Characteristic gene expression profiles of benign prostatic hypertrophy and prostate cancer

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Abstract. The molecular mechanism playing a role in the development of benign prostate hypertrophy (BPH) and prostate cancer (PC) is not well defined. We performed microarray analysis to assess the gene expression change in BPH and PC, and performed network analysis. Normal prostate, BPH and PC tissues were obtained from patients who underwent an operation at Chiba University Hospital. Using Affymetrix Human Genome U133 Plus2.0 Array, we identified genes differentially expressed. The identified genes were analyzed using the Ingenuity Pathway Analysis (IPA) to investigate the functional network and gene ontology. The microarray analysis identified 402 genes in BPH and 141 genes in PC, which were up- or down-regulated at least 5.0-fold change in PC at all dose points. Analysis using IPA software revealed eight networks in BPH and five networks in PC. We narrowed these down to the top five genes, which were up- or down-regulated on the networks in their characteristic manner. From this new perspective, comparing BPH and PC in microarray studies, our data showing gene expression profiles provide candidate genes for better understanding of disease and new therapeutic targets.

Introduction

Benign prostatic hypertrophy (BPH) is the most common benign neoplasm among aging men. BPH is a disease characterized by prostatic enlargement, and the prevalence of histologically identifiable BPH is >50% for 60-year-old men and ~90% by age 85 years (1). The development of BPH is multifactorial, but two of the primary conditions required are the effect of androgens and aging (2,3).

Prostate cancer (PC) is the most common cancer among men in Western countries (4), and the second leading death in men (5). The clinical course of this cancer shows a great variability due to its biological heterogeneity (6).

Serum PSA concentration is useful for PC screening, but it cannot differentiate accurately between early-stage PC and non-cancer such as BPH, prostatitis, and so on (7). With the current suggested PSA threshold of 4 ng/ml ~15.2% of PCs are missed (8), while conversely only 20-25% have positive histological findings of PC (9,10). Therefore, numerous studies of novel biomarkers for PC are carried out, but there is no conclusive biomarker yet. Many of the molecular-biological studies are focusing on features relating to bone metastasis and androgen dependence.

In this study, we performed microarray analysis using RNAs isolated from BPH and PC tissues to assess the gene expression change in BPH and PC. Further, the genes identified were analyzed for network and gene ontology by Ingenuity Pathway Analysis (IPA) software (Ingenuity Systems, Mountain View, CA) to identify networks of interacting genes, other functional groups, and corresponding canonical pathways. In addition, comparing between the genes of BPH and PC identified, we narrowed down the candidate genes that are characteristic for BPH and PC.

Materials and methods

Normal prostate, prostate cancer and BPH tissues. Tissue samples were obtained from patients who underwent their operation at Chiba University Hospital. Normal prostate tissue sample (n=1) was obtained from total cystectomy for bladder cancer, and BPH tissues (n=2) were from transurethral prostatectomy and suprapubic prostatectomy for BPH, and PC tissues (n=3) were obtained from radical prostatectomy for PC. Pathological evaluation of these tissues was confirmed. Freshly acquired tissues were stored immediately at -80˚C until use.

Isolation of RNA. Total RNA was extracted with TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA) from BPH and PC tissues according to the manufacturer's

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Key words: benign prostate hypertrophy, prostate cancer, microarray analysis, network analysis

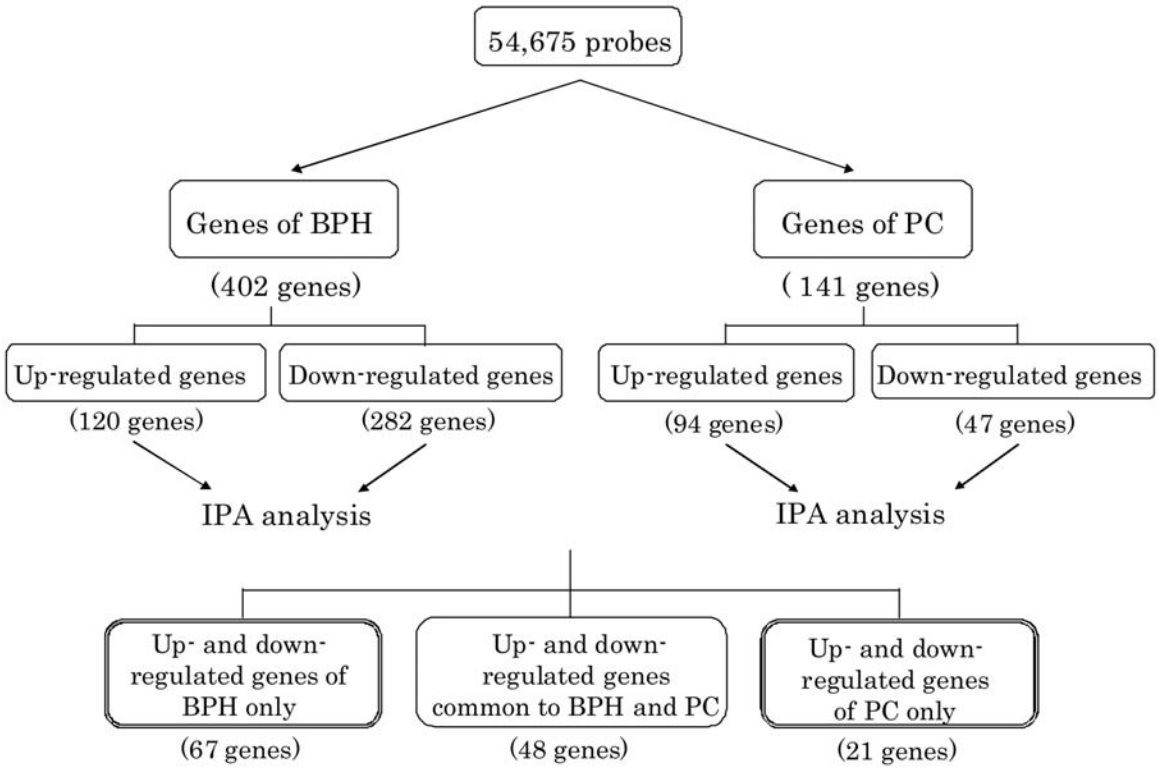

Figure 1. Flowchart of BPH or PC genes. The gene expression levels were compared with the fold-changes of normal prostate and BPH or PC tissues.

