Published in final edited form as:

Prostate. 2009 April 1; 69(5): 505–519. doi:10.1002/pros.20898.

Quantification of phase I/II metabolizing enzyme gene expression and polycyclic aromatic hydrocarbon-DNA adduct levels in human prostate

Kaarthik John¹, Narasimhan Ragavan², M. Margaret Pratt¹, Paras B. Singh², Salah Al-Buheissi³, Shyam S. Matanhelia², David H. Phillips³, Miriam C. Poirier¹, and Francis L. Martin^{2,*}

¹ Carcinogen-DNA Interactions Section, LCBG, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, MD, 20892-4255, USA

² Lancaster Environment Centre, Lancaster University, Bailrigg, Lancaster LA1 4YQ, UK

³ Institute of Cancer Research, Brookes-Lawley Building, Sutton, Surrey SM2 5NG, UK

Abstract

BACKGROUND—Studies of migrant populations suggest that dietary and/or environmental factors play a crucial role in the aetiology of prostatic adenocarcinoma (CaP). The human prostate consists of the peripheral zone (PZ), transition zone (TZ) and central zone (CZ); CaP occurs most often in the PZ.

METHODS—To investigate the notion that an underlying differential expression of phase I/II genes, and/or the presence of polycyclic aromatic hydrocarbon (PAH)-DNA adducts might explain the elevated PZ susceptibility, we examined prostate tissues (matched tissue sets consisting of PZ and TZ) from men undergoing radical retropubic prostatectomy for CaP (n=26) or cystoprostatectomy (n=1). Quantitative gene expression analysis was employed for cytochrome P450 (*CYP*) isoforms *CYP1A1*, *CYP1B1* and *CYP1A2*, as well as *N*-acetyltransferase 1 and 2 (*NAT1* and *NAT2*) and catechol-*O*-methyl transferase (*COMT*).

RESULTS—*CYP1B1*, *NAT1* and *COMT* were expressed in all tissue sets; levels of *CYP1B1* and *NAT1* were consistently higher in the PZ compared to TZ. Immunohistochemistry confirmed the presence of *CYP1B1* (nuclear-associated and primarily in basal epithelial cells) and *NAT1*. Tissue sections from 23 of these aforementioned 27 matched tissue sets were analyzed for PAH-DNA adduct levels using antiserum elicited against DNA modified with r7, t8-dihydroxy-t-9,10-oxy-7,8,9,10-tetrahydro-benzo[*a*]pyrene (BPDE). PAH-DNA adduct levels were highest in glandular epithelial cells, but a comparison of PZ and TZ showed no significant differences.

CONCLUSION—Although expression of activating and/or detoxifying enzymes may be higher in the PZ, PAH-DNA adduct levels appear to be similar in both zones. Therefore, factors other than PAH-DNA adducts may be responsible for promotion of tumour formation in the human prostate.

*Correspondence to: Dr Francis L Martin PhD, Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ, UK; Tel.: +44 1524 594505; Fax: +44 1524 593192; E-mail: f.martin@lancaster.ac.uk.

Conflict of interest statement – None declared. None of the authors have financial interests or other interests that might be prejudicial to the interpretation or presentation of the work contained within this manuscript.

Keywords

Automated Cellular Imaging System; Immunohistochemistry; Peripheral zone; Radical retropubic prostatectomy; RT-PCR; Transition zone

---

INTRODUCTION

Prostatic adenocarcinoma (CaP) is one of the commonest male cancers in the developed world [1,2]. The aetiology and pathogenesis of this malignancy remains obscure, while its clinical invasiveness varies markedly between individuals. It is diagnosed predominantly in men >65 yr, and incidence varies among ethnic groups; the lowest rates occur in Asia and the highest rates occur in North America [1,3]. Increasing incidence in high-risk countries (*e.g.*, USA) may be partially due to increased serum prostate specific antigen (PSA) screening, while in traditionally low-risk countries (*e.g.*, Japan) this may be due to Westernization of diet and life-style [3,4]. However, PSA testing with earlier detection of organ-confined disease might give rise to the trend of decreasing mortality rates in some Western populations [5,6]. Among migrant populations originating from areas of low incidence and moving to higher-risk regions, levels rise to more closely match those of the host population within three generations [7–9]. Genetic factors may have a modifying effect on risk [7–10], but epidemiology suggests that there is a complex interplay among with environmental factors [11,12].

The prostate is an acorn-shaped gland located at the lowermost part of the pelvic cavity [13]. The urethra, which passes through the prostate, is an important anatomical reference point, as nearby tissue is designated as the transition zone (TZ), and that further away the peripheral zone (PZ). The prostate tissue surrounding the ejaculatory ducts (entering from behind) makes up the central zone (CZ) [14]. These zonal components have different embryologic origins, with the PZ, TZ and urethra developing from the uro-genital sinus, while the CZ, seminal vesicles and ejaculatory ducts are from mesonephric duct [14,15]. This morphology has clinico-pathological significance in that benign prostate hypertrophy occurs in the TZ, whereas CaP primarily arises in the PZ; the CZ appears to be relatively immune to such conditions [16].

One might hypothesize that differences in the expression of enzymes responsible for metabolism of environmental pro-carcinogens may underlie the increased susceptibility of the PZ to CaP. To this end, we investigated the zone-specific (PZ *vs.* TZ) gene expression of the phase I enzymes *CYP1A1*, *CYP1A2* and *CYP1B1*, and the phase II enzymes *N*-acetyltransferase 1 (*NAT1*), *NAT2* and catechol-*O*-methyltransferase (*COMT*) in matched tissue sets (stored at –85°C for RNA extraction) derived from 27 individual patients. In addition, in the same tissue sets (paraffin-embedded blocks; n=23) we set out to determine the presence, localization and quantity of polycyclic aromatic hydrocarbons (PAH)-DNA adducts. Such observations might shed light on individual endogenous activation mechanisms, in addition to steady-state adduct levels induced by environmental exposures. To the best of our knowledge, there are no prior studies that have examined differential gene expression and PAH-DNA adduct formation in the context of the two differentially cancer susceptible zones of the human prostate (PZ and TZ). The aim of this study, therefore, was to ascertain whether the differential gene expression of candidate phase I/II metabolizing enzymes and quantifiable PAH-DNA adduct levels might vary according to the expected cancer susceptibility of the PZ and TZ.

PARTICIPANTS AND METHODS

Study Participants

Statutory approval for collection of prostate tissue for research was obtained from the Preston, Chorley and South Ribble Local Research Ethics Committee (LREC; no. 2003.6.v), with consent forms signed by each participant. Study participants were selected from patients undergoing radical retropubic prostatectomy (RRP) for CaP, discovered on the basis of a low PSA (<20 µg/ml serum) and low volume of disease (≤two core biopsies positive for CaP per eight taken), or cystoprostatectomy (CyP) for bladder cancer. A single pathologist assigned the Gleason grade [17] after histopathological examination of the prostate. The Gleason score was expressed as a sum of the most common grade seen in the whole specimen and the next most common grade, each scored out of five. Demographic and clinical details are shown in the Supplementary data (Table S1).

Tissue Retrieval and Storage

After surgical resection, prostate was transported to the pathology laboratory (<3 min) for dissection using aseptic techniques. For tissue obtained by RRP, this comprised material from the prostate lobe that showed no or low-volume cancer on prostate biopsy. For tissue obtained by CyP, this comprised material from one of the lobes. Prostate tissue was sliced from the upper part of the gland starting just above the area of the verumontanum. The material (2 cm in length and 0.3 cm in width) isolated from the most peripheral and posterolateral aspect of the gland was designated PZ, while that (1.5 cm in length and 0.3 cm in width) isolated from the area immediately lateral to the urethra (peri-urethral) was designated as TZ. Tissues sets destined for gene expression analysis were immediately placed in RNAlater (QIAGEN Ltd., Valencia, CA), kept at 4°C for 24 h and then transferred for storage at −85°C. Matched sets consisting of PZ and TZ per individual patient, as well as the remaining prostate, were formalin-fixed and paraffin-embedded for enzyme and PAH-DNA adduct localization by immunohistochemistry (IHC). H&E-stained sections were checked retrospectively to assess for CaP.

