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Inhibition of Cell Growth by Nuclear Receptor COUP-TFI: Possible Involvement of Decorin in Growth Inhibition

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Abstract

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Introduction

Chicken Ovalbumin Upstream Promoter-Transcription Factors (COUP-TFs) [1] are the member of the steroid/thyroid hormone-activated nuclear receptor superfamily. Since no ligand has yet been identified, COUP-TFs are regarded as orphan receptors. Two distinct genes for COUP-TFs, COUP-TFI/Ear3 [1,2] and COUP-TFII/ARP-1 [3], have been identified, and their sequence homology was 98 and 96% in the DNA-binding domain and in the putative ligand-binding domain, respectively [4]. Although COUP-TFI was originally shown to stimulate transcription of the ovalbumin gene [5], a number of studies have demonstrated that COUP-TFI antagonistically represses transactivation by other nuclear receptors [6-10], mainly through competitive occupancy of a target binding sequence. However, it has been recently reported that COUP-TFs can function as transactivators [11-13] as well as an auxiliary cofactor for other transcription factors [14,15].

Targeted disruption of the COUP-TFI gene resulted in defects in neurogenesis, axon guidance, and arborization [16], whereas targeted deletion of COUP-TFII resulted in embryonic lethality with defects in angiogenesis, vascular remodeling, and fetal heart development [17]. Therefore, COUP-TFs are essential for early development and cell differentiation [18,19], and could be associated with cell proliferation.

Recent studies have reported that COUP-TFI was required for growth inhibition and apoptosis in response to retinoic acid in some cancer cell lines [20], and that ectopic expression of COUP-TFII in breast cancer cell lines caused growth arrest associated with modulation in expression of cell-cycle regulatory proteins such as p21Cip1 and cyclin D1 [21]. On the other hand, COUP-TFI stimulates proliferation [22] and migration [23] of breast cancer cells by activating estrogen signaling or EGF signaling.

In the present study, we stably introduced COUP-TFI into Ha-ras-transformed NIH3T3 cells and examined its effect on cell growth. Ectopic expression of COUP-TFI led to decrease in cell proliferation rates, reduction of anchorage-independent growth, and delay of progression through mid-S to G2/M phase. Results of cDNA microarray analysis and reporter assay suggested that decorin is one of the transcriptional targets of COUP-TFI and mediates the growth-inhibitory effects of COUP-TFI.

Materials and Methods

Cells and culture

A cell strain, Ha-ras-transformed NIH3T3 (ras-NIH3T3, clone F25) was kindly supplied by T. Sekiya [24] and cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 5% calf serum.

Stable transfection

Transfection of plasmids into ras-NIH3T3 cells and selection of stable transfectants were done essentially as described [25]. Cells were plated at a density of 2×10^5 cells in 35 mm dishes. Twenty-four hours later, the cells were co-transfected with the plasmid expressing human COUP-TFI cDNA [7] under the control of the elongation factor 1 promoter of pEF-BOS [26], in addition to neo gene-bearing plasmid pcDNA3 (Thermo Fisher Scientific, Waltham, MA) at a ratio of 10:1.

Control cells were co-transfected with pEF-BOS and pcDNA3. For transfection, FuGENE6 reagent (Roche Diagnostics, Mannheim, Germany) was used according to manufacturer's protocol. The next

Results

Isolation of stable transfectants of COUP-TFI

To examine the biological activity of COUP-TFI, Ha-ras-transformed NIH3T3 (ras-NIH3T3) cells were stably transfected with COUP-TFI-expressing plasmids. Among G418-selected clones, COUP-TFI-transfected clones (FCO-3, 7 and 10) showed higher expression of COUP-TFI at both mRNA and protein levels than the parental cells and a vector-transfected clone (Figure 1a and b).

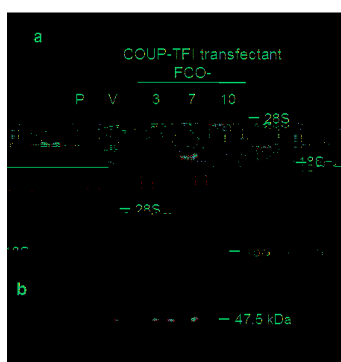


Figure 1: Isolation of stable transfectants of COUP-TFI. COUP-TFI cDNA was transfected into ras-NIH3T3 cells, and G418-resistant stable transfectants (FCO-3, 7 and 10) were isolated. The expression of COUP-TFI in FCO-3, 7 and 10, control vector-transfected cells (V) and the parental cells (P) was examined by Northern blotting (a) and Western blotting (b). A lower panel in a represents ethidium bromide staining of rRNAs in the agarose gel prior to blotting.

Reduced proliferation rates and delay of cell cycle progression through mid-S to G2 phase in COUP-TFI transfected cells

When the proliferation rates were examined by direct counting of cell numbers, all of COUP-TFI-transfected cells grew much more

mid-S to G2/M phase is responsible for growth inhibition in COUP-TFI-transfected cells.

