Hormone Therapy Failure in Human Prostate Cancer: Analysis by Complementary DNA and Tissue Microarrays

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Background: **The molecular mechanisms underlying the progression of prostate cancer during hormonal therapy have remained poorly understood. In this study, we developed a new strategy for the identification of differentially expressed genes in hormone-refractory human prostate cancer by use of a combination of complementary DNA (cDNA) and tissue microarray technologies.** ***Methods:*** **Differences in gene expression between hormone-refractory CWR22R prostate cancer xenografts (human prostate cancer transplanted into nude mice) and a xenograft of the parental, hormone-sensitive CWR22 strain were analyzed by use of cDNA microarray technology. To validate the data from cDNA microarrays on clinical prostate cancer specimens, a tissue microarray of specimens from 26 prostates with benign prostatic hyperplasia, 208 primary prostate cancers, and 30 hormone-refractory local recurrences was constructed and used for immunohistochemical detection of protein expression.** ***Results:*** **Among 5184 genes surveyed with cDNA microarray technology, expression of 37 (0.7%) was increased more than twofold in the hormone-refractory CWR22R xenografts compared with the CWR22 xenograft; expression of 135 (2.6%) genes was reduced by more than 50%. The genes encoding insulin-like growth factor-binding protein 2 (IGFBP2) and 27-kd heat-shock protein (HSP27) were among the most consistently overexpressed genes in the CWR22R tumors. Immunohistochemical analysis of tissue microarrays demonstrated high expression of IGFBP2 protein in 100% of the hormone-refractory clinical tumors, in 36% of the primary tumors, and in 0% of the benign prostatic specimens (two-sided** ***P*** **= .0001). Overexpression of HSP27 protein was demonstrated in 31% of the hormone-refractory tumors, in 5% of the primary tumors, and in 0% of the benign prostatic specimens (two-sided** ***P*** **= .0001).** ***Conclusions:*** **The combination of cDNA and tissue microarray technologies enables rapid identification of genes associated with progression of prostate cancer to the hormone-refractory state and may facilitate analysis of the role of the encoded gene products in the pathogenesis of human prostate cancer. [J Natl Cancer Inst 1999;91:1758–64]**

Despite the widespread use of prostate-specific antigen screening for early detection, prostate cancer remains the second leading cause of cancer-related death among men in western countries *(1)*. Metastatic, hormone-refractory prostate cancer is the end-stage, lethal form of the disease. Defining the molecular mechanisms underlying the transition of an androgen-responsive prostate cancer to a hormone-refractory prostate cancer represents both an intriguing biologic question and a critical clinical problem *(2)*. It is important to better understand the biologic basis of prostate cancer progression, since no effective therapies exist for end-stage, hormone-refractory disease.

There are several *in vitro* and *in vivo* models for the study of hormone-refractory prostate cancer. For example, numerous hormone-independent strains of the LNCaP human prostate cancer cell

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See "Notes" following "References."

line have been developed *(3)*. Several hormone-refractory xenograft model systems also exist. Human xenografts are constructed by the introduction of human prostate tissue or cells into immunodeficient mice where they can be serially transplanted. For example, the CWR22 xenograft tumor grows in nude mice and recurs as hormone-refractory disease after castration of the mice *(4)*. The availability of such model systems will become increasingly powerful, as high-throughput genomic technologies, such as large-scale parallel gene expression analysis with complementary DNA (cDNA) microarrays or serial analysis of gene expression *(5,6)*, become more widely available. The quantity of information obtained from the analysis of the expression of thousands of genes at once creates unique opportunities for research but also poses substantial challenges. For example, which of the hundreds of differentially expressed genes identified in large-scale gene expression surveys are important primary events and which are downstream or secondary changes? Furthermore, are novel genes discovered from experimental model systems of cancer progression also involved in the cancer progression of human patients? By use of traditional methods in molecular pathology, substantial work is required to analyze the frequency of involvement or the clinical significance of just a single gene or protein. We recently developed a tissue microarray-based technology for high-throughput molecular analyses of human cancer *(7)*. This tumor tissue microarray ("tissue chip") technique is based on the arraying of cylindrical biopsy specimens from hundreds of different tumors into a single paraffin block. Consecutive sections of this tissue microarray block can then be used for the analysis of multiple molecular alterations at the DNA, RNA, and protein levels in hundreds of tumors per experiment.

In this study, we combined the cDNA and tissue microarray technologies to identify molecular alterations associated with the progression of human prostate cancer. First, the CWR22/CWR22R human prostate cancer xenograft model *(4)* was used to screen for differential messenger RNA (mRNA) expression of more than 5000 genes between hormone-refractory and hormone-responsive prostate cancers. Two consistently overexpressed genes, insulin-like growth factor-binding protein 2 (IGFBP2) and the 27-kd heat-shock protein (HSP27), were then validated to be involved in clinical prostate cancer progression on the basis of immunohistochemical analysis of the encoded proteins in a prostate cancer tissue microarray containing 264 clinical specimens from various stages of tumor progression.

Materials and Methods

Xenograft tumors. CWR22 is a serially transplantable, human prostate cancer that was derived from a Gleason score 9 primary prostate cancer with osseous metastasis *(8)*. CWR22 is highly responsive to androgen deprivation, with marked tumor regression after castration *(4)*. About half of the treated animals develop recurrent tumors (CWR22R) over a time from a few weeks to several months. CWR22R is not dependent on androgen and is able to grow in castrated animals *(4)*. Nude mice were housed and cared for as described earlier *(8,9)*. Their care was in accord with institutional guidelines. Fresh-frozen human prostate xenograft tissues (one sample from CWR22 and four independent hormone-refractory CWR22R strains) were obtained.

Comparative genomic hybridization. Comparative genomic hybridization was used to characterize the tumor progression in this model system and was carried out essentially as described previously *(10)*, with some modifications. In brief, tumor (test) and normal male (reference) DNAs were labeled by nick translation incorporating either SpectrumGreen or SpectrumRed deoxyuridine diphosphates (Vysis Inc., Downers Grove, IL). Labeled DNAs were hybridized to denatured normal peripheral blood metaphase slides. After acquisition of digital images on wavelengths matching the 4′,6-diamidinophenylindole, SpectrumGreen and SpectrumRed emissions, green-to-red-ratio profiles were quantitated with Quips XL program (Vysis Inc.). Green and red intensities were normalized so that the average green-to-red ratio in each metaphase was set to 1.0. Chromosomal regions where ratios exceeded 1.2 were considered as gained, and those regions where the ratio was less than 0.8 was considered as lost.

cDNA microarrays. RNA was prepared from CWR22/CWR22R xenografts as described by Chirgwin et al. *(11)*, with minor modifications. mRNA was purified with the use of oligo(dT) selection with DynaBeads (Dynamic Analysis Inc., Huntsville, AL) according to the manufacturer's instructions. Two different cDNA microarray formats were used (Clontech Laboratories, Inc. [Palo Alto, CA], and Research Genetics, Inc. [Huntsville, AL]). The Atlas human cDNA expression array from Clontech Laboratories, Inc. contains 588 duplicate spots on a single membrane, each representing 8–10 ng of cDNA of known and sequence-verified genes. These arrays were hybridized with [^{32}P]deoxycytidine triphosphate (dCTP)-labeled cDNA probes prepared from 2 μg of polyadenylic acid–RNA. In addition, we used cDNA array filters from Research Genetics, Inc. (Prostate array, version I), with transcripts known to be expressed in the prostate on the basis of expressed sequence tag (EST) sequences found in normal or malignant cDNA libraries. These filters contained 5184 spots (each with 5 ng of cDNA) of known genes (n = 1960) or expressed sequence tags (ESTs; n = 3224), which were not sequence verified. These arrays were hybridized with [^{33}P]dCTP-labeled cDNAs derived from 50 μg of total RNA. After overnight hybridization at 68 °C in ExpressHyb solution (Clontech Laboratories, Inc.), the filters were washed and exposed to a high-resolution screen (Molecular Dynamics, Sunnyvale, CA) for 3 days and scanned on a Storm PhosphorImager® (Molecular Dynamics). The spot intensities reflecting gene expression levels on the Atlas human cDNA array filter were quantified with ImageQuant® software (Molecular Dynamics), and those on the Research Genetics prostate-specific filter were quantified with a custom software (Dearray software: Y. Chen). The normalization of the spot intensities within an experiment (CWR22R versus CWR22) was done on the basis of the average of the intensities of all spots. The gene expression profiles of the CWR22Rs were compared with the gene expression profile of CWR22. To define genes/ESTs as underexpressed or overexpressed, an at least two-fold expression difference was required. In addition, visual confirmation of all differentially expressed spots on filters was performed. The gray-scale images were pseudocolored (red for hormone refractory and green for hormone responsive) and overlaid for better visualization of the relative expression intensities with Adobe Photoshop software (Adobe Systems Inc., San Jose, CA).

