Published in final edited form as:

Prostate. 2009 July 1; 69(10): 1077–1090. doi:10.1002/pros.20960.

Unique Patterns of Molecular Profiling between Human Prostate Cancer LNCaP and PC-3 Cells

Mikhail G. Dozmorov¹, Robert E. Hurst^{1,2}, Daniel J. Culkin^{1,3}, Bradley P. Kropp¹, Mark Barton Frank⁴, Jeanette Osban⁴, Trevor M. Penning⁵, and Hsueh-Kung Lin^{1,3,6}

¹Department of Urology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104

²Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104

³Oklahoma City Veterans Affairs Medical Center, Oklahoma City, OK 73104

⁴Oklahoma Medical Research Foundation Microarray Research Facility, Oklahoma City, OK 73104

⁵Center of Excellence in Environmental Toxicology, Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

⁶Department of Physiology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104

Abstract

BACKGROUND—Human prostate cancer LNCaP and PC-3 cell lines have been extensively used to study prostate cancer progression and to develop therapeutic agents. Although LNCaP and PC-3 cells are generally assumed to represent early and late stages of prostate cancer, respectively, there is limited information regarding gene expression patterns between these two cell lines and its relationship to prostate cancer.

METHODS—Comprehensive gene expression analysis was performed. Total RNA was isolated from cultured cells and hybridized to Illumina human BeadChips representing 24,526 transcripts. Bioinformatics analysis was applied to identify cell line specific genes as well as biological mechanisms, pathways, and functions related to the genes.

RESULTS—A total of 2,198 genes were differentially expressed between LNCaP and PC-3 cells. Using a robust statistical analysis and high significance criteria, 115 and 188 genes were identified to be unique to LNCaP and PC-3 cells, respectively. LNCaP cells maintained various metabolic pathways including a gene cluster that encodes UDP-glucuronosyltransferases. Several transcription factors including Tal α / β , GATA-1, and c-Myc/Max may be responsible for regulating LNCaP cell specific genes. By contrast, PC-3 cells were characterized by their unique expression of cytoskeleton-related genes and other genes including VEGFC, IL8, and TGF β 2.

CONCLUSIONS—This study showed that LNCaP and PC-3 cells represent two distinct prostate cancer cell lineages. LNCaP cells retain many prostate cell specific properties, whereas PC-3 cells

Address all correspondence to: Hsueh-Kung Lin, Ph.D. Department of Urology University of Oklahoma Health Sciences Center 800 Research Parkway, Room 462 Oklahoma City, OK 73034 TEL: (405) 271-6900 FAX: (405) 271-3118 e-mail: hk-lin@ouhsc.edu.

Supplemental Material Supplementary data 1. Excel spreadsheet containing complete list of genes uniquely expressed and overexpressed in LNCaP and PC-3 cells.M

Supplementary data 2. Excel spreadsheet containing complete list of genes for ontological clusters and potential TREs in LNCaP and PC-3 cells

have acquired a more aggressive phenotype. Future studies for prostate cancer research need to consider similarities and differences between these two cells and their relationship to prostate cancer.

Keywords

prostate cancer; prostate cancer cells; microarray

INTRODUCTION

To understand pathological characteristics of prostate cancer, experimental model systems of this disease should precisely or at least closely reflect the disease conditions. Two human prostate cancer cell lines, LNCaP and PC-3, have been extensively used as cell models for prostate cancer studies. LNCaP was established from a supraclavicular lymph node metastatic lesion of human prostate adenocarcinoma (1,2). These cells express the androgen receptor (AR) and exhibit androgen sensitive cell growth. Despite the absence of receptors for estradiol and progesterone, LNCaP cells respond to both estrogen and progestagens with elevated proliferation (3). In addition, antiandrogens such as flutamide were also growth stimulators (4). These aberrant responses have been attributed to a threonine to alanine substitution at amino acid position 868 in the steroid binding domain of the AR (5,6). LNCaP cells gradually lose their androgen requirement for growth upon passage, mimicking androgen-independent progression in prostate cancer (7).

The human prostate cancer PC-3 cell line was established from a prostatic adenocarcinoma metastatic to bone (8). Electron microscopic studies showed that PC-3 cells retain features common to neoplastic cells of epithelial origin including numerous microvilli, junctional complexes, abnormal nuclei and nucleoli, abnormal mitochondria, annulate lamellae, and lipoidal bodies (8). PC-3 cells do not respond to androgen, glucocorticoid, epidermal growth factor (EGF), or fibroblast growth factor (FGF) for elevated cell growth regardless of the presence of glucocorticoid receptor (GR), EGF receptor, and FGF receptor in these cells (9-11).

LNCaP and PC-3 cells share some common biochemical features. For example, both cell lines retain the expression of a prostate marker, prostate specific antigen (PSA) (12); and they are similar in the expression of cell proliferation-associated markers (13). However, the two cell lines show marked differences other than the well-known difference in androgen sensitivity. LNCaP and PC-3 cells express different levels of angiogenic factors (14,15), pro-inflammatory cytokines (16), and prostate cancer markers (17-19). These cells also exhibit different capabilities in angiogenesis and tumor aggressiveness (14,15,20), as well as their responses to pro-inflammatory modulators (20,21) and environmental stress (22). Thus, there are many concerns regarding these two cell lines leading to the following question: do these two cell lines maintain prostate phenotypes and represent different stages of cancer progression?

In this communication, we compare global gene expression profiles between LNCaP and PC-3 cells. With thousands of differentially expressed genes being identified between these two lines, our data show that LNCaP cells retain metabolic functions and resemble prostatic epithelium, whereas PC-3 cells seem to have diverged from the original prostatic phenotype. Future molecular and biochemical studies comparing LNCaP and PC-3 cells need to consider not only the existence of phenotypic differences between these two cell lines but also their fundamental differences in gene expression profiles and associated biological functions.

MATERIALS AND METHODS

Human Prostate Cancer Cell Lines

Androgen sensitive human metastatic prostate adenocarcinoma LNCaP-FGC (LNCaP) cells (CRL-1740) and human bone metastasis-derived prostate cancer PC-3 cells (CRL-1435) were obtained from the ATCC (Manassas, VA). LNCaP cells were routinely maintained in a complete growth medium consisting of phenol red-positive RPMI1640 (Invitrogen; Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS; Invitrogen) and 100 units/ml penicillin-100 µg/ml streptomycin (Invitrogen) (23). PC-3 cells were maintained in phenol red-positive F-12 nutrient mixture (Invitrogen) containing 7% FBS and 100 units/ml penicillin-100 µg/ml streptomycin (24). LNCaP cells were passaged once a week, whereas PC-3 cells were passaged twice a week. In this experiment, LNCaP cells were harvested between passages 31 and 34, which are considered to be low passage numbers. PC-3 cells were used between passages 16 and 19.

RNA Extraction and Quality Evaluation

Total RNA was isolated from prostate LNCaP and PC-3 cells for probe preparation and microarray hybridization. Briefly, 5×10^5 prostate cells were seeded in 60 mm tissue culture plates and cultured for 48 hours in their growth media. Total RNA was isolated from 4 consecutive passages from each cell line using RNeasy® Mini total RNA isolation kit following the manufacturer's recommendations (Qiagen, Valencia, CA). The concentration of the isolated total RNA was determined with a Nanodrop scanning spectrophotometer and then qualitatively assessed for their integrity using the ratio of 28:18s rRNA by a capillary gel electrophoresis system (Agilent 2100 Bioanalyzer, Agilent Technologies, Santa Clara CA).

