Expression Profiling Reveals Hepsin Overexpression in Prostate Cancer

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Abstract

Prostate cancer is the most commonly diagnosed neoplasm among men. Despite this fact, many of the genetic changes that coincide with prostate cancer progression remain enigmatic. We have addressed this problem by characterizing the expression profiles of several benign and malignant human prostate samples, and we have identified several genes that are differentially expressed between benign and malignant glands. One gene that was overexpressed encodes the serine protease hepsin. We used an independent sample set to confirm that hepsin is overexpressed in prostate tumors, and in situ hybridization demonstrates that hepsin is specifically overexpressed in the carcinoma cells themselves. These facts, together with the molecular properties of hepsin, make it an ideal target for prostate cancer therapy.

Introduction

Prostate cancer poses a significant clinical challenge both in terms of its prevalence and its complexity. In the year 2001, ~198,000 new cases of prostate cancer will be diagnosed in the United States, and ~32,000 American males will die of this disease (1). These patients will harbor heterogeneous tumors that dramatically vary in their rate of progression and their response to therapy. The scope of this problem has prompted intense scrutiny into the molecular genetics of prostate cancer. Recent studies have identified the PTEN, p27, and Nkx3.1 gene products as tumor suppressors in prostate epithelia (2, 3). Moreover, the well-characterized oncogenes c-myc and bcl-2 are commonly overexpressed in prostate tumors (2). Efforts to identify additional prostate cancer-related genes will be facilitated by microarray-based gene expression analyses used previously to analyze other tumor types. For example, Golub et al. (4) have used microarrays to discriminate between acute myeloid leukemias and acute lymphoblastic leukemias according to their expression profiles. More recently, microarray analysis has been used to characterize the molecular profiles of non-Hodgkin’s lymphomas, breast cancer, colon cancer, and glioblastomas (5–8). Here we present the expression profiles of prostate tumor specimens of varying histological grades and clinical stages. By comparing the profiles of benign and malignant prostate specimens, we noted considerable heterogeneity between tumors but also identified several genes that are over- or underexpressed in a majority of tumors relative to normal prostate tissues. Among these tumor-associated genes was the gene hepsin. Hepsin encodes a transmembrane serine protease that is also overexpressed in ovarian carcinomas and renal cell carcinomas (9, 10). Its overexpression in prostate cancer was confirmed molecularly on an independent panel of prostate specimens and by in situ hybridization. Thus, hepsin offers an enticing drug target for prostate cancer therapy.

Materials and Methods

Tissue Preparation and Probe Synthesis. Frozen tissue specimens were obtained from the Siteman Cancer Center Tissue Procurement Core at Washington University School of Medicine and from the University of Washington Medical Center under an Institutional Review Board-approved protocol. The specimens were blocked, and frozen sections were cut and analyzed by a single pathologist (P. A. H.) for Gleason grade and percentage of tumor. The tissues were then sectioned into Trizol (Life Technologies, Inc.) for RNA preparation. Antisense biotinylated riboprobes were synthesized from benign and tumor RNA samples as per the Affymetrix GeneChip protocol. Probes were then hybridized to Human 6800 Affymetrix GeneChips representing 7088 full-length human transcripts.

GeneChip Data Analysis. Average difference values from each individual chip were scaled such that the average intensity of any given chip was 1500. We discarded genes that were scored “absent” in every sample according to the Affymetrix Array Suite software package, leaving a filtered set of 4712 genes that were scored “present” on at least one chip. We established a lower boundary of 70.8 for the scaled average difference values of the remaining genes. This baseline represents the scaled noise of the “noisiest” chip included in the analysis.

To identify specific genes that were misexpressed in tumors relative to normal samples, we generated a computer algorithm allowing us to select genes exhibiting a ≥3-fold expression change in all of the 11 tumors relative to all of the four normal glands. We applied this algorithm to the scaled average difference values of the 4712-gene filtered data set. P values were calculated by a two-tailed t test for independent data sets of unequal size and variance.

Quantitative RT-PCR Analysis. RNA was isolated from tissue samples as described above. Total RNA was reverse transcribed, and quantitative PCR was performed using the ABI sequence detection system 7700 machine. Amplicons were detected by SYBR green I fluorescence as described elsewhere (11). Data were analyzed with standard curves to determine relative gene expression levels.

In Situ Hybridization. Sense and antisense digoxigenin-labeled RNA probes for in situ hybridization were transcribed from a fragment of the hepsin cDNA corresponding to nucleotides 1430–1730. The probe was hybridized to fresh frozen tissue samples as described previously (12). Briefly, frozen sections were fixed with 4% paraformaldehyde in 1× PBS and treated with active 0.1% diethyl pyrocarbonate for 2 × 15 min. Probes were hybridized at 58°C for 40 h in 50% formamide/5× SSC (pH 7.0). After washing, signal was detected by alkaline phosphatase-conjugated antidigoxigenin antibodies (Roche) followed by incubation with nitroblue tetrazolium and 5-bromo-4-chloro-3-indoly phosphate.