Reverse transcription–polymerase chain reaction (RT–PCR). cDNA was prepared by reverse transcriptase reaction by use of oligo(dT) primer (Research Genetics, Inc.). PCR was carried out with specific primers for the IGFBP2 (Gene Bank #M35410) and HSP27 gene (Gene Bank #M54079) at an annealing temperature of 55 °C for 27 cycles generating 391-base-pair (bp) and 260-bp products, respectively. Aliquots of the reaction products were subjected to electrophoresis on a 2% agarose gel and visualized by staining with ethidium bromide. Amplification of the human asparagine synthetase gene by use of specific primers was used as a control.

Prostate tissue microarray. Formalin-fixed and paraffin-embedded tumor and benign control specimens were obtained from the archives of the Institutes for Pathology, University of Basel (Switzerland) and the Tampere University Hospital (Finland). All sections of tumors and controls were reviewed by one pathologist (L. Bubendorf). Tumor grading was performed according to the method of Gleason *(12)*. The specimens included 208 primary prostate cancers, 30 transurethral resection specimens from locally recurrent hormone-refractory cancers operated on from 1976 through 1997, and 26 transurethral resections for benign prostatic hyperplasia as benign controls. The group of primary (non-hormone-refractory) prostate cancers consisted of 56 incidentally detected tumors in transurethral resections for presumed benign prostatic hyperplasia (stage T1a or b), 137 radical prostatectomy specimens from patients with clinically localized disease (stage T2), and specimens from 15 patients with locally extensive disease (stage T3 or T4) *(13)*. More than one sample per tumor specimen was arrayed in 34 of the 238 patients. In these cases, the sample with the strongest immunohistochemical staining was chosen for the immunohistochemical classification. The array also included 114 autopsy specimens from hormone-refractory metastatic prostate can-

cers. These were excluded from this analysis, since immunohistochemistry is often unreliable in tissues from routine autopsies because of protein degradation. The prostate tissue microarray was constructed as previously described *(7).* In brief, core tissue biopsy specimens (diameter, 0.6 mm) were taken from the least differentiated regions of individual paraffin-embedded prostate tumors (donor blocks) and precisely arrayed into a new recipient paraffin block (35 × 20 mm) with a custom-built precision instrument (Beecher Instruments, Silver Spring, MD). After the block construction was completed, 5-μm sections were cut with a microtome by use of an adhesive-coated tape sectioning system (Instrumedics, Hackensack, NJ) to support the adhesion of the array elements. The presence of tumor tissue on the arrayed samples was verified on an hematoxylin–eosin-stained section.

Immunohistochemistry. Antigen retrieval was performed by treatment in a pressure cooker for 5 minutes. Standard indirect immunoperoxidase procedures were used for immunohistochemistry (ABC-Elite; Vector Laboratories, Inc., Burlingame, CA). A goat polyclonal antibody, C-18 (1 : 1000; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) was used for detection of IGFBP2. HSP27 protein was detected by use of a monoclonal mouse antibody HSP27 (1 : 100; BioGenex Laboratories, San Ramon, CA). The reactions were visualized by diaminobenzidine as a chromogen. The primary antibodies were omitted for negative staining controls. The intensity of the cytoplasmic IGFBP2 and HSP27 staining was classified into four groups (negative, weak, intermediate, and strong staining). The number of tumors that could be analyzed for IGFBP2 and HSP27 expression differed slightly from each other because of loss of representative prostate cancer tissue on consecutive sections of some punch samples.

Statistical analysis. Contingency table analysis was used to analyze the relationship between immunohistochemical staining, grade, and stage (total chi-squared test). All *P* values were two-sided.

RESULTS

Analysis of Chromosomal Alterations by Comparative Genomic Hybridization

The hormone-sensitive CWR22 xenograft contained five chromosomal aberrations, including gain of 1q, gain of whole chromosomes 7, 8, and 12, and loss of 2q. The same five aberrations were also present in the hormone-refractory CWR22R xenograft, indicating that the recurrent tumor was a clonal derivative of the primary CWR22. In addition, the CWR22R showed a gain of chromosome 14q, which was not present in the primary CWR22 (data not shown).

cDNA Microarray Analysis of Gene Expression Changes

cDNA microarray experiments were first performed with a nylon filter-based 588 clone array (Clontech Laboratories, Inc.). This analysis revealed 10 overexpressed and 14 underexpressed genes in at least two or more of the four hormone-refractory CWR22R xenografts as compared with the hormone-responsive CWR22 xenograft (Table 1). Among these, HSP27 was substantially overexpressed in three of the four CWR22R strains (median ratio, 2.6) and IGFBP2 in all four CWR22Rs (median ratio, 2.6). Two other members of the insulin-like growth factor (IGF) pathway—insulin-receptor and IGF-II—were also markedly overexpressed in two of the four CWR22R xenografts. RT–PCR analysis confirmed the finding that the expression of IGFBP2 and HSP27 was increased in hormone-refractory CWR22R strains as compared with hormone-sensitive CWR22 strains (Fig. 1).

