行政院國家科學委員會專題研究計畫　成果報告

由動物實驗模式探討雄性素如何改變雄性素受體的輔助因子之表現

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英文摘要

Abstract

Background and purpose: Androgen receptor (AR) coactivators modulate the transcriptional activity of AR and may activate AR-associated genes upon androgen deprivation. Changes of expression patterns may help reveal the role of AR cofactors in androgen-independent prostate cancer. This study investigated changes of expression patterns of several AR coactivators after androgen deprivation in an androgen-dependent prostate cancer cell line (LNCaP).

Methods: LNCaP cells were cultured in RPMI medium with charcoal/dextran-treated FBS for 28 days. Samples of total RNA collected at one-week intervals were analyzed using a non-isotopic multi-probe ribonuclease protection assay system with an hAR multi-probe template set. Expression changes of 10 mRNAs of interest (AR, Rb, ARA160, ARA24, ARA54, ARA55, ARA70, BRCA1, F-SRC-1, and RAC3), were analyzed simultaneously as AR and AR-associated cofactors in one hybridization reaction.

Results: Seven of ten cofactors tested were expressed in LNCaP cells. Only Rb, ARA55 and BRCA1 were not detected. LNCaP cells cultured in charcoal-treated serum for 28 days clearly showed more than 1.5-fold increases in AR, ARA160, and ARA70 expression, while Expression of ARA24 and ARA54 increased less than 1.5-fold. Only RAC3 and F-SRC-1 decreased in RNA expression. Cell morphology features changed gradually into neuron-like shapes with elongated cytoplasm during the culture period. Cell growth almost ceased 28days after the start of culture.

Conclusions: The changes in expression pattern of AR and AR cofactors and in particular the remarkable increase in AR, ARA160 and ARA70 support the role of AR cofactors in modulating prostate cancer cell growth from androgen dependence to androgen independence.

KEY WORDS:

androgen receptor, coactivators, prostate cancer, ribonuclease protection assay.

中文摘要

截斷雄性激素能相當有效的抑制前列腺癌細胞之生長。雖然荷爾蒙治療相當有效，但會漸漸的失去對荷爾蒙治療之感受性。為何前列腺癌細胞會由荷爾蒙依賴型轉成荷爾蒙不依賴型?現有許多假說及實驗證據解釋其原因。在這其中，以 AR 的輔助因子（cofactor）最受重視。許多分子生物學的實驗證據顯示在雄性激素相當低量微弱下，AR 的 cofactor 可以增強 androgen 與其 receptor 接合後啟動下游基因之生物效應、或甚至在 cofactor 存在下使 estrogen 也能啟動 AR 下游基因之生物效應.第一個被發現的雄性激素受體的輔助因子是 1996 年的 ARA70。基於 AR cofactor 的特別功能，新的 AR 調節的轉錄途徑因此被發現.在 ARA70 或是 ARA55 輔助下，抗雄性激素藥物例如 Hydroxyflutamide 或 Casodex 可能變成雄性激素。在 AR cofactor ARA70 協同作用下，天然的雌性激素(17beta-estradiol)，可作用類似雄性激素，並活化雄性激素受調控基因。這說明抗雄性激素藥物從拮抗性變成同質性(agonist)的途徑可受到 AR cofactor 的調節.簡而言之，AR cofactor 很可能就是前列腺癌轉變成雄性素不依賴型的關鍵蛋白質。過去的研究報告，是屬於基因層次的證據，進一步必須要有細胞生物學及組織學上的佐證。因此本研究將 androgen dependent 的前列腺癌之細胞株(LNCaP)培養於 androgen deprivated Charcoal treated FBS medium，以瞭解細胞生長在缺乏雄性素環境下，這些 AR cofactor 是否其 mRNA 表達的量是否會有變化, 本實驗完全採用 non-isotope methods 進行 Multi-probe RNase protection assay: 選取 AR 相關之 cofactors 的基因序列設計一組多重探針之模版，可以同時間定量分析一系列之 RNA。這種變化,將是支持進一步研究 AR cofactor 是前列腺癌轉變成雄性素不依賴型的關鍵.

