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14

Inhibition of Advanced Prostate Cancer by Androgens and Liver X Receptor Agonists

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1. Introduction

Prostate cancer is the most frequently diagnosed non-cutaneous tumor of men in western countries. National Cancer Institute estimated that more than 217,000 people were diagnosed and 32,000 people died of prostate cancer in the United States in 2010. Currently, primary therapies for prostate cancer include radical prostatectomy, radiation therapy, high-intensity focused ultrasound, chemotherapy, cryosurgery, hormonal therapy, and combination of different treatments. Approximately 20-40% of patients treated with radical prostatectomy will have tumor recurrence and elevation of serum prostate-specific antigen (PSA) (Sadar 2011). More than 80% of patients who died from prostate cancer developed bone metastases, primary metastatic sites include bones and lymph nodes (Bubendorf et al 2000, Ibrahim et al 2010, Keller et al 2001).

In 1941, Huggins and Hodges reported that androgen ablation therapy caused regression of primary and metastatic prostate cancer (Huggins C 1941). Since then, androgen ablation therapy, using luteinizing hormone-releasing hormone agonists (LH-RH) or bilateral orchiectomy, has become one of the primary treatment for prostate cancer (Seruga and Tannock 2008). More than 80% of men with these advanced prostate cancers respond to androgen ablation therapy, resulting in tumors shrinkage and reduction of serum PSA (Seruga and Tannock 2008). Anti-androgens are frequently used in conjunction with androgen ablation therapy as a combined androgen blockade to improve therapeutic outcome (Klotz et al 2004). However, 80-90% of the patients who receive androgen ablation therapy ultimately develop recurrent tumors in 12-33 months. The median overall survival of patients after tumor relapse is 1-2 years (Fowler et al 1998, Hellerstedt and Pienta 2002). In addition, androgen deprivation therapy is associated with several undesired side-effects, including sexual dysfunction, osteoporosis, hot flashes, fatigue, gynecomastia, anemia, depression, cognitive dysfunction, increased risk of diabetes, and cardiovascular diseases (Keating et al 2006, Keating et al 2010, Saigal et al 2007, Seruga and Tannock 2008). Androgen deprivation therapy using LH-RH agonists was reported to increase risk of incident diabetes, incident coronary heart disease, myocardial infarction, sudden cardiac death, and stroke (Keating et al 2006, Keating et al 2010, Saigal et al 2007). Combined

androgen blockade was associated with increased risk of incident coronary heart disease (Keating et al 2010). Orchiectomy was associated with coronary heart disease and myocardial infarction (Keating et al 2010). Therefore, shortening the period of androgen ablation therapy may protect the patients.

Liver X receptors (LXRs) are ligand-activated transcriptional factors that belong to the nuclear receptor superfamily. LXRs are important regulators of cholesterol, fatty acid, and glucose homeostasis (Chuu et al 2007). There are two LXR isoforms. LXRα expression is most abundant in liver, kidney, intestine, fat tissue, macrophages, lung, and spleen, while LXRβ is ubiquitously expressed (Chuu et al 2007, Edwards et al 2002, Willy et al 1995). A specific group of oxysterols are natural ligands for LXRs (Chuu et al 2007, Forman et al 1997, Janowski et al 1996). LXR agonists are effective for treatment of murine models of atherosclerosis, diabetes, and Alzheimer's disease (Alberti et al 2001, Blaschke et al 2004, Cao et al 2003, Chuu et al 2007, Edwards et al 2002, Efanov et al 2004, Joseph et al 2002, Joseph et al 2003, Koldamova et al 2005, Peet et al 1998, Song et al 2001, Song and Liao 2001). Our and other groups' previous studies suggested that androgen and LXR agonists may suppress tumor growth of hormone-refractory prostate cancer cells (Chuu et al 2006, Chuu et al 2007, Chuu and Lin 2010, Fukuchi et al 2004b). We thus discuss the possibility of manipulating androgen/androgen receptor (AR) signaling and LXR signaling as a treatment for advanced prostate cancers.

2. Androgens and androgen receptor in prostate cancer

Androgens include testosterone, dehydroepiandrosterone, androstenedione, androstenediol, androsterone, and dihydrotestosterone (DHT). Androgens are mainly produced by testes, while the rest amount of androgens are produced from the adrenal glands. Androgens are important for growth and survival of the prostate cells. Testosterone is the main circulating androgen in human body, while DHT is the more potent androgen (Anderson and Liao 1968, Kokontis and Liao 1999, Liang and Liao 1992). 90% of the free testosterone enters prostate cells is converted to dihydrotestosterone (DHT) by the enzyme 5α-reductase (Liang and Liao 1992). The average serum testosterone level declines with age from approximately 620-670 ng/dl at age 25-44 to 470-520 ng/dl at age 65-84 (Vermeulen 1996). Low serum testosterone level was associated with an increased risk of prostate cancer (Morgentaler and Rhoden 2006), and prostate tumors arising in a low testosterone environment appeared to be more aggressive (Hoffman et al 2000, Lane et al 2008), suggesting a potential therapeutic role for androgen in advanced prostate cancer treatment.

Androgen receptor (AR) is an androgen-activated transcription factor and belongs to the steroid nuclear receptor family. AR is composed of an N-terminal domain, a central DNA-binding domain, and a C-terminal ligand-binding domain (Chang et al 1988a, Chang et al 1988b, Feldman and Feldman 2001). After binding ligand DHT, AR dissociates from heat-shock proteins, phosphorylates, dimerizes, transocates into the nucleus, and binds to androgen-response elements (ARE) in the promoter regions of its target genes under the regulation of co-activators and co-repressors (Feldman and Feldman 2001). Target genes of AR regulate growth, survival, and the production of prostate-specific antigen (PSA) in prostate cells.

