

Ferritin: A multidimensional bio marker

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Abstract

Ferritin is a protein that plays a very important role in the storage of iron in the body. Ferritin is now emerging as a very important factor in the pathogenesis of diseases such as atherosclerosis, cancer, neuropsychiatric disorders to name a few. A number of mechanisms such as pro oxidant and pro inflammatory pathways are responsible for this. Ferritin performs diverse functions in the body besides iron storage. Alterations in the structure and concentration of ferritin have been observed in various diseases, establishing it as an important marker. The present review aims to understand the mechanisms involved in the cyto toxicity of iron and discuss some important disorders in which ferritin has emerged as an important bio marker. As ferritin has been established as an etiological factor in several disorders: pharmacological interventions to reduce its levels may prove beneficial. Indeed iron chelation therapy has been tried in various cancers with promising results.

FERRITIN : A MULTIDIMENSIONAL BIOMARKER

Ferritin and iron homeostasis have been implicated in the pathogenesis of many diseases, including disease involved in iron absorption transport and storage, atherosclerosis, cancer, neuropsychiatric disorders and diabetes.

FERRITIN STRUCTURE

Ferritin is the major intracellular iron storage protein in all organisms. The ferritin molecule is a hollow protein shell (outside diameter 12-13 nm, inside 7-8 nm, Mr about 500KDa) that permits storage of upto 4500 Fe(III) atoms [1].

Each apoferritin shell is assembled from 24 polypeptide chains of 2 species, the heavy subunit (H-subunit) and the light subunit (L-subunit). The H subunit has a molecular weight of 21KDa and has a relatively acidic electrophoretic mobility, whereas the L subunit is a smaller protein with a molecular weight of 19KDa. The H-subunit functions as a ferroxidase that oxidizes iron to the Fe(III) form, whereas the L-subunit is associated with iron nucleation, mineralization and long term iron storage [23]. The ratio between H and L subunit in a ferritin shell varies widely in different tissues. L-subunit rich ferritin predominates in iron storage organs such as the heart and pancreas [4567].

The H to L ratio is not fixed, but is rather quite plastic: it is readily modified in many inflammatory and infectious conditions and in response to xenobiotic stress,

differentiation and developmental transitions [8].

FERRITIN:GENETICS

About 16 copies of the H-gene and 5 copies of the L-gene have been identified in humans, but the most of them are intronless pseudogenes. A single functional human H gene and L gene have been identified and are localized on chromosome 11q 23 and 19 q 13.3 respectively [91011].

A novel mitochondrial ferritin (MtF) is encoded by an intronless gene on chr 5q23.1. The MtF may have a role in regulating mitochondrial iron homeostasis and heme synthesis [12].

REGULATION OF FERRITIN LEVEL ROLE OF IRON

The content of cytoplasmic ferritin is regulated by the translation of ferritin H and L mRNAs in response to an intracellular pool of chelatable or labile iron. Thus, when iron levels are low, ferritin synthesis is decreased; conversely when iron levels are high, ferritin synthesis increases [131415161718].

This process is mediated by interaction between RNA binding proteins and a region in the 5 untranslated region of ferritin H and L mRNA termed the iron responsive element (IRE) that has a stem loop secondary structure [19].

There are two RNA binding proteins, iron regulatory proteins, 1 and 2 (IRP1 and IRP2), that bind to this stem

loop structure and inhibit mRNA translation [20].

ROLE OF CYTOKINES AND INFLAMMATION[]

TNF- α and interleukin 1- α transcriptionally induce the H chain of ferritin, suggesting that pathway related to inflammation and stress can impact on ferritin regulation. The observation that ferritin H could be selectively transcriptionally regulated provided a molecular model to explain the linkage between inflammation and the modulation of subunit composition and content of ferritin, largely inexplicable based on posttranscriptional regulation alone.

Ferritin H is regulated by TNF- α hme a cis-acting region (FER2) located 4.8 kb upstream of the transcriptional start site that binds the transcription factor NF κ B.

Cytokines also regulated ferritin postranscriptionally. In the Hep G 2 hepatic cell line, induction of ferritin synthesis was observed with a number of cytokines: IL-1 α , IL-6, TNF- α [2324].