instructions. The quality of total RNA was determined by Bioanalyzer (Agilent Technologies, Palo Alto, CA).

Hybridization of RNAs to oligonucleotide arrays and data analysis. We used Human Genome U133 Plus2.0 Array (Affymetrix). This gene chip, containing 54,675 probe sets, analyzes the expression level of over 47,000 transcripts and variants, including 38,500 well-characterized human genes. For hybridization, 1 μg of total RNA per sample was prepared according to the manufacturer's protocols (Affymetrix). Fragmented cRNA (15 μg) was hybridized to the Human Genome U133 Plus2.0 Array. The arrays were stained with phycoerythrin-streptavidin and the signal intensity was amplified by treatment with a biotin-conjugated anti-streptavidin antibody, followed by a second staining using phycoerythrin-streptavidin. The arrays stained a second time were scanned using the Affymetrix GeneChip Scanner 3000.

GeneChip analysis was performed based on the Affymetrix GeneChip Manual (Affymetrix Inc.) with GeneChip-operating software (Affymetrix Inc.). All genes on the GeneChip were globally summarized and normalized. The GeneChip-operating software used Wilcoxon's test to generate detected (present or absent) calls and used the calls to statistically determine if a transcript was expressed or not. After being filtered through a 'present' call ($P<0.05$), the expression data were analyzed using GeneSpring 7.3.1 (Silicon Genetics, Redwood City, CA). Fold changes were calculated by comparing transcripts between normal prostate and BPH or PC tissues. We identified 402 genes in BPH and 141 genes in PC tissues differentially expressed by ≥5.0-fold.

Genetic network analysis and canonical pathway analysis. The genes, which were identified by microarray analyses, were used for network and gene ontology analysis. Gene accession numbers were imported into the IPA software. Using this software, the genes were categorized based on biochemical, biologic, and molecular functions. The identified genes were also mapped to genetic networks available in the Ingenuity database and then ranked by score, which is the probability that a collection of genes equal to or greater than the number in a network could be achieved by chance alone. A score of ≥3 had a 99.9% confidence level of not being generated by random chance alone. This score was used as the cut-off for identifying gene networks. Moreover, relationships between the network generated in IPA and the known pathways that were associated with metabolism and signaling were investigated by canonical pathway analysis.

Results

cDNA microarray analysis. The gene expression profiles of BPH and PC tissues were analyzed using the high-throughput gene chip. We sought to identify genes consistently expressed in BPH and PC tissues. A total of 120 genes were over-expressed ≥5.0-fold in BPH, while 282 genes were down-regulated ≥5.0-fold change in BPH at all dose points (Fig. 1). A total of 94 genes were overexpressed ≥5.0-fold in PC, while 47 genes were down-regulated ≥5.0-fold in PC at all dose points (Fig. 1).

Genetic network and canonical pathway analyses. We then investigated whether the 402 genes of BPH or the 141 genes