由初步的結果可知當 androgen dependent prostate cancer cell (LNCaP)培養於 androgen deprivation 的環境下其 AR 及 ara24 的 mRNA 會增加.而 ara54 ara70 及 RAC3 的 mRNA 則減少. 這個結果不同於已有的文獻報告認為 ara70 可能是前列腺癌轉變成雄性素不依賴性的關鍵蛋白質的理論.

關鍵詞：雄性素受體，雄性素受體輔助因子，前列腺癌

計畫成果自評:

研究成果的學術或應用價值: 以 Non-isotope method 進行 multiple RPA assay methods 是高難度的定量檢測 RNA 的方法,不但可同時定量多組樣本且可以同時比較一系列多達十種之 mRNA 這是文獻上尚未見過的報告,值得一般實驗室採用.

主要發現: 由初步的結果可知於 androgen deprivation 的環境下其 AR 及 ara24 的 mRNA 會增加.而 ara54 ara70 及 RAC3 的 mRNA 則減少.

本次研究之方法及結果已刊載於 J Formos med Assoc 2005 Vol 104 No9 Page 652—658. 學術期刊發表。這個結果不支持已有的文獻報告的理論認為 ara70 可能是前列腺癌轉變成雄性素不依賴型的關鍵蛋白質. 因此如何看待 AR cofactor 在 androgen independent prostate cancer cell 形成過程中扮演的角色,將需要不同的分子生物學的解釋.

In Vitro Gene Expression Changes of Androgen Receptor Coactivators after Hormone Deprivation in an Androgen-Dependent Prostate Cancer Cell Line

Hong-Chiang Chang, Shyh-Chyan Chen, Jun Chen, and Ju-Ton Hsieh

Background and Purpose: Androgen receptor (AR) coactivators modulate the transcriptional activity of AR and may activate AR-associated genes upon androgen deprivation. Changes of expression patterns may help reveal the role of AR cofactors in androgen-independent prostate cancer. This study investigated changes of expression patterns of several AR coactivators after androgen deprivation in an androgen-dependent prostate cancer cell line (LNCaP).

Methods: LNCaP cells were cultured in RPMI medium with charcoal/dextran-treated fetal bovine serum for 28 days. Samples of total RNA collected at 1-week intervals were analyzed using a non-isotopic multi-probe ribonuclease protection assay system with a human AR multi-probe template set. Expression changes of 10 mRNAs of interest (AR, Rb, ARA160, ARA24, ARA54, ARA55, ARA70, BRCA1, F-SRC-1, and RAC3), were analyzed simultaneously as AR and AR-associated cofactors in 1 hybridization reaction.

Results: Seven of 10 cofactors tested were expressed in LNCaP cells. Only Rb, ARA55 and BRCA1 were not detected. LNCaP cells cultured in charcoal-treated serum for 28 days clearly showed more than 1.5-fold increases in AR, ARA160, and ARA70 expression, while expression of ARA24 and ARA54 increased less than 1.5-fold. Only RAC3 and F-SRC-1 decreased in RNA expression. Cell morphology features changed gradually into neuron-like shapes with elongated cytoplasm during the culture period. Cell growth almost ceased 28 days after the start of culture.

Conclusion: The changes in expression pattern of AR and AR cofactors and in particular the remarkable increase in AR, ARA160 and ARA70 support the role of AR cofactors in modulating prostate cancer cell growth from androgen dependence to androgen independence.

