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Cardiomyopathy in Friedreich's Ataxia

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Abstract

Friedreich's ataxia (FRDA), an autosomal recessive disorder, is characterized by spinocerebellar degeneration and cardiomyopathy. Here we explore some of the putative mechanisms underlying the cardiomyopathy in FRDA that have been elucidated using different experimental models. FRDA is characterized by a deficiency in frataxin, a protein vital in iron handling. Iron accumulation, lack of functional iron-sulphur clusters, and oxidative stress seem to be among the most important consequences of frataxin deficiency explaining the cardiac abnormalities in FRDA.

Key words: Friedreich's ataxia; cardiomyopathy.

Abbreviations

FRDA = Friedreich's Ataxia, Iron-sulphur clusters (ISC), superoxide dismutase (SOD), induced pluripotent stem (iPS).

Introduction

Friedreich's ataxia (FRDA) is the most common inherited ataxia, affecting 2-3 in 100,000 live births in Caucasians. This autosomal recessive disorder is characterised by spinocerebellar degeneration leading to truncal and limb ataxia that typically becomes progressively severely debilitating. In addition to the neurological symptoms and signs, cardiomyopathy, which is the most common cause of death, is often a feature of FRDA. Although FRDA was first described more than a century ago in 1863 by the German neurologist Nicholaus Friedreich, the mechanisms underlying the different aspects of the condition, including the potentially lethal cardiomyopathy, remain inadequately understood. In addition, it often is difficult to predict the long-term progression of the different effects of the disease. In this review, the important cardiac features and potential causative mechanisms are discussed to explore these issues.

Methods

In February and March 2010, the First Author conducted systematic searches of published work in PubMed and Scopus on Friedreich's ataxia and cardiomyopathy. Clinicaltrials.gov was searched for any trials of FRDA treatment.

Studies have been conducted in a wide range of models such as cell culture lines, yeast and other unicellular organisms, mouse knockout models, and patients. There is a lack of well-designed, large randomised placebo-controlled trials of putative therapeutic agents that would strengthen the evidence base of theories. Further, there is a need for long-term studies that can follow the progress of the disease and effect of treatments.

What are the cardiac findings in FRDA?

Cardiomyopathy, diabetes mellitus and skeletal abnormalities such as scoliosis are some of the common non-neurological FRDA presentations. Hypertrophic cardiomyopathy affects at least 40% of FRDA patients and is the most common cause of death (1). The frequency of this cardiomyopathy, characterised by concentric ventricular hypertrophy or asymmetrical septal hypertrophy as seen on echocardiography, increases with the size of the GAA expansion (2). In a retrospective study of patient notes, it was demonstrated that cardiac hypertrophy and ejection fraction worsens with age but the rate of decline is greater after adolescence. Both are unrelated to the severity of neurological disability (3). Severe cases progress onto cardiac dilatation and heart failure.

Although echocardiography abnormalities may not always be present, almost all patients demonstrate electrocardiography (ECG) anomalies such as hypertrophy, Q waves, conduction blocks, non-specific ST and T wave changes, and/or arrhythmias (4).

Similarly, patients may range from being asymptomatic to having more significant symptoms such as chest pain, shortness of breath and palpitations (5). These symptoms reflect the varying severity and progression of the heart disease in FRDA, which is often hard to predict. It has been recommended that all patients should receive echocardiography and ECG follow-up to pick up cardiac diastolic or systolic dysfunction, conduction abnormalities and arrhythmias, and ischaemia and hypokinesis (5).

What is the genetic abnormality and its consequences?

Over 95% of FRDA cases are caused by a hyper-expansion of GAA trinucleotide repeats located in the first intron, i.e. a non-coding region, of the FXN gene on chromosome 9. Less commonly, patients are compound heterozygotes for the expanded GAA repeat and a different loss-of-function mutation, such as a point mutation or a deletion. In all cases, patients still express structurally and functionally normal frataxin, but in a reduced amount (6). Complete loss of function appears to be lethal during embryonic development in mouse knockout models (7). The reduced level of frataxin in FRDA patients causes cell dysfunction and death only in specifically vulnerable cell types, such as sensory, cerebellar and pyramidal neurons and cardiomyocytes. FXN is widely expressed, with the highest levels in the heart and the spinal cord (6), possibly indicating a correlation between the normal level of its expression and specific vulnerability to FRDA.

The exact function of the highly conserved mitochondrial protein frataxin, and the sequence of events and their consequences in FRDA remain hotly debated. However, frataxin's role in iron metabolism is considered to be vital in the pathophysiological process. There is a consensus that frataxin contributes to the biogenesis of iron-sulfur clusters in mitochondria, but other postulated functions in mitochondrial iron-storage, heme synthesis, and free radical generation remain controversial.

Frataxin and its yeast homologue, yfh1, appear to have several low affinity iron binding sites, whose functional roles and relative importance still need to be clarified (7). Furthermore, the physiological relevance of high molecular weight frataxin-iron complexes, more easily formed by yfh1 than mammalian frataxins, has not yet been determined. In any case, yeast models, conditional mouse knockouts and FRDA patient tissues, in particular the heart, demonstrate mitochondrial iron accumulation (8, 9). In the mouse model of FRDA cardiomyopathy, the iron accumulation was promoted by increased transferrin receptor 1 expression and therefore transferrin iron uptake (10). A pathogenic role of increased mitochondrial iron, particularly as a source of free radicals, is postulated but not yet definitely proven.

