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Review

The significance of ferritin in cancer: Anti-oxidation, inflammation and tumorigenesis

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ABSTRACT

The iron storage protein ferritin has been continuously studied for over 70 years and its function as the primary iron storage protein in cells is well established. Although the intracellular functions of ferritin are for the most part well-characterized, the significance of serum (extracellular) ferritin in human biology is poorly understood. Recently, several lines of evidence have demonstrated that ferritin is a multi-functional protein with possible roles in proliferation, angiogenesis, immunosuppression, and iron delivery. In the context of cancer, ferritin is detected at higher levels in the sera of many cancer patients, and the higher levels correlate with aggressive disease and poor clinical outcome. Furthermore, ferritin is highly expressed in tumor-associated macrophages which have been recently recognized as having critical roles in tumor progression and therapy resistance. These characteristics suggest ferritin could be an attractive target for cancer therapy because its down-regulation could disrupt the supportive tumor microenvironment, kill cancer cells, and increase sensitivity to chemotherapy. In this review, we provide an overview of the current knowledge on the function and regulation of ferritin. Moreover, we examine the literature on ferritin's contributions to tumor progression and therapy resistance, in addition to its therapeutic potential.

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1. Introduction

Ferritin is the oldest known protein involved in iron metabolism. It was first described in 1894 by the German pharmacologist Oswald Schmiedeberg who noted an iron-rich component in horse livers [1]. However, it was not until 1937 that ferritin was purified from horse spleen by the Czech biologist Vilém Laufberger who proposed that it "must be a substance which serves as a depot for iron in the organism" [1,2]. The early isolation of ferritin was facilitated by several distinct biochemical characteristics: its stability at high temperatures (>80 °C), relative insolubility in ammonium sulfate solutions, and its crystallization with cadmium salts.

Ferritin is a 450 kDa hollow nano-cage (outside diameter 12–13 nm; inside diameter 8 nm) capable of incorporating up to 4500 iron atoms in a non-toxic but bioavailable form [3,4]. In mammals, each ferritin complex is composed of 24 subunits that form a spherical symmetrical protein shell. Each ferritin subunit folds into a 4-helix bundle with a fifth short helix in close proximity to the C-terminus [5,6]. In its assembled form, the ferritin complex has eight hydrophilic channels which have been proposed to serve as entry ports for ferrous iron [7,8]. Iron is stored within the ferritin cavity as mineralized ferrihydrite ($Fe_2O_3 \cdot 9H_2O$) with traces of phosphorus and nitrogen [9].

Two functionally and genetically distinct ferritin subunits exist: L-ferritin and H-ferritin (also known as light chain and heavy chain ferritin). In humans, but not all species, their molecular masses are 19 and 21 kDa respectively [10,11]. Although the two subunits share approximately 55% of their sequence as well as their multi-helical three dimensional structures, they are functionally distinct [11–13]. The H subunit possesses enzymatic activity and can oxidize ferrous iron into ferric iron. The ferroxidase center in H-ferritin is composed of several residues (mainly glutamic acid) which are buried within the H-ferritin helical bundle and serve as metal ligands [5,14]. The ferroxidase activity of H-ferritin is not dependent on the assembly of the full-ferritin complex and can be detected in the monomeric form [15]. The presence of a ferroxidase center within the ferritin subunit is essential and sufficient for rapid iron uptake [13,14,16]. A mutant of H-ferritin generated by mutating two residues within the ferroxidase center (Glu62 and His65) was capable of forming stable ferritin complexes but lacked detectable ferroxidase activity [17]. Furthermore, introduction of several glutamic acid residues necessary for the ferroxidase center into L-ferritin was sufficient to increase its iron incorporation capacity to similar levels as H-ferritin [18].

L-ferritin lacks enzymatic activity and thus does not contribute to iron oxidization and uptake. However, it has a higher number of carboxy groups lining the ferritin cavity which serve as iron nucleation sites [16,19]. In vitro experiments with recombinant L-ferritin homopolymers showed that it is capable of mineralizing iron faster than H-ferritin homopolymers [19]. Moreover, the L-ferritin monomer contains a salt bridge within its helical fold which confers greater stability on the ferritin complex in acidic and reducing conditions [20].

2. Serum ferritin

In addition to its intracellular form, ferritin is also an abundant protein in circulation. This form of ferritin, termed serum ferritin, was first detected in 1948 in animals experiencing hepatic cirrhosis or shock [21]. This original observation was later confirmed in humans with various forms of liver disease [22]. Serum ferritin showed similar immunologic reactivity, molecular size and isoelectrical focusing characteristics as that of ferritin extracted from the liver or spleen [23–26]. Furthermore, serum ferritin was surprisingly iron poor with approximately 4–20% of the iron content of liver or spleen ferritin [23,24]. This relatively low iron content persisted even in patients with iron overload [23].

