[CANCER RESEARCH 64, 7682–7685, November 1, 2004]

Advances in Brief

Androgenic Suppression of ATP-binding Cassette Transporter A1 Expression in LNCaP Human Prostate Cancer Cells

Junichi Fukuchi,[1] Richard A. Hiipakka,[1] John M. Kokontis,[1] Stephen Hsu,[1] Andrew L. Ko,[1] Michael L. Fitzgerald,[2] and Shutsung Liao[1]

[1]Ben May Institute for Cancer Research and Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, Illinois; and [2]Lipid Metabolism Unit, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts

Abstract

Alteration of lipid metabolism is commonly observed in sex hormone-dependent cancer cells, yet its mechanistic involvement in cancer cell proliferation and progression is unclear. We have found that the expression of the cholesterol transporter, ATP-binding cassette transporter A1 (ABCA1), was 15- to 20-fold higher in androgen-dependent than in androgen-independent LNCaP human prostate cancer cells, indicating a possible relationship between the expression levels of ABCA1 and prostate cancer progression. On the basis of real-time quantitative PCR and Western blot analysis, expression of ABCA1 in androgen-dependent cells was inhibited by androgen. The antiandrogen Casodex blocked the effect of androgen, implicating the androgen receptor in regulation of ABCA1 expression by androgens. Using an ABCA1 promoter-reporter gene assay, androgenic suppression was observed at the transcriptional level in androgen-dependent but not in androgen-independent prostate cancer cells. ABCA1 appears to have a role in modulating cell proliferation because knockdown of ABCA1 expression by RNA interference in androgen-dependent cells increased their rate of proliferation. Therefore, a suppressive effect of androgen on ABCA1 expression may be one of the mechanisms by which androgens regulate proliferation in prostate cancer cells. Attenuated ABCA1 expression in androgen-independent cells thus may contribute, in part, to prostate cancer progression.

Introduction

Prostate cancer is a significant source of morbidity and mortality, especially in many Western societies. More than 60 years ago, Charles Huggins showed that androgens regulate the growth of advanced prostate tumors, and this finding ushered in hormonal therapy for treatment of this disease (1). However, in most cases, androgen ablation is only a temporary respite from this disease, and soon, the cancer becomes androgen-independent and continues to grow. Other targets are urgently needed to control the growth and progression of prostate cancer. Recently, Ettinger *et al.* (2) reported that sterol response element-binding proteins (SREBPs), the transcriptional regulators that control the metabolic pathway of lipogenesis and cholesterol, are activated in androgen-independent tumors. The normal prostate produces and secretes cholesterol in prostatic fluid, and the level of both intracellular and secreted cholesterol are 2- to 10-fold higher in benign prostatic hyperplasia and carcinoma of the prostate (3, 4). Therefore, exploring the mechanism of alteration of lipid metabolism in prostate cancer may be helpful in elucidating the molecular mechanism responsible for prostate cancer cell progression.

Received 7/16/04; revised ; accepted 9/15/04.

Grant support: NIH Grants CA58073 and AT00850.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Shutsung Liao, Ben May Institute for Cancer Research and Department of Biochemistry and Molecular Biology, University of Chicago, MC6027, 5841 South Maryland Avenue, Chicago, IL 60637. Phone: (773) 702-6999; Fax: (773) 834-1770; E-mail: sliao@huggins.bsd.uchicago.edu.

To mimic prostate cancer progression, we established a cell culture system with an androgen-dependent LNCaP clonal cell line, LNCaP 104-S. After long-term culture in androgen-depleted medium, LNCaP 104-S cells progress to androgen-independent LNCaP 104-R1 and 104-R2 cells (5, 6). Using our progression model, we analyzed the expression levels of genes that play important roles in cholesterol and lipid homeostasis in the androgen-dependent 104-S and androgen-independent 104-R1 and 104-R2 cells. We report that the expression levels of ATP-binding cassette transporter A1 (ABCA1), which functions as a cholesterol exporter (7), is altered during prostate cancer progression, is regulated by androgens, and can regulate cell proliferation.

