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A potential alternate pathway for transferrin mediated iron delivery to mitochondria involves moonlighting Glyceraldehyde-3-Phosphate Dehydrogenase

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Abstract

We describe the existence of an potential evolutionarily conserved, ancient but as yet undefined pathway for Transferrin (TF) mediated iron delivery into mitochondria that involves intracellular traffickingvia,the moonlighting glycolytic enzyme Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH). Utilizing cell free as well as cell culture model systems we observed that GAPDH aids in the trafficking of Tf to deliver iron into mitochondria.This could contribute to the vital cellular catabolic processes of heme and other iron sulfur cluster bio-synthesis.

Key words: Mitochondria, iron,GAPDH, moonlighting, trafficking

Introduction

Iron is an essential element for all organisms due to its unparalleled versatility as a Co-factor. It forms a major constituent of heme proteins, myoglobin, cytochromes, Iron-sulfur clusters and other proteins that utilize iron in functional groups [1]. Cellular Iron deficiency arrests cell growth and leads to cell death, while excess iron can catalyze the production of reactive oxygen species. Circulating iron-bound TF (holo- TF) is delivered to different tissues and cells of the body by binding to the membrane-Bound transferrin receptor 1 (TFR1 also known as CD71). The resulting holo-TF- TFR1 complex is internalized by receptor-mediated endocytosis. Within the endosome, low pH causes the iron to dissociate and is made available in the cytosol [2]. Together with cytosolic iron-storage protein ferritin, the major destination for delivery inside mammalian cells is mitochondria. The mitochondria are a critical intracellular site of iron utilization. More than 90% of transferrin derived iron enters the mitochondrial matrix of erythroid cells. Inside mitochondria, iron is inserted into protoporphyrpin IX for heme synthesis [3]. Though erythrocytes contain a majority of the body's heme in the form of hemoglobin along with high concentrations of free heme, nonerythroid cells also possess significant quantity of cytosolic labile heme with the highest levels present in the liver [4, 5]. Regulated availability of heme is crucial for diverse essential processes that are vital to maintaining life. These range from ubiquitous, housekeeping pathways such as respiration, to highly cell-specific ones such as oxygen transport by hemoglobin [6]. Non-availability of iron limits the heme synthesis rate. Maintaining a continuous supply of iron for utilization in heme and other iron containing macromolecules is critical as mitochondria cannot accumulate this strategic and vital element [6]. Prior investigations have shown that the only physiologically active chelate that can provide iron for their hemoglobin synthesis is transferrin [7]. Therefore, for synthesis of adequate quantities of heme proteins the mitochondria of both erythroid as well as nonerythroid cells need a continuous and adequate supply of transferrin iron. Earlier it had been hypothesized that iron could be taken up directly by mitochondrion from cytosol as Fe^{2+} utilizing a process driven by membrane potential [8, 9] however the exact mechanisms as to how this occurs has remained unclear. A recent hypothesis suggests that iron may be transported into mitochondria by a mechanism that involves docking of transferrin containing endosomes with mitochondria. This "iss and run" hypothesis has been proposed for the iron routing directly from TF-containing endosomes to mitochondrial matrix bypassing the oxygen rich cytosol and without involvement of the cytosolic iron pool [2]. It has been postulated that iron is imported to mitochondria through direct contact of Tf-laden endosomes with the outer mitochondrial membrane via transient interactions of these organelles [3]. Various compelling experimental evidences for the kiss and run hypothesis, come from studies on erythroid cells where the TF-TFR pathway is unavailable to cytoplasmic chelators. Moreover, in reticulocytes with high biosynthesis of heme, it has been demonstrated that "free" cytoplasmic iron is not used efficiently for hemoglobin synthesis [7, 10] and a direct transfer of iron from Tf endosomal-mitochondrial interactions could contribute to increased mitochondrial iron [11]. In a recent study that utilized lock–hTF (transferrin which is unable to Release Fe)

