Dicarbonyl/L-Xylulose Reductase: A Potential Biomarker Identified by Laser-Capture Microdissection-Micro Serial Analysis of Gene Expression of Human Prostate Adenocarcinoma

Jeong Hee Cho-Vega,[3] Spiridon Tsavachidis,[2] Kim-Anh Do,[2] Junichi Nakagawa,[4] L. Jeffrey Medeiros,[1] and Timothy J. McDonnell[1]

Departments of [1]Hematopathology and [2]Biostatistics, The University of Texas M. D. Anderson Cancer Center; [3]Departments of Pathology, The Methodist Hospital, Houston, Texas; and [4]Molecular Biology Laboratory, Taisho Pharmaceutical Co., Saitama, Japan

Abstract

To identify genes involved in prostate carcinogenesis, we used laser-capture microdissection-micro serial analysis of gene expression to construct libraries of paired cancer and normal cells from human tissue samples. After computational comparison of the two libraries, we identified dicarbonyl/L-xylulose reductase (DCXR), an enzyme that catalyzes α-dicarbonyl and L-xylulose, as being significantly up-regulated in prostate cancer cells. The specificity of DCXR up-regulation for prostate cancer tissues was confirmed by quantitative real-time reverse transcriptase-PCR, virtual Northern blot, and Western blot analyses. Furthermore, DCXR expression at the protein level was assessed using fresh-frozen tissues and a tissue microarray consisting of 46 cases of organ-confined early-stage prostate cancer and 29 cases of chemohormonally treated prostate cancer. In most normal prostate epithelial cells, DCXR was expressed at low levels and was localized predominantly in the cytoplasmic membrane. In contrast, in virtually all grades of early-stage prostate cancer and in all chemohormonally treated cases, DCXR was strikingly overexpressed and was localized predominantly in the cytoplasm and nucleus. In all samples, the stromal cells were completely devoid of DCXR expression. Based on these findings, we suggest that DCXR overexpression has the potential to be an additional useful biomarker for prostate cancer. (Cancer Epidemiol Biomarkers Prev 2007;16(12):2615–22)

Introduction

Increased understanding of the molecular pathogenesis of prostate cancer will allow recognition of molecular markers for disease detection, provide insights into prostate cancer development and behavior, and identify potential therapeutic targets (1). Progress in identifying such markers has been markedly accelerated by recent advances in molecular biology technologies such as cDNA array (2). This approach enables analysis of the expression of thousands of genes in a single experiment and has been applied by others to the study of prostate cancers, leading to improved understanding of this disease (3-7). An alternative, relatively less explored approach to the study of prostate cancer is the serial analysis of gene expression (SAGE) technique (8). Compared with the cDNA array technique, SAGE provides an unbiased view of gene expression profiles (8-10). This feature is particularly important in the analysis of cells that constitute only a small fraction of the tissue under study because transcripts from these cells are unlikely to be well represented in the estimated sequence tag database.

Laser-capture microdissection (LCM) is a recently developed technique that allows for the reliable and accurate procurement of cells from specific microscopic regions of tissue sections. LCM now permits molecular genetic analysis on pure populations of cancer cells obtained from their native tissue environment (11, 12). We previously showed the feasibility of combining LCM with microSAGE to generate cell-specific expression profiles from human tissue samples (13).

Using the same LCM-microSAGE technique, in this study we generated cell-specific expression profiles of human prostate cancer and normal prostate cells obtained from their actual tissue milieu to identify genes differentially regulated in cancer and normal cells. We identified dicarbonyl/L-xylulose reductase (DCXR), an enzyme that catalyzes α-dicarbonyl and L-xylulose, as an up-regulated gene in prostate cancer cells. The *dcxr* gene, which is located on chromosome 17q25.3, encodes an ~100-kDa homotetramer with NADPH-linked reductase activity for both α-dicarbonyl compounds and L-xylulose (14). The specificity of DCXR mRNA overexpression in prostate cancer tissue was confirmed by quantitative real-time reverse transcriptase-PCR (RT-PCR) and virtual Northern blot analysis. Furthermore, by Western blot analysis and immunohistochemical study of a tissue

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Requests for reprints: Timothy J. McDonnell, Department of Hematopathology, Unit 89, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030. E-mail: tmcdonn@mdanderson.org

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Table 1. A list of genes showing significantly ($P < 0.05$) up-regulated or down-regulated in prostate cancer cells