In addition to these consistently differentially regulated genes in two or more xenograft specimens, 47 genes were overexpressed and 89 genes were underexpressed in only one of the four hormone-refractory CWR22R xenografts.

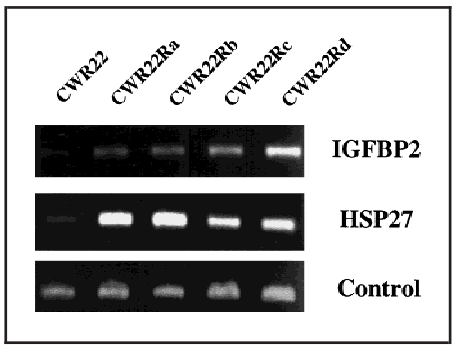

Fig. 1. Reverse transcription–polymerase chain reaction analysis of insulin-like growth factor-binding protein 2 (IGFBP2), 27-kd heat-shock protein (HSP27), and asparagine synthetase (internal control) expression in one hormone-responsive (CWR22) and in four hormone-refractory prostate cancer xenografts (CWR22Ra–d).

To further explore the differential gene expression patterns in hormone-refractory prostate cancer, we analyzed the same tumors with a much larger cDNA microarray (5184 spots, Research Genetics, Inc.) containing a comprehensive collection of genes and ESTs found to be expressed in

Table 1. Most consistently overexpressed and underexpressed genes in the complementary DNA microarray experiments and the ratios of gene expression in hormone-refractory human prostate cancer xenografts (CWR22Ra–d) compared with gene expression in a xenograft of the hormone-sensitive strain CWR22

Gene name	Chromosomal location	Ratios				
		CWR22 Ra	CWR22 Rb	CWR22 Rc	CWR22 Rd	Median
Overexpressed						
IGFBP2	2q33–q34	2.7	2.4	2.6	5.3	2.6
Heat-shock 27-kd protein	7q	2.6	2.7	1.5	4.8	2.6
Insulin receptor	19p13.3–p13.2	1.8	1.5	2.9	5.3	2.4
Transcription factor LCR-F1	7q32	3.2	3.1	0.8	0.9	2.0
BSP-1	4q28	2.8	2.9	1.1	1.1	2.0
P14-cyclin dependent kinase inhibitor	9p21	2.4	2.7	1.2	1.3	1.9
Insulin-like growth factor-II	11p15.5	1.1	0.7	2.7	2.1	1.6
Homeobox protein HOX-A4	7p15–p14	2.0	2.1	1.2	1.1	1.6
Tumor suppressor protein DCC	18q21.1	2.2	2.6	0.7	0.7	1.5
ETS variant gene 3	1q21–q23	2.1	2.1	0.9	0.9	1.5
Underexpressed						
Oncostatin M	22q12.1–q12.2	0.2	0.3	1.1	0.5	0.4
Integrin alpha 2B	17q21.32	0.5	0.3	1.4	0.2	0.4
T-lymphocyte-secreted protein I-309	17	0.3	0.6	0.7	0.5	0.5
CD40 ligand	Xq26	0.4	0.6	0.8	0.3	0.5
Acyl-COA-binding protein	2q12–q21	0.4	1.2	0.5	0.4	0.5
Interleukin 9 receptor	Xq28 or Yq12	0.4	0.4	0.8	0.6	0.5
E-selectin	1q22–q25	0.8	0.6	0.3	0.4	0.5
Fms-like tyrosine kinase 4	5q34–q35	0.3	0.7	0.5	0.4	0.6
Interleukin 2 receptor alpha chain	10p15–p14	0.4	0.5	1.1	1.3	0.8
Hepatoma-derived growth factor	Xq25	0.4	0.5	1.0	1.8	0.8
Interleukin 7 receptor alpha chain	5p13	0.4	0.5	1.0	1.9	0.8
Cyclin H	5q13.3–q14	1.0	1.7	0.5	0.3	0.8
SHB adaptor protein	9p12–p11	1.4	1.7	0.5	0.5	1.0
Clusterin	8p21–p12	0.4	0.3	2.0	2.0	1.2

