Identification of Differentially Expressed Prostate Genes: Increased Expression of Transcription Factor ETS-2 in Prostate Cancer

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BACKGROUND. Little is known about the genetic events in the malignant transformation of prostatic cells. This is due in large measure to the cellular heterogeneity of the prostate.

METHODS. An amplification method was devised to synthesize cDNA from small samples of cancer and benign tissues of the same resected glands. Differential gene expression of candidate informative markers between cancer and benign was screened by the polymerase chain reaction with gene-specific oligonucleotide primers.

RESULTS. The expression of a transcription factor, ETS-2, was shown to be elevated in some cancer specimens. Elevated expression was also noted for neuron-specific enolase (NSE) and another transcription factor, SEF2.

CONCLUSIONS. Our method can be used to identify quickly genes that are differentially expressed between benign and cancerous prostate cells. Transcription factors, such as ETS-2, may play a significant role in cancer progression. Prostate 30:145-153, 1997.

KEY WORDS: prostate cancer; cDNA amplification; transcription factors; neuron-specific enolase

INTRODUCTION

Prostate cancer (CaP) is the second leading cause of cancer death and has recently become the most frequently diagnosed cancer in males, in part because of the increased use of diagnostic strategies involving the tumor marker prostate-specific antigen (PSA). Yet CaP sometimes grows very slowly, and nearly 40% of older men dying of other causes have this cancer in their prostate [1]. Because there is no good way to assess the aggressiveness of CaP once diagnosed and no way to cure it once metastatic, it is important to understand better the molecular events in the progression of this disease.

Our goal is to find the differences in gene expression between normal and malignant prostatic epithelial cells in order to generate useful markers for disease stratification and prognosis, and to develop new therapies. The approach we have chosen entails the construction of prostate cDNA libraries and isolation of many clones, followed by analysis of their expression in various prostate specimens through serial hybridization. In this way a pool of candidate differentially expressed sequences is created, from which individual sequences are taken for further study. These studies involve the use of many pairs of normal and cancerous prostate tissue samples taken from patients’ resected glands. With standard stained slides as a guide, prostate specimens of approximately 1 mm3 are cut out of frozen sections for RNA isolation. A technique of cDNA amplification, originally developed for the construction of single-cell cDNA libraries [2], is then applied to synthesize enough cDNA from

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the RNA for expression analysis. This analysis uses polymerase chain reaction (PCR) with gene-specific oligonucleotide primers to detect the presence of particular transcripts in the cDNA population. In the cDNA amplification method, poly (A) RNA is converted to cDNA, and a short homopolymeric segment of (C)_n is added to the cDNA by the enzyme terminal transferase. With the use of two DNA primers, one containing T residues, the other G residues, cDNA sequences in between can be amplified. In our design, we minimized the number of buffer changes for the various enzymatic reactions and chose oligonucleotide sequences that allowed directional cloning of the PCR products.

We show here that the expression of transcription factors ETS-2 and SEF2, together with neuron-specific enolase (NSE), appears to be elevated in cancer specimens. The dysregulation of transcription factors in cancer cells is significant because these factors can activate other genes, some of which are likely to confer growth advantages on the cancer cells.

MATERIALS AND METHODS

Large-Scale Prostate cDNA Sequence Analysis

One microgram of poly (A^+) RNA isolated from a normal human prostate was used to construct a cDNA library in λZapII (Stratagene, San Diego, CA) according to the protocol supplied by the vendor. En masse excision of the λZapII library was performed in *Escherichia coli* XL1BlueMRF' with ExAssist helper phage, and the rescued phagemid was plated on *E. coli* SOLR cells. Clones were picked randomly and plasmid DNA was extracted by a 96-sample alkaline lysis procedure [3]. Plasmid DNA was tested for inserts by either restriction digestion or PCR. Single-pass fluorescence DNA sequencing was performed with AmpliTaq polymerase and T7 fluorescent dye primers (Perkin Elmer, Norwalk, CT) with an AmpliTaq DNA polymerase kit (Perkin Elmer, Norwalk, CT) with an AmpliTaq DNA polymerase kit (Perkin Elmer, Norwalk, CT). Oligonucleotide primers to detect the presence of particular transcripts in the cDNA population. In the cDNA amplification method, poly (A) RNA is converted to cDNA, and a short homopolymeric segment of (C)_n is added to the cDNA by the enzyme terminal transferase. With the use of two DNA primers, one containing T residues, the other G residues, cDNA sequences in between can be amplified. In our design, we minimized the number of buffer changes for the various enzymatic reactions and chose oligonucleotide sequences that allowed directional cloning of the PCR products.

