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Original Article

T-cadherin in prostate cancer: relationship with cancer progression, differentiation and drug resistance

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Abstract

Prostate cancer represents the second leading cause of cancer-related death in men. T-cadherin (CDH13) is an atypical GPI-anchored member of the cadherin family of adhesion molecules. Its gene was reported to be downregulated in a small series of prostate tumours. T-cadherin protein expression/localisation in prostate tissue has never been investigated. The purpose of our study was to analyse CDH13 gene and protein levels in large sets of healthy and cancer prostate tissue specimens and evaluate CDH13 effects on the sensitivity of prostate cancer cells to chemotherapy. Analysis of CDH13 gene expression in the TCGA RNAseq dataset for prostate adenocarcinoma ($N = 550$) and in tissue samples ($N = 101$) by qPCR revealed weak positive correlation with the Gleason score in cancer and no difference between benign and malignant specimens. Immunohistochemical analysis of tissue sections ($N = 12$) and microarrays ($N = 128$ specimens) demonstrated the presence of CDH13 on the apical surface and at intercellular contacts of cytokeratin 8-positive luminal cells and cells double-positive for cytokeratin 8 and basal marker p63. T-cadherin protein expression was markedly upregulated in cancer as compared to benign prostate hyperplasia, the increase being more prominent in organ-confined than in advanced hormone-resistant tumours, and correlated negatively with the Gleason pattern. T-cadherin protein level correlated strongly with cytokeratin 8 and with an abnormal diffuse/membrane localisation pattern of p63. Ectopic expression of CDH13 in metastatic prostate cancer cell line DU145 reduced cell growth in the presence of doxorubicin. We conclude that CDH13 protein, but not its gene expression, is strongly upregulated in early prostate cancer, correlates with changes in luminal/basal differentiation and p63 localisation, and promotes sensitivity of cancer cells to doxorubicin. These data identify CDH13 as a novel molecule relevant for prostate cancer progression and response to therapy.

Keywords: prostate cancer; T-cadherin; cadherins; basal and luminal cells; doxorubicin; drug resistance

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Introduction

Prostate cancer (PCa) represents the second leading cause of cancer-related death in men. Early-stage localised tumours are successfully treated by surgery, radiation and androgen-deprivation therapy. However, in advanced stage PCa, the effects of these treatments in more advanced cases are frequently temporary, and after 18–24 months the disease progresses to its lethal stage characterised by insensitivity to hormone deprivation, metastasis and a short survival period [1]. Therefore, the search for alternative mechanisms of tumour growth and novel markers that can enable early identification of patients at high risk for relapsing aggressive disease remains a central objective in PCa research.

The inner epithelial layer of the healthy prostate gland is constituted of terminally differentiated luminal secretory cells characterised by expression of several luminal markers including cytokeratin 8 (CK8). The outer basal compartment contains basal cells and is believed to be a niche for prostate stem cells. Amongst established basal cell markers is p63, a member of the p53 gene family which plays a key role in regulating epithelial differentiation in many organs including the prostate [2–4]. The cellular origin of PCa is still debated [5]. Luminal cells comprise acinar adenocarcinoma, the predominant variant of PCa [6], and can initiate tumours *in vivo* [7]. However, there is evidence that basal cells display significant plasticity and can give rise to luminal-type tumours after differentiation into luminal cells [8–10]. A subset of prostate tumours which express basal markers [11] and are likely to originate from a basal cell population that has undergone partial differentiation towards the luminal phenotype has been recently identified. The identification of molecular mechanisms underlying prostate tumour heterogeneity is of major importance, since tumours with different molecular and histological features have different clinical outcomes. Some studies suggest that tumours of luminal origin are more aggressive and have molecular signatures predictive of poor survival and treatment response [10]; others show that aggressive prostate cancer shares a conserved transcriptional programme with adult prostate basal stem cells [12].

Amongst molecular markers differentially expressed in luminal and basal cells in PCa are the cadherins, a superfamily of adhesion molecules mediating homophilic intercellular adhesion [13]. PCa progression is characterised by the so-called 'cadherin switch', transcriptional repression of epithelial E- and P-cadherins and upregulation of mesenchymal N-cadherin and cadherin-11 (OB-cadherin), which has strong independent prognostic value for PCa progression [14]. Downregulation of E- and P-cadherins is associated with PCa invasive potential, high Gleason score and poor prognosis [15–25] whilst patients retaining E-cadherin expression had a longer recurrence-free interval after radical prostatectomy [26]. Data on epithelial cadherin levels in metastatic PCa are inconsistent showing either loss [27,28] or re-expression in bone metastases [14,29–32]. Upregulation of mesenchymal cadherins promotes invasion and metastasis and enables metastatic cells to survive outside of the epithelial environment. Upregulation of N- and OB-cadherins correlates with higher Gleason patterns and promotes migration, metastasis and resistance to therapy [33–42]. Targeting of N- and OB-cadherin limits PCa growth, metastasis, castration resistance and angiogenesis [42–44].

T-cadherin (CDH13 or H-cadherin) is an atypical member of the cadherin superfamily. It lacks transmembrane and cytoplasmic domains and is attached to the plasma membrane via a glycosylphosphatidylinositol anchor. Described functions for T-cadherin include stimulation of angiogenesis and neuron guidance during embryonic development [45]. Interest in T-cadherin as a marker of cancer progression arose after its cloning from embryonic nervous tissue [46]. By analogy with the prototype superfamily member E-cadherin, T-cadherin was suggested to be a tumour suppressor. Indeed, downregulation of T-cadherin gene expression due to allelic loss of chromosome bands 16q24.1-q24.2 or aberrant promoter methylation was demonstrated in several types of human cancers including breast, colon, lung carcinomas, *inter alia* [45]. To date only two studies have analysed T-cadherin gene expression in PCa. CDH13 promoter was frequently methylated in serum of patients with PCa [47], and mRNA was underexpressed in 15 out of 18 analysed prostate tumour specimens [48]. T-cadherin was identified as an androgen-responsive gene and reported to inhibit PCa cell proliferation and anchorage-independent growth [48]. Our own recent investigation unexpectedly found that T-cadherin overexpression promoted migration and invasion of benign prostate cell line BPH-1 and metastatic cell line DU145 [49]. These functional phenomena were accompanied by loss of epithelial morphology and were likely due to a change in activities of epidermal growth factor receptor (EGFR) and insulin-like growth factor-1 receptor (IGF-1R). A pilot analysis of human prostate tissue demonstrated that some adenocarcinoma samples exhibit very high level of T-cadherin protein expression as compared to benign tissue, confirming that T-cadherin upregulation in PCa cells is a pathophysiologically relevant phenomenon [49]. Analysis of the expression levels and specific cellular localisation patterns of T-cadherin protein in PCa has never been conducted. To assess potential roles of T-cadherin in PCa we performed a comprehensive quantitative analysis of T-cadherin expression at gene and protein levels in human benign and malignant prostate tissue using large sets of clinical specimens and tissue microarrays.