Materials and Methods

Materials. The synthetic androgen 17β-hydroxy,17α-methylestratrien-3-one (R1881) was obtained from New England Nuclear (Boston, MA). The antiandrogen Casodex [ICI 176,334; (2RS)-4′-cyano-3-(4-fluorophenylsulfonyl)-2-hydroxy-2-methyl-3′-(trifluoromethyl)-propionanilide; bicalutamide] was from AstraZeneca Pharmaceuticals (Wilmington, DE). A polyclonal antibody against ABCA1 was prepared as described previously (8).

Cell Culture. LNCaP 104-S, 104-R1, and 104-R2 cells were maintained and cultured as described previously (5, 6). To observe androgenic effects, cells were grown in DMEM supplemented with 10% dextran-coated charcoal-stripped fetal bovine serum.

Real-Time Quantitative PCR. Total RNA was isolated with the TRIZOL Reagent (Invitrogen, Carlsbad, CA) and was treated with DNase I (DNA-free, Ambion, Austin, TX). Reverse transcription was performed with random hexamers and Moloney murine leukemia virus reverse transcriptase (Omniscript, Qiagen, Valencia, CA). The TaqMan primer/probe was designed using Primer Express (Applied Biosystems, Foster City, CA). The sequences of primers and probes are as follows: ABCA1 primers, 5′-TGTCCAGTCCAGTAATGGTTCTGT-3′ and 5′-AAGCGAGATATGGTCCGGATT-3′, and ABCA1 probe, 5′-ACACCTGGAGAGAAGCTTTCAACGAGACTAACC-3′; ABCG1 primers, 5′-CCAGAAGTCGGAGGCCATC-3′ and 5′-AAGTCCAGGTACAGCTTGGCA-3′, and ABCG1 probe 5′-TGCGGGAGCTGGACGTGGAAA-3′; LXRα primers, 5′-GCCCTGCATGCCTACGTCT-3′ and 5′-TCATTAGCATCCGTGGGAACA-3′, and LXRα probe, 5′-CATCCACCATCCCCATGACCGACT-3′; LXRβ primers, 5′-GGAGCTGGCCATCATCTCA-3′ and 5′-GTCTCTAGCAGCATGATCTCGATAGT-3′, and LXRβ probe, 5′-CAGGAGATCGTGGACTTCGCTAAGCAA-3′; SREBP-1a primers, 5′-TGCTGACCGACATCGAAGAC-3′ and 5′-CTGTCTTGGTTGTTGATAAGCTGAA-3′, and SREBP-1a probe, 5′-ATCGCGGAGCCATGGATTGCACT-3′; SREBP-1c primers, 5′-GGTAGGGCCAACGGCCT-3′ and 5′-CTGTCTTGGTTGTTGATAAGCTGAA-3′, and SREBP-1c probe, 5′-ATCGCGGAGCCATGGATTGCACT; The 5′-end of the probe was labeled with the reporter-fluorescent dye, carboxyfluorescein. The 3′-end of probe was labeled with the quencher dye, 6-carboxytetramethylrhodamine. Real-time PCR was performed on an ABI PRISM 7700 system (Applied Biosystems) with the QuantiTect Probe PCR protocol (Qiagen). The rRNA Control Kit (Applied Biosystems) was used to normalize transcript levels between samples.

Western Blot Analysis. A cellular membrane fraction was prepared with Mem-PER (PIERCE, Rockford, IL), according to the manufacturer's instruc-

tions. Protein concentration was determined with the Bradford reagent (Bio-Rad Laboratories, Hercules, CA) with a BSA standard. Proteins were separated on 6% polyacrylamide gels containing SDS. Electrophoresis and blotting were performed as described previously (5).