Serum ferritin is a reliable indicator of the body's iron stores [27–29]. Its levels are significantly lower in individuals suffering from iron-deficiency anemia or undergoing phlebotomy [27,28]. In contrast, serum ferritin levels are higher in patients with iron-overload disease and hemochromatosis [27,30]. Generally, women tend to have lower levels of serum ferritin than men [27,28,31], possibly due to loss of hemoglobin during menstruation. Serum ferritin values in healthy individuals show some variability [31]. Serum ferritin correlated positively with age, body mass index, iron supplement, and heme-iron intakes, but was inversely correlated with physical activity and aspirin use in postmenopausal women [32]. Another study examining serum ferritin in men demonstrated that serum ferritin is positively correlated with body mass index, but not with the use of dietary iron supplement [33].

Serum ferritin is elevated during chronic and acute inflammation [34–36]. Its rise correlates with the rise in other acute phase proteins such as C-reactive protein (CRP) and α_1-acid glycoprotein (AGP) [36,37]. Consistently, chronic use of aspirin lowers serum ferritin and other parameters of inflammation in patients with various inflammatory diseases [38].

The source of serum ferritin is still unclear. Several lines of evidence have demonstrated that hepatocytes, macrophages and microglia are capable of ferritin secretion in vitro [39–42]. This secretion was reflective of their intracellular iron levels and was responsive to iron loading and chelation [39,41,42]. Although the secreted ferritin in those experiments contained both the H and L subunits, their ratios varied greatly between animal strains and cell types [39–41].

Although different cells are capable of ferritin release in vitro, serum ferritin seems to be primarily derived from macrophages in vivo [43,44]. The macrophage-specific ablation of the iron response protein 2 (IRP2) – a negative regulator of ferritin expression – increased serum ferritin levels, whereas hepatocyte or intestinal epithelial-specific ablation did not affect serum ferritin levels in mice [44]. Moreover, the size and immunological reactivity of serum ferritin in mice were similar to the ferritin found in organs with major macrophage populations such as the bone marrow and spleen [43] and serum ferritin levels fell by 75% after splenectomy suggesting the macrophages from the spleen were the source of ferritin in serum [43]. Recently, a study utilizing a mouse model with macrophage-specific deletion in the iron exporter ferroportin showed a robust increase in serum ferritin levels as well as increased iron accumulation in spleen and liver macrophages [45]. This study also suggests that macrophages contribute significantly to serum ferritin. Overall, although multiple cells are capable of ferritin secretion in response to various stimuli, there is increasing evidence that, macrophages may represent a primary systemic source for serum ferritin.

The existing literature on ferritin's secretory pathway is conflicted, as evidence exists to suggest both classical and non-classical pathways. For example, a truncated and unglycosylated ferritin similar to ferritin found within lysosomes was detected in mouse serum and splenic macrophages suggesting macrophage-specific release of lysosomal ferritin [43]. In other studies, the secretion of ferritin by hepatocytes and macrophages was inhibited by brefildin A (BFA) which is a potent blocker of protein transport from the endoplasmic reticulum (ER) to the Golgi apparatus [39,40]. This study also demonstrated that the entry of the ferritin monomers into the ER system occurs under the relative absence of free cytosolic iron and that iron can induce ferritin cellular retention [39].

3. Ferritin in cancer

3.1. Ferritin expression and localization in tumor tissue

Ferritin is differentially over-expressed in tissues from multiple malignancies, including: hepatocellular carcinoma [46,47], Hodgkin's lymphoma [48], breast cancer [49–53], and pancreatic cancer [50]. Structural, immunological, and isoelectric analyses demonstrated that tumor ferritins differ in their subunit composition and are most likely composed of different ratios of the L and H subunits [54].

In breast tumors, the increase was specific to L-ferritin and was approximately six-fold higher compared to benign breast tissue [49,52,53,55]. This increase was correlated with greater epithelial proliferation, histopathological dedifferentiation and shorter survival. Histological examination of ferritin expression and distribution showed strong ferritin expression in epithelial ductal cells from normal breast tissue, moderate to weak staining in breast cancer cells, and relatively strong staining in the tumor stroma [53,55,56]. Intriguingly, ferritin levels within epithelial tumor cells decreased with tumor transformation and progression possibly indicating a decrease in iron levels within cancer cells. It has been previously noted that breast cancer cells display increased expression of the iron importer transferrin receptor [57,58] and decreased expression of the iron exporter ferroportin [59]. Because intracellular iron can regulate the expression of both transferrin receptor and ferroportin on a post-transcriptional level, these two observations may suggest that cancer cells have low iron levels or a high rate of iron utilization with little export.

