Biochemistry and Biophysics Reports 4 (2015) 141–147

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

Characterization of the retinal pigment epithelium in Friedreich ataxia

Duncan E. Crombie [a], Nicole Van Bergen [a], Kathryn C. Davidson [a], Sara Anjomani Virmouni [b], Penny A. Mckelvie [c], Vicki Chrysostomou [a], Alison Conquest [a], Louise A. Corben [d,e], Mark A. Pook [b], Tejal Kulkarni [a], Ian A. Trounce [a], Martin F. Pera [f], Martin B. Delatycki [d,e,g], Alice Pébay [a,*]

[a] Centre for Eye Research Australia, Royal Victorian Eye and Ear Hospital & Department of Surgery, The University of Melbourne, East Melbourne, Australia
[b] Division of Biosciences, Department of Life Sciences, College of Health & Life Sciences & Synthetic Biology Theme, Institute of Environment, Health & Societies, Brunel University London, Uxbridge, UK
[c] St. Vincents Hospital, Fitzroy , Australia
[d] Bruce Lefroy Centre for Genetic Health Research, Murdoch Childrens Research Institute, Parkville Victoria, Australia; Department of Paediatrics, The University of Melbourne, Australia
[e] School of Psychological Sciences , Monash University, Clayton, Australia
[f] Department of Anatomy and Neurosciences, The University of Melbourne, Florey Neuroscience and Mental Health Institute, Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia
[g] Clinical Genetics, Austin Health, Heidelberg, Australia

ARTICLE INFO

Article history:
Received 7 June 2015
Received in revised form
31 August 2015
Accepted 9 September 2015
Available online 11 September 2015

Keywords:
Friedreich ataxia
Induced pluripotent stem cells
Retinal pigment epithelium
Human eye
Mouse models
Oxidative phosphorylation

ABSTRACT

We assessed structural elements of the retina in individuals with Friedreich ataxia (FRDA) and in mouse models of FRDA, as well as functions of the retinal pigment epithelium (RPE) in FRDA using induced pluripotent stem cells (iPSCs). We analyzed the retina of the FRDA mouse models YG22R and YG8R containing a human FRATAXIN (FXN) transgene by histology. We complemented this work with post-mortem evaluation of eyes from FRDA patients. Finally, we derived RPE cells from patient FRDA-iPSCs to assess oxidative phosphorylation (OXPHOS) and phagocytosis. We showed that whilst the YG22R and YG8R mouse models display elements of retinal degeneration, they do not recapitulate the loss of retinal ganglion cells (RGCs) found in the human disease. Further, RPE cells differentiated from human FRDA-iPSCs showed normal OXPHOS and we did not observe functional impairment of the RPE in Humans.

1. Introduction

Friedreich ataxia (FRDA) is a hereditary degenerative disease that presents with both neuronal and non-neuronal manifestations including ataxia, dysarthria, cardiomyopathy, diabetes mellitus, and impairment of auditory and visual functions [1]. Cardiomyopathy is the leading cause of death in FRDA and usually presents as a hypertrophy, which can commonly progress to dilated cardiomyopathy and arrhythmias [1]. The defective gene *FRATAXIN* (*FXN*) has been identified in the majority of affected individuals, as containing an unstable GAA repeat mutation within the first intron [2]. FXN is a nuclear-encoded mitochondrial protein involved in iron–sulfur cluster assembly, heme synthesis, and intracellular iron homeostasis [1]. The levels of FXN are reduced in individuals with FRDA, as a consequence of its reduced transcription due to interference caused by the expanded GAA repeats [1].

In FRDA, ophthalmic manifestations including optic neuropathy and *retinitis pigmentosa*-like syndrome have been described in some patients, suggesting that retinal ganglion cells (RGCs), photoreceptors and the retinal pigment epithelium (RPE) may be affected [3–5]. Most data have implicated degeneration of the optic nerve [5–8] but very little is known of the RPE layer. The RPE plays a major role in the health of the retina. Photoreceptor death can be associated with RPE impairment, as this layer plays a supportive role to the photoreceptors. Further, the RPE accumulates iron, which increases with aging and has been implicated in retinal degeneration [9–11] and linked to impairment of RPE functions such as phagocytosis [12]. Given the central role of iron in the pathogenesis of FRDA, we questioned if the RPE could also be an affected tissue in FRDA.

The difficulty in obtaining human tissue and the paucity of functional assays specific to RPE function renders the study of the RPE layer in Humans challenging. A potential way to study RPE

* Correspondance to: Centre for Eye Research Australia. 1/32 Gisborne Street, East Melbourne, VIC 3002, Australia. Fax: +61 3 9662 3859.
E-mail address: apebay@unimelb.edu.au (A. Pébay).

http://dx.doi.org/10.1016/j.bbrep.2015.09.003

cells *in vitro* is through the differentiation of human induced pluripotent stem cells (iPSCs). iPSCs have previously been derived from individuals with FRDA [13–16], showing that the iPSCs model intergenerational repeat expansion/contraction [14], and suggesting mitochondrial abnormalities in the iPSC-derived-cardiomyocytes [13,16]. We previously reported the generation of two FRDA-iPSC lines, FA3 and FA4, which were respectively derived from individuals with 527/1058 and 751/1027 GAA repeats and showed instability in repeat numbers in culture [15]. So far, there has been no study of RPE cells from FRDA-iPSCs.

