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Molecular Features of the Transition from Prostatic Intraepithelial Neoplasia (PIN) to Prostate Cancer: Genome-wide Gene-expression Profiles of Prostate Cancers and PINs

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ABSTRACT

To characterize the molecular feature in prostate carcinogenesis and the putative transition from prostatic intraepithelial neoplasia (PIN) to invasive prostate cancer (PC), we analyzed gene-expression profiles of 20 PCs and 10 high-grade PINs with a cDNA microarray representing 23,040 genes. Considering the histological heterogeneity of PCs and the minimal nature of PIN lesions, we applied laser microbeam microdissection to purify populations of PC and PIN cells, and then compared their expression profiles with those of corresponding normal prostatic epithelium also purified by laser microbeam microdissection. A hierarchical clustering analysis separated the PC group from the PIN group, except for three tumors that were morphologically defined as one very-high-grade PIN and two low-grade PCs, suggesting that PINs and PCs share some molecular features and supporting the hypothesis of PIN-to-PC transition. On the basis of this hypothesis, we identified 21 up-regulated genes and 63 down-regulated genes commonly in PINs and PCs compared with normal epithelium, which were considered to be involved in the presumably early stage of prostatic carcinogenesis. They included *AMACR*, *OR51E2*, *RODH*, and *SMS*. Furthermore, we identified 41 up-regulated genes and 98 down-regulated genes in the transition from PINs to PCs; those altered genes, such as *POV1*, *CDKN2C*, *EPHA4*, *APOD*, *FASN*, *ITGB2*, *LAMB2*, *PLAU*, and *TIMP1*, included elements that are likely to be involved in cell adhesion or the motility of invasive PC cells. The down-regulation of *EPHA4* by small interfering RNA in PC cells lead to attenuation of PC cell viability. These data provide clues to the molecular mechanisms underlying prostatic carcinogenesis, and suggest candidate genes the products of which might serve as molecular targets for the prevention and treatment of PC.

INTRODUCTION

Prostate cancer (PC) is the most common malignancy in males and the second-leading cause of cancer-related deaths in the United States and Europe (1). Despite surgery and radiation therapy that often cure localized disease, and the possibility of early diagnosis through testing for prostate-specific antigen in serum, up to 30% of treated PC patients suffer relapse (2–4). Most patients with relapsed or advanced disease initially respond to androgen-ablation therapy because early PC growth is androgen dependent, but they eventually progress to androgen-independent disease, which no longer responds to androgen ablation. The most important issue to be solved in this context is that this advanced stage of PC does not respond to other therapies either.

Investigations designed to clarify the molecular mechanisms that underlie the initiation and progression of PC have revealed altered expression of *PTEN*, *p27KIPS*, and *Nkx3.1* tumor suppressor genes, the *c-myc* oncogene, and the *Bcl-2* antiapoptotic gene in human and/or mouse PCs (5, 6). However, the overall molecular events are still largely unknown. High-grade PIN (prostatic intraepithelial neoplasia) has been considered to be a putative precursor of cancer (7), and some clinical and scientific evidence supports a relationship between high-grade PINs and PCs. However, that hypothesis is still controversial (8, 9). Added to the difficulties of proving a PIN-to-PC transition is that human PCs are histopathologically very heterogeneous, and PIN is generally a very small lesion. Most previous molecular-based studies have analyzed bulk cancer tissues that were contaminated by a large proportion of noncancerous cells including fibromascular, microvasculature, and inflammatory cells, but such strategies do not yield precise molecular profiles of the respective lesions.

To overcome that limitation, we obtained purified populations of PC and PIN cells, as well as normal prostatic epithelial cells as a control, by LMM (laser microbeam microdissection), before analyzing genome-wide gene-expression profiles of 20 PCs and 10 PINs on a cDNA microarray representing 23,040 genes. This is the first report to show precise expression profiles of PC and PIN tumors, and to disclose molecular features confirming a relationship between PINs and PCs.

MATERIALS AND METHODS

Patients and Tissue Samples. Tissue samples were obtained with informed consent from 26 cancer patients undergoing radical prostatectomy. All of the surgical specimens were at clinical stages T_{2a} to T_{3a} with or without N_1, and their Gleason scores were 5–9. All of the samples were embedded in TissueTek OCT medium (Sakura, Tokyo, Japan) immediately after surgical resection and stored at −80°C until use. Histopathological diagnoses were made by a single pathologist (M. F.) before LMM. H&E-stained sections from adjacent frozen tissues were prepared to confirm the histological diagnosis. High-grade PINs included in the surgical specimen of PC were characterized and identified by the following criteria: (*a*) a basal cell layer consistently enveloped the intraductal/acinar proliferation; and (*b*) atypical cells that had large nuclei of relatively uniform size increased the chromatin content, which might be irregularly distributed, and had prominent nucleoli that were similar to those of carcinoma cells (7, 10). Once high-grade PIN cells, PC cells, and normal epithelium were identified by H&E study, we microdissected them by LMM from the adjacent frozen slides. Normal prostatic epithelial cells were microdissected from the prostatic lobe opposite to the prostate cancer, or the normal prostatic tissue apparently far from the cancer. From among the 26 resected tissues, 20 cancers and 10 high-grade PINs had sufficient amounts and quality of RNA for microarray analysis.

Laser Microbeam Microdissection and T7-based RNA Amplification. LMM and T7-based RNA amplification were performed as described previously (11). Prostate tumor cells and normal prostatic epithelial cells were

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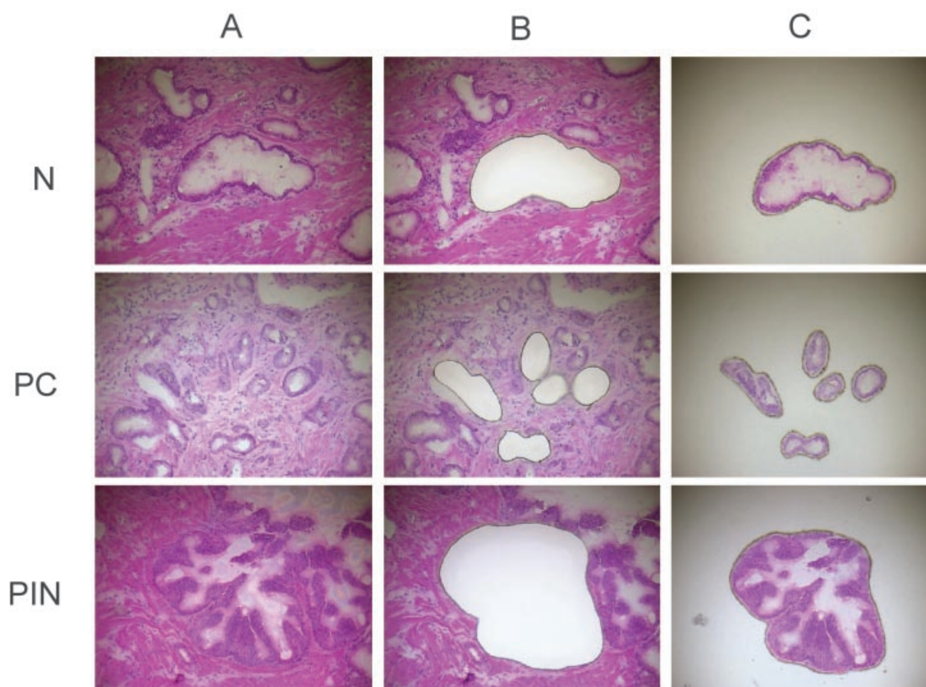

Fig. 1. Laser microbeam microdissection. Normal prostatic ductal epithelial cells (*N*), prostate cancer cells (*PC*), and prostatic intraepithelial neoplasia cells (*PIN*) from a single specimen (patient 16) were microdissected from H&E-stained sections. *Lane A*, premicrodissected tissue; *Lane B*, postmicrodissected tissue; *Lane C*, microdissected cells.

