The ABCA5 Protein: A Urine Diagnostic Marker for Prostatic Intraepithelial Neoplasia
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Abstract

Purpose: To develop a urine diagnostic test for preneoplastic intraepithelial neoplasia of the prostate.

Experimental Design: We have used a DNA-binding assay and electrophoretic mobility shift assays (EMSA) to screen for novel duplexed DNA-binding sequences, which bind protein(s) overexpressed in crude protein extracts from high-grade prostatic intraepithelial neoplasia (HGPIN). EMSAs, immunohistochemistry, and ELISAs were used to measure expression of the ABCA5 protein identified as a specific marker in prostate tissue and patient urine.

Results: Following screening of 4,096 sequences, an 8-bp dsDNA sequence (i.e., TCCAGCGA) was identified, which binds the ABCA5 protein, a member of the ATP-binding cassette multidrug resistant family. EMSAs showed that ABCA5 was overexpressed in HGPIN tissue (n = 11/11) and in the urine of patients with HGPIN (n = 18/18) but was not expressed in prostate cancer, benign prostatic hyperplasia, or stroma. Immunohistochemistry indicated that ABCA5 was overexpressed in foci of intermediate basal cells in normal glands and in HGPIN. ABCA5 was faintly expressed in prostate cancer glands. ELISAs showed in ‘blinded studies’ that ABCA5 was a highly sensitive (98% sensitivity) urine diagnostic marker for HGPIN in biopsy-positive patients (n = 107) at a cutoff of 25 ng/mL. ABCA5 was present at very low levels (i.e., <25 ng/mL) in the urine of patients diagnosed with benign prostatic hyperplasia (n = 79) or prostatitis or kidney and bladder cancer (86% specificity).

Conclusions: The data indicate that ABCA5 might be a specific urine marker for diagnosis of patients with HGPIN.

Frank's (1) first proposed that atypical hyperplasia was a precursor of prostatic carcinoma. Helpap (2) showed by [3H]thymidine uptake that “severe atypical primary hyperplasia is a precancerous lesion.” In a key study, McNeal and Bostwick (3) showed an association between high-grade prostatic intraepithelial neoplasia (HGPIN) and prostatic adenocarcinoma. Bostwick and Brawer (4) subsequently provided a detailed description of the architectural features of HGPIN where they pointed out that >75% of all HGPIN exhibits a dome-like architecture. Since these initial studies, Epstein et al. (5) provided further linkage of HGPIN with organ-confined carcinoma, and multiple studies have now described HGPIN and showed a significant association with cancer (6–17). In a series of 249 autopsy cases, 77% of prostates with HGPIN harbored invasive adenocarcinoma compared with only 24% without HGPIN (10). Autopsy studies also showed that the development of HGPIN predated the development of clinically detectable cancer by 5 to 10 years, consistent with the concept that HGPIN is a premalignant lesion (18). Other studies have provided strong support for the association of HGPIN with the incidence of prostate cancer. For example, Gokden et al. (19) retrospectively identified 190 men with HGPIN and 1677 men with only benign prostatic hyperplasia (BPH) in needle biopsy tissue. The cumulative risk of detection of carcinoma on serial sextant follow-up biopsies was 30.5% for those with isolated HGPIN compared with 26.2% for the control group (P < 0.001). HGPIN found on the first repeat biopsy was associated with a 41% risk of subsequent detection of carcinoma compared with an 18% risk if benign prostatic tissue was found on the first repeat biopsy. The results suggest that the risk of prostate cancer is 30.5% after a diagnosis of isolated HGPIN in a needle biopsy. Likewise, Bishara et al. (20) showed that the multiple core involvement by HGPIN, both on initial and first repeat biopsy, defines a subset of men that is at increased risk of harboring synchronous invasive carcinoma. Studies by Bostwick and Brawer (4) and Bostwick (21) have shown that HGPIN, patient age, and prostate-specific antigen (PSA) are all highly significant predictors of prostate cancer, with HGPIN having the highest risk ratio (4, 21). In fact, HGPIN is more predictive of prostate cancer in older patients and those with a serum PSA of >4 ng/mL (4, 21). Because HGPIN predate the appearance of prostate cancer by approximately 5 to 10 years (4, 21), these reports suggest that patients with HGPIN need to be aggressively monitored for the development of cancer.
Despite this evidence, a considerable controversy remains about the importance of HGPIN as a precursor of prostate cancer. Thus, more work is required to confirm the relationship of HGPIN with the onset of prostate cancer. Because HGPIN is not always apparent in biopsies due to the multifocal nature of the condition, a sensitive and specific marker capable of uniformly detecting HGPIN with improved accuracy is required. For these reasons, we have developed methods for identification of a HGPIN diagnostic marker. These efforts have led to the identification of ABCA5 as a potential tissue and urine marker for HGPIN. ABCA5 is a member of the ATP-binding cassette multidrug resistant family, which is overexpressed in prostate tissue samples and urine of patients with PIN but is not present at detectable levels in the urine of patients with BPH or prostatitis. ELISAs were developed for detection and quantification of ABCA5. A preliminary evaluation of the ELISA data in a blinded study indicated that, at 25 ng/mL used as a ‘cutoff’ value, the sensitivity was >98% and the specificity was >86%. The data indicate, therefore, that ABCA5 is an excellent urine diagnostic marker for HGPIN in biopsy-confirmed patients (n = 107).