Das and co-workers have shown direct endosome e-mitochondria interaction at nanometer resolution using 3D direct stochastic optical reconstruction microscopy [12]. Evidently, for mitochondria to acquire iron by this route, cells would first have to internalize holo-transferrin into endosomes which would then go on to dock with mitochondria. The canonical pathway for this process is via TFR1 based receptor mediated endocytosis. However studies have shown that TFR1-/- mice demonstrate heme synthesis and even show increased reticulocyte count, indicating existence of a TFR1 independent pathway [13]. Another transferrin receptor TFR2, a paralog of TFR1 also exists, the expression of which is restricted mainly to hepatocyte and erythroid progenitor cells, however, its expression is unaffected by intracellular iron concentrations and its binding affinity to diferric transferrin is 30 times lower than TFR1, suggesting its role in iron sensing than transport [14-16]. Additionally, TfR2 is not expressed in numerous tissues and cell lines where a significant amount of transferrin is internalized [17, 18]. Studies involving knock down of TFR1 in the Human hepatoma cell line HuH7 that expresses both TFRI and TFR2 revealed that TfR2 was not involved in uptake of transferring [19]. Over the past decade it has been revealed the multifunctional, glycolytic enzyme Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) moonlights to traffic iron carrier proteins into cells [20]. GAPDH has been demonstrated to be present on the surface of a large number of diverse cell types where it was observed to bind and internalize the iron

carrier proteins transferrin and lactoferrin (Lf) into cells leading to iron delivery into the cell [21-23]. Apart from functioning as a cell surface receptor, cells also internalize transferrin and the closely related iron carrier lactoferrin via a secreted form of GAPDH (sGAPDH). In this process when cellular iron levels are depleted they secrete GAPDH which captures iron replete Tf and Lf to traffic them into cells via macropinocytosis and lipid raft-mediated endocytosis. The delivery of iron carrier proteins via soluble GAPDH has been shown to be significantly more efficient than delivery via the cannonical cell surface receptors and is uPAR dependent [24, 25]. GAPDH is a known fusogenic protein that promotes vesicular delivery of transferrin and lactoferrin [26]. Prior investigations have revealed that the extracellular recruitment and secretion of GAPDH is enhanced in responsive to cellular stresses like iron starvation and hypoxia [27, 28]. Recently GAPDH has been shown to be a critical middleman in a dynamic intracellular network that makes heme bioavailable for directed mobilization to down-stream targets. It binds and buffers cytosolic heme to shuttle it for insertion into various apo-hemoproteins [29]. In the current study we have explored the role of GAPDH as a means to enhance transferrin-Fe delivery into mitochondria for utilization of transferrin bound iron and incorporation into the mitochondrial iron pool. This could contribute to the processes of heme bio-synthesis and iron sulfur cluster synthesis. In the current study we have focused primarily on studying the delivery of iron carrier protein transferrin via GAPDH into mitochondria.

Materials and Methods

Reagents, Antibodies and Cell lines

Rabbit muscle Glyceraldehyde-3-phosphate dehydrogenase, human holo-Transferrin, Tween-20, RPM1 1640 media, Dulbecco's modified eagle media (DMEM) media, HEPES, succinylacetone (SA) and phenyl hydrazine (PHZ) were all obtained from SIGMA. Foetal Bovine Serum (FBS) was from Gibco. Transferrin was conjugated with Alexa-647/Alexa568 using commercial kits (Molecular Probes Invitrogen). Polyclonal Rabbit anti GAPDH, Monoclonal mouse β-actin and Rabbit anti mouse-

HRP and Goat anti rabbit–HRP were all obtained from Sigma. Goat anti Rabbit Alexa 647and Goat anti Rabbit Alexa 568 was from Invitrogen. Anti-Mouse CD71/isotype control APC labelled and Monoclonal anti COX2 were from BD Biosciences. Monoclonal mouse anti GAPDH, Anti-Cytochrome C and Anti-Lamin B1 antibody were from Calbiochem, Abeam and Cell Signaling Technology respectively. Nitrocellulose membrane and LuminataTM forte western HRP substrate for western blot were from MDI and Merck Millipore respectively. To radio-label transferrin, 10 mg of the protein was dissolved in 400 µl Tris/ Bicarbonate buffer. Separately a solution of 143 µM nitrilotriacetic acid and 143 µM $^{55}FeCl_3$ (ARC), in 600 µl buffer was equilibrated for 30 min at room temperature. The two solutions were then mixed and allowed to equilibrate for 30 min and excess free iron was removed on a desalting column [23].

