

Role of Ferritin in Cancer Biology

Min Pang BS and James R Connor*

Department of Neurosurgery, Pennsylvania State University College of Medicine, Milton S. Hershey Medical Center, Hershey, PA 17033, USA

Abstract

As the major iron storage protein, ferritin has been linked with iron metabolism for many years. However, recent studies have discovered new functions for this protein. Our current review summarizes new findings suggesting the link between ferritin and many pathways related with cancer, such as cell proliferation, growth suppressor evasion, cell death inhibition, immortalization, angiogenesis, invasion and metastasis, and immunomodification. Most of the evidence revealed suggested that elevated ferritin in cancer cells may be related with cancer progression, resistance to therapies, or poor prognosis. By either directly or indirectly participating in cancer related pathways, ferritin proposes itself as a promising target for cancer therapy. Ongoing and prospective preclinical and clinical research will further explore this new strategy that is currently underappreciated.

Conclusion: Ferritin is proving to be a much more versatile protein than simply iron storage. It may have signaling properties and reporter properties for some cancers. Data are mounting that ferritin may be a promising target in cancer therapy.

Keywords: Ferritin; Iron; Cancer hallmarks

Ferritin: The Iron Management Protein

As the most abundant transition metal element in our body, iron is ubiquitously distributed and widely involved in various physiological processes, such as oxygen transport, energy metabolism, electron transport, cell cycle regulation, and DNA synthesis [1-3]. Despite its role as a co-factor in such biochemical activities, excessive iron can be toxic by generating Reactive Oxygen Species (ROS) through the Fenton reaction. This reaction converts less stable ferrous ions into the ferric form, while consuming hydrogen peroxide (H_2O_2) [4]. The main byproducts of this reaction are hydroxyl radicals ($\bullet OH$), the most reactive species in biological systems that are capable of inducing intracellular oxidative damage. Thus, excessive iron accumulation is considered a major risk factor of cell damage and tight regulation of iron metabolism is required to maintain cellular homeostasis and viability [2].

In mammalian cells, the major iron storage protein is ferritin. Ferritin is a hollow, spherical-structured protein complex composed of 24 subunits [5]. These 24-mers consist of two gene products: H-ferritin and L-ferritin, sharing a multihelical three-dimensional structure and a homology of 50-56% in amino acid sequence but exhibiting distinct functions [1,6]. The H-ferritin subunit exhibits ferroxidase and anti-oxidant activity, which converts toxic ferrous ions into less toxic ferric ions. On the other hand, L-ferritin has no ferroxidase activity but can modify the microenvironment to facilitate iron storage. It was reported that the iron uptake process requires the cooperation of both H- and L-subunits [7]. The composition of the ferritin complex varies in different tissues based on their iron requirements and metabolism patterns. For example, H-ferritin is abundant in muscle, brain, and heart while L-ferritin is rich in liver and spleen [8].

Ferritin's capacity for iron storage is only second to hemoglobin [9]. It can store up to 4500 iron atoms per ferritin molecule, but no more than 3000 atoms are stored under normal conditions [1]. For circulating serum ferritin, the iron storage capacity was quantified as 1100-1200 iron per molecule in healthy individuals and around 800 iron per molecule in patients with inflammatory diseases [10,11]. Clinically, elevated serum ferritin has been linked with inflammatory response, such as cancer and autoimmune diseases [12]. Moreover,

higher level of serum ferritin is also correlated with poor disease outcome [13]. Therefore, ferritin not only functions as an iron delivery and recycling, but a role as diagnostic and prognostic biomarker could be implied as well.

More recently, a new gene product exhibiting high homology with ferritin was discovered in mitochondria, which is a homopolymer of 24 subunits and regulates mitochondrial iron homeostasis [14]. The crucial role of iron in mitochondria oxidative homeostasis has greatly highlighted the importance of the function of mitochondrial ferritin in cell metabolism. Unlike H- and L-ferritin, mitochondrial ferritin has both ferroxidase (though with a slower rate) and iron binding activity [15]. It is highly expressed in the testes and erythrocytes and is expressed at a lower level in other cells [16]. It has been shown that the overexpression of mitochondrial ferritin leads to an increased iron uptake from cytosol to mitochondria [17,18]. Despite the above findings about the functions of mitochondrial ferritin, less is known about its role in cancer.