Gene microarray study of seven different human prostate cancer xenograft models demonstrated that increase of AR mRNA is the only change consistently associated with the

development of androgen-independency phenotype following androgen ablation therapy, and elevation of AR mRNA and protein are both necessary and sufficient progression of prostate cancer towards androgen-independency (Culig et al 1999, Joly-Pharaboz et al 1995). Elevated AR expression in androgen-independent prostate cancer cells or recurrent hormone-refractory tumors has been observed in our progression model (Chuu et al 2005, Chuu et al 2006, Kokontis et al 1994, Kokontis et al 1998, Kokontis et al 2005, Umekita et al 1996) and several other groups (Chen et al 2004a, de Vere White et al 1997, Edwards et al 2003, Ford et al 2003, Gregory et al 2001, Hara et al 2003, Holzbeierlein et al 2004, Kim et al 2002, Linja et al 2001, Shi et al 2004, Singh et al 2004, Visakorpi et al 1995, Wang et al 2001, Zhang et al 2003). Mechanisms contribute to the progression towards androgen-independency including AR gene amplification, AR mutation, bypass of androgenic activation of AR, or bypass AR signaling for cell survival and proliferation (Feldman and Feldman 2001).

3. Androgenic suppression of advanced prostate cancer cells

3.1 Androgenic suppression *in vitro*

LNCaP is one of the most commonly used cell line for prostate cancer research, which was derived from a human lymph node metastatic lesion of prostate adenocarcinoma (Chuu et al 2007, Horoszewicz et al 1980). LNCaP expressed AR and inducible PSA. Previously, we cultured androgen-sensitive LNCaP 104-S cells in androgen-depleted conditions *in vitro* to establish relapsed hormone-refractory prostate cancer cells mimic clinical situation in which prostate cancer recurs during androgen deprivation (Kokontis et al 1994a, Kokontis et al 1998b). After 3 months in medium depleted with androgens, most LNCaP 104-S cells underwent G1 cell cycle arrest and apoptosis. A few colonies of cells, named 104-I cells, evolved that proliferated very slowly in the absence of androgen (Kokontis et al 1994). After approximately 11 months, cells called 104-R1 cells emerged that grew much more rapidly in the absence of androgen. After 20 months, 104-R2 cells evolved which proliferated in the absence of androgen at a rate comparable to the proliferation rate of 104-S cells grown in androgen (Kokontis et al 1994, Kokontis et al 1998).

During the transition of 104-S cells to 104-R1 and 104-R2 cells, AR mRNA and protein level elevated several folds (Chuu et al 2005, Chuu et al 2006, Kokontis et al 1994, Kokontis et al 1998). Proliferation of 104-R1 and 104-R2 cells is androgen-independent but is unexpectedly suppressed by physiological concentrations of androgen both *in vitro* and *in vivo* (Chuu et al 2005, Chuu et al 2006, Kokontis et al 1994, Kokontis et al 1998b, Kokontis et al 2005, Umekita et al 1996). When 104-R1 cells were incubated for several weeks in a high concentration of synthetic androgen R1881 (20 nM), cells named R1Ad adapted after a period of growth arrest (Kokontis et al 1998). Growth of R1Ad cells is slow and not dependent on androgen but is stimulated by 10 nM R1881.

To further mimic the clinical situation of combined androgen deprivation and anti-androgen therapy, LNCaP 104-S cells were incubated with 5 μM Casodex (biculatimide) in androgen-deprived medium. After four weeks, Casodex-resistant colonies appeared at low frequency (1 in $1.4x10^5$) as most of the cells appeared to undergo senescent cell death. The relapsed cells, called CDXR, had increased AR expression and were repressed by androgen (Kokontis et al 2005). Unlike 104-R1 cells, most CDXR cells grown in 10 nM R1881 underwent apoptosis 6 to 8 days after R1881 exposure. However, 1 in $1.9x10^3$ cells relapsed as androgen-insensitive that were not repressed by R1881 or Casodex. These sublines,

designated IS, showed greatly reduced AR expression (Kokontis et al 2005). Growth of IS cells was not stimulated by R1881 or suppressed by Casodex. 104-R2 cells, like CDXR cells, gave rise to androgen-insensitive cells after androgen treatment (unpublished data). Therefore, during progression from 104-R1 to 104-R2 stages, the cells appear to pass a point where cells can no longer recover responsiveness to androgen, but instead progress to androgen insensitivity (Liao et al 2005). Direct progression of 104-S cells to the CDXR stage by selection in androgen-depleted medium containing anti-androgen seems to bypass this intermediate 104-R1 stage. Androgen-suppressive phenotype and elevated AR of hormone-refractory LNCaP cells was observed by several other groups (Culig et al 1999, Joly-Pharaboz et al 1995, Joly-Pharaboz et al 2000, Shi et al 2004, Soto et al 1995). The progression model of LNCaP is shown in Figure 1.

