients(58) and that disrupted regulation of these pathways is associated with poorer survival of patients with prostate cancer(56) implies a role for iron in human metastasis, since mortality from these cancers is primarily due to metastatic disease. Mouse models demonstrating that iron depletion inhibits metastases also support a role for iron in tumor dissemination. For example, treatment with the iron chelator Dp44mT reduced bone metastasis of human metastatic breast cancer cells (MDA-MB231-BoM) in intracardially injected mice(77). Dp44mT also inhibited the formation of lung tumors in a metastatic model created by injection of osteosarcoma cells in nude mice(78), and augmented the efficacy of a nanoparticle-delivered cisplatin prodrug in reducing mammary metastasis(79). The iron chelator desferiorox inhibited invasion of pancreatic cancer cells(80) and treatment with the iron chelator DFO inhibited cancer cell growth in the leptomeningeal metastatic site(16). In studies using induction of ferroportin rather than a chelator to deplete iron, our group observed a decrease in the number of tumors in a mouse model of ovarian cancer metastasis(57). In this model, ovarian cancer cells were injected directly into the peritoneum (a major site of ovarian cancer metastases); a reduction in both tumor number and mass was

observed with tumor cells that overexpressed ferroportin. Similarly, ferroportin overexpression impeded not only tumor growth but EMT and the metastasis of 4T1 breast cancer cells to lung and liver in mice(81). Supporting these findings, global knockout of hepcidin, a regulator of ferroportin that hinders iron egress by promoting ferroportin degradation, decreased tumor formation and increased survival following tail vein injection of Lewis lung cancer cells in mice(81).

Effects of iron deprivation on metastasis have been linked to the upregulation of the metastasis suppressor NDRG1 as well as STAT3 signaling. NDRG1 is a metastasis suppressor that is downregulated in multiple cancer types(77). NDRG1 is induced transcriptionally following treatment with iron chelators(82). In ovarian cancer, STAT3 signaling was an important link between changes in cellular iron status and cancer: a FPN-mediated decrease in ovarian peritoneal tumors was associated with a decrease in invasion of ovarian cancer stem cells mediated by a decrease in IL6 and phosphorylated STAT3(57).

Iron and Molecular Signaling Pathways

Iron both regulates and is regulated by oncogenic and tumor suppressor signaling pathways.

Iron-regulated signaling.

WNT: One of the strongest epidemiological associations between high dietary iron and cancer is in colorectal carcinoma (CRC)(26,83). The expression of multiple iron metabolism genes is altered in CRC leading to an accumulation of intracellular iron(20,84). Several studies have implicated Wnt signaling in this process. For example, iron augments Wnt signaling in cells with aberrant APC (a tumor suppressor frequently inactivated in CRC(20,84)). Further, high throughput screening for Wnt inhibitors identified iron chelators as top hits (85,86).

STAT3.—Genetic or pharmacological inhibition of the iron importer DMT1 reduced sporadic and colitis-associated colon tumors in mice(21). Unexpectedly, STAT3 rather than Wnt appeared critical to this process. Elevated levels of iron or DMT1 activated STAT3, and inhibition of this pathway reduced tumor burden, implying a critical role for activation of DMT1-mediated JAK/STAT signaling in iron-mediated enhancement of colonic tumorigenesis. Mechanistic studies revealed that iron binds directly to CDK1 kinase and potentiates STAT3 activity(21). A study in lung cancer similarly implicated iron-dependent activation of CDK1 and STAT3 signaling(87). The relative contributions of Wnt and STAT3 to iron signaling may depend on tissue type and requires further evaluation. Since hepcidin is downstream of the IL6/JAK/STAT3 pathway, it will be of interest to determine whether DMT1-mediated activation of STAT3 also contributes to iron retention in tumor cells by upregulating hepcidin.

EGFR.—EGFR binds to and regulates the cell surface distribution of TFR1 in lung adenocarcinoma cells and thereby influences iron import, illustrating an additional mechanism by which cancer cells may increase iron uptake(88) and linking EGFR to iron proteins in cancer.

NDRG1, a metastasis suppressor, is upregulated by iron chelation, implying a role for iron in regulating NDRG1. NDRG1 acts by multiple mechanisms(89), including attenuating signaling of EGFR family members by inhibiting the formation of HER2/EGFR and HER2/HER3 heterodimers and by stimulating degradation of EGFR(90).

ERK1/2 and Akt.—In head and neck carcinoma, iron stimulates ERK1/2 and AKT pathways, activates AP1, and increases expression of the metalloprotease MMP9(91), potentially contributing to invasion.