Materials and methods

Ethics statement

Use of clinical samples for immunohistochemical and qPCR analyses was approved by the Ethical Committees of both cantons of Basel (EKBB) and of the

University of Basel. Written informed consent was obtained from patients with the requirements of the Committees.

The Cancer Genome Atlas (TCGA) database screening for T-cadherin gene expression in prostate cancer

IlluminaHiSeq-RNASeqV2 expression data (level 3) for 550 samples of prostate adenocarcinoma and corresponding clinical data were downloaded from the UCSC Cancer Browser (TCGA_PRAD_exp_HiSeqV2-2015-02-24, https://genome-cancer.ucsc.edu). Analysis and data visualisation were performed using R software (version 3.2.3).

Analysis of gene expression in prostatic tissues and cell lines by qPCR

A consecutive series of 101 specimens were obtained from men diagnosed for benign prostatic hyperplasia (BPH, n = 44) or PCa ($n = 57$) at the Urology Department, Basel University Hospital. Patients with BPH underwent conventional transurethral resection (TUR-P), whilst patients with PCa underwent either palliative TUR-P or endoscopic extraperitoneal radical prostatectomy (EERP). Relevant clinical data were collected by reviewing patients' files. PCa tissues were screened for the presence of tumours by an experienced pathologist. Total RNA from tissues or cultured cells was isolated using RNeasy® MiniKit (Qiagen, Basel, Switzerland), treated with deoxyribonuclease I and reverse transcribed using M-MLV Reverse Transcriptase (Invitrogen, Carlsbad, California). cDNA was amplified by qPCR as previously detailed [50]. Gene expression was normalised to the level of endogenous housekeeping gene GAPDH. Primer sequences are available on request.

Tissue microarrays

Tissue microarrays (TMAs) have been constructed from formalin-fixed and paraffin-embedded (FFPE) tissue specimens from the archives of the Institute of Pathology, University Hospital Basel. The histological grading and classification of tumours were performed by experienced pathologists. In construction of the TMAs, for each specimen three cores with Ø 0.6 mm from representative tumour regions were included. Consecutive 4-μm sections from any given TMA block were stained with hematoxylin and eosin for standard histological documentation and using indirect immunoperoxidase and immunofluorescent procedures to detect T-cadherin, CK8 and p63 (supplementary material and methods). Quantitative data were obtained for 128 specimens comprising 18 BPH cases from TUR-P and 110 PCa cases that included organ-confined cancer from TUR-P ($n = 24$), organ-confined cancer from EERP ($n = 49$) and castration-resistant PCa from TUR-P ($n = 37$).

Tissue sections

T-cadherin protein expression was also explored in larger sections from a small series of prostate tissue samples from the archives of the Institute of Pathology Basel. Immunohistochemical staining for T-cadherin, CK8 and p63 (supplementary material and methods) was performed on 4 μm sections from FFPE samples. In total 9 BPH tissue specimens from TUR-P and 5 adenocarcinoma specimens from core needle biopsies were analysed. Representative images are shown.

Cells and vectors

T-cadherin was stably overexpressed (Tcad+) in metastatic human PCa cell line DU145 (LGC Standards, Wesel, Germany) using pLVX-puro vector carrying full length human T-cadherin cDNA [51]. Empty pLVX-puro-vector (E) served as control. Cell culture and sensitivity assay protocols are detailed in supplementary material and methods.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA, USA). Differences between sample groups were determined using one-way repeated measures ANOVA with Tukey's multiple comparison. Pearson's correlation coefficient was calculated to determine relationships between cell marker expression levels. Differences with p-value of <0.05 were considered significant.

Additional materials and methods are provided as supplementary material and methods.

Results

The cancer genome atlas screening for T-cadherin gene expression in prostate cancer

T-cadherin/CDH13 gene expression in PCa tissue was analysed using data included within The Cancer Genome Atlas (TCGA) database. The TCGA RNA-seq dataset for PCa (TCGA-PRAD) contains 550 samples of prostate adenocarcinoma. For 29 samples

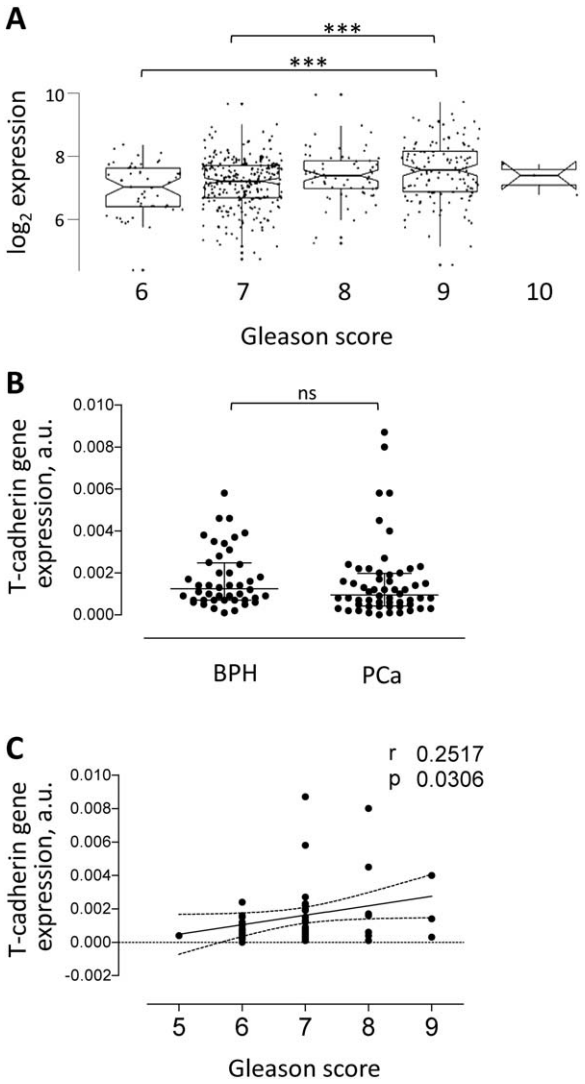

Figure 1. T-cadherin gene expression in prostate tissue. T-cadherin gene expression was analysed (A) in the TCGA RNA-seq dataset (TCGA-PRAD) containing 550 samples of prostate adenocarcinoma, and (B) in tissue samples obtained from patients with benign prostate hyperplasia (BPH, $n = 44$) and or prostate cancer (PCa, $n = 57$) by qPCR, normalised to GAPDH and expressed as arbitrary units (a.u.). T-cadherin gene expression determined by qPCR in 57 PCa samples was correlated with the Gleason score (C). Pearson's correlation coefficient (r) and p-values are shown.