K562 (Human Chronic Myelogenic Leukemic cell) and Hela (Human ovarian cell line) HepG2 were obtained from European Collection of Authenticated Cell Cultures (ECACC), UK. HepG2 (human hepatoma) was from ATCC. CHO-TRVb cell line, a variant of CHO cell line which lacks both transferrin receptors (TfR1 and TfR2) but can acquire transferrin via GAPDH [23, 30] was a kind gift from Prof T. McGraw (Weill Cornell Medical College, New York) and Dr. S. Mayor NCBS Bangalore. All the cells were maintained in RPMI medium supplemented with 10% FBS. For transfection with Mito-GFP plasmid (Addgene), 4x105 CHO-TRVb or HepG2 cells were plated in 6 well tissue culture plates and maintained overnight in an incubator at 37°C, 5% CO_2. The next day cells were washed and transfection was performed using Lipofectamine® 2000 (Life Technologies) as per manufacturer's instructions. Stable cell lines were selected using G418 selection pressure.

Flow subcytometry to analyse GAPDH mediated Tf-endosome docking to mitochondria

The procedure for flow subcytometry of mitochondrial Tf uptake was adapted from previously published protocols [11]. Briefly, all cell types tested were allowed to internalize 10 μg Tf-A647 in the presence of 20 μg of GAPDH. Cells were incubated for different time points at 37°C in the dark. Control cells were incubated with only TF-A647. After completion of each time interval, samples were immediately placed on ice and were extensively washed three times with ice-cold PBS to stop endocytosis. After three washes, the cells were re-suspended and treated with 1 mg/ml pronase (Roche) to remove any residual fluorescent probe bound to cell surface [22, 31]. Samples were re-suspended in 1 ml STM mitochondrial isolation buffer and processed for isolation of mitochondria as described in supplementary methods and were then analysed by flow cytometry for the mitochondrial associated endosomal Tf-A647 fluorescence signal (Figure S1 C&D). Comparison was made with mitochondria isolated from cells wherein GAPDH had been omitted (incubation with only TF-A647).

In vivo Colocalization of GAPDH and Tf containing vesicles with mitochondria

HepG2 cells stably expressing mito-GFP were cultured in 35mm confocal dishes ($5x10^5$ cells/ dish). After 30 minutes of incubation in serum free medium they were allowed to internalize 10 μg Tf-A568 in the presence of 20 μg of GAPDH-A647 at 37˚C for 20 min [25]. Cells were washed and fixed in 4% paraformaldehyde and imaged in a Nikon A1R confocal microscope with a 60X oil immersion, 1.49 NA objective lens using 1 Airy unit aperture.

GAPDH modulated uptake of Tf ^{55}Fe and Incorporation into mitochondria

Reticulocytes were isolated from mice in which experimental anemia had been induced by injection of Phenylhydrazine (PHZ) as described in Supplementary 1methods. Cells were allowed to internalize 50 μg Tf^{55}Fe in presence of GAPDH for different time points at 37°C. After extensive washing, cells were treated with 0.1% pronase to remove surface-bound Tf^{-55}Fe. Mitochondria were isolated and the associated 55Fe radioactivity was counted by liquid scintillation in the H^3 channel of a β-counter.

Surface CD71 staining of reticulocytes from PHZ treated mice

High reticulocyte population in circulation was verified by the occurrence of an elevated CD71 positive population of cells as determined by flow cytometry analysis. Cells from PHZ treated mice as well as control mice were washed with FACS buffer and blocked using FACS block (FACS buffer supplemented with 5% normal human serum and normal goat serum) at 4°C for 30 min. Surface CD71, staining was carried out using anti CD71 (TfR1)-APC/ isotype control, for 1 hour at 4°C. After extensive washing, cells were fixed using 4% paraformaldehyde and florescent signal evaluated by flow cytometry.

Statistical analysis

Statistical analysis was performed using unpaired Student's t-test. All images were analyzed using Image J software.

Animal Ethics

All animal handling protocols were as per the Institute of Microbial Technology Experimental Animal Facility protocols. The procedures were approved by the statutory Institutional Animal Ethics committee vide approval No. IAEC 18/13 and were performed strictly as per guidelines of the Committee for the purpose of Control and Supervision of Animal Experiments on Animals (CPCEA), Govt. of India.

