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Endo180 expression with cofunctional partners MT1-MMP and uPAR–uPA is correlated with prostate cancer progression ☆

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ABSTRACT

Endo180 (CD280; MRC2; uPARAP) regulates collagen remodelling and chemotactic cell migration through cooperation with membrane type-1 matrix metalloproteinase (MT1-MMP), urokinase-type plasminogen activator receptor (uPAR) and urokinase-type plasminogen activator (uPA). One hundred and sixty nine prostate tissue sections clinically graded as benign prostatic hyperplasia (BPH) ($n = 29$) or prostate cancer (PCA) with Gleason scores indicating low ($\leqslant 7(3+4)$; $n = 26$), intermediate ($7(4+3)$–8; $n = 96$) or high (9–10; $n = 19$) clinical risk were immunofluorescently stained for Endo180, pan-cytokeratin (pCk), vimentin, MT1-MMP and uPAR–uPA. Quantification of % Endo180$^+$/pCk$^-$ and Endo180$^+$/pCk$^+$ cells in entire tissue cores revealed stromal ($p = 0.0001$) and epithelial ($p = 0.0001$) upregulation of Endo180 in PCA compared to BPH. Epithelial Endo180 expression was significantly different between the three clinical risk groups of PCA ($p < 0.05$). Correlations with MT1-MMP and uPAR–uPA confirmed the functionality of Endo180 during PCA progression. This molecular evaluation is the first step in the exploration of Endo180 in PCA diagnosis and therapy.

1. Introduction

Prostate cancer (PCA) is commonly diagnosed upon presentation with clinical symptoms relating to its metastatic spread to bone or during screening assessment for prostate specific antigen (PSA). Inaccurate diagnosis, based on serum levels of this secreted protease,[1] and lack of effective treatment for metastasis necessitate identification of improved diagnostics for screening campaigns and new therapeutic targets. Extracellular matrix (ECM) regulatory systems function in both stromal and epithelial compartments of many solid tumours as part of the cancer degradome.[2] Components of this degradome are currently used or under investigation for diagnosis or prognosis of cancer and as therapeutic targets for metastatic disease prevention.[1,3] The multifunctional receptor-protease complex of urokinase-type plasminogen activator receptor and urokinase-type plasminogen activator (uPAR–uPA),[2,4,5] and the plasma membrane associated membrane

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type-1 matrix metalloproteinase (MT1-MMP; MMP-14)[6] are two such components with diagnostic, prognostic and therapeutic potential in PCA.

In recent work by ourselves and others, the recycling collagen-binding receptor Endo180 (CD280, MRC2; urokinase-type plasminogen activator receptor-associated protein, uPARAP) was shown to cofunction with uPA–uPAR in chemotactic tumour cell migration[7] and MT1-MMP in stromal collagen remodelling.[8,9] The ability of Endo180 to disrupt epithelial cell–cell interactions *in vitro* has potential functional relevance during the epithelial-to-mesenchymal transition (EMT) associated with cancer progression.[10,11] Contrary to this, the histopathological analysis of head and neck cancer tissue indicated that Endo180 is only associated with the reactive tumour stroma.[12] This restricted stromal expression of Endo180 was also reported for the most common, luminal, subtype of breast cancer.[11] However, this stromal restriction was not the case in a small subset of highly invasive basal-like breast tumours, where high expression of Endo180 was detected on the myoepithelial-like tumour cells.[11]

The objectives of this study were to address the current lack of information regarding Endo180 protein expression during PCA progression. The study was designed to determine whether Endo180 in PCA: (a) is expressed by stromal or epithelial cells and correlates with clinical risk according to Gleason score and/or serum PSA; (b) is localised on tumour cells with a high metastatic potential and/or reactive stromal cells; (c) correlates with its cofunctional partners MT1-MMP and uPA-uPAR. A tissue microarray (TMA) containing 169 core biopsies of prostate samples graded as non-malignant benign prostatic hyperplasia (BPH) or malignant PCA was employed for this purpose. The predicted strong association of Endo180 expression with the stroma was addressed by costaining for pan-cytokeratin (pCk) as a marker for cells of epithelial origin. This enabled generation of an extensive antigen expression data set that was then subjected to whole field quantification of stromal (pCk^-) and epithelial (pCk^+) antigen expression levels.

2. Materials and methods

2.1. Tissue microarray

PCA TMAs (PR951 & PR952) obtained from US Biomax Inc., (USA) were used in accordance with the regulations of the local Research Ethics Committee of Imperial College NHS Healthcare Trust. Analysis included non-malignant adjacent tissue graded as BPH ($n = 29$) and tumour tissue cores graded according to Gleason score (MMW) and categorised according to previously validated clinicopathological outcomes[13,14] as low (Gleason score: $\leqslant 7(3 + 4)$, $n = 26$), intermediate (Gleason score: $7(4 + 3)$–$8(4 + 4)$, $n = 96$) or high (Gleason score: 9–10, $n = 18$) clinical risk. The mean patient age after grouping into the four clinical risk groups was: BPH (65.0 ± 1.8), low risk (67.2 ± 2.6), intermediate risk (64.9 ± 0.9) and high risk (65.9 ± 1.1).

