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Analysis of Gene Expression Identifies Candidate Markers and Pharmacological Targets in Prostate Cancer

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Abstract

Detection, treatment, and prediction of outcome for men with prostate cancer increasingly depend on a molecular understanding of tumor development and behavior. We characterized primary prostate cancer by monitoring expression levels of more than 8900 genes in normal and malignant tissues. Patterns of gene expression across tissues revealed a precise distinction between normal and tumor samples, and revealed a striking group of about 400 genes that were overexpressed in tumor tissues. We ranked these genes according to their differential expression in normal and cancer tissues by selecting for highly and specifically overexpressed genes in the majority of cancers with correspondingly low or absent expression in normal tissues. Several such genes were identified that act within a variety of biochemical pathways and encode secreted molecules with diagnostic potential, such as the secreted macrophage inhibitory cytokine, *MIC-1*. Other genes, such as *fatty acid synthase*, encode enzymes known as drug targets in other contexts, which suggests new therapeutic approaches.

Introduction

Prostate cancer is the most common malignancy in men and the second most frequent cause of cancer death in the United States (1). Its development proceeds through a series of defined states, including preinvasive disease [prostatic intraepithelial neoplasia (PIN)], invasive cancer, and androgen-dependent or androgen-independent metastases (2). The early stages of organ-confined prostate cancer are generally curable by surgery or radiation therapy, and detection efforts based on PSA[2] screening (3) have led to the identification of thousands of men with localized disease. Although serum PSA is widely recognized as the best prostate tumor marker currently available, levels between 4 and 10 ng/ml are seen in men with obstructive or inflammatory uropathies, lowering the specificity of PSA as a cancer marker (4). Other biomarkers, such as glandular kallikrein-2 (hK2), have been proposed as adjuncts to PSA to increase diagnostic specificity (5) and to reduce the number of men subjected to unnecessary biopsy.

A molecular understanding of prostate cancer development and progression is an important step toward the identification of additional biomarkers with increased specificity for invasive prostate cancer and of new tumor-specific therapeutic targets. Previous systematic interrogations of gene expression levels in human cancers by microarray hybridization (6) suggest that this is a broad and efficient approach toward identifying candidate markers and drug targets. Here, we analyzed the expression levels of more than 8900 different human genes in a set of normal and malignant prostate tissues. Quantitative and qualitative differences in gene expression seen in tissue samples led to a molecular definition of prostate cancer. We identified several genes as overexpressed in most or all of the tumors. Some of these may form the basis for new diagnostic or therapeutic approaches as well as provide insight into prostate tumor development or behavior.

Materials and Methods

Cell Culture. PC3, LNCaP, and Du145 cells (American Type Culture Collection) were grown in DMEM supplemented with 10% FCS and 100 μg/ml streptomycin (Life Technologies, Inc.). LNCaP cells were also grown in androgen-depleted medium in which dialyzed charcoal-stripped FCS was substituted for FCS. Normal prostate epithelial cells (PrEC; Clonetics) and a human papillomavirus (HPV) E6-transfected derivative (hPr1) were grown in serum-free keratinocyte growth medium (Life Technologies, Inc.). Fibroblastic cell strains CAF1598, CAF1303, CAF1852, and CAF2585 were expanded from fragments of prostates removed for adenocarcinoma. Frozen sections were stained with H&E at the time of surgery and were determined to contain malignant epithelium; adjacent fragments were minced and expanded in supplemented DMEM. BPHF1598 fibroblastic cells were propagated from fragments of prostate tissue that contained only benign hypertrophic glands. Prostate stromal cells (PrSC) A and B (Clonetics) from cancer-free men were grown according to the manufacturer's recommendations. Adherent cells were expanded and harvested at 60–70% confluence after 4–8 passages. LNCaP grown in the absence of androgens and two cultures of HUVECs were each sampled twice.

