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Friedreich Ataxia: New Pathways

Massimo Pandolfo, MD

Université Libre de Bruxelles - Hôpital Erasme

Abstract

Friedreich ataxia is a rare disorder characterized by an autosomal recessive pattern of inheritance. The disease is noted for a constellation of clinical symptoms, notably loss of coordination and a variety of neurological and cardiac complications. More recently, scientists have focused their research on an array of general investigations of the underlying cellular basis for the disease, including mitochondrial biogenesis, iron-sulfur cluster synthesis, iron metabolism, antioxidant responses, and mitophagy. Combined with investigations that have explored the pathogenesis of the disease and the function of the protein frataxin, these studies have led to insights that will be key to identifying new therapeutic strategies for treating the disease.

Keywords

Friedreich ataxia; induced pluripotent stem cells; iron-sulfur cluster; metabolic pathways

Introduction

Friedreich ataxia, a severe disorder with autosomal recessive inheritance,¹ is a rare disease. A survey in France detected 11 carriers of this recessive disorder per 1000 tested from the general population,² leading to an estimated incidence of 2 to 3 out of 100 000 births. Neurological symptoms dominate the clinical picture of Friedreich ataxia and correlate with the underlying degenerative neuropathology.³ Atrophy of sensory and cerebellar pathways causes ataxia, dysarthria, fixation instability, deep sensory loss, and loss of tendon reflexes. Corticospinal degeneration leads to muscular weakness and extensor plantar responses. With progression, patients lose the ability to walk and become dependent on others for all activities. In some cases, and more commonly in advanced disease, visual loss and neurosensory deafness further increase disability. In addition to neurological involvement, hypertrophic cardiomyopathy, present in most cases, may become symptomatic and even cause premature death. About 10% of these patients develop diabetes, but subclinical abnormalities in glucose and insulin metabolism are universal. Other problems include skeletal abnormalities such as kyphoscoliosis, and, less commonly, pes cavus.⁴

Friedreich ataxia is caused by reduced expression of a small mitochondrial protein, frataxin.⁵ Most patients carry a homozygous mutation consisting of the expansion of GAA trinucleotide repeat within the first intron of the frataxin (FXN) gene,⁶ leading to a partial silencing of transcription through epigenetic mechanisms affecting the packaging of chromatin.⁷ A few patients with Friedreich ataxia (about 4%) are compound heterozygotes for the expanded repeat mutation in one allele and a deleterious point mutation in the other.⁸ The degree of frataxin expression reduction tightly correlates with the severity of clinical symptoms: frataxin amounts in symptomatic patients range between 5% and 35% of the

levels in healthy individuals, while heterozygous subjects with no sign of disease have approximately 50%. These data suggest that partially restoring frataxin expression in affected cells may slow or stop disease progression and stabilize or reduce the severity of disabilities.

Although the exact function of frataxin is still the subject of debate, available evidence supports a role in iron metabolism.⁹ Accordingly, the major cellular consequences of its deficiency include impairment of iron-sulfur clusters biogenesis, altered cellular iron metabolism, mitochondrial dysfunction due to iron overload, and increased oxidative stress. Gene expression profiling experiments indicate these abnormalities affect intracellular signaling pathways in a cell-specific manner, resulting in homeostatic adaptation in some cases, but in cell dysfunction and eventual cell death in others.^{10,11} We know from constitutional knockout experiments that a complete lack of frataxin is embryonic lethal for all investigated multicellular organisms (mice,¹² worms,¹³ flies,¹⁴ plants¹⁵). Similarly, conditional knockouts in the mouse result in progressive degeneration and death of all cells where the frataxin gene has been deleted, even when the targeted tissues are not affected in the human disease.^{16,17} Furthermore, cells without frataxin, isolated from conditional knockout mice, can only be maintained in culture for a short time and are unable to grow.¹⁸ Only unicellular eukaryotes such as yeast can survive without frataxin, but eventually they lose the ability to carry out oxidative phosphorylation.¹⁹

Although these data clearly show that frataxin is essential for the long-term survival of all cells from higher eukaryotes, very little is currently known about the biological basis for the selective vulnerability to low levels of frataxin found in patients with Friedreich ataxia, which underlies the specific pathology of the condition. One consequence of our lack of understanding specific cell vulnerability in Friedreich ataxia is our inability to design and test potential therapeutics targeting pathogenic mechanisms that operate in affected cell types. Adequate cellular and animal models are an essential tool to investigate the pathways affected by frataxin deficiency. Current models have been very useful to this purpose, but none of them is ideal and all have limitations, as discussed in the next section.