HIF1α and HIF2α.—Iron exerts a major influence on signaling in cancer cells by regulating the activity of HIF1α and HIF2α. These transcription factors are frequently upregulated in cancer and are associated with poor prognosis(92). Both HIF1α and HIF2α can complex with constitutively active HIF1β to transcriptionally induce many genes important to survival of tumor cells in a hypoxic environment, including *VEGFA*, *EPO, GLUT1* and others. Levels of the alpha subunits of HIF1 are post-translationally controlled by prolyl hydroxylases, which are iron-, 2-oxoglutarate- and oxygen-dependent enzymes that modify HIF1 and target it for proteasomal degradation. Among other activities, HIF1 induces genes that increase iron uptake and intracellular iron release, notably *TFRC*, *HO1* and ceruloplasmin(93–95). Thus, HIF1α and HIF2α, which are held in check by iron in normoxic cells, may be co-opted to increase iron availability in the hypoxic tumor environment. Further, studies in IRP1 knockout mice showed that HIF2α is repressed by IRP1, linking iron regulation to oxygen sensing, and suggesting that IRP1 may be a target for controlling HIF2 activity(96).

Ferritin.—Best known for its role as an intracellular iron storage protein, ferritin is also secreted into the serum, where it may have signaling activity(64,65,97). In hepatic stellate cells, exogenous ferritin was observed to induce NFKB (98), a transcription factor that induces multiple tumor-promoting cytokines including IL6. These observations are consistent with the association between elevated ferritin and poor outcome observed in a number of cancers, including AML (99).

Regulation of iron by oncogenic and tumor suppressive signaling pathways.

c-myc.—The earliest evidence of an association between oncogenic pathways and iron was the observation that adenoviral early gene product, E1A, a viral oncogene with immortalizing properties similar to those of the oncogene *c-myc*, represses ferritin at the level of transcription(35). Subsequently, it was shown in EBV-immortalized B cells that *c-myc* also represses transcription of ferritin and in addition transcriptionally induces IRP2 (100); induction of IRP2 further decreased ferritin and increased transferrin receptor post-transcriptionally. These effects of *c-myc* increased intracellular iron and were required for cell transformation by *c-myc;* IRP2 was later demonstrated to exert pro-tumorigenic activity through mechanisms in addition to its role in iron metabolism(48). *C-myc* was further shown to activate *TFRC* to enhance proliferation and tumorigenesis in *in vitro* and *in vivo* models of B cell lymphoma(101).

p53.—Iron influences the activity of the p53 tumor suppressor. Iron in the form of heme directly binds to p53, stimulating its degradation and interfering with its interaction with DNA, thus reducing the anti-tumor effects of p53(102,103). However, other iron regulatory pathways appear to have opposite effects on p53, suggesting that the final p53 status of cancer cells is influenced by multiple iron-regulatory interactions. Thus, supplementation of cell cultures with exogenous inorganic iron reduces levels of MDM2, the ubiquitin ligase responsible for p53 degradation(51,104), which would be expected to increase levels of p53, and in fact did so in one report(104). The iron-mediated decrease in MDM2 was shown to occur by an IRP2-dependent mechanism, in which stabilization of MDM2 mRNA by IRP2 binding to its 3' UTR was disrupted by excess iron(51). Whether iron increases or decreases p53 may depend on the form of iron (inorganic iron versus heme), the relative rates of these apparently competing pathways, or the particular cell types involved.

There is also evidence that p53 in turn regulates iron: p53 translationally increases ferritin, reducing the availability of intracellular iron during p53-mediated cell cycle arrest(105). The effects of p53 on ferritin may occur through direct transcriptional induction of ISCU2 (iron sulfur cluster assembly enzyme) by p53 (106). Upregulation of ISCU2 favors the formation of iron-sulfur clusters in IRP1, diminishing the activity of IRP1 as an RNA binding molecule (i.e. reducing its activity as a translational repressor of ferritin)(106).

NRF2.—Iron signaling in cancer is also influenced by NRF2, a transcription factor that drives the chemopreventive response but also exhibits paradoxical oncogenic activity(107,108). NRF2 is upregulated and associated with poor prognosis in several cancers, including non-small cell carcinoma, papillary renal cell carcinoma, papillary thyroid cancer, esophageal and serous ovarian cancer (109). Among its targets, NRF2 induces HMOX1 (heme oxygenase 1), an enzyme that catabolizes heme into carbon monoxide, biliverdin and ferrous iron. NRF2 also reduces sensitivity to ferroptosis by transcriptionally inducing ferritin(108), which sequesters intracellular iron and limits its ability to catalyze formation of lipid radicals.

H-RAS.—Early studies demonstrated that cell cycling and proliferation in cells expressing oncogenic H-RAS was dependent on labile iron and could be increased by diverting iron from intracellular iron storage in ferritin to the labile iron pool(110,111). Highlighting the exquisite dependence of cancer cells on maintaining iron balance, excess iron was later shown to induce cell death in ovarian cancer cells expressing mutant H-ras, at least in part via a MAPK signaling pathway(112). Parallels between effects of iron and those of oncogenic H-RAS have also been suggested by the observation that in combination with MYC, H-RAS induced genetic changes in ovarian cancer precursor cells similar to those induced by chronic iron exposure, including increased expression of EVI1 (ecotropic virus integration site 1), a transcription regulator located at 3q26.2, a locus frequently amplified in ovarian cancer(113). These intriguing but limited results require further mechanistic study.