We show here that the expression of transcription factors ETS-2 and SEF2, together with neuron-specific enolase (NSE), appears to be elevated in cancer specimens. The dysregulation of transcription factors in cancer cells is significant because these factors can activate other genes, some of which are likely to confer growth advantages on the cancer cells.

Isolation of Candidate Differentially Expressed cDNA

DNA from 49 clones with and 45 without homology to database sequences was probed in a dot-blot format for their representation in the cDNA libraries of DU145 prostate adenocarcinoma cells, a human prostatic adenocarcinoma, a human prostate adenocarcinoma xenograft, and a normal prostate. DNA of the 94 clones was denatured in 0.4 M NaOH, 10 mM EDTA and replica blotted onto nylon membranes (Micron Separations, Westborough, MA). A 5 μl aliquot of each cDNA library (titers >10^6 pfu/μl) was subjected to PCR to amplify the insert sequences for 40 cycles of 93°, 1 min; 55°, 1 min; and 72°, 2 min, with a 5-sec autoextension per cycle. The products were analyzed on a 0.7% agarose gel and DNA larger than 0.5 kb was excised. The DNA was purified with Sephadras BP (Pharmacia, Piscataway, NJ) and labeled with digoxigenin-11-dUTP (Boehringer Mannheim, Indianapolis, IN). The dot-blot replica membranes were prehybridized in 5X SSC, 1% (w/v) blocking reagent, 0.1% N-lauroylsarcosine, 0.02% SDS. Labeled probes were added and hybridization was performed at 65° overnight. After hybridization, the membranes were washed in 2X SSC, 0.1% SDS at room temperature followed by 0.5X SSC, 0.1% SDS at 65°. The membranes were treated with blocking reagent, incubated with anti-digoxigenin alkaline phosphatase-conjugated Fab fragments, and reacted with LuminPhos 530 (Boehringer Mannheim). The membranes were exposed for 16 hr, and the autoradiograms were visually inspected for differential signal intensity.

Synthesis of Amplified cDNA

Oligonucleotide sc3a, GGCCACAGCTGTGCACT-GCAGT\_15VN (V = G, A, C; N = G, A, T, C), was used to prime first-strand cDNA synthesis. One microliter of 2 pmol/μl primer sc3a was mixed with the RNA, and the solution was heated to 70° for 10 min. Standard reverse-transcriptase buffer and MMLV-RT (GIBCO-BRL, Gaithersburg, MD) were added, and the reaction was incubated at 39° for 1 hr. The RNA was degraded in 13 mM EDTA, 50 mM NaOH at 65°, 1 hr. The solution was neutralized by the addition of...
Tris-HCl, pH 8.0, and HCl to 0.2 M. The single-stranded cDNA was cleaned of primer sc3a on Wizard PCR Preps Minicolumn (Promega, Madison, WI) according to the manufacturer’s instructions. For the amplification step, the cDNA was resuspended in standard PCR buffer (20 mM Tris-HCl, pH 8.4, 50 mM KCl, 2 mM MgCl2) with 0.15 mM dCTP and 7 U Taq DNA pol (GIBCO-BRL) were added. After 94°, 1 sec and 57°, 5 min, 5 pmol primer sc3b, (CAU)4GGCCACAGCTGTGCAC-TGCAG, 10 m Tm, and 2 U Taq DNA pol (GIBCO-BRL) were added. After 94°, 1 sec and 57°, 30 sec; 69°, 1 min each except for PSA, CD44, Gsα, hXBP-1, PIP, FAU, AXL, and PAG. To follow up on these observations, we investigated the expression of these genes in CaP specimens obtained from radical prostatectomies. The cellular heterogeneity of the prostate gland and the small size of many cancers, however, presented obstacles to such an analysis. The technique of “single-cell” cDNA cloning was used to surmount these problems. In a pilot experiment, RNA was isolated from LNCaP cells, and cDNA was synthesized and amplified separately from 1, 0.1, 0.01, 0.001, 0.0001 μg of this RNA, representing the amount present in 30,000, 3000, 300, 30, and 3 cells, respectively. This calculation was based on the rough assumption that a eukaryotic cell has 100,000-200,000 gene transcripts with a complexity of 5,000-10,000. The amplified cDNA was tested for the presence of several gene sequences. PSMA, which is a very abundant transcript in LNCaP, was detected by agarose gel electrophoresis in the cDNA amplified from a 30-cell equivalent of RNA, while a much less abundant transcript, p53, was detected in the cDNA amplified from a 3,000-cell equivalent of RNA (Fig. 1).