Gleason score data are not available; other samples are classified as tumours with Gleason score 6–10. Analysis of T-cadherin gene expression (Figure 1A) demonstrated no difference in expression level between groups with Gleason score 6, 7, 8 and 10. T-cadherin level was slightly increased in group with Gleason score 9 versus Gleason score 6 ($\log_2$ difference = 0.58, $p = 0.0004$) and Gleason score 7 ($\log_2$ difference = 0.38, $p = 0.0002$). No correlations were observed between T-cadherin expression and prostate-specific antigen (PSA) values ($r = 0.062$, $p = 0.187$), overall survival ($r = -0.009$, $p = 0.831$) or recurrence-free survival ($r = 0.001$, $p = 0.986$).

T-cadherin gene expression in benign and malignant prostate tissue

T-cadherin gene expression in BPH ($n = 44$) and PCa ($n = 57$) tissues was measured by qPCR. Prostate adenocarcinoma samples included tumours with Gleason score 5–9 and 1 case of hormone-resistant cancer. The average T-cadherin gene expression level in cancer samples was not different from benign disease (Figure 1B). In accordance with the TCGA analysis results, T-cadherin gene expression in PCa tissue showed weak positive correlation with the Gleason score ($r = 0.2517$, $p = 0.0306$) (Figure 1C).

T-cadherin protein expression pattern in benign prostatic tissue

T-cadherin protein expression pattern in non-malignant prostatic tissue was evaluated by horseradish peroxidase (HRP)-immunostaining (Figure 2A) or by immunofluorescence labelling for T-cadherin, luminal cell marker CK8 and basal cell marker p63 (Figure 2B,C). Paraffin sections from individually processed specimens (Figure 2B) and TMA samples of BPH (Figure 2A,C) were used. T-cadherin protein was detected in all layers of the gland. In the luminal compartment, it was localised on the apical surface and intercellular contacts of CK8-positive luminal cells (Figure 2B,C). In the basal compartment T-cadherin was present on cells double-positive for CK8 and p63 (Figure 2B,C). As expected, T-cadherin was also detected on blood vessels (Figure 2A).

T-cadherin protein expression pattern in prostate cancer tissue

T-cadherin protein expression in prostatic tissue was evaluated by HRP-immunostaining (Figure 3A) and by triple immunofluorescence staining for T-cadherin, CK8 and p63 (Figure 3B,C). Figure 3 shows a selection of 12 different specimens labelled I–XII where I is high-grade prostatic intraepithelial neoplasia (PIN) and II–XII are PCa adenocarcinomas. Sections of TMA blocks (Figure 3A,C) and individually processed samples (Figure 3B) were used for the analysis. Specimens exhibited a high range of differentiation including tumours with partially retained glandular-like structure (samples II, VII-X) and