The ferritin-rich cells in the breast tumor stroma are of the macrophage lineage [53,55]. In light of the decrease in ferritin expression in cancer cells, the increase in tumoral ferritin may thus be a consequence of increased infiltration of ferritin-rich macrophages. Although the localization of ferritin within other solid tumors has not been explored, it is reasonable to speculate that it may be also due to macrophage infiltration — a hallmark of many solid tumors [60,61].

The recent studies demonstrating that tumor-associated macrophages (TAMs) are rich in ferritin provide further insights into the molecular phenotype of TAMs and allow for speculation about the functional significance of ferritin in cancer biology [53,55]. The high expression of ferritin in TAMs may protect them from iron induced damage and may stimulate their survival or proliferation [62]. Because macrophages are one of the few cell types capable of ferritin secretion and in light of evidence to suggest local ferritin secretion within tumors [53,63,64], extracellular ferritin secreted by macrophages could stimulate tumorigenesis on multiple levels. It can increase proliferation in cancer cells directly [53], increase angiogenesis [65], and suppress lymphocyte responses [66,67]. Collectively, these effects would all contribute to the many aspects of tumorigenesis attributed to TAMs and suggests the secretion of ferritin could have a direct role in promoting and maintaining the tumor.

Furthermore, ferritin could be playing a role in preventing macrophages from initiating an effective pro-inflammatory (M1) phenotype and thus maintaining a wound-healing (M2) phenotype by sequestering intracellular iron. The accumulation of free iron in macrophages can cause an increase in the production and secretion of several inflammatory cytokines [45,68–70]. Iron loading of macrophage can also cause unrestrained inflammation in vivo through increased cytokine and hydroxyl radical production which may impair wound healing and the resolution of inflammation [71]. Moreover, the jumonji family of histone demethylases is iron-dependent and is essential for LPS-mediated transcriptional up-regulation of a wide-range of inflammatory cytokines including TNFα and IL-1β [72].

3.2. Serum ferritin in cancer patients

Serum ferritin is elevated in patients with Hodgkin's lymphoma [73–75], hepatocellular carcinoma [46,47,76], neuroblastoma [77,78], glioblastoma [79], squamous cell carcinoma of the head and neck [80], renal cell carcinoma [81], melanoma [82,83], non-small-cell lung cancer [84], pancreatic cancer [85–88], and breast cancer [89–93]. This increase is often associated with more progressive disease and shorter survival [74,78,80,82,85,91]. Pre-treatment levels of serum ferritin were also a strong predictor of response to trastuzumab-containing therapy in patients with HER2/neu-overexpressing breast tumors [91]. Moreover, serial measurement of serum ferritin in cancer patients receiving chemotherapy indicated that a return to normal level is associated with therapy response [47,76,80].

The increase in serum ferritin observed in breast cancer patients is primarily in L-ferritin [53,92], although H-ferritin was also detected, albeit in small quantities, in sera of approximately 50% of advanced breast cancer patients compared to only 7% in healthy controls [92,94]. The marked difference in circulating levels may be due to differences in half-life, clearance, complex stability between the two ferritin subunits or even types of antibodies used. The difference in type of ferritin present in the serum could be biologically important as the H and L subunits do appear to have unique receptors [67,95,96].

Although all of the studies examining serum ferritin in cancer patients showed statistically-significant increases in patients with both localized and metastatic diseases [89,90,92,94,97], one study showed an association between serum ferritin levels and metastatic breast cancer but not localized disease [93]. The elevation in serum ferritin seemed to be more pronounced in patients with metastases to the viscera [93], more specifically the liver [98]. These reports, however, did not demonstrate a causative link and only speculated that liver metastases might be causing liver damage and thus ferritin release into circulation. This possibility has been rejected by studies that demonstrated that cancer-associated elevation in serum ferritin does not correlate with serum biomarkers of liver damage [46,77].

3.3. Serum ferritin and cancer risk

Serum ferritin is associated with a higher risk for some cancers. For example, an increase in serum ferritin but a decrease in transferrin was associated with a three-fold increase in hepatocellular carcinoma incidence and mortality in a Taiwanese cohort [99]. A similar trend in serum ferritin and transferrin levels was associated with overall cancer incidence in women, but not men, participating in a large French study [100]. Interestingly, increased dietary iron intake was not associated with increased cancer risk raising the possibility that the increase in serum ferritin is not due to increased iron stores [100]. Moreover, constant elevation in serum ferritin in patients with chronic liver disease is associated with a higher risk for the development of hepatocellular carcinoma than patients whose levels normalize after diagnosis [101]. Conversely, the decrease in serum ferritin by phlebotomy in patients with peripheral arterial disease was associated with decreased cancer incidence and mortality [102]. However, there was little evidence to suggest that serum ferritin is associated with a higher risk for colorectal cancer or recurrence of colorectal adenomas [103–105], despite strong evidence suggesting that increased iron stores (as measured by iron parameters that excluded serum ferritin) is associated with a higher risk of colorectal cancer [106].