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isolated selectively by the EZ cut system with a pulsed UV narrow beam-focus laser (SL Microtest GmbH, Germany) in accordance with the manufacturer's protocols. After DNase treatment, total RNAs were subjected to two rounds of T7-based amplification, which yielded 50 to 100 μg of amplified RNA (3) from each sample. Then 2.5-μg aliquots of amplified RNA from PC or PIN cells and from normal prostatic ductal epithelial cells were labeled by reverse transcription with Cy5-dCTP (tumor cells) or Cy3-dCTP (normal cells (Amersham Biosciences, Buckinghamshire, United Kingdom), as described previously (12).

cDNA Microarray Analysis and Acquisition of Data. We fabricated a genome-wide cDNA microarray with 23,040 cDNAs selected from the UniGene database (build no.131) of the National Center for Biotechnology Information (NCBI).[7] Construction, hybridization, washing, and scanning were carried out according to methods described previously (11, 12). Signal intensities of Cy3 and Cy5 from the 23,040 spots were quantified and analyzed by substituting backgrounds with ArrayVision software (Imaging Research, Inc., St. Catharines, Ontario, Canada). Subsequently, the fluorescent intensities of Cy5 (tumor) and Cy3 (control) for each target spot were adjusted so that the mean Cy3/Cy5 ratio of 52 housekeeping genes was equal to one. Because data derived from low-signal intensities are less reliable, we determined a cutoff value on each slide (12), and we excluded genes from further analysis when both the Cy3 and the Cy5 dyes yielded signal intensities lower than that of the cutoff. For other genes, we calculated the Cy5/Cy3 ratio using the raw data of each sample.

Cluster Analysis of 20 Prostate Cancers and 10 PINs According to Gene-expression Profiles. We applied a hierarchical clustering method to both genes and tumors, excluding genes whose Cy3- and Cy5-fluorescence intensities were both below the cutoff value. To obtain reproducible clusters for classification of the 30 tumors, we selected 63 genes for which valid data were obtained in 80% of the experiments and whose expression ratios varied by SDs of more than 1.5. The analysis was performed with web-available software (Cluster and TreeView) written by Eisen.[8] Before applying the clustering algorithm, we log-transformed the fluorescence ratio for each spot and then median-centered the data for each sample to remove experimental biases.

Identification of Genes That Were Up- or Down-regulated Commonly from Normal Epithelium to PINs and PCs. We identified genes the expression of which was altered from normal epithelium to 10 PINs and 20 PCs according to the following criteria: (*a*) genes for which we were able to obtain expression data in more than 50% of the cases examined; and (*b*) genes the expression ratio of which was more than 3.0 or less than 0.33 in more than 50% of informative cases.

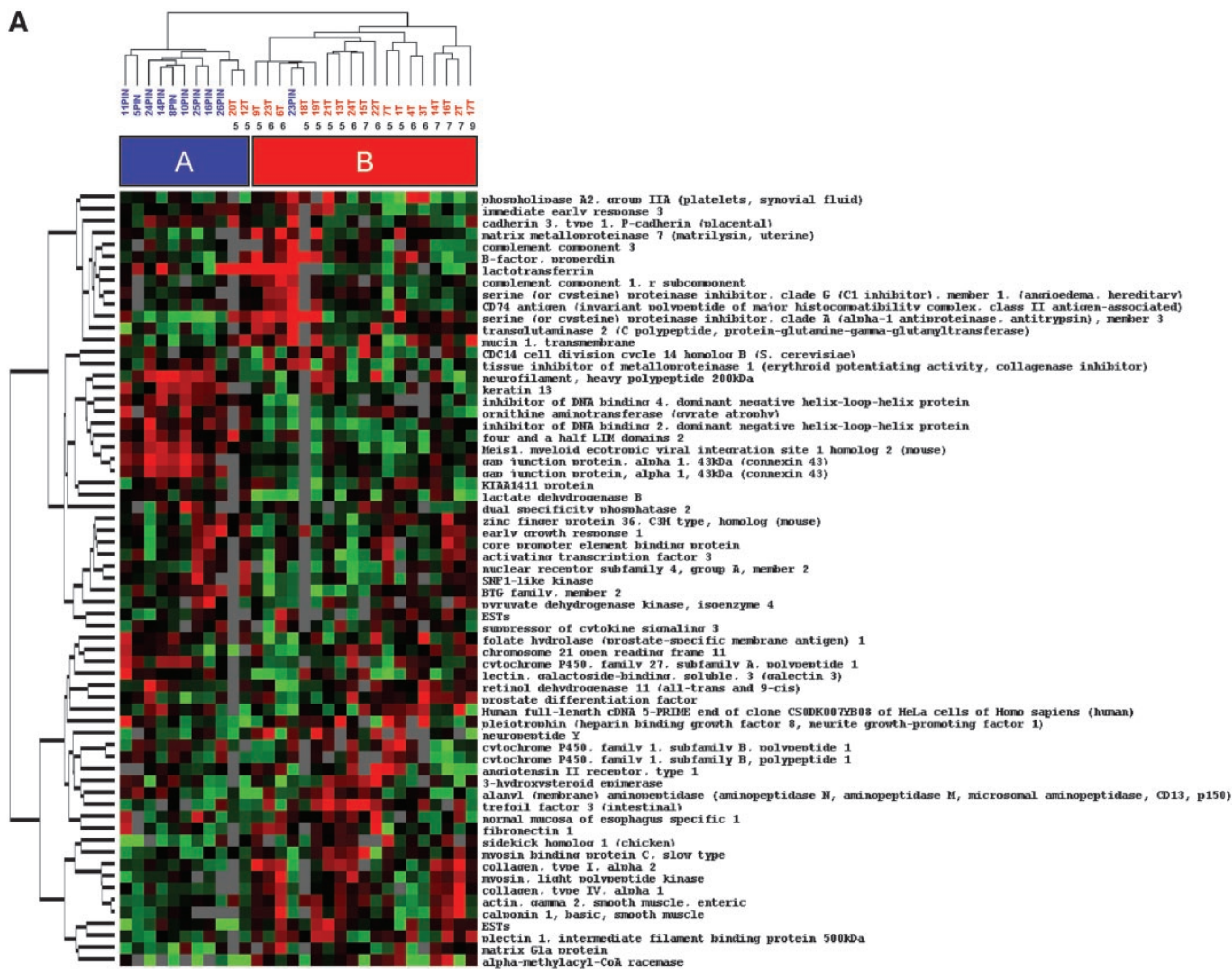
Identification of Genes That Were Up- or Down-regulated from PINs to PCs. We identified genes with changed expression in 20 PCs and 10 PINs according to the following criteria: (*a*) genes for which we were able to obtain expression data in more than 50% of the cases examined; and (*b*) genes the expression ratio of which was more than 3.0 in PCs and between 0.5 and 2.0 in PINs (defined as up-regulated genes) or genes whose expression ratio was less than 0.33 in cancers and between 0.5 and 2.0 in PINs (defined as down-regulated genes) in more than 50% of informative cases.

Immunohistochemistry. Formalin-fixed and paraffin-embedded prostatic tumor sections were immunostained with a mouse anti-APOD (apolipoprotein D) monoclonal antibody (NeoMarkers, Fremont, CA) or a rabbit anti-EPHA4 (EphA4) polyclonal antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA) for APOD or EPHA4 expression. PC tissues included PC cells, PIN cells, and normal prostatic epithelium heterogeneously. Deparaffinized tissue sections were placed in 10 mmol/L citrate buffer (pH 6.0) and were heated to 108°C in an autoclave for 15 minutes for antigen retrieval. Sections were incubated with a 1:10 dilution or a 1:100 dilution of primary antibody for APOD or EPHA4, respectively, in a humidity chamber for 1 hour at room temperature and were developed with peroxidase labeled-dextran polymer followed by diaminobenzidine (DAKO Envision Plus System; DAKO Corporation, Carpinteria, CA). Sections were counterstained with hematoxylin. For negative controls, primary antibody was omitted.

