Differentially Expressed Genes in Two LNCaP Prostate Cancer Cell Lines Reflecting Changes during Prostate Cancer Progression

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SUMMARY: Prostate cancer tends to become transformed to androgen-independent disease over time when treated by androgen-deprivation therapy. We used two variants of the human prostate cancer cell line LNCaP to study gene expression differences during prostate cancer progression to androgen-independent disease. Production of prostate-specific antigen was regarded as a marker of androgen-dependence and loss of prostate-specific antigen was regarded as a marker of androgen-independence. mRNA from both cell lines was used for cDNA microarray screening. Differential expression of several genes was confirmed by Northern blotting. Monoamine oxidase A, an Expressed Sequence Tag (EST) similar to rat P044, and EST AA412049 were highly overexpressed in androgen-dependent LNCaP cells. Tissue-type plasminogen activator, interferon-inducible protein p78 (MxB), an EST similar to galectin-1, follistatin, fatty acid-binding protein 5, EST AA609749, annexin I, the interferon-inducible gene 1–8U, and phospholipase D1 were highly overexpressed in androgen-independent LNCaP cells. All studied genes had low or no expression in PC-3 cells. The EST similar to rat P044, the EST similar to galectin-1, follistatin, annexin I, and the interferon-inducible gene 1–8U were also expressed in benign prostatic hyperplasia tissue. The Y-linked ribosomal protein S4, Mat-8, and EST AA307912 were highly expressed in benign prostatic hyperplasia tissue. Additionally, both confirmation of differential expression in Northern blots and in situ hybridization were carried out for monoamine oxidase A, the EST similar to rat P044, the EST similar to galectin-1, fatty acid-binding protein 5, and the interferon-inducible gene 1–8U. We identified several potential prostate cancer markers, indicating that the method used is a useful tool for the screening of cancer markers, but other methods, such as in situ hybridization, are needed to further investigate the observations. (*Lab Invest 2000, 80:1259–1268*).

*P*rostate cancer is the most commonly diagnosed cancer among men in Western industrialized countries. The growth of prostate cancer is usually androgen-dependent at the beginning, but tends to become transformed to androgen-independent disease over time when treated by androgen-deprivation therapy (Schroder, 1998). The transition from hormone-dependent to hormone-independent tumorigenesis is believed to be a consequence of multiple genetic alterations. This multistep process is thought to include a cascade of genetic alterations caused by activation of oncogenes and/or inactivation of tumor suppressor genes (Fearon and Vogelstein, 1990; Knudson, 1993). A widely used in vitro model of prostate cancer is the LNCaP cell line (Horoszewicz et al, 1980). Androgens are essential for growth of LNCaP cells, unless they have become transformed to androgen-independent clones (van Steen-

brugge et al, 1991). Androgen-dependent and androgen-independent LNCaP cell lines have previously been used for studying differentially expressed genes in the respective stages of prostate carcinoma. B cell translocation gene 1, the UDP glucuronosyl transferase gene 2B15, and two unknown genes are differentially expressed (Chang et al, 1997). The cell lines used expressed both prostate-specific antigen (PSA) and prostatic acid phosphatase and contained androgen receptors (although one cell line was androgen-independent). Differential expression of fibronectin, E2 ubiquitin-conjugating enzyme, metalloproteinase-related collagenase, and breast basic conserved gene was shown in another androgen-independent LNCaP cell line in a study by Stubbs et al, 1999. Recently, differential display analysis was successfully used for identification of a cDNA (DD3) with prostate cancer–specific expression (Bussemakers et al, 1999).

To study gene expression changes during prostate cancer progression, we used two LNCaP cell line variants. Loss of PSA production in LNCaP cells was regarded as a marker of androgen-independent growth of the cells. The non–PSA-producing LNCaP cell line (LNCaP−) served as a model of progressive prostate cancer, whereas the PSA-producing LNCaP

cell line (LNCaP+) served as a model of well-differentiated prostate cancer. The aim of this study was to identify differentially expressed genes in LNCaP+/− cells using cDNA array technology. With this technology, the expression of several thousand genes can be observed in single hybridization screening. Thus, this technology is a powerful tool in cancer research with potential for the identification of new cancer markers. Several genes with prominent expression in one of the cell line variants were analyzed by Northern blot hybridization in the LNCaP+/−, PC-3, and DU-145 cell lines and in benign prostatic hyperplasia (BPH) tissue, and some promising markers were investigated by in situ hybridization (ISH).

Results

Overview of the Screening Results

In gene expression microarray analysis, 7075 human cDNAs were screened. Among genes expressed at high levels in both LNCaP cell lines there were several genes known to take part in the regulation of cell growth, apoptosis, or oncogene action. These genes are presented in Table 1. Many genes were expressed at low level: signal values below 200 (range: 76 to 199) were detected for 2013 genes. Of these genes, 1252 (62%) were EST (Expressed Sequence Tags) with no homology to known sequences; 207 (10%) were EST with similarity to some known sequences; and 554 (28%) were known clones different from EST.

Differentially Expressed Genes

The genes expressed most prominently in LNCaP+ and LNCaP− cells are presented in Tables 2 and 3, respectively. Androgen receptor was expressed in LNCaP+ cells with a signal value of 2941, 7.9 times overexpressed in these cells (Table 2). The respective value in LNCaP− cells was 373, indicating the presence of androgen receptor transcripts in these cells as well.