Germline mutations in the High Iron (*HFE*) gene are associated with increased overall iron stores [30,107]. The HFE gene encodes a MHC class-I protein that associates with the iron importer transferrin receptor and decreasing its affinity to transferrin. There are two common loss-of-function mutations in HFE that can cause iron overload: a cysteine to tyrosine substitution at residue 282 (C282Y), and a histidine to aspartic acid substitution at residue 63 (H63D) [30,107,108]. C282Y, but not H63D, homozygosity is associated with increased risk for breast cancer in women and colorectal cancer in men [109]. The C282Y allele is also more prevalent in breast cancer patients than healthy individuals [110]. Interestingly, individuals homozygous for the C282Y mutation have higher levels of serum ferritin than patients with wild type or H63D alleles [30]. It is unclear if the cancer risk associated with the C282Y polymorphism is connected to its effects on serum ferritin levels but in the context of existing literature there is a compelling argument for a connection.

4. Source of the cancer-associated elevation in serum ferritin

The cancer-associated elevation in serum ferritin is most likely induced by an inflammatory state and is not due to liver damage or to alterations in the body's iron stores. For example, the increase in

serum ferritin observed in patients with Hodgkin's disease was inversely correlated with serum iron, transferrin, transferrin saturation levels, and hemoglobin [74,75], but was positively correlated with a higher erythrocyte sedimentation rate (ESR) — a marker of inflammation [75]. Moreover, serum ferritin levels in hepatocellular carcinoma and neuroblastoma patients did not correlate with the hepatic marker transaminase indicating that the rise in serum ferritin is not caused by liver damage [46,77]. The iron content of serum ferritin is below normal range in approximately 90% of non-small-cell lung cancer patients, and shows positive correlation with other iron parameters but negative correlations with serum ferritin levels and ESR [84]. In breast cancer patients with metastatic disease, serum ferritin was moderately correlated with the hepatic inflammatory biomarker C-reactive protein (CRP) [91]. When patients were stratified based on both serum ferritin and CRP, patients with elevation in both inflammatory biomarkers had the worst clinical outcome indicating that their prognostic value is reflective of the broader state of cancer-associated inflammation [91]. Overall, these findings support a role for inflammation-induced production of ferritin while arguing strongly against the involvement of body iron or liver damage.

The elevation in serum ferritin is partly due to localized release within the site of the tumor. In patients with elevated serum ferritin, surgical resection of tumors lowered serum ferritin levels by approximately 50% implicating a relationship between the tumor mass and the elevation in serum ferritin [97]. Moreover, analysis of intraductal fluid in breast cancer patients revealed a five-fold elevation in ferritin levels compared to healthy controls suggesting localized ferritin release within the breast tumor microenvironment [63,64]. In patients with renal tumors, the concentration of serum ferritin measured from the renal vein was higher than the levels in inferior vena cava or the preoperative systemic levels [81]. Most convincingly, cerebrospinal fluid (CSF) ferritin increased approximately ten-fold in patients with glioblastoma compared to patients without neurological abnormalities or enteroviral meningitis [79]. Although CSF contains little ferritin under normal conditions, almost 70% of the glioblastoma patients had CSF ferritin levels exceeding those in serum providing strong support for local synthesis and release [79].

Cancer cells from different malignancies have been reported to secrete ferritin or ferritin-like molecules in vitro [77,111–114]. Moreover, transplantation of human neuroblastoma or hepatocellular carcinoma cell lines into nude mice led to the detection of circulating human ferritin in the majority of tumor-bearing animals [115]. All of the animals showed an increase in circulating levels of L-ferritin but few showed detectable levels of H-ferritin. However, it is still unclear whether or not tumor secretion alone could account for the high levels of serum ferritin seen in many cancer patients.

5. Regulation of ferritin expression

The importance of iron in biological systems and its detrimental effects on cells when present in excess have led to the evolution of multiple layers of regulation to control the expression of the ferritin genes. Probably the most prominent and best understood mode of ferritin regulation occurs on the mRNA levels in response to the labile iron pool through the activity of iron regulatory proteins (IRPs). This type of regulation has been thoroughly covered in several excellent reviews [4,116]. Here, we will briefly address the multiple stimuli that induce ferritin expression in the context of cancer.

5.1. Inflammation

Several inflammatory cytokines (i.e. TNFα and IL-1β) are capable of inducing H-ferritin transcription via the activation of NFκB signaling [117–121]. Interestingly, IL-1β also increased the expression of L-ferritin on a translational level by increasing the association of L-ferritin mRNA with ribosomes in human hepatoma cells [118]. Although this effect was blocked by iron chelation, it is still unclear if it was dependent on the increase in H-ferritin transcription and the possible saturation of the IRP system [118]. Therefore, inflammatory cytokines can regulate the expression of ferritin on two levels: a transcriptional level (mainly H-ferritin) and a translational level (both H- and L-ferritin). Deletion mapping of the H-ferritin promoter regions identified a cis-acting region 4.8 kb upstream of the transcription start site [121]. This region contained two binding sites for members of the NFκB transcription factor family.