Regulation of Ferritin Expression

Like some of the other proteins involved in iron metabolism, the expression of ferritin is regulated transcriptionally by an Iron-Responsive Element (IRE) [19-21]. The IRE is a highly-conserved, hairpin-structured RNA sequence located within 5' or 3' untranslated regions (UTR) of the mRNA encoding such proteins. Iron Regulatory Protein (IRP), an RNA binding protein, binds to the IRE with an iron-dependent affinity. When cellular iron is low, IRP is activated and binds to an IRE in the 5' UTR of proteins in charge of iron export and storage, such as ferritin and ferroportin, and triggers the degradation

*Corresponding author: James R. Connor, Department of Neurosurgery, Pennsylvania State University College of Medicine, Milton, S. Hershey Medical Center, Hershey, PA 17033, USA, Tel: 717-531-4541; Fax: 717-531-0091; E-mail: jconnor@psu.edu

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of their mRNA. Conversely, IRP binding to the IRE in the 3' UTR for proteins in charge of iron uptake, such as transferrin receptor, and stabilizes their mRNA and elevates their protein expression level. IRP is inactivated under iron-rich conditions and the expression of proteins in charge of iron export and storage is up-regulated, and the expression of iron import proteins is repressed.

In addition to the canonical regulation mechanism, ferritin expression can also be regulated by other factors commonly observed in cancer, such as oxidative stress and hypoxia. Other than its role as an iron management protein, ferritin, especially H-ferritin, has been shown to have an intricate relationship with oxidative stress-related signaling pathways. Pham, et al. demonstrated that the up-regulation of H-ferritin, mediated by the inflammatory cytokine NF- κ B, suppressed the generation of ROS. The iron sequestering activity of ferritin is essential to its protection against oxidative stress based on the observation that the overexpression of wild-type H-ferritin significantly reduced the generation of ROS by iron sequestration, while the overexpression of the mutant H-ferritin without iron binding activity led to no inhibition in ROS induction [22]. The prompt response to intracellular oxidative stress is accomplished by the regulation of antioxidant proteins at the transcriptional level by a gene regulator named Antioxidant Responsive Element (ARE) [23]. In human L-ferritin gene, ARE was found overlapping with a Maf Recognition Element (MARE) and the activation of both resulted in an elevated gene expression more potent than IRE regulation [24]. Gene enhancer functions similar to an ARE was discovered in human H-ferritin gene as well. The transcription of H-ferritin gene can be activated by H₂O₂ in a JunD-dependent manner, which is essential in detoxification against oxidation insult [25,26].

Hypoxia is commonly observed in human solid tumors as a result of rapid proliferation of cancer cells and poorly developed vasculature formation. This hypoxic microenvironment benefits cancer cells by supporting angiogenesis, metastasis, resistance to therapies, and by providing the niche favoring the maintenance of cancer stem cells. Several lines of evidence support the idea that hypoxia is closely related to the local iron status, such as the finding that hypoxia leads to the differential activation of IRP, which in turn affects ferritin expression. It was first found in human oligodendroglioma cells that 6 hours of hypoxia treatment induced ferritin synthesis, without an elevation in mRNA level [27,28]. Later, two reports not only confirmed this finding in HEK293 cells, but also attributed it to the decrease in IRP1 binding activity under short-term hypoxia. In contrast, their study of long-term hypoxia (16-21 hours) showed an increase in the IRP2 binding activity, due to an up-regulated IRP2 protein level. This increase in IRP2 activity, along with an increase in iron uptake and decrease in ferritin synthesis, resembled an iron deficient phenotype [29,30]. Further study showed that under long-term hypoxia (24 hours), the induction of ferritin expression by iron treatment occurred both transcriptionally and transcriptionally, and was more remarkable in L-ferritin than H-ferritin, suggesting different regulation mechanisms between the two subunits [31].