Small Interfering RNA-expressing Constructs and Colony Formation/MTT Assay. We used small interfering RNA (siRNA)-expression vector (psiU6BX) for RNA interference effect to the target genes. The U6 promoter was cloned upstream of the gene-specific sequence (19-nucleotide sequence from the target transcript, separated by a short spacer TTCAAGAGA from the reverse complement of the same sequence) and five thymidines as a termination signal as well as neocassette for selection with Geneticin (Sigma). The target sequences for *EphA4* are 5′-TCCGAACCTACCAAGTGTG-3′ (198si), 5′-TCATGAAGCTGAACACCGA-3′ (468si) and 5′-GCAGCACCATCATCCATTG-3′ (1313si). The sequence of 5′-GAAGCAGCACGACTTCTTC-3′ (EGFPsi) corresponding to EGFP was as a negative control. PC3 PC cells were plated onto 10-cm dishes (5×10^5 cells/dish) and transfected with psiU6BX containing EGFP target sequence

[7] Internet address of database: http://www.ncbi.nlm.nih.gov.

[8] Internet address: http://genome-www5.stanford.edu/MicroArray/SMD/restech.html.



B

Fig. 2. *A*, dendrogram of unsupervised clustering analysis of 63 genes (*vertical axis*) across 30 prostatic tumors (*horizontal axis*). *Red*, the transcript level above the median for that gene across all samples; *green*, below the median for that gene across all samples; *black*, unchanged expression; *gray*, no detectable expression. In the horizontal axis, PCs and PINs were separated in two trunks (*column A on left* and *column B on right*); *above top of dendrogram, numbers below patient identification numbers,* Gleason scores. *In the vertical axis*, the 63 genes were clustered in different branches according to similarities in relative expression ratios. *Genes that appear more than once*, identical genes spotted on different sets of slides. *B*, histological examination of samples from patient 23PIN, which fell in the PC cluster on the basis of its molecular profile. This tumor was later confirmed to be a very-high-grade PIN.

(*EGFP*) or psiU6BX containing EPHA4 target sequence with LipofectAMINE 2000 (Invitrogen) according to the manufacturer's instruction. Cells were selected by 500 μg/mL Geneticin for 1 week, harvested 48 hours after transfection, and analyzed by reverse transcription (RT)-PCR to validate knockdown effect on *EPHA4*. The primers of RT-PCR were 5′-GAAGGCGTGGTCACTAAATGTAA-3′ and 5′- TTTAATTTCAGAGGGCGAAGAC-3′ for *EPHA4*, and 5′-GAGAGAGAATGAAAAGTGGAGCA-3′ and 5′-GATTAACCACAACCATGCCTTAC-3′ for *β2-MG*, used to quantify the amount of cDNA input. These cells were also stained by Giemsa solution and applied for 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay to evaluate the colony formation and the cell number, respectively.

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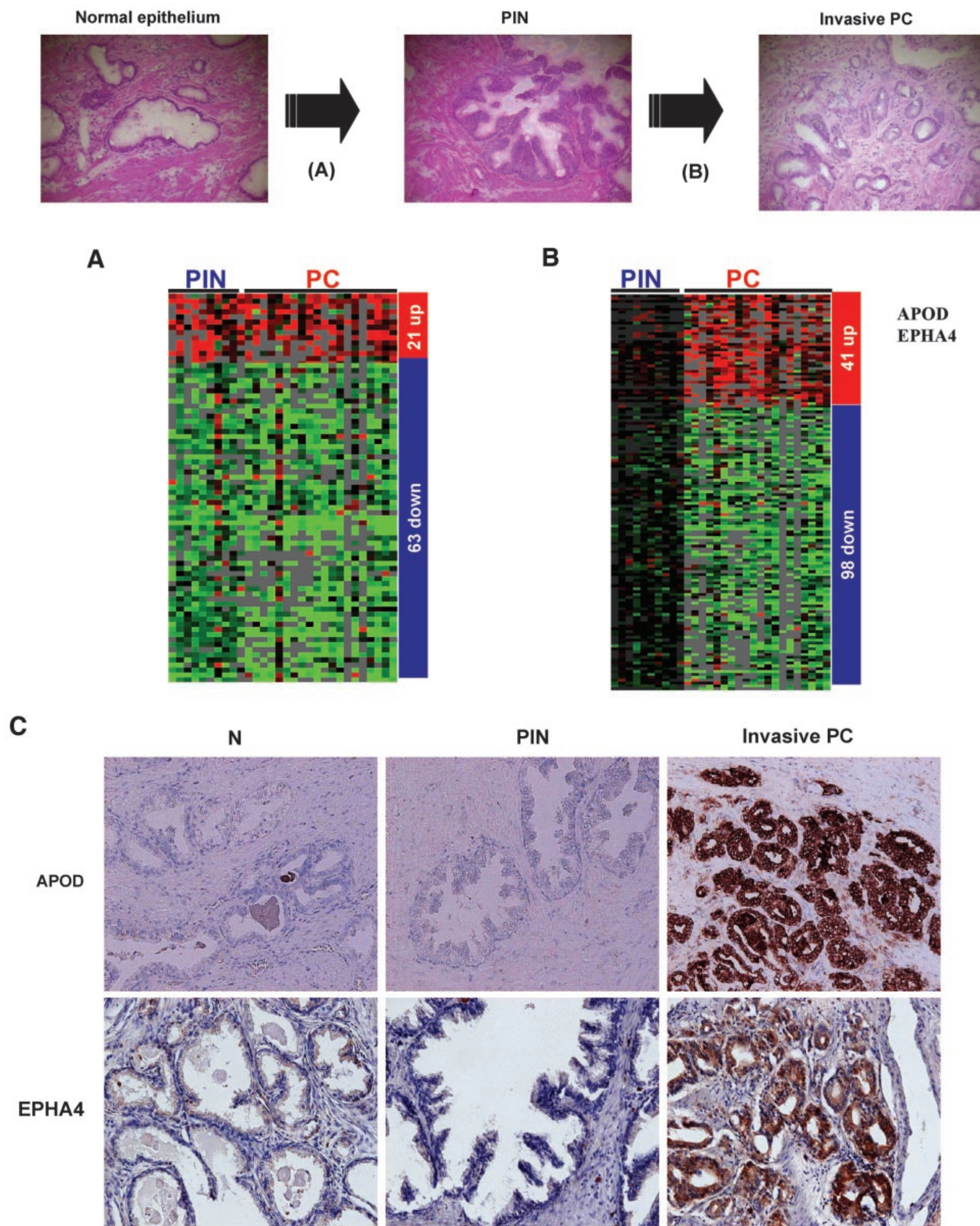

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Fig. 3. In *A* , 21 genes were up-regulated (*21 up*) and 63 genes were down-regulated (*63 down*) commonly in PINs and PCs as genes involved in an early step of prostatic carcinogenesis. In *B*, on the other hand, 41 genes were up-regulated (*41 up*) and 98 genes were down-regulated (*98 down*) in the transition from PINs to PCs. *Red*, the transcript level up-regulated compared with normal prostatic epithelial cells; *green*, the transcript level down-regulated compared with normal prostatic epithelial cells; *black*, unchanged expression; *gray*, no detectable expression. The detail of the genes in the gene lists that were differentially expressed in each step of this prostatic carcinogenesis hypothesis are shown in Tables 1 to 4. *C*, immunohistochemical analysis of genes, apolipoprotein D (*APOD*) and *EPHA4*, which were identified to be differentially expressed in the transition from PIN to PC in *B*. APOD was abundantly expressed in PC cells, whereas PINs and normal prostatic epithelium (*N*) from the same patient showed no expression of APOD protein. The EPHA4 protein was also strongly expressed in PC cells, whereas PINs and normal prostatic epithelium (*N*) from the same patient showed no, or very weak, expression of EPHA4 protein. The PC, PIN, and normal prostate epithelium were included on one PC tissue. ×200.