Iron and cancer stem cells

Tumor-initiating cells (often termed cancer stem cells (CSCs)), constitute a small but biologically important fraction of tumor cells. Their properties include unlimited self-

renewal, the ability to differentiate into cells that constitute the bulk of the tumor, mesenchymal characteristics, drug- and radiation-resistance, and the ability to seed metastases(114). Evidence from a variety of cancer types suggests that addiction to iron may be particularly evident in cancer stem cells. It should be noted that many studies use cells grown as spheroids (tumorspheres) as a surrogate for cancer stem cells due to technical challenges in isolating and propagating cancer stem cells; conclusions drawn may reflect the limitations of this experimental model.

Tumor-initiating cells and iron have been linked in many cancer types. RNA sequencing and enhancer mapping revealed that gliobastoma stem-like cells upregulate transferrin (TF), and preferentially require TFR1 and ferritin to form tumors in vivo(115). In these cells, increased TF-mediated influx of iron upregulated ferritin and activated STAT3 and FOXM1 signaling, fostering cell cycle progression. Similarly, proteomic profiling of CSCs derived from tumors of *HER2/neu* transgenic mice identified H-ferritin as upregulated in CSCs compared to non-cancer stem cells(116). In contrast, in SKOV3 ovarian cancer cells, silencing of H-ferritin promoted EMT and spheroid formation(117). Silencing H-ferritin similarly promoted EMT in breast cancer stem cells, possibly due to increased ROS (expected due to the decreased iron sequestration and increased labile iron in cells with lowered ferritin), or to mechanisms unrelated to the function of ferritin in iron storage, such as control of miRNA profile(118).

Cholangiocarcinoma stem-like cells similarly exhibited dysregulated iron metabolism, with a profile of iron retention possibly tied to low expression of ferroportin(119). Accordingly, expression of CSC markers was reduced by iron chelation. Iron was also found to induce CSC in human lung cancer cells by an unexpected mechanism involving induction of the transcription factor SOX9(120). In concert with SOX2, SOX9 is a key participant in orchestrating and maintaining the breast cancer stem cell state(121).

A consistent theme in studies of iron and cancer stem cells has been the sensitivity of CSCs to iron deprivation induced either by exposure to iron chelators or by ferroportin overexpression. The sensitivity of cancer stem cells to iron chelation has been observed in virtually every instance that it has been studied. For example our laboratory observed that a genetic model of ovarian cancer stem cells exhibited an iron-retention profile, with upregulation of TfR and downregulation of FPN relative to non-cancer stem cells(57). These cells exhibited enhanced sensitivity to iron chelation. In a CSC model derived from induced pluripotent stem cells, iron chelation suppressed proliferation and decreased expression of stemness markers such as NANOG and SOX2 *in vitro* and *in vivo*(122). Other examples exist(119,123,124).

Breast cancer tumor initiating cells exhibit increased labile iron; along with prostate cancer tumor initiating cells they also exhibit a unique iron gene expression signature that distinguishes them from bulk tumor cells(125). Of the 10 genes identified in this signature, 4 (*TFRC, CYBRD1, EPAS1, HFE*) overlapped with the 16 member IRGS (iron regulatory gene signature) that predicts prognosis in breast cancer patients(58). Although specific genes in these two signatures are not identical, they exhibit substantial functional overlap, with roles in iron uptake, Fe-S cluster formation, and the hypoxic response(125), suggesting

parallels in the iron dependencies of bulk cancer cells and the tumor initiating cell population.

Three-dimensional tumor models and the tumor microenvironment.

It is now commonly accepted that the tumor microenvironment affects the behavior of cancer cells. The tumor microenvironment includes the extracellular matrix (ECM) as well as non-tumor cells, including immune cells (such as macrophages and T cells), fibroblasts, endothelial cells, and others(126). These cells not only supply iron to tumor cells, but depend on iron to maintain extracellular matrix stiffness, as well as influence macrophage polarization and T-cell promoted tumor cell death (Figure 1).

Iron and immune cells in the tumor microenvironment.

Monocytes can be polarized into classically activated, proinflammatory (M1) macrophages and alternatively activated (M2) macrophages, although this dichotomy is likely an oversimplification. The M1 macrophage phenotype is characterized by the production of high levels of pro-inflammatory cytokines, production of reactive nitrogen and oxygen intermediates, and promotion of Th1 responses. In contrast, M2 macrophages are characterized by efficient phagocytic activity and a role in tumor promotion(127). However, macrophage differentiation is plastic, and tumor-associated macrophage switching to an M1 phenotype has been proposed as an anti-cancer strategy(127).

M1 and M2 macrophages differ in their iron phenotype: M2 exhibit an “iron donor” phenotype with enhanced expression of the iron efflux pump ferroportin, and the ability to deliver iron to cancer cells(128,129). The *in vivo* relevance of this finding is supported by the analysis of human samples that demonstrate high levels of both ferroportin and iron in macrophages in breast tumor tissue(130). Iron-laden tumor-associated macrophages (TAMs) were observed in human breast cancer samples using FeMRI; depletion of these iron-laden cells was associated with tumor reduction following treatment with anti-colony stimulating factor 1 receptor (CSF1R) in murine tumor models, supporting the concept that macrophage iron can play a tumor supportive role(131).