Reduced anchorage-independent growth in COUP-TFI transfectants

Colony-forming ability in soft agar medium is well established as a useful mean to assess the transformed state of a given cell population, and was examined on COUP-TFI transfectants.

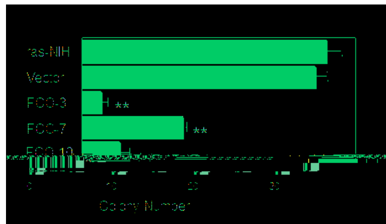
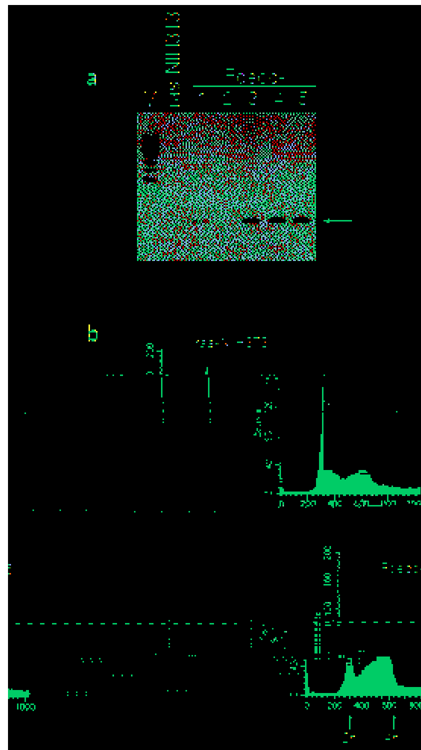


Figure 4: Reduced anchorage-independent growth in COUP-TFI transfectants. Cells (600 cells per plate) of indicated clones were seeded in soft agar medium in triplicate, cultured for 3 weeks, and the number of colonies was counted under a phase-contrast microscope. Mean colony numbers of three independent experiments are shown. Error bars represent S.E. Significant difference from parental ras-NIH3T3 clone was w „ ansfectbrÚ

that decorin inhibits cell proliferation by neutralizing transforming growth factor (TGF) activity [35,44] and/or by inducing p21Cip1 through epidermal growth factor receptor (EGFR) activation [45-47]. The latter mechanism may not account for the growth inhibition in the present study because EGFR is not expressed in ras-NIH3T3 cells (data not shown) and also because p21 was not induced in COUP-TFI transfectants (data not shown). On the other hand, it is plausible that suppression of TGF activity by decorin is the cause of growth inhibition in response to COUP-TFI introduction, because TGF stimulates the proliferation of NIH3T3 cells [48]. It is noteworthy that decorin-overexpressing COUP-TFI transfectants showed apparent reduction of anchorage-independent growth (Figure 4). This observation is concordant with the notion that decorin plays a role in suppression of some transformed phenotypes of tumor cells. Indeed, it has been demonstrated that de novo decorin gene expression suppressed the malignant phenotype in human colon cancer cells [49]. We also isolated stable decorin transfectants, which showed similar cell cycle distribution to that of COUP-TFI transfectants (Supplemental Figure S1).

The accumulation at mid-S to G2/M phase was more prominent than COUP-TFI-tranfectants, possibly due to the enforced overexpression of decorin.

Accession Number	Name	Ratio (Cy3/Cy5)	Ratio (Cy4/Cy3)



Supplemental Figure S1: Isolation and characterization of decorin transfectants. A) Isolation of stable decorin transfectants. ras-NIH3T3 cells were transfected with decorin expression plasmid, and G418-resistant clones were isolated. The expression levels of decorin mRNA in decorin-transfected clones, Fdeco-1 to 5, and the parental ras-NIH3T3 cells were examined by RT-PCR. An arrow indicates the PCR products of decorin. B) Mid-S to G2/M arrest of cell cycle pregression in decorin-transfected cells. Cell cycle distribution of the parental ras-NIH3T3 and decorin transfected clone Fdeco-5 was examined by flow cytometry.

20. Lin B, Chen GQ, Xiao D, Kolluri SK, Cao X, et al. (2000) Orphan receptor COUP-TF is required for induction of retinoic acid receptor beta, growth inhibition, and apoptosis by retinoic acid in cancer cells. Mol Cell Biol 20: 957-970.
21. Nakshatri H, Mendonca MS, Bhat-Nakshatri P, Patel NM, Goulet RJ Jr, et al. (2000) The orphan receptor COUP-TFII regulates G2/M progression of breast cancer cells by modulating the expression/activity of p21(WAF1/CIP1), cyclin D1, and cdk2. Biochem Biophys Res Commun 270: 1144-1153.
22. Le Dily F, Métivier R, Guéguen MM, Le Péron C, Flouriot G, et al. (2008) COUP-TFI modulates estrogen signaling and influences proliferation, survival and migration of breast cancer cells. Breast Cancer Res Treat 110: 69-83.
23. Boudot A, Kerdivel G, Lecomte S, Flouriot G, Desille M, et al. (2014) COUP-TFI modifies CXCL12 and CXCR4 expression by activating EGF signaling and stimulates breast cancer cell migration. BMC Cancer 14: