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Cardiolipin deficiency affects respiratory chain function and organization in an induced pluripotent stem cell model of Barth syndrome☆

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Abstract Barth syndrome (BTHS) patients carrying mutations in *tafazzin* (*TAZ1*), which is involved in the final maturation of cardiolipin, present with dilated cardiomyopathy, skeletal myopathy, growth retardation and neutropenia. To study how mitochondrial function is impaired in BTHS patients, we generated induced pluripotent stem cells (iPSCs) to develop a novel and relevant human model system for BTHS. BTHS–iPSCs generated from dermal fibroblasts of three patients with different mutations in *TAZ1* expressed pluripotency markers, and were able to differentiate into cells derived from all three germ layers both in vitro and in vivo. We used these cells to study the impact of tafazzin deficiency on mitochondrial oxidative phosphorylation. We found an impaired remodeling of cardiolipin, a dramatic decrease in basal oxygen consumption rate and in the maximal respiratory capacity in BTHS–iPSCs. Simultaneous measurement of extra-cellular acidification rate allowed us a thorough assessment of the metabolic deficiency in BTHS patients. Blue native gel analyses revealed that decreased respiration coincided with dramatic structural changes in respiratory chain supercomplexes leading to a massive increase in generation of reactive oxygen species. Our data demonstrate that BTHS–iPSCs are capable of modeling BTHS by recapitulating the disease phenotype and thus are important tools for studying the disease mechanism.
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Introduction

Barth syndrome (BTHS, MIM# 302060) is a recessive disorder characterized by dilated cardiomyopathy, attended with skeletal myopathy, neutropenia, growth retardation and increased urinary excretion of 3-methylglutaconic acid in early childhood (Barth et al., 1983; Spencer et al., 2005; Takeda et al., 2011). The disease-causing gene was mapped to the *TAZ1* locus in the q28 region of the X chromosome (Xq28) encoding for the mitochondrial protein tafazzin. Tafazzin is an evolutionary conserved CoA independent phospholipid acyltransferase, involved in remodeling of cardiolipin (CL), the hallmark lipid of mitochondria (Neuwald, 1997; Xu et al., 2006). CL is a constituent of the inner (75%) and outer (25%) membranes where it plays pleiotropic roles in the maintenance of membrane complexes and cristae morphology (Xu et al., 2005; Acehan et al., 2007; Gebert et al., 2009). After its synthesis CL is deacylated to monolysocardiolipin (MLCL) and subsequently reacylated by tafazzin. This remodeling process maintains the normal content and composition of cardiolipin in mitochondria. Although tafazzin is ubiquitously expressed in all human tissues, the remodeled molecular species of cardiolipin are cell-type and tissue specific. Heart and skeletal muscle, tissues that require high mitochondrial metabolic activity, contain mainly tetra-linoleoyl-CL ($(C18{:}2)_4$-CL) in mitochondria whereas a broader species composition is found in other tissues, especially in the brain (Schlame et al., 2003; Schlame et al., 2002; Valianpour et al., 2002; Mckenzie et al., 2006; Houtkooper et al., 2009). It has been suggested that the different molecular species of cardiolipin observed in different cells and tissues are tailored to match the functional requirements and/or energetic demands of that cell or tissue (Houtkooper et al., 2009).

