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Peroxisome Proliferator-Activated Receptor γ Coactivator-1α Enhances Antiproliferative Activity of 5′-Deoxy-5-Fluorouridine in Cancer Cells through Induction of Uridine Phosphorylase

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ABSTRACT

Peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) is capable of coactivating several nuclear receptors and transcription factors that participate in the regulation of multiple metabolic processes, including gluconeogenesis, mitochondrial biogenesis, and adaptive thermogenesis. Uridine phosphorylase (UPase) catalyzes the reversible conversion of uridine into uracil and contributes to the antineoplastic activity of 5′-deoxy-5-fluorouridine (5′-DFUR) and homeostasis of uridine levels in plasma and tissues. This study demonstrates uridine phosphorylase as a novel target gene of PGC-1α, which induces the transcription and enzymatic activity of UPase in various cancer cells and thus augments their susceptibility to 5′-DFUR. PGC-1α-induced activation of UPase expression occurs at its transcription level that is mediated by an estrogen-related receptor (ERR) binding site (−1078 to −1070 base pairs) mapped in the promoter region of UPase gene. Our mutational studies using luciferase reporter construct together with electrophoretic mobility shift assays confirm the binding of ERR to PGC-1α-responsive element. Moreover, the inhibition of PGC-1α/ERRα-dependent signaling by 3-[4-(2,4-bis-trifluoromethylbenzyloxy)-3-methoxyphenyl]-2-cyano-*N*-(5-trifluoromethyl-1,3,4-thiadiazol-2-yl)acrylamide (XCT790) compromises the ability of PGC-1α to induce the transcript of UPase, indicating PGC-1α-dependent and ERRα-mediated up-regulation of UPase. Finally, the overexpression of PGC-1α sensitizes breast and colon cancer cells to growth inhibition by 5′-DFUR presumably by inducing apoptosis in tumor cells and XCT790 can inhibit the process. Taken together, our results corroborate the regulatory function of PGC-1α in uridine homeostasis and imply its links with the energy metabolism. The mechanistic elucidation of this association between both cellular pathways should advance the clinical use of 5-fluorouracil-based chemotherapy.

Peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) is a master regulator of cellular energy homeostasis and plays important roles in adaptive thermogenesis in skeletal muscle and brown fat, mitochondrial biogenesis and respiration in muscle cells, and liver gluconeogenesis. PGC-1α expression is highly induced in liver and heart during fasting period, in brown adipose tissue after the exposure to cold temperature, and in skeletal muscle while exercising (Lin et al., 2005; Finck and Kelly, 2006). PGC-1α can coactivate a variety of transcription factors, including but not limited to all ERR and PPAR subtypes, nuclear respiratory factor, forkhead box O1, and hepatocyte nuclear factor 4 (Lin et al., 2005). The PGC-1α knockout mice exhibit multiple metabolic defects, indicating its involvement in numerous pathogenic conditions, such as obesity, diabetes, neurodegen-

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ABBREVIATIONS: PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α; ERR, estrogen-related receptor; UPase, uridine phosphorylase; 5′-DFUR, 5′-deoxy-5-fluorouridine; 5-FU, 5-fluorouracil; TPase, thymidine phosphorylase; TNF, tumor necrosis factor; NF-κB, nuclear factor-κB; bp, base pair(s); Ad, adenovirus; PCR, polymerase chain reaction; EMSA, electrophoretic mobility shift assay; FACS, fluorescence-activated cell sorting; GFP, green fluorescent protein; TLC, thin layer chromatography; DMSO, dimethyl sulfoxide; XCT790, 3-[4-(2,4-bis-trifluoromethylbenzyloxy)-3-methoxyphenyl]-2-cyano-*N*-(5-trifluoromethyl-1,3,4-thiadiazol-2-yl)acrylamide; ERRE, estrogen-related receptor element.

eration, and cardiomyopathy (Lin et al., 2004; Leone et al., 2005).

Estrogen-related receptors (ERRs) belong to a family of orphan nuclear hormone receptors consisting of ERRα, ERRβ, and ERRγ. ERRα participates in the metabolic regulation and is expressed in various tissues. Its expression is elevated in tissues with increased metabolic demands such as the heart, kidney, skeletal muscle, and adipose tissue (Horard and Vanacker, 2003; Tremblay and Giguère, 2007). ERRα-ablated mice are resistant to obesity that is induced by a high-fat diet and exhibit reduced lipogenesis in their adipose tissue (Luo et al., 2003). ERRα-regulated genes include pyruvate dehydrogenase kinase 4, a key enzyme of glucose metabolism, and medium-chain acyl-CoA dehydrogenase, a rate-limiting enzyme catalyzing the mitochondrial β-oxidation of fatty acids (Wende et al., 2005). In addition to coactivating ERRα, PGC-1α can also strongly induce ERRα mRNA expression, which in turn stimulates its own expression, thereby generating a positive feedback loop (Laganière et al., 2004; Mootha et al., 2004). ERRα has also been shown to be associated with osteoporosis, metabolic disorders, and cancers. For example, ERRα contributes to the breast cancer and progression of ovarian malignancies and serves as a potential therapeutic target (Stein and McDonnell, 2006).

Uridine phosphorylase (UPase) catalyzing the formation of uracil from uridine participates in the regulation of pyrimidine salvage pathway and activates fluoropyrimidines, for example, 5′-deoxy-5-fluorouridine (5′-DFUR) (Cao et al., 2002, 2005). UPase activity is amplified in many tumors, including colorectal carcinomas, breast cancer, melanoma, and lung adenocarcinomas, suggesting that this enzyme is an important target for tumor-specific modulation of fluoropyrimidines reactivity (Wan et al., 2006). 5-FU is one of the active antitumor agents used in treatment of multiple tumors. Capecitabine is an oral agent that is designed to generate 5-FU preferentially in the tumor tissues. 5′-DFUR, an intermediate metabolite of capecitabine, is converted to 5-FU by two pyrimidine phosphorylases, UPase and thymidine phosphorylase (TPase), both of which are elevated in tumor tissues (Pizzorno et al., 2002; Wan et al., 2006).

It has been demonstrated that tumor necrosis factor (TNF)-α induces UPase expression through NF-κB pathway and enhances the cell sensitivity to 5′-DFUR (Wan et al., 2006). Together with interleukin-1α and interferon-γ, TNF-α accentuates the tumor cytotoxicity of 5-FU and 5′-DFUR by 2.7- and 12.4-fold, respectively. In contrast, p53 decreases UPase expression by physically interacting with its promoter region (Zhang et al., 2001). EWS/ETS fusion proteins, playing a dominant oncogenic role in cellular transformation, can stimulate UPase gene expression through interaction with the UPase promoter. In addition, the up-regulation of UPase by EWS/FLI 1 sensitizes cells to in vitro growth inhibition by 5′-DFUR (Deneen et al., 2003).

