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Development of a stable cell line with an intact PGC-1α/ERRα axis for screening environmental chemicals

Christina T. Teng [a,*], Burton Beames [a], B. Alex Merrick [a], Negin Martin [b], Charles Romeo [b], Anton M. Jetten [c]

[a] DNTP, BioMolecular Screening Branch, Division, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC 27709, United States
[b] DIR, Viral Core Lab, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC 27709, United States
[c] DIR Laboratory of Respiratory Biology, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC 27709, United States

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ABSTRACT

The estrogen-related receptor α (ERRα) and the peroxisome proliferator-activated receptor γ (PPARγ) coactivator 1α (PGC-1α) play critical roles in the control of several physiological functions, including the regulation of genes involved in energy homeostasis. However, little is known about the ability of environmental chemicals to disrupt or modulate this important bioenergetics pathway in humans. The goal of this study was to develop a cell-based assay system with an intact PGC-1α/ERRα axis that could be used as a screening assay for detecting such chemicals. To this end, we successfully generated several stable cell lines expressing PGC-1α and showed that the reporter driven by the native ERRα hormone response unit (AAB-Luc) is active in these cell lines and that the activation is PGC-1α-dependent. Furthermore, we show that this activation can be blocked by the ERRα selective inverse agonist, XCT790. In addition, we find that genistein and bisphenol A further stimulate the reporter activity, while kaempferol has minimal effect. These cell lines will be useful for identifying environmental chemicals that modulate this important pathway.

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1. Introduction

Between conception and death, we are exposed chronically to countless environmental chemicals [1]. Data from *in vitro* and *in vivo* experimental studies and epidemiological investigations all point to the contribution of environmental chemicals in the development of many complex diseases [2]. Understanding how environmental chemicals impact on human health and protecting individuals from unnecessary exposure to toxic chemicals remains a critical need. In meet this need, the National Institute of Environmental Health Sciences (NIEHS)/National Toxicology Program (NTP), the U.S. Environmental Protection Agency's National Center for Computational Toxicology (EPA's NCCT), the National Human Genome Research Institute (NHGRI)/National Institutes of Health Chemical Genomics Center (NCGC), and U.S. Food and Drug Administration (FDA) formed the Tox21 partnership to better identify compounds that could pose a health hazard to humans [3]. Recent advances in molecular biology techniques and the availability of robotic handling systems enable the screening of tens of thousands of compounds for biological activity in individual biochemical- or cell-based assays within a few days. In Phase II of Tox21, a 10,000 compound library is being screened against a panel of nuclear receptors and stress response pathway assays [3]. However, few of the current assays are designed to screen pathways that play an important role in maintaining metabolic homeostasis. The pleiotropic PPARγ coactivator (PGC)-1α is a key regulator of several metabolic pathways, such as oxidative phosphorylation, energy homeostasis, and glucose and lipid metabolism and is a major regulator of mitochondria function and biogenesis [10,11]. PGC-1α deficient mice exhibit several abnormalities related to defects in energy homeostasis, including abnormal weight control, muscle dysfunction, and hepatic steatosis [4,5]. One of the major partners for PGC-1α is the estrogen-related nuclear receptor α (ERRα) [6–10]. ERRα belongs to the orphan member (NR3B) of the nuclear receptor superfamily [11] and its activity is primarily controlled by its level of expression, cellular localization, and interactions with coactivators, including PGC-1α [6,12–14]. The PGC-1α/ERRα axis is a powerful signaling pathway for energy homeostasis. Environmental chemicals that serve as ligands to the receptor or that affect the interaction or stability of the receptor or coactivator will enhance or reduce the activity of this signaling pathway, and consequently influence energy balance and therefore susceptibility to metabolic syndromes, diabetes, obesity, and cancer. In this study, we developed stable cell lines with an intact PGC-1α/ERRα pathway and characterized one of the clones for its utility to detect chemicals that modulate pathway activity.

* Corresponding author. Address: DNTP, BSB, NIEHS, PO Box 12233, MD K2-17, 111 Alexander Dr. RTP, NC 27709, United States. Fax: +1 919 541 3715.
E-mail address: teng1@niehs.nih.gov (C.T. Teng).

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2. Materials and methods

2.1. Reagents

XCT790 (Chemical Abstracts Services Registry Number, CASRN: 725274-18-7) was obtained from Tocris (Bristol, United Kingdom). Stock solution (10 mM in dimethyl sulfoxide, DMSO) for Bisphenol A (BPA, CASRN: 80-05-7), 4-hydroxytamoxifen (4-OHT, CASRN: 68047-06-3), diethylstilbesterol (DES, CASRN: 56-53-1), genistein (Gen, CASRN: 446-72-0), and kaempferol (Kaemp, CASRN: 520-18-3) were obtained and provided by MRIGlobal (Kansas City, MO, USA) under contract to the NTP. The antibody to ERRα peptide (P3) was described previously [15]. Antibodies to the HA tag and β-actin were obtained from Cell Signaling Technology (Danver, MA, USA). Standard culture medium and reagents were obtained form Thermo Fisher Scientific (Walthan, MA, USA).