This technique was next used to amplify cDNA from prostate specimens taken from resected glands. In Patient A, a piece of cancer tissue 1 mm3 in size and a similarly sized piece of normal tissue were scooped out of a frozen section for RNA isolation after the cancer area was identified under the microscope. cDNA was synthesized and amplified. The respective PCR products, labeled CP and NP, were tested for the presence of relevant gene sequences. The results are shown in Figure 2. The gene-specific primer sequences used and their PCR product sizes are listed in Table I. No differences in the intensity of the ethidium bromide-stained DNA bands were detected for PSA, PSMA, CD44, Gsα, hXBP-1, PIP, FNRβ, and Znα2GP. Failure to detect certain gene sequences could be due either to their low abundance or to the fact that they may not be prostate epithelial cell-specific genes. A somewhat higher level of C3PD was evident in CP. Increased expression of this gene has been reported in cancer [15]. The increase in NSE was pronounced. Presence of this neuroendocrine marker in CaP correlates with a poor prognosis [25]. Increases were also detected for SEF2 and ETS-2.

SEF2 and ETS-2 are DNA-binding transcription factors. Among the genes regulated by ETS-2 are those that encode enzymes that degrade the extracellular matrix, such as STROM and COL [26]. These

Gene-Specific Oligonucleotide Primers

The sequences of the primers used to evaluate the representation of the amplified cDNA are shown in Table I. All oligonucleotide primers were purchased from Integrated DNA Technologies (Coralville, IA). Most of the primer sequences were selected to give PCR product bands of 400-1,200 bp, with Tm around 73°-75° by the 4° GC, 2° AT formula, 40-60% GC content, no 6 bp palindromes or homopolymeric stretches. Working solutions of the primers were made at 5 pmol/μl. The markers included NSE [5]; ETS-2 and ETS-1 [6]; SEF2 [7]; epithelial glycoprotein (EGP) [8]; PSA [9]; prostate-specific membrane antigen (PSMA) [10]; CD44 [11]; Zn glycophorin (Znα2GP) [12]; G protein activator of adenylyl cyclase (Gsα) [13]; fibronectin receptor (FRNβ) [14]; glyceraldehyde-3-phosphate dehydrogenase (G3PD) [15]; collagenase (COL) [16]; stromelysin (STROM) [17]; X-box binding protein (hXBP-1) [18]; prolactin-inducible protein (PIP) [19]; p53 [20]; FAU, a ubiquitin-like-S30 fusion protein [21]; AXL, receptor tyrosine kinase [22]; proliferation-associated antigen (PAG) [23]; and BCL-2 [24]. PCR was carried out for 45 cycles of 94°, 1 sec; 69°, 1 min each except for PSA, PSMA, G3PD, and BCL-2 (94°, 1 sec; 57°, 30 sec; 69°, 1 min) in a volume of 50 μl with a Hybaid OmniGene thermocycler (Woodbridge, NJ). Ten microliters of the PCR products was analyzed by agarose gel electrophoresis.

RESULTS

Ninety-four cDNA clones from an NP library were sequenced and screened for differential expression. Of the 94 cDNA clones that were probed, 13 appeared to show differential expression as defined by probe hybridization signal-intensity differences among the prostate samples of cell line, xenograft, normal, and cancer tested. Ten of them were chosen for this study: ETS-2, SEF2, Znα2GP, Gsα, FNRβ, hXBP-1, PIP, FAU, AXL, and PAG. To follow up on these observations, we investigated the expression of these genes in CaP specimens obtained from radical prostatectomies. The cellular heterogeneity of the prostate gland and the small size of many cancers, however, presented obstacles to such an analysis. The technique of “single-cell” cDNA cloning was used to surmount these problems. In a pilot experiment, RNA was isolated from LNCaP cells, and cDNA was synthesized and amplified separately from 1, 0.1, 0.01, 0.001, 0.0001 μg of this RNA, representing the amount present in 30,000, 3000, 300, 30, and 3 cells, respectively. This calculation was based on the rough assumption that a eukaryotic cell has 100,000-200,000 gene transcripts with a complexity of 5,000-10,000. The amplified cDNA was tested for the presence of several gene sequences. PSMA, which is a very abundant transcript in LNCaP, was detected by agarose gel electrophoresis in the cDNA amplified from a 30-cell equivalent of RNA, while a much less abundant transcript, p53, was detected in the cDNA amplified from a 3,000-cell equivalent of RNA (Fig. 1).

