Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 357 (2007) 231–236
BBRC
www.elsevier.com/locate/ybbrc

Estrogen-related receptor α is essential for the expression of antioxidant protection genes and mitochondrial function

Shamina M. Rangwala [a,*], Xiaoyan Li [a], Loren Lindsley [a], Xiaomei Wang [a], Stacey Shaughnessy [a], Thomas G. Daniels [a], Joseph Szustakowski [b], N.R. Nirmala [b], Zhidan Wu [a], Susan C. Stevenson [a]

[a] *Diabetes and Metabolism Disease Area, Novartis Institutes of BioMedical Research Institutes, 100 Technology Square, Cambridge, MA 02139, USA*
[b] *Genome and Proteome Sciences, Novartis Institutes of BioMedical Research Institutes, 500 Technology Square, Cambridge, MA 02139, USA*

Received 6 March 2007
Available online 28 March 2007

Abstract

Estrogen-related receptor α (ERRα) is an important mediator of mitochondrial biogenesis and function. To investigate the transcriptional network controlling these phenomena, we investigated mitochondrial gene expression in embryonic fibroblasts isolated from ERRα null mice. Peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) stimulated mitochondrial gene expression program in control cells, but not in the ERRα null cells. Interestingly, the induction of levels of mitochondrial oxidative stress protection genes in response to increased PGC-1α levels was dependent on ERRα. Furthermore, we found that the PGC-1α-mediated induction of estrogen-related receptor γ and nuclear respiratory factor 2 (NRF-2), was dependent on the presence of ERRα. Basal levels of NRF-2 were decreased in the absence of ERRα. The absence of ERRα resulted in a decrease in citrate synthase enzyme activity in response to PGC-1α overexpression. Our results indicate an essential role for ERRα as a key regulator of oxidative metabolism.

Keywords: Estrogen-related receptor; Mitochondrial gene expression; Peroxisome proliferator-activated receptor γ coactivator-1α; Oxidative metabolism; Type 2 diabetes; Nuclear respiratory factor 2

The members of the estrogen-related receptor (ERR; NR3B1-3) family of orphan nuclear hormone receptors, ERRα, ERRβ and ERRγ, show constitutive, ligand-independent transcriptional activity [1]. ERRα and ERRγ are expressed in tissues with high energetic demand, such as the heart, skeletal muscle and the brain, while the β isoform is prevalent during embryonic development [2]. These proteins are coactivated by the PGC-1 family of transcriptional regulators; PGC-1α is often referred to as a protein ligand for the ERRs [3]. Recent evidence shows that the ERRs partner with PGC-1α to regulate mitochondrial gene expression, thus implicating these nuclear receptors as important modulators of mitochondrial function and metabolism [4,5].

Studies in human subjects have demonstrated a strong correlation between reduced mitochondrial number/function and the incidence of type 2 diabetes [6,7]. Microarray analysis of muscle biopsies from type 2 diabetic individuals displayed a decreased expression of OXPHOS genes regulated by PGC-1α in muscle [8,9]. Studies *in vitro* and *in vivo* suggest that ERRα is necessary for the activation of mitochondrial genes as well as increased mitochondrial biogenesis in response to elevated PGC-1α levels; this

Abbreviations: ERR, estrogen-related receptor; PGC-1, peroxisome proliferator-activated receptor γ coactivator-1; WT, wildtype; NRF-2, nuclear respiratory factor 2; OXPHOS, oxidative phosphorylation; MEFs, mouse embryonic fibroblasts; COX-4, cytochrome *c* oxidase-4; UCP-3, uncoupling protein 3; CPT-1b, carnitine palmitoyl transferase 1b; SOD2, superoxide dismutase 2; TXN2, thioredoxin 2; PRDX-3, peroxiredoxin 3; PRDX-5, peroxiredoxin 5; PPAR, peroxisome proliferator-activated receptor; B2M, beta-2 microgobulin; UQCRb, ubiquinol c reductase b; ROS, reactive oxygen species.

* Corresponding author. Fax: +1 617 871 3044.
E-mail address: shamina.rangwala@novartis.com (S.M. Rangwala).

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.03.126

occurs through a direct interaction of ERRα with promoter regions of mitochondrial genes [4,10]. Additionally, NRF-2 and ERRα co-regulate the expression of each other; however, the hierarchical nature of the interaction between these transcription factors is unclear.

Although ERRα null mice do not exhibit an increased susceptibility to weight gain or lipid accumulation in response to a high fat diet, they do have an impairment in adaptive thermogenesis [10,11]. We used mouse embryonic fibroblast cells (MEFs) from these mice as a tool to demonstrate that the loss of ERRα results in a significant diminution in PGC-1α-driven mitochondrial gene expression. We find that ERRα, through its partnership with PGC-1α, is a pleiotropic regulator of mitochondrial pathways and plays a central role in controlling multiple aspects of mitochondrial metabolism.

