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Antiviral Activities of Ferrous and Ferric Iron in Viral Prevention, Entry and Uncoating, Replication, mRNA Degradation and DNA/RNA Virus Replication, and Release and Budding along Viral Life Cycle

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Abstract

Iron is important trace element in the human body that the absorption of iron is strictly controlled through hepcidin, while there are no efficient physiologic mechanisms to excrete iron from the body, in which cellular iron homeostasis is controlled by iron uptake at the plasma membrane. Viruses are the smallest pathogens and consisted of a single nuclei acid (RNA or DNA) encased in a protein shell which are covered with a lipid-containing membrane. A virus infection depends on factors both in the virus and the host that the most important factor in a virus is genomic alterations which the viral life cycle of a virus starts with its entry into a host. Iron regulator hepcidin has a direct antiviral activity against HCV replication. Iron is released from transferrin within the endocytic vesicle and reduced, Fe(Ⅱ) is transported across the endosomal membrane by the permease DMT1. Fe^{2+} chelator (BIP) inhibits DNA virus replication. Fe(Ⅲ) inhibits replication of DNA and RNA viruses.

Lactoferrin has effective antiviral activity for viral prevention, enveloped and naked viruses such as rotavirus, enterovirus, and adenovirus. TfR1 plays a role in HCV infection at the level of glycoprotein-mediated entry. Iron can be toxic when present in excess for its capacity to donate electrons to oxygen, thus causing the ROS generation of superoxide anions and hydroxyl radicals, superoxide hydrogen, and copious ROS and RNS are synthesized. Oxygen consumption in the peroxisome leads to H_2O_2 production, and H_2O_2 releases into the cytosol which contributes to oxidative stress. Chelators of DFO and deferiprone are ideal candidates for use during co-infection and excess iron situations because they have been implicated in HIV replication inhibition. The evolution of iron chelators from a range of primordial siderophores and aromatic heterocyclic ligands has led to the formation of a new generation of potent and efficient iron chelators.

Keywords: *Ferrous and ferric ions, Iron deficiency and overload, HIV replication, HCV infection, Lactoferrin, Ferroportein, DMT, DFO, ROS, Iron chelation.*

Abbreviations

AU=adenylate-uridylate, BIP=bipyridyl, BLf=bovine lactoferrin, BLV=bovine leukemia virus, BVDV=bovine diarrhoea virus, CHC=chronic hepatitis C, DENV=dengue virus serotypes, DFO=desferrioxamine, DMT=divalent metal transporter, DST=DownSTream, Dcytb=duodenal cytochrome b ferrireductase, EDTA=ethylene diamine tetraacetic acid, EGF=epidermal growth factor, EV=Enterovirus, EVs=extracellular vesicles, FAC=ferric ammonium citrate, FHC=ferritin heavy chain, FLC=ferritin light chain, FPN=ferroportin, HBC=hepatitis B virus, HCC=hepatocellular carcinoma, HCMV=human cytomegalovirus, HCV=hepatitis C virus, HEV=Hepatitis E virus, HH=hereditary hemochromatosis, HIV=human immunodeficiency virus, HO=heme oxygenase, HSCs=hepatic stellate cells, HSV=Herpes Simplex Virus type-1 (HSV-1), IAV=influenza A virus, IFNγ=interferon gamma, IONPs=iron oxide nanoparticles, IREs=iron-responsive elements,

IRP=iron regulatory protein, JEV=Japanese encephalitis virus, MHC=major histocompatibility, NADPH= nicotinamide adenine dinucleotide phosphate, PDGFR-**β=**platelet-derived growth factor receptor beta, PPI= protein-protein interaction, RBCs=red blood cells, RE=reticulo-endothelial, ROS=reactive oxygen species, RNS=reactive nitrogen species, RR=ribonucleotide reductase, RT=reverse transcription, SCD=sickle cell disease, TfR=transferrin receptor, XO=Xanthine oxidase, XD=xanthine dehydrogenase, XOR=xanthine oxido-reductase, ZIKV=Zika virus,

Introduction

Iron is an essential but potentially hazardous bioelement from bacteria to mammals that its importance lies in its ability to mediate electron transfer which in the ferrous (Fe^{2+}) state, iron acts as an electron donor, while in the ferric (Fe^{3+}) state it acts as an acceptor. Iron plays a vital role in the catalysis of enzymatic reaction that involve electron transfer (redox reaction). In healthy individuals, the amount of human body iron is maintained within a range of 4～5 g by a strict control of its absorption, mobilization, storage, and recycling that Fe excretion is not actively controlled and skin desquamation is the major mechanism described so far, accounting for about 1～2 mg/day [1]. Iron is required for many vital functions including oxygen transport and energy metabolism that about 3/4 of total body iron is present in heme associated with hemoglobin, myoglobin and cytochromes, while nonheme iron is either stored in tissues or transported in the circulation bound to the serum protein transferrin in which low body iron status results in iron-deficient anemia, impaired motor activity and poor brain development [2]. The other, excess iron is toxic and high iron stores promote oxidative stress with inflammatory responses and cellular injury that eventually leads to cell damage and death which high iron status is related to many infectious diseases and inflammatory responses, as exemplified by malaria, viral infection and neurodegeneration [2].

On the other hand, virus structures have been shown that viruses are comprised of a protein coat, or capsid that encloses either DNA or RNA, the genetic code for the virus which some viruses also a membrane made up of lipids that covers the capsid, but not all viruses have this membrane. In virus and pathogen, not only can the nutritional status of the host affect the immune response, but it can also affect the viral pathogen that the oxidative stress status of the host can have a profound influence on a viral pathogen [3]. As viruses are obligate intracellular pathogens they cannot replica without the machinery and metabolism of a host cell. Although the replicative life cycle of viruses differs greatly, there are six basic stages that are essential for viral replication [4]; ① Attachment: Viral proteins on capsid and phospholipid envelope interact with specific receptors on the host cellular surface, ② Penetration: Fusion of viral and cellular membranes, ③ Uncoating: viral capsid is removed and degraded by viral enzymes or host enzymes releasing the viral genomic nucleic acid, ④ Replication: Transcription or translation of the viral genome is initiated after uncoating of the viral genome that the viral replication stage differs greatly between DNA and RNA viruses and viruses with opposite nucleic acid polarity, ⑤ Assembly: Viral proteins are packaged with newly replicated viral genome into new virions that are ready for release from the host cell, ⑥ Virion release: Lysis and budding.

