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Prostate cancer lesions are surrounded by FOXP3$^+$, PD-1$^+$ and B7-H1$^+$ lymphocyte clusters

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ABSTRACT

The immune response against prostate cancer seems to be inefficient although tumour cells show an over-expression of tumour-associated antigens suggesting that regulatory networks inhibit immune cell function locally. To address this proposition, lymphocytes within prostate cancer-inflicted tissue were analysed for the expression of markers associated with negative regulatory function and exhaustion.

Prostate cancer, benign prostatic hyperplasia and healthy prostate tissues were investigated by immunohistology for CD25, FOXP3, PD-1 and B7-H1.

We had previously documented that prostate cancer islets are surrounded by clustered accumulations of CD3$^+$ lymphocytes, which lack perforin and interferon-gamma (IFNγ) expression, thus are apparently quiescent. Here, we report that these clusters contain numerous CD25$^+$ and FOXP3$^+$ cells. These markers are associated with regulatory T cells, and their presence in lymphocyte clusters near prostate cancer regions indicates an environment with negative impact on immune response against cancer cells. Consistent with this hypothesis, cells expressing PD-1 and its ligand B7-H1, which are markers associated with exhaustion of lymphocyte function, were also detected in the lymphocyte clusters.

Expression of molecules associated with inhibition and exhaustion of lymphocytes may reflect events contributing to ineffective immune responses against cancer cells.

1. Introduction

Prostate cancer is the most common solid-organ cancer of men in Western countries and follows lung cancer as the second most frequent cancer-related cause of death. Among all malignant urological tumours, prostate cancer is the leading cause of death.[1,2] Despite its high incidence, the prognosis for patients with prostate cancer is excellent when the carci-

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noma is detected at a stage when curative treatments, like prostatectomy or radiation therapy, can be implemented.

Prostate cancer cells express several tumour-associated antigens, like PSMA. Nevertheless, the immune response against prostate cancer is inefficient. Investigating tumour-infiltrating lymphocytes is an important strategy to improve our understanding about immunological events that occur in the tumour environment. Infiltration of lymphocytes has been described for a number of solid tumours.[3,4] The infiltration of lymphocytes into the tumour microenvironment seems to play a role in anti-tumour immunity.[5] Furthermore, infiltrating lymphocytes have been associated with prognosis in some cancer patients. In colorectal cancer, for instance, a low density of $CD3^+$ infiltrating lymphocytes was associated with reduced survival time compared to patients with a high density of $CD3^+$ infiltrating lymphocytes.[6] Despite the presence of infiltrating lymphocytes, the question remains why efficient tumour cell elimination does not occur.

$CD4^+$ $CD25^+$ T cells are consistently observed among the total $CD4^+$ lymphocyte population in mice and humans. These cells have been associated with regulatory activity in immune responses in both species. The leading function of regulatory T cells (Tregs) is to control immune recognition to self-antigens, thereby preventing autoimmunity.[7,8] Tregs have also been seen in human cancers. Nevertheless, their role in cancer pathogenesis is not yet clarified, and has become a central focus of research during the past several years.[9,10]

A second form of immune suppression can be mediated by the PD-1-receptor and its ligand B7-H1.[11] The presence of PD-1 or B7-H1 was associated with disease progression in chronic viral infections in mice and in HIV-infected individuals.[12,13] Moreover, PD-1-deficient mice developed autoimmune diseases.[14] Thus, the expression of PD-1 and B7-H1 plays an important role in immune regulation.

In this study, freshly resected prostate carcinoma tissues, benign prostatic hyperplasia (BPH) and healthy prostate gland tissues were analysed for the presence of regulatory T lymphocyte markers using antibodies CD25 and FOXP3, as well as PD-1 and B7-H1.

We had previously described a clustered accumulation of $CD3^+$ and $CD4^+$ lymphocytes around prostate cancer islets with only few lymphocytes scattered within the tumour area. The lymphocytes were predominantly negative for perforin and IFNγ suggesting that they were functionally inactive.[15] Now, we report that $CD25^+$ and $FOXP3^+$ cells are abundantly present within these lymphocyte clusters. Additionally, we document the presence of PD-1^+ and B7-$H1^+$ cells within the lymphocyte clusters. The presence of cells with negative immune regulatory activity and functionally exhausted cells may in part explain the previously observed quiescent lymphocyte phenotype. These findings together provide novel insight and a more detailed understanding of the *in situ* immune regulation to prostate cancer.