In LNCaP+ cells, the genes for four serine proteases, PSA (Accession number (AC): M24543), prostate-specific glandular kallikrein (hK2, AC: S39329), TMPRSS2 (AC:

Table 1. Oncogenes, Genes Related to Regulation of Oncogene Action, Cell Growth or Apoptosis Expressed at High Level in Both LNCaP+ and LNCaP− Cells

Signal Value in LNCaP+	Signal Value in LNCaP−	Gene Name	Accession
8489	7057	RAB7, member of RAS oncogene-like family 1	D84488
6675	5689	Synovial sarcoma, translocated to X chromosome	X79201
4631	7110	RAN, member RAS oncogene family	AF052578
5527	5198	CASP8 and FADD-like apoptosis regulator	AF015450
6742	3934	Human mRNA for hepatoma-derived growth factor	AI720570
6871	3324	Myeloid leukemia factor 2	AF070539
5463	4643	v-crk avian sarcoma virus CT10 oncogene homolog	D10656
3905	4692	RAB interacting factor	U74324
2437	5575	non-metastatic cells 1, protein (NM23A)	AA399275
3754	3801	Mutated in colorectal cancers	M62397
4115	3391	Proliferation-associated 2G4, 38kD	U59435
2404	4699	Proliferation-associated gene A	X67951
3473	3468	Teratocarcinoma-derived growth factor 1	M96955
3380	3368	Caspase 10, apoptosis-related cysteine protease	U60519
3800	2869	tumor protein D52	U18914
3676	2991	v-myc avian myelocytomatosis viral oncogene homolog	V00568
3474	3144	mouse double minute 4; p53-binding protein	AF007111
3368	3207	ras homolog gene family, member G (rho G)	X61587
4221	2316	G1 to S phase transition 1	U95742
2735	3684	Defender against cell death 1	U84214
3260	3132	Caspase 6, apoptosis-related cysteine protease	U20536
2099	4244	Human transformation-sensitive protein	M86752
2285	3977	SHC (Src homology 2 domain-containing) transforming protein 1	X68148
2682	3247	v-raf-1 murine leukemia viral oncogene homolog 1	X03484
3193	2646	Proliferating cell nuclear antigen	AI631255
3074	2710	Neuronal apoptosis inhibitory protein	U80017
2922	2620	Forkhead (Drosophila) homolog (rhabdomyosarcoma)-like 1	AF032886
2583	2622	Ewing sarcoma breakpoint region 1	Y07848
2031	2374	v-crk avian sarcoma virus CT10 oncogene homolog-like	X59656
2014	2011	RAB13, member RAS oncogene family	X75593

Signal values (arbitrary units), gene identification, and accession number are presented in descending order of total signal values.

Table 2. Genes Overexpressed in LNCaP+ Cells

Overexpression	Signal Value in LNCaP+	Signal Value in LNCaP−	Gene Name	Accession
105.1	26165	249	Kallikrein 3 (prostate specific antigen, PSA)	M24543
49.3	20147	409	Kallikrein 2, prostatic (hK2)	S39329
22.9	3342	146	Transmembrane protease, serine 2 (TMPRSS2)	U75329
20.8	7984	384	Monoamine oxidase A (MAOA)	M69226
15.6	4962	319	Myosin VI (MYO6)	AB002387
14.7	1838	125	Epithelial V-like antigen	AI819274
13.4	2526	188	Desmoplakin (DPI, DPII)	M77830
12.6	2148	171	EST	AA412049
12.2	3010	247	Phospholemman-like (Mat-8)	AA826766
11.8	1325	112	EST	N51335
11.3	4239	376	Catenin alpha 1 (102 kD)	AF102803
10.6	8086	764	Ribosomal protein S4, Y-linked (RPS4Y)	AI929331
9.9	2835	287	Selenium-binding protein 1	U29091
8.7	5864	675	Protein phosphatase 3 catalytic subunit (PPP3CA)	AA504039
8.6	1063	124	EST	AA781244
8.5	2550	299	Protein kinase, cAMP-dependent, catalytic, beta	M34181
8.5	2266	268	Cyclin-dependent kinase inhibitor 1A (p21, Cip1)	L25610
8.3	5213	629	Creatine kinase, mitochondrial 1	J04469
8.1	2181	269	3-oxoacid CoA transferase	U62961
7.9	2941	373	Androgen receptor	M27430
7.7	1333	174	Islet cell autoantigen 1 (69 kD)	U26591
7.0	1379	198	T-cell receptor, gamma cluster	M16768
6.8	1347	197	H. sapiens gene from PAC 295C6, similar to rat P044	AI92351
6.8	1652	243	Hexosaminidase A (alpha polypeptide)	S62076
6.4	7972	1241	Caldesmon 1	M64110
6.3	2177	343	EST	AI536671
5.9	874	148	EST	AA641953
5.7	1102	194	Kallikrein 1, renal/pancreas/salivary	M12706

Overexpression, signal values (arbitrary units) in LNCaP+/−, gene identification and respective accession number are presented in descending order of overexpression.