Materials and Methods

Isolation of protein from prostate tissues. After surgical removal, human prostates were collected within 30 min and dissected to remove pieces of tissue containing prostate cancer, HGPIN, stroma, BPH, and seminal vesicles. The diagnosis of these tissues was confirmed by Dr. F.U. Garcia (Drexel University, Philadelphia, PA) by H&E-stained sections. In addition, freshly frozen prostate tissues with histologically confirmed HGPIN (n = 10) were purchased from a private vendor (Genomics Collaborative, Inc., Boston, MA). Protein extracts were prepared from the tissue samples (~100-200 mg) containing at least 80% HGPIN, and there was no evidence of prostate cancer (based on histologic examinations of tissue sections) using a modification of the method of Singh et al. (22) as previously described by our laboratory (23). In brief, tissue was washed thrice with PBS and cut to small pieces and then suspended in 2 mL cold buffer A [10 mmol/L HEPES (pH 7.9), 10 mmol/L KCl, 0.1 mmol/L EDTA, 0.1 mmol/L EGTA, 1 mmol/L DTT, 0.5 mmol/L phenylmethylsulfonyl fluoride]. The pellet was sonicated for 30 s and centrifuged (10,000 rpm for 15 min). The supernatant was collected and the protein was precipitated with 4 volumes of cold acetone (~20°C). The protein was then snap frozen in liquid nitrogen and stored at ~80°C for <1 year. For DNA-binding assays, the samples were thawed and 2 μL of the protein extract (~5 μg protein) were incubated with 1 μg of poly(deoxyinosinic-deoxyctydilic acid) for 15 min followed by incubation with the 32P-labeled DNA probe (~100,000 dpm) for 30 min at 4°C. DNA binding to the protein(s) was then examined by gel electrophoretic mobility shift assays (EMSA; see below). Tissue was obtained by Dr. Garcia in accordance with Institutional Review Board–approved protocol at Drexel University College of Medicine.

Identification of the DNA sequence that binds ABCA5. An EMSA (23) was used to screen 32P-labeled duplexed DNA sequences (8 bp), which preferentially bind a protein(s) expressed by HGPIN compared with prostate cancer, BPH, and stroma tissue. Accordingly, we used EMSAs and screened ~4,096 sequence combinations to identify the tissue-specific binding sequence (i.e., TCCAGCGA), which binds a single protein uniquely expressed by HGPIN. HGPIN-specific cDNA expression library. mRNA was isolated from freshly dissected HGPIN tissue according to the protocol described by PolyATtract System 1000 (Promega, Madison, WI). A phage vector, ZAP Express Expression Vector (Stratagene, La Jolla, CA), was used and construction of the cDNA library was according to a protocol of the ZAP Express cDNA Synthesis kit and ZAP Express cDNA Gigapack III Gold Cloning kit (Promega).