2. Patients, materials and methods

2.1. Human prostate tissue samples

Tissues were collected from 17 untreated patients who underwent radical curative prostatectomy (Department of Urology, Ludwig-Maximilians-University-Hospital, Munich). All prostate cancer samples showed histopathological evidence of prostate carcinoma with a differentiation grade of 6–9 according to Gleason (Table 1). Four tissues with BPH were collected from patients who underwent surgical adenoma resection (Table 2). Prostate tissues from eight healthy men were obtained from autopsies (Table 3). These tissue samples were available through the Institute of Legal Medicine (Ludwig-Maximilians-University, Munich). Autopsy tissues and tumour specimens were collected by a procedure securing anonymity.

Cancer and healthy tissue samples were frozen immediately in liquid nitrogen and stored at –80 °C until further analysis. An experienced pathologist (G.B.) evaluated all samples for the existence of infiltrative carcinoma in the prostatectomy tissue or for the absence of pathological findings in the cases of the autopsy-derived tissue samples. For this pathological investigation, 5 μm cryosections were prepared and

Table 1 – Clinical and pathological characteristics of prostate carcinoma patients.

	n
Number of samples (n)	17
Age (y): median (range)	66 (59–75)
Tumour stage: TNM (acc. to UICC 2002)	
T1	0
T2	11
pT2a	3
pT2b	1
pT2c	7
T3	6
pT3a	3
pT3b	3
T4	0
Gleason score	
Gleason 6	7
Gleason 7	5
Gleason 8	4
Gleason 9	1

Table 2 – Clinical and pathological charcteristics of patients with benign prostatic hyperplasia (BPH).

Sample number (n)	4
Age: median (range)	67 (63–71)
Pathological findings	BPH

Table 3 – Clinical and pathological characteristics of patients from healthy prostate gland tissue.

Number of samples (n)	7
Age: median (range)	39 (24–51)
Cause of death	
Accident	2
Suicide	2
Head injury	2
Aneurysma bleeding	1
No pathology of prostate	7

stained with haematoxylin eosin. The ethics committee of the Ludwig-Maximilians-University, Munich consented to the tissue collection.

2.2. Immunohistological analysis

Tissue samples were embedded in Tissue Tek® compound (Sakura, Zoeterwoude, The Netherlands) for the preparation of cryosections. Immunohistology was performed using the alkaline phosphatase anti-alkaline phosphatase (APAAP) method. Serial 5 μm cryosections were fixed in 100% (v/v) ice-cold acetone. After fixation and three washing steps with phosphate-buffered saline (PBS) (Invitrogen, Karlsruhe, Germany), a blocking step using 2% (w/v) bovine albumin (Sigma–Aldrich, St. Louis, USA) was employed before applying the primary antibody, diluted in 8% (v/v) human, type AB, male serum (Cambrex, Verviers, Belgium) in PBS, with subsequent incubation for 1 h at room temperature. After three washing steps with PBS, two consecutive antibody incubation steps followed, first using polyclonal rabbit anti-mouse immunoglobulin (dilution 1:20) and then applying monoclonal mouse APAAP (dilution 1:40) (both antibodies from DAKO, Glostrup, Denmark). For visualisation of antibody binding, a developing solution consisting of 40 mg levamisole, 50 mg naphthol AS phosphate disodium salt, 600 μl dimethylformamide (all Sigma), 40 mg sodium nitrite (Merck, Darmstadt, Germany) and 200 μl New Fuchsin (Sigma–Aldrich) dissolved in tris(hydroxymethyl)amino-methane (Merck) buffer was prepared. Nuclear counterstain was performed with Mayer's hemalum solution (Merck).[15]

Primary antibodies were anti-human CD3 (clone UCHT-1; Dako; Glostrup, Denmark), anti-human CD4 (clone: MT 310; BD Pharmingen, Heidelberg, Germany), anti-human CD25 (clone 2A3, BD Pharmingen), anti-human FOXP3 (clone 259D, Biolegend, San Diego, USA), anti-human PD-1 (clone MIH4, BD Pharmingen) and anti-human B7-H1 (clone MIH1, BD Pharmingen) antibody. All antibodies were diluted in PBS (Invitrogen) containing 8% (v/v) human, type AB, male serum (Cambrex). The number of $CD25^+$, $FOXP3^+$, $PD\text{-}1^+$ and $B7\text{-}H1^+$ cells was evaluated semi-quantitatively in serial tissue cryosections by estimating the number of positively stained cells (in case of lymphocyte clusters) or by counting positive cells (in cases where positively stained cells were rare). Tissues were grouped according to the estimated cell count. The term '>1000 positive cells' describes a dense cluster of positive cells which exceeded the visual field at magnification 100×. '100–1000' describes clustered accumulations not exceeding the visual field of magnification 100×. '50–100' or '20–50' describes differently sized groups of positively stained cells or is the number of cells found scattered throughout the tissue section. '<10' represents the counted number of positively stained cells throughout the tissue section.