RNA Labeling, Microarray Hybridization, and Scanning

A total of 250 ng of total RNA from each passage and from each cell line was labeled using the Illumina Total Prep RNA Amplification Kit following manufacturer's directions (Ambion, Austin, TX). Briefly, cDNA was reverse transcribed from the total RNA after priming with T7-oligo-dT, and cRNA was synthesized *in vitro* from the T7 promoter with the incorporation of biotinylated UTP. Biotinylated cRNA was hybridized overnight to Illumina human Ref-8 version 3 BeadChips containing probes for a total of 24,526 transcripts. Microarray chips were washed at high stringency and labeled with streptavidin - Cy3 (Amersham Biosciences, Piscataway, NJ) prior to scanning on an Illumina BeadArray Reader.

Data Normalization and Comparison of Gene Expression

The data were first normalized based on normal distribution of low-level expressed genes using the previously described procedure (25,26). Normalization was performed for each array by first plotting a frequency histogram of the raw expression data for all genes. The histograms showed a right-skewed unimodal distribution curve with the mode around zero. A normal distribution curve representing variability of the data around zero was then fitted around the mode, mirroring Gaussian profile of the left part of the histogram. Two parameters [mean and standard deviation (SD)] were defined for this distribution; and the data were normalized to the SD of the noise after subtraction of the zero point. The arrays were then adjusted to each other by robust linear regression (27,28). Genes with expression value less than 3.0 were considered to be not expressed. This is equivalent to setting a threshold at 3 SD above noise level. Each experiment identified approximately 10,000 genes that were expressed below the noise level. Raw and normalized expression values are available on Gene Expression Omnibus (GEO) with accession number GSE13131.

To identify differentially expressed genes between the two experimental groups, we used the associative analysis as described by Dozmorov *et al.* (29). Briefly, in pooled microarray datasets, a reference group of genes expressed above background with low variability of expression was identified by an F-test. A *t*-test termed “associative *t*-test” was applied to test if a given gene belongs to or differs from this group. The associative *t*-test is a standard Student *t*-test applied to the comparison of expression variability. Due to a large number of genes that are differentially expressed between LNCaP and PC-3 cells, a very stringent criterion was applied to identify genes with the most dramatic differences. Only genes expressed below the noise level in one group and greater than 50 SD above the noise level in another group were selected for further analysis. For genes that were expressed in both cell lines, genes that have a greater than 10-fold difference in expression level and expressed greater than 100 SD above the noise level in at least one cell line were also selected. These genes can be considered to be “beacons” that point to pathways or gene networks that are altered.

Analysis of Significantly Overrepresented Ontologies, Pathways and Networks

Lists of differentially expressed genes were analyzed for overrepresented gene ontologies using the Database for Annotation, Visualization and Integrated Discovery (DAVID) (30). The Gene Functional Classification tool in DAVID clusters genes with significantly similar ontologies as tested against whole lists of genes in microarray. Medium stringency (default for DAVID) was used to yield comprehensive sets of clusters.

To relate functions, pathways, networks, and unique features to genes that are differentially expressed between two prostate cancer cell lines, Ingenuity Pathway Analysis (IPA; Ingenuity® Systems, Redwood City, CA, <http://www.ingenuity.com>), a web-based bioinformatics application, was used. IPA maps the identified genes to its human-curated database, tests what functions and pathways are overrepresented by the genes as compared to the whole list of genes in the microarray, and assembles biologically relevant networks based on reported interactions between target genes. Due to the high complexity of genetic interactions, IPA groups networks with no more than 35 genes in a single network. IPA also provides tests for significant differences between functions and pathways overrepresented in given lists of genes. These analyses were run with default statistical significance at $p < 0.05$. In addition, IPA identifies potential biomarkers among differentially expressed genes for each cell line. Biomarker filters for differentially expressed genes between LNCaP and PC-3 cells using IPA were set for Homo Sapiens genes, identified in prostate gland, and in urine as well as for possessing cancer characteristics.

Analysis of 5'-flanking Regions of Differentially Expressed Genes

The promoter analysis and interaction network toolset (PAINT) was used to identify overrepresented *cis*-acting elements within the genes of interest against similar or identical transcription regulatory elements (TREs) selected randomly from the whole set of genes on microarray (<http://www.dbi.tju.edu/dbi/tools/paint/>) (31). Statistical significance for testing the presence of TREs was set at $p < 0.05$; and additional filtering was set by false discovery rate (FDR) as described in the text.

Data Comparison

Genes that were differentially expressed between LNCaP and PC-3 cells were compared with published results from other studies. Unique genes between LNCaP and PC-3 cells reported by Yang *et al.* (32) were mapped into Illumina Human Ref-8 v.3 annotations and compared to correspondent genes identified by the Illumina expression array. Differentially expressed genes between primary prostate cancer and androgen-independent prostate cancer bone marrow metastases identified by Affymetrix oligonucleotide microarrays (33) were

manually compared to tables of differentially expressed genes between LNCaP and PC-3 cells by gene symbols.

RESULTS

Identification of Differentially Expressed Genes between LNCaP and PC-3 Cell Lines

An initial two-tailed *t*-test with Bonferroni correction ($p < 0.00004$) identified a total of 2,198 differentially expressed genes between these two cell lines. To minimize false positives, an associative analysis was applied to these two groups of arrays, four arrays for each of LNCaP and PC-3 cells. Statistical parameters of the associative analysis were set to identify statistically significant genes serving as “beacons” of differences between LNCaP and PC-3 cell lines. A total of 115 genes were highly expressed in LNCaP cells but expressed below noise levels in PC-3 cells, and classified as uniquely expressed genes in LNCaP cells. Similarly, a total of 188 genes were identified as uniquely expressed in PC-3 cells (genes that are highly expressed in PC-3 cells but expressed below noise levels in LNCaP cells). Additionally, groups of overexpressed genes, defined as genes that are expressed in both cell lines, but showed greater than 10-fold and higher than 100 SD above noise levels in one cell line versus the other, were identified. Based on these criteria, 85 genes were found to be overexpressed in LNCaP cells, whereas 113 genes were overexpressed in PC-3 cells (Supplementary data 1). Since combined analysis for both uniquely expressed and overexpressed genes did not significantly add information to the interpretation of the results, only uniquely expressed genes are presented.

Unique Ontological Signatures for LNCaP and PC-3 Cells

The Gene Functional Classification tool in DAVID identified 7 ontological clusters among the 115 genes uniquely expressed in LNCaP cells, and 9 ontological clusters among the 188 genes uniquely expressed in PC-3 cells (Table 1, complete lists of genes for all clusters are provided in Supplementary data 2).

Overrepresented ontologies differed significantly between LNCaP and PC-3 cell lines. For example, in LNCaP cells the UDP-glucuronosyltransferases was the first cluster of 7 genes overexpressed in this cell line that provided a unique gene signature. In addition, there was cluster of genes representing peptidase activity in LNCaP cells containing a group of kallikreins (KLK2, 3, 4). Interestingly, a differentiation signature was represented by three genes identified in neural tissues, RTN4, SLITRK3, and SPON2. Other ontological clusters contained various genes coding transmembrane proteins, domains, and channels.

In contrast, genes that are uniquely expressed by PC-3 cells exhibited a different set of ontologies. The first cluster of genes presented in this cell line contained several keratins, nestin and plasminogen activator. All molecules comprise cytoskeleton structural filaments. Two clusters contained genes from ubiquitin pathways and genes related to peptidase activity. Other ontology clusters included various transmembrane proteins and receptors, glycoproteins, and kinase/phosphatase activity. The most notable genes, among the genes unique to PC-3 cells, were receptors for TNF (TNFRSF18) and TGF- β (TGFB2). TGF β s including TGF β 2 and TGFBI (TGF β induced) were also present as genes specifically expressed in PC-3 cells.

