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CYP1B1 expression in prostate is higher in the peripheral than in the transition zone

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Abstract

Prostate cancer (CaP) mostly occurs in the peripheral zone whereas benign prostatic hypertrophy (BPH) occurs in the transition zone. Human prostates ($n=12$) were obtained, with ethical approval, from radical retropubic prostatectomies. Following resection, tissue sets consisting of peripheral zone and transition zone were isolated from a lobe pre-operatively identified as negative for CaP. Real-time RT-PCR was employed to quantitatively examine *CYP1A1*, *CYP1A2* and *CYP1B1*. Quantifiable *CYP1A1* expression was observed (in nine out of twelve tissue sets) whilst *CYP1A2* mRNA transcripts, although detectable (in six out of twelve tissue sets), were unquantifiable. In ten tissue sets, 2- to 6-fold higher *CYP1B1* expression in peripheral zone as compared to transition zone was observed. In the other two, equal *CYP1B1* expression levels were observed; retrospective examination identified malignancy in one of the zones. Inter-individual variations (up to 10-fold) in *CYP1B1* were also noted. Immunohistochemistry for CYP1B1 showed epithelial and stromal nuclear staining. Since CYP1B1 metabolises hormones and carcinogens our results, if confirmed, suggest that this enzyme may influence susceptibility to CaP.

Keywords: *CYP1B1*; Human prostate; Nuclear staining; Peripheral zone; Radical retropubic prostatectomy; Transition zone

1. Introduction

Although only a small acorn-shaped gland (~50 g) in the genitourinary tract, the human prostate is the most common site of pathology in the male [1]. Situated beneath the bladder and surrounding the urethra, it is a composite organ consisting of non-glandular (fibromuscular) and glandular components [2]. Using the urethra as the key anatomical reference

Abbreviations: BPH, benign prostatic hypertrophy; BSAT, 0.2% bovine serum albumin in Tris-buffered saline (pH 7.6); CaP, prostate cancer; DAB, 3,3′-diaminobenzidine; DRE, digital rectal examination; HAAs, heterocyclic aromatic amines; NV, non-vegetarian; PAHs, polycyclic aromatic hydrocarbons; PSA, prostate specific antigen; RT, reverse transcriptase; TBS, tris-buffered saline.

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point, the prostate is conventionally divided into different regions or tightly-fused zones [1]. This morphology is of clinical significance in the development of age-associated conditions such as benign prostatic hypertrophy (BPH) and prostate cancer (CaP) [3]. BPH is a non-malignant overgrowth that appears to arise exclusively within the transition zone [2]. CaP is a multi-focal entity and is mainly confined to the peripheral zone [4].

Approximately 70% of the glandular tissue of the prostate is found in the peripheral zone and some 5% in the transition zone; the remaining 25% occurs in a third zone, the central zone [1]. CaP rarely originates in the central zone but the reasons for this remain obscure. Ducts and acini in the central zone are distinctively larger than those found in the transition and peripheral zone [2]. The identification of the factors responsible for the progression of latent CaP to clinically-invasive disease [5] remains a challenge, especially in the light of controversies over the relative merits of prostate specific antigen (PSA) screening and 'watch and wait' strategies [6,7].

CaP is the second most common cause of male cancer mortality in the UK and US whereas it is only ranked eleventh in Japan [8]. These demographic variations are paralleled by epidemiological studies showing that incidence rates rise amongst populations migrating from lower-risk to higher-risk areas. Dietary and/or environmental changes are probably responsible, and similar increases in incidence have been observed for female breast cancer [8]. Similarities in demographic incidence of CaP and breast cancer [9], and a high rate of co-incidence amongst spouses [10] point to common aetiological factors. Carcinogenic chemicals present in the human diet and environment include the polycyclic aromatic hydrocarbons (PAHs) and the heterocyclic aromatic amines (HAAs). Target tissues may metabolically activate these pro-carcinogens to DNA-binding reactive species [11].