Construction of Luciferase-Reporter Plasmid. A DNA fragment containing a 1.7-kb portion of the human *ABCA1* gene (representing nucleotides −211 to +1439 relative to the transcriptional start site) was amplified by PCR from 104-S genomic DNA. The primers were designed from the published *ABCA1* gene sequence and consisted of 5′-CTGGAGATCCTGTTGACTG-TAGCAT-3′ and 5′-CACTTTTGTGTTTGCGTCTCTT-3′. The 1.7-kb amplification product was cloned into the pSTBlue-1 cloning vector (Novagen, Madison, WI). After the sequence was verified, the ABCA1 promoter fragment was transferred from pSTBlue-1 to the pGL3 firefly luciferase reporter plasmid (Promega, Madison, WI). The c-fos-Ren plasmid consists of nucleotides −56 to +109 of the human c-fos minimal promoter (9) inserted into pGL3 and substitution of the firefly luciferase gene with a *Renilla* luciferase gene from phRL-SV40 (Promega).

Transient Transfection Assay. Cells were seeded at 1×10^5 cells per well on a 12-well plate, and then, the indicated compounds were added. Forty-eight hours later, cells were transiently transfected with 0.2 μg/well of the ABCA1 promoter-reporter plasmid and 0.1 μg/well of the c-fos-Ren plasmid (normalization reporter plasmid) with Effectene (Qiagen). After 12 hours, the medium was changed, and the indicated compounds added again. After an additional 24 hours, cells were harvested and luciferase activity was measured with a Dual-Luciferase kit (Promega) with a Monolight luminometer (PharMingen, San Diego, CA).

RNA Interference (RNAi) Experiments. The RNA interference sequence was designed by using the AA Scanning program from OligoEngine (Seattle, WA). DNA coding for a RNAi for human ABCA1 was prepared with the following oligonucleotides: 5′-GATCCCCTGAGTTTAGGTATGGCGGCT-TCAAGAGAGCCGCCATACCTAAACTCATTTTTGGAAA-3′ and 5′-AG-CTTTTCCAAAAATGAGTTTAGGTATGGCGGCTCTCTTGAAGCCGCC-ATACCTAAACTCAGGG-3′. These 64-mer oligonucleotides were annealed and ligated into the pH1RP vector (10), and the construct was verified by sequencing. The ABCA1-RNAi expression plasmid was stably transfected into 104-S cells with Effectene and selection for G418 resistance.

