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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