In light of the aforementioned facts, we were prompted to investigate the association of PGC-1α and ERR with the cellular transformation and UPase expression. Here, we demonstrate that PGC-1α regulates the expression of UPase by means of ERR binding to its promoter. Moreover, the ectopic overexpression of PGC-1α sensitizes breast and colon cancer cells to growth inhibition by 5′-DFUR and induces apoptosis.

Materials and Methods

Plasmids and Adenoviral Vectors. The mouse UPase gene promoter (−1108 to +143 bp) was amplified as described previously (Cao et al., 1999) using mouse genomic DNA and cloned at HindIII and XhoI sites of the firefly luciferase reporter plasmid (pGL3-Basic). To amplify different promoter regions, corresponding forward primers 5′-CTAGTTctcgagGCCTCTGTCCTTGGGTGACCTTAGC-3′ (−1108 bp), 5′-CTAGTTctcgagACAAAGGGCTGGTACTCGTCTC-3′ (−924 bp), or 5′-ATAGTTctcgagGGCAGTTCTGCTACGGCTCATG-3′ (−413 bp) were used with the identical reverse primer (5′-CTACGGaagcttTGGAAGTCGCTGTATGGCTACC-3′; +143 bp). The site-direct mutagenesis was performed using the primer 5′-CCTTGGGTGAttTTACCGAGAG-3′ and QuikChange kit (Stratagene, La Jolla, CA). All clones were verified by sequencing.

Human PGC-1α cDNA was amplified using 5′-TCAGCTGTgtcgacATGGCTTGGGACATGTGCAG-3′ and 5′-AGCTGACTgatatcTTACCTGCGCAAGCTTCTCTG-3′ primers and pcDNA3.1-PGC-1α (kindly provided by Daniel P. Kelly, Washington University School of Medicine, St. Louis, MO). Adenovirus-PGC-1α (Ad-PGC-1α) was constructed by cloning full-length PGC-1α cDNA into pAd-Track cytomegalovirus vector at SalI and EcoRV sites. Likewise, Ad-ERRα was prepared by PCR cloning of full-length human ERRα at BglII and EcoRV sites of pAd-Track cytomegalovirus vector. The cDNA of ERRα encoding a C-terminal FLAG epitope was generated using 5′-TGCACagatctGCCATGTCCAGCCAGGTGGTG-3′ and 5′-TCGATgatatcTCACTTGTCATCGTCGTCCTTGTAGTCGTCCATCATGGCCTCGAGC-3′ primers and pcDNA3.1-ERRα template. Recombination with pAdEasy-1 and propagation of adenovirus expressing PGC-1α and ERRα in the 293A cell line was performed as described previously (He et al., 1998).

Cell Culture, Luciferase Assay, and Adenovirus Infection. HepG2 cells were maintained in minimal essential medium-nonessential amino acids supplemented with 10% fetal bovine serum (37°C; 5% CO_2). Transient transfection was performed with cells plated on 24-well plates as recommended by the manufacturer (Vigorous, Beijing, China). Indicated amounts of each expression construct were cotransfected with 200 ng of luciferase reporter plasmid. To normalize for transfection efficiency, 20 ng of pRL-TK reporter plasmid was added to each transfection. As required, the empty vector was used to ensure that each transfection received the equivalent amount of total DNA. Twenty-four to 48 h after transfection, dual-specific luciferase reporter assays were performed using commercial luciferase assay kits (Promega, Madison, WI). The firefly luciferase activities were normalized based on the activity of *Renilla reniformis* luciferase. Breast cancer cell lines (MCF-7 and SK-BR-3) were maintained in Dulbecco's modified Eagle's medium, and colon cancer cell lines (Colo320 and HCT116) were cultured in RPMI 1640 medium and Iscove's modified Dulbecco's medium, respectively (10% fetal bovine serum; 37°C; 5% CO_2). The cells were infected at a multiplicity of infection sufficient to infect >95% of cells as determined by GFP fluorescence.

Enzymatic Activity Assay. UPase activity (uridine conversion to uracil) was determined by TLC chromatographic separation as described previously (Liu et al., 1998; Zhang et al., 2001). In brief, cell lysates were prepared in 50 mM Tris-Cl, pH 7.5, and supernatant (20,000*g*; 1 h; 4°C) was used to estimate the UPase activity. The enzyme reaction was carried out in a total volume of 100 μl of 50 mM Tris-Cl buffer, pH 7.5, containing 1 mM potassium phosphate and 200 μM [5-^{3}H]uridine for 30 min at 37°C. Subsequently, 10-μl aliquots of the reactions were applied onto TLC plates (Kieselgel 60; Merck, Darmstadt, Germany) prespotted with unlabeled mixture of uracil, uridine, and UMP as the markers. TLC plates were developed in chloroform/methanol/acetic acid [85:15:5, v/v/v], and the radioactivity was measured by a scintillation counter (Beckamn Coulter, Fullerton, CA). Protein concentrations were determined by the bicinchoninic acid method (Pierce Chemical, Rockford, IL).

Electrophoretic Mobility Shift Assay. LightShift chemiluminescent EMSA kit (Pierce Chemical) was used according to the manufacturer's instructions. In brief, 293A cells were seeded in 100-mm dish followed by transfection with ERR-expressing plasmid (10 μg). After 24 h, the nuclear proteins were extracted according to the manufacturer's protocol (Pierce Chemical). The sequence of UPase-ERRE-wild-type oligonucleotide was 5′-CCTGGCCTCTGTCCTTGGGTGACCTTAGCCGAG-3′, whereas the sequence of the UPase-ERRE-mutant was 5′-CCTGGCCTCTGTCCTTGGGTGAttTTAGCCGAG-3′. Equal amounts of complementary oligonucleotides were annealed and labeled by 5′-end labeling with biotin. The binding reactions were executed in a total volume of 20 μl containing 50 mM KCl, 5 mM $MgCl_2$, 10 mM EDTA, 5% glycerol, 3 μl of nuclear extract, 1 μg of poly(dI-dC), and 2 μl of binding buffer. To perform the competition analysis, 200-fold molar excess of unlabeled competitor DNA was added to the reaction before the addition of the nuclear extracts. After preincubation for 10 min at room temperature, 20 fmol of biotin-labeled probe was added, and the incubation was continued for an additional 20 min. The electrophoresis was done with a 6% polyacrylamide gel in 0.5× Tris borate-EDTA buffer at 100 V. The samples were blotted onto a nylon membrane using an electrophoretic transfer unit (380 mA; 60 min). Subsequently, the DNA was cross-linked to membrane (120 mJ/cm^2; 60 s), and the biotin-labeled DNA was detected by chemiluminescence reagent.