How are iron-sulfur clusters (ISC) important?

One of frataxin's important functions is thought to be its role in the assembly of complexes of iron and sulfur atoms called iron-sulfur clusters (ISC), which act as prosthetic groups for various enzymes. Bacterial and yeast frataxin homologues interact in multiprotein complexes with essential factors for the assembly of ISC (11, 12). It has been proposed that frataxin acts as an iron donor in ISC biogenesis (13), however this has never been directly proven in vivo or in an in vitro reconstructed complete ISC synthesis assembly complex. A regulatory role in ISC synthesis has also been postulated, mostly on the basis of in vitro data with partially reconstructed enzymatic systems (14) and of structural data (15).

Whatever the primary role of frataxin in ISC biogenesis, reduced activities of multiple ISC-containing enzymes are systematically observed in cells and tissues where frataxin is deficient. In post-mortem cardiac samples and endomyocardial biopsies from patients with FRDA, there was a significant reduction in the function of ISC dependent complexes I, II and III of the electron transport chain and the Krebs' cycle aconitase (8, 16). A deficiency of these mitochondrial proteins would be expected to disrupt ATP production, which was confirmed by 31P magnetic resonance spectroscopy in skeletal muscle and myocardium of patients (17, 18). Further, the cardiac hypertrophy correlated significantly with the cardiac energy deficiency suggesting that the energy production dysfunction may explain the hypertrophy in FRDA (18). However, this does not necessarily mean that energy deficiency or cardiac hypertrophy correlate with mortality in FRDA.

Puccio *et al*. reported, in conditional mouse knockouts, that the deficiency in function of these proteins and cardiac hypertrophy precede mitochondrial iron accumulation, suggesting that the primary pathogenic event may be altered ISC synthesis rather than toxicity from accumulated iron (9). On the other hand, it is possible that the initial stages of FRDA may cause iron elevation in particular microenvironments that result in mitochondrial dysfunction, which was not detectable with techniques used. In the future, more sensitive measures may help uncover more details, such as a fluorescent indicator protein dependent imaging technique that gives good temporal and spatial resolution. Nevertheless, Puccio *et al*.'s finding is consistent with another report

demonstrating abnormal cardiac ATP levels in a small group of FRDA patients without hypertrophy or cardiac failure (1). In fact, the ISC enzyme deficiency (and therefore probably energy generation) precede echocardiography changes in the FRDA cardiomyopathy mouse models which lack cardiac FXN expression (19). This would support the hypothesis that cardiac hypertrophy results from a dysfunction in energy production in a process that may be similar to other mitochondrial conditions associated with both electron transport chain dysfunction and cardiac hypertrophy (1).

Although frataxin is a mitochondrial protein, it is also important in the assembly of Fe-S clusters of nuclear and cytosolic enzymes, at least in mouse knockout models (20). In a mouse model with cardiac deleted FXN, the activity of a nuclear DNA repair enzyme with ISC, Nth1, was reduced (20) suggesting that pathophysiology of FRDA may not be limited to the mitochondria. The varying severity of cardiac disease in FRDA may reflect not only the severity of the mitochondrial dysfunction but damage to these other pathways that are vital for cell survival such as DNA repair.

What is the contribution of oxidative stress?

Frataxin deficiency increases the production of highly toxic free radicals that can damage proteins, lipids and nucleic acids (21). The free radical damage could also contribute to the energy generation problems mentioned earlier. Frataxin deficiency increases hydrogen peroxide (H2O2) and iron due to the impairment of ISC synthesis and the subsequent impairment in mitochondrial respiration (7). Hydrogen peroxide generates hydroxyl radicals by reacting with reduced iron (Fe2+) in the Fenton reaction.

Yeast with particular Yfh1 mutations that spared the postulated iron donor capabilities for ISC synthesis, nevertheless hadincreased iron-induced oxidative stress, impaired function and increased mortality (22). This finding has been attributed to the ability of frataxin to detoxify iron under iron excess conditions. Fibroblast cell lines from FRDA patients were more sensitive to oxidative stress from H2O2 and iron and desferrioxamine, an iron chelator that removes membrane-bound iron, had a protective effect (23). However, desferrioxamine in heart homogenates impairs aconitase function (24) making it unclear if in vivo chelation would be beneficial. In fact, in the conditional mouse knockout model, chelation therapy with desferrioxamine and PIH (a membranepermeable chelator) reduced cardiac iron accumulation and the extent of cardiac hypertrophy but did not improve ventricular function (10). This lack of improvement may be due to the detrimental effect on other structures by desferrioxamine, or may indicate that iron reduction by itself is not sufficient to treat FRDA cardiomyopathy. Currently, results are expected from a randomised, placebo-controlled double-blind study of deferiprone, an iron chelator, administered for 6 months. This may help to shed further light on this issue.