Key words: Androgen receptors; Nuclease protection assays; Prostate neoplasms; Transcription factors

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Several androgen receptor (AR) cofactors have been found, including ARA70,[1] ARA55,[2] and ARA54[3] for the C-terminal AR-ligand binding domain, ARA160,[4] and ARA24[5] for the N-terminal. Other AR cofactors have also been shown to be associated with the AR DNA-binding domain or AR N-terminal domain, such as RB, SRC-1, and TFIIH. ARA70, ARA55 and ARA54 can enhance 5α-dihydrotestosterone (DHT)-mediated AR transcriptional activity and these interactions between AR and cofactors are androgen-dependent.

Androgen ablation has been the cornerstone of treatment for advanced prostate cancer, but the effect is often short-lived, as hormone-refractory elements continue to proliferate. Several theories postulate why prostate cancer can transform from hormone-dependent to hormone-independent status. These theories include: AR mutations; post-receptor alterations; and alterations in down-stream AR target genes. However, AR cofactors have assumed increased importance since Yeh and Chang's studies suggested their role in 1996.[1] Their evidence showed that the AR cofactors can enhance the ability of AR to activate the AR target gene during androgen ablation or androgen blockage.[2] It was also suggested that some selective AR coactivators can modulate the specificity of sex hormones and antiandrogens.[6,7] Because of this phenomenon, the prostate cancer cell can keep growing in an androgen-deprived status.

Thus, AR coactivators could be the key protein supporting prostate cancer cell growth in an androgen-independent mode. Specific changes in the gene expression profile of AR coactivators could explain the molecular mechanism of prostate cancer cells becoming hormone independent. We hypothesized

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that the expression of AR coactivators, e.g., ARA70 or ARA55, may increase in prostate cancer cells after long-term androgen deprivation, enhancing AR function and converting the AR antagonist to agonist.

In order to mimic hormone-refractory prostate cancer cell growth, an androgen-dependent prostate cancer cell line (LNCaP) was cultured in hormone-deprived status. The expression of mRNAs collected serially from the culture was analyzed. In order to simultaneously analyze multiple targets, we used a multiple ribonuclease protection assay (RPA) template with 10 of the AR cofactors and applied the non-isotope multi-probe RNAse protection assay to investigate the effect of androgen deprivation on mRNA expression of AR cofactors. This technique enabled collection of data to determine whether the increased expression of AR cofactors is compatible with transition from androgen dependence to androgen independence.

Methods

Materials

RPMI-1640 medium, penicillin/streptomycin, fetal bovine serum (FBS) and trypsin/ethylenediamine tetra-acetic acid were purchased from Life Technologies, Inc. (Grand Island, NY, USA). Charcoal/dextran-treated certified FBS (lot no. AKD11642, C-FBS) was from Hyclone (Logan, UT, USA), with a certificate that the concentration of testosterone was below 3.0 ng/dL.

Cell culture and RNA isolation

The prostate cancer cell line, LNCaP, was purchased from the American Type Culture Collection (Rockville, MD, USA). Cells were grown in phenol red-positive RPMI-1640 medium supplemented with 10% FBS, 1% glutamine and penicillin/streptomycin at 37°C in an atmosphere of 5% carbon dioxide in air. We developed a subline of LNCaP cells grown in phenol red-free RPMI-1640 medium supplemented with 10% C-FBS. Cells were fed twice per week and split once per week with trypsinization. We developed this subline of LNCaP cells under conditions which mimic androgen-deprived growth. RNAs of this LNCaP subline were collected serially on the 7th, 14th, 21st, and 28th day of the culture.

Total RNA was extracted from the cells using TRIzol (Invitrogen,Carlsbad, CA, USA) according to the manufacturer's specifications. Total RNA was extracted in chloroform, ethanol precipitated, and stored at –80°C. A total of 5 RNA samples were collected for serial analysis in 1-week intervals, 1 before and 4 after androgen deprivation.

Non-isotopic multi-probe ribonucleic protection assay

Our multi-probe RPA successfully created non-isotopic conditions by combining the human AR (hAR) Multiple-Probe Template set (BD Pharmingen, Franklin Lakes, NJ, USA), the MAXIscript T7™ in vitro transcription kit (Ambion, Austin, TX, USA), Biotin-14-CTP (GIBCO, Carlsbad, CA, USA) for nucleotide labeling, the RPAIII kit and the BrightStar™ BioDect™ kit (Ambion). This novel non-radioisotopic method can simultaneously and rapidly measure mRNA changes of 10 AR-associated coactivators.