Iron induced viral activity is decreased that iron overload toxicity is shown to associate with chronic liver diseases which lead to hepatic fibrosis and subsequently the progression to cancer through oxidative stress and apoptotic pathways, in which in human and animal research models, iron overload may be related with hereditary hemochromatosis, thalassemia, and hepatic diseases such as chronic viral hepatitis, alcoholic hepatitis that iron is stored within liver cells in various forms including Fe containing enzymes, ferritin, hemosiderin, and heme [5].

In this review, firstly cellular iron metabolism, iron homeostasis, and inflammation are dealt with important roles in pathophysiologic conditions. Secondly iron deficiency and overload, and iron-host cell-virus pathogen interactions are described for both host and pathogen, complex systems of acquisition and utilization. Thirdly, antiviral activities of iron ions occur by the inhibitions of viral entry, replication, mRNA degradation and DNA/RNA virus replication, and release and budding along viral life cycle, and antiviral actions of ferritin, lactoferrin, and

ferroportin are taken up specially. Lastly, iron induced ROS production and iron chelation lead to oxidative stress and iron-chelator efficiency, subsequent viral inhibition of replication, DNA/RNA damages are discussed.

Cellular Iron Metabolism, Iron Homeostasis, and Inflammation

Iron serves important functions for mammalian cells as it is involved in cell proliferation, metabolism and growth that these processes are controlled by a variety of iron- and heme-containing proteins, including enzymes involved in DNA stability and cell cycle progression, mitochondrial enzymes involved in respiratory complexes, and detoxifying enzymes. The processes of Fe uptake and recycling supply the daily need for hemoglobin(Hb) synthesis (25-30 mg) that degradation of senescent red blood cells (RBCs) by splenic macrophages accounts for 90% of total Fe recycling, remaining 10% comes from the diet which this process occurs in the duodenum where the duodenal cytochrome b ferrireductase (Dcytb) reduces it to Fe^{2+}, which is then offered to the divalent metal transporter-1 (DMT-1) for cellular uptake [1]. DMT-1 is revealed by the pathologic conditions caused by its mutations including a severe form of microcytic hypochromic anemia. Inflammation plays a critical role in controlling Fe metabolism, as the pro-inflammatory cytokines released upon immune cell activation alter the levels of proteins regulating iron homeostasis that Fe is essential for proliferation of both prokaryotes and eukaryotes, disruption of its homeostasis may either favor the establishment of the infection or act as a host defense mechanism to defeat pathogen invasion [1].

Cellular iron homeostasis is controlled by iron uptake at the plasma membrane, eliciting balanced iron distributions that patients with an inflammatory process may present with decreased, normal or increased total body iron stores which decreased iron stores in inflammatory disease and infection can either represent true preexisting iron deficiency or functional iron deficiency and inflammatory states increase serum ferritin levels as well as the release of inflammatory cytokines and may ultimately lead to hypoferraemia through an increase in hepcidin with consecutive iron sequestration in macrophages and duodenal enterocytes [6]. Further, inflammation reduces erythropoietin and bone marrow activity through inflammatory cytokines, including tumor necrosis factor α, interleukin 1 and hepcidin [6].

Iron Deficiency and Overload

Iron deficiency or overload drives to the development of several pathologies that the organism controls the dietary iron absorption by enterocytes which iron homeostasis is maintained [7]. These processes are controlled by hepcidin, a liver-derived hormone which synthesis is regulated by iron levels, inflammation, infection, anemia, and erythropoiesis. Iron deficiency anemia is the most common nutritional deficiency that it occurs more frequently in determined groups of individuals, such as the premenopausal woman, children, hospitalized individuals that require frequent diagnostic blood sampling and blood loosing patients [7]. During inflammation, macrophages produce in high levels, the pro-inflammatory cytokine that triggers the expression of hepcidin and, consequently, lowers dietary iron absorption and stored iron release [7]. Iron deficiency and its associated anemia may contribute to reduced energetic, lower aerobic capacity, decreased endurance, and fatigue that in practical terms, the functional limitations of iron deficiency and iron-deficiency anemia may affect the ability of women to participate in work, school, social, and family activities [8]. Iron deficiency may contribute to the cycle of poverty by limiting the ability of women to work that the use of iron supplements needs to be approached with caution in women with human immunodeficiency virus (HIV) and hepatitis C virus (HCV) infections. The effects of infectious HCV on iron metabolism in permissive Huh7.5.1-HCV cells have been studied, resulting that these cells express inappropriately low hepcidin mRNA levels and develop an iron deficient phenotype in response to HCV infection [9]. Iron deficiency and host defence will be described below [17].

On the other hand, iron overload is known to be toxic to many organs, particularly to the liver that is the major site of storage of excess iron which the most common form of iron overload is that related to classic hereditary hemochromatosis, in which due to mutations in the HFE hemochromatosis gene, there is excessive uptake of iron into enterocytes [10]. Thus, iron can cause or exacerbate liver damage, including

viral hepatitis that effects of iron and iron chelators on liver cell, some of which also expressed genes and proteins of the HCV which iron increased oxidative stress and led to up-regulation of heme oxygenase-1 (HMOX1) gene, a key cytoprotective gene. In mild iron overload, HFE S65C mutation was enriched in patients with high serum ferritin or increased transferrin (Tf) saturation compared with healthy controls that half of patients carrying the S65C mutation had evidence of mild or moderate hepatic iron overload but no signs of extensive fibrosis [11]. Further, chronic hepatitis C (CHC) leads to iron overload, and iron affects the HCV life cycle in turn that the mechanism involved in the complicated interaction between iron and HCV which excessive iron deposition occurs in the liver, heart, and endocrine organs, resulting in reactive oxygen species (ROS) through the Haber-Weiss and Fenton reactions [12]. Iron overload induced mitochondrial injury and increases the risk of hepatocellular carcinoma (HCC) development in transgenic mice expressing the HCV polyprotein [13]. Iron overload also down-regulates HIV-1 gene expression by decreasing the levels of viral RNAs that iron overload modulates the expression of many viral cofactors which among them, the downregulation of the REV cofactor elF5A may correlate with the iron-induced inhibition of HIV-1 gene expression [14]. HFE is major histocompatibility class I-like (MHC) molecule that has a function in cell and body iron metabolism which HFE is unable to bind iron but indirectly modulates the rate of iron accumulation in cells that are key in body iron trafficking and absorption [15]. In hemochromatosis of iron overload, individuals with otherwise intrinsically normal iron absorption machinery and erythroid iron needs have a positive iron balance of 1 to 2 mg of iron daily from birth that mild to moderate iron overload is common among patients with chronic hepatitis C(CHC): Up to 30%-40% may show increased serum transferrin-iron saturation and serum ferritin or increased hepatic iron concentration, the latter finding being particularly common in subjects with end-stage liver disease [15]. Iron overload is common in patients with chronic hepatitis C (CHC) that the positive role of iron on HCV translation, the mechanisms of which involve increased expression levels of factors associated with HCV internal ribosome entry site-dependent translation, such as cukaryotic initiation factor 3 and La protein [16]. Hemochromatosis exists both in primary and secondary form that primary or hereditary hemochromatosis (HH) is the most common inherited disease in Caucasians which a state of iron excess in hereditary hemochromatosis has different implications, since it involves preferential iron loading of the parenchymal cells and not the reticuloendothelial system, which in turn hinders the growth of many intercellular organisms [16].