Secretion of ferritin is stimulated by cytokines. In primary cultured human hepatocytes, IL-1 α and IL-6 induced a transient secretion of ferritin at 24 hr followed by a decline to baseline, whereas TNF treatment result in a sustained increase in ferritin secretion, reaching a level 10 times that found in untreated cells. Cytokines play a pivotal role in the cellular response to infection and ferritin plays a prominent role in the cytokine response. Lipopolysaccharide (LPs), a component of the outer membrane of gram-negative bacteria, elicits a variety of reactions that involve ferritin LPS administered endotracheally to rats induced ferritin protein expression.

ROLE OF NITRIC OXIDE (NO) AND OXIDATIVE STRESS.

There is strong experimental support for ferritin as a protectant against oxidant stress. In tumour cell lines, sensitivity to oxidants was inversely correlated with ferritin protein levels. Increased ferritin levels reduce the low molecular weight iron pool. These findings are consistent with observations that a reduction in ferritin sensitizes cells to pro oxidant cytotoxicity, that over-expression of ferritin reduces oxidant species in cells challenged with oxidants and reduces oxidant toxicity as well as the importance of ferritin H ferroxidase activity in limiting oxidant toxicity [25]. Oxidants induce ferritin transcription by directly targeting conserved region of ferritin genes. Oxidative stress can also contribute to ferritin induction by inactivating IRPI through

reversible oxidation of critical cysteine residues.

Oxidants may also alter ferritin transcription and translation through release of iron from cellular proteins. Oxidants, including ROS and nitric oxide, may release iron from ferritin, IRPI or hemoglobin, either directly or through heme oxygenase. This can lead to ferritin induction through IRP inhibition, and perhaps through direct iron-mediated transcriptional regulation of ferritin. Cytokines may also affect ferritin translation indirectly through their ability to induce iNOS and increase NO. No in turn causes the activation of both IRP 1 and IRP2. Mechanisms hypothesized to underlie NO-mediated induction of IRP binding activity include cluster dis assembly (IRP1), intracellular iron chelation (IRP1 and IRP2), or increased denovo synthesis (IRP2) [2627].

BIOCHEMICAL BASES OF IRON CYTO-TOXICITY

IRON HYPOTHESIS

Iron donates of reactive oxygen species (ROS), such as the hydroxyl radical (OH) from H₂O₂ via the fenton reaction.²⁸ Excessive iron in tissue may catalyze the formation of highly reactive forms of oxygen free radicals. Lipids proteins and DNA are biomolecules targeted by iron-mediated ROS. These radicals can causes oxidation of low density lipoprotein (LDL). Ox LDL can activate endothelial cells to produce a variety of cell adhesion molecules and chemotactic factors that promote migration and binding of monocytes and lymphocytes to the arterial wall. Macrophages are then formed and further inflammation leads to the formation of foam cells and development of atherosclerosis.

ROS produced by iron may specifically target some tumour suppressor genes, leading to a novel concept of 'genomic sites vulnerable to Fenton reactions'. To support this concept, it has been shown that lipid peroxidation derived aldehydes such as 4-hydroxynonenal (4-HNE) can interact with DNA to form exocyclic guanine adducts and 4-HNE-DNA adducts are preferentially formed at the third base of codon 249 in the p53gene.²⁹ Administration of Fe-NTA, a renal carcinogen, can specifically cause allelic loss of the p16 tumour suppressor gene in renal tubular cells [282930].

IRON-INDUCED OXIDATIVE-RESPONSIVE TRANSCRIPTION FACTORS.

Besides the direct attack, of iron-mediated ROS on DNA, it has recently been proposed that iron can induce early

signaling pathways that may modulate activities of several oxidative-responsive transcription factor, such as activator protein-1 (Ap-1) and nuclear factor kappa B (NF-KB) [31].

Since putative AP-1 and NFKB binding sites are found in the promoters of many genes, such as interleukin-6 (IL-6) and IL-8, activation of these transcription factors may contribute to the up-regulation of those cytokine genes [3233].