Growth Inhibition Assays and FACS Analysis. The antiproliferative activity of 5′-DFUR (Sigma-Aldrich, St. Louis, MO) was assessed by means of the CellTiter 96 proliferation assay kit (Promega, Madison, WI). In brief, 5×10^3 cells were seeded in 96-well plates and allowed to adhere for 24 h. The following day, cells were infected with adenovirus expressing GFP (Ad-GFP), PGC-1α (Ad-PGC-1α), and/or ERRα (Ad-ERRα) under the control of a cytomegalovirus promoter and treated with DMSO or 3-[4-(2,4-bis-trifluoromethylbenzyloxy)-3-methoxyphenyl]-2-cyano-*N*-(5-trifluoromethyl-1,3,4-thiadiazol-2-yl)acrylamide (XCT790) (Sigma-Aldrich). Infection rate of ~90% was achieved by 24 h as determined by quantification of GFP-expressing cells. Next, cells were treated with 5′-DFUR for 72 h. The number of viable cells was determined by CellTiter 96 proliferation assay kit (Promega). For FACS analysis, cells were harvested by trypsin-EDTA digestion, washed once with ice-cold phosphate-buffered saline, and fixed with 70% ethanol at 4°C for at least 8 h. Then, the fixed cells were collected by brief centrifugation and resuspended in phosphate-buffered saline containing 100 μg/ml RNase A and 50 μg/ml propidium iodide. After 30-min incubation at room temperature, samples were analyzed by flow cytometric analysis for the presence of apoptotic cells (subG1) according to their DNA content.

Quantitative PCR. Total RNA was extracted by TRIzol-based method and subjected to the cDNA synthesis using random hexamers and reverse transcription reagent kit (Invitrogen, Carlsbad, CA). The forward and reverse primers 5′-GGCAGAGTTTGAGCAGATTGT-3′ and 5′-CAGATACGCCTGCTTGTCCTT-3′, respectively, were used to measure the UPase transcript. The mRNA of β-actin was used as an internal control using 5′-AGCGGGAAATCGTGCGTGAC-3′ and 5′-CGGACTCGTCATACTCCTGCT-3′ primers. The real-time PCR was performed with SYBR Green PCR Master Mix (Invitrogen) on a IQ5 system (Bio-Rad Laboratories, Hercules, CA).

Statistical Analysis. The data are reported as the mean ± S.D. of at least three independent experiments. Differences between groups were compared by Student's t test, and P values <0.05 are considered to be statistically significant.

Results

PGC-1α Induces the Expression of UPase in Breast and Colon Cancer Cells. The published microarray data (www.diabetesgenome.org) suggest uridine phosphorylase as a potential target gene of PGC-1α in hepatocyte and muscle cells. To investigate whether PGC-1α plays a direct role in the regulation of UPase gene, we examined the effect of adenovirus-mediated PGC-1α overexpression on UPase mRNA in cancer cells. As depicted in Fig. 1A, we detected an up-regulation of UPase mRNA upon overexpression of PGC-1α in different cancer cells compared with the control cells expressing GFP. The level of UPase mRNA was increased approximately 7-fold in MCF-7 cells, 6.1-fold in SK-BR-3 cells, approximately 3-fold in HCT116 cells, and 1.9-fold in Colo320 cells. Relative induction of UPase transcript paralleled those observed in microarray analysis. These data indicated that PGC-1α functions as an upstream activator of UPase gene. We also measured the enzymatic activity of cellular UPase in cancer cells after their infection with Ad-PGC-1α. As anticipated, PGC-1α also augmented the UPase activity in breast cancer cells (SK-BR-3) as well as in the colon cancer cells (Colo320) (Fig. 1B). The mRNA of TPase gene was not influenced by PGC-1α as determined by quantitative PCR (data not shown).

PGC-1α-Induced UPase Transcription Is Mediated by Binding of a Nuclear Receptor. To determine whether PGC-1α-dependent induction of UPase gene occurs at its transcriptional level, we performed promoter-reporter transfections in HepG2 cells. First construct (UPase.Luc-1108) contained the ~1.1-kilobase promoter region of the UPase gene governing the expression of luciferase protein. Cotransfection of UPase.Luc-1108 with PGC-1α expression vector resulted in a robust (7-fold) activation of the reporter gene (Fig. 2). To map the *cis*-acting region conferring the PGC-1α-dependent activation of luciferase, we executed cotransfection experiments with reporter constructs harboring serial deletions at the 5′-end of the UPase promoter (UPase.Luc-924 and UPase.Luc-413). PGC-1α-mediated reporter activation was compromised upon deletion of the promoter region from −1108 to −924 bp (Fig. 2).

To deduce the molecular mechanism, we searched for the sequence motif in the PGC-1α-responsive nucleotide region. A screen of 185 bp between −1108 and −924 bp revealed the presence of a putative ERRE (TGACCTTAG, −1078 to −1070 bp). The fact that PGC-1α can induce ERRα expression and coactivate ERR subtypes (Laganière et al., 2004; Mootha et al., 2004) prompted us to speculate the participation of ERRs in PGC-1α-induced activation of UPase. It is interesting that further truncation of the promoter between −924 and −413 bp did not accentuate the process. We also observed that PGC-1α was still competent in stimulating the UPase promoter (UPase.Luc-924 and UPase.Luc-413) compared with the empty vector (pcDNA3.1), which implicates that factors other than ERR also contributes to the observed residual transcriptional activation by PGC-1α.

PGC-1α-Dependent Activation of UPase Promoter Is Mediated by ERR. Mutational studies were performed to examine the participation of ERR subtypes in the PGC-1α-dependent activation of the UPase promoter. The potential ERRE (TGACCTTAG, −1078 to −1070 bp) within the UPase promoter was abolished using a mutation that has been shown previously to inactivate this element (Wende et al., 2005). As shown in Fig. 3A, cotransfection of a PGC-1α expression vector with UPase.Luc-1108 resulted in approximately 7-fold induction of UPase promoter. However, PGC-1α-mediated transcription through UPase promoter is considerably reduced with ERRE mutant (Fig. 3A), suggesting the involve-

ment of ERRs in PGC-1α action. It is notable that we again observed the modest activation of mutant UPase promoter, which further confirmed the participation of other factors in the above-mentioned function of PGC-1α.