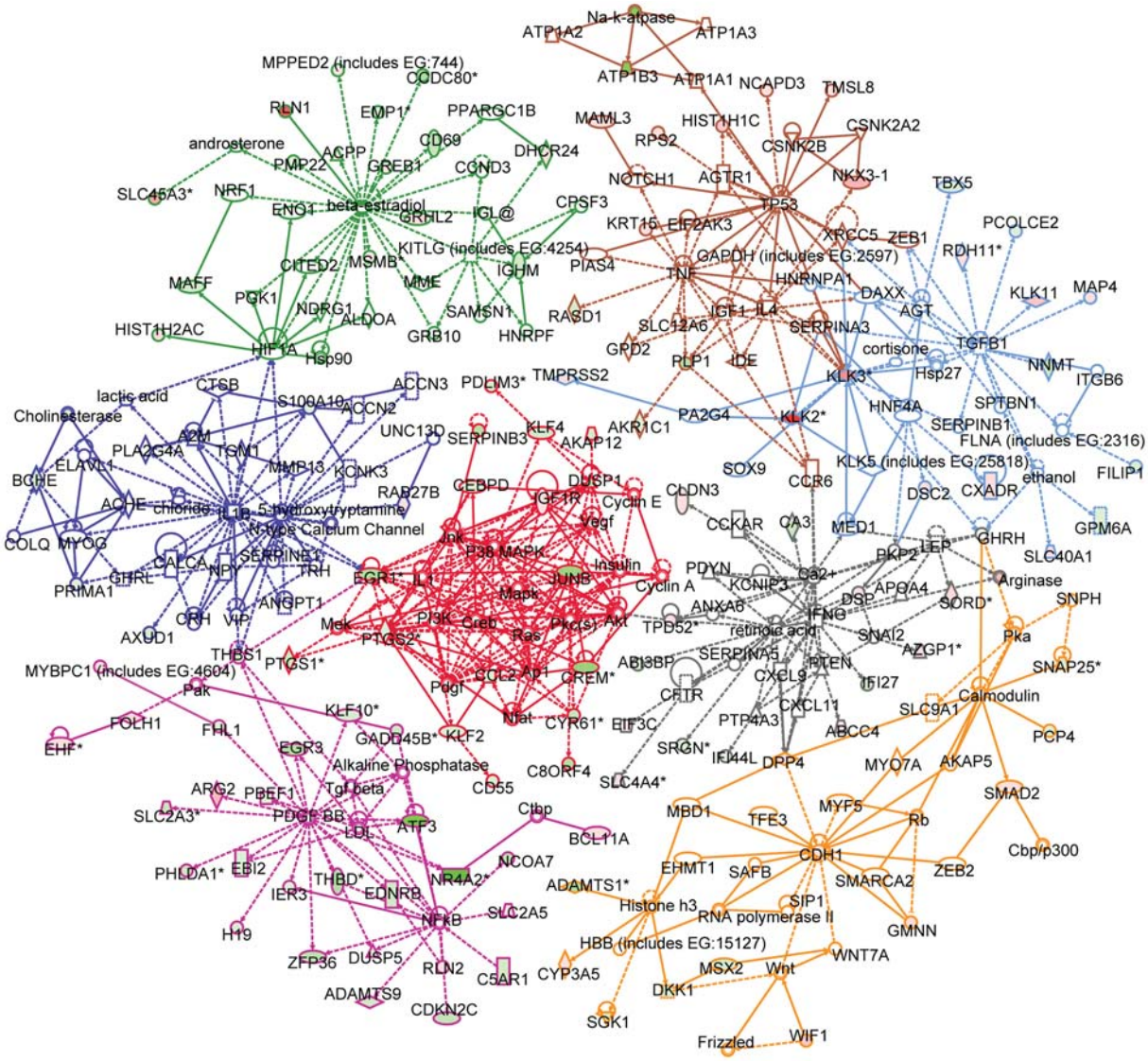

Figure 2. Networks of genes of BPH. IPA software was used to analyze the identified genes (n=115). Eight networks were identified by overlapping genes (purple, network1; red, network2; green, network3; aqua, network4; gray, network5; orange, network6; brown, network7; blue, network8). The intensity of node color indicates the degree of overexpression (red), and the degree of down-regulation (green). Genes in uncolored notes were not identified as differentially expressed in our experiment and were integrated into the computationally generated networks on the basis of the evidence stored in the IPA knowledge memory indicating a relevance to this network. The node shapes denote cytokines (▭), enzymes (◇), G-protein coupled receptors (▯), growth factors (▭), ion channels (▯), kinases (▽), nuclear receptors (▭), peptidases (◁▷), phosphates (△), transcription factors (⬭), translation factors (⬭), transmembrane receptors (⬯), transporters (▱), and others (⬭).

of PC interacted biologically. Genetic network analysis of these genes was performed using the IPA tool. Among these genes, we discovered 115 genes of BPH and 69 genes of PC that mapped to networks which had at least one gene in common and were merged for display (Figs. 2 and 3). The 115 genes of BPH were mapped to eight genetic networks, and the 69 genes of PC were mapped to five genetic networks. These networks indicated functional relationships between gene products based on known interactions in the literature. The results of the IPA tool are shown in Tables I and II. The results of canonical pathway analysis are shown in Tables III and IV. In this study, it revealed that hepatic fibrosis/hepatic stellate cell activation was most important in BPH, and folate biosynthesis was most important in PC.

Characteristic genes differentially expressed in BPH and PC. We selected the top five genes which were up-regulated or down-regulated in BPH or PC only (Fig. 1). The list of top five genes are shown in Table V. These genes are considered candidate genes to characterize BPH or PC.

Discussion

Many molecular methods have been used in the search to determine the mechanism of development of PC, and to find new diagnostic and prognostic markers (11,12). Our study demonstrated candidate genes both up- and down-regulated. To assess gene expression profiles of BPH and PC we compared them with each other, we narrowed down the candidate genes which characterize the disease. We next summarized up- and down-regulated genes in BPH and PC.

The top five genes up-regulated in BPH. TMSL8 is a newly discovered 5300-Da protein that binds actin monomers and