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SEF2 and ETS-2 are DNA-binding transcription factors. Among the genes regulated by ETS-2 are those that encode enzymes that degrade the extracellular matrix, such as STROM and COL [26]. These
enzymes play an important role in tumor metastasis [27]. Accordingly, primers were designed to detect stromelysin-1 and collagenase sequences in the NP and CP cDNA (Fig. 2, panels “STROM” and “COL”). There was a detectable level of STROM RNA in CP and an elevated level of COL in CP. In addition, we examined the expression of ETS-1, a close relative of ETS-2, which recognizes similar DNA elements [28]. The presence of ETS-1 is associated with angiogenesis [29]. Panel “ETS-1” of Figure 2 shows that expression of ETS-1 was elevated in CP.

In Patient B, three small adjacent pieces of cancer and a piece of normal tissue were excised from frozen sections after histological examination. The four tissue samples were processed as before and their cDNA after amplification was tested for EGP, Znα₂GP, NSE, SEF2, ETS-1, ETS-2, STROM, and COL. As shown in Figure 3 the band signal intensity

<table>
<thead>
<tr>
<th>Marker</th>
<th>Primer pair sequence</th>
<th>Map coordinates</th>
<th>PCR band size (bp)</th>
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<td></td>
<td>CTTAAAGGCTCTTATTTGAGCCGCTGATC</td>
<td>1017–1870</td>
<td>850</td>
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<tr>
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<td>TCGGTCAGCTCCTCAGTCCGCTCAC</td>
<td>833–1609</td>
<td>780</td>
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<td>TCGGTCAGCTCCTCAGTCCGCTCAC</td>
<td>982–1782</td>
<td>890</td>
</tr>
<tr>
<td>SEF2</td>
<td>CAGAGTGTCCTCCTCCGAGCCTGC</td>
<td>213–1098</td>
<td>890</td>
</tr>
<tr>
<td>EGP</td>
<td>TTGCGCGAGCTCGAGGAAGAATGTC</td>
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<td>490</td>
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<tr>
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<tr>
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<tr>
<td>Znα₂GP</td>
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<td>376–1353</td>
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<td>G3PD</td>
<td>CCTAGGAGAAGGCTGGG</td>
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<td>COL</td>
<td>ACAAAATCCCTTACTCCCGAAAGTGAAGT</td>
<td>757–1612</td>
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<tr>
<td>STROM</td>
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<tr>
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<td>GTGGTTAATGTCCTGCGAGTTCGTGG</td>
<td>1814–2377</td>
<td>560</td>
</tr>
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</table>

TABLE I. Gene-Specific Primers
for EGP and Znα2GP was equivalent in the four cDNA-labeled NP, CP-1, CP-2, and CP-3. The expression of NSE, ETS-2, and SEF2 was increased in cancer, as was the case with the first cancer discussed above. Expression of COL was also increased, but that of STROM was not detected. Variation in band intensity among the three CP samples was observed for ETS-1, and to some extent for COL. This could indicate cellular heterogeneity within the tumor nodule, which probably would have been obscured if the entire tumor were processed. In Patient C, multiple foci of cancer and prostatic intraepithelial neoplasia (PIN), a putative premalignant lesion [30], were available for study. The cDNA-PCR analysis revealed a substantial increase in ETS-2 sequences again in all five of the multifocal cancer samples, but not in PIN (Fig. 4). The increase of SEF2 was more modest, and ETS-1 was detected in only one cancer sample. This suggests that the increase in ETS-2 and SEF2 expression is associated with cancer and probably not withpreneoplastic abnormality. Note that while the five cancer samples CP-1 to CP-5 were taken from different areas of the gland, all showed elevation in ETS-2 expression but different expression patterns of SEF2 and ETS-1. These findings argue for independent origins and strengthen the supposition that elevation of ETS-2 expression characterizes this cancer phenotype.