2.2. Cell culture and transient transfection

Human embryonic kidney (HEK 293T) cells obtained from the ATCC (Manassas, VA, USA) were maintained in high-glucose Dulbecco's Modified Essential Medium (DMEM), supplemented with glutamine and 10% fetal bovine serum (FBS), 100 IU/mL penicillin and 100 μg/mL streptomycin at 37 °C under 5% CO_2. To evaluate PGC-1α activity, the HEK293T cells were transiently transfected with pcDNA3/HA-hPGC1α [16] expression plasmids, pCMV-renilla (internal control), and the AAB-Luc reporter [17] using Lipofectamine 2000 reagent according to the manufacturer's instruction (Invitrogen, Grand Island, NY, USA). Five hours after transfection, the culture medium was changed to phenol red-free DMEM and 10% charcoal/dextran treated FBS (CD-FBS). The cells were then treated with various compounds for 16 h before luciferase activities were determined with the Dual-Glo Luciferase Assay System (Promega Corporation, Madison, WI, USA) according to the manufacturer's protocol. The normalized luciferase activities were plotted using GraphPad Prism (La Jolla, CA, USA).

2.3. Western blot

Whole cell lysates were prepared using Passive Lysis Buffer according to the manufacturer's instructions (Promega Corporation). Protein concentrations were determined using Bio-Rad Protein Assay Reagents (Pierce, Rockford, IL, USA). Samples were heated at 95 °C for 5 min and then separated on a 4–12% polyacrylamide gel. The proteins were then electrotransferred onto polyvinylidene difluoride membranes followed by blocking in TRIS-buffered saline containing 0.05% Tween 20 (TTBS) and 5% nonfat milk for 2 h. Blots were incubated with primary antibodies in TTBS at 4 °C overnight, rinsed with TTBS, and then incubated with appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies at a dilution of 1:5000 in TTBS/milk at room temperature for 1 h. Immunoreactive products were detected by the Enhanced Chemiluminescence (ECL) System (Amersham Pharmacia, Piscataway, NJ, USA). β-actin was used as a loading control.

2.4. Construction of pCDH510-hPGC expression vector and production of lentivirus

The HA-tagged hPGC1α was excised by *Bam*H1/*Not*1 from pcDNA3/HA-hPGC1 expression plasmids [16] and subcloned into pCDH-promoter-MCS-EF1 Lentivector series 510B-1, an expression vector with a puromycin resistance marker (System Biosciences, Mountain View, CA, USA). The construct was verified by sequencing and packaged into lentivirus that were used to infect the cells. All lentivirus were packaged in HEK293T/17 cells according to published Current Protocols [18]. Briefly, HEK293T cells were transiently transfected with pMD2G, psPAX2, and transfer vector containing the desired gene using Lipofectamine 2000 (Invitrogen). Supernatant was collected 48 h post transfection and cleared from debris before storage. All titers were determined by performing quantitative PCR to measure the number of lentiviral particles that integrated into the host genome.

3. Results

3.1. Establishing the cell system

HEK293T cells are commonly used in high throughput cell-based assays, including those used in Tox21 Phase II to screen the 10 K library [19]. These cells are estrogen receptor (ER) α and β negative, but contain high levels of endogenous ERRα and low levels of ERRγ [17]. Relying on the existing level of endogenous ERRα, we tested PGC-1α/ERRα function in this cell line. For the luciferase reporter, rather than using the consensus synthetic ERRE as the response element, we used the pleiotropic nuclear receptor enhancer MHRE [17] of the ERRα gene promoter as the response element. This enhancer is composed of three TCAAGGTCA (ERRE) binding sites, originally identified in the human lactoferrin gene to bind ERRα and the steroidogenic factor (SF)-1 [20], and two half-motifs (AGGTCA) of the estrogen response element (ERE). We cloned a 57 bp region of the MHRE that contains 2 copies of the 23 bp ERRE and one ½ ERE (named as A) followed by an 11 bp ERRE (named as B) into a luciferase reporter (AAB-Luc). Individual or combined mutations in any of these elements reduced PGC-1α dependent Luc-reporter activity [17]. The ability of PGC-1α to activate the AAB-Luc reporter in HEK293T cells was assessed by transient transfection experiments. Although ERRα was considered to be constitutively active [21], the endogenous ERRα activated the AAB-Luc reporter in these cells only when PGC-1α was ectopically expressed (Fig. 1A). PGC-1α induced activity was repressed in a dose-dependent manner by the ERRα-selective inverse agonist, XCT790. Furthermore, addition of DES and 4-OHT, which act as inverse agonists for ERRγ and ERRβ, but not ERRα [22], did not affect PGC-1α-induced activity. Treatment with Kaemp, a dietary flavonoid reported to be an ERRα and ERRγ inverse agonist [23], cause a slight but not statistically significant increase in activity (Fig. 1B). These results suggest that the PGC-1α-dependent transactivation is likely mediated by the endogenous ERRα. We reasoned that generating a stable HEK293T cell line expressing PGC-1α would be a useful tool for analyzing the effects of chemicals on the activity of the PGC-1α/ERRα axis.