Network and Canonical Pathway Analysis

Among the 115 genes unique to LNCaP cells, 65 were eligible for building five non-overlapping networks, while the other 50 were insufficiently annotated to participate in Ingenuity analysis. IPA identified five significant networks containing 35 genes each (Ingenuity limitation, see Methods). UDP-glucuronosyltransferases formed a solid group in

the first network and shared lipid metabolism and small molecule biochemistry functions (Fig. 1). The same set of UDP-glucuronosyltransferases was also overrepresented in multiple canonical metabolic pathways, including retinol, androgen, and estrogen metabolism (Table 2). The other five networks lacked clear “hubs” of genes at the center of a set of interactions. Such hubs identified key regulatory genes; and the poorly organized nature of other networks suggested that little is known about the functional activities of the genes comprising the networks.

Genes that are unique to PC-3 cells built more detailed networks; 135 out of 188 well-annotated genes from the literature formed 9 established networks and pathways. These genes shared some major functions, such as cancer, cellular movement, cellular growth, and proliferation, based on the IPA database. The most significant network comprised of genes responsible for cancer, cellular development and movement. Focus genes (grayed in Fig. 2) concentrated around NF κ B, P38 MAPK, IL12, Smad 2/3, LDL, ERK1/2, IGFBP and vitamin D3-VDR-RXR central hubs (Fig. 2A). Although not identified as differentially expressed genes, parts of these hubs were expressed on microarrays, but the differences in the expression levels were not sufficient to pass our stringent selection criteria. However, their presence on mRNA level indicates that the network is functional and such hubs participate in signal transduction. Network 2 contained cancer as well as renal and urological disease genes, and featured PDGF, TGF β , TIMP1, and MET expressed hubs, as well as other well characterized genes such as AP-1, ERK, IL-1, JAK, and JNK (Fig. 2B). Of particular interest are VEGFC, PDGFC, TGF β I, and JAK1. Network 3 consisted of VEGF, IL6, Ras homolog, and Hsp70 (Fig. 2C); and these genes interconnected with other well known genes such as AKT and PKC(s), cyclins A and E, insulin and interferon α , histone H3 and RNA polymerase II. Although none of these were selected as unique genes based on our statistical criteria, they nonetheless were expressed in PC-3 cells and can be key regulatory players.

Several canonical pathways provided additional clues of signaling networks characterizing PC-3 cells. Aryl hydrocarbon receptor signaling was overrepresented by 9 genes and was the most significant canonical pathway. However, this is a very complex canonical pathway, and the 9 genes were scattered around the web of interactions in this pathway rather than highlighting one or two interaction sequences. Thus it is unclear which branches of this pathway are affected. Additional 8 genes previously identified with intracellular signaling involved with hepatic fibrosis/hepatic stellate cell activation were also seen. VEGF, TGF β , and IL-6 ligands, as well as c-Met and IL-1R, which represent angiogenesis, differentiation, and cytokine activity, were also identified. Wnt/ β -catenin canonical pathway (9 genes) showed an initial activation of Frizzled and TGF β R (Table 3).

Comparison Analysis of Gene Expression by LNCaP and PC-3 Cells

Ingenuity comparison analysis identified several unique functions and pathways between LNCaP and PC-3 cells. Using this analysis, lists of differentially expressed genes were compared with each other for overrepresented parameters, and unique features for each cell line were selected. When *p*-value was set at less than 0.05, ontologies that are unique to LNCaP cells were grouped into several categories including metabolic functions, cell cycle, and cell signaling, as well as developmental processes. Canonical pathways that are unique to LNCaP cells were all related to metabolism, predominantly represented by UDP-glucuronosyltransferases. In accordance with androgen dependency of LNCaP cells, androgen and estrogen metabolism was part of these canonical pathways (Table 2).

In contrast, genes that are unique to PC-3 cells showed different functions and pathways. Ontologies that are unique to PC-3 cells were organismal survival as well as visual system development and function (Table 3). Unique canonical pathways in PC-3 cells showed more

diversified features ranging from metabolism, retinoic acid signaling pathway, to cell proliferation and differentiation pathways (Table 3).

To identify potential biomarkers for each cell line, the uniquely expressed genes for LNCaP and PC-3 cells was filtered by IPA software. Among 115 genes identified in LNCaP, only 7 genes were classified as expressed in human prostate gland, identifiable in urine samples, and cancer related (Table 4). Among the 188 genes identified to be unique to PC-3 cells, 16 genes were classified as biomarkers based on the same criteria.

Transcription Regulatory Networks

Lists of unique genes for LNCaP and PC-3 cells were analyzed for presence of significantly overrepresented TREs. All TREs in a given list of genes were tested against the set of TREs in the entire Illumina HumanRef v3 gene list. Using stringent criteria of $p < 0.05$ and $FDR < 0.1$, 4 *trans*-acting elements were identified as overrepresented among genes expressed uniquely in LNCaP cells. Tal- α :E47 and Tal- β :E47 heterodimer had multiple TRE sequences in the *TP53TG3* gene. GATA-1 and c-Myc/Max regulated a group of G antigens (GAGE6, GAGE7B), as well as testis and cancer specific antigens. In addition, NCAM2, PAFAH1B3, C21orf100, PLA2G4F, and TRPM8 shared the c-Myc:Max heterodimer *trans*-acting element exclusively. PAINT analysis did not consider if the gene is annotated and identified both well-annotated genes appearing in Ingenuity networks and ontological analyses (i.e. NCAM2 or PAFAH1B3) and less known players (i.e. TP53TG3, C21orf100, or TRM8) which contain a given TRE in their 5'-flanking regions.

Results of the PAINT analysis are provided in Supplementary data 2. Genes that are uniquely expressed in PC-3 cell shared several TREs using $p < 0.05$. Most of the 188 genes uniquely expressed in PC-3 cells shared AP-1, CP-2, and FOXD3 TREs with $p < 0.05$. Other TREs regulating small sets of genes were NF κ B, GR, Brn-2, CP-2, and FOXD3. However, the significance of these findings was not clear because additional filtering with a $FDR < 0.3$ showed none of these TREs passing the test.

Data Validation and Comparison

The current findings were compared with a previous study of gene expression patterns between LNCaP and PC-3 cells. Yang *et al.* established subtracted cDNA libraries between LNCaP and PC-3 cells (32), and reported a list of 13 differentially expressed genes between these two cell lines. Our results demonstrated a high degree of consistency between these two assays (Table 5).

Based on gene expression profiles of these cell lines and microdissected primary prostate cancer and prostate cancer metastasized to bone marrow (33), LNCaP cells showed some resemblance to primary prostate cancer, whereas gene expression profiles of PC-3 cells were similar to cells derived from bone metastases (Table 6). However, expression of some genes by the cultured cells did not totally match expression in tumors. For example, KRT18 was highly expressed in primary prostate cancer, while a group of keratin genes (KRT6A and KRT7) was identified as uniquely expressed genes in androgen insensitive PC-3 cells. In addition, while UGT2B15 was highly expressed in metastatic prostate cancer cells, the expression of UDP-glucuronosyltransferases was found to be uniquely present in androgen sensitive LNCaP cells.