U75329), and kallikrein 1 (AC: M12706), were among the thirty most overexpressed genes, whereas in LNCaP− cells there was only one serine protease, trypsin 3 (AC: X71345), among the ninety most overexpressed genes. Furthermore, genes for three transmembrane proteins, TM-PRSS2, Mat-8 (AC: AA826766) and nma (AC: U23070), were among the ninety most overexpressed genes in LNCaP+ cells. In LNCaP− cells, there were several interferon-inducible genes that were overexpressed. Among the ninety most overexpressed genes were cDNAs for 2′-5′ oligoadenylate synthetase 2 (OAS2, AC: M87284), MxA (AC: M30817), interferon-inducible protein 17 (AC: AA428847), a 56 kDa protein (AC: X03557), interferon-α–inducible protein 27 (AC: AA302123), interferon-γ–inducible protein 16 (AC: S75433), MxB (AC: M33883), and the interferon-inducible gene 1–8U (AC: X57352). Protein phosphatase 3 and myosin VI were differentially expressed in the whole array, but they were expressed at comparable levels in both LNCaP+ and LNCaP− cells according to the results of Northern blotting (data not shown).

Analysis of Overexpressed Genes in LNCaP+ Cells

The Y-linked ribosomal protein S4 was expressed strongly in BPH tissue, was expressed at lower levels in LNCaP+, and was hardly detectable in PC-3 or LNCaP− cells (Table 4, Fig. 1a). Mono-amine oxidase A (MAOA) was expressed in LNCaP+ cells and at lower levels in BPH tissue and LNCaP− cells (Table 4, Fig. 1a), and was not seen in PC-3 cells. ISH showed the localization of MAOA transcripts in both benign and malignant epithelium (Fig. 2, a and b). Mat-8 was expressed in BPH tissue and in LNCaP+ cells, whereas transcripts encoding Mat-8 were absent in PC-3 and LNCaP− cells (Table 4, Fig. 1a). An mRNA for an EST similar to rat P044 was most prominently expressed in LNCaP+ cells and BPH tissue, and was weakly expressed in LNCaP− cells (Table 4, Fig. 1a). It was expressed at a low level in ISH, but was detectable in benign and malignant epithelia (Fig. 2, c and d). ISH showed that EST AA781244 was expressed at similar levels in benign and malignant epithelia (Fig. 2, e and f). EST AA781244 was weakly expressed in LNCaP+ cells and BPH tissue in Northern blots (Table 4). The gene encoding EST AA412049 was expressed only in LNCaP+ cells in Northern blots (Table 4), but in ISH it was detectable in both benign and malignant epithelia (Fig. 2, g and h).

Table 3. Genes Overexpressed in LNCaP− Cells

Overexpression	Signal Value in LNCaP−	Signal Value in LNCaP+	Gene Name	Accession
40.9	18986	464	Plasminogen activator, tissue (tPA)	D01096
34.6	6461	187	2-5A synthetase 2 (OAS2)	M87284
34.2	8345	244	Myxovirus resistance 1 (interferon-inducible protein p78)	M30817
25.9	7567	292	ESTs, Moderately similar to GALECTIN-1	AA340061
25.1	4262	170	Phospholipase D1, phosphatidylcholine-specific (PLD1)	U38545
21.0	3672	175	Follistatin	M19481
21.0	7675	366	Interferon-induced protein 17	AA428847
20.9	3623	173	Homo sapiens clone 23916 mRNA sequence	AF035283
19.0	4119	217	Human mRNA for 56-KDa protein induced by interferon	X03557
18.2	4284	235	Annexin I (lipocortin I)	X05908
18.0	2735	152	SH3-domain GRB2-like 2	AF036268
17.6	2693	153	Human mRNA for unknown product, partial cds	D29810
17.6	4513	257	Cytochrome P450, subfamily XXVIIB	AB006987
16.5	3545	215	EST	AI352370
15.8	4838	307	EST	AA307912
15.1	2308	153	EST	AI809937
14.9	3606	242	Fatty acid-binding protein 5 (psoriasis-associated) (FABP5)	AA888264
14.7	3664	250	Defensin, alpha 1, myeloid-related sequence	M26602
14.4	9620	669	Human butyrophilin (BTF1) mRNA, complete cds	U90543
13.6	2448	180	Human enkephalin B (enkB) gene	AL034562
13.6	2471	182	Interferon, alpha-inducible protein 27	AA302123
12.9	2307	179	Human chromosome 16 BAC clone CIT987SK-A-101F10	AC002550
12.8	4510	351	Heparin-binding growth factor-binding protein	M60047
12.8	3964	309	Protease, serine, 3 (trypsin 3)	X71345
12.7	1761	139	EST	AI422824
12.1	2950	243	TNF receptor-associated factor 3	U19260
11.9	2389	201	Tumor necrosis factor receptor superfamily, member 8	D86042
11.9	4253	358	Activin A receptor, type I	L02911

Overexpression, signal values (arbitrary units) in LNCaP+/−, gene identification and respective accession number are presented in descending order of overexpression.

