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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

In prostate cancer, androgen receptor (AR) supervises several key genes expressions. In the cell. AR exerts its regulatory control on a target cell only in the presence of its cognate ligand, androgen. The regulatory functions of AR are more complex and are fine-tuned by accessory proteins. These proteins are required for the maximum biological impact by androgen. These modulators, called coactivators, provide a positive stimulus for receptor action. Our laboratory has cloned the first nuclear receptor coactivators such as SRC-1, -2, and -3. They have the capacity to activate the transcriptional activity of steroid receptor. However, the role of steroid receptor coactivators in prostate gland is still unclear. To know these genes function in the human prostate cancer, we have performed *in situ* hybridization on human prostate cancer samples, construction for over- and under-expressing SRC-3 gene to get stable cell line. During this 1st year's work, we have examined the SRC-3 is highly expressed in the prostate tumor area and its expression is highly correlated with tumor genesis. Also, we have successfully established stable SRC-3 expressing cell line using RU486 induction system. We are now investigating the role of SRC-3 in the prostate cancer using this SRC-3 stable cell line.

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Introduction

Prostate cancer is the most frequently diagnosed solid tumor and the second leading cause of cancer death in man in the United State (1). Cancer initially arises from abnormal expression of certain key genes, which oversee vital cellular processes. In prostate cancer, it is the task of the androgen receptor (AR) to supervise the expression of these key and vital genes in the cell. AR exerts its regulatory control on a target cell only in the presence of its cognate ligand, androgen. In the cell, the regulatory functions of AR are more complex and are fine-tuned by accessory proteins. These accessory proteins are required for the maximum biological impact by androgen. These modulators, called coactivators, provide a positive stimulus for receptor action (2-9). Our laboratory has successfully cloned the first nuclear receptor coactivator, steroid receptor coactivator, SRC-1 (2). Subsequently, the other two members were cloned later (2, 7, and 8). They all have the capacity to activate the transcriptional activity of steroid receptors. However, the role of steroid receptor coactivators (SRCs) in prostate gland and other reproductive organs is still unclear. It has been shown that SRC-3 is overexpressed and amplified in 60% and 18% of breast cancer samples respectively. Also, we have demonstrated that in human breast and prostate cancer cell lines, SRC could enhance the AR transcriptional activity and DNA synthesis. Therefore, we hypothesize that the SRC may play an important role in the initiation and progression of prostate cancer. To test this hypothesis, we have performed 1) *in situ* hybridization on human prostate cancer samples to examine the expression pattern of SRC-3 in prostate cancer, 2) construction of over- and under-expressing vectors for SRC-3 in prostate cell lines to examine its role in cell growth, and 3) construction of SRC-3 expression vector to generate transgenic mice overexpress SRC-3 in the prostate.

Body

This work was carried out according to the following three specific aims;

Aim 1. Study the expression of steroid receptor coactivators in human prostate cancer samples.

Expression analysis of endogenous steroid receptor coactivators and AR in human prostate cancer tissue samples. They will be correlated with each other.

Aim 2. Study the effect of over- and under-expression of steroid receptor coactivator-3 on prostate cancer cell cycle progression in Cells.

Design and development of *in vitro* models of SRC-3 over-expression in the prostate cancer cell lines. Then analyze the growth properties of these stably transfected cell lines that over-expressed SRC-3.

Aim 3. Evaluate the role of steroid receptor coactivator-3 in prostate cancer progression in vivo.

We propose to generate transgenic mouse expressing SRC-3 in prostate epithelial cells under the control of the prostate specific antigen (PSA) promoter. This promoter has been shown to be highly specific to direct its target to the lateral prostate. The transgenic mouse obtained will then be induced to produce prostate tumors by mating with murine strains exhibiting susceptibility to prostate cancer. The ability of SRC-3 to activate androgen receptor will be examined in correlation with the tumor growth in these animals.

During the first year's work, we have accomplished the following subjects;

1. Expression analysis of SRC expression in human prostate tumor samples

To investigate the role of SRC in the prostate tumors, first, we analyzed SRC-3 expression profile in the prostate tumor samples using *In situ* hybridization technique. Human prostate tumor samples were used for this experiment. The SRC-3 expression level was then used to correlate with the combined Gleason score and stages of prostate caner.

Antisense probe for *in situ* hybridization was generated for the specific region of SRC-3 gene, which is specific for the transcripts of SRC-3 in the prostate. Sense probe was also generated as a negative control (data not shown). *In situ* hybridization analysis have reveled that transcripts of SRC-3 gene abounded in the tumor area of prostate but not in the adjacent normal area of prostate.