The rationale for further exploring the link between ferritin and hypoxia resides in the findings that poly(rC)-binding protein 1 (PCBP1), initially discovered as a heterogeneous ribonucleoprotein, serves as a shared iron chaperone for ferritin and prolyl hydroxylase (PHD), the enzyme that destabilizes hypoxia-inducible factors (HIFs) [32,33]. PCBP1 binds to ferritin and facilitates the iron loading process, while the iron delivery to PHD elevates its hydroxylase activity. Therefore, it is possible that the disruption of iron metabolism through targeting ferritin will also affect the HIFs and the genes that HIFs regulate.

Ferritin and Cancer

Cancer has remained one of the major causes of death in the past several decades mainly attributed to its remarkable diversity and complexity in the progression to malignancy. In their two excellent reviews one decade apart, Hanahan and Weinberg have summarized the recent achievement in the area of cancer research into a set of hallmarks. These hallmarks include: sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, along with the recent addition of reprogramming of energy metabolism and evading immune destruction [34,35].

Because of its versatility in multiple crucial physiological processes related with cell growth and proliferation, iron has been suspected as one of the culprits in carcinogenesis as well as cancer progression. Although more investigations are required to establish a solid causal relationship between excessive iron and cancer, various reviews throughout the decades have speculated the potential significance of iron in cancer as well as targeting iron in cancer treatment [1,36-40]. Meanwhile, accumulating evidence has suggested that ferritin may be a relevant factor in most, if not all of such cancer hallmarks.

The rapid rate of cancer cell proliferation is driven by hyper-activated growth-promoting signals. Pro-survival pathways such as MAPK and PI3K, while tightly controlled in normal cells, are usually de-regulated in malignant cells. Tesfay, et al. reported that treating endothelial cells with ferritin enhanced activity of Erk and Akt signaling, promoting pro-angiogenic effects [41]. In breast cancer cells, the binding and uptake of ferritin was observed along with increased cell growth, independent of the iron status of ferritin [42].

In addition to promoting growth, cancer cells also commonly repress growth-suppressing genes. TP53, the well-investigated tumor suppressor gene encoding for p53 protein, is found mutated in almost all types of cancers with varying frequencies. It was previously reported that iron depletion up-regulates TP53 expression post-transcriptionally, mediated by the elevation of HIF-1 α stability [43,44]. Based on the antioxidant function of ferritin through iron regulation, Lee, et al. reported a direct link between ferritin and p53 protein, which also controls oxidative stress. An immunoprecipitation assay demonstrated a direct binding of p53 with both H- and L- ferritin in HEK293T cells. This binding with ferritin activates p53, which was indicated by an enhanced reporter activity of p53 after binding. This p53 activation is independent of the ferroxidase activity of ferritin, but in cells with H-ferritin down-regulation, it is sharply repressed [45]. It was also shown that the expression level of H-ferritin was regulated by p53, a process mediated by the multiprotein complex Bbf and the trimeric transcription factor NF-Y [46]. These studies established a well delineated feedback loop between p53 and ferritin, which also subscribed the potential of ferritin in cancer therapy.

Another mechanism by which ferritin could promote tumor growth is through anti-apoptotic properties. There is accumulating evidence that has also implicated ferritin as an anti-apoptotic protein, presumably based on its anti-oxidant property. It was first reported in astrocytes that the overexpression of the apoptosis inhibitor, Bcl-xL led to up-regulation of both H- and L-ferritin as part of the anti-apoptotic mechanism [47]. In breast cancer cells, Yang, et al. have shown that down-regulation of ferritin in MCF-7 cells resulted in cell growth inhibition through increased apoptosis, mediated by decreased levels of Bcl-2 mRNA [48]. Several other studies have suggested an antagonistic role of ferritin in tumor necrosis factor- α (TNF- α) induced apoptosis.