*IGFBP2 = insulin-like growth factor-binding protein 2; LCR-F1 = locus control region F1; BSP-1 = transforming growth factor-β signaling protein-1; DCC = deleted in colorectal carcinoma; ETS = E-twenty-six specific; SHB = src homology B.

cDNA libraries from normal or malignant prostate. Altogether, 172 overexpressed or underexpressed genes or ESTs (approximately 3%) in at least three of the four hormone-refractory derivatives were discovered as compared with the untreated, hormone-sensitive human prostate cancer xenograft. Thirty-seven transcripts (0.7%) were substantially (ratio >2) elevated and 135 (2.6%) were underexpressed (ratio <0.5) in the CWR22R xenografts. A pseudocolored overlay of one CWR22/CWR22R comparison and the corresponding ratio distribution are shown in Fig. 2.

Histology and Immunohistochemistry

To evaluate whether the gene expression changes seen in the hormone-refractory CWR22R tumors reflected molecular changes involved in tumor progression in patients with prostate cancer, we created a tissue microarray to analyze the expression of two overexpressed genes, IGFBP2 and HSP27, at the protein level in 238 different human prostate cancers and in 26 benign prostate tissues. The total number of evaluable specimens on the tissue microarray was 264 for the IGFBP2 and 258 for the HSP27 immunostaining.

In these arrayed clinical specimens, a strong association was seen between increased IGFBP2 and HSP27 protein expression and the progression of prostate cancer to hormone-refractory disease (Fig. 3). A strong cytoplasmic IGFBP2 staining was present in all of the 30 locally recurrent, hormone-refractory prostate cancers, in 74 (36%) of the 208 primary tumors, and in none of the 26 benign prostate specimens (Fig. 3; $P = .0001$, two-sided). HSP27 was strongly expressed in nine (31%) of 29 recurrent tumors, in 11 (5%) of 204 primary tumors, but never in the secretory prostate epithelial cells of 25 benign prostatic hyperplasia specimens (Fig. 3; $P = .0001$, two-sided). There was no statistically significant association between IGFBP2 or HSP27 expression and tumor grade or T stage in the primary tumors (data not shown). A subgroup of 36 patients had received primary neoadjuvant endocrine therapy before radical prostatectomy, but their IGFBP2 and HSP27 expression data were similar to those of the untreated patients (data not shown).

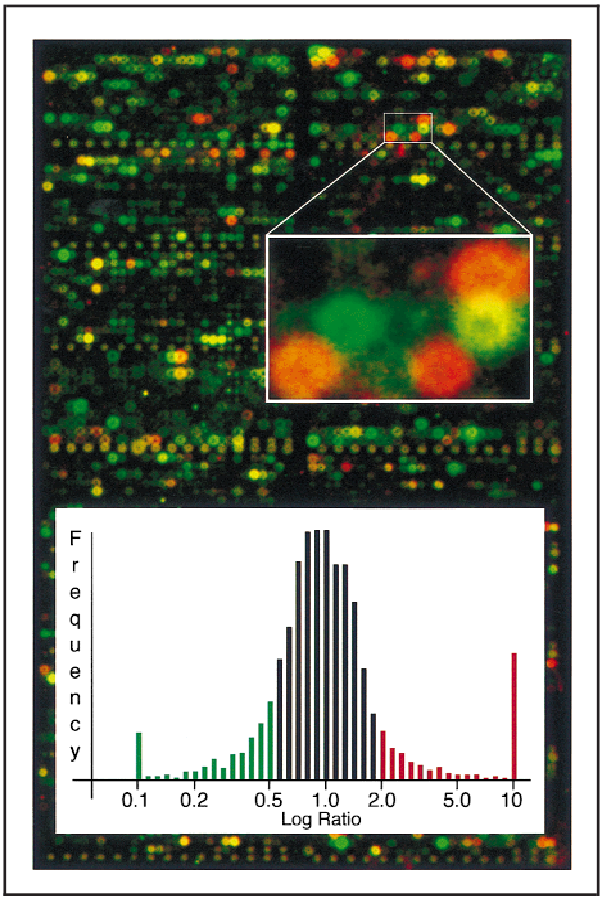

Fig. 2. Hybridization of the prostate complementary DNA microarray containing 5184 genes (Research Genetics, Inc.). A color image overlay of the CWR22 hybridization (**green**) and CWR22R recurrent xenograft (**red**) is shown. Spots with more **red** color represent transcripts overexpressed in the hormone-refractory tumor in comparison to the primary tumor, **yellow** spots indicate genes that were equally abundant, and **green** spots indicate underexpressed genes in CWR22R. Genes that were not expressed in either of the two tissues appear in the **black** background color. Inset (histogram) shows a normal frequency distribution of the $\log_{10}$ intensity ratios for CWR22R versus CWR22 for all of the 5184 spots on the microarray. The ratios are displayed on the *x*-axis, and the relative frequency of genes with the given ratios is indicated on the *y*-axis. Ratios of genes that have a twofold or higher expression in the recurrent than in the primary xenograft tumor are shown as **red bars** and those with a 50% or more reduction as **green bars.**