5.2. Hormonal induction

The thyroid-stimulating hormone (TSH or thyrotropin) stimulates H-ferritin transcription in rat thyroid cells [122,123]. Many of the functions of thyrotropin are mediated by the second messenger cyclic adenosine monophosphate (cAMP). The treatment of thyroid cells with cAMP increased H-ferritin transcription to the same levels as thyrotropin [124,125]. Moreover, the pancreatic hormone insulin and its homologous protein insulin-like growth factor 1 (IGF-1) stimulated the transcription of both H- and L-ferritin in a dose-dependent manner [124,126]. IGF-1 was approximately 10 times more potent than insulin, and its K_d was 10 times lower [126]. The hormonal regulation of ferritin in human cells has not been thoroughly examined.

5.3. Oxidative stress

Cells respond to oxidative damage by increasing the transcription of genes that play a role in anti-oxidative protection. Due to its ferroxidase activity and iron storage capacity, ferritin may have beneficial roles during oxidative insult. Exposure to various oxidants increased the levels of H and L-ferritin mRNA in vitro [127–130]. A 75-bp segment located 4.1 kb upstream of the transcription initiation site for mouse H-ferritin was identified as an electrophile response element (EpRE) and was shown to be essential for the oxidative stress-induced ferritin transcription [128]. In addition to the induction of ferritin transcription, oxidative stress modifies ferritin expression on a translational level through its effects on IRPs. Therefore, oxidative stress affects ferritin expression on both transcriptional and translational levels allowing cells to respond quickly to oxidative insult and thus minimizing ROS-induced cellular damage.

5.4. Hypoxia

Hypoxia is a defining characteristic of many solid tumors and is believed to contribute to disease progression and therapy resistance [131]. Early studies in the brain have demonstrated that hypoxia induces the specific and reversible expression of ferritin [132,133]. This induction occurs primarily on a post-transcriptional level due to decreased mRNA binding capacity of IRPs [134,135]. Consistently, hypoxic conditions can increase the accumulation of ferritin in alveolar cells (including cells derived from lung tumors) without altering their iron content [136].

6. Intracellular functions: Iron storage and anti-oxidation

As a consequence of its ability to sequester iron, ferritin plays a key protective role against oxidative stress. Unbound intracellular iron is capable of generating reactive oxygen species (ROS) through Fenton chemistry causing lipid peroxidation, DNA breaks and other forms of cellular damage [4,137,138]. Several over-expression and deletion studies have demonstrated the effect of ferritin, specifically H-ferritin, on survival under conditions of oxidative stress. In *C. elegans*, deletion of a mammalian H-ferritin homolog reduced lifespan under conditions of iron overload [139]. In HeLa cells, the overexpression of H-ferritin was protective against oxidative stress and hydrogen peroxide-induced cytotoxicity [14]. The expression of H-ferritin was also speculated to

be a possible mechanism of resistance to chemotherapeutic agents that induce oxidative stress [140].

The role of ferritin as an anti-oxidant is also evident in its protection against TNFα- and TGF-β1-induced oxidative stress. Overexpression of H-ferritin, but not L-ferritin, protected HeLa cells from TNFα-induced apoptosis [141]. Furthermore, NFκB-mediated up-regulation of H-ferritin protected cells from the accumulation of ROS caused by TNFα exposure [121,142]. Remarkably, expression of H-ferritin in NFκB-null cells was sufficient to block TNFα-induced apoptosis. H-ferritin was also shown to be a downstream target for TGF-β1 [143]. In this study, TGF-β1 was shown to transiently increase the labile iron pool and thus increase the generation of reactive oxygen species. The induction of H-ferritin and the subsequent sequestration of free iron protected cells from the TGF-β1-induced spike in ROS.

Deletions of H-ferritin in mice are embryonically lethal signifying its protective role in the vulnerable early stages of development [144]. Mice heterozygous for the H-ferritin deletion lacked any apparent abnormalities but had elevated levels of L-ferritin in serum and tissue [145]. This elevation in L-ferritin is unlikely to be reflective of a compensatory role for L-ferritin in iron storage and is probably a consequence of increased intercellular free iron levels caused by the decrease in the iron storage capacity of H subunit-deficient ferritin complexes. Furthermore, a closer look at the brains of heterozygous mice revealed an increase in oxidative stress and apoptosis, indicating a possible connection between iron accumulation in excess of ferritin in the brain and neuronal diseases [146].