The hAR multi-probe template set contains DNA templates that can be used for the T7 RNA polymerase-directed synthesis of a biotin-labeled, anti-sense RNA probe set that can hybridize with target human mRNAs encoding ARs,[8] RB,[9] ARA160,[4] ARA24,[5] ARA54,[3] ARA55,[2] ARA70,[1] BRCA1,[10] F-SRC-1,[11] RAC3,[12] as well as 2 housekeeping gene products, L32 and GAPDH (Table). The multiple hAR RNA probes were synthesized using the MAXIscript T7™ in vitro transcription kit. Additionally, 0.4 μL biotin-14-CTP (10 mM) in the ratio of 40% biotin-CTP: 60% CTP was used as the labeling nucleotide in each 20 μL in vitro transcription reaction. NucAway™ spin columns (Ambion) were used to remove unincorporated nucleotides and salts after probe synthesis reactions.

The multi-probe RNAse protection assay was performed using the RPAIII kit. For the purpose of non-isotopic design, a biotin-labeled anti-sense RNA probe set was used according to the manufacturer's protocol. The probe set is hybridized in excess to target RNA in solution, after which free probes and other single-stranded RNAs are digested with RNase. The remaining RNase-protected probes are purified and analyzed by resolving on denatured polyacrylamide gels.

Table. Twelve human androgen receptor (AR) multi-probe templates with expected sizes of full-length probes and protected fragments.

Template	Size (bp)	
	Full-length probe	Protected probe
AR	429	400
Rb	381	352
ARA160	349	320
ARA24	315	286
ARA54	284	255
ARA55	256	227
ARA70	231	202
BRCA1	211	182
F-SRC-1	190	161
RAC3	170	141
L32	141	112
GAPDH	126	97

Briefly, after denaturing at 90-95°C for 2-3 minutes, dried total RNA (10 µg) with 1 µL biotin-labeled multi-probe sets was hybridized (56°C; 12 hours) in 9 µL RPAIII hybridization buffer. Reaction mixtures were then treated with 100 µL (500X diluted) RNase A/RNase T1 Mix (Ambion) and incubated 45 minutes at 30°C. Digestion and precipitation were performed by adding 150 µL RPAIII inactivation/precipitation III solution (Ambion). RNA was precipitated from each reaction and pellets were washed with ethanol, air dried, resuspended in gel loading Buffer II (Ambion), heated (95°C, 3 minutes), and resolved on a denatured 5% polyacrylamide gel at 250 volts for 3.5 hours. The RNase-protected probes on the polyacrylamide gel were then electro-blotted on to a positively charged nylon membrane. The biotinylated RNA probe on the nylon membrane was then detected by BrightStar™ BioDect™ kit and the film was exposed by light emission for 1-2 hours. Protected bands on the X-ray film were quantified by densitometry. Relative levels of protected probes were calculated by normalizing the specific band to the GAPDH band included in the multi-probe kits.

For each multi-probe RPA assay, negative controls included 5 µL of probe mixture incubated with 10 µg of yeast tRNA. The probes-only control was treated with 1X RNase digestion buffer in the absence of RNase A/RNase T1 Mix.

Results

Cell growth properties of LNCaP cells in charcoal-treated serum

We examined the in vitro growth properties of LNCaP cells under steroid-reduced culture conditions. With continuous passage of LNCaP parental cells in the medium, supplemented with 10% charcoal-treated FBS, we found that cell morphology features and growth rate changed gradually (Fig. 1A-1C). The LNCaP cells are epithelial cancer cells and the typical doubling time is 2 days. After culture for 2 weeks in charcoal-treated serum, the cell growth rate decreased and the size of cell bodies became smaller. During the fourth week, obvious morphologic changes in LNCaP cells occurred, with loosening of the attachment to cell culture dishes, with cells changing to neuron-like shapes with elongated cytoplasm, and cell growth almost ceased.