Cloning of the ABCA5 gene. Cloning of the ABCA5 gene was carried out using a HGPIN-specific cDNA library [made by Dr. Y. Hu (Drexel University) in our laboratory] according to methods modified by our laboratory (23) from Singh et al. (22). In brief, the 32P-labeled HGPIN-specific binding sequence (TCCAGCGA) was used as a probe to screen the HGPIN cDNA library. The phage was plated at 1 × 10^5 plaque-forming units/150-mm plate. The replica filters were incubated in binding buffer containing 32P-labeled probe and poly(deoxyinosinic-deoxyctydilic acid) for 30 min at room temperature with gentle shaking. The filters were washed four times with the binding buffer, dried, and exposed to Kodak (Rochester, NY) X-OmatAR film overnight at ~80°C. The positive clones were picked and resuspended in SM buffer [5.8 g NaCl, 2 g MgSO4·7H2O, 1 mol/L Tris-Cl (pH 7.5) plus 5 mL 0.2% gelatin] and then put on a shaker overnight at 4°C. Three positive cDNA clones that expressed ABCA5 were purified by three sequential repetitions of the above protocol. Sequencing and blast analysis revealed that the cDNAs (~3,321 bp) were encoded for the full-length ABCA5 protein (968 amino acids). A glutathione S-transferase (pGEX) fusion protein was generated to purify the protein according to published methods (24, 25).

Reverse transcription-PCR analysis. RNA was isolated from different patient tissues according to methods described above and subsequently prepared for reverse transcription-PCR (RT-PCR). RT-PCR was carried out with primers for the ABCA5 gene (see below). This work was carried out by Dr. M. Wang (Drexel University) in our laboratory or by Dr. Celia Chang (Human Genomics Laboratory, Wistar Research Institute, Philadelphia, PA). cDNA (~100 bp) was extracted from the gels for sequencing. Blast analysis and comparisons on Genbank were carried out for each RNA specimen.

Real-time quantitative RT-PCR. Total RNA was isolated from tissues following a protocol described by RNAGents Total RNA Isolation System (Promega). Custom-made primers were obtained from Integrated DNA Technologies (Coralville, IA). Brilliant SYBR Green qPCR was used to do quantitative PCR amplification for ABCA5-specific and glyceraldehyde-3-phosphate dehydrogenase–specific regions. ABCA5 gene was amplified using the primers (26) 5′-GGCTGCTATTCTGGAC-CACCTACATATA-3′ (forward) and 5′-TTAATGCGACCACACATGAT-3′ (reverse). Glyceraldehyde-3-phosphate dehydrogenase was amplified using the primers 5′-TGGTACCACTGCGGACGAC-3′ (forward) and 5′-AAGGAAAGCTGGCAAATAAACAAGCC-3′ (reverse).

Western blot and immunoprecipitation studies. Rabbit polyclonal antibodies were raised against an NH2-terminal peptide (termed P1), 5′-MSTAIREGGVVRQIRTRLLIKN-3′ (i.e., +1 to +22), and a COOH-terminal peptide (termed P2), 5′-ISGLFALDSHSN4LICG-3′ (i.e., +854 to +876), unique to the ABCA5 protein. The antibodies were partially purified using a protein A column. Whole-cell lysates were prepared and subjected to Western blot analysis as described previously (27). Blots were probed with rabbit polyclonal anti-ABCA5 (1:2,000 dilution). A secondary horseradish peroxidase–conjugated IgG anti-rabbit antibody (Pierce, Rockford, IL) was applied for 1 h (1:5,000 dilution) followed by SuperSignal Western Pico Chemiluminescent substrate detection (Pierce) and exposure on Chemidox XRS 5 (Bio-Rad, Richmond, CA) to visualize immunoreactive bands. Immunoprecipitation assays were carried out by adding 1 μg/mL of the rabbit polyclonal antibody (P1) to 1 mL volume of the supernatants of the bacteriophage or an ABCA5 phagemid clone or to 10 mL volumes of human urine from patients diagnosed with HGPIN, prostate cancer, or BPH. The immunoprecipitates were run on 10% SDS-polyacrylamide gels and Western blotted with the anti-ABCA5 (P1) polyclonal antibodies according to methods described above.