2.2. Antibodies

The Previous studies describe the characteristics of mouse monoclonal antibody (mAb) A5/158[7] and mouse mAb 39.10.[11] Rabbit anti-cow cytokeratin polyclonal antibody (pAb) (pan, wide spectrum screening; N1512) and mouse anti-human uPAR (Clone R4; M7294) (DAKO, UK). Mouse anti-human vimentin (Clone LN-6; V2258) (Sigma, UK). Rabbit anti-human MT1-MMP catalytic domain (ab51074) and rabbit anti-human uPA (ab24121) (Abcam, UK). Mouse IgG_1 control mAb (MCA928F) (Serotec, UK). Anti-mouse and anti-rabbit Alexa Fluor 405-, Alexa Fluor 488-, Alexa Fluor 555 and Alexa Fluor 633-conjugated secondary mAbs, DAPI, TO-PRO-3 and Xenon Alexa-Fluor 555-labelling kits (Molecular Probes, UK).

2.3. Immunohistochemistry

The 1.5 mm diameter and 5 μm thick paraffin-embedded tissue cores on the TMA were deparaffinised in xylene and were rehydrated in graded ethanol (100%, 95% and 70% v/v). Antigen retrieval was performed by incubation with 20 μg/ml Proteinase K for 30 min at 37 °C. Endogenous peroxidase activity was quenched in 3% v/v H_2O_2 for 10 min after which sections were incubated in blocking buffer (1% w/v bovine serum albumin, 2% v/v foetal calf serum, 20% v/v normal goat serum) for 1 h at room temperature. Sections were probed with antibodies against Endo180 (50 μg/ml) followed by pan-cytokeratin (pCk) (1:500 dilution), vimentin (1:40 dilution), MT1-MMP (1:50 dilution), uPAR mAb (1:500 dilution) or uPA (1:50 dilution) for 1 h at 37 °C. Slides were washed in phosphate buffered saline and were incubated with Alexa Fluor-conjugated anti-mouse or anti-rabbit IgG for 1 h at room temperature. Unless otherwise stated Endo180 was detected using A5/158 and secondary Alexa Fluor 488-conjugated anti-mouse IgG. Other antigens were detected using appropriate primary mAb with secondary Alexa Fluor 555 or 633-conjugated IgGs. To enable sequential staining with two mouse anti-human mAbs, the first mAb was labelled using a standard Alexa Fluor-conjugated anti-mouse IgG, and the second mAb was labelled using the direct IgG-labelling system Zenon Alexa Fluor® (Molecular Probes). Cell nuclei were counterstained using TO-PRO-3 or DAPI, and were mounted in fluorescent medium (DAKO) before signal detection by epifluorescent microscopy.

2.4. Histological analysis

Whole field analysis was carried out for each core on the TMA. Stromal/epithelial cells were characterised by location within the ECM and pCk absence (pCk^-) or presence (pCk^+), respectively. For each core, the cellular expression pattern of Endo180, vimentin, MT1-MMP, uPAR and uPA was obtained, and cell nuclei visualised by counterstaining with TO-PRO-3 or DAPI. For total tissue, Endo180 signal analysis intensities were calculated as arbitrary units using Metamorph® software (Molecular Devices, UK) with corrections for cell number. For coexpression analysis, sequential images from the same field of view were acquired in separate channels for: (a) Endo180; (b) vimentin, MT1-MMP, uPAR or uPA; (c) pCk and (d) nuclear stain, using an ImageXpress® Micro screening microscope with ×10 lens and MetaXpress software (Molecular Devices, UK). Channels were merged using Metamorph® software. Whole field quantification was carried out under blinded conditions (G.K. and M.M.W.) with a manual threshold set using Metamorph® software. Cells were scored as

positive, when a distinct cytoplasmic signal above the pre-set threshold could be observed. Control staining with species and isotype-matched control IgG verified that secondary antibodies did not cross react with inappropriate primary antibodies or produce non-specific tissue staining. Images of H&E sections for clinical grading were captured using an automated microscope with ×10 lens (Automated Cell Image System).

2.5. *Statistical analysis*

Statistical analysis was carried out using SPSS 15.0 statistical software (SPSS Ltd., UK). Parametric tests were used throughout the study following logarithmic transformation of all values, which allowed distribution of data to be nearly normal (Gaussian). Analysis of variance and post-hoc Tukey tests were used to test differences between all clinical grades and BPH, this allowed comparison between more than two means at once and reduced the error associated with multiple *t*-tests. Simple linear regression analysis was used to calculate *R* square (R^2) values and Pearson correlation coefficients (PC) between the intensity of staining or the % positive cell staining for each antigen, clinical grade of tissue and serum PSA levels. A p value of 0.05 or less is considered statistically significant. All statistical tests were two sided and all confidence intervals are 95%.