The cytochrome P-450 (CYP) multigene family consists of isoenzymes that are expressed mainly in the liver but also to varying degrees in extra-hepatic sites [12]. These constitutively expressed and inducible enzymes are involved in the biotransformation of endogenous compounds, such as hormones, or exogenous agents, such as PAHs and HAAs. The detection of *CYP* mRNA transcripts in prostate tissues has previously been reported [13,14], suggesting that metabolically active cells within this gland could be susceptible to reactive metabolites formed from pro-carcinogens.

Reasons for the varying susceptibilities of prostate zones to different pathologies remain obscure. In this study, we have quantitatively investigated the relative, inter- and intra-individual, levels of *CYP1A1*, *CYP1A2* and *CYP1B1* expression in prostate tissues ($n=12$) taken from either the transition zone or peripheral zones. Differences in the capacity to metabolically activate endogenous/exogenous agents may underlie the in situ occurrence of chromosomal damage, eventually resulting in CaP.

2. Materials and methods

2.1. Prostate tissues

Study participants were selected from patients undergoing radical retropubic prostatectomy for CaP on the basis of a low PSA (<20 μg/l serum) and low volume of disease ($<$ two core biopsies positive for CaP per eight taken). Informed consent to obtain tissue for research was obtained (LREC no. 2003.6.v; Preston, Chorley and South Ribble Ethical Committee).

After surgical resection, prostate was transported to the pathology laboratory (<3 min) and dissected using aseptic techniques. Provisionally, tissue assumed to be cancer-free was selected from the lobe from which biopsy cores were negative for cancer; formalin-fixed sections stained with H&E were checked retrospectively in order to confirm the absence of CaP. Using a forceps and scalpel, the prostate tissue was sliced from the upper part of the gland starting just above the area of the verumontanum. To obtain peripheral zone tissue, a slice approximately 2 cm in length and 0.3 cm in width was isolated from the most peripheral and posterolateral aspect of the gland. Tissue slices approximately 1.5 cm in length and 0.3 cm in width were isolated from the area identified immediately lateral to the urethra (peri-urethral); this was designated as the transition zone. Tissue sets (peripheral zone and transition zone from one individual patient) were

labelled 1–12. Remaining tissue was formalin fixed for histopathology.

2.2. Quantitative real-time reverse transcriptase (RT)-PCR

Tissues were immediately placed in RNAlater (QIAGEN Ltd, Crawley, West Sussex, UK) and stored at −85 °C. For subsequent RNA extraction, tissues were ground to a fine powder under liquid nitrogen using a pestle and mortar. Total RNA extraction was performed using the Qiagen RNeasy® Kit in combination with the Qiagen Rnase-free DNase kit (QIAGEN Ltd, Crawley, West Sussex, UK). RNA (0.4 μg) was reverse transcribed in a final volume of 20 μl containing Taqman® reverse transcription reagents (Applied Biosystems, Warrington, Cheshire, UK): 1×Taqman RT buffer; $MgCl_2$ (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 then 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 (Table 1) for *CYP1A1*, *CYP1A2*, *CYP1B1* and the endogenous control *β-ACTIN* were chosen using Primer Express software 2.0 (Applied Biosystems, Warrington, UK) and designed so that one primer spanned an exon boundary (Table 1). 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, Warrington, UK). Reaction mixtures contained 1×SYBR® Green PCR master mix (Applied Biosystems, Warrington, UK); forward and reverse primers (Invitrogen, Paisley, UK) at a concentration of 300 nM; for *CYP1A1*, *CYP1A2* or *CYP1B1* amplification 20 ng cDNA template or for *β-ACTIN* amplification 5 ng cDNA template; made to a total volume of 25 μl with sterile H_2O. Thermal cycling parameters included activation at 95 °C (10 min) followed by 40 cycles each of denaturation at 95 °C (15 s) 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 [15].