Results and Discussion

Microscopy confirms co-localization of endosomestrafficking Tf with Mitochondria

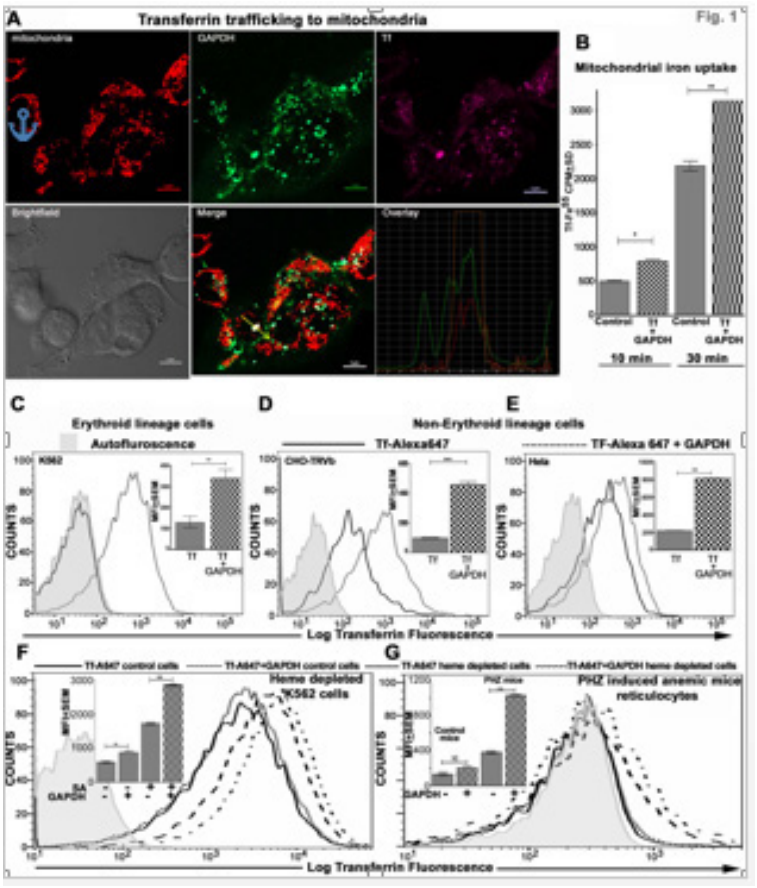
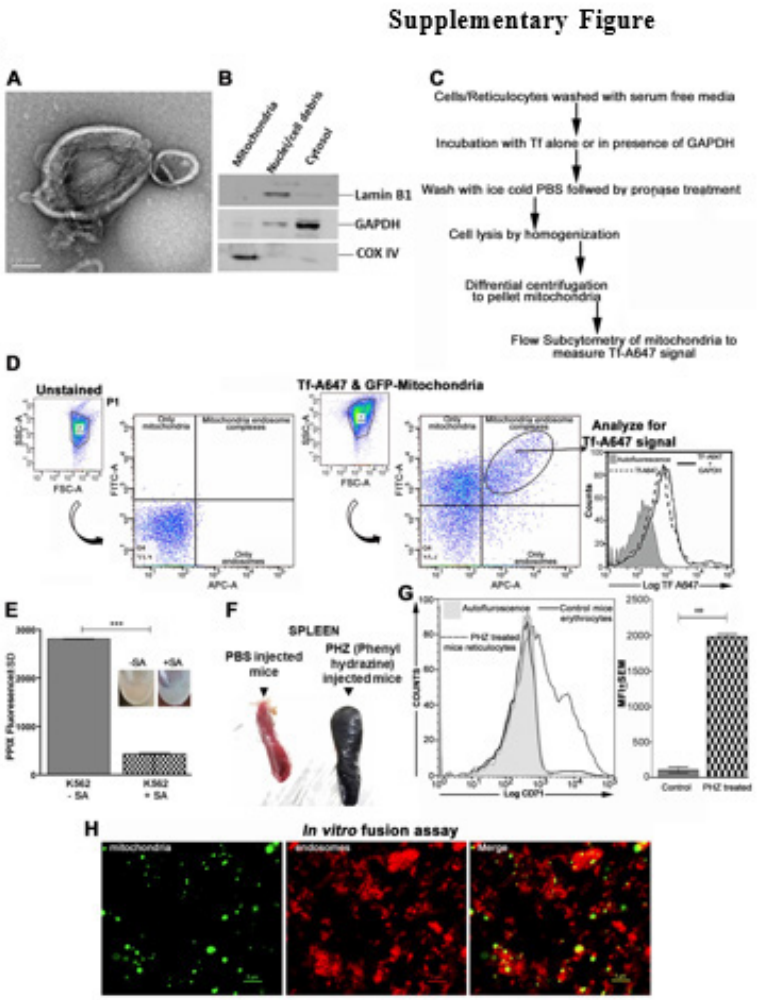