Table 4. Semi-Quantitative Analysis of Genes Overexpressed in LNCaP+/− Cells

Gene Name	LNCaP+	LNCaP−	BPH	PC−3
Ribosomal protein S4, Y-linked	+	(+)	+++	(+)
Monoamine oxidase A	+++++	(+)	+	−
Mat-8	+	−	+++	−
EST similar to rat P044	+++	(+)	++	−
EST AA781244	+	−	+	−
EST AA412049	+++	−	−	−
Plasminogen activator, tissue type	−	+++	−	−
MxB	−	+++	−	−
EST AA307912	−	+	+++	+
EST similar to galectin-1	−	+++	++	+
Follistatin	−	+++	++	+
Fatty acid-binding protein 5	−	++	−	+
EST AA609749	−	+++	+	(+)
Annexin I	−	++	+++	(+)
Interferon-inducible gene 1-8U	−	+++	++++	−
Phospholipase D1	−	+++	−	(+)

Intensities of hybridization signals for different mRNAs obtained by Northern blot analysis were quantified by densitometric scanning and normalized against signals obtained for GAPD. Northern blots with LNCaP+/− cell, BPH tissue and PC-3 cell mRNAs were used. Expression levels of different genes are not comparable.
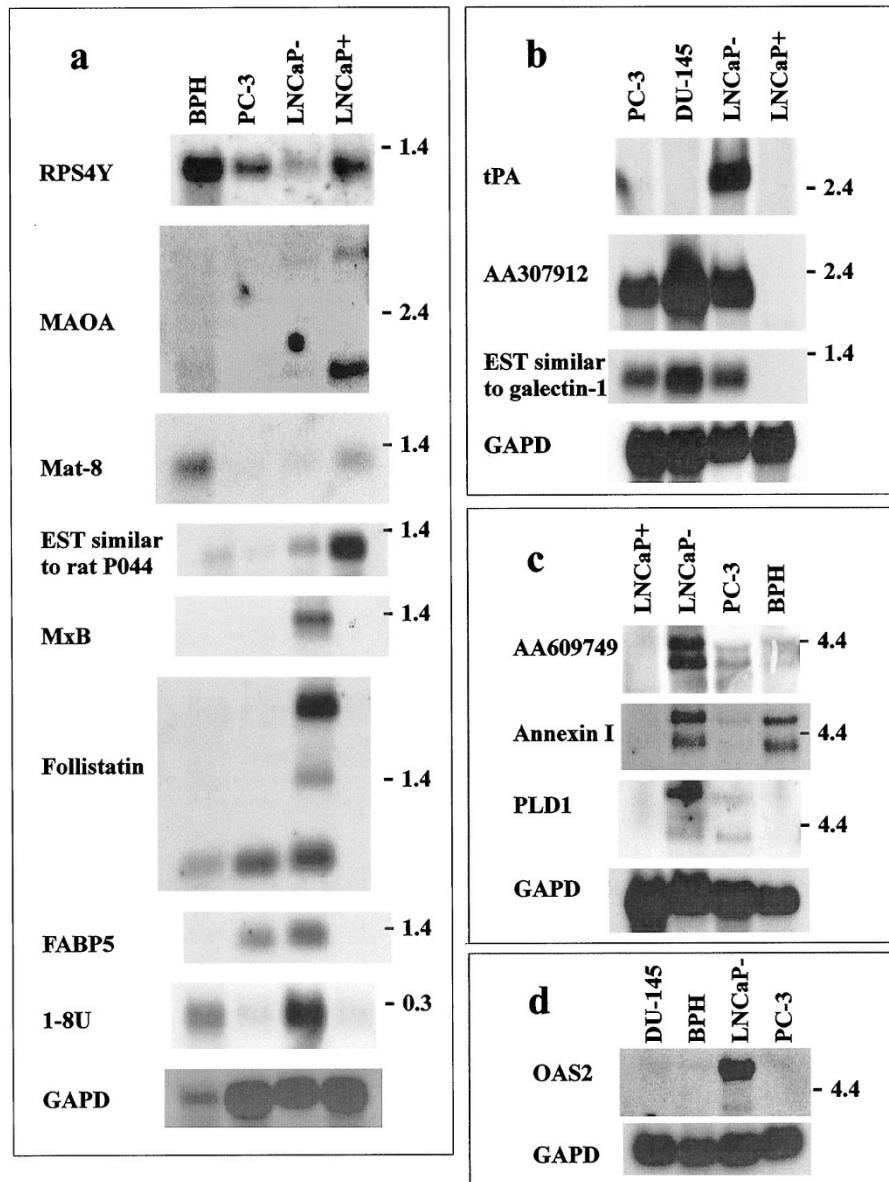
Figure 1.
Northern blot analysis of selected genes differentially expressed in LNCaP+/− cells according to cDNA array. *a*, Expression of Y-linked ribosomal protein S4 (*RPS4Y*), monoamine oxidase A (*MAOA*), Mat-8, expressed sequence tag (EST) similar to rat P044, MxB, follistatin, fatty acid-binding protein 5 (*FABP5*), interferon-inducible gene 1–8U (*1–8U*), and GAPD in BPH tissue, PC-3, LNCaP−, and LNCaP+ cells. *b*, Expression of tissue-type plasminogen activator (*tPA*), EST AA307912, EST similar to galectin-1, and GAPD in PC-3, DU-145, LNCaP−, and LNCaP+ cells. *c*, Expression of EST AA609749, annexin I, phospholipase D1 (*PLD1*), and GAPD in LNCaP+ cells, LNCaP− cells, PC-3 cells, and BPH tissue. *d*, Expression of 2′-5′ oligoadenylate synthetase 2 (*OAS2*) and GAPD in DU-145 cells, BPH tissue, LNCaP− cells, and PC-3 cells. RNA size markers are indicated on the right hand side. Five micrograms of mRNA was loaded on the gel in *a* and 10 μg in *b* to *d*.