Animal and Cellular Models

Conditional knockouts do not faithfully recapitulate the human disease with its chronically and severely reduced frataxin levels due to the effect of large intragenic GAA repeat expansions. The main challenge for the development of better animal models derives from the technical difficulties of inserting large GAA repeats by homologous recombination or by transgenesis. Relatively small repeats (about 230 GAA triplets) are carried by the only current knock-in mouse model. These are insufficient to reduce frataxin levels enough to cause a full-blown phenotype even when in compound heterozygosity with an Fxn null allele (KIKO mice, which express 25% to 35% of the frataxin levels of wild-type mice).²⁰ A YAC transgenic rescue mouse model carrying about 200 GAA triplets in 2 copies of the human frataxin gene on a mouse *fxn*^{-/-} background does develop a motor phenotype and some Friedreich ataxia-like pathology.²¹ However, in addition to the genetic difference from the human disease, these animals still have rather elevated human frataxin levels and their phenotype may be in part due to lower activity of human frataxin in the mouse system.

As for cellular models directly obtained from patients with Friedreich ataxia, fibroblasts, peripheral blood mononuclear cells, and lymphoblastoid cell lines have been used to elucidate some aspects of pathogenesis and to test drug effects, but the metabolic and epigenetic characteristics of these clinically unaffected cell types differ from those of the specifically vulnerable cells such as neurons and cardiomyocytes.

Stable transfection of normal or mutated forms of frataxin into mouse fibroblasts carrying homozygously delete Fxn has been used as well.²² This model has been useful to address some aspects of the biochemical cascades triggered by frataxin deficiency, but again it makes use of non-specifically vulnerable cells and lacks expanded GAA repeats.

RNA interference-based knockdown of frataxin can be performed in animals, via viral transduction of short hairpin RNA constructs or transgene technology, in primary cultures of animal cells, in easily accessible human cells (eg, fibroblasts), and in immortalized cell lines. Downregulation by RNA interference, particularly when using inducible constructs, is a powerful approach to studying the consequences of frataxin deficiency and their reversibility. In practice, however, this approach is hardly applicable to human vulnerable cells such as neurons and cardiomyocytes, because of the difficulty in obtaining and maintaining long-term primary cultures of these cells. Furthermore, lack of expanded GAA repeats excludes this important area of investigation and of therapeutic intervention.

New Models: Induced Pluripotent Stem Cells

The possibility of reprogramming somatic human cells to acquire an embryonic stem cell-like phenotype is a powerful innovative method to develop new tools to study genetic diseases.²³ Induced pluripotent stem cells are pluripotent in nature and capable of indefinite self-renewal in vitro. This technique is technically relatively simple and is associated with fewer ethical issues, compared with those raised by somatic cell nuclear transfer and embryo manipulation. These characteristics make induced pluripotent stem cells a highly advantageous source for deriving any cell types, including the nervous system cells and cardiomyocytes affected in Friedreich ataxia. Several laboratories have generated induced pluripotent stem cells from patients with Friedreich ataxia that are now under intensive investigation. Already published results have helped to shed light on the mechanisms of GAA repeat instability, demonstrating a connection between repeat instability and the level of expression of some DNA repair enzymes.²⁴ Novel information on the consequences of frataxin deficiency in vulnerable cell types is now expected from these studies.