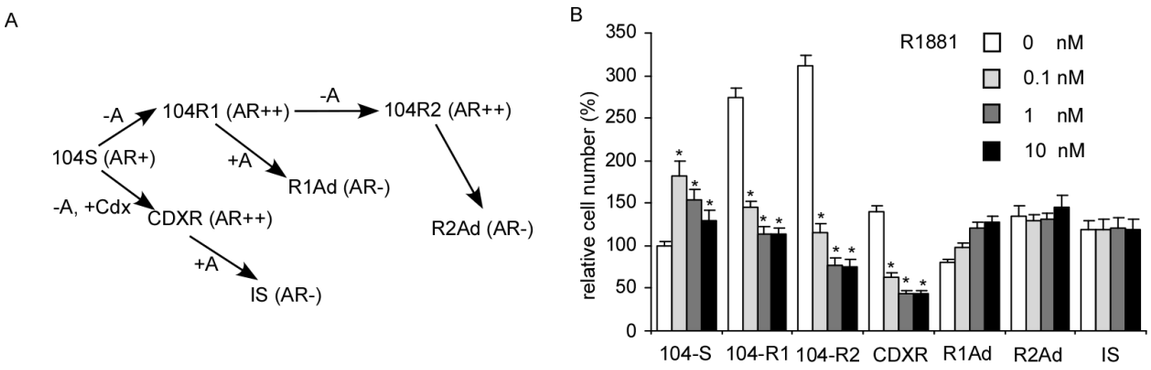

Fig. 1. The LNCaP cell line progression model. (A) AR expression level increases during the progression from androgen-dependent LNCaP 104-S cells to androgen-independent 104-R1, 104-R2, and CDXR cells. Proliferation of LNCaP 104-R1, 104-R2, CDXR cells are suppressed by androgen, but these cells can adapt to androgenic suppression and evolve as R1Ad, R2Ad, and IS cells. R1Ad, R2Ad, and IS cells express very little AR. (B) Effect of 96 h treatment of synthetic androgen (0, 0.1, 1, 10 nM) R1881 on 104-S, 104-R1, 104-R2, CDXR, R1Ad, R2Ad, IS cells was assayed by 96-well proliferation assay.

LNCaP cells express a mutant AR (T877A) that displays relaxed ligand binding specificity (Kokontis et al 1991, Veldscholte et al 1990), however, androgenic suppression is not limited to LNCaP cells. ARCaP is an AR-positive, tumorigenic, and highly metastatic cell line derived from the ascites fluid of a patient with advanced metastatic disease. Proliferation of ARCaP cells is suppressed by androgen (Zhau et al 1996). MDA PCa 2b-hr was generated *in vitro* from bone metastasis-derived, androgen-dependent MDA PCa 2b human PC cells with higher AR proteins. Proliferation of MDA PCa 2b-hr was inhibited by testosterone concentration higher than 3.5 nM or Casodex (Hara et al 2003). PC-3 is a commonly used human prostate cancer cell lines established from bone-derived metastases with no AR expression (Chuu et al 2007). Physiological concentration of DHT caused growth inhibition, G1 cell cycle arrest, and apoptosis in PC-3 cells over-expressing full length wild-type AR (Heisler et al 1997, Litvinov et al 2004, Yuan et al 1993).

3.2 Androgenic suppression *in vivo*

Castration causes regression of 104-S xenografts but tumor relapsed after 8 weeks as androgen-independent relapsed tumors 104-Rrel with elevated mRNA and protein

expression of AR (Chuu et al 2006). Low serum level of testosterone (130 ± 60 ng/dl), stop tumor growth of 104-Rrel tumors but tumor growth resumed in 4 weeks. High serum level of testosterone (2970 ± 495 ng/dl), which is approximately 5-fold higher than normal level, caused regression of 104-Rrel tumors growth. However, all 104-Rrel cells adapted to androgen and relapsed after 4 weeks as androgen-stimulated 104-Radp tumors (Chuu et al 2006) (Figure 2). Growth of the LNCaP 104-R1 tumors was also suppressed by androgen, but all tumors adapted to androgenic suppression and relapsed as androgen-stimulated R1Ad tumors in 5-6 weeks (Chuu et al 2005). Growth of R1Ad tumors was stimulated by testosterone and removal of testosterone totally stopped the tumor growth (Chuu et al 2005, Chuu et al 2006). Both 104-Radp and R1Ad tumors express very little AR and PSA mRNA and protein or serum PSA level (Figure 2), similar to R1Ad cells observed in cell culture (Chuu et al 2005, Chuu et al 2006, Kokontis et al 1998).

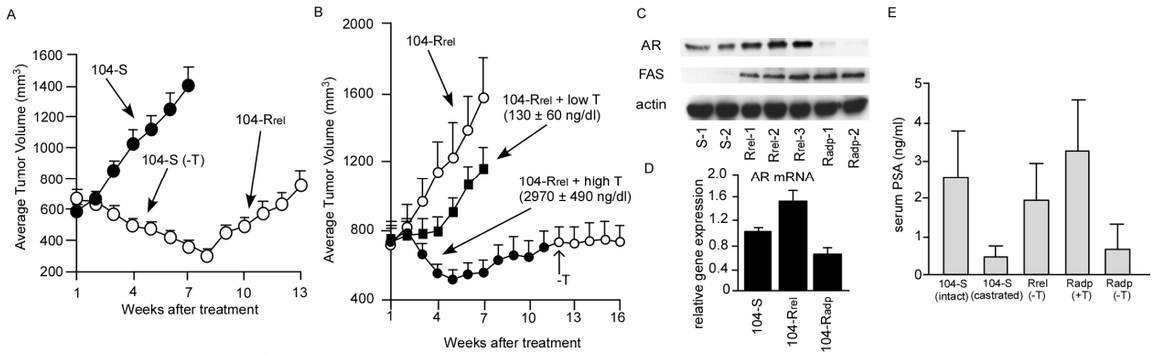

Fig. 2. Progression of androgen-dependent LNCaP 104-S tumors to androgen-independent 104-Rrel tumors, and androgenic growth suppression of 104-Rrel tumors. (A) Mice were injected subcutaneously with androgen-dependent 104-S cells. After allowing tumors to grow for 7 weeks, mice were separated into control (filled circles, 14 mice with 19 tumors) and castration groups (open circles, 24 mice with 36 tumors) and the time was designated as week 1 (Chuu et al 2006). (B) Mice in the castrated group in (A) at the 14th week were separated into 3 groups including a control group (open circles, 6 mice with 9 tumors), a low dosage testosterone treatment group that received a subcutaneous implant of a 20 mg TP/cholesterol (1:9) pellet (filled squares, 9 mice with 12 tumors), and a high-dosage testosterone treatment group that received a subcutaneous implant of a 20 mg pure TP pellet (filled circles, 10 mice with 12 tumors) (Chuu et al 2006). Tumor volumes are expressed as the mean + standard error. (C) PSA, AR, and actin protein levels in 104-S tumor (in intact mice), 104-Rrel-T tumors, 104-Radp-1+T tumors, and 104Radp-T were assayed by Western blot (Chuu et al 2005). (D) Serum PSA level of mice with 104-S tumors (in intact mice), 104-Rrel-T tumors, 104-Rrel+T tumors, Radp+T tumors, Radp-T tumors was determined by Elisa kit (Chuu et al 2005).