We have examined a total of 134 patient samples and results are summarized in Table 1. As shown in Table 1, we observed that approximately 47% of prostate tumor samples overexpressed SRC-3 in the tumor area (n=134) but only 8.2% of samples overexpressed SRC-3 in the adjacent normal areas (n=61).

Table 1. Incidences of SRC-3 expression in the prostate cancer patients

	Total Sample	SRC-3 Positive (%)
Tumor Area	134	63 (47.0%)
Non-tumor Area	61	5 (8.2%)

We then correlated the SRC-3 expression with the combined Gleason score. This combined Gleason score counting method is commonly used in prostate area when prostate cancer is diagnosed. As shown in table 2, SRC-3 expression level correlate well with the Gleason score. This result suggests that SRC-3 is an excellent marker for late stage of prostate cancer.

Table 2. Correlation of SRC-3 expression with combined Gleason Score

Gleason Score	5	6	7	8	9
SRC Positive Sample	2 (12%)	14 (43%)	39 (54%)	6 (55%)	2 (100%)
Total Analyzed Sample	17	32	72	11	2

Also, we have correlated SRC-3 expression with stage of prostate caner. As shown in the table 3, SRC-3 expression was most prominent in the T3b stage, seminal vesicle invasion stage. We could see moderate level of SRC-3 expression in T3a and D1 stage as well as low level expression in T2 stage.

Table 3. Correlation of SRC-3 expression with stage of cancer

Stage*	T2	T3a	T3b	D1
SRC-3 positive	28 (36%)	18 (53%)	16 (80%)	1 (50%)
Total Analyzed	78	34	20	2

* T2: organ confined
T3a: extracapsular extension
T3b: seminal vesicle invasion
D1: pelvic lymph node with metastatic disease and distant metastasis

2. Construction and analysis of SRC-3 over-expressing vector

One of our goals of this work is to construct expression plasmids for coactivators to generate stable cell lines overexpressing SRC-3. We elected to use RU486 inducible two vector expression system developed in our laboratory to expressed SRC-3 (10). Regulator plasmid was constructed in the mammalian expression vector pcDNA3 (Invitrogen). pcDNA3 was used for this construction because this vector contains strong constitutive cytomegalovirus (CMV) enhancer-promoter sequence for the high level of expression of the regulator protein. The regulator consists of Gal4-DNA binding domain (DBD), PR LBDΔ891 domain, and p65-activation domain (AD) (Fig. 1A). As shown in Fig. 1, we have made this regulator driven by CMV promoter. It is expressed constitutively but is inactive in the absence of RU486. When RU486 was added in the cultured cell, inactive form of regulator undergoes a conformational change and becomes active. This activated regulator dimerizes and binds to the GAL4-DNA binding site of target gene in front of SRC-3 to induce SRC-3 target gene expression (Fig. 1B). An influenza hemagglutinin (HA) was tagged to the N-terminal region of SRC-3 gene as positive expression control (Fig. 1B).

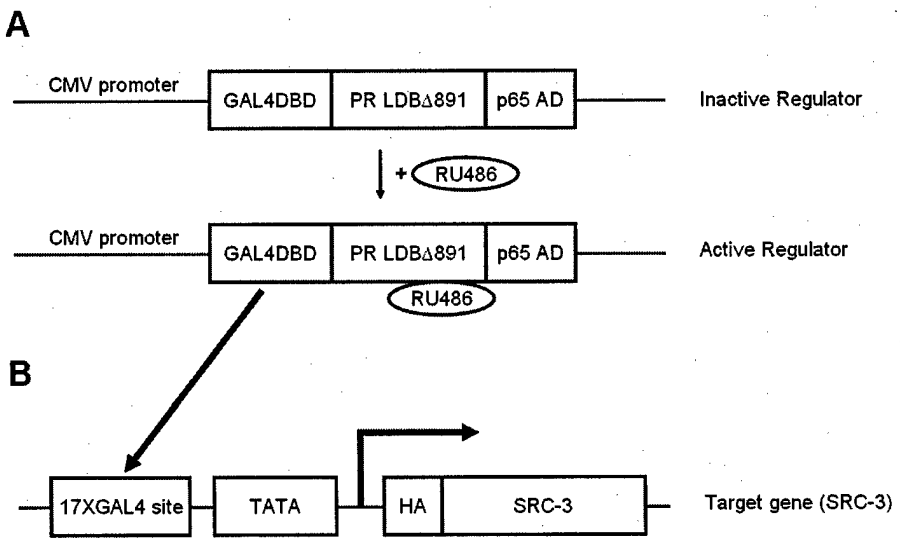

Figure 1. A schematic diagram of RU486 induction system for SRC-3 overexpression.

