Published in final edited form as:

Ultrastruct Pathol. 2015 ; 39(5): 336–339. doi:10.3109/01913123.2015.1042610.

Megamitochondria in cardiomyocytes of a knockout (*Klf15*^{−/−}) mouse

Bernard Tandler^{1,2}, Hisashi Fujioka^{2,3}, Charles L. Hoppel^{2,4}, Saptarsi M. Haldar⁵, and Mukesh K. Jain⁵

¹ Department of Biological Sciences, School of Dental Medicine, Case Western Reserve University, Cleveland, Ohio

² Center for Mitochondrial Disease, School of Medicine, Case Western Reserve University, Cleveland, Ohio

³ Electron Microscopy Facility, School of Medicine, Case Western Reserve University, Cleveland, Ohio

⁴ Departments of Pharmacology and of Medicine, School of Medicine, Case Western Reserve University, Cleveland, Ohio

⁵ Case Cardiovascular Research Institute, School of Medicine, Case Western Reserve University, and Harrington Heart & Vascular Institute, University Hospitals /Case Medical Center, Cleveland, Ohio

Abstract

The Kruppel-like Factors (KLF) family of zinc-finger transcriptional regulators control many aspects of cardiomyocyte structure and function. Deletion of *Klf15* from the nuclear genome in mice affects cardiac mitochondria. Some become grossly enlarged, extending many sarcomeres in length. These display many sites of incipient pinching, but there is little attenuation of the megamitochondria at these sites; there are no examples of organelles that clearly have reached the point where further membrane encroachment will cause separation into smaller daughter mitochondria. It is clear that deletion of *Klf15* interferes with nuclear control of mitochondrial fission, whereas fusion appears to be unaffected.

Keywords

Kruppel-like Factor (KLF); cardiac mitochondria; megamitochondria; mitochondrial fission; mitochondrial fusion

Introduction

Megamitochondria (giant mitochondria) are a relatively common feature of heart cells in some types of human cardiomyopathy (Arbustini et al, 1998; Sekiguchi et al, 1970; Sluga and Monneron, 1970; Tandler et al., 2002). For example Arbustini et al. (1998) found that of

601 patients with cardiomyopathy, ~14% had megamitochondria in their heart cells. In addition, it is possible to produce great enlargement of mouse heart mitochondria by rigorous endurance training (Coleman et al., 1987).

In our continuing study of murine cardiac mitochondria, we have examined the hearts of Kruppel-like factor 15 (*Klf15*) knockout mice. Here, germline deletion of *Klf15* in mice direct genetic manipulation has resulted in the formation of scattered, mostly interfibrillar, megamitochondria in the heart that exhibit morphological signs of incipient division. The structure of these outsized organelles is described in this report.

Materials and methods

Animal studies were approved by the Institutional Animal Care and Use Committees of Case Western Reserve University School of Medicine. Mice with systemic deletion of *Klf15* have been previously described (Gray et al., 2002). All mice were adult littermate males (12-14 weeks old) in a pure C57Bl/6 background. The mice were allowed food and water *ad libitum*. They were killed by decapitation and specimens of left ventricle obtained. These were prepared for electron microscopy using a protocol described in an earlier publication (Fujioka et al., 2012). Thin sections were observed and photographed either in a JEOL 1200EX or a FEI Technai Spirit (T12) electron microscope.

Results

In most fields examined by electron microscopy, normal-sized mitochondria were closely apposed to one another. A number of very large mitochondria (megamitochondria) were sporadically present in both a subsarcolemmal and interfibrillar position, although they were much more common in the latter. These megamitochondria were up to 14 μ m in length and spanned as many as 10 sarcomeres and could be three times as wide as ordinary cardiac mitochondria in the same cells (Figs. 1, 2). Cristae orientation in these megamitochondria was more-or-less transverse, but in some the cristae were arrayed in an extremely regular grid (Fig. 3). None of the enlarged organelles contained single-membrane vacuoles that enclosed glycogen particles, as has been reported to occur in some cases of human cardiomyopathy (Tandler et al., 2002). Some of the large organelles were intimately associated with sparse lipid droplets.