Tissue Procurement, cRNA Synthesis, and Hybridization to Oligonucleotide Arrays. The use of human tissue samples at the University of Virginia (UVA) was approved by the UVA Human Investigation Committee. Fresh samples of adenocarcinoma were obtained from men with elevated PSA levels. H&E-stained sections from prostatectomy specimens or (in one case) from a lymph node metastasis were examined to assess the relative amounts of tumor, benign epithelium, stroma, and lymphocytes. Tissues containing cancer were trimmed to enrich for neoplastic cells. All of the samples were stored at −80°C before processing for microarray analysis. The set of prostate tissues included 23 primary cancer tissues, 1 lymph node metastasis, and 9 nonneoplastic tissues. Eight of the cancers were paired with normal tissue obtained from the same patient. One cancer tissue (case 13) was divided and processed as two independent samples. In each case, several milligrams of tissue were sharply dissected and homogenized with a rotary homogenizer (Omni International) in RNeasy lysis buffer (Qiagen). RNA was prepared from tissues and cells using the RNeasy Mini kit (Qiagen). mRNAs from T-lymphoblastic MOLT4 and myeloleukemic HL60 cells were purchased (Clontech), and RNA from endothelial cells and from three individual isolates of activated B-cells were gifts from Drs. Akira Kawamura (The Scripps Research Institute, La Jolla, CA) and Michael Cooke (Genomics Institute of The Novartis Research Foundation, San Diego, CA) respectively. Labeled cRNA was prepared and hybridized to oligonucleotide arrays ("U95a"; Affymetrix) as described previously (7).

Data Analysis. Scanned image files were visually inspected for artifacts and analyzed with GENECHIP 3.1 (Affymetrix). Each image was then scaled to an average hybridization intensity of 200, which corresponds to ~3–5

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[2] The abbreviations used are: FASN, fatty acid synthase; HUVEC, human umbilical vein endothelial cell; PSA, prostate-specific antigen.

Table 1 *Clinicopathological information and selected transcript levels in normal and malignant prostate tissues*

Percentage of various cell types is estimated from sections adjacent to the tissue that was profiled. Cases N9 and T9 also contained 15% PIN.

		Clinicopathological					Hybridization intensity $\times 10^{-3}$		
Case	Age	Grade[a]	Stage	% T	% N	% S+L	PSA[b]	*hepsin*	*MIC-1*
N1[c]	65			0	50	50	11.0	0.2	1.7
N2	60			0	60	40	8.9	0.1	0.2
N3[c]	65			0	25	75	3.1	0.1	0.2
N4[c]	66			0	60	40	12.1	0.4	1.5
N5[c]	53			0	25	75	8.4	0.2	0.5
N7[c]	63			0	25	75	8.8	0.1	0.3
N8[c]	59			0	40	60	14.4	0.3	0.6
N9[c]	57			0	25	60	10.8	0.3	0.4
N10[c]	58			0	35	65	10.3	0.1	0.4
T1[c]	65	8	$T_{2a}N_0M_0$	75	5	20	13.1	0.5	1.7
T3[c]	65	7	$T_{2b}N_0M_0$	85	5	10	13.7	1.8	0.5
T4[c]	66	9	$T_{2b}N_0M_0$	80	10	10	9.5	1.6	6.6
T5[c]	53	8	$T_{2a}N_0M_0$	80	5	15	14.1	1.1	0.7
T6	62	6	$T_{2b}N_0M_0$	70	10	20	9.8	2.0	3.8
T7[c]	63	7	$T_{3a}N_0M_0$	60	10	30	11.4	0.9	2.3
T8[c]	59	6	$T_{3b}N_0M_0$	70	15	15	19.6	1.4	3.1
T9[c]	57	7	$T_{2b}N_0M_0$	65	5	10	13.8	1.5	6.5
T10[c]	58	7	$T_{2a}N_0M_0$	85	5	10	17.4	1.3	5.3
T11	60	7	$T_{2a}N_0M_0$	80	2	18	11.2	1.7	5.9
T12	63	6	$T_{3a}N_0M_0$	65	15	20	14.1	2.0	2.5
T13	54	6	$T_{3a}N_0M_0$	75	15	10	13.0	1.9	10.5
T16	43	8	$T_{2b}N_0M_0$	75	0	25	22.8	2.4	3.6
T17	65	8	$T_XN_1M_X$	90	0	10	17.5	1.4	7.4
T19	63	8	$T_3N_0M_0$	70	10	20	20.5	2.6	4.3
T21	65	7	$T_XN_XM_0$	85	2	13	13.5	2.9	2.6
T22	60	7	$T_{3a}N_0M_0$	75	2	23	13.5	3.2	6.0
T23	57	6	$T_{3a}N_0M_0$	70	15	15	18.3	1.5	8.5
T24	65	9	$T_XN_0M_0$	85	2	13	23.6	2.2	2.6
T26	60	6	$T_{3a}N_XM_0$	70	15	15	22.8	1.9	7.6
T27	56	7	$T_XN_XM_0$	65	10	25	15.5	2.3	4.8
T29	61	5	$T_{2a}N_0M_0$	50	25	25	15.3	1.4	4.4
T30	47	6	$T_{3b}N_0M_0$	80	10	10	18.5	1.2	0.4
T31	55	7	$T_{2b}N_0M_0$	65	10	25	17.6	2.2	9.8