Pathways Affected by Frataxin Deficiency

Iron-Sulfur Cluster Biogenesis

Frataxin has been implicated in one of the most important iron-related metabolic pathways, the synthesis of iron-sulfur clusters. Iron-sulfur clusters are ensembles of 2 or more iron atoms bridged by sulfide centers. A number of metalloproteins (iron-sulfur proteins) in both prokaryotes and eukaryotes contain iron-sulfur clusters, which are essential for their function, structure and stability.²⁵ Biological iron-sulfur clusters are most commonly of the [2Fe-2S] type or of the [4Fe-4S] type. [2Fe-2S] clusters consist in 2 iron atoms bridged by 2 sulfides, which are bound to the protein backbone through 4 cysteines (2 cysteines and 2 histidines in Rieske proteins), [4Fe-4S] clusters have a cubane structure and are bound to the protein backbone through cysteine residues.

Iron-sulfur proteins are found in different cell compartments and have diverse functions. In mitochondria, iron-sulfur proteins include, among others, several subunits of respiratory complexes I, II, and III and a complex I assembly factor (*NUBPL*), the Krebs cycle enzyme aconitase, a ferredoxin, ferrochelatase (the enzyme that inserts Fe into protoporphyrin IX to form heme), the molybdenum cofactor synthesis enzyme MOCS1A, and the membrane protein MitoNEET, a target of the thiazolidinedione antidiabetic drugs. Iron-sulfur proteins are present also in the cytosol and in the nucleus, where they are involved in different biological processes, including control of iron metabolism, various metabolic pathways,

signaling pathways, and DNA repair. Such a wide distribution and functional variety of iron-sulfur proteins clearly show how their importance cannot be overstated.

In simple eukaryotes such as yeast, iron-sulfur cluster biogenesis takes place in the mitochondria. In higher eukaryotes, iron-sulfur cluster biogenesis takes place in mitochondria and in the cytosol, but cytosolic iron-sulfur cluster synthesis appears to depend on the integrity of the mitochondrial assembly system and on the export of a still unknown component from the mitochondria through a carrier known as ABCB7 in mammals. Mitochondrial iron-sulfur cluster biogenesis in higher eukaryotes is carried out by an assembly machinery composed by many factors.²⁵ The list is probably not yet complete and the function of all components is not known with certainty. The main factors involved in iron-sulfur cluster biogenesis in humans are the scaffold protein ISCU, where the nascent iron-sulfur cluster is assembled; the cysteine desulfurase enzyme (EC 2.8.1.7) NFS1, which acts as sulfur donor by catalyzing the reaction that converts L-cysteine into alanine and a highly reactive persulfide group; the small protein ISD11, which forms a complex with NFS1 and is needed for its stability and function; the ferredoxin FDX1 and the ferredoxin reductase FDXR, which provide reducing equivalents; and NFU, which may be an alternative scaffold protein. Cluster transfer from ISCU to the apoproteins to form holo-iron sulfur proteins requires a further set of factors: the chaperones HSCB and mortalin; the glutaredoxin GLRX5; the GrpE-L1/2 nucleotide exchange factor; and a set of specific assembly factors for certain iron sulfur proteins, including Ind1 for complex I, and ISCA1, ISCA2, and IBA57 for SAM-dependent proteins and aconitase.

Recent work has shown that frataxin is part of the iron-sulfur cluster assembly complex and functions as an allosteric activator. The complex without frataxin is essentially in an off state, only when frataxin is present iron-sulfur cluster synthesis can proceed at a significant rate.²⁶ As a result, when frataxin levels are too low, the function of multiple iron-sulfur proteins is impaired. Reduced activities of aconitase and of complexes I, II, and III of the respiratory chain were first detected in endomyocardial biopsies of children with Friedreich ataxia-related cardiomyopathy,²⁷ then deficiencies in iron-sulfur protein activities in mitochondria and other cellular compartments²⁸ were confirmed in various tissues from patients with Friedreich ataxia, as well as in cellular and animal models.^{16,17,29}

Reduced iron-sulfur protein activities affect many cellular functions. Energy production is expected to be impaired because of the presence of iron-sulfur proteins in the Krebs cycle and in the respiratory chain. Accordingly, preliminary data show that neurons obtained from Friedreich ataxia induced pluripotent stem cells have decreased mitochondrial membrane potential compared with controls (our unpublished results and J. Gottesfeld, personal communication). Among the several extramitochondrial iron-sulfur proteins, at least 3 (xanthine oxido-reductase, glutamine phosphoribosylpyrophosphate amidotransferase, and the DNA repair protein Nth1) have been shown to be affected in frataxin-deleted mouse tissues,²⁸ with an expected impact on the respective pathways.