2.3. Immunohistochemical staining

Tissues were fixed in formalin prior to wax-embedding. Immunohistochemical staining of tissue sections 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% H_2O_2 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 (anti-CYP1B1 antibody (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 tertiary anti-sera (avidin–biotin complex) in BSAT for 30 min and washed again with TBS for 5 min. 3,3′-Diaminobenzidine (DAB) chromogen in 0.05 M

Table 1
Primers used for quantitative real-time RT-PCR analyses

Assay	Name	Sequence (5′→3′)
CYP1A1	*CYP1A1*-F	ACT TCA TCC CTA TTC TTC GCT ACC T
	CYP1A1-R	CGG ATG TGG CCC TTC TCA
CYP1A2	*CYP1A2*-F	GAC ATC TTT GGA GCA GGA TTT GA
	CYP1A2-R	CTT CCT CTG TAT CTC AGG CTT GGT
CYP1B1	*CYP1B1-F*	GTA CCG GCC ACT ATC ACT GAC A
	CYP1B1-R	CAC ATC AGG ATA CCT GGT GAA GAG
β-ACTIN	*β-ACTIN*-F	CCT GGC ACC CAG CAC AAT
	β-ACTIN-R	GCC GAT CCA CAC GGA GTA CT

F, forward primer; R, reverse primer.

Tris/HCl buffer (pH 7.6) with 0.1% H_2O_2 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 coverslips using Styrolite mounting medium (VWR International, Poole, UK).

2.4. Electron microscopy

Following dissection (see earlier), prostate tissue fragments ($\sim <50$ mg) were placed in glutaraldehyde (4% in 0.1 M sodium cacodylate buffer). After fixation, tissues were washed in sodium cacodylate buffer, post-fixed in osmium tetroxide, dehydrated in an alcohol series and embedded in araldite resin. Ultrathin sections (70 nm) were cut on a Reichert Ultracut E ultramicrotome and stained with 2% uranyl acetate and lead citrate prior to being examined on a JEOL JEM-1010 transmission electron microscope.

3. Results

3.1. Patient details prior to study participation

Tissues from twelve patients undergoing radical retropubic prostatectomy were obtained (Table 2). The age range was 53–71 years, all participants were non-vegetarian (NV), seven were current or ex-smokers and all but one (no. 4) consumed alcohol. PSA (μg/l serum) levels ranged from 4.4 to 19.1 (mean $\pm$ SD of 8.9 ± 5.2). On digital rectal examination (DRE), carried out by a single assessor, seven participants were characterised as benign (non-cancerous-feeling glands—Stage T_1c) and the remaining patients ($n=5$) had a malignant-feeling gland in one lobe (Stage T_2). A single pathologist assigned the Gleason grade [16] after histopathological examination of the prostate. The Gleason score was expressed as a sum of most common grade seen in the whole specimen and the next most common grade (each scored out of five); the participants in this study were found to have moderately-differentiated cancer (Gleason score (grade) of 6 (3+3) or 7 (4+3)).

3.2. Quantitative gene expression analyses in the peripheral and transition zones of the human prostate

Tissues were isolated from a lobe thought to be negative for CaP on the basis of pre-operative prostate biopsies. A portion of each tissue specimen was routinely subjected to normal H&E examination. Ten of the twelve tissue sets were found to be cancer free whilst, in two tissue sets (nos. 3 and 8), CaP was found in either the peripheral or the transition zone (Table 3). Real-time RT-PCR gene expression

Table 2
Details of study participants and tissue samples examined

Patient no.	Age	PSA (μg/ml)	DRE	Smoking status	Alcohol	Diet	Gleason grade
1	71	8.6	Benign	Yes	Yes	NV	3+3
2	61	6.7	T_2 Left lobe	Yes	Yes	NV	3+3
3	65	16.1	Benign	No	Yes	NV	4+3
4	65	8	T_2 Right lobe	Yes	No	NV	4+3
5	54	4.6	T_2 Right lobe	No	Yes	NV	4+3
6	68	4.4	Benign	No	Yes	NV	3+4
7	58	4.6	Benign	No	Yes	NV	3+3
8	53	19.1	T_2 Right lobe	Yes	Yes	NV	4+3
9	53	8.1	T_2 Left lobe	Yes	Yes	NV	3+3
10	67	8.1	Benign	No	Yes	NV	3+4
11	68	6.9	Benign	Yes	Yes	NV	3+4
12	69	8.0	Benign	Yes	Yes	NV	3+3

Patients who donated prostate tissue for the research purposes of this study were chronologically numbered 1–12. Tumours were distinguished histologically according to Geason grade [16]. Abbreviations: DRE, digital rectal examination; NV, non-vegetarian; PSA, prostate specific antigen.

