Ironing Out Cancer

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Abstract

New insights into the roles of proteins that regulate cellular iron in cancer growth, angiogenesis, and metastasis have recently emerged. Discoveries of the roles of ferroportin, hepcidin, lipocalin 2, and members of the six transmembrane epithelial antigen of the prostate (STEAP) and iron regulatory protein (IRP) families in cancer have provided specificity and molecular definition to the role of iron homeostasis in cancer growth and metastasis. A number of studies directly support a role of these proteins in modifying bioavailable iron, whereas other studies suggest that at least some of their effects are independent of their role in iron biology. Cancer Res 71(5): 1511–4. ©2011 AACR.

Cancer and iron homeostasis have been occasional bedfellows for some time. However, it is only in the past few years that insights into mechanisms of normal iron regulation have enabled focused interrogation of basic mechanisms, biological rationale, and pathophysiologic implications of changes in iron metabolism in cancer.

Strong evidence exists for the parsimonious regulation and distribution of iron in cells and tissues. In fact, metabolic studies of iron in human volunteers done by Finch and colleagues in the late 1950s remain some of the most elegant human biodistribution studies conducted (1). However, it was not until the recent explosion in the identification of proteins involved in iron regulation (e.g., transporters, reductases, oxidases, regulatory proteins, and an iron efflux pump; ref. 2) that the trafficking of iron in cells and tissues could be understood precisely. Although our knowledge is not complete, we can now begin to account for many previously ill-defined aspects of iron biology, such as the complex shifts in redox state of iron between Fe[II] and Fe[III] as iron is "chaperoned" from the duodenal enterocyte to the plasma and then to various cellular compartments, including endosomes, cytosol, and mitochondria, which require iron for important processes such as hemoglobin synthesis, DNA synthesis, and energy metabolism. These advances were greatly facilitated by mouse and human genetics, as well as an occasional zebrafish (2).

An important intersection in the study of normal iron biology and disease occurred with the discovery of the plasma membrane protein ferroportin and the secreted liver protein hepcidin. Ferroportin is an iron export pump and the only known mechanism for the export of nonheme iron from vertebrate cells. Ferroportin is expressed on enterocytes and macrophages, where it facilitates iron delivery to the plasma. Hepcidin is a 25–amino acid peptide hormone that is a master regulator of systemic iron homeostasis. In conditions of excess iron, hepcidin binds to ferroportin and triggers its degradation, thereby both preventing intestinal iron absorption and restricting iron release from macrophages (3). Hepcidin not only responds to fluctuations of iron as part of normal iron homeostasis, but also to inflammation. Hepcidin is transcriptionally induced in response to inflammatory cytokines such as interleukin-6 (IL-6) as well as bacterial pathogens and lipopolysaccharide. This connection between hepcidin and inflammatory pathways has provided a molecular explanation for the pathophysiology of the anemia of chronic disease, a common and heretofore poorly understood consequence of many common medical conditions, including cancer.

New links between the ferroportin–hepcidin regulatory axis and cancer were recently reported by our group (4). These results begin to provide insight into fundamental ways in which iron is perturbed in cancer and suggest that the cancer cell has subverted this tightly regulated physiologic pathway to accumulate iron. We observed that ferroportin protein levels are decreased in malignant breast cell lines when compared with normal mammary epithelial cells. Two mechanisms were responsible: a decrease in ferroportin mRNA and an increase in hepcidin. Our observation shows that hepcidin is expressed and regulates ferroportin in breast cells and indicates that the regulatory connection between hepcidin and ferroportin, so important in maintenance of body iron homeostasis, also plays a local role in peripheral tissues. Further, these changes in ferroportin and hepcidin have functional consequences to the iron metabolism of cancer cells; they result in an increase in bioavailable iron (the so-called "labile iron pool"). Importantly, reduced ferroportin seems to drive aggressive growth, because reintroduction of ferroportin into breast cancer cells reduced their growth following orthotopic implantation into mice. Nor were these observations confined to experimental systems; ferroportin protein levels were also reduced in malignant tissue from breast cancer patients.