Quantitative Real-Time Reverse Transcriptase (RT)-PCR

Total RNA extraction was performed using the Qiagen RNeasy® kit in combination with the Qiagen RNase-free DNase kit (QIAGEN Ltd.) RNA (0.4 µg) was reverse transcribed in a final volume of 20 µl containing Taqman® reverse transcription reagents (Applied Biosystems, Foster City, CA): 1 × Taqman RT buffer; MgCl₂ (5.5 mM); oligo d(T)₁₆ (2.5 µM); dNTP mix (dGTP, dCTP, dATP and dTTP; each at a concentration of 500 µM); RNase inhibitor (0.4 U/µl); reverse transcriptase (MultiScribe™) (1.25 U/µl) and RNase-free water. Reaction mixtures were incubated at 25°C (10 min), 48°C (30 min) and 95°C (5 min).

cDNA samples were stored at −20°C prior to use. Primers (see Supplementary data, Table S2) for *CYP1A1*, *CYP1A2*, *CYP1B1*, *NAT1*, *NAT2*, *COMT* and the endogenous control β -ACTIN were chosen using Primer Express software 2.0 (Applied Biosystems, Foster City, CA) and designed so that each primer spanned an exon boundary. Specificity was confirmed using the NCBI BLAST search tool. Quantitative real-time PCR was performed using the ABI Prism 7000 Sequence Detection System (Applied Biosystems). Reaction mixtures contained 1 × SYBR® Green PCR master mix (Applied Biosystems); forward and reverse primers (Invitrogen) at a concentration of 300 nM; for *CYP1A1*, *CYP1A2*, *CYP1B1*, *NAT1*, *NAT2*, *COMT* amplification 20 ng cDNA template or for β -ACTIN amplification 5 ng cDNA template; made to a total volume of 25 µl with sterile H₂O. Thermal cycling parameters included activation at 95°C (10 min) followed by 40 cycles each of denaturation at 95°C (15 sec) and annealing/extending at 60°C (1 min). Each reaction was performed in triplicate and “no-

template” controls were included in each experiment. Dissociation curves were run to eliminate non-specific amplification, including primer-dimers [18].

Immunohistochemical Staining for Metabolizing Enzymes

Immunohistochemical staining was performed on a BioGenex Optimax Plus (A. Menarini Diagnostics, Wokingham, Berkshire, UK) immuno-stainer. Staining took place following de-waxing and re-hydration, and endogenous peroxidase was blocked by immersing the tissue sections in 4% H₂O₂ in methanol for 15 min. High temperature antigen retrieval was performed by heating the tissue sections in citrate buffer (pH 6.0) for 2 min, under pressure and at full power (800 W) in a microwave oven. The antiserum specific for CYP1B1 (ME001, Alpha Diagnostic, San Antonio, TX) was diluted 1:100 in 0.2% bovine serum albumin in tris-buffered saline (pH 7.6) (BSAT). The tissue sections were incubated with primary antibody for 30 min at room temperature. Following the manufacturer's instructions for the StreptABComplex duet kit (DakoCytomation, Ely, Cambridgeshire, UK), the tissue sections were washed with tris-buffered saline (TBS) for 5 min, incubated for 30 min with secondary anti-sera (goat anti-rabbit) in BSAT and washed with TBS for 5 min. The tissue sections were then incubated with avidin-biotin complex in BSAT for 30 min and washed again with TBS for 5 min. 3,3'-Diaminobenzidine chromogen in 0.05 M Tris/HCl buffer (pH 7.6) with 0.1% H₂O₂ was applied to preparations for 15 min after which they were washed for 5 min with tap water. Finally, slides were stained (15 s) with Harris' haematoxylin rinsed with tap water, blued in warm tap water (15 s) and rinsed again. Preparations were dehydrated with graded alcohol solutions through to xylene and mounted with cover-slips using Styrolite mounting medium (VWR International, West Chester, PA). The polyclonal antibody for NAT1 was a generous gift from Prof Edith Sim (University of Oxford, UK). Immunohistochemistry and staining of sections was carried out as previously described [19].

Cultured Human Cervical Keratinocytes Exposed to r7,t8-Dihydroxy-t-9,10-oxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BPDE) for Generation of a Standard Curve

Normal human cervical keratinocytes (NHCK), immortalized with HPV 16 [20], were cultured in serum-free MCDB153 medium (Sigma-Aldrich, St. Louis, MO) supplemented with sodium bicarbonate (1.2 g/l), insulin (5 µg/ml), hydrocortisone (0.5 µg/ml), transferrin (10 µg/ml), *O*-phosphoethanolamine (0.1 mM), ethanolamine (0.1 mM), epidermal growth factor (10 ng/ml) and bovine pituitary extract (40–70 µg/ml) (all from Sigma-Aldrich). BPDE (CAS no. 58917-67-2, Chemical Carcinogen Reference Standard Repository, Kansas city, MO) was dissolved in dimethylsulfoxide (DMSO) and immediately added to the cell culture medium of 90–95% confluent NHCK to give final concentrations of 0, 0.25, 0.50, 0.75 or 1.0 µM. After 1 h incubation, cells were washed with 1× phosphate-buffered saline (PBS) and harvested by scraping. Each treatment group contained the combined cells from twelve 15-cm plates, which were centrifuged 5 min at 1200 rpm. The cell pellet was re-suspended in an equal volume of PBS, and 75% of the suspension was used for paraffin-embedding and IHC, while the remaining 25% was frozen for subsequent DNA extraction and quantitation of r7, t8, t9-trihydroxy-c-10-(N²deoxyguanosyl)-7,8,9,10-tetrahydro-benzo[a]pyrene (BPdG). The portion of cell pellet to be used for IHC was embedded in 3% Seaplaque agarose (Lonza Inc., Walkersville, MD), fixed in 10% phosphate-buffered formalin (Fisher Scientific, Pittsburgh, PA) and embedded in paraffin. Three 1-mm (in diameter) cores were taken from each treatment group and embedded in a single piece of paraplast paraffin to form an array containing 3 cores from each exposure group. This 'standard curve' array could be cut and analyzed as a single slide; so for each group of unknown samples, a standard curve array was stained simultaneously as detailed below.

BPDE-DNA Antiserum and Immunohistochemical Staining of Human Prostate

Rabbit polyclonal antibodies, elicited against BPDE-modified DNA (rabbit #30 bleed 6/30/78) [21,22], were employed for detection of BPdG and PAH-DNA adducts. These antibodies exhibit specificity for the different enantiomers of benzo[a]pyrene (BP) bound to DNA, but not for BP alone, its metabolites, or DNA alone [21]. However, cross-reactivity of these antibodies with several other carcinogenic PAH-DNA adducts has been reported [22]. For IHC, the BPDE-DNA antiserum was pre-absorbed with calf thymus DNA to reduce non-specific background staining. In addition, some of the specific BPDE-DNA antiserum was absorbed with the immunogen BPDE-modified DNA (1.3% modified, 13.9 nmol BPdG adducts) so as to eliminate the specific BPDE-DNA immunoglobulins. This was used as a control for unknown samples.

Three serial 5- μ m sections (a, b, c) of paraffin-embedded prostate tissue and the standard array were mounted on positively-charged glass slides, which were incubated at 60°C for 1h, deparaffinized by a series of xylene/ethanol washes, and subjected to antigen retrieval by microwaving in the presence of Antigen Retrieval Citra solution (Biogenex, San Ramon, CA). Subsequently, slide “a” was stained with specific BPDE-DNA antiserum, absorbed only with calf thymus DNA; slide “b” was stained with haematoxylin, for visualization of the nuclei; and, slide “c” was stained with BPDE-DNA antiserum absorbed with immunogen BPDE-DNA. Rabbit antisera were diluted 1:20,000 in antibody diluent (Ventana Medical Systems Inc., Tucson, AZ) before use.

