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Peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) upregulated E-cadherin expression in HepG2 cells

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Abstract **Peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α), a highly inducible transcriptional coactivator regulating energy homeostasis, is down-regulated in hepatoma tissues. To dissect its role in hepato-tumorigenesis, Ingenuity® Pathway Analysis was applied to construct pathways affected by PGC-1α upregulation in HepG2 hepatoma cells based on our microarray data. Interestingly, migration of these cells was markedly diminished by PGC-1α overexpression in consistency with Ingenuity® results. Moreover, a deduced expression increase of E-cadherin was also observed in PGC-1α-overexpressing HepG2 cells. Finally, transient transfection and chromatin-immunoprecipitation assays suggested that increased histone acetylation might be responsible for PGC-1α-mediated transactivation of a minimal E-cadherin promoter.**

Keywords: PGC-1α; E-cadherin; Hepatocellular carcinoma; Migration; Transcription; Histone modification

1. Introduction

PGC-1α was first identified as a peroxisome proliferator-activated receptor gamma (PPARγ)-interacting protein that regulates adaptive thermogenesis in murine [1]. Human PGC-1α was first described by Esterbauer et al. as a potential regulator of insulin sensitivity [2]. This energy-athirst induced transcription coactivator is highly responsive to a variety of environmental cues, including temperature changes, nutritional status and physical activity where it cooperates with nuclear receptors in a tissue-specific manner [3]. Therefore, various diseases with underlying dysregulation of metabolism may occur because of aberrant PGC-1α expression [4].

Cancer cells preferentially utilize glycolytic pathways for energy generation while down-regulating their aerobic respiratory activity, known as the "Warburg effect" [5]. In other words, tumor cells may have impaired mitochondrial function which results in an elevation of glycolytic ATP generation and a simultaneous decrease of oxidative phosphorylation [6,7]. In a previous study, we demonstrated that PGC-1α expression, the copy number of mitochondrial DNA and the content of mitochondrial respiratory proteins were reduced in hepatocellular carcinoma (HCC) as compared with the adjacent non-tumorous tissues [8], suggesting that dysregulated expression of this transcription coactivator may be involved in hepatocarcinogenesis.

By docking on several nuclear receptors, PGC-1α greatly enhances their transcriptional activity through the recruitment of histone acetyltransferase (HAT)-containing proteins as well as interacting with RNA polymerase II complex [9,10]. Among the nuclear partners of PGC-1α, PPARγ has been shown to inhibit the growth of a variety of cancer cells including breast, colon, prostate and liver [11]. The malignancy suppressive effect of PPARγ agonists has been postulated to result from an increased expression of E-cadherin [12,13], a transmembrane protein of the adherens junctions that mediates cell–cell interaction which is involved in the morphogenesis and establishment of tissues [14]. While a loss of E-cadherin expression has been reported to be correlated with the progression of non-invasive tumor cells into malignant, metastatic carcinomas including HCC [14,15], reintroduction or upregulation of this cell adhesion molecule has been shown to suppress the in vivo [16] and in vitro invasion of tumor cells [17]. These results together emphasize that PPARγ pathway upregulated E-cadherin expression plays a crucial role in suppressing tumor progression.

The decrease of PGC-1α is linked to the variation of PPARγ expression level in breast cancer [18] and ovarian cancer cells [19]; however, the potential role of PGC-1α in the pathogenesis of liver cancer remains to be examined. Here, we transduced PGC-1α into human hepatoma HepG2 cells and employed cDNA microarray analysis to dissect the role of this transcription coactivator in regulation of cellular function in hepatoma cells. Our results suggested that PGC-1α upregulation is

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Abbreviations: ChIP, chromatin-immunoprecipitation; GFP, green fluorescence protein; HAT, histone acetyltransferase; HCC, hepatocellular carcinoma; MOI, multiplicity of infection; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator-1 alpha; PPAR, peroxisome proliferator-activated receptor; ROS, reactive oxygen species; SEAP, secreted alkaline phosphatase

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involved in reducing migration ability in HepG2 cells. Moreover, a unique finding in transcriptional regulation of E-cadherin by PGC-1α in HepG2 cells was described and discussed in details.

2. Materials and methods

2.1. Cell culture

HepG2 human hepatoblastoma cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 10 μg/ml gentamycin, 2 mM L-glutamine, 0.1 mM non-essential amino acid at 37 °C in a humidified atmosphere containing 5% CO_2. Cell growth was determined by trypan blue exclusion assay.

2.2. Construction of the recombinant adenoviral genome and production of Ad-PGC-1α virus

Full length PGC-1α cDNA fragment was amplified by PCR from HepG2 cDNA. Ad-PGC-1α virus was produced according to manufacturer's protocol (AdEasy™ XL Adenoviral vector system; Stratagene, La Jolla, CA). Large preparation of virus and its infection in HepG2 cells were performed as described previously [20].

2.3. Microarray analysis and pathway identification

Total RNA was isolated from 2 days post-infected HepG2 cells using Trizol® reagent (Invitrogene, Carlsbad, CA). RNA samples were processed for hybridization on Affymetrix Human Genome U133 plus 2.0 gene chips (Affymetrix, Santa Clara, CA). The differentially expressed (>2-fold) probe sets were overlaid on a cellular pathway map in the Ingenuity® Pathway Analysis.