Table 3
Relative *CYP* expression in prostate by real time RT-PCR

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

Patients who donated prostate tissue were chronologically numbered 1–12. Following resection, tissue sets consisting of peripheral zone and transition zone 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 peripheral zone or transition zone derived from the first patient number was arbitrarily taken as the calibrator (c). For intra-individual variations, mRNA transcript levels in peripheral zone tissue for each individual tissue set were taken as the (c). Quantitative gene expression was carried out exactly as previously described [15]. (c), calibrator for respective tissues; nd, not determined; 0 represents undetected or unquantifiable relative levels of mRNA transcripts; *, Indicates samples containing detectable but unquantifiable mRNA transcripts.

analyses were carried out without prior knowledge of these histopathological findings.

The results of quantitative gene expression analyses of phase I metabolising enzymes (*CYP1A1*, *CYP1A2* and *CYP1B1*) in the peripheral and transition zones of prostate tissues from each patient ($n = 12$) are shown in Table 3. Expression analyses for the three genes using tissue sets from three patients were examined in individual experiments. *CYP1A1* expression was quantifiable in either or both of the zones in six of nine tissue sets (nos. 1–6 and 10–12) and was unquantifiable in three other tissue sets (nos. 7–9). Tissue sets nos. 1–3 exhibited quantifiable expression only in the transition zone whereas levels of gene expression were equal in the peripheral and transition zones of tissue sets nos. 4 and 5; a 2-fold higher level of gene expression was observed in transition zone compared to peripheral zone in tissue set no. 6. There was a 2-fold higher level of *CYP1A1* expression in peripheral compared to transition zone from tissue set no. 10 whilst mRNA transcripts were only detectable in transition or peripheral zone, respectively, of tissue sets nos. 11 and 12. Approximately a 3-fold inter-individual variation between tissue sets nos. 4–6 and 7-fold inter-individual variation between tissue sets nos. 10–11 in quantitative *CYP1A1* expression was also observed. *CYP1A2* was not quantifiably expressed in any of the tissues examined (Table 3).

CYP1B1 expression was detected in both the peripheral and transition zones of all tissue sets

examined in this study (Table 3). Inter-individual variation in *CYP1B1* expression levels in peripheral zone (0.5–2-fold) and transition zone (0.5–5-fold) were noted. In the ten tissue sets found to be cancer free, *CYP1B1* expression was consistently observed to be 2- to 6-fold higher in the peripheral zone compared to the transition zone. In the other two tissue sets (nos. 3 and 8) there was approximately equal gene expression levels in both zones; histopathology showed CaP in one of the zones in both these tissue sets (Table 3).

3.3. CYP1B1 expression as detected using immunohistochemistry

Immunohistochemical staining of the tissues using a polyclonal anti-CYP1B1 antibody showed the presence of a distinct pattern of nuclear staining. Fig. 1(A)–(D) shows staining of cancer-free glandular tissue (1(A) and (C)) and stromal tissue (1(B)) from tissue set no. 6 in comparison with the staining pattern seen in cancer tissue (1(D)) obtained from the same individual. In the cancer-free tissue, CYP1B1 staining in the glands (Fig. 1(A) and (C)) appeared to be stronger in the basal epithelial cells than in the cells adjacent to the lumen; nuclear staining was also observed in the stroma (Fig. 1(B)). In cancer tissue from the same individual, sheaths of cells exhibiting both nuclear staining and cytoplasmic staining were observed (Fig. 1(D)).