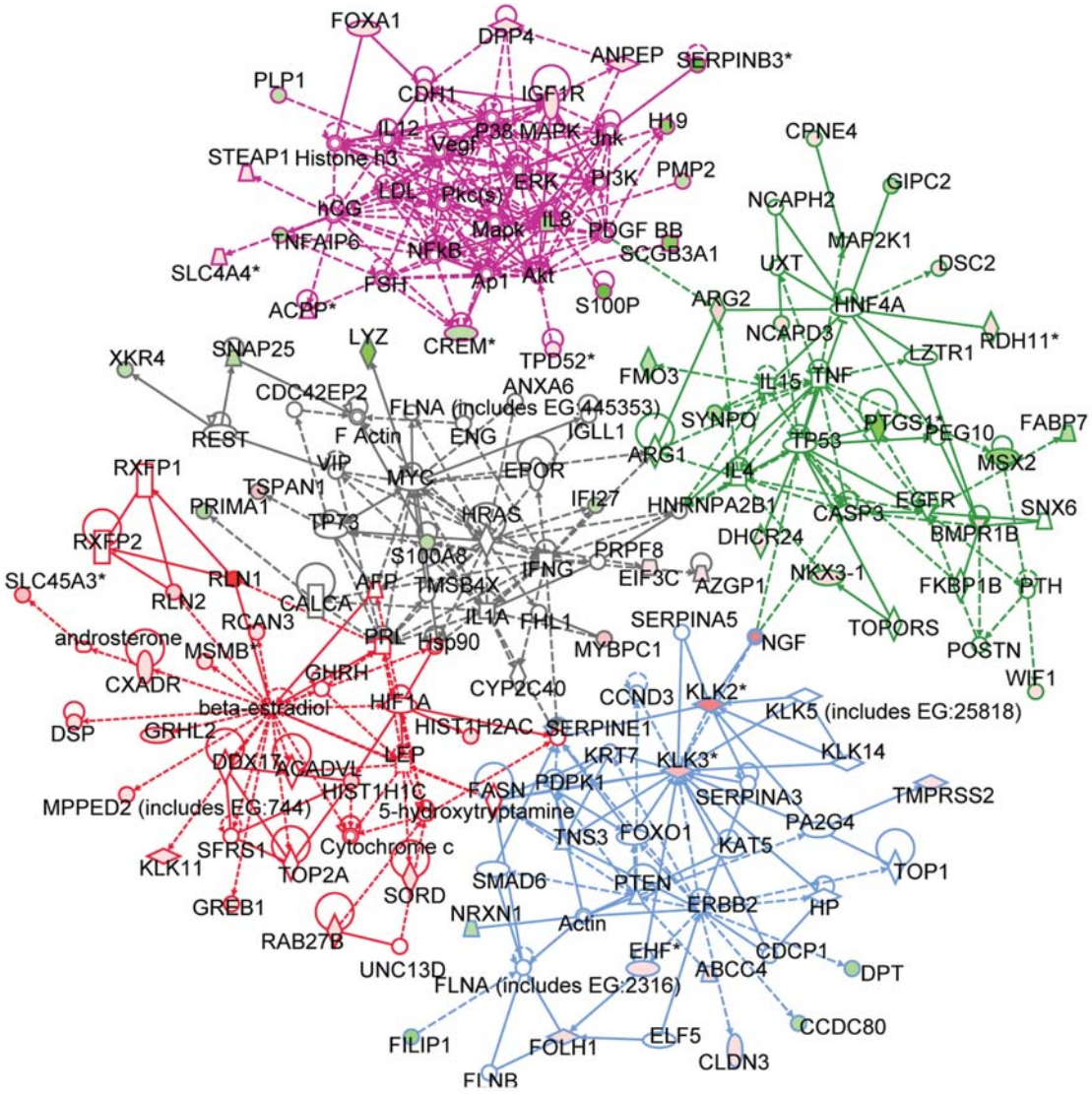

Figure 3. Networks of genes of PC. IPA software was used to analyze the identified genes (n=69). Five networks were identified by overlapping genes (purple, network1; red, network2; green, network3; aqua, network4; gray, network5). The intensity of node color indicates the degree of overexpression (red), and the degree of down-regulation (green). Genes in uncolored notes were not identified as differentially expressed in our experiment and were integrated into the computationally generated networks on the basis of the evidence stored in the IPA knowledge memory indicating a relevance to this network. The node shapes denote cytokines (▭), enzymes (◇), G-protein coupled receptors (▯), growth factors (▭), ion channels (▯), kinases (▽), nuclear receptors (▭), peptidases (⌓), phosphates (△), transcription factors (⬭), translation factors (⬭), transmembrane receptors (⬯), transporters (▱), and others (⬭).

inhibits actin polymerization and might thus increase cellular motility. Thymosin ß15 levels increase in human prostate cancer, correlating positively with Gleason tumor grade (13). But in relation to BPH, we could find no report.

CYP3A5 catalyzes 6ß-hydroxylation of testosterone, producing a metabolite, 6ß-hydroxytesterone, which is less biologically and more readily eliminated, thus inhibiting the metabolism of testosterone to more biologically active forms of androgens. CYP3A5 is highly expressed in normal prostate cells but absent in prostate cancer (14). It is considered that endogenous function of CYP3A5 is related to the metabolism of intra-prostatic androgens and cell growth, and that polymorphisms affecting CYP3A5 activity may result in altered PC risk and aggressiveness. But no relationships to BPH remain unknown.

SMAD2 protein is a signal transducer and transcriptional modulator that mediates multiple signaling pathways. This protein mediates the signal of the TGF-ß, and thus regulates multiple cellular processes, such as cell proliferation, apoptosis, and differentiation. In BPH tissues, TGF-ß expression and secretion is increased (15). TGF-ß1 is produced by basal cells that also express both corresponding receptors (16). While low concentrations support proliferation, high TGF-ß1 concentrations have been shown to inhibit prostate stromal cell growth (17). Prostatic fibroblasts undergo transdifferentiation into myofibroblasts/smooth muscle cells (SMCs) upon stimulation with TGF-ß1 (18,19). Thus, TGF-ß1 is believed to generate a 'reactive stroma' composed of myofibroblasts and fibroblasts that strongly express extracellular matrix components (20). TGF-ß1 treatment of *in vitro* cultures of human prostatic stromal cells induces overexpression of SMC-α-actin calponin and tenascin, known markers for myofibroblasts/SMCs in prostate stroma. Recently, a detailed profiling of TGF-ß1 target genes was performed on human

Table I. Genetic networks in BPH tissues.