[a] Grade, Gleason score; N, normal epithelium; T, malignant epithelium; S+L, stroma and lymphocytes.
[b] GenBank accession no. X07730.
[c] Paired case.

transcripts per cell (7). The hybridization intensity for each gene was ranked according to intersample variability (SD), and 3530 genes with the most variable expression across all of the samples were median-centered and normalized with respect to other genes in the sample and corresponding genes in other samples. Genes and samples were subjected to hierarchical clustering essentially as described previously (8). Differential expression of genes in benign and malignant prostate tissues was estimated using an algorithm (9) based on equally weighted contributions from the difference of hybridization intensities ($\mu_{Tumor}-\mu_{Normal}$), the quotient of hybridization intensities (μ_{Tumor}/μ_{Normal}), and the result of an unpaired *t* test between expression levels in tumor and normal tissues. The genes were scored with respect to each of the three metrics, then ranked according to the sum of the three scores.

Reverse Transcription-PCR Analysis of *hepsin* and *MIC-1* Expression. cDNA was prepared using 1 μg of total RNA isolated from prostate tissues. Primers used to amplify specific gene products were: hepsin sense, 5′-CGGGACCCCAACAGCGAGGAGAAC-3′; hepsin antisense, 5′-TCGGGGTAGCCAGCACAGAACATC-3′; MIC-1 sense, 5′-CGCGCAACGGGGACGACT-3′; and MIC-1 antisense, 5′-TGAGCACCATGGGATTGTAGC-3′. PCR conditions for hepsin and MIC-1 comprised 95°C for 10 min, 30 cycles of 95°C for 30 s, 55°C for 30 s (annealing), and 72°C for 30 s, and a final elongation step of 72°C for 7 min. All of the PCR reactions used a volume of 20 μl, with 1 unit of AmpliTaq Gold (Perkin-Elmer). Amplification products (10 μl) were separated by 2% agarose gel electrophoresis.

Immunohistochemistry. The avidin-biotin immunoperoxidase method was performed on deparaffinized zinc formalin-fixed, paraffin-embedded sections. Slides placed in citrate buffer were heated with a microwave for 20 min prior to the application of the anti-FASN antibody for 1 h at room temperature.

Results and Discussion

Gene Expression Profiles in Prostate Tissue Samples and Cell Lines. We monitored the expression levels of genes in tissues and cells by hybridization of RNA samples to oligonucleotide microarrays representing ~8920 different genes. In total, we hybridized 55 RNA samples derived from 25 prostate cancer tissues (24 unique samples), 9 nonmalignant prostate tissues, and 21 cell line samples (18 unique lines).[3] Clinical and pathological information on the tumors is detailed in Table 1.

To reveal distinctions between individual tissue samples and cells, we selected a subset of 3530 genes that varied most across the samples and grouped both the genes and the samples according to their overall similarities in levels of expression (8). These samples were separated on a dendrogram in which their overall similarity was proportional to the length of the vertical branches between them (Fig. 1*a*). As expected, independently prepared samples of tumor 13 (13A and 13B) and replicate samples of LNCaP cells and HUVECs were highly correlated, demonstrating the overall reproducibility of the technique. This analysis revealed a major division between cells grown *in vitro* and the human tissue specimens, with highly divergent gene expression patterns. Within the "tissue" branch of the dendrogram, samples were further subdivided into two distinct groups of normal and malignant samples.