DISCUSSION

The exact molecular mechanisms by which prostate cancer progresses to androgen-independent stages and bone metastasis are not fully understood and remain the focus of intensive investigation in the field of prostate cancer research. Human prostate cancer

LNCaP and PC-3 cell lines have been extensively used as cell models to study the progression of this disease. LNCaP cells are considered to represent early stages of prostate cancer development based on their androgen-sensitive cell growth (2,23) and progression to AR-independent growth (34). In contrast, PC-3 cells are considered to represent advanced disease based on their source of origin and androgen-insensitive characteristics (8). Although androgen-insensitivity with a functional AR usually characterizes advanced prostate cancer (35), in some patients, androgen-insensitivity is due to a lack of AR expression (36), which produces more aggressive phenotypes (37). The differences between these two established cell lines and the consequences of loss of AR expression remain unknown. In this report, we identified genes that are uniquely expressed in either LNCaP or PC-3 cells as a mean to assess the similarity and differences between these two commonly used lines at a global transcriptome level. This study demonstrates that these two cell lines represent two distinct prostate cancer cell lineages; LNCaP cells retained numerous prostate cells properties, whereas PC-3 cells acquired aggressive characteristics following bone metastasis.

LNCaP cells continue to preserve prostate specific genes. For example, UDP-glucuronosyltransferases formed one of the most significant ontology clusters from genes that are unique to LNCaP cells. UGT2B15 and UGT2B17 have been found in the prostate and are responsible for glucuronidation of 5 α -dihydrotestosterone (5 α -DHT) and its metabolites, androstane-3 α , 17 β -diol and androsterone; and their expression is regulated by androgens (38,39). Unique expression of UGT2B15 and UGT2B17 in LNCaP but not in PC-3 cells is consistent with our biochemical characterization in which androgen metabolites are rapidly glucuronidated in LNCaP, but not in PC-3 cells (40). In addition, unique expression of UGT2B11 and UGT2B28 in LNCaP cells is in agreement with previous reports (41,42). Expression of TMEFF2, a transmembrane protein with EGF-like and two follistatin-like domains 2, is an androgen responsive gene (43) and has been confirmed in LNCaP cells (44). A group of genes responsible for peptidase activity identified in this study are prostate-specific (KLK2, KLK3, KLK4) (45) and their expression is regulated by androgen metabolism (46,47). From the group of peptidase activity, FOLH1, a gene coding for prostate-specific membrane antigen, is prostate specific and highly expressed in LNCaP cells (48).

PC-3 cells have acquired a more aggressive phenotype that is highly angiogenic. We showed that VEGFC, TGF β 2, and TGF β I are uniquely expressed in PC-3 cells and IL-8, MMP-9, and TGF α are expressed at higher levels than in LNCaP cells. Previous studies have shown that VEGF, TGF β 2, MMP-9, and IL-8 are elevated in PC-3 cells (15,16). A recent report showing cross-talk between the AR and TGF β signaling suggested a role of androgens in suppressing TGF β -induced apoptosis and progression of prostate cancer to androgen-independent state (49). Our results consistently indicated the total absence of TGF β expression in LNCaP cells with elevated levels of TGF β 2, TGF β 2 receptor, and TGF β I genes in AR-independent PC-3 cells. Identification of Wnt/ β -catenin pathway and NF κ B/IL6 signaling in prostate cancer skeletal (50) and bone (51) metastasis, respectively, further support aggressive nature of PC-3 cells identified in this report.

Ontological analysis identified that STAT3 and PTEN are involved in multiple processes unique to LNCaP cells. The PTEN tumor suppressor gene is a negative regulator of STAT3, whose expression is required for epithelial cell differentiation (52). In addition, expression of PTEN has been shown to inhibit tumor angiogenesis through suppressing VEGF and HIF-1 expression (53). Consistent with these results, we demonstrated that PTEN expression is unique to LNCaP cells with no detectable VEGF expression, whereas PTEN is not expressed in PC-3 cells with highly elevated expression of VEGFC. Although ontological analysis becomes an indispensable tool for our understanding of functional roles of genes of

interest (54,55), results obtained from such analysis should be interpreted with caution. For example, the axonogenesis cluster in LNCaP cells may indicate more general morphogenesis and differentiation related processes. In addition, incoherent names across different databases remains a major problem for bioinformatics analysis because a particular gene may have only been investigated within a particular biological context and may not be recognized within other tissues (56). The identification of visual system development and function unique for PC-3 cells by Ingenuity analysis may be due to the fact that the particular set of genes has been intensively studied only in visual development and not in other systems.

The cytokeratin complement of each cell line was slightly different but retained the phenotype of the luminal cell. Our results demonstrated that LNCaP and PC-3 cells have different keratin expression patterns. Consistently, using two-dimensional gel electrophoresis, Nagle *et al.* showed that LNCaP cells express cytokeratins 8 and 18, whereas PC-3 cells show cytokeratins 7, 8, 18, and 19 (57). In addition, the expression of intermediate filament protein nestin, originally identified in stem cells of the central nervous system but later in the PC-3 cells (32), is implicated in cell motility and invasion (58). The lack of nestin expression in LNCaP cells suggests that the intermediate filament could be a potential biomarker for metastatic risk by identifying it in cells that might have high metastatic potential.

The two cell lines possess considerable differences in transcriptional regulatory networks. GATA-1 and other members of the GATA family, identified by promoter over-representation in LNCaP cells in this study, have been shown to be responsible for optimal androgen regulation of the PSA (59), and may account for highly elevated PSA expression in LNCaP cells versus PC-3 cells. c-Myc has been reported to respond to mitogenic stimuli and regulate not only the transition of cells from resting states to proliferation but also regulate cell cycle (60). Interaction of GATA-1 with c-Myc has been established (61) and may be implicated in LNCaP cell growth.

The transcriptional regulatory networks in PC-3 cells were less clear. Two modifications of NFκB TFs (NFκB and NFκB p65) are consistent with NFκB regulating genes in the first Ingenuity network. Recent findings corroborated the role of NFκB in controlling the progression of prostate cancer to androgen-independent growth (62). Other TFs, AP-1, GR, along with NFκB were reported to participate in steroid signaling (63), and may potentially be targets for treating metastatic prostate cancer. GR is of particular interest because it has been associated with tumor suppressive functions (64) and could potentially prove useful in gene therapy.

Our microarray results confirmed a previous report identifying uniquely expressed genes between LNCaP and PC-3 cells by establishing subtracted cDNA libraries (32). However, NPG-1, NPG-2, and NPG-3 were not mapped to microarray annotation. Another question concerning these cells is how closely do they resemble the original tumors? Comparison between established prostate cells and microdissected primary prostate tumors and bone metastasis demonstrates that LNCaP and PC-3 cells are related to primary tumors and bone metastasis, respectively. These cells have also gained and lost some features after being cultured *in vitro*. A microRNA analysis also demonstrated that LNCaP cells and primary prostate cancer share a similar pattern of microRNA profile (65). However, these results do not suggest that cells derived from a lymph node metastasis such as LNCaP cells are more similar to primary tumor or a bone metastasis. An attempt was also made to correlate differential gene expression patterns between LNCaP and PC-3 cells to Gleason scores of prostate cancer reported by True *et al.* (66), who identified 736 genes expressed

differentially between cancer and benign prostate tissues. The subset of genes that correlated with Gleason scores was not recapitulated by cultured LNCaP and PC-3 cells.

In this study we report, for the first time, a genome-wide comparison of LNCaP and PC-3 cells, the two major cell models for low and high metastatic potential, respectively. The two cell lines are vastly different with several thousand genes being expressed differentially. Functional analysis identified the major differences between the two lines; and androgen sensitivity is associated with many of the differences in gene expression. Others are derived from the different microenvironments where cells were isolated. PC-3 cells seem to have acquired new characteristics that are different from prostate cells. How many of these changes are present in the primary tumors that later become a bony metastasis and how many appear as a result of the different environment are unknown and are awaiting further investigation. The comprehensive gene expression analysis in this study provides a detailed understanding of the use of these cell lines for prostate cancer research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIH R01 DK069808 to REH and R01-CA90744 and P30-ES013580 to TMP as well as by the US Department of Veterans Affairs Merit Award to HKL.