Genes up-regulated in cancer cells

Tag	Normal	Cancer	*P*	Unigene	Gene	Description
TTGTAATCGT	3*	12	0 0.00717706	281960	*OAZ1*	Ornithine decarboxylase antizyme 1
AGGGTCTGGG	0	6	0.008964801	2985	*EMD*	Emerin (Emery-Dreifuss muscular dystrophy)
AAGAAGCAAG	0	5	0.01966907	75249	*ARL6IP*	ADP-ribosylation factor-like 6 interacting protein
AGACATCAGG	0	5	0.01966907			
CAGGATGGCC	0	5	0.01966907			
CGTGAGCCAC	0	5	0.01966907	2441	*BIMLEC*	Estrogen-responsive B box protein
CTGTTGATTG †	0	5	0.01966907	376844	*HNRPA1*	Heterogeneous nuclear ribonucleoprotein A1
GCATCCCCGA	0	5	0.01966907			
GTCCGCCCGC	0	5	0.01966907			
GTGGTACAGG	0	5	0.01966907	31731	*PRDX5*	Peroxiredoxin 5
GGGCCCCAAA	2	8	0.02982691	256301	*MGC13170*	MGC13170 gene
CCGTGCTCAT	1	6	0.03823766	9857	*DCXR*	Dicarbonyl/L-xylulose reductase
GAGTCAGGAG	1	6	0.03823766	181271	*COPZ1*	CGI-120 protein
AAAACATTAT	0	4	0.0431546	80917	*AP3S1*	Adaptor-related protein complex 3, σ_1 subunit
AACCCGGGAG †	0	4	0.0431546	173936	*IL10RB*	Interleukin-10 receptor, β
ATATGAATGT	0	4	0.0431546	7862	*FLJ20312*	Hypothetical protein FLJ20312
CAACTGCCCC	0	4	0.0431546	11801	*IRF6*	IFN regulatory factor 6
CACGAACGGT	0	4	0.0431546			
CATCTGTTAC	0	4	0.0431546	270591	—	Estimated sequence tags
CATTTCAATA †	0	4	0.0431546	32587	*SRA1*	Steroid receptor R activator 1
CCAAGAAGGA	0	4	0.0431546			
CTCATTCAGC	0	4	0.0431546	180139	*SMT3H2*	SMT3 suppressor of mif two 3 homologue 2 (yeast)
GAAGAATGGT	0	4	0.0431546	181357	*LAMR1*	Laminin receptor 1 (ribosomal protein SA, 67 kDa)
GCCACTACCC	0	4	0.0431546	71475	*ACP33*	Acid cluster protein 33
GCTTCCATCT †	0	4	0.0431546	55296	*BAT1*	HLA-B associated transcript 1
GGATATGGCC	0	4	0.0431546			
GGGAGAATGA	0	4	0.0431546			
TAACAGCTAC	0	4	0.0431546	180139	*SMT3H2*	SMT3 suppressor of mif two 3 homologue 2 (yeast)
TGAAAAGCTT	0	4	0.0431546	2384	*TPD52*	Tumor protein D52
TTACTGCACT	0	4	0.0431546			
CACCCCTGAT	4	10	0.04677713	173724	*CKB*	Creatine kinase, brain
ACTAACTGTG	1	3	0.08 ‡	16003	*RBBP4*	Retinoblastoma binding protein 4

Genes down-regulated in cancer cells

Tag	Normal	Cancer	*P*	Unigene	Gene	Description
TGTGTTGAGA †	20	2	1.66e−04	422118	*EEF1A1*	Eukaryotic translation elongation factor 1 α1
GGCAAGAAGA †	16	3	0.004262236	83321	*NMB*	Neuromedin B
AGGTCAGGAG †	11	1	0.00436658	59498	*CDC2L5*	Cell division cycle 2-like 5 (cholinesterase-related cell division controller)
GAAAAATGGT †	20	5	0.004889212	181357	*LAMR1*	Laminin receptor 1 (ribosomal protein SA, 67 kDa)
GAAATAAAGC †	7	0	0.007694597	413826	*IGHG3*	Immunoglobulin heavy constant γ3 (G3m marker)
GGGTTGGCTT	16	5	0.02389573	73818	*UQCRH*	Ubiquinol-cytochrome *c* reductase hinge protein
TTGGGGTTTC †	12	3	0.02531239	418650	*FTH1*	Ferritin, heavy polypeptide 1
AAACATTAAA †	5	0	0.02598002	103521	*SR-A1*	Actin, γ2, smooth muscle, enteric
CGGCCCAACG	5	0	0.02598002	20521	*HRMT1L2*	HMT1 hnRNP methyltransferase-like 2 (*S. cerevisiae*)
GAAGATGTGT	5	0	0.02598002	112318	*TOM7*	Homologue of Tom7 (*S. cerevisiae*)
TGACAGAAAC	5	0	0.02598002	200412	*EPPK1*	Epiplakin 1
GTGAAACCCC †	19	7	0.02956758	182378	*CSF2RA*	Colony stimulating factor 2 receptor, α, low-affinity (granulocyte-macrophage)

(*Continued on the following page*)

Table 1. A list of genes showing significantly ($P < 0.05$) up-regulated or down-regulated in prostate cancer cells (Cont'd)