Stable transfectants were generated from androgen dependant human prostate cancer cell line, LNCaP. They were obtained through selection with 400ug/ml G418 at 37°C CO_2 incubator for 2 to 3 weeks. All of the G418 positive stable clones were then incubated for 24 hours in the presence or absents of 10^{-8}M RU486 for the induction test. RU418 untreated clones were used as negative control. As shown in figure 2A, RU486 treated clones expressed SRC-3 as detected by anti-SRC-3 antibody. In contrast, RU486 untreated clones did not express SRC-3 (compare Fig. 2A, middle panels). As expected, SRC-3 N-terminal tagged marker, HA, was detected only in the RU486 treated samples (Fig. 2A, upper panels). This result suggests that the recombinant SRC-3 were generated correctly by activated regulator but not generated by inactive regulator. Therefore, clone number 6, 9, 12, 24, and 48 were regarded as correct clones since SRC-3 expression is induced only in the presence of RU486. Anti-β-tubulin antibody was used as control (Fig. 2A, bottom panels).

Among these positive clones, the clone number 48 was subjected to time course induction experiment (Fig. 2B). As shown in figure 2B, SRC-3 expression was induced after 2 hours RU486 treatment and remains elevated levels until 28 hours after treatment.

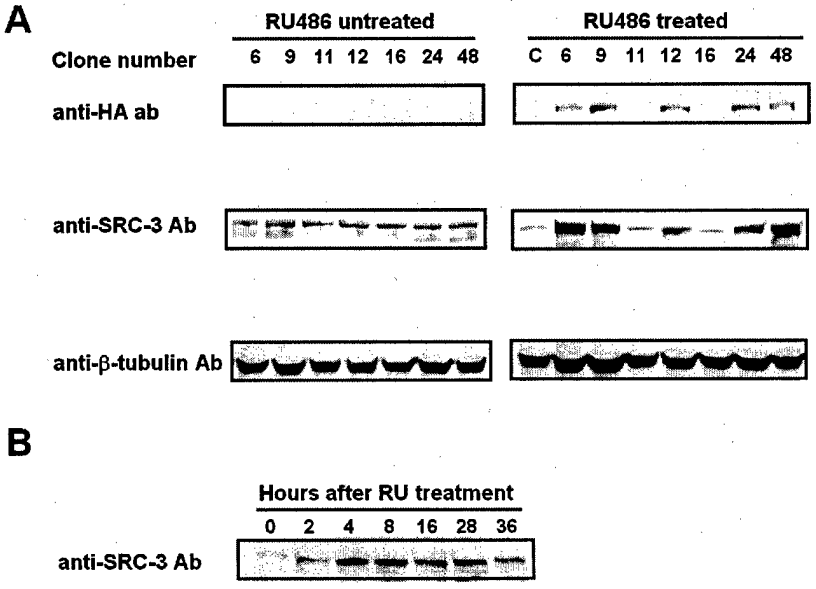

Figure 2. Inducible expression of SRC-3 in LNCaP cell lines by RU486 treatment.

3. Generation of transgenic mouse line expressing SRC-3

DNA for SRC-3 expressing in transgenic mouse has been constructed. The construct will be injected into the animal in the coming year (data now shown).

Key Research Accomplishments:

1. We detected SRC-3 overexpression in human prostate tumors but not in the adjacent non-tumor area.
2. We found SRC-3 level is highly correlated with Gleason score and tumor stages.
3. We have successfully established the RU486 inducible expression system in prostate cell lines.
4. We have constructed transgenic expressing construct for SRC-3 in the prostate gland.

Reportable Outcomes

1. We have established the RU486 inducible expression system in prostate cell lines.
2. We have generated SRC-3 overexpressing stable cell line.
3. We have constructed transgenic expressing construct for SRC-3 in the prostate gland.
4. Because of the support of this award, I have research opportunities as a postdoctoral fellow in Dr. Ming-Jer Tsai's group in Baylor College of Medicine during past one year, which contributed my personal and professional career.

Conclusions

During the 1st year's work, entitled in "The role of steroid receptor coactivators in the developmental cancer", we have accomplished several important results as presented in above. Briefly, SRC-3, one of steroid receptor coactivators, is overexpressed in prostate cancer patients, especially in tumor area of prostate cancer (47%). Also, SRC-3 overexpression is correlated with the increasing severity of prostate cancer. Furthermore, we have established a SRC-3 inducible stable cell lines. These SRC-3 inducible stable cell lines will be analyzed in the second year of this grant.

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Appendices

- Figures and tables are embedded in the text.