Iron Accumulation in Mitochondria and Oxidative Stress

Impaired iron-sulfur cluster synthesis alters iron metabolism in at least 2 major ways. First, iron that enters mitochondria for iron-sulfur cluster synthesis and is not efficiently used tends to accumulate in the organelle. Iron is imported into mitochondria by 2 membrane carriers, called mitoferrin 1 and 2.³⁰ Mitoferrin 1 is erythroid cell-specific; mitoferrin 2 is present in all cells. Mitoferrins carry reduced (ferrous) iron, which, if not rapidly used in a biosynthetic process, can rapidly become oxidized and form insoluble precipitates. Such precipitates have been detected in mitochondria from frataxin-depleted yeast, from conditional frataxin KO mice, and also in the myocardium of patients with Friedreich ataxia.³ Iron oxidation in mitochondria is likely to be damaging, because of the toxic free

radicals it generates. Ferrous iron is oxidized by reactive oxygen species, in particular by hydrogen peroxide (H_2O_2) through the Fenton reaction ($\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^\bullet$), which produces the highly toxic hydroxyl radical ($\text{OH}^\bullet$). In addition to the presence of excess iron, reactive oxygen species also become more abundant in mitochondria of frataxin-deficient cells because of respiratory chain dysfunction. Iron-sulfur clusters in complexes I, II, and III are involved in electron transport. Insufficient iron-sulfur cluster synthesis leads to impaired electron flow, increasing leakage of electrons from the respiratory chain before they can reach complex IV (cytochrome c oxidase), where normally they reduce molecular oxygen to water. These prematurely leaked electrons directly react with molecular oxygen to form superoxide, which is turned into hydrogen peroxide by mitochondrial superoxide dismutase, feeding the Fenton reaction with its other ingredient in addition to ferrous iron. Although direct evidence for these mechanisms is still partial,³¹ there are observations of increased superoxide in Friedreich ataxia fibroblasts³² and of oxidative damage in several cellular and animal models of Friedreich ataxia.^{33–36} The absence of signs of oxidative damage in frataxin-deleted mouse tissues³⁷ remains puzzling, but it may well be the consequence of a nearly complete respiratory chain shutdown in cells entirely devoid of frataxin.

Increased production of reactive oxygen species has a direct damaging effect on many cellular components (proteins, nucleic acids, lipids), but also activates signaling pathways, which may be protective or cell death-promoting according to the intensity and kind of oxidative stress and cell type. An example is the activation of the “stress kinase” pathway, with increased levels of mitogen-activated protein kinase kinase 4 and increased phosphorylation of c-Jun N-terminal kinase, which has been detected in frataxin-deficient cells.³⁸ This basally “hyperactive” stress signaling pathway increases vulnerability to stress-induced apoptotic cell death, eg, by serum withdrawal,³⁸ and may play a role in Friedreich ataxia pathogenesis. In addition, protective antioxidant responses normally triggered by oxidative stress are blunted in frataxin-deficient cells.^{39,40} There is evidence that 2 major pathways involved in antioxidant responses, the nuclear factor-erythroid 2-related factor 2⁴¹ and the peroxisome-proliferator activated receptor gamma coactivator 1-alpha³² pathways, are both impaired. Though the responsible mechanisms are still unclear, increased H_2O_2 production and altered iron metabolism are thought to play a role (see below).

