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Transcriptional activity of c-Jun is critical for the suppression of AR function

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ABSTRACT

Androgen receptor (AR) signaling plays a pivotal role in growth and survival of prostate cancer cells. c-Jun is an important member of the activator protein 1 (AP-1) family and was shown to interact with AR. However, the role of c-Jun in AR signaling remains controversial, with being a coactivator or a corepressor reported. Here, utilizing multiple approaches, we show that c-Jun efficiently inhibits AR activity and the growth of prostate cancer cells. Overexpression of c-Jun inhibits not only the activities of various androgen-responsive promoters but also the transcripts of multiple AR target genes. Interestingly, long-term c-Jun overexpression also down-regulates AR expression at both the protein and mRNA levels. Molecular analysis suggests that c-Jun inhibits AR transactivation potential via an unknown target gene. The inhibition of AR by c-Jun occurs in both hormone naïve and castration-resistant prostate cancer cells. Our results unravel a novel mechanism by which c-Jun antagonizes the AR signaling.

1. Introduction

Prostate cancer represents the most common non-cutaneous human cancer and is the second leading cause of cancer deaths among men in the US (Jemal et al., 2010). Like normal prostate gland, the proliferation and survival of prostate cancer cells rely on androgens, which signal through the androgen receptor (AR). Thus, androgen ablation therapy, also known as hormone therapy, is the most effective way to control advanced prostate cancer (Salesi et al., 2005). Despite the success of hormone therapy, most tumors eventually relapse and develop into castration-resistant prostate cancer (CRPC) due to the aberrant restoration of AR activity (Feldman and Feldman, 2001). Interestingly, numerous studies have showed that AR signaling axis remains essential for the development and maintenance of CRPC (Chen et al., 2004; Gao et al., 2006; Snoek et al., 2009; Yuan et al., 2006; Zegarra-Moro et al., 2002).

Similar to other steroid hormone receptors, AR is composed of an N-terminal domain (NTD) which contains a major activation domain, AF-1, a DNA-binding domain (DBD), a hinge region and a C-terminal ligand binding domain (LBD) containing a weak activation domain, AF-2 (Dehm and Tindall, 2007). Unliganded AR is sequestered in the cytoplasm by heat shock proteins (Marivoet et al., 1992). Upon binding to testosterone or dihydrotestosterone (DHT), the two major physiology androgens, AR dissociates from heat shock proteins and translocates to the nucleus where it functions as a transcription factor by binding as a homodimer to the androgen-response element (ARE) in the promoter and/or enhancer regions of target genes. c-Jun is a basic region leucine zipper (bZIP) transcription factor and is an important member of the activator protein 1 (AP-1) family (Vogt, 2001). The basic region of c-Jun is required for DNA binding while the leucine zipper enables c-Jun to form a homodimer or a heterodimer with other AP-1 members, such as Fos and activating transcription factor 2 (ATF2). Depending on the dimerization partner, c-Jun/AP-1 complex binds to TPA-responsive elements (TREs) or cyclic AMP-responsive elements (CREs) in the promoter region of target genes that are involved in several cellular responses including proliferation, apoptosis and differentiation (Eferl and Wagner, 2003). Phosphorylation at residues Ser-63 and Ser-73 by c-Jun N-terminal kinases (JNKs) was shown to enhance the transactivation activity of c-Jun (Karin, 1995; Smeal et al., 1991).

Many molecular and genetic studies have provided evidence that AP-1 activity may also be implicated in the development and progression of prostate cancer. The expression of JunB and Fos was found to be up-regulated in primary prostate tumors but down-regulated in metastatic samples (Chandran et al., 2007). Conversely, Ouyang et al. (2008) reported that while both c-Jun and Fos are up-regulated in metastatic prostate cancer, only high c-Jun expression is associated with poor prognosis. However, in the same report, it was found that only few cases (3–4%) of prostate cancer showed high expression of the AP-1 proteins. On the

Abbreviations: AP-1, activator protein 1; CRPC, castration-resistant prostate cancer; DBD, DNA-binding domain; ARE, androgen-response element; DHT, dihydrotestosterone; bZIP, basic region leucine zipper; TRE, TPA-responsive elements; CRE, cyclic AMP-responsive elements; Dox, doxycycline; TMPRSS2, transmembrane protease, serine 2; CBP, CREB binding protein.