Figure 1. (A) GAPDH and transferrin co-traffic to mitochondria. HepG2 cells expressing MitoGFP co-internalized TF-A568 and GAPDH-A647. Vesicles containing transferrin along with GAPDH are in very close proximity to mitochondria. Arrows in the merged panel indicate a triple-localization event. (B) Iron uptake into mitochondria of reticulocytes is enhanced by GAPDH. Mitochondria of reticulocytes isolated from phenylhydrazine-treated mice demonstrate significantly greater iron uptake from transferrin in the presence of GAPDH. Data are presented as CPM ± SEM, $^{**}p < 0.001$, $^{*}p < 0.02$, n = 3. (C–E) Transferrin delivery to mitochondria of both erythroid and non-erythroid cell lines is significantly enhanced by GAPDH. Flow cytometry overlay histograms reveal a large increase in transferrin signal associated with mitochondria isolated from all cell types tested when transferrin was incubated along with GAPDH. Insets present the data as bar graphs of mean fluorescence intensity (MFI ± SEM), $^{**}P < 0.001$, $^{***}P < 0.0001$, n = 10^4 cells. All experiments were repeated multiple times, and representative overlay histograms are shown. (F) GAPDH enhances delivery of transferrin to mitochondria in heme-depleted cells. K562 cells were first treated with 100 μM succinylacetone to deplete cellular heme. Subsequently, aliquots of 10^6 cells (heme-depleted as well as control cells) were allowed to internalize Tf-Alexa 647 and GAPDH for 30 minutes, after which the cells were processed for mitochondrial isolation. The associated transferrin signal was quantified using flow cytometry and compared with that of mitochondria obtained from cells in which GAPDH was omitted. Flow cytometry overlay histograms reveal a significant increase in transferrin signal associated with mitochondria in the presence of GAPDH. The increase is further enhanced in the heme-depleted cell samples. Inset data are presented as bar graphs of mean fluorescence intensity (MFI ± SEM), $^{**}P < 0.001$, $^{***}P < 0.0001$, n = 10^4 cells. The experiment was repeated multiple times. (G) Delivery of transferrin to mitochondria of anemic mouse reticulocytes is enhanced by GAPDH. Reticulocytes from phenylhydrazine (PHZ)-treated mice demonstrate significantly enhanced mitochondria-associated transferrin in the presence of GAPDH. Data from a control group of mice (administered PBS instead of PHZ) are also presented

for comparison. Mitochondria of reticulocytes from anemic mice exhibit enhanced transferrin uptake. Inset data are presented as bar graphs of mean fluorescence intensity (MFI ± SEM). NS, not significant; ***P < 0.0001, n = 10^4. All experiments were repeated multiple times, and representative overlay histograms are presented.



The purpose of iron carrier trafficking within cells is to effect iron delivery to the site of its utilization. Transferrin-bound iron is efficiently used for heme synthesis within mitochondria. It has been recently been proposed that iron trafficking leading to its accumulation into ferritin occurs with the help of certain accessory proteins [32]. To confirm that the enhanced trafficking of Tf to mitochondria by GAPDH also results in enhanced Fe delivery to the mitochondrial iron pool, we utilized Fe^{55} labeled Tf. A significant increase in Fe^{55} levels were observed in mitochondria when cells were incubated with radio-labeled Tf in the presence of GAPDH. This confirmed that the delivery of more Tf via GAPDH results in not only more of protein trafficking but, it also increases the iron pool of mitochondria in a time-dependent manner (Figure 1B). GAPDH enhances transferrin delivery to mitochondria of both erythroid and non-erythroid cells. In the cell mitochondria are the sole site for heme synthesis and transferrin is the principal iron delivery molecule into cells. Though the major tissue for production are erythroid precursor cells, however synthesis of heme also occurs in non-erythroid cells and plays a variety of essential roles in many cellular processes. Although the molecular pathways involved in the generation of heme and ISCs are well known [33], only more recently have some of the molecular players responsible for mitochondrial iron transport been identified. Once heme is formed in the mitochondria it is exported out into the cytosol where GAPDH acts to both buffer and shuttle heme to apo- hemoproteins in both erythroid as well as non-erythroid cells [34]. To explore if GAPDH also moonlights to enhance transferrin-Fe delivery into mitochondria we assayed for transferrin delivery into these organelles by incubating erythroid as well as non-erythroid cells (K562, Hela and CHOTRVb) with either, transferrin alone or transferrin in the presence of GAPDH. Mitochondria were then