The effects of iron deficiency and overload on host defence suggest that iron deficiency is remarkably protective against high-grade parasitaemia and severe malaria, perhaps explaining why more effective iron absorption and storage mechanisms conferred by haemochromatosis mutations are less common in the tropical regions than in northern Europe, and that iron overload has high protective effects for a host defence function of hepcidin in protecting host from infection by hepcidin-induced iron sequestration [17].

Iron-Host Cell-Virus Pathogen Interactions

Viruses are the smallest pathogens and consisted of a single nuclei acid (RNA or DNA) encased in a protein shell which are covered with a lipid-containing membrane. Clinical illness following a virus infection depends on factors both in the virus and the host that the most important factor in a virus is genomic alterations which the viral life cycle of a virus starts with its entry into a host; it reaches the susceptible target cell, enters it, replicates and causes cell injury, and may be cell death. Iron is essential for both host and pathogen, and complex systems of acquisition and utilization have evolved in competition that iron is a key regulator of host pathogen interactions. Iron deficiency significantly impairs cell proliferation and immune function, whereas, iron overload is equally detrimental, and because no iron excretory pathways exist, cellular homeostatic mechanisms must balance needs vs. overload and redox utility vs. toxicity [18]. The host occurs that iron absorption and release from reticulo-endothelial (RE) macrophages must be linked to erythroblastic demand and the enterocyte and macrophage are central to iron homeostasis in the absence of an excretory pathway. The regulation of gut absorption is the primary homeostatic mechanism for total body iron content, the transport of iron between sites of utilization and storage represents a major internal homeostatic mechanism.

On the other hand, in intracellular pathogen, IFN-γ is crucial in the control of intracellular infections that

it activates macrophages to promote the maturation and acidification of phagosomes, to decrease the expression of transferrin receptor on the phagosome membrane and decrease the access of the virus to transferrin bound iron [18].

Thus, iron is a key regulator of the host-pathogen interaction that host homeostasis adapts during deficiency, overload, and infection to balance requirement against toxicity and availability to potential pathogens. The virulence of pathogenic organisms in the mammalian host is related to the availability of iron, therefore, microbial iron acquisition mechanisms are an important determinant of infection potential that iron levels regulate 10-20% of all genes present on microbial genomes, including iron acquisition mechanism and virulence factor encoding genes [19]. Iron has the ability to be readily oxidized and reduced, which makes it essential to be capable of catalyzing the conversion of hydrogen peroxides to free radicals as the Fenton reactions; $\mathbf{H_2O_2 + Fe^{2+} = Fe^{3+} + \cdot OH + OH^-}$ [19]. The radicals can react with DNA, RNA, proteins, and lipids and membranes as thereby causing substantial damages. The consideration of host-pathogen dynamic interaction systems as innate and adaptive loops and subsequent comparisons of inferred innate and adaptive networks suggested roles of immunological memory in the coordination of host defensive and offensive molecular mechanisms to achieve specific and powerful defense against pathogens which pathogens enhance intraspecific crosstalk and abrogate host apoptosis to accommodate enhanced host defense mechanisms during the adaptive phase [20]. Iron ions present in phage tails enable phages to exploit their host's iron-uptake mechanism, where the apparent gift of iron leads to cell lysis that if the host iron stores are recycled during the assembly of progeny phases, as much as 14% of the cellular iron released into the water column upon lysis would already be incorporated into new phage tails which the potential role of phages as iron-binding ligands has significant implications for both oceanic trace metal biogeochemistry and marine phage-host interactions [21]. Hepatitis E virus (HEV) protein leads to an imbalance coagulation and fibrinolysis by interacting with human host proteins and triggering the corresponding pathological processes [22]. During infectious course, interaction between the viral and host proteins play essential roles, a clear understanding of which is essential to decode the life cycle of the virus that protein-protein interactions are key mediators of host-pathogen interactions which HEV is a major cause of viral hepatitis in humans. Protein-protein interactions (PPIs) are key mediators of host-pathogen interactions. The host interaction partners of proteins encoded by geno-type1 HEV and constructed the virus-host PPI network, and analysis of the network indicated a role of HEV proteins in modulating multiple host biological processes such as stress and immune responses, the ubiquitin-proteasome system, energy and iron metabolism, and protein translation have been identified, in which this investigation revealed the presence of multiple host translation regulatory factors in the viral translation/replication complex [23]. One of the dangers of excessive iron is its ability to favor animal viral infections that the metal is essential for host cell synthesis of virions and can also impair defense cell function and increase oxidative stress which in both animal models and humans, viral infections cause upregulation of the iron withholding defense system [24]. Hepcidin was first described as a cationic antimicrobial peptide with microbicidal properties against many micro-organism that the peptide hepcidin is the master regulator of iron homeostasis in vertebrates [25]. Hepcidin is strongly induced during inflammation and emerging data support its role in the pathogenesis of number of infections. Thus, hepcidin induces iron accumulation in macrophages and may be detrimental in defense against pathogens that occupy this intracellular niche that this effect has been demonstrated convincingly but is not supported by in vivo data which interrogating the role of hepcidin in animal models of intracellular infections should further clarify the complex relationship between iron distribution and pathogenesis of such infections in humans [26]. Understanding the interaction between host and virus seems to be a prerequisite for any new therapeutic approach that multiple axes of interaction have been suggested: cytokines, alterations of intracellular signaling and metabolism e.g. of fatty acids [26]. An imbalanced iron homeostasis is another one; pathological iron deposits have been observed in about 50% of patients with chronic HCV infection that iron overload is one of the most profibrogenic and carcinogenic factors increasing the risk of HCC by approximately 200-fold which the combination of free iron and reactive oxygen species (ROS) leads to

the production of highly toxic hydroxyl radicals via the Fenton reaction causing severe cell damage [26]. Thus, HCV patients with hereditary iron overload due to mutations in HFE, respond better to antiviral therapy, and potential disturbances in macrophage function due to iron overload are tempting to speculate that increased hepatic iron content may contribute to viral RNA clearance and antagonize the relapse of HCV infection following therapy, while increasing intracellular iron levels reduced HCV replication, preloading of Huh7.5.1 cells with iron failed to protect them against HCV infection, suggesting that the iron bound to ferritin is unavailable for binding to NS5B and the active agent is free intracellular iron only [26].