Immunolabeling. To determine the subcellular localization of ABCA5, CD44, and ABCG2 proteins, whose normal prostate sections and pieces of human brain (as a positive control) were incubated for 45 min at 37°C with the polyclonal rabbit anti-ABCA5 (P1), CD44 (Abcam, Inc., Cambridge, United Kingdom), or ABCG2 (Chemicon, Madison, WI) antibodies digested with Proteinase K and dried before exposure to Kodak X-OmatAR film.
DNA probe (20-50 ng) completely blocked the bandshift assays, cold competition experiments with excess unlabeled the urine of patients with biopsy results showing HGPIN (Fig. 1C, lane 1, 3, 5, 7, and 9) but was not found in the urine of patients diagnosed with BPH (Fig. 1D, lanes 1 and 2) or in the urine of patients diagnosed with prostate cancer only (i.e., no indication of HGPIN; Fig. 1D, lanes 6, 8, 10, and 11). Again, the specific affinity of the 32P-labeled TCCAGCGA probe for the urine ABCA5 protein was shown by ‘supershift’ assays. EMSAs with increased amounts of the ABCA5 antibody produced protein–antibody complexes near the tops of the gels, indicating that the 31P-labeled TCCAGCGA probe specifically binds the ABCA5 protein (data not shown). In addition, ‘cold competition’ experiments with the TCCAGCGA probe (i.e., 20-50 ng) eliminated the ‘bandshift’ normally observed. Cold competition assays where 1 base was changed or with a random oligonucleotide sequence failed to block the bandshift observed with urine protein (5 μg total protein; data not shown).

Western blots. Rabbit polyclonal antibodies were raised against two different peptide domains unique to the ABCA5 protein and partially purified using a protein A column. Western blots showed that both antibodies specifically recognized recombinant ABCA5 protein and neither antibody cross-reacted with other multidrug resistant genes (i.e., ABCG2, ABCB1, and mdr-1) or other proteins present in recombinant phagmid extracts or whole-cell extracts of prostate cells (data not shown). Immunoprecipitation assays (combined with SDS gel electrophoresis; Fig. 2, top bands) with excess amounts of ABCA5 antibody (i.e., 1 μg/mL) raised against the P1 peptide showed that the 160-kDa ABCA5 protein was present in the urine of patients with HGPIN (Fig. 2, lanes 1-3) and was faintly expressed in the urine of patients with prostate cancer (Fig. 2, lane 6). More importantly, ABCA5 was not found in the urine of patients with BPH (Fig. 2, lanes 4 and 5) or in crude extracts of the bacteriophage (Fig. 2, lane 7). ABCA5 was expressed by the ABCA5 phage clone, however (Fig. 2, lane 8). Western blots (bottom bands) of the immunoprecipitates confirmed that ABCA5 was expressed in the urine of patients with HGPIN (Fig. 2, lanes 1-3), was faintly expressed in the urine of prostate cancer patients (Fig. 2, lane 6), and was present in protein extracts of the ABCA5 phage clone (Fig. 2, lane 8). Western blots confirmed that ABCA5 was not expressed in the urine of patients with BPH (Fig. 2, lanes 4 and 5) and in the bacteriophage (Fig. 2, lane 7).

Immunolabeling studies. Immunolabeling of human prostate glands (n = 10 whole-mount prostates) confirmed that the ABCA5 protein was selectively expressed in glands with HGPIN lesions (n = 10). In contrast, ABCA5 was not expressed in BPH or was faintly expressed in normal glands and prostate cancer (Fig. 3A). Note that, in normal glands, the occasional foci of intermediate basal cells (arrow) were strongly labeled with the ABCA5 antibodies, suggesting that these cells might be the origin of HGPIN (Fig. 3A). Because basal cells are thought to express CD44, as a positive control, adjacent sections were labeled with CD44 antibodies. We found that CD44 antibodies labeled the vast majority of the
Fig. 1. A to D. EMSAs showing the bandshift (arrow) observed following incubation of the radiolabeled probe (i.e., TCCAGCGA) with crude protein extracts. The $^{32}$P-labeled duplexed DNA (100,000 dpm) was incubated for 10 min with the crude protein extract (5 μg protein/well) before electrophoresis. A, protein extracts from stroma (lanes 1 and 2), BPH (lanes 3 and 4), HGPIN (lanes 5-7), and prostate cancer (lanes 8-10) tissues, respectively. B, protein from the urine (1 mg protein) of patients diagnosed with HGPIN (lanes 1, 2, 3, and 6) and BPH and prostate cancer (lanes 4 and 5). Lane 6, the patient had HGPIN and prostate cancer. C, protein extracts from HGPIN tissue (lane 1) and recombinant proteins (lanes 2-4) isolated from three different clones, respectively. D, protein (1 mg) from the urine of patients diagnosed with HGPIN (lanes 1, 3, 5, 7, and 9), BPH (lanes 2 and 4), and prostate cancer (lanes 6, 8, 10, and 11).
mRNA isolated from different types of prostate tissue (and the bacteriophage (protein (55 kDa. Arrow, ABCA5 was expressed by the ABCA5 phagemid clone (lane 8). Western blots of the immunoprecipitates confirmed that the 160-kDa ABCA5 protein (bottom band) was expressed in the urine of patients with HGPIN (lanes 1-3), prostate cancer (lane 6), and the ABCA5 phagemid clone (lane 8). ABCA5 was not expressed in the urine of patients with BPH (lanes 4 and 5) and the bacteriophage (lane 7).