Evidence of increased oxidative damage due to frataxin deficiency has led to the trial of antioxidants as treatment for FRDA. These trials provide a test of the argument that oxidative stress is central to cardiomyopathy (and possibly neurodegeneration) in FRDA. In a patient study for 47 months, coenzyme Q10 and vitamin E therapy improved myocardial energy generation and fractional shortening without affecting cardiac hypertrophy (25). The synthetic analogue of coenzyme Q10, idebenone (a free radical scavenger), protects membrane lipids and mitochondrial proteins from Fe2+ induced injury in heart homogenates from FRDA patients (24). In small patient studies, idebenone, unlike coenzyme Q10, improved septal thickness, ventricular hypertrophy, shortening fraction and any outflow obstruction in patients treated for at least 4 months when followed up for up to 1 year (24, 26). On the other hand in a larger study with a follow-up of up to 7 years, posterior wall thickness decreased but septal thickness did not improve and ejection fraction deteriorated (27). However, the study did not have a control group and therefore it is unclear how protective idebenone is. In fact, idebenone slows down but does not arrest the cardiac remodelling in mouse models and therefore one would continue to expect a deterioration albeit slower. In these models life span was improved by 1 week but it must be noted that in the murine models cardiac frataxin expression was abolished (19). Therefore, in FRDA where there is some frataxin activity, idebenone may be more effective. The benefit in the mice occurred without affecting the ISC enzyme deficiency, which is in contrast to findings in a single patient report (28). This requires clarification in a larger patient study.

It is important to notice that a pathogenic role of oxidative stress in FRDA has not yet been definitely proven and some contradicting data exists. In particular, the group that generated frataxin conditional KO mice reported that increased superoxide dismutase activity to reduce superoxide in the murine FRDA cardiomyopathy model did not improve survival. They also did not find any increase in oxidised proteins in frataxindeficient cerebellar mice tissue. They conclude that oxidative stress is a minor player in FRDA (28). These controversial findings may have alternative explanations as well: SOD will

increase H2O2 and therefore may promote damage caused by the Fenton reaction, the MnTBAP used may have a toxic effect or SOD may not tackle the oxidative damage pathways important in FRDA, or, more generally, complete lack of frataxin, as occurring in conditional KO mice, may substantially differ from the human disease, where frataxin is present at low levels. In this regard, the residual level of activity of the respiratory chain may be critical, because an abnormally low activity, as in FRDA, leads to increased free radical production, while a complete shutdown, likely to occur when frataxin is absent, prevents the generation of oxygen radicals.

In comparison to the response of the heart hypertrophy, idebenone is unable to demonstrate a consistent improvement in debilitating ataxia of FRDA, though one study showed a possible dose-related improvement at least in young ambulatory patients (24). This highlights the need to further understand the anti-oxidant and/or other beneficial effects of idebenone. Further, it suggests that the predominant mechanism of disease vary between cardiac and other tissues

What are the current limitations and future directions?

The various models of FRDA have been powerful tools in gaining insight into the many putative mechanisms that may contribute to the cardiac manifestations. However, our interpretations need to always consider the potential differences between these models and patients. For example, an unicellular organism model, or a mouse model with no myocardial frataxin are likely to behave somewhat differently to FRDA patients. This underlines the need to develop new cellular and animal models that more closely reproduce the features of the human disease. For cellular models, a promising possibility is offered by the technology of induced pluripotent stem (iPS) cells. iPS cells are embryonic-stem cell like cells than can be derived from easily obtained adult cells, like skin fibroblasts. They can then be differentiated into many, potentially all somatic cell types, including neurons and cardiomyocytes, providing a source of vulnerable cell types that would otherwise be impossible to obtain from human subjects. In addition, patient studies have further helped to delineate the underlying mechanisms in FRDA. Long-term prospective patient studies of the progression of different pathological pathways and its relation to the phenotype would provide exciting new details to develop our understanding.

What remains unclear is if any of the treatments that show promise can prevent deteriorations in cardiac ejection fraction and improve mortality in FRDA patients. Another important question is if early treatment can prevent the occurrence of cardiomyopathy. These are both questions that need to be tackled. Large randomized placebo-controlled double blind trials with an appropriate length of follow-up would be ideal and could lead to future treatment strategies. The multiple suggested mechanisms might mean that trials have to use multiple agents that tackle the different pathways of damage.

Conclusion

In conclusion, FRDA patients show electrophysiological abnormalities and/or hypertrophic cardiomyopathy that may progress to cardiac dilatation and heart failure. This review has explored frataxin's several likely significant roles in the pathophysiology of the cardiac disease. However, it is still not clear if the lack of frataxin causes systems to fall apart through the collective effects of numerous problems or due to a principal pathway. It seems likely that a combined effect of loss of ISC enzymes, iron accumulation and oxidative stress contribute to the cardiac manifestations. If the cardiacrelated mortality is due to a particular mechanism is still unclear. Although not discussed in detail here, there are numerous other reported abnormalities that are less well understood but may be important, such as defects in heme synthesis. What is clear is that we require much more in depth study in both models and patients to develop the best therapeutic approach.

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