Abbreviations

AR	androgen receptor
EGF	epidermal growth factor
FGF	fibroblast growth factor
GR	glucocorticoid receptor
PSA	prostate specific antigen
SD	standard deviation
TF	transcription factor
TRE	transcription regulatory elements

REFERENCES

1. Horoszewicz JS, Leong SS, Chu TM, Wajsman ZL, Friedman M, Papsidero L, Kim U, Chai LS, Kakati S, Arya SK, Sandberg AA. The LNCaP cell line--a new model for studies on human prostatic carcinoma. *Progress in clinical and biological research*. 1980; 37:115–132. [PubMed: 7384082]
2. Horoszewicz JS, Leong SS, Kawinski E, Karr JP, Rosenthal H, Chu TM, Mirand EA, Murphy GP. LNCaP model of human prostatic carcinoma. *Cancer Res*. 1983; 43:1809–1818. [PubMed: 6831420]
3. Veldscholte J, Voorhorst-Ogink MM, Bolt-de Vries J, van Rooij HC, Trapman J, Mulder E. Unusual specificity of the androgen receptor in the human prostate tumor cell line LNCaP: high affinity for progestagenic and estrogenic steroids. *Biochimica et biophysica acta*. 1990; 1052:187–194. [PubMed: 2322591]
4. Veldscholte J, Ris-Stalpers C, Kuiper GG, Jenster G, Berrevoets C, Claassen E, van Rooij HC, Trapman J, Brinkmann AO, Mulder E. A mutation in the ligand binding domain of the androgen

- receptor of human LNCaP cells affects steroid binding characteristics and response to anti-androgens. *Biochem Biophys Res Commun.* 1990; 173:534–540. [PubMed: 2260966]
5. Veldscholte J, Berrevoets CA, Ris-Stalpers C, Kuiper GGJM, Jenster G, Trapman J, Brinkmann AO, Mulder E. The androgen receptor in LNCaP cells contains a mutation in the ligand binding domain which affects steroid binding characteristics and response to antiandrogens. *J Steroid Biochem Mol Biol.* 1992; 41:665–669. [PubMed: 1562539]
 6. Veldscholte J, Berrevoets CA, Brinkmann AO, Grootegoed JA, Mulder E. Anti-androgens and the mutated androgen receptor of LNCaP cells: differential effects on binding affinity, heat-shock protein interaction, and transcription activation. *Biochemistry.* 1992; 31:2393–2399. [PubMed: 1540595]
 7. Langelier EG, van Uffelen CJ, Blankenstein MA, van Steenbrugge GJ, Mulder E. Effect of culture conditions on androgen sensitivity of the human prostatic cancer cell line LNCaP. *The Prostate.* 1993; 23:213–223. [PubMed: 7694266]
 8. Kaighn ME, Narayan KS, Ohnuki Y, Lechner JF, Jones LW. Establishment and characterization of a human prostatic carcinoma cell line (PC-3). *Investigative urology.* 1979; 17:16–23. [PubMed: 447482]
 9. Reyes-Moreno C, Frenette G, Boulanger J, Lavergne E, Govindan MV, Koutsilieris M. Mediation of glucocorticoid receptor function by transforming growth factor beta I expression in human PC-3 prostate cancer cells. *The Prostate.* 1995; 26:260–269. [PubMed: 7753711]
 10. Morris GL, Dodd JG. Epidermal growth factor receptor mRNA levels in human prostatic tumors and cell lines. *J Urol.* 1990; 143:1272–1274. [PubMed: 1692888]
 11. Nakamoto T, Chang CS, Li AK, Chodak GW. Basic fibroblast growth factor in human prostate cancer cells. *Cancer Res.* 1992; 52:571–577. [PubMed: 1732045]
 12. Papsidero LD, Kuriyama M, Wang MC, Horoszewicz J, Leong SS, Valenzuela L, Murphy GP, Chu TM. Prostate antigen: a marker for human prostate epithelial cells. *J Natl Cancer Inst.* 1981; 66:37–42. [PubMed: 6935463]
 13. Zhong W, Peng J, He H, Wu D, Han Z, Bi X, Dai Q. Ki-67 and PCNA expression in prostate cancer and benign prostatic hyperplasia. *Clinical and investigative medicine.* 2008; 31:E8–E15. [PubMed: 18312749]
 14. Tesan T, Gustavsson H, Welen K, Damber JE. Differential expression of angiopoietin-2 and vascular endothelial growth factor in androgen-independent prostate cancer models. *BJU international.* 2008; 102:1034–1039. [PubMed: 18489523]
 15. Aalinkeel R, Nair MP, Sufrin G, Mahajan SD, Chadha KC, Chawda RP, Schwartz SA. Gene expression of angiogenic factors correlates with metastatic potential of prostate cancer cells. *Cancer Res.* 2004; 64:5311–5321. [PubMed: 15289337]
 16. Araki S, Omori Y, Lyn D, Singh RK, Meinbach DM, Sandman Y, Lokeshwar VB, Lokeshwar BL. Interleukin-8 is a molecular determinant of androgen independence and progression in prostate cancer. *Cancer Res.* 2007; 67:6854–6862. [PubMed: 17638896]
 17. Saleem M, Adhami VM, Zhong W, Longley BJ, Lin CY, Dickson RB, Reagan-Shaw S, Jarrard DF, Mukhtar H. A novel biomarker for staging human prostate adenocarcinoma: overexpression of matriptase with concomitant loss of its inhibitor, hepatocyte growth factor activator inhibitor-1. *Cancer Epidemiol Biomarkers Prev.* 2006; 15:217–227. [PubMed: 16492908]
 18. Laidler P, Dulinska J, Lekka M, Lekki J. Expression of prostate specific membrane antigen in androgen-independent prostate cancer cell line PC-3. *Archives of biochemistry and biophysics.* 2005; 435:1–14. [PubMed: 15680901]
 19. Banerjee AG, Liu J, Yuan Y, Gopalakrishnan VK, Johansson SL, Dinda AK, Gupta NP, Trevino L, Vishwanatha JK. Expression of biomarkers modulating prostate cancer angiogenesis: differential expression of annexin II in prostate carcinomas from India and USA. *Molecular cancer.* 2003; 2:34. [PubMed: 14613585]
 20. Wang X, Klein RD. Prostaglandin E₂ induces vascular endothelial growth factor secretion in prostate cancer cells through EP2 receptor-mediated cAMP pathway. *Molecular carcinogenesis.* 2007; 46:912–923. [PubMed: 17427962]
 21. Menschikowski M, Hagelgans A, Gussakovsky E, Kostka H, Paley EL, Siegert G. Differential expression of secretory phospholipases A2 in normal and malignant prostate cell lines: regulation