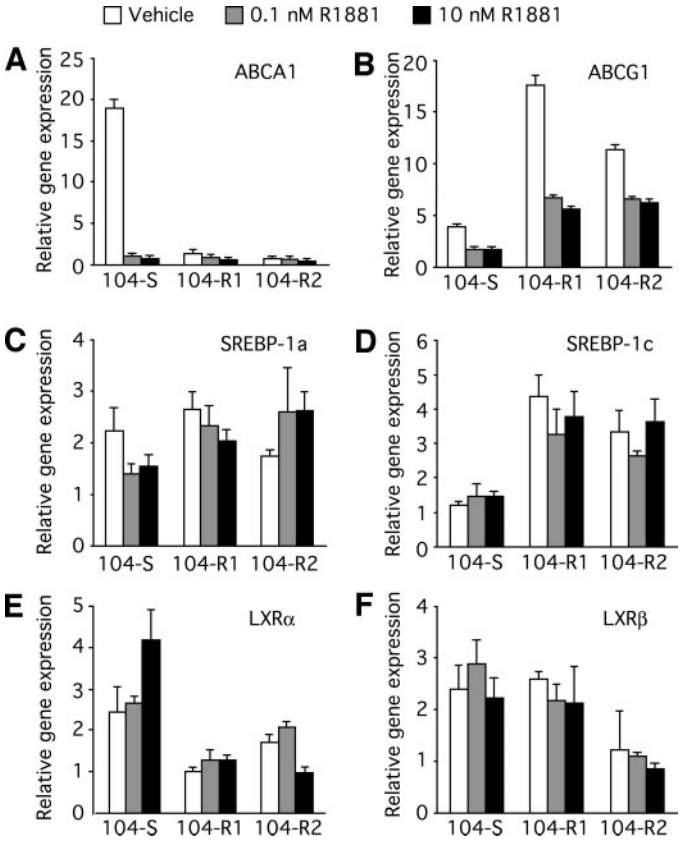

Fig. 1. Effect of R1881 on the mRNA level of genes related to cholesterol/lipid metabolism in LNCaP sublines. Androgen-dependent 104-S and androgen-independent 104-R1 and 104-R2 cells were grown in DMEM supplemented with 10% charcoal-stripped fetal bovine serum in the presence or absence of the synthetic androgen R1881 (□, 0 nmol/L; ▩, 0.1 nmol/L; and ■, 10 nmol/L, respectively) for 72 hours. Total RNA was isolated, and target gene transcripts were analyzed by real-time quantitative PCR. Gene expression was normalized to 18S rRNA content as described in Materials and Methods. *A*, ABCA1; *B*, ATP-binding cassette transporter G1 (ABCG1); *C*, SREBP-1a; *D*, SREBP-1c; *E*, LXRα; *F*, LXRβ.

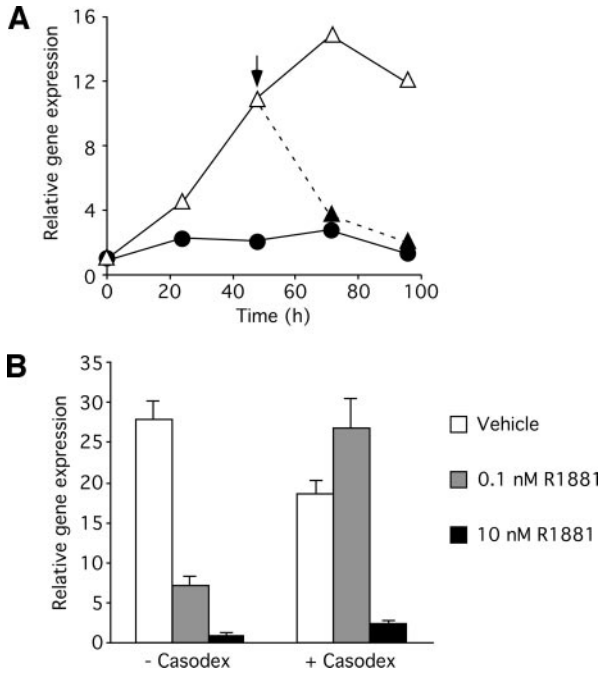

Fig. 2. Time course analysis and effect of Casodex on the levels of ABCA1 expression in LNCaP 104-S cells. *A*, 104-S cells cultured in DMEM supplemented with 10% charcoal-stripped fetal bovine serum and 0.1 nmol/L R1881 (●) were switched (0 hour) to media without (△) or with (▲) 0.1 nmol/L R1881. At 48 hours, 0.1 nmol/L R1881 were added to some of the culture medium without androgen (▲ with *dashed line*). *B*, effect of the antiandrogen Casodex on the androgenic suppression of ABCA1 expression. Cells were grown in the presence or absence of R1881 and/or 5 μmol/L Casodex for 72 hours. At the indicated time points, total RNA was isolated and gene expression was analyzed by real-time quantitative PCR and normalized to 18S rRNA content as described in Materials and Methods.

Cell Proliferation Assay. Cell number was analyzed by measuring DNA content with fluorescent dye Hoechst 33258 as described previously (10).

Data Analysis. Data are presented as the mean +/− SD or SE of three experiments or are representative of experiments repeated at least three times.

Results and Discussion

To investigate the relationship between prostate cancer progression and the expression levels of the genes playing important roles in cholesterol and lipid homeostasis, we analyzed gene expression levels by using real-time quantitative PCR analysis. In androgen-dependent LNCaP 104-S cells, treatment with the synthetic androgen, R1881, drastically suppressed ABCA1 gene expression (15- to 20-fold; Fig. 1*A*). ABCG1 also has been proposed to play a role in cholesterol and phospholipid efflux (7). ABCG1 mRNA levels in 104-R1 and 104-R2 cells were 2.5-fold higher than in 104-S cells (Fig. 1*B*). As with ABCA1, androgen down-regulated ABCG1 mRNA levels in 104-S cells by ~50% compared with vehicle-treated cells. Neither the expression levels of mRNA for SREBP-1a, which preferentially regulates cholesterol synthesis, nor the mRNA expression of SREBP-1c, which controls expression of various lipogenic enzymes (11), were affected by androgens (Fig. 1, *C* and *D*). Consistent with the studies by Ettinger *et al.* (2), the expression of SREBP-1c was increased in androgen-independent 104-R1 and 104-R2 cells. We also measured

