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Small molecules affecting transcription in Friedreich ataxia

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Abstract

This review concerns the development of small molecule therapeutics for the inherited neurodegenerative disease Friedreich ataxia (FRDA). FRDA is caused by transcriptional repression of the nuclear *FXN* gene, encoding the essential mitochondrial protein frataxin and accompanying loss of frataxin protein. Frataxin insufficiency leads to mitochrondrial dysfunction and progressive neurodegeneration, along with scoliosis, diabetes and cardiomyopathy. Individuals with FRDA generally die in early adulthood from the associated heart disease, the most common cause of death in FRDA. While antioxidants and iron chelators have shown promise in ameliorating the symptoms of the disease, there is no effective therapy for FRDA that addresses the cause of the disease, the loss of frataxin protein. Gene therapy and protein replacement strategies for FRDA are promising approaches; however, current technology is not sufficiently advanced to envisage treatments for FRDA coming from these approaches in the near future. Since the *FXN* mutation in FRDA, expanded GAA•TTC triplets in an intron, does not alter the amino acid sequence of frataxin protein, gene reactivation would be of therapeutic benefit. Thus, a number of laboratories have focused on small molecule activators of *FXN* gene expression as potential therapeutics, and this review summarizes the current status of these efforts, as well as the molecular basis for gene silencing in FRDA.

Keywords: Frataxin; Friedreich ataxia; Transcription; Polyamides; Histone deacetylase inhibitor

Abbreviations: FRDA, Friedreich ataxia; *FXN*, frataxin gene; HDAC, histone deacetylase.

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1. Introduction: Friedreich ataxia, the gene mutation and molecular basis for the disease

Friedreich ataxia (FRDA) is the most prevalent inherited ataxia (reviewed in Pandolfo, 1999) affecting approximately 1:50,000

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individuals. FRDA is caused by deficiency of the protein frataxin, a highly conserved nuclear-encoded, mitochondrial protein. Frataxin is believed to be involved in iron homeostasis, storage and transfer of iron–sulfur clusters to partner proteins and energy production in the cell, but its precise cellular function is controversial (Bulteau et al., 2004; Seznec et al., 2005; Bencze et al., 2006). Frataxin insufficiency leads to progressive spinocerebellar neurodegeneration, causing symptoms of gait and limb incoordination, slurred speech, muscle weakness and loss of position and vibration sense, with extraneural scoliosis, cardiomyopathy and diabetes. At present, there is no effective treatment for FRDA, and generally within 15–20 years after the first appearance of symptoms, affected individuals are confined to a wheelchair. Individuals with FRDA generally die in early adulthood from the associated heart disease.

FRDA is an autosomal recessive disease in which the majority of patients (~98%) inherit an expanded region of GAA●TTC triplet-repeats in the first intron of the frataxin (*FXN*) gene from both parents (Campuzano et al., 1996). Typically, the parents of FRDA patients are themselves asymptomatic heterozygous carriers of this expansion in one *FXN* allele. A small number of patients have one expanded allele and a second allele harboring a premature stop codon or point mutation (Bidichandani et al., 1997). FRDA patients have a marked deficiency of frataxin mRNA (Campuzano et al., 1996; Bidichandani et al., 1998), providing evidence that the expanded repeats cause gene silencing. The extent of frataxin deficiency is related to the length of the GAA●TTC repeats (Pianese et al., 2004). Individuals who are heterozygous for the mutation also have reduced levels of frataxin, but are asymptomatic. Two models have been put forward to explain *FXN* gene silencing by triplet expansion. In the first model, transcription inhibition is believed to be caused by an unusual DNA structure adopted by the GAA●TTC repeats (see below), which interferes with transcriptional elongation. In the second model, the GAA●TTC repeats, which resemble transcriptionally silent satellite DNA, recruit heterochromatin binding proteins and cause gene silencing through an inactive chromatin structure. Normal *FXN* alleles have 6–34 repeats, while FRDA patient alleles have 66–1700 repeats. Longer repeats cause a more profound frataxin deficiency and are associated with earlier onset and increased severity of the disease. Yeast cells deficient in the frataxin homologue accumulate iron in mitochondria and show increased sensitivity to oxidative stress (Babcock et al., 1997). This suggests that FRDA is caused by mitochondrial dysfunction and free radical toxicity, with consequent mitochondrial damage, axonal degeneration, and cell death. Human frataxin is likely involved in similar processes, since it is a mitochondrial protein, and FRDA patients have abnormal myocardial iron deposits (Harding, 1993). Based on these findings, antioxidant and iron chelation-based strategies appear promising in counteracting the course of the disease (Rotig et al., 2002; Richardson, 2003; Seznec et al., 2004; Hart et al., 2005; Boddaert et al., 2007). However, these strategies only treat the symptoms of the disease and not its cause; thus, pursuit of other approaches that address the cause of the disease are worthwhile. In this regard, stem cell therapy (Galvin & Jones, 2006), protein replacement (Mackenzie & Payne, 2007), and gene therapy (Gomez-Sebastian et al., 2007) could certainly correct frataxin deficits in affected individuals; however, these technologies are not sufficiently advanced to expect success for a neurological disease in the near term. Thus, pharmacological reactivation of the silenced *FXN* gene has received considerable attention over the past few years.