Cellular Iron Metabolism

The second major consequence of impaired iron-sulfur cluster synthesis on iron metabolism is the abnormal activation of iron regulatory protein 1, a factor controlling the expression of a set of proteins involved in iron uptake, storage, and use.⁴² Iron regulatory protein 1 is localized in the cytosol and contains a cubane iron-sulfur cluster. When this iron-sulfur cluster is entirely assembled, the protein function as a cytosolic aconitase (eg, it enzymatically converts citrate into isocitrate). When cytosolic iron is low (ie, when the cell is iron-deficient), the iron regulatory protein 1 iron-sulfur cluster loses a labile iron atom and disassembles, leading to a conformational changes that turns iron regulatory protein 1 into an RNA-binding protein.

A second iron regulatory protein, iron regulatory protein 2, is also an RNA-binding protein, but does not contain an iron-sulfur cluster. Iron regulatory protein 2 undergoes iron-triggered degradation so, when cytosolic iron is low, its level increases along with its binding to the target RNAs. Iron regulatory proteins bind to specific motifs in messenger RNAs, called iron-responsive elements, which are located either in the 5' untranslated region, usually as a single iron-responsive element, or in multiple copies in the 3' untranslated region. Iron regulatory protein binding to a 5' iron-responsive element blocks translation, thus decreasing protein level, while iron regulatory protein binding to 3' iron-responsive elements stabilizes the messenger RNA, thus increasing translation and protein

levels. Proteins with 3' iron-responsive elements whose levels increase after iron regulatory protein activation promote iron uptake (eg, the transferrin receptor [TfR]), while proteins with a 5' iron-responsive element are iron storage proteins (eg, ferritin, and proteins that use iron, including some iron-sulfur cluster-containing proteins like mitochondrial aconitase). Therefore, iron regulatory protein activation eventually induces an increase in cytosolic iron, leading to iron regulatory protein 2 degradation and to restoration of the iron regulatory protein 1 iron-sulfur cluster, which turns it back into a cytosolic aconitase.

Under normal circumstances, this negative feedback mechanism assures cellular iron homeostasis. Frataxin deficiency alters iron regulatory protein function primarily as a consequence of impaired iron-sulfur cluster biogenesis, which results in a larger proportion of iron regulatory protein 1 molecules that are devoid of an iron-sulfur cluster and, therefore, in their RNA-binding conformation.⁴³ Furthermore, frataxin-deficient cells show cytosolic iron depletion, which has been attributed to iron being accumulated and trapped in mitochondria.^{19,44,45} Together, these mechanisms activate an iron regulatory protein 1-mediated cellular response occurring during iron deficiency, whose first consequence is the upregulation of cellular iron uptake through increased translation of TfR messenger RNA. In normal conditions, iron is then transported into the mitochondria, where it is used for heme and iron-sulfur cluster synthesis, the iron-sulfur cluster of iron regulatory protein 1 is restored, cytosolic iron levels increase and the iron regulatory protein response stops. Frataxin deficiency, by impairing iron-sulfur cluster synthesis, prevents termination of the iron regulatory protein response and leads to oxidation and accumulation of iron in mitochondria, turning a homeostatic response into a pathogenic vicious cycle.⁴⁵

Alterations in Metabolic Pathways

Gene expression profiling studies by microarray analysis in tissues from frataxin-deficient mice and in peripheral blood mononuclear cells from patients with Friedreich ataxia and carriers have consistently shown that frataxin deficiency affects several metabolic pathways. Studies in animals that do not develop any obvious degenerative process (such as KIKO mice) and in peripheral blood mononuclear cells are of particular interest, because the observed gene expression phenotypes are likely to be a direct consequence of frataxin deficiency rather than the nonspecific result of cellular stress and cell death. In the central nervous system of KIKO mice, this phenotype followed the known regional susceptibility in this disease, with most changes occurring in the spinal cord, and gene ontology analysis identified a clear mitochondrial component.⁴⁶ In heart and skeletal muscle of the same mouse model there was molecular evidence of increased lipogenesis in skeletal muscle, and alteration of fiber-type composition in heart, consistent with insulin resistance and cardiomyopathy, respectively.