the mRNA levels of liver X receptor (LXR) α and β because these nuclear receptors are activated by oxysterols and directly up-regulate the transcription of the mRNA for ABCA1, ABCG1, SREBP-1c, and LXRα itself (12). LXRα is preferentially expressed in the liver, intestine, adipose tissue, and macrophages (12), whereas LXRβ, which was discovered in our laboratory, is expressed ubiquitously and was named ubiquitous receptor (13). LXRα mRNA expression was 2-fold lower in androgen-independent 104-R1 and 104-R2 cells than in 104-S cells (Fig. 1*E*), and LXRβ mRNA expression was similar in 104-S cells and 104-R1 cells but was much lower in 104-R2 cells (Fig. 1*F*). Treatment of cells with androgen did not significantly alter the mRNA levels for LXRs.

The effect of androgen treatment or withdrawal on the kinetics of ABCA1 mRNA expression in 104-S cells was studied. The maximum level of ABCA1 mRNA was achieved at 72 hours after the onset of androgen depletion (Fig. 2*A*). Addition of androgen to the androgen-depleted culture decreased the maximum level of ABCA1 expression with kinetics similar to induction (solid triangle with dashed line in Fig. 2*A*). The androgenic suppression of ABCA1 expression by 0.1 nmol/L R1881 was reversed by 5 μmol/L Casodex (Fig. 2*B*). Casodex was unable to block the suppressive effects of 10 nmol/L R1881. This is most likely because Casodex has a 50- to 100-fold lower affinity for androgen receptor than R1881. The effect of Casodex suggests that androgenic suppression is mediated by the androgen receptor. In androgen-independent 104-R1 and 104-R2 cells, *ABCA1* gene expression was low relative to 104-S cells and was not significantly affected by R1881 or Casodex (Fig. 1 and data not shown). Consistent with mRNA expression studies, Western blot analysis showed that ABCA1 was expressed in androgen-dependent 104-S cells in the absence of R1881 or in the presence of Casodex (Fig. 3*A*). Expression of ABCA1 protein, with or without androgen, was not detected in androgen-independent 104-R1 and 104-R2 cells.

