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Hemin rescues adrenodoxin, heme *a* and cytochrome oxidase activity in frataxin-deficient oligodendroglioma cells

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Abstract

Mutations in the frataxin gene cause neurodegeneration and demyelination in Friedreich's ataxia. We showed earlier that frataxin deficiency causes primary iron–sulfur cluster defects, and later causes defects in heme and cytochrome *c* hemoprotein levels. Iron–sulfur (Fe/S) clusters are required in two enzymes of heme biosynthesis in humans i.e. in ferrochelatase and adrenodoxin. However, decreases in ferrochelatase activity have not been observed in frataxin-deficient HeLa cells or patient lymphoblasts. We knocked down frataxin in oligodendroglioma cells using siRNA, which produced significant defects in the activity of the Fe/S cluster enzymes adrenodoxin and aconitase, the adrenodoxin product heme *a*, and cytochrome oxidase, for which heme *a* serves as a prosthetic group. Exogenous hemin produced a significant rescue of adrenodoxin, aconitase, heme *a* levels and cytochrome oxidase activity. Thus hemin rescues iron–sulfur cluster defects that are the result of frataxin-deficiency, perhaps as a consequence of increasing the pool of bioavailable iron, and thus should be more fully tested for beneficial effects in Friedreich's ataxia models.

Keywords: RNAi; Frataxin; Iron–sulfur cluster; Heme; Adrenodoxin; Hemin; Theraphy

1. Introduction

Mutations in the frataxin gene result in deficiency of the frataxin protein, expressed in mitochondria, causing the neurodegenerative disease Friedreich's ataxia (FRDA). Although the primary physiological role of frataxin has been debated, a demonstrated function of frataxin is the support of iron–sulfur (Fe/S) clusters [1–3], which function in iron–sulfur enzymes inside mitochondria.

A microarray study carried out with lymphoblasts and fibroblasts from FRDA patients, human neural cells, and cardiac cells from knockout mice showed that the most consistent transcriptional consequence of frataxin deficiency was an inhibition of the heme pathway transcript, coproporphyrinogen oxidase [4].

Furthermore, we observed elevated protoporphyrin IX and heme *b* levels, and deficiency of hemes *a* and *c* and cytochrome oxidase in lymphoblasts from Friedreich's patients [4,5]. Work with an inducible knockdown model showed that Fe/S functions decline rapidly after frataxin deficiency, whereas the heme defect occurs after the decline of Fe/S function in the mitochondria. This is consistent with the idea that heme deficiency is a consequence of a mitochondrial Fe/S cluster defect [6].

There are two known Fe/S enzymes in mammalian heme biosynthesis, ferrochelatase and adrenodoxin. Recently we demonstrated that the activity of ferrochelatase in cells from patients with FRDA is not decreased [5], consistent with work from other groups [7].

The only other Fe/S enzyme besides ferrochelatase known to participate in the heme biosynthetic pathway is adrenodoxin, which carries out the first step of the conversion of heme O to heme *a,* that is required for cytochrome oxidase activity. Thus we knocked down frataxin levels using siRNA in a human

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oligodendroglial cell line (HOG), and observed decreased adrenodoxin activity, decreased heme *a* levels, decreased cytochrome oxidase activity, and decreased heme *c* level.

To see if exogenous heme could rescue these frataxin-dependent effects, we supplemented the frataxin-deficient HOGs with hemin, and observed a significant rescue of adrenodoxin, aconitase, and increased cytochrome oxidase activity and heme *a* levels, suggesting that hemin supplementation may be a rational therapeutic strategy for FRDA patients.

2. Results

2.1. There are two iron–sulfur cluster enzymes in the heme biosynthetic pathway

Frataxin is important for Fe/S cluster biogenesis in the mitochondria [1–4,6,8,9]; its deficiency in human cells causes a defect in heme *a,* a defect in the activity of the heme *a*-containing enzyme cytochrome oxidase, and also in cytochrome *c* and heme *c* levels [4,5]. There are only two enzymes of the heme biosynthetic pathway known to have Fe/S clusters, ferrochelatase and adrenodoxin (Fig. 1). Frataxin-deficiency does not cause a decrease in the activity of the Fe/S cluster enzyme ferrochelatase in FRDA lymphoblasts or HeLa cells [1,4]. It has been proposed that the yeast homolog of the human adrenodoxin (ferredoxin) is specifically involved in the first step of conversion of heme O to heme *a* in the heme biosynthetic pathway [10]. Heme *a* is in turn inserted in cytochrome oxidase and acts as the electron transport prosthetic group of the enzyme. Thus, we transfected human HOG cells with frataxin siRNA to see if frataxin level has an effect on the Fe/S enzyme adrenodoxin that participates in the heme pathway.