Slides were stained using the Nexes IHC (Ventana Medical Systems Inc.) automated slide staining system as previously described [23]. The antiserum incubation was for 20 min (for slides “a” and “c”) with no counterstaining. Slide “b” was incubated with normal rabbit serum and counterstained with modified Gill haematoxylin (Ventana Medical Systems Inc.) and bluing reagent (Ventana Medical Systems Inc.). Stained slides were rinsed for 1 min in water containing Dawn dishwashing liquid soap (Proctor and Gamble, Cincinnati, OH), and rinsed 3–4 times in deionized H₂O to remove the soap. The stained slides were subsequently air dried, and mounted with cover-slips using Permount mounting media (Fisher Scientific).

All of the sections were cut at the same time but were randomized to determine the overall order of staining. This usually, but not always, meant that the replicates were stained on different days. An effort was made to find and count the same areas in the replicate slides, but this was not always possible due to histological variations in the tissue.

Semi-quantitation of BPDE-DNA nuclear staining intensity by Automated Cellular Imaging System (ACIS)

The pink colour intensity of nuclear PAH-DNA adduct staining was measured by the ACIS II (Clariant, Aliso Viejo, CA) [24–26]. The colour threshold parameters (hue, luminosity and saturation) for the PAH-DNA adduct staining were manually set so as to eliminate background, and were as follows: hue = 222–237, luminosity = 49–255, saturation = 14–145. To further enhance accuracy, scoring parameters were set to count only round or oblong pixels with a diameter of 5 μ m–10 μ m. The nuclear count was obtained manually from haematoxylin (blue)-stained slides.

The prostate regions chosen for scoring, typically located in the glandular epithelial cells, were 300 μ m–400 μ m in length and no more than 1 or 2 cell layers deep. A minimum of 3 regions and 100 cells per tissue, chosen manually using the ACIS II “region selection” tools, were scored. The occasional sample possessed few or no scorable regions and/or cells. For the standard curve array, 2 arrays and 3 cores/dose were scored. For the samples, where 3 regions

were scored on one slide, every attempt was made to score exactly the same area in the slides stained with specific antibody, absorbed antibody and haematoxylin.

Quantitation of PAH-DNA adduct staining was carried out on a high-resolution, composite digital scan of each section, constructed using the ACIS II microscope and software. For each region scored, the total intensity of nuclear pink colour from all cells scored was designated as integrated optical density (IOD) by the ACIS II software. IODs from all scored regions were added and divided by the total number of nuclei, obtained by manually counting corresponding regions on the haematoxylin-stained slides. The data for each sample were expressed as total OD/nucleus. A reduction in OD/nucleus in samples stained with the BPDE-DNA-absorbed BPDE-DNA antiserum indicated that the specific staining signal was due to the presence of PAH-DNA adducts (in the samples) or BPdG (in the standard curve).

Quantification of BP-DNA Adducts in Cultured NHCKs by Chemiluminescence Immunoassay (CIA)

DNA from NHCKs was extracted using the QiaAmp Blood DNA Midi kit (QIAGEN Ltd.). DNA was measured by absorbance at 260 nm and samples were stored at -20°C until assayed. The methodological details for the BPDE-DNA CIA are detailed elsewhere [24]. Limitrac 600 high-binding microtitre LIA plates (Greiner Bio-one, Monroe, NC), coated with 1.0 fmol of BP-DNA adducts, were employed in the present study. Five ng of sample DNA from the each of the treatment groups was assayed in each well. Each sample was assayed twice in triplicate wells on two separate microtitre plates.

Statistical Methods

The correlation between staining intensity determined by IHC and BP-DNA adduct levels determined by BPDE-DNA CIA was evaluated using the least-squares method. Comparisons of PAH-DNA adduct levels determined by IHC/ACIS and gene expression levels in the PZ vs. the TZ were determined by a two-tailed *t*-test and the Wilcoxon signed-rank test (two-tailed), respectively.

RESULTS

Quantitative Gene Expression Analyses in PZ vs. TZ

Efforts were made to obtain cancer-free prostate tissues *i.e.*, a lobe believed to be negative for CaP per patient undergoing RRP. Real-time RT-PCR gene expression analyses of phase I / II metabolising enzymes (*CYP1A1*, *CYP1A2*, *CYP1B1*, *NAT1*, *NAT2*, *COMT*) were carried out without prior knowledge of histopathology and were limited to patients from whom matched tissue sets (PZ and TZ) were obtained [RRP (n=26) and CyP (n=1)] (see Supplementary data). Expression analyses for matched PZ and TZ tissue sets were analysed in groups of three patients for *CYP1A1*, *CYP1A2*, *CYP1B1*, *NAT1* and *NAT2*, and in groups of six patients for *COMT*. In 19 of the 27 patients, CaP-free tissue sets were obtained while in the rest (n=8) cancer was found in one of the zones. *CYP1B1*, *NAT1* and *COMT* were quantifiable in all patients in both the zones, while the detection and quantification for the other genes varied.

Marked differences in inter-individual expression were observed (Tables 1 and 2). For *CYP1A2*, expression varied 7-fold, and for *CYP1B1*, expression varied 20-fold. *CYP1A1*, *NAT1*, *NAT2* and *COMT* each showed a 10-fold inter-individual variation. Assessment of intra-individual differences amongst the cancer-free tissue sets consistently demonstrated markedly higher expression levels in the PZ compared to the TZ for *CYP1B1* (18 out of 19) and *NAT1* (14 out of 19). The intra-individual differences in the other genes examined were not found to follow the same pattern. In the tissue sets where CaP was identified retrospectively to be present in one of the zones, the aforementioned gene expression pattern was less robust. However,

even in these tissue sets *CYP1B1* expression levels were often found to be higher in the PZ, the CaP-susceptible zone (in 7 out of 8).

IHC for CYP1B1 and NAT1

Immunohistochemical staining of prostate tissues was performed using polyclonal anti-CYP1B1 or anti-NAT1 antibodies (Fig. 1). The anti-CYP1B1 staining exhibited a distinct pattern of nuclear-associated staining in cancer-free glandular tissue (Fig. 1A, C) and stromal tissue (1A) which was stronger in the basal epithelial cells than in the cells adjacent to the lumen. In cancer tissue, sheaths of cells exhibiting both nuclear staining and cytoplasmic staining were observed (Fig. 1B). In the tissue obtained from the patient undergoing CyP for invasive bladder cancer, it was also possible to obtain CZ and identify typical more-rounded glandular structures. Of note was the lack of anti-CYP1B1 staining in the epithelial cells of these glandular elements (Fig. 1D). Anti-NAT1 staining also exhibited prominent staining (nuclear-associated and cytoplasmic) in the basal epithelial cells lining glandular elements (Fig. 1 E–H) but, there was an absence of positive staining in surrounding stroma.

BPDE Exposure of NHCKs for the IHC Standard Curve

The standard curve, generated by treating NHCKs with increasing doses of BPDE, exhibited a dose-dependent increase in nuclear staining intensity, as measured by IHC using the BPDE-DNA antiserum (Fig. 2A). Cells stained with specific BPDE-DNA antiserum (top row) are compared with those stained with BPDE-DNA-absorbed BPDE-DNA antiserum (bottom row). The specific BPDE-DNA antiserum staining showed a dose response for intensity of nuclear pink colour that was quantified by ACIS and expressed (numbers on photomicrographs) as OD/nucleus. The absorbed serum showed very little pink nuclear staining, indicating that almost all of the original staining was due to the presence of BPdG adducts.

In addition to the IHC/ACIS analysis, some of the BPDE-exposed cultured NHCKs were used for DNA extraction and assay by BPDE-DNA CIA. For this study, the standard curve 50% inhibition for CIA was 0.118 ± 0.04 fmol BPdG /well (mean $\pm$ range) ($n = 2$), and using 5 ng DNA per well. The BPDE-DNA CIA also showed a dose-dependent increase in BPdG adducts with increasing BPDE dose (Fig. 2B). By expressing BPdG values, determined by IHC/ACIS, as a function of those determined by CIA (Fig. 2C) a strong correlation was observed ($R^2 = 0.94$) between the two methodological approaches. Therefore, subsequent quantification in the PZ and TZ tissue sets of BPdG adducts/ 10^8 nucleotides was achieved by comparison of IHC/ACIS values with the standard curve (Fig. 2C).