Multi-probe RPA study

Four samples of RNA were collected from LNCaP cells cultured in charcoal-treated FBS for 1, 2, 3 and 4 weeks, and 1 sample of RNA from LNCaP in regular FBS. The results of chemiluminescent exposure of

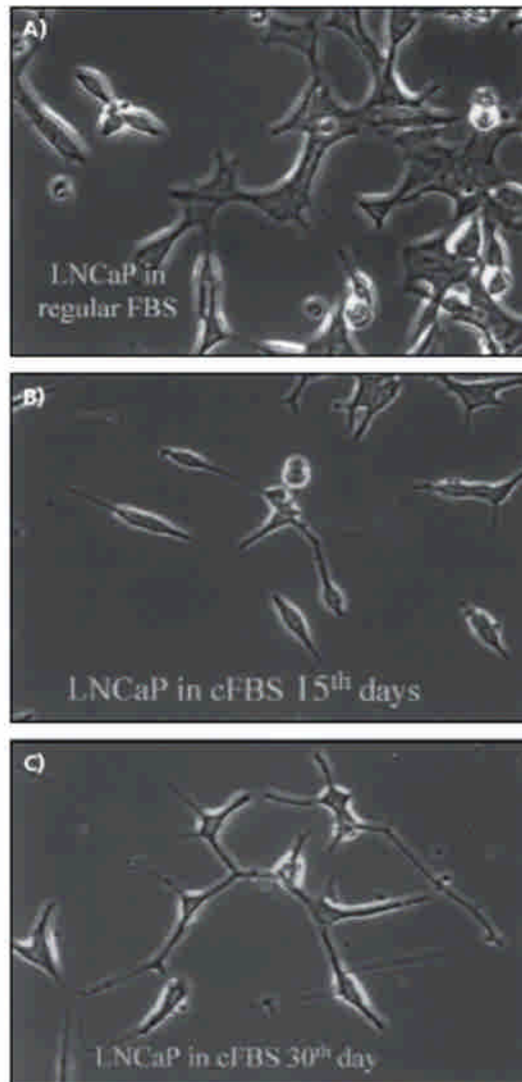

Fig. 1. Phase-contrast micrographs of different androgen-dependent prostate cancer cell line (LNCaP) cells (× 200). A) LNCaP cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS). B) LNCaP cells were cultured in RPMI-1640 medium supplemented with 10% charcoal-treated FBS for 2 weeks. C) LNCaP cells were cultured in the same condition as in part B, for 4 weeks.

samples on film are shown in Fig. 2 and include the lane of the hAR multi-probe not treated with RNases (lane 1). Fig. 2 also shows results for the corresponding RNase-protected probes following hybridization with RNA from LNCaP (10 µg, lane 4-8) and BrightStar™ Biotinylated RNA century™ size markers (Ambion) [1 µg, lane 3]. Each probe band (lane 1)

migrated more slowly than its protected band (lanes 4-8). Lane 2 of Fig. 2 shows the negative controls for each multi-probe RPA assay. The expressions of mRNA are indicated in relative light units of CDP-Star™ chemiluminescent substrate exposure on film. The band was normalized to GAPDH RNA level in each lane. The data were collected from 3 sets of independent experiments and showed as mean ± standard deviation (SD). Our data demonstrated the serial changes of AR cofactors expression from androgen-dependent to androgen-independent status. As shown in Fig. 3, we found that LNCaP cells cultured in charcoal-treated serum for 28 days would induce more than 1.5-fold expression in AR, ARA160, and ARA70. Another 2 mRNAs, ARA24 and ARA54, also showed an increased pattern but the ratio was less than 1.5-fold. Only RAC3 and F-SRC-1 decreased in RNA expression. The 3 bands of Rb, ARA55, and BRCA1 were too low to be detected.