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other hand, it has also been observed that some AP-1 proteins are also down-regulated in a subset of prostate cancer patients. In fact, Edwards et al. (2004) found that while 16% of CRPC patients showed c-Jun up-regulation, 20% of CRPC patients exhibited c-Jun down-regulation. Moreover, Tamura et al. (2007) showed that transcripts of both c-Jun and Fos were down-regulated in CRPC. Although these studies examined the expression level of AP-1 proteins in prostate cancer tissues, it remains unclear whether and how their transcriptional activity correlates to the development and progression of prostate cancer.

In addition to functioning as an AP-1 transcription factor, AP-1 proteins also interact with other family of transcription factors such as NF-kappaB (Fujioka et al., 2004; Shyu et al., 2008), NFAT (Macian et al., 2000) and nuclear hormone receptors (Herrlich, 2001; Lamph, 1991). In fact, several studies have suggested that c-Jun may physically interact with AR and modulate the AR activity. Sato et al. (1997) reported that c-Jun can interact with the DNA-binding domain of AR via its leucine zipper region to inhibit the DNA-binding as well as the transcriptional activity of AR. Recently, Mulholland and the coworkers proposed that the up-regulation of c-Jun in PTEN null murine prostate cancer cells contributes to CRPC progression by suppressing AR function and thus reducing the androgen-dependence (Mulholland et al., 2011). Conversely, it was also shown that c-Jun functions as an AR coactivator by enhancing the intramolecular interaction between amino and carboxyl termini of AR (Bubulya et al., 2001, 2000, 1996; Chen et al., 2006; Shemshedini et al., 1991; Wise et al., 1998). Despite the controversy of being an AR coactivator or corepressor, it remains unclear if transcriptional activity of c-Jun is involved in these regulations. Because of the critical role of AR in prostate cancer development and progression and because of the potential regulatory role of AP-1 in the AR signaling, we took a different approach to evaluate the impact of the transcriptional activity of c-Jun on the AR signaling. We found that the DNA binding and transcriptional activities of c-Jun, rather than its physical interaction with AR, are required for the maximal inhibition of the AR signaling. Taken together, our results suggest that an unknown target gene of c-Jun is required for the inhibition of the AR activity and future identification of such a target gene will provide new insight into the regulatory role of AP-1 in the AR signaling and prostate cancer development and progression.

2. Materials and methods

2.1. Antibodies

Polyclonal anti-AR antibody (sc-816) and monoclonal anti-phospho-c-Jun antibody (sc-822) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Monoclonal anti-PSA antibody (1984-1) was purchased from Epitomics (Burlingame, CA). Monoclonal Anti-β-Tubulin (T0198) and anti-Flag M2 (F3165) antibodies were from sigma. Monoclonal Anti-Human PARP antibody (4C10-5) was purchased from BD Biosciences (San Diego, CA).

2.2. Cell culture

LNCaP and COS-1 cells were purchased from American Type Culture Collection (Manassas, VA). C4-2 cells were obtained from the University of Texas MD Anderson Cancer Center (Houston, TX). LNCaP cells were cultured in RPMI1640 medium with 10% fetal bovine serum (FBS) and C4-2 cells were maintained in T-medium with 10% FBS (Gleave et al., 1991; Wu et al., 1994). COS-1 cells were cultured in DMEM medium supplemented with 5% FBS. For androgen treatment, LNCaP or C4-2 cells were cultured in phenol red-free RPMI1640 with 10% charcoal/dextran-stripped FBS (designated androgen-depleted medium) for 24 h before transient transfection or doxycycline (Dox) induction of c-Jun expression for another 24 h. Cells were then treated with 10 nM R1881 for 24 h. To determine the effect of c-Jun on the expression of endogenous AR-regulated genes, cells were cultured in regular medium (RPMI 1640 with 10% FBS for LNCaP cells; T-medium with 10% FBS for C4-2 cells) before induction of c-Jun expression for indicated periods of time.