7. Functions of serum ferritin

7.1. Iron delivery

Iron is an essential micronutrient in cells and is required for energy production and DNA synthesis. Cancer cells with their high proliferative potential are expected to have a higher demand for iron especially under poorly vascularized conditions. Ferritin with its high iron storage capacity could serve as a very efficient iron delivery molecule.

The existence of ferritin as a non-transferrin iron delivery system has been convincingly demonstrated by several groups. Injection of ^{59}Fe-loaded H- or L-ferritin into adult mice led to differential uptake by some organs. Radioactivity was detected in the liver, spleen, kidney, and lung [95]. More ^{59}Fe was detected in the spleen, lung, and brain when loaded into H-ferritin than L-ferritin [95]. Mice embryos with total transferrin receptor deletions can still initiate organogenesis – a process that requires cellular iron accumulation – via ferritin endocytosis [96,147]. Addition of ferritin to culturing conditions led to increased cellular iron levels in oligodendrocytes [148,149], erythrocyte progenitor cells [150–153], and renal cells [96].

7.2. Immunosuppression

Immunosuppression in cancer patients represents a major obstacle facing the development of effective immunotherapy for cancer. Several lines of evidence have demonstrated that extracellular ferritin may exert immunosuppressive effects on lymphocytes and myeloid cells through its modulation of iron availability [66,67]. Using recombinant H- and L-ferritin, Fargion et al. demonstrated saturable specific binding for H-ferritin but not L-ferritin on human peripheral lymphocytes. The binding of H-ferritin increased markedly in PHA-stimulated lymphocytes and its presence in culture suppressed PHA-induced proliferation [67]. Furthermore, immuno-screening of a cDNA library from the MM200 melanoma cell line identified H-ferritin as a secreted factor with immunosuppressive properties [114]. H-ferritin is elevated in the sera of melanoma patients, and this elevation is associated with increased numbers of circulating CD4+ CD25+ regulator T cells [83].

H-ferritin has suppressive effects on myelopoiesis as well [154,155]. Injecting recombinant H-ferritin into mice led to a decrease in hematopoietic progenitor cells in the bone marrow and spleen in vivo. The same group also generated several H-ferritin mutants to gain more insight into its binding sites and mechanism of action. Interestingly, only one mutant lacked the immunosuppressive activity [156]. This mutant had an inactive ferroxidase center and thus was incapable of efficient iron incorporation [17]. When colony-forming unit granulocyte-macrophage (CFU-GM) cultures were incubated with excess hemin as an iron source, the ferritin-mediated suppression on colony formation was blocked [156]. Therefore, the immunosuppressive effects of ferritin on myelopoiesis seem to be iron-dependent and are specific to H-ferritin with an active ferroxidase center.

Although, serum ferritin consists primarily of L-ferritin-rich complexes, slight elevations in H-ferritin are observed in some cancer patients. Therefore, elevation in H-ferritin could be a possible mechanism of immunosuppression in some tumor types. In light of the extensive infiltration of lymphocytes into tumors [157,158], it is also possible that the relatively low levels of H-ferritin in circulation are due to sequestration by activated lymphocytes.

7.3. Angiogenesis

Angiogenesis is a tightly-regulated process that requires the cooperation of many factors to ensure sufficient, but not excessive, vascularization of tumors. High molecular weight kininogen (HK) is a plasma glycoprotein that plays a role in the intrinsic coagulation pathway [159,160]. It functions as a cysteine protease inhibitor and is a precursor to the vasodilator nanopeptide bradykinin [161,162]. Ferritin can bind HK and reduce its proteolytic cleavage and subsequent production of bradykinin [163–165]. Ferritin can also bind the other HK cleavage product — the anti-angiogenic 2-chain high molecular weight kininogen (HKa) [65]. The binding of HKa to ferritin occurs at a Kd approximately 10 times lower than to HK indicating that the binding of ferritin to HKa may be more physiologically significant [65,163]. The incubation of endothelial cells with HKa and L subunit-rich ferritin protected them from HKa-induced apoptosis and allowed them to migrate and form vessel structures [65]. This protective effect was not dependent on the iron content of ferritin. Furthermore, deletion mapping of HKa revealed that ferritin specifically binds domain 5 in HKa which is necessary for its anti-angiogenic functionality [65]. In a tumor mouse model, the subcutaneous injection of a mixture of prostate cancer cells with HKa and ferritin induced normal vessel formation within the tumor. The protective effects of ferritin on endothelial cells are due to the restoration of survival and adhesion signaling, such as AKT, ERK1/2, FAK and paxillin, which are blocked by the binding of HKa to UPAR [166].

7.4. Ferritin as a signaling factor

The increase in serum ferritin during inflammatory conditions has led Ruddell et al. to speculate a role for ferritin as a pro-inflammatory cytokine [167]. This group has demonstrated that the addition of L subunit-rich ferritin complexes to primary rat hepatic stellate cells led to a time- and concentration-dependent increase in the phosphorylation of IKK α/β and the subsequent activation of NFκB transcription factor. Ferritin increased the expression of several NFκB-response genes including the pro-inflammatory cytokine IL-1β. The effects of ferritin were independent of iron as iron-rich and iron-depleted ferritins had similar effects.