Although most of the megamitochondria had relatively smooth silhouettes, a few showed irregularities in the form of surface membrane invaginations of varying depth (Fig. 2): some were shallow and some were moderately deep, but no megamitochondria were observed wherein two organelle moieties were held together by a slender isthmus. The observed surface morphology is consonant with the phenomenon of “pinching”, which is thought to be a mechanism for mitochondrial fission (Yoon and McNiven, 2001; Chan, 2006). Irrespective of the depth of the surface incursions, each had several elements of sarcoplasmic reticulum (SR) at its mouth. For the most part, there was no SR in relation to the smooth portions of the surface of the megamitochondria.

Discussion

Although megamitochondria can be engendered by various experimental procedures (see compendium by Tandler and Hoppel, 1986), this is the first example of such organelles resulting from genetic manipulation. *Klf15* is important in cardiac lipid development (Prosdocimo, 2014) and the heart's response to pathological stress (Fisch, 2007; Haldar, 2010); its role in other aspects of cardiovascular biology are only beginning to be understood (Haldar et al., 2007). Cultured cardiomyocytes deficient in *Klf15* are hypertrophied (Fisch et al., 2007), an enlargement that might in part be due to an expansion of the mitochondrial compartment.

It seems likely that the megamitochondria than we show in the *in situ* cardiomyocytes arise by fusion of their smaller companions. Fusion of conventional mitochondria probably is a regular event in cardiomyocytes (and more than likely in skeletal muscle)), ensuring that each organelle is furnished with all of the requisite factors (mitochondrial contents and phospholipid membranes) for cellular homeostasis (Chan, 2006; Bereiter-Hahn et al., 2008; Chen et al., 2010). If mitochondrial fusion continued without compensatory fission, the result would be cells crowded with megamitochondria (Parone et al., 2008). This is not the case in cardiomyocytes in conventional mice, and certainly not in the *Klf15*-knockout mice in our study. In the latter animals, megamitochondria were noted-- and then only rarely-- in some, but not all cardiac cells, principally among interfibrillar organelles.

In the *KLF*-knockout mice, it appears as if the fusion-fission combination is out of balance, permitting fusion at the expense of fission, although the resultant megamitochondria display signs of incipient pinching. The latter operation requires dynamin or a dynamin-like protein (Smirnova et al., 2001), which acts like a tightening belt to cut the organelle (Yoon and McNiven, 2001; Chan, 2006). Despite what likely are only abortive attempts to divide, the incursions of the outer surface of the megamitochondria in our study are almost always associated with SR. A similar relationship exists between SR and normal-size mitochondria in cardiac muscle (Fujioka et al, 2012) and between SR and string mitochondria in skeletal muscle (Fujioka et al., 2013). It seems probable that the SR conducts proteins, phospholipids, calcium, etc. necessary for organelle division to specific points on the megamitochondrial surface.

Ultimately, the number, size, and shape of mitochondria are under nuclear control (Virbasius and Scarpulla., 1994; Xia et al., 1997). Deletion of *Klf15* may be associated with, among other effects, impairment of the gene(s) that control mitochondrial division. Interestingly, it is the IFM that appear mostly to be affected-- there were relatively few megamitochondria among the SSM. These two disparate populations (IFM and SSM) have been shown to have differing oxidative capacities in the cardiomyocytes of untreated rats (Palmer et al., 1977) and in a number of other species as well. A strain of hamsters-- BIO14.6-- exhibits congenital dystrophic myopathy that eventually evolves into cardiomyopathy with a defect solely in IFM in which oxidative phosphorylation is decreased by 40-50 %; the SSM are unaffected by the disease (Hoppel et al., 1982). Clearly, different genes direct SSM and IFM in the heart.

In rats fed a copper-deficient diet, the number of mitochondria in the heart rises significantly (Medeiros and Jennings, 2002). This increase probably is brought about by division of IFM through the agency of partition formation (Tandler and Hoppel, 1974). In a recent study (Fujioka et al, 2012), It was found that normal-size mitochondria in the hearts of untreated rats divide by two distinct modalities: partition formation and pinching. The former was restricted to SSM, the latter occurs predominantly in IFM. It appears that the division pathway employed by cardiac mitochondria depends on whether they are normal and unsullied or whether they have been experimentally compromised. The interfibrillar megamitochondria in *Klf15* knockout mice are attempting to follow the conventional trajectory of pinching, but are unsuccessful in achieving fission.

References

- Arbustini E, Diegoli M, Fasani R, Grasso M, Morbini P, Banchieri N, Bellini O, Bello B, Pilotto A, Magrini G, Campana C, Fortina P, Gavazzi A, Narula J, Vigano M. Mitochondrial DNA mutations and mitochondrial abnormalities in dilated cardiomyopathy. *Am J Pathol.* 1998; 153:1501–1510. [PubMed: 9811342]
- Bereiter-Hahn J, Vöth M, Mai S, Jendrach M. Structural implications of mitochondrial dynamics. *Biotechnol J.* 2008; 3:765–780. [PubMed: 18512869]
- Chan DC. Mitochondrial fusion and fission in mammals. *Annu Rev Cell Dev Biol.* 2006; 22:79–99. [PubMed: 16704336]
- Chen H, Vermulst M, Wang YE, Chomyn A, Prolla TA, McCaffery M, Chan DC. Mitochondrial fusion is required for mtDNA stability in skeletal muscle and tolerance of mtDNA mutations. *Cell.* 2010; 141:280–289. [PubMed: 20403324]
- Coleman R, Silbermann M, Gershon D, Rexnik AZ. Giant mitochondria in the myocardium of aging and endurance-trained mice. *Gerontology.* 1987; 33:34–39. [PubMed: 3596262]
- Fisch S, Gray S, Heymans S, Haldar SM, Wang B, Pfister O, Cui L, Kumar A, Lin Z, Sen-Banerjee S, Das H, Petersen CA, Mende U, Burleigh BA, Zhu Y, Pinto YM, Liao R, Jain MK. Kruppel-like factor 15 is a regulator of cardiomyocyte hypertrophy. *Proc Natl Acad Sci USA.* 2007; 104:7074–7079. [PubMed: 17438289]
- Fujioka H, Tandler B, Haldar SM, Jain MK, Hoppel CL. String mitochondria in mouse soleus muscle. *Microsc Res Tech.* 2013; 76:237–241. [PubMed: 23174930]
- Fujioka H, Tandler B, Hoppel CL. Mitochondrial division in rat cardiomyocytes: An electron microscope study. *Anat. Rec.* 2012; 295:1455–61.
- Gray S, Feinberg MW, Hull S, Kuo CT, Watanabe M, Sen-Banerjee S, DePina A, Haspel R, Jain MK. The Kruppel-like factor Klf15 regulates the insulin-sensitive glucose transporter GLUT4. *J Biol Chem.* 2002; 277:34322–34328. [PubMed: 12097321]
- Haldar SM, Ibrahim OA, Jain MK. Kruppel-like factors (KLFs) in muscle biology. *J Mol Cell Cardiol.* 2007; 41:1–10. [PubMed: 17531262]
- Haldar SM, Lu Y, Jeyaraj D, Kawanami D, Cui Y, Eapen SJ, Hao C, Li Y, Doughman YQ, Watanabe M, Shimizu K, Kuivaniemi H, Sadoshima J, Margulies KB, Cappola TP, Jain MK. *Klf15* deficiency is a molecular link between heart failure and aortic aneurysm formation. *Sci Transl Med.* 2010; 2:6ra26. doi: 10.1126/scitranslmed.3000502.
- Hoppel CL, Tandler B, Parland W, Turkaly JS, Albers LD. Hamster cardiomyopathy—A defect in oxidative phosphorylation in the cardiac interfibrillar mitochondria. *J Biol Chem.* 1982; 257:1540–1548. [PubMed: 6460026]
- Medeiros DM, Jennings D. Role of copper in mitochondrial biogenesis via interaction with ATP synthase and cytochrome *c* synthase. *J Bioenerg Biomembr.* 2002; 34:389–395. [PubMed: 12539966]
- Palmer JW, Tandler B, Hoppel CL. Biochemical properties of subsarcolemmal and interfibrillar mitochondria isolated from rat cardiac muscle. *J Biol Chem.* 1977; 252:8731–8739. [PubMed: 925018]