In HeLa cells, Cozzi, et al. found that both H- and L-ferritin were induced in response to TNF- α treatment. Overexpression of H-, but not L-ferritin led to a ~50% decrease in apoptosis. Again, this anti-apoptosis activity was independent of the ferroxidase activity, as the overexpression of the mutant ferritin with inactivated ferroxidase center showed a similar effect [49]. Additionally, it was found that H-ferritin is an essential mediator of the anti-apoptotic activity of NF- κ B during the inflammatory response. This activity is fulfilled by iron sequestration and ROS inhibition and the subsequent repression of the proapoptotic c-JNK signaling [22]. In human malignant mesothelioma cells, it was also reported that constitutively expressed H- ferritin protects cells from H₂O₂ induced apoptosis, while the down-regulation of H-ferritin led to increased sensitivity [50]. A recent study investigated the mechanism of action in artesunate- induced toxicity in cancer cells and found that ferritin degradation is an essential step. Artesunate accumulates in the lysosomes and activates lysosomal degradation of iron loaded ferritin. As a result, iron is released, mitochondrial ROS is induced, and apoptosis pathway is activated. The overexpression of H-ferritin protects cells from artesunate-induced cell death [51]. In contrast, results from our laboratory showed that H-ferritin down-regulation led to an increase in caspase-3 activity as an indicator for apoptosis [52]. However, a contradictory finding was reported that the placental isoform, presumably an H- and L- heterodimer, promoted intrinsic apoptosis activity through Fas signaling in rat hepatocytes [53]. More studies are necessary to investigate the intricate functions of ferritin in apoptosis, but if a dual role of ferritin may be established in normal and malignant cells, as suggested by the above research, it is possible that targeting ferritin may provide a powerful tool by differentially killing cancer cells.

Almost all types of human tumors exhibit abnormal vasculature development. The rapid proliferation of cancer cells requires the reactivation of the quiescent angiogenic signal from the pre-existing vasculature. Interestingly, several recent studies have revealed a potential role of ferritin in angiogenesis. Coffman, et al. reported that ferritin, independent of the iron loading status, binds to cleaved high molecular weight kininogen (HKa). HKa is an inhibitor of angiogenesis and can be inactivated upon binding with ferritin [54]. A follow-up study further revealed that both H- and L-ferritin are capable of blocking the anti-angiogenic effect of HKa and that the survival of endothelial cells can be enhanced by ferritin treatment [41].

One of the most important angiogenic factors and a major target for cancer therapy is vascular endothelial growth factor (VEGF). This protein can recruit vascular endothelial cells and consequently facilitate the angiogenesis process [55]. Evidence supports iron is among the regulators of VEGF and ferritin is likely mediating this regulation process. Iron depletion through the widely utilized iron chelator, deferoxamine, although widely considered as an antitumor agent, promoted both expression and secretion of VEGF in cancer cells [56]. Recent studies in breast cancer also showed that iron depletion led to decreased ferritin levels and increased VEGF expression, which was mediated by elevated HIF-1 α levels and enhanced angiogenic activity *in vivo*. Analysis of clinical data suggested that the relationship of iron deficiency and angiogenesis may contribute to the high recurrence of breast cancer in young patients [57,58]. In a study on lens epithelial cells, H-ferritin siRNA transfection was associated with decreased VEGF secretion, [59] establishing a potential relationship between H-ferritin and VEGF that should be further explored in cancer cells.