Moreover, extracellular ferritin was also shown to stimulate the proliferation of epithelial breast cancer cells. The addition of ferritin extracted from the liver or spleen to the culturing media increased the proliferation of both MCF7 and T47D cells [53]. Ferritin was taken up by breast cancer cells in a temperature-dependent manner indicating a direct interaction. Ferritin's proliferative influence was seemingly independent of iron as 1) iron-poor ferritin had similar effects to iron-rich ferritin, and 2) ferritin did not increase intracellular iron levels [53].

8. Ferritin membrane receptors

Many normal and malignant cell lines specifically bind ferritin [67,150,168–171]. Most of those studies focused on H-ferritin and demonstrated that it is internalized through receptor-mediated endocytosis with a binding association constant ranging from 5 to 30 nM [169–172].

The first ferritin receptor to be identified was the murine T cell immunoglobulin and mucin domain 2 (TIM-2) [173]. TIM-2 shows specific affinity to H-ferritin but not L-ferritin and is expressed on splenic B cell, renal tubule cells, liver cells, and oligodendrocytes [148,173]. Moreover, it is capable of internalizing ferritin into endosomes and lysosomes [170,171,173], and its protein levels are responsive to iron loading and chelation [148]. TIM-2 does not have a human ortholog, and thus the question of the human H-ferritin receptor is still unresolved. Recently, Li et al. have argued that transferrin receptor 1 (TfR1) is responsible for most of the H-ferritin binding in human lymphocytes [174]. Using cell line and cDNA library screens, Li et al. identified TfR1 as a differentially expressed membrane protein in the cells capable of H-ferritin binding. However, whether or not transferrin receptor can account for the immunosuppressive effects observed with H-ferritin in lymphocyte cultures is yet to be determined [67]. Intriguingly, the extracellular domain of chemokine receptor CXCR4 seemed to directly interact with H-ferritin [175] suggesting further exploration of CXCR4 as a human H-ferritin receptor is warranted.

Recently, scavenger receptor class A member 5 (Scara5) was identified as the L-ferritin receptor [96]. Scara5 shows sequence and structural homology to class A scavenger receptors, but unlike other class A receptors it is unable to endocytose acetylated or oxidized low density lipoprotein [176]. Earlier expression profiling of Scara5 in mice has demonstrated that its expression is restricted to populations of epithelial cells primarily in the lung, trachea, testis, and bladder [176]. Scara5 was expressed in the murine developing kidney and was capable of delivering iron through ferritin endocytosis and thus replacing transferrin receptor as the major iron delivery pathway. Although its expression has not been comprehensively examined in tumors tissue, Scara5 expression correlated with favorable prognosis in breast and hepatocellular carcinoma patients [177,178].

9. Future and therapeutic considerations

The increase in intratumoral and serum ferritin in cancer patients raises questions about whether or not these two phenomena are connected. In addition to the evidence supporting that ferritin is released locally with tumors, ferritin is localized primarily within tumor-associated macrophages which are one of the few cell types capable of ferritin secretion. Although other sites, such as the liver and bone marrow, could be contributing to the serum ferritin increase, the relative contribution of TAMs to serum ferritin should be explored. There are currently no reliable biomarkers that allow for the non-invasive detection of TAM recruitment and activation.

On a molecular level, the prognostic value of ferritin within tumor tissue is still unclear. It is tempting to speculate that ferritin is acting as a factor with a multifunctional role during tumorigenesis. For example, a single ferritin complex can include both H and L subunits and therefore could have functional significance as a proliferative/angiogenic (primarily due to L-ferritin) or immunosuppressive factor (primarily due to H-ferritin). The H:L ratio within a single complex can modulate its functional potential in a context-dependent manner. Therefore, extracellular ferritin may represent a multifunctional molecule that simultaneously contributes to the many aspects of tumorigenesis. The coupling of angiogenic and immunosuppressive programs has been speculated previously in the context of inflammation and wound-healing [179]. Future studies should aim to manipulate ferritin levels in TAMs and examine their effects on the different aspects of tumor development (Fig. 1).