3.2. Generating stable cell lines expressing PGC-1α

To construct the viral expression vector, the HA-tagged PGC-1α was excised from the pcDNA3/HA-hPGC1 plasmid [16] and inserted into the pCDH-Promoter-MCS-EF1 lentivector series CMV-Puro vector pCDH510-hPGC-1α. First, we compared the functional activity of the pCDH510-hPGC-1α with the original pcDNA3/HA-hPGC-1α on AAB-Luc activation and the response to XCT790 treatment (Fig. 2A). Consistent with the original expression plasmids, the HA-PGC-1α in the lentiviral vector was functional but with lower activity probably due to a lower transfection efficiency associated with the larger size of the viral constructs. Nonetheless, XCT790 effectively blocked the AAB-Luc activity induced by the viral expression plasmids. We next infected HEK293T cells with various concentrations of pCDH510-hPGC-1α lentivirus. As shown in Fig. 2B, PGC-1α stimulated AAB-Luc activity correlated with the amount of infected virus. This activation was repressed by the addition of 10 μM of XCT790 (comparing control vs XCT)

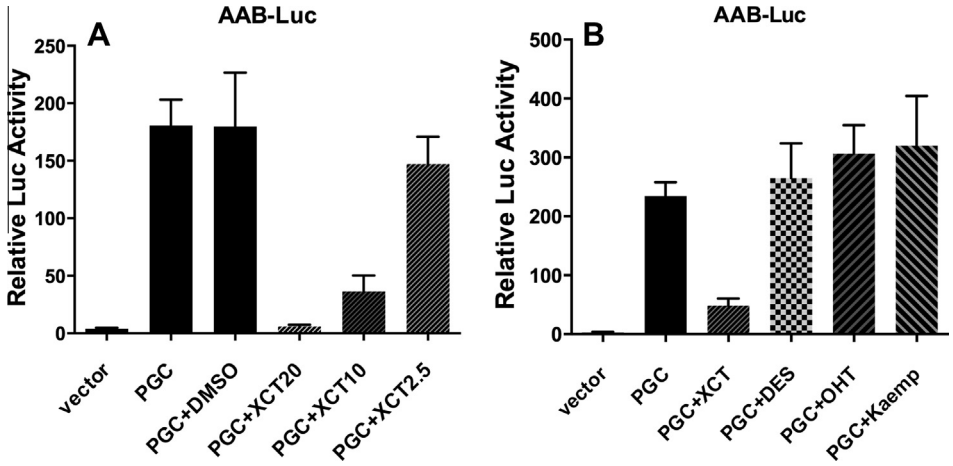

Fig. 1. PGC-1α induces AAB-Luc reporter activity in HEK293T cells. Expression plasmids of PGC-1α or empty vector were transiently transfected along with the AAB-Luc reporter and the internal control CMV-Renilla into HEK293T cells by lipofectamine. After transfection, the cells were transferred to phenol red-free, 10% charcoal-stripped bovine fetal serum and treated with DMSO or XCT790 (20, 10 or 2.5 μM) in (A) and 10 μM of XCT, DES, OHT or Kaemp in (B) and incubated for 16 h before the Luc activities were measured. The data were presented as means ± standard deviation (sd) of 4 technical and three biological replicates.