Table 1 *Up-regulated genes from normal epithelium to prostate tumors (PINs and PCs)*

Accession no.	UniGene No. Hs.	Symbol	Name
Function known			
AF071202	139336	*ABCC4*	*ATP-binding cassette, sub-family C (CFTR/MRP), member 4*
S77410	89472	*AGTR1*	*Angiotensin II receptor, type 1*
AJ130733	128749	*AMACR*	*α-methylacyl-CoA racemase*
AF039918	80975	*ENTPD5*	*Ectonucleoside triphosphate diphosphohydrolase 5*
BF106962	20415	*FAM3B*	*Chromosome 21 open reading frame 11*
AI302799	68583	*MIPEP*	*mitochondrial intermediate peptidase*
NM_012342	78776	*NMA*	*Putative transmembrane protein*
AI805082	303171	*OR51E2*	*Olfactory receptor, family 51, subfamily E, member 2*
NM_021200	380812	*PLEKHB1*	*Pleckstrin homology domain containing, family B (evectins) member 1*
AF044588	344037	*PRC1*	*Protein regulator of cytokinesis 1*
AK025460	286049	*PSA*	*Phosphoserine aminotransferase*
U89281	11958	*RODH*	*3-Hydroxysteroid epimerase*
U80456	27311	*SIM2*	*Single-minded homolog 2 (Drosophila)*
AD001528	89718	*SMS*	*Spermine synthase*
L15203	82961	*TFF3*	*Trefoil factor 3 (intestinal)*
Function unknown			
AI133467	95612		*ESTs*
H17800	438858		*ESTs*
AI700341	110406		*ESTs, Weakly similar to hypothetical protein FLJ20489 [Homo sapiens]*
AA743348	120591		*Homo sapiens cDNA FLJ35632 fis, clone SPLEN2011678.*
AA206763	7991	*C20orf102*	*Chromosome 20 open reading frame 102*
AW135763	6375	*HT010*	*Uncharacterized hypothalamus protein HT010*

Abbreviation: EST, expressed sequence tag.

RESULTS

Hierarchical Clustering Analyses of Expression Patterns in PCs and PINs. To avoid contamination of PC or PIN tissues with stromal cells as much as possible, we performed LMM of 26 surgical specimens of PCs. Because PCs and PINs originate from prostatic epithelial ductal cells, we also purified normal ductal epithelial cells as controls for our microarray study. Normal prostatic ductal cells, PC cells (PC), and PIN cells (PIN) were clearly microdissected from each clinical specimen as shown (*N*) in Fig. 1.

RNA from each microdissected specimen was amplified for analysis on a genome-wide cDNA microarray. We generated the expression profiles of 30 prostatic tumors (20 PCs and 10 PINs) from 26 patients (cancer and PIN coexisted in four patients, in which cases both lesions were examined). On the basis of expression patterns of 63 genes that we selected applying strict conditions (*i.e.,* valid data obtained in 80% of the experiments and expression ratios that varied by SDs of more than 1.5), all of the tumors fell into two major groups according to their status of malignancy in an unsupervised way, except for three cases (Fig. 2*A*) in which two PCs (12T and 20T) with low Gleason scores (score 5) were included in the branch corresponding to the PIN group, and one PIN (23PIN) belonged to the PC group. Histological re-examination of this misplaced PIN diagnosed it as a very-high-grade PIN (Fig. 2*B*) that pathologically resembled PC and probably represented a transitional lesion to invasive PC. These experiments showed that, although it was generally possible to distinguish PC from PIN by molecular classification on the basis of expression profiles, the distinction was not as obvious as we expected, and some molecular features were commonly shared. These results offered considerable validity to the concept that high-grade PINs can become invasive PCs.

Identification of Genes That Were Up- or Down-regulated Commonly in PINs and PCs As Compared with Normal Epithelium. Because we have obtained supportive evidence for the concept that PIN is a precursor of invasive PC through our hierarchical-clustering analysis of molecular profiles, it seemed likely that genes involved in an early stage of prostatic carcinogenesis would be deregulated from normal epithelium to PIN and also their deregulation should be retained in PCs (Fig. 3*A*). On the basis of this hypothesis of prostatic carcinogenesis, we identified 21 genes that were commonly up-regulated in PINs and PCs according to the criteria mentioned in Materials and Methods (Fig. 3*A*; Table 1); they included *AMACR*, *OR51E2*, *RODH* and *SMS*. On the other hand, 63 genes were selected as commonly down-regulated in PINs and PCs (Fig. 3*A*; Table 2); that list included several proteinases such as *SERPINB1, SERPING1*, and *MMP7*.

Identification of Genes Up- or Down-regulated during Malignant Transformation from PINs to Prostate Cancers. We then focused on differential expression patterns between PINs and PCs to search for genes likely to be involved in the transition from non-invasive precursor PINs to malignant cancers (Fig. 3B). Comparing the expression profiles of 20 PCs with those of 10 PINs, we identified 41 up-regulated genes (Table 3) and 98 down-regulated genes (Table 4). The list included *POV1*, *CDKN2C*, *EPHA4*, *APOD, FASN,* and *VWF* as up-regulated, and *ITGB2*, *LAMB2*, *PLAU*, and *TIMP1* as down-regulated; the altered genes might be involved with cell adhesion or motility in invasive PC cells.

To validate the differential expression pattern in the transition from PIN to PC, we performed immunohistochemical analysis of apolipoprotein D (APOD) and EPHA4, listed in Table 3 as up-regulated genes in the PIN-to-PC transition. In general, PC tissues include PC cells, PIN cells, and normal prostatic epithelium heterogeneously, and we compared the staining pattern of each kind of cells associated with prostatic carcinogenesis on the same tissues from the same patient. As shown in Fig. 3*C*, APOD was abundantly expressed in PC cells, whereas PINs and normal prostatic epithelium from the same patient had no expression of APOD protein. The EPHA4 protein was also strongly expressed in PC cells while PINs and normal prostatic epithelium from the same patient had no, or very weak, expression of EPHA4 protein.

Growth Suppression Mediated by EPHA4-specific siRNA in Prostate Cancer Cells. To investigate the growth or survival effect of *EPHA4* on PC cells, we knocked down their endogenous expression by mammalian vector-based RNA interference technique by using the PC cell line. The transfection of one of the siRNA-expressing vectors, 1313si, clearly reduced the endogenous expression of *EPHA4* (Fig. 4*A*). This knocking-down effect by the siRNA on

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Table 2 *Down-regulated genes from normal epithelium to prostate tumors (PINs and PCs)*