isolated and evaluated for associated transferrin by flow subcytometry. As compared to mitochondria isolated from cells which had been incubated with only transferrin, the mitochondria isolated from cells which had been incubated with transferrin in presence of GAPDH demonstrated a significantly higher Tf signal (Figure 1 C-E). An inhibition of intracellular heme biosynthesis would render the cells being starved for heme and lead to stimulating transferrin-Fe import to the site of heme production [6, 10]. If GAPDH is a facilitator of Tf-Fe delivery to mitochondria then under these conditions, its effects could be expected to be enhanced. A similar result of enhanced demand and trafficking has been observed in case of cells from anemic subjects. We proceeded to first test this

hypothesis using succinylacetone. to inhibit heme synthesis in K562 cells. After confirming heme depletion we evaluated transferrin delivery to mitochondria and observed a significantly enhanced Tf-endosome delivery to mitochondria in the presence of GAPDH. These results confirmed that the total transferrin loaded endosomes associated with mitochondria is increased upon heme

Starvation and GAPDH significantly enhances its delivery and especially in the cells where heme synthesis is inhibited (Figure 1F). We then proceeded to confirm this observation utilizing active heme synthesizing reticulocytes isolated from PHZ treated anemic mice. We assayed for transferrin uptake and once again observed that, GAPDH significantly enhances the delivery of Tf loaded endosomes to mitochondria. The effect being much more pronounced, in case of cells from anemic mice as compared to those from control animals (Figure 1G).

A part from mammalian cells, several bacterial pathogens utilize transferrin and lactoferrin as sources of iron [35]. In numerous bacterial species it has also been shown that GAPDH is responsible for iron acquisition via internalization of these mammalian iron carriers [36, 37]. GAPDH is an essential multifunctional molecule which appeared early in the evolution of life forms and has been relatively well conserved across species. GAPDH mediated trafficking of transferrin into cells has been described to represent a primitive mechanism for the uptake of iron transport proteins that has been conserved across prokaryotic to mammalian cells [22, 38]. Mitochondria are believed to have evolved from a prokaryotic ancestor and retain several of their ancestral characteristics [39]. Building upon these prior observations we hypothesized regarding the existence of an evolutionarily conserved, ancient but as yet undefined pathway for TF-Fe delivery into mitochondria that involves intracellular traf-ficking via GAPDH. Our current investigations indicate that GAPDH by facilitating the delivery of transferrin to the mitochondria may act in conjunction with ion channels such as VDACs, outer mitochondrial proteins such as DMT1 and inner mitochondrial proteins such as mitoferrins to facilitate the trafficking of iron into the organelle Iron uptake by mitochondria is intimately coupled with heme biosynthesis. Heme is formed in almost all living systems, except for a few obligatory anaerobes and certain unicellular organisms auxotrophic for porphyrins and/or heme. It plays a fundamental role in many crucial biochemical reactions and its biosynthesis is finely tuned to these requirements which vary significantly among various cells and tissues. Hemoproteins exist in almost every subcellular and are integral for essential cellular processes such as; oxidative amino acid metabolism, phosphorylation transcriptional regulation, responding to NO signaling, circadian rhythm regulation and mediating the macrophage response to LPS [34]. Besides serving as the prosthetic group of numerous hemoproteins, heme plays an important role in controlling protein synthesis and cell differentiation. Cellular heme levels are tightly controlled via a fine balance betweenheme biosynthesis and catabolism. Consequently, any dysregulation of heme synthesis could have myriad consequences on the organism's homeostatic processes. Imbalance in the regulation of heme in an organism is closely related to maintenance in the iron metabolism homeostasis and an understanding of the processes by which iron is delivered to mitochondria are vital in dealing with any dysbiosis. Both iron deficiency and iron excess promote oxidative stress and damage to mitochondrial DNA. This leads to mitochondrial dysfunction and manifests as decreased mitochondrial respiratory efficiency. The increased oxidative stress has also been observed also in aging and associated degenerative diseases. [40,41].

Conclusions

Mitochondrial dysfunction is known to impair reticulocyte maturation, enhance their fragility and cause rupture thereby elevatingcirculating free heme levels that promotes pulmonary arterial hypertension [42] and our observations though very preliminary could have implications for more detailed investigations leading to a deeper understanding of the mechanisms involved. Increased insights on the mitochondrial-iron carriers and their ability to communicate with other compartments or organelles will aid in shaping the investigation of iron metabolism in health and disease.