Network	Gene	Top functions	Score[a]
1	ADAMTS9, Alkaline phosphatase, ARG2, ATF3, BCL11A, C5AR1, CDKN2C, Ctbp, DUSP5, EBI2, EDNRB, EGR3, EHF, FHL1, FOLH1, GADD45B, H19, IER3, KLF10, LDL, MYBPC1 (includes EG:4604), NCOA7, NF-κB, NR4A2, Pak, PBEF1, PDGF BB, PHLDA1, RLN2, SLC2A3, SLC2A5, Tgf beta, THBD, THBS1, ZFP36	Connective tissue disorders, immunological disease, inflammatory disease	58
2	AKAP12, Akt, Ap1, C8ORF4, CCL2, CD55, CEBPD, Creb, CREM, Cyclin A, Cyclin E, CYR61, DUSP1, EGR1, IGF1R, IL1, Insulin, Jnk, JUNB, KLF2, KLF4, Mapk, Mek, Nfat, P38 MAPK, Pdgf, PDLIM3, PI3K, Pkc(s), PTGS1, PTGS2, Ras, SERPINB3, TPD52, Vegf	Cellular growth and proliferation, cell cycle, cancer	32
3	ACPP, ALDOA, androsterone, beta-estradiol, CCDC80, CCND3, CD69, CITED2, CPSF3, DHCR24, EMP1, ENO1, GRB10, GREB1, GRHL2, HIF1A, HIST1H2AC, HNRPF, Hsp90, IGHM, IGL@, KITLG (includes EG:4254), MAFF, MME, MPPED2 (includes EG:744), MSMB, NDRG1, NRF1, PGK1 PMP22, PPARGC1B, RLN1, SAMSN1, SLC45A3	Connective tissue disorders, inflammatory disease, skeletal and muscular disorders	25
4	AGT, cortisone, CXADR, DAXX, DSC2, ethanol, FILIP1, FLNA (includes EG:2316), GHRH, GPM6A, HNF4A, HNRNPA1, Hsp27, ITGB6, KLK2, KLK3, KLK11, KLK5 (includes EG:25818), MAP4, MED1, NNMT, PA2G4, PCOLCE2, PKP2, RDH11, SERPINA3, SERPINB1, SLC40A1, SOX9, SPTBN1, TBX5, TGFB1, TMPRSS2, XRCC5, ZEB1	Cancer, cellular growth proliferation, gene expression	23
5	ABCC4, ABI3BP, ANXA6, APOA4, Arginase, AZGP1, CA3, Ca^{2+}, CCKAR, CCR6, CFTR, CLDN3, CXCL9, CXCL11, DPP4, DSP, EIF3C, GHRH, IFI27, IFI44L, IFNG, KCNIP3, LEP, MED1, PDYN, PKP2, PTEN, PTP4A3, retinoic acid, SERPINA5, SLC4A4, SNAI2, SORD, SRGN, TPD52	Cellular movement, cell signaling, molecular transport	23
6	ADAMTS1, AKAP5, Calmodulin, Cbp/p300, CDH1, CYP3A5, DKK1, DPP4, EHMT1, Frizzled, GHRH, GMNN, HBB (includes EG:15127), Histone h3, MBD1, MSX2, MYF5, MYO7A, PCP4, Pka, Rb, RNA polymerase II, SAFB, SGK1, SIP1, SLC9A1, SMAD2, SMARCA2, SNAP25, SNPH, TFE3, WIF1, Wnt, WNT7A, ZEB2	Embryonic development organismal development cell morphology	19
7	AGTR1, AKR1C1, ATP1A1, ATP1A2, ATP1A3, ATP1B3, CCR6, CSNK2A2, CSNK2B, DAXX, EIF2AK3, GAPDH (includes EG:2597), GPD2, HIST1H1C, HNRNPA1, IDE, IGF1, IL4, KLK3, KRT15, MAML3, Na-k-atpase, NCAPD3, NKX3-1, NOTCH1, PIAS4, PLP1, RASD1, RPS2, SERPINA3, SLC12A6, TMSL8, TNF, TP53, XRCC5	Cell death, nervous development and function, cell cycle	19
8	5-hydroxytryptamine, A2M, ACCN2, ACCN3, ACHE, ANGPT1, AXUD1, BCHE, CALCA, chloride, Cholinesterase, COLQ, CRH, CTSB, EGR1, ELAVL1, GHRL, HIF1A, IL1B, KCNK3, lactic acid, MMP13, MYOG, N-type calcium channel, NPY, PLA2G4A, PRIMA1, RAB27B, S100A10, SERPINE1, TGM1, THBS1, TRH, UNC13D, VIP	Cellular growth and proliferation, tissue development, tissue morphology	9

Underlining indicates genes identified by microarray analysis; other genes were or were not in the expression array or did not change significantly. [a]A score >3 is considered significant.

Table II. Genetic networks in PC tissues.