Subdivisions in the "cell line" branch of the dendrogram (Fig. 1*a*) largely reflected the embryological origin of the cells. For example, profiles of activated B cells from three different donors were highly correlated with one another and with cells derived from two hematological cancers (MOLT4 and HL60). Six mesenchymal lines derived from normal or malignant prostates of different individuals (designated as BPHF, PrSC, or CAF) were highly related. Similarity was also

[3] The complete dataset is available on our web site, address: http://www.gnf.org/cancer/prostate.

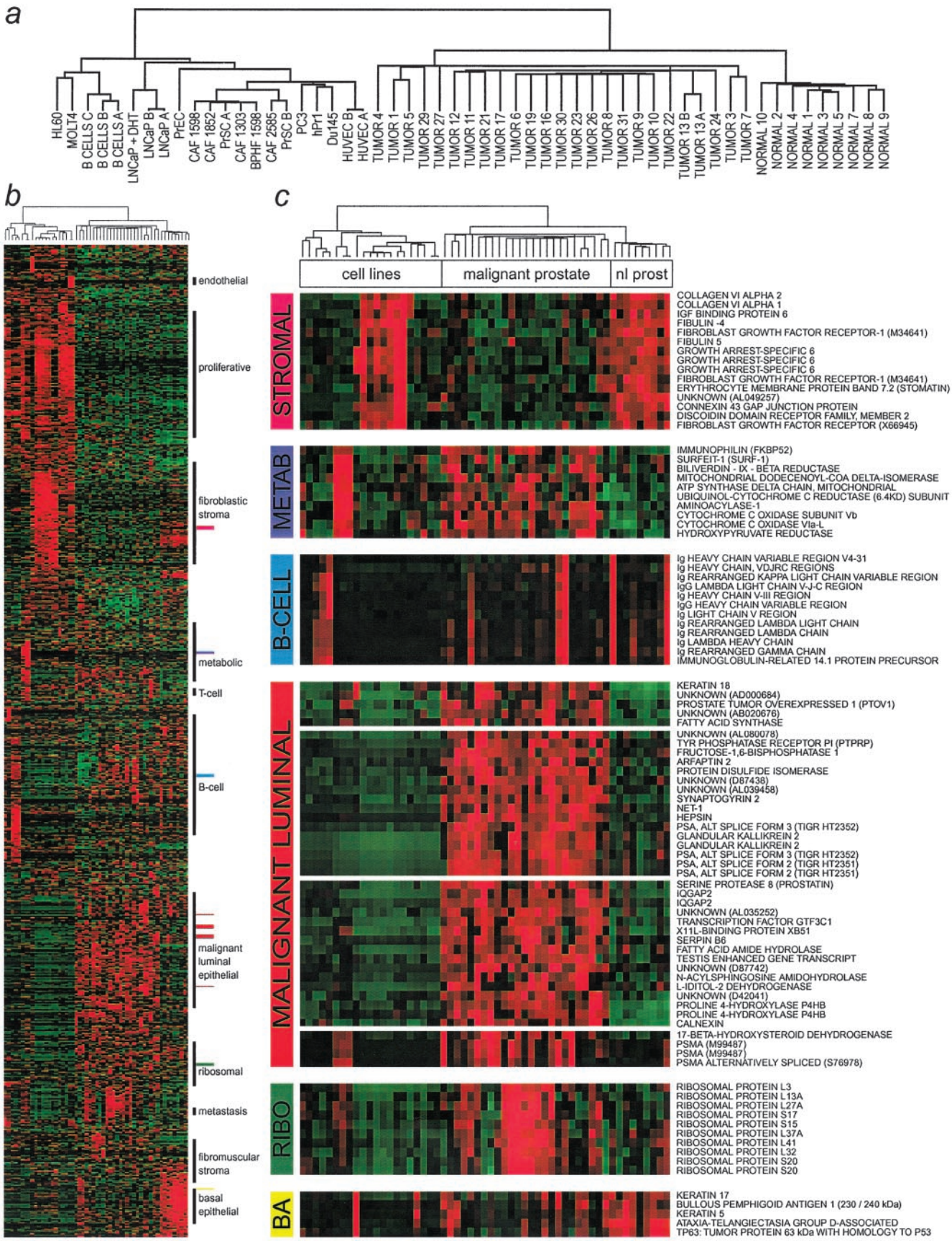

Fig. 1. Expression of 3530 genes in 55 experimental samples. *Rows*, individual genes; *columns*, individual samples. Each cell in the matrix represents the expression level of a single transcript in a single sample; *red* and *green*, transcript levels above and below, respectively, the median for that gene across all of the samples. Color saturation is proportional to the magnitude of the difference from the mean. *a*, dendrogram of samples showing overall similarity in gene expression profiles across the samples. *b*, demonstration of overall groupings of genes and samples. *Black bars to the right*, the extent of cell type-specific or functionally relevant gene clusters (*identified next to the bars*). *Colored bars*, gene clusters of special interest. *c*, enlarged view of selected clusters of genes. Three groups of columns are designated by the predominant sample type; *nl prost*, normal prostate.

seen among LNCaP cells grown in the presence or absence of dihydrotestosterone (DHT).

Expression of Genes in Malignant and Cancer-associated Cell Types. We identified a striking group of ~400 genes as being highly and specifically expressed in malignant samples (Fig. 1*b*, *malignant luminal epithelial*; expanded in *MALIGNANT LUMINAL*, Fig. 1*c*). Among this group, we identified keratins 8 and 18, which strongly suggested that this cluster of genes signified malignant luminal epithelium within the cancer tissue (10). We identified several other clusters of genes for which expression levels were similar in some of the cells and tissues. These clusters suggested the presence of varying amounts of B cells, normal basal epithelial cells, and stromal