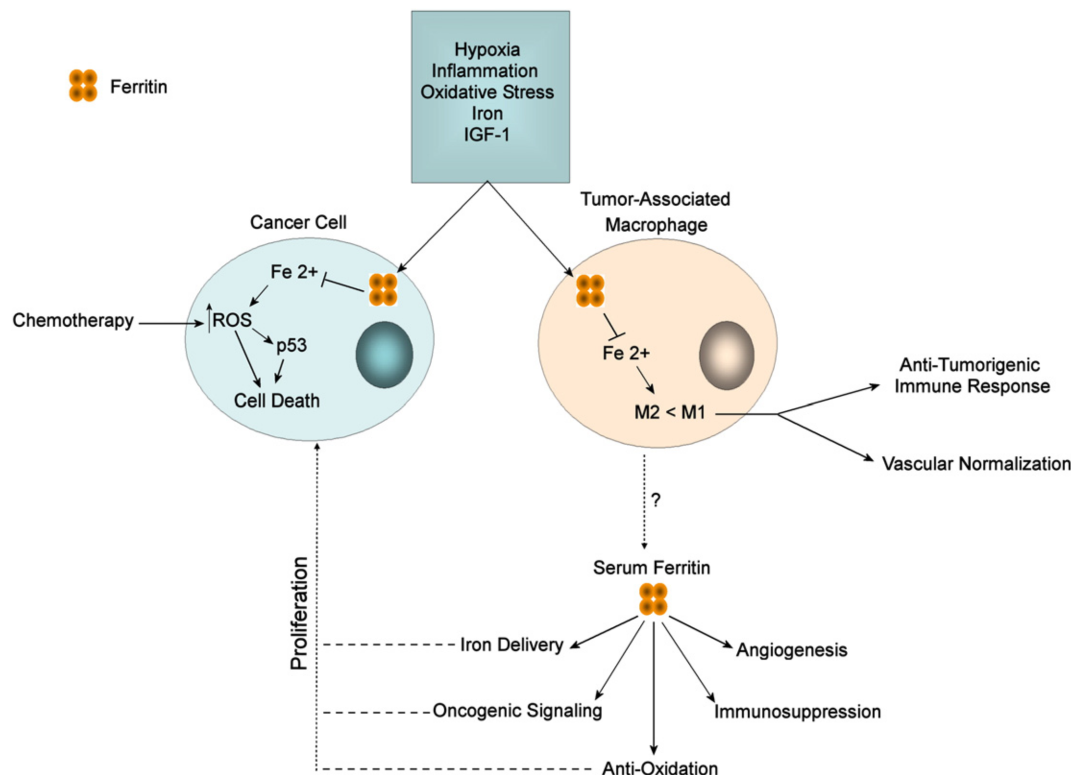

Fig. 1. The function and regulation of ferritin in tumors. Ferritin protects cancer cells from the iron-induced generation of reactive oxygen species (ROS) thus increasing their resistance to chemotherapy. In tumor-associated macrophages, ferritin plays a role in maintaining a pro-tumorigenic (M2) program. Aside from its intracellular roles, serum (extracellular ferritin) can stimulate angiogenesis, immunosuppression, and proliferation through various mechanisms.

From a therapeutic perspective, the down-regulation of ferritin within tumor-associated macrophages would impair their ability to safely maintain their iron stores which can lead to cell death. The ablation of tumor-associated macrophages has been shown to sensitize breast tumors to chemotherapy and impair their vascularization [180–182]. In addition to the resulting cytotoxic effects from unbound intracellular iron, the down-regulation of ferritin and the subsequent increase in free iron may induce an unrestrained pro-inflammatory (M1) state in macrophages. When loaded with iron, macrophages have been shown to secrete more pro-inflammatory cytokines (TNFα and IL-6) and hydroxyl radicals which damage and induce apoptosis in cancer cells [45,71]. Therefore, down-regulation of ferritin in macrophages and the subsequent decrease in their iron storage capacity could lead to the reprogramming of tumor-associated macrophages from a pro-tumorigenic wound-healing (M2) to an anti-tumor pro-inflammatory (M1) program. Moreover, the reprogramming of tumor-associated macrophages may improve the structure of tumor vascular and thus improve drug delivery [183].

The antioxidant nature of ferritin may decrease the efficacy of many chemotherapeutic drugs (such as anthracyclines and alkylating agents) as their cytotoxicity is induced partly by the production of ROS and the induction of oxidative stress [184,185]. It is important to note that ROS is capable of inducing cell death via p53-dependent and p53-indepdent mechanisms [186–189]. Therefore, it is logical to speculate that the knockdown of ferritin may be a viable approach to sensitize cancer cells to the cytotoxic effects of chemotherapy as it would increase the generation of free radicals from Fenton chemistry. This strategy has been successfully demonstrated in ferritin-rich glioma cells as liposomes carrying H-ferritin siRNA sensitized cancer cells to the DNA-alkylating agent BCNU [190]. Alternatively, exploration of the regulation of the proteasomal degradation of ferritin may produce several therapeutic targets that may cause an acute increase in intracellular iron levels and ROS. One interesting hint for this largely unknown regulatory circuit is the interesting connection between the iron exporter ferroportin and ferritin. The overexpression of ferroportin can cause the mobilization of ferritin's iron and the proteasomal degradation of the ferritin shell [191].

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