Genes down-regulated in cancer cells						
Tag	Normal	Cancer	*P*	Unigene	Gene	Description
TAGTTGAAGT	7	1	0.03575104	131255	*UQCRB*	Ubiquinol-cytochrome *c* reductase binding protein
AAGAATCTGA	4	0	0.04773821	183435	*NDUFB1*	NADH dehydrogenase (ubiquinone) 1β subcomplex 1, 7 kDa
AGTGCAAGAC	4	0	0.04773821			
ATTTGAGGAG	4	0	0.04773821	366	*MGC27165*	Hypothetical protein MGC27165
CAGTTTGTAC	4	0	0.04773821	1023	*PDHA1*	Pyruvate dehydrogese (lipoamide) α1
CTTGATTCCC †	4	0	0.04773821	77266	*QSCN6*	Quiescin Q6
GCGAAACCCT †	4	0	0.04773821	84285	*UBE2I*	Ubiquitin-conjugating enzyme E2I (UBC9 homologue, yeast)
GCTAGGTTTA	4	0	0.04773821			
GGGGAAATCG	4	0	0.04773821	76293	*TMSB10*	Thymosin, β10
GTATCCGGAC	4	0	0.04773821			
TACTCGAATA	4	0	0.04773821	349397	—	*Homo sapiens* cD FLJ32123 fis, clone PEBLM1000174.
TTCACTGTGA	4	0	0.04773821	621	*LGALS3*	Lectin, galactoside-binding, soluble, 3 (galectin 3)
TTCTAATTTT †	4	0	0.04773821	112830	*FLJ32312*	Hypothetical protein FLJ32312

NOTE: According to Monte Carlo simulation, 56 transcripts revealed differential expression at significantly different levels ($P < 0.05$); 31 of these were up-regulated in prostate cancer cells whereas 25 were down-regulated. Twelve tags had no matched gene entries. Blank entries indicate that no matching gene was found. Normal, microdissected normal prostate epithelial cells; Cancer, microdissected prostate cancer epithelial cells.
Abbreviaton: UID, Unigene identification number.
*A copy number of each gene.
†Tags matching with more than one gene.
‡A gene with no statistically significantly different level between normal and cancer cells.

microarray, DCXR overexpression at the protein level and its subcellular localization were confirmed in fresh-frozen prostate cancers and a spectrum of organ-confined and chemohormonally treated prostate cancers. The consistent up-regulation of DCXR observed at both the mRNA and protein levels suggests that DCXR is a potential new biomarker for prostate cancer.

Materials and Methods

LCM-microSAGE Library Construction. For the LCM-microSAGE library construction, one paired cancerous and normal tissues were obtained from a single prostatectomy specimen from a patient with prostatic adenocarcinoma, Gleason score 7 (3 + 4), and pathologic stage $pT_2N_0M_0$. The patient was not treated before surgery. The prostate samples were obtained fresh, immediately snap frozen, and stored at −70°C. For this study, frozen tissues were retrieved and histologically examined by a pathologist (J.H.C-V.) using cryostat sections.

Using a PixCell laser capture microscope with an IR diode laser (Arcturus Engineering), 5-μm-thick serial sections of fresh frozen tissues were stained with HistoGene LCM Frozen Section Staining Kit (Arcturus) and LCM was done immediately. The mRNAs of cancer cells from 12 serial frozen sections and normal cells from 10 serial frozen sections were isolated using 70,000 and 100,000 laser pulses, respectively, from a laser beam that was 30 μm in diameter.

Total RNA was extracted and purified following the protocol of the PicoPure RNA Isolation Kit (Arcturus). RNA integrity and quantity were checked by spectrophotometry (DU^R7400 Spectrophotometer, Beckman). Cells captured by ~10,000 pulses were used to extract total RNA in a 10-μL volume. We collected 10 samples to prepare a total of 100 μL (20-30 ng mRNA) ready for microSAGE analysis. Using the I-SAGE kit (Invitrogen), microSAGE analysis with a modified ditag PCR method was done according to previous reports (15).

A total of 1,253 concatemer clones (678 of prostate cancer cells, 575 of normal cells) were sequenced by direct sequencing and the ABI 3700 instrument (Applied Biosystems) according to the manufacturer's instructions. The sequence and occurrence of each transcript tag were determined with SAGE 2000 version 4.1.[5] The tags were then matched to the SAGE reliable map.[6]

For the sequence data processing and tag extraction strategies, we used a Monte Carlo simulation with adjustment for multiple comparisons to determine statistical significance and calibrate the appropriate threshold value (16). On the basis of these comparisons, a maximum value of 0.05 was chosen for P_{chance}. This yielded a false-positive rate that was no higher than 0.02 for the least significant P_{chance} value below the cutoff. The P_{chance} was double checked with another method as described previously (17).

Validation of DCXR Up-Regulation in Prostate Cancer Tissues and Cell Lines

Quantitative Real-time RT-PCR. One-step quantitative real-time RT-PCR was done to validate the LCM-microSAGE results. Prostate cancer and paired normal prostate specimen analyzed by LCM-microSAGE were used in this validation.

