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Correlation between the expression of integrins in prostate cancer and clinical outcome in 1284 patients

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ABSTRACT

The aim of this study was to investigate the expression of a panel of integrins in prostate cancer in order to explore their potential for tumor biology. Formalin-fixed and paraffin-embedded tissue samples of 1284 prostate cancer patients were retrieved from the archive of the Department of Pathology. Immunostaining was done with rabbit monoclonal antibodies directed against αvβ3, αvβ5, αvβ6, αvβ8, β3, and αv-pan. Staining results were correlated with clinicopathologic patient characteristics and patient survival. Immunostaining of tumor cells performed on whole tissue sections of 52 patients was sparse for αvβ3, αvβ6, and αvβ8, and more prevalent for αvβ5 and αv-pan. αvβ5, αvβ8, and αv-pan were selected for further analyses in tissue microarrays representing the entire study cohort. αvβ8 staining was generally observed in peripheral nerves. αvβ5 and αv-pan provided strong evidence for the differential expression of these integrins in prostate cancer. The expression was variable with regard to the histoanatomical/cytoanatomical localization, cell type, intensity of immunolabeling, and Gleason pattern. αvβ5 and αv-pan are differentially expressed in prostate cancer, and the differentiation of prostate cancer seems to influence integrin expression and subcellular distribution.

1. Introduction

Prostate cancer is the second most common cancer in men and the sixth most common cause of cancer death in the world [1,2]. The most important factors affecting patient outcome are tumor stage, tumor grade according to the Gleason Score (GS) and serum levels of prostate-specific antigen [3].

Recently, several markers like galectin 3, circulating microRNAs, and integrins were discussed as new prognostic biomarkers [4–7]. Integrins are transmembrane receptors that mediate cell signaling pathways. Because of their various physiological functions in cell survival and differentiation, they play important roles in the pathology of tumor progression and metastasis [8,9]. During the last decades, systematic investigations have been hampered by the lack of antibodies suitable for formalin-fixed and paraffin-embedded (FFPE) tissue, and current knowledge about integrins is mainly derived from cell line analyses [10].

Lately, integrins, particularly αvβ3 and αvβ5, became putative novel targets for the treatment of several cancer entities, which has spurred research on integrins in cancer biology [11]. For this reason, the characterization of integrin distribution in human tumors is of great interest. Among the integrins, αvβ3 and αvβ5 are expressed among others in endothelial cells and promote cell survival [12]. They play an important role in angiogenesis, which is essential for tumor progression and metastasis [13]. In bone metastasis, αvβ3 is responsible for bone turnover in the interaction with osteopontin [14].

αvβ6 and αvβ8, in turn, interact with TGF-β and play an important role in the immune response. αvβ6 influences regulatory T cells and seems to be involved in the avoidance of immune reaction in colorectal cancer, which promotes tumor spread [15,16]. αvβ8 has a key part in the blood vessel development during embryogenesis and is expressed in several human tumors [17]. Moreover, the up-regulation of some integrin subunits in prostate cancer has been previously described [18,19].

The aim of this study was to investigate the expression of a panel of integrins (αvβ3, αvβ5, αvβ6, αvβ8, β3, αv-pan) in prostate cancer in order to explore their potential significance for tumor biology. For this purpose, a large retrospective cohort of prostate cancer specimens was retrieved and immunohistochemistry was applied using newly established rabbit monoclonal integrin antibodies that have previously been shown to react specifically in FFPE tissue. Results of immunostaining were correlated with clinicopathologic patient characteristics.

2. Material and methods

2.1. Ethics statement

This project was approved by the local ethics committee of the University Hospital in Kiel, Germany (AZ 110/99). All patient data were pseudonymized before study inclusion.

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2.2. Study population

From the archive of the Department of Pathology, Christian-Albrechts-University Kiel, we retrieved all cases that had undergone radical prostatectomy for prostate cancer spanning the period from 1997 to 2011. All specimens had been fixed in formalin, embedded in paraffin (FFPE), and stored at room temperature. Study inclusion criterion was prostatectomy with histologically confirmed prostate cancer. Patients were excluded if clinical data were incomplete and prostate cancer featured less than 10% of tissue samples or offered retraction artifacts of the tumor glands due to autolysis. Biopsy samples and transurethral resection specimens were excluded. Date and cause of patient death were obtained from the *Epidemiological Cancer Registry* of the state of Schleswig-Holstein, Germany. Follow-up data of patients who are still alive were retrieved from hospital records.

2.3. Histology

De-paraffinized tissue sections were stained with hematoxylin and eosin. Tumor stage was reclassified according to the seventh edition of the TNM classification of the Union internationale contre le cancer (UICC). Tumor type and histologic grading were classified according to the World Health Organization classification of prostate cancer and the revised Gleason grading system [20,21]. The Gleason grading was separately applied to whole tissue sections (WTS) and tissue microarrays (TMA).

2.4. Tissue microarray construction

Formalin-fixed and paraffin-embedded tissue samples were used to generate TMAs as described previously [22]. Briefly, 3 morphologically representative regions of a single paraffin "donor" block were chosen per cancer sample. Tissue cylinders of 1.5-mm diameter were punched from these areas, precisely arrayed into a new "recipient" paraffin block using a custom-built instrument (Beecher Instruments, Silver Spring, Maryland). Serial sections of 2.5 μm were cut for further analysis.