Accession no.	UniGene No. Hs.	Symbol	Name
Function known			
NM_005159	118127	*ACTC*	*Actin, α, cardiac muscle*
D00017	217493	*ANXA2*	*Annexin A2*
L20688	83656	*ARHGDIB*	*Rho GDP dissociation inhibitor (GDI) β*
AK027126	160786	*ASS*	*Argininosuccinate synthetase*
S67310	69771	*BF*	*B-factor, properdin*
AB004066	171825	*BHLHB2*	*Basic helix-loop-helix domain containing, class B, 2*
NM_001733	1279	*C1R*	*Complement component 1 r subcomponent*
K02765	284394	*C3*	*Complement component 3*
AF134640	7235	*CACNG3*	*Calcium channel, voltage-dependent, gamma subunit 3*
K01144	84298	*CD74*	*CD74 antigen*
X63629	2877	*CDH3*	*Cadherin 3, type 1, P-cadherin (placental)*
L02870	1640	*COL7A1*	*Collagen, type VII, α1 (epidermolysis bullosa, dystrophic, dominant and recessive)*
U16306	81800	*CSPG2*	*Chondroitin sulfate proteoglycan 2 (versican)*
L12579	147049	*CUTL1*	*Cut-like 1, CCAAT displacement protein (Drosophila)*
NM_002996	80420	*CX3CL1*	*Chemokine (C-X3-C motif) ligand 1*
AF245505	72157	*DKFZp564I1922*	*Adlican*
D83407	156007	*DSCR1L1*	*Down syndrome critical region gene 1-like 1*
AW002941	339283	*ERAP140*	*Endoplasmic reticulum associated protein 140 kDa*
J04162	176663	*FCGR3A*	*Fc fragment of IgG, low-affinity IIIa, receptor for (CD16)*
M87770	278581	*FGFR2*	*Fibroblast growth factor receptor 2*
L42176	8302	*FHL2*	*Four and a half LIM domains 2*
NM_001456	195464	*FLNA*	*Filamin A, α (actin binding protein 280)*
AW949747	169946	*GATA3*	*GATA binding protein 3*
M55543	171862	*GBP2*	*Guanylate binding protein 2, interferon-inducible*
NM_002081	2699	*GPC1*	*Glypican 1*
NM_002083	2704	*GPX2*	*Glutathione peroxidase 2 (gastrointestinal)*
NM_002084	386793	*GPX3*	*Glutathione peroxidase 3 (plasma)*
NM_000186	250651	*HF1*	*H factor 1 (complement)*
M15178	318720	*HLA-DRB4*	*Major histocompatibility complex, class II, DR β4*
AA490691	421136	*HOXD11*	*Homeo box D11*
S81914	76095	*IER3*	*Immediate early response 3*
J02770	36602	*IF*	*i factor (complement)*
M31159	77326	*IGFBP3*	*Insulin-like growth factor binding protein 3*
NM_002198	80645	*IRF1*	*Interferon regulatory factor 1*
X52186	85266	*ITGB4*	*Integrin, β4*
X03212	23881	*KRT7*	*Keratin 7*
U07643	105938	*LTF*	*Lactotransferrin*
Z68179	77667	*LY6E*	*Lymphocyte antigen 6 complex, locus E*
NM_005928	3745	*MFGE8*	*Milk fat globule-EGF factor 8 protein*
AW298180	2256	*MMP7*	*Matrix metalloproteinase 7 (matrilysin, uterine)*
J05581	89603	*MUC1*	*Mucin 1, transmembrane*
J02854	9615	*MYL9*	*Myosin, light polypeptide 9, regulatory*
M13692	572	*ORM1*	*Orosomucoid 1*
AF027208	112360	*PROML1*	*Prominin-like 1 (mouse)*
AL045876	430637	*PTGDS*	*Prostaglandin D2 synthase 21 kDa (brain)*
NM_003979	194691	*RAI3*	*Retinoic acid induced 3*
M93056	183583	*SERPINB1*	*Serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 1*
M13690	151242	*SERPING1*	*Serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), member 1, (angioedema, hereditary)*
U59299	90911	*SLC16A5*	*Solute carrier family 16 (monocarboxylic acid transporters), member 5*
AI423028	71622	*SMARCD3*	*SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 3*
BF514189	345728	*SOCS3*	*Suppressor of cytokine signaling 3*
U54831	75248	*TOP2B*	*Topoisomerase (DNA) IIβ 180 kDa*
X63187	2719	*WFDC2*	*WAP four-disulfide core domain 2*
AF122922	284122	*WIF1*	*WNT inhibitory factor 1*
Function unknown			
AA632025	444752		*ESTs*
AI027791	132296		*ESTs*
AI566720	380045		*Homo sapiens cDNA FLJ34528 fis, clone HLUNG2008066*
N58556	323053		*Homo sapiens mRNA full-length insert cDNA clone EUROIMAGE 26539.*
BE539165	355793	*DKFZp313M0720*	*Hypothetical protein DKFZp313M0720*
AB002319	8663	*KIAA0321*	*KIAA0321 protein*
T78873	9587	*KIAA2002*	*KIAA2002 protein*
AA180145	351270	*LOC152485*	*Hypothetical protein LOC152485*
AW888223	59384	*MGC3047*	*Hypothetical protein MGC3047*

Abbreviation: EST, expressed sequence tag.

EPHA4 mRNA resulted in drastic growth suppression in colony formation assay as well as in MTT assay (Fig. 4*B* and *C*). These findings strongly suggested that overexpression of *EPHA4* in PC cells was associated with the enhanced growth of cancer cells.

DISCUSSION

PC is one of the most common malignant diseases in Western countries. A number of research groups have investigated gene-expression profiles of PCs (13–16), but the results presented here are markedly different from theirs. Compared with a meta-analysis of four representative microarray datasets (17), only three (7.5%) of the 40 genes listed as being commonly up-regulated in PCs, and seven (17.5%) of the 40 down-regulated genes, coincided with our data. We attribute these discrepancies to the fact that prostate tumors are histopathologically very heterogeneous, containing a large proportion of stromal cells, and expression profiles from bulk tissues could be substantially influenced by contamination with surrounding noncan-

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Table 3 *Up-regulated genes in the transition from PIN to PC*

Accession no.	UniGene No. Hs.	Symbol	Name
Function known			
X12433	99364	*ABHD2*	*Abhydrolase domain containing 2*
AF039018	135281	*ALP*	*α-Actinin-2-associated LIM protein*
H61951	12152	*APMCF1*	*APMCF1 protein*
J02611	75736	*APOD*	*Apolipoprotein D*
AA633487	108708	*CAMKK2*	*Calcium/calmodulin-dependent protein kinase kinase 2, β*
AI357641	4854	*CDKN2C*	*Cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)*
NM_004938	153924	*DAPK1*	*Death-associated protein kinase 1*
NM_004405	419	*DLX2*	*Distal-less homeo box 2*
T78186	241565	*DNMT3A*	*DNA (cytosine-5-)-methyltransferase 3 α*
W94051	336678	*DTNA*	*Dystrobrevin, α*
L36645	73964	*EPHA4*	*EphA4*
M16967	30054	*F5*	*Coagulation factor V (proaccelerin, labile factor)*
U29344	83190	*FASN*	*Fatty acid synthase*
AF100143	6540	*FGF13*	*Fibroblast growth factor 13*
D14446	107	*FGL1*	*Fibrinogen-like 1*
BE747327	7644	*HIST1H1C*	*Histone 1, H1c*
BG163483	76907	*HSPC002*	*HSPC002 protein*
AF064493	4980	*LDB2*	*LIM domain binding 2*
U21128	79914	*LUM*	*Lumican*
U07620	151051	*MAPK10*	*Mitogen-activated protein kinase 10*
NM_002465	169849	*MYBPC1*	*Myosin binding protein C, slow type*
AI767296	123655	*NPR3*	*Natriuretic peptide receptor C/guanylate cyclase C*
X76770	49007	*PAPOLA*	*Poly(A) polymerase α*
AF045584	18910	*POV1*	*Prostate cancer overexpressed gene 1*
AI298501	21192	*SDK1*	*Sidekick homolog 1 (chicken)*
AB020335	181300	*SEL1L*	*sel-1 suppressor of lin-12-like (Caenorhabditis elegans)*
BE735822	75069	*SHMT2*	*Serine hydroxymethyltransferase 2 (mitochondrial)*
N21096	99291	*STXBP6*	*Syntaxin binding protein 6 (amisyn)*
AF091395	367689	*TRIO*	*Triple functional domain (PTPRF interacting)*
AK000235	31608	*TRPM4*	*Transient receptor potential cation channel, subfamily M, member 4*
NM_000552	110802	*VWF*	*von Willebrand factor*
Function unknown			
N66442	135971		*ESTs*
BE274422	380933		*Homo sapiens mRNA; cDNA DKFZp586O1224*
AI304487	171443		*Homo sapiens, clone IMAGE:3354344, mRNA, partial cds*
AA830405	403857		*Homo sapiens, clone IMAGE:5932767, mRNA*
D14657	81892	*KIAA0101*	*KIAA0101 gene product*
W63676	356547	*LOC129642*	*Hypothetical protein BC016005*
AW295100	5957	*LOC201562*	*Hypothetical protein LOC201562*
AL137707	103422	*LOC220115*	*Hypothetical protein LOC220115*
AI057614	293845	*LOC89944*	*Hypothetical protein BC008326*
AW972144	422113	*MGC30006*	*Hypothetical protein MGC30006*

Abbreviation: EST, expressed sequence tag.

cerous cells and by the proportions of various cell types. For example, genes that are highly expressed in stromal cells, but not in epithelial cells, may be selected as down-regulated elements in microarray analyses of bulk tissues, because stromal components are less abundant in cancer tissues than in normal prostate (~17 *versus* 60%; ref. 15). *SDF1* (stromal cell-derived factor 1), which is highly expressed in stroma, showed low signal (under the cutoff) in our experiments (data not shown). However, Stamey *et al.* (15) reported that gene as down-regulated in PCs, probably because stromal components were present in the normal control. Similarly, genes that are expressed in normal epithelial cells and cancer cells, but not in stromal cells, may be selected as up-regulated elements during microarray analysis of bulk tissue if noncancerous epithelial cells are enriched in the tumor tissue. For instance, *PRSS8* (prostasin) was reportedly up-regulated in many microarray studies that used bulk tissues (17), but in our experiments, expression of this gene was either unchanged or down-regulated in some PCs as compared with normal epithelium. *PRSS8* is predominantly expressed in normal prostatic epithelial cells; several studies using *in situ* techniques have revealed its down-regulation in PC cells (18, 19). These observations make clear that contamination of cancer tissues with stromal cells or normal prostate epithelium can mislead efforts to understand the molecular pathology of PC; therefore, microdissection is definitely required for precision in microarray analyses of PCs. In this study, we applied the LMM system (11) to purify populations of cancer cells, PIN cells, and normal prostatic epithelial cells from surgical specimens, in an effort to obviate influences from stromal components and normal prostate.