[5] http://www.sagenet.org
[6] ftp://ftp.ncbi.nih.gov/pub/sage/map/Hs/NlaIII/

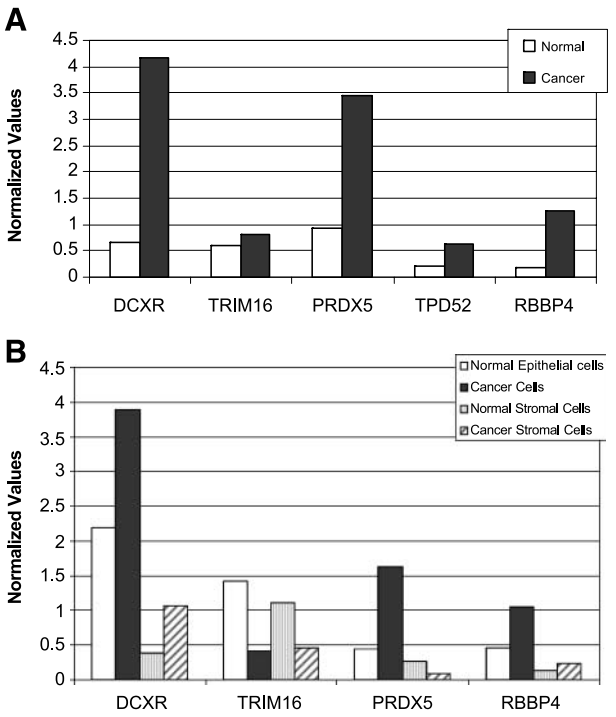

Figure 1. Quantitative real-time RT-PCR validation of five candidate genes, *DCXR, TRIM16* (*BIMLEC*), *PRDX5, TPD52*, and *RBBP4*, which were up-regulated in a cancer cell LCM-microSAGE library. **A.** By RT-PCR, four of five genes were up-regulated in nondissected prostate cancer tissues compared with normal tissues. **B.** Only DCXR mRNA was expressed at considerably higher level in microdissected cancer cells compared with normal and stromal cells. The mRNA expression level of each gene was normalized to β-actin (normalized values).

Total RNA was isolated using the PicoPure RNA Isolation Kit (Arcturus) and checked for integrity and quantity by spectrophotometry (DU^{R}7400 Spectrophotometer, Beckman). After we compared prostate cancer and normal prostate tissue libraries, we selected five genes [*DCXR, TRIM16* (*BIMLEC*), *PRDX5, TPD52*, and *RBBP4*] for further analysis. Total RNAs of whole tissue sections (300 ng/μL) and LCM samples (30 ng/μL) were used for one quantitative real-time RT-PCR reaction. Quantitative real-time RT-PCR was done on the ABI PRISM 7900HT Sequence Detection System by using One-Step RT-PCR Master Mix Reagents (Applied Biosystems) and Assays-on-Demand Gene Expression Probes (Applied Biosystems). The relative standard curve method (Applied Biosystems) was used to calculate the amplification difference between prostate cancer and normal prostate tissue for each primer set. mRNA expression of each gene was normalized to mRNA expression of β-actin.

Virtual Northern Blot. The virtual Northern blot was done using an electronic database available at the Cancer Genome Anatomy Project website.[7] The sequence tags were used to determine levels of mRNA expression in the 186 functional libraries in the SAGE database at the Cancer Genome Anatomy Project. *Nla*III was used as the anchoring enzyme.

Fresh-Frozen Tissues. Paired normal prostate and prostate cancer tissues from eight patients (Gleason score 6-7 in seven patients, Gleason score 9 in one patient), one specimen of normal mouse liver tissue, and a purchased normal human liver lysate (Pierce Biotech) were used for the immunohistochemical and Western blot analyses.

Western Blot Analysis. Whole-cell protein (M-PER mammalian protein extraction reagents) was isolated from three pairs of normal and prostate cancer tissue (all of Gleason score 6-7), two prostate cancer cell lines (LNCaP and PC3), and one normal liver lysate following the manufacturer's recommendations (Pierce Biotech).

Protein concentrations were determined with a protein assay (Bio-Rad). Techniques for running SDS-PAGE and Western blots were described in previous reports (14, 18). Prestained molecular weight standards were obtained from Bio-Rad. On completion of electrophoresis, proteins were transferred onto nitrocellulose (Bio-Rad) and probed in 5% nonfat dried milk/PBS/0.2% Tween 20. After washing, the membranes were probed with horseradish peroxidase–conjugated secondary antibody (anti-IgG). Enhanced chemiluminescence was done with the enhanced chemiluminescence kit (Amersham-Pharmacia) followed by autoradiography. For appropriate blots, β-actin levels were also determined to verify equal loading.

Immunohistochemical Analysis. Immunohistochemical analysis was done with a primary antibody specific for DCXR (polyclonal, rabbit anti-human, Taisho Pharmaceutical, Inc.) as described previously (14).

Eight fresh-frozen paired normal and prostate cancer tissues were initially analyzed. Fresh mouse liver tissue was used as a positive control to confirm antibody specificity and sensitivity.

Tissue Microarray Analysis. Tissue specimens from 75 patients with prostate cancer retrieved from M.D. Anderson Cancer Center between 1994 and 2001 were included in tissue microarrays. These specimens were further subdivided into two groups. Forty-six cases were organ-confined prostate cancer, stage $pT_2N_0M_0$. These patients underwent radical prostatectomy with no prior treatment. The Gleason score of this group of tumors was 5 (n = 10), 6 (n = 13), 7 (n = 10), 8 (n = 10), and 9 (n = 3). Twenty-nine patients received neoadjuvant chemohormonal therapy as part of a phase II study at our institution. These patients were either stage $T_{2\text{-}3}N_0M_0$ (n = 18) or stage $T_3N_1M_0$ (n = 11). Cases of lymph nodes involved by metastasis (n = 7) were included in this group of chemohormonally treated patients.

