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Research Article

MEIS1 functions as a potential AR negative regulator

Liang Cui[a,b,1], *Mingyang Li*[c,1], *Fan Feng*[d], *Yutao Yang*[e], *Xingyi Hang*[f], *Jiajun Cui*[g,*], *Jiangping Gao*[a,**]

[a]*Department of Urology, Chinese PLA Medical School/Chinese PLA General Hospital, Beijing 100853, PR China*
[b]*Department of Urology, Civil Aviation General Hospital/Civil Aviation Medical College of Peking University, Beijing 100123, PR China*
[c]*Department of Gastroenterology, Nan Lou Division, Chinese PLA Medical School/Chinese PLA General Hospital, Beijing 100853, PR China*
[d]*Department of Pharmacy, General Hospital of Shenyang Military Command, Shenyang 110016, PR China*
[e]*Beijing Institute for Neuroscience, Capital Medical University, Beijing 100069, PR China*
[f]*National Scientific Data Sharing Platform for Population and Health, Beijing 100730, PR China*
[g]*Department of Cancer and Cell Biology, University of Cincinnati College of Medicine, Cincinnati, OH 45267, USA*

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ABSTRACT

The androgen receptor (AR) plays critical roles in human prostate carcinoma progression and transformation. However, the activation of AR is regulated by co-regulators. MEIS1 protein, the homeodomain transcription factor, exhibited a decreased level in poor-prognosis prostate tumors. In this study, we investigated a potential interaction between MEIS1 and AR. We found that overexpression of MEIS1 inhibited the AR transcriptional activity and reduced the expression of AR target gene. A potential protein–protein interaction between AR and MEIS1 was identified by the immunoprecipitation and GST pull-down assays. Furthermore, MEIS1 modulated AR cytoplasm/nucleus translocation and the recruitment to androgen response element in prostate specific antigen (*PSA*) gene promoter sequences. In addition, MEIS1 promoted the recruitment of NCoR and SMRT in the presence of R1881. Finally, MEIS1 inhibited the proliferation and anchor-independent growth of LNCaP cells. Taken together, our data suggests that MEIS1 functions as a novel AR co-repressor.

Introduction

The prostate gland is one of the most predominant sites of male neoplasia. Researchers have identified the critical role deregulations of the androgen receptor (AR) pathway plays in both oncogenesis and eventual resistance to castration. Although there are newer and more effective therapy strategies targeting the AR, the majority of male continue to have disease progression [1]. The androgen and AR hold the central roles in the prostatic carcinoma maintenance and development [2]. In mammal cells, androgen is recognized by AR. Like other members of nuclear receptor superfamily, AR contains three major functional domains, including N-terminal transactivation domain (NTD), DNA-binding domain (DBD), and C-terminal ligand-binding domain (LBD) [2]. Two

*Corresponding author.
**Corresponding author. Fax: +86 10 66938110.
E-mail addresses: cuijn@ucmail.uc.edu (J. Cui), jpgao@163.com (J. Gao).
[1] These authors contributed equally to this work.

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transactivation functions of AR, a constitutively active activation function (AF-1) exerted by NTD and a ligand-dependent activation function (AF-2) exerted by LBD, are required for the signal transduction [3]. AR is arrested in the cytosol in the absence of androgen; however, it would be accumulated in nucleus in response to androgen [4]. In cell nucleus, AR needs to bind the androgen responsive element (ARE) sequences to regulate the expression of downstream genes responding to the androgen. These genes may be involved in AR activity and human prostatic carcinoma cell proliferation. Some co-regulators including coactivators and corepressors were found to be recruited by AR in this process. Thus, it is critical to identify novel molecular regulators in the androgen/AR signaling [1].