3. Results

3.1. *Endo180 is positively correlated with clinical risk in PCA*

Sequential TMA sections containing non-malignant adjacent tissue clinically graded as BPH or PCA with low, intermediate or high clinical risk were immunostained with A5/158 and 39.10 (Figs. 1 and 2). Both mAbs bind with high affinity and specificity to human Endo180, and were used in our previous study to stain both normal and malignant human breast tissues.[11] Similar increases in total tissue staining intensity obtained with both mAbs (Fig. 2) and lack of signal for isotype-matched IgG (Fig. 1) validated their suitability for use in human prostate. Total tissue staining with A5/158 displayed slightly better correlation ($p = 0.00002$, PC = 0.538, $R^2 = 0.294$) than 39.10 ($p = 0.0002$, PC = 0.368, $R^2 = 0.192$), and was used in all subsequent immunostaining.

Because our recent data suggests that the previous focus on the role of stromal Endo180 in cancer pathology should be extended to consider the role of tumour cell-associated Endo180 expression,[11] we made a distinction between stromal and epithelial contributions to total tissue staining (Fig. 3A) by costaining with pCk (Fig. 1). Quantification of Endo180$^+$/pCk$^-$ cells (Fig. 3B) confirmed a significant correlation between the ⩽5-fold increase in Endo180$^+$ cells of mesenchymal/stromal

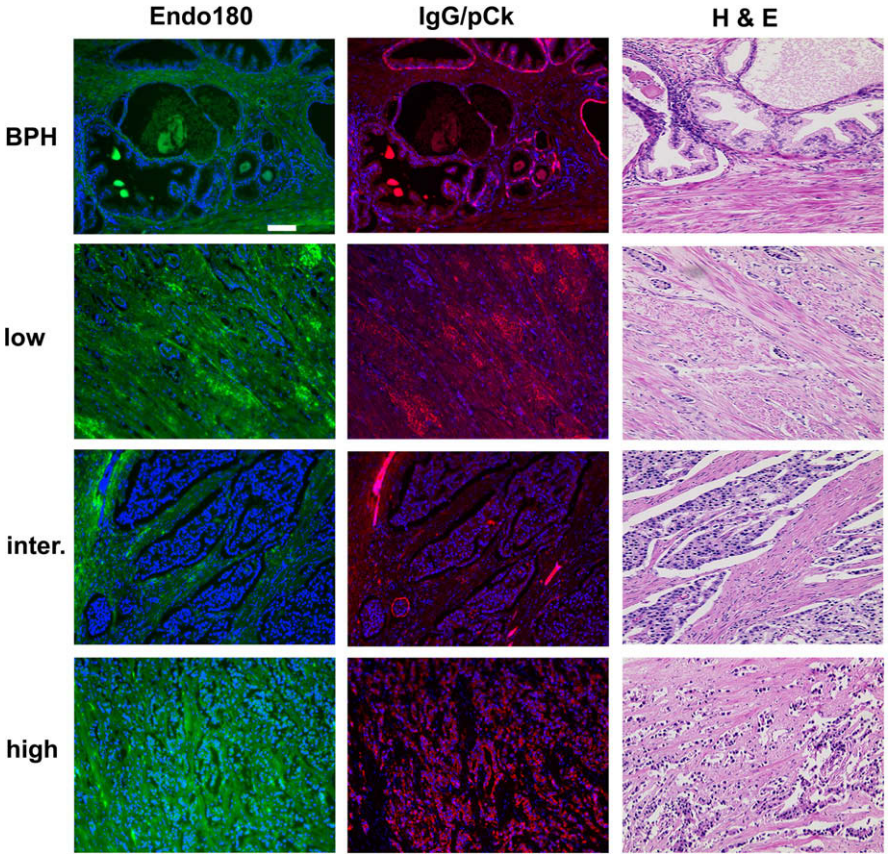

Fig. 1 – Endo180 staining in TMA cores comprising BPH, low, intermediate and high clinical risk PCA. Immunofluorescent staining for: Endo180 (green, first panel), IgG negative control/pCk (green/red, second panel). H&E-staining of sequential sections (third panel). Nuclear stain, DAPI (blue, first and second panels). Bar = 100 μm.

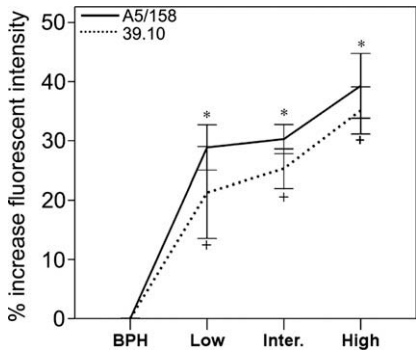

Fig. 2 – Immunostaining of TMA cores with A5/158 and 39.10 mAbs and quantification for total fluorescent staining intensity. Datasets grouped as BPH or low, intermediate and high clinical risk PCA. Results presented are mean values with 95% confidence intervals; * and + indicate statistical significance compared to BPH, $p < 0.05$, two-sided Tukey test.