fibroblasts within normal and tumor tissues (Fig. 1*c*, *B-CELL*, *BA*, and *STROMAL*, respectively). The varying proportion of cancer-associated cells implied by expression profiling was consistent with our histological estimates of these cell types in each of the tissue samples. High expression of B-cell genes in normal and malignant samples from cases 9 and 10 (Fig. 1*c*, *B-CELL*) was consistent with histologically diagnosed prostatitis in these patients. A fourth group of genes was highly expressed in LNCaP cells and in most of the tumor samples (Fig. 1*b* and *METAB*, Fig. 1*c*) and included multiple genes the products of which are involved in intermediary metabolism, such as ATP synthase and cytochrome C oxidase. Expression of genes within this cluster likely reflects accelerated metabolism of the malignant cells.

Genes with Potential Diagnostic and Therapeutic Utility in Prostate Cancer. The strong molecular distinction between normal and tumor tissues and the relative homogeneity of gene expression in malignant epithelial cells when compared with normal prostate samples suggested that we could identify genes that were overexpressed in the majority of tumors. The tumor samples examined here contained, on average, about twice as many epithelial cells as the normal tissues. Thus, we focused on methods that could rank highest those genes with the greatest and most uniform differences in gene expression. We used a metric that would take into account the equal importance of average hybridization intensities and fold differences, as well as uniformity of expression within a tissue type (penalizing genes with broad and overlapping ranges of expression; Ref. 9). This metric returned genes the expression of which was generally low in normal tissues, elevated in tumors (average, >5-fold for the top 20 genes; Fig. 2) with nonoverlapping expression ranges. Among the 20 highest scoring genes were ones encoding known tumor markers such as the carcinoma-associated antigen GA733–2 (TACSTD1) and FASN. We also identified *MIC-1*, which encodes a secreted cytokine (11), and *hepsin*, which encodes a membrane-bound extracellular serine protease involved in cell growth control (12). Expression levels of *PSA*, *MIC-1*, and *hepsin* transcripts are given in Table 1.