The human oligodendroglioma cell line (HOG) was transfected with frataxin-specific short interfering RNA (RNAi) [3], which resulted in an overall strong inhibition of the targeted message, and about 40% residual frataxin protein level (Fig. 2) relative to transfection with the 'scrambled' RNAi, i.e. an RNAi of identical nucleotide content but in randomized order.

2.2. Frataxin depletion reduces adrenodoxin and cytochrome oxidase activity in oligodendroglioma cells

Adrenodoxin and cytochrome oxidase activities were measured in scrambled and si-ftx HOGs; frataxin -deficiency produced a similar and significant reduction, of about 35%, in the activities of these two enzymes, compared with cells transfected with the scrambled oligonucleotide (Fig. 3).

2.3. Frataxin-deficiency produces heme a and cytochrome c heme deficiency

We previously observed that both hemes *a* [4] and cytochrome *c* (i.e.'heme *c*'), were deficient in cells from Friedreich's patients, the latter perhaps as the result of some feedback from the adrenodoxin deficiency [4,5].

Using HPLC we measured heme *a* levels in frataxin-deficient HOG cells and observed that it was significantly reduced with respect to the scrambled oligonucleotide (Fig. 4A). Cytochrome *c* heme amount was evaluated as previously described [6] (Fig. 4C); and was significantly decreased in the frataxin-depleted cells (Fig. 4B).

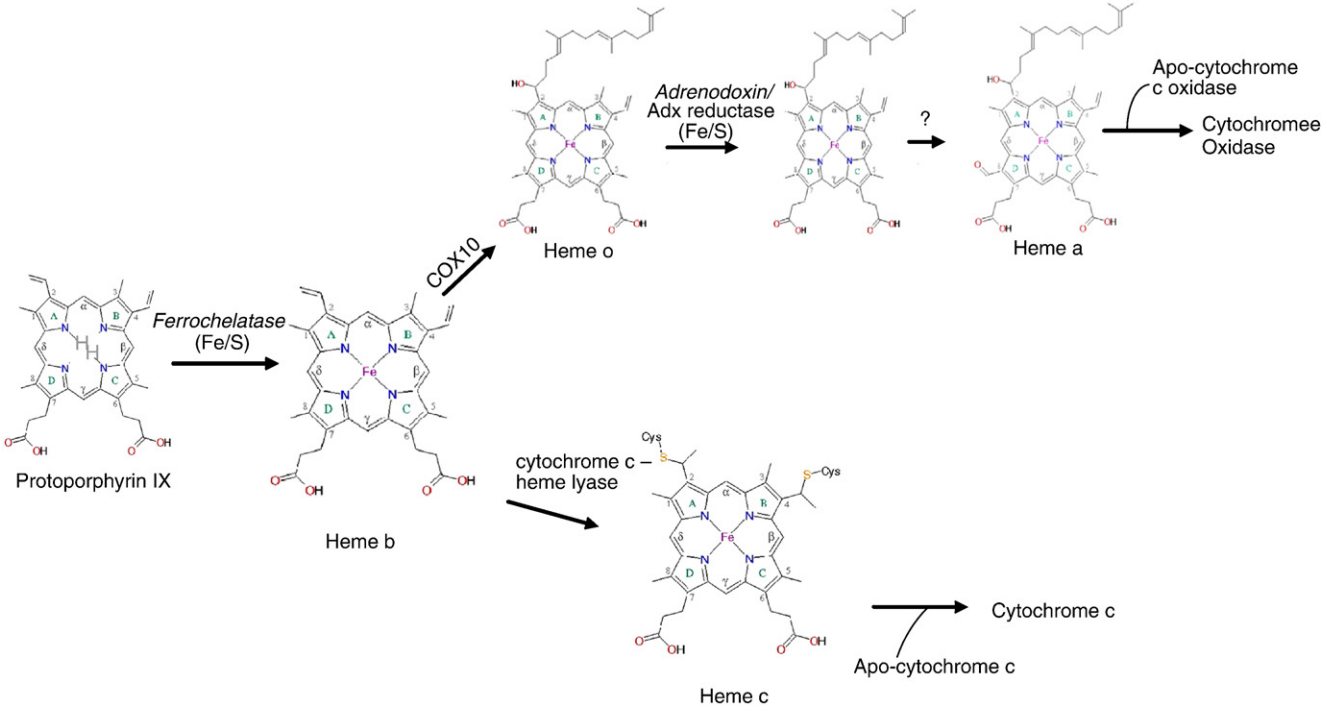

Fig. 1. Heme *a* and *c* biosynthetic pathways. Adapted from Schoenfeld et al. [4].