origin (these could include fibroblasts, myofibroblasts, pericytes, endothelial cells or infiltrating leukocytes) and all clinical risks of PCA compared to BPH ($p = 0.0001$, PC = 0.547, $R^2 = 0.299$). Quantification of Endo180$^+$/pCk$^+$ cells (Fig. 3C) confirmed a significant correlation between the ⩽6-fold increase in Endo180$^+$ cells of epithelial lineage and all cancer grades compared to BPH ($p = 0.0001$, PC = 0.501, $R^2 = 0.240$). Similar results were obtained using 39.10 (data not shown). Moreover, significant differences in number of Endo180$^+$ tumour cells in PCA with different clinical risk (Fig. 3C) confirmed a positive linear correlation between receptor expression and disease progression. Comparison of Gleason 7 subgroups 3 + 4 and 4 + 3 revealed no significant difference in stromal expression, but a significant difference in epithelial expression ($p = 0.005$) between these two clinical risk subgroups.

3.2. *Endo180 is a stronger predictor of Gleason score than serum PSA*

PSA, a well-characterised secreted enzyme produced by the prostate gland, is widely used for PCA detection.[1] Serum PSA levels for 125 biopsy cores on the TMA were available and grouped according to PSA cut-offs with different clinicopathological outcomes: (a) ⩽4 ng/ml indicates absence of disease, BPH or a low clinical risk tumour ($n = 13$); (b) ⩽10 ng/ml indicates an aggressive high clinical risk tumour ($n = 24$); and (c) 4–10 ng/ml indicates a possible tumour with suggested referral for biopsy ($n = 88$).[15] Table 1 shows a poor correlation between mean serum PSA and clinical grade ($p = 0.05$, PC = 0.174), when grouping was made according to these PSA cut-offs. In contrast, there was a negative correlation for % Endo180$^+$/pCk$^+$ cells and serum PSA ($p = 0.006$, PC = –0.79), but a strong positive correlation for mean% Endo180$^+$/pCk$^+$ cells and increased clinical risk ($p = 0.0001$, PC = 0.59). The poor prognostic value of PSA was further revealed by its three-dimensional graphical representation according to grouping for serum PSA and clinical grade. In this analysis, both BPH and low clinical risk tumours were associated with 4–10 ng/ml and ⩽10 ng/ml serum PSA, and intermediate and high clinical risk tumours were associated with ⩽4 ng/ml PSA (Fig. 4A). A similar three-dimensional graphical representation of mean% Endo180$^+$/pCk$^+$ cells verified the improved reliability of this parameter as a determinant of clinical grade compared to serum PSA (Fig. 4B).

3.3. *Reactive stromal and migratory tumour cells express Endo180 in PCA*

Endo180 can drive an EMT-like phenotype via contractile signals that promote the disassembly of cell–cell adhesions and

Table 1 – Correlations between Endo180$^+$ PCA epithelial cells, serum PSA and clinical risk.

	p-Value	PC
Serum PSA versus		
Serum PSA group	0.0001	0.419
Clinical risk group	0.0502	0.174
Endo180$^+$ PCA epithelial cells versus		
Serum PSA group	0.0055	–0.79
Clinical risk group	0.0001	0.590

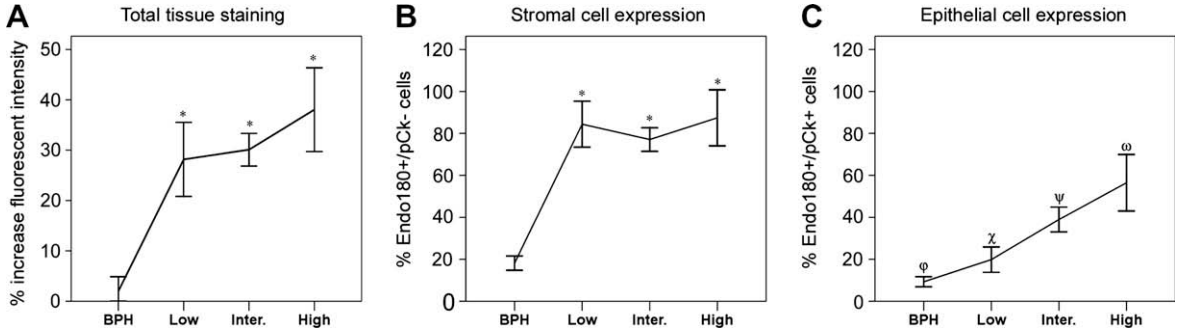

Fig. 3 – Immunostaining of TMA using A5/158 and quantification for: (A) total tissue fluorescent staining intensity; (B) % Endo180$^+$/pCk$^-$ stromal cells; and (C) % Endo180$^+$/pCk$^+$ epithelial cells. Datasets grouped as BPH or low, intermediate and high clinical risk PCA. Results presented are mean values with 95% confidence intervals; * indicates statistical significance compared to BPH; φ, χ, ψ and ω indicate statistical difference compared to all other clinical risk groups, $p < 0.05$, two-sided Tukey test.

