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Nuclear tristetraprolin acts as a corepressor of multiple steroid nuclear receptors in breast cancer cells

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ABSTRACT

Tristetraprolin (TTP) is a 34-kDa, zinc finger-containing factor that in mammalian cells acts as a tumor suppressor protein through two different mechanisms. In the cytoplasm TTP promotes the decay of hundreds of mRNAs encoding cell factors involved in inflammation, tissue invasion, and metastasis. In the cell nucleus TTP has been identified as a transcriptional corepressor of the estrogen receptor alpha (ERα), which has been associated to the development and progression of the majority of breast cancer tumors. In this work we report that nuclear TTP modulates the transactivation activity of progesterone receptor (PR), glucocorticoid receptor (GR) and androgen receptor (AR). In recent years these steroid nuclear receptors have been shown to be of clinical and therapeutical relevance in breast cancer. The functional association between TTP and steroid nuclear receptors is supported by the finding that TTP physically interacts with ERα, PR, GR and AR *in vivo*. We also show that TTP overexpression attenuates the transactivation of all the steroid nuclear receptors tested. In contrast, siRNA-mediated reduction of endogenous TTP expression in MCF-7 cells produced an increase in the transcriptional activities of ERα, PR, GR and AR. Taken together, these results suggest that the function of nuclear TTP in breast cancer cells is to act as a corepressor of ERα, PR, GR and AR. We propose that the reduction of TTP expression observed in different types of breast cancer tumors may contribute to the development of this disease by producing a dysregulation of the transactivation activity of multiple steroid nuclear receptors.

1. Introduction

Tristetraprolin (TTP) is a 34-kDa protein characterized by the presence of two tandemly located CCCH-type zinc finger domains and three proline rich motifs [1–4]. In mammalian cells TTP is localized both in the nucleus and cytoplasm and its total protein expression and organelle-specific distribution are regulated by signal transduction pathways activated by interleukins, growth factors and glucocorticoids [5–9]. Functional analysis performed in animal models and cells in culture revealed that the cytoplasmic form of TTP uses its zinc finger domains to interact with AU-rich elements (AREs) present in the 3′-untranslated region of mRNAs [10–13]. The binding of TTP accelerates mRNA degradation by contributing to the recruitment of the Ccr4/Caf1/Not deadenylation complex [14].

Although AREs are present in 8% to 10% of all mammalian transcripts, TTP has been shown to selectively target around 250 mRNAs encoding factors involved in regulation of cell growth, inflammation, metastasis and apoptosis [15–17]. These results have led different investigators to suggest that TTP is involved in the control of neoplasic development in diverse tissues [18]. The function of TTP as a tumor suppressor protein has been supported by the finding that its protein expression levels negatively correlate with tumor progression in different types of cancer tumors [15,19,20].

Recently, our laboratory demonstrated that the nuclear form of TTP acts as a corepressor of estrogen receptor a (ERα), a ligand activated transcription factor associated to the development of 70%–80% of all breast cancer tumors [4,21]. Mechanistically, TTP was shown to repress ERα transactivation by disrupting its interaction with the steroid receptor coactivator 1 (SRC-1) and by facilitating the recruitment of histone deacetylase 1 (HDAC1) to the promoter region of estradiol-responsive genes [4]. This study demonstrated that TTP overexpression reduces

Abbreviations: TTP, tristetraprolin; ERα, estrogen receptor; PR, progesterone receptor; AR, androgen receptor; GR, glucocorticoid receptor.

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the proliferation rate and tumorigenicity potential of human breast cancer cells in mice [4]. These results allowed us to suggest that nuclear TTP also acts as a tumor suppressor protein through a mechanism that involves its ability to regulate ERα transactivation [4].

In this work, we sought to gain better insight into the function of nuclear TTP as a transcriptional corepressor by exploring its effect on the activity of other hormone nuclear receptors that have been recently shown to be of clinical or therapeutical relevance in breast cancer [22–25]. Our results show that TTP is physically associated to progesterone receptor (PR), glucocorticoid receptor (GC) and androgen receptor (AR) in the nucleus of breast cancer MCF-7 cells *in vivo*. We show further that TTP overexpression reduces ERα, PR, GR and AR transactivation activity. In contrast, siRNA-mediated reduction of endogenous TTP protein levels produced a significant increase in the transcriptional activity of these steroid nuclear receptors without affecting their protein levels in MCF-7 cells. These results suggest that the corepressor activity of nuclear TTP may participate in the regulation of multiple hormone-dependent signaling pathways that play a key role in the development of human normal mammary gland epithelium and breast cancer tumors.

2. Materials and methods

2.1. Reagents and Antibodies

Estradiol (E8875, 17β-estradiol), progesterone (*P*-0130, 4-Pregnene-3,20 dione) and dexamethasone (D1756), were obtained from Sigma-Aldrich. Androgen (10300, 5α-Androstan-17β-ol-3-one) was obtained from Fluka Chemika. Human ERα antibody (Sc *D*-12, Sc HC-20) and Human TTP antibody (Sc-12565) were purchased from Santa Cruz Biotechnology, Inc. and TTP polyclonal (T5327) antibody was from Sigma-Aldrich. Human PR (ab-68195), GR (ab-2768) and AR (ab-74272) antibodies were from Abcam. TTP knockdown assays were performed using TTP siRNA mixture and control siRNA from Santa Cruz Biotechnology (Sc-36760, Sc-37007). Lipofectamine 2000 was purchased from Invitrogen by life Technologies (11668-019).