- Parone PA, Da Cruz S, Tondera D, Mattenberger Y, Di J, Maechler P, Barja F, Martinou JC. Preventing mitochondrial fission impairs mitochondrial function and leads to loss of mitochondrial DNA. *PLoS One*. 2008; 3:e3257. [PubMed: 18806874]
- Prosdocimo DA, Anand P, Liao X, Zhu H, Shelkay S, Artero-Calderon P, Zhang L, Kirsh J, Moore D, Rosca MG, Vazquez E, Kerner J, Akat KM, Williams Z, Zhao J, Fujioka H, Tuschli T, Bai X, Schulze PC, Hoppel CL, Jain MK, Haldar SM. 2014 Kruppel-like factor 15 is a critical regulator of cardiac lipid metabolism. *J Biol Chem*. 2014;5914–5924. [PubMed: 24407292]
- Sekiguchi M, Yu ZX, Take M, Hiroe M, Hirose K, Shirai T, Ishide T, Takahashi T. Ultrastructural features of endomyocardium in patients with eosinophilic heart disease. An endomyocardial biopsy study. *Jpn Circ J*. 1984; 48:1375–1382. [PubMed: 6512945]
- Sluga E, Monneron A. Über die Feinstruktur und Topochemie von Riesenmitochondrien und deren Einlagerungen bei Myopathien. *Virchows Arch A*. 1970; 350:250–260.
- Smirnova E, Griparic L, Shurland D-L, van der Bliek AM. Dynamin-related protein Drp1 is required for mitochondrial division in mammalian cells. *Mol Biol Cell*. 2001; 12:2245–2256. [PubMed: 11514614]
- Tandler B, Dunlap M, Hoppel CL, Hassan M. Giant mitochondria in a cardiomyopathic heart. *Ultrastruct Pathol*. 2002; 26:1–7. [PubMed: 12028651]
- Tandler B, Hoppel CL. Possible division of cardiac mitochondria. *Anat Rec*. 1972; 173:309–324. [PubMed: 5039085]
- Tandler B, Hoppel CL. Studies on giant mitochondria. *Ann NY Acad Sci*. 1986; 488:65–81. [PubMed: 3555262]
- Virbasius JV, Scarpulla RC. Activation of the human mitochondrial transcription factor A gene by nuclear and respiratory factors: A potential regulatory link between nuclear and mitochondrial gene expression in organelle biogenesis. *Proc Nat Acad Sci USA*. 1994; 91:1309–1313. [PubMed: 8108407]
- Xia Y, Buja LM, Scarpulla RC, McMillin JB. Electrical stimulation of neonatal cardiomyocytes results in the sequential activation of nuclear genes governing mitochondrial proliferation and differentiation. *Proc Nat Acad Sci USA*. 1997; 94:11399–11404. [PubMed: 9326621]
- Yoon Y, McNiven MA. Mitochondrial division: new partners in membrane pinching. *Curr. Biol*. 2001; 11:R67–R70. [PubMed: 11231145]

Fig. 1.
An interfibrillar megamitochondrion that is at least 10 sarcomeres long and much wider than its normal companion organelles. The boxed area is shown at higher magnification in Fig. 2.
Scale bar = 1 μ m.

Fig. 2.

A higher magnification of a portion of Fig. 1 showing several surface incisures that in normal mitochondria are associated with the phenomenon of pinching. Note the elements of SER in relation to the openings of these invaginations, especially of the one at the left. Scale bar = 1 μ m.

Fig. 3.
A portion of the interior of a megamitochondrion illustrating the abundance and parallel orientation of its cristae. Scale bar = 1 μ m.