

Valproic Acid Induces Neuroendocrine Differentiation and UGT2B7 Up-Regulation in Human Prostate Carcinoma Cell Line^S

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ABSTRACT:

Prostate cancer originates as an androgen-dependent hyperproliferation of the epithelial cells of the gland and it evolves in an androgen-independent, highly aggressive cancer for which no successful therapy is available to date. Neuroendocrine (NE) differentiation plays an important role in the progression of prostate cancer to an androgen-independent state with profound impact on prostate cancer (CaP) therapies. Actually, new approaches on treating advanced prostate cancer are focused on modulators of epigenetic transcriptional regulation. A new class of antitumoral agents is emerging: histone deacetylase (HDAC) inhibitors are interesting for their ability to arrest cell growth, to induce cell differentiation, and in some cases, to induce apoptosis of cancer cells. We studied the effect of valproic acid (VPA), an inhibitor of HDAC, in the human prostate androgen-dependent cancer cell line LNCaP. We observed that VPA promotes neuroendocrine-like differentiation associated with an increase in the expression of neu-

ron-specific enolase, a decrease in prostate-specific antigen, and a down-regulation of androgen receptor protein, suggesting a modulation in the responsiveness to androgen therapy. Furthermore, selective gene expression profiling using a low-density microarray showed that VPA was able to modulate the expression of different androgen metabolism genes. We observed a down-regulation of androgen receptor coregulator (ARA24) and prostate-specific antigen, and an up-regulation of some of the UDP-glucuronosyltransferases (UGT2B11 and UGT2B7) implicated in catabolism of dihydrotestosterone (DHT) was detected. Even though UGT2B7 has only about one-tenth to one-hundredth the activity of UGT2B15 and 2B17 toward active androgens and we did not find any modulation in gene expression of these enzymes, it can be hypothesized that VPA might enhance DHT catabolism in this in vitro model and induces NE differentiation. Our data seem to raise concern about CaP treatment with VPA.

Recently, modulators of epigenetic transcriptional regulation have been suggested to be useful for treating advanced prostate cancer. Indeed, it is well known that acetylation and deacetylation of histones play an important role in the epigenetic regulation of gene expression (Roth and Allis, 1996; Minucci and Pelicci, 2006). There are at least two classes of enzymes involved in determining the acetylation state of histones, histone acetyl transferases and histone deacetylases. Altered states of these enzymes have been associated with various malignancies (Blaheta et al., 2005; Angelucci et al., 2006). New data provide evidence that prostate cancer may particularly benefit from

valproic acid (VPA) treatment. In fact, some authors demonstrated that VPA in vitro is able to induce apoptosis in the LNCaP prostate cancer cell line (Thelen et al., 2004; Angelucci et al., 2006), inducing a dramatic decrease of prostate-specific antigen (PSA) mRNA expression (Thelen et al., 2004). Moreover, recent studies demonstrate that chronic VPA treatment results in statistically significant reduction of tumor xenograft growth in vivo, independently of androgen regulation (Xia et al., 2006).

Androgens play a central role in promoting the development and progression of prostate cancer, and androgen ablation through the androgen receptor (AR) has been the cornerstone of treatments for advanced prostate cancer. In this context, the identification of pharmacological targets and the investigation of the mechanisms underlying the regulation of AR function is of critical importance for the design and development of new therapies (Chen et al., 2005). Moreover, it has been reported that after long-term antiandrogen therapy, tumor cell populations become enriched for neuroendocrine (NE)

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ABBREVIATIONS: VPA, valproic acid; CaP, prostate cancer; NE, neuroendocrine; UGT, UDP-glucuronosyltransferase; PSA, prostate-specific antigen; NSE, neuron-specific enolase; AR, androgen receptor; TBS-T, Tris-buffered saline/Tween 20 (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.05% Tween 20); RT-PCR, reverse transcription-polymerase chain reaction.

cells and that NE differentiation is associated with progression of CaP toward an androgen-independent state, a condition for which there is currently no successful therapy (Abrahamsson and Lilja, 1989). In fact, neuroendocrine differentiation has been implicated in prostate cancer progression and hormone therapy failure since neuroendocrine cells are nonproliferating and then avoid apoptotic cell death (Cox et al., 1999).