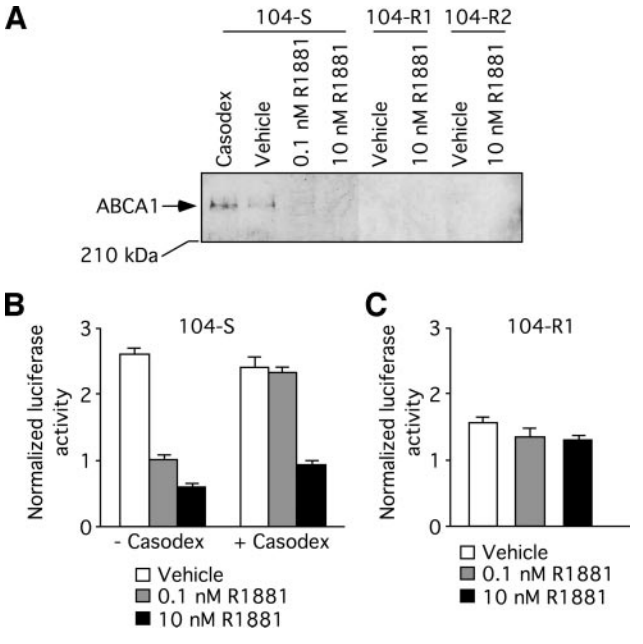

Fig. 3. Effect of Casodex and R1881 on ABCA1 protein expression and promoter activity in LNCaP cells. *A*, Western blot analysis of ABCA1. 104-S, 104-R1, and 104-R2 cells were treated for 72 hours with the indicated compound, and membrane fractions were prepared, and 15 μg of protein were separated on a 6% polyacrylamide gel containing SDS. ABCA1 protein was detected by polyclonal anti-ABCA1 antibody. The position for ABCA1 protein and 210-kDa molecular marker are shown. *B*, LNCaP 104-S cells were grown under the indicated conditions with or without 10 μmol/L Casodex for 48 hours. A 1.7-kb human ABCA1 promoter/firefly luciferase reporter plasmid and c-fos minimal promoter/*Renilla* luciferase control plasmid were cotransfected using Effectene. After 36 hours, firefly luciferase activity was measured and normalized by *Renilla* luciferase activity. *C*. LNCaP 104-R1 cells were grown under the indicated conditions. Transient transfection assays were performed as described above.

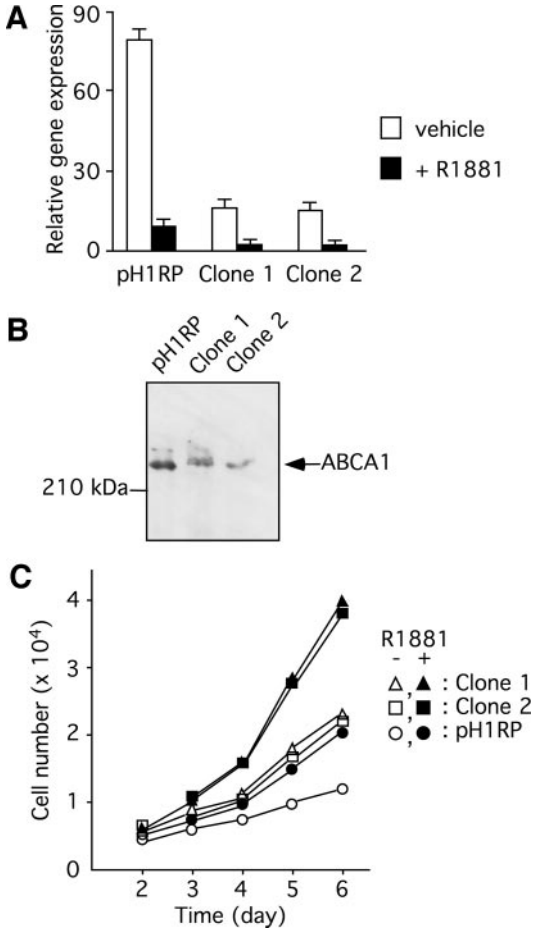

Fig. 4. Effect of ABCA1 RNAi expression on cell growth of androgen-dependent LNCaP 104-S cells. An expression plasmid for RNAi for ABCA1 was constructed and stably transfected into 104-S cells as described in Materials and Methods. *A*, effect of RNAi on the expression of ABCA1. The expression of ABCA1 was analyzed with real-time quantitative PCR. Two clones that express ABCA1 mRNA at levels less than the control (104S-pH1RP) are clone 1 and clone 2. *B*, Western blot analysis of ABCA1 protein levels in cells treated with RNAi. Cells were grown in the absence of androgen. The position of ABCA1 protein is indicated by an *arrow*. *C*, effect of R1881 on the growth of ABCA1 RNAi-expressing cells. Cells numbers were measured with a fluorometric DNA assay. *Error bars* were omitted for clarity. SDs were <10% of mean values.