The TALE (Triple amino acid loop extension) transcription factors containing homeodomain, including Meis and Pbx families, are generally recognized for their roles in growth and differentiation during vertebrate embryogenesis and tumorigenesis [5]. As a member of TALE factors, MEIS1 belongs to homeodomain-containing MEIS family and is well-defined in modulating certain leukemia [6–8]. Aberrant expression of MEIS1 was detected in normal prostate, compared with tumor; whereas it would be a useful biomarker or therapeutic target in human prostate carcinoma [9,10]. Recent evidences suggested that appropriate MEIS/Hox/Pbx1 protein interactions would be involved in cancer etiology [11]. By establishing the data that MEIS1 would interact with homeodomain containing transcriptional factors, and play potential roles in prostate carcinoma regulation, we hypothesized that MEIS1 would interact with AR and regulate the AR signaling. In this study, we found that MEIS1 interacted with AR in vitro and in vivo. Overexpression of MEIS1 inhibited the ligand-mediated AR transcriptional activity, whereas knockdown of endogenous MEIS1 protein level via its small interfering RNA (siRNA) significantly enhanced AR transcriptional activity in response to R1881. Multiple lines of data had suggested that MEIS1 inhibits AR transcriptional activity via affecting AR cytosol/nucleus translocation and the recruitments of NCoR and SMRT in PSA promoter. More important, MEIS1 plays an important role in regulation of LNCaP cell proliferation and anchor-independent growth.

Materials and methods

Plasmids

The expression vectors of AR, NCoR, NCoR siRNA, SMRT, SMRT siRNA, ARE-Luc or PSA-Luc reporter gene were described previously [9,10]. The full length sequences of MEIS1 were amplified by PCR and cloned into pcDNA3.1 vector linked without or with a FLAG tag. Control siRNA and AR siRNA were from reference [9,10]. The MEIS1 siRNA expression constructs were prepared as follows: TCCAGAACTGGATAACTTG (targeted to sequence in MEIS1 mRNA nt 871-889) and CTTGATGATTCAAGCCATA (targeted to sequence in MEIS1 mRNA nt 886-904) following reference [12]. Plasmid pSilencer2.1-U6 negative control (Ambion) was used as a control vector. All of the vectors were confirmed by DNA sequencing.

Cell culture and reagents

Synthetic androgen R1881 was from Sigma (St. Louis, MO, USA), bicalutamide was from Melonepharma Co. Ltd., Dalian, China, and other agents from Amersham Biosciences, Piscataway, NJ, USA were used. Human prostate cancer cells LNCaP and PC-3 were from reference [9,10] and were cultured in complete Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA) with 10% fetal bovine serum (Invitrogen, Carlsbad, CA). Cells were cultured in a sterile incubator maintained at 37 °C with 5% CO_2.

Luciferase assay

PC-3 or LNCaP cells were seeded in 24-well plates (Corning, NY, USA) in DMEM supplemented with 0.5% charcoal-stripped FBS (Hyclone, Logan, UT, USA). Transfection was performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). Cells were cotransfected with plasmids or luciferase reporters and then harvested for analysis of luciferase and β-galactosidase activities, as described previously [13,14]. The luciferase assays were performed with or without androgen. Similar results were obtained from three independent experiments.

Antibodies and immunoblotting analysis (western blotting)

Antibodies against AR, MEIS1, PSA, NCoR1, SMRT, Lamin A/C, β-actin, and GAPDH were from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and reference [9,10,15]. A polyclonal anti-rabbit IgG antibody and anti-Flag monoclonal antibody both conjugated with the horseradish peroxidase (HRP) were from Sigma (St. Louis, MO, USA). LNCaP cells were seeded and cultured in six-well plates (Corning, NY, USA). The cells were transfected with plasmids or treated with compounds, harvested after 48 h. Total protein samples were performed by SDS-PAGE and IB assays following reference [16]. The blots were performed on three independent occasions with similar results.

Immunoprecipitation

LNCaP cells were transfected with FLAG-AR or FLAG-MEIS1 using Lipofectamine 2000. Cells were harvested and lysed in the immunoprecipitation buffer after 18–24 h culture at 4 °C. The co-immunoprecipitation analysis was performed and then detected by immunoblotting assays treated without or with 10 nM R1881 following reference [17].

GST-pull down assay

AR or MEIS1 was expressed as GST-fusion proteins in *Escherichia coli* (*E. coli*) strain BL21 and bound to Glutathione–Sepharose beads purified as described by the manufacturer (Amersham Biosciences, USA). The expression plasmid for FLAG-AR or FLAG-MEIS-1 was used for expression in HEK293T cells and purified by FLAG-beads. FLAG-AR or FLAG-MEIS-1 was incubated with GST alone, or GST-MEIS-1 or GST-AR fusion protein bound to GST-beads following reference [9,10]. The beads were precipitated, washed three times with binding buffer, and subjected to SDS-PAGE and WB assays.

ChIP

The ChIP assay was described previously [18]. LNCaP cells were transfected with plasmids or treated with compounds, and fixed by adding formaldehyde to the medium at a final concentration of