Delivery of iron to tumor cells may be accomplished by ferroportin-mediated efflux from macrophages followed by TFR1-dependent uptake by tumor cells. Alternatively or additionally, lipocalin-2 secreted by macrophages may serve to deliver iron to breast and other cancer cells(132). Ferritin, an iron-containing protein secreted by macrophages(64), may also serve as a mechanism of iron delivery, as noted previously. Further, because ferritin inhibits cleavage of kininogen to HKa, an endogenous inhibitor of angiogenesis, ferritin secretion may also affect endothelial cells and promote angiogenesis in the tumor microenvironment(133,134).

Iron itself may modulate the interplay between immune and cancer cells in the tumor microenvironment by influencing macrophage polarization. For example, a high iron diet promoted M2 macrophage polarization in vivo and dampened the inflammatory response(135). However, opposite results were observed in a mouse macrophage cell line, where iron induced polarization to a proinflammatory (M1) phenotype by increasing

ROS(136). In line with these observations, tumor-associated macrophages (which have an M2-like phenotype) exposed to hemolytic RBCs or iron-containing nanoparticles were converted to pro-inflammatory macrophages capable of killing lung cancer cells in vitro and in vivo(137). The shift to an M1 phenotype was frequently observed in hemorrhagic areas within tumors. Thus, macrophage polarization and consequent effects on tumor behavior may vary within subdomains of the tumor.

In addition to providing tumor cells with iron, immune cells in the tumor microenvironment may also modulate susceptibility to ferroptosis, an iron dependent form of cell death (see Iron and Ferroptosis section). CD8+ tumor-killing T cells activated by treatment with PD-L1, an immune checkpoint inhibitor, were observed to drive ferroptosis of ovarian or melanoma cancer cells by secreting interferon gamma, which downregulates SLC7A11, a component of an anti-ferroptotic pathway(138).

Iron and fibroblasts in the tumor microenvironment.

Our work with breast cancer spheroids demonstrated that fibroblasts up-regulate synthesis of hepcidin in breast cancer cells by secreting IL6(139). Since hepcidin triggers ferroportin degradation, this pathway increases intracellular iron in tumor cells.

Fibroblasts present in the tumor microenvironment also produce a collagenous matrix that stiffens the tumor stroma and creates a hypoxic environment that activates tumor cell invasion(140). Iron-dependent enzymes play a critical role in this process: matrix stiffening is mediated by collagen crosslinking, a process driven by lysyl hydroxylase LH2, an Fe^{2+}- and 2-oxoglutarate-dependent oxygenase. Fe^{2+} is required not only for the catalytic activity but for the stability of prometastaic collagen lysyl hydroxylase(141).

Iron and oxidative damage

The ability of iron to redox cycle between Fe^{2+} and Fe^{3+} oxidation states is central to its function in catalyzing biological reactions(142). In this regard iron differs from most biologically important transition metals except copper (e.g. zinc, magnesium, manganese), which do not redox cycle under physiological conditions. However the redox property of iron also underlies its mutagenic potential, since it enables iron to participate in the Fenton reaction, in which iron transfers an electron to hydrogen peroxide to generate highly reactive radical species (the hydroxyl radical $OH^{\cdot}$, and/or FeO^{2+} (143)). These radical species can directly damage DNA by producing oxidatively modified bases, abasic sites, and DNA strand breaks. Aberrant repair of such lesions leads to mutations. The ability of iron to directly damage DNA establishes a direct link between iron and cancer, a disease characterized by the accumulation of oncogenic and tumor suppressor mutations. Iron can also oxidatively damage lipids, a property whose significance in cancer has grown with the discovery of ferroptosis.

Iron and Ferroptosis

In 2012, Stockwell and colleagues coined the term ferroptosis to describe an iron-dependent form of cell death characterized by the accumulation of oxidized lipids in membranes(144–

146). Ferroptosis was so named due to its iron dependence: iron chelators inhibit ferroptosis, and high levels of iron promote ferroptosis(144,147). (Iron has been implicated in other forms of cell death such as apoptosis and necrosis through its participation in the formation of ROS(148); however, iron is not an essential constituent of the mechanism of these cell death pathways, as it is in ferroptosis). Ferroptosis is fundamentally different from other regulated cell death pathways such as apoptosis, necrosis, autophagy, and pyroptosis (149), and is most similar to oxytosis, a form of cell death largely studied in neuronal cells that is characterized by the accumulation of reactive oxygen species (ROS)(150).

Because an essential feature of ferroptosis is the accumulation of peroxidized membrane phospholipids, small molecules that inactivate glutathione peroxidase 4 (GPX4), an enzyme that detoxifies oxidized lipids, also induce ferroptosis(151); changes in membrane lipid composition similarly modulate sensitivity ferroptosis(152,153). In addition to candidate small molecule drugs, approved chemotherapeutic drugs, such as cisplatin and sorafinib, induce ferroptosis as part of their mechanism of action(154). Notably, cancer stem cells appear particularly vulnerable to ferroptosis, at least in part due to the activity of YAP, which antagonizes Hippo signaling to upregulate expression of ferroptosis modulators such as TFR1 and ACSL4 (155). These observations have stimulated an ongoing search for ferroptosis inducers for cancer treatment.