basal cells and intermediate basal cells in normal glands and strongly labeled the epithelial cells in HGPIN. Like ABCA5, the CD44 antibodies faintly labeled the luminal epithelial cells of prostate cancer. In comparison, ABCG2, a homologue of ABCA5, failed to label the basal cells or intermediate basal cells or HGPIN and prostate cancer cells in any of the specimens examined (n = 10).

PCR and quantitative PCR assays. PCR analysis to amplify mRNA isolated from different types of prostate tissue (n = 5; i.e., normal, BPH, HGPIN, and prostate cancer) combined with sequencing of the 100-base fragments revealed that ABCA5 was highly expressed in HGPIN, whereas it was only faintly expressed in tissue specimens of prostate cancer. Quantitative RT-PCR and comparisons of the relative levels of expression of ABCA5 in different tissues clearly showed that the ABCA5 gene was overexpressed in HGPIN but was not expressed in BPH, prostate cancer, normal prostate, or prostate stroma (Fig. 4A). Likewise, ABCA5 was not expressed in bladder, kidney cancer, and lung cancer (Fig. 4A) or in prostate cancer cells collected by centrifugation (10,000 × g, 10 min) from 10 mL of normal urine (n = 5 specimens; ref. 31). However, ABCA5 was overexpressed in seminal vesicle, kidney, liver, and testis tissue (but not in testis cancer; Fig. 4A), in agreement with a previous report of Langmann et al. (26). In addition, assays of RNA extracts from BPH tissue of patients with prostatitis (n = 5) plus bladder (n = 4), kidney (n = 4), and testis (n = 2) cancer specimens revealed that ABCA5 mRNA was not expressed in any of these pathologies (Fig. 4B). The positive controls confirmed that ABCA5 was overexpressed in HGPIN and normal testis specimens (Fig. 4B). Note that, in all the above studies, the specimens were freshly collected and stored frozen at −80°C for <1 year before extraction of RNA for quantitative RT-PCR assays.

ELISAs. Using the P1 polyclonal antibodies, we initially developed an ELISA with the purified recombinant ABCA5 protein. The standard curve (Fig. 5) showed that the limit of detection of ABCA5 was ~1 ng/0.1 mL. A linear increase in absorbance was observed for increased amounts of ABCA5 ranging from 1 to 23 ng/0.1 mL with a correlation coefficient of 0.54. Note that an absorbance (450 nm) of 0.2 nm corresponded to ~2.0 ng/0.1 mL ABCA5 P1 peptide. The addition of increased amounts of P1 peptide to the negative urine yielded a standard curve identical to that observed with PBS (data not shown).

In Fig. 6A, we assayed the levels of ABCA5 protein in freshly collected/frozen urine specimens (biopsy negative for HGPIN, bladder cancer, kidney cancer, prostate cancer, normal prostate, or prostate stroma (Fig. 4A), in agreement with a previous report of Langmann et al. (26). The sensitivity was >98.1% and the specificity was >86% at a ‘cutoff’ of 25 ng/mL. That is, the levels of ABCA5 were above the ‘cutoff’ of 25 ng/mL in the biopsy-positive HGPIN patients and below 25 ng/mL in the BPH patients biopsy negative for HGPIN. The range of detection was from approximately 25 to 230 ng/mL in the HGPIN patients. The patients with prostate cancer and HGPIN all exhibited elevated levels of ABCA5 in the urine. The assay conditions were identical to the methods described for the standard curve (Fig. 5).

Accuracy and precision. Repeat assays with 10 different specimens positive for HGPIN and 10 BPH specimens (i.e., negative for HGPIN) have shown that all the HGPIN were positive for ABCA5 and all the BPH were negative for ABCA5. More importantly, the measurements from repeat
assays were consistent (SE, <0.5 ng/0.1 mL and <0.1 ng/0.1 mL, respectively).