IRON INDUCED RESPONSE TO HYPOXIA.

the formation of new blood vessels and angiogenesis is well known as a crucial step in tumour growth and progression. Angiogenesis can be induced by hypoxic conditions and regulated by the hypoxia-inducible factors (HIF-1) [34].

HIF is a heterodimer composed of α and β subunits. HIF-1 α hydroxylase that hydroxylates HIF-1 α at proline 564 is iron dependent. This process mediates the ubiquitination of HIF-1 α for proteosomal degradation. Iron depletion or hypoxia prevents hydroxylation of proline 564 that leads to increased p53 levels [35].

OTHER MECHANISMS

There are at least two more plausible mechanisms for iron carcinogenesis, that is:

- iron serves as a nutrient for cell growth
- iron may affect the immune system

Iron is an absolute requirement for cell proliferation, as iron-containing proteins catalyze key reactions involved in oxygen sensing, energy metabolism, respiration and DNA synthesis. Without iron, cells are unable to proceed from G1 to the S phase of cell cycle [36].

Iron also modulates immune effector mechanisms, such as cytokine activities (INF-gamma effector pathway towards macrophages), nitric oxide formation or immune cell proliferation, and thus hosts immune surveillance [37]. The immuno-regulatory balance induced by iron may increase growth rate of cancer cells and infectious organisms leading to cancer development [38394041].

Ferritin iron represents approximately 20% of total iron in the body and serum ferritin has a relative high reliability coefficient among pre-and post menopausal women tested[4243]. Since serum ferritin concentration correlates well with various parameters: it could be a good biomarker for body iron status. Yet, ferritin concentration can be

influenced by sex, age, infection and frequency of blood donation. Therefore, a molar ration of iron per ferritin may provide better iron indicators for cancer risk evaluation than ferritin itself [44].

FERRITIN IN HUMAN DISEASES

FERRITIN IN INHERITED HUMAN DISEASE

Genetic mutations of the ferritin IRE region as well as coding regions of ferritin cause inherited human diseases. Ferritin L IRE mutations cause the hereditary hyperferritinaemia-cataract syndrome, which is a cataract syndrome, which is an autosomal dominant disease that is, characterized the elevated serum ferritin levels and an early onset bilateral cataract [1].

Neuroferritinopathy, a dominantly inherited movement disorder that is characterized by a decrease in serum ferritin and an abnormal deposition of ferritin and iron in the brain, is caused by a mutation in the C-terminus of the ferritin gene.

A single A to U mutation at position 49 in the second residue of the 5-bse IRE loop sequence of H-ferritin leads to an increased affinity of the IRE for IRP, which reduces ferritin H protein and leads to iron overload [1].

FERRITIN IN ATHEROSCLEROSIS

Results from animal studies support the notion that iron plays a significant role in the progression of atherosclerosis. Several studies supported our association of free iron with lipid induced atherosclerotic lesion formation. Fatty streak resistant mice as opposed to fatty streak-susceptible mice showed significantly low intracellular free iron and high levels of liver apoferritin in response to an atherogenic diet. A number of studies have been carried out to assess the role of ferritin in the pathogenesis of CAD in humans.

Solonen et al first reported a significant association between the serum ferritin level and the risk of MI in a Finnish Kuopio Inchaemic Heart Disease Risk factor study (KIHD) of 1931 middle-aged men during an average followup of 3 years [45]. They found that Finnish men with a serum ferritin $\geq 200 \mu\text{g/L}$ had a 2.2 higher risk of MI than did men with lower serum ferritin. In a study that analysed the relationship between sonographically assessed carotid atherosclerosis and body iron stores, Klechl et al reported an increase in the odds ratio of 1.54 per $100\mu\text{g/L}$ of s. ferritin ($p < 0.001$) in a cross-sectional analysis of 847 men and women aged 40 to 79 years [46].

Iron depletion has also been shown to reduce the risk of MI and other cardiovascular disease events. Facchin and Saylor explored the effect of iron depletion on cardiovascular risk factors in 31 patients who were phlebotomized monthly or bimonthly to achieve iron depletion state near to deficiency levels. In this study, they observed a significant increase in HDL and reductions in LDL, triglycerides, fibrinogen and blood pressure ($p < 0.001$) [47].