Several lines of clinical and pathological evidence have linked PINs to PCs, and high-grade PIN has been widely considered the precursor of invasive PC (8, 10). PINs and PCs can show certain genetic alterations in common (20). However, some data have argued against this hypothesis; for example, early-stage PCs are not always accompanied by PIN (9, 10) and some genetic markers of PINs are not always observed in PCs (21). Hence, considerable controversy has arisen about the natural history of high-grade PIN and the mechanism of PIN development, and the putative transition from PIN to invasive PC has remained unsettled.

We analyzed the precise molecular profiles of purified populations of PC and PIN cells to investigate the molecular mechanism of prostate carcinogenesis and to examine the molecular linkage between PINs and PCs. Our unsupervised hierarchical clustering analysis was able to distinguish most PCs from PINs on the basis of their expression profiles, although two low-grade PCs with Gleason scores of 5 fell in the PIN cluster and one high-grade PIN was classified into the PC group. The latter turned out to be a very-high-grade PIN in a retrospective histological review and probably represented a transitional lesion to invasive PC. These results implied that the gene-expression profile of very-high-grade PIN is very similar to that of PC, supporting the concept that at least some high-grade PINs are precursors of PC. It should also be added that prostate pathologists

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Table 4 *Down-regulated genes in the transition from PIN to PC*

Accession no.	UniGene No. Hs.	Symbol	Name
Function known			
AI827230	374481	*APCDD1*	*Adenomatosis polyposis coli down-regulated 1*
BF965257	74120	*APM2*	*Adipose specific 2*
AA156854	114309	*APOL1*	*Apolipoprotein L, 1*
NM_004024	460	*ATF3*	*Activating transcription factor 3*
M94345	82422	*CAPG*	*Capping protein (actin filament), gelsolin-like*
AF035752	139851	*CAV2*	*Caveolin 2*
D13639	75586	*CCND2*	*Cyclin D2*
M16445	89476	*CD2*	*CD2 antigen (p50), sheep red blood cell receptor*
AI750036	22116	*CDC14B*	*CDC14 cell division cycle 14 homolog B (Saccharomyces cerevisiae)*
AK021865	173380	*CKIP-1*	*CK2 interacting protein 1; HQ0024c protein*
X15880	108885	*COL6A1*	*Collagen, type VI, α1*
L16510	297939	*CTSB*	*Cathepsin B*
U03688	154654	*CYP1B1*	*Cytochrome P450, family 1, subfamily B, polypeptide 1*
M62401	82568	*CYP27A1*	*Cytochrome P450, family 27, subfamily A, polypeptide 1*
X90579	166079	*CYP3A5P2*	*Cytochrome P450, family 3, subfamily A, polypeptide 5 pseudogene 2*
X93920	180383	*DUSP6*	*Dual specificity phosphatase 6*
NM_001421	151139	*ELF4*	*E74-like factor 4 (ets domain transcription factor)*
AF275945	116651	*EVA1*	*Epithelial V-like antigen 1*
AW300770	61265	*FAM3D*	*Family with sequence similarity 3, member D*
D84239	111732	*FCGBP*	*Fc fragment of IgG binding protein*
AF182316	234680	*FER1L3*	*fer-1-like 3, myoferlin (C. elegans)*
NM_001924	80409	*GADD45A*	*Growth arrest and DNA-damage-inducible, α*
W91908	6079	*GALNAC4S-6ST*	*B cell RAG associated protein*
AA666119	92287	*GBP3*	*Guanylate binding protein 3*
NM_000165	74471	*GJA1*	*Gap junction protein, α1, 43 kDa (connexin 43)*
J03004	77269	*GNAI2*	*Guanine nucleotide binding protein (G protein), α inhibiting activity polypeptide 2*
J03817	301961	*GSTM1*	*Glutathione S-transferase M1*
M33906	198253	*HLA-DQA1*	*Major histocompatibility complex, class II, DQ α1*
NM_018950	110309	*HLA-F*	*Major histocompatibility complex, class I, F*
U26726	1376	*HSD11B2*	*Hydroxysteroid (11-β) dehydrogenase 2*
BF793633	180919	*ID2*	*Inhibitor of DNA binding 2, dominant negative helix-loop-helix protein*
AV646610	34853	*ID4*	*Inhibitor of DNA binding 4, dominant negative helix-loop-helix protein*
M15395	83968	*ITGB2*	*Integrin, β2*
U25138	93841	*KCNMB1*	*Potassium large conductance calcium-activated channel, subfamily M, β member 1*
AB012955	129867	*KIP2*	*DNA-dependent protein kinase catalytic subunit-interacting protein 2*
X72760	90291	*LAMB2*	*Laminin, β2 (laminin S)*
Y00711	234489	*LDHB*	*Lactate dehydrogenase B*
M36682	621	*LGALS3*	*Lectin, galactoside-binding, soluble, 3 (galectin 3)*
L13210	79339	*LGALS3BP*	*Lectin, galactoside-binding, soluble, 3 binding protein*
X03444	377973	*LMNA*	*Lamin A/C*
AA779709	7457	*MAGE-E1*	*MAGE-E1 protein*
L08895	78995	*MEF2C*	*MADS box transcription enhancer factor 2, polypeptide C*
AF017418	104105	*MEIS2*	*Meis1, myeloid ecotropic viral integration site 1 homolog 2 (mouse)*
AF203032	198760	*NEFH*	*Neurofilament, heavy polypeptide 200 kDa*
M12267	75485	*OAT*	*Ornithine aminotransferase (gyrate atrophy)*
AW051593	189999	*P2RY5*	*Purinergic receptor P2Y, G-protein coupled, 5*
BG028573	64056	*PAK1*	*p21/Cdc42/Rac1-activated kinase 1 (STE20 homolog, yeast)*
BF969355	8364	*PDK4*	*Pyruvate dehydrogenase kinase, isoenzyme 4*
AA253194	303125	*PIGPC1*	*p53-induced protein PIGPC1*
M22430	76422	*PLA2G2A*	*Phospholipase A2, group IIA (platelets, synovial fluid)*
D00244	77274	*PLAU*	*Plasminogen activator, urokinase*
X56134	297753	*RPLP2*	*Ribosomal protein, large P2*
W73992	132792	*SDCCAG43*	*Serologically defined colon cancer antigen 43*
AW965789	66450	*SENP1*	*Sentrin/SUMO-specific protease*
AF029082	184510	*SFN*	*Stratifin*
U44403	75367	*SLA*	*Src-like-adaptor*
AV705470	380991	*SNF1LK*	*SNF1-like kinase*
Y08110	101657	*SORL1*	*Sortilin-related receptor, L(DLR class) A repeats-containing*
BE439695	160483	*STOM*	*Stomatin*
AB042646	94785	*TGIF2*	*TGFB-induced factor 2 (TALE family homeobox)*
NM_003241	2387	*TGM4*	*Transglutaminase 4 (prostate)*
U21847	82173	*TIEG*	*TGFB inducible early growth response*
M12670	5831	*TIMP1*	*Tissue inhibitor of metalloproteinase 1*
AA837002	9741	*TJP4*	*Tight junction protein 4 (peripheral)*
M35252	84072	*TM4SF3*	*Transmembrane 4 superfamily member 3*
M19309	73980	*TNNT1*	*Troponin T1, skeletal, slow*
W72411	137569	*TP73L*	*Tumor protein p73-like*
H99016	171501	*USP11*	*Ubiquitin specific protease 11*
AF077197	74669	*VAMP5*	*Vesicle-associated membrane protein 5 (myobrevin)*
AW137980	115659	*VIK*	*vav-1 interacting Kruppel-like protein*
D88154	103665	*VILL*	*Villin-like*
M92843	343586	*ZFP36*	*Zinc finger protein 36, C3H type, homolog (mouse)*
BF055342	326801	*ZNF6*	*Zinc finger protein 6 (CMPX1)*
Function unknown			
AI769569	112472		*ESTs*
AW510657	156044		*ESTs*
BF111819	21470		*ESTs*
T79422	119237		*ESTs*
AI304862	12867		*ESTs*

