Integrin B6 – A Potential Marker for the Early Malignant Transformation in Prostate Cancer

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Received: June 20, 2016; Accepted: July 6, 2016; Published: July 8, 2016

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Abstract
Background: Integrins play important role in Prostate Cancer (PCa) initiation and progression and also may serve as biomarkers in its detection.

Objective: To evaluate and assess the αv, β6 and αvβ6-integrin expression in different prostatic lesions, as well as to explore the prostate malignant transformation.

Design, setting, and participants: Immunohistochemical assessment of the integrin αv, β6 and αvβ6 expression in 48 prostatectomy and 11 bone metastasis samples was performed. The expression was examined in normal, atrophic, Low-Grade Intraepithelial Neoplasia (LGPIN), High-Grade Intraepithelial Neoplasia (HGPIN) and PCa tissues.

Outcome measurements and statistical analysis: Kruskal-Wallis tests were used to evaluate the integrins αv, β6 and αvβ6 expression and their associations along with clinical variables and distribution in tissues.

Results: αv highest expression was detected in bone metastasis samples. Integrin β6 had weak staining in normal tissues, with a peak in LGPIN and HGPIN. The majority of PCa demonstrated integrin β6 negative/weak staining. Integrin β6 in the atrophy-LGPIN, -HGPIN and -PCa merging lesions was elevated. The expression of αvβ6 was remarkable in the prostatic atrophy and PCa bone metastasis.

Conclusions: Our results suggest that the prostate atrophy can be another candidate precursor of PCa. The elevated expression of integrin β6 in LGPIN provides new evidence about the role of LGPIN as a risk factor for PCa. Our data shed light on possible role of the integrin β6 as a specific biomarker for the early malignant prostatic development and warrant further investigations in the intricate process of prostatic neoplasia.

Keywords: Atrophy; Integrins; Precursor; Low-grade intraepithelial neoplasia; Prostate cancer

Abbreviations
IHC: Immunohistochemistry; GS: Gleason Score; H&E: Hematoxylin/Eosin; HPF: High Field Power; H2O2: Hydrogen Peroxide; DAB: 3, 3’-Diaminobenzidine Tetra Hydrochloride; PBS: Phosphate Buffered Saline; LGPIN: Low-Grade Intraepithelial Neoplasia; HGPIN: High-Grade Intraepithelial Neoplasia; PSA: Prostate Specific Antigen; Pca: Prostate Cancer; PIA: Proliferative Inflammatory Atrophy; TRUS: Transrectal Ultrasound; SD: Standard Deviation; WHO: World Health Organization

Introduction
Prostate Cancer (PCa) is the most common neoplasm among men worldwide and second leading cause of cancer death after the lung cancer [1]. It is also a significant economic burden, associated with impaired quality of life [2]. Despite of significant progress in diagnosis and treatment, the PCa etiology is not completely elucidated. Detected at early stage, PCa is curable and the prompt diagnosis is essential, improving the efficiency of treatment. Therefore, the need of identification of the earliest step in malignant transformation of PCa from its non-malignant precursors and developing of new biomarkers is imperative. Prostate cancer is a step-wise process that develops from its forerunners or precursors. These defined states arise through multiple transformations in normal cell functions [3, 4]. To date, between several proposed forerunners, only the HGPIN appears to be a true precursor