To determine whether ERR protein binds to the PGC-1α-responsive element identified within the UPase promoter, EMSAs were performed using the nuclear extracts and the synthetic double-stranded oligonucleotides. Figure 3B demonstrates that the nuclear proteins extracted from 293A cells bind to the labeled oligonucleotide probe and that a 200-fold excess of unlabeled wild-type probes abolishes the formation of protein-DNA complex. In contrast, 200-fold excess of unlabeled mutant probes did not affect the formation of designated complex, indicating the ERR specificity of this interaction. Taken together, these results confirm that the observed PGC-1α-dependent activation of the UPase promoter is mediated by ERRE and presumably requires its direct interaction with ERR.

PGC-1α-Dependent Induction of UPase Gene in Cancer Cells Is Mediated by ERRα. To resolve the function of ERRα in mediating the induction of UPase transcription by PGC-1α, we carried out adenovirus-mediated individual or collective overexpression of PGC-1α and/or ERRα (Ad-PGC-1α and Ad-ERRα) in MCF-7 cells. As anticipated, we detected approximately 6-fold induction of UPase mRNA by PGC-1α, whereas ERRα increased it by a modest 2.5-fold. The cotransfection of both PGC-1α and ERRα resulted in approximately 13-fold induction of UPase transcript (data not shown).

Furthermore, we treated plasmid-recipient cells with XCT790, which is a potent and selective ERRα antagonist and interferes with PGC-1α/ERRα-dependent signaling (Willy et al., 2004). We observed a significant reduction in

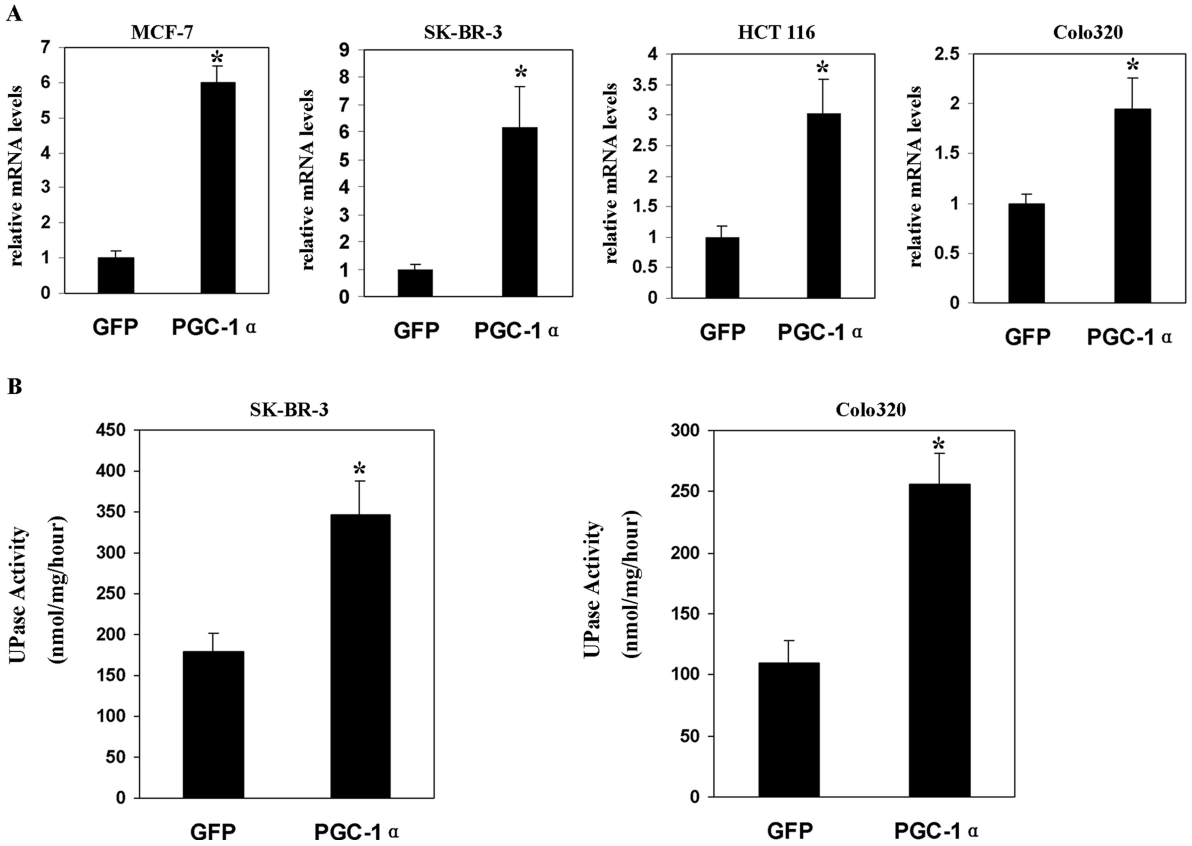

Fig. 1. PGC-1α induces the transcription and activity of UPase. A, cells were infected with Ad-GFP (control) or Ad-PGC-1α adenoviruses followed by RNA isolation at 24 h (HCT116) or 48 h (MCF-7, SK-BR-3, and Colo320) after infection. The UPase transcripts were quantified by quantitative PCR, normalized to β-actin mRNA, and expressed relative to the GFP-expressing control cells. B, SK-BR-3 and Colo320 cells were infected with Ad-GFP or Ad-PGC-1α and subjected to the measurements of UPase activity as described under *Materials and Methods*. ∗, $P < 0.05$.

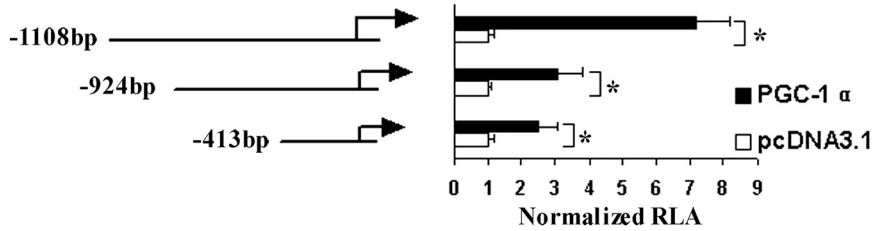

Fig. 2. PGC-1α can regulate the promoter of UPase gene in HepG2 cells. The DNA constructs harboring the 5′-deletion series of UPase promoter fused with luciferase reporter (UPase.Luc-1108, UPase.Luc-924, and UPase.Luc-413) were cotransfected with pcDNA3.1 (control) or PGC-1α into HepG2 cells. The graph depicts relative luciferase activity (RLA) corrected for *R. reniformis* luciferase activity and normalized to the control activity of UPase. Luciferase activity was measured as described under *Materials and Methods*. All values represent at least three independent transfections, each conducted in triplicate. ∗, $P < 0.05$.