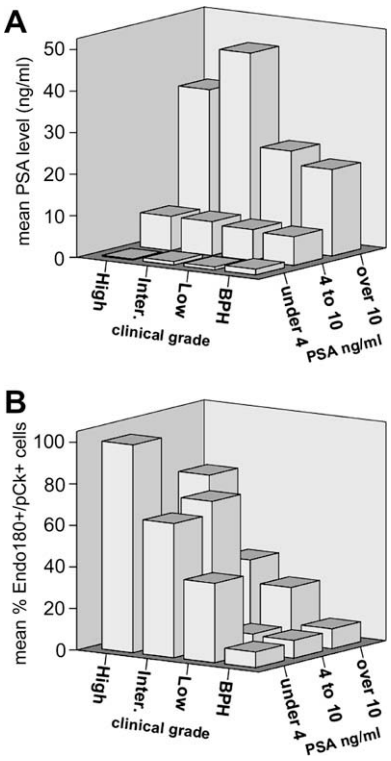

Fig. 4 – Epithelial Endo180 expression is a better predictor of clinical grade than serum PSA. Three-dimensional graphs show (A) mean serum PSA levels (ng/ml) (*y*-axis), or (B) mean% Endo180$^+$/pCk$^+$ cells (*y*-axis), against clinical risk group (*x*-axis) and PSA cut-offs grouped as $\leqslant$4 ng/ml (n = 13), 4–10 ng/ml (n = 24) and $\leqslant$10 ng/ml (n = 88) (*z*-axis).

increased cell migration,[10] and is also upregulated by the EMT-promoting factor TGF-β_1.[11] Here, we use vimentin as a marker associated with increased prostate tumour cell migration and metastatic potential,[16] and the reactive prostate stroma associated with malignancy.[17] Analysis of the TMA costained with vimentin and pCk (Fig. 5) confirmed a significant correlation between clinical grade, vimentin$^+$/pCk$^-$ cells (Fig. 6A) and vimentin$^+$/pCk$^+$ cells (Fig. 6B). To determine whether the population of Endo180$^+$ epithelial cells present in PCA exhibited increased migratory and metastatic potentials, or whether the population of Endo180$^+$ stromal cells represented activated myofibroblasts, the TMA was costained for Endo180, vimentin and pCk (Fig. 5). Accordingly, correlations between % Endo180$^+$/vimentin$^+$/pCk$^-$ cells and % Endo180$^+$/vimentin$^+$/pCk$^+$ cells and all clinical risk groups of PCA compared to BPH were found (Table 2).

3.4. *The collagen degradome in PCA – Endo180 and MT1-MMP coexpression*

The cell-associated collagen degradome can remodel the ECM through two major pathways: the pericellular pathway driven by collagenases, such as MT1-MMP, that directly cleave collagen fibrils in the extracellular milieu[18]; and the intracellular pathway[19] regulated by Endo180 located on the plasma membrane, which mediates the endocytosis of cleaved collagen and subsequent delivery to lysosomes for degradation.[20,21] To investigate whether the collagen degradome contributes to PCA progression, the TMA was costained for Endo180, MT1-MMP and pCk (Fig. 5). Almost negligible numbers of MT1-MMP$^+$/pCk$^-$ and Endo180$^+$/MT1-MMP$^+$/pCk$^-$ cells (Fig. 6C), or MT1-MMP$^+$/pCk$^+$ and Endo180$^+$/MT1-MMP$^+$/pCk$^+$

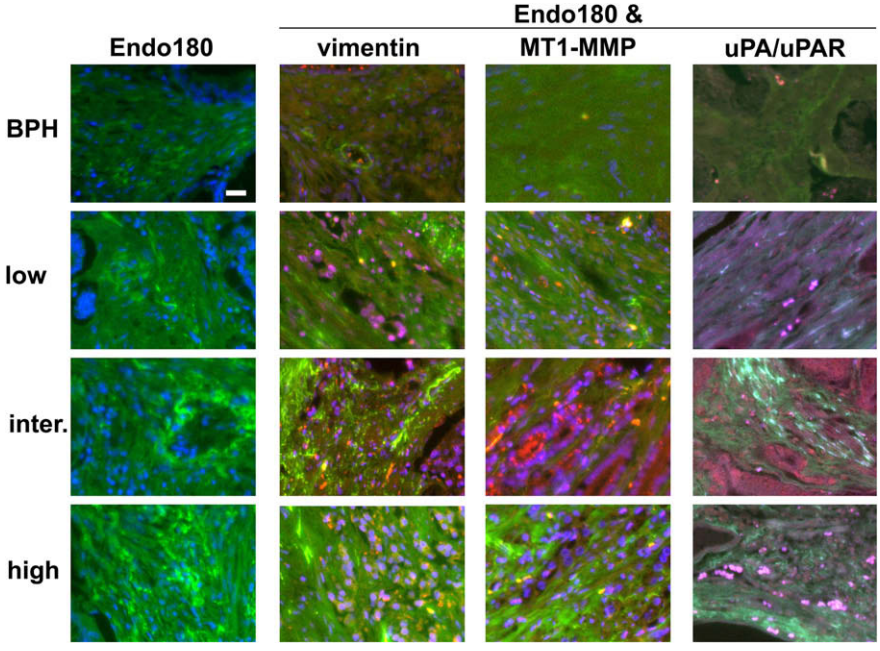

Fig. 5 – Endo180 and cofunctional partner staining in TMA cores comprising BPH, low, intermediate and high clinical risk PCA. Immunofluorescent staining for: Endo180 (green, first panel), Endo 180/vimentin (red, second panel), Endo180/MT1-MMP (green/red, third panel) and Endo180/uPA/uPAR (green/red/blue, fourth panel). Nuclear stain, DAPI (blue, all panels). Bar = 25 μm.