Another way to determine whether the RPE is involved in FRDA is by using animal models of the disease. The double mutant *Fxn*^{tm1Mkn}Tg(FXN)YG22Pook/J mice (YG22R), which carry a *Fxn* knock-out and a human *FXN* transgene, exhibit progressive retinal degeneration but the pathophysiology of this abnormality has not been fully characterised (https://www.jax.org/strain/010963). Using these mice and comparing them to other double mutants and controls could provide information on the pathophysiology of retinal degeneration in FRDA. In this study, we used the YG22R mice, the *Fxn*^{tm1Mkn}Tg(FXN)YG8Pook/J (YG8R) and the *Fxn*^{tm1Mkn} Tg (FXN)Y47Pook/J (Y47R) mice. The YG8R and the YG22R are homozygous for the Fxnt^{m1Mkn} (*Fxn*) targeted allele and hemizygous for the human *FXN* transgene, each with a pure GAA expansion in the first intron [17–20]. These mice differ in the GAA expansion repeats, with the YG22R having 190 GAA repeats and the YG8R having 90 and 190 GAA repeats. The Y47R control mice contain a normal GAA repeat length (9 repeats). A recent analysis of the YG22R, YG8R and Y47R mice show reduced levels of human FXN protein to 60% in the YG22R and to 76% in the YG8R in comparison to the levels observed in the control Y47R in the brain with some gender variability [21]. Little is known on the impact the genetic mutations in these models have on the RPE.

Here we used three approaches: mouse models, human post mortem eyes and iPSC-derived RPE cells-to gain a better picture on the health of the RPE in FRDA and to determine if any obvious defects could be modeled *in vitro*.

2. Material and methods

2.1. Ethics

All experimental work performed in this study was approved by the Human Research Ethics committees of the Eye and Ear Hospital (09/921H, 11/1031H, 12/1091H) and the University of Melbourne (0605017, 0829937) and the UK Home Office animal licence PPL30/3031, and completed in accordance with the requirements of the National Health & Medical Research Council of Australia and the Declaration of Helsinki. This research also adheres to the ARVO statement for the use of animals in ophthalmic and vision research. For retrieval of human tissue, autopsies and eye removal were performed after a legally responsible family member gave formal permission. The Institutional Review Board of Veterans Affairs Medical Center in Albany, New York, USA, approved the collection of the autopsy samples for research, their processing and distribution to researchers, and the posthumous collection of clinical data.

2.2. Mouse tissue collection and processing

The following mouse models of FRDA were used: *Fxn*^{tm1Mkn}Tg(*FXN*)YG8Pook/J mouse (YG8R), the *Fxn*^{tm1Mkn} Tg(*FXN*)YG22Pook/J mouse (YG22R) and the *Fxn*^{tm1Mkn} Tg(FXN) Y47Pook/J (Y47R) control mice [17–20]. C57BL/6 mice were also used as background controls. Including background and transgene controls, 40 age– (16 ± 0.18 months, 10 per group, 5 males and 5 females) and sex- matched mouse eyes were immersion-fixed in 4% PFA and processed to paraffin.

Table 1
Assessment of stability of expression of housekeeping genes in PSC-derived RPE cells.

Targets	RPE 1	RPE 2	RPE 3
18s	13.96	13.98	11.96
GAPDH	20.98	21.97	20.99
HPRT1	28.96	28.95	27.95
GUSB	27.98	27.96	27.97
ACTB	**21.98**	**21.98**	**21.00**
B2M	23.95	23.95	22.95
RPLP0	23.95	23.95	22.94
HMBS	29.94	29.94	29.95
TBP	30.94	29.94	29.95
PGK1	23.93	24.93	22.94
UBC	23.95	24.94	23.94
PPIA	22.96	23.96	22.96

qRT-PCR were performed using three different PSC-derived RPE cells from three independent experiments. Data are shown as raw Ct values. ACTB (bold) was chosen as the reference housekeeping gene.

2.3. Morphometric analysis of mouse retinal sections

Sagittal sections were stained with haematoxylin and eosin (H&E) and the thicknesses of various retinal layers and RPE were measured using Image J (Image J v1.46r, NIH, USA). Cell counts were also performed on the RGC layer. All analysis was performed blinded to the identity of the sections. Four measurements from four equivalent regions across each retina were obtained in triplicate serial sections for each animal. For analysis, measurements were normalized to the thickness of the entire retina (INL-ONL) except for the inner retina layers which were normalized across the inner retina, to account for obliquely cut sections.

2.4. Collection of human eyes

Six human eyes from individuals with FRDA were collected during autopsy, together with other tissues, and were de-identified before delivery to the researchers. Briefly, eyes were removed either through enucleation or through the orbital roof after removal of the brain. They were then fixed by immersion in formalin. After 1 h, a frontal cut was performed through the eyes to allow formalin to reach the retina.

2.5. Pathology of post-mortem FRDA eyes

The six fixed post-mortem eyes from FRDA patients were hand processed through graded alcohols and xylene to paraffin. Sagittal sections were cut at 7 μm and stained with H&E. transversely sectioned at 5 μm and stained with haematoxylin and eosin and Luxol fat blue. The sixth eye had no retrobulbar optic nerve, so optic atrophy was assessed from the intrabulbar portion. All sections were assessed for abnormalities in RPE, retina, optic disc and nerve by a pathologist.

2.6. Cell culture

The FRDA iPSC lines FA3 and FA4 [15] and the hESC line H9 [22] were maintained as described in [15]. PSCs were differentiated into RPE cells according to [23]. After 30–60 days, pigmented cells were manually isolated and transferred to organ culture dishes in 15% fetal bovine serum (FBS) medium containing taurine, hydrocortisone and triiodothyronine (15% RPE medium) [23]. After 24 h the medium was replaced with RPE medium containing 5% FBS