2.2. Plasmids

ERα transactivation activity was determined using a luciferase reporter gene driven by a promoter containing three estrogen responsive elements (ERE-Tk-LUC) that has been previously described [4,26]. To test the transactivation activity of PR, AR. and GR, human cells were transfected with the PGL3-MMTV in which the luciferase reporter gene is regulated by a promoter containing a palindromic sequence recognized by PR, GR and AR. pcDNA3.1-ERα and ERE-Tk-LUC vectors were kindly provided by Dr. W. Lee Kraus (Cornell University). Vectors PRE-dbCAT (PR), pSVhARo-BHEXE (AR), pcDNA3.1-GR and PGL3-MMTV were a gift from Dra. Rocío Ángeles García Becerra (Instituto Nacional de Ciencias Médicas y Nutrición-México). Human full-length TTP cDNA (GenBank™ accession no. NM_003407.3) was cloned into the mammalian expression vector pCMV-3Tag-1 A (Agilent Technologies, Santa Clara, CA) as previously described [4]. The sequences of all constructs were verified by DNA sequencing at LARAGEN Inc. (Culver City, CA).

2.3. Cell culture and transfection assays

MCF-7 cells (ATCC HTB-22, American Type Culture Collection, Manassas, VA) were maintained in Minimum Essential medium Alpha Medium (11900-024, Gibco) supplemented with 5% FBS, 100 units/ml penicillin, and 100 mg/ml streptomycin in a humidified atmosphere containing 5% CO2 at 37 °C. Cells were seeded into tissue culture dishes containing phenol red-free Minimum Essential Medium Eagle (M3024, Sigma-Aldrich) supplemented with 5% charcoal/dextran-treated FBS and cultured for 36–40 h before all experimental treatments with hormone. Cells were transfected using Lipofectamine 2000 with 2 mg of ERE-Tk-LUC reporter, 1 mg of pcDNA3. 1-ERα, PR, GR, AR and 0.5 mg pCMV-3Tag-1 A-TTP or 0.006 mg of Renilla vector (transfection control). After 4 h, the cells were washed twice with phosphate-buffered saline and treated with 100 nM of estradiol, progesterone, dexamethasone or androgens for 48 h in phenol red-free MEM supplemented with 5% stripped FBS. Cells were then washed and harvested in Passive Lysis Buffer (Promega). Control cells were incubated under the same experimental conditions in the absence of hormones. Luciferase and Renilla activities were measured using a Dual-Luciferase Reporter Assay System (E1960, Promega) and Glomax Multi JR Detection System (Promega). TTP knockdown assays were performed using TTP siRNA mixture and control siRNA from Santa Cruz Biotechnology and transfected using Lipofectamine 2000 (Invitrogen). The specificity of the TTP knockdown assay was determined by analyzing the expression of TTP, ERα, PR, GR and AR in MCF-7 cells transfected with a control siRNA or with a specific siRNA-TTP.

2.4. Immunoprecipitation and Western blot

MCF-7 cells were lysed with TNTE buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.5% Triton X-100 plus a mixture of protease and phosphatase inhibitors). Proteins were immunoprecipitated with mouse anti-ERα, anti-GR or rabbit anti-PR and anti-AR antibodies. Immunoprecipitated proteins were separated by SDS-PAGE, and Western blot analysis was performed using a specific antibodies anti-TTP and anti-mouse or anti-rabbit secondary HRP-conjugated antibody (Pierce). Proteins were visualized using an enhanced chemiluminescence assay (SuperSignal West Pico Chemiluminescent Substrate, Thermo Scientific).

2.5. Statistical analysis

Each transfection assay was performed by triplicate in three different experiments using different cell cultures. Data are presented as mean ± S.E. Statistical significance was analyzed at 0.05 levels of significance using Student's *t*-test.

3. Results

3.1. Correlation between TTP mRNA expression levels and breast carcinoma using the Oncomine cancer microarray database

In this study, we set out to determine the effect of TTP on the transactivation activity of the steroid nuclear receptors PR, GR and AR that along with ERα have been associated to cell proliferation, tumor development, apoptosis control and response to hormonal therapy in breast cancer. To explore the relationship between TTP expression and breast cancer tumorigenesis, we compared TTP mRNA levels in normal tissues and different types of breast carcinomas using the Oncomine database (http://www.oncomine.org, Compendia Bioscience, Ann Arbor, MI). The analysis of data sets containing gene chip profiles classified as normal or breast carcinoma tissues, showed a significant reduction in TTP mRNA levels in breast carcinomas compared to normal tissue. Two representative results of two independent data sets [27] are shown in Fig. 1.

3.2. TTP interacts with different steroid nuclear receptors in MCF-7 cells in vivo

To study the functional interaction between TTP and steroid nuclear receptors we used breast cancer MCF-7 cells that had previously shown to express multiple steroid nuclear receptors [25,28–36]. To explore the physical interaction between TTP and the steroid nuclear receptors ERα, PR, GR and AR, we performed coimmunoprecipitation assays in MCF-7 cells. Endogenous nuclear hormone receptors present in MCF-7 protein