**Antigen characteristics.** Preliminary studies have been carried out to evaluate antigen stability and the precision of the assay for ABCA5 in the urine. The data from repeated measurements of urine specimens showed that ABCA5 is a very stable protein and that the signal is not diminished >0.5 ng/0.1 mL if urine is kept at 4°C for 1 week or frozen for 1 year or frozen and thawed thrice. Dilution of the urine with PBS (1:1) slightly diminished the signal by ~10%. Repeated assays on the sample indicated that ~70% of the ABCA5 protein was captured in the first assay and that <5% remained after two assays. Finally, the dilution of positive HGPIN urine with negative urine specimens from prostate cancer or BPH patients did not eradicate the signal but simply reduced the level of ABCA5 in accordance with dilution effects of the added urine, indicating that these urines did not contain an interference factor.

**Discussion**

Development of a diagnostic marker for HGPIN is important, as several major studies have shown that HGPIN is a precursor of prostate cancer. It is expected that ~35% of patients diagnosed with HGPIN will develop prostate cancer within the first year after diagnosis and ~50% will develop cancer by year 2. In this article, we have identified the ABCA5 gene and ABCA5 protein as being overexpressed in prostate glands with HGPIN. In contrast, the ABCA5 protein was absent or only faintly expressed in prostate cancer, BPH, BPH with prostatitis,
50 ng of total RNA was used for each PCR assay. mRNA in different human prostate tissue DNA preparations. cDNA equivalent to cancer (www.aacrjournals.org ClinCancerRes2007;13(3)February1,2007935

Fig.4.

A
Fig.5. ELISA standard curve with the ABCA5 peptide (P1) using a rabbit polyclonal antibody raised against P1 (1:5,000) and a goat anti-rabbit horseradish peroxidase (1:10,000) secondary antibody.

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and stromal tissue of the prostate. Interestingly, immunolabeling indicated that ABCA5 was highly expressed in histologically defined intermediate basal cell foci found in normal glands and was overexpressed in all the cells of HGPIN glands. It was faintly expressed in prostate cancer as well. Although further work is required at this juncture, we speculate that the ABCA5-positive intermediate basal cells might constitute a highly proliferative cell type that is the precursor of HGPIN.

In this study, we have also identified the ABCA5 protein as a urine biomarker for HGPIN, presumably as a direct result of the proliferation and sloughing of HGPIN cells in the lumen of glands and subsequently shedding in the urine. The ABCA5 protein was found in urine at levels ranging from 25 to 230 ng/mL, presumably as a direct result of the lysis of HGPIN cells in the hypotonic urine. Further, we found in a ‘blinded study’ of

>195 patients (of which 107 patients were categorized as “HGPIN positive” by biopsy) that the ABCA5 protein was present in the urine at elevated levels compared with the biopsy-negative patients (n = 79). In the latter population, we found that the ABCA5 protein was either not present in the urine or present at very low levels (i.e., <25 ng/mL). The overall sensitivity and specificity of the ABCA5 marker was >98.1% and 86%, respectively, with a limit of detection of 25 ng/mL at an absorbance of 450 nm. Additional control studies showed by Western blots and ELISAs confirmed that the ABCA5 protein was not present in the urine of patients with BPH and prostatitis and in the urine of patients with other cancers, including testis, kidney, and bladder cancer. In summary, by identifying a marker that is specific for HGPIN, not only for the prostate, the assay for the ABCA5 protein offers a means to improve on the early detection of premalignant prostate cancer (i.e., HGPIN), thereby reducing biopsies when used as an adjunct to current methods. Moreover, a urine assay, such as ABCA5, is convenient and noninvasive because a needle puncture is not required to collect urine and therefore can readily be added to existing diagnostic tools. It is important to emphasize that, although the ABCA5 protein was overexpressed in all the cells of the HGPIN lesions, ABCA5 was not only expressed by the HGPIN glands. That is, the ABCA5 protein was expressed by unique foci of cells, termed intermediate basal cells, in normal glands. It was not expressed by other basal cells or luminal epithelial cells of normal glands, however. In addition, ABCA5 was faintly expressed by the luminal epithelial cells of prostate cancer (see Fig. 3A), indicating that the HGPIN cells, which express ABCA5, may cause prostate cancer. This means that, although the urine levels of ABCA5 are significantly elevated in patients with HGPIN, ABCA5 may be found in the urine of patients with prostate cancer. Moreover, we cannot rule out that patients with biopsy-confirmed HGPIN might harbor undetected prostate cancer, which also contributes to the presence of ABCA5 in the urine.