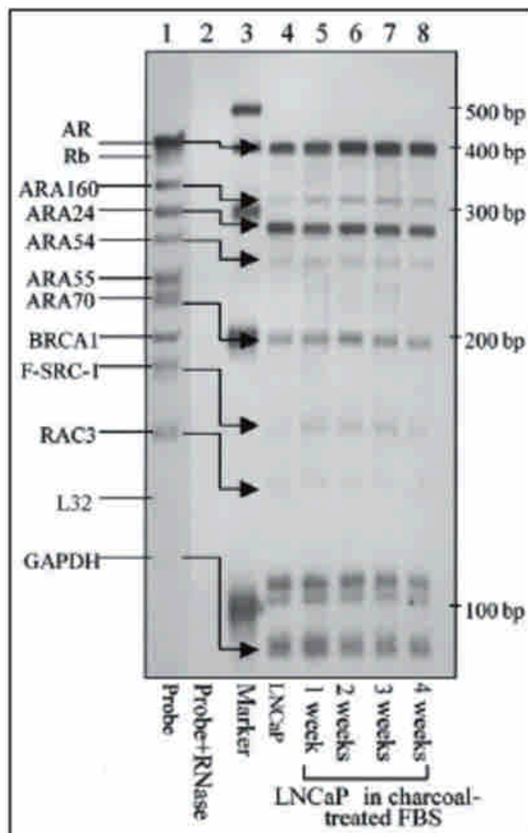

Fig. 2. Multi-probe ribonuclease protection assay study for androgen receptor and its associated cofactors of androgen-dependent prostate cancer cell line (LNCaP) cells influenced by steroid-reduced culture medium. LNCaP cells were cultured in RPMI-1642 with charcoal-treated fetal bovine serum (FBS) for 4 weeks. Samples of total RNA (10 μg, lanes 5-8) were isolated weekly. A GAPDH probe was used as a control for equivalent mRNA loading.

Discussion

Prostate cancer has become the most frequently diagnosed neoplasm in the United States, and the sixth most frequently diagnosed cancer in Taiwan. Androgen ablation has been the cornerstone of treatment for advanced prostate cancer, but the mechanisms responsible for androgen independence remain uncharacterized. In the past 10 years, knowledge of AR-associated proteins has provided a new direction to study the mechanism of androgen-independent prostate cancer cell growth. Much evidence in molecular biology supports the linkage between AR cofactors and androgen-independent growth.[6,7,13,14]

Miyamoto et al reported that antiandrogens (hydroxyflutamide) can activate androgen target genes in the presence of ARA70 or ARA55 in the DU145 cell line.[6] AR coactivators may be the key trigger factors between androgen-dependent and androgen-independent status. However, hydroxyflutamide can also behave as a partial agonist depending on the cell and tissue context. This suggests that the mechanism of action is complex and tissue-specific. Furthermore, prostate cancer cells may develop resistance to hydroxyflutamide or may even cycle between phases of resistance and responsiveness. This varied response along with abundant other evidence, suggests that hydroxyflutamide resistance is due to a cellular adaption phenomenon rather than to permanent genetic alterations.[7]

Yeh et al reported that E_2-AR-ARA70 also plays an essential role in AR function.[13] ARA70 can induce AR transcriptional activity in the presence of 10 nM E_2. ARA70 is an essential factor to modulate this pathway; without ARA70, this pathway would be closed.[13] Androstenediol, a natural estrogen, can activate AR target genes in the presence of AR. ARA70 can further enhance this androstenediol-induced AR transcriptional activity. Hydroxyflutamide fails to block androstenediol-mediated induction of AR transcriptional activity in prostate cancer cells.[15] This may raise critical questions about the possible role of Adiol and ARA70 in overcoming the effects of androgen ablation therapy for prostate cancer.[15-17]