Although the precise role of mitochondria in ferroptosis remains unclear, alterations in mitochondrial morphology and membrane potential are observed in ferroptosis, and iron management in this organelle may play a role in ferroptosis. For example, recent work suggests that NEET proteins may contribute to protection from ferroptotic cell death: mitoferrin-1 and mitoferrin-2 reduced mitochondrial lipid peroxidation and ferroptosis in cultured hepatocellular carcinoma cells(156) and head and neck cancer cells(157). Conversely, inhibition of NEET proteins sensitized cells to ferroptosis by increasing lipid ROS and disrupting mitochondrial iron. Similarly, mitochondrial ferritin, which inhibits tumor growth by sequestering mitochondrial iron and causing cytosolic iron depletion(158), protects against ferroptosis in cultured neuroblastoma cells(159).

The discovery of ferroptosis as an iron-dependent form of cell death coupled with its potential role in cancer therapy have led to two critical questions: (1) what is the role of iron in ferroptosis? (2) given that non-cancer cells also contain iron, can ferroptosis inducers be selectively cytotoxic to cancer cells?

The role of iron in ferroptosis.

Does the ability of iron to redox cycle in Fenton chemistry explain the contribution of iron to ferroptosis? Since the redox activity of iron renders it capable of oxidizing lipids, it is reasonable to posit that non-specific iron-catalyzed lipid peroxidation through Fenton chemistry may underlie the dependence of ferroptosis on iron. Although this presumption is widely supported by the literature, the picture may be more nuanced.

The critical role of lipid peroxides in precipitating ferroptosis is documented by multiple independent lines of evidence(153,160–162). Current debate focuses on whether ferroptosis is attributable to a specific lipid (in particular hydroperoxy derivatives of arachidonoyl

phosphatidyl ethanolamine (HOO-AA-PE) or adrenoyl phosphatidylethanolamine), or rather reflects the accumulation of numerous different oxidized lipid species (see (160,163) for review). As recently noted(160), these possibilities imply two different roles for iron. If a specific lipid is key, then the major role of iron may be as a cofactor in the enzyme(s) that produces it. There is evidence for this, since LOX15 (product of the *ALOX15* gene), a lipoxygenase implicated in ferroptosis that catalyzes the peroxidation of arachidonic acid(164) contains a non-heme mono iron center that is required for its enzymatic activity(165). In addition, fatty acid desaturases, which contribute to the formation of readily oxidizable PUFA-containing lipids, contain iron in a diiron center(166). On the other hand, genetic studies demonstrating the inability of *ALOX15* knockout to protect mice from ferroptosis suggests that LOX15 is not absolutely required for ferroptosis(167). Further, the demonstration of multiple oxidized lipid species in cells undergoing ferroptosis(161) suggests a less specific mechanism – i.e., iron-catalyzed Fenton reaction-mediated lipid oxidation. To date, technical challenges in characterizing and quantifying temporal changes in oxidized lipid species have limited the ability to distinguish between these mechanisms, and it remains possible, and perhaps likely, that both are involved.

What intracellular source of iron is involved in ferroptosis? Cellular redox active iron -- generally referred to as the labile iron pool (LIP) -- is a small, metabolically available fraction of total intracellular iron that is the likely form of iron for pro-ferroptotic processes, either directly or indirectly. LIP is defined functionally by its ability to be bound by permeable low molecular weight iron chelators(168). The LIP is believed to exist in a Fe^{2+} oxidation state that is coordinated by small intracellular ligands (possibly glutathione(169,170), although debate as to the nature of the intracellular ligand(s) that coordinate “free” iron has raged for decades). As such, the LIP can participate directly in the generation of oxygen and lipid radicals through Fenton chemistry. Iron in the LIP is also available to PCBP1, a chaperone that delivers iron to client proteins such as ferritin, prolyl hydroxylases, and others(171,172). Notably, PCBP1 can transfer iron from the LIP to enzymes containing mono-iron centers such as that found in LOX15(171,172), supporting a connection between this pool of iron and ferroptotic processes.

Iron redistribution into and out of the labile iron pool profoundly affects ferroptosis. Early studies documenting the general dependence of ferroptosis on iron using iron chelators to limit overall iron availability(144) were subsequently supported by experiments that manipulated levels of intracellular iron using genetic rather than pharmacologic methods: overexpression of ferroportin, an iron efflux pump, sensitized ovarian cancer cells to ferroptosis(57), and knockdown of ferroportin accelerated erastin-induced ferroptosis in neuroblastoma(173). More recent work has provided additional mechanistic detail by demonstrating that ferritin repartitions iron among intracellular compartments to modulate iron’s ferroptotic activity. Specifically, liberation of iron from ferritin, an intracellular iron storage protein, is associated with ferroptosis: (1) treatment of breast cancer stem cells with salinomycin or a synthetic derivative ironomycin leads to lysosomal degradation ferritin, liberation of iron and ferroptosis(174); (2) inhibition of NCOA4-mediated ferritin degradation reduces sensitivity of cancer cells to ferroptosis (41); (3) elimination of ferritin from breast cancer cells by the prominin-2 dependent, MVB-mediated, pathway protects

them from ferroptosis(66). Underlining the link between iron and ferroptosis, TFR1 emerged as a candidate marker to identify ferroptotic tissue *in vivo* (175).