PAH-DNA Adduct Levels Determined by IHC/ACIS and Comparison to the Standard Curve

In the prostate, PAH-DNA adduct staining (pink colour) was localized primarily in nuclei of the glandular epithelial cells. Of the 27 tissue sets, 2 PZ and 4 TZ blocks had no scorable tissue and were excluded because ACIS measurements require intact tissue architecture. The final analysis of representative stained areas (Fig. 3) comprised paired PZ and TZ tissue sets ($n=23$). The left panels of Fig. 3 show specific PAH-DNA adduct staining in prostate epithelial cells lining glandular elements in a matched set of PZ and TZ tissues. The ACIS II software calculated the IODs from all the designated areas for a particular sample, and divided this value by the total number of nuclei contained in the same areas, which were determined from the haematoxylin-stained slides (Fig. 3, right panels). The resulting OD/nucleus values (boxes in the 4 left panels, Fig. 3) were used to calculate PAH-DNA adducts/ 10^8 nucleotides using the standard curve (Fig. 2C). Loss of staining intensity with the 'absorbed' anti-BPDE antiserum (Fig. 3, middle panels) confirmed that positive staining is specific for PAH-DNA adducts.

Fig. 4 shows staining intensity values for matched PZ (Fig. 4A) and TZ (Fig. 4B) tissue sets ($n=23$), where the specific nuclear staining is indicated by grey bars and the corresponding

absorbed-serum values are shown as black bars. The mean OD/nucleus values for staining of the PZ and TZ with the specific antiserum were 15,845 and 19,244, respectively. The corresponding absorbed antiserum values were 1,477 and 1,945 for PZ and TZ, respectively. The limit of detection (LOD) was defined as the half value between zero and the lowest OD/nucleus (306 OD/nucleus for the absorbed serum of Patient 7 PZ). Therefore, 153 OD/nucleus, was designated the LOD and used to calculate the non-detectable samples. In this study, the highest consistent OD/nucleus values were seen with Patient 5, which gave 40,450 in the PZ, and Patient 16 which gave 43,432 in the TZ.

The PAH-DNA adduct values obtained upon subtraction of the absorbed serum values and comparison with the standard curve (Fig. 2C) are shown in Fig. 5. Again, Patient 5 had the highest adduct value, with 1,812 PAH-DNA adducts/ 10^8 nucleotides in the PZ (Fig. 5A) and Patient 16 had the highest value, with 2,214 PAH-DNA adducts/ 10^8 nucleotides in the TZ (Fig. 5B). Overall, there were no statistically-significant differences between PAH-DNA adduct levels in the PZ versus the TZ when evaluated by Wilcoxon signed rank test ($P = 0.66$).

DISCUSSION

The role of environmental and genetic factors in the aetiology of CaP remains obscure. Although, genetic factors are likely to play a role in the inter-ethnic differences observed in the incidence of CaP [8] they are unlikely to be wholly responsible for its increasing incidence [27,28]. Migration studies point to an important role for environmental factors [3,7,9], but endogenous hormones, such as oestrogen, may also play an important role [29–31]. Quantitative gene expression analysis demonstrated that *CYP1B1*, *NAT1* and *COMT* are consistently expressed in prostate tissue; of interest was the additional observation that the pro-carcinogen bio-activating and detoxification enzymes *CYP1B1* and *NAT1*, respectively tended to be expressed at a higher level in the PZ compared to TZ. IHC confirmed the presence of CYP1B1 and NAT1 to be nuclear-associated (NAT1 was also cytoplasmic-associated) and primarily in basal epithelial cells of the glandular elements. PAH-DNA adduct levels were also highest in glandular epithelial cells, but a comparison of PZ and TZ showed no significant differences between the zones. These results might suggest that over time, accumulation of persistent, bulky DNA adducts might occur independent of levels bio-activating enzyme activity at the target site.

Environmental pro-carcinogens, such as PAHs and heterocyclic aromatic amines (HAAs), undergo bio-activation in target tissues, and endogenous hormones (*e.g.*, oestrogens) could also result in genotoxic compounds [31–35]. These compounds undergo metabolism by both phase I and phase II enzymes. HAAs [*e.g.*, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)] are present in cooked meat and fish. In rats, PhIP induces cancers in colon and mammary pads and, at high doses, in prostate (in high doses) [36,37]. Human prostate tissues also possess the metabolic capacity to activate PhIP [34,38]. PAHs (*e.g.*, BP) are generated by incomplete combustion of organic matter and are present in charcoal-broiled steaks, cigarette smoke and fossil fuels [39]. BP is genotoxic in mammary cells lines [35] and prostate cells lines with or without hormones [34,40,41]. Primary cell cultures of prostate epithelial cells (PECs) activate BP to give rise to BP-DNA adducts [34]. PAH-DNA adducts have also been observed in CaP tissues by IHC [42]. mRNA transcripts of *CYP1A1*, *CYP1A2* or *CYP1B1* [41] as well as *NAT1* and *NAT2* and *SULT2B1* have been detected in prostate [43,44]. Loss of *GSTP1* expression in human prostate appears to enhance susceptibility to carcinogens such as *N*-OH-PhIP [45].

CYP enzymes (multi-function oxidases) have overlapping substrate specificity and may metabolise exogenous compounds such as PAHs and HAAs as well as endogenous substrates such as steroid hormones [34,46]. Exogenous pro-carcinogens are metabolised predominantly

by CYP1A1 and CYP1A2, whereas endogenous hormones (*e.g.*, oestrogens) are metabolised by CYP1B1 giving rise to 2-*OH* and 4-*OH* metabolites [47,48]. 4-*OH* Oestrogen metabolites are highly genotoxic [46]. These inducible isoenzymes also deactivate drugs such as flutamide that are used in CaP treatment [49]. Phase II enzymes have high affinity for lipophilic substances, catalyse endogenous substances, have broad substrate specificity, and may generate DNA-damaging metabolites [50]; they include glutathione-*S*-transferase (GST), UDP-glucuronyltransferase (UGT), sulfotransferase (SULT), NATs and COMT [51–53]. Metabolic activation of PhIP through CYP1A2 is subsequently followed by *O*-esterification by NATs or SULTs [54].

Phase I and II enzymes are expressed in the prostate; however, to our knowledge, only a small number of studies have addressed the differences between the cancer-susceptible PZ and the TZ [14,16]. Following exposure, non-susceptible zones of rodent prostate, when compared to susceptible, were found to have higher levels of pro-carcinogen conjugates, thereby pointing to higher levels of COMT/GST in the non-susceptible zones. Immunoblot detection of CYP1A1 and CYP1A2 was possible after induction by BP in normal and tumour cells, but not in untreated cells [55]. IHC staining for CYP1B1 is contradictory; nuclear [54] and cytosolic [57] staining have both been reported as has the presence [18,56] and complete absence [57] of staining in normal prostate. *N*-Acetylation activity has also been detected in the prostate [58].

We found that mRNA transcripts for phase I and II enzymes were detectable and mostly quantifiable in the human prostate. *CYP1B1*, *NAT1* and *COMT* were readily quantifiable in all the tissue sets in both zones, while *CYP1A2* was the least quantifiable. IHC for CYP1B1 and NAT1 in the normal prostate glands showed mostly nuclear-associated (and some cytoplasmic) staining, probably due to the nuclear-associated nature of sex-steroid activity. Large inter-individual differences were noted probably due to the inducible nature of these genes. Intra-individual differences in the normal prostate tissues sets showed higher levels of *CYP1B1* and *NAT1* in PZ, which is the cancer-susceptible zone. Gene expression of *CYP1A1*, *CYP1A2* and *NAT2* was less quantifiable. CYP1A2-catalysed metabolism accounts for most of the elimination of ingested HAAs [59,60].