3. Results

3.1. $CD25^+$ lymphocytes reside in the lymphocyte clusters which surround prostate cancer areas

We had previously documented that pronounced clusters of $CD3^+$ cells are formed adjacent to islets of tumour cells (for clarity documented in Fig. 1a and b).[15] Within these lymphocyte clusters, $CD4^+$ cells were the dominant cell type. There was no evidence for functional activity, defined by the absence of perforin and IFNγ, markers for cytotoxicity and effector function suggesting that these cells were quiescent.[15]

In this study, we report the presence of cells with negative regulatory function within these clusters. $CD25^+$ expression was used as the first marker to detect potentially regulatory T cells. Many $CD25^+$ cells were observed in the clusters (Figs. 1c and 2a), while few $CD25^+$ lymphocytes were detected in the carcinoma areas (Fig. 2c). The CD25 count per cluster was more than 50 cells ($n = 10$) or often much higher (>100 positive cells, $n = 7$) (Table 4). A correlation to the Gleason score or the TNM status was not evident (Table 4).

In the healthy tissues, $CD25^+$ lymphocytes were very rare (<10 cells per tissue section, Table 4). The few detectable lymphocytes were scattered throughout the healthy prostate tissue without apparent cluster formation and preferentially found within the glandular structures of the prostate (Fig. 2e). In BPH tissues, $CD25^+$ lymphocytes formed small

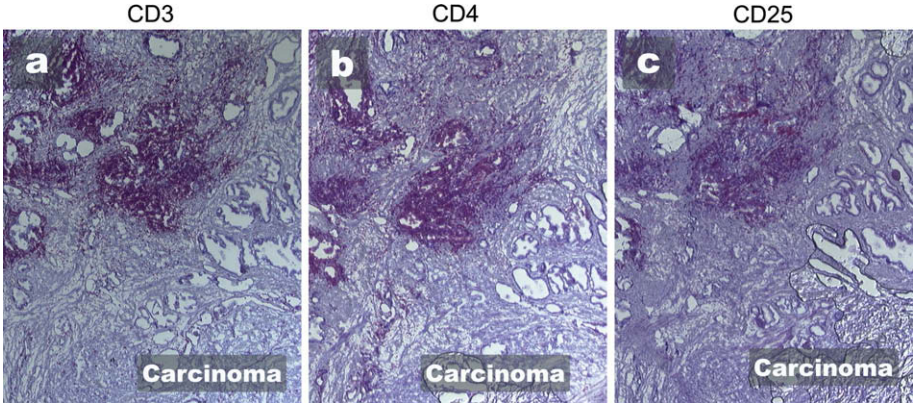

Fig. 1 – Lymphocyte clusters surround prostate cancer lesions. Serial 5 μm cryosections of prostate cancer-inflicted tissues were stained with anti-human CD3 (dilution 1:5000) (a), anti-human CD4 (dilution 1:1000) (b) and anti-human CD25 (dilution 1:10) (c). (a–c) are overviews (magnification 25×) of prostate cancer-inflicted tissue to demonstrate the cluster formation of tissue-infiltrating lymphocytes adjacent to the prostate cancer lesions (patient 6, Gleason 6, pT2a, as representative example). A dense stromal compartment separates the carcinoma area (lower right corner) and the lymphocyte clusters.[15]