2.5. Immunohistochemistry

Immunohistochemical stainings were performed with a Ventana Benchmark ULTRA (Roche Diagnostics, Mannheim, Germany), using the ULTRAView Universal DAB Kit (Roche Diagnostics). Formalin-fixed and paraffin-embedded material from each tumor was stained with 6 recently established monoclonal rabbit antibodies (Table 1) directed against integrin complexes or individual chains, as previously described [23]. The biochemical specificity of the antibodies against integrins, which were used in this study, has been precisely defined [24,25]. They detect the αvβ3 (EM22703), αvβ5 (EM09902), αvβ6 (EM052), and αvβ8 (EM13309) heterodimeric complexes; the αv chain in all the αv heterodimeric complexes (EM01309); or the β3 chain cytoplasmic domain (EM00212).

2.6. Study design

To evaluate the immunostaining characteristics of the different antibodies with regard to the staining pattern and intensity, a test cohort of 52 samples, represented on WTS, was set up from the entire cohort, which represented in equal amounts the different GS of prostate cancer. For those antibodies that showed no positive staining results in WTS, a cohort of 112 cases, represented on TMAs, was stained to see if the primary staining results were confirmed. For those antibodies that showed positive staining results on the WTS, staining was performed for the entire cohort using TMAs. Staining results were correlated among themselves and with clinicopathologic data.

2.7. Read-outs

The quantity, intensity, and localization of immunoreactivity within the tumor cells were assessed for each antibody. Localization of immunoreactivity was evaluated as (1) membranous linear intercellular staining, (2) basal staining localized at the interface between tumor cell complexes and stroma, and/or (3) cytoplasmic staining.

Immunostaining was evaluated using the HistoScore (Hscore) as previously described [26]. The first parameter was based on the intensity of the stained cells. A score of 0 (no evidence of staining) to 3 (strong staining reaction) was applied. The second parameter (P) estimates the distribution of the stained cells in percentage. Finally, an Hscore was calculated according to the following formula: $\text{HScore} = (0 \times P) + (1 \times P) + (2 \times P) + (3 \times P)$, resulting in an Hscore ranging from 0 to 300.

Moreover, an optional integrin expression in other tumor components than cancer cells (eg, perineural sheets and nonneoplastic prostate tissue) was documented as side notes, but not systematically analyzed.

2.8. Statistical analysis

The statistical analysis was performed with SPSS Statistics 18.0 (SPSS Institute, Chicago, Illinois). Fisher exact test, Kendall τ, and log-rank test were used to correlate the integrin expression with clinicopathologic patient characteristics as well as for the comparison of WTS with the corresponding TMA staining results. Survival data of the patients were illustrated by Kaplan-Meier curves and compared using the log-rank test. Every test was rated by the P value. A P value less than .05 was considered statistically significant.

3. Results

3.1. Study population

A total of 1284 male patients fulfilled all study inclusion criteria (Table 2). In 1272 cases (99.1%), a GS could be evaluated. The GS represented the major prognostic factor. Follow-up period ranged from 0.03 to 189.5 months (mean [SD], 70.7 [41.7]).

3.2. Expression of integrins in prostate cancer

Because of the rather low expression of integrin αvβ3, β3, αvβ6, and αvβ8 in prostate cancer cells in a test cohort of 52 WTS, evaluation of the entire cohort was neglected for these antibodies. Only 112 tumor samples, represented on TMAs, were evaluated to see if the primary staining results found in WTS were confirmed.

αvβ5 and αv-pan showed a distinctive immunoreaction in prostate cancer cells, and subsequently, the entire cohort was studied using TMAs.

Table 1
Staining protocols

Antigen	Clone	Source	Pretreatment	Antibody dilution	Detection system
αvβ3	EM22703	Merck, Darmstadt, Germany	Protease 2	1:100	Ventana Benchmark ULTRA
αvβ5	EM09902	Merck	Protease 2	1:5000	Ventana Benchmark ULTRA
β3	EM00212	Merck	CC1	1:80	Ventana Benchmark ULTRA
αvβ6	EM05201	Merck	Protease 2	1:1000	Ventana Benchmark ULTRA
αvβ8	EM13309	Merck	Protease 2	1:500	Ventana Benchmark ULTRA
αv-pan	EM01309	Merck	CC1	1:20.000	Ventana Benchmark ULTRA

Table 2
Clinicopathologic patient characteristics

Parameter	n (% of valid)
Patient no.	1284
Age (y), mean ± SD	65.1 ± 6.2
Follow-up data	1255
Alive	1101 (87.7)
Dead	154 (12.3)
Prostate-specific death	24 (1.9)
Local tumor growth	
T2a[a]	161 (12.6)
T2b	90 (7.2)
T2c	568 (44.4)
T3a	254 (19.8)
T3b	186 (14.5)
T4	19 (1.5)
Lymph node metastases	
N0	1098 (89.1)
N1	135 (10.9)
Distant metastases	
M0	5 (55.6)
M1	4 (44.4)
UICC tumor stage	
I	147 (11.6)
II	642 (50.5)
III	335 (26.3)
IV	148 (11.6)
Lymphatic vessel invasion	
L0	800 (89.9)
L1	90 (10.1)
Blood vessel invasion	
V0	904 (98.0)
V1	18 (2)
Perineural invasion	
Pn0	67 (12.6)
Pn1	466 (87.4)
Resection margin	
R0	967 (77.7)
R1/R2	277 (22.2)
Tumor grade	
G1	21 (1.6)
G2	818 (64)
G3	440 (34.4)
GS	
6	427 (33.6)
7 (low risk)[b]	425 (33.4)
7 (high risk)[b]	186 (14.6)
8	122 (9.6)
9	106 (8.3)
10	6 (0.5)

Gleason 7 high-risk group contains all cases with a GP4 + 3 and 5 + 2.
[a] T1a and T1b were summarized to T2a, diagnosed at the prostatectomy specimen.
[b] Gleason 7 low-risk group contains all cases with a GP3 + 4 and 2 + 5.