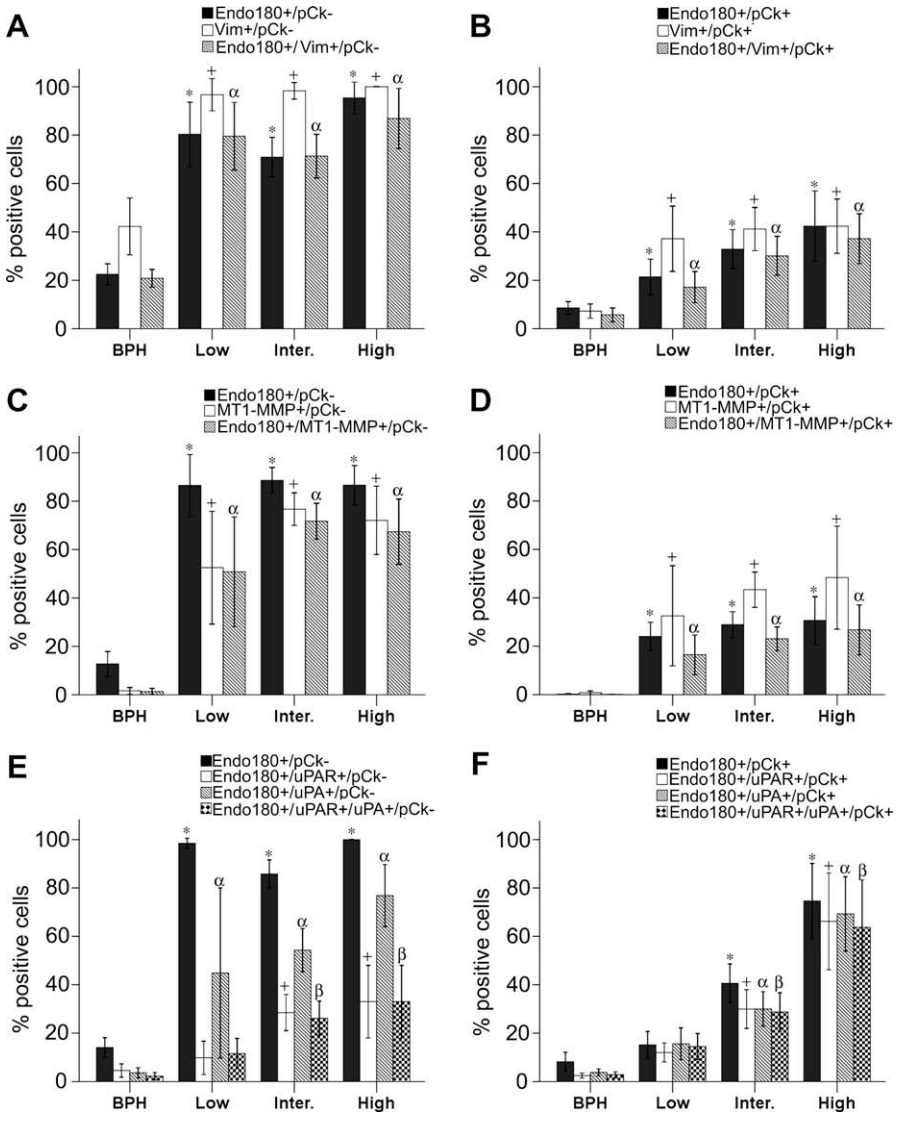

Fig. 6 – Entire field quantification of Endo180 expression in the reactive stroma/EMT, the collagen degradome and the uPAR–uPA chemotactic axis. Fluorescently immunostained TMA cores (see Fig. 5) were quantified for: (A, C, E) % stromal (B, D, F) and % epithelial Endo180 expressions together with: (A, B) vimentin (vim); (C, D) MT1-MMP and (E, F) uPAR–uPA. Datasets grouped as BPH or low, intermediate and high clinical risk PCA. Results presented are mean values with 95% confidence intervals. *, +, α and β indicate statistical significance compared to BPH, $p < 0.05$, two-sided Tukey test.

cells (Fig. 6D) were detected in BPH. The respective striking increases of ⩽25-fold and ⩽35-fold in MT1-MMP$^+$/pCk$^-$ and Endo180$^+$/MT1-MMP$^+$/pCk$^-$ cells (Fig. 6C), and ⩽50-fold and ⩽75 fold in MT1-MMP$^+$/pCk$^+$ and Endo180$^+$/MT1-MMP$^+$/pCk$^+$ cells (Fig. 6D) were significantly correlated with all clinical risk groups of PCA compared to BPH (Table 2).