Both early and late treatment of androgen caused regression of CDXR3 tumors. 70% of tumors regress completely and the rest of tumors relapse after 60-90 days of treatment (Kokontis et al 2005) (Figure 3). The relapsed tumors show diminished expression of AR and no longer require androgen for growth, essentially identical to the behavior of IS3 cells that emerged after androgen exposure *in vitro* (Kokontis et al 2005). It is worthwhile to notice that 100% of 104-R1 tumor being treated with testosterone relapsed in 4-5 weeks, while only 30% of CDXR tumors relapsed after 9-13 weeks after testosterone treatment (Chuu et al

2005, Kokontis et al 2005). This is probably due to the apoptosis induced in CDXR cells but not in 104-R1 cells by androgen (Kokontis et al 1998, Kokontis et al 2005). Regression and relapse after androgen treatment of LNCaP xenograft was also observed by other group (Joly-Pharaboz et al 2000) and ARCaP xenograft (Zhau et al 1996).

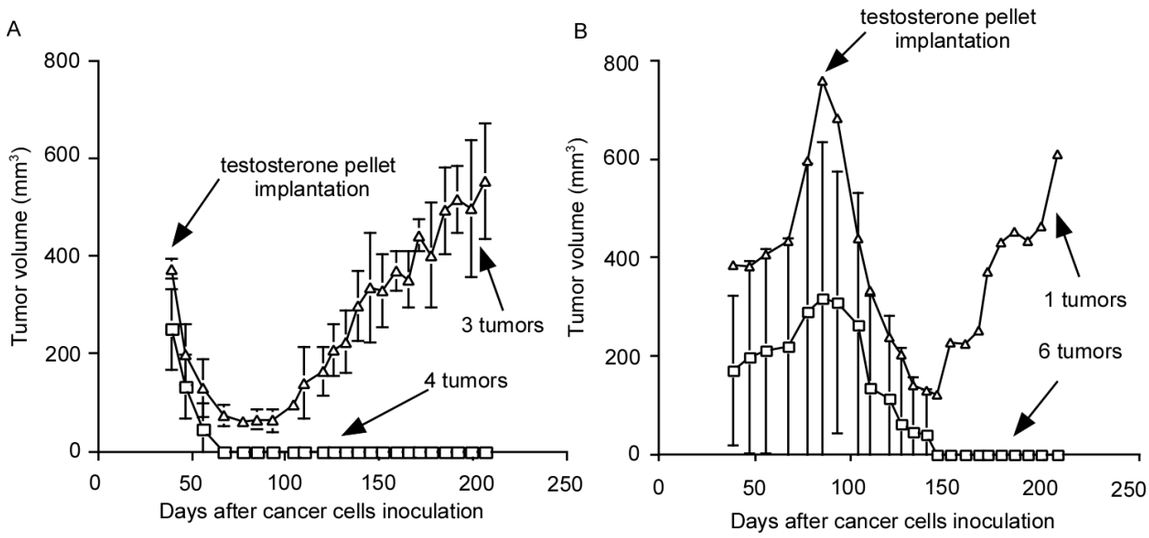

Fig. 3. Regression and relapse of LNCaP CDXR-3 tumor xenografts in nude mice treated with testosterone (A) LNCaP CDXR-3 tumor xenografts in castrated male nude mice were allowed to grow until they reached an average volume of 400 mm^3 on the 38th day. All mice carrying tumors received a subcutaneous implant of a 20mg testosterone. The mice in the control group were implanted with a 20 mg testosterone pellet either at early stage (50 days after inoculation) or late stage (92 days after inoculation). Open triangle represent tumors relapsed, while open squares represent tumors disappeared after androgen treatment. Tumor volumes are expressed as the mean ± standard error.

3.3 Molecular mechanism of androgenic suppression

Antiandrogen Casodex (bicalutamide) does not affect proliferation of 104-R1 and 104-R2 cells but blocked androgenic repression of growth as well as androgenic induction of PSA (Kokontis et al 1998). Knockdown of AR expression in CDXR3 cells by shRNA relieved androgenic repression of growth (Kokontis et al 2005). Retroviral overexpression of AR in IS cells restored the androgen-repressed phenotype in these cells (Kokontis et al 2005). These observations confirmed that androgen cause growth inhibition via AR.

Synthetic androgen R1881 increases S phase population in androgen-dependent LNCaP 104-S cells but induces G1 arrest in androgen-independent LNCaP cells (such as 104-R1m 104-R2, CDXR, etc.) within 24 hours of treatment (Joly-Pharaboz et al 2000, Kokontis et al 1994, Kokontis et al 1998, Kokontis et al 2005, Soto et al 1995) (Figure 4). Cell cycle inhibitors $p21^{waf1/cip1}$ and $p27^{Kip1}$ were induced by androgen in 104-R1 and 104-R2 cells (Kokontis et al 1998a) (Figure 4). In contrast, expression of $p21^{waf1/cip1}$ and $p27^{Kip1}$ was repressed by androgen in 104-S cells. Androgen down-regulates F-box protein S phase kinase-associated protein 2 (Skp2), a protein mediating the ubiquitination and degradation of $p27^{Kip1}$. Androgen also decreases c-Myc at the protein and mRNA level in hours in 104-R1 cells (Figure 5). Enforced retroviral overexpression of c-Myc blocks androgenic repression of 104-

R1 growth (Kokontis et al 1994). Therefore, androgen regulate cell cycle and proliferation of LNCaP cells via AR, Skp2, c-Myc, and $p27^{Kip1}$.

