# Inhibition of Androgen Responsive Element Decoy DNA on the Promoter of Prostate Specific Antigen in Prostate Cancer Cell PC–3–M

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**Abstract** **Objective** To study the effect of androgen responsive element decoy DNA on the promoter of prostate specific antigen. **Methods** pGL3-PSA luciferase expression vector, containing 640bp DNA fragment of prostate specific antigen(PSA) gene 5' promoter region, was constructed. Meanwhile, a 23-mer phosphorothioated ARE decoy based on the deduced ARE sequence at the promoter of PSA gene was synthesized. The human androgen receptor (hAR) expression plasmid, pGL3-PSA, pRL-TK and ARE decoy DNA were co-transfected into PC3-M by lipofectaminTM2000. Through detecting the activity of luciferase, the effect of ARE decoy on AR was studied. **Results** Activity of luciferase was significantly reduced in the ARE decoy transfected cells, but not in the cells transfected with the control decoy. **Conclusions** ① The ARE decoy DNA can suppress the function of AR as a transcription factor. ② The utility of decoy strategy combined with reporter gene method provides a powerful tool for research, especially to evaluate the specific function of target gene regulation.

**Key Words** Decoy strategy; androgen responsive element; transcription factor; reporter gene

Androgen is essential for the development, differentiation and normal function of prostate gland. Androgen exerts its function via the intracellular androgen receptor (AR). AR is a ligand-mediated transcriptional factor that belongs to the superfamily of nuclear receptors. Activated by androgen binding, AR moves into the nucleus, binds to the specific DNA sequences—androgen responsive element (ARE) and regulates the transcriptional activity of target genes[1,2].

Prostate-specific antigen (PSA) is expressed in epidermic cells of prostate specifically[3], which is regulated by androgen[4]. The PSA promoter contains the ARE sequence AGAACAGCAAGTGTC at position-170. Activated AR can recognize and bind this responsive element[5].

The transcription factor "decoy" strategy is that applying double-strands oligodeoxynucleotides (ODNs) which has consensus sequence with endogenous cis-element and high-affinity to the transcription factor to transfect target cells and compete with the endogenous cis-element for binding to transcription factor. This will result in the attenuation of authentic cis-trans interaction, leading to the removal of transfactors from the endogenous cis-elements with subsequent modulation of gene expression, thus leading to prevention of the endogenous gene expression[6].

We combined decoy strategy with reporter gene method to observe the effect of exogenous androgen responsive element decoy DNA on the androgen receptor.

## *MATERIALS AND METHODS*

### Materials

All kinds of enzymes were purchased from Takara. RPMI1640 was the product of Hyclone. Dual-Luciferase Reporter Assay System was obtained from Promega. The human prostate carcinoma cell line PC3-M and the human AR expression plasmid hAR were established by our laboratory. Plasmid pGL3-Basic, pGL3-Control and pRL-TK were kindly provided by professor Gong yao-qin..

### Methods

#### Plasmid constructs

A 640bp fragment of the PSA promoter was generated using the polymerase chain reaction(PCR). The plasmid pPSA-EGFP was used as a template and a pair of specific primers were synthesized which containing digestion site of Sac I and Kpn I respectively. The se-

Grant sponsor: National Natural Science Foundation of China (No.39970807)

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quence of upstream primer was 5'GGGGTACCATTGGAATTCCACATTGTTTTGC3', the sequence of downstream primer was 5'GGGAGCTCAAGCTTGGGGCTGGGGAG3'. The PCR product was digested with Sac Ⅰ and Kpn Ⅰ restriction enzymes, agarose gel purified, and ligated into the vector pGL3-Basic pre-cut with Sac Ⅰ and Kpn Ⅰ enzymes. The 640bp DNA fragment was inserted into the upstream of luciferase gene. The recombinant plasmid was transformed into E.coli DH-5α, then was isolated and purified. After the plasmid was identified by double digestion and electrophoresis, it was sent to Boya co.for sequence measuring. The recombinant was named after pGL3-PSA.