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Table 4 *Continued*

Accession no.	UniGene No. Hs.	Symbol	Name
AA705222	119880		*ESTs*
AA768607	122926		*ESTs*
AI052358	131741		*ESTs*
AW888225	250723		*ESTs, Weakly similar to hypothetical protein FLJ20378 [Homo sapiens]*
BF223679	118747		*Homo sapiens cDNA FLJ33407 fis, clone BRACE2010535.*
AI821113	292781		*Homo sapiens cDNA FLJ36327 fis, clone THYMU2005748.*
AL360198	22870		*Homo sapiens mRNA insert cDNA clone EUROIMAGE 34988.*
AL050204	28540		*Homo sapiens mRNA; cDNA DKFZp586F1223 (from clone DKFZp586F1223)*
AV733210	367688		*Homo sapiens, clone IMAGE:4794726, mRNA*
U57961	181304	*13CDNA73*	*Hypothetical protein CG003*
AL050289	7446	*C6orf4*	*Chromosome 6 open reading frame 4*
AW956111	79404	*D4S234E*	*DNA segment on chromosome 4 (unique) 234 expressed sequence*
AK001021	22505	*FLJ10159*	*Hypothetical protein FLJ10159*
R43725	98927	*FLJ13993*	*Hypothetical protein FLJ13993*
D42047	82432	*KIAA0089*	*KIAA0089 protein*
NM_014766	75137	*KIAA0193*	*KIAA0193 gene product*
AA921341	3610	*KIAA0205*	*KIAA0205 gene product*
AB007903	113082	*KIAA0443*	*KIAA0443 gene product*
BF431643	15420	*KIAA1500*	*KIAA1500 protein*
AA706316	32343	*ZD52F10*	*Hypothetical gene ZD52F10*

Abbreviation: EST, expressed sequence tag.

have long recognized a phenomenon in which PC spreads along prostatic ducts to mimic PIN (22). In fact, an early controversy that arose when the concept of PIN was first introduced was that the lesions merely reflected intraductal spread of invasive cancer. Except for the one PIN case, our microarray data comparing PIN cells with PC cells rejects this criticism and clearly supports the concept that PINs are precursor lesions of PC, not ductal spread of PC cells.

We identified genes that were up- or down-regulated (Tables 1 and 2) commonly in PINs and PCs comparing with normal epithelium; those genes may be involved in an early step of prostatic carcinogenesis. The up-regulated elements in PINs and PCs included *AMACR* (*α-methyl acyl-CoA racemase*), *OR51E2* (*olfactory receptor, family 51, subfamily E, member 2*), *RDOH* (*3-hydroxysteroid epimerase*), and *SMS* (*spermine synthase*). *AMACR* has a key role in β-oxidation of dietary branched-chain fatty acids and is often overexpressed in PCs (23, 24). *OR51E2* is also overexpressed in PCs (25), but its function is unknown. *RDOH* functions in androgen catabolism and is likely to be associated with androgen-dependent prostate tumorigenesis. *SMS* is a polyamine-biosynthesis enzyme; polyamines such as spermine have been implicated in the growth of PC cells and protection from apoptosis (26). On the other hand, *SERPIN-B1* [*serine (or cysteine) proteinase inhibitor, clade B* (*ovalbumin*) *member 1*] and *SERPIN-G1* [*serine* (*or cysteine*) *proteinase inhibitor, clade G* (*ovalbumin*), *member 1*] were down-regulated from normal prostate epithelium to PIN. The pigment epithelium-derived factor encoded by *SERPIN-F1* was recently reported to be a key inhibitor of stromal vasculature and growth of epithelial tissue in mouse prostate (27); in pigment epithelium-derived factor-deficient mice, stromal vessels were increased and associated with epithelial-cell hyperplasia. Those observations suggest to us that *SERPIN-B1* and *SERPIN-G1* may also be key regulators of prostate growth. Almost all genes that were up- or down-regulated in PINs were also up- or down-regulated, respectively, in PCs compared with normal prostate epithelium, implying that PCs retain the molecular features of their putative precursor PINs. These data supported the notion of PIN-to-PC transition.

Next, we focused on genes showing differential expression after the transition from PINs to PCs (see Fig. 3*B*; Tables 3 and 4). The list included *POV1*, *CDKN2C*, *EPHA4*, *APOD*, and *FAM* as up-regulated genes and *LAMB2*, *ITGB2*, *PLAU*, and *TIMP1* as down-regulated genes. *EPHA4* is one of the receptor tyrosine kinase receptors and is likely to play a critical role in neuronal circuit development and angiogenesis by regulating cell shape and motility (28). However, EPHA4 overexpression in PCs is novel and may involve PC cell viability and motility. Some of the latter are associated with cell adhesion and proteinase activity, suggesting that their expression changes may contribute to the invasive phenotype by abolishing ductal structures during the transition from PIN to PC. We validated the differential expression of APOD and EPHA4 by the immunohistochemical analysis on several PC tissues (Fig. 3*C*), which was consistent with our precise RNA profiles and indicated that our profile analysis was highly reliable.

Furthermore, we investigated by the small interfering RNA strategy whether overexpression of EPHA4 was associated with enhanced growth of PC cells. As shown in Fig. 4, down-regulation of *EPHA4* in PC cell line resulted in a drastic reduction in PC cell viability, suggesting that the overexpression of EPHA4 in the transition of PIN to PC cells is likely to be essential in PC cell growth, indicating that targeting to EPHA4 may be a promising approach to develop novel PC treatments.

Overall, it is interesting that our lists of differentially expressed genes in prostate carcinogenesis via PINs to invasive PCs included many genes associated with lipid metabolism, including *AMACR*, *FASN* (*fatty acid synthase*), *MFGE8* (*milk fat globule-EGF factor 8 protein*), *APOD* (*apolipoprotein D*), *APOL1* (*apolipoprotein L1*), *PLA2G2A* (*phospholipase A2, group IIA*), and *SORL1* (*sortilin-related receptor L*). Epidemiological aspects suggest that the development of PC is strongly associated with high fat intake (1); our data concerning these genes may help to explain the apparent association between fat intake and prostate carcinogenesis.

In conclusion, our extensive list of genes derived from precise expression profiles of PCs and PINs should provide useful information for identifying molecular targets for the prevention and treatment of PC, as well as for understanding the molecular mechanism of prostatic carcinogenesis, especially the transition from PIN to PC.