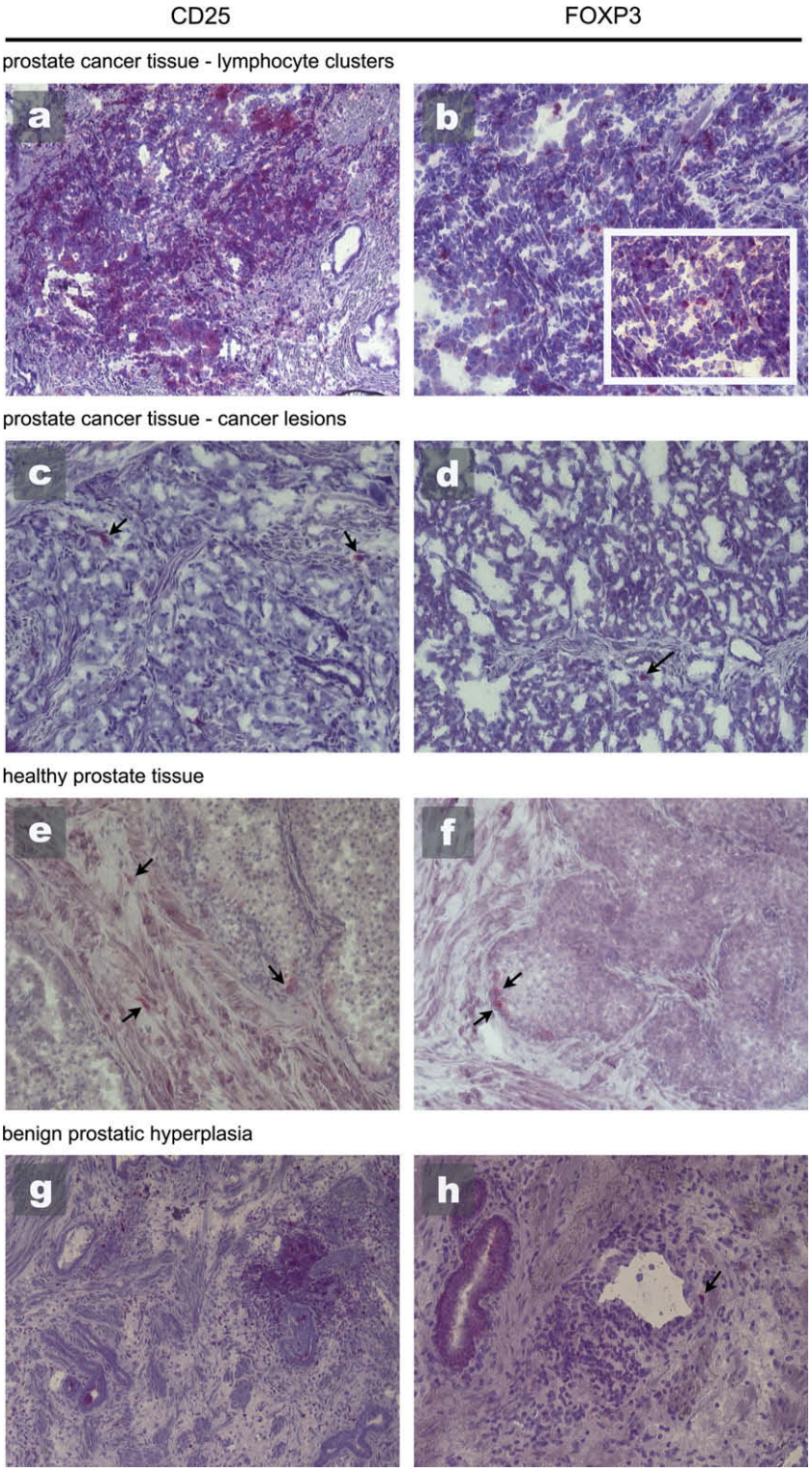

Fig. 2 – Immunohistological staining of prostate tissue for CD25 and FOXP3. Serial 5 μm thick cryosections of prostate cancer tissue (a–d) of representative patient 6, healthy prostate gland tissue (e, f) of donor 2 and benign prostatic hyperplasia (g, h) of patient 4 were stained with anti-human CD25 (dilution 1:10) (a, c, e, g) and anti-human FOXP3 (dilution 1:50) (b, d, f, h). (a) magnification 100× and (b) magnification 200× depict representative areas of lymphocyte clusters surrounding the cancerous lesion. The inset in (b) (magnification 630×; oil immersion) reveals the nuclear staining of FOXP3. (c) and (d) depict the carcinoma area (magnification 200×). (g) magnification 100× and (h) magnification 200× reveal the specific staining of lymphocytes with CD25 and FOXP3 in the small lymphocyte clusters in the benign prostatic hyperplasia tissue without any relationship to pathological morphological changes in their neighbourhood. Arrows indicate positive cells. The depicted patterns of locoregional distribution and lymphocyte composition are representative of all 17 tissues derived from prostate cancer patients, the eight healthy prostate gland tissues and the four benign prostatic hyperplasia tissues.

Table 4 – Semi-quantitative evaluation of the frequency of T lymphocytes expressing regulatory markers in prostate tissues.