Discussion

The transition from a hormone-sensitive human prostate cancer to a hormone-refractory recurrent strain in the CWR22 xenograft model system resembles the clinical progression of human prostate cancer *(4)*. As shown in this study by comparative genomic hybridization, there was a close clonal genetic relationship between the primary and recurrent xenograft tumors. Furthermore, many of the alterations seen by comparative genomic hybridization in this model system, such as gains of chromosome 7 and 8, are similar to those commonly found in clinical specimens from patients with prostate cancer. The cDNA microarray technology allows rapid, large-scale screening of expression of hundreds or thousands of genes in a single experiment *(5)*. Here, up to 170 genes (3.3%) were identified to be differentially expressed between the primary and recurrent (hormone-sensitive and hormone-refractory) xenograft tumors. This high number of differentially expressed genes illustrates the complex molecular basis of prostate cancer progression. The regrowth of the hormone-refractory tumor during androgen deprivation therapy may necessitate a complex reprogramming of multiple key regulatory mechanisms involving cell growth, apoptosis (i.e., programmed cell death), and other signaling pathways. It will be important to identify the molecular mechanisms that contribute to the development of recurrent tumors and to examine if some of the signaling pathways involved would provide starting points for the development of novel diagnostic or therapeutic approaches for patients with advanced, hormone-refractory prostate cancers.

The translation of gene-expression findings from model systems to human patients with cancer presents several challenges. First, although this xenograft model system displayed phenotypic properties resembling human prostate cancer progression, it remains important to validate whether the same alterations of gene expression and the same signaling pathways contribute to the disease progression in human cancer patients. Second, to utilize the cDNA microarray data for the development of improved diagnostic or therapeutic approaches, it remains critically important not only to screen for expression of many different genes but also to screen many different tumor tissues and establish an accurate frequency of in-