The tissue microarrays were constructed with a Beecher Instruments tissue arrayer. A H&E-stained section of each donor block was used to identify representative areas of tumor to be included in the 1- to 2-mm-diameter cores (19, 20). A total of 3 to 12 cores were arrayed from each of 75 surgical specimens. The completed array consisted of 377 cores from prostate cancer specimens: 232 cores from organ-confined cancers

[7] http://www.ncbi.nlm.nih.gov/SAGE/index.cgi

and 145 cores from chemohormonally treated cancers. Lymph nodes involved by metastasis were included in chemohormonally treated cases ($n = 7$).

The images of each core were acquired with the Bacus Laboratories Incorporated Imaging System (BLISS; Bacus Laboratories, Inc.). Core images were hardlinked to a tissue array database using Active X application programming interface (Bacus). DCXR protein levels were scored manually. The percentage of tumor cells exhibiting detectable staining was scored semiquantitatively as 0 (no staining), 1 (up to 25% of cells positive), 2 (25-75%), or 3 (>75%). The intensity of staining was scored as 0 (negative), 1 (weak), 2 (mixed with weak and strong), or 3 (strong). Both variables were dichotomized as low (a score of 1 or 2) and high (a score of 3) and the final DCXR score for each core summarized these variables as low (low/low), intermediate (low/high or high/low), and high (high/high) values. In addition, the subcellular distribution (cytoplasmic membrane, cytosol, or nucleus) of DCXR was observed. With Fisher's exact test, the staining score and subcellular localization of DCXR were compared between each group. On the basis of this comparison, a maximum value of 0.05 was chosen for P_{chance}.