3.4. Electron microscopic detection of pre-existing in situ chromosomal damage

Electron microscopy of various features of tissue no. 4 are shown in Fig. 2(A)–(F). Features of a typical secretory epithelium, characterised as a monolayer of cuboidal cells with surface microvilli and numerous internal vesicles, are apparent in Fig. 2(A) and (E). In situ evidence of ‘extra-nuclear chromosomal

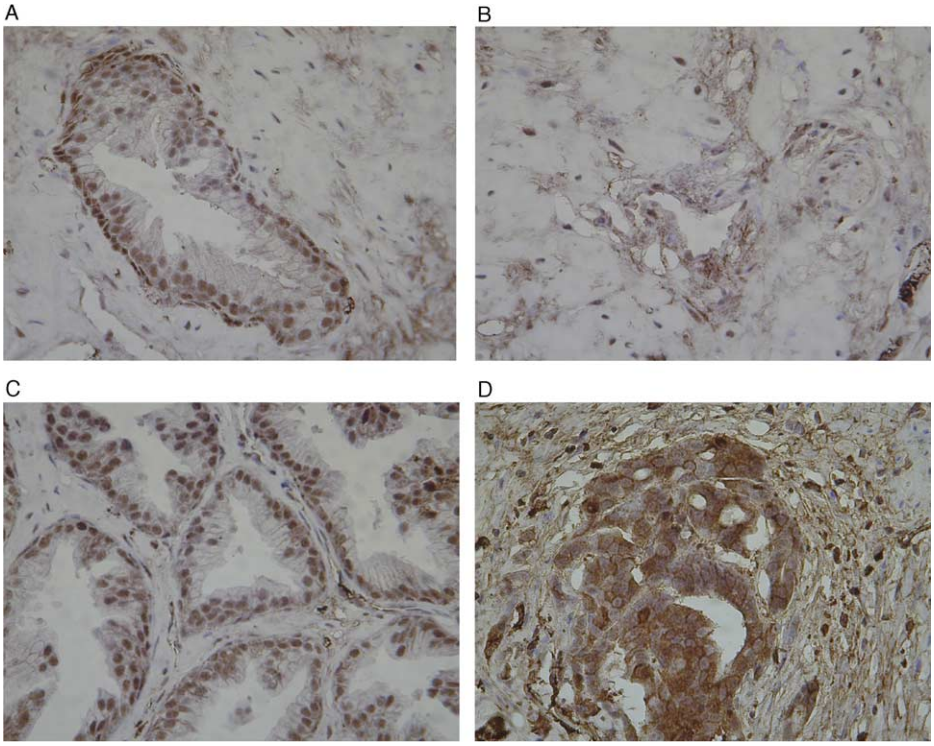

Fig. 1. Immunohistochemical analysis of CYP1B1 in cancer-free tissue and cancer tissue of prostate from patient no. 6. Photomicrographs show (A) cancer-free glandular element, (B) cancer-free stroma, (C) cancer-free glandular elements, and (D) cancer tissue. Original magnification×200