We investigated the effect of androgen on ABCA1 promoter activity with transient transfection of 104-S cells with a luciferase reporter gene linked to a 1.7-kb fragment of the ABCA1 gene promoter adjacent to the transcriptional start. R1881 suppressed ABCA1 promoter activity in 104-S cells (Fig. 3*B*) but not in 104-R1 cells (Fig. 3*C*). This suppression was reversed by 10 μmol/L Casodex in 104-S cells (Fig. 3*B*). No obvious androgen-response element was present in the 1.7-kb promoter fragment. Because strong androgenic suppression of ABCA1 promoter activity was observed in androgen-dependent 104-S cells but not in androgen-independent 104-R1 cells (Fig. 3), a 104-S cell-specific *trans*-acting factor may be involved in androgenic regulation of ABCA1 in 104-S cells. Although the induction of ABCA1 expression by cyclic AMP is well characterized (14), the intracellular levels of cyclic AMP were not altered by androgen (data not shown), and there is no cyclic AMP response element in this 1.7-kb ABCA1 promoter. This region contains several binding sites for *trans*-acting factors. Among these binding sites, the Oct1 site is a potentially interesting candidate because Oct1 has been reported to be related to androgenic suppression of the rat dehydroepiandrosterone sulfotransferase gene (15). Additional studies will be required to

identify *trans*-acting factors that may be involved in prostate cancer progression and how they mediate the androgenic suppression of ABCA1 expression.

Because the expression of ABCA1 mRNA was drastically suppressed by androgen in 104-S cells and was decreased during progression from104-S to 104-R1 cells, we examined the role of ABCA1 in 104-S cell proliferation with RNAi to decrease the ABCA1 expression level. An RNAi sequence for human ABCA1 was expressed in 104-S cells with the RNAi-expression vector pH1RP. Cells transformed with pH1RP-ABCA1-RNAi (clone 1 and 2) showed significant decreases in ABCA1 mRNA expression measured by real-time quantitative PCR, compared with control cells transformed with pH1RP empty vector (Fig. 4*A*). Decreases in ABCA1 expression in these ABCA1-RNAi transformants were confirmed by Western blot analysis (Fig. 4*B*). ABCA1-RNAi transformants had increased growth rates compared with control transformants (Fig. 4*C*). Although androgen still stimulated the proliferation of these transformants, the growth rate of the ABCA1-knockdown cells was faster than control cells. In the absence of androgen, these transformants grew as fast as control cells treated with R1881, although cell cycle distribution in ABCA1-knockdown cells was not significantly changed (data not shown). We observed that in the absence of androgen, 104-S cells express a high level of ABCA1 (Fig. 1). The observations that 104-S cells grow faster when the expression of the ABCA1 gene is downregulated (Fig. 4*C*) is consistent with ABCA1 expression inhibiting LNCaP growth. The molecular mechanism by which ABCA1 affects proliferation is not clear at present. One possibility is that a decrease in ABCA1 expression may modulate cholesterol and/or phospholipid levels in lipid raft domains, which function as membrane platforms for cellular signal transduction (16). Additional exploration of ABCA1 function in our system may also enable us to define how cholesterol and/or lipids directly contribute to proliferation. Another possibility is that the interaction of the ABCA1 with Fas-associated death domain protein (17) may affect LNCaP 104-S cell proliferation because Fas-associated death domain protein has been reported to be involved in proliferation, as well as apoptosis (18). Recently, several ABCA1-interacting proteins involved in multiple cellular functions have been identified (19, 20). Some of these interactions may contribute to ABCA1-mediated regulation of proliferation in LNCaP 104-S cells.

Our findings point to an important role for ABCA1 in human androgen-dependent prostate cancer LNCaP cells, and androgen regulation of LNCaP cell proliferation may involve transcriptional suppression of ABCA1. Our results also suggest that it may be possible to modulate prostate cancer growth by induction of ABCA1, for example, through use of LXR agonists because the ABCA1 gene is a direct transcriptional target of LXR.

Acknowledgments

We thank Dr. Itsuko Ishii of Chiba University for valuable advice.