Analysis of Overexpressed Genes in LNCaP− Cells

In the prostate cell lines studied, tissue-type plasminogen activator (tPA) was expressed only in LNCaP− cells; no expression was seen in BPH tissue (Table 4, Fig. 1b). MxB, like tPA, was expressed only in LN-CaP− cells (Table 4, Fig. 1a). Expression of EST AA307912 was detected in the androgen-independent cell lines, DU-145 and PC-3, as well as in LNCaP− cells (Fig. 1b). However, EST AA307912 was also expressed in BPH tissue (Table 4). The EST similar to galectin-1 was expressed most strongly in LNCaP− cells (Table 4, Fig. 1b), otherwise, its expression

pattern was comparable to that of EST AA307912. In previous studies, galectin-1 expression was observed only in androgen-independent DU-145, PC-3, and metastatic PC-3M cell lines (Ellerhorst et al, 1999a), similar to the expression pattern of the EST similar to galectin-1. In prostate tissue, the EST similar to galectin-1 was expressed in the stroma (Fig. 2, i and j), where the expression of galectin-1 has also been localized by immunohistochemistry (Ellerhorst et al, 1999b).

Follistatin and fatty acid-binding protein 5 (Table 4, Fig. 1a) were expressed in LNCaP− cells and in the
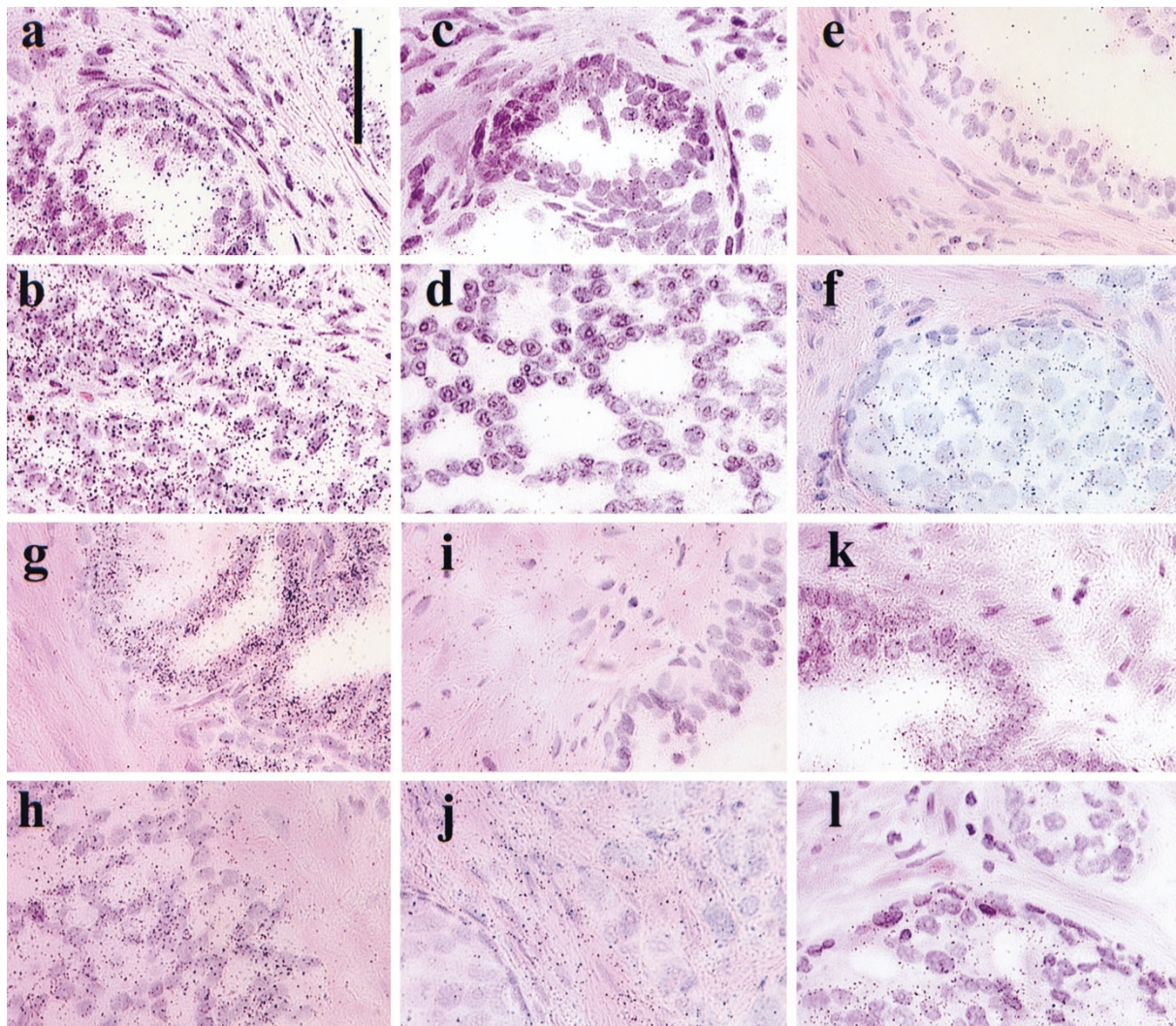
Figure 2.