Coordinate dysregulation of the transcriptional co-activator peroxisome-proliferator activating receptor gamma coactivator 1-alpha (downregulated) and of the transcription factor sterol responsive element binding-protein 1 (upregulated) could explain at least in part the observed changes and was confirmed in other cellular and animal models of frataxin deficiency, and in cells from patients with Friedreich ataxia (fibroblasts and peripheral blood mononuclear cells).¹¹ As for gene expression changes in human peripheral blood mononuclear cells, these were not only observed in patients, but also in Friedreich ataxia carriers, indicating that even mild frataxin deficiency that does not cause disease impacts cellular homeostasis. Changes in gene expression were related to the mitochondria, lipid metabolism, cell cycle, and DNA repair.⁴⁷ Finally, the occurrence of metabolic dysregulation in patients with Friedreich ataxia is also supported by the observation that these patients as a group show reduced insulin sensitivity, which cannot be attributed to their motor impairment.¹¹

Putting It All Together: New Pathways

Despite remarkable progress in defining the consequences of frataxin deficiency, we have not yet understood the pathogenesis of Friedreich ataxia. Two related questions need to be addressed. First, how do we combine all the observations into a coherent picture, in which each pathogenetic step is mechanistically explained? Second, how do we explain specific vulnerability, or, in other terms, how frataxin deficiency differentially affects distinct cell types? The answers to these questions are likely to come from studies on new animal and cellular models. In particular, induced pluripotent stem cells from patients with Friedreich ataxia, already available in several laboratories, appear to be a very promising tool for this purpose. We can, however, define some hypotheses to direct future investigations. The basic assumption, now supported by many data, is that frataxin is an activator of mitochondrial iron-sulfur cluster biogenesis. The similarity between the phenotype of frataxin-deficient yeast and yeast depleted of other iron-sulfur cluster biogenesis factors has been the first indirect evidence in this regard.⁴⁸ The observation of biochemical and genetic interactions with the highly conserved NFS1/ISCU complex, the 2 main components of the iron-sulfur cluster assembly machinery,^{49–51} has further supported this hypothesis. Yet there are still several controversies surrounding the function of frataxin.

One important point is the identification of the functionally relevant form of frataxin and the confirmation of its exclusive subcellular localization in mitochondria. Concerning the first point, the 210 amino acid frataxin precursor synthesized on cytosolic ribosomes is imported into mitochondria, then processed by mitochondrial processing peptidase, with the generation of several isoforms that differ in the number of amino acids that have been removed from the N-terminus. Frataxin 81–210 is clearly the most abundant isoform, retaining all structural features needed to interact with the iron-sulfur cluster assembly factors. Controversy concerns frataxin 42–210, considered by some to be just a processing intermediate, but by others a functionally relevant isoform because, through interactions involving the extra amino acids at the N-terminus, it is able to oligomerize.⁵² Clearly, this is crucial to establish if oligomerization is an accidental, dispensable property only shown by incompletely processed frataxin, or it is an important property for the full functionality of the protein and, in particular, to provide antioxidant and iron-detoxifying properties, as proposed by some researchers.^{53–55} Another controversy concerns the presence and functional relevance of a cytosolic pool of frataxin, possibly with a cytoprotective and antiapoptotic function.⁵⁶ Despite these controversies, there is little doubt that mature frataxin has an intramitochondrial localization and that the primary function of frataxin is in mitochondrial iron-sulfur cluster synthesis. While the causal links between defective iron-sulfur cluster synthesis and abnormal iron metabolism have been in part identified, as described above, the mechanisms leading to other functional changes are much less clear. Here, some possibly involved pathways will be suggested.

First, a feedback loop between frataxin expression and some homeostatic changes following frataxin deficiency is emerging from the data. Reduced levels of the transcriptional co-activator peroxisome-proliferator activating receptor gamma coactivator 1-alpha, which are found in many frataxin-deficient tissues and cell types (fibroblasts, skeletal muscle, liver, neural precursor cells, peripheral blood mononuclear cells), with the notable exception of the heart, not only may contribute to mitochondrial dysfunction and impaired energy production, but also to further reduction of frataxin levels, as shown by peroxisome-proliferator activating receptor gamma coactivator 1-alpha knock-down experiments.^{11,32} Cytosolic iron depletion also causes downregulation of frataxin expression.⁵⁷ Iron is an important ingredient for mitochondrial biogenesis; it is needed by mitochondria for heme and iron-sulfur cluster synthesis and it is contained in many mitochondria hemoproteins and iron-sulfur proteins.