3.2.1. αvß3 integrin

In WTS obtained from 51 patients (1 missing value), most tumor cells were immunonegative for αvβ3. Subsequently, TMA samples obtained from 112 separate patients were immunohistochemically stained, and findings made in WTS were confirmed (Table 3).

3.2.2. β3 integrin

β3 was not expressed by prostate cancer cells either in WTS or in TMAs, but β3 expression was found in extratumoral blood vessel walls of 48 (94.1%) of 51 patients (WTS) and in 19 (17.3%) of 110 patients (TMAs), respectively.

3.2.3. αvß6 integrin

Ten cases showed a basal immunostaining and 3 cases showed a cytoplasmic immunostaining of the tumor cells in WTS (Table 3). Moreover, the stratified epithelium of excretory ducts showed a mainly strong immunolabeling. All other tumor components (eg, tumor stroma and blood vessels) were immunonegative.

Table 3
Distribution of expression of αvβ3, αvβ6, and αvβ8 in prostate cancer cells, separated into basal, cytoplasmic, and membranous staining (mean ± SD)

	Tumor cells	Integrins		
		αvβ3	αvβ6	αvβ8
WTSs	n (missing)	52 (1)	52 (2)	52 (0)
	Basal	0 ± 0	12.1 ± 29.0	0 ± 0
	Cytoplasmic	7.8 ± 36.9	4.7 ± 24.0	2.1 ± 6.3
	Membranous	0 ± 0	0 ± 0	0 ± 0
TMAs	n (missing)	112 (0)	112 (1)	1211 (0)
	Basal	0 ± 0	6.8 ± 29.6	0 ± 0
	Cytoplasmic	0.09 ± 0.95	8.3 ± 31.5	1.7 ± 11.7
	Membranous	0 ± 0	5.1 ± 22.8	0.01 ± 0.29
	Nontumor cells	Integrins		
		αvβ3		αvβ8
WTSs	n (missing)	52 (1)		52 (0)
	Blood vessels	138.0 ± 95.0		
	Perineural sheaths			112.0 ± 85.0
TMAs	n (missing)	112 (0)		691 (520)
	Blood vessels	75.5 ± 83.0		
	Perineural sheaths			192.0 ± 140.0

In addition, the integrins αvβ3 and αvβ8 were found in intratumoral blood vessels and perineural sheaths, respectively (mean ± SD).

Because of the predominantly weak staining of tumor cells, only 111 patients (1 missing value) were evaluated on TMAs. Previous findings were confirmed (Table 3).

3.2.4. αvß8 integrin

The anti-αvβ8 antibody showed a strong immunostaining of perineural sheaths (Pn) with and without tumor cell infiltration. The 52 WTS demonstrated that the tumor cells were mainly immunonegative, as well as the prostatic stroma (Table 3). The nonneoplastic glands showed a weak immunoreaction in 25 (50%) patients.

3.2.5. αvß5 and αv-pan integrins

Most tumor cells, stroma cells, and nonneoplastic glands showed a positive immunoreaction for both αvβ5 and αv-pan in WTS. The basal cells of nonneoplastic glands constantly showed a mainly strong immunolabeling. Hence, the expression of αvβ5 and αv-pan was evaluated for WTS (52 cases) as well as for the entire cohort (1255 cases) using TMAs. Staining results of αvβ5 and αv-pan were correlated with the Gleason pattern (GP), clinicopathologic patient characteristics, and survival.

3.2.5.1. Evaluation of αvβ5 and αv-pan expression in WTSs. αvβ5 showed a predominantly weak basal immunoreaction in tumor cells. The cytoplasmic and membranous expression increased with an advanced GP (Fig. 1; Table 4).

Basal expression of αv-pan increased with advanced GP. Cytoplasmic immmunostaining of αv-pan was usually weak with no significant differences between the different GPs. Membranous immunostaining decreased from GP3 to GP5 (Fig. 2; Table 4).

3.2.5.2. Evaluation of αvβ5 and αv-pan expression in TMAs. Both antibodies showed a basal, cytoplasmic, and membranous staining of tumor cells. Because of the different growth patterns and the loss of cellular structure of GP4 and GP5, the membranous and basal staining of αv-pan was not detectable in every patient (Table 4).

As shown in Table 5, GP4 and GP5 showed different staining results depending on the different phenotypes (fusiform, cribriform, ill-defined, papillary, mucinous, and solid with necrosis). The basal layer of the cribriform glands and the solid tumor nests with necrosis showed the strongest immunoreaction with both antibodies.