Can ferroptosis inducers be selectively cytotoxic to cancer cells?

Ferroptosis is not restricted to cancer cells. For example, ferroptosis has been implicated in the pathophysiology of neurodegenerative diseases and acute brain injury(176). Further, all cells have iron stores and labile iron pools that can potentially contribute to ferroptosis. However, several factors weight the scales towards preferential cytotoxicity of ferroptosis inducers to cancer cells. First, because the levels of labile iron are dependent on iron uptake and efflux and the proteins that regulate these properties are often expressed in cancer cells in a way that increases labile iron, the pool of metabolically available iron is frequently higher in malignant cells than non-malignant cells of the same histologic type. Second, oxidative stress is enhanced in cancer cells relative to their non-malignant counterparts, rendering them less tolerant to the increased oxidative burden imposed by ferroptosis inducers. Third, reduced levels of lipid radical detoxifying enzymes in some cancers augment their susceptibility to ferroptosis-inducing agents(177).

Nevertheless, some caution will be required in deploying ferroptosis inducers for cancer treatment, since increased autophagy, particularly selective autophagy, can contribute to ferroptotic cell death(178). In one report, it was demonstrated that pancreatic cancer cells with mutant KRASs released KRAS in exosomes, and that this release was fostered by ferroptosis(179). Uptake of released exosomes by macrophages promoted macrophage polarization to the M2 phenotype and accelerated tumor growth, suggesting that in selected contexts, induction of ferroptosis may foster rather than inhibit tumor growth. Thus additional research will be required to identify specific genetic characteristics that render tumors potentially susceptible to ferroptosis inducers, as has been suggested more broadly regarding targeting autophagy in cancer(180).

Iron and Cancer Therapeutics

It is rather extraordinary that approaches to target cancer by modulating intracellular iron involve two diametrically opposed strategies: iron depletion, including chelation, on the one hand, and purposeful use of excess iron to generate toxic free radicals on the other. In yet a third strategy, iron transporters are used to deliver cytotoxic payloads preferentially to tumor cells. Below we provide a brief overview and some current examples of these strategies; more detailed information can be found in recent reviews(181,182).

Early studies aimed at targeting iron for cancer therapy took advantage of an existing, clinically approved iron chelator used to treat iron overload, desferoxamine. This highly effective and specific iron chelator is used to treat patients with thalassemia and other conditions of iron overload. In small numbers of patients with hepatocellular carcinoma and leukemia(183,184), modest therapeutic success was attained, encouraging the search for new iron chelators with improved activity. Some of the more successful anti-cancer iron chelators have been thiosemicarbazone derivatives, such as Dp44mT(185). Mechanistic studies revealed that this tridentate chelator not only binds intracellular iron but does so in a way

that permits iron to redox cycle, generating ROS that contribute to tumor cell killing. Dp44mT has entered clinical trials(181).

Gallium salts also represent a variant of an iron depletion strategy for cancer therapeutics. Due to the chemical similarly between iron and gallium, gallium incorporates into proteins and enzymes that use iron as a cofactor(186), inactivating enzymes that require iron for their function, such as ribonucleotide reductase. The anti-cancer activity of gallium maltolate or tris(8-quinolinolato)gallium III (KP46), which may be more effective than the nitrate salt used in the original clinical formulation of gallium, is currently being explored(187).

In a different approach, apolactoferrin, an Fe^{3+}-binding protein with bactericidal properties present in mammalian milk, inhibited the growth of squamous cell carcinoma cell lines in vitro, and inhibited the growth of head and neck tumors following oral administration in mice(188). Whether the therapeutic benefit of lactoferrin depends on its iron-binding activity remains uncertain(188); however, in patients with colorectal cancer receiving chemotherapy, oral lactoferrin improved a number of clinical parameters, although survival benefit was not evaluated(189).

The opposite approach to iron-based cancer therapeutics is to provide excess iron. This supplemental iron, coupled with the high levels of labile iron already present in tumor cells, can produce sufficient oxidative stress to eliminate tumor cells, which are oxidatively stressed at baseline(190). A recent study with ferumoxytol (Feraheme), a clinically approved iron oxide nanoparticle used to treat iron deficiency, suggested that this approach may hold promise in AML. In this study, Feraheme induced oxidative stress and reduced tumor burden in cells from patients and in a murine leukemia model(62,191).

A novel variation on this approach has been to use supra-physiological levels of ascorbate to trigger iron dependent oxidative stress, an approach proposed for patients with non-small cell lung cancer and glioblastoma (192) as well as other malignancies. This approach is currently being pursued in multiple clinical trials(193) (e.g. NCT03508726, NCT02905578, NCT02420314, NCT02344355, NCT02905591, NCT03602235, and NCT03799094 in clinicaltrials.gov).