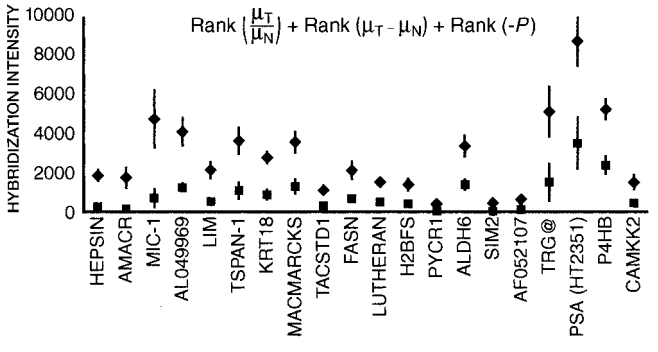

Fig. 2. Expression levels of selected genes in normal and malignant prostate tissues. Each gene is represented by two mean values derived from its expression level in 24 malignant (◆) and 9 normal (■) samples. *Error bars*, 99% confidence intervals. Shown are 20 genes ranked most highly by a hybrid metric (9).

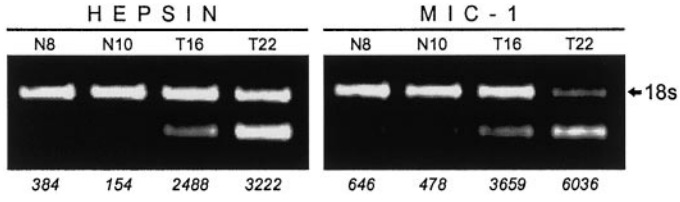

Fig. 3. Amplification of *hepsin* and *MIC-1* transcripts from selected prostate tissues. Transcripts from normal (*N8*, *N10*) and tumor (*T16*, *T22*) tissues were coamplified with 18s rRNA. *Number beneath the PCR product generated from each case*, hybridization intensities of *hepsin* and *MIC-1* on corresponding microarrays.

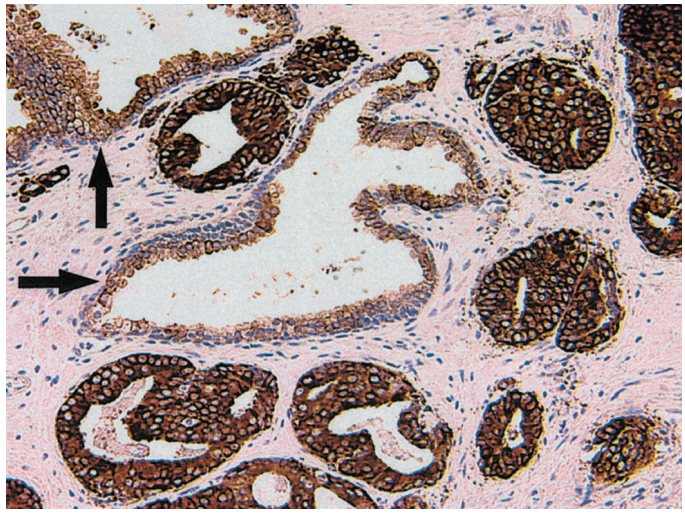

Fig. 4. Immunohistochemical staining of prostate tissue showing differential reactivity of anti-FASN antibody to normal (*arrows*) and malignant glands. ×200.

Differential expression of genes that were ranked high by our method was validated in several ways. First, tumor-specific overexpression of *MIC-1* and *hepsin* transcripts in selected RNAs was confirmed by reverse transcription-PCR amplification (Fig. 3). Second, the public "Gene-to-Tag" database[4] was queried for differential expression of the genes in Fig. 2 in normal and prostate cancer samples. Reliable estimates of the expression levels in a single microdissected pair of normal and malignant prostate samples were available for 12 of the 20 genes. Of these 12, 10 showed >5-fold overexpression in cancer tissue. Third, we stained tissue sections with a monoclonal antibody against FASN (13) and found strong and specific immunopositivity in malignant epithelium in all 10 cases of prostate cancer that we examined (Fig. 4).

Differences in Gene Expression among Prostate Tumors. The apparent molecular similarity among prostate cancers (typified by the malignant luminal epithelial cluster, Fig. 1, *b* and *c*) resulted from the initial analysis of 3530 genes the expression of which varied most across normal tissues, malignant tissues, and cell lines (Fig. 1*b*). To better estimate the extent of the similarity among prostate cancers, we selected 788 of these genes the expression of which was inferred to be specific to the malignant cells. Clustering of the data showed a dichotomy among the tumors, which was largely attributable to differential expression of a group of ribosomal genes (Fig. 1*a*, *Ribo*). This division roughly corresponded to the degree of differentiation among the tumors, with significantly higher Gleason scores ($P < 0.01$) in the tumor samples with lower ribosomal gene expression. We next sought genes the expression of which might better differentiate low- and high-grade tumors.