2.3. Plasmids

Human c-Jun was amplified from a cDNA library originated from HEK 293T cells and was cloned in frame into the EcoRI/KpnI site of pFlag-CMV2 (sigma). Plasmids encoding c-Jun63A/73A, c-JunΔLZ (c-JunΔ280-317), c-Jun A→D^{265} In265 and TAM67 (c-JunΔ3-122) were generated by PCR or ligation PCR (Ali and Steinkasserer, 1995). To clone pLVX-Tight-Puro-Flag-c-Jun, the cDNA encoding Flag-c-Jun was amplified from pFlag-c-Jun by primer sets: BamHI-Kozak-Flag F (5′-CGG GAT CCG CCG CCA CCA TGG ACT ACA AAG ACG ATG ACG-3′) and c-Jun-stop-EcoRV R (5′-GGG ATA TCT TAA AAT GTT TGC AAC TGC TGC G-3′). The amplified cDNA was then cloned into BamHI and Klenow-blunted EcoRI sites of pLVX-Tight-Puro (Clontech). Similar strategy was used to clone all other Flag-c-Jun mutants into pLVX-Tight-Puro. All constructs generated by the PCR-based method were confirmed by DNA sequencing. For generation of c-Jun short hairpin RNA (shRNA) plasmid, annealed oligonucleotides (The RNAi Consortium TRCN0000010366) targeting TAGTACTCCTTAAGAACACAA in the 3′ untranslated region of c-Jun were cloned into pLKO-Tet-On (Wiederschain et al., 2009) to produce pLKO-Tet-On-c-JunKD.

2.4. Generation of Dox-inducible stable cell lines

LNCaP or C4-2 stable cell lines with inducible wild-type or mutant c-Jun were generated by Lenti-X Tet-On Advanced Inducible Expression System (Clontech) according to the manufacturer's protocol with the following modifications. Cells were first infected with viral particles constitutively expressing rtTA-advanced protein (a mutant Tetracycline-repressor). To generate viral particles, HEK 293T cells cultured in 10-cm culture dish were cotransfected with 2 μg of pLVX-Tet-On, 1.5 μg of pHR′-CMV-ΔR8.20vpr, and 0.5 μg of pHR′-CMV-VSV-G using Fugene HD reagent (Roche Applied Science). The supernatant containing viruses was harvested 2 days post-transfection and was then filtered through a 0.45 μm filter to remove cell debris. Infection was carried out by applying 4 ml of viral supernatant to LNCaP or C4-2 cells cultured in 11 ml complete medium. Polybrene was added at a final concentration of 8 μg/ml to facilitate infection. Two days after infection, cells were selected with 500 μg/ml G418 for more than one week. The cells stably expressing rtTA-advanced protein (LNCaP- or C4-2-rtTA cells) were then transduced with lentiviral particles packaged with pLVX-Tight-Puro-Flag-c-Jun (wild type or mutants) using similar procedures as described above. Following transduction for 2 days, cells were then selected with 2 μg/ml puromycin for 2 additional days. To generate LNCaP cells with inducible c-Jun knockdown, cells were infected with lentiviral particles packaged with pLKO-Tet-On-c-JunKD followed by puromycin selection using similar methods described above.

2.5. Luciferase reporter gene assay

LNCaP or C4-2 cells were trypsinized and washed with phosphate-buffered saline (PBS) once, followed by seeding in a 12 well plate at a density of 1×10^5 cells/well in androgen-depleted medium. Twenty-four hours later, cells were transfected with 0.5 μg of an androgen-responsive luciferase reporter construct, 100 ng of