Declarations

Ethics approval and consent to participate

Not applicable

1. Consent for publication

All authors have seen the manuscript and consent to its publication

Availability of data and materials

All data generated or analyzed during this study are included in this published article [including its supplementary information files]. Further enquiries can be directed to the corresponding author.

Competing Interests

The authors have no conflicts of interest to declare.

Author Contributions

A.D and M.R. conceptualized the work. A.D. and S.T. contributed equally to the manuscript. They carried out most of the experiments, compiled the data and wrote the initial draft. R.S.M., G.K.C. and R.D. assisted with the tissue culture and animal related experiments. C.I.R. validated some of the experiments and data analysis. M.R. provided the resources, carried out the overall supervision of the work and wrote the final manuscript.

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References

1. Wilson M, Britigan B (1998) Iron acquisition by parasitic protozoa. Parasitology Today 14: 348-353.

2. Richardson DR, et al. (2010) Mitochondrial iron trafficking and the integration of iron metabolism between the mitochondrion and cytosol. Proceedings of the National Academy of Sciences 107: 10775-10782.

3. Hamdi A (2016) Erythroid cell mitochondria receive endosomal iron by a "kiss-and-run" mechanism. Biochimica et Biophysica Acta - Molecular Cell Research 1863: 2859-2867.

4. Aich A, Freundlich M, Vekilov PG (2015) The free heme concentration in healthy human erythrocytes. Blood Cells, Molecules, and Diseases 55: 402-409.

5. Donegan RK, et al. (2019) Handling heme: The mechanisms underlying the movement of heme within and between cells. Free Radical Biology and Medicine 133: 88-100.

6. Yien YY, Perfetto M (2022) Regulation of heme synthesis by mitochondrial homeostasis proteins. Frontiers in Cell and Developmental Biology 10: 895521.

7. Ponka P (1997) Tissue-specific regulation of iron metabolism and heme synthesis: Distinct control mechanisms in erythroid cells. Blood 89: 1-25.

8. Lange H, Kispal G, Lill R (1999) Mechanism of iron transport to the site of heme synthesis inside yeast mitochondria. Journal of Biological Chemistry 274: 18989-18996.

9. Ohgami RS, et al. (2005) Identification of a ferrireductase required for efficient transferrin-dependent iron uptake in erythroid cells. Nature Genetics 37: 1264-1269.

10. Richardson DR, Ponka P, Vyoral D (1996) Distribution of iron in reticulocytes after inhibition of heme synthesis with succinylacetone: Examination of the intermediates involved in iron metabolism.

11. Sheftel AD, et al. (2007) Direct interorganellar transfer of iron from endosome to mitochondrion. Blood 110: 125-132.

12. Das A (2016) Endosome-mitochondria interactions are modulated by iron release from transferrin. Journal of Cell Biology 214: 831-845.

13. Khalil (2017) A specialized pathway for erythroid iron delivery through lysosomal trafficking of transferrin receptor 2. Blood Advances 1: 1181-1194.

14. Wong YC. (2019) Regulation and function of mitochondria-lysosome membrane contact sites in cellular homeostasis. Trends in Cell Biology 29: 500-513.

15. Kawabata H, et al. (2004) Analyses for binding of the transferrin family of proteins to the transferrin receptor 2. British Journal of Haematology 127: 464-473.

16. West AP, et al. (2000) Comparison of the interactions of transferrin receptor and transferrin receptor 2 with transferrin and the hereditary hemochromatosis protein HFE. Journal of Biological Chemistry 275: 38135-38138.

17. Calzolari A, et al. (2007) Transferrin receptor 2 is frequently expressed in human cancer cell lines. Blood Cells, Molecules, and Diseases 39: 82-91.

18. Kawabata H, et al. (1999) Molecular cloning of transferrin receptor 2: A new member of the transferrin receptor-like family. Journal of Biological Chemistry 274: 20826-20832.

19. Trinder D, Morgan EH (1997) Inhibition of uptake of transferrin-bound iron by human hepatoma cells by nontransferrin-bound iron. Hepatology 26: 691-698.

20. Boradia VM, Raje M, Raje CI (2014) Protein moonlighting in iron metabolism: Glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Biochemical Society Transactions 42: 1796-1801.