Materials and methods

Animals. All mice used in this study were maintained on 12 h light/dark cycle and in accordance with regulations under an approved institutional Animal Care and Use Committee protocol in the Novartis animal facility. ERRα null mice were obtained from Deltagen (San Carlos, CA). The endogenous ERRα gene locus was targeted such that amino acids 92–119 in the DNA binding domain of the protein were deleted. Genotyping analysis was performed as described using the following primers. For the wildtype allele, the primers 5′-TCA TGG AAT CCT GCT CTC CCT TTC C-3′ and 5′-GTG CTC ACC CTC CTT GAG CAT GC-3′ generate a 263-base-pair band. For the knockout allele, the primers 5′-GGG TGG GAT TAG ATA AAT GCC TGC TCT-3′ and 5′-GTG CTC ACC CTC CTT GAG CAT GC-3′ generate a 447-base-pair band. The primary rabbit anti-mouse ERRα antibody for immunoblotting was kindly provided by Dr. Vincent Giguere, McGill University, Montreal, Canada [11].

MEF studies. Mouse embryonic fibroblast cells (MEFs) were isolated from ERRα null mice. Briefly, timed heterozygote matings were set up. On day 13.5 *post coitum*, pregnant females were euthanized and the embryos isolated in PBS. Each embryo was individually dissected and homogenized in PBS using 3 ml syringes and 18.5 gauge needles. These crude suspensions were then plated in 10 cm cell culture dishes. At the second passage, similar genotypes were pooled, and cryopreserved.

Adenoviral transduction and gene expression analysis. MEFs were plated in a 6-well plate at 0.5×10^6 cells per well. Cells were then transduced with adenovirus expressing either (1) GFP, (2) mouse PGC-1α, (3) human ERRα or (4) human ERRα in combination with mouse PGC-1α. For each adenovirus, MEFs were transduced at a viral titer of $12–20 \times 10^9$ viral particles per well. When using two adenoviruses in combination, the adenoviral titer was doubled, resulting in a viral titer of $24–40 \times 10^9$ viral particles per well.

Total RNA was extracted from MEFs using TRIzol reagent (Invitrogen, Carlsbad, CA). cDNA synthesis performed with Superscript III RNase H Reverse Transcriptase (Invitrogen, Carlsbad, CA). QPCR was performed using Taqman (Applied Biosystems, Foster City, CA) as described by the manufacturer. Relative mRNA expression levels were calculated comparing the level of expression of target genes to a control transcript (B2M); further, data were expressed as a fold change relative to cells transduced with adenoviral GFP. Samples were assayed in triplicate and expressed as means ± SEM of the fold change relative to the control (set at 100%).

Total RNA was analyzed using the Affymetrix whole-genome microarray, MOE430 Plus 2.0. RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA) and cRNA synthesis and hybridizations were performed as per the manufacturer's instructions (Affymetrix, Santa Clara, CA). For each condition, the RNA from each of three wells was hybridized to individual chips. Raw expression data were normalized using the RMA procedure [12] with default settings. Gene Set Enrichment Analysis (GSEA) was performed to identify pathways that underwent coordinated modulation as previously described elsewhere [13,14]. Briefly, probe sets were sorted for differential expression as estimated by the LIMMA moderated *t*-test [15]. One thousand two hundred eighty-two gene sets were then projected onto the sorted list of probe sets and evaluated for coordinated modulation with the Wilcoxon ranked sum test. These gene sets were culled from several sources including: Jubilant/Pathart (717), Celera Panther (232), Celera Public Pathways (59), KEGG (128), and the literature [13]. Finally, the False Discovery Rate correction [16,17] was applied to all 1283 *p*-values to account for multiple hypothesis testing.

Citrate synthase activity. Citrate synthase (CS) activity was determined in MEF lysates at 72 h post-transduction as previously described [18]. Each sample was assayed in triplicate. CS activity was normalized for protein content and expressed as means ± SEM of the fold change relative to the control, which was set at 100%.

Experimental setup and data analysis. In each experiment, the transduction and analysis was performed in three independent wells of cells. Each well was assayed in triplicate for gene expression and enzyme activity. Each experiment was independently repeated a minimum of two times, to ensure reproducibility.

Data analyses were performed using Microsoft Excel and GraphPad Prism 4 software (GraphPad Software, San Diego, CA). Data are presented as means ± SEM. Statistical analysis was performed using either a Student's *t*-test for direct comparison of two groups or a one way ANOVA followed by Bonferroni or Newman–Keul's multiple comparison post tests for more than two groups. A *p*-value of <0.05 was considered to be significant.