PGC-1α induction of UPase transcription upon treatment of MCF-7 cells with XCT790. Although substantial, the inhibition of ERRα protein by XCT790 did not entirely abolish the action of PGC-1α on the UPase gene (Fig. 4A). These data were also corroborated in HepG2 cells using luciferase reporter construct where the activity of PGC-1α was significantly decreased but not eliminated (Fig. 4B). Collectively, our data suggest the active involvement of ERRα, at least in part, in facilitating the function of PGC-1α.

Overexpression of PGC-1α Sensitizes Cancer Cells to 5′-DFUR. UPase is strongly induced in a variety of human tumors and transformed cells that underline the tumor-specific reactivity of fluoropyrimidines, including 5′-DFUR and capecitabine. Based on these data, we reasoned that the overexpression of PGC-1α should sensitize cancer cells to antiproliferative effect of 5′-DFUR. To validate this presumption, cell proliferation assays were performed on breast and colon cancer cell lines. After infection of SK-BR-3 with Ad-PGC-1α, the drug susceptibility of these breast cancer cells to 5′-DFUR was increased by 2.4-fold that corresponds to a reduction in its IC_{50} value from 120 to 50 μM (Fig. 5A). However, cells infected with Ad-PGC-1α but treated with XCT790 were less susceptible to 5′-DFUR than untreated cells harboring the Ad-PGC-1α (IC_{50} value increases from 50 to 100 μM) (Fig. 5A). Similar results were obtained with the colon cancer cells Colo320 (Fig. 5B). These data underscore the contribution of ERRα in sensitizing the breast and colon cancer cells to 5′-DFUR.

To elucidate whether PGC-1α-augmented antiproliferative activity of 5′-DFUR was due to increased apoptosis of the cancer cells, the apoptotic index was determined by flow cytometry. Indeed, the overexpression of PGC-1α alone stimulated apoptosis rates in breast as well as in the colon cancer cells as determined by the fraction of cells with subG0/G1 DNA content (Fig. 6). As anticipated, coinfection of cancer cells with adenoviruses expressing both PGC-1α and ERRα inflicted a higher susceptibility to 5′-DFUR, and XCT790 reversed the phenomenon (Fig. 6). Taken together, these results confirm that PGC-1α-stimulated antiproliferative effect of 5′-DFUR is due to an enhanced apoptosis in the recipient cancer cell lines and that the process is mediated by ERRα.

Discussion

This study demonstrates PGC-1α as a novel regulator of UPase gene transcription, whose effect is mediated by ERRα binding to its motif present in the promoter region. The expression of UPase, a rate-limiting enzyme involved in pyrimidine synthesis, is also up-regulated in various tumors such as colorectal and breast carcinomas, melanoma tissue, and lung adenocarcinomas (Pizzorno et al., 2002; Wan et al., 2006). In tumor cells, UPase catalyzes the transformation of 5′-DFUR, the intermediate product of capecitabine metabolism, to 5-FU, which inhibits their proliferation (Cao et al., 2002, 2005). Capecitabine (fluoropyrimidine carbonate) is an orally administered anticancer drug, which is converted to 5-FU in three steps. The first and second reactions leading to the formation of 5′-DFUR are catalyzed by carboxyl esterase and cytidine deaminase. The final step, producing a reactive uracil analog 5-FU from 5′-DFUR primarily in cancer cells, is catalyzed by UPase and TPase, both of which are highly induced in several tumor tissues. UPase- and/or TPase-mediated formation of 5-FU in cancer cells confers tumor specificity to the antiproliferative activity of capecitabine (Miwa et al., 1998; de Bruin et al., 2003). Our results reveal that PGC-1α selectively induces the UPase tran-

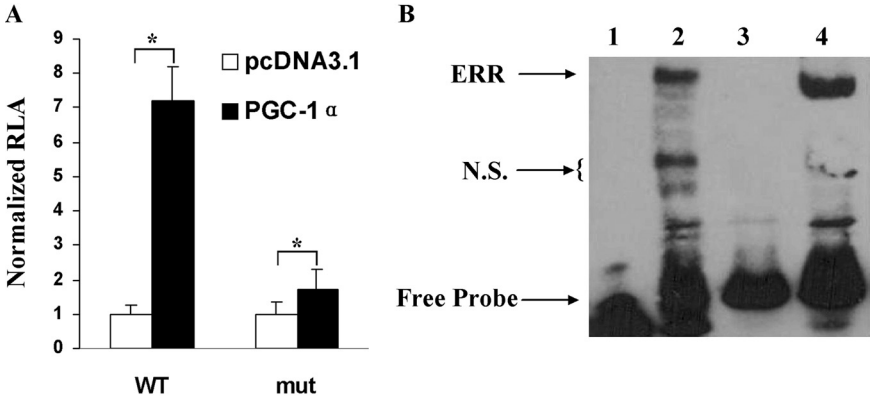

Fig. 3. PGC-1α-mediated induction of the UPase promoter involves ERR element and its interaction with ERR. A, HepG2 cells were cotransfected with luciferase reporter under the control of UPase-1.1 kilobase (WT, wild type) or its mutant (mut) and equivalent amounts of PGC-1α expression or control vectors. Luciferase activity was measured as described under *Materials and Methods*. The results represent median of three experiments. *, $P < 0.05$. B, EMSA was executed as described by the manufacturer (Pierce Chemical). Lane 1, DNA probe only; lane 2, DNA probe incubated with the nuclear protein extract of 293A cells; lane 3, same as lane 2, but with a 200-fold excess of unlabeled wild-type DNA probe; and lane 4, same as lane 2, but with a 200-fold excess of mutant unlabeled DNA probe. N.S., nonspecific binding.