3.5. *Endo180–uPAR–uPA chemotactic axis associates with PCA progression*

The mechanistic regulatory function of Endo180 during directional cell migration via its cooperation with the uPAR–uPA system[7] has not yet been validated in the pathology of human malignant disease. Quadruplicate immunostaining of the TMA for Endo180, uPAR, uPA and pCk (Fig. 5) confirmed that the Endo180–uPAR–uPA chemotactic signalling axis is correlated with PCA progression. The general pattern of uPAR and uPA expression was similar to that found previously,[22] but the more complete quantification carried out here revealed distinct patterns of expression during the disease progression. Relatively low numbers of uPAR$^+$/pCk$^-$ cells and uPAR$^+$/pCk$^+$ cells were detected in BPH (data not shown). The ⩽25-fold increases in uPA$^+$/pCk$^-$ cells (data not shown) and Endo180$^+$/uPA$^+$/pCk$^-$ cells (Fig. 6E) confer uPA to be a predominantly stromal factor (Table 3). In contrast, the ⩽27-fold increases in uPAR$^+$/pCk$^+$ cells (data not shown) and Endo180$^+$/uPAR$^+$/pCk$^+$ cells (Fig. 6F) confirm that uPAR expression predominates on epithelial cells in PCA with high clinical risk (Table 3). Interestingly, Endo180$^+$/pCk$^+$ cells that coexpress uPA and/or uPAR follow an expression profile during PCA progression that was almost identical to Endo180 alone (Fig. 6F).

Table 2 – Correlation between clinical risk of PCA and expression of Endo180 with its cooperative partners in stromal and epithelial cells.

Antigenic marker(s)	Stromal (pCk^-) cell expression			Epithelial (pCk^+) cell expression		
	p-Value	PC	R^2	*p*-Value	PC	R^2
Reactive stroma/EMT						
Endo180	0.0001	0.268	0.300	0.0001	0.501	0.240
Vimentin	0.0001	0.663	0.440	0.048	0.213	0.045
Endo180/Vimentin	0.0106	0.273	0.074	0.0001	0.432	0.187
Collagen degradome						
Endo180	0.0001	0.746	0.556	0.0001	0.502	0.252
MT1-MMP	0.0001	0.705	0.497	0.0001	0.517	0.267
Endo180/MT1-MMP	0.0001	0.665	0.443	0.0001	0.481	0.231
Chemotactic axis						
Endo180	0.0001	0.761	0.578	0.0001	0.578	0.334
uPAR	0.0001	0.368	0.135	0.0001	0.437	0.191
uPA	0.0001	0.571	0.326	0.0001	0.504	0.253
uPAR/uPA	0.0001	0.393	0.155	0.0001	0.453	0.205
Endo180/uPAR	0.0001	0.380	0.144	0.0001	0.528	0.279
Endo180/uPA	0.0001	0.589	0.346	0.0001	0.570	0.325
Endo180/uPAR/uPA	0.0001	0.405	0.164	0.0001	0.510	0.261

Table 3 – Comparison between stromal and epithelial cell antigen expression in prostate tissue with different clinical risks.

Antigen(s)	*p*-Value Clinical risk			
	BPH	Low	Intermediate	High
Reactive stroma/EMT				
Endo180	0.887	0.0001(S)	0.0001(S)	0.007(S)
Vimentin	0.0006(S)	0.0001(S)	0.0001(S)	0.0001(S)
Endo180/vimentin	0.7267	0.0001(S)	0.0001(S)	0.0018(S)
Collagen degradome				
MT1-MMP	1	0.8495	0.0001(S)	0.31
Endo180/MT1-MMP	1	0.0808	0.0001(S)	0.0002(S)
Chemotactic axis				
uPAR	0.9997	0.308	0.2708	0.05(E)
uPA	0.9992	0.4722	0.0035(S)	0.9972
uPAR/uPA	0.9966	0.9999	0.9994	0.0972
Endo180/uPAR	0.9999	1	0.9999	0.05(E)
Endo180/uPA	1	0.5535	0.0001(S)	0.9978
Endo180/uPAR/uPA	1	0.9999	0.9994	0.0753

S = stromal expression predominates; E = epithelial expression predominates.

4. Discussion

This is the first validation of Endo180 protein expression in human BPH and PCA. A strong positive linear correlation of $Endo180^+$ expression in epithelial tumour cells with clinical risk is confirmed. These promising results suggest that the detailed analysis of tumour tissue sections by immunofluorescent staining can be successfully applied in future studies that investigate the association between Endo180 and a number of clinicopathological outcomes related to PCA disease status and prognosis. It should be noted that this study adopted current trends for the use of Gleason Grade as a prognostic indicator, where Gleason 7(3 + 4) and Gleason 7(4 + 3)–8 provide well-defined groups for low and intermediate risk outcomes.[13,14] The expression profile of Endo180 and cooperative partners obtained here also reveals that there are temporal changes in its molecular functionality as PCA progresses to become more invasive and metastasises to secondary sites, such as bone, where we have clearly identified $Endo180^+$ tumour cells (Appendix 1).