Tissues	Marker	Cell counts[a]	Number of tissues (n)	Gleason scores (n)	Tumour stages T (n): TNM (acc. to UICC 2002)
Prostate cancer-inflicted tissue			*n* = 17		
	CD25	>1000	2	6 (1); 7 (1)	pT2a (1); pT2c (1)
		100–1000	5	6 (1); 7 (3); 9 (1)	pT2c (1); pT3a (2); pT3b (2)
		50–100	10	6 (5); 7 (1); 8 (4)	pT2a (2); pT2b (1); pT2c (5); pT3a (1); pT3b (1)
		10–50	0		
		<10	0		
	FOXP3	>1000	0		
		100–1000	6	6 (1); 7 (3); 8 (1); 9 (1)	pT2a (2); pT2c (1); pT3a (2); pT3b (1)
		50–100	11	6 (6); 7 (2); 8 (3)	pT2a (1); pT2b (1); pT2c (6); pT3a (1); pT3b (2)
		10–50	0		
		<10	0		
Benign prostatic hyperplasia tissue			*n* = 4		
	CD25	>1000	0		
		100–1000	0		
		50–100	0		
		10–50	3		
		<10	1		
	FOXP3	>1000	0		
		100–1000	0		
		50–100	0		
		10–50	3		
		<10	1		
Healthy prostate tissues			*n* = 8		
	CD25	>1000	0		
		100–1000	0		
		50–100	0		
		10–50	0		
		<10	8		
	FOXP3	>1000	0		
		100–1000	0		
		50–100	0		
		10–50	0		
		<10	8		

a Estimated number of positively stained cells (see Section 2).

groups (*n* = 3) or were rare and scattered (*n* = 1) (Table 4). Their location had no relationships to pathological changes in the neighbouring tissues (Fig. 2g).

3.2. *FOXP3$^+$ lymphocytes are present in the lymphocyte clusters*

Cells with clear nuclear staining for FOXP3$^+$ were detected in prostate cancer tissues. The distribution of FOXP3$^+$ cells followed that of the CD3$^+$, CD4$^+$ and CD25$^+$ lymphocytes with a predominant localisation in the clusters (Fig. 2b). Only few FOXP3$^+$ cells were localised in the carcinoma areas themselves (Fig. 2d). In all prostate cancer samples, the lymphocyte clusters contained very high number of FOXP3$^+$ cells (100–1000 for *n* = 6, 50–100 for *n* = 11; Table 4). In nine of 17 prostate cancer tissues, FOXP3$^+$ cells formed clusters adjacent to the carcinoma. In the other eight tissues, FOXP3$^+$ cells were more diffusely scattered around the prostate cancer lesions. A correlation between the extent of FOXP3$^+$ infiltration and the Gleason score or TNM status was not evident (Table 4). FOXP3$^+$ cells were also encountered in healthy prostate tissue, but their numbers were very low (<10 cells per tissue section, Table 4), and they were preferentially found in the glandular structures (Fig. 2f). BPH tissue also had FOXP3$^+$ cells (10–50, *n* = 3; <10, *n* = 1) which were either in small lymphocyte clusters (Fig. 2h) or scattered.

Collectively, a comparison between prostate cancer-inflicted tissue, BPH and healthy prostate tissue revealed that CD25$^+$ and FOXP3$^+$ cells are much more frequent in the cancer-inflicted tissue. None of the BPH or healthy tissues reached numbers of 10–50 of cells positive for these markers, whereas these high numbers were observed in all analysed cancerous samples (Table 4). In the cancer-inflicted tissues, the CD25$^+$ and FOXP3$^+$ cells were observed within the lymphocyte clusters adjacent to the carcinoma indicating that T cells with regulatory capacity reside close to, yet outside, the prostate cancer regions. The higher frequency of CD25$^+$ and FOXP3$^+$ cells observed in BPH tissue compared to healthy tissue might reflect the attempt of the tissue environment to limit collateral tissue damage within the inflammatory milieu of BPH.

3.3. *PD-1 and its ligand B7-H1 are present in the prostate cancer environment but are not expressed by cancer cells*

Immunohistological staining with PD-1 antibody detected positive-stained cells in the lymphocyte clusters adjacent to the