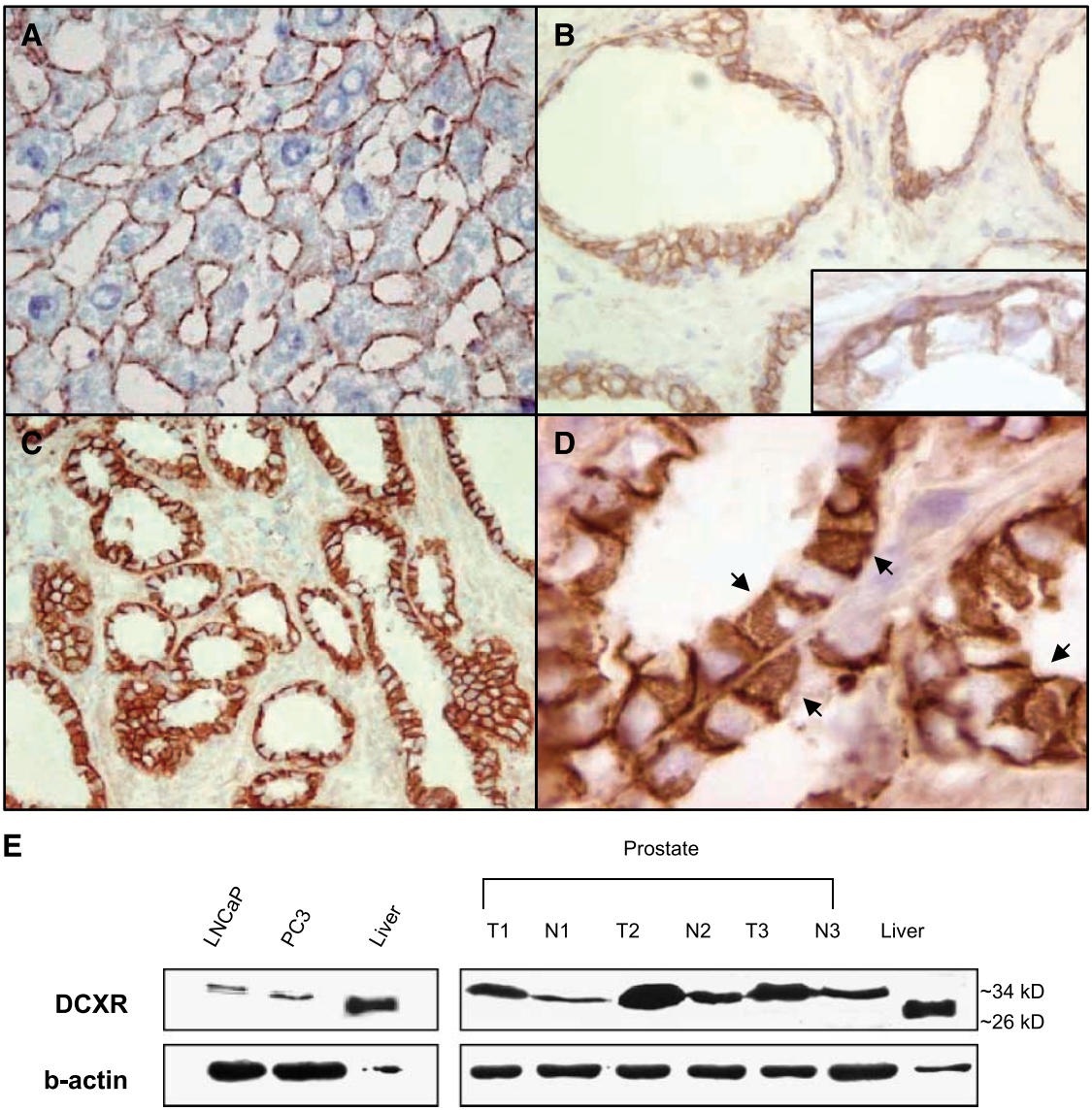

Figure 2. Immunohistochemical results for DCXR expression in fresh-frozen mouse liver (**A**), normal human prostate (**B**), and matched human prostate cancer (**C** and **D**). The expression of DCXR was localized in the cytoplasmic membrane of mouse hepatocytes and normal human prostate epithelial cells (**A** and **B**). Increased expression of DCXR in the cytoplasmic membrane, cytoplasm, and nucleus (**D**, *arrows*) was found in human prostate cancer cells (**C** and **D**). DCXR was not expressed in surrounding stromal cells. Original magnification: **A**, *inset* for **B**, and **D**, ×400; **B** and **C**, ×100. **E,** Western blot analysis of DCXR from whole-cell lysates of LNCaP, PC3, human liver, and human prostate tissues. Two different sizes of DCXR protein, ~26 kDa in human liver and ~34 kDa in prostrate tissues and cell lines, were noted. *T1* to *T3,* tumor tissues; *N1* to *N3,* normal tissues.

Results

Genes Are Differentially Expressed in a Prostate Cancer Cell LCM-microSAGE Library. LCM-microSAGE libraries were generated from prostate cancer cells and paired normal epithelial cells. A total of 6,462 unique tags were procured after 1,253 concatemer clones were sequenced. After matching the tags to genes in the SAGE map, 2,057 tags each matched with single genes and 1,463 tags matched with more than one gene. Three hundred forty-six tags (5.4% of 6,467 analyzed) were increased and 374 tags (5.8%) were decreased >4-fold in cancer cells (data not shown). Fifty-six transcripts revealed statistically significant differential expression ($P < 0.05$); 31 of these were up-regulated and 25 were down-regulated in cancer cells (Table 1). Twelve tags had no matching genes. The *dcxr* gene was up-regulated in cancer cells 6-fold compared with normal epithelial cells.

DCXR mRNA Was Consistently Up-Regulated in Prostate Cancer Tissue. Five genes, *DCXR, TRIM16* (*BIMLEC*), *PRDX5, TPD52*, and *RBBP4*, which are up-regulated in the cancer cell LCM-microSAGE library and not yet studied extensively in prostate cancer, were subjected to quantitative real-time RT-PCR. In non-dissected cancer and normal tissues from the same patient, all five genes were up-regulated in prostate cancer tissues (Fig. 1A). However, after microdissection, only *DCXR* showed two to eight times overexpression in cancer compared with normal and stromal cells (Fig. 1B). The other four genes showed a <3-fold overexpression in cancer cells compared with normal and stromal cells or no difference at all.

High Level of dcxr Gene Transcripts in Registered Prostate Cancer SAGE Libraries. The virtual Northern blot from 186 SAGE libraries revealed that *dcxr* gene transcripts were significantly higher in prostate cancer (263 DCXR tags of total 491,794 tags) compared with normal prostate (59 of 266,949 tags; $P < 0.001$). Cancer tissues from breast, lung, and colon also expressed significantly more *dcxr* gene transcripts than corresponding normal tissues (all $P < 0.001$) but to a lesser extent than prostate cancer: breast, 467 of 1,329,651 versus 24 of 434,551; lung, 44 of 122,803 versus 28 of 159,917; and colon, 25 of 32,836 versus 18 of 98,089 (cancer versus normal, respectively). DCXR was expressed ubiquitously in all organs at a relatively very low level.

DCXR Overexpression at the Protein Level Was Tightly Linked to Prostate Cancer Cells. DCXR protein