The progression of CaP from androgen-responsive to an androgen-unresponsive state remains one of the major obstacles in the treatment of this disease. Therefore, VPA might represent an effective therapy of androgen-unresponsive cancer (Angelucci et al., 2006).

In this study, we evaluated the expression profile of genes involved in the biosynthesis and metabolism of androgens in LNCaP cells after VPA treatment. To this purpose, we developed a low-density oligo-array containing 103 genes able to delineate the effects of VPA treatment on the androgen-signaling pathway. Using this approach, we were able to provide additional findings to understand the cellular and molecular effects of VPA in androgen-responsive CaP cells in vitro.

Materials and Methods

Chemicals and Cells. Human prostate cancer cell line LNCaP was grown in RPMI-1640 (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (Invitrogen), 5% L-glutamine (GIBCO), and antibiotics, under standard conditions (37°C temperature, 5% CO₂ in a humidified atmosphere). Valproic acid (Sigma, St. Louis, MO) dissolved in sterile water was administered to the cells 24 h after seeding, at the final concentrations of 1 mM and 5 mM.

PSA and Neuron-Specific Enolase Assay. The Elecsys total PSA immunoassay (Roche Diagnostics, Indianapolis, IN) was used to quantify the PSA in medium of cells treated or not treated with valproic acid. The neuron-specific enolase (NSE) immunoassay (Roche Diagnostics) was used to measure the quantity of NSE in medium of cells treated or not treated with VPA. The electrochemiluminescence immunoassays (ECLIA) were used on the Modular Analytics (Elecsys module; Roche Diagnostics). The results of PSA and NSE levels are related to the cell number.

Western Blotting. Total cell lysates were obtained by resuspending the cells in buffer containing 1% Triton, 0.1% SDS, 2 mM CaCl₂, 100 µg/ml phenylmethylsulfonyl fluoride. Protein content was determined using the Protein Assay Kit 2 (Bio-Rad Laboratory, Hercules, CA). Proteins (30 µg) were electrophoresed in 10% SDS-polyacrylamide gel and then electrotransferred to nitrocellulose membrane (GE Healthcare, Chalfont St. Giles, Buckinghamshire, UK), which was then blocked overnight with 10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.05% Tween 20 (TBS-T) containing 5% nonfat dry milk. The membrane was then incubated with 1 µg/ml primary antibody in TBS-T [AR (clone G122-434; BD PharMingen, San Diego, CA) or UGT2B7 (BD Gentest, Woburn, MA)] and with specific horseradish peroxidase-conjugated secondary antibodies in TBS-T. Protein bands were visualized using a chemiluminescent detection system (GE Healthcare).

Microarray Design and Printing. We developed a low-density homemade oligo-array composed of 103 genes selected on the basis of their proven or potential role in prostate cancerogenesis related to androgen signaling ("AndroChip"). The genes have been subdivided into different classes, according to their molecular function or the biological process in which they are active: genes regulating the androgen metabolism within the prostate, androgen receptor (AR), and genes that bind to the AR-complex (such as *ARA70*), genes whose expression is androgen-regulated (ARGs), genes involved in transcriptional regulation, and genes in the signaling pathway.

A complete list of genes present on the AndroChip is available as Supplemental Data. The oligonucleotide set (MWG Biotech, Ebersberg, Germany) was printed in triplicate onto UltraGAPS glass slides (Corning, Schiphol-rijk, The Netherlands) using TheRoboArrayer (Microgrid Compact Plus, BioRobotics, Ann Arbor, MI). Printed slides were dried overnight and cross-linked with UV light at 600 mJ using a Strata-linker 2400 (Stratagene, Glensville, VA) and stored in a desiccator at room temperature. Before hybridization, each slide was incubated in a prehybridization buffer (5× standard saline citrate, 0.1% SDS, and 0.1 mg/ml bovine serum albumin) at 42°C for 45 to 60 min.

RNA Extraction, Labeling, and Hybridization. Total RNA was isolated from untreated and VPA-treated LNCaP cells for 48 h using the TRIzol standard protocol (Invitrogen). A small aliquot of RNA was then used for quantification and quality control using, respectively, a spectrophotometer (NanoDrop Technologies, Wilmington, DE) and agarose gel electrophoresis.