Antiviral Activity along Viral Life Cycle

Prevention on Viral Infections

Lactoferrin and its family's transferrin play important roles of inhibitory viral activities, host defense, viral prevention and entry. Lactoferrin, a multifunctional iron binding glycoprotein, play an important role in immune regulation and defense mechanism against bacteria, fungi and viruses that lactoferrin withholding ability is related to inhibition of microbial growth as well as to modulation of motility, aggregation and biofilm formation of pathogenic bacteria [27]. Lactoferrin antiviral activity is associated to the prevention of Echovirus 6- and H3N2 influenza virus-induced apoptosis that lactoferrin antiviral activity is also strongly related to its binding to viral particles or to host cells or both [27]. Iron-binding glycoprotein lactoferrin exhibits inhibitory activities against a wide range of viruses in vitro that the protective role of the lactoferrin oral administration against common viral infections was researched, in which lactoferrin consumption may protect the host from viral infections through inhibiting the attachment of a virus to the cells, replication of the virus in the cells, and enhancement of systemic immune functions [28]. The results have been revealed that the beneficial effects of lactoferrin have been found in common viral infection including the common cold, influenza, viral gastroenteritis, summer cold, and herpes which lactoferrin is easily consumed by an individual to prevent these infections [28]. In addition, among the 58 compounds evaluated, 3'-fluoro3'-deoxythymidine (FLT), interferon γ, Ro 5-3335(a TAT inhibitor) and desferrioxamine were modest and selective inhibitors for prevention of activation of HIV-1 infection [29].

Inhibition of Entry/Fusion/Uncoating

Many viruses take advantage of receptor-mediated endocytosis in order to enter target cells, and most entry inhibitors target host cell components with high genetic barriers and eliminate viral infection from the very beginning of the viral life cycle [30]. Viruses recruit host proteins, called entry factors, to help gain entry to host cells that identification of entry factors can provide targets for developing antiviral drugs which by exploring the concept that short linear peptide motifs involved in human protein-protein interactions may be mimicked by viruses to hijack certain host cellular processes and thereby assist viral infection/survival [31]. Protein kinase Cβ II (PKCβ II) is important regulator of a late endosomal sorting event needed for influenza virus entry and infection that the trafficking of two cellular ligands, transferrin and epidermal growth factor (EGF) in which PKCβ II (T500V) expression specifically blocked EGF receptor trafficking and degradation, without affecting transferrin receptor recycling [32]. Transferrin receptor 1(TfR1) plays a role in HCV infection at the level of glycoprotein-mediated entry, acts after CD81, and possibly is involved in HCV particle internalization, namely host cell entry factors are internalized with the viral particle [33].

Replication

The iron chelators of deferoxamine, deferiprone, and bleomycin were investigated against HIV-1 replication in human mononuclear blood cells that viral inhibition of deferoxamine and deferiprone is closely linked to a decrease in cellular proliferation, and that clinically relevant bleomycin concentrations reduced p24 levels by 50% without affecting proliferation, in which these iron chelators with different mechanisms of action could be of additional benefit in antiretroviral combination theory [34]. Iron impairs HCV replication by inactivating the RNA polymerase NS5B and that its negative effects in chronic hepatitis C may be primarily due to attenuation of antiviral immune responses which these findings have implications for the control of HCV replication and may aid in the design of antiviral therapies [35]. Iron regulator hepcidin also had a direct antiviral activity against HCV replication in cell culture that the antiviral effects is associated

with STAT3 activation [36]. IFN-γ-induced nitric oxide (NO) inhibits viral late gene protein synthesis, DNA replication, and virus particle formation that putative enzymatic targets of NO were identified by reversing the NO-mediated inhibition of the poxviruses ectromelia and vaccinia (VV) replication in the 293 cells with exogenous ferrous sulfate and L-cysteine [37].

mRNA Degradation and DNA/RNA Virus Replication

Comparison of kinetic and thermodynamic properties of iron regulatory protein1 (IRP1) binding to ferritin and aconitase2 IRE-RNAs revealed differences specific to each IRE-RNA that IRE-RNA structures are noncoding and bind Fe^{2+} to regulate biosynthesis rates of the encoded, iron homeostatic proteins, in which IRP1 protein binds IRE-RNA, inhibiting mRNA activity; Fe^{2+} decreases IRE-RNA/IRP1 binding, increasing encoded protein synthesis [38]. From these thermodynamic and kinetic analyses, the results indicated that decreased RNA hydrogen bonding and changed RNA conformation upon IRP1 binding and illustrate how small, conserved, sequence differences among IRE-mRNAs selectively influence thermodynamic and kinetic selectivity of the protein/RNA interactions [38]. Transferrin receptor mRNA interactions contribute to uncontrollable iron homeostasis that contains an instability element that is protected from degradation during iron depletion through interactions of iron regulatory proteins (IRPs) with five iron-responsive elements (IREs), in which these results are supportive of a mechanism for a graded response to the intracellular iron resulting from a progressive loss of IRP protection [39]. Using pharmacological and genetic approaches, the DownSTream (DST) cis-acting element in the 3'-untranslated region of the iron-induced ferritin AtFER1 mRNA was shown to be involved in the degradation of this transcript, and oxidative stress triggers this destabilization that iron-regulated genes containing putative DST sequences also display altered expression [40]. Thus, the DST-dependent mRNA stability control appears to be an essential mechanism that allows plants to cope with adverse environmental conditions.