One other group of investigators has examined PAH-DNA adduct formation in human prostate, and prostate tumour, using IHC [61,62]. Semi-quantitative values for intensity of nuclear staining were obtained upon examination of 100 cells in tumour and non-tumour areas from the same specimen, and the data showed that PAH-DNA adducts were higher in Caucasians who smoked, compared with non-smokers. A similar relationship was not observed in African-Americans. These investigators further showed that 1 yr after surgery, individuals with PAH-DNA adducts above the median in normal prostate tissue had a 3.22-fold (3.79-fold for African-Americans) increased hazard ratio for elevated PSA levels, indicating the possibility of cancer recurrence [62]. Similar to the studies described above, we found measurable values for PAH-DNA adduct formation in virtually all of the samples examined. Whereas others calculated their data based on staining absorbance [61,62], we attempted a further quantitation of the absorbance values by comparison with a standard curve containing known amounts of DNA adduction determined in the same cells by BPDE-DNA CIA and IHC/ACIS. This refinement provides the added capability of adduct comparison between studies and among different tissues examined.

The scientific question posed by our studies - are localization of PAH-DNA adducts and metabolizing enzymes different in PZ and TZ? - is different from than that examined by others [61,62], who did not consider the zonal localization of adducts. Although CaP appears to arise predominantly in the PZ as opposed to the TZ or CZ [15], and we and others have found higher PAH-activating CYP1B1 expression in the PZ as opposed to the TZ, our study found no

significant differences in PAH-DNA adduct levels between the two zones. Even when stratified on the basis of smoking status and diet, no significant differences in PAH-DNA adduct levels were observed across both the PZ and TZ (data not shown). Race was not a variable in this study as all the subjects were Caucasian. Our study points to a higher expression of CYP1B1 in the PZ as opposed to the TZ, and suggest that diffusion/distribution of the ultimate metabolites occur independently of metabolite generation. It is also possible that contributing factors include: differential DNA repair and/or tissue turnover rates in the two zones, differential expression of detoxification enzymes (NAT1 expression was also higher in the PZ vs. the TZ in our study), local hormonal effects that might stimulate proliferation of initiated cells and, a role for polymorphisms in Phase I, Phase II or DNA repair genes. Our study demonstrates that the progression of CaP is complex and that multiple factors need to be considered in order to elucidate the underlying mechanisms responsible for transformation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We would like to thank Drs. Stephen Hewitt and Kris Ylaya, Advanced Technology Center (ATC), Gaithersburg, MD, for their guidance and assistance with the preparation of the 'standard curve' arrays. This work was funded by: the Rosemere Cancer Foundation (Ragavan, N., Singh, P.B., and Martin, F.L.); Cancer Research UK (Phillips, D.H.); and the intramural program of the US National Institutes of Health, National Cancer Institute, Center for Cancer Research (Poirier, M.C., John, K., and Pratt, M.M.).

References

1. Cancer Research UK. <http://info.cancerresearchuk.org/cancerstats/types/prostate/>
2. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005;55:74–108. [PubMed: 15761078]
3. Hsing AW, Tsao L, Devesa SS. International trends and patterns of prostate cancer incidence and mortality. *Int J Cancer* 2000;85:60–67. [PubMed: 10585584]
4. Sim HG, Cheng CW. Changing demography of prostate cancer in Asia. *Eur J Cancer* 2005;41:834–845. [PubMed: 15808953]
5. Collin SM, Martin RM, Metcalfe C, et al. Prostate-cancer mortality in the USA and UK in 1975–2004: an ecological study. *Lancet Oncol* 2008;9:445–452. [PubMed: 18424233]
6. Oberaigner W, Horninger W, Klocker H, Schonitzer D, Stuhlinger W, Bartsch G. Reduction of prostate cancer mortality in Tyrol, Austria, after introduction of prostate-specific antigen testing. *Am J Epidemiol* 2006;164:376–384. [PubMed: 16829552]
7. Cook LS, Goldoft M, Schwartz SM, Weiss NS. Incidence of adenocarcinoma of the prostate in Asian immigrants to the United States and their descendants. *J Urol* 1999;161:152–155. [PubMed: 10037388]
8. Kolonel LN. Cancer patterns of four ethnic groups in Hawaii. *J Natl Cancer Inst* 1980;65:1127–1139. [PubMed: 6933244]
9. Shimizu H, Ross RK, Bernstein L, Yatani R, Henderson BE, Mack TM. Cancers of the prostate and breast among Japanese and white immigrants in Los Angeles County. *Br J Cancer* 1991;63:963–966. [PubMed: 2069852]
10. Nomura AM, Kolonel LN. Prostate cancer: a current perspective. *Epidemiol Rev* 1991;13:200–227. [PubMed: 1765112]
11. Albano JD, Ward E, Jemal A, et al. Cancer mortality in the United States by education level and race. *J Natl Cancer Inst* 2007;99:1384–1394. [PubMed: 17848670]
12. Kolonel LN, Altshuler D, Henderson BE. The multiethnic cohort study: exploring genes, lifestyle and cancer risk. *Nat Rev Cancer* 2004;4:519–527. [PubMed: 15229477]

13. Brooks, JD. Anatomy of lower urinary tract and male genitalia. In: Wein, AJ.; Kavoussi, LR.; Novick, AC.; Partin, AW.; Peters, CA., editors. Campbell-Walsh Urology. Vol. 1. New York: Saunders; 2007. p. 61-63.
14. McNeal JE. The zonal anatomy of the prostate. *Prostate* 1981;2:35–49. [PubMed: 7279811]
15. McNeal JE. Normal histology of the prostate. *Am J Surg Pathol* 1988;12:619–633. [PubMed: 2456702]
16. McNeal JE. Origin and development of carcinoma in the prostate. *Cancer* 1969;23:24–34. [PubMed: 5763258]
17. Gleason DF, Mellinger GT. Prediction of prognosis for prostatic adenocarcinoma by combined histological grading and clinical staging. *J Urol* 1974;111:58–64. [PubMed: 4813554]
18. Ragavan N, Hewitt R, Cooper LJ, et al. *CYP1B1* expression in prostate is higher in the peripheral than in the transition zone. *Cancer Lett* 2004;215:69–78. [PubMed: 15374634]
19. Al-Buheissi SZ, Patel HR, Meinel W, et al. *N*-Acetyltransferase and sulfotransferase activity in human prostate: potential for carcinogen activation. *Pharmacogenet Genomics* 2006;16:391–399. [PubMed: 16708048]
20. Ohta Y, Tsutsumi K, Kikuchi K, Yasumoto S. Two distinct human uterine cervical epithelial cell lines established after transfection with human papillomavirus 16 DNA. *Jpn J Cancer Res* 1997;88:644–651. [PubMed: 9310137]
21. Poirier MC, Santella R, Weinstein IB, Grunberger D, Yuspa SH. Quantitation of benzo(a)pyrene-deoxyguanosine adducts by radioimmunoassay. *Cancer Res* 1980;40:412–416. [PubMed: 7356524]
22. Weston A, Manchester DK, Poirier MC, et al. Derivative fluorescence spectral analysis of polycyclic aromatic hydrocarbon-DNA adducts in human placenta. *Chem Res Toxicol* 1989;2:104–108. [PubMed: 2519708]
23. van Gijssel HE, Schild LJ, Watt DL, et al. Polycyclic aromatic hydrocarbon-DNA adducts determined by semiquantitative immunohistochemistry in human esophageal biopsies taken in 1985. *Mutat Res* 2004;547:55–62. [PubMed: 15013699]
24. Divi RL, Beland FA, Fu PP, et al. Highly sensitive chemiluminescence immunoassay for benzo[a]pyrene-DNA adducts: validation by comparison with other methods, and use in human biomonitoring. *Carcinogenesis* 2002;23:2043–2049. [PubMed: 12507927]
25. Pratt MM, Sirajuddin P, Poirier MC, et al. Polycyclic aromatic hydrocarbon-DNA adducts in cervix of women infected with carcinogenic human papillomavirus types: an immunohistochemistry study. *Mutat Res* 2007;624:114–123. [PubMed: 17583755]
26. van Gijssel HE, Divi RL, Olivero OA, et al. Semiquantitation of polycyclic aromatic hydrocarbon-DNA adducts in human esophagus by immunohistochemistry and the automated cellular imaging system. *Cancer Epidemiol Biomarkers Prev* 2002;11:1622–1629. [PubMed: 12496053]
27. Grönberg H. Prostate cancer epidemiology. *Lancet* 2003;361:859–864. [PubMed: 12642065]
28. Lichtenstein P, Holm NV, Verkasalo PK, et al. Environmental and heritable factors in the causation of cancer--analyses of cohorts of twins from Sweden, Denmark, and Finland. *N Engl J Med* 2000;343:78–85. [PubMed: 10891514]
29. Cavalieri E, Frenkel K, Liehr JG, Rogan E, Roy D. Estrogens as endogenous genotoxic agents--DNA adducts and mutations. *J Natl Cancer Inst Monogr* 2000;75–93. [PubMed: 10963621]
30. Cavalieri EL, Devanesan P, Bosland MC, Badawi AF, Rogan EG. Catechol estrogen metabolites and conjugates in different regions of the prostate of Noble rats treated with 4-hydroxyestradiol: implications for estrogen-induced initiation of prostate cancer. *Carcinogenesis* 2002;23:329–333. [PubMed: 11872641]
31. Singh PB, Matanhelia SS, Martin FL. A potential paradox in prostate adenocarcinoma progression: oestrogen as the initiating driver. *Eur J Cancer* 2008;44:928–936. [PubMed: 18381236]
32. Cavalieri EL, Rogan EG. A unified mechanism in the initiation of cancer. *Ann N Y Acad Sci* 2002;959:341–354. [PubMed: 11976208]
33. Lijinsky W. The formation and occurrence of polynuclear aromatic hydrocarbons associated with food. *Mutat Res* 1991;259:251–261. [PubMed: 2017211]
34. Martin FL, Cole KJ, Muir GH, et al. Primary cultures of prostate cells and their ability to activate carcinogens. *Prostate Cancer Prostatic Dis* 2002;5:96–104. [PubMed: 12496996]