Network	Gene	Top functions	Score[a]
1	ACPP, Akt, ANPEP, Ap1, ARG2, CDH1, CREM, DPP4, ERK, FOXA1, FSH, H19, hCG, Histone h3, IGF1R, IL8, IL12, Jnk, LDL, Mapk, NF-κB, P38 MAPK, PDGF BB, PI3K, Pkc(s), PLP1, PMP2, S100P, SCGB3A1, SERPINB3, SLC4A4, STEAP1, TNFAIP6, TPD52, Vegf	Cancer, cellular growth and proliferation, reproductive system disease	40
2	5-hydroxytryptamine, ACADVL, AFP, androsterone, beta-estradiol, CXADR, Cytochrome c, DDX17, DSP, FASN, GHRH, GREB1, GRHL2, HIF1A, HIST1H1C, HIST1H2AC, Hsp90, KLK11, LEP, MPPED2 (includes EG:744), MSMB, PRL, RAB27B, RCAN3, RLN1, RLN2, RXFP1, RXFP2, SERPINE1, SFRS1, SLC45A3, SORD, TOP2A, UNC13D	Reproductive system development and function, tissue morphology, cell signaling	29
3	ARG1, ARG2, BMPR1B, CASP3, CPNE4, DHCR24, DSC2, EGFR, FABP7, FKBP1B, FMO3, GIPC2, HNF4A, HNRNPA2B1, IL4, IL15, LZTR1, MAP2K1, MSX2, NCAPD3, NCAPH2, NGF, NKX3-1, PEG10, POSTN, PTGS1, PTH, RDH11, SNX6, SYNPO, TNF, TOPORS, TP53, UXT, WIF1	Drug metabolism, lipid metabolism, small molecule biochemistry	29
4	ABCC4, Actin, CCDC80, CCND3, CDCP1, CLDN3, DPT, EHF, ELF5, ERBB2, FASN, FILIP1, FLNA (includes EG:2316), FLNB, FOLH1, FOXO1, HP, KAT5, KLK2, KLK3, KLK14, KLK5 (includes EG:25818), KRT7, NGF, NRXN1, PA2G4, PDPK1, PTEN, SERPINA3, SERPINA5, SERPINE1, SMAD6, TMPRSS2, TNS3, TOP1	Cancer, reproductive system disease, cellular movement	20
5	AFP, ANXA6, ARG1, AZGP1, CALCA, CDC42EP2, CYP2C40, EIF3C, ENG, EPOR, F Actin, FHL1, FLNA (includes EG:445353), HNRNPA2B1, HRAS, Hsp90, IFI27, IFNG, IGLL1, IL1A, LYZ, MYBPC1, MYC, PRIMA1, PRL, PRPF8, REST, S100A8, SERPINE1, SNAP25, TMSB4X, TP73, TSPAN1, VIP, XKR4	Cellular growth and proliferation, cellular movement, cell signaling	17

Underlining indicates genes identified by microarray analysis; other genes were or were not in the expression array or did not change significantly. [a]A score > 3 is considered significant.

prostate fibroblasts to identify important genes/factors involved in the transdifferentiation process into myofibroblasts/SMCs. These investigations indicated elevation of a variety of factors influencing extracellular matrix production and secretion, similar to the *in vivo* situation in the stroma of BPH patients (21). Our canonical pathway analysis revealed that this gene possibly is one of the key genes in BPH.

SLC2A5, also known as GLUT5, is a high-affinity fructose transporter. SLC2A5 expression levels and fructose uptake rates are significantly affected by diabetes, hypertension, obesity, and inflammation and seem to be induced during carcinogenesis, particularly in the mammary glands (22). However, no genetic relatedness to prostate is reported.

MAML3 is one of the evolutionarily conserved elements of Notch signaling (23). It also has no relationship to BPH, even to prostate.

The top five genes down-regulated in BPH. EGR1 is a transcription factor, and associated with the inducible transcription of the heparanase gene. Heparanase degrades heparin sulfate and has been implicated to tumor invasion and metastasis. There is a report that indicates increasing heparanase expression in PC tissues is due to promoter hypomethylation and up-regulation of transcription factor EGR1 (24). In this study, mRNA transcripts of EGR1 were inversely lower in BPH samples than in PC samples. Our microarray analysis shows a comparable result.

JUNB, one of the Jun proteins, is a core member of the activator protein-1 (AP-1), a dimeric transcription factor complex consisting of homo- and heterodimers of the Jun, Fos, activating transcription factor (ATF) and musculoaponeurotic fibrosarcoma (Maf) families (25). Growth factors, hormones and a variety of environmental stress activates mitogen activated protein kinase (MAPK) cascades that enhance Jun/AP-1 activity, e.g., through phosphorylation thereby regulating cell proliferation, differentiation, transformation and/or apoptosis. In controlling prostate carcinogenesis, JUNB plays an important role and may be a new target for cancer prevention and therapy (26). The role of JUNB in BPH development and progression is unknown.

Table III. Canonical pathway of BPH genes.

Canonical pathway	-log (P-value)	Molecules
Hepatic fibrosis/hepatic stellate cell activation	1.47E+00	CCL2, EDNRB, IGF1R, SMAD2
Complement system	1.36E+00	C5AR1, CD55
Nicotinate and nicotinamide metabolism	1.20E+00	NNMT, NAMPT, SGK1
Eicosanoid signaling	8.88E-01	PTGS2, PTGS1
LXR/RXR activation	8.66E-01	CCL2, ARG2
Arachidonic acid metabolism	8.64E-01	CYP3A5, PTGS2, PTGS1
Folate biosynthesis	8.00E-01	FOLH1
VDR/RXR activation	7.40E-01	KLF4, THBD
Riboflavin metabolism	7.36E-01	ACPP
cAMP-mediated signaling	7.03E-01	DUSP1, AKAP12, CREM
C21-steroid hormone metabolism	6.99E-01	AKR1C1
IGF-1 signaling	6.75E-01	IGF1R, CYR61
Wnt/ß-catenin signaling	6.70E-01	DKK1, WIF1, CDH1
p53 signaling	6.60E-01	THBS1, GADD45B
Urea cycle and metabolism of amino groups	5.69E-01	ARG2
Phenylalanine metabolism	5.46E-01	DHCR24
Coagulation system	5.24E-01	THBD
Notch signaling	4.95E-01	MAML3
Bile acid biosynthesis	4.68E-01	AKR1C1
Nitrogen metabolism	4.51E-01	CA3
Huntington's disease signaling	4.46E-01	SNAP25, IGF1R, SGK1
Retinol metabolism	4.43E-01	RDH11
Tryptophan metabolism	4.14E-01	CYP3A5, DHCR24
Metabolism of xenobiotics by cytochrome P450	4.06E-01	AKR1C1, CYP3A5
Fructose and mannose metabolism	3.93E-01	SORD
IL-10 signaling	3.51E-01	ARG2
Glucocorticoid receptor signaling	3.49E-01	CCL2, DUSP1, SMAD2
Propanoate metabolism	3.41E-01	DHCR24
PXR/RXR activation	3.02E-01	CYP3A5
NRF2-mediated oxidative stress response	2.89E-01	MAFF, JUNB
Chemokine signaling	2.89E-01	CCL2
Arginine and proline metabolism	2.89E-01	ARG2
TGF-ß signaling	2.65E-01	SMAD2
Androgen and estrogen metabolism	2.51E-01	AKR1C1
G-protein coupled receptor signaling	2.50E-01	DUSP1, EDNRB
LPS/IL-1 mediated inhibition of RXR function	2.48E-01	CYP3A5, ABCC4
TR/RXR activation	2.44E-01	AKR1C1
Linoleic acid metabolism	2.22E-01	CYP3A5
p38 MAPK signaling	2.13E-01	DUSP1