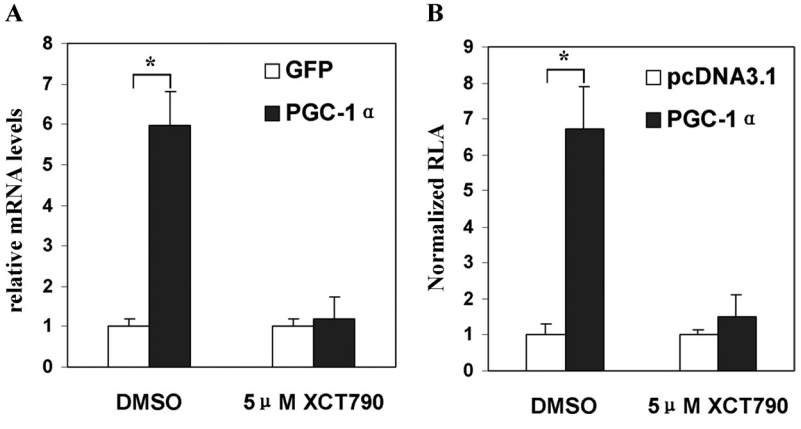

Fig. 4. The inhibitor of ERRα XCT790 interferes with PGC-1α-dependent induction of UPase mRNA. A, MCF-7 cells were preinfected with Ad-GFP or Ad-PGC-1α. Six hours after infection, cells were treated with 5 μM XCT790 or DMSO for 24 h. The quantitative PCR values of UPase transcript were normalized to β-actin mRNA. The data represent at least three infections conducted in triplicates. B, inhibition of PGC-1α-induced UPase promoter by XCT790. HepG2 cells were cotransfected with UPase.Luc-1108 reporter plasmid and equivalent amounts of PGC-1α expression construct or empty vector (pcDNA3.1), and treated with 5 μM XCT790 or DMSO. Luciferase activity was measured as described under Materials and Methods. *, $P < 0.05$.

script through ERRα and augments the cytotoxicity of 5′-DFUR in a variety of cancer cells. In addition, PGC-1α does not influence the mRNA of TPase, indicating the participation of UPase but not of TPase in PGC-1α-induced reactivity of 5′-DFUR against the cancer cells.

Induction of UPase activity in tumors provides a rationale to target this enzyme for the tumor-specific reactivity of fluoropyrimidines-based drugs. Therefore, there is considerable interest in understanding the mechanisms of UPase induction. Zhang et al. (2001) have reported that p53 represses the expression of UPase by its interaction with a consensus p53 binding site (−303 to −294 bp) located in UPase promoter. TNF-α is also known to induce the UPase expression through p65 (NF-κB)-dependent pathway, which

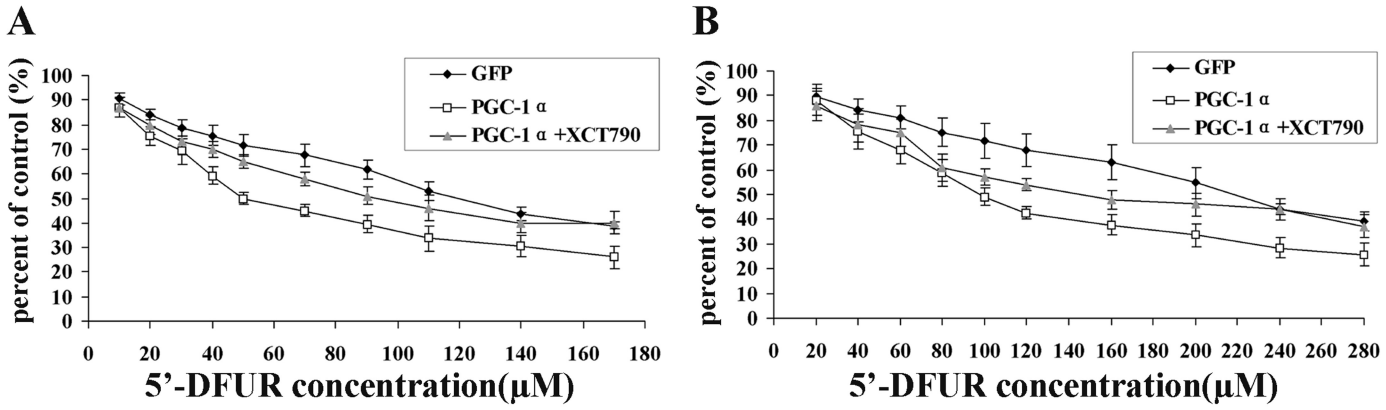

Fig. 5. Overexpression of PGC-1α sensitizes SK-BR-3 and Colo320 cells to inhibition by 5′-DFUR. SK-BR-3 (A) and Colo320 (B) cells were preinfected with Ad-GFP or Ad-PGC-1α and treated with DMSO or 5 μM XCT790 for 24 h followed by treatment of cultures with 5′-DFUR for 72 h. The cell viability was determined by CellTiter 96 proliferation assay kit (Promega).