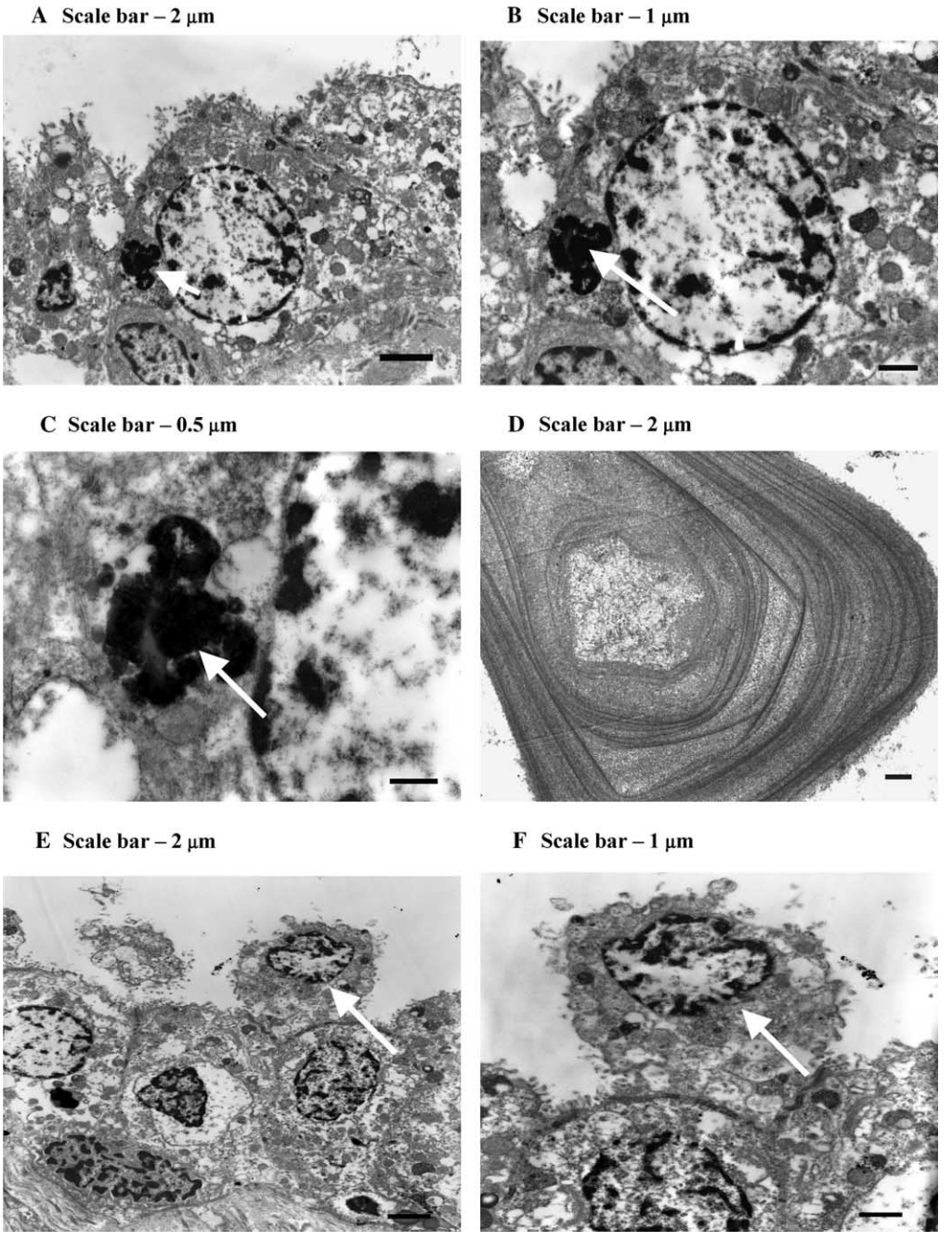

Fig. 2. Transmission electron microscopic analysis of cancer-free tissue from patient no. 4. Photomicrographs show (A) secretory cell of a cancer-free gland containing 'extra-nuclear chromosomal material', (B) secretory cell of cancer-free gland containing 'extra-nuclear chromosomal material', (C) 'extra-nuclear chromosomal material' found in a secretory cell of a cancer-free gland, (D) aggregated glycoprotein deposit known as corpora amylacea present in a gland lumen, (E) a cell exfoliating from the secretory layer of a gland in cancer-free tissue, and (F) a cell exfoliating from the secretory layer of a gland in cancer-free tissue. White arrows in photomicrograph (A), (B) and (C) point to 'extra-nuclear chromosomal material', in (E) and (F) to the exfoliating cell. Scale bars indicate relative sizes.

material', indicated by a white arrow, in a cell adjacent to the glandular lumen is shown in Fig. 2(A)–(C). The nucleus of this cell appears to contain a normal distribution of heterochromatin peripheral to electron-lucent euchromatin. The 'extra-nuclear chromosomal material' appears to consist mostly of dense, inactive heterochromatin with a small boundary of active euchromatin. This suggests the presence of a micronucleus in a prostate epithelial cell. Fig. 2(D) shows the ultrastructure of a typical lamellated mass known as the corpora amylacea; a characteristic feature of ageing prostate. Finally, Fig. 2(E) and (F) show what appears to be an epithelial cell exfoliating into the glandular lumen; a removal mechanism for pre-malignant prostate epithelial cells may be an important protective mechanism against the development of CaP.