In situ hybridization of overexpressed genes in LNCaP+ and LNCaP− cells. MAOA, EST AA781244, and fatty acid-binding protein 5 were expressed in both benign (*a, e,* and *k,* respectively) and malignant (*b, f,* and *l,* respectively) epithelium of human prostate. Expression of the EST similar to rat P044 was weak, but still detectable in epithelium of BPH (*c*) and prostate cancer (*d*). EST AA412049 tended to be slightly more highly expressed in benign epithelial cells (*g*) compared with malignant epithelium (*h*). The EST similar to galectin-1 was expressed in the stroma of both BPH and prostate cancer samples (*i* and *j,* respectively). Hematoxylin and eosin staining. Black vertical bar in right upper corner of *a* corresponds to 50 μm for all panels.

androgen-independent PC-3 cell line. Several mRNA species for follistatin were detected in LNCaP− cells (Fig. 1a) and one was also expressed in BPH tissue. Fatty acid-binding protein 5 was expressed in both benign and malignant epithelium by ISH (Fig. 2, k and l), although it was not expressed in BPH tissue in Northern blot. EST AA609749 was most abundantly expressed in LNCaP− cells, weakly expressed in PC-3 cells and BPH tissue, whereas the gene encoding this EST was silent in LNCaP+ cells (Table 4, Fig. 1c).

Annexin I (Fig. 1c) and 1–8U (Fig. 1a) were expressed in both LNCaP− cells and BPH tissue (Table 4). Annexin I was also detectable in PC-3 cells. According to the results of ISH, the expression of 1–8U was interesting (Fig. 3, a to e) because it was concentrated in some cells of cancer areas. In benign tissue, 1–8U was mainly localized in epithelial cells. However, in some areas, expression was also detected in the stroma. Phospho-

lipase D1 was strongly expressed in LNCaP− cells and low amounts of mRNA were detected in PC-3 cells, whereas LNCaP+ cells and BPH tissue totally lacked expression (Table 4, Fig. 1c).

According to the cDNA expression array analysis, OAS2 was 34.6 times overexpressed in LNCaP− cells (Table 3). In Northern blot (Fig. 1d), OAS2 transcripts were clearly observed only in LNCaP− cells, not in BPH tissue, DU-145, or PC-3 cell lines. In ISH, expression of OAS2 (Fig. 3, g and h) was detected in some prostate cancer areas in the samples analyzed. Expression was also seen in non-malignant prostate tissue, but the sparsely distributed transcript level was low.

Discussion

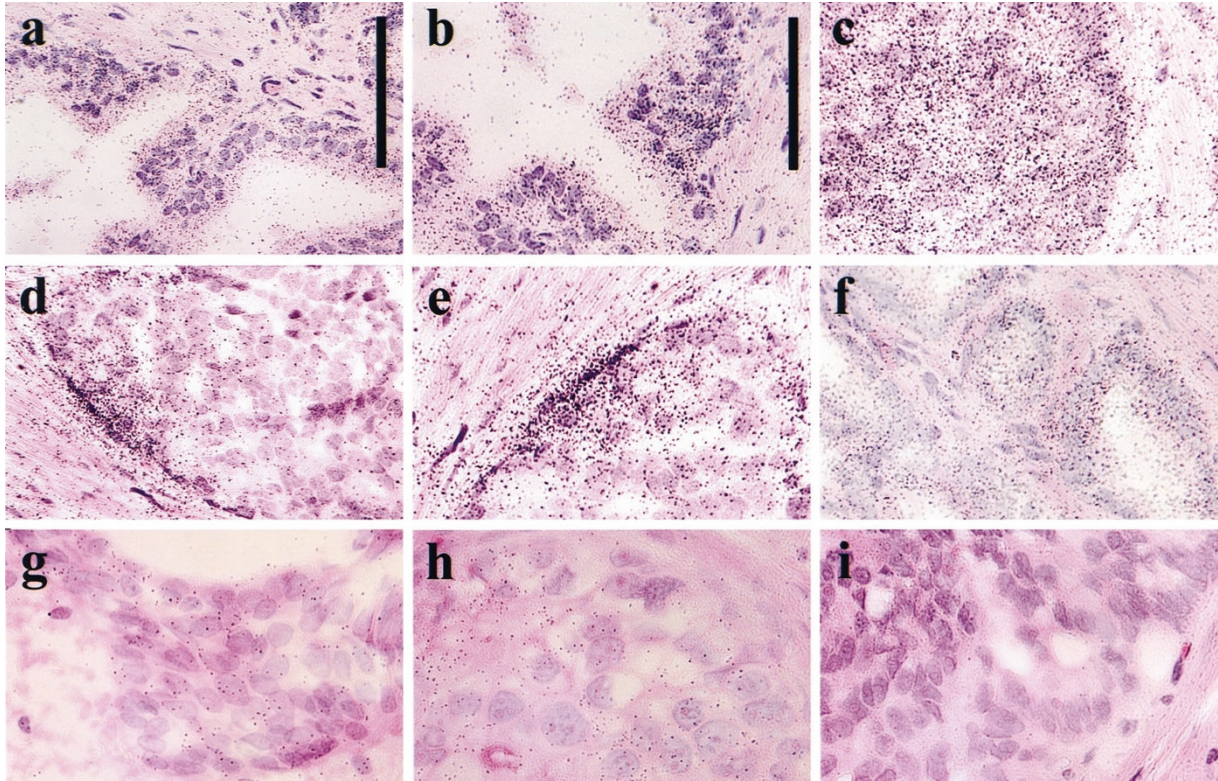Here we describe the results of cDNA microarray screening of two LNCaP cell line variants. Sixteen

Figure 3.