Fig.4. A and B, quantitative RT-PCR assays for quantitative detection of ABCA5 mRNA in different human prostate tissue DNA preparations. cDNA equivalent to 50 ng of total RNA was used for each PCR assay. C, values obtained with different tissues were used to calculate the input amount of template by the calibration curve method. After normalization to the endogenous control glyceraldehyde-3-phosphate dehydrogenase (data not shown), these values were expressed relative to the calibrator (tissue with lowest expression) and displayed as relative levels of expression. Columns, average of three independent assays; bars, SD. A, tissues included five different HGPIN (columns 1-5), prostate cancer (columns 6 and 7), BPH (columns 8 and 9), seminal vesicle (column 10), kidney (column 11), testis (column 12), testis cancer (column 13), liver (column 14), bladder (column 15), kidney cancer (column 16), lung cancer (column 17), prostate stroma (column 18), and normal prostate (column 19). B, tissues included HGPIN (column 1), BPH with prostatitis inflammation (columns 2-6), bladder cancer (columns 7-10), kidney cancer (columns 11-14), testis cancer (columns 15 and 16), and normal testis (column 17). ABCA5 primers were 5’-GGCGTCTATTCGACACACGATA-3’ (forward) and 5‘-TTAAGTCGAGATCTTATGTG-3’ (reverse).

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That is, the majority of prostates with HGPIN harbor invasive adenocarcinoma (i.e., 77%; ref. 10), and because epithelial cells of prostate cancer express ABCA5, it is possible that cancer glands might account, in part, for the elevated levels of ABCA5 in the urine specimens of HGPIN patients. Thus, before drawing firm conclusions, more exhaustive biopsy evaluation of prostate cancer patients is required to assess their HGPIN status (and vice a versa). This information in combination with ELISAs of the urine specimens would help establish whether elevated urine levels of ABCA5 are specifically associated with HGPIN or are released by prostate cancer glands as well. In addition, differentials of ABCA5 urine levels to serum PSA and/or digital rectal examination data plus patient age may be developed to better stage patients with premalignant or early-stage cancer, for example.

The cloning protocol used to identify the ABCA5 gene was based on a procedure previously developed to isolate a diagnostic marker for prostate cancer, termed PCADM-1 (23). In these studies, a novel duplexed probe was identified that binds a leucine zipper domain of a mutant S2 ribosomal protein (23). Here, we identified the duplexed TCCAGCGA probe that presumably binds a specific consensus domain of the ABCA5 protein. Although the ABCA5 protein might selectively bind DNA or RNA in vivo, we believe that the DNA-protein binding assays in vitro may reflect the specific buffer conditions that do not normally occur in vivo.

The ATP-binding cassette transporter superfamily is a large gene family comprising at least 48 genes and encodes a functionally diverse group of multispans membrane proteins involved in energy-dependent transport of a wide variety of substrates across membranes (32–34). Some multiple drug resistance phenotypes in tumor cells have been associated with the gene encoding the multidrug resistance protein, which also has an ATP-binding cassette transporter structure. Several ATP-binding cassette proteins in the human system are responsible for drug exclusion in compound-treated tumor cells, providing

![ELISAs of ABCA5 levels in the urine from patients. See Fig. 5 for antibody dilutions. A, patients with serum PSA levels of <1.5 ng/mL (■) and >10 ng/mL (▲). BPH with prostatitis (n = 12) or bladder cancer (n = 6) or kidney cancer (n = 8). B, patients diagnosed as normal (n = 79; ■) and with prostate cancer (n = 9; ▲) and HGPIN (n = 107; ▼) patients.](image-url)
cellular mechanisms for the development of multidrug resistance (33–35). One group contains five genes (ABCA5, ABCA6, ABCA8, ABCA9, and ABCA10) arranged in a cluster on chromosome 17q24. Two members of this subfamily, the ABCA1 and ABCA4 (ABCR) proteins, have been studied extensively. The ABCA1 protein is involved in disorders of cholesterol transport and high-density lipoproteins biosynthesis (see below). The ABCA4 protein transports vitamin A derivatives in the outer segments of photoreceptor cells and therefore does a crucial step in the visual cycle (35).