Synthesis of the labeled first-strand cDNA was conducted using the Superscript Indirect cDNA labeling system (Invitrogen) with starting material of 10 µg of total RNA. The amino-allyl-labeled deoxynucleoside-5'-triphosphate mix was added to the reaction to generate amino-allyl-labeled second-strand cDNA. After the hydrolysis reaction, single-stranded cDNA probes were purified using a Purification Module (Invitrogen). Probe mixtures were then evaporated in a vacuum centrifuge, and the cDNA pellet was resuspended in 3 µl of water. The dye-coupling reactions were performed by mixing the cDNA samples with AlexaFluor Dye 555 or 647 and were incubated overnight in the dark. The reactions were purified with a Purification Module (Invitrogen) to remove the unincorporated/quenched dyes. After the purification, samples were combined for hybridization. The labeled cDNAs were cohybridized to microarrays in duplicate. Slides were scanned on the GenePix 4000B Microarray Scanner (Molecular Devices, Sunnyvale, CA) at the optimal wavelength for the AlexaFluor 555 (F532) and 647 (F635) (Invitrogen) using lasers.

Image Analysis and Processing Bioinformatics. The acquired images were analyzed with GenePix Pro 5.0 software (Molecular Devices). Oligonucleotide spots were automatically segmented, local backgrounds (B635 and B532) were subtracted, and total intensities (F635 and F532) as well as the fluorescence ratios of the two dyes were calculated for each spot. The spots were rejected when they exhibited poor hybridization signals or when they were saturated (F635 median = 65,534 or F532 = 65,534). Genes with signal to background ratio below 2 were flagged, together with genes strongly disagreeing by -fold change: if two of three replicates were overexpressed, whereas the third had a -fold change below the cutoff -2, the gene was flagged; underexpressed genes were treated similarly.

Data have been normalized with a separate global normalization for each slide. This permits a better stabilization of the MA plots than multi-slide or intensity-dependent normalizations do. Genes were selected through *t*-testing, and we corrected for the multiplicity by controlling the false discovery exceedance of Van der Laan et al. (2004), to avoid an explosion of the number of false rejections. False discovery exceedance control was given by the GAUGE procedure and guarantees that the proportion of erroneously selected genes is below 10% with a probability at least 95%. Finally, among the significant genes, we considered only the ones with a -fold change at least equal to ±1.5.

Reverse Transcription and Quantitative Real-Time RT-PCR. cDNA synthesis was performed from 1 µg of total RNA for each sample, by using AMV reverse transcriptase and as primers oligo(dT) (1st Strand cDNA synthesis kit for RT-PCR (AMV); Roche Applied Science, Indianapolis, IN).

Real-time quantitative RT-PCR was carried out using the Mx3000P (Stratagene), which detects the signal from the fluorogenic SYBR Green during PCR. The volume of each reaction was 25 µl containing 5 µl of the cDNA sample and 5 pmol of each primer: UGT2B7 (5'-AGAATTCATCATGCAACAG, 3'-GTTATGTCACCAATATTG), ARA24 [5'-CCACCAGAAGTTGTCATGGAC, 3'-ACAAGGGATGAGTTCACCTGC), UGT2B11 (5'-TTCCATTC-TTTTGTATCCCAATGATG, 3'-TAGGTATGAGGAAGGGGAAAAA-TC), UGT2B15 (5'-GTGTTGGGAATATTGACTACTACAGTAAC, 3'-TCA-GCCAGTAGCTACCACAGGG), UGT2B17 (5'-TGTTGGGAATATTCT-GACTATAATATA, 3'-CAGGTACATAGGAAGGAGGGAA); and for β₂-microglobulin (5'-GAGTATGCCTGCCGTGTG, 3'-AATCCAATGCGGC-ATCT), 12.5 µl of SYBR Green Universal PCR Master Mix (Applied Biosystems, Foster City, CA). The specificity of the primers used for UGTs was confirmed by the BLAST program considering the Query coverage of 100%. The thermal cycling conditions comprised 10 min at 95°C. Thermal cycling consisted of 44 cycles at 95°C for 30 s, 55°C for 1 min, and 72°C for 30 s. Each assay included a standard curve (from 1 µg to 125 ng of cDNA) in duplicate, a no-template control (water), and 250 ng of calibrator cDNA in triplicate (from DU145 cells). All samples with a coefficient of variation (CV) higher than 10% were retested.

