A recent study proposes novel functions of the long known mitochondrial endonuclease, endonuclease G (ENDOG). ENDOG was identified via quantitative trait loci analysis as a mediator of blood-pressure–independent cardiac hypertrophy and identified as a novel component in the mitochondrial biogenesis signaling network.

Blood-pressure-independent cardiac hypertrophy is commonly observed in subjects with obesity and Type 2 diabetes mellitus. It is likely that multiple predisposing factors and genetic modifiers interact in a complex fashion, to induce left ventricular (LV) hypertrophy. In the study by McDermott-Roe et al, the authors analyzed quantitative trait loci for blood pressure-independent cardiac mass in rats using an intercross of spontaneously hypertensive rats (SHR) and Brown Norway rats and identified ENDOG as a novel determinant of LV mass and cardiac function. Expression of ENDOG was reduced in SHR rats due to a frame-shift insertion in exon 1 of ENDOG that correlated with LV hypertrophy and impaired cardiac function. In subsequent studies, the authors demonstrated that shRNA-mediated knockdown of ENDOG in isolated neonatal rat cardiomyocytes resulted in increased cardiomyocyte size and that cardiomyocyte area was increased in mice lacking ENDOG. Thus, ENDOG may be a novel determinant of blood-pressure-independent LV hypertrophy in rodents. To determine the potential relevance to cardiac hypertrophy in humans, a genome-wide coexpression network analysis in a large human cardiac expression data set was performed. ENDOG was identified as participating in a network that was highly enriched for mitochondrial genes and oxidative metabolism processes. Subsequently, the authors demonstrated that ENDOG is a direct target of ERRα and PGC-1α, master regulators of mitochondrial biogenesis. Overexpression of ENDOG increased mitochondrial mass in HEK293 and H9C2 cardiomyocytes. ENDOG directly binds to mitochondrial DNA suggesting a role in the regulation of mitochondrial gene transcription. Functionally, ENDOG−/− mice showed impaired mitochondrial respiration rates and increased reactive oxygen species (ROS) production, which was associated with a marked elevation of triglyceride levels in their hearts. Thus it is hypothesized that ENDOG deficiency may promote cardiac hypertrophy on the basis of persistent oxidative stress.

ENDOG is a nuclear-encoded endonuclease that belongs to the large family of DNA/RNA nonspecific nucleases. The most prominent and intensively studied function of ENDOG is its participation in nucleosomes degradation during programmed cell death, a scenario in which ENDOG is thought to be released from the mitochondrial intermembrane space in a caspase-independent fashion and to migrate to the nucleus where it facilitates chromatin degradation. The study by McDermott-Roe et al identified a novel role of ENDOG in the regulation of mitochondrial DNA transcription. Moreover, ENDOG is an important regulator of mitochondrial energetics and may play a role in the regulation of LV mass and cardiac function, presumably via ROS. This finding was unexpected, given that endonuclease activity was previously believed to participate in prodeath pathways. By contrast, maintenance of ENDOG levels may also play an important role in maintaining mitochondrial energetics and normal cardiac physiology, consistent with the heart’s high-energy demands. Studies some 20 years ago suggested that ENDOG may play a role in mitochondrial DNA replication but this aspect of ENDOG biology was not pursued further and was practically put to rest by initial phenotyping of ENDOG-deficient mice indicating no effect on mitochondrial DNA copy number, structure, or mutation rates.

The findings of McDermott-Roe et al have several important implications. Mitochondrial energy metabolism is essential for maintaining cardiac function, and the frequent observation of mitochondrial dysfunction in various models of cardiac hypertrophy and failure has led to the general assumption that impaired mitochondrial energetics contribute to or may even predispose to the development of hypertrophy and heart failure. Although an association between cardiac hypertrophy, heart failure, and impaired mitochondrial function has primarily been made in models of pressure-overload dependent cardiac hypertrophy, it is likely that a similar relationship could also exist in pressure-overload independent cardiac hypertrophy associated with obesity and diabetes mellitus. To date, a rigorous analysis of the contribution of impaired mitochondrial biogenic signaling or bioenergetics to
the pathogenesis of obesity and diabetes-associated cardiac hypertrophy has not been performed. The present study should stimulate an examination of this issue in the context of obesity and diabetes. It will also be of interest to determine if changes in the expression levels of ENDOG play any role in mitochondrial dysfunction, increased ROS production, and LV hypertrophy in these models.