[4] Internet address: http://www.ncbi.nlm.nih.gov/SAGE/.

ANOVA was performed on tumors divided into subgroups with different Gleason scores (group 1: Gleason score, 5 or 6; group 2: Gleason score, 7; group 3: Gleason score, 8 or 9). Among the 20 most significantly different genes identified by this method, we found high expression of *IGFBP-2* and *IGFBP-5* in group 3 (Gleason score, 8 or 9) compared with groups 1 and 2 (Gleason scores, 5, 6, or 7; data not shown). Both of these genes have been reported as elevated in high-grade tumors (14).

In this study, we exploited the observed similarities among prostate tumors to identify genes with potential clinical use. The list of genes highly ranked by our metric (Fig. 2) is functionally diverse and includes several genes involved in fatty acid, sugar, nucleotide, and steroid intermediary metabolism. *FASN* up-regulation is consistent with a previous report (15) and is of particular interest because pharmacological inhibition of FASN results in dose-dependent growth inhibition in a prostate cancer xenograft model (13). The serine protease, hepsin, which we and others (16) find up-regulated in all of the primary tumors examined, is a potential drug target (17). The potential benefit of hepsin inhibition is shown by growth arrest induced in hepatoma cells using anti-hepsin antibodies and antisense oligonucleotides (12). We also found overexpression of the gene encoding the secreted cytokine *MIC-1*, a member of the transforming growth factor-β superfamily (18) in 21 of the 24 cancers examined (Table 1). High MIC-1 levels are found in amniotic (19) and cerebrospinal (20) fluids, and recent studies suggest its overexpression in the serum of patients with metastatic breast, colon, and prostate carcinomas.[5]

We profiled androgen-dependent and -independent prostate cancer-derived cells grown *in vitro* to evaluate the fidelity with which these cells replicate features of malignant prostate cells *in vivo*. Overall, we found only a small number of genes with concordant expression in cell lines and malignant tissues, which suggests that these cell lines have lost many features that characterize prostate cancer *in vivo*. Thus, it is not surprising that many of the genes that we identified as overexpressed in this study have not been reported as overexpressed in prostate cancer cell types. The fact that we, and others (16), now find genes such as *hepsin* to be overexpressed in all of the primary tumors examined underscores the usefulness of profiling primary tumors and the likelihood that these data will provide new insight into prostate cancer.

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[5] S. N. Breit, personal communication.