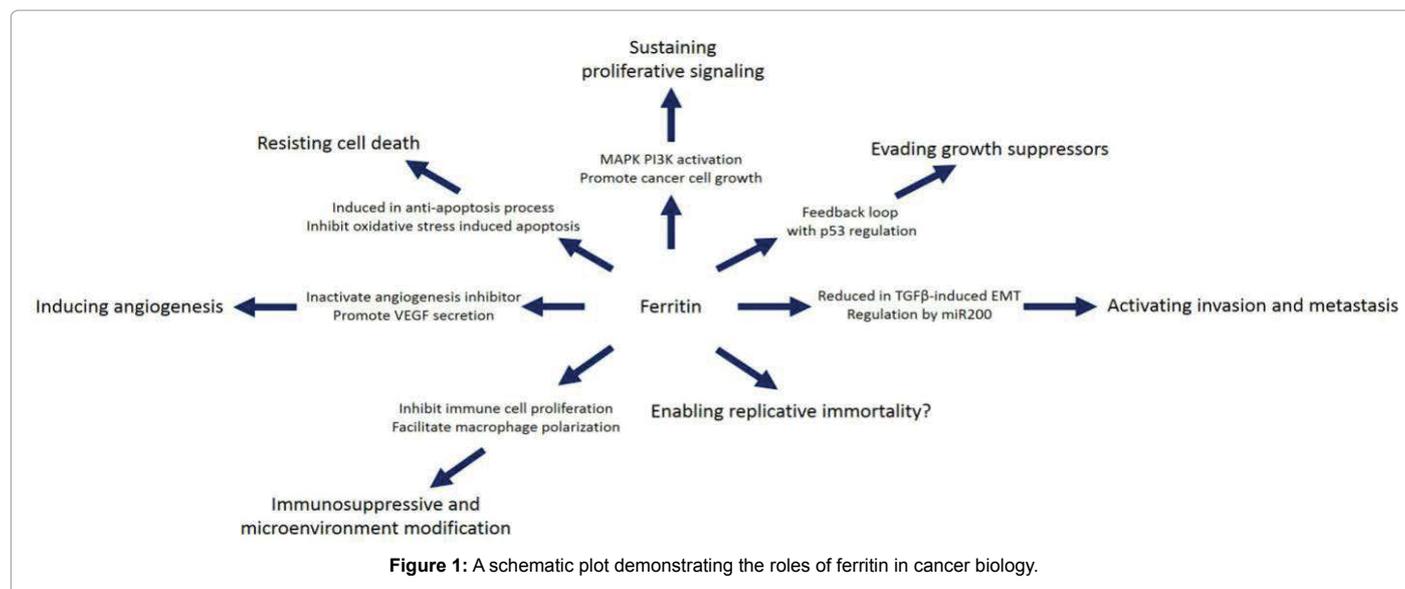
Within the tumor, the transition of cancer cells from epithelial phenotype to mesenchymal phenotype (epithelial-mesenchymal

transitions, or EMT), has been identified as a marker of metastasis and gain of invasiveness [60] and may be influenced by ferritin. In murine hepatocytes, a significantly reduced H-ferritin level was observed in cells with TGF- β induced EMT. This decrease of H-ferritin led to increased labile iron pool and ROS generation, both of which contributed to EMT [61]. However, another study in breast cancer cells reported that epithelial cell lines express low levels of both H- and L-ferritin but elevated expression in the more aggressive mesenchymal cell lines [62]. This study also revealed that miR-200, whose down-regulation was previously observed in mesenchymal breast cancer cells [63], targets and antagonizes ferritin expression. Therefore, the data may suggest a cell type dependent effect for ferritin on EMT. It is possible that in normal cells, where cellular metabolism and oxidative stress levels are relatively low, the down-regulation of H-ferritin leads to increased ROS and therefore triggers EMT; while in cancer cells, where the iron demand and oxidative stress level are elevated, the up-regulation of H-ferritin promotes EMT and cell proliferation. Researching oxidative stress and ferritin in cancer cells may be the key to understand these observations.

The most remarkable and most intriguing characteristic of cancer cells is replicative immortality. Cancer cells have developed mechanisms that enable sustained deregulated replications, while their non-malignant counterparts will be stalled by senescence or cell death after a limited number of cell divisions. A number of investigators have suggested that ferritin may play a role in these mechanisms. A comparison between the mortal human breast epithelial cells, S-130, and the chemically immortalized cell line, MCF-10F, derived from S-130 revealed that a cDNA encoding H-ferritin was preferentially expressed along with an elevated mRNA and protein level in MCF-10F [64]. This study also supported the hypothesis that ferritin may play a role in early malignant transformation in breast cancer cells. Interestingly, within the same system, another study reported a shift of the p53 gene in exon 7 of MCF-10F cells [65]. These studies also corroborated the link between ferritin and p53 as described above.