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Perhaps the most compelling relationship between breast cancer and iron metabolism emerged when we examined gene expression profiles from more than 800 breast cancer patients in whom ferroportin and hepcidin mRNA had been measured (4). Unlike the laboratory investigations, in which the end point of the experiments was cell growth, the readout for these women was the propensity to develop metastases. We observed that decreased ferroportin gene expression was associated with a significant reduction in metastasis-free and disease-specific survival, which was independent of other breast cancer risk factors. Particularly striking was that among patients with both high ferroportin and low hepcidin transcript levels, we could identify a group with highly favorable outcome (approximately 90% metastasis-free survival at 10 years). Remarkably, 40% of the patients in this favorable group were lymph-node positive, a group that generally has unfavorable outcome and is traditionally treated with chemotherapy. Thus, ferroportin activity, as approximated in a 2-gene model of ferroportin and hepcidin, may be clinically useful in guiding breast cancer therapy. Further, because mortality in breast cancer is related to metastasis, an additional implication of these results is a connection between iron metabolism and metastasis. How iron relates to metastatic processes or whether ferroportin and hepcidin have additional, non–iron-related functions in cancer is largely unknown and unexplored.

Another link between iron and cancer relates to the six transmembrane epithelial antigen of the prostate (STEAP) family of metalloreductases. This multigene family includes STEAP1, STEAP2, STEAP3, and STEAP4 (Steap 1–4 in the mouse; ref. 5). STEAP1, STEAP2, and STEAP3 are highly expressed in prostate tumors. POSITIONAL cloning identified Steap3 as the gene responsible for iron deficiency anemia in the mouse mutant nm1054; it encodes a ferri- and cupric reductase that is required for efficient delivery of transferrin-iron (6). STEAP family members colocalize with transferrin receptor 1 (TfR1), and ectopic expression of Steap2, Steap3, or Steap4 increases cellular iron uptake, although Steap1 does not (5). It is believed that these transmembrane proteins reduce the ferric iron delivered into the endosome by transferring it to ferrous iron, thus facilitating subsequent exit of iron from the endosome into the cytosol via divalent metal transporter 1 (DMT1; ref. 5). The increased expression of STEAP2 and STEAP3 in cancers may relate to their ability to promote iron assimilation in cancer cells.

Although aggressive acquisition and handling of iron seems to mark at least some cancer cells, outright thievery was only recently identified as another cancer trait; this work also suggests that bacterial and mammalian mechanisms of iron acquisition may be more directly related than previously suspected. Lipocalins are a family of proteins that bind small hydrophobic ligands and are characterized by an 8-stranded antiparallel beta barrel that forms the ligand-binding site. Lipocalin 2 (also termed NGAL, 24p3) is a member of this family that binds bacterial siderophores. Siderophores are organic molecules synthesized and used by microorganisms for iron acquisition. Lipocalin 2 binds Fe(III)-containing enterobactin, the primary siderophore of enteric bacteria, blocking growth of Escherichia coli and thus acting as a potent bacteriostatic agent (7). Two recent reports provide evidence that mammalian cells also produce siderophore-like molecules that circulate bound to lipocalin 2 (8, 9). Because lipocalin 2 can bind to specific cell surface receptors (megalin, 24p3R) and deliver its associated iron, these results suggest that lipocalin 2 may represent a previously unrecognized mechanism of iron acquisition by mammalian cells, including cancer cells. Interestingly, lipocalin 2 can also bind siderophores that are not complexed with iron; cellular uptake of the iron-free siderophore–lipocalin 2 complex induces a state of iron deprivation and can promote apoptosis. Thus, the cellular effect of lipocalin 2 is dependent on whether its associated ligand contains iron or is iron free. Lipocalin 2 is upregulated in a number of cancers, including breast cancer and leukemia. Two separate reports indicate that knockout of Lcn2, the gene encoding lipocalin 2, suppresses mammary tumor formation in mice (10, 11). Thus, tumors induced in both MMTV-ErbB2 (V664E; ref. 10) and MMTV-PyMT (11) models of spontaneous breast cancer were delayed in onset, multiplicity, and size when crossed with an Lcn2 knockout mouse. However, lipocalin 2 has additional effects apart from its role in iron scavenging and delivery. For example, it promotes the activity of matrix metalloproteinase 9 (MMP-9), a protease involved in tumor invasion. Thus, whether the effects of lipocalin 2 on tumorigenesis are primarily linked to its effects on iron or are attributable to its other properties has yet to be determined.