Tuomainen et al conducted a prospective, 5.5 year followup study involving a cohort of 2682 finnish men, and showed that MI was decreased 86% in blood donors as compared with non-donors ($p < 0.001$) [48].

However, some epidemiological studies have failed to confirm the presence of a direct association between the iron status and the risk of developing cardiovascular disease.

In the US Physician study, Stampfer et al found that men with serum ferritin levels $\geq 200\mu\text{g/L}$ were not at a higher risk of MI [49]. Moore et al examined the association between serum ferritin and carotid arterial intimal thickening by carotid duplex ultrasound in a case control study from the Atherosclerosis Risk in communities (ARIC). They found no association between serum ferritin concentrations and the severity of arterial intimal thickening [50].

FERRITIN IN CANCER

Iron-induced malignant tumours were first reported in 1959 by repeated intramuscular injection of iron dextran complex in rats. Beginning in the 1980s, some epidemiological reports have associated increased iron exposure with elevated cancer risk in either prospective or ratio-prospective studies.

COLORECTAL CANCER

A study by Stevens et al and the follow-up study by Wurzelman et al on the cohort of the National Health and Nutrition Examination survey I showed a positive association between dietary and body iron stores with colorectal cancer risk [51,52]. Neison et al and Bired et al showed that body iron stores were positively associated with the development of precancerous lesions in the colon, colonic adenoma or polyps [53,54].

Increasing iron concentration in human intestinal Caco-2 cells resulted in increased protein and DNA oxidative damage, as shown by the immunoreactivity for 4-hydroxy-2-nonenal modified proteins and 8-oxo-2-deoxy guanosine.

LIVER CANCER

Most experimental and human data support the hypothesis that iron overload is a risk factor for liver cancer. It is the excessive accumulation of iron in hepatocytes that causes hepatocellular injury, which leads to the development of fibrosis, cirrhosis and hepatoma [55].

In chemical induced hepatocarcinogenesis, iron was shown to greatly sensitize mice to the induction of hepatic porphyria by hexachlorobenzene [56]. Levels of lipid peroxides as well as 8-hydroxy-2 deoxyguanosine and oxidative DNA damage, were significantly increased in mice following combined iron/hexachlorobenzene.

In attempts to study the cancer initiating, promoting and/or progressing effects of excess hepatic iron, dietary iron overload in combination with fusonisin B1, or polychlorinated biphenyls, or diethylnitrosamine, were tested in animal models. Generally speaking, iron depletes intracellular antioxidants such as ubiquinones, and acts at least as a promoter of already initiated hepatocytes in HCC development [57,58,59,60,61,62,63].

KIDNEY CANCER

Numerous studies have lined increased risk of kidney cancer in workers of iron and steel industry.

Ferric nitrilotriacetate (Fe-NTA) is a renal carcinogen in rodents [64]. Neither H, K and N-Ras oncogenes nor were p53 tumour suppressor genes found mutated in the RCC tissues induced by NTA indicating that iron is responsible for RCC development [65,66]. In contrast p16 tumour suppressor gene was shown to be vulnerable. Ferric ion complexed with NTA in this model is thought to be a tumour initiator as well as promoter through the production of reactive oxygen species and free radicals [64].

The role of iron in estrogen induced renal carcinogenesis was studied by investigating the effects of iron content of hamster diets on tumour induction by estradiol. The renal tumour incidence increased significantly in hamsters treated with estradiol plus a diet enriched in iron [67].

Redox cycling of catechol estrogen metabolites between quinone and catechol forms can cause the reduction of Fe^{3+} to Fe^{2+} and release Fe^{2+} from ferritin, which in turn generates hydroxyl radicals by iron-catalyzed reactions [68].

BREAST CANCER

Elevation of serum ferritin in breast carcinoma is well

known, and levels suggest the severity of the disease. Weinstein et al found that malignant tissue had 6 times the ferritin concentration, as did benign breast tissue [69]. Malignancies with the highest ferritin concentrations were more anaplastic suggesting that the major site of the increased ferritin was the malignant epithelium. The postulated that ferritin may be a marker of neoplasia. Studying ferritin levels in breast carcinoma tissue may give us information about anaplasia and the proliferation index.