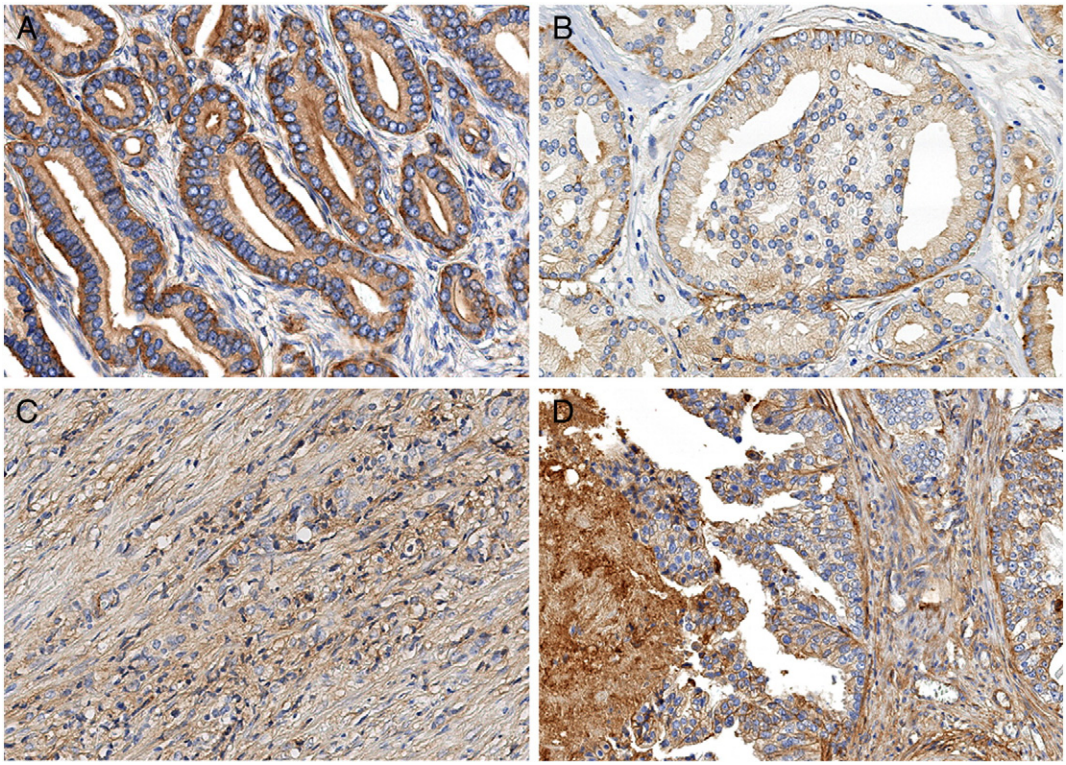

Fig. 1. Expression of αvβ5 in prostate cancer. This figures illustrates prostate carcinoma with a GP3 with strong basal and moderate cytoplasmic expression of αvβ5 (A), GP4 cribriform growth pattern (B), GP4 with ill-defined glands and small gland fusion (C), and GP5 solid with comedonecrosis (D). Original magnification 200-fold.

3.2.5.3. Correlation with GP and other clinicopathologic patient characteristics. Membranous immunostaining of αv-pan correlated significantly inversely with the GP. Moreover, cytoplasmic αvβ5 expression correlated significantly with the GP and with the tumor grade (Table 6).

There was no correlation with any other clinicopathologic patient characteristic.

3.2.5.4. Correlation between immunostaining of αvβ5 and αv-pan. As shown in Table 7, significant coincidental expression was found for basal, cytoplasmic, and membranous immunostaining of αvβ5 and αv-pan, respectively.

3.3. Survival analyses

One hundred fifty-four patients (12.0%) died during the study period. Twenty-four (1.9%) patients died of prostate cancer, 106 (8.3%) of other diseases, and 24 (1.9%) of unknown reason. Correlation between clinicopathologic patient characteristics and overall survival or tumor-specific survival is illustrated in Table 8.

Because of the small number of cancer-related deaths, it was impossible to analyze prostate cancer–specific patient survival using the Kaplan-Meier curves, although the log-rank test revealed a significant correlation between tumor-specific survival and basal expression of αvβ5 as well as overall survival with αvβ5 membranous expression.

4. Discussion

This study was designed to investigate integrin expression in prostate cancer. Integrins belong to a family of heterodimeric cell surface receptors and interact through an RGD binding domain with extracelluar ligands [27]. Within their function to facilitate cell survival and differentiation, they play an important role in tumor progression and metastasis [13,10]. Ideas about pharmacologic treatment based on the inhibition of integrins already exist; therefore, a verification of integrin expression in different tumors would be preferable.

Table 4
Distribution of expression of αvβ5 and αv-pan in prostate cancer cells, separated into basal, cytoplasmic, and membranous staining