**Decoy oligo deoxynucleotides synthesis and annealing**

The ARE decoy was designed based on the DNA sequence(-173 to -153) of the ARE in the promoter region of the human PSA gene. The control decoy had two mutations in the consensus ARE sequence and was used in the control studies. TEN buffer (10mmol/L Tris-HCl, 0.1mmol/L EDTA,0.1mol/L NaCl, PH 8.0) diluted oligo deoxynucleotides respectively. Complement oligo deoxynucleotides were mixed as equal mol, 95℃ 10min, then cooled down to room temperature slowly.

ARE decoy

5'-TGC AGA ACA GCA AGT GCT AGC-3'

3'-ACG TCT TGT CGT TCA CGA TCG-5'

Control decoy

5'-GTC TGA TAA AGG GTG TTC TTT TT-3'

3'-CAT ACT ATT TCC CAC AAG AAA AA-5'

**Cells culture**

PC3-M cells were maintained in RPMI1640 medium supplemented with 5% cattle bovine serum, 100 units/ml penicillin, and 0.1mg/ml streptomycin at 37℃ in a 5% $CO_2$ incubator.

**Tansfection experiments**

The day before transfection, PC3-M cells were seeded at a density of $2\times10^5$/per well in a 12 well. 18-24 hour later, when cells reached 90%-95% confluency, the decoy ODNs and a number of plasmids were co-transfected into PC3-M cells by Lipofectamin TM2000 according to the manufacturer's instructions. 0.4-1.6μg plasmid/decoy DNA and 1-4μl lipofectamin were diluted into 100μl medium without serum respectively. Then the above two were combinded and layed in room temperature for 20 minute. The complexes were added onto the cells cultured with 0.8ml serum-free medium. Plasmid pRL-TK was used as a internal control. The ratio of pRL-TK and experimental vector was 1:25.

**Cells harvest and luciferase activity detection**

Dual-luciferase Reporter Assay System was used for this procesure. The transfected cells were washed once by PBS and were harvested following the addition of 1×PLB (passive lysis buffer) by scrapping vigorously with a rubber policeman. The luminometer was programmed to perform a 2-second premeasurement delay , followed by a 10-second measurement period for each reporter assay. 100μl Luciferase Assay Reagent(LAR) and 20μl cell lysate were mixed in the luminometer tube and were placed in the luminometer to initiate reading. The figure was M1, which represented the firefly luciferase activity. Then another 100μl Stop&Glo Reagent was added into the above tube. The renilla luciferase activity carried by pRL-TK, recorded M2, was detected in the same way. To eliminate the influence of the efficiency of transfection, M1/M2 was used for representing the luciferase relative activity.

**Statistical analysis**

All numerical data were expressed as mean±standard error(SE) and results were considered significant if $P<0.05$ was obtained by appropriate analysis of variance (ANOVA) procedures and Students t-test. Every experiment was repeated three times.

## *RESULTS*

**PGL3–PSA construction and identification**

A 640 bp DNA fragment of the promoter of PSA gene was generated using PCR. It was connected with pGL3-Basic and lay the upstream of Luc+ gene (Fig 1). The recombinant could be cut into 640bp and 4.8kb fragments by Sac Ⅰ and Kpn Ⅰ enzymes. The results of digestion and sequence measurement were consist with those of anticipation.

In the recombinant plasmid the upstream promoter could regulate the expression of luciferase, that is to say, the activity of luciferase reflected function of the promoter directly. The same amount of pGL3-Basic, pGL3-Control and pGL3-PSA was respectively co-transfected with pRL-TK into PC3-M. pGL3-PSA, hAR and pRL-TK were cotransfected into PC3-M cultured with or without testosterone. The results (Fig 2) showed that luciferase activity of pGL3-PSA lay between that of pGL3-Basic and pGL3-Control. When hAR and testosterone existed, the activity of luciferase increased significantly.