- by cytokines, cell signaling pathways, and epigenetic mechanisms. *Neoplasia*. 2008; 10:279–286. (New York, NY. [PubMed: 18320072])
22. Klossner DP, Baust JM, VanBuskirk RG, Gage AA, Baust JG. Cryoablative response of prostate cancer cells is influenced by androgen receptor expression. *BJU international*. 2008; 101:1310–1316. [PubMed: 18261151]
 23. Nunlist EH, Dozmorov I, Tang Y, Cowan R, Centola M, Lin HK. Partitioning of 5 α -dihydrotestosterone and 5 α -androstane-3 α , 17 β -diol activated pathways for stimulating human prostate cancer LNCaP cell proliferation. *J Steroid Biochem Mol Biol*. 2004; 91:157–170. [PubMed: 15276623]
 24. Wang S, Yang Q, Fung KM, Lin HK. AKR1C2 and AKR1C3 mediated prostaglandin D₂ metabolism augments the PI3K/Akt proliferative signaling pathway in human prostate cancer cells. *Mol Cell Endocrinol*. 2008; 289:60–66. [PubMed: 18508192]
 25. Dozmorov I, Knowlton N, Tang Y, Centola M. Statistical monitoring of weak spots for improvement of normalization and ratio estimates in microarrays. *BMC Bioinformatics*. 2004; 5:53. [PubMed: 15128432]
 26. Knowlton N, Dozmorov IM, Centola M. Microarray Data Analysis Toolbox (MDAT): for normalization, adjustment and analysis of gene expression data. *Bioinformatics*. 2004; 20:3687–3690. [PubMed: 15271778]
 27. Hua J, Balagurunathan Y, Chen Y, Lowey J, Bittner ML, Xiong Z, Suh E, Dougherty ER. Normalization benefits microarray-based classification. *EURASIP journal on bioinformatics & systems biology*. 2006:43056. [PubMed: 18427588]
 28. Gusnanto A, Calza S, Pawitan Y. Identification of differentially expressed genes and false discovery rate in microarray studies. *Current opinion in lipidology*. 2007; 18:187–193. [PubMed: 17353668]
 29. Dozmorov I, Centola M. An associative analysis of gene expression array data. *Bioinformatics*. 2003; 19:204–211. [PubMed: 12538240]
 30. Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, Lempicki RA. DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome biology*. 2003; 4:P3. [PubMed: 12734009]
 31. Vadigepalli R, Chakravarthula P, Zak DE, Schwaber JS, Gonye GE. PAINT: a promoter analysis and interaction network generation tool for gene regulatory network identification. *OMICS*. 2003; 7:235–252. [PubMed: 14583114]
 32. Yang M, Loda M, Sytkowski AJ. Identification of genes expressed differentially by LNCaP or PC-3 prostate cancer cell lines. *Cancer Res*. 1998; 58:3732–3735. [PubMed: 9721886]
 33. Stanbrough M, Bubley G, Ross K, Golub TR, Rubin MA, Penning TM, Febbo PG, Balk SP. Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. *Cancer Res*. 2006; 66:2815–2825. [PubMed: 16510604]
 34. Lin HK, Hu YC, Yang L, Altuwaijri S, Chen YT, Kang HY, Chang C. Suppression versus induction of androgen receptor functions by the phosphatidylinositol 3-kinase/Akt pathway in prostate cancer LNCaP cells with different passage numbers. *J Biol Chem*. 2003; 278:50902–50907. [PubMed: 14555644]
 35. Culig Z, Bartsch G. Androgen axis in prostate cancer. *J Cell Biochem*. 2006; 99:373–381. [PubMed: 16598769]
 36. Kinoshita H, Shi Y, Sandefur C, Meisner LF, Chang C, Choon A, Reznikoff CR, Bova GS, Friedl A, Jarrard DF. Methylation of the androgen receptor minimal promoter silences transcription in human prostate cancer. *Cancer Res*. 2000; 60:3623–3630. [PubMed: 10910077]
 37. Bonaccorsi L, Nosi D, Quercioli F, Formigli L, Zecchi S, Maggi M, Forti G, Baldi E. Prostate cancer: a model of integration of genomic and non-genomic effects of the androgen receptor in cell lines model. *Steroids*. 2008; 73:1030–1037. [PubMed: 18358509]
 38. Barbier O, Belanger A. Inactivation of androgens by UDP-glucuronosyltransferases in the human prostate. *Best practice & research*. 2008; 22:259–270.
 39. Chouinard S, Barbier O, Belanger A. UDP-glucuronosyltransferase 2B15 (UGT2B15) and UGT2B17 enzymes are major determinants of the androgen response in prostate cancer LNCaP cells. *J Biol Chem*. 2007; 282:33466–33474. [PubMed: 17848572]

40. Rizner TL, Lin HK, Peehl DM, Steckelbroeck S, Bauman DR, Penning TM. Human type 3 α -hydroxysteroid dehydrogenase (aldo-keto reductase 1C2) and androgen metabolism in prostate cells. *Endocrinol.* 2003; 144:2922–2932.
41. Beaulieu M, Levesque E, Hum DW, Belanger A. Isolation and characterization of a human orphan UDP-glucuronosyltransferase, UGT2B11. *Biochem Biophys Res Commun.* 1998; 248:44–50. [PubMed: 9675083]
42. Levesque E, Turgeon D, Carrier JS, Montminy V, Beaulieu M, Belanger A. Isolation and characterization of the UGT2B28 cDNA encoding a novel human steroid conjugating UDP-glucuronosyltransferase. *Biochemistry.* 2001; 40:3869–3881. [PubMed: 11300766]
43. Gery S, Sawyers CL, Agus DB, Said JW, Koeffler HP. TMEFF2 is an androgen-regulated gene exhibiting antiproliferative effects in prostate cancer cells. *Oncogene.* 2002; 21:4739–4746. [PubMed: 12101412]
44. Chen Q, Watson JT, Marengo SR, Decker KS, Coleman I, Nelson PS, Sikes RA. Gene expression in the LNCaP human prostate cancer progression model: progression associated expression in vitro corresponds to expression changes associated with prostate cancer progression in vivo. *Cancer letters.* 2006; 244:274–288. [PubMed: 16500022]
45. Vickers AJ, Cronin AM, Aus G, Pihl CG, Becker C, Pettersson K, Scardino PT, Hugosson J, Lilja H. A panel of kallikrein markers can reduce unnecessary biopsy for prostate cancer: data from the European Randomized Study of Prostate Cancer Screening in Goteborg, Sweden. *BMC Med.* 2008; 6:19. [PubMed: 18611265]
46. Biancolella M, Valentini A, Minella D, Vecchione L, D'Amico F, Chillemi G, Gravina P, Bueno S, Prosperini G, Desideri A, Federici G, Bernardini S, Novelli G. Effects of dutasteride on the expression of genes related to androgen metabolism and related pathway in human prostate cancer cell lines. *Investigational new drugs.* 2007; 25:491–497. [PubMed: 17636412]
47. Sardana G, Jung K, Stephan C, Diamandis EP. Proteomic analysis of conditioned media from the PC3, LNCaP, and 22Rv1 prostate cancer cell lines: discovery and validation of candidate prostate cancer biomarkers. *J Proteome Res.* 2008; 7:3329–3338. [PubMed: 18578523]
48. Watt F, Martorana A, Brookes DE, Ho T, Kingsley E, O'Keefe DS, Russell PJ, Heston WD, Molloy PL. A tissue-specific enhancer of the prostate-specific membrane antigen gene, FOLH1. *Genomics.* 2001; 73:243–254. [PubMed: 11350116]
49. Song K, Wang H, Krebs TL, Kim SJ, Danielpour D. Androgenic control of transforming growth factor-beta signaling in prostate epithelial cells through transcriptional suppression of transforming growth factor- β receptor II. *Cancer Res.* 2008; 68:8173–8182. [PubMed: 18829577]
50. Robinson DR, Zylstra CR, Williams BO. Wnt signaling and prostate cancer. *Current drug targets.* 2008; 9:571–580. [PubMed: 18673243]
51. Paule B, Terry S, Kheuang L, Soyeux P, Vacherot F, de la Taille A. The NF- κ B/IL-6 pathway in metastatic androgen-independent prostate cancer: new therapeutic approaches? *World J Urol.* 2007; 25:477–489. [PubMed: 17541600]
52. Wu R, Sun S, Steinberg BM. Requirement of STAT3 activation for differentiation of mucosal stratified squamous epithelium. *Mol Med.* 2003; 9:77–84. [PubMed: 12865943]
53. Fang J, Ding M, Yang L, Liu LZ, Jiang BH. PI3K/PTEN/AKT signaling regulates prostate tumor angiogenesis. *Cellular signalling.* 2007; 19:2487–2497. [PubMed: 17826033]
54. Khatri P, Draghici S. Ontological analysis of gene expression data: current tools, limitations, and open problems. *Bioinformatics.* 2005; 21:3587–3595. [PubMed: 15994189]
55. Zhang B, Schmoyer D, Kirov S, Snoddy J. GOTree Machine (GOTM): a web-based platform for interpreting sets of interesting genes using Gene Ontology hierarchies. *BMC Bioinformatics.* 2004; 5:16. [PubMed: 14975175]
56. Draghici S, Sellamuthu S, Khatri P. Babel's tower revisited: a universal resource for cross-referencing across annotation databases. *Bioinformatics.* 2006; 22:2934–2939. [PubMed: 17068090]
57. Nagle RB, Ahmann FR, McDaniel KM, Paquin ML, Clark VA, Celniker A. Cytokeratin characterization of human prostatic carcinoma and its derived cell lines. *Cancer Res.* 1987; 47:281–286. [PubMed: 2431765]