Do all links between the proteins of iron regulation and cancer involve changes in cellular iron? In our recent report, the labile iron pool was increased in breast cancer cells compared with normal breast epithelium (4). This finding supports observations dating from the 1980s that TfR1, a cell surface receptor responsible for transferrin-mediated iron uptake, is upregulated in many cancers. TfR1 was soon identified as a target for cancer therapy and remains a popular strategy for the delivery of anticancer agents. Also indirectly supporting iron as the link between proteins of iron metabolism and cancer have been discoveries of the anticaner properties of iron chelators, which continue to be explored as potential chemotherapeutics (12). Yet, roles for at least 2 proteins that regulate iron metabolism, the iron regulatory proteins IRP1 and IRP2, seem complex and may not be entirely related to their function in iron homeostasis. IRP1 and IRP2 are well-studied post-transcriptional regulators of mRNAs encoding a number of proteins important in iron metabolism, such as TR1, the iron storage protein ferritin, ferroportin, and DMT1 (reviewed in refs. 13, 14). These regulatory proteins act both as translational repressors, inhibiting translation of mRNAs such as ferritin and ferroportin, and mRNA stabilizers, stabilizing mRNAs of TR1 and DMT1. The effect of overexpression of IRP1 and IRP2 on tumor growth was recently examined, with some surprising results (15, 16). Although overexpression of IRP1 was able to increase levels of TR1 (as anticipated) in H1299 lung cancer xenografts, in this setting it did not modulate levels of ferritin or ferroportin and, unexpectedly, led to suppression rather than growth of tumor xenografts (15).
contrast, overexpression of IRP2 stimulated growth of H1299 lung cancer xenografts (16), an effect mediated by a specific 73–amino acid domain not present in IRP1. Similar to observations with IRP1-overexpressing tumors, TfR1 was increased in IRP2-overexpressing xenografts, but ferritin, ferroportin, and DMT1 were not affected. Thus, despite their similar functions as iron regulatory proteins, IRP1 acts like a tumor suppressor, whereas IRP2 acts as an oncogene. Western blot and microarray analyses revealed differences in IRP2-overexpressing xenografts, including increased levels of c-myc and ERK1/2 phosphorylation. These results suggest that IRPs may have additional functions that are independent of their roles in iron metabolism.

Cancers, of course, are comprised of more than tumor cells; they exist in a rich microenvironment that includes stroma, endothelial cells, and inflammatory cells including macrophages. New evidence suggests that cells in the microenvironment may provide iron to tumor cells. Tumor-promoting macrophages are characterized by a specific pattern of cytokine secretion and resemble M2-polarized macrophages. Two recent reports suggest that M2-polarized macrophages express ferroportin and downregulate ferritin and heme oxygenase, all of which promote iron release (18). Further, conditioned media from M2 macrophages promote tumor cell proliferation, an effect inhibited by iron chelation. These findings suggest that iron is involved in the cross-talk between tumor cells and their environment. A model that incorporates some of these observations is depicted in Fig. 1.

Blood vessels are another critical element of the tumor microenvironment, and proteins of iron metabolism may also influence the angiogenic process that recruits these blood vessels to support tumor growth. Ferritin is a multi-subunit protein, primarily known for its intracellular role in iron storage and detoxification. However, this protein also exists in extracellular compartments, where its role is less clear. Ferritin can bind to specific cell surface receptors, including TIR1 (19), and thus has the potential to function in iron delivery. In addition, our group observed that ferritin may promote angiogenesis (20). Ferritin binds to and inhibits the activity of cleaved high-molecular-weight kininogen (HKa), an endogenous antiangiogenic protein that induces endothelial cell apoptosis (20, 21). However, the extent to which ferritin serves these roles in the tumor microenvironment is still uncertain.
More remains to be explained than has been explained. Cancer biologists have known for some time that "cancer" is not one disease, but many. It would be naïve to believe that there is only one mechanism by which cancer cells interact with cellular iron and the proteins that regulate iron absorption, transport, and redox state. Nonetheless, themes are beginning to emerge, including the preferential transport and accumulation of iron in at least some cancer cells. The connections between cancer cells, iron, and the complex roles of the proteins that regulate iron homeostasis seem to be hard wired.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**References**


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