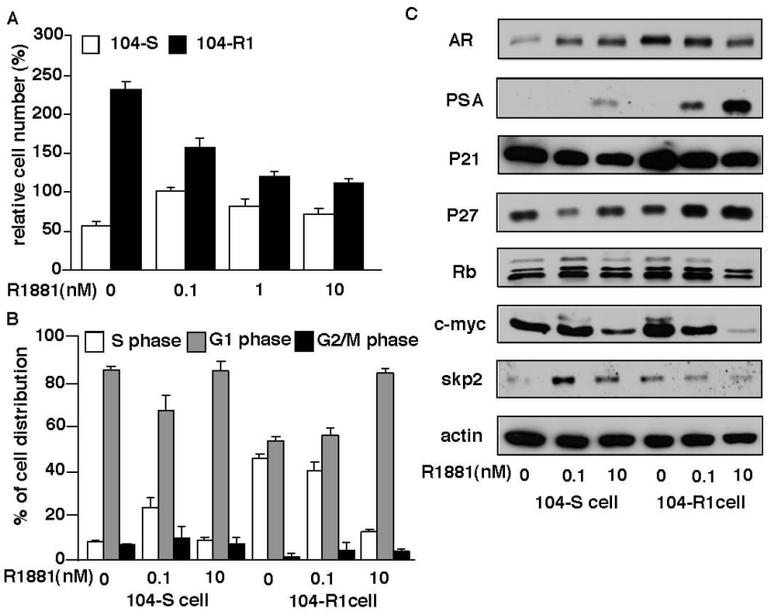

Fig. 4. Effect of androgen on cell proliferation, cell cycle, and cell cycle-related proteins in androgen-dependent 104-S and androgen-independent 104-R1 cells. (A) LNCaP 104-S and 104-R2 cells were treated with increasing concentration of synthetic androgen R1881 for 96 hours. Relative cell number was determined by 96-well proliferation assay and was normalized to cell number of 104-S cells at 0.1 nM R1881. (B) Percentage of 104-S and 104-R1 cells in S phase determined by flow cytometry. LNCaP 104-S and 104-R2 cells were treated with increasing concentration of synthetic androgen R1881 for 96 hours. Values represent the mean +/- Standard Error derived from 5 independent experiments. (C) Protein expression of androgen receptor (AR), prostate specific antigen (PSA), $p21^{cip}$, $p27^{Kip}$, retinoblastoma protein (Rb), c-myc, S phase kinase-associated protein 2 (Skp2) were determined by Western bloting assay in 104-S and 104-R1 cells treated 96 hrs with different concentration of R1881. β-actin was used as loading control.

4. Androgen treatment of advanced prostate cancer in clinical

Clinical and basic studies showed that in comparison with continuous androgen ablation (CAB) therapy, intermittent androgen suppression (IAS) therapy substantially prolongs the time to development of castration-resistant prostate cancer (Akakura et al 1993, Mathew 2008, Sato et al 1996, Szmulewitz et al 2009). Intermittent androgen ablation therapy is a strategy to periodically perform and terminate the androgen ablation therapy, allowing the endogenous testosterone level to elevate during the period between ablation therapies. IAS therapy delayed the androgen-independent progression of Shionogi mammary carcinoma (Akakura et al 1993) and LNCaP xenograft (Sato et al 1996). Pether et al. reported in a clinical trial of 102 patients that there is a trend toward extended times to progression and death compared to CAB treatment, and growth of advanced prostate tumors was delayed in ~50% patients treated with IAS (Pether et al 2003). Bruchovsky et al. showed that IAS

therapy cause repeated differentiation of tumor with recovery of apoptotic potential, inhibition of tumor growth by rapid restoration of serum testosterone, and restraint of tumor growth by subnormal levels of serum testosterone (Bruchovsky et al 2000). They concluded that IAS is a viable treatment option for men with prostate cancer which affords an improved quality of life as well as reduced toxicity and costs (Bruchovsky et al 2000, Morris et al 2009, Pether et al 2003).

A few studies have shown that androgen is safe and potentially effective for treatment of advanced prostate cancer. Mathew reported that the testosterone level in a prostate cancer patient undergone radical prostatectomy and LH-RH therapy remained at castrated levels and serum PSA was undetectable for 15 years. PSA levels then began to rise and the patient was given testosterone replacement therapy to attain a normal range of serum testosterone. After an initial flare, PSA levels gradually declined over 18 months. After 27 months, PSA level started to increase. When testosterone replacement therapy was discontinued, PSA levels dropped (Mathew 2008). The observation was similar to the transition from 104-R1 to R1Ad phenotype under androgen treatment in our LNCaP progression model (Chuu et al 2005, Kokontis et al 1998). Szmulewitz et al. reported that 15 prostate cancer patients with progressive disease following androgen ablation, anti-androgen therapy, and withdrawal without minimal metastatic disease were randomized to treatment with three doses of transdermal testosterone of 2.5, 5.0, or 7.5 mg/day, resulting in increase of serum testosterone concentrations to 305 ng/dl, 308 ng/dl, and 297 ng/dl, respectively. The conclusion of this study is that testosterone is a feasible and reasonably well-tolerated therapy for men with early hormone-refractory prostate cancer (Szmulewitz et al 2009). Morris et el. performed a phase 1 clinical trial to determine the safety of high-dose exogenous testosterone in patients with castration-resistant metastatic prostate cancer. Cohorts of 3-6 patients with progressive castration-resistant prostate cancer who had been castrated for at least 1 yr received testosterone by skin patch or topical gel for 1 week, 1 month, or until disease progression. No adverse effect was reported. The serum testosterone ranged from 330-870 ng/dl (Morris et al 2009). This study suggested that patients with advanced prostate cancer can be safely treated with exogenous testosterone. Researchers suggested that maximizing testosterone serum levels in selected patients with androgen receptor over-expression may improve the treatment outcome.