Quantitative Real-Time PCR Validation Experiment. The amount of target, normalized to the endogenous reference (β₂-microglobulin) and relative to the calibrator, was performed by the 2^{-ΔΔCT} method (Livak and Schmittgen,

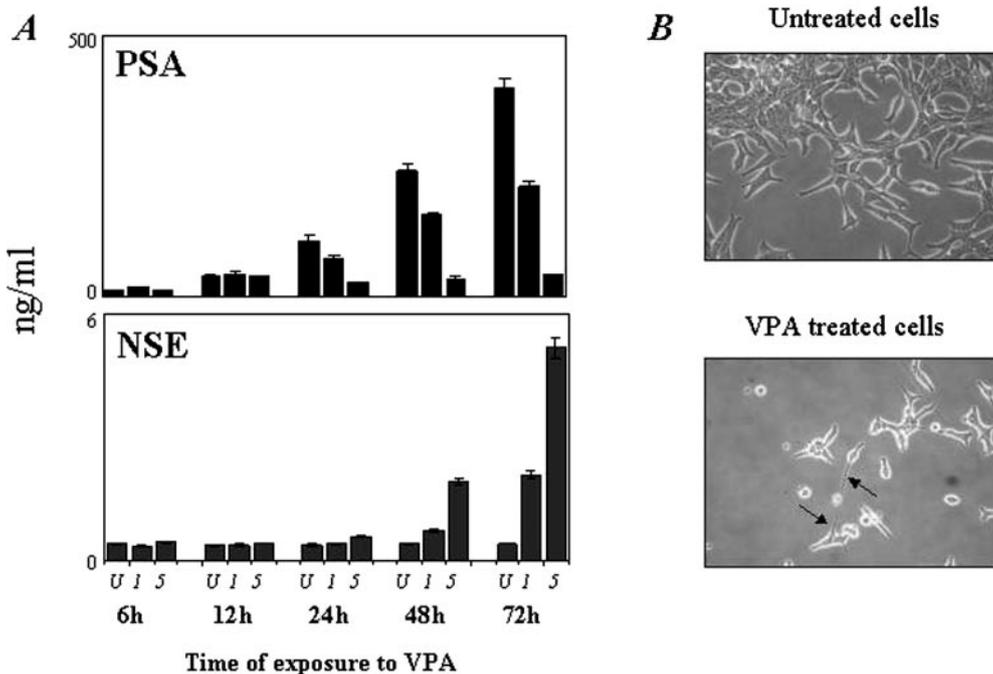


FIG. 1. A, PSA and NSE analysis is expressed as ng/ml protein in culture medium of LNCaP cells after different times of exposure to valproic acid and results are related to the cell number for each point. The electrochemiluminescence immunoassay (ECLIA) was used on the Modular Analyt-ics (Elecsys module; Roche). U, untreated cells; 1, 1 mM VPA; 5, 5 mM VPA. Results are expressed as mean \pm S.D. for $n = 3$ independent experiments. B, fewer attached cells that showed long dendritic processes (arrows) remained after 72 h of VPA treatment (lower panel) compared with untreated cells (upper panel).

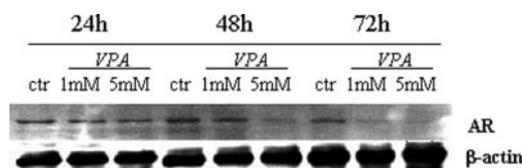


FIG. 2. Representative Western blot illustrating the expression of AR protein in LNCaP cells after different times of exposure to VPA. β -Actin has been used as a control for sample loading.

2001). The relative expression was calculated with the “delta-delta Ct method” ($\Delta\Delta Ct = \Delta Ct_{\text{sample}} - \Delta Ct_{\text{calibrator}}$). The Ct (threshold cycle) values were normalized against the endogenous reference gene β_2 -microglobulin ($\Delta Ct = Ct_{\text{target gene}} - \Delta Ct_{\beta_2\text{-microglobulin}}$) and were compared with the calibrator. The $\Delta\Delta Ct$ validation required equal efficiencies of target genes and reference amplification. Therefore, standard curve assays were obtained for the target gene and reference by a cDNA sample diluted at 2-fold intervals. For each dilution, cycle threshold was plotted against the log value of the input cDNA concentration. Quantitative PCR efficiencies (E) were calculated from the slopes, where one cycle in the exponential phase yielded the efficiency: $E = 10^{[-1/\text{slope}]}$.