	GP	αv-pan WTS Hscore			αv-pan TMA Hscore			αvβ5 WTS Hscore			αvβ5 TMA Hscore		
		n	Mean		n	Mean		n	Mean		n	Mean	
Basal	3	23	69.0 ± 59.0	$P = .207$; $\tau = 0.172$	707	62.0 ± 55.0	$P = .002$; $\tau = 0.080$	23	88.0 ± 80.0	$P = .604$; $\tau = 0.059$	733	64.0 ± 57.0	$P = .772$; $\tau = 0.008$
	4	14	89.0 ± 75.0		286	75.0 ± 65.0		16	96.0 ± 54.0		293	73.0 ± 71.0	
	5	2	120.0 ± 0		34	74.0 ± 76.0		11	85.0 ± 47.0		35	62.0 ± 68.0	
Cytoplasmic	3	23	91.0 ± 69.0	$P = .591$; $\tau = -0.062$	706	62.0 ± 55.0	$P = .003$; $\tau = 0.073$	23	92.0 ± 62.0	$P = .962$; $\tau = -0.007$	732	49.0 ± 54.0	$P < .001$; $\tau = 0.089$
	4	16	69.0 ± 40.0		366	70.0 ± 55.0		14	79.0 ± 67.0		374	62.0 ± 63.0	
	5	11	94.0 ± 83.0		103	71.0 ± 58.0		3	117.0 ± 78.0		108	61.0 ± 62.0	
Membranous	3	23	27.0 ± 46.0	$P = .880$; $\tau = -0.022$	704	40.0 ± 46.0	$P = .001$; $\tau = -0.092$	23	8.7 ± 23.0	$P = .429$; $\tau = 0.118$	728	11.0 ± 32.0	$P = .881$; $\tau = -0.004$
	4	14	24.0 ± 36.0		289	33.0 ± 45.0		14	11.0 ± 26.0		290	12.0 ± 35.0	
	5	2	2.5 ± 3.5		35	26.0 ± 42.0		4	32.0 ± 47.0		34	1.5 ± 4.4	

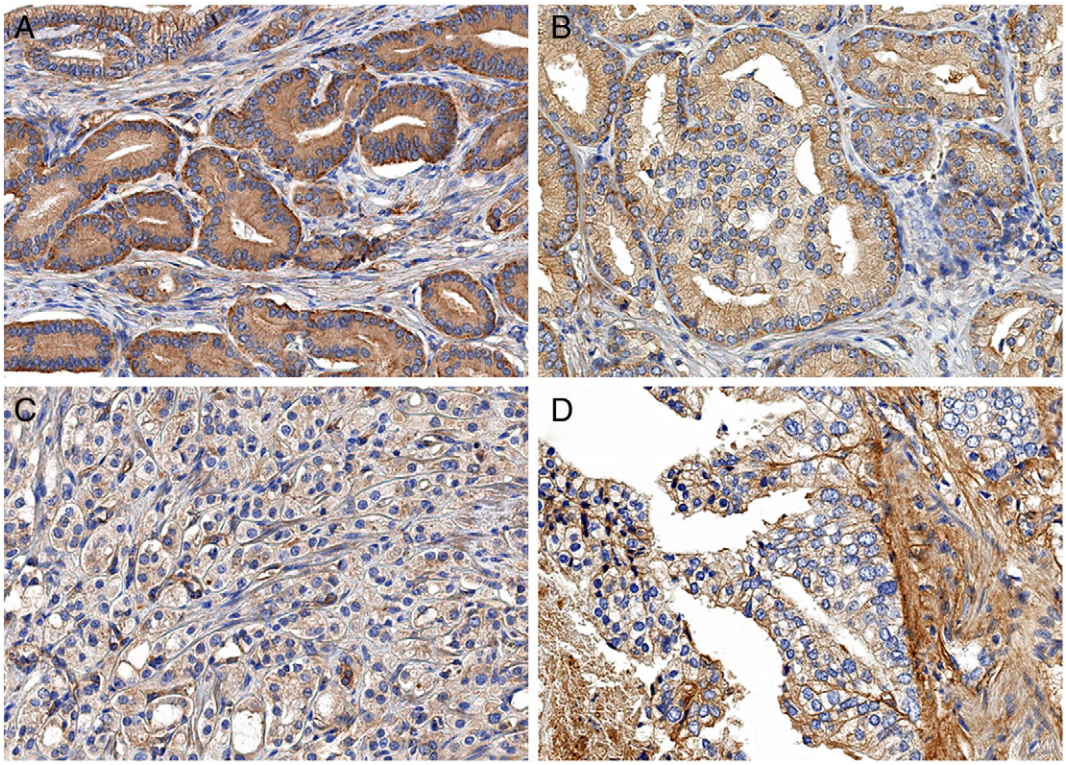

Fig. 2. Expression of αv-pan in prostate cancer. This figure illustrates prostate carcinoma with a GP3 with strong basal and moderate cytoplasmic expression of αvβ5 (A), GP4 cribriform growth pattern (B), GP4 with ill-defined glands and small gland fusion (C), and GP5 solid with comedonecrosis (D). Original magnification 200-fold.

Until now, systemic investigations have been hampered by the lack of antibodies suitable for FFPE tissue. We report the first extensive longitudinal investigation of the expression of the main αv integrins in a cohort of prostate cancer, using novel rabbit monoclonal antibodies suitable for FFPE tissue. In addition, putative prognostic patient characteristics such as GS and TNM status were compared with patient survival.

One of the most interesting findings was the variability of the expression of all investigated markers. In our study, αvβ3 was expressed in blood vessels as previously described [25]. αvβ3 is known to be an important factor of tumor progression and metastasis in prostate cancer, although the direct expression by tumor cells in prostate was not confirmed. Therefore, it is plausible to speculate that αvβ3 may be involved in bone metastases despite an absent extensive expression in prostate cancer cells [28]. β3 showed no expression in intratumoral blood vessels, but in peripheral extratumoral blood vessels near the prostate capsule. Because of the different staining results in blood vessels, β3 offers the possibility to differentiate between nonneoplastic and tumor-associated blood vessels.