5. Liver X receptor (LXR) signaling

5.1 LXRα and LXRβ

Liver X receptors are ligand-activated transcriptional factors that belong to the nuclear receptor superfamily. There are two LXR isoforms, LXRα and LXRβ (Chuu et al 2007). Although LXRα and LXRβ share high similarity in their DNA- and ligand-binding domains, expression of these proteins in various tissues differs. LXRα expression is restricted to liver, kidney, intestine, fat tissue, macrophages, lung, and spleen (Edwards et al 2002, Willy et al 1995). LXRβ is ubiquitously expressed (Song et al 1994). LXRα and LXRβ form heterodimers with the obligate partner 9-cis retinoic acid receptor (RXR) (Chuu et al 2007, Song et al 1994, Willy et al 1995). The LXR/RXR heterodimer can be activated with either an LXR agonist (oxysterols) or a RXR agonist (cis-retinoic acid). Oxysterols are oxygenated derivatives of cholesterol. Oxysterols, such as 22(R)-hydroxycholesterol, 24(S)-hydroxycholesterol, and cholestenoic acid, are natural ligands for LXR (Chuu et al 2007, Forman et al 1997, Janowski

et al 1996). A few synthetic LXR agonists have been developed, including non-steroidal LXR agonists T0901317 (Schultz et al 2000) and GW3965 (Collins et al 2002), and steroidal LXR agonists hypocholamide (Song and Liao 2001) and YT-32 (Kaneko et al 2003)].

5.2 Role of LXR signaling in metabolism

LXRs are important regulators of cholesterol, fatty acid, and glucose homeostasis (Chuu et al 2007). Oral administration of an LXR agonist has an overall hypolipidemic effect in hypercholesterolemic rats, mice, and hamsters (Song and Liao 2001). LXRα-/- mice are healthy when fed with a low-cholesterol diet. However, LXRα-/- mice develop enlarged fatty livers, hepatocellular degeneration, high hepatic cholesterol levels, and impaired liver function when fed a high-cholesterol diet (Alberti et al 2001, Edwards et al 2002, Peet et al 1998). LXRβ-/- mice are unaffected by a high-cholesterol diet, suggesting that LXRα and LXRβ have separate roles. LXRα and LXRβ regulate cholesterol transport. LXRs induces expression of the cholesterol transporters ATP-binding cassette transporter A1 and G1 (ABCA1 and ABCG1) (Edwards et al 2002, Nakamura et al 2004, Venkateswaran et al 2000) as well as cholesterol acceptor apolipoprotein E (ApoE) (Chawla et al 2001). Treatment with LXR agonists (hypocholamide, T0901317, or GW3965) lowers the cholesterol level in serum and liver and inhibits the development of atherosclerosis in murine disease models (Blaschke et al 2004, Joseph et al 2002, Song et al 2001, Song and Liao 2001).

LXRs regulate fatty acid synthesis by modulating the expression of sterol regulatory element-binding protein-1c (SREBP-1c) (Repa et al 2000, Yoshikawa et al 2001) and downstream lipogenic genes, including acetyl CoA carboxylase and FAS (Liang et al 2002). LXRs also regulate insulin signaling in liver (Chen et al 2004b, Tobin et al 2002). LXRα-/- LXRβ-/- double knockout mice lack insulin-mediated induction of an entire class of enzymes involved in both fatty acid and cholesterol metabolism (Tobin et al 2002). Treatment with T0901317 stimulates insulin secretion in pancreatic beta cells, reduces plasma glucose, and improves glucose tolerance and insulin resistance in murine and rat obesity models (Cao et al 2003, Efanov et al 2004, Joseph et al 2003).

LXR signaling is important for brain function as well. LXRs regulate lipid homeostasis in the brain. LXRα-/- LXRβ-/- mice develop neurodegenerative changes in brain tissue (Wang et al 2002). Knockout of LXRβ, but not LXRα, results in adult-onset motor neuron degeneration in male mice (Andersson et al 2005), suggesting a different role of LXRβ from LXRα. Treatment with T0901317 decreases amyloidal beta production in an Alzheimer's disease mouse model (Koldamova et al 2005).

6. Anti-cancer effect of LXR agonists

6.1 Anti-proliferative effect of LXR agonists in cancer cells

Based on our recent observations using several prostate cancer cell lines, we discovered that LXR agonists suppress proliferation of human prostate cancer cell lines. Treatment of PC-3, DU-145, and LNCaP sublines (104-S, 104-R1, 104-R2, CDXR, R1Ad, IS) cells with LXR agonists (22(R)-hydroxycholesterol, 24(S)-hydroxycholesterol, or T0901317) suppresses the proliferation of these cells (Chuu and Lin 2010, Fukuchi et al 2004b, Vigushin et al 2004).

LXR agonists treatment causes growth inhibition in prostate cancer cells via induction of G1 cell cycle arrest (Chuu and Lin 2010, Fukuchi et al 2004b). T0901317 decreases the percentage of cells in S-phase and increases the percentage of cells in G1-phase. T0901317 suppresses

the expression of Skp2 and causes the accumulation of $p27^{Kip1}$. Overexpression of Skp2 in PC-3 cells or knockdown of $p27^{Kip1}$ in LNCaP cells increases the resistance of cells to T0901317 treatment (Chuu and Lin 2010, Fukuchi et al 2004b). Daily oral administration of T0901317 (10 mg/kg) suppresses growth of androgen-dependent LNCaP 104-S prostate tumors in athymic mice, resulting in a 2-fold difference in mean tumor volume between the control and the T0901317 treatment group (Fukuchi et al 2004b) (Figure 5).