The downregulation of Endo180 by small interference RNA in invasive tumour cell lines, which normally express high endogenous levels of the receptor, decreases their normal cell migratory responses.[7,10,11] More specifically in our previous studies, we have determined that Endo180 acts to spatially localise the contractile signals required to promote deadhesion from the ECM during cell migration.[10] By using vimentin as a marker, we have now provided the first evidence that

Endo180 is upregulated on tumour cells with an increased migratory and metastatic potential *in vivo*.

Studies in mouse models of cancer pathology indicate that endogenous expression of Endo180 by tumour stromal cells,[23] or overexpression by tumour epithelial cells,[11] results in extensive collagen remodelling and tumour progression. MT1-MMP upregulation in human PCA[6] is linked to the enhanced migratory, invasive and metastatic behaviour of tumour cells.[24–26] Here, we find that Endo180/MT1-MMP coexpression is strongly upregulated in the stroma of PCA with low clinical risk, indicating that tumour-associated stromal fibroblasts can acquire the ability for effective collagen degradation and internalisation at the early stages of tumour development. In this context, there is potential for similar cooperative functionality for Endo180/MT1-MMP-mediated collagen turnover, as previously shown in bone development[9] and tissue remodelling,[8] in the stromal mechanisms that govern the initiating, as well as progressive, steps of cancer pathology. It will be of particular interest to determine the contribution of collagen degradome components in tumour-associated stromal fibroblasts to actively drive epithelial transformation towards an invasive phenotype, given that there is a key role for such fibroblasts in driving the progression of certain carcinomas,[27,28] including those arising in the prostate.[17]

The association of Endo180 with uPAR occurs at the cell membrane upon binding of uPA[29] and this interaction enables activation of intracellular Rho GTPases, which promote the chemotactic migration of tumour cells *in vitro*.[7] The first *bona fide* evidence that this trimolecular association of Endo180–uPAR–uPA has relevance in disease pathology is provided here. The data obtained indicate that autocrine or paracrine signals can be generated by this chemotactic axis to help promote localised invasion and metastatic spread of prostate tumour cells. This has particular relevance to the late stages of PCA progression, when organ-specific metastasis is most likely to occur.

The unexpected finding that epithelial Endo180 expression and serum PSA are negatively correlated may relate to a common link in their regulation and mechanistic functions at the cellular level during disease progression. However, it is also possible that poor correlation of PSA with clinical grade and negative correlation with Endo180 reflect its differential secretion by distinct subsets of prostate tumours,[30] for which no data were available in the current study.

Current therapeutic intervention in PCA is largely aimed at targeting the androgen-dependent epithelial component of the disease, but this target eventually evades susceptibility to hormone-based treatment,[31] leaving the reactive stroma unhindered in its activation of tumourogenesis and disease progression.[28,32] Our data suggest that Endo180 can function together with its cooperative partners MT1-MMP and uPAR–uPA in both of these tissue compartments. This provides future potential in exposing PCA to a single-target, double-hit, therapeutic approach. Such a strategy could help improve clinical outcome in the large number of PCA patients, whose disease develops to an androgen-independent and chemoresistant state. Antibodies could be considered as suitable tools for this type of intervention, since they have already been shown to effectively block the chemotactic migratory responses that involve the Endo180–uPAR–uPA signalling axis, and also have the added potential of blocking collagen uptake via direct interference with the fibronectin type II collagen-binding domain of Endo180.[7] Other strategies may include targeting malignant cells with Endo180 antibodies conjugated to radiolabelled isotopes or knock down of Endo180 by either gene therapy or combination therapy.

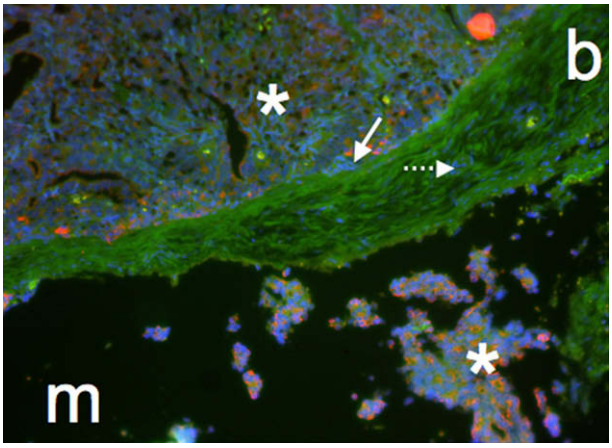

Fig. A1 – Fluorescent immunostaining with A5/158 (green) and pCk (red) reveals both Endo180$^+$ epithelial tumour cells and bone stromal cells in a prostatic bone lesion. Nuclear stain, DAPI (blue). * Tumour epithelial cells; b = bone tissue; m = marrow space; solid arrow = osteocyte; dashed arrow = osteoblast.

Conflict of interest statement

None declared.

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Appendix 1

See Fig. A1.

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