Results

VPA Induced PSA Decrease and Neuron-Endocrine Differentiation in LNCaP. We analyzed the effect on PSA secretion after VPA treatment (1 mM and 5 mM) at different times of exposure. Figure 1A shows that the effect of VPA on PSA secretion was dose-dependent starting from 24 h of exposure. A reduction of PSA secretion of about 50% was detected after 24 h of treatment with 1 mM VPA as well as after 48 and 72 h. At the concentration of 5 mM VPA, stronger reduction in PSA production was observed (Fig. 1A).

After 48 h of treatment with VPA (5 mM), we observed a dose-dependent increase of NSE, indicating that VPA is able to induce an early neuron-endocrine differentiation in LNCaP. In addition, after 72 h of VPA exposure, the secretion of NSE increased even in cells treated with 1 mM VPA. The NSE increase is consistent with morphological changes observed in VPA-treated cells (Fig. 1B); indeed, fewer attached cells that showed long dendritic processes remained after 72 h of VPA treatment (Fig. 1B, lower panel) compared with untreated cells (Fig. 1B, upper panel).

Down-Regulation of Androgen Receptor Protein Expression Induced by VPA. Since valproic acid is expected to modulate AR activity with downstream consequences as suggested by PSA decrease (Thelen et al., 2004), we investigated the effect of VPA treatment on AR expression in LNCaP cells by Western blot analysis. Figure 2 shows that the down-regulation of AR protein is VPA dose-dependent. After 48 h of exposure to valproic acid, a decrease of AR protein

expression was evident, with a complete down-regulation of AR protein expression after 72 h of exposure for both the doses used, 1 and 5 mM.

Gene Expression Profile of LNCaP Cells Treated with VPA. To monitor changes in gene expression, we performed microarray experiments in LNCaP cells treated with 5 mM VPA after 48 h. Experiments were done in duplicate using reverted labeled RNA. After data normalization, we considered only genes showing a ± 1.5 -fold change at least equal to ± 1.5 . A total of 15 differentially expressed genes were detected (Table 1). Of these, 10 were underexpressed and 2 (UGT2B7 and UGT2B11) were overexpressed.

Microarray results were confirmed by quantitative real-time PCR for both UGT2B7 and UGT2B11 genes (Fig. 3A). In addition, as shown in Fig. 3B the UGT2B7 protein up-regulation was evaluated. Using $\Delta\Delta Ct$ gene expression quantification, we observed that the up-regulation of UGT2B7 and UGT2B11 genes was VPA dose-dependent. In fact, as shown in Fig. 3 at the concentration of 1 mM VPA, we observed a 2-fold up-regulation of UGT2B7 gene expression, whereas at 5 mM, a 7-fold higher increase was detected. Analogously, UGT2B11 was up-regulated 2.2-fold after 1 mM VPA exposure and 5-fold after 5 mM VPA. ARA24 was found to be down-regulated 2-fold after 1 mM VPA and 5.2-fold after 5 mM VPA exposure. Moreover, we analyzed the expression of UGT2B15 and UGT2B17, implicated in DHT glucuronidation, and we did not find any modulation induced by VPA treatment. The relative expression level of gene in VPA-treated versus untreated cells, reported as $2^{-\Delta\Delta Ct}$, was 1.0 for both genes.

TABLE 1

Genes differentially expressed in LNCaP treated with valproic acid

Gene	Accession Number	Gene Title	-Fold Change
Down-Regulated Genes			
<i>ARA24</i>	NM_006325	Androgen receptor coregulator	-2.89
<i>AR</i>	NM_000044	Androgen receptor	-1.64
<i>CYP11A</i>	NM_000781	Cytochrome P450, family 11, subfamily A, polypeptide 1	-1.73
<i>KLF6</i>	NM_001300	Krappel-like factor 6	-1.63
<i>CGI-10</i>	NM_0152940	CGI-10 protein	-1.69
<i>FDPS</i>	NM_002004	Farnesyl diphosphate synthase-like 1 (farnesyl pyrophosphate synthetase-like 1, cholesterol-repressible protein 39A)	-2.02
<i>ID11</i>	NM_004508	Isopentenyl-diphosphate delta isomerase 1	-2.01
<i>PART1</i>	NM_016590	Prostate androgen-regulated transcript 1	-2.01
<i>PDE5A</i>	NM_001083	Phosphodiesterase 5A, cGMP-specific	-1.94
<i>PSA</i>	NM_001648	Kallikrein 3, (prostate-specific antigen)	-6.19
Up-Regulated Genes			
<i>UGT2B7</i>	NM_001074	UDP-glucuronosyltransferase 2 family, polypeptide B7	1.90
<i>UGT2B11</i>	NM_0010173	UDP-glucuronosyltransferase 2 family, polypeptide B11	2.46