HFE is a nonclassical class I molecule that associates with β2-microglobulin(β2m) and with the transferrin receptor. Human cytomegalovirus (HCMV) US2 targeted HFE for proteasomal degradation that both HFE and classical class I molecules are targeted to degradation via a similar pathway which this HCMV US2-mediated degradation of HFE leads to increased intracellular iron pools as indicated by reduced synthesis of TfR and increased ferritin synthesis [41]. In response to Fe deficiency, the *Saccharomyces cerevisiae* Cth2 protein specifically downregulates mRNAs encoding proteins that participate in many Fe-dependent processes, mRNA turnover requires the binding of Cth2, an RNA binding protein conserved in plants and mammals, to specific adenylate-uridylate (AU)-rich elements (AREs) in the 3' untranslated region of mRNAs targeted for degradation [42]. The endonuclease Regnase-1 has been shown to be critical for the degradation of mRNAs involved in iron metabolism in vivo, in which these results of Regnase-1 promotion of TfR1 mRNA decay, severe iron deficiency anemia induced a defect in duodenal iron uptake, and a HIF2α-inducible activation have demonstrated that Regnase-1-mediated regulation of iron-related transcripts is essential for the maintenance of iron homeostasis [43].

Viral ribonucleotide reductase (RR) is an early gene product of both vaccinia and herpes simplex virus that for productive infection, the apoprotein must scavenge iron from the endogenous, labile iron pool [44]. Intracellular Fe^{2+} chelator, 2,2'-bipyridine (bipyridyl, BIP), is known to sequester iron from this pool that BIP strongly inhibits the DNA virus replication of both vaccinia and herpes simplex virus type I (HSV-1) which the diiron prosthetic group in vaccinia RR is assembled from iron taken from the BIP-accessible, labile iron pool that is sampled also by ferritin and the iron-regulated protein found in the cytosol of mammalian cells [44]. Iron(Ⅲ) inhibits replication of DNA and RNA viruses that the IC_{50} for the iron chloride was 1200 μM for Hep-2 cells and more than 1400 μM for BT cells, and that the concentration-dependent antiviral activity of iron chloride against HSV-1 and Bovine Diarrhoea virus (BVDV) viruses was observed, in which significant inhibitory effects of iron (Ⅲ) against HSV and BVDV, at non-cytotoxic concentrations in the Hep-2 and BT cells [45].

Release and Budding

Viral release of iron has been demonstrated to strongly influence both primary and secondary production in pelagic as well as coastal upwelling high-nutrient low-chlorophy Ⅱ regime. An iron salt ferric ammonium

citrate (FAC) inhibited the endosomal release of viruses and the infection of influenza A virus (IAV), human immunodeficiency virus (HIV), Zika virus (ZIKV), and Enterovirus 71(EV71), suggesting that iron is involved not only in nutritional metabolism but also in antiviral immunity, in which both ferric ion and citrate ion are essential for antiviral effects [50]. As FAC directly targets virus and inhibits early viral infection events, the viral release of iron is useful to be applied as vital-prevention agents that alternative administration route should be considered for iron application to human body, which may include but not limited to spray, liniment or suppository [46]. Mechanistically, FAC inhibited viral infection through including viral fusion and blocking endosomal viral release that FAC induced liposome aggregation and intracellular vesicle fusion, which was associated with a unique iron-dependent cell death [47]. The release of extracellular vesicles (EVs) is important for both normal physiology and disease. However, a basic understanding of the targeting of the EV cargoes, composition and mechanism of release is lacking. The divalent metal ion transporter (DMT1) is unexpectedly regulated through release in EVs that this process involves the ubiquitin ligase, and the adaptor proteins via different membrane budding mechanism [48]. DMT1 release from the plasma membrane into EVs may represent a novel mechanism for the maintenance of iron homeostasis, which may also be important for the regulation of other membrane proteins [48]. Iron is released from transferrin within the endocytic vesicle and reduced, Fe(Ⅱ) is transported across the endosomal membrane by the permease DMT1. When transferrin-iron uptake in mammalian cells similar to siderophore-iron uptake in budding yeast occurs, then an intracellular ferrireductase may exist [49]. Similarly, the considerable evolutionary distance between budding and fission yeast resulted in substantial diversion in the regulation of iron homeostasis [50].

Antiviral Activities Iron Oxide Nanoparticles, Ferritin, Lactoferrin, and Hepcidin-Mediated Ferroportin

Iron oxide nanoparticles (IONPs, < 20 nm size) inhibit for gram-positive and gram-negative bacteria, and common infectious diseases [51], and it is effective for field development of binding and internalization of IONPs targeted to nuclear oncoprotein [52]. However, it is remained unclear whether IONPs play functional role for antiviral activity during viral infections.

Ferritin that an iron storage protein, is the primary iron storage mechanism and is critical to iron homeostasis which ferritin of an iron-binding protein exists in both intracellular compartments [53]. Induction of the heme oxygenase 1(HO-1)/ferritin system prevent inorganic phosphate (Pi)-mediated calcification and osteoblastic differentiation of human smooth muscle cells mainly via the ferroxidase activity of ferritin [54]. Developing organs require iron a myriad of functions, but embryos deleted of the major adult transport proteins, transferrin or its receptor transferrinreceptor1(TfR1), still initiate organogenesis, suggesting that non-transferrin pathways are important that these tested data implicate cell-type-specific mechanisms of iron traffic in organogenesis, which alternatively utilize transferrin or non-transferrin iron delivery pathways [55]. The anti-BLV antibody titers of Bovine leukemia virus (BLV)-infected daily cows were significantly higher in serum than in milk; iron and ferritin concentrations were significantly higher in serum than in milk, therefore the BLV infection affects iron homeostasis through iron metabolism in the daily cow mammary gland [56]. Ferritin is a spherical molecule composed of 24 subunits of two types, ferritin heavy chain (FHC) and ferritin light chain (FLC) that ferritin stores iron within cells, but it also circulates and binds specifically and saturable to a variety of cell types. This binding and uptake can be mediated by ferritin composed only of FHC, indicating that binding of ferritin to cells is mediated by FHC but not FLC [57]. Ferritin reduction sensitizes cells to pro-oxidant cytotoxicity that overexpression of ferritin reduces oxidant species in cells challenged with oxidants and reduces oxidant toxicity, as well as the importance of FHC ferroxidase activity in limiting oxidant toxicity [58]. The FHC ferroxidase oxidizes Fe^{2+} to catalytically inactive Fe^{3+} inside ferritin that FHC overexpression has been shown to be cytoprotective which a human wild-type-ferritin heavy chain (wt-hFHC) ferroxidase activity enhances cytoprotective Nrf2-regulated proteins inducing heme oxygenase-1 (HO-1), thereby resulting in decreased NF-κB-activation adhesion molecules, and microvascular stasis in transgenic SCD mice [59]. On the other hand, the FLC, a key protein in iron metabolism, is associated with the survival

of glioblastoma multiform (GBM) patients; ① FTC knockdown substantially decreased the expression of two Wnt target genes, Cyclin D1 and c-mic, ② a possible explanation for the role of FTC in mitosis was provided, and ③ human platelet-derived growth factor (**PDGF**) receptor **beta** (PDGFR-**β)** expression decreased after FTL knockdown in GBM cells. Thus, FTC silencing inhibits glioblastoma cell proliferation via the GADD45/JNK pathway, however, the molecular mechanisms underlying this association remain largely unclear [60].

