

SUMMARY OF FINAL REPORT

Understanding the role of frataxin in cell survival

Principal researcher: Dr Roberto Testi, University of Rome, Italy

April 2007 – April 2009

Background and aims:

We know that Friedreich's ataxia (FA) is caused by the inherited deficiency of a protein called frataxin, which is found in the mitochondria of cells. Frataxin deficiency likely generates oxidative stress in a specific set of neurons and cardiac cells, which eventually die prematurely, causing FA. Consequently, current therapy is based on anti-oxidants. However, more specific and effective therapeutic approaches are needed. To design a definitive cure we need to understand better why frataxin-defective cells die prematurely. This project directly investigated the role of frataxin in the protection of human cells from oxidative damage and cell death.

Our preliminary observations suggested that frataxin may reside outside the mitochondria (extramitochondrial frataxin) as well as inside, and based on this we investigated the metabolic function of this extramitochondrial frataxin and its possible role in cell survival. We used a combination of genetic and biochemical approaches to look for proteins that interact with extramitochondrial frataxin to reveal relevant molecular partners. We also generated a simple animal model (a nematode worm, *C.elegans*) of frataxin deficiency by a technique called RNA interference (RNAi), and observed that these animals live longer and have altered sensitivity to oxidative stress. We used variations of these nematode models to study the stress response elicited by frataxin deficiency and performed a detailed analysis to map the role of frataxin in cell death.

Results:

Characterisation of extramitochondrial frataxin

We expressed wild type frataxin in HEK-293 cells, isolated the extramitochondrial frataxin and the mature mitochondrial frataxin and performed sequence analysis on the separate samples. The results showed that extramitochondrial frataxin is found in the same form as mature mitochondrial frataxin. Thus, we propose a view in which mature frataxin is first produced in the mitochondria, by a proteolytic process, and subsequently exported outside the mitochondria by a still unknown mechanism.

As mitochondrial frataxin binds to mitochondrial aconitase, we hypothesised that extramitochondrial frataxin might bind to cytosolic aconitase. We investigated this by co-immunoprecipitation experiments and the interaction was confirmed. To better understand the physiological function of extramitochondrial frataxin, the effects of its

interaction with aconitase were investigated. Mitochondrial and cytosolic aconitase enzymes are well characterised iron-sulphur cluster-dependent enzymes that are known to be defective in FA. We investigated the effects of wild type frataxin and extramitochondrial frataxin protein on the function of aconitase enzymes.

Wild type frataxin was able to restore the function of defective mitochondrial and cytosolic aconitase enzymes from FA cell lysates. However, extramitochondrial frataxin was only able to restore function of the cytosolic aconitase, although it was more effective at this than the wild type frataxin protein.

These results provide the first evidence of the molecular control of cytosolic aconitase/IRP1 activity by extramitochondrial frataxin. These data shed new light on the pathophysiology of FA and the role of extramitochondrial frataxin in the still unknown pathways governing cytosolic iron-sulphur cluster biogenesis/regeneration in humans.

The C.elegans model of frataxin deficiency

In an attempt to create a *C.elegans* model of FA, we treated nematodes with frataxin RNAi. Initially we were surprised to find that treated animals displayed an increased lifespan. We hypothesized that the life extension resulting from this modest decrease in frataxin expression could result from a hormesis-like mitochondrial phenomenon. We therefore predicted that mild mitochondrial dysfunction induced protective responses that could increase longevity, but in the case of severe mitochondrial dysfunction these pathways would be no longer sufficient to protect animal viability. In support of our hypothesis, we increased RNAi efficacy and showed that further reduction of frataxin expression in fact decreased animal lifespan.

We also investigated the gene expression profile underlying this protective response. This was done by tagging the promoters of certain stress response genes with green fluorescent protein and carrying out epistatic analyses. The results show that mild reduction of frataxin expression produces a robust protective stress response and requires expression of the *C.elegans* p53 homologue, Cep-1, to prolong lifespan.