The use of ferroptosis-inducing agents is based on a similar rationale of leveraging excess iron in cancer therapy. Early evidence suggests that ferroptosis inducers may be effective anti-cancer drugs, and that they target cancer stem cells to reduce not only tumor growth but metastasis. Studies in our laboratory using a model of ovarian cancer metastasis driven by cancer stem cells demonstrated that erastin, a ferroptosis-inducing agent, effectively reduced tumor number and mass in the peritoneal cavity, the major site of metastatic dissemination in ovarian cancer(57). In another example, salinomycin triggered a ferroptotic cell death pathway in cancer stem cells by causing lysosomal degradation of ferritin, the release of ferritin-bound iron, and ensuing liberation of iron into the cytosol(174). Pursuing this observation, inhibitors of DMT1 that preferentially targeted CSCs were developed to block the translocation of iron out of the lysosome, leading to lysosomal iron accumulation, production of ROS, and ferroptotic cell death(194).

Apart from using small molecules to directly target iron, other approaches that use overexpression of TFR1 in cancer cells either as a target or as a portal for the delivery of anti-cancer therapeutics have been explored. For example, chemotherapeutic drugs such as doxorubicin and cisplatin have been conjugated to transferrin to preferentially deliver these drugs to cancers. Further, cellular toxins such as diphtheria toxin and ricin, as well as anti-neoplastic nucleic acids(195), have been linked to transferrin to deliver the toxin preferentially to malignant tissues. Because toxicity to normal tissues was observed in early trials, recent attention has focused on the development of new antibodies that may improve the therapeutic index(196,197). In addition, conjugation to lactoferrin (LF) enhances dendrimer-mediated gene delivery to tumors in mice, suggesting that like TF LF may have a role in cancer targeting(198).

Conclusions, limitations, unanswered questions, and future directions

The last ten years have seen extraordinary developments in understanding the biology of iron homeostasis in normal cells and the pathobiology of iron metabolism in cancer. Nonetheless, some fundamental questions remain. Can iron-dependent enzymes and pathways be prioritized in terms of their necessity for cancer cell growth? Can the level at which iron switches from nutrient to oxidative toxicant be quantified? Is the level different in different cell types? Can the amount of iron required by tumors and normal tissues be quantified, and can this information facilitate the more effective deployment of iron-targeting agents in cancer? Answers to these questions have been hindered in part because metabolically available iron (the labile iron pool) is a small fraction of total cellular iron and remains difficult to quantify. Levels of labile iron in specific intracellular compartments are even more difficult to measure but may be of crucial importance. Developing robust quantitative methods to measure metabolically available iron in the cytosol and in organelles as well as developing organelle-targeted iron chelators will help to address these issues. It also appears that tumors can deploy multiple strategies to extract iron from their environment; thus, tumor profiling may be necessary to identify tumor-specific iron targets. Systems biology approaches also hold substantial promise in identifying critical nodal points in tumor iron metabolism (199–201).

Despite the converging evidence that implicates iron in cancer, it should be remembered that experimental systems have their limits. Notably, individual reports generally focus on one type of cancer; the generality of results across malignancies cannot be assumed without confirmation. Additionally, the specifics of experimental systems can shape results: for example, we observed that mechanisms of hepcidin regulation are different when breast cancer cells are cultured in three dimensional spheroids when compared to two dimensional plates(139). However, in general there has been remarkable concordance between experimental results and predictions derived from databases of human gene expression; such databases have had the added benefit of focusing enquiry in new and often unexpected directions, and will likely continue to serve as important resources for future discovery.

Overall, the fundamental concept that iron is a critical nutrient that is essential for cancer cell survival appears well established. The next years promise not only continued discovery, but the successful leveraging of cancer cell iron addiction into effective cancer therapies.

There are abundant possibilities: bringing ferroptosis-inducing drugs to the clinic, fostering oxidative cytotoxicity in already oxidatively fragile cancer cells, turning the unique iron metabolic properties of cancer stem cells to therapeutic advantage, developing better targeted and more effective anti-cancer iron chelators, or using recent insights into the contributions of the tumor microenvironment to limit the growth and metastatic potential of cancer.

Acknowledgements:

Supported by NCI grants R01 CA233636 (F.M.T), R01 CA188025 (S.V.T).