Results and discussion

ERRα is required for PGC-1α-mediated induction of mitochondrial gene expression

Mitochondrial number and function are regulated by the transcriptional coactivator, PGC-1α. One class of transcription factors coactivated by PGC-1α are the NRFs, which are nuclear encoded proteins that control mitochondrial gene expression [19]. Bioinformatic analysis of promoters of genes upregulated by PGC-1α identified the ERRs as transcriptional regulators of the mitochondrial gene expression program [5]. These authors showed that Gabpa (NRF-2) and ERRα function in an integrated fashion to form a feed-forward loop, inducing the expression of each other as well as that of PGC-1α. Numerous approaches in cell culture systems have reported that a reduction in the expression and/or the function of ERRα, using either genetic or pharmacological tools, results in the reduction of mitochondrial gene expression. We obtained a strain of ERRα null mice from Deltagen (San Carlos, CA). These mice are healthy and viable and are indistinguishable from their control littermates on a normal chow diet (data not shown), in agreement with data reported by Luo et al. [11]. In order to investigate the transcriptional factors that cooperate with PGC-1α to control mitochondrial gene expression, we transduced wildtype and ERRα null embryonic fibroblasts from these mice with PGC-1α adenovirus. Equivalent levels of PGC-1α expression were obtained in these cells (data not shown). As previously reported, ERRα levels were increased in response

to PGC-1α expression; this was reflected in both the mRNA (Supplemental Figure S1A) and protein (Supplemental Figure S1B) levels. The PGC-1α-mediated induction of mitochondrial genes such as cytochrome *c* (Fig. 1A), UQCRB (Fig. 1B) and CPT-1b (Fig. 1C), was abolished in the absence of ERRα. A similar effect was reported in SAOS2 cells with siRNA-mediated ERRα knockdown [4]. Microarray experiments provide a global, unbiased view of the transcriptional changes in ERRα null MEFs relative to wildtype MEFs in the presence of PGC-1α. The GSEA results (Supplemental Table S2) indicate that, in this system, the absence of ERRα results in broad and profound changes in the transcriptional regulation of several mitochondrial pathways. These changes are consistent with the results seen using the candidate gene approach. Specifically, gene sets describing mitochondrial pathways such as electron transport, oxidative phosphorylation, the TCA cycle and fatty acid oxidation were observed to undergo coordinated down-regulation in ERRα null MEFs (Supplemental Table S2). While many studies have looked at genes that are positively regulated by the ERRs, interaction of these proteins with transcriptional repressors has recently come into focus [20,21]. Interestingly, expression of pathways of genes regulating the cell cycle was increased in ERRα null cells (Supplemental Table S2). This may be indicative of a direct transcriptional repression of these pathways by ERRα, or, alternatively, may result indirectly due to reduced proliferation and exit from cell cycle caused by increased ERRα/PGC-1α activity. This switch in gene expression patterns reflects the balance between differentiation and proliferation controlled by the ERRα/PGC-1α heterodimer. Increased expression of PGC-1α in fibroblasts was shown to decrease cell growth previously [22].