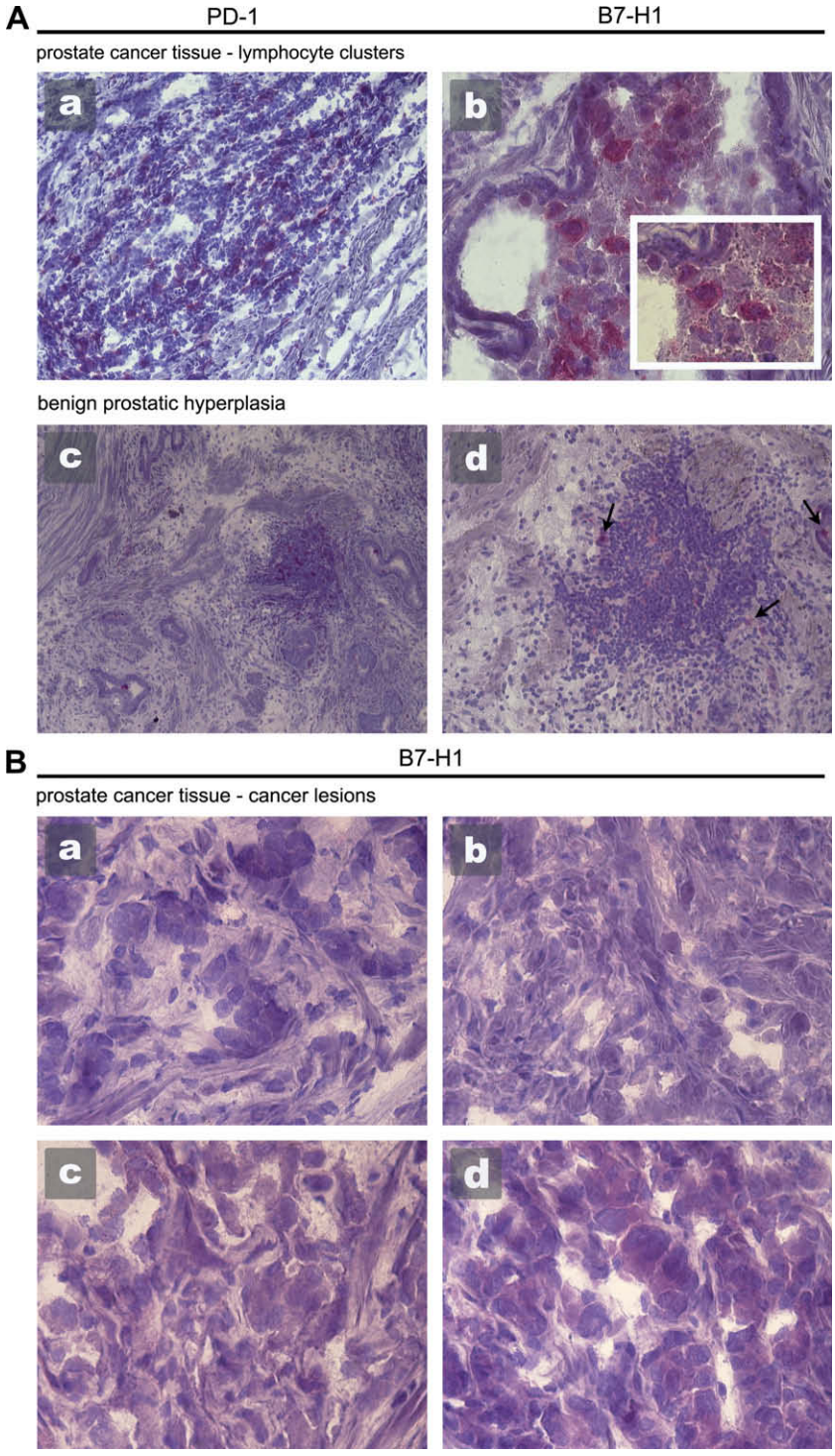

Fig. 3 – Immunohistological staining for PD-1 and B7-H1. Serial 5 μm cryosections of prostate cancer tissue or BPH tissue stained with anti-human PD-1 (dilution 1:50) and B7-H1 (dilution 1:30) antibodies. A: (a) PD-1$^+$ (magnification 200×) and (b) B7-H1$^+$ cells (magnification 400×) (inset: magnification 630×, oil immersion) in the lymphocyte clusters adjacent to the prostate cancer lesions. Tissue of patient 6 is shown as representative example of 17 carcinoma tissues. (c) PD-1$^+$ (magnification 100×) and (d) B7-H1$^+$ (magnification 200×) cells in small lymphocyte clusters in benign prostatic hyperplasia tissue. Their location has no relationship to pathological changes in the surrounding tissue. Patient 4 is shown as representative example. B: No B7-H1 expression by prostate cancer cells. Cancer areas of four different individuals (a–d corresponding to patient 4–7) (magnification 630×, oil immersion) are shown.

carcinoma lesions (Fig. 3A/a). Within the carcinoma regions or in healthy tissue, only few PD-1$^+$ cells were found (data not shown). BPH also had PD-1$^+$ lymphocytes (Fig. 3A/c). The ligand of the PD-1-receptor is B7-H1. B7-H1$^+$ cells were rare

Table 5 – Semi-quantitative evaluation of the frequency of PD-1 and B7-H1 expressing cells.