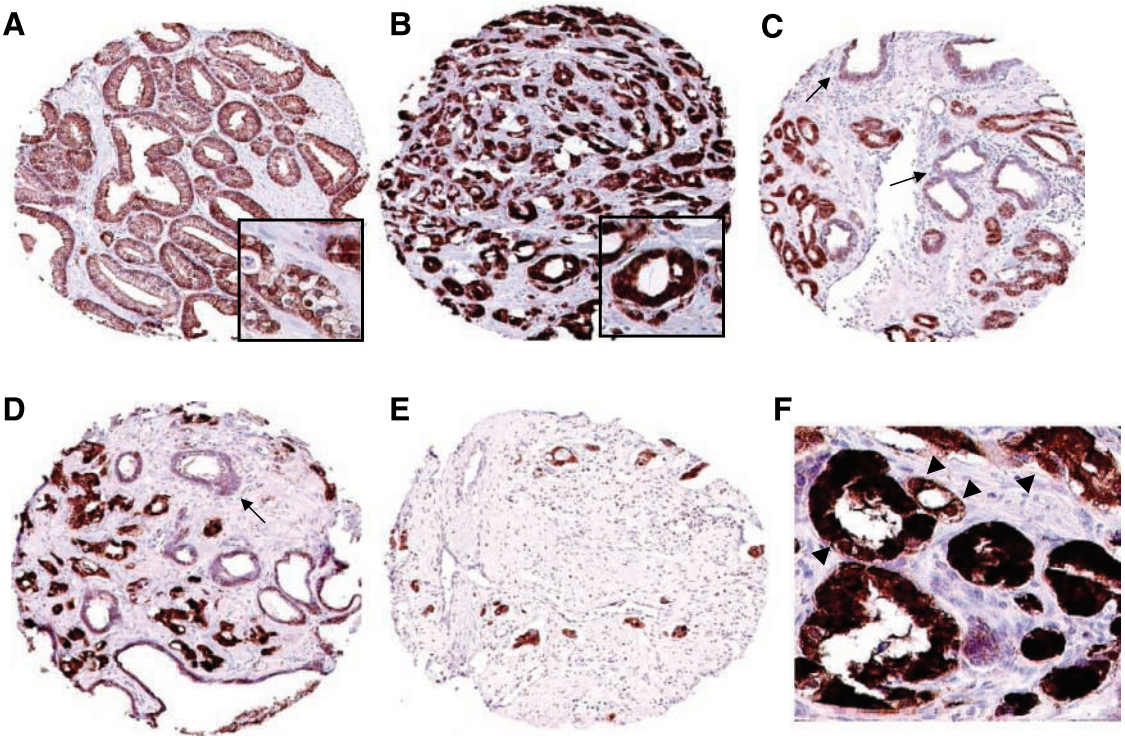

Figure 3. Immunohistochemical results of DCXR expression using a tissue microarray. *Top,* representative cases of organ-confined prostate cancer. In low-grade cancers (Gleason score 6; **A**) and high-grade cancers (Gleason score 9; **B**), the cancer cells showed diffuse cytoplasmic membranous, cytoplasmic, or nuclear staining for DCXR (*insets*). Increased intensity of DCXR expression was associated with high-grade cancers. Intertwining nonneoplastic epithelial cells (**C** and **D**, *arrows*) generally showed weaker DCXR expression that was limited to the cytoplasmic membrane. In all samples, the stromal cells did not express DCXR. *Bottom,* representative cases of chemohormonally treated prostate cancer. **D** to **F,** all cancer cells showed DCXR overexpression at a high level, predominantly in the cytoplasm and nucleus (**F**, *arrows*). DCXR overexpression was also observed in individual or clustered cancer cells in the surrounding fibrous stroma (**E**). Original magnification: **A** to **E**, ×10; *insets* for **A** and **B**, ×400; **F**, ×100.

Table 2. Immunohistochemical results of DCXR protein expression by tissue microarray for prostate cancers

Cases	Tissue microarray					Total
	Intensity + cell proportion			Subcellular localization (%)		
	L	I	H	C/CM	C/N	
GS 5-6	1	22	2	13 (56)	10 (44)	23
GS 7	5	2	3	3 (30)	7 (70)	10
GS 8-9	0	4	9*	4 (30)	9 (70)	13
CHx	2	3	24†	5 (14)	24† (86)	29

Abbreviations: GS, Gleason score; CHx, patients are under phase II clinical trial of chemohormonal therapy. C, cytosol; CM, cytoplasmic membrane; N, nucleus. L, low (low/low); I, intermediate (low/high or high/low); H, high (high/high).
*$P < 0.05$, compared with Gleason scores 5-6 and 7.
†$P < 0.05$, compared with Gleason score 8-9.

expression was initially assessed using fresh-frozen mouse liver and human prostate tissues. DCXR expression was predominantly localized in the cytoplasmic membrane of hepatocytes (Fig. 2A). In normal human prostate epithelial cells, DCXR expression was mainly localized in the cytoplasmic membrane of both luminal and basal epithelial cells and less so in the cytoplasm (Fig. 2B). Higher levels of cytoplasmic, membranous, cytoplasmic, and nuclear DCXR expression were observed in all prostate cancer cells compared with the matched normal epithelial cells (Fig. 2C and D). Stromal cells and infiltrating lymphocytes were completely devoid of DCXR expression.

Quantitative expression analysis by Western blot also revealed a >2-fold increase in DCXR expression in prostate cancer tissues compared with paired normal tissues. There was no difference of DCXR protein expression between the LNCaP and PC3 cell lines (Fig. 2E). DCXR protein from human liver had a lower molecular weight (~26 kDa) than that from prostate tissues and cell lines (~34 kDa).

To further characterize DCXR expression in a spectrum of prostate cancers, we used immunohistochemistry to assess tissue microarrays constructed from specimens of 75 prostate cancer patients as described above. Forty-six organ-confined prostate cancers showed higher DCXR expression in both low-grade and high-grade cancer cells compared with surrounding normal epithelial cells (Fig. 3A-C). The stronger intensity of DCXR expression was observed in high-grade (Gleason score >8) cancers compared with low-grade (Gleason score 5-6) and intermediate-grade (Gleason score 7) cancers ($P < 0.05$; Table 2). In cancer cells, DCXR was expressed in the cytoplasmic membrane, cytoplasm, and nucleus with varying degrees of intensity (Fig. 3A-C, *insets*). In contrast, in normal epithelial cells, DCXR was expressed weakly and predominantly within the cytoplasmic membrane (Fig. 3C).