LUNG AND STOMACH CANCERS

Workers of iron ores and steel foundries have an elevated risk of lung and stomach cancers.

In a nested case control study comprising of 144 male lung cancer cases and 558 controls in a large iron and steel foundry in Austria and Spain, workers having ever been employed in the blast furnace had an excess lung cancer risk (odds ratio = 2.55) as compared to a reference group of workers not employed in a metal producing department [70].

It has been shown that some mineral dusts from iron are mines are very active in an oxidative process in aqueous medium, implying the formation of radical oxygen species.

The presence of a Fe²⁺ ion on the surface of the particles or its desolution from the surface may be responsible for the oxidant formation. Similarly, it was shown that redox activities of coal dusts, coal fly ashes and asbestos correlated well with levels of bioavailable iron in the dusts, extent of ferritin induction by the dusts as well as levels of lipid peroxidation in cells treated with the dusts [71727374757677].

The role of ferritin has also been noticed in neuroblastomas[78] and melanomas [79].

FERRITIN IN NEUROPSYCHIATRIC DISEASES

ALZHEIMER'S DISEASE

Pinero et al reported that iron accumulates in the brain in alzheimer's disease without a concomitant increase in ferritin. An increase in iron without proper sequestration can increase the vulnerability of cells to oxidative stress. A more stable IRE/IRP complex in the AD brain could increase stability of the transferrin receptor mRNA and inhibit ferritin synthesis [80].

PARKINSON'S

Semiquantitative histological evaluation of brain iron and ferritin in parkinson's disease was performed. Results indicated a significant increase in Fe(III) and ferritin in the

substantia nigra of parkinsonian brains [81].

RESTLESS LEG SYNDROME

In a study conducted by Mizuno et al, a positive correlation was observed between serum and cerebrospinal fluid ferritin levels. They concluded that low iron concentration caused by the dysfunction of iron transportation from serum to CNS led to the pathogenesis of restless leg syndrome [82].

ATTENTION DEFICIT HYPERACTIVITY SYNDROME (ADHD)

ADHD is one of the most common neuropsychiatric disorders of childhood. It consists of two symptom domains, hyperactivity and inattentiveness. Lower ferritin levels are associated with higher hyperactivity scores [83].

MULTIPLE SCLEROSIS

Deposition of iron has been recognized recently as an important factor of pathophysiologic change including neurodegenerative process in multiple sclerosis. Quantitative measurements of iron content with magnetic field correlation imaging which demonstrate increased accumulation of iron in the deep gray matter in patients with multiple sclerosis which may be associated with the disrupted iron outflow pathway [84].

FERRITIN IN DIABETES MELLITUS 2

The level of ferritin in DM type 2 was evaluated in the E-PIC – Norfolk prospective study. Forouhi et al found that baseline serum ferritin was higher among cases than control participants (men 96.6 v/s 67.8 ng/ml, $p < 0.001$, women 45.9 v/s 34.8 ng/mL, $p = 0.005$). In analysis, adjusted for known risk factors (age, BMI, Sex, family history, physical activity, smoking habit and dietary factors measured by 7-day food diary, the risk of diabetes was markedly elevated in participants with clinically raised ferritin compared with the lowest quartile. This shown that serum ferritin is an important and independent predictor for the development of diabetes. This finding may have important implication for understanding the actiology of diabetes [85].

IRON CHELATION: ROLE IN THERAPEUTICS.

The increased dependence of tumour cells on iron has led to the suggestion that depleting iron may represent a strategy to limit tumour growth. Indeed, tumour cells in a highly proliferative state have a high density of transferrin receptors, and antisense cDNA for the transferrin receptors, was shown to reduce TFR mRNA and expression, resulting in more inhibition of growth of human breast

carcinoma cells than normal breast cells. Monoclonal antibodies against TFR severely restricted the growth of lymphoma tumours in mice [86].

The chelator currently used to treat iron overload disease, deferoxamine has shown anti-proliferative activity against leukemia and neuroblastoma activity against leukemia and neuroblastoma cells in vitro, in vivo, suggesting that iron deprivation may be a useful anti cancer strategy.

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