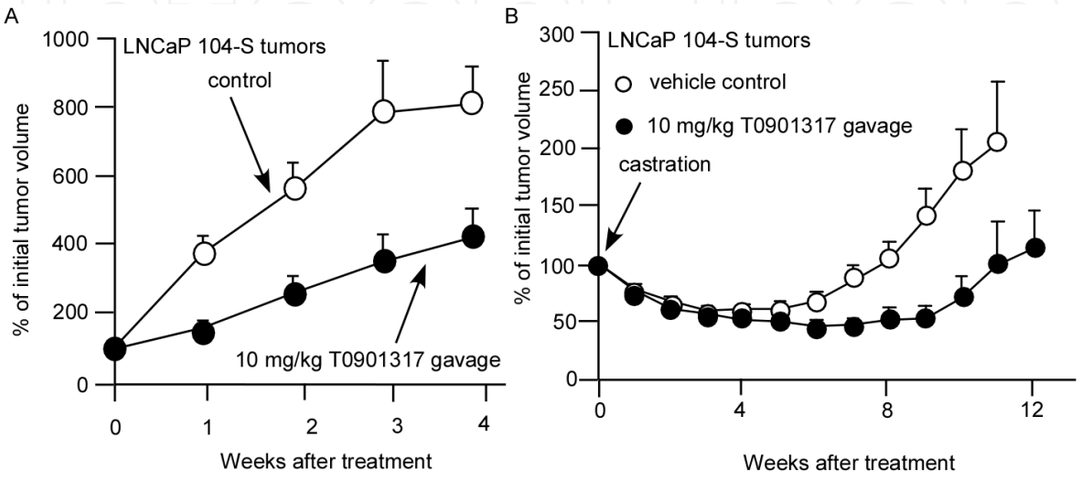

Fig. 5. Inhibition of proliferation and progression of prostate cancer by the LXR agonists T0901317. (A) Mice carrying 104-S tumors were administered 10 mg/kg T0901317 (filled circle, 10 mice with 13 tumors) or vehicle alone (open circle, 10 mice with 15 tumors) by gavage once a day during the experiment period, resulting in a more than 2-fold difference in mean tumor volume between vehicle and T0901317-treated tumors after 4 weeks. Relative tumor volumes were expressed as mean ± SE. (Fukuchi et al 2004b). (B) After castration, mice carrying 104-S tumors were administered 10 mg/kg T0901317 (filled circle, 9 mice with 15 tumors) or vehicle alone (open circles, 9 mice with 13 tumors) by gavage five times a week during the experiment period, resulting in a 4-week delay in time required for development of androgen-independent relapsed tumors between vehicle and T0901317-treated group. Relative tumor volumes were expressed as mean ± SE. See reference 8 for details.

T0901317 and 22(R)-hydroxycholesterol also suppresses the proliferation of several commonly used human cancer cell lines, including breast cancer MCF-7 cells, hepatoma HepG2 cells, non-small lung cancer H1299 cells, cervical cancer HeLa cells, epidermoid carcinoma A431 cells, osteosarcoma saos-2 cells, melanoma MDA-MB-435 cells, squamous carcinoma SCC13 cells, CAOV3 and SKOV3 ovarian cancer cells, as well as T and B cells of chronic lymphoblastic leukemia (CLL) (Chuu and Lin 2010, Fukuchi et al 2004b, Geyeregger et al 2009, Scoles et al 2010, Vedin et al 2009). Expression of LXRα mRNA in these cancer cells correlates with the cancer cells' sensitivity to 22(R)-hydroxycholesterol treatment (Chuu and Lin 2010), suggesting that G1 cell cycle arrest induced by LXR agonists in cancer cells is partially mediated through LXRα gene regulation (Fukuchi et al 2004b).

The EC_{50} for 22(R)-hydroxycholesterol in suppressing the proliferation of cancer cells (Chuu and Lin 2010) is comparable to the concentration required for 22(R)-hydroxycholesterol to activate LXRα (1.5 μM) (Janowski et al 1996), this may explain why the level of LXRα

correlates with the sensitivity of different cancer cells to 22(R)-hydroxycholesterol treatment. The effective concentrations for 22(R)-hydroxycholesterol to suppress cancer cell growth is within its known physiological range and is much lower than the concentrations to activate other nuclear receptors (Janowski et al 1996). LXRβ-ABCG1 signaling was reported to regulate sterol metabolism (Bensinger et al 2008). Activation of LXRβ inhibited the proliferation of T-cells but had no effect on cell viability (Bensinger et al 2008). Since T0901317 did not inhibit the proliferation of CAOV3 ovarian cancer cells treated with siRNA against LXRα or LXRβ (Scoles et al 2010), it is possible that 22(R)-hydroxycholesterol inhibited cell proliferation mainly through activation of LXRα, while inhibition of T0901317 may be caused by both LXRα and LXRβ activation. We did not observe T0901317 to cause cancer cell growth inhibition at 300 nM (data not shown). It is unclear why the concentration needed for T0901317 to suppress the proliferation of human cancer cells is 15-fold higher than the effective concentration for T0901317 to activate LXRα (20 nM) (Schultz et al 2000). The concentration of T0901317 observed to cause growth inhibition of ovarian cancer cell lines by Scoles et al. was 10-50 nM when the researchers used 0.1% FBS (Scoles et al 2010). We used 10% FBS in our study, it is possible that some proteins or growth factors in serum may hinder the suppressive effect of T0901317.