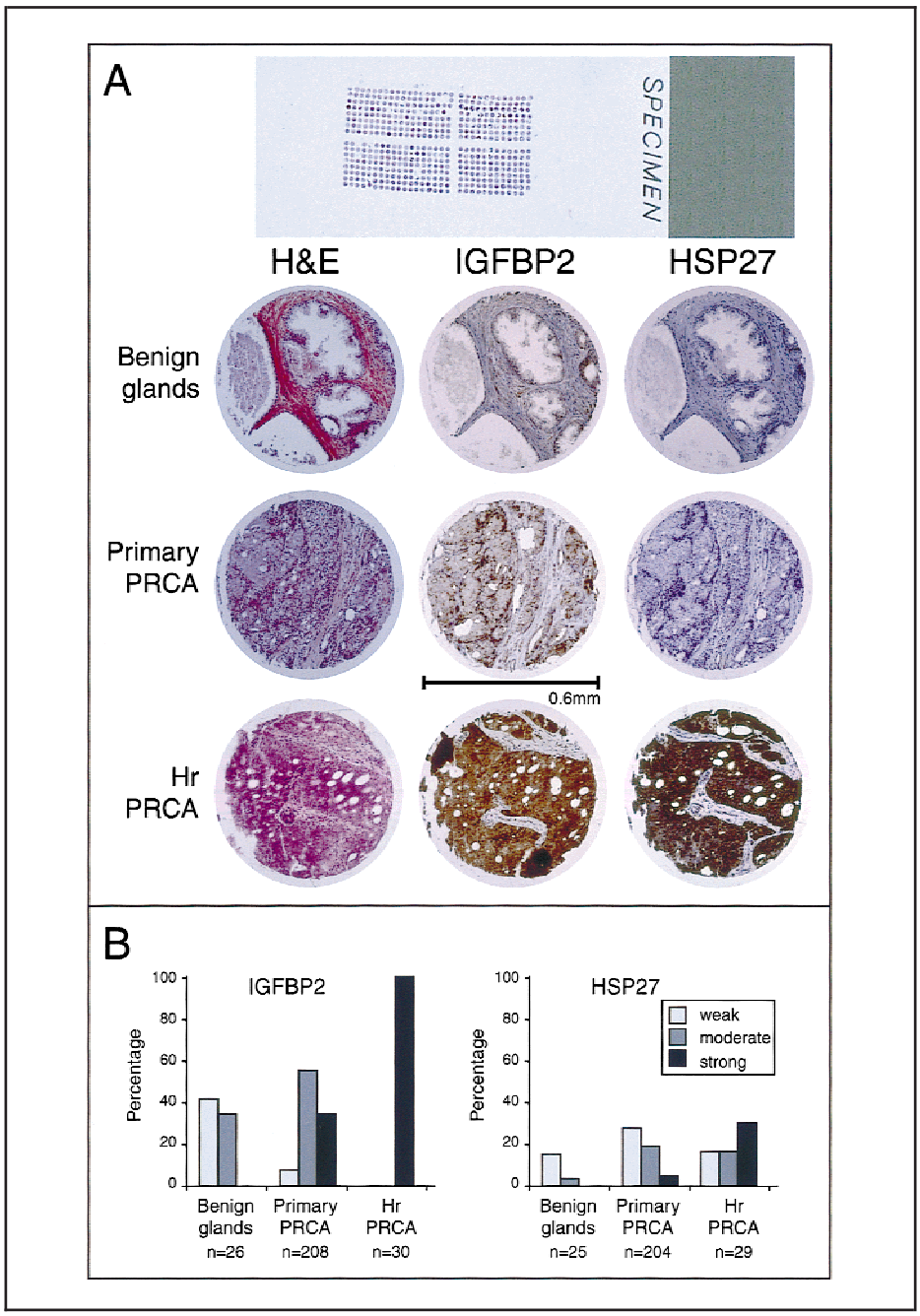

Fig. 3. A) Hematoxylin–eosin and immunohistochemical staining of insulin-like growth factor-binding protein 2 (IGFBP2) and 27-kd heat-shock protein (HSP27) on the prostate cancer tissue microarray (original magnification ×200). Benign prostate glands show no immunoreactivity. Primary untreated prostate cancer (PRCA) demonstrates weak immunostaining of IGFBP2 but no immunoreactivity of HSP27. In contrast, hormone-refractory prostate cancer with local recurrence (Hr PRCA) shows strong expression of both IGFBP2 and HSP27. **B**) Frequency distribution of expression of IGFBP2 and HSP27 during progression to hormone-refractory prostate cancer as measured by immunohistochemistry on a prostate cancer tissue microarray.

volvement of these genes in different stages of the prostate cancer progression. A substantial amount of work is required to fully explore the role of just a single gene in cancer. Before performing full-length cDNA cloning, functional analyses, and other tedious experiments, one would have to prioritize the long list of potential target genes that always emerges from cDNA microarray experiments and to perform large-scale studies of clinical specimens. In this study, we first took advantage of the fact that the pattern of gene expression in the recurrent xenograft tumors was different from one animal to another. Therefore, we decided to first concentrate on those genes that were differentially expressed in two or more recurrent xenograft tissues. One would expect that such genes are more likely to be associated with hormone therapy failure, whereas genes that are only overexpressed in one case may be important only for that particular tumor. The decisive step was the evaluation of gene expression patterns in clinical specimens by use of our newly developed tissue microarray technology.

Evaluation of all candidate genes emerging from the present cDNA microarray experiments in a large series of uncultured clinical tumors would take years if traditional methods were used. Furthermore, after a few hundred genes had been analyzed, one would run out of the available tumor tissues. Tissue microarray technology substantially facilitates the translation of basic research findings to clinical applications *(7)* and makes it possible to perform *in situ* analysis of hundreds of tumors either at the DNA, the RNA, or the protein level. This study was done with immunocytochemical techniques, but expression analyses of newly identified genes could also be analyzed by mRNA *in situ* hybridization when antibodies are not available. Such a strategy allows one to quickly validate and further explore in a large number of clinical specimens the *in vivo* significance of candidate genes discovered with the cDNA microarrays. Only minute amounts of tissues are required to make the tissue microarray blocks, causing minimal damage to the original tumor blocks. Since one can generate multiple replicate tissue microarray blocks, each of which can be sectioned 200–300 times, one could easily generate thousands of tissue microarray sections from the same set of clinical tumor material. Each section can be utilized for the analysis of a different molecular marker.