Results

Expression Profiling of Benign and Malignant Prostate Specimens. We analyzed the gene expression profiles of 11 malignant and four nonmalignant prostate samples. Tumor specimens included five low-grade primary tumors (Gleason grade 6), three high-grade samples (Gleason grade 7–9), one sample with local extraprostatic invasion.
sion into the seminal vesicle, and two lymph node metastases. To minimize the contributions of benign glands to the tumor profiles, we dissected the tumor samples to achieve >50% tumor within the sample for the primary tumors and 90–100% tumor for the metastases. We synthesized probes for the Affymetrix Hu6800 GeneChips and analyzed the expression data as described in the “Materials and Methods.”

We generated a list of 4712 probe sets that were scored present on at least one chip by the Affymetrix Gene Expression Suite software. From this list, we sought to identify genes that are misregulated in tumors relative to benign specimens or misregulated in metastases relative to primary tumors. In comparing tumors with benign samples, we identified genes of which the scaled average difference values varied by ≥3-fold in all 11 tumor samples relative to all four of the benign samples (Table 1). In comparing metastases to primary tumors, we identified genes of which the scaled average difference values varied by ≥3-fold in all three of the metastases relative to all eight of the primary tumors (Table 1).

The initial comparison of tumors to benign glands revealed only a single up-regulated gene, hepsin, and no down-regulated genes (Fig. 1A). Therefore, we relaxed the stringency of our selection criteria to identify genes that exhibited a 3-fold expression difference in 9 of 11 tumors relative to all of the four benign samples. These criteria identified three additional misregulated genes (Fig. 1B). The serotonin receptor 2B and a cdk-related gene, cdk10/PISSLRE, were both up-regulated in tumors relative to benign glands, whereas the single down-regulated gene encodes a catalytically inactive phosphoglucomutase-related protein, Pgm-5. Statistically, the scaled average difference values of all four of the genes significantly varied between benign and tumor sample sets (P < 0.05). However, comparing expression levels in low-grade tumors to high-grade tumors to metastases did not reveal any correlations between pathological grade and gene expression (data not shown).

The comparison of metastases to primary tumors revealed three genes that were down-regulated in all three of the metastases relative to all eight of the primary tumors: G protein α16, pyridoxal kinase, and diacylglycerol kinase (Table 1). We relaxed the selection criteria to include genes exhibiting a 3-fold difference among all three of the metastases and seven of eight tumor samples. These criteria identified

### Table 1 Misregulated genes in benign and malignant prostate samples

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession</th>
<th>Mean avg difference</th>
<th>Mean avg difference</th>
<th>Mean fold difference</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>cdk 10</td>
<td>L33264</td>
<td>$71 \pm 0^b$</td>
<td>$950 \pm 519^c$</td>
<td>13.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hepsin</td>
<td>X07732</td>
<td>$186 \pm 230^b$</td>
<td>$7915 \pm 4939^c$</td>
<td>42.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serotonin receptor 2B</td>
<td>X77307</td>
<td>$71 \pm 0^a$</td>
<td>$322 \pm 161^c$</td>
<td>4.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Phosphoglucomutase 5</td>
<td>L40933</td>
<td>$1136 \pm 373^b$</td>
<td>$109 \pm 83^c$</td>
<td>10.4</td>
<td>0.011</td>
</tr>
<tr>
<td>HMG-CoA reductase</td>
<td>M11058</td>
<td>$80 \pm 27^f$</td>
<td>$335 \pm 73^f$</td>
<td>4.2</td>
<td>0.021</td>
</tr>
<tr>
<td>Rad50</td>
<td>U63159</td>
<td>$82 \pm 24^f$</td>
<td>$333 \pm 36^f$</td>
<td>4.1</td>
<td>0.003</td>
</tr>
<tr>
<td>TYRO3 protein tyrosine kinase</td>
<td>U18934</td>
<td>$952 \pm 301^f$</td>
<td>$134 \pm 57^f$</td>
<td>7.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diacylglycerol kinase</td>
<td>X62535</td>
<td>$1294 \pm 527^f$</td>
<td>$127 \pm 97^f$</td>
<td>10.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pyridoxal kinase</td>
<td>U99686</td>
<td>$628 \pm 487^f$</td>
<td>$71 \pm 0^a$</td>
<td>8.8</td>
<td>0.014</td>
</tr>
<tr>
<td>Stabilin 1/JIAA0246</td>
<td>D87433</td>
<td>$1240 \pm 506^f$</td>
<td>$98 \pm 24^f$</td>
<td>12.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Protein kinase C, β 1</td>
<td>X07109</td>
<td>$1125 \pm 541^f$</td>
<td>$80 \pm 16^f$</td>
<td>14.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Butyrophilin</td>
<td>U90352</td>
<td>$390 \pm 196^f$</td>
<td>$71 \pm 0^a$</td>
<td>5.5</td>
<td>0.002</td>
</tr>
<tr>
<td>Early growth response 3</td>
<td>X63741</td>
<td>$596 \pm 374^f$</td>
<td>$71 \pm 0^a$</td>
<td>8.0</td>
<td>0.005</td>
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<tr>
<td>Early growth response 2</td>
<td>J04076</td>
<td>$881 \pm 615^f$</td>
<td>$101 \pm 12^f$</td>
<td>8.7</td>
<td>0.009</td>
</tr>
<tr>
<td>G protein α16</td>
<td>M63904</td>
<td>$913 \pm 752^f$</td>
<td>$71 \pm 0^a$</td>
<td>12.9</td>
<td>0.016</td>
</tr>
</tbody>
</table>