In situ hybridization results of overexpressed genes in LNCaP− cells. The interferon-inducible gene 1–8U was expressed in both benign (*a* and *b*) and malignant (*c*) epithelium. Expression was also detected in the stroma. Furthermore, the expression level varied within any one sample (*d* and *e*). *b* and *e* represent higher magnifications of *a* and *d*, respectively. OAS2 was expressed in epithelia of BPH (*g*) and prostate cancer (*h*), but only in some of the samples analyzed. Hybridization with sense OAS2 probe gave no signal (*i*). Preservation of RNA in the samples was confirmed by hybridization with GAPD probe (*f*). Hematoxylin and eosin staining. Black vertical bars in right upper corners of *a* and *b* correspond to 100 and 50 μm, respectively. *d* has same magnification as *a*; *c, e, f, g, h,* and *i* have same magnifications as *b*.

genes were selected from the array; differential expression of fourteen of these genes was confirmed by Northern blotting. Among differentially expressed genes, there are many potential prostate cancer markers. Several genes studied more closely here have previously been investigated with regards to their overall association with cancer. These genes are tPA, OAS, follistatin, annexin I, 1–8U, PSA, hK2, TMPRSS2, MAOA, and Mat-8. Of these, only PSA, hK2, TMPRSS2, tPA, and follistatin have been studied specifically in prostate cancer. Additionally, six EST clones or clones without a known product or function were found to be differentially expressed (EST similar to rat P044, AC: AI192351; EST similar to galectin-1, AC: AA340061; AC: AA781244; AC: AA412049; AC: AA307912; and AC: AA609749). Our results indicate that the well known androgen-regulated prostatic proteins PSA and hK2 are highly expressed in LNCaP+ cells, whereas they are down-regulated in LNCaP− cells.

The galectins are a family of lectins that probably mediate cell adhesion, regulate cell growth, and trigger or inhibit apoptosis. The expression pattern of some galectins is altered in breast, colon, prostate, and thyroid carcinomas (Perillo et al, 1998). Our finding of overexpression of an EST similar to galectin-1 in LNCaP− cells suggests that these cells are trans-

formed to a more progressive stage of prostate cancer compared with galectin-1, which was expressed in DU-145, PC-3, and PC-3M cells but not in LNCaP cells analyzed by Northern blotting and immunoblotting (Ellerhorst et al, 1999a). Furthermore, another protein sharing similarity with the galectin family, PCTA-1, is expressed in prostate cancer tissue (Su et al, 1996).

Overexpression of follistatin in LNCaP− cells is in accordance with the theory of follistatin overproduction favoring androgen-independent prostate cancer growth (Thomas et al, 1997). Activin inhibits the growth of DU-145 and LNCaP cells, even after androgen-stimulation (Wang et al, 1999), but not PC-3 cell lines. This inhibition may be prevented by follistatins (Thomas et al, 1997). An interesting question is how to overcome the growth-promoting influence of follistatin in prostate cancer cells.

MxA and MxB belong to the family of large GTPases. Cytoplasmic human MxA protein mediates resistance to multiple RNA viruses, whereas no antiviral activity has been found for human MxB protein (Melen et al, 1996). The genes for MxA and MxB are located in the same chromosomal area as the TMPRSS2 gene, 21q22.3 (Paoloni-Giacobino et al, 1997). Interestingly, MxA and MxB were up-regulated in the androgen-independent cell line (LNCaP−), whereas

TMPRSS2 was down-regulated in these cells. Thus, deletion of this chromosomal area is not likely to clarify the differential expression of these genes in the cell line variants, and up- or down-regulation is due to specific regulatory factors. Additionally, expression of TMPRSS2 is androgen-regulated (Lin et al, 1999; Vaarala et al, unpublished data) and low amounts of TMPRSS2 as well as other androgen-dependent serine proteases in LNCaP− cells reflect a change in the hormonal status/response of this cell line variant.

The antitumor effectiveness of interferons in vivo and in vitro is well documented. OAS is an interferon-inducible enzyme that polymerizes 2′-5′ oligoadenylates from adenosine triphosphate. 2′-5′ oligoadenylates, in turn, are responsible for RNaseL activation, which is needed for RNA degradation and antiviral action (Stryer, 1988). However, only some of the interferon-inducible genes activated in LNCaP− cells are known to belong to the OAS-RNaseL pathway. The uneven distribution of OAS2 mRNA in prostate samples found here may reflect participation in cell growth regulation or an as yet unknown process involved in the development of cancer cell populations with different endocrine characteristics. The cytocidal effect of treating the HT29 human colon carcinoma cell line with both TNF-α and interferon-γ is reported to be accompanied by an increase in OAS levels and apparent rRNA breakdown (Chapekar and Glazer, 1988). However, the antiproliferative effects of interferons did not correlate with induction of OAS in human lung cancer (Martyre et al, 1988). The gene encoding 1–8U has been observed to be strongly expressed in ulcerative colitis-associated colon cancers (Hisamatsu et al, 1999). This gene is highly interferon-inducible, but its function is unclear. There is some evidence of a link between prostate cancer and colon cancer (Moore et al, 1998) and, furthermore, the expression of certain genes is similarly altered in cancers of different tissues (eg, KAI1). It is therefore possible that 1–8U also has some association with prostate cancer. The activation of interferon-inducible genes in LNCaP− cells seems to be involved in the transition to more aggressive growth or malignant transformation, as in the case of 1–8U in colon cancer (Hisamatsu et al, 1999). The possibility of this induction being a result of *mycoplasma* contamination was ruled out by regular testing of the cells.