It has been suggested that the theory of post-receptor alterations can explain the relative expression of coactivators modulating the regulation of androgen and antiandrogen for AR transcriptional activity.[18] Agonist/antagonist receptor interactions

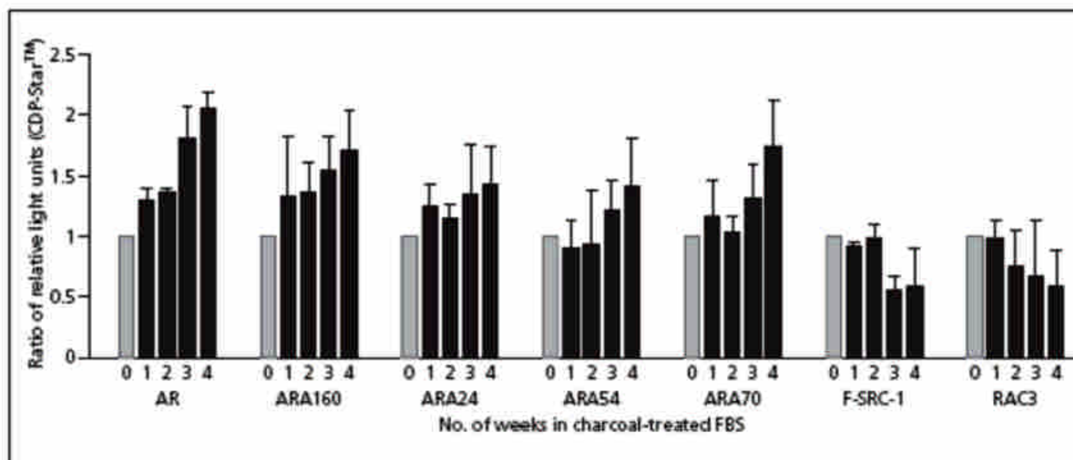

Fig. 3. Bands from Fig. 2 were quantified by densitometry. The expressions of mRNA are indicated in relative light units of CDP-Star™ chemiluminescent substract exposure on film. Expression of GAPDH gene was analyzed for standardization purposes and the expression level in lane 4 was set as 100% for comparison with lanes 5-8. The data represent the mean ± standard deviation from 3 independent experiments. FBS = fetal bovine serum; AR = androgen receptor.

alone may not be able to explain the AR function, and the interaction between the ligand-receptor complex and cofactors could be essential for steroid hormone function and sensitivity.

In summary, AR cofactors might be the critical proteins that enhance the effect of minimizing androgen, turn on the ARs activated via estrogen, or promote agonist activity of antiandrogens. Although previous evidence for these postulations is well demonstrated at the transcriptional level by in vitro study, this study has provided more data on cell biology to support them. In this study, the expression of AR coactivators was investigated in the human prostate to provide a better understanding of androgen deprivation action in prostate cancer. Some of those coactivators (e.g., ARA70 and ARA160) were shown to be up-regulated by the androgen deprivation; this evidence supports the critical role of coactivators in androgen-independent growth. We also found 2 AR cofactors (RAC3 and F-SRC-1) presenting down-regulation in the hormone-deprived environment. The variable expression change in different cofactors is also illustrated by several observations in other studies. Yeh et al reported that while ARA70, F-SRC-1 and RAC3 could enhance AR transcriptional activity at 1 nM DHT, only ARA70 could induce a 30-fold AR transcriptional activity increase in the presence of 10 nM E_2, but not diethylstilbestrol.[13] Katzenellenbogen et al proposed a new tripartite system (ligand-receptor-cofactor) to explain the molecular interaction of steroid receptors that may define the potency and biological character of steroid hormones.[14]