35. Pfau W, Martin FL, Cole KJ, et al. Heterocyclic aromatic amines induce DNA strand breaks and cell transformation. *Carcinogenesis* 1999;20:545–551. [PubMed: 10223180]
36. Ito N, Hasegawa R, Sano M, et al. A new colon and mammary carcinogen in cooked food, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). *Carcinogenesis* 1991;12:1503–1506. [PubMed: 1860171]
37. Shirai T, Sano M, Tamano S, et al. The prostate: a target for carcinogenicity of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) derived from cooked foods. *Cancer Res* 1997;57:195–198. [PubMed: 9000552]
38. Lawson T, Kolar C. Human prostate epithelial cells metabolize chemicals of dietary origin to mutagens. *Cancer Lett* 2002;175:141–146. [PubMed: 11741741]
39. Phillips DH. Polycyclic aromatic hydrocarbons in the diet. *Mutat Res* 1999;443:139–47. [PubMed: 10415437]
40. Kooiman GG, Martin FL, Williams JA, Grover PL, Phillips DH, Muir GH. The influence of dietary and environmental factors on prostate cancer risk. *Prostate Cancer Prostatic Dis* 2000;3:256–258. [PubMed: 12497073]
41. Williams JA, Martin FL, Muir GH, Hewer A, Grover PL, Phillips DH. Metabolic activation of carcinogens and expression of various cytochromes P450 in human prostate tissue. *Carcinogenesis* 2000;21:1683–1689. [PubMed: 10964100]
42. Rybicki BA, Rundle A, Savera AT, Sankey SS, Tang D. Polycyclic aromatic hydrocarbon-DNA adducts in prostate cancer. *Cancer Res* 2004;64:8854–8859. [PubMed: 15604244]
43. Wang CY, Debiec-Rychter M, Schut HA, et al. *N*-Acetyltransferase expression and DNA binding of *N*-hydroxyheterocyclic amines in human prostate epithelium. *Carcinogenesis* 1999;20:1591–1595. [PubMed: 10426812]
44. Her C, Wood TC, Eichler EE, et al. Human hydroxysteroid sulfotransferase SULT2B1: two enzymes encoded by a single chromosome 19 gene. *Genomics* 1998;53:284–295. [PubMed: 9799594]
45. Nelson CP, Kidd LC, Sauvageot J, et al. Protection against 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine cytotoxicity and DNA adduct formation in human prostate by glutathione *S*-transferase P1. *Cancer Res* 2001;61:103–109. [PubMed: 11196146]
46. Yager JD. Endogenous estrogens as carcinogens through metabolic activation. *J Natl Cancer Inst Monogr* 2000:67–73. [PubMed: 10963620]
47. Felton JS, Knize MG. Occurrence, identification, and bacterial mutagenicity of heterocyclic amines in cooked food. *Mutat Res* 1991;259:205–217. [PubMed: 2017208]
48. Jefcoate CR, Liehr JG, Santen RJ, et al. Tissue-specific synthesis and oxidative metabolism of estrogens. *J Natl Cancer Inst Monogr* 2000:95–112. [PubMed: 10963622]
49. Schaufli K, Haslmayer P, Jager W, Pec M, Thalhammer T. The environmental toxin 2,3,7,8-tetrachlorodibenzo-*p*-dioxin induces cytochrome P450 activity in high passage PC 3 and DU 145 human prostate cancer cell lines. *Int J Mol Med* 2002;9:411–416. [PubMed: 11891538]
50. Bradfield C. UNIT III: Metabolism and modifiers of chemical carcinogenesis. 2005:2000.
51. Mannisto PT, Kaakkola S. Catechol-*O*-methyltransferase (COMT): biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. *Pharmacol Rev* 1999;51:593–628. [PubMed: 10581325]
52. Sheweita SA. Drug-metabolizing enzymes: mechanisms and functions. *Curr Drug Metab* 2000;1:107–132. [PubMed: 11465078]
53. Sheweita SA, Tilmsany AK. Cancer and phase II drug-metabolizing enzymes. *Curr Drug Metab* 2003;4:45–58. [PubMed: 12570745]
54. King RS, Teitel CH, Shaddock JG, Casciano DA, Kadlubar FF. Detoxification of carcinogenic aromatic and heterocyclic amines by enzymatic reduction of the *N*-hydroxy derivative. *Cancer Lett* 1999;143:167–171. [PubMed: 10503898]
55. Sterling KM Jr, Cutroneo KR. Constitutive and inducible expression of cytochromes P4501A (CYP1A1 and CYP1A2) in normal prostate and prostate cancer cells. *J Cell Biochem* 2004;91:423–429. [PubMed: 14743400]
56. Muskhelishvili L, Thompson PA, Kusewitt DF, Wang C, Kadlubar FF. In situ hybridization and immunohistochemical analysis of cytochrome P450 1B1 expression in human normal tissues. *J Histochem Cytochem* 2001;49:229–236. [PubMed: 11156691]

57. Carnell DM, Smith RE, Daley FM, et al. Target validation of cytochrome P450 CYP1B1 in prostate carcinoma with protein expression in associated hyperplastic and premalignant tissue. *Int J Radiat Oncol Biol Phys* 2004;58:500–509. [PubMed: 14751521]
58. Di Paolo OA, Teitel CH, Nowell S, Coles BF, Kadlubar FF. Expression of cytochromes P450 and glutathione *S*-transferases in human prostate, and the potential for activation of heterocyclic amine carcinogens *via* acetyl-coA-, PAPS- and ATP-dependent pathways. *Int J Cancer* 2005;117:8–13. [PubMed: 15880531]
59. Boobis AR, Lynch AM, Murray S, et al. CYP1A2-catalyzed conversion of dietary heterocyclic amines to their proximate carcinogens is their major route of metabolism in humans. *Cancer Res* 1994;54:89–94. [PubMed: 8261468]
60. Finnström N, Bjelfman C, Söderström TG, et al. Detection of cytochrome P450 mRNA transcripts in prostate samples by RT-PCR. *Eur J Clin Invest* 2001;31:880–886. [PubMed: 11737226]
61. Nock NL, Tang D, Rundle A, et al. Associations between smoking, polymorphisms in polycyclic aromatic hydrocarbon (PAH) metabolism and conjugation genes and PAH-DNA adducts in prostate tumors differ by race. *Cancer Epidemiol Biomarkers Prev* 2007;16:1236–1245. [PubMed: 17548691]
62. Rybicki BA, Neslund-Dudas C, Bock CH, et al. Polycyclic aromatic hydrocarbon--DNA adducts in prostate and biochemical recurrence after prostatectomy. *Clin Cancer Res* 2008;14:750–757. [PubMed: 18245535]