The small size of the samples makes tissue microarrays a powerful screening tool. However, the small tissue samples may not always be representative of the whole tumor and, therefore, the prevalence of a molecular alteration in a tissue microarray analysis may be underestimated. However, sampling bias may not be a serious concern if the tumor areas are carefully selected for punching. In our previous studies *(7,14)*, we found a high concordance between gene-amplification frequencies on tissue microarrays when compared with the data from the literature. The representativeness of tissue microarray data could be improved by in-

cluding several samples from different sites of a tumor on each array. Furthermore, comparisons of the involvement of one gene against another on the same array or comparisons of one molecular alteration between two different stages of tumor progression will generate relative frequency estimates that are not biased by the sampling method. Nevertheless, tissue microarray technology should be regarded as a rapid, high-throughput tool to survey many different genes and markers to identify those that are most promising for clinical applications. These would then have to be tested on conventional tissue specimens before clinical application.

The tissue microarray results validated that overexpression of IGFBP2 may be an important event in hormone-refractory prostate cancer, not only in the CWR22 xenograft model system but also in patients who had developed a recurrent tumor during androgen deprivation therapy. This finding is in agreement with recent experimental and clinical studies *(15–19)* indicating that the IGF system may be a key growth regulatory pathway in prostate cancer. IGFBP2 is a member of the IGF growth factor system, which involves two growth factors (IGF-I and IGF-II), two IGF receptors (type I and II), seven IGF-binding proteins (IGFBP1–7), as well as IGFBP proteinases *(16,20)*. IGF-I stimulates growth and inhibits apoptosis in normal and transformed epithelial cells *(21–24)*. High plasma levels of IGF-I were recently shown to be associated with increased risk of getting prostate cancer *(17)*. Moreover, IGF-I has been shown to enhance androgen receptor-mediated gene transcription in the prostate cancer cell lines DU 145 (after cotransfection with an androgen-inducible reporter gene and an androgen receptor expression vector) and LNCaP in the absence of androgen, suggesting that IGF-I may drive the androgen-signaling pathway in hormone-refractory prostate cancer *(25)*. IGFBPs can enhance or inhibit the bioactivity of IGFs (IGF-I and IGF-II) by modulating the availability of free IGFs for their receptors *(26,27)*. IGFBP2 has also been suggested to be an enhancer of IGF-I function *(22)*. It can be speculated that overexpression of IGFBP2 promotes survival and androgen-independent growth of prostate cancer by increasing the bioavailability of IGFs. Members of the same pathway (IGF-II and insulin receptor) were also overexpressed in some of the hormone-refractory xenograft tissues. However, IGFBP2 was systematically and most highly overexpressed, suggesting that it may perhaps have a central role in modulating the IGF signaling in hormone-refractory prostate cancer. Alterations of IGFBP2 may also play a role in the development and progression of other tumor types, such as breast, colorectal, and ovarian cancers *(28–30)*. Overexpression of IGFBP2 has also been observed in cell lines established from several solid tumors *(31,32)*.

The overexpression of HSP27 in about one third of hormone-refractory prostate cancers but in only 5% of primary tumors is intriguing in light of the fact that HSP27 has been shown to increase resistance to apoptosis induced by several drugs such as doxorubicin *(33–36)*. Blockage of apoptosis may be an important feature of hormone-refractory prostate cancer and has been associated with the differential expression of the Bcl-2 gene family *(37–39)*. It was recently suggested that HSP27 and Bcl-2 act at different levels to prevent apoptosis in immortalized embryo fibroblasts, depending on the type of apoptotic stimulus *(40)*. The role of HSP27 as a predictor of patient outcome or response to therapy has received attention in breast cancer *(41–44)*, but it has not been extensively studied in prostate cancer. In one study *(45)*, variable HSP27 immunostaining was found in 13 prostate tumors derived from transurethral resection specimens, but no information about the hormonal treatment status was provided. Another study *(46)* did not find HSP27 immunoreactivity in radical prostatectomy specimens from patients with clinically localized disease. On the basis of this study, HSP27 expression is unlikely to play a major role in primary prostate cancer but may be important in hormone therapy failure.

In summary, we describe a new strategy based on the combination of cDNA and tissue microarray technologies to explore the molecular basis of human prostate cancer progression. Our results indicate that multiple gene expression changes may contribute to prostate cancer progression and hormonal therapy failure and that at least some of the mechanisms involved in the CWR22 xenograft model system may be similar to those contributing to therapy failure and hormone-refractory prostate cancer growth in patients. We detected an association between increased expression of IGFBP2 and HSP27 and the hormone therapy failure in both the xenograft model system and in patients' specimens. Further studies are needed to evaluate these molecules as well as dozens of other differentially expressed genes as diagnostic or therapeutic targets for hormone-refractory prostate cancer.

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