Respiratory chain complexes are organized into higher oligomeric structures called supercomplexes or respirasomes. In mammalian cells, complex I (NADH:ubiquinone oxidoreductase) is associated with dimeric complex III (ubiquinol: cytochrome *c* oxidoreductase) and multiple copies of complex IV (cytochrome *c* oxidoreductase) (Schägger and Pfeiffer, 2001; Schägger and Pfeiffer, 2000; Moreno-Lastres et al., 2012). The formation of supercomplexes was suggested to increase efficiency of respiration and prevent the generation of reactive oxygen species (Acín-Pérez et al., 2008; Vukotic et al., 2012; Strogolova et al., 2012; Chen et al., 2012). CL binding has been shown for all individual complexes of the respiratory chain and available crystal structures reveal CL in critical positions in complexes III and IV and the ADP–ATP carrier (Schwall et al., 2012; Shinzawa-Itoh et al., 2007; Lange et al., 2001; Pebay-Peyroula et al., 2003). CL molecules at the interface between individual complexes are thought to be essential for assembly of these complexes into supercomplexes (Brandner et al., 2005; Zhang et al., 2002; Wenz et al., 2009; Pfeiffer et al., 2003; Zhang et al., 2005; Bazán et al., 2012). Previous studies show that cardiomyocytes contain a unique composition of CL-dependent membrane complexes. A well-studied example is the respiratory chain complex IV with its cardiac tissue-specific subunits VIa, VIIa and VIII (Bachman et al., 1997; Grossman and Lomax, 1997; Arnaudo et al., 1992; Lomax et al., 1995). Experiments with yeast as a model organism or lymphoblasts from BTHS patients showed destabilization of respiratory chain supercomplexes in these cells and a decrease in respiratory chain activity in fibroblasts from BTHS patients (Mckenzie et al., 2006; Barth et al., 1996; Sedlák and Robinson, 1999).

Although mitochondrial respiratory dysfunction and destabilization of supercomplexes have been reported in fibroblasts or lymphoblasts from BTHS patients, it has remained open how these individual effects integrate into cellular metabolism, and how the different mutations in the tafazzin gene influence the cardiolipin composition and mitochondrial function in different cells and tissues.

To address the pathological changes of cardiac function in a suitable model system, a tafazzin knock down mouse model has been designed. However, the late onset of the cardiac phenotype after 8 months in adult mice did not reflect the early onset of the disease in human and raised the question of how well a mouse model resembles the situation in human tissue (Acehan et al., 2011; Soustek et al., 2011; Phoon et al., 2012). The recent successes in generation of human induced pluripotent stem cells (iPSCs) allow us modeling a disease in a culture dish, which is based on the unique capacity of these cells to continuously self-renew and to give rise to all cell types in the human body (Robinton and Daley, 2012). Indeed, there have been a number of studies reporting on the successful in vitro modeling of diseases using iPSCs generated from patients with a full range of genetically inherited as well as sporadic diseases (Wu and Hochedlinger, 2011).

In this study we therefore aimed to establish a human iPSC model system from dermal fibroblasts of BTHS patients. We found an impaired CL remodeling in BTHS–iPSCs, which recapitulates the disease phenotype. Assessment of mitochondrial function revealed a dramatic decrease in mitochondrial oxygen consumption in BTHS–iPSCs compared to control cells, which was not compensated by increased glycolysis. We found that structural changes in the respiratory chain supercomplexes are causing defects in energetic coupling and an increase of reactive oxygen species (ROS), thus, providing a possible explanation for the pathogenesis of the disease.

Material and methods

Generation and culture of BTHS–iPSCs

The study was approved by the ethical commission of Universitätsmedizin Göttingen. Dermal fibroblasts were obtained from 3 patients diagnosed with BTHS (Table 1) and 1 healthy donor (Houtkooper et al., 2009). Cells were grown in Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen) containing 10% inactivated fetal bovine serum (FBS, Sigma), 2 mM L-glutamine (Invitrogen), 1× Non-Essential Amino Acid (NEAA; Invitrogen), and 50 μM β-mercaptoethanol (Serva). For transduction of dermal fibroblasts into iPSCs, a standardized protocol was applied as previously described (Streckfuss-Bomeke et al., 2012). Briefly, fibroblasts were infected with the lentivirus encoding all four human transcription factors OCT4, SOX2, KLF4 and c-MYC using the STEMCCA system and cultivated on mitomycin C-inactivated mouse embryonic fibroblasts (MEFs) in human iPSC medium composed of DMEM/F12 (Invitrogen) containing 20% knockout serum replacement (Invitrogen), 1× NEAA, 50 μM β-mercaptoethanol and 10 ng/ml basic fibroblast growth factor (bFGF, Peprotech)

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