In most of our cases, anti-αvβ6 immunostained the basal layer of benign glands without staining tumor cells or stroma cells. Anti-αvβ8 showed a strong staining of peripheral nerve sheaths or neural axons, but did not correlate with any of the tested parameters. Thus, the expression of αvβ6 and αvβ8 does not seem to be tumor biologically relevant in the primary prostate cancer.

Among the integrins studied herein, only αvβ5 and αv-pan were almost ubiquitously expressed in prostate cancer cells, and their expression varied with regard to histopathologic localization. To date, only little is known about the biological significance of αvβ5 and αv-pan in prostate carcinoma, as most of the previous studies focused on αvβ3 instead. In cell culture studies, an enhanced expression of αvβ3 and αvβ5 resulted in a more spread morphology and in a survival advantage of the cells in culture via a delay of apoptosis [29]. Another study describes that αv and αvβ5, respectively, show a similar expression pattern in cells isolated from either prostate carcinoma or normal tissue, and especially, αvβ5 was found to be poorly expressed [30]. Moreover, αv integrins, including αvβ3 and αvβ5, are known to promote survival of prostate cancer cells in bone metastasis via adherence to and migration

Table 5
Correlation of the αv-pan and αvβ5 integrin expression with the different growth patterns of Gleason grades 4 and 5

Growth pattern		Hscore αv-pan					Hscore αvβ5				
		n	% of valid	Basal, mean ± SD	Cytoplasmic, mean ± SD	Membranous, mean ± SD	n	% of valid	Basal, mean ± SD	Cytoplasmic, mean ± SD	Membranous, mean ± SD
GP4	Small gland fusion	82	17.4	–	70.0 ± 54.0	0 ± 0	82	16.9	–	57.0 ± 54.0	0
	Cribriform	182	38.7	91.0 ± 69.0	76.0 ± 56.0	36.0 ± 49.0	185	38.1	96.0 ± 74.0	71.0 ± 69.0	15.0 ± 41.0
	Ill-defined glands	99	21.2	49.0 ± 45.0	62.0 ± 54.0	28.0 ± 38.0	105	21.6	34.0 ± 42.0	50.0 ± 58.0	6.0 ± 17.0
	Papillary	1	0.2	20.0	20.0	0	1	0.2	20.0	60.0	0
	Mucinous	3	0.6	43.0 ± 51.0	0	37.0 ± 55.0	4	0.8	30.0 ± 48.0	25.0 ± 50.0	0
GP5	Solid with comedonecrosis	7	1.5	127.0 ± 62.0	73.0 ± 40.0	14.0 ± 38.0	7	1.4	84.0 ± 69.0	23.0 ± 36.0	0
	Fusion/Solid sheets	69	14.7	0	72.0 ± 56.0	0	73	15.1	–	62.0 ± 57.0	–
	Cribriform/papillary without necrosis	27	5.7	63.0 ± 74.0	68.0 ± 67.0	30.0 ± 44.0	28	5.8	57.0 ± 68.0	69.0 ± 77.0	2.0 ± 5.0
Total		470	100.0				485	100.0			

Table 6
Expression of αvβ5 and αv-pan in prostate cancer cells compared with clinicopathologic patient characteristics investigated in TMAs

			αvβ5 TMA			αv-pan TMA		
			Basal, n (%)$^{(m)}$	Cytoplasmic, n (%)$^{(z)}$	Membranous, n (%)$^{(m)}$	Basal, n (%)$^{(m)}$	Cytoplasmic, n (%)$^{(z)}$	Membranous, n (%)$^{(m)}$
T category	n	p$^{(2)}$	.130	.685	.795	.169	.579	.721
T2a			61 (50.4)	77 (57.5)	23 (19.2)	62 (52.1)	69 (53.1)	57 (47.9)
T2b			45 (63.4)	58 (70.7)	16 (22.5)	36 (54.5)	41 (53.9)	34 (50.7)
T2c			235 (49.6)	321 (62.3)	63 (13.4)	230 (49.6)	231 (45.8)	256 (55.5)
T3a			105 (50.5)	145 (61.4)	42 (20.4)	92 (47.4)	116 (52.0)	100 (51.0)
T3b			56 (45.9)	110 (64.0)	20 (16.7)	53 (44.5)	76 (45.8)	64 (53.3)
T4			4 (30.8)	12 (70.6)	3 (23.1)	6 (54.5)	8 (53.3)	6 (54.5)
N category	n	p$^{(1)}$	1.000	.846	.100	.827	.568	.442
N0			439 (50.0)	620 (62.8)	149 (17.1)	414 (49.1)	457 (48.2)	448 (53.1)
N1			47 (50.5)	81 (63.8)	9 (9.9)	44 (47.8)	64 (51.2)	53 (57.6)
UICC stage	n	p$^{(2)}$	.612	.441	.024$^{(1)}$	.198	.950	.826
I			55 (49.5)	69 (56.6)	22 (20.0)	58 (53.2)	63 (53.4)	51 (46.8)
II			272 (51.2)	367 (63.5)	78 (14.8)	257 (49.9)	264 (47.1)	284 (55.4)
III			124 (48.8)	196 (63.2)	55 (21.8)	109 (45.6)	144 (49.0)	121 (50.0)
IV			50 (48.5)	87 (63.0)	11 (10.9)	48 (48.0)	67 (50.0)	56 (56.0)
Lymphatic invasion	n	p$^{(1)}$	.700	.402	.358	.148	1.000	.507
L0			327 (52.6)	459 (64.5)	86 (14.0)	322 (52.5)	342 (48.9)	354 (58.0)
L1			33 (50.0)	60 (69.8)	12 (18.2)	27 (42.2)	41 (49.4)	34 (53.1)
Venous invasion	n	p$^{(1)}$	.755	.198	.166	.753	.620	1.000
V0			370 (52.2)	529 (65.1)	100 (14.3)	359 (51.4)	390 (48.9)	404 (58.1)
V1			6 (60.0)	14 (82.4)	3 (30.0)	6 (60.0)	9 (56.2)	6 (60.0)
Perineural invasion	n	p$^{(1)}$	.566	.486	.539	.775	.787	.064
Pn0			30 (52.6)	36 (58.1)	6 (10.7)	26 (45.6)	31 (50.0)	35 (61.4)
Pn1			207 (57.2)	267 (62.8)	53 (15.0)	172 (48.6)	199 (48.1)	169 (47.7)
Resection status	n	p$^{(2)}$	.274	.674	.470	.204	.886	.352
R0			388 (51.0)	536 (62.5)	131 (17.3)	363 (49.7)	400 (48.6)	380 (52.1)
R1			100 (46.7)	168 (63.9)	32 (15.2)	93 (44.5)	127 (49.4)	118 (55.9)
R2			0 (0)	1 (100.0)	0 (0)	0 (0)	0 (0.0)	0 (0)
Gleason	n	p$^{(2)}$	.713	.001	.901	.342	.575	<.001
3			346 (49.6)	411 (58.9)	116 (16.7)	321 (48.0)	328 (49.1)	379 (56.9)
4			146 (52.5)	241 (67.3)	47 (17.1)	145 (53.1)	170 (48.3)	128 (46.4)
5			14 (41.2)	73 (70.9)	4 (12.1)	13 (40.6)	44 (45.4)	11 (33.3)