6.2 Inhibition of prostate cancer progression by LXR agonists

In our progression model, expression of LXRα and its target gene ABCA1 is higher in androgen-dependent LNCaP 104-S cells than in androgen-independent LNCaP 104-R1 and 104-R2 cells (Fukuchi et al 2004a). Expression of the LXRα, ABCA1, and sterol 27-hydroxylase (CYP27) genes, all target genes of LXRα, decreases during prostate cancer progression towards androgen-independency in athymic mice (Chuu et al 2006). The change in expression of genes involved in LXR signaling suggests a potential role of LXR signaling during prostate cancer progression. LXR agonists treatment on LNCaP sublines suggested that androgen-dependency and expression of AR level did not affect the growth inhibition caused by LXR agonists, thus LXR agonists may inhibit different progression stages of prostate tumors in patients (Chuu and Lin 2010).

We found that suppression of ABCA1 expression by androgen coincided with increased proliferation of androgen-dependent LNCaP 104-S cells (Fukuchi et al 2004a). Thus, under androgen-depleted conditions, ABCA1 levels are high and proliferation of 104-S cells is inhibited. During progression, the surviving androgen-independent relapsed tumor cells appear to escape ABCA1 suppression by down-regulating expression of LXR target genes. T0901317 induces expression of the ABCA1 gene in 104-S tumors in athymic mice (Fukuchi et al 2004b). Compared to the control group, T0901317 treatment delays the development of androgen-independent relapsed tumors for 4 weeks in athymic mice bearing 104-S tumors after castration (Chuu et al 2006) (Figure 5). This result indicates that treatment with an LXR agonist may retard development of androgen-independent prostate cancer.

7. Conclusion

Our LNCaP progression model may provide the molecular explanation for IAS treatment. As most relapsed prostate tumors after androgen ablation therapy express AR and expression of mRNA and protein level of AR are frequently elevated (de Vere White et al 1997, Ford et al 2003, Linja et al 2001), restoration of endogenous testosterone level by IAS

treatment or treatment with exogenous testosterone will suppress the proliferation of the AR-rich relapsed prostate cancer cell according, similar to the observations in LNCaP 104-R1, 104-R2, CDXR, and in other relapsed prostate cancer cell models. Patients showed no response to IAS treatment might have tumors with very low or no AR expression. At the beginning of IAS or testosterone treatment, serum PSA level will increase dramatically (Mathew 2008), similar to the stimulated PSA expression in 104-R1, 104-R2, and CDXR cells. The AR-rich relapsed prostate cancer cells will then undergo G1 cell cycle arrest and/or apoptosis, causing the regression of tumor and decrease of serum PSA level. The regression of tumors can continue for weeks or months before the prostate cancer cells adapt to the androgenic suppression, possibly by down-regulating AR. The adapted cells are probably similar to R1Ad cells in patients receiving androgen ablation therapy (LH-RH agonists) or similar to IS cells in patients receiving combined treatment of LH-RH agonists and anti-androgens. The PSA secretion stimulated by androgen in R1Ad or IS cells is very low, so the serum PSA level will remain low until the adapted tumors start to grow, either stimulated by testosterone like R1Ad cells or by androgen-insensitive growth like IS cells. IAS will delay the growth of R1Ad-like but not IS-like tumors, therefore, only the subgroup of patients carrying R1Ad-like tumors will respond to the subsequent cycles of IAS treatment. As 104-R1 cells will progress to 104-R2 cells in androgen-depleted medium and 104-R2 cells, like CDXR cells, will generate IS-like cells following androgen treatment, patients receiving a few cycle of IAS treatment will ultimately develop IS-like tumors which don't respond to further IAS treatment. Alternative therapies, such as green tea catechin epigallocatechin 3-gallate (EGCG) or liver X receptor agonists might be able to suppress growth of these androgen-insensitive prostate tumors.

Patients develop relapsed androgen-independent prostate tumors after androgen ablation therapy should be biopsied for expression level of AR protein in tumors. IAS and/or administration of androgen at a concentration 5-fold higher than the physiologic concentration will benefit patients with AR-rich relapsed tumors by suppressing tumor growth, improving quality of life, and reducing risks for cardiovascular diseases and diabetes. Combined treatment of androgen ablation therapy with anti-androgen may cause a more rapid and irreversible selection of CDXR-like advanced prostate cancer cells, although androgen treatment may cause regression and disappearance of these tumors (Kokontis et al 2005). Androgen deprivation therapy alone, on the other hand, may promote a slow adaptation to androgen-independence. LXR agonists suppress the proliferation of multiple human prostate cancer cell lines via reduction of Skp2 and induction of $p27^{Kip}$, thus cause G1 cell cycle arrest. LXR agonist T0901317 treatment also delays the progression of androgen-dependent LNCaP xenograft towards androgen-independency in castrated nude mice. It is therefore possible to modulate LXR signaling as an adjuvant therapy for treatment of all stages of prostate cancer. In conclusion, manipulating androgen/AR might be a potential therapy for AR-positive advanced prostate cancer, and LXR agonists might be an adjuvant therapy for treatment of advanced prostate cancer.

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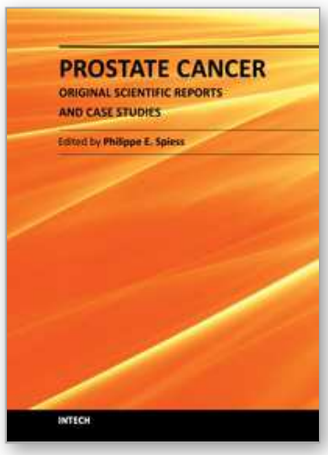

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This book encompasses three sections pertaining to the topics of cancer biology, diagnostic markers, and therapeutic novelties. It represents an essential resource for healthcare professionals and scientist dedicated to the field of prostate cancer research. This book is a celebration of the significant advances made within this field over the past decade, with the hopes that this is the stepping stone for the eradication of this potentially debilitating and/or fatal malignancy.

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