After these initial three CaP cases we narrowed our analysis to ETS-2 and NSE. All samples were first assayed for EGP (an epithelioid marker) and PSA (a prostatic marker) to monitor the amplified cDNA products. EGP and PSA were expressed in all samples. The results for 16 specimens (including patients A, B, and C) are shown in Table II. ETS-2 and NSE sequences were detected in 6 of the 16 CP samples. The combined Gleason’s scores of the cancers (primary) in the radical prostatectomy specimens from which the samples were obtained were between 6 and 8. The Gleason grade of each sample that was analyzed was 3. The range of the tumor volumes was between 0.3 and 4.1 cc. However, there appeared to be no correlation between the presence of the two markers and any of the characteristics listed in Table II. Furthermore, ETS-2 was not detected in samples of nodular hyperplasia, or in one of stromal elements, or one with inflammatory infiltrate (data not shown).

**DISCUSSION**

One of the most pressing issues in CaP is in our inability to differentiate aggressive cancers from the more indolent ones. This is due in part to a lack of understanding of neoplastic transformation of prostate cells. An understanding of the lineage relationship among the several component cellular types that constitute the prostatic epithelium could be of great help in deciphering this process. How do we go about identifying the important genetic markers, which might be useful either for typing cancer behavior or for lineage analysis? One criterion these markers must satisfy is that their expression differs in distinct cell types. To search for such markers we have performed large-scale cDNA sequence determination of clones isolated from a prostate cDNA library. The
cDNA clones were then screened by serial hybridization with probes prepared from various sources of prostate tissue such as cell lines, xenografts, and resected glands. One reservation about using cultured cells and xenografts as a source of probes is that they represent an artificially narrow spectrum of the cancer progression pathway. Furthermore, only a few cell lines and xenografts are available. Changes in

![Fig. 2. Gene expression analysis of Patient A's CaP. The genes tested are identified at the top of the panels. λHindIII is the DNA size marker. PCR products were resolved by 1% agarose gel electrophoresis in Tris-borate buffer and the DNA stained by ethidium bromide. The lane labeled "H2O" in the G3PD panel represents a negative control for PCR in which cDNA was omitted in the reaction. NP is normal tissue and CP cancer tissue.](image)

![Patient A](image)

![Fig. 3. Gene expression analysis of Patient B's CaP. The genes tested are identified below the panels. CP-1, CP-2, and CP-3 are pieces of the same tumor nodule.](image)

![Patient B](image)
gene expression may also arise from in vitro growth. The problem with resected glands as a source of probes on the other hand is the heterogeneous cellular composition of the tissue, as well as the small size of most tumors. To overcome tissue heterogeneity and small tumor size, we have devised a cDNA amplification method, previously used to generate so-called single-cell cDNA libraries. By means of this technique we can rapidly analyze expression of 50 genes in pairs of NP and CP from the same gland. This method can also allow us to synthesize cDNA libraries of purer populations of CaP cells prepared, for example, by cell sorting.

Initially, two candidate cDNA, ETS-2 and SEF2, showed evidence of differential expression. Both are transcription factors. *ETS* sequences were first identified in the chicken E26 retrovirus, and they were later shown to be members of a sizeable family [28]. The transcription factor *ETS* can bring about cellular transformation when overexpressed [31]. Reciprocal translocations involving *ETS* have been found in acute myelogenous leukemia [28]. ETS-2 recognizes DNA sequence elements containing a GGA core and cooperates with the AP-1 factor and others in activating transcription [32]. Hence, deregulation of *ETS* will in turn affect the expression of other genes controlled through ETS recognition sequences such as *COL*, the gene product which can degrade the extracellular matrix. Given the fact that ETS proteins are especially effective in activating promoters of genes involved in the early response to growth stimuli, their own activation appears to be a key event in cellular transformation [32]. Since the specimens studied by us, though small, could still have contained more than one cell type, we cannot formally rule out the possibility that these genetic changes might occur in nonepithelial cells. However, unlike normal glandular structures, CaP specimens usually contain predominantly cancer epithelial cells. It is likely, therefore, that expression of *ETS* is localized to epithelial cells. Support for this claim comes from the finding of *ETS* in the human CaP cell lines LNCaP and PC3, as well as in prostate xenografts (unpublished results). The other transcription factor, SEF2, contains a basic helix-loop-helix motif and recognizes enhancer elements first identified in the mouse leukemia virus SL3-3 [7]. The recognition sequence is related to elements found in the immunoglobulin heavy chain and insulin gene enhancers. SEF2 is nearly identical to the immunoglobulin transcription factor ITF2 [33]. The involvement of transcription factors in cancer cells is noteworthy because these factors can by themselves orchestrate an altered gene expression program favorable for the neoplastic cells.