References

1. Huggins C, Stevens RE, Hodges CV. Studies on prostate cancer: II. The effects of castration on advanced carcinoma of the prostate gland. Arch Surg 1941;43:209–223.
2. Ettinger SL, Sobel R, Whitmore TG, et al. Dysregulation of sterol response element-binding proteins and downstream effectors in prostate cancer during progression to androgen independence. Cancer Res 2004;64:2212–21.
3. Swyer GIM. The cholesterol content of normal and enlarged prostates. Cancer Res 1942;2:372–5.
4. Acevedo HF, Campbell EA, Saier EL, et al. Urinary cholesterol. V. Its excretion in men with testicular and prostatic neoplasms Cancer (Phila.) 1973;32:196–205.
5. Kokontis J, Takakura K, Hay N, Liao S. Increased androgen receptor activity and altered c-myc expression in prostate cancer cells after long-term androgen deprivation. Cancer Res 1994;54:1566–73.
6. Kokontis JM, Hay N, Liao S. Progression of LNCaP prostate tumor cells during androgen deprivation: hormone-independent growth, repression of proliferation by androgen, and role for p27Kip1 in androgen-induced cell cycle arrest. Mol Endocrinol 1998;12:941–53.
7. Tall AR, Costet P, Wang N. Regulation and mechanisms of macrophage cholesterol efflux. J Clin Investig 2002;110:899–904.
8. Fitzgerald ML, Mendez AJ, Moore KJ, Andersson LP, Panjeton HA, Freeman MW. ATP-binding cassette transporter A1 contains an NH2-terminal signal anchor sequence that translocates the protein's first hydrophilic domain to the exoplasmic space. J Biol Chem 2001;276:15137–45.
9. Gilman MZ, Wilson RN, Weinberg RA. Multiple protein-binding sites in the 5′-flanking region regulate c-fos expression. Mol Cell Biol 1986;6:4305–16.
10. Fukuchi J, Hiipakka RA, Kokontis JM, Nishimura K, Igarashi K, Liao S. TATA-binding protein-associated factor 7 regulates polyamine transport activity and polyamine analog-induced apoptosis. J Biol Chem 2004;279:29921–9.
11. Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Investig 2002;109:1125–31.
12. Joseph SB, Tontonoz P. LXRs: new therapeutic targets in atherosclerosis? Curr Opin Pharmacol 2003;3:192–7.
13. Song C, Kokontis JM, Hiipakka RA, Liao S. Ubiquitous receptor: a receptor that modulates gene activation by retinoic acid and thyroid hormone receptors. Proc Natl Acad Sci USA 1994;91:10809–13.
14. Bortnick AE, Rothblat GH, Stoudt G, et al. The correlation of ATP-binding cassette 1 mRNA levels with cholesterol efflux from various cell lines. J Biol Chem 2000; 275:28634–40.
15. Song CS, Jung MH, Kim SC, Hassan T, Roy AK, Chatterjee B. Tissue-specific and androgen-repressible regulation of the rat dehydroepiandrosterone sulfotransferase gene promoter. J Biol Chem 1998;273:21856–66.
16. Zhuang L, Lin J, Lu ML, Solomon KR, Freeman MR. Cholesterol-rich lipid rafts mediate akt-regulated survival in prostate cancer cells. Cancer Res 2002;62:2227–31.
17. Buechler C, Bared SM, Aslanidis C, Ritter M, Drobnik W, Schmitz G. Molecular and functional interaction of the ATP-binding cassette transporter A1 with Fas-associated death domain protein. J Biol Chem 2002;277:41307–10.
18. Zhang J, Cado D, Chen A, Kabra NH, Winoto A. Fas-mediated apoptosis and activation-induced T-cell proliferation are defective in mice lacking FADD/Mort1. Nature (Lond.) 1998;392:296–300.
19. Tsukamoto K, Hirano K, Tsujii K, Ikegami C, Zhongyan Z, Nishida Y, et al. ATP-binding cassette transporter-1 induces rearrangement of actin cytoskeletons possibly through Cdc42/N-WASP. Biochem Biophys Res Commun 2001;287: 757–65.
20. Buechler C, Boettcher A, Bared SM, Probst MC, Schmitz G. The carboxyterminus of the ATP-binding cassette transporter A1 interacts with a beta2-syntrophin/utrophin complex. Biochem Biophys Res Commun 2002;293:759–65.