Lactoferrin can be considered not only a primary defense factor against mucosal infections, but also a polyvalent regulator which interacts in vital infectious processes. Its antiviral activity, demonstrated against both enveloped and naked viruses such as rotavirus, enterovirus and adenovirus, lies in the early phase of infection, thus preventing viral entry in the host cell that this activity is exerted by binding to heparan sulfate glycosaminoglycan cell receptors [27]. Lactoferrin from bovine milk (BLf) possesses a dual role, preventing virus attachment to intestinal cells by binding to viral particles, and inhibiting a post adsorption step that the BLf effect towards poliovirus is due to the interference with an early infection step, it is also capable of inhibiting viral replication after the viral adsorption phase which lactoferrin is an excellent candidate against viral enteric diseases, as it acts by hindering adsorption and internalization into cells through binding to cell receptors and viral particles [61]. BLf inhibits Toscana virus infection by binding to heparan sulphate that lactoferrin was capable of inhibiting Toscana virus replication in a dose-dependent manner which the anti-Toscana virus action of lactoferrin took place on virus attachment to the cell membrane, mainly through a competition for common glycosaminoglycan receptors [62].

Ferroportin (FPN) is the only known iron exporter in vertebrates that hepcidin, a peptide secreted by the liver in response to iron or inflammation, binds to FPN, including its internalization and degradation, in which after binding of hepcidin, FPN is tyrosine phosphorylated at the plasma membrane [63]. An inability to ubiquitinate FPN does not prevent hepcidin-induced internalization, but it inhibits the degradation of FPN that depletion of protein involved in multivesicular body trafficking, by small-interfering RNA, reduces the trafficking of FPN-green fluorescent to the lysosome [63]. All iron transfer to plasma occurs, through the iron exporter FPN that the concentration of functional membrane-associated FPN is controlled by its ligand, the iron-regulatory hormone hepcidin, and fine-tuned by regulatory mechanisms serving iron homeostasis, oxygen utilization, host defense, and erythropoiesis [64]. Excessive hepcidin expression inhibits dietary iron absorption and iron availability for erythroblasts and contributes to the development of anemias with iron-restricted erythropoiesis. Pharmacological targeting of the hepcidin/ferroportin axis may offer considerable therapeutic benefits by correcting iron traffic that underlies the development of hepcidine-based therapies for the treatment of iron-related disorders and discusses the emerging strategies for manipulating hepcidin pathways [65]. FPN knockdown in hepatic stellate cells (HSCs) prohibits TGFβ1-inducible Smad3 phos-phorylation, whereas FPN over-expression has the opposite effect. HSC-specific FPN deletion ameliorates liver fibrosis that hepcidin suppresses liver fibrosis by impeding TGFβ1-induced Smad3 phosphorylation in HSCs, which depends on Akt activated by a deficiency of FPN [66]. It is also resulting that FPN as a trigger of HIV-1 restriction in sickle cell disease (SCD) setting, linking reduced intracellular iron levels to the inhibition of CDK2 activity, reduction of SAM domain and HD domain-containing protein1 (SAMHD1) phosphorylation, increased IKBαexpression, and inhibition of HIV-1 reverse transcription (RT) and transcription had been pointed out [67].

Iron Induced ROS Production

Iron, an essential element for cell growth and proliferation, is a component of fundamental processes such as DNA replication and energy production. However, iron can be toxic when present in excess for its capacity to donate electrons to oxygen, thus causing the generation of reactive oxygen species (ROS), such as superoxide anions($\cdot O_2^-$) and hydroxyl radicals($\cdot OH^-$), superoxide hydrogen (H_2O_2). These ROS cause tissue injury and organ failure by damaging number of cellular components, including DNA, proteins and membrane lipids that this dichotomy of iron, able to gain and loss electrons, has led to the evolution of tight controls on iron uptake to minimize iron deficiency, as well as iron excess. ROS production

has been demonstrated in several viral infections, including those caused by human immunodeficiency virus, influenza virus, and Japanese encephalitis virus (JEV). ROS and superoxide hydrogen production formations may occur during viral infection in the following chemical reactions [68];

$$NADPH + 2O_2 \rightarrow NADP^+ + 2 \cdot O_2^- + H^+$$

$$2 \cdot O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

Haber-Weiss reaction; $H_2O_2 + O_2^- \rightarrow \cdot OH + OH^- + O_2$

Fenton reaction; $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^-$

$(k=63.0\ L/mol \cdot s[69])$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + \cdot O_2H + H^+$$

$(k=3.1 \times 10^{-3}\ L/mol \cdot s[69])$

$$Fe^{3+} + \cdot O_2^- \rightarrow Fe^{2+} + O_2$$

ROS pervade all facets of cell biology with both detrimental and protective properties that activation of the NADPH oxidase 2 (NOX2) isoform of the NADPH oxidase family of ROS-producing enzymes promotes lung oxidative stress, inflammation, injury, and dysfunction resulting from influenza A viruses of low to high pathogenicity, as well as impeding virus clearance, in which NADPH oxidase inhibitors and the molecular features of the NADPH oxidase enzymes that could be exploited by drug discovery for development of more specific and novel inhibitors to prevent or treat disease caused by influenza can be considered [70]. ROS are a by-product of normal cell metabolism in plant that ROS rapidly inactive enzymes, damage vital cellular organelles in plant, and destroy membranes by inducing the degradation of pigments, proteins, lipids and nucleic acids which ultimately results in cell death, in which the generation, origin, and role of ROS and the removal of ROS by antioxidative defense systems in plants during various developmental pathways were investigated [71]. ROS production is a key mechanism involved in the neuronal damage caused by viral encephalitis that the capability of dengue virus serotypes 2 (DENV2) and DENV4 to induce ROS production was investigated in a rat microglial cell line, highly aggressively proliferating immortalized (HAPI) cells, in which DENV2 and DENV4 have the capability to induce ROS production and active microglia, which have been reported as the key components of neuronal damage [72]. In addition, all viruses activated microglia and induced ROS production. According to the mechanisms of ROS production, there may be two phases of ROS production [72]: in the first phase, for example during viral attachment and entry, components of virus particle per sec may induce rapid ROS production and trigger signaling pathways of pro-inflammation cytokine production and iNOS expression, and further the second phase could be a consequence of the release of oxidative products such as cytokines, NO, and other components from damaged cell involved in iron metabolism and mitochondrial functions of the host cells. Although increased ROS production resulted in oxidative stress and cell death, the oxidative susceptibility and cell responses were different upon exposure to the different viruses. The effective use of ROS-mediated antioxidants as therapeutic agent against particular infections is a realistic possibility that is beginning to be applied against viruses [73].