Lay summary of the results:

Understanding the function of frataxin, the protein defective in Friedreich's Ataxia patients, is crucial for the rational design of specific therapeutic approaches. We have previously demonstrated the existence of an extramitochondrial pool of functional frataxin in human cells, and in the last two years we focused on defining its role. We first confirmed that the extramitochondrial pool derives from the mitochondrial frataxin; it is exported from the mitochondria into the cytosol. Then we identified a protein that physically interacts with extramitochondrial frataxin. This is a cytosolic aconitase, an enzyme that is involved in iron metabolism, and whose activity is known to be defective in people with FA. We found that extramitochondrial frataxin interacts with and restores the activity of cytosolic aconitase in cells derived from people with FA. These data reveal a specific function of extramitochondrial frataxin and help define targets of frataxin that are suitable for possible pharmacological interventions.

We also created a simple animal model of frataxin deficiency, using the soil worm *Caenorhabditis elegans* (*C.elegans*). We previously observed that, by slightly

reducing frataxin expression in the worms, the animals lived longer than usual. However, when however frataxin suppression was strong, the animals died prematurely. This suggested that mild frataxin deficiency, and consequent mild mitochondrial dysfunction, might induce a healthy protective response in the animals, while marked deficiency is eventually overwhelming. Since a mild mitochondrial dysfunction is observed in the early, asymptomatic phases of FA, a protective response might be at play also in humans, but go unrecognized. To get insights on the molecular basis of this response, a genetic analysis was performed in the worms that allowed us to identify a protein, whose human counterpart is named p53, as a central player of the animal protection and consequent life extension. This study opens the possibility that, by understanding the molecular basis of the protective response, we might be able to pharmacologically exploit it, in order to delay the onset of symptoms in patients.

Benefits to people with ataxia arisen/likely to arise from this research:

One of the goals of this project was to gather further information about the function of human extramitochondrial frataxin. Extramitochondrial frataxin can substitute for mitochondrial frataxin in granting stress resistance to cells, yet its cytosolic targets are in principle more easily accessible than mitochondrial targets. Therefore they are likely to be easier to manipulate pharmacologically and may prove to be good targets for future FA therapy. We have now identified one such target, the cytosolic aconitase/IRP1, an enzyme that plays a key role in iron metabolism and cell survival. Further study will clarify whether or not cytosolic aconitase/IRP1 represents a possible target for FA therapy.

The *C.elegans* model offers a convenient genetic system to study the function of frataxin at the organismal level. Frataxin-deficient worms can also be used to screen for lead compounds that are able to revert relevant phenotypes. We refined our model system by clarifying that opposite phenotypes, in terms of life extension, can be obtained by modulating frataxin suppression. In particular, it is now clear that mild frataxin suppression elicits a protective stress response at the organismal level and that this is mediated by the p53 homologue Cep-1. A protective stress response could be also at play in the asymptomatic phases of the human disease, when the frataxin defect and the mitochondrial dysfunction is still mild. Further study is necessary to identify more components of such response and to understand whether it could be exploited to delay the onset of symptoms in FA.

Publications arisen from this project:

p53/CEP-1 increases or decreases lifespan, depending on level of mitochondrial bioenergetic stress. Ventura N, Rea SL, Schiavi A, Torgovnick A, Testi R, Johnson TE. *Aging Cell*. 2009 Aug;8(4):380-93. Epub 2009 Apr 22.

Molecular control of the cytosolic aconitase/IRP1 switch by extramitochondrial frataxin. Condò I, Malisan F, Guccini I, Serio D, Rufini A, Testi R. *Hum Mol Genet*. 2010 Jan 20. [Epub ahead of print]

A role for p53 in mitochondrial stress response control of longevity in *C. elegans*. Torgovnick A, Schiavi A, Testi R, Ventura N. *Exp Gerontol*. 2010 Feb 18. [Epub ahead of print]

Conferences/ meetings where this research has been presented:

- 1st Italian *C. elegans* meeting, Naples, Italy (2007)
- 16th International *C. elegans* meeting, Los Angeles, USA (2007)
- Keystone Symposia on Metabolic Pathways of Longevity, Keystone, USA (2008)
- European Worm Meeting, Seville, Spain (2008)
- XI Congress of Italian Society of Human Genetics, Genova, Italy (2008)
- Telethon Convention, Riva del Garda, Italy (2009)

**For more support or information please contact: Ataxia UK, Lincoln House,
Kennington Park, 1 – 3 Brixton Road. London SW9 6DE**

Website: www.ataxia.org.uk.

Helpline: 0845 644 0606 **Tel:** +44 (0)20 7582 1444 **Fax:** +44 (0)20 7582 9444

Email: helpline@ataxia.org.uk