In the 29 patients who received neoadjuvant chemohormonal therapy, prostate cancer cells showed intense DCXR overexpression predominantly in the nucleus and cytoplasm (Fig. 3D-F; Table 2). This characteristic pattern made it possible to observe single cancer cells (Fig. 3E). There was no significant difference of DCXR expression levels between matched primary tumors and nodal metastases (data not shown). Stromal cells and infiltrating lymphocytes were completely devoid of DCXR expression in all cases assessed.

Discussion

With the development of microarray technology, a number of genes have been identified that are consistently up-regulated in prostate cancer cells compared with normal and hyperplastic prostate cells (21, 22). Even so, SAGE presents considerable technical challenges in profiling specific cell populations within heterogeneous human tissue (23, 24). We previously showed the feasibility of combining LCM with micro SAGE to generate cell-specific expression profiles and to assess the expression levels of known and unknown transcripts in specific cell types obtained from human tissue samples (13). Our LCM-microSAGE technique circumvents the limitations associated with a traditional whole tissue approach and produces cell-specific expression profiles from limited human tissue samples. Although this technique is labor-intensive, expensive, and time-consuming, identification of prostate cancer genes by using this approach may complement or perhaps replace currently used diagnostic methods for prostate cancer.

Using this technology, we discovered that the *dcxr* gene was substantially up-regulated in a prostate cancer cell LCM-microSAGE library. Quantitative real-time RT-PCR and both virtual Northern blot and Western blot analyses confirmed that DCXR mRNA and protein are specifically overexpressed in prostate cancer cells. Furthermore, immunohistochemical analysis of a large group of prostate cancers showed that marked DCXR overexpression occurred in virtually all grades of early-stage prostate cancer and in all chemohormonally treated cases. These findings reveal that DCXR overexpression is a potential molecular marker with high sensitivity for prostate cancer.

High levels of DCXR expression have been reported in normal mouse liver, kidney, and hamster epididymis (14). DCXR has three, apparently unrelated, functions: (*a*) it has a crucial role in detoxification as a NADPH-dependent reductase in mouse liver (14, 25); (*b*) it has a role in carbohydrate metabolism as an L-xylulose reductase of the uronate cycle, participating in cellular osmoregulation in mouse proximal renal tubules (14); and (*c*) it is proposed to be involved in the human

sperm-zona pellucida interaction process (26). In addition, the subcellular localization of DCXR is different in various organs; for example, the luminal membrane in mouse kidney proximal tubules (14), perinuclear in hamster epididymis (P31h; ref. 27), and covering the acrosome (P34H) in human sperm (26). In our virtual Northern blot analysis, DCXR was expressed ubiquitously in all organs at a relatively very low level. Therefore, DCXR may be another ''moonlighting'' protein; in other words, a protein that plays different functions in different organs. α-Methyl CoA racemase (28), which was initially identified by RNA subtraction hybridization and cDNA microarray as a prostate cancer marker (3), is also expressed in multiple normal organs and in various cancers.

In human prostate, we observed a different subcellular localization of DCXR protein between normal epithelial cells and cancer cells. In normal epithelium, DCXR was located predominantly in the cytoplasmic membrane, raising the possibility that DCXR has an additional function as a cell adhesion molecule. The ExPASy proteomics server[8] predicted that DCXR is a membrane-associated protein and probably recruited to membrane via an interaction with phosphatidylinositol. One transmembrane domain and several myristoylation sites are also predicted in the DCXR protein (TMAP and TMPRED). Membranous DCXR expression has been reported in mouse kidney proximal convoluted tubules and human sperm (14, 26). We colocalized DCXR with E-cadherin and β-catenin in human normal and cancer prostate epithelial cells.[9] In addition, we recently published morphologic findings supporting a possible cell adhesion function of DCXR in human melanocytic lesions (29). However, further molecular evidence supporting a cell adhesion function of DCXR remains to be clarified.

In contrast, 75 prostate cancers showed altered DCXR subcellular localization with increased DCXR expression. In particular, nuclear localization was associated with cancer cells. Based on our virtual Northern blot results, we also immunohistochemically assessed a variety of other human cancers including pulmonary carcinomas, colorectal adenocarcinomas, and cutaneous melanomas. Compared with that seen in prostate cancers, DCXR expression was relatively lower in these cancers and was rarely localized to the nucleus.[9] Translocation to the nucleus of β-catenin is associated with increased androgen receptor–mediated transcription of target genes in prostate cancer (30). Although the functional significance of DCXR in prostate cancer remains unknown, it is conceivable that aberrant DCXR expression in prostate cancer correlates with molecular events involving the *dcxr* gene. Indeed, we found two different sizes of DCXR protein products in human liver (~26 kDa) and prostate (~34 kDa) by Western blot analysis.

In summary, using the LCM-microSAGE technique, we identified DCXR as being significantly up-regulated in a prostate cancer cell library, suggesting that DCXR can be a new biomarker for prostate cancer, useful in detecting and diagnosing this disease.

[8] http://us.expasy.org/uniprot/Q7Z4W1
[9] Unpublished data.

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Jeong Hee Cho-Vega, Spiridon Tsavachidis, Kim-Anh Do, et al.

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