**ARE decoy DNA transfection results**

To ensure the suitable ratio of pGL3-PSA and hAR expression plasmid,hAR fixed 1.5μg/ml was co-transfected with different dose of pGL3-PSA and pRL-TK into PC3-M(Fig 3). Then the pGL3-PSA fixed 0.19μg/ml and pRL-TK 7.6ng/ml were co-transfected with different amount of hAR into PC3-M (Fig 4). In the end, 0.19μg/ml hAR, 0.19μg/ml pGL3-PSA and 7.6ng/ml pRL-TK were used for co-transfection with different dose of ARE decoy DNA and took function in different time by harvesting cells 24h, 48h, 72h respectively after transfection. It turned out that ARE decoy DNA could inhibit the activity of luciferase significantly at the dose of 1.98μg/ml, in which the inhibition rate reached 95% (Fig 5).There were no significant difference in different action time of ARE decoy(Fig 6).

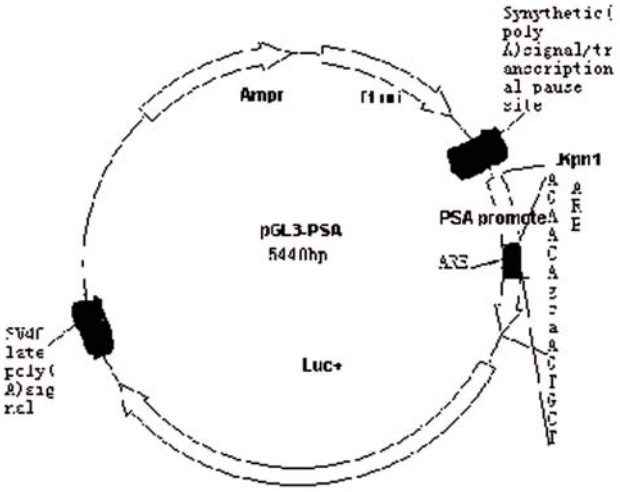


**Fig 1** pGL3-PSA plasmid map

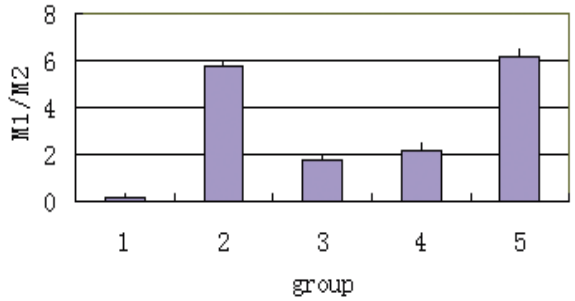


**Fig 2** Mesurment of Luciferrase activities in PC3-M
1. pGL3-Basic; 2. pGL3-Control; 3. pGL3-PSA;
4. pGL3-PSA+hAR; 5. pGL3-PSA+hAR+testerone