Many studies of the expression pattern of AR cofactors in normal prostate tissue or cancer cells have been reported.[19-21] Fujimoto et al found that ARA55 and SRC-1 were higher in the cancer specimens with a higher grade.[21] Alen et al found no alteration in the expression level of ARA70 in LNCaP cells treated with androgen.[19] Mestayer et al reported that AR coactivator expression was down-regulated by DHT.[20] In addition, ARA55 expression was decreased in tumor cell relative to normal tissue, and not expressed in LNCaP cancer cells. In our multiple RPA study, ARA55 was not expressed before or after androgen deprivation. The different expression changes of AR cofactors may contribute to the varying response of prostate cancer in different stages of androgen deprivation.[21] The cofactors of steroid receptors can play the role of either coactivator or corepressor. Our findings of either up-regulation or down-regulation in different AR cofactors are thus reasonable.

Although previous reports demonstrated the expression patterns of cofactors in different prostate tissue or cancer cell lines, they did not compare the dynamic change of expression patterns in a single prostate cancer cell line throughout the process from androgen-dependent to androgen-independent growth by androgen deprivation. Although a study by Nessler-Menardi et al had the same purpose as this study, they used semi-quantitative reverse transcriptase-polymerase chain reaction to analyze the cofactor expression over a long-term period (> 75 passages) in an androgen-deprivated model of LNCaP cells.[22] The cofactor expression pattern (e.g., ARA70, ARA54, SRC-1) showed no dramatic difference between

parental LNCaP cells and the sublines established after long-term androgen ablation.[22] In this study, we demonstrated that the expressions of AR and AR cofactors (e.g., ARA160, ARA24, ARA54 and ARA70) are up-regulated after androgen deprivation. This result was significant in a serial time course pattern, as shown in Fig. 3. The differences in results between long-term androgen deprivation and short-term androgen deprivation need to be clarified.

In this study, we found that the LNCaP cell stopped growing after more than 8 passages in charcoal-treated serum medium. During the early phase of LNCaP cell survival in the androgen-deprivated environment, a dramatic expression change of AR cofactors was demonstrated to be a short-term androgen deprivation effect for androgen-dependent prostate cancer cells. Many AR cofactors, either coactivators or corepressors, have been found, and the role of AR and AR cofactors in prostate cancer cell growth is complex. It has been thought that no single mechanism could comprehensively explain the related theory. Our results help clarify the molecular mechanisms of AR cofactor expression during short-term androgen deprivation.

This study is the first to use a multi-probe RPA to measure expression of AR and AR-associated cofactors mRNA influenced by androgen deprivation growth. The multi-probe RPA is a highly sensitive and specific method for the detection and quantitation of mRNA species, and can simultaneously evaluate several mRNAs of interest at different time points. The amount of RNA needed for a single Northern blotting is all that is required for the multi-probe RPA to evaluate as many as 10 targets in 1 procedure. This allows the study of multiple mRNA expression patterns of cofactors in androgen-deprived growth and their comparison in a single hybridization experiment. These data further clarify preliminary molecular biologic findings, and may be useful in helping to forecast the responsiveness of androgen-deprivation therapy in prostate cancer.

In conclusion, we used a novel non-isotopic multi-probe RPA assay to study AR coactivator expression patterns in an LNCaP. From the results of this study, we were able to compare the expression change of several AR cofactor proteins in a hormone-deprived environment. The results indicate that the increased expression of AR cofactors is compatible with the transition from androgen dependence to androgen independence, suggesting that some AR coactivators could play a role in androgen-deprived growth of prostate cancer cells. Previous reports have shown that AR cofactors may amplify or change the AR transcription pathway.[6,7,13] In this study, culture of androgen-dependent LNCaP cells in charcoal-treated serum resulted in increased expression of AR cofactors (e.g., ARA160, ARA24, ARA54, ARA70). This expression change indicated that these AR coactivators not only enhance the ordinary AR transcription pathway by increasing the amount of AR coactivator proteins, but also can activate AR through different pathways or by means of another ligand, such as estradiol or antiandrogen.[6,13] Additional studies are needed to further clarify the mechanism of AR coactivators in prostate cancer cell growth.

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