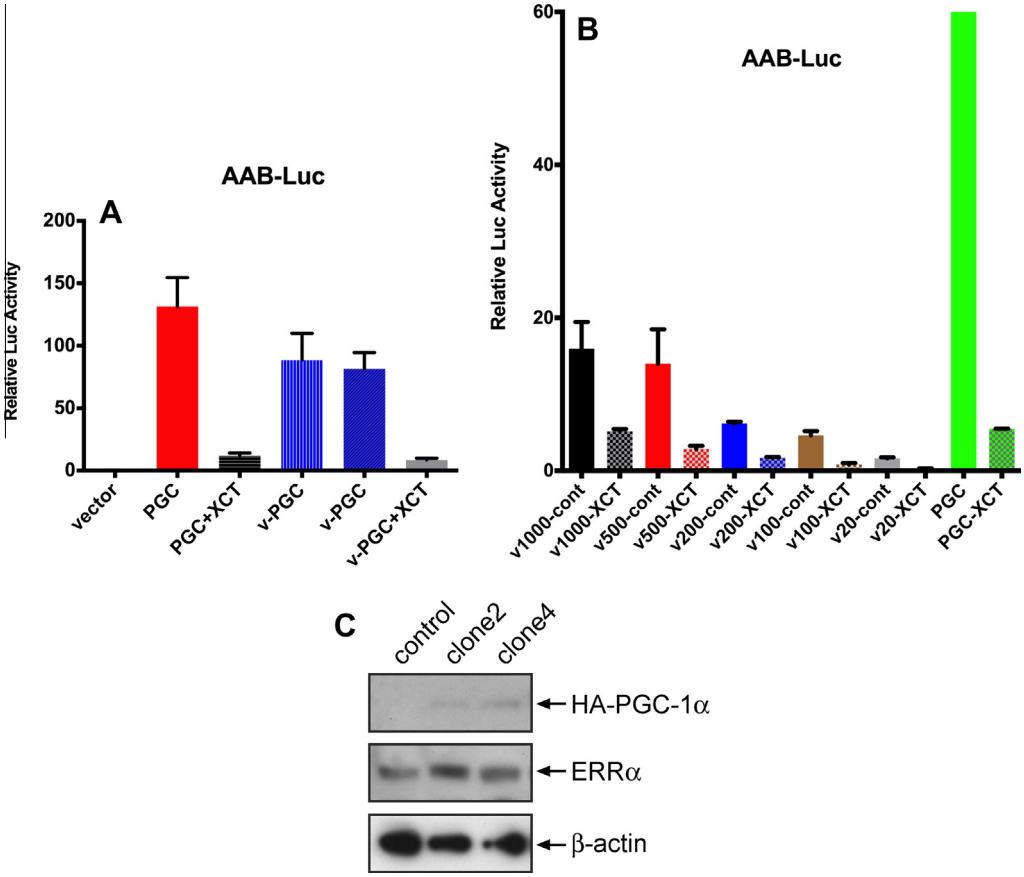

Fig. 2. The transactivation capacity of PGC-1α expression plasmids on reporter is compared. (A) Both PGC (pcDNA3/HA-hPGC-1α) and v-PGC (PGC-1α lentiviral expression plasmids, pCDH510-hPGC-1α) induced strong activation of AAB-Luc reporter and the activation was blocked by 10 μM of XCT790. (B) HEK293T cells were infected with different amount (v1000, v500, v200, v100 and v20) of virus containing PGC-1α viral expression constructs. The infected cells (maintained in the presence of 1 μg/mL of puromycin) were either treated with vehicle (cont) or 10 μM XCT790 (XCT). The far right-hand columns (green) were transient transfection experiment as described in Fig. 1, with 40 ng PGC-1α/well in a 24-well plate format. Data were presented as means ± standard deviation (sd) of 4 repeated samples and the experiments were repeated. (C) Western blots. Cell lyses protein (35 μg) from HEK293T (control) clone 2 and clone 4. The blot was sequentially probed with HA, ERRα peptide P3, or β-actin antibodies as described in Section 2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

suggesting that ERRα was mediating the PGC-1α-dependent activation. Compared to transiently transfected cells (last two columns of Fig. 2B, PGC vs PGC-XCT), PGC-1α-dependent AAB-Luc activation was less robust in virus-infected cells and may reflect a lower level of expressed PGC-1α in the stable cell line than in the transiently transfected cells. Of the five different viral infections, we used the viral dose 500 in clonal selection (Fig. 2B). Seven clones were randomly selected and expanded in medium containing 1 μg/mL of puromycin. The clones showed a variable level of AAB-Luc reporter activities and the activity could be blocked by XCT790 in all selected clones (data not shown). As expected, western blot analysis detected the expression of HA-PGC-1α in clone 2 (low