It is, therefore, tempting to link cytosolic iron deficiency, as occurring in frataxin-depleted cells, with suppressed mitochondrial biogenesis, including further downregulation of the expression of frataxin itself. A possible mechanism may involve the hypoxia-inducible factors-1 α and -2 α . These factors control the cellular response to hypoxia. Under normoxic conditions, they are hydroxylated by a set of enzymes (PHDs, FIH) that are iron Fe^{2+} and 2-oxoglutarate-dependent. Hydroxylated hypoxia-inducible factors bind to the Von Hippel-Lindau protein, which is a component of a ubiquitin ligase complex that delivers them to the proteasome system for degradation.⁵⁸ Under hypoxia, PHDs and FIH cannot hydroxylate hypoxia-inducible factors, which remain stable and transfer to the nucleus where they act as transcription factors. Cytosolic iron depletion may impair PHD/FIH function, leading to hypoxia-inducible factor activation even under normoxia. Hypoxia-inducible factor 1 α changes the transcription profile of the cell from one supporting aerobic to one supporting anaerobic metabolism, suppressing mitochondrial biogenesis and increasing glycolytic enzymes. Interestingly, one of hypoxia-inducible factor 1 α -induced genes encodes a micro-RNA, miR210, which downregulates ISCU,^{59,60} a key factor in iron-sulfur cluster synthesis and a frataxin interactor. Hypoxia-inducible factor 2 α has a similar, but not overlapping set of target genes, which, at least in the mouse, includes frataxin.⁶¹ Although hypoxia-inducible factor 2 α should also be stabilized when PHD/FIH lose activity, the presence of a 5' iron-responsive element in its messenger RNA may actually lead to block of its translation and consequent depletion when cytosolic iron is low and iron regulatory proteins are activated.⁶² Although there are no published data in this regard, the postulated increase in hypoxia-inducible factor 1 α and decrease in hypoxia-inducible factor 2 α in frataxin deficiency make a relatively easily testable hypothesis.

A second pathway that may link cytosolic iron depletion with impaired mitochondrial biogenesis and accumulation of altered mitochondria, as has been seen in frataxin-depleted cells and animal tissues, involves the energy sensor AMP-dependent protein kinase. Adenosine monophosphate-dependent protein kinase is allosterically activated by adenosine monophosphate, whose levels increase when the cell has a low energy level, reflected in low adenosine triphosphate and high adenosine diphosphate (which may be converted to adenosine monophosphate). Adenosine monophosphate-bound adenosine monophosphate-dependent protein kinase is much more efficiently phosphorylated by the constitutively active kinase LKB-1, and phospho-adenosine monophosphate-dependent protein kinase in turn phosphorylates several substrates, including peroxisome proliferator-activated receptor- γ coactivator 1- α , to promote mitochondrial biogenesis.⁶³ Recently, it has been shown that adenosine monophosphate-dependent protein kinase also activates mitophagy,⁶⁴ the autophagic process that removes abnormal mitochondria. Preliminary evidence (our unpublished results) suggests that cytosolic iron depletion reduces levels of total and phospho-adenosine monophosphate-dependent protein kinase, suggesting another testable hypothesis about Friedreich ataxia pathogenesis.

Conclusion

Studies on Friedreich ataxia pathogenesis and frataxin function have converged with more general investigations about fundamental cellular processes such as mitochondrial biogenesis, iron-sulfur cluster synthesis, iron metabolism, antioxidant responses, and mitophagy. This has created a highly fruitful interaction between basic scientists and researchers interested in understanding and eventually treating this dreadful disease. The progressive dissection of the iron-sulfur cluster synthesis machinery is a clear example of this convergence of interests. This process is continuing and promises to lead to new, exciting discoveries and to the identification of new therapeutic targets.

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