21. Rawat P, et al. (2012) The multifunctional glycolytic protein glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a novel macrophage lactoferrin receptor. Biochemistry and Cell Biology 90: 329-338.

22. Raje CI, et al. (2007) The macrophage cell surface glyceraldehyde-3-phosphate dehydrogenase is a novel transferrin receptor. Journal of Biological Chemistry 282: 3252-3261.

23. Kumar S, et al. (2012) Characterization of glyceraldehyde-3-phosphate dehydrogenase as a novel transferrin receptor. The International Journal of Biochemistry & Cell Biology 44: 189-199.

24. Chauhan AS, et al. (2015) Secreted multifunctional glyceraldehyde-3-phosphate dehydrogenase sequesters lactoferrin and iron into cells via a non-canonical pathway. Scientific Reports 5: 1-10.

25. Sheokand N, et al. (2013) Secreted glyceraldehyde-3-phosphate dehydrogenase is a multifunctional autocrine transferrin receptor for cellular iron acquisition. Biochimica et Biophysica Acta - General Subjects 1830: 3816-3827.

26. Malhotra H, et al. (2016) Exosomes: Tunable nano vehicles for macromolecular delivery of transferrin and lactoferrin to specific intracellular compartment. Journal of Biomedical Nanotechnology 12: 1101-1114.

27. Malhotra H, et al. (2019) Moonlighting protein glyceraldehyde-3-phosphate dehydrogenase: A cellular rapid-response molecule for maintenance of iron homeostasis in hypoxia. Cellular Physiology and Biochemistry 52: 517.

28. Chauhan AS, et al. (2019) Trafficking of a multifunctional protein by endosomal microautophagy: Linking two independent unconventional secretory pathways. The FASEB Journal 33: 5626-5640.

29. Sweeny EA, et al. (2018) Glyceraldehyde-3-phosphate dehydrogenase is a chaperone that allocates labile heme in cells. Journal of Biological Chemistry 293: 14557-14568.

30. McGraw TE, Greenfield L, Maxfield FR (1987) Functional expression of the human transferrin receptor cDNA in Chinese hamster ovary cells deficient in endogenous transferrin receptor. The Journal of Cell Biology 105: 207-214.

31. Malhotra H, et al. (2017) Mycobacterium tuberculosis glyceraldehyde-3-phosphate dehydrogenase (GAPDH) functions as a receptor for human lactoferrin. Frontiers in Cellular and Infection Microbiology 7.

32. Dietz JV, Fox JL, Khalimonchuk O (2021) Down the iron path: Mitochondrial iron homeostasis and beyond. Cells 10: 2198.

33. Kumari A (2023) Sweet Biochemistry: Remembering Structures, Cycles, and Pathways by Mnemonics. Elsevier.

34. Dunaway LS, et al. (2024) Heme metabolism in non-erythroid cells. Journal of Biological Chemistry: 107132.

35. Caza M, Kronstad JW (2013) Shared and distinct mechanisms of iron acquisition by bacterial and fungal pathogens of humans. Frontiers in Cellular and Infection Microbiology 3: 80.

36. Patidar A, et al. (2022) Host glyceraldehyde-3-phosphate dehydrogenase-mediated iron acquisition is hijacked by intraphagosomal Mycobacterium tuberculosis. Cellular and Molecular Life Sciences 79: 1-17.

37. Kopeckova M, Pavkova I, Stulik J (2020) Diverse localization and protein binding abilities of glyceraldehyde-3-phosphate dehydrogenase in pathogenic bacteria: The key to its multifunctionality? Frontiers in Cellular and Infection Microbiology 10: 89.

38. Boradia VM, et al. (2014) Mycobacterium tuberculosis acquires iron by cell-surface sequestration and internalization of human holo-transferrin. Nature Communications 5.

39. Müller M, Martin W (1999) The genome of Rickettsia prowazekii and some thoughts on the origin of mitochondria and hydrogenosomes. BioEssays 21: 377-381.

40. Walter PB, et al. (2002) Iron deficiency and iron excess damage mitochondria and mitochondrial DNA in rats. Proceedings of the National Academy of Sciences 99: 2264-2269.

41. Wood MJ, Skoien R, Powell LW (2009) The global burden of iron overload. Hepatology International 3: 434-444.

42. James J, et al. (2022) Dysfunctional mitochondria and hemolysis are linked by impaired maturation of reticulocytes: Possible role in pulmonary hypertension. The FASEB Journal 36.