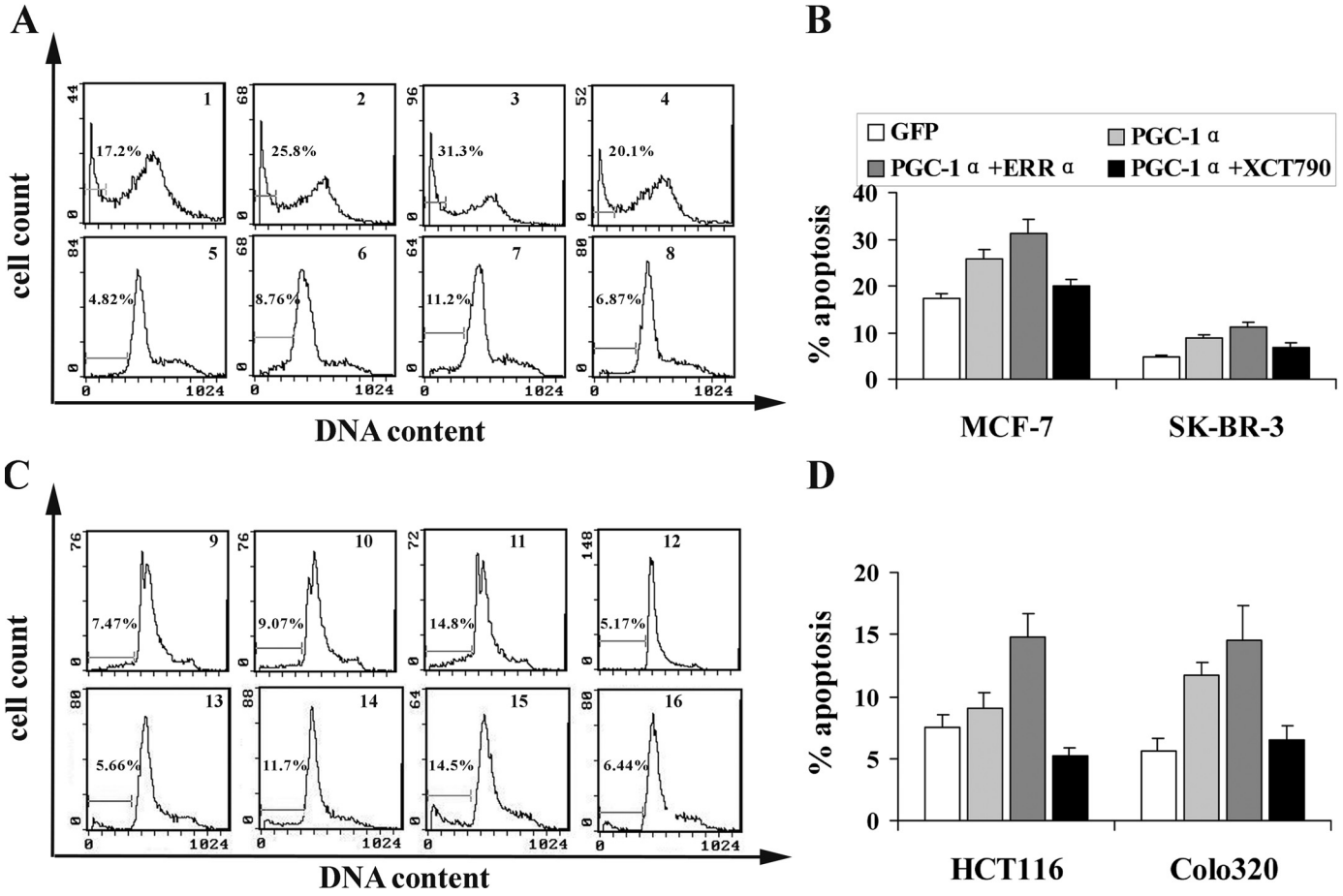

Fig. 6. PGC-1α/ERRα-mediated antiproliferative activity of 5′-DFUR against cancer cells is due to enhanced apoptosis. The breast cancer cell lines (MCF-7 and SK-BR-3) (A) and the colon cancer cell lines (HCT116 and Colo320) (C) were infected with Ad-GFP, Ad-PGC-1α, and/or Ad-ERRα followed by their treatment with DMSO or 5 μM XCT790 and 40 or 80 μM 5′-DFUR for 24 or 48 h, respectively. Cells were harvested, stained with propidium iodide, and analyzed by FACS. Panels 1, 5, 9, and 13, infected with Ad-GFP; panels 2, 6, 10, and 14, infected with Ad-PGC-1α; panels 3, 7, 11, and 15, coinfected with Ad-PGC-1α and Ad-ERRα; and the rest of the panels were infected with Ad-PGC-1α and treated with XCT790. The FACS data of 5′-DFUR-treated cells were quantified from three independent experiments and are depicted as graphs. Right (B and D), FACS analysis of cancer cells.

enhances the cell sensitivity to 5′-DFUR (Wan et al., 2006). Furthermore, Deneen et al. (2003) have shown that EWS/FLI1 fusion proteins, which play a dominant oncogenic role in cellular transformation by transcriptional modulation of several target genes, can directly associate with UPase promoter and activate its expression, resultantly sensitizing the cells to growth inhibition by 5′-DFUR. In accordance, this report confirms the PGC-1α/ERR-dependent up-regulation of UPase as an additional mechanism that contributes to an enhanced enzymatic activity in the cancer cells.

To investigate the mechanistic action of PGC-1α on UPase activity, a series of UPase promoter-luciferase reporter, PGC-1α/ERR inhibition, and gel mobility shift assays were performed. The results confirm that ERR binding to its element in UPase promoter is required for PGC-1α-dependent induction of its transcription. Motif analysis of UPase promoter indicated the presence of a potential ERR binding site located between −1078 and −1070 bp. Huss et al. (2002) have demonstrated that PGC-1α can interact and coactivate the nuclear receptor, ERRα and ERRγ. ERRα and ERRγ share high sequence homology in their DNA binding domains and interact with their response elements in a similar manner. Both, ERRα as well as ERRγ are coexpressed in metabolically active tissues, including heart, kidney, and muscles. The typical ERR binding motif is TCAAGGTCA (Tremblay and Giguère, 2007), and the nucleotide sequence between −1078 and −1070 bp in UPase promoter region resembles the consensus ERR element. Based on these data, we postulate that PGC-1α-induced expression of UPase is mediated by coactivation of a nuclear receptor ERR. Our results also indicate that PGC-1α retains a modest activity in the absence of ERR binding to its motif suggesting the involvement of regulatory factors other than ERRs in regulating the UPase expression. Because NF-κB, EWS/ETS, and p53 can also stimulate or inhibit the UPase promoter activity (Zhang et al., 2001; Deneen et al., 2003; Wan et al., 2006), the possibility of these factors in mediating the PGC-1α effect cannot be excluded. Zhang et al. have (2007) reported that PGC-1α induces apoptosis in the ovarian epithelial cancers; however, the mechanism of its action remains unclear. Here, we show that PGC-1α can predispose the colon and breast cancer cells to growth inhibition and apoptosis by 5′-DFUR in an ERR-dependent manner.

PGC-1α is a master regulator of cellular energy metabolism. Its dysregulation is associated with the insulin resistance and mitochondrial dysfunction in type 2 diabetic patients (Ek et al., 2001; Andrulionytè et al., 2004). This study underlines the regulatory function of PGC-1α in nucleotide metabolism that is quite discernible as the malignant transformation is often linked to the modulation of metabolic genes such as UPase. The current chemotherapeutic treatments of cancers mostly involve combination therapies; hence, the proposed mechanistic action of 5′-DFUR (PGC-1α-ERR-UPase) should be considered, when coupling fluoropyrimidine-based drugs with other anticancer agents. Further research on PGC-1α, ERR, and UPase in different tumors should facilitate the design of new therapeutics that exploit the tumor-specific activation of fluoropyrimidines.

References

Andrulionytè L, Zacharova J, Chiasson JL, Laakso M, and STOP-NIDDM Study Group (2004) Common polymorphisms of the PPAR-gamma2 (Pro12Ala) and PGC-1alpha (Gly482Ser) genes are associated with the conversion from impaired glucose tolerance to type 2 diabetes in the STOP-NIDDM trial. *Diabetologia* **47:**2176–2184.

Cao D, Leffert JJ, McCabe J, Kim B, and Pizzorno G (2005) Abnormalities in uridine homeostatic regulation and pyrimidine nucleotide metabolism as a consequence of the deletion of the uridine phosphorylase gene. *J Biol Chem* **280:**21169–21175.

Cao D, Nimmakayalu MA, Wang F, Zhang D, Handschumacher RE, Bray-Ward P, and Pizzorno G (1999) Genomic structure, chromosomal mapping, and promoter region analysis of murine uridine phosphorylase gene. *Cancer Res* **59:**4997–5001.