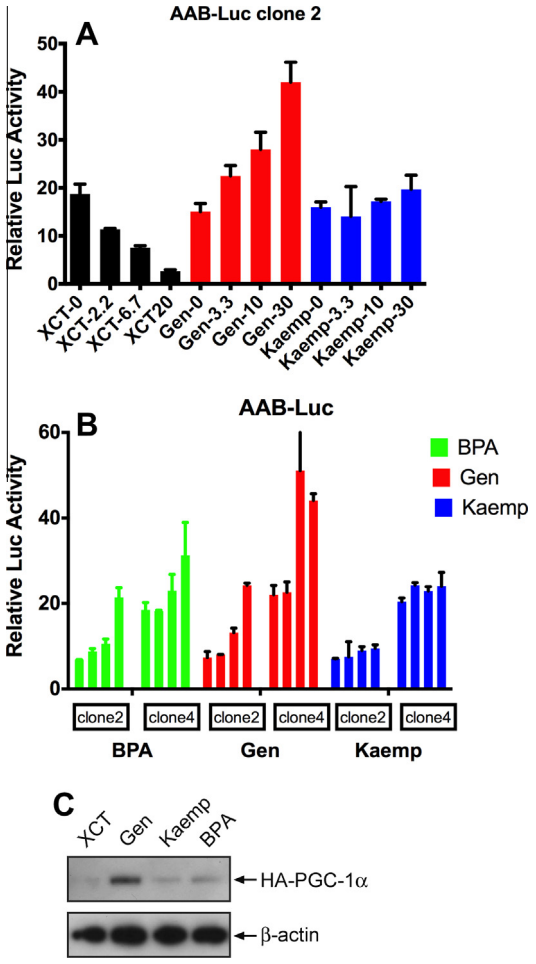

Fig. 3. AAB-Luc activity in various pCDH510-hPGC-1α lentiviral infected stable cell lines. (A) Stable cell clone 2 was treated with various concentrations of XCT790 (XCT-0, 2.2, 6.7 and 20 μM), Genistein (Gen-0, 3.3, 10 and 30 μM) or Kaempferol (Kaemp-0, 3.3, 10 and 30 μM) for 16 h before the Luc reporter activities were measured. (B) Stable cell clone 2 and clone 4 were treated as described in A; bisphenol A (BPA-0, 3.3, 10 and 30 μM) was included in this experiment. Data were presented as means ± standard deviation (sd) of 4 repeated samples and the experiments were repeated. (C) Western blots. Cells lyses protein (35 μg) of clone 2 treated with 30 μM of compounds as indicated. The blot was probed with HA or β-actin antibodies. β-actin served as a loading control.

expression) and clone 4 (high expression) and the endogenous ERRα protein in these two clones was increased accordingly (compare the intensity of band of clone 2 and clone 4 to control, Fig. 2C). Clone 2 with moderate PGC-1α expression was selected for further analysis. Consistent with our previous finding, XCT790 inhibited AAB-Luc activity in a dose-dependent manner. Treatment with Kaemp did not significantly alter AAB-Luc activity, while another dietary isoflavone, Gen, strongly enhanced its activity (Fig. 3A). When examined by Western blotting, the PGC-1α level in clone 2 was significantly increased by the presence of 30 μM Gen (Fig. 3C). Although clone 4 has about a 3-fold higher basal AAB-Luc activity, the results with Gen and Kaemp in clone 2 could be confirmed in clone 4 (Fig. 3B). BPA, a well-studied endocrine disruptor (EDC) with a broad spectrum of biologic effects, also enhanced AAB-Luc activity in both selected clones.

4. Discussion

The PGC-1α/ERRα axis has been implicated in many cellular pathways and pathologies. The extent to which ERRα activity is regulated by endogenous ligands is not yet known; rather, ERRα appears to be constitutively active in a cell type-specific manner [24] and its activity is increased by interaction with the coactivator PGC-1α. The crystal structure of the ERRα ligand-binding domain bound to a coactivator peptide from PGC-1α revealed a transcriptionally active conformation even in the absence of a ligand [21]. In addition, the ERRα ligand-binding pocket is rather small compared to other nuclear receptors [21]. However, phytoestrogens have been reported to modulate ERRα activity [23,25]. This led to the possibility that ERRα can function as a ligand (either small molecule or protein)-dependent transcriptional regulator [12]. Thus, environmental chemicals that bind to ERRα might disrupt or enhance its interaction with coregulators. These interactions might have profound effects on PGC-1α/ERRα-dependent transcriptional regulation and subsequently the physiological functions they control. Such changes may influence susceptibility to various diseases, including obesity, metabolic syndrome, diabetes, heart disease, and cancer. Previously, most molecular mechanism studies were carried out using transient transfected cells. Recently, it was realized that overexpression of regulatory proteins could adversely affect signaling pathways and therefore biological interpretation of the observed responses [26]. Generating a stably expressed PGC-1α in HEK293T cells is the first step toward developing an assay that could be used to identify compounds that modulate this pathway. In this study, using a lentivirus delivery system, we describe the generation of HEK293T cells stably expressing human PGC-1α at a level acceptable for cell survival and function. These cells express high levels of endogenous ERRα. By Western blot analysis, we showed that clones with detectable HA-tagged PGC-1α exhibit a higher level of endogenous ERRα expression (Fig. 2C) demonstrating the co-dependent relationship of the receptor and the coactivator [14]. This suggests intracellular mechanisms are controlling their endogenous expression in a form of homeostasis. We demonstrate that these stable cell clones have an intact PGC-1α/ERRα axis and can be used to detect environmental chemicals that act on this signaling pathway.