58. Kleeberger W, Bova GS, Nielsen ME, Herawi M, Chuang AY, Epstein JI, Berman DM. Roles for the stem cell associated intermediate filament Nestin in prostate cancer migration and metastasis. *Cancer Res.* 2007; 67:9199–9206. [PubMed: 17909025]
59. Perez-Stable CM, Pozas A, Roos BA. A role for GATA transcription factors in the androgen regulation of the prostate-specific antigen gene enhancer. *Mol Cell Endocrinol.* 2000; 167:43–53. [PubMed: 11000519]
60. Kitaura H, Shinshi M, Uchikoshi Y, Ono T, Iguchi-Ariga SM, Ariga H. Reciprocal regulation via protein-protein interaction between c-Myc and p21(cip1/waf1/sdi1) in DNA replication and transcription. *J Biol Chem.* 2000; 275:10477–10483. [PubMed: 10744738]
61. Rylski M, Welch JJ, Chen YY, Letting DL, Diehl JA, Chodosh LA, Blobel GA, Weiss MJ. GATA-1-mediated proliferation arrest during erythroid maturation. *Molecular and cellular biology.* 2003; 23:5031–5042. [PubMed: 12832487]
62. Jin RJ, Lho Y, Connelly L, Wang Y, Yu X, Saint Jean L, Case TC, Ellwood-Yen K, Sawyers CL, Bhowmick NA, Blackwell TS, Yull FE, Matusik RJ. The nuclear factor- κ B pathway controls the progression of prostate cancer to androgen-independent growth. *Cancer Res.* 2008; 68:6762–6769. [PubMed: 18701501]
63. Yemelyanov A, Czwarnog J, Gera L, Joshi S, Chatterton RT Jr. Budunova I. Novel steroid receptor phyto-modulator compound a inhibits growth and survival of prostate cancer cells. *Cancer Res.* 2008; 68:4763–4773. [PubMed: 18559523]
64. Yemelyanov A, Czwarnog J, Chebotaev D, Karseladze A, Kulevitch E, Yang X, Budunova I. Tumor suppressor activity of glucocorticoid receptor in the prostate. *Oncogene.* 2007; 26:1885–1896. [PubMed: 17016446]
65. Tong AW, Fulgham P, Jay C, Chen P, Khalil I, Liu S, Senzer N, Eklund AC, Han J, Nemunaitis J. MicroRNA profile analysis of human prostate cancers. *Cancer gene therapy.* 2009; 16:206–216. [PubMed: 18949015]
66. True L, Coleman I, Hawley S, Huang CY, Gifford D, Coleman R, Beer TM, Gelmann E, Datta M, Mostaghel E, Knudsen B, Lange P, Vessella R, Lin D, Hood L, Nelson PS. A molecular correlate to the Gleason grading system for prostate adenocarcinoma. *Proc Natl Acad Sci U S A.* 2006; 103:10991–10996. [PubMed: 16829574]

Figure 1.

Figure 2.

Table 1

Unique ontologies and corresponding genes for LNCaP or PC-3 cells*

LNCaP cells	PC-3 cells
UDP-glucuronosyltransferase: IFI27, UGT2B10, UGT2B11, UGT2B15, UGT2B17, UGT2B28, UGT2B7	Intermediate filament cytoskeleton: KRT15, KRT6A, KRT6A, KRT6A, KRT7, KRTHA4, NES, PLAUR
Transmembrane: ALDH3B2, IFI27, MANSC1, PIK3IP1, ZPLD1	Transmembrane: GLIPR1, IL13RA2, SUSD3, TMEM30B
Axonogenesis/cellular morphogenesis during differentiation: RTN4, SLITRK3, SPON2	Peptidase S1A, chymotrypsin: KLK6, PLAT, PLAU, PRSS3
Transmembrane: ADAM7, ALDH3B2, CSAG2, FZD9, GHR, M160, MANSC1, NCAM2, NETO1, TMEFF2, ZPLD1	EGF-like calcium-binding: EFEMP1, EGFL9, FBLN1, FBN2
Peptidase activity: ADAM7, FOLH1, HPN, KLK2, KLK3, KLK4, MME, PGC	Glycoprotein: ADAM1 9, ADAM8, ALDH3A1, ANPEP, CD44, CDH11, EDG2, EGFL9, FZD2, GPRC5A, HAS3, HOXA9, IER3, IL13RA2, IL1R2, OPN3, P2RY2, PTPNS1, SGCE, SLC2A10, TNFRSF18
Ionic channel: BCHE; CSAG2; KCNN2; TRPM8	Protein kinase, core: AXL, JAK1, MET, TGFB2
Transcription regulation: IRX4, KLF9, KLF9,MAFB,ZBTB16	Nucleoside-triphosphatase activity: ARL4, NAV2, PCOLCE2, RAB34, RND3, TMEM16A
	Ubiquitin cycle: ASB9, FHOD3, TMEM166, TMEM16A
	Transcription regulation: BHLHB3, CTHRC1, EPAS1, ETS1, ETV4, ETV5, EYA2, FOXA2, FOXQ1, HMGA2, HOXA9, NR2F1, OSR1, PEG10, PGM5, RUNX1, RUNX3, SOX21, SP8, TFCEP2, TSPYL5, ZNF22

* Poorly annotated genes (ORFs, hypothetical proteins etc.) were not included.