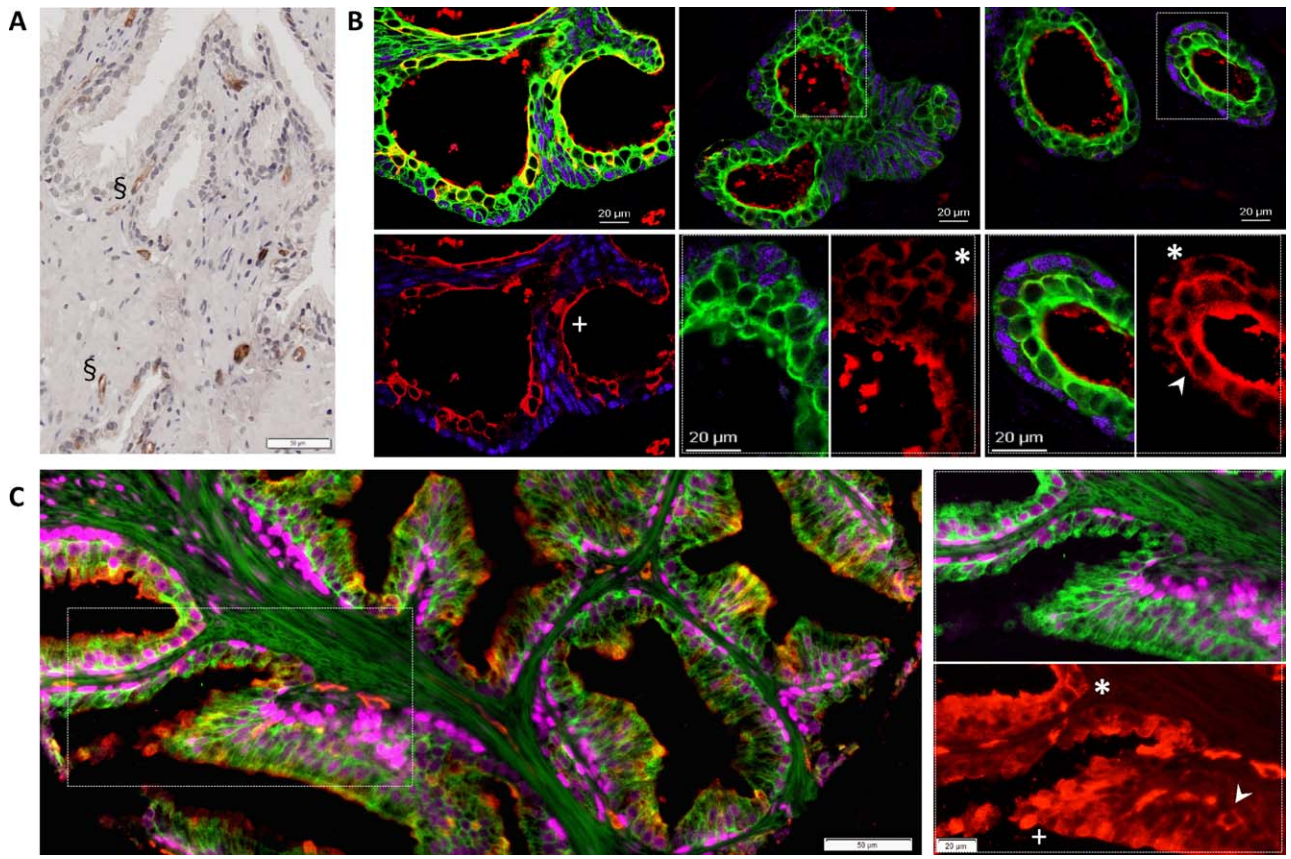

Figure 2. T-cadherin protein expression in benign prostate hyperplasia. T-cadherin protein expression in BPH was analysed by horseradish peroxidase (HRP)-immunostaining (A) or by immunofluorescence labelling for T-cadherin (red), cytokeratin 8 (CK8, green) and p63 (purple) (B, C) in paraffin-embedded sections of individually processed (B) or TMA (A, C) samples. Boxes outlined with dashed line show high magnification fragments of full-frame images (B, C). Note T-cadherin staining on the apical surface (+) and in intercellular contacts (arrow) of luminal CK8-positive cells, as well as in the basal compartment on cells double-positive for CK8 and p63 (*). §, blood vessels.

tumours with diffuse and solid growth patterns (III-VI, XI, XII). With respect to expression of luminal/basal phenotype markers, we observed tumours with high CK8 levels (VII, VIII, IX), predominantly high p63 expression (XI) or mixed patterns where both markers were present at comparable levels (X, XII).

T-cadherin was detected in PIN, as well as in both glandular-like and disorganised tumours (Figure 3A–C). The expression patterns of T-cadherin ranged from localisation to the apical surface and intercellular contacts of the epithelial lining, more typical for tumours forming glandular structures (samples VII-IX), to a more diffuse or mixed pattern, more typical for tumours with diffuse growth pattern (X, XI, XII). Both CK8- and p63-positive specimens were positive for T-cadherin (Figure 3B,C). Levels of T-cadherin were heterogeneous: some specimens were negative (IV) or almost negative (X), whereas other tumours (II, III, VII-IX, XI, XII), as well as PIN (I), displayed prominent T-cadherin expression on the epithelial lining of the gland or in the tumour mass.

T-cadherin, cytokeratin 8 and p63 proteins are upregulated in prostate cancer

To establish the relationship between T-cadherin expression and PCa progression we performed quantitative analysis of T-cadherin levels in TMAs which were stained for T-cadherin using either HRP- (Figure 4A,B) or immunofluorescence (Figure 4C,D) procedures. Outcomes for T-cadherin expression levels were quantitatively comparable with either procedure, with the slight differences likely attributable to higher sensitivity of the fluorescent staining (compare Figure 4A,B versus 4C,D). T-cadherin expression was strongly upregulated in PCa specimens as compared to BPH (Figure 4A,C). Within the PCa sample set, specimens of organ-confined cancer displayed

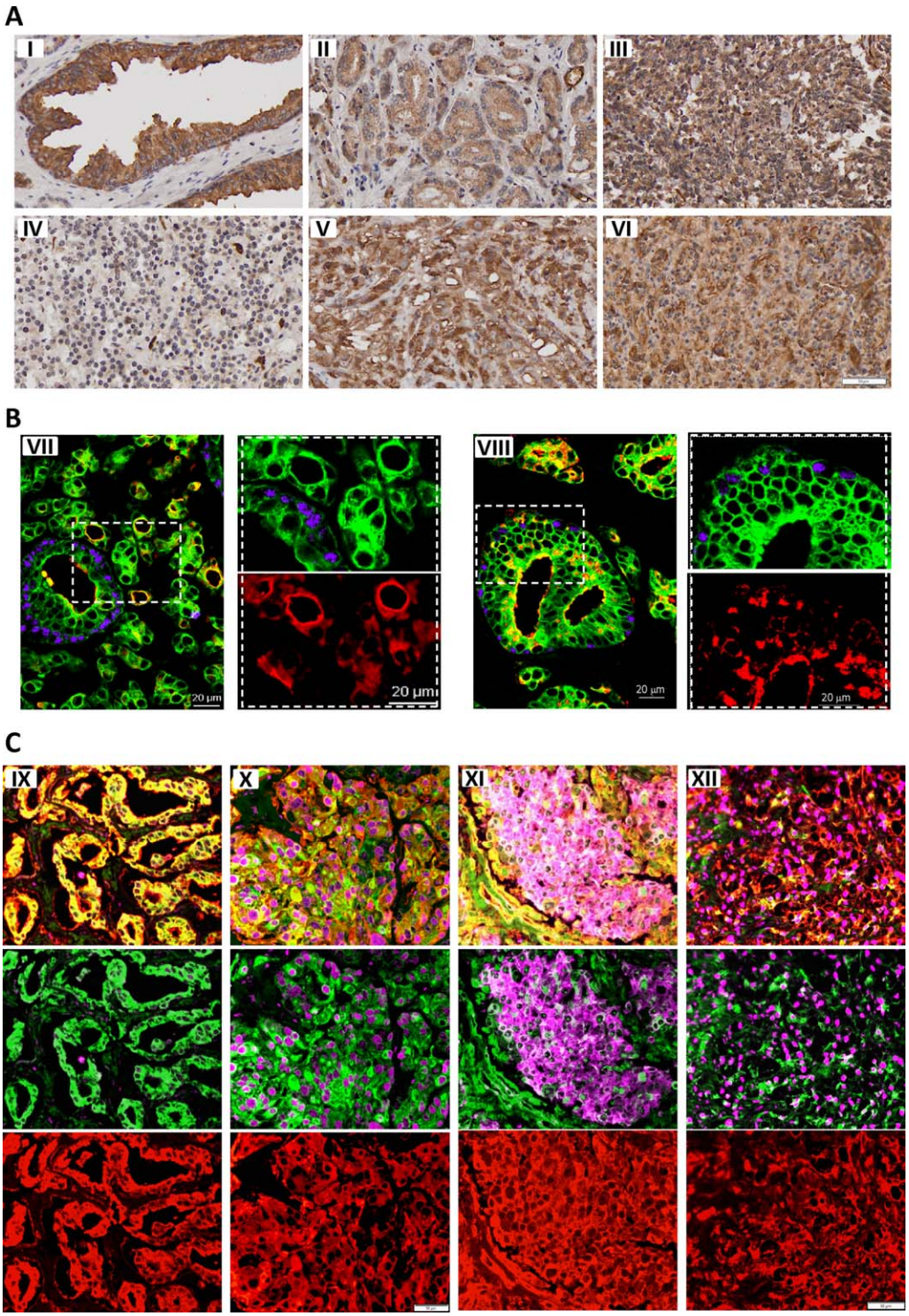

Figure 3. T-cadherin protein expression pattern in prostate cancer. Twelve different specimens derived from TMA (A, C) or individually processed tumours (B) including PIN (I) and PCa (II–XII) were analysed by HRP-staining for T-cadherin protein (A) and by triple immunofluorescence-staining for T-cadherin (red), CK8 (green) and p63 (purple) (B, C). Dashed boxes show high magnification fragments of full-frame images (B).