FDPS, farnesyl pyrophosphate synthase; ID11, isopentenyl-diphosphate δ -isomerase; PART1, prostate androgen-regulated transcript.

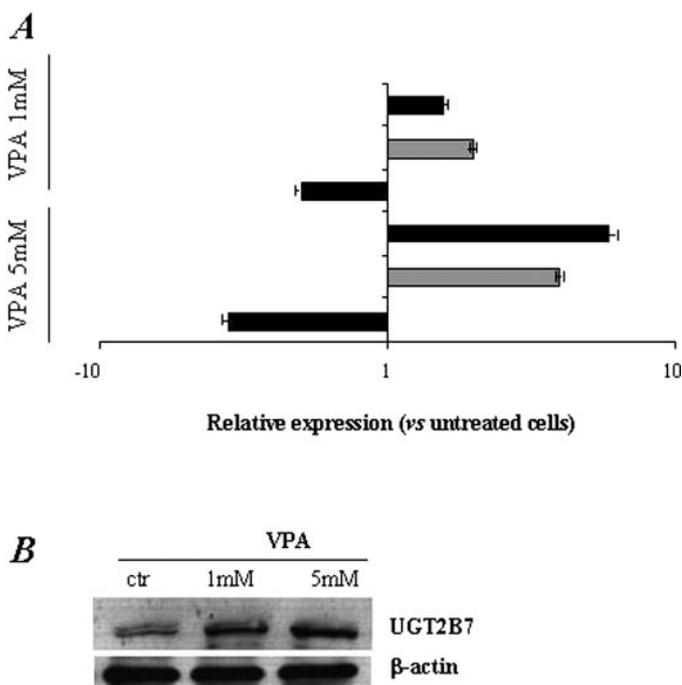


FIG. 3. A, UGT2B7 (black columns) and UGT2B11 (gray columns) gene expression increased and ARA24 (dark gray columns) decreased in the LNCaP cells exposed to VPA for 48 h. Target mRNA levels were evaluated by quantitative RT-PCR and standardized to β_2 -microglobulin mRNA levels ($\Delta\Delta CT$ method). Expression levels in the control group were designated as 1.0, and relative expression levels in the LNCaP VPA 1 mM (upper)- and VPA 5 mM (lower)-treated cells are shown. Data are represented as the mean \pm S.D. from three independent experiments. B, representative Western blotting illustrating the expression of UGT2B7 protein in LNCaP cells after 48 h of exposure to VPA. β -Actin has been used as a control for sample loading.

Discussion

We report that VPA promotes neuroendocrine-like differentiation of androgen-dependent LNCaP prostate cancer cells. This differentiation is associated with an increase in the secretion of NSE, a decrease in PSA, and down-regulation of AR protein, suggesting a role of VPA in the mechanism of androgen responsiveness of prostate cancer. Because AR expression is influenced by post-translational and epigenetic modifications, it could be of interest to better understand the role of VPA in modulating this protein. Inhibition of cell proliferation and of AR protein expression has been observed in prostate cancer cells after exposure to chemopreventive compounds, such as vitamin E,

resveratrol, and VPA, as well (Culig et al., 2005; Angelucci et al., 2006). Since elevated NSE levels in prostate carcinomas during androgen ablation therapy correlate with hormone-refractory growth and poor prognosis (Hvamstad et al., 2003), the ability of the histone deacetylase inhibitor VPA to induce NE differentiation in LNCaP-derived prostatic carcinoma cell lines should be elucidated by studying genes involved in homeostasis of prostate cancer cells.