The production of ROS during viral infection leads to oxidative stress, which increases muscle fatigue and decreases athletic performance. The role of ROS in the pathogenesis of viral infections was focused on DNA viruses, RNA viruses, and retroviruses, with particular attention to influenza viruses, HBV, and HIV that the effects of the virus on activation of phagocytic cells to release ROS and pro-oxidant cytokines such as tumor necrosis factor, the virus on the pro-/antioxidant balance in host cells, the redox state of the cell on the genetic composition of the virus as well as ROS-mediated release of host cell nuclear transcription factor-kappa-B, and efficacy of antioxidants as therapeutic agents in viral diseases were analyzed [74]. An important mechanism of nanotoxicity is the generation of ROS that overproduction of ROS can induce oxidative stress, resulting in cells failing to maintain normal physiological redox-regulated functions which these ROS leads DNA damage, unregulated cell signaling change in cell motility, cytotoxicity, apoptosis, and cancer initiation [75]. Both HBV and HCV infections are characterized by accumulation of oxidative stress in lever and blood of the patients that both viruses trigger ROS production in the infected hepatocytes due to mitochondrial dysfunction and unfolded protein response, in which HBV and HCV-triggered ROS production was shown to promote expression and secretion of proinflammatory

cytokines and to drive liver inflammation [76]. Thus, a better and more detailed understanding of how HBV and HCV alter ROS producing and ROS scavenging events and to assess their impact on fibrosis and neoplastic transformation may be needed. Supplementary reactive radicals derived from oxygen that can be produced in living systems are peroxyl radicals (ROO·) that hydroperoxyl radical initiates fatty acid peroxidation by two parallel pathways: fatty acid hydroperoxide-independent (LOOH-independent) and LOOH-dependent, in which the LOOH-dependent pathway of HO_2^-· initiated fatty acid peroxidation may be significant to mechanisms of initiation of lipid peroxidation [77]. Xanthine oxidase (XO) and xanthine dehydrogenase (XD) are inter-comvertible forms of same enzyme, known as xanthine oxidoreductase (XOR) that has viral functions as a cellular defense enzyme against oxidative stress, in which with both XO and XOR forms, but predominantly with the XO form, copious ROS and reactive nitrogen species (RNS) are synthesized [77]. Oxygen consumption in the peroxisome leads to H_2O_2 production, which is then used to oxidize a wide array of molecules that when peroxisomes are damaged and their H_2O_2 consuming enzymes are down regulated, H_2O_2 releases into the cytosol which drastically contributes to oxidative stress [77].

Iron Chelation

Iron chelation with desferrioxamine (DFO) B enhances the clearance of *Plasmodium falciparum* infection and the iron chelation may provide a new strategy to be developed for the treatment of malaria [78]. The inhibition of self-cleavage of EDTA-TAR is due to two effects of TAT binding: (ⅰ) Tat binds in the bulge and protects residues in the vicinity of the bulge from self-cleavage and (ⅱ) RNA goes through a structural change where EDTA-U24 is rigidly positioned out of the helix and cannot get access to other nucleotides, which are not protected by the Tat peptide that visualizing tertiary RNA folding and RNA-protein interactions, in which Fe^{2+}-EDTA-mediated RNA self-cleavage can be applied to study RNA tertiary structures and RNA-protein interactions [79]. By coordinating with intracellular and extracellular iron, these ligands promote the excretion and subsequent depletion of this transition metal in biological systems that iron-chelating agents consist of a range of bidentate, tridentate, and hexadentate ligands in which two, three, or six atoms, respectively, can coordinate with iron, forming octahedral complexes, but molecular interaction mechanism between iron chelation and virus is remained unclear. The membrane-permeant, intracellular Fe^{2+} chelator strongly inhibits for DNA virus replication of vaccinia and HSV-1 that the strong inhibition was observed only when the Fe^{2+} chelator had been added within 3 h post-infection, in which is due to inhibition of viral late protein and DNA synthesis by Fe^{2+} chelator [44]. The evolution of iron chelators from a range of primordial siderophores and aromatic heterocyclic ligands has led to the formation of a new generation of potent and efficient iron chelators, in which are evolving novel iron chelators from their initial lead compounds with regards to their pharmacological actions and structure-activity relationships for treatment of iron overload diseases [80].

Chelators of desferrioxamine (DFO) and deferiprone (L1) in clinical use are ideal candidates for use during *in vitro* analysis of co-infection and excess iron situations because they have already been implicated in HIV replication inhibition that DFO has also been successfully tested as inhibition of mycobacterium infections even though desferri-exochelins were able to inhibit DNA replication and RR activity at lower concentration than the former chelator [81]. This research provided brief commentary on the possibility of iron chelators presently in clinical use influencing simultaneous HIV-*Mycobacterium tuberculosis* infections during iron loading and the feasibility of evaluating this *in vitro*. HIV-1 replication is induced by the excess iron chelation with DFO that inhibits viral replication by reducing proliferation of infected cells, in which DFO and 2-hydroxy-1-naphthy-laldehyde isonicotinoyl hydrazine (311) inhibit expression of proteins that regulate cell-cycle progression [82]. Iron chelator also of 4-[3,5-bis-(hydroxyphenyl)-1,2,4-triazol-1-yl]-benzoic acid (ICL670) induced cytotoxicity at concentrations that inhibited HIV-1 transcription [82].