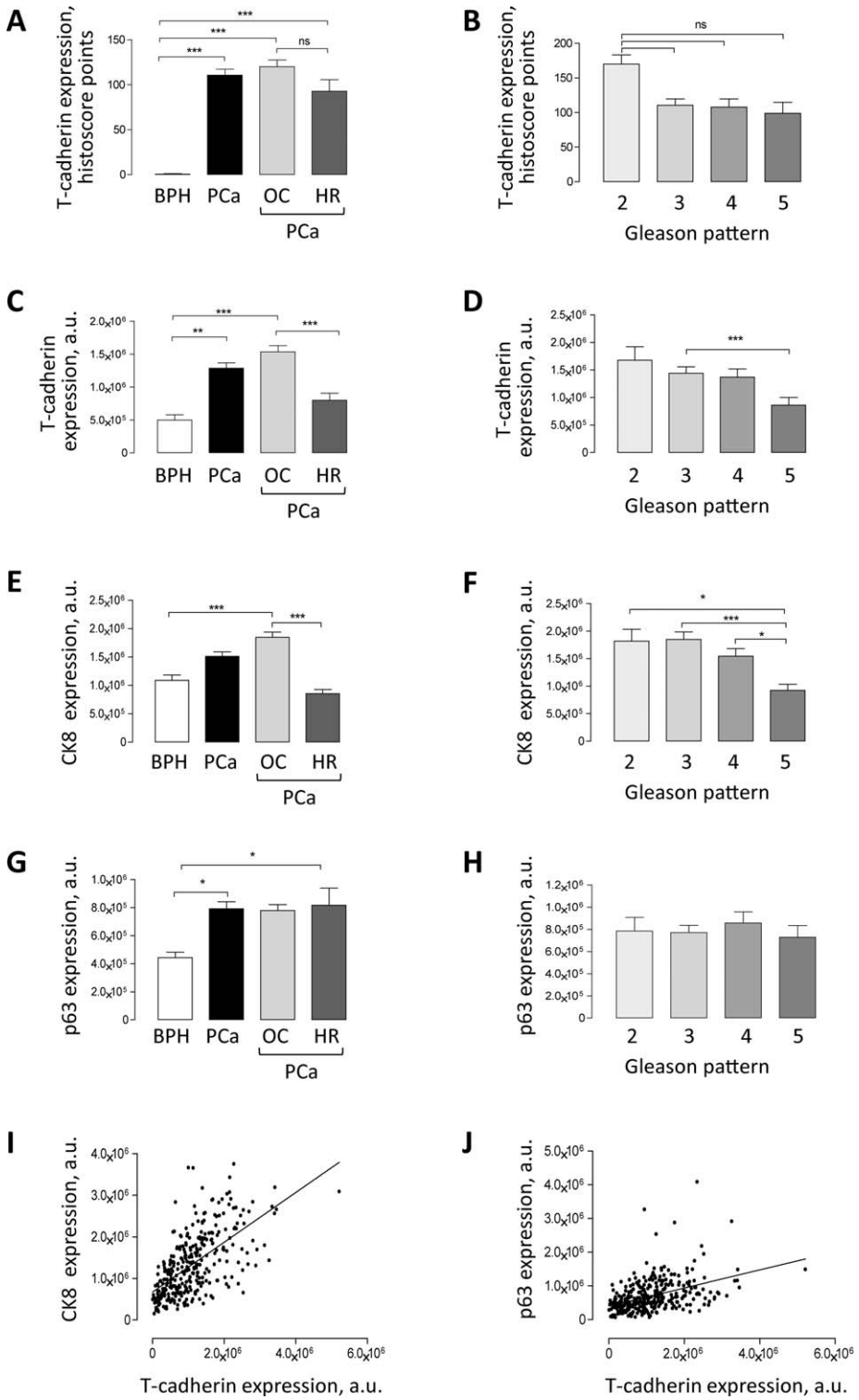

Figure 4. Protein expression levels of T-cadherin, cytokeratin 8 and p63 in prostate cancer. PCa TMA was used to quantify expression of T-cadherin protein (A–D, I, J), cytokeratin 8 (E, F, I) and p63 (G, H, J) using HRP-staining (A, B, quantified with H-score) or immunofluorescence-staining (C–J, calculated as staining intensity). Protein levels were compared between BPH, all PCa samples (PCa), PCa separated into organ-confined (OC) and hormone-resistant (HR) tumour groups (A, C, E, G) or between tumours with different Gleason pattern (B, D, F, H). Correlations between expression levels of T-cadherin and CK8 (I) or between T-cadherin and p63 (J). a.u., arbitrary units.*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns, not significant.

higher T-cadherin expression than advanced hormone-resistant tumours (Figure 4A,C). For both datasets T-cadherin expression weakly and negatively correlated with the Gleason pattern (Figure 4B,D). The correlation was significant for data derived from immunofluorescence-staining ($r = -0.289$, $p = 0.003$) and close to significance for data derived from HRP-staining ($r = -0.149$, $p = 0.062$).

Immunofluorescence analysis of prostate cell marker levels in the same TMA set revealed that, and similarly to T-cadherin, both luminal marker CK8 and basal marker p63 were upregulated in PCa as compared to benign tissue (Figure 4E,G). The CK8 intensity profile within the PCa sample set resembled that of T-cadherin: CK8 expression was increased in organ-confined cancer versus BPH and decreased in advanced hormone-resistant tumours (Figure 4E) and tumours with higher Gleason pattern (Figure 4F). Expression of p63 was increased in adenocarcinoma (Figure 4G) but without any evident correlation with the cancer stage (Figure 4G) or Gleason pattern (Figure 4H). In accordance with these observations overall T-cadherin expression displayed a strong positive correlation with CK8 (Figure 4I; $r = 0.614$, $p < 0.0001$) and a weaker correlation with p63 (Figure 4J; $r = 0.424$, $p < 0.0001$).