Table 2

Unique ontologies and pathways overrepresented among LNCaP specific genes

Unique ontologies	<i>p</i> -value	Genes
Metabolic processes		
Amino Acid Metabolism	1.09E-03-1.12E-02	DDC,HGD,GCG
Carbohydrate Metabolism	5.62E-03-1.68E-02	CXCL10,MMP7,GHR,GCG,PTEN
Nutritional Disease	9.99E-03-1.22E-02	STAT3,PTEN
Psychological Disorders	9.99E-03-9.99E-03	STAT3,PTEN
Nucleic Acid Metabolism	5.62E-03-5.62E-03	STAT3
Vitamin and Mineral Metabolism	5.62E-03-1.68E-02	FAAH,FOLH1,UGT2B7
Cell cycle/Signaling		
Cell Cycle	9.31E-05-1.68E-02	CXCL10,KLF9,GHR,STAT3,ZBT B16,PTEN
Cell Signaling	5.62E-03-5.62E-03	MB
Developmental processes		
Reproductive System Development and Function	6.42E-04-1.68E-02	FAAH,KLF9,MMP7,GHR,STAT3,ZBTB16,AMHR2,PTEN,PAFAH1B3
Respiratory System Development and Function	9.99E-03-1.12E-02	MAFB,NDN,PTEN
Unique pathways		
Retinol Metabolism	1.95E-07	UGT2B7,UGT2B15,UGT2B17,UGT2B10,UGT2B11,UGT2B28
Pentose and Glucuronate Interconversions	2.88E-07	UGT2B7,UGT2B15,UGT2B17,UGT2B10,UGT2B11,UGT2B28
Androgen and Estrogen Metabolism	5.37E-06	UGT2B7,UGT2B15,UGT2B17,UGT2B10,UGT2B11,UGT2B28
Starch and Sucrose Metabolism	1.15E-05	UGT2B7,UGT2B15,UGT2B17,UGT2B10,UGT2B11,UGT2B28
Phenylalanine Metabolism	8.13E-04	FAAH,ALDH3B2 (includes EG:222),DDC
Tyrosine Metabolism	4.37E-03	ALDH3B2 (includes EG:222), DDC,HGD
Histidine Metabolism	2.40E-02	ALDH3B2 (includes EG:222), DDC
Neuregulin Signaling	1.26E-02	PRKD1,PTEN,TMEFF2

Table 3

Unique ontologies and pathways overrepresented among PC-3 specific genes

Unique ontologies	<i>p</i> -value	Genes
Organismal Survival	1.3E-03-5.73E-03	TGM2,TNFRSF18,FST,EDG2,CD44,DKK1,IL6,CTSC,B3GALNT1,HMGA2,IL11,PLAT
Visual System Development and Function	3.62E-03-5.73E-03	TIMP1,PLAU
Unique pathways		
Metabolic pathways		
Ascorbate and Aldarate Metabolism	2.04E-02	ALDH3A1,ALDH7A1
Glycolysis/Gluconeogenesis	2.40E-02	PGM5,FBP1,ALDH3A1,ALDH7A1
Glutathione Metabolism	7.76E-03	GSTM1 (includes EG:2944),GSTP1,GSTM2,ANPEP
Retinoic acid related pathways		
LPS/IL-1 Mediated Inhibition of RXR Function	3.98E-04	IL1R2,GSTM1 (includes EG:2944),GSTP1,GSTM2,ACSL4,PLTP,ABCC3,ALDH3A1,ALDH7A1
LXR/RXR Activation	4.68E-02	IL1R2,PLTP,IL6
PXR/RXR Activation	4.68E-02	GSTM1 (includes EG:2944),IL6,ABCC3
Proliferation, differentiation, and cytoskeleton signaling pathways		
Aryl Hydrocarbon Receptor Signaling	5.62E-07	TGM2,NR2F1,GSTM1 (includes EG:2944),GSTP1,GSTM2,CDK6,TGFB2,IL6,ALDH3A1,CYP1B1,ALDH7A1
Hepatic Fibrosis / Hepatic Stellate Cell Activation	1.05E-04	IL1R2,MET,TGFB2,IGFBP4,TIMP1,TGFB2,VEGFC,IL6
IL-10 Signaling	3.72E-02	IL1R2,JAK1,IL6
Wnt/ β -catenin Signaling	3.98E-02	TGFB2,CD44,TGFB2,DKK1,FZD2
PDGF Signaling	4.47E-02	JAK1,SPHK1,PDGFC

Table 4

Unique biomarkers for LNCaP and PC-3 cells

LNCaP biomarkers	
Name	Description
ACPP	acid phosphatase, prostate
APOD	apolipoprotein D
KLK3	kallikrein-related peptidase 3
MME	membrane metallo-endopeptidase
MMP7	matrix metallopeptidase 7 (matrilysin, uterine)
PIP	prolactin-induced protein
RTN4	reticulon 4

PC-3 biomarkers	
ANPEP	alanyl (membrane) aminopeptidase (aminopeptidase N, aminopeptidase M, microsomal aminopeptidase, CD13, p150)
AXL	AXL receptor tyrosine kinase
CD44	CD44 molecule (Indian blood group)
CDH11	cadherin 11, type 2, OB-cadherin (osteoblast)
EFEMP1	EGF-containing fibulin-like extracellular matrix protein 1
GPRC5A	G protein-coupled receptor, family C, group 5, member A
IGFBP4	insulin-like growth factor binding protein 4
IGFBP6	insulin-like growth factor binding protein 6
IL6	interleukin 6 (interferon, beta 2)
LGALS3	lectin, galactoside-binding, soluble, 3
NOV	nephroblastoma overexpressed gene
PLAT	plasminogen activator, tissue
PVRL3	poliovirus receptor-related 3
S100A6	S100 calcium binding protein A6
SIRPA	signal-regulatory protein alpha
SLIT2	slit homolog 2 (Drosophila)

Table 5

Comparison of selected genes expression between LNCaP and PC-3 cells

Genes reported by Yang et al.	Ratio *	Current finding	Ratio *
PSA	>100	KLK3, kallikrein 3, prostate-specific antigen	>100
Vimentin	<0.01	VIM, vimentin	<0.1
Annexin II	<0.01	ANXA2, Annexin A2	<0.1
p16ink4 (MTS1)	<0.01	CDKN2A ; p14ARF; TP16; p16; CDKN2; MTS1; INK4a	<0.5
hSP56	>100	SELENBP1; FLJ13813; LPSB; hSP56; SP56; hSBP, selenium binding protein 1	>69
Uridine phosphorylase	<0.01	UPP1, uridine phosphorylase 1	<0.07
Keratin K7	0.07	KRT7, keratin 7	<0.01
Ferritin, light chain	0.25	FTL, ferritin, light polypeptide	0.6
α -Enolase	0.33	ENO1, enolase 1, (alpha)	0.88

* Ratios were calculated by the levels of gene expression of LNCaP versus PC-3 cells.

Table 6

Gene expression comparison between established prostate cancer cell lines and prostate cancer tissues

Elevated in LNCaP cells vs. PC-3 cells	Elevated in primary tumors vs. metastatic tumors	Elevated in PC-3 cells vs. LNCaP cells	Elevated in metastatic tumors vs. primary tumors
ACPP	ACPP		
KLK2, KLK3, KLK4	KLK2, KLK3		
GHR	GHR		
HGD	HGD		
TRPM8	TRPM8		
ALDH3B2	ALDH1A3, ALDH3A2, ALDH6A	ALDH3A1, ALDH7A1	
	ANXA3	ANXA2, ANXA2P1, ANXA3, ANXA8	
	TMEM30B	TMEM16A, TMEM30B, TMEM46	
	KRT18	KRT6A, KRT7, KRT15, KRT34	
		CDH11	CDH11
		CHN1	CHN1
		PLOD2 , PCOLCE2	PLOD2
		COL13A1, CTHRC1	COL1A2 and more
		RAFTLIN	RAFTLIN
		SERPINB1	SERPINE, SERPINF
		LAMC2	LAMA4, LAMB1, LAMC1
SMA4			SMA4
UGT2B10, UGT2B11, UGT2B15, UGT2B17, UGT2B7, UGT2B28			UGT2B15
AKR1C2			AKR1C2
APOD			APOE
IFI27			IFI30
KIF1A			KIF4A, KIF20A
MMP7			MMP9

Genes that are matched between LNCaP-primary tumor and PC-3-metastasized tumor pairs are highlighted in bold. Genes that show opposite matches are presented as normal font.