Tissues	Marker	Cell counts[a]	Number of tissues (n)	Gleason scores (n)	Tumour stages T (n): TNM (acc. to UICC 2002)
Prostate cancer-inflicted tissue			*n* = 17		
	PD-1	> 1000	1	6 (1)	pT2a (1)
		100–1000	5	6 (3); 7 (1); 8 (1)	pT2b (1); pT2c (3); pT3a (1)
		50–100	9	6 (2); 7 (4); 8 (2); 9 (1)	pT2c (4); pT3a (2); pT3b (3)
		10–50	2	6 (1); 8 (1)	pT2a (2)
		<10	0		
	B7-H1	>1000	0		
		100–1000	3	6 (2); 7 (1)	pT2a (1); pT2c (1); pT3a (1)
		50–100	11	6 (4); 7 (3); 8 (3); 9 (1)	pT2b (1); pT2c (5); pT3a (2); pT3b (2)
		10–50	3	6 (1); 7 (1); 8 (1)	pT2a (2); pT2c (1)
		<10	0		
Benign prostatic hyperplasia tissue			*n* = 4		
	PD-1	>1000	0		
		100–1000	0		
		50–100	0		
		10–50	3		
		<10	1		
	B7-H1	>1000	0		
		100–1000	0		
		50–100	0		
		10–50	3		
		<10	1		
Healthy prostate tissues			*n* = 8		
	PD-1	> 1000	0		
		100–1000	0		
		50–100	0		
		10–50	3		
		<10	5		
	B7-H1	>1000	0		
		100–1000	0		
		50–100	0		
		10–50	3		
		<10	5		

a Estimated number of positively stained cells (see Section 2).

in healthy tissue and when present they were only seen in glandular structures (data not shown). In cancer-inflicted tissues, PD-1$^+$ and B7-H1$^+$ cells were abundantly present in the lymphocyte clusters surrounding the carcinoma area (Fig. 3A/b). B7-H1$^+$ cells were larger than CD3$^+$ cells and showed a mononuclear morphology (Fig. 3A/b). In the BPH tissues, some PD-1$^+$ or B7-H1$^+$ cells were present within small lymphocyte clusters (Fig. 3A/c and d). In the 17 investigated prostate cancer tissues, none showed expression of B7-H1 on the prostate cancer cells themselves (Fig. 3B/a–d).

Semi-quantitative evaluation of PD-1$^+$ and B7-H1$^+$ cells revealed that most tumour tissues (n = 14/17) had clusters rich in PD-1$^+$ cells, whereas B7-H1$^+$ cells were slightly less abundant (Table 5). A correlation to the Gleason score or TNM status was not evident (Table 5). In BPH and healthy prostate tissues, these markers expressing cells were rare (Table 5).

4. Discussion

The aim of this study was to analyse prostate cancer tissues for the infiltration of T cells with negative immune regulatory capacity. During the past several years, more information about the negative regulatory function of a small subpopulation of CD4$^+$ T lymphocytes expressing FOXP3 and CD25 have emerged. This small population was recognised to play a role in tolerance to self-antigens.[7,8] In various studies, the role of Tregs for cancer is discussed and the results seem to be similar, namely that Tregs may play a substantial role in the poor development of immune responses to cancer.[9,10]

Regulatory T cells belong to the CD3$^+$–CD4$^+$–CD25$^+$ population but this phenotype also characterises activated T cells and only a very small fraction of these cells are truly T lymphocytes with regulatory function. One credible marker for the identification of Tregs is the forkhead box protein P3 (FOXP3). In mice, Foxp3 was identified as an essential molecule involved in the development and function of Tregs. The expression of Foxp3 in mice led to suppression of proliferation of wild type CD4$^+$ CD25$^-$ responder cells.[16] Foxp3-mutated mice, scurfin mice, die 16–25 d after birth due to their inability to regulate T cell activity leading to hyperproliferation of lymphocytes.[17] Furthermore, the development of diabetes was observed in Foxp3-mutated mice.[18] In humans,

the absence of intact FOXP3 is responsible for the IPEX syndrome, which is a serious autoimmune syndrome in children which includes several different autoimmune phenomena. This syndrome, which is not associated with gene mutations other than FOXP3, reveals the key role of this transcription factor in self-tolerance.[19] The presence of FOXP3$^+$ cells is a common signature of many cancer types.[10] For prostate cancer, Foxp3 transcripts were detected in tumour-infiltrating lymphocytes obtained through serial needle biopsies.[20] Further, CD4$^+$ and CD8$^+$ FOXP3$^+$ Treg cells were found in prostate tumour-derived TILs.[21,22]