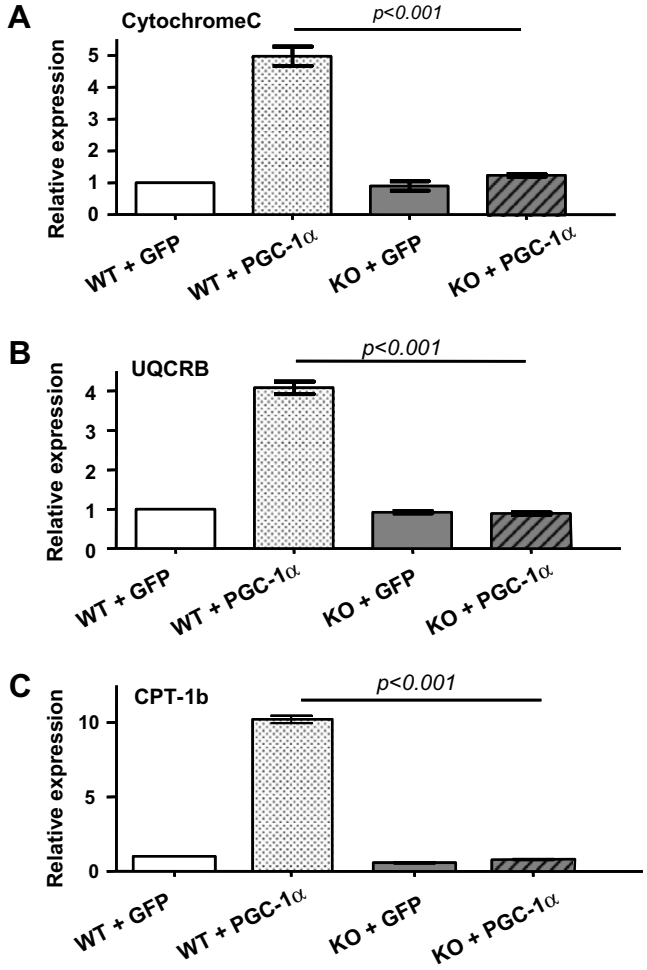

Fig. 1. ERRα is essential for PGC-1α-mediated induction of genes of OXPHOS and fatty acid oxidation in mitochondria. MEFs were transduced with GFP or PGC-1α-GFP adenovirus and gene expression was examined at 72 h. An increase in (A) cytochrome *c* (B) UQCRB and (C) CPT-1b mRNA was observed in WT cells, but not in the ERRα null (KO) cells. Expression levels were measured by Q-PCR and normalized to B2M expression. The data are presented as means ± SEM and normalized to expression in the WT cells transduced with GFP virus. Statistical significance is as indicated.

ERRα mediates the effects of PGC-1α on the ROS protection system

Reactive oxygen species (ROS) are implicated in the etiology of several debilitating conditions such as cancer and neurodegeneration. The role of ROS in the incidence of vascular and neurologic diabetic complications is well established [23]; further, new evidence suggests that the generation of ROS may play a central role in causing insulin resistance [24]. Recent studies have shown that PGC-1α is a critical regulator for mitochondrial as well as non-mitochondrial ROS detoxification pathways [25]. Expression of PGC-1α in human vascular endothelial cells was recently shown to be critical for the induction of detoxification genes that protect from cellular damage caused by ROS [26]. The identity of the transcription factor partner mediating the effects of PGC-1α on ROS detoxification genes is currently unknown. Interestingly, we find that the induction of genes of the mitochondrial ROS protection system, such as superoxide dismutase 2 (Fig. 2A), thioredoxin 2 (Fig. 2B), peroxiredoxin 3 (Fig. 2C) and peroxiredoxin 5 (Fig. 2D) by PGC-1α was dependent on the expression of ERRα. In contrast, the expression of catalase, a peroxisomal enzyme, was not affected by the loss of ERRα (data not shown). Our data suggest that the genes of the mitochondrial ROS detoxification pathway are under the control of ERRα; thus, enhancing the activity of this nuclear receptor may have therapeutic impact in diverse disease states.

Transcriptional control of mitochondrial gene expression

ERRα, β, and γ show a high degree of homology in their DNA binding domains [27]. ERRα and ERRγ are both expressed in the adult, with highest expression levels in the brain, heart, kidney and muscle [2]. We found that levels of ERRγ in the PGC-1α-expressing wildtype MEFs was increased as compared to the GFP-expressing cells (Fig. 3A). Interestingly, in the absence of ERRα, the