2.4. Reverse transcription-polymerase chain reaction (RT-PCR) analysis

Total RNA and cDNA was prepared as described previously [8]. PCR primer sets were: for PGC-1α, sense: 5′-CCAAATGACCCCAAGGGTTC-3′ and antisense: 5′-TATGAGGAGGAGTGGTGGGTG-3′; for glucose-6-phosphatase (G6Pase), sense: 5′-GACTCCCAGGACTGGTTCATC-3′ and antisense: 5′-TCAGCTGCACAGCCCAGAATC-3′; for carnitine palmitoyltransferase I (CPTI), sense: 5′-CAAGGTCTGGCTCTACCATG-3′ and antisense: 5′-GACTTGTCAAACCACCTGTCG-3′; for cytochrome *c* (Cyt. *c*), sense: 5′-GATGGAGTATTTGGAGAATCC-3′ and antisense: 5′-GATCTGAATTCTGGTGTATGAG-3′; for E-cadherin, sense: 5′-CAGCCTGTC GAAGCAGGATTGC-3′ and antisense: 5′-GAGCTCAGACTAGCAGCTTCGG-3′; for β-actin, sense: 5′-TGGCATTGCCGACAGGAT-3′ and antisense: 5′-GCTCAGGAGGAGCAATGATCT-3′.

SyBr green intercalating real-time quantitative PCR (qPCR) was performed by ABI PRISM 7700 (PE Applied Biosystems, Foster city, CA). E-cadherin primer sets for qPCR were sense: 5′-TGAAGGTGACAGAGCCTCTGGAT-3′ and antisense: 5′-TGGGTGAATTCGGGCTTGTT-3′.

2.5. Western blot analysis

Cells were harvested 3 days post-infection and Western blotting was performed as described previously [21] using the following antibodies: PGC-1α, cytochrome *c* (Santa Cruz Biotechnology Inc., Santa Cruz, CA), cyclin D1, E-cadherin (BD Biosciences, San Diego, CA) and β-actin (Sigma, Saint Louis, MO).

2.6. Immunofluorescence staining and confocal imaging

Infected cells were grown on coverslips for 48 h, then performed immunofluorescence double staining using PGC-1α and E-cadherin antibodies as described previously [21]. A confocal microscope (Zeiss inverted, Axiovert 200M) with a dip in lens Plan Fluor objective (×100 oil) was used. Images were acquired using LSM 5 PASCAL software.

2.7. Transwell cell migration analysis

Two days post-infection cells (1×10^5) were added to 8 μm pore size non-coated transwell top chambers (Corning Costar, Acton, MA) and mobility analysis was performed as described previously [21].

2.8. E-cadherin promoter derived secreted alkaline phosphatase (SEAP) reporter gene construction and SEAP reporter activity analysis

Human E-cadherin promoter −1001 to +67 fragment phE-cad(-1kb) was obtained by PCR amplification of the genomic DNA prepared from human HepG2 cells and inserted into pSEAP2-Basic (Clontech Laboratories, Inc., Mountain View, CA). The serial deletion mutant fragments were amplified by PCR from phE-cad(-1kb). SEAP activity assay was performed as described previously [22].

2.9. Chromatin-immunoprecipitations (ChIPs)

DNA–protein complex was cross-linked two day post-infection; chromatin was sheared by sonication (4 W, 4 s; break, 10 s for 30 times on ice). The following analysis was performed by Chromatin Immunoprecipitation Assay Kit (Upstate, Lake Placid, NY) according to manufacturer's recommendation. E-cadherin promoter fragment amplification was performed by nested-PCR using the following primer sets: 1st – sense, pE-cad(-239)-F, 5′-GGATTCGAACCCAGTGGAATCAG-3′; antisense, pE-cad(+67)-R, 5′-CTGGAGCGGGCTGGAGTCTGAACTGAC-3′; 2nd – sense, pE-cad(-192)-F, 5′-CTAGACCCTAGCAACTCCAG-3′; antisense, pE-cad(+9)-R, 5′-GACGCCACTGAGAGG-3′.

2.10. Statistical analysis

Arithmetic means and standard deviations (S.D.) were obtained from three independent experiments, and statistical significance was considered when $P < 0.05$ using Student's *t*-test.

3. Results

3.1. PGC-1α transduced by recombinant adenovirus is functional in HepG2 cells

To identify genes whose expression in hepatocyte might be affected by PGC-1α upregulation, recombinant adenovirus carrying the corresponding gene (Ad-PGC-1α) was generated and used to infect HepG2 cells. Expression of several well-known PGC-1α-inducible genes including glucose-6-phosphatase (G6Pase), carnitine palmitoyl transferase I (CPTI) and cytochrome *c* (Cyt. *c*) was examined by RT-PCR analysis. As shown in Fig. 1a, all three genes were induced by PGC-1α upregulation. Fig. 1b shows that the growth of HepG2 cells was not altered by PGC-1α overexpression in the first 2 days post-infection but slightly decreased on day 3. Overexpression of PGC-1α did not induce apoptosis of HepG2 cells (data not shown). Fig. 1c shows that the expression of cyclin D1 protein was reduced in PGC-1α-overexpressing cells at the same time.

3.2. Assessment of the pathways in HepG2 cells influenced by PGC-1α upregulation

Having validated the effects of PGC-1α overexpression in HepG2 cells, we then used microarray analysis to identify more thoroughly its target genes. In comparison with cells infected by the control virus (Ad-green fluorescence protein (GFP)), Ad-PGC-1α-infected cells exhibited different expression patterns in genes mainly involved in energy homeostasis such as gluconeogenesis, lipid metabolism and mitochondrial respiration (Fig. 2a). After reconstruction of the pathway networks by Ingenuity® Pathway Analysis, we found that the altered pathway with second highest score was associated with cancer and cellular movement (Fig. 2b).

3.3. PGC-1α overexpression decreases migration ability of HepG2 cells

Since cellular movement has been suggested to be affected by PGC-1α overexpression, transwell migration analysis was