As mentioned-above, each of reactions on antiviral activity of Fe^{3+}, Fe^{2+} ions are summarized in ***Table 1*** that function in viral prevention, entry and uncoating, replication, mRNA/RNA/DNA virus, release and budding along viral life cycle.

Table 1. *Antiviral activities of Fe^{3+}, Fe^{2+} ions and iron-induced ROS production for viral prevention, entry and uncoating, replication, mRNA/DNA/RNA damage, and release and budding along viral life cycle*

Fe^{3+}, Fe^{2+}	Antiviral Activities of Fe^{3+}, Fe^{2+} Ions along Viral Life Cycle					
	Viral Prevention	Entry and Uncoating	Replications	mRNA Degradation	DNA/RNA	Release, Budding
	→	→	→	→	→	→
	Fe^{3+} (ROS ?)	Fe^{2+}, Fe^{3+} O_2^-, H^+ H_2O_2	Fe^{3+}, Fe^{2+} O_2^-, ·OH, OH^-, H_2O_2	Fe^{2+}, Fe^{3+} O_2^-, ·OH, H_2O_2	Fe^{3+}, Fe^{2+} O_2^-, ·OH, H_2O_2	Fe^{2+}, Fe^{3+} O_2^-, ·OH, OH^-, H_2O_2
Fe^{3+} or Fe^{2+}	· Lactoferrin · FLT, interferonγ, Ro 53335 (TAT inhibitor) and desferrioxamine	· PKCβ Ⅱ needed for influenza virus entry · TfR1 plays a role in HCV infection	· Iron chelators deferoxamine, deferiprone, and bleomycin against HIV-1 · Iron regulator hepcidin against HCV · IFN-γ-induced nitric oxide against poxviruses ectromelia and vaccinia (VV)	· Iron regulatory protein (IRP1), DownS-Tream (DST) iron-induced ferritin AtFER1, HFE with the transferrin receptor, and endonuclease · Regnase-1 lead to mRNA degradation or decay.	· BIP(Fe^{2+}) strongly inhibits the DNA virus replication · Iron(Ⅲ) inhibits replication of DNA and RNA viruses	· Iron salt ferric ammonium citrate (FAC) inhibits the endosomal release of viruses of influenza A virus (IAV), HIV, Zika virus(ZIKV), and Enterovirus 71(EV71) · Transferrin-iron uptake inhibits budding in mammalian cells

Conclusions

Cellular iron homeostasis is controlled by iron uptake at the plasma membrane, eliciting balanced iron distributions that patients with an inflammatory process may present with decreased, normal or increased total body iron stores which decreased iron stores in inflammatory disease and infection can either represent true preexisting iron deficiency or functional iron deficiency and inflammatory states increase serum ferritin levels. On the other hand, viruses are the smallest pathogens and consisted of a single nuclei acid (RNA or DNA) encased in a protein shell which are covered with a lipid-containing membrane. A virus infection depends on factors both in the virus and the host that the most important factor is genomic alterations which the viral life cycle starts with its entry into a host; it reaches the susceptible target cell, enters it, replicates and causes cell injury, and may be cell death. Iron is essential for both host and pathogen, and complex systems of acquisition and utilization have evolved in competition that iron is a key regulator of host-pathogen interactions.

Iron regulator hepcidin had a direct antiviral activity against HCV replication in cell culture that the antiviral effects is associated with STAT3 activation. Iron(Ⅲ) inhibits replication of DNA and RNA viruses that the IC_{50} for the iron chloride was 1200 μM for Hep-2 cells and more than 1400μM for BT cells, and that the concentration-dependent antiviral activity of iron chloride against HSV-1 and BVDV viruses was observed, in which significant inhibitory effects of iron (Ⅲ) against HSV and BVDV, at non-cytotoxic concentrations in the Hep-2 and BT cells. The other, DMT1 release from the plasma membrane into EVs

may represent a novel mechanism for the maintenance of iron homeostasis, which may also be important for the regulation of other membrane proteins. Iron is released from transferrin within the endocytic vesicle and reduced, Fe(Ⅱ) is transported across the endosomal membrane by the permease DMT1. Iron is released from transferrin within the endocytic vesicle and reduced, Fe(Ⅱ) is transported across the endosomal membrane by the permease DMT1.

Lactoferrin antiviral activity is associated to the prevention of Echovirus 6- and H3N2 influenza virus-induced apoptosis that lactoferrin antiviral activity is also strongly related to its binding to viral particles or to host cells or both. TfR1 plays a role in HCV infection at the level of glycoprotein-mediated entry, acts after CD81, and possibly is involved in HCV particle internalization, namely host cell entry factors are internalized with the viral particle. HFE is a nonclassical class I molecule that associates with β2-microglobulin (β2m) and with the transferrin receptor. HCMV US2-mediated degradation of HFE leads to increased intracellular iron pools as indicated by reduced synthesis of TfR and increased ferritin synthesis. In response to Fe deficiency, the *Saccharomyces cerevisiae* Cth2 protein specifically downregulates mRNAs encoding proteins that participate in many Fe-dependent processes, mRNA turnover requires the binding of Cth2, an RNA binding protein conserved in plants and mammals, to specific AU-rich elements in the 3' untranslated region of mRNAs targeted for degradation. Bipyridyl, BIP strongly inhibits the DNA virus replication of both vaccinia and HSV-1 which the diiron prosthetic group in vaccinia RR is assembled from iron taken from the BIP-accessible, labile iron pool that is sampled by ferritin and the iron-regulated protein found in the cytosol of mammalian cells. When transferrin-iron uptake in mammalian cells similar to siderophore-iron uptake in budding yeast occurs, then an intracellular ferrireductase may exist. It is also resulting that FPN as a trigger of HIV-1 restriction in SCD setting, linking reduced intracellular iron levels to the inhibition of HIV-1 RT and transcription had been pointed out.

Iron, an essential element for cell growth and proliferation, is a component of fundamental processes such as DNA replication and energy production. However, iron can be toxic when present in excess for its capacity to donate electrons to oxygen, thus causing the generation of ROS, such as superoxide anions($\cdot O_2^-$) and hydroxyl radicals($\cdot OH^-$), superoxide hydrogen (H_2O_2) with both XO and XOR forms, but predominantly with the XO form, copious ROS and RNS are synthesized. Oxygen consumption in the peroxisome leads to H_2O_2 production, which is then used to oxidize a wide array of molecules that when peroxisomes are damaged and their H_2O_2 consuming enzymes are down regulated, H_2O_2 releases into the cytosol which contributes to oxidative stress.

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