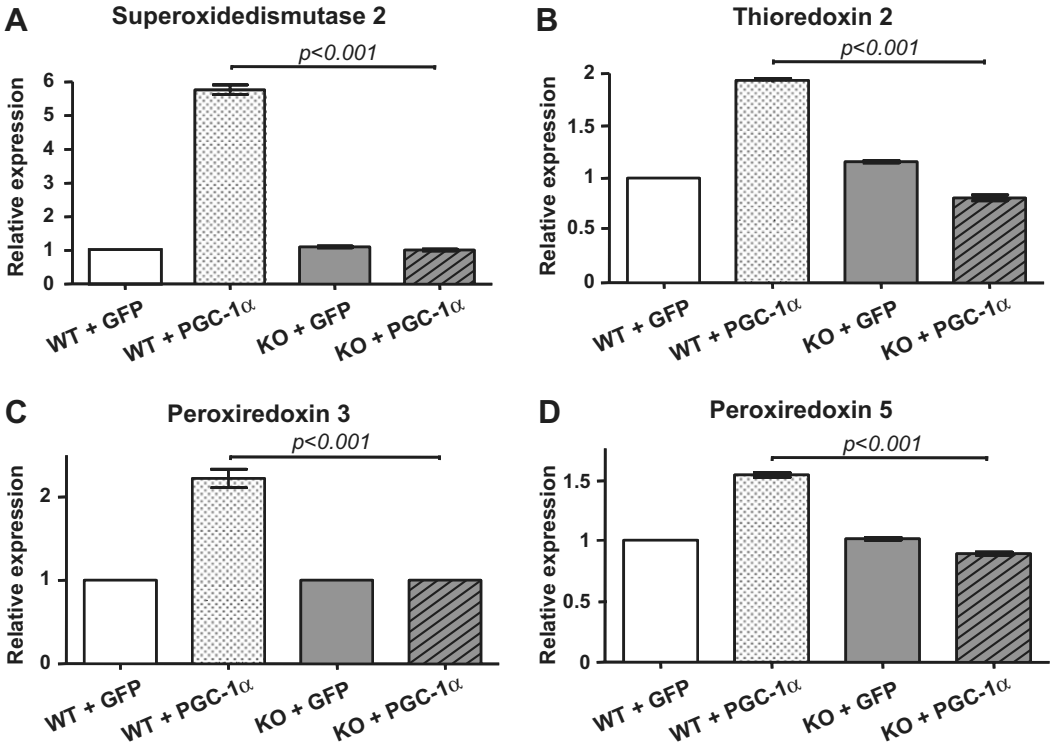

Fig. 2. ERRα is essential for PGC-1α-mediated induction of genes of involved in detoxification of ROS in mitochondria. MEFs were transduced with GFP or PGC-1α-GFP adenovirus and gene expression was examined at 72 h. An increase (A) superoxide dismutase 2, (B) thioredoxin 2, (C) peroxiredoxin 3 and (D) peroxiredoxin 5 was observed in WT cells, but not in the ERRα null (KO) cells. Expression levels were measured by Q-PCR and normalized to B2M expression. The data are presented as means ± SEM and normalized to expression in the WT cells transduced with GFP virus. Statistical significance is as indicated.

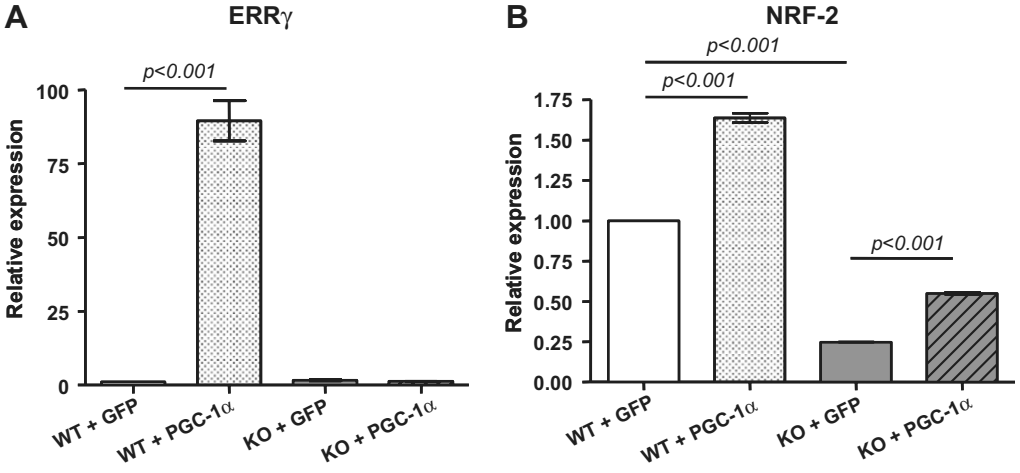

Fig. 3. ERRα is necessary for PGC-1α-mediated induction of ERRγ, but not NRF-2. MEFs were transduced with GFP or PGC-1α-GFP adenovirus and gene expression was examined at 72 h. An increase (A) ERRγ was observed in WT cells, but not in the ERRα null (KO) cells. In contrast, the expression of (B) NRF-2 was diminished in the ERRα null (KO) cells, but not absent. Expression levels were measured by Q-PCR and normalized to B2M expression. The data are presented as means ± SEM and normalized to expression in the WT cells transduced with GFP virus. Statistical significance is as indicated.

increase in ERRγ levels was not observed, suggesting that the presence of ERRα is required to initiate the expression of ERRγ. Thus, while *in vivo*, the loss of ERRα results in an increase [28] or decrease [10] in ERRγ expression depending on the tissue examined, we found that the induction of ERRγ by PGC1α in a our cellular system is dependent upon the presence of ERRα. In HEC-1B cells, the expression of ERRα was shown to depend on ERRγ [29]. These data indicate that the two isoforms regulate each other's expression in a coordinated fashion. The levels of NRF-2 were attenuated in PGC-1α-expressing ERRα null MEFs as compared to the control cells. (Fig. 3B). Further,

the basal expression of NRF-2 was lower in the null MEFs. Thus, our data suggest that NRF-2 is not sufficient for the induction of mitochondrial gene expression mediated by PGC-1α; ERRα is an important component of this pathway.