Our immune system is capable of detecting and eliminating neoplastic cells. However, these cells generally develop certain immunoevasive mechanisms during tumorigenesis [35]. Immunosurveillance activity is performed mostly by immune cells, whose proliferation depends on cofactors, including iron [66,67]. By moderating the local iron availability, ferritin is involved in many mechanisms of immunosuppression. Early studies showed that H-ferritin can bind T lymphocytes and inhibit their proliferation [68]. It is reported that H-ferritin induces IL-10 production from CD4+/CD25+ regulatory T (Treg) cells, as part of the immunosuppression mechanism [69]. This induction of Treg cells by elevated H-ferritin in circulation was also observed in melanoma patients [70]. Several other lines of evidence suggest a distinctive pattern of ferritin expression and presumably iron metabolism is essential in the polarization of macrophages. In M2-polarized macrophages, which facilitate tumor growth, H-ferritin expression is low and ferroportin level is high, which is reflective of an iron secretion phenotype. While elevated circulating H-ferritin may repress immune response, low H-ferritin in macrophages causes decreased iron storage ability, enhanced iron release, and a microenvironment that favors tumor progression [71,72]. Further investigation is required to clarify the detailed mechanisms of ferritin-mediated immunoevasion, but the data suggests that targeting ferritin as a novel immunomodulator in cancer treatment is promising.

Other than the hallmarks mentioned above, genomic instability is an important enabling characteristic of cancer cells [35]. Random



genetic mutations are generated as a result, and some of the oncogenic ones are considered as leading players in tumorigenesis and acquisition of cancer hallmarks. Genomic instability is obtained mainly by defect in DNA-maintenance machinery or increased sensitivity to mutagens. TP53 is a key player in maintaining DNA stability and we discussed its link to ferritin in the previous paragraph. A series of recent discoveries with regard to the role of ferritin localized in the nucleus suggests ferritin can be directly involved in DNA protection and stability. It was reported that H-ferritin, but not L-ferritin or ferroxidase-deficient H-ferritin mutant, is capable of DNA binding [73]. H-ferritin was also found preferentially translocated into the nucleus and protect DNA from iron-induced oxidative damage [74]. Along with the findings in epithelial cells that nuclear ferritin protects DNA from oxidative stress and UV-radiation [75,76], it could be extrapolated that over-expressed ferritin resulted in enhanced DNA protection and an indirect relationship between ferritin and genomic instability may be implied in cancer cells.

Therapeutics and Further Issues

Iron's role in so many crucial pathways and evidence that iron metabolism is preferentially elevated in cancer cells, has made iron a target of great interest to cancer researchers. Various iron chelators have been validated as potent anti-cancer agents. Based on its key position in intracellular iron regulation and potential involvement in cancer related pathways, ferritin may also serve as another target in cancer treatment. Indeed, several studies have shown promising perspectives of the therapeutic effect by targeting ferritin.

Following an early study of apoptosis induction by ferritin down-regulation in breast cancer cells [48], Shpyleva, et al. reported that the down-regulation of H-ferritin induced by miR-200 was sufficient to sensitize MDA-MB-231 mesenchymal breast cancer cells to doxorubicin [62]. A more comprehensive study was recently performed in melanoma cells. The H-ferritin gene was down-regulated in human metastatic melanoma cell line, MM07m, in which H-ferritin is consistently elevated. A subsequent proteomic screening identified clusters of proteins differentially expressed with the H-ferritin gene silenced. Many of the silenced proteins are related to tumor progression and metastasis. Down-regulation of H-ferritin in MM07m cells also led to remarkably reduced tumor growth capacity in a subcutaneous

mouse model [77]. Recent data from our laboratory showed that H-ferritin down-regulation sensitized high-grade glioma cells to chemotherapeutic agents both in vitro and in vivo [52], expanding a role for ferritin as an anticancer target in brain tumor as well.

Ferritin is playing a significant role in many of the cancer related pathways, as summarized in Figure 1. Some of these functions are directly related with iron, while others may not necessarily be iron-dependent. The discovery of these novel functions, as well as expanded understanding of its canonical iron management function, position ferritin as a promising target in cancer therapy.

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