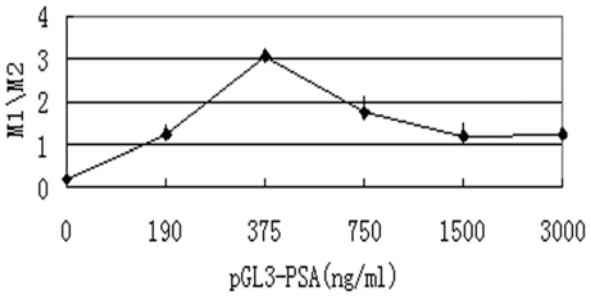


**Fig 3** Mesurement of Luciferase activities in PC3-M
Different amount of pGL3-PSA cotransfected with hAR

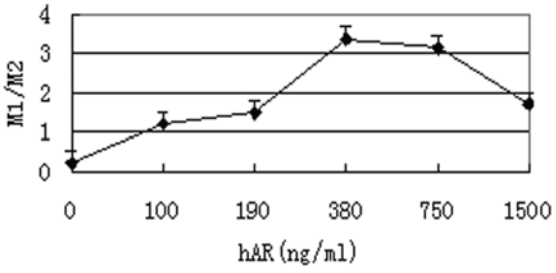


**Fig 4** Measurement of Luciferase activites in PC3-M
Different amount of hAR cotransfected with pGL3-PSA

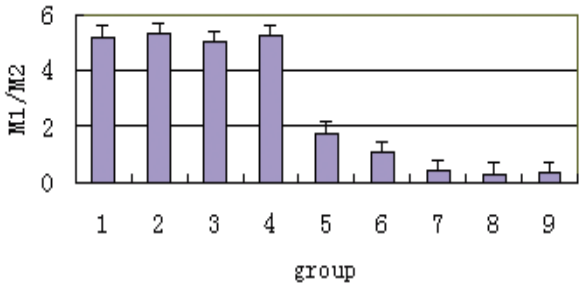


**Fig 5** Measurement of Luciferase activities in PC3-M
1. treated without any decoy DNA; 2-4 treated with 0.66μg/ml,1.32μg/ml, 1.98μg/ml control decoy DNA respectively; 5-9 treated with 0.66μg/ml,0.99μg/ml, 1.32μg/ml, 1.98μg/ml, 2.64μg/ml ARE decoy DNA respectively

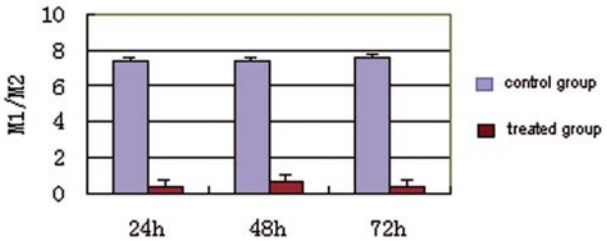


**Fig 6** Measurement of Luciferase assay activities in PC3-M in different time
Control group represented cotransfected pGL3-PSA, pRL-TK,hAR without any decoy DNA; experiment group represented pGL3-PSA,pRL-TK, hAR cotransfected with 1.98μg/ml ARE decoy DNA.

## DISCUSSION

Androgen regulates the growth and development of the prostate gland and is necessary for the maintenance of its functions. Androgen exerts its function through androgen receptor. In the absence of androgens, AR stays in the cytoplasm and binds to hot-shock protein in an inactive form. When activated by androgen binding, it changes its structure to form dimmer, moves into nucleolus and binds to a specific DNA sequence-androgen responsive element in the promoter/enhancer of target gene to initiate gene transcription. PSA is a differentiation marker for the human prostate. The expression of PSA is mainly induced by androgens at the transcriptional level. In the proximal PSA promoter, there are two functionally active androgen receptor-binding sites (ARs).

Recently, transfection of cis-element double-stranded oligodeoxy-nucleotides (ODNs), reffered to as "decoy" ODNs, has been reported to be a powerful tool in a new class of anti-gene strategies. Transfection of double-stranded ODNs corresponding to the cis sequence will result in the attenuation of authentic cis-trans interaction, leading to the removal of transfactors from the endogenous cis element and the suppression of related gene. In this experiment, we constructed luciferase gene eukaryotic expressin vector by linking the PSA promoter to luciferase gene(reporter gene). The effect of ARE decoy on transcription factor hAR was studied after co-transfection it with the recombinant hAR plasmid into PC3-M cells. The results showed that ARE decoy could suppress the transcriptional activity of AR by blocking its binding sites to the endogenous ARE sequence resulting in the inhibition of PSA promoter and luciferase expression.

Different cis-element decoy DNA can inhibit the correspondent transcrioption factor after transfected into cells so it can be used for studying the mechanisms of cis-element and transcription factor in transcriptional regulation as well as the interaction of cis-element and transcription factor. On the other hand, transfection of decoy DNA can induce the suppression of cell growth and the cell apoptosis by inhibiting the expression of some genes such as survivine gene and Bcl-2 gene. So, Transcription factor "decoy" stratedy has been a powerful tool that is useful for the study of transcriptional regulation and for gene therapy[7,8].

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