Impact of ERRα ablation on oxidative metabolism in MEFs

In addition to looking at changes in gene expression, we examined the influence of ERRα ablation on PGC-1α-mediated substrate selection for energy production. One of the important functions of mitochondria is the complete oxidation of three-carbon substrates such as pyruvate and lactate. Citrate synthase is a key regulatory step of the tricarboxylic acid cycle that catalyzes the conversion of acetyl CoA and oxaloacetate to citrate. The level of citrate synthase activity was elevated in the presence of PGC-1α, as previously reported [30]. This increase was not observed in the absence of ERRα (Fig. 4). Thus, ERRα is critical for the PGC-1α-mediated mitochondrial function in MEFs.

ERRα and PGC-1α function in an integrated manner to control mitochondrial gene expression

The levels of PGC-1α are tightly regulated and change acutely in response to physiologic stress. This property of PGC-1α to serve as a sensor for "adaptive" mitochondriogenesis in response to ATP deficit is reflected in the inability of PGC-1α null mice to withstand stress such as cold exposure and exercise [31]. Exercise studies in rats have shown that the elevation in PGC-1α results in an induction in the complete substrate oxidation of lipids which provides the energy to sustain this stress [32]. Increased intramyocellular lipid accumulation in muscle is linked to an increase in insulin resistance, which can be alleviated by the improvement in the ability of mitochondria to perform complete oxidation of lipid substrates [33]. In our studies, we have clearly demonstrated that ERRα is critical for the regulation of adaptive mitochondrial gene expression induced by elevated PGC-1α function. Recently published data show the importance of ERRα in regulating mitochondrial gene expression in brown adipose tissue *in vivo* [10]. Thus, PGC-1α and ERRα form a well-regulated network for the control of adaptive mitochondrial biogenesis that enables the organism to respond to energy demands imposed by diverse physiological conditions. This axis provides a potential node for therapeutic intervention in conditions of obesity-linked insulin resistance, which represent an imbalance between nutrient intake and energy demand.

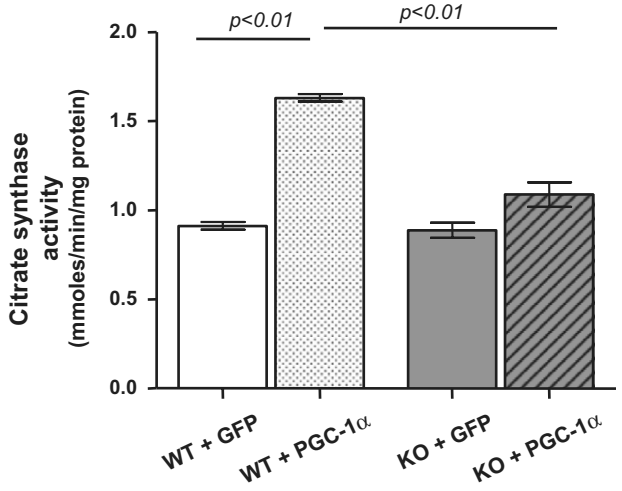

Fig. 4. ERRα is critical for PGC-1α -mediated induction of mitochondrial function. MEFs were transduced with GFP or PGC-1α -GFP adenovirus for 72 h, after which cells were harvested and activities in lyates were measured. An increase in citrate synthase activity was observed in WT cells, but not in the ERRα null (KO) cells. The data are presented as means ± SEM. Statistical significance is as indicated.

Acknowledgments

We thank Dr. Bruce Spiegelman for the PGC-1α adenovirus, Xueming Huang, Olivia Bare and Sandhya Punreddy for technical assistance, and Drs. Thomas Hughes, Brigitte Fournier, Richard Vega and Jennifer Calvo for critical reading of the manuscript and helpful discussions.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc. 2007. 03.126.