Cao D, Russell RL, Zhang D, Leffert JJ, and Pizzorno G (2002) Uridine phosphorylase (−/−) murine embryonic stem cells clarify the key role of this enzyme in the regulation of the pyrimidine salvage pathway and in the activation of fluoropyrimidines. *Cancer Res* **62:**2313–2317.

de Bruin M, van Capel T, Van der Born K, Kruyt FA, Fukushima M, Hoekman K, Pinedo HM, and Peters GJ (2003) Role of platelet-derived endothelial cell growth factor/thymidine phosphorylase in fluoropyrimidine sensitivity. *Br J Cancer* **88:**957–964.

Deneen B, Hamidi H, and Denny CT (2003) Functional analysis of the EWS/ETS target gene uridine phosphorylase. *Cancer Res* **63:**4268–4274.

Ek J, Andersen G, Urhammer SA, Gaede PH, Drivsholm T, Borch-Johnsen K, Hansen T, and Pedersen O (2001) Mutation analysis of peroxisome proliferator-activated receptor-gamma coactivator-1 (PGC-1) and relationships of identified amino acid polymorphisms to Type II diabetes mellitus. *Diabetologia* **44:**2220–2226.

Finck BN and Kelly DP (2006) PGC-1 coactivators: inducible regulators of energy metabolism in health and disease. *J Clin Invest* **116:**615–622.

He TC, Zhou S, da Costa LT, Yu J, Kinzler KW, and Vogelstein B (1998) A simplified system for generating recombinant adenoviruses. *Proc Natl Acad Sci U S A* **95:**2509–2514.

Horard B and Vanacker JM (2003) Estrogen receptor-related receptors: orphan receptors desperately seeking a ligand. *J Mol Endocrinol* **31:**349–357.

Huss JM, Kopp RP, and Kelly DP (2002) Peroxisome proliferator-activated receptor coactivator-1α (PGC-1α) coactivates the cardiac-enriched nuclear receptors estrogen-related receptor-α and -γ. Identification of novel leucine-rich interaction motif within PGC-1α. *J Biol Chem* **277:**40265–40274.

Laganière J, Tremblay GB, Dufour CR, Giroux S, Rousseau F, and Giguère V (2004) A polymorphic autoregulatory hormone response element in the human estrogen-related receptor α (ERRα) promoter dictates peroxisome proliferator-activated receptor gamma coactivator-1α control of ERRα expression. *J Biol Chem* **279:**18504–18510.

Leone TC, Lehman JJ, Finck BN, Schaeffer PJ, Wende AR, Boudina S, Courtois M, Wozniak DF, Sambandam N, Bernal-Mizrachi C, et al. (2005) PGC-1alpha deficiency causes multi-system energy metabolic derangements: muscle dysfunction, abnormal weight control and hepatic steatosis. *PLoS Biol* **3:**e101.

Lin J, Handschin C, and Spiegelman BM (2005) Metabolic control through the PGC-1 family of transcription coactivators. *Cell Metab* **1:**361–370.

Lin J, Wu PH, Tarr PT, Lindenberg KS, St-Pierre J, Zhang CY, Mootha VK, Jäger S, Vianna CR, Reznick RM, et al. (2004) Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1alpha null mice. *Cell* **119:**121–135.

Liu M, Cao D, Russell R, Handschumacher RE, and Pizzorno G (1998) Expression, characterization, and detection of human uridine phosphorylase and identification of variant uridine phosphorolytic activity in selected human tumors. *Cancer Res* **58:**5418–5424.

Luo J, Sladek R, Carrier J, Bader JA, Richard D, and Giguère V (2003) Reduced fat mass in mice lacking orphan nuclear receptor estrogen-related receptor alpha. *Mol Cell Biol* **23:**7947–7956.

Miwa M, Ura M, Nishida M, Sawada N, Ishikawa T, Mori K, Shimma N, Umeda I, and Ishitsuka H (1998) Design of a novel oral fluoropyrimidine carbamate, capecitabine, which generates 5-fluorouracil selectively in tumours by enzymes concentrated in human liver and cancer tissue. *Eur J Cancer* **34:**1274–1281.

Mootha VK, Handschin C, Arlow D, Xie X, St Pierre J, Sihag S, Yang W, Altshuler D, Puigserver P, Patterson N, et al. (2004) Erralpha and Gabpa/b specify PGC-1alpha-dependent oxidative phosphorylation gene expression that is altered in diabetic muscle. *Proc Natl Acad Sci U S A* **101:**6570–6575.

Pizzorno G, Cao D, Leffert JJ, Russell RL, Zhang D, and Handschumacher RE (2002) Homeostatic control of uridine and the role of uridine phosphorylase: a biological and clinical update. *Biochim Biophys Acta* **1587:**133–144.

Stein RA and McDonnell DP (2006) Estrogen-related receptor alpha as a therapeutic target in cancer. *Endocr Relat Cancer* **13 (Suppl 1):**S25–S32.

Tremblay AM and Giguère V (2007) The NR3B subgroup: an ovERRview. *Nucl Recept Signal* **5:**e009.

Wan L, Cao D, Zeng J, Yan R, and Pizzorno G (2006) Modulation of uridine phosphorylase gene expression by tumor necrosis factor-α enhances the antiproliferative activity of the capecitabine intermediate 5′-deoxy-5-fluorouridine in breast cancer cells. *Mol Pharmacol* **69:**1389–1395.

Wende AR, Huss JM, Schaeffer PJ, Giguère V, and Kelly DP (2005) PGC-1alpha coactivates PDK4 gene expression via the orphan nuclear receptor ERRalpha: a mechanism for transcriptional control of muscle glucose metabolism. *Mol Cell Biol* **25:**10684–10694.

Willy PJ, Murray IR, Qian J, Busch BB, Stevens WC Jr, Martin R, Mohan R, Zhou S, Ordentlich P, Wei P, et al. (2004) Regulation of PPARgamma coactivator 1alpha (PGC-1alpha) signaling by an estrogen-related receptor alpha (ERRalpha) ligand. *Proc Natl Acad Sci U S A* **101:**8912–8917.

Zhang D, Cao D, Russell R, and Pizzorno G (2001) p53-dependent suppression of uridine phosphorylase gene expression through direct promoter interaction. *Cancer Res* **61:**6899–6905.

Zhang Y, Ba Y, Liu C, Sun G, Ding L, Gao S, Hao J, Yu Z, Zhang J, Zen K, et al. (2007) PGC-1alpha induces apoptosis in human epithelial ovarian cancer cells through a PPARgamma-dependent pathway. *Cell Res* **17:**363–373.

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