ATP1B3, one of the Na^+ -K^+-ATPase enzymes, is vital in skeletal muscle function (27). Na^+/K^+-ATPase is an integral membrane protein responsible for establishing and maintaining the electrochemical gradients of Na and K ions across the plasma membrane. No relationship to prostate is reported.

ATF3 is a member of the mammalian activation transcription factor/cAMP responsive element-binding (CREB) protein family of transcription factors (28). ATF3 is rapidly up-regulated under various stress conditions including hepatotoxicity, UV/ionizing radiation, exposure to DNA damaging agents, ischemia and hypoxia (29-32). It was shown that ATF3 overexpression promoted invasiveness of prostate tumor cells *in vitro* and significantly enhanced spontaneous lung metastasis without affecting primary

Table IV. Canonical pathway of PC genes.

Canonical pathway	-log (P-value)	Molecules
Folate biosynthesis	1.06E+00	FOLH1
IL-6 signaling	1.04E+00	IL8, TNFAIP6
LPS/IL-1 mediated inhibition of RXR function	1.02E+00	FMO3, FABP7, ABCC4
Riboflavin metabolism	9.63E-01	ACPP
Huntington's disease signaling	9.18E-01	IGF1R, KLK2, SNAP25
Hepatic fibrosis/hepatic stellate cell activation	7.93E-01	IL8, IGF1R
Urea cycle and metabolism of amino groups	7.86E-01	ARG2
Phenylalanine metabolism	7.61E-01	DHCR24
NF-κB signaling	7.17E-01	BMPR1B, KLK2
Glutamate metabolism	7.16E-01	FMO3
Retinol metabolism	6.41E-01	RDH11
Wnt/ß-catenin signaling	6.39E-01	CDH1, WIF1
IL-8 signaling	6.08E-01	IL8, CDH1
Fructose and mannose metabolism	5.94E-01	SORD
Aminosugars metabolism	5.94E-01	FMO3
TREM1 signaling	5.52E-01	IL8
Propanoate metabolism	5.34E-01	DHCR24
IL-10 signaling	5.28E-01	ARG2
Eicosanoid signaling	5.22E-01	PTGS1
Neurotrophin/TRK signaling	5.10E-01	KLK2
Glutathione metabolism	4.94E-01	ANPEP
LXR/RXR activation	4.88E-01	ARG2
Arginine and proline metabolism	4.73E-01	ARG2
BMP signaling pathway	4.59E-01	BMPR1B
TGF-ß signaling	4.45E-01	BMPR1B
FXR/RXR activation	4.11E-01	FOXA1
IGF-1 signaling	4.07E-01	IGF1R
PTEN signaling	4.03E-01	BMPR1B
Arachidonic acid metabolism	2.86E-01	PTGS1
Tryptophan metabolism	2.74E-01	DHCR24
Hepatic cholestasis	2.72E-01	IL8
Synaptic long term depression	2.64E-01	IGF1R
cAMP-mediated signaling	2.32E-01	CREM
Tight junction signaling	2.31E-01	CLDN3
RAR activation	2.09E-01	RDH11
Calcium signaling	2.06E-01	RCAN3

tumorigenicity in a severe combined immunodeficient mouse model (33).

NR4A2 encodes a member of the steroid-thyroid hormone-retinoid receptor superfamily, and the encoded protein may act as a transcription factor (34). Mutations in this gene have been associated with disorders related to dopaminergic dysfunction, including Parkinson disease, schizophrenia, and manic depression. No relationship to BPH or PC was reported.

The top five genes up-regulated in PC. The protein encoded by TSPAN1 is a member of the transmembrane 4 superfamily, also known as the tetraspanin family. Most of these members are cell-surface proteins that are characterized by the presence of four hydrophobic domains. The proteins mediate signal transduction events that play a role in the regulation of cell development, activation, growth and motility. There are reports that TSPAN1 was frequently expressed in HCC and the peritumor tissue (35), and strongly expressed in cervical intraepithelial neoplasm and cervical cancer tissue (36).

BMPR1B encodes a member of the bone morphogenetic protein (BMP) receptor family of transmembrane serine/threonine kinases. The ligands of this receptor are BMPs,

Table V. Top five genes of up-regulated or down-regulated in BPH only or PC only.