Human ABCA5 and rat Abca5 represent recently identified subfamily members (36). Petry et al. (36) reported cloning of cDNA sequences encoding rat and human ABCA5. Up-regulation of Abca5 mRNA expression was observed during culture of primary rat hepatocytes (37). Quantitative RT-PCR showed that an ABCA5 splice variant was expressed in numerous tissues (including tests, brain, and lung; ref. 26). The substrate spectrum remains to be defined and its normal function in different cell types and tissues is unknown. It has been shown to colocalize with a marker protein for the Golgi apparatus in Leydig cells (37), and it has been suggested that it may play a role in intracellular sterol/steroid trafficking. Whether it also plays a role in drug resistance is not known.

We are the first to clone the ABCA5 gene from a human prostate HGPIN cDNA expression library (patent pending). Because ATP-binding cassette genes are prone to be involved in cancer progression and drug resistance, we believe that the overexpression of ABCA5 in cells of HGPIN lesions may ultimately reveal a role of this protein in drug or sterol trafficking in the cells or another as of yet unidentified role of this protein in these premalignant cells. Thus, ABCA5 may be a promising target molecule for the development of a therapeutic approach for treating HGPIN (34–37) and possibly reduce HGPIN progression to prostate cancer.

Because HGPIN is more predictive of prostate cancer in older patients and those with a serum PSA of >4 ng/mL (4, 21), a therapeutic approach for treating HGPIN may be of tremendous benefit to these individuals at high risk for prostate cancer (i.e., older men, those with first-degree relatives having prostate cancer, and men of African descent). Currently, there is great interest in developing treatments for precancerous lesions of prostate. Due to the proposed role of HGPIN in the direct pathway to prostate cancer, several researchers believe that it is a suitable target for putative chemopreventive agents, such as green tea polyphenols, soy isoflavones, and selective estrogen receptor modulators. Thus, a noninvasive assay, such as the ABCA5 test, would be of tremendous benefit. This is especially true in light of the ongoing debate in the clinical diagnostic community as to whether HGPIN can be routinely detected and diagnosed by pathologists. Detecting HGPIN by biopsy is extremely difficult, and this problem is compounded by the fact that HGPIN lesions tend to be small and scattered throughout the prostate gland. If ABCA5 proves to be a precursor to prostate cancer as some clinicians believe, a specific HGPIN assay would be invaluable not only for early detection of cancer but also for monitoring the progression from HGPIN to prostate cancer. Likewise, the assay (i.e., for ABCA5) would be of potential use in monitoring the response of promising novel therapies aimed at preventing the conversion of HGPIN to prostate cancer (i.e., toremifene citrate; ref. 38).

In an ongoing trial, toremifene citrate, which is a selective estrogen receptor modulator compound, was originally developed as an antiestrogen and is currently marketed as Fareston for advanced breast cancer in women. Toremifene has been convincingly shown to prevent HGPIN-like lesions and prevent or delay the onset of prostate tumors in the mouse prostate transgenic adenocarcinoma of mouse prostate model. In addition, a placebo-controlled phase IIb clinical trial was conducted by GTx for the treatment of HGPIN using prostate cancer on a follow-up biopsy as a primary end point (38). The study included 514 cancer-free patients with affirmed HGPIN. Results showed that the risk of prostate cancer incidence was 24.4% in the 20 mg toremifene treatment group (n = 114) compared with 31.2% in the placebo group (n = 109), representing a 21.8% (P = 0.048) cumulative risk reduction in prostate cancer. The phase IIb trial was the largest study of the natural history of prostate cancer to date and included the collection of specimens, both blood and urine, for the purpose of biomarker discovery to improve screening and detection of HGPIN. Based on the promising results of the phase IIb study, a double-blind, randomized, multicenter, phase III trial is ongoing with ~1,260 patients to confirm the efficacy of 20 mg/d toremifene versus placebo for the prevention of invasive prostatic adenocarcinoma in high-risk men. We will participate in this trial to determine whether the ABCA5 protein is a diagnostic marker that can predict HGPIN and/or predict the failure of HGPIN to progress to prostate cancer in toremifene-treated patients.

References

GTx, Inc., unpublished data.


The ABCA5 Protein: A Urine Diagnostic Marker for Prostatic Intraepithelial Neoplasia

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