T-cadherin protein is upregulated in tumours with abnormal p63 localisation

Besides changes in marker expression levels, tumour specimens on the TMA set exhibited heterogeneity regarding cellular localisation of p63. In some samples p63 was localised only in cell nuclei (Figure 5A, left), resembling the staining pattern detectable in basal cells of BPH tissues (Figure 2A,C). Other tumours exhibited abnormal p63 localisation patterns: p63 was either absent from the nuclei and distributed diffusely over the cell body with occasional slight enrichment at intercellular contacts (Figure 5A, right), or showed an intermediate partly nuclear and partly diffuse distribution pattern (Figure 5A, centre). Of the 128 specimens analysed, normal, diffuse and intermediate p63 localisation patterns were detected in 32 (25%), 34 (26%) and 58 (45%) specimens, respectively. In 4 (3%) samples p63 expression was below detection levels. The incidence of abnormal p63 localisation was markedly increased in PCa tissue. The frequency of the diffuse staining pattern increased to 31% in PCa versus 5% in BPH, and the frequency of the intermediate staining pattern (ie nuclear and diffuse in any given sample) increased to 81% (50%+31%) in PCa versus 33% (28%+5%) in BPH (Figure 5B). No substantial differences regarding p63 pattern incidence were observed between organ-confined and hormone-resistant PCa (Figure 5B). Tumours with Gleason patterns 3–5 had a higher incidence of diffuse p63 pattern than specimens with Gleason pattern 2 (Figure 5C). T-cadherin expression was increased in tumours exhibiting intermediate or diffuse p63 patterns as compared to those with normal nuclear localisation (Figure 5D).

T-cadherin overexpression does not induce the 'cadherin switch' in PCa cells

The onset of cancer is often characterised by epithelial-to-mesenchymal transition (EMT) which is associated with the 'cadherin switch', namely concomitant transcriptional repression of epithelial E- and P-cadherins and upregulation of mesenchymal N-cadherin and cadherin-11. To establish whether T-cadherin upregulation in early PCa is related to cadherin switching we analysed effects of T-cadherin overexpression on cadherin levels in cultured PCa cells. PCa cell line DU145 derived from brain metastasis, which does not express T-cadherin mRNA or protein [49], was selected as the experimental PCa model. DU145 cells were stably transduced to express human T-cadherin gene (Tcad+) and compared with control, empty vector (E) transductants. Control DU145 cells express only epithelial E- and P-cadherins and no mesenchymal N-cadherin and cadherin-11 (Figure 6A) thus displaying an epithelial phenotype. This is consistent with the current view suggesting that metastatic cancer cells need to undergo the 'reverse' mesenchymal-to-epithelial (MET) transition to successfully seed metastasis. T-cadherin overexpression induces neither upregulation of N-cadherin and cadherin-11, nor downregulation of E- and P-cadherins. On the contrary, E-cadherin level is slightly increased in Tcad+ cells. These data suggest that T-cadherin does not induce cadherin switching in DU145 cells.

T-cadherin overexpression increases sensitivity of PCa cells to doxorubicin

The phenomenon of prostate tumour heterogeneity is of major clinical importance, since tumour initiation from different cell types in the lineage hierarchy may give rise to tumour subtypes with different clinical outcomes and different degrees of resistance to treatment [10]. To establish whether T-cadherin expression not only correlates with PCa progression and differentiation status but may also modulate response to chemotherapy, we analysed the impact of T-cadherin overexpression on the sensitivity of PCa cells to doxorubicin *in vitro*. Growth of DU145 transductants in the absence or presence of doxorubicin was evaluated by