Mat-8 is an 8-kd transmembrane protein homologous to phospholemman (Morrison et al, 1995). It is differentially expressed in the MCF-7 breast cancer cell line and in a progressive variant of MCF-7 (Schiemann et al, 1998). The kallikrein gene family and Mat-8 genes are located in chromosomal region 19q, suggesting that this genetic area could be vulnerable to regulatory changes during transformation.

The Y-linked ribosomal protein S4 was most prominently expressed in BPH tissue and was also detectable in LNCaP+ cells. The gene encoding this protein is overexpressed in a non-metastatic variant of rat rhabdomyosarcoma, SMF-Da (Daigneault et al, 1995). The expression of several other ribosomal proteins is also altered in prostate cancer and prostate cancer cell lines (Vaarala et al, 1998).

Angiostatin, an inhibitor of angiogenesis and growth of tumor metastases, is generated by urokinase-mediated proteolysis of plasminogen in the PC-3 cell line (Gately et al, 1997). Recently, PSA has also been shown to have antiangiogenic activity (Fortier et al, 1999). Another plasminogen activator, tPA, was over-expressed in LNCaP− cells. Down-regulation of PSA production possibly leads to accumulation of plasminogen in these cells, which might then promote up-regulation of the gene encoding tPA. However, according to Gately et al, 1997, tPA seems not to have similar activity to urokinase and PSA (Fortier et al, 1999), and the concentration of tPA is not altered in prostate cancer tissue compared with normal prostate tissue (Plas et al, 1998). Unfortunately, cDNA for urokinase was not available in the array. The plasminogen gene was expressed at a low level with signal values of 575 and 281 in LNCaP+ and LNCaP− cells, respectively.

Several electronic databanks now offer tissue- or cell line-specific expression data for cDNA clones. A sophisticated model for expression studies in prostate tissue specimens, using the microarray technique, has been presented recently (Cole et al, 1999). Information given by cDNA arrays, as in the present study, is a valuable tool in cancer research, because genes with prominent or very low expression can be compared with expression data available on another disease states. For example, finding a gene with high expression in LNCaP+ and LNCaP− cells, with no or low expression in BPH or normal prostate tissue, would reveal a potential new prostate cancer marker. Careful examination of clones is still needed, because there are differences within and between LNCaP cell lines and prostate cancer, as indicated by ISH analyses.

Materials and Methods

Cells and Tissues

LNCaP-FGC, PC-3, and DU-145 cells were purchased from ATCC (Rockville, Maryland). Production of PSA by LNCaP cells was followed by using DELFIA PSA kits (Wallac, Turku, Finland); cell clone variants with high PSA production and undetectable PSA production were selected. Lack of *mycoplasma* contamination was confirmed regularly (Gen-Probe, San Diego, California). Tissues for ISH were taken from prostatic adenocarcinoma patients treated by radical prostatectomy or transurethral electroresection of the prostate. BPH tissue was obtained from patients treated by radical/total prostatectomy. These treatments are widely used and accepted in cases of BPH and prostate cancer. Tissues were removed only for treatment purposes and were used for this study only after diagnostic confirmation by histopathology.

RNA Isolation, cDNA Microarray, and Clone Sequencing

Total RNA was isolated from the cells using TRIzol (Life Technologies, Gaithersburg, Maryland). Poly-A

RNA was isolated using a QuickPrep Micro mRNA Purification Kit (AmershamPharmacia, Uppsala, Sweden). mRNA was used for Human UniGEM V v1.0 Custom Screening (GenomeSystems Inc., St. Louis, Missouri). mRNAs from LNCaP+/− cells were labeled with different fluorescent labels. Average signals for the elements in the array were achieved for both probes. For details of screening and clone selection, see http://www.genomesystems.com. Results are presented as either probe average signal values (arbitrary units) or as overexpression, when signal values of a cell line are divided by signal values in the other cell line. Histologically confirmed tissue samples of BPH were used for the isolation of total RNA by the CsCl gradient method. Poly-A RNA was isolated using oligo(dT)-cellulose (AmershamPharmacia) and standard methods. Clones selected according to screening were purchased from GenomeSystems Inc. and were further sequenced using an ABI377 automatic sequencer (Applied Biosystems, Branchburg, New Jersey) to confirm the sequence data and clone orientation.

Northern Blot Analysis

mRNA isolated from the cells and tissues were loaded on the gel. Northern blots were carried out as recommended by the membrane manufacturer (Boehringer Mannheim, Mannheim, Germany). Hybridization was carried out with ^{32}P-labeled cDNA and GAPD in plasmid vectors. The filters were washed under high stringency conditions and exposed to Kodak MS film (Eastman Kodak, Rochester, New York) with intensifying screens. The filters were stripped and removal of probes was confirmed by exposure of the blots before each hybridization.

In Situ Hybridization

Antisense and sense [α-^{35}S]CTP-labeled RNA probes were transcribed with T7 or SP6 RNA polymerases using linearized plasmids as templates. The specific activities of the synthesized RNA probes were approximately 6×10^6 cpm/μl. ISH was carried out as previously described (Mustonen et al, 1998) using tissue samples obtained from transurethral electroresection of prostate cancer patients and tissue blocks containing BPH tissue and prostate cancer tissue. The hybridization temperature was 60° C. The slides were stained with hematoxylin and eosin, and mounted with Pertex (Histolab, Göteborg, Sweden). Each probe was analyzed in a minimum of two BPH tissue samples and two prostate cancer samples.

Acknowledgements

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