* Up-regulated in tumors relative to normal.
* Normal.
* Tumor.
* Down-regulated in tumors relative to normal.
* Up-regulated in metastases relative to primaries.
* Primaries.
* Mts.
* Down-regulated in metastases relative to primaries.
Discussion

The advent of microarray technology has prompted significant change in cancer research. By analyzing the expression of thousands of genes concurrently, the genetic changes that accompany tumorigenesis can be efficiently identified. We have used microarray-based expression profiling, quantitative RT-PCR, and in situ hybridization to identify and validate prostate cancer-associated genetic changes. Whereas these analyses yielded only two consistent, cancer-associated gene expression changes, we believe this reflects the histological and biological heterogeneity of the prostate cancer specimens. To minimize the occurrence of false positive genes, we selected genes that were misexpressed in >80% of tumors relative to all of the benign samples. However, given the fact that prostate cancer exhibits dramatic clinical variability and complex intratumoral cellular heterogeneity, we expect these samples to exhibit diverse molecular profiles. Indeed, hierarchical cluster analysis failed to segregate the prostate samples into meaningful clinical or pathological subgroups (data not shown). Furthermore, several prostate cancer-associated genes, including c-myc, insulin-like growth factor-I, p27, and hepatocyte growth factor, all displayed variable expression across all of the tumor samples.

One gene that is consistently down-regulated in tumor samples, Pgm-5, shares close homology with members of the phosphoglucomutase family. These genes encode proteins that convert glucose-1-phosphate to glucose-6-phosphate. However, Pgm-5 lacks a functional catalytic domain (17). Rather, Pgm-5 associates with the structural proteins dystrophin and utrophin, predominantly in smooth muscle cells (17). Whereas the observed difference between benign and tumor Pgm-5 expression may simply reflect the altered cellular compositions of tumors, other smooth muscle markers such as smooth muscle myosin and dystrophin did not differ as dramatically or consistently. We suggest that the Pgm-5 expression changes may reflect a bona fide smooth muscle response to tumorigenesis.

The most notable misregulated gene, hepsin, belongs to a family of trypsin-like transmembrane serine proteases that also includes TMPRSS2, corin, and enterokinase (14). Characteristic features of these type II integral membrane proteins include an extracellular serine protease domain, a scavenger receptor cysteine-rich domain, and an intracellular domain (14). Previous studies have reported hepsin expression predominantly in the liver, though low levels are also observed in the prostate gland (18). Interestingly, pathological overexpression of hepsin has also been reported in renal cell carcinoma and in most ovarian carcinomas (9, 10). These observations, together with our results, strongly imply a role for hepsin in the progression of several epithelial cancers.

Whereas the role of hepsin in prostate cancer progression awaits additional investigation, its homology to other serine proteases does offer tantalizing hypotheses. In general, extracellular proteases serve crucial functions in tumor progression, because they regulate growth factor availability and trigger the angiogenic switch. Furthermore, a related transmembrane serine protease, corin, cleaves and activates proatrial natruretic peptide (19). Thus, hepsin may cleave a currently unknown substrate, such as a growth factor propeptide, to promote tumor growth.

Several properties of hepsin make it an ideal target for prostate cancer therapy. The trypsin-like catalytic domain of hepsin is extracellular, thus facilitating drug delivery. Furthermore, the dramatic difference between hepsin expression in benign glands and malignant tissues suggests that prostate carcinomas would be acutely sensitive to hepsin inhibitors. Finally, hepsin-specific inhibitors should exhibit minimal toxicities, because hepsin-deficient mice lack an overt phenotype (20). Genetic studies with
mouse tumor models should help define the requirement for hepsin in tumor progression, and the genetic changes responsible for tumor-specific hepsin overexpression also warrant additional exploration. Given the known biological properties of hepsin and the several outstanding questions regarding its function in prostate cancer, the results presented here bring to light an exciting new avenue for the study and treatment of prostate cancer.

References

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