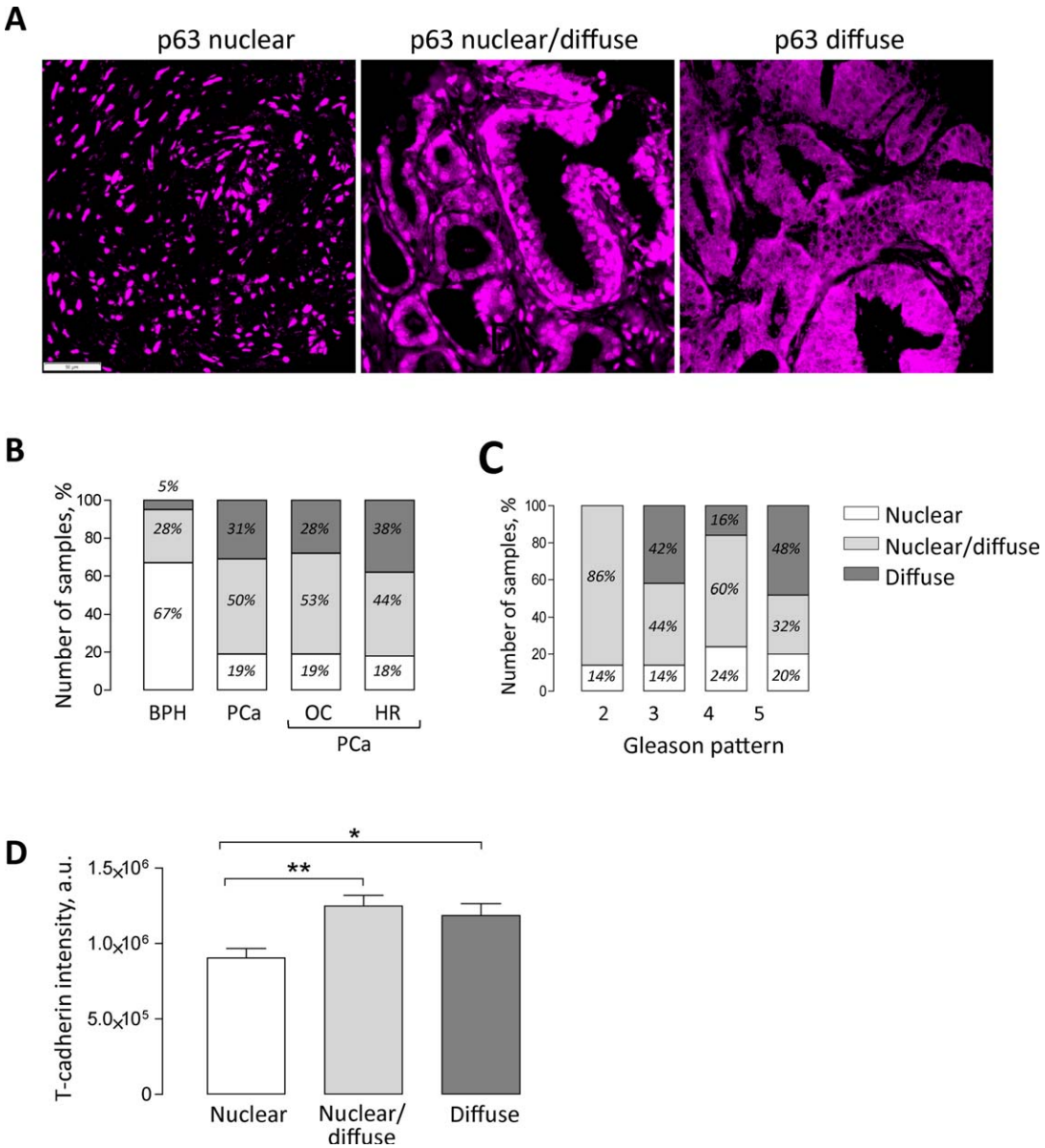

Figure 5. Correlation between T-cadherin protein expression and abnormal p63 localisation. Representative examples of three p63 localisation patterns variously observed in TMA specimens (A): nuclear localisation (left panel), diffuse pattern (right panel) and an intermediate pattern combining both types of localisation (central panel). The incidence of these patterns was evaluated in BPH, all PCa samples (PCa), PCa separated into organ-confined (OC) and hormone-resistant (HR) tumour groups (B) or in tumours with different Gleason pattern (C) and expressed as % of the total number of samples in each group. T-cadherin protein expression in tumours with different p63 localisation was evaluated in immunofluorescence-stained TMA samples (C). *$p < 0.05$; **$p < 0.01$.

cell enumeration. Doxorubicin dose-dependently reduced growth in both E- and Tcad+ DU145 cells, but the latter were significantly more sensitive to the inhibitory effects of the drug (Figure 6B). Accordingly, doxorubicin dose-dependently reduced cell viability, as assessed by propidium iodide flow cytometric assay and, in comparison with control E cells, the Tcad+-DU145 exhibited increased sensitivity to the drug (Figure 6C).

Discussion

In the present study we have characterised T-cadherin gene and protein expression in a large set of human prostate tissue specimens. Whilst T-cadherin gene expression is not different between benign and malignant tissues and shows weak positive correlation with the Gleason score in tumours, its protein level is

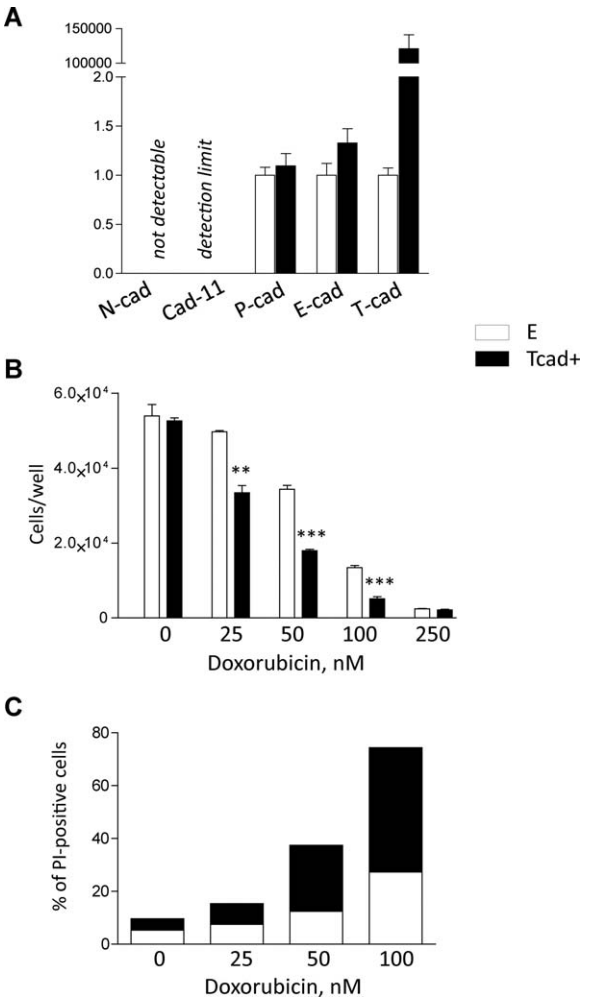

Figure 6. Effects of T-cadherin expression on the 'cadherin switch' and sensitivity of prostate cancer cells to doxorubicin. DU145 cells were transduced either with lentiviral vector to overexpress human T-cadherin (Tcad+) or with control empty vector (E). Expression of E, P, N-cadherins and cadherin-11 was measured by RT-qPCR (A). Sensitivity of DU145 transductants to indicated concentrations of doxorubicin was evaluated by cell counting (B) or by propidium iodide flow cytometry quantification of non-viable cells (C).

dramatically upregulated in PCa as compared to benign disease. T-cadherin protein is at highest levels in early organ-confined tumours with low Gleason pattern and decreases in advanced hormone-resistant and high Gleason pattern tumours. T-cadherin protein is also high in PIN. These data suggest that elevation of T-cadherin protein might be early event in prostate tumourigenesis, whilst its subsequent decrease may mark transition to more advanced PCa stages.

Accumulating data show that mRNA and protein expression in human tissue samples and cell lines often do not match, with average correlation coefficients not exceeding 0.4 [52], with discrepancies between levels of mRNA and protein variously reflecting complex post-transcriptional, translational and protein degradation regulatory processes [53]. Our data support that T-cadherin mRNA expression cannot be reliably used as a surrogate for corresponding protein levels without verification. Similar observations have been made by Kuphal *et al* who demonstrated discrepancies between T-cadherin gene and protein levels in melanoma cell lines and tissues [54]. These findings underscore the necessity for immunohistochemical analysis of T-cadherin protein expression and cellular distribution pattern to correctly assess its prognostic and predictive significance.