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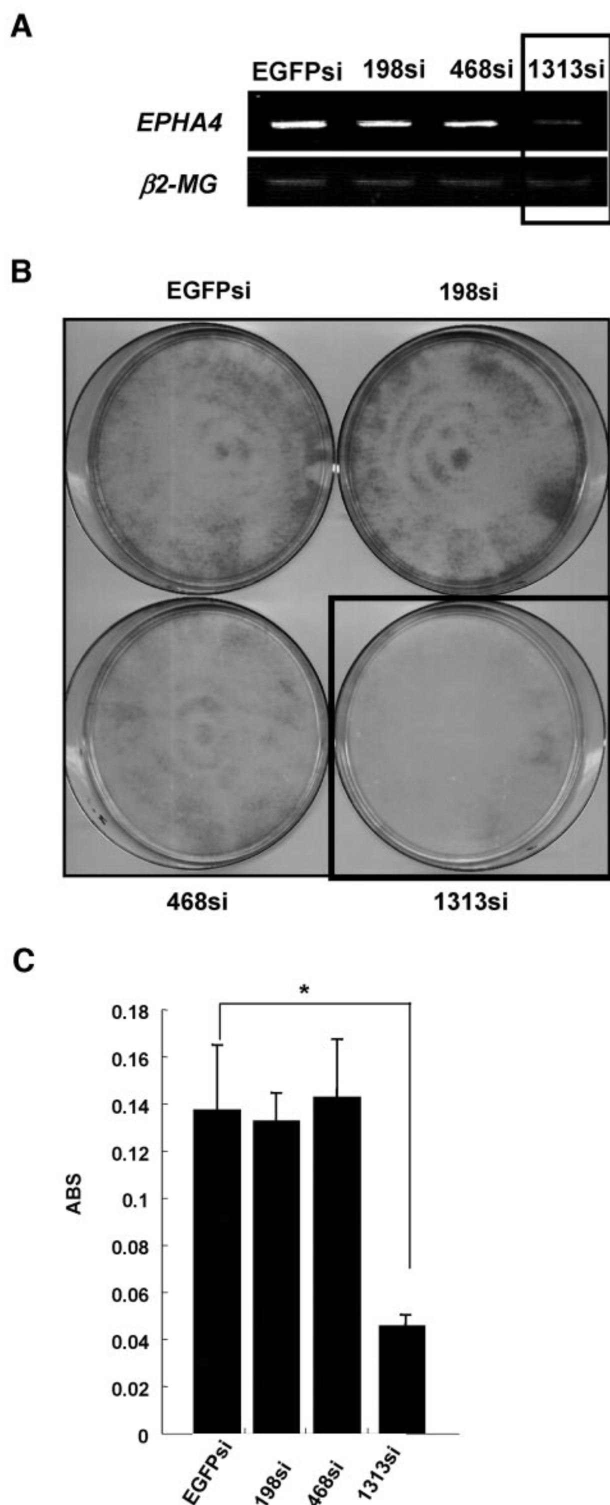

Fig. 4. Knocking-down effect of *EPHA4* in PC cell line by siRNA. In *A*, RT-PCR experiments indicate knockdown effect of *EPHA4* mRNA by transfection of siRNA expression vectors 1313si, but not 198si, 468si1, or EGFPsi. Each of the sequences of 198si, 468si, and 1313si corresponded to a part of the *EPHA4* sequence, and that of EGFPsi corresponded to a part of the *EGFP* sequence. Cells were harvested 48 hours after transfection and analyzed. *β2-MG* was used as the quantitative standard. In *B*, colony formation assay showed a drastic decrease of colony numbers in PC cells 1 week after transfection with 1313si. In *C*, MTT assay also showed the drastic decrease of the number of PC cells transfected with 1313si, but not with 198si, 468si1, or EGFPsi. *, *P* 0.01. (ABS, antibodies)

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REFERENCES

1. Gronberg H. Prostate cancer epidemiology. Lancet 2003;361:859–64.
2. Han M, Partin AW, Piantadosi S, Epstein JI, Walsh PC. Era specific biochemical recurrence-free survival following radical prostatectomy for clinically localized prostate cancer. J Urol 2001;166:416–9.
3. Roberts SG, Blute ML, Bergstralh EJ, Slezak JM, Zincke H. PSA doubling time as a predictor of clinical progression after biochemical failure following radical prostatectomy for prostate cancer. Mayo Clin Proc 2001;76:576–81.
4. Roberts WW, Bergstralh EJ, Blute ML, et al. Contemporary identification of patients at high risk of early prostate cancer recurrence after radical retropubic prostatectomy. Urology 2001;57:1033–7.
5. Abate-Shen C, Shen MM. Molecular genetics of prostate cancer. Gene Dev 2000; 14:2410–34.
6. Di Cristofano A, De Acetis M, Koff A, Cordon-Cardo C, Pandolfi PP. Pten and p27KIP1 cooperate in prostate cancer tumor suppression in the mouse. Nat Genet 2001;27:222–4.
7. McNeal JE, Bostwick DG. Intraductal dysplasia: a premalignant lesion of the prostate. Hum Pathol 1986;17:64–71.
8. DeMarzo AM, Nelson WG, Isaacs WB, Epstein JI. Pathological and molecular aspects of prostate cancer. Lancet 2003;361:955–64.
9. Sakr WA, Haas GP, Cassin BF, Pontes JE, Crissman JD. The frequency of carcinoma and intraepithelial neoplasia of the prostate in young male patients. J Urol 1993;150: 379–85.
10. Montironi R, Mazzucchelli R, Scarpelli M. Precancerous lesions and conditions of the prostate: from morphological and biological characterization to chemoprevention. Ann N Y Acad Sci 2002;963:169–84.
11. Nakamura T, Furukawa Y, Nakagawa H, et al. Genome-wide cDNA microarray analysis of gene expression profiles in pancreatic cancers using populations of tumor cells and normal ductal epithelial cells selected for purity by laser microdissection. Oncogene 2004;23:2385–400.
12. Ono K, Tanaka T, Tsunoda T, et al. Alterations of gene expression during colorectal carcinogenesis revealed by cDNA microarrays after laser-capture microdissection of tumor tissues and normal epithelia. Cancer Res 2000;60:5007–11.
13. Dhanasekaran SM, Barrette TR, Ghosh D, et al. Delineation of prognostic biomarkers in prostate cancer. Nature (Lond) 2001;412:822–6.
14. Magee JA, Araki T, Patil S, et al. Expression profiling reveals hepsin overexpression in prostate cancer. Cancer Res 2001;61:5692–6.
15. Stamey TA, Warrington JA, Caldwell MC, et al. Molecular genetic profiling of Gleason grade 4/5 prostate cancers compared to benign prostatic hyperplasia. J Urol 2001;166:2171–7.
16. Welsh JB, Sapinoso LM, Su AI, et al. Analysis of gene expression identifies candidate markers and pharmacological targets in prostate cancer. Cancer Res 2001;61:5974–8.
17. Rhodes DR, Barrette TR, Rubin MA, Ghosh D, Chinnaiyan AM. Meta-analysis of microarrays: interstudy validation of gene expression profiles reveals pathway dysregulation in prostate cancer. Cancer Res 2002;62:4427–33.
18. Chen LM, Hodge GB, Guarda LA, Welch JL, Greenberg NM, Chai KX. Down-regulation of prostasin serine protease: a potential invasion suppressor in prostate cancer. Prostate 2001;48:93–103.
19. Takahashi S, Suzuki S, Inaguma S, et al. Down-regulated expression of prostasin in high-grade or hormone-refractory human prostate cancers. Prostate 2003;54: 187–93.
20. Qian J, Jenkins RB, Bostwick DG. Genetic and chromosomal alterations in prostatic intraepithelial neoplasia and carcinoma detected by fluorescence in situ hybridization. Eur Urol 1999;35:479–83.
21. Emmert-Buck MR, Vocke CD, Pozzatti RO, et al. Allelic loss on chromosome 8p12–21 in microdissected prostatic intraepithelial neoplasia. Cancer Res 1995;55: 2959–62.
22. Kovi J, Jackson MA, Heshmat MY. Ductal spread in prostatic carcinoma. Cancer (Phila) 1985;56:1566–73.
23. Rubin MA, Zhou M, Dhanasekaran SM, et al. α-Methylacyl coenzyme A racemase as a tissue biomarker for prostate cancer. JAMA 2002;287:1662–70.
24. Luo J, Zha S, Gage WR, et al. α-Methylacyl-CoA racemase: a new molecular marker for prostate cancer. Cancer Res 2002;62:2220–6.
25. Xia C, Ma W, Wang F, Hua S, Liu M. Identification of a prostate-specific G-protein coupled receptor in prostate cancer. Oncogene 2001;20:5903–7.
26. Thomas T, Thomas TJ. Polyamines in cell growth and cell death: molecular mechanisms and therapeutic applications. Cell Mol Life Sci 2001;58:244–58.
27. Doll JA, Stellmach VM, Bouck NP, et al. Pigment epithelium-derived factor regulates the vasculature and mass of the prostate and pancreas. Nat Med 2003;9:774–80.
28. Kullander K, Klein R. Mechanisms and functions of Eph and ephrin signaling. Nat Rev Mol Cell Biol 2002;3:475–86.

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