Fig. 1. Immunohistochemical staining of the prostate tissues. Anti-CYP1B1 staining in (A) PZ of Patient no. 2 showing the presence of a distinct pattern of nuclear-associated staining in cancer-free glandular epithelial cells (blue arrows) and stroma (yellow arrows); (B) in adjacent CaP tissue (identified by the pathologist in the separate lobe) from Patient no. 2, sheaths of cells exhibiting both nuclear-associated staining and cytoplasmic staining (red arrows); (C) TZ of Patient no. 2 showing the presence of a distinct pattern of nuclear-associated staining in cancer-free glandular epithelial cells (blue arrows) and stroma (yellow arrows); and (D) CZ of Patient no. 25 who underwent a cystoprostatectomy, showing an apparent absence of positive staining in the typical large glandular elements (green arrows). Anti-NAT1 staining in (E) CZ (green

arrows) compared to PZ (blue arrows) of Patient no. 25 who underwent a cystoprostatectomy; (**F**) PZ of Patient no. 25 showing the presence of a distinct pattern of nuclear-associated and cytosolic staining in cancer-free glandular epithelial cells (blue arrows); (**G**) TZ of Patient no. 25 showing the presence of a distinct pattern of nuclear-associated and cytosolic staining in cancer-free glandular epithelial cells (yellow arrows); and (**H**) TZ of Patient no. 25 showing the presence of a distinct pattern of nuclear-associated and cytosolic staining in cancer-free glandular epithelial cells (yellow arrows), at a higher magnification than before.

Scale bar = 50 μ m

Fig. 2.

Standard curve consisting of cultured human keratinocytes exposed to 0, 0.25 μ M, 0.50 μ M, 0.75 μ M or 1.00 μ M BPDE for 1 h. **(A)** **Top row** of IHC photos shows nuclear staining of cells that were formalin-fixed, embedded in paraffin arrays, and stained with specific BPDE-DNA antiserum, a secondary antibody and Fast Red (pink color). Inserts contain staining intensity values (OD/nucleus) for the pink color determined by ACIS. **Bottom row** of photos shows cells cultured and fixed the same as cells in the top row, but stained with specific BPDE-DNA antiserum absorbed with the immunogen BPDE-DNA, a secondary antibody and Fast Red. Inserts contain staining intensity values (OD/nucleus) for the pink color determined by ACIS. **(B)** Portions of the cultured keratinocytes shown in **(A)** were analyzed by BPDE-DNA CIA after DNA extraction, and the BPdG adducts/10⁸ nucleotides are shown as a function of dose. Values are mean $\pm$ range for 2 assays each with triplicate wells. **(C)** Standard curve consisting of BPdG adducts visualized by IHC/ACIS (OD/nucleus, ordinate), plotted as a function of BPdG adducts/10⁸ nucleotides determined by BPDE-DNA CIA (abscissa). Values represent mean $\pm$ range for 2 CIAs (each with triplicate wells), and mean $\pm$ range for 2 IHC arrays, with staining of 3 cores and 3 regions/core for each experimental group. Each major division on the scale represents 100 μ m with each sub-division within representing 10 μ m.

Fig. 3.

Immunostaining for PAH-DNA adducts in selected areas of PZ and TZ obtained from Patient no. 2. The **left** panels show staining of one region with BPDE-DNA antiserum. The **middle** panels show staining of the same region with BPDE-DNA-absorbed BPDE-DNA antiserum, and the **right** panels show staining of the same region with hematoxylin. These are identical regions obtained from serial cross-sections. Inserts contain values for OD/nucleus for the region outlined in blue. The numbers of nuclei were obtained by manually counting the blue cells within the demarcated area.

Fig. 4. Staining intensity (OD/nucleus) determined by IHC/ACIS for matched sets of PZ (**A**) and TZ (**B**) tissues from all 23 individuals, stained with either specific BPDE-DNA antiserum (grey bars) or BPDE-DNA-absorbed BPDE-DNA antiserum (black bars).

Fig. 5. PAH-DNA adducts/10⁸ nucleotides, obtained from the IHC/ACIS OD/nucleus values (with absorbed serum subtracted) by calculation from the standard curve (Figure 2C). Shown here are adduct values for PZ (**A**) and TZ (**B**) for all 23 individuals. There were no statistically-significant differences between PAH-DNA adduct levels in the two groups when evaluated by Wilcoxon signed rank test ($P = 0.66$).

TABLE 1

CYP quantitative expression in prostate zones

Patient no. (zone)	Inter-individual gene expression			Intra-individual gene expression		
	<i>CYP1A1</i>	<i>CYP1A2</i>	<i>CYP1B1</i>	<i>CYP1A1</i>	<i>CYP1A2</i>	<i>CYP1B1</i>
1 (peripheral)	0	0	1 (c)	nd	nd	1 (c)
(transition)	1 (c)	0	1 (c)			0.24
2 (peripheral)	0	0	2.35	nd	nd	1 (c)
(transition)	0.1	0	3.64			0.36
3 (peripheral)	0	0	0.93	nd	nd	1 (c)
(transition)	1.9	0	4.74			1.19
4 (peripheral)	1 (c)	0	1 (c)	1 (c)	nd	1 (c)
(transition)	1 (c)	0	1 (c)	1.15		0.4
5 (peripheral)	0.69	0	0.89	1 (c)	nd	1 (c)
(transition)	0.56	0	1.10	0.93		0.5
6 (peripheral)	0.21	0	0.83	1 (c)	1 (c)	1 (c)
(transition)	0.39	0	0.67	2.16	1.63	0.32
7 (peripheral)	0	0	1 (c)	nd	nd	1 (c)
(transition)	0	0	1 (c)			0.3
8 (peripheral)	0	0	0.44	nd	nd	1 (c)
(transition)	0	0	2.13			1.4
9 (peripheral)	0	0	0.62	nd	nd	1 (c)
(transition)	0	0	0.66			0.31
10 (peripheral)	1 (c)	0	1 (c)	1 (c)	nd	1 (c)
(transition)	1 (c)	0	1 (c)	0.58		0.16
11 (peripheral)	0	0	1.48	nd	nd	1 (c)
(transition)	7.24	0	1.40			0.16
12 (peripheral)	0.94	0	1.92	nd	nd	1 (c)
(transition)	0	0	2.01			0.17

Patient no. (zone)	Inter-individual gene expression			Intra-individual gene expression		
	CYP11A1	CYP11A2	CYP11B1	CYP11A1	CYP11A2	CYP11B1
13 (peripheral) (transition)	1 (c)	0	1 (c)	1 (c)	nd	1 (c)
	1 (c)	0	1 (c)	0.12		0.15
14 (peripheral) (transition)	0.73	0	1.42	1 (c)	nd	1 (c)
	0.74	0	0.86	0.12		0.16
15 (peripheral) (transition)	0.21	0	0.83	1 (c)	nd	1 (c)
	0.27	0	1.05	0.26		0.20
16 (peripheral) (transition)	0	0	1 (c)	nd	nd	1 (c)
	0	0	1 (c)			0.24
17 (peripheral) (transition)	1 (c)	0	0.31	1 (c)	nd	1 (c)
	1 (c)	0	1.32	0.26		1.02
18 (peripheral) (transition)	1.04	0	0.80	1 (c)	nd	1 (c)
	10.58	0	0.90	2.40		0.72
19 (peripheral) (transition)	0	0	1 (c)	nd	1 (c)	1 (c)
	0	0	1 (c)		0.24	0.07
20 (peripheral) (transition)	0	0	0.31	nd	nd	1 (c)
	0	0	3.07			0.68
21 (peripheral) (transition)	0	0	0.33	nd	nd	1 (c)
	0	0	4.74			1.00
22 (peripheral) (transition)	1 (c)	0	1 (c)	1 (c)	nd	1 (c)
	0	0	1 (c)	0		0.02
23 (peripheral) (transition)	0	0	0.05	nd	nd	1 (c)
	0	0	1.11			0.44
24 (peripheral) (transition)	9.55	0	0.17	1 (c)	1 (c)	1 (c)
	0	0	1.40	0.48	2.19	0.17
25 (peripheral)* (transition)	1 (c)	0	1 (c)	1 (c)	nd	1 (c)
	1 (c)	0	1 (c)	3.35		0.23