References

1. Greenlee, R. T., Murray, T., Bolden, S., and Wingo, P. A. Cancer statistics, 2000. CA Cancer J. Clin., *50:* 7–33, 2000.
2. Scher, H. I., and Heller, G. Clinical states in prostate cancer: toward a dynamic model of disease progression. Urology, *55:* 323–327, 2000.
3. Catalona, W. J., Smith, D. S., Ratliff, T. L., and Basler, J. W. Detection of organ-confined prostate cancer is increased through prostate-specific antigen-based screening. JAMA, *270:* 948–954, 1993.
4. Brawer, M. K. Prostate-specific antigen. Semin. Surg. Oncol., *18:* 3–9, 2000.
5. Nam, R. K., Diamandis, E. P., Toi, A., Trachtenberg, J., Magklara, A., Scorilas, A., Papnastasiou, P. A., Jewett, M. A., and Narod, S. A. Serum human glandular kallikrein-2 protease levels predict the presence of prostate cancer among men with elevated prostate-specific antigen. J. Clin. Oncol., *18:* 1036–1042, 2000.
6. Liotta, L., and Petricoin, E. Molecular profiling of human cancer. Nat. Rev. Genet., *1:* 48–56, 2000.
7. Wodicka, L., Dong, H., Mittmann, M., Ho, M. H., and Lockhart, D. J. Genome-wide expression monitoring in *Saccharomyces cerevisiae*. Nat. Biotechnol., *15:* 1359–1367, 1997.
8. Eisen, M. B., Spellman, P. T., Brown, P. O., and Botstein, D. Cluster analysis and display of genome-wide expression patterns. Proc. Natl. Acad. Sci. USA, *95:* 14863–14868, 1998.
9. Welsh, J. B., Zarrinkar, P. P., Sapinoso, L. M., Kern, S. G., Behling, C. A., Monk, B. J., Lockhart, D. J., Burger, R. A., and Hampton, G. M. Analysis of gene expression profiles in normal and neoplastic ovarian tissue samples identifies candidate molecular markers of epithelial ovarian cancer. Proc. Natl. Acad. Sci. USA, *98:* 1176–1181, 2001.
10. Hudson, D. L., Guy, A. T., Fry, P., O'Hare, M. J., Watt, F. M., and Masters, J. R. Epithelial cell differentiation pathways in the human prostate: identification of intermediate phenotypes by keratin expression. J. Histochem. Cytochem., *49:* 271–278, 2001.
11. Bootcov, M. R., Bauskin, A. R., Valenzuela, S. M., Moore, A. G., Bansal, M., He, X. Y., Zhang, H. P., Donnellan, M., Mahler, S., Pryor, K., Walsh, B. J., Nicholson, R. C., Fairlie, W. D., Por, S. B., Robbins, J. M., and Breit, S. N. MIC-1, a novel macrophage inhibitory cytokine, is a divergent member of the TGF-β superfamily. Proc. Natl. Acad. Sci. USA, *94:* 11514–11519, 1997.
12. Torres-Rosado, A., O'Shea, K. S., Tsuji, A., Chou, S. H., and Kurachi, K. Hepsin, a putative cell-surface serine protease, is required for mammalian cell growth. Proc. Natl. Acad. Sci. USA, *90:* 7181–7185, 1993.
13. Pizer, E. S., Pflug, B. R., Bova, G. S., Han, W. F., Udan, M. S., and Nelson, J. B. Increased fatty acid synthase as a therapeutic target in androgen-independent prostate cancer progression. Prostate, *47:* 102–110, 2001.
14. Figueroa, J. A., De Raad, S., Tadlock, L., Speights, V. O., and Rinehart, J. J. Differential expression of insulin-like growth factor binding proteins in high versus low Gleason score prostate cancer. J. Urol., *159:* 1379–1383, 1998.
15. Shurbaji, M. S., Kalbfleisch, J. H., and Thurmond, T. S. Immunohistochemical detection of a fatty acid synthase (OA-519) as a predictor of progression of prostate cancer. Hum. Pathol., *27:* 917–921, 1996.
16. Luo, J., Duggan, D. J., Chen, Y., Sauvageot, J., Ewing, C. M., Bittner, M. L., Trent, J. M., and Isaacs, W. B. Human prostate cancer and benign prostatic hyperplasia: molecular dissection by gene expression profiling. Cancer Res., *61:* 4683–4688, 2001.
17. Leung, D., Abbenante, G., and Fairlie, D. P. Protease inhibitors: current status and future prospects. J. Med. Chem., *43:* 305–341, 2000.
18. Hromas, R., Hufford, M., Sutton, J., Xu, D., Li, Y., and Lu, L. PLAB, a novel placental bone morphogenetic protein. Biochim. Biophys. Acta, *1354:* 40–44, 1997.
19. Moore, A. G., Brown, D. A., Fairlie, W. D., Bauskin, A. R., Brown, P. K., Munier, M. L., Russell, P. K., Salamonsen, L. A., Wallace, E. M., and Breit, S. N. The transforming growth factor-β superfamily cytokine macrophage inhibitory cytokine-1 is present in high concentrations in the serum of pregnant women. J. Clin. Endocrinol. Metab., *85:* 4781–4788, 2000.
20. Strelau, J., Sullivan, A., Bottner, M., Lingor, P., Falkenstein, E., Suter-Crazzolara, C., Galter, D., Jaszai, J., Krieglstein, K., and Unsicker, K. Growth/differentiation factor-15/macrophage inhibitory cytokine-1 is a novel trophic factor for midbrain dopaminergic neurons *in vivo*. J. Neurosci., *20:* 8597–8603, 2000.