A decade's long search for compounds that function as agonist or antagonist for ERRα has not led to the identification of an endogenous agonist or antagonist. However, a few synthetic inverse agonists which repress the constitutive activity of the receptor have been reported [27–29]. These inverse agonists not only promote coactivator dissociation but also enhance proteasome-mediated protein degradation [29,30]. XCT790, an ERRα-selective inverse agonist with no effect on other ERR subtypes or ERs, strongly inhibited AAB-Luc activity in our assay system, suggesting that this activation is mediated by endogenous ERRα in HEK293T cells. Supporting this notion, AAB-Luc activity was not affected by the presence of DES and 4-OHT, inverse agonists specific to both ERRγ and ERRβ [22] (Figs. 1 and 2). The combination of structure-based virtual screening and transient transfection experiments revealed that flavone and isoflavone phytoestrogens, including the dietary isoflavone Gen, were ERRα agonists [25]. In the present study, we also found that Gen stimulates the PGC-1α/ERRα pathway with increasing reporter activity and PGC-1α protein (Fig. 3).

The mechanism by which Gen is able to stimulate this pathway is unclear. Virtual screening suggested that it might dock to the ligand-binding pocket [25]; however, this phenomenon needs to be demonstrated with binding studies. Recent work has shown that PGC-1α activity is controlled by several post-translational modifications [31], including phosphorylation by several kinases. Phosphorylation by the Akt pathway on Ser570 has an inhibitory effect on PGC-1α function by preventing the recruitment of PGC-1α to the cognate promoter [32,33]. Furthermore, ERK8, a downstream target of the tyrosine kinase pathway, was found to be a specific ERRα repressor by promoting translocation of the nuclear ERRα to the cytoplasm [13]. Gen is a known tyrosine kinase inhibitor [34] and in our assay system Gen might repress Akt and

ERK8 function and increase reporter activity by PGC-1α and as such promote the activation of ERRα target genes [32] or (Fig. 3). An alternative explanation is stabilizing the protein-protein interaction between the receptor/coactivator since PGC-1α is a very labile protein with a half-life of ~30 min [35,36]. The endocrine disruptor BPA has a broad spectrum of biologic effects mediated through both ER-dependent [37] and ER-independent pathways [38]. It may not be surprising that BPA also activates the PGC-1α/ERRα pathway in our study and this could relate to additional biological effects of this compound. Unexpectedly, Kaemp, an antagonist for both ERRα and ERRγ, had a minimal affect in our assay system [23].

Finally, a stable cell line with an intact PGC-1α/ERRα transactivation reporter will be useful for the large scale screening of environmental chemicals or drugs to discover compounds that modulate the PGC-1α/ERRα dependent signaling pathway either positively or negatively. In addition, it will be a helpful model to better understand the impact of modulating this metabolic axis on cellular bioenergetics.