A low-density microarray, such as our custom AndroChip, is a reliable technique through which, in a single assay, the quantitative expression of each gene in response to a change in the cellular state can be measured simultaneously. Gene profiling by AndroChip of LNCaP treated with valproic acid showed that genes differentially expressed are mostly involved in steroid metabolism. In particular, we observed a down-regulation of androgen receptor coregulator (ARA24), farnesyl pyrophosphate synthase, which is a cholesterol-repressible protein, isopentenyl-diphosphate δ -isomerase, prostate androgen-regulated transcript, and PSA, and up-regulation of UDP-glucuronosyltransferases (UGT2B11 and UGT2B7) involved in the catabolism of DHT.

It should be pointed out that ARA24 is a cofactor implicated in the enhancement of AR transactivation, in AR nuclear translocation, and in chromatin organization. The AR coactivator effect of ARA24 is seen not only at the physiological concentration of DHT but also at low DHT levels (10^{-10} M) usually found in patients undergoing androgen ablation therapy (Hsiao et al., 1999). Moreover, ARA24 is significantly higher in prostate tumor tissue compared with non-neoplastic prostate tissue (Li et al., 2002). In vitro studies have shown that altered expression of AR coregulators may significantly modify the transcriptional activity of AR, suggesting that these coregulators could also contribute to the progression of prostate cancer. AR coactivators, showing histone acetyltransferase activity, interact with one or more domains of the AR, thus leading to remodeling the chromatin structure through the acetylation of histones (Culig et al., 2005). Not only expression, but also functional interactions of coregulatory proteins may be altered in the late stage of prostate cancer. This mechanism might be active with other cofactors in prostate cancer, thus enhancing expression of AR target genes in late tumor stages. In a cellular context, AR overexpression is clearly associated with malignant phenotype.

Furthermore, among VPA up-regulated genes, we have found UDP-glucuronosyltransferases. Valproic acid is a specific substrate for glucuronidation through UGT2B7 (Hum et al., 1999; Ethell et al., 2003); in addition, UGT2B7 catalyzes the conjugation of the 17 β -hydroxy position of DHT, testosterone, and 3 α -diol, and gluco-

ronidates androsterone (Turgeon et al., 2001). UGT2B7 has only about one-tenth to one-hundredth the activity of UGT2B15 and UGT2B17 toward these active androgens. Although it has been demonstrated that human prostate and LNCaP cells express both UGT2B15 and UGT2B17, which are two widely distributed UGT isoforms implicated in steroid glucuronidation (Lévesque et al., 1998), we did not find any modulation in gene expression of these two enzymes in LNCaP cells treated with VPA (data not shown). Thus, it could be hypothesized that VPA might enhance DHT catabolism in this in vitro model. Glucuronidation is catalyzed by UGTs, which transfer the polar moiety from UDP-glucuronic acid to a wide variety of endogenous compounds, including steroid hormones. UGT2B7 has been demonstrated to glucuronidate estrogens, catechol estrogens, and androstane-3 α ,17 β -diol more efficiently than any other human UGTB isoform (Turgeon et al., 2001).

Moreover, major pathways of valproate metabolism include glucuronidation by UGTs, mitochondrial β -oxidation and a minor cytochrome P450-dependent oxidation pathway, and desaturation (Baillie and Sheffels, 1995). In androgen-sensitive tissues, such as skin and breast, UGT2B7 probably contributes to the conjugation of phase I DHT metabolites, particularly 3 α -diol and androsterone (Bélanger et al., 2003). So far, it is known that UGT2B11 is involved in xenobiotic metabolism (Jinawath et al., 2006), and recently, it has been demonstrated that treatment of the prostate cancer cells LNCaP with natural and synthetic androgens, interleukin-1 α , or epidermal growth factor differently modulates the glucuronidation of androgen and bioactive lipid metabolites. Indeed, glucuronidation of 5 α -androstane-3 α ,17 β -diol and 13-hydroxyoctadecadienoic acid was drastically reduced, whereas 12-hydroxyeicosatetraenoic acid conjugation by UGT2B11 was increased after androgen treatment. In LNCaP cells, UGT2B11 mRNAs are detected and are regulated by androgens in a manner similar to that in human prostate epithelial cells (Chouinard et al., 2006). Bélanger (2003) has postulated that UGT activities may be implicated as a means to control androgen levels in target tissues.

However, because VPA is a short-chain branched fatty acid and hence a substrate of UGT2B7, we cannot exclude a competitive effect of VPA in steroid catabolism (Ethell et al., 2003). Thus, the action of VPA must be further investigated before considering it for therapeutic protocols in prostate cancer.

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