The role of ROS in the pathogenesis of cardiac hypertrophy is complex. A relationship between mitochondrial ROS overproduction and cardiac hypertrophy has been previously described. For example, studies in mice lacking one allele of MnSOD (sod2) or with inducible knockout of sod2 support a role for mitochondrial ROS as a cause of cardiac hypertrophy, and increasing mitochondrial antioxidant defenses reduces the extent of cardiac remodeling following myocardial infarction. Moreover, genetic models of altered mitochondrial gene transcription are also associated with cardiac hypertrophy. Thus genetic deletion of mitochondrial transcription factors A (TFAm) leads to progressive LV hypertrophy, with doubling of LV mass by 8 weeks of age. Conversely, a reduction in ROS production may increase cardiac hypertrophy as was observed in NOX4-deficient hearts, which had an exaggerated hypertrophic response to pressure overload. Therefore, whether or not the association between ENDOG and LV hypertrophy is ROS-dependent remains to be established and critical proof of the ROS hypothesis of cardiac hypertrophy in ENDOG mice remains to be provided. Future studies to determine the effect of ROS scavenging on cardiomyocyte hypertrophy in ENDOG-deficient mice, or in cells with ENDOG silencing, will be required to provide definitive proof of this mechanism.

Although the mutation in SHR rats within the ENDOG gene may indeed contribute to cardiac hypertrophy in that model, it remains unclear whether mitochondrial dysfunction is the cause or consequence of LV hypertrophy in ENDOG-deficient rodents. Many studies have shown that mitochondrial dysfunction often develops in response to pathological cardiac hypertrophy. Data in the present study were presented at a single age in ENDOG mice and a cross-sectional analysis might not prove a direct cause-and-effect relationship. Thus it would be of great interest to examine the relationship between mitochondrial function and LV hypertrophy from birth to adulthood (in ENDOG-deficient mice or SHR rats) to determine if mitochondrial dysfunction precedes LV hypertrophy in these rodents. Additional mechanisms for the association between ENDOG deficiency and cardiac hypertrophy could also exist. For example, cardiomyocyte lipid deposition as is observed in models of obesity and diabetes mellitus can lead to cardiac hypertrophy in surviving myocytes as a consequence of lipid-induced apoptotic cell death. Interestingly, the lipid overload that was associated with ENDOG deficiency was independent of any differences in apoptosis. However, cardiomyocyte lipid accumulation can also lead to increased levels of highly reactive acyl-CoA species, which may promote cellular injury via various mechanisms, including lipid peroxidation, ceramide accumulation, or phospholipid remodeling, or promote cardiac hypertrophy by activating signaling pathways such as protein kinase C. Thus, a lipotoxic mechanism for pathogenesis of LV hypertrophy in ENDOG mice remains a possibility.