4. Discussion

As the apparent incidence of CaP increases in developed countries as a result of increased screening [17], early recognition and treatment of individuals with invasive disease whilst, at the same time, avoiding the unnecessary treatment of those with latent CaP is an important priority. The prostate is a hormone-responsive organ in which androgens are believed to stimulate growth and secretory functions whilst oestrogens act as growth inhibitors; the ratio between the two hormonal effects may be critical to disease progression [18]. The chemoprevention of CaP either through administration of finasteride (a 5α-reductase inhibitor) [19] or selective oestrogen receptor modulators [20] are currently being investigated in human clinical trials.

Although many CYP enzymes are involved in hormone and carcinogen hydroxylation, the CYP1A1, CYP1A2 and CYP1B1 isoforms have been subjected to the most intense scrutiny [13,14,21,22]. *CYP1A2* mRNA transcripts, although detectable at very low levels, were unquantifiable in the tissues examined in the present study; *CYP1A1* expression was also observed but again at low levels. This is in contrast to previous reports [13,14] where mRNA transcripts were readily measurable. Differences in the various tissue-collection protocols may account for this; firstly, our tissue originated exclusively from radical retropubic prostatectomies as opposed to transurethral resection [13] and, secondly, our tissues were placed in an RNA-stabilising solution rather in liquid nitrogen [14]. The prostate exhibits a high degree of anatomical heterogeneity and the differential levels of *CYP1A1* mRNA transcripts in transition and peripheral zones underscore the importance of highlighting the exact point of tissue retrieval. In six of the nine prostate tissue sets in which *CYP1A1* mRNA transcripts were detected, higher levels of expression were observed in the transition zone compared to the peripheral zone (Table 3).

CYP1B1 is inducible by compounds such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin acting through receptor ligands [12,21]. It preferentially catalyses the 4-hydroxylation of 17β-oestradiol [23], metabolically activates a wide spectrum of exogenous pro-carcinogens [12] and is also believed to inactivate some anticancer agents used in the treatment of CaP [24]. Single nucleotide polymorphisms at codon 119 (G→T) of *CYP1B1* have been associated with an elevated risk of CaP [21]. An apparent up-regulation of *CYP1B1* in CaP suggests that it may be a suitable target for therapy [25]. Our preliminary results demonstrate the presence of *CYP1B1* mRNA transcripts (Table 3) and protein (Fig. 1(A)–(C)) in cancer-free tissues. This is despite speculation that post-transcriptional CYP1B1 regulation, possibly involving altered mRNA stability, reduces the levels in normal tissues [26]. Intriguingly, we have observed 2- to 6-fold higher intra-individual levels of *CYP1B1* expression in peripheral zone as compared to transition zone (Table 3). Whether such differences underlie the varying susceptibilities of prostate zones remains to be ascertained.

An increasing awareness of the presence of pre-existing DNA damage measured either as single-strand breaks [27] or micronuclei [28] suggests that these may be useful biomarkers of pre-malignant cells. The identification, using transmission electron microscopy, of what appears to be a micronucleus in a secretory cell of a prostate gland (Fig. 2(A)–(C)) strongly supports the view that the removal of such cells, for instance by exfoliation (Fig. 2(D) and (E)), may be an important protective mechanism [8]. Interestingly, it appears that a small portion of the extra-nuclear chromosomal material identified in Fig. 2(A)–(C) remains actively transcribed.

Up-regulation of CYP1B1 expression has also been associated with cancers in other hormone-responsive tissues such as breast [29] and ovary [30]. Oestrogens are potentially genotoxic at physiological concentrations [31] and it has been suggested that cancer-susceptible tissues in a rat model have lower levels of detoxification enzymes than other prostatic tissues [32]. Determining whether the CYP1B1 enzyme can act as a primary regulator in the progression to clinically invasive disease would be of great importance. Identification of such a primary target could also result in the development of either chemoprevention strategies to delay cancer progression or a novel enzyme inhibition for the treatment of hormone-resistant cancer. Future experiments will aim to further quantify gene and protein expression levels in the heterogeneous cell populations of cancer-free tissue in comparison with cancer tissue.

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