Our study is the first to describe cellular localisation and expression pattern of T-cadherin protein in prostate tissue. Immunohistochemical studies of T-cadherin protein levels and expression patterns in human tissues and organs are scarce. We have shown that T-cadherin is often found on simple and multilayered epithelium such as cutaneous basal and squamous keratinocytes [51,55] or basal keratinocytes of esophagus and small intestine (unpublished observations). Its cellular localisation differs from that of classical cadherins, which are normally present only in cell-cell contact areas, and ranges from intercellular/diffuse membrane localisation in keratinocytes and smooth muscle cells to strictly apical expression in polarised endothelial cells [51,56]. In healthy skin T-cadherin is highly expressed in the deepest basal layer of the stratified epithelia where keratinocyte stem cells reside. As these cells give rise to transient amplifying cells and differentiated keratinocytes which move upward towards the skin surface to create new strata layers, T-cadherin expression decreases and is eventually completely lost. In skin tumours its expression level varies depending on tumour type and cell of origin: it is strongly expressed in basal cell carcinoma [55], but downregulated in squamous cell carcinoma and leads to increased tumour growth, invasion and metastasis [51,57,58], Together, these observations suggest that T-cadherin may be involved in coordination of epithelial differentiation and structural organisation of polarised tissues. Whilst the relationship between prostate cell types is still debated, lineage tracing studies [8–10] and the identification of cells with an intermediate phenotype (transit-amplifying cells) expressing both basal and luminal markers [59] support that basal cells serve as precursors for luminal cells. Our data demonstrate that T-cadherin is expressed in both epithelial compartments. In healthy prostate tissue its expression is weak in the basal compartment on cells positive for

both CK8 and p63 and gradually increases towards the luminal side where it is detected on the apical surface and at intercellular contacts of luminal cells exhibiting the staining pattern typical for the polarised epithelium. Gradual changes in T-cadherin expression across epithelial layers may suggest a role in differentiation of luminal cell progenitors from basal and transiently amplifying precursors and in maintenance of polarised architecture of the gland.

Disturbances in the normal process of epithelial differentiation due to mutations or alterations in the tissue environment may lead to initiation of cancer [60]. Prostate tumours exhibit predominantly a luminal phenotype [6]; *CK8* gene expression is elevated in primary tumours and downregulated in metastases [61]. p63 expression in PCa is infrequent and has been mainly reported on the rare basal subtype. However, an increasing number of studies show the presence of basal markers and specifically p63 in PCa tissue and demonstrate correlations between a luminal/basal cell ratio and various degrees of tumour aggressiveness [4,6]. Further, a subset of prostate tumours has been described which, in contrast to normal nuclear p63 immunoreactivity, exhibit aberrant diffuse p63 cellular localisation [11,62]. Consistent with these data, we detected both CK8 and p63 in prostate tumours. That the incidence of p63 positivity is higher than previously reported [6] may be explained by our immunofluorescence-staining approach which is more sensitive than the HRP procedure commonly used in routine clinico-pathological analysis. We found that whilst both CK8 and p63 markers are upregulated in PCa as compared to the benign tissue, their expression profiles during cancer progression differed. CK8 was upregulated in early organ-confined cancer and then decreased in hormone-resistant and high Gleason score tumours, whereas p63 remained upregulated in all tumour groups. Importantly, we determined an association between abnormal p63 cellular localisation and PCa progression: the incidence of diffuse immunostaining p63 pattern was strikingly increased in tumours as compared to BPH tissue. T-cadherin protein expression closely followed the expression pattern of CK8: it was strongly elevated in early organ-confined tumours and decreased gradually during transition to high Gleason score- and hormone-resistant stages. The relationship between T-cadherin and p63 was more complex and defined not only by protein level but also pattern: T-cadherin was more strongly elevated in tumours with abnormal cellular localisation of p63. Together, these data suggest that changes in T-cadherin expression in PCa are associated with deregulated differentiation of prostate epithelial cells. In support of this hypothesis is the evidence that T-cadherin controls PCa cell epithelial polarity via regulation of EGFR and IGF-1R activities which are important for prostate differentiation and tumour growth [49].

Many studies utilised transcriptome analysis in attempt to identify PCa cell of origin. Of great interest are recent publications which identified T-cadherin as one of the three highest positive-weighted genes predicting prostate basal stem cell molecular signature shared by aggressive PCa [12], showed enrichment of T-cadherin gene in human benign prostatic basal epithelial cells compared to luminal cells [63] and demonstrated that T-cadherin knockdown impairs basal cell proliferation and stemness [63]. These data might appear contradictory to our findings showing (1) expression of T-cadherin protein in both basal and luminal compartments in benign prostate tissue, and (2) a marked increase in T-cadherin protein at early stages of PCa which correlates better with upregulation of luminal marker CK8 than with changes in basal marker p63. However, as demonstrated herein, T-cadherin protein levels in prostate cells do not exactly mirror its mRNA expression. Therefore, regulatory mechanisms at the post-translational level may sustain T-cadherin protein expression in luminal cells even when its gene expression is reduced. Differential expression of T-cadherin in basal stem versus luminal cells and in normal versus cancer tissue may shed light on molecular pathways involved in PCa initiation. Upregulation of T-cadherin in PIN and at early stages of PCa may indicate either expansion of a certain cell population or activation of differentiation programmes such as embryonic/stem cell signalling pathways which are often dysregulated in cancer [63].

Although chemotherapy remains the main treatment option for hormone-resistant PCa, its survival benefits are limited due to frequent development of chemo-resistance. Evolving evidence suggests that effectiveness of therapies depends on the differentiation status of tumour cells. Whilst chemotherapeutic agents preferentially target differentiated cells, their progenitors are less sensitive and may sustain tumour growth [64]. We found that T-cadherin impacts PCa cell sensitivity to doxorubicin, an antibiotic of the anthracycline family which is widely used as a chemotherapy agent to treat many types of neoplasia including PCa. PCa cell line DU145 used in the study derives from brain metastasis, exhibits significant resistance to doxorubicin [65] and does not express T-cadherin [49]. Ectopic expression of T-cadherin sensitises DU145 cells to doxorubicin reducing cell viability. These data suggest that T-cadherin

expression not only correlates with PCa progression, but also directly affects PCa cell survival and may influence outcome of the treatment. Further studies are needed to establish the molecular mechanisms linking T-cadherin to prostate cell differentiation and plasticity and to identify T-cadherin as potential molecular target and prognostic biomarker in PCa.

Author contributions

Study conception and design: MP, TJR, LB. Acquisition of data: BD, TV, CM, CR, LB, GS, SW, MP. Analysis and interpretation of data: BD, TV, CM, LB, MP. Drafting of manuscript: MP, TJR. Critical revision: TV, CM, GS, SW, PE, TJR, MP. All authors have agreed with the submission in its present form.

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SUPPLEMENTARY MATERIAL ONLINE

Supplementary material and methods