In our study, a pronounced presence of CD25$^+$ and FOXP3$^+$ cells was detected in the CD3$^+$ and CD4$^+$ lymphocyte clusters surrounding prostate cancer lesions, which we had previously described.[15] Only very few FOXP3$^+$ cells were found in the tumour lesions themselves. Because we applied immunohistological staining using FOXP3 antibody instead of determining relative transcript levels in isolated tumour RNA, we were able not only to reveal the presence of FOXP3$^+$ cells but additionally to define their locoregional distribution, which would have not been possible using mRNA expression analysis.

Recently, *in vitro* experiments have provided evidence that tumour-infiltrating lymphocytes in prostate cancer are non-reactive.[23] Additionally, our own *in situ* analysis revealed a lack of markers for functional activity, such as perforin and IFNγ.[15] Collectively, these data suggest a cancer-induced immune suppression. Now, the detection of FOXP3$^+$ cells adjacent to the prostate cancer lesion provides further evidence that the immune system may sustain tolerance against the malignant cells.

Furthermore, we observed high numbers of PD-1$^+$ and B7-H1$^+$ cells in the lymphocyte clusters. PD-1 expression on lymphocytes is thought to indicate functional exhaustion, as PD-1 expressing cells detected in chronic viral-infected mice and HIV-infected humans were no longer able to react against specific antigens *in vitro*.[12,13] The detection of PD-1$^+$ and B7-H1$^+$ cells in the lymphocyte clusters surrounding the prostate cancer regions indicates that functional exhaustion of lymphocytes may be an additional factor that limits an anti-tumour response.

In a murine cancer model, the PD-1/B7-H1 pathway was associated with resistance against cancer vaccination immunotherapy. Anti-tumour response was enhanced when blocking this pathway with anti-B7-H1 antibody or anti-PD-1 antibody.[24] Our demonstration of PD-1 and B7-H1 expression indicates that the PD-1/B7-H1 pathway may be a target for prostate cancer therapy.

Recent studies documented B7-H1 expression on tumour cells of different human cancers including lung carcinoma, ovarian carcinoma, colon carcinoma, melanoma and glioblastoma.[25,26] It is thought that through B7-H1 expression, tumour cells directly inactivate infiltrating lymphocytes, thereby escaping immune destruction. However, studies investigating the relevance of B7-H1 expression by tumour cells for prognosis of patients provided conflicting results: studies of non-small-cell-lung-cancer (NSCLC) detected no correlation between B7-H1 expression and clinicopathological variables or postoperative survival of patients. In contrast, a negative correlation between B7-H1 expression and prognosis was observed for renal cell carcinoma.[27–29]

B7-H1 was reported to be expressed on antigen-presenting cells and tumour cells. Due to single marker analysis in our study it was not possible to precisely define the cell type expressing B7-H1, and multiparameter staining is required to clarify whether B7-H1$^+$ cells are antigen-presenting cells. Yet, our immunohistological analysis clearly demonstrates that the prostate cancer cells themselves do not express B7-H1, consistent with another recent publication.[30] Thus, the cancer cells do not impact on immune cells through a direct PD-1/B7-H1 pathway. Rather our results suggest an indirect influence of the cancerous lesions on the immune system, which is manifested by an accumulation of lymphocytes with negatively regulatory function (FOXP3) around but not within malignant lesions and an environment that leads to functional inhibition or exhaustion of lymphocytes (PD-1/B7-H1). The observation of several inhibitory markers including FOXP3, B7-H1 and PD-1 may help to explain why the immune responses directed against prostate tumour cells are ineffective. Investigating whether there is a correlation between infiltrating lymphocytes subsets or select marker expression and prognosis should help to shed further light into the potential importance of cells expressing these markers in cancer progression. Our patient collective was not suitable to address this issue, as all patients had received radical prostatectomy as a curative treatment and 5-year-survival of this patient group is the rule. The tissue collection was implemented three years ago and, thus, the time period was not long enough for our patients to experience progression of their cancer disease. This issue should be investigated in future studies and is also interesting for other kind of cancers.

Conflict of interest statement

None declared.

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