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References

[1] L.S. Birnbaum, When environmental chemicals act like uncontrolled medicine, Trends Endocrinol. Metab. 24 (2013) 321–323.
[2] C.J. Patel, A.J. Butte, Predicting environmental chemical factors associated with disease-related gene expression data, BMC Med. Genomics 3 (2010) 17.
[3] R.R. Tice, C.P. Austin, R.J. Kavlock, J.R. Bucher, Improving the human hazard characterization of chemicals: a tox21 update, Environ. Health Perspect. 121 (2013) 756–765.
[4] J. Lin, P.H. Wu, P.T. Tarr, K.S. Lindenberg, J. St-Pierre, C.Y. Zhang, V.K. Mootha, S. Jager, C.R. Vianna, R.M. Reznick, L. Cui, M. Manieri, M.X. Donovan, Z. Wu, M.P. Cooper, M.C. Fan, L.M. Rohas, A.M. Zavacki, S. Cinti, G.I. Shulman, B.B. Lowell, D. Krainc, B.M. Spiegelman, Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1alpha null mice, Cell 119 (2004) 121–135.
[5] 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. Chen, J.O. Holloszy, D.M. Medeiros, R.E. Schmidt, J.E. Saffitz, E.D. Abel, C.F. Semenkovich, D.P. Kelly, PGC-1alpha deficiency causes multi-system energy metabolic derangements: muscle dysfunction, abnormal weight control and hepatic steatosis, PLoS Biol. 3 (2005) e101.
[6] 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 (ERRalpha), J. Biol. Chem. 278 (2003) 9013–9018.
[7] V. Giguere, Transcriptional control of energy homeostasis by the estrogen-related receptors, Endocr. Rev. 29 (2008) 677–696.
[8] J.A. Villena, A. Kralli, ERRalpha: a metabolic function for the oldest orphan, Trends Endocrinol. Metabol. 19 (2008) 269–276.
[9] L.J. Eichner, V. Giguere, Estrogen related receptors (ERRs): a new dawn in transcriptional control of mitochondrial gene networks, Mitochondrion 11 (2011) 544–552.
[10] C.Y. Chang, D.P. McDonnell, Molecular pathways: the metabolic regulator estrogen-related receptor alpha as a therapeutic target in cancer, Clin. Cancer Res. 18 (2012) 6089–6095.
[11] G. Benoit, A. Cooney, V. Giguere, H. Ingraham, M. Lazar, G. Muscat, T. Perlmann, J.P. Renaud, J. Schwabe, F. Sladek, M.J. Tsai, V. Laudet, International Union of Pharmacology. LXVI. Orphan nuclear receptors, Pharmacol. Rev. 58 (2006) 798–836.
[12] Y. Kamei, H. Ohizumi, Y. Fujitani, T. Nemoto, T. Tanaka, N. Takahashi, T. Kawada, M. Miyoshi, O. Ezaki, A. Kakizuka, PPARgamma coactivator 1beta/ERR ligand 1 is an ERR protein ligand, whose expression induces a high-energy expenditure and antagonizes obesity, Proc. Natl. Acad. Sci. USA 100 (2003) 12378–12383.
[13] M. Rossi, D. Colecchia, C. Iavarone, A. Strambi, F. Piccioni, A. Verrotti di Pianella, M. Chiariello, Extracellular signal-regulated kinase 8 (ERK8) controls estrogen-related receptor alpha (ERRalpha) cellular localization and inhibits its transcriptional activity, J. Biol. Chem. 286 (2011) 8507–8522.
[14] V.K. Mootha, C. Handschin, D. Arlow, X. Xie, J. St Pierre, 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, Proc. Natl. Acad. Sci. USA 101 (2004) 6570–6575.
[15] H. Shigeta, W. Zuo, N. Yang, R. DiAugustine, C.T. Teng, The mouse estrogen receptor-related orphan receptor alpha 1: molecular cloning and estrogen responsiveness, J. Mol. Endocrinol. 19 (1997) 299–309.
[16] D. Knutti, A. Kaul, A. Kralli, A tissue-specific coactivator of steroid receptors, identified in a functional genetic screen, Mol. Cell. Biol. 20 (2000) 2411–2422.
[17] D. Liu, Z. Zhang, C.T. Teng, Estrogen-related receptor-gamma and peroxisome proliferator-activated receptor-gamma coactivator-1alpha regulate estrogen-related receptor-alpha gene expression via a conserved multi-hormone response element, J. Mol. Endocrinol. 34 (2005) 473–487.
[18] I. Bardi, P. Salmon, D. Trono, Production and titration of lentiviral vectors in: J. N. Crawley (Ed.), Current Prototcols in Neuroscience, 2010, pp. 21 (Chapter 4).
[19] R. Huang, M. Xia, M.H. Cho, S. Sakamuru, P. Shinn, K.A. Houck, D.J. Dix, R.S. Judson, K.L. Witt, R.J. Kavlock, R.R. Tice, C.P. Austin, Chemical genomics profiling of environmental chemical modulation of human nuclear receptors, Environ. Health Perspect. 119 (2011) 1142–1148.