References

[1] H. Hong, L. Yang, M.R. Stallcup, Hormone-independent transcriptional activation and coactivator binding by novel orphan nuclear receptor ERR3, Journal of Biological Chemistry 274 (1999) 22618–22626.
[2] D.J. Heard, P.L. Norby, J. Holloway, H. Vissing, Human ERR gamma, a third member of the estrogen receptor-related receptor (ERR) subfamily of orphan nuclear receptors: Tissue-specific isoforms are expressed during development and in the adult, Molecular Endocrinology 14 (2000) 382–392.
[3] S.N. Schreiber, D. Knutti, K. Brogli, T. Uhlmann, A. Kralli, The transcriptional coactivator PGC-1 regulates the expression and activity of the orphan nuclear receptor estrogen-related receptor alpha (ERR alpha), Journal of Biological Chemistry 278 (2003) 9013–9018.
[4] S.N. Schreiber, R. Emter, M.B. Hock, D. Knutti, J. Cardenas, M. Podvinec, E.J. Oakeley, A. Kralli, The estrogen-related receptor alpha (ERR alpha) functions in PPAR gamma coactivator 1 alpha (PGC-1 alpha)-induced mitochondrial biogenesis, Proceedings of the National Academy of Sciences of the United States of America 101 (2004) 6472–6477.
[5] V.K. Mootha, C. Handschin, D. Arlow, X. Xie, P.J. St, S. Sihag, W. Yang, D. Altshuler, P. Puigserver, N. Patterson, P.J. Willy, I.G. Schulman, R.A. Heyman, E.S. Lander, B.M. Spiegelman, Erralpha and Gabpa/b specify PGC-1alpha-dependent oxidative phosphorylation gene expression that is altered in diabetic muscle, Proceedings of the National Academy of Sciences of the United States of America 101 (2004) 6570–6575.

[6] K.F. Petersen, D. Befroy, S. Dufour, J. Dziura, C. Ariyan, D.L. Rothman, L. DiPietro, G.W. Cline, G.I. Shulman, Mitochondrial dysfunction in the elderly: Possible role in insulin resistance, Science 300 (2003) 1140–1142.

[7] K.F. Petersen, S. Dufour, D. Befroy, R. Garcia, G.I. Shulman, Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes New England, Journal of Medicine 350 (2004) 664–671.

[8] V.K. Mootha, C.M. Lindgren, K.F. Eriksson, A. Subramanian, S. Sihag, J. Lehar, P. Puigserver, E. Carlsson, M. Ridderstrale, E. Laurila, N. Houstis, M.J. Daly, N. Patterson, J.P. Mesirov, T.R. Golub, P. Tamayo, B. Spiegelman, E.S. Lander, J.N. Hirschhorn, D. Altshuler, L.C. Groop, PGC-1 alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes, Nature Genetics 34 (2003) 267–273.

[9] M.E. Patti, A.J. Butte, S. Crunkhorn, K. Cusi, R. Berria, S. Kashyap, Y. Miyazaki, I. Kohane, M. Costello, R. Saccone, E.J. Landaker, A.B. Goldfine, E. Mun, R. DeFronzo, J. Finlayson, C.R. Kahn, L.J. Mandarino, Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: Potential role of PGC1 and NRF1, Proceedings of the National Academy of Sciences of the United States of America 100 (2003) 8466–8471.

[10] J.A. Villena, M.B. Hock, W.Y. Chang, J.E. Barcas, V. Giguere, A. Kralli, Orphan nuclear receptor estrogen-related receptor alpha is essential for adaptive thermogenesis, Proceedings of the National Academy of Sciences of the United States of America 104 (2007) 1418–1423.

[11] J.M. Luo, R. Sladek, J. Carrier, J.A. Bader, D. Richard, V. Giguere, Reduced fat mass in mice lacking orphan nuclear receptor estrogen-related receptor alpha, Molecular and Cellular Biology 23 (2003) 7947–7956.

[12] R.A. Irizarry, B. Hobbs, F. Collin, Y.D. Beazer-Barclay, K.J. Antonellis, U. Scherf, T.P. Speed, Exploration, normalization, and summaries of high density oligonucleotide array probe level data, Biostatistics 4 (2003) 249–264.

[13] V.K. Mootha, C.M. Lindgren, K.F. Eriksson, A. Subramanian, S. Sihag, J. Lehar, P. Puigserver, E. Carlsson, M. Ridderstrale, E. Laurila, N. Houstis, M.J. Daly, N. Patterson, J.P. Mesirov, T.R. Golub, P. Tamayo, B. Spiegelman, E.S. Lander, J.N. Hirschhorn, D. Altshuler, L.C. Groop, PGC-1 alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes, Nature Genetics 34 (2003) 267–273.

[14] J.D. Szustakowski, J.H. Lee, C.A. Marrese, P.A. Kosinski, N.R. Ninnala, D.M. Kemp, Identification of novel pathway regulation during myogenic differentiation, Genomics 87 (2006) 129–138.

[15] G.K. Smyth, Linear models and empirical Bayes methods for assessing differential expression in microarray experiments, Statistical Applications in Genetics and Molecular Biology 3 (1) (2004). Article 3.

[16] J.D. Storey, A direct approach to false discovery rates, Journal of the Royal Statistical Society Series B-Statistical Methodology 64 (2002) 479–498.

[17] J.D. Storey, R. Tibshirani, Statistical significance for genomewide studies, Proceedings of the National Academy of Sciences of the United States of America 100 (2003) 9440–9445.