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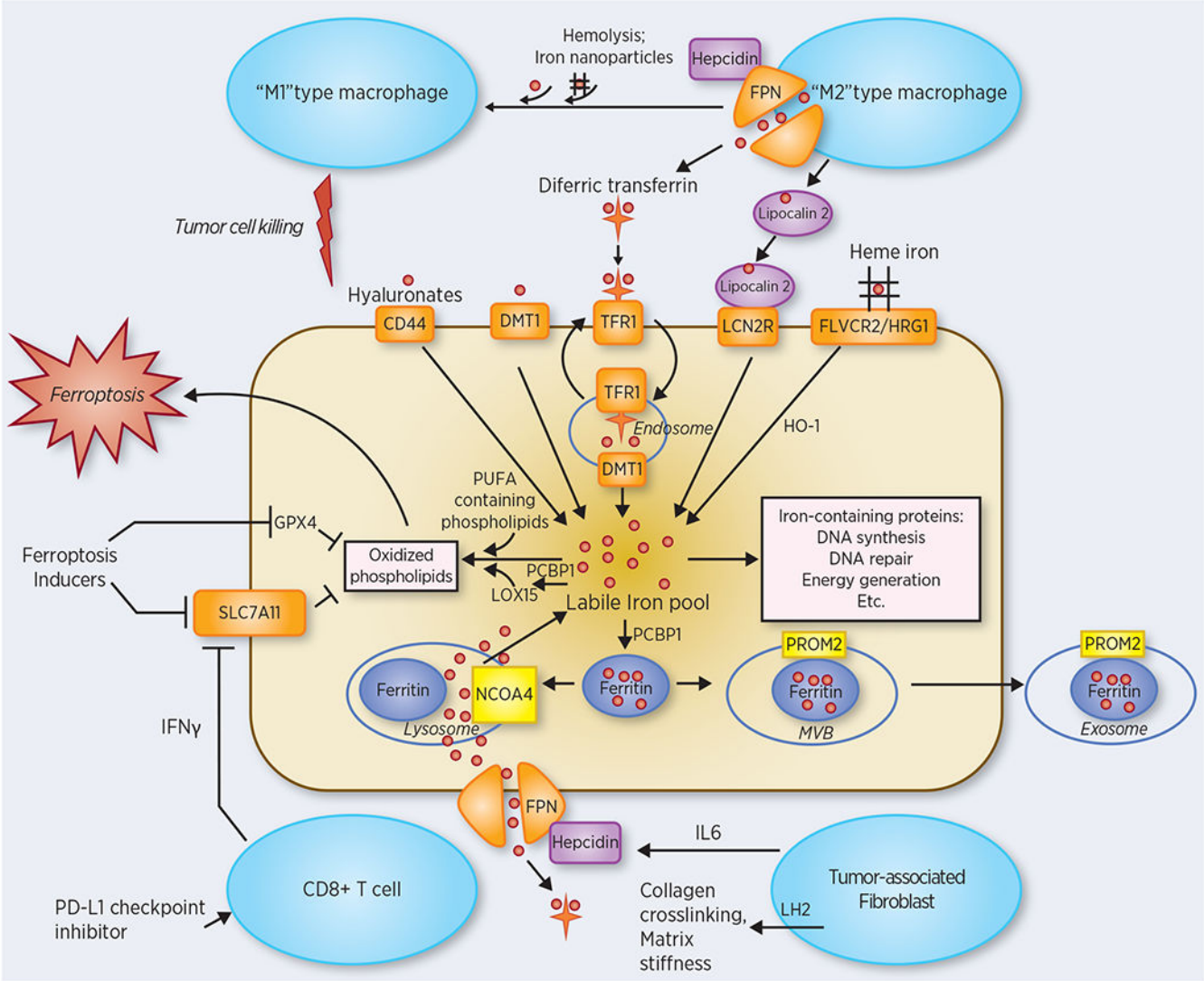

Figure 1. Intracellular and intercellular iron trafficking in the tumor microenvironment.

Intracellular trafficking: Plasma membrane proteins involved in iron uptake are heme transporters FLVCR2 and HRG1; lipocalin 2 receptor LCN2R (also called 24p3R), which binds a lipocalin 2-siderophore-iron complex; DMT1 (which is also involved in endosomal iron efflux), which transports Fe^{2+}; CD44, which binds an iron-hyaluronate complex; and TFR1, which binds diferric transferrin. Iron taken up by these pathways is delivered to a pool of redox-active iron termed the labile iron pool to supply iron to iron-containing proteins. The chaperone PCBP1 delivers iron to ferritin and LOX15, among other targets. Ferritin stores excess iron in a non-toxic bioavailable form. Iron in ferritin can also be redistributed following binding to NCOA4 and lysosomal degradation (ferritinophagy), or ferritin-bound iron can be effluxed from the cell via a multivesicular body (MVB)/exosome pathway. The major route of iron efflux is ferroportin (FPN), which is degraded by the secreted peptide hepcidin. Iron participates in producing the oxidized membrane lipids that mediate ferroptotic cell death. Ferroptosis inducers disable endogenous pathways that reduce oxidized lipids, including the peroxidase GPX4 and the cystine/glutamate antiporter

SLC7A11. ***Intercellular trafficking:*** Pro-tumorigenic effects are mediated by tumor-associated macrophages, which provide iron to tumor cells, as well as by tumor-associated fibroblasts (TAFs). TAFs express the iron-dependent enzyme lysyl hydroxylase 2 (LH2) an enzyme that enhances matrix stiffness; TAFs also secrete IL6, which upregulates hepcidin, degrades ferroportin, and limits iron efflux from tumor cells. Anti-tumorigenic effects are exerted by PD-L1-stimulated CD8+ T cells, which promote ferroptosis by secreting interferon gamma and inhibiting SLC7A11. Excess exogenous iron resulting from hemolysis or provided by iron-containing nanoparticles can trigger a shift of M2-type tumor associated macrophages to a tumoricidal M1 phenotype. Note that this figure consolidates findings from multiple different tumor types; not all pathways are operant in all cancers.