	BPH only	PC only
1	TMSL8	TSPAN1
2	CYP3A5	BMPR1B
3	SMAD2	FOXA1
4	SLC2A5	STEAP1
5	MAML3	RCAN3
:	:	:
5	EGR1	DPT
4	JUNB	IL8
3	ATP1B3	SCGB3A1
2	ATF3	LYZ
1	NR4A2	S100P

which are members of the TGF-ß superfamily. BMPs have been shown to be important during development and in the regulation of various cellular processes such as migration, proliferation, and differentiation of many different cell types, including neuroepithelial and endothelial cells (37,38). Several lines of evidence have suggested that BMP signals play important roles in the generation and progression of prostate cancers. It is considered that human prostate cancer cells frequently exhibit loss of expression of BMPRs and suggest that loss of BMPRs may play an important role during the progression of prostate cancer (39,40), but details were not given.

FOXA1 encodes a member of the forkhead class of DNA-binding proteins. These hepatocyte nuclear factors are transcriptional activators for liver-specific transcripts such as albumin and transthyretin, and they also interact with chromatin. It has been shown to be expressed in the epithelia of the prostate gland, and has been determined to regulate the transcription of prostate-specific genes (41,42). Moreover, it plays a pivotal role in controlling prostate morphogenesis and cell differentiation (43). FOXA1 interacts with the androgen receptor to regulate prostate and epididymal genes differentially, and possibly is a co-repressor of the androgen receptor (44,45). The role of FOXA1 proteins in prostate cancer development and progression is unknown.

STEAP1 is predominantly expressed in prostate tissue and is found to be up-regulated in multiple cancer cell lines. The gene product is predicted to be a six-transmembrane protein, and was shown to be a cell surface antigen significantly expressed at cell-cell junctions. Biochemical and secondary structure analyses suggested that this protein could function as a channel, receptor, or transporter protein; however, its function is currently unknown (46).

RCAN3 is a five-exon gene mapped on chromosome 1 and belongs to the human RCAN gene family. The efforts to determine Calcineurin endogenous inhibitors have led to the identification of several proteins (47), including the members of the RCAN family. Calcineurin is a key enzyme that links Ca^{2+} extracellular signals to transcriptional activation of many target genes, but its detailed function was not revealed.

The top five genes down-regulated in PC. DPT is an extracellular matrix protein with possible functions in cell-matrix interactions and matrix assembly (48-50). The protein is found in various tissues and many of its tyrosine residues are sulphated. DPT is postulated to modify the behavior of TGF-ß through interaction with decorin (51). *In vitro* experiment using the PC-3 cell line, indicated that DPT may be involved in the pathogenesis and growth of prostate cancer (52), but details were not shown.

IL-8 is a CXC chemokine, the overexpression of which is associated with the angiogenesis, tumorigenicity and lymph node metastasis of androgen-independent prostate cancer (AIPC) in athymic nude mice (53,54). Elevated serum levels of IL-8 have been reported in patients with localized disease and AIPC (55,56). IL-8 may have a potential role in promoting the progression of the disease to the androgen-independent state. However, our data showed that IL-8 was down-regulated in PC. In canonical pathway analysis, however, this molecule was important, so we think that this gene plays an important role.

SCGB3A1 encoding a small, secreted protein is silenced due to methylation in a substantial fraction of breast, prostate, lung, and pancreatic carcinomas, suggesting a potential tumor suppressor function (57,58). It is reported that SCGB3A1 expression is down-regulated in the majority of breast, lung, prostate, pancreatic, and nasopharyngeal cancers, this down-regulation is associated with hypermethylation of the SCGB3A1 promoter (59-62). Silencing of SCGB3A1 expression by methylation is considered an early and frequent event in a number of cancers, and along with the *in vitro* data on growth inhibition, suggests that SCGB3A1 is a candidate tumor suppressor gene.

LYZ encodes the human lysozyme, whose natural substrate is the bacterial cell wall peptidoglycan. Lysozyme is one of the anti-microbial agents found in human milk, and is also present in spleen, lung, kidney, white blood cells, plasma, saliva, and tears.

The S100P protein was originally isolated from placenta and is one of the largest sub-families of the 20 human EF-hand calcium binding proteins (63). S100 gene family members have several functions including regulation of cytoskeletal interactions, protein phosphorylation, transcriptional control and Ca^{2+} homeostasis. Members of this gene family have been implicated in various disease processes and are considered as possible targets for development of clinical diagnostics and therapy. Studies on prostate cancer have indicated that S100P expression is regulated by androgens (64). A large number of evidence exists concerning the structure and expression of S100P but little is known about its function in general, or in prostate cancer.

The results obtained in network analyses in BPH and PC were completely different. In PC networks, functions of 'cancer' and 'cellular growth and proliferation' were mainly occupied as expected, and existed in BPH also. But in BPH, function of 'connective tissue disorders' was found in BPH

networks, and was not found in PC. This result reflects histological features of not only muscle or gland tissue cells, but also connective tissue cell growth was recognizable in BPH tissues. Function of 'reproductive system disease', 'reproductive system development and function' and 'drug metabolism' were found in PC, not in BPH. This difference may provide some clues to unravel causes for BPH and PC.

Our data comparison between BPH and PC provide new gene expression profiles and candidate genes for better understanding of diseases and new therapeutic targets. It should be kept in mind that there are certain limits to *in silico* analysis. We considered that several combined genomic studies such as real-time RT-PCR analysis and immunostaining using multitude of patient samples and so on, are necessary for determining whether these genes play important roles in diseases.

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