[18] C.D. Moyes, O.A. MathieuCostello, N. Tsuchiya, C. Filburn, R.G. Hansford, Mitochondrial biogenesis during cellular differentiation, American Journal of Physiology. Cell Physiology 41 (1997) C1345–C1351.

[19] Z.D. Wu, P. Puigserver, U. Andersson, C.Y. Zhang, G. Adelmant, V. Mootha, A. Troy, S. Cinti, B. Lowell, R.C. Scarpulla, B.M. Spiegelman, Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1, Cell 98 (1999) 115–124.

[20] A. Castet, A. Herledan, S. Bonnet, S. Jalaguier, J.M. Vanacker, V. Cavailles, Receptor-interacting protein 140 differentially regulates estrogen receptor-related receptor transactivation depending on target genes, Molecular Endocrinology 20 (2006) 1035–1047.

[21] P. Augereau, E. Badia, P. Balaguer, S. Carascossa, A. Castet, S. Jalaguier, V. Cavailles, Negative regulation of hormone signaling by RIP140, The Journal of Steroid Biochemistry and Molecular Biology 102 (2006) 51–59.

[22] D. Xu, T. Finkel, A role for mitochondria as potential regulators of cellular life span, Biochemical and Biophysical Research Communications 294 (2002) 245–248.

[23] T. Nishikawa, D. Edelstein, X.L. Du, S. Yamagishi, T. Matsumura, Y. Kaneda, M.A. Yorek, D. Beebe, P.J. Oates, H.P. Hammes, I. Giardino, M. Brownlee, Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage, Nature 404 (2000) 787–790.

[24] N. Houstis, E.D. Rosen, E.S. Lander, Reactive oxygen species have a causal role in multiple forms of insulin resistance, Nature 440 (2006) 944–948.

[25] J. St-Pierre, S. Drori, M. Uldry, J.M. Silvaggi, J. Rhee, S. Jager, C. Handschin, K.N. Zheng, J.D. Lin, W.L. Yang, D.K. Simon, R. Bachoo, B.M. Spiegelman, Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators, Cell 127 (2006) 397–408.

[26] I. Valle, A. varez-Barrientos, E. Arza, S. Lamas, M. Monsalve, PGC-1 alpha regulates the mitochondrial antioxidant defense system in vascular endothelial cells, Cardiovascular Research 66 (2005) 562–573.

[27] B. Horard, J.M. Vanacker, Estrogen receptor-related receptors: orphan receptors desperately seeking a ligand, Journal of Molecular Endocrinology 31 (2003) 349–357.

[28] J.M. Huss, I.P. Torra, B. Staels, V. Giguere, D.P. Kelly, Estrogen-related receptor alpha directs peroxisome proliferator-activated receptor at signaling in the transcriptional control of energy metabolism in cardiac and skeletal muscle, Molecular and Cellular Biology 24 (2004) 9079–9091.

[29] Z. Zhang, C. Teng, Interplay between estrogen-related receptor alpha (ERRalpha) and gamma (ERRgamma) on the regulation of ERR-alpha gene expression, Molecular and Cellular Endocrinology 264 (2007) 128–141.

[30] O.H. Mortensen, L. Frandsen, P. Schjerling, E. Nishimura, N. Grunnet, PGC-1 alpha and PGC-1 beta have both similar and distinct effects on myofiber switching toward an oxidative phenotype, American Journal of Physiology. Endocrinology and Metabolism 291 (2006) E807–E816.

[31] T.C. Leone, J.J. Lehman, B.N. Finck, P.J. Schaeffer, A.R. Wende, S. Boudina, M. Courtois, D.F. Wozniak, N. Sambandam, C. Bernal-Mizrachi, Z.J. Chen, J.O. Holloszy, D.M. Medeiros, R.E. Schmidt, J.E. Saffitz, E.D. Abel, C.F. Semenkovich, D.P. Kelly, PGC-1 alpha deficiency causes multi-system energy metabolic derangements: Muscle dysfunction, abnormal weight control and hepatic steatosis, PLoS Biology 3 (2005) 672–687.

[32] T.R. Koves, P. Li, J. An, T. Akimoto, D. Slentz, O. Ilkayeva, G.L. Dohm, Z. Yan, C.B. Newgard, D.M. Muoio, Peroxisome proliferator-activated receptor-gamma co-activator 1 alpha-mediated metabolic remodeling of skeletal myocytes mimics exercise training and reverses lipid-induced mitochondrial inefficiency, Journal of Biological Chemistry 280 (2005) 33588–33598.

[33] K.F. Petersen, G.I. Shulman, Etiology of insulin resistance, American Journal of Medicine 119 (2006) 10S–16S.