Patient no. (zone)	Inter-individual gene expression			Intra-individual gene expression		
	CYP1A1	CYP1A2	CYP1B1	CYP1A1	CYP1A2	CYP1B1
26 (peripheral)	0.01	0	0.6	1 (c)	nd	1 (c)
(transition)	0.03	0	0.8	9.73		0.31
27 (peripheral)	0.02	0	0.56	1 (c)	nd	1 (c)
(transition)	0.03	0	0.28	7.24		0.11

All patients are RRP except * who had cystoprostatectomy. Some of the data contained in this table was previously published,¹⁸ this is an expanded dataset.

Patients who donated prostate tissue were chronologically numbered 1–27. Following resection, tissue sets consisting of PZ and TZ were isolated. Levels of *CYP1A1*, *CYP1A2* and *CYP1B1* mRNA transcripts were compared in tissue sets from groups of three patients per experiment. For inter-individual variations, the mRNA transcript levels in PZ or TZ derived from the first patient number was arbitrarily taken as the calibrator (c). For intra-individual variations, mRNA transcript levels in PZ tissue for each individual tissue set were taken as the (c). Quantitative gene expression was carried out exactly as described. (c), calibrator for respective tissues; nd, not determined; 0 represents undetected or unquantifiable relative levels of mRNA transcripts.

TABLE 2

NAT and COMT quantitative expression in prostate zones

Patient no. (zone)	Inter-individual gene expression			Intra-individual gene expression		
	NAT1	NAT2	COMT	NAT1	NAT2	COMT
1 (peripheral)	1 (c)	1 (c)	1 (c)	1 (c)	1 (c)	1 (c)
(transition)	1 (c)	1 (c)	1 (c)	0.22	0.58	0.28
2 (peripheral)	2.45	1.09	1.85	1 (c)	1 (c)	1 (c)
(transition)	1.90	0.67	2.73	0.17	0.36	0.40
3 (peripheral)	0.23	0.11	0.67	1 (c)	1 (c)	1 (c)
(transition)	1.43	0.37	2.50	1.39	1.90	1.03
4 (peripheral)	1 (c)	1 (c)	1.57	1 (c)	1 (c)	1 (c)
(transition)	1 (c)	1 (c)	3.43	0.37	1.07	0.60
5 (peripheral)	0.83	0.75	0.53	1 (c)	1 (c)	1 (c)
(transition)	1.15	1.20	2.42	0.52	1.72	1.26
6 (peripheral)	0.12	0.26	0.22	1 (c)	1 (c)	1 (c)
(transition)	0.33	0.29	1.945	1.00	1.21	2.37
7 (peripheral)	1 (c)	1 (c)	1 (c)	1 (c)	1 (c)	1 (c)
(transition)	1 (c)	1 (c)	1 (c)	0.54	0.66	0.48
8 (peripheral)	0.51	0.42	0.75	1 (c)	1 (c)	1 (c)
(transition)	0.73	2.75	1.42	0.78	4.31	0.90
9 (peripheral)	0.69	0.82	0.60	1 (c)	1 (c)	1 (c)
(transition)	0.56	0.21	0.25	0.44	0.17	0.20
10 (peripheral)	1 (c)	1 (c)	0.75	1 (c)	1 (c)	1 (c)
(transition)	1 (c)	1 (c)	0.53	0.33	0.83	0.34
11 (peripheral)	3.66	0.89	0.68	1 (c)	1 (c)	1 (c)
(transition)	1.33	0.88	0.84	0.12	0.85	0.59
12 (peripheral)	1.65	0.64	1.24	1 (c)	1 (c)	1 (c)
(transition)	5.76	0.94	3.18	1.16	1.22	1.22

Patient no. (zone)	Inter-individual gene expression			Intra-individual gene expression		
	NAT1	NAT2	COMT	NAT1	NAT2	COMT
13 (peripheral) (transition)	1 (c)	1 (c)	1 (c)	1 (c)	1 (c)	1 (c)
	1 (c)	1 (c)	1 (c)	0.16	2.30	0.13
14 (peripheral) (transition)	1.31	1.86	0.82	1 (c)	1 (c)	1 (c)
	0.96	1.93	0.70	0.11	2.40	0.11
15 (peripheral) (transition)	0.17	0.46	0.06	1 (c)	1 (c)	1 (c)
	1.04	1.03	1.50	0.96	5.12	2.99
16 (peripheral) (transition)	1 (c)	1 (c)	1.64	1 (c)	1 (c)	1 (c)
	1 (c)	0	2.31	0.35	nd	0.18
17 (peripheral) (transition)	0.75	0	0.64	1 (c)	nd	1 (c)
	0.40	0	4.03	0.19	nd	0.82
18 (peripheral) (transition)	0.73	0.07	0.58	1 (c)	1 (c)	1 (c)
	0.68	0	2.41	0.33	2.99	0.53
19 (peripheral) (transition)	1 (c)	1 (c)	1 (c)	1 (c)	1 (c)	1 (c)
	1 (c)	1 (c)	1 (c)	1.53	1.59	0.75
20 (peripheral) (transition)	2.56	0.88	1.09	1 (c)	1 (c)	1 (c)
	1.69	0.45	1.03	1.01	0.80	0.71
21 (peripheral) (transition)	0.61	0.12	0.70	1 (c)	1 (c)	1 (c)
	0.84	0	1.09	2.11	nd	1.17
22 (peripheral) (transition)	1 (c)	1 (c)	0.89	1 (c)	1 (c)	1 (c)
	1 (c)	1 (c)	0.06	0.41	1.53	0.05
23 (peripheral) (transition)	0.13	0.34	0.12	1 (c)	1 (c)	1 (c)
	0.52	0.75	0.11	1.59	3.33	0.70
24 (peripheral) (transition)	0.68	0.36	0.40	1 (c)	1 (c)	1 (c)
	0.93	0.40	0.41	0.56	1.73	0.77
25 (peripheral)* (transition)	1 (c)	1 (c)	1 (c)	1 (c)	1 (c)	1 (c)
	1 (c)	1 (c)	1 (c)	0.54	1.03	2.02

Patient no. (zone)	Inter-individual gene expression			Intra-individual gene expression		
	NATI	NAT2	COMT	NATI	NAT2	COMT
26 (peripheral)	2.02	0.67	1.33	1 (c)	1 (c)	1 (c)
(transition)	3.83	5.11	0.81	1.02	7.83	1.23
27 (peripheral)	3.08	1.24	2.87	1 (c)	1 (c)	1 (c)
(transition)	1.15	2.65	0.75	0.20	2.20	0.53

All patients are RRP except * who had cystoprostatectomy

Patients who donated prostate tissue were chronologically numbered 1–27. Following resection, tissue sets consisting of PZ and TZ were isolated. Levels of *NATI*, *NAT2* and *COMT* mRNA transcripts were compared in tissue sets from groups of three patients per experiment (except for *COMT*, which was done in groups of six). For inter-individual variations, the mRNA transcript levels in PZ or TZ derived from the first patient number was arbitrarily taken as the calibrator (c). For intra-individual variations, mRNA transcript levels in PZ tissue for each individual tissue set were taken as the (c). Quantitative gene expression was carried out exactly as described. (c), calibrator for respective tissues; nd, not determined; 0 represents undetected or unquantifiable relative levels of mRNA transcripts.