p(1), *P* value of Fisher exact test; p(2), *P* value of Kendall τ test; (z), dichotomized at zero (Hscore = 0: negative; Hscore > 0: positive); (m), dichotomized at the median (Hscore < median: negative; score > median: positive); nc, cannot be calculated.

on their ligand vitronectin [30–32]. Nevertheless, to date nothing is described regarding the localization of αvβ5 and αv-pan immunostaining and its significance in prostate carcinoma. In the present study, membranous immunostaining of αv-pan correlated significantly inversely with the GP. Moreover, cytoplasmic immunostaining of tumor cells with anti-αvβ5 correlated significantly with the GP. Thus, the differentiation of prostate cancer may influence integrin expression and subcellular distribution, for example, via integrin trafficking [33]. GP4 and GP5, as a result of ill-defined glands, lose their membranous cell borders. Consequently, the number of tumors with positive membranous staining and the intensity of immunostaining decreased. These results appear to be partly contradictory to former findings that say that an increased αvβ5 expression comes along with a more aggressive tumor behavior, as it is known to be on hand for tumors with a GP4 or GP5. Nevertheless, processes like trafficking could influence the subcellular distribution of the immunostaining: it might be conceivable that in ill-defined glands, the integrin heterodimers got endocytosed from the plasma membrane in to the cytoplasm; this mechanism could be a possible explanation for the change from membranous to cytoplasmic immunostaining within ill-defined glands. However, further investigations in this interesting field of cancer research are needed. However, our observations lead to the conjecture that integrin expression may also serve as a novel immunohistochemical marker of tumor cell differentiation.

Because both integrins are closely tied to the GS, αv-pan and αvβ5 are no independent prognostic markers. The cancer-specific biological relevance is unknown and, in this case, not measurable. The expression of αvβ5 in the basal cell compartment and its correlation with tumor-

Table 7
Correlation of αvβ5 and αv-pan integrin expression in prostate cancer

αv-pan			αvβ5 TMA		αvβ5 TMA		αvβ5 TMA	
			Basal$^{(m)}$		Cytoplasmic$^{(z)}$		Membranous$^{(m)}$	
			Negative, n (%)	Positive, n (%)	Negative, n (%)	Positive, n (%)	Negative, n (%)	Positive, n (%)
Basal	n	p$^{(1)}$	960	<.001				
Negative			333 (68.5)	153 (31.5)				
Positive			139 (29.3)	335 (70.7)				
Cytoplasmic	n	p$^{(1)}$			1104	<.001		
Negative					293 (51.5)	276 (48.5)		
Positive					108 (20.2)	427 (79.8)		
Membranous	n	p$^{(1)}$					950	<.001
Negative							411 (93.2)	30 (6.8)
Positive							378 (74.3)	131 (25.7)

p(1), *P* value of Fisher exact test; (z), dichotomized at zero (Hscore = 0: negative; Hscore > 0: positive); (m), dichotomized at the median (Hscore < median: negative; Hscore > median: positive.