[20] N. Yang, H. Shigeta, H. Shi, C.T. Teng, Estrogen-related receptor, hERR1, modulates estrogen receptor-mediated response of human lactoferrin gene promoter, J. Biol. Chem. 271 (1996) 5795–5804.
[21] J. Kallen, J.M. Schlaeppi, F. Bitsch, I. Filipuzzi, A. Schilb, V. Riou, A. Graham, A. Strauss, M. Geiser, B. Fournier, Evidence for ligand-independent transcriptional activation of the human estrogen-related receptor alpha (ERRalpha): crystal structure of ERRalpha ligand binding domain in complex with peroxisome proliferator-activated receptor coactivator-1alpha, J. Biol. Chem. 279 (2004) 49330–49337.
[22] P. Coward, D. Lee, M.V. Hull, J.M. Lehmann, 4-Hydroxytamoxifen binds to and deactivates the estrogen-related receptor gamma, Proc. Natl. Acad. Sci. USA 98 (2001) 8880–8884.
[23] J. Wang, F. Fang, Z. Huang, Y. Wang, C. Wong, Kaempferol is an estrogen-related receptor alpha and gamma inverse agonist, FEBS Lett. 583 (2009) 643–647.
[24] B. Horard, J.M. Vanacker, Estrogen receptor-related receptors: orphan receptors desperately seeking a ligand, J. Mol. Endocrinol. 31 (2003) 349–357.
[25] M. Suetsugi, L. Su, K. Karlsberg, Y.C. Yuan, S. Chen, Flavone and isoflavone phytoestrogens are agonists of estrogen-related receptors, Mol. Cancer Res. 1 (2003) 981–991.
[26] T.J. Gibson, M. Seiler, R.A. Veitia, The transience of transient overexpression, Nat. Meth. 10 (2013) 715–721.
[27] B.B. Busch, W.C. Stevens Jr., R. Martin, P. Ordentlich, S. Zhou, D.W. Sapp, R.A. Horlick, R. Mohan, Identification of a selective inverse agonist for the orphan nuclear receptor estrogen-related receptor alpha, J. Med. Chem. 47 (2004) 5593–5596.
[28] P.J. Willy, I.R. Murray, J. Qian, B.B. Busch, W.C. Stevens Jr., R. Martin, R. Mohan, S. Zhou, P. Ordentlich, P. Wei, D.W. Sapp, R.A. Horlick, R.A. Heyman, I.G. Schulman, Regulation of PPARgamma coactivator 1alpha (PGC-1alpha) signaling by an estrogen-related receptor alpha (ERRalpha) ligand, Proc. Natl. Acad. Sci. USA 101 (2004) 8912–8917.
[29] M.J. Chisamore, M.E. Cunningham, O. Flores, H.A. Wilkinson, J.D. Chen, Characterization of a novel small molecule subtype specific estrogen-related receptor alpha antagonist in MCF-7 breast cancer cells, PloS One 4 (2009) e5624.
[30] O. Lanvin, S. Bianco, N. Kersual, D. Chalbos, J.M. Vanacker, Potentiation of ICI182,780 (Fulvestrant)-induced estrogen receptor-alpha degradation by the estrogen receptor-related receptor-alpha inverse agonist XCT790, J. Biol. Chem. 282 (2007) 28328–28334.
[31] J.T. Rodgers, C. Lerin, Z. Gerhart-Hines, P. Puigserver, Metabolic adaptations through the PGC-1 alpha and SIRT1 pathways, FEBS Lett. 582 (2008) 46–53.
[32] X. Li, B. Monks, Q. Ge, M.J. Birnbaum, Akt/PKB regulates hepatic metabolism by directly inhibiting PGC-1alpha transcription coactivator, Nature 447 (2007) 1012–1016.
[33] C. Canto, J. Auwerx, Clking on PGC-1alpha to inhibit gluconeogenesis, Cell Metab. 11 (2010) 6–7.
[34] T. Akiyama, J. Ishida, S. Nakagawa, H. Ogawara, S. Watanabe, N. Itoh, M. Shibuya, Y. Fukami, Genistein, a specific inhibitor of tyrosine-specific protein kinases, J. Biol. Chem. 262 (1987) 5592–5595.
[35] P. Puigserver, J. Rhee, J. Lin, Z. Wu, J.C. Yoon, C.Y. Zhang, S. Krauss, V.K. Mootha, B.B. Lowell, B.M. Spiegelman, Cytokine stimulation of energy expenditure through p38 MAP kinase activation of PPARgamma coactivator-1, Mol. Cell 8 (2001) 971–982.
[36] J. Trausch-Azar, T.C. Leone, D.P. Kelly, A.L. Schwartz, Ubiquitin proteasome-dependent degradation of the transcriptional coactivator PGC-1{alpha} via the N-terminal pathway, J. Biol. Chem. 285 (2010) 40192–40200.
[37] B.S. Rubin, Bisphenol A: an endocrine disruptor with widespread exposure and multiple effects, J. Steroid Biochem. Mol. Biol. 127 (2011) 27–34.
[38] T. N'Tumba-Byn, D. Moison, M. Lacroix, C. Lecureuil, L. Lesage, S.M. Prud'homme, S. Pozzi-Gaudin, R. Frydman, A. Benachi, G. Livera, V. Rouiller-Fabre, R. Habert, Differential effects of bisphenol A and diethylstilbestrol on human, rat and mouse fetal leydig cell function, PloS One 7 (2012) e51579.