The most interesting finding of the study by McDermott-Roe is the discovery of a role for ENDOG in the complex signaling network involved in mitochondrial biogenesis. Although ERRα signaling regulates both expression of oxidative phosphorylation and fatty acid oxidation genes, ENDOG seems to specifically regulate mitochondrial replication and/or transcription independent of the nuclear actions of ERRα, in a manner that is analogous to the mitochondrial transcription factors A (TFAm), B1 and B2 (TFB1, TFB2). Thus, ENDOG may allow more precise fine-tuning of nucleo-mitochondrial crosstalk in the regulation of mitochondrial biology. Although the mitochondrial genome only encodes for a small number of genes, loss of such control mechanisms is detrimental to the cell, as evidenced by the development of severe heart failure in mice lacking TFAm. In contrast, the degree of LV hypertrophy in ENDOG-deficient mice is relatively mild at a similar age of 8 weeks, with no changes in metabolic enzyme expression. Of note, a compensatory increase in TFAm expression was not observed in ENDOG-deficient mice. Thus, the effects of TFAm deficiency on LV mass and cardiac dysfunction are much more pronounced than the effects of ENDOG. Only following a challenge with angiotensin II was the degree of hypertrophy clearly observable, although it still remained much less pronounced than in TFAm-deficient mice under basal conditions. Thus, in comparison with other regulators of mitochondrial gene transcription, ENDOG exhibits a smaller quantitative impact on cardiac hypertrophy. Because of the minor effect of altered ENDOG on organismal survival in contrast to the more severe phenotypes of other mitochondrial mutations on survival, it is possible that defects in this pathway are more likely to be genetically transmitted. Therefore it will be of interest to see whether polymorphisms in ENDOG correlate with LV mass in human studies.

The phenotype of ENDOG mice, relative to that of its upstream regulators ERRα and PGC-1α, warrants discussion. Mice lacking PGC-1α or PGC-1β, respectively, have relatively mild cardiac phenotypes and do not develop cardiac hypertrophy in nonstressed hearts, despite reduced expression of many genes involved in mitochondrial oxidative phosphorylation and fatty acid metabolism and baseline mitochondrial dysfunction. Thus it will be of interest to determine the status of ENDOG expression in these models. Of interest, both PGC-1α and PGC-1β deficient mice exhibit accelerated LV remodeling and more severe heart failure following transverse aortic constriction, which is associated with increasing oxidative stress. A role for ENDOG in this process is unknown. Thus determining the role of ENDOG in the transition to heart failure in PGC-1 deficient models would be of interest. Furthermore, given the association between pressure overload hypertrophy and repression of PGC-1α expression in many studies, it would be of interest to determine if ENDOG-deficient hearts will exhibit a more rapid transition to heart failure following pressure overload. The observation that ENDOG mice develop more pronounced hypertrophy following angiotensin treatment may support the idea that these hearts may fail following pro-
longed pressure overload. In contrast, ERRα-deficient mice exhibit more severe phenotypes than individual knockouts of PGC-1 isoforms and manifest signatures of heart failure including LV chamber dilatation, reduced fractional shortening, and defects in mitochondrial function and gene expression. It would therefore be of great interest to determine the specific contribution of ENDOG to heart failure in the context of ERRα deficiency and to determine whether maintaining ENDOG expression would be sufficient to reverse or ameliorate these effects. If this is not the case, then such an observation would indicate that additional targets of ERRα and PGC-1 are essential mediators of the mitochondrial adaptations to pressure overload cardiomyopathy.

In conclusion, the study by McDermott-Roe et al provides novel insights into the complex regulation of mitochondrial biogenesis and function. Using an elegant methodological approach, the authors identified ENDOG as a regulator of mitochondrial function, ROS production, and mitochondrial genome replication/transcription, and convincingly demonstrated a direct regulation of ENDOG expression by PGC-1α and ERRα. In addition, they show that ENDOG participates in the development of blood pressure-independent LV hypertrophy. The extent of cardiac hypertrophy is rather mild in mice lacking ENDOG. However, these findings raise the intriguing possibility that ENDOG expression may represent an underestimated contributor to pressure-overload independent cardiac hypertrophy. The association between ENDOG and cardiac hypertrophy, which may play a role in the regulation of cardiac mass in rodents will need to be replicated in human studies to determine if variations in ENDOG regulation of cardiac mass in rodents will need to be replicated in human studies to determine if variations in ENDOG expression are associated with pressure overload-independent hypertrophy, such as obesity and Type 2 diabetes mellitus.

References
Endonuclease G: The Link Between Mitochondria and Cardiac Hypertrophy?
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doi: 10.1161/RES.0b013e318249dcc8

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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