Table 8
Overall patient survival and tumor-specific survival

Parameter	Overall survival					Tumor-specific survival			
	Total n	Events n	Mean	95% CI	*P*	Events n	Mean	95% CI	*P*
Patient no.	1255	154	156.2 ± 2.5	151.4-161.1		24	183.3 ± 1.4	180.6-186.0	
Age group					.001				.152
<66 y	624	66	162.0 ± 3.0	156.1-167.9		17	181.1 ± 1.9	177.2-185.0	
≥66 y	631	88	149.3 ± 4.0	141.4-157.1		7	186.3 ± 1.3	183.7-188.8	
T category					.181				<.001
T2a	156	22	155.6 ± 6.3	143.3-167.9		1	187.6 ± 1.5	184.7-190.5	
T2b	88	13	140.9 ± 5.1	130.9-150.9		1	160.1 ± 1.9	156.5-163.7	
T2c	561	52	157.7 ± 4.9	148.0-167.3		4	186.7 ± 1.2	184.2-189.1	
T3a	250	33	157.7 ± 4.6	148.6-166.8		8	179.5 ± 3.2	173.2-185.8	
T3b	180	29	144.8 ± 7.2	130.8-158.9		9	175.2 ± 5.0	165.4-185.1	
T4	16	3	147.6 ± 18.0	112.3-182.9		1	170.6 ± 0.0	170.6-170.6	
N category					.004				.009
N0	1077	122	158.6 ± 2.6	153.5-163.7		19	183.6 ± 1.5	180.7-186.5	
N1	128	19	125.6 ± 8.9	108.1-143.1		5	162.8 ± 4.8	153.3-172.3	
UICC stage					.087				<.001
I	142	20	155.9 ± 6.6	143.0-168.8		0	nc	nc	
II	631	62	161.1 ± 3.6	154.1-168.1		5	186.9 ± 0.9	184.9-188.8	
III	330	47	157.3 ± 4.1	149.2-165.3		13	179.2 ± 2.9	173.6-184.8	
IV	140	21	130.1 ± 7.9	114.6-145.6		6	162.4 ± 4.5	153.7-171.2	
Lymphatic invasion					.257				.197
L0	789	63	143.4 ± 2.4	138.6-148.1		8	158.8 ± 1.2	156.4-161.2	
L1	83	9	109.3 ± 5.3	99.0-119.7		2	123.1 ± 2.4	118.4-127.8	
Venous invasion					.003				.015
V0	888	70	144.4 ± 2.16	140.1-148.6		10	158.9 ± 1.1	156.7-160.9	
V1	15	4	77.2 ± 9.2	59.2-95.2		1	91.1 ± 7.0	77.3-104.8	
Perineural invasion					.951				.439
Pn0	65	4	102.1 ± 3.2	95.8-108.4		0	nc	nc	
Pn1	453	44	153.3 ± 3.3	146.8-159.9		9	168.9 ± 2.5	164.1-173.8	
Resection status					.238				.001
R0	945	110	157.4 ± 2.9	151.7-162.9		11	185.9 ± 0.9	183.9-187.8	
R1	270	39	153.2 ± 5.0	143.3-163.1		12	176.2 ± 3.7	168.9-183.5	
R2	1	0	nc	nc		0	nc	nc	
GS					.005				<.001
6	416	43	157.0 ± 5.2	146.9-167.1		1	187.9 ± 0.6	186.7-189.1	
7 (3 + 4)	420	48	159.6 ± 3.9	151.9-167.2		7	184.7 ± 1.7	181.4-188.1	
7 (4 + 3)	180	22	154.9 ± 6.7	141.7-168.1		2	181.2 ± 4.8	171.8-190.5	
8	120	28	139.2 ± 7.3	124.8-153.6		10	168.5 ± 5.8	157.2-179.9	
9	101	10	158.6 ± 9.6	139.7-177.4		3	179.7 ± 5.6	168.8-190.7	
10	6	3	31.0 ± 6.1	19.1-42.9		1	36.9 ± 6.4	24.2-49.5	
Integrin expression									
αvβ5 basal +	501	45	162.8 ± 3.8	155.4-170.2	.149	3	186.9 ± 1.2	184.7-189.3	.037
αvβ5 cytoplasmatic +	718	77	155.5 ± 3.6	148.6-162.5	.728	13	181.2 ± 2.3	176.6-185.8	.971
αvβ5 membranous +	165	33	146.0 ± 5.8	134.6-157.5	.012	5	180.9 ± 3.7	173.6-188.2	.211
αv-pan basal +	472	47	160.5 ± 4.2	152.2-168.8	.852	3	187.7 ± 1.1	185.6-189.8	.050
αv-pan cytoplasmatic +	535	59	155.8 ± 3.9	148.1-163.5	.984	9	181.9 ± 2.5	177.1-186.8	.465
αv membranous +	510	46	161.3 ± 4.1	153.3-169.3	.473	7	183.9 ± 2.3	179.5-188.3	.996

Survival is denoted in months. "+" denotes positive status, parameter divided by the median. nc denotes not calculated.

specific death lead to the speculation of a potential prognostic marker in the course of pharmacologic therapy.

Two general methodical issues need to be considered. Only 24 patients died of prostate cancer, and a mean follow-up period of 70 months is too short to capture the time of death of the whole cohort. However, the small number of tumor-specific deaths is similar to other clinical studies with a median observation period of 10 years [34]. The comprehension of biochemical cancer recurrence, instead of or in addition to death from prostate cancer, is another important surrogate end point. Unfortunately, the postoperative serum prostate-specific antigen levels were unavailable for this study, and only tumor-specific survival could be considered as an end point.

Integrin expression is heterogeneous in tumor cells, regardless of the different GPs. Strong staining can exist in close vicinity to weak staining. The expression depends on the individual growth pattern of the prostate cancer itself. This putatively limits the value of TMAs, which carry the risk of a sampling error by harboring a GP which is not representative for the entire tumor and may need further consideration of future investigations on integrins in prostate cancer.

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