To date, we have detected overexpression of *ETS* in seven CaP specimens (Table II), while no expression was observed in the normal prostate tissue obtained from a 19-year-old man. Nine other cancer cases did not exhibit detectable expression of *ETS* (Table II). These cases, perhaps not coincidentally, also did not show expression of *NSE*. Positive immunohistochemical staining for *NSE* is often encountered in CaP. Its presence in CaP has been associated

![ETS-2](image1)

![SEF2](image2)

![ETS-1](image3)

**Patient C**

Fig. 4. Gene expression analysis of Patient C’s CaP. The genes tested are identified below the panels. NP-1 and NP-2 are samples of normal tissue while CP-1, CP-2, CP-3, CP-4, and CP-5 are samples of cancer tissue taken from different areas of the resected gland. “PIN” represents PIN tissue of grades II and III from one area. The last lane in each panel represents a negative control for PCR.
TABLE II. Frequency of ETS-2 in Prostate Cancer*

<table>
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<tr>
<th>Patient</th>
<th>Age</th>
<th>Weight</th>
<th>Grade</th>
<th>Score</th>
<th>Vol</th>
<th>ETS-2</th>
<th>NSE</th>
<th>PSA</th>
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<td>+</td>
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<td>66</td>
<td>47</td>
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<td>+</td>
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<td>72</td>
<td>79</td>
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<td>38</td>
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<tr>
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<td>3</td>
<td>3 + 3</td>
<td>1.7</td>
<td>-</td>
<td>-</td>
<td>4.48</td>
</tr>
</tbody>
</table>

*Weight is the weight of the resected prostate in grams; grade is the Gleason grade of the sample of cancer that was analyzed; score is the combined Gleason’s score of each cancer; Vol is the estimated volume of the cancer in cubic centimeters. Preoperative serum PSA values are reported in ng/ml.

aPatient RH was on LUPRON-Flutamide therapy a month before surgery, his original PSA value was 4.7.

with a poor prognosis [25]. It is possible that the expression of NSE and the transcription factors are interconnected, and their dual presence in CaP may indicate a more aggressive type of cancer. No correlation is, however, evident between ETS-2/NSE expression and the other observable characteristics (e.g., cancer volume) listed in Table II. Complications in our analysis may come from the presence of lymphocytes, which may express ETS-2, in our samplings. Moreover, a minority of normal prostatic epithelial cells express NSE in the intact prostate. Interestingly, both ETS-2 and NSE were consistently detected in cells from short-term cultures of NP samples, which were ETS-2− when taken from the gland. The culture medium was chosen to suppress selectively the outgrowth of fibroblastic cells. The resultant cells appeared epithelioid. Upon analysis, these cells were shown to be EGP+, CD44+ (most similar to the basilar epithelioid phenotype), but PSA−. Furthermore, NSE and ETS-2 sequences were detected in xenograft LuCaP23.1 harvested from castrated hosts, and a brain metastasis from a patient at the late stages of his disease.

From these observations, we propose that the activation of the NSE program in CaP cells could lead to the appearance of aggressive variants of the cancer cells. It is suggestive that the 5′ regulatory sequences of NSE contain an SEF2 site at −500 CAGAGAACA-GAGGATGCTTG (matching nucleotide residues are underlined) [7]. The following questions are raised by our findings. Could up-regulation of NSE be mediated by SEF2? How are ETS-2 and SEF2 themselves activated? Sequences upstream of SEF2 have not been characterized, but those of ETS-2 have been in some detail [34]. In future experiments, the upstream sequences of NSE, ETS-2, and SEF2 will be used to identify factors that control their expression in prostatic cells.

We are currently using in situ hybridization to evaluate the expression pattern of ETS-2 and others. If the expression of ETS-2, SEF2, and NSE is linked and plays an important role, the localization of their expression to specific cells needs to be determined. The in situ study may succeed in settling this question. Our first in situ results have localized ETS-2 expression to the basal cells in the prostatic epithelium with little or no expression in the luminal cells. Expression was detected in cancers with combined Gleason’s scores from 6 to 9 (M. Tennant and S. Plymate, personal communication).

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