Extensive biochemical studies have documented that expanded GAA●TTC repeats adopt an unusual DNA structure (Bidichandani et al., 1998; Ohshima et al., 1998; Sakamoto et al., 1999; and additional references therein). Long GAA●TTC repeats form triplexes containing 2 purine (R) GAA strands along with 1 pyrimidine (Y) TTC strand, flanking a single-stranded pyrimidine region. Formation of the RRY triplex in plasmid DNA requires divalent metal ions and negative supercoiling. A high-resolution NMR structure of a GAA●TTC●GAA triplex has been presented (Mariappan et al., 1999). Additionally, RRY triplexes have been shown to adopt "sticky" DNA structures in vitro (Sakamoto et al., 1999). Sticky DNA is detected by reduced mobility of DNA restriction fragments containing GAA●TTC repeats on gel electrophoresis and likely arises from intramolecular association of long GAA●TTC repeat triplexes. An excellent cartoon visualization of a model for the formation of intramolecular sticky DNA is provided as supplementary on-line information to the paper by Sakamoto et al. (1999). Sticky DNA formation has been shown to inhibit transcription both in vitro and in transfection assays in mammalian cells (Sakamoto et al., 2001). Using cloned repeat sequences from FRDA patients, GAA●TTC repeats have been found to interfere with in vitro transcription in a length-dependent manner (Bidichandani et al., 1998; Ohshima et al., 1998; Grabczyk & Usdin, 2000b). This interference is most pronounced in the physiological orientation of transcription (i.e., synthesis of the GAA-rich transcript). These results are consistent with the observed correlation between repeat length, triplex formation and the age at onset and severity of disease. GAA●TTC repeat-mediated inhibition of replication in mammalian cells also provides strong evidence for the formation of RRY triplexes and sticky DNA in cells (Krasilnikova et al., 2007).

In contrast to the DNA structure-based mechanism for gene silencing by long GAA●TTC repeats, a study employing artificial transgenes for the lymphoid cell surface marker protein hCD2 has shown that expanded GAA●TTC repeats induce repressive heterochromatin in vivo, in a manner reminiscent of position effect variegated gene silencing (PEV; Saveliev et al., 2003). PEV occurs when a gene is located within or near regions of heterochromatin, and silent heterochromatin is characterized by the presence of particular types of histone modifications (e.g., H3-K9 trimethylation and histone tail hypoacetylation), the presence of histone deacetylases, DNA methyltransferases, chromodomain proteins, such as members of the HP-1 family of repressors, and polycomb group proteins (Elgin & Grewal, 2003). To investigate the role of chromatin structure in GAA●TTC repeat-associated gene silencing, Festenstein and coworkers Saveliev et al. (2003) found that the hCD2 transgene harboring GAA●TTC repeats is resistant to DNase I digestion at the promoter element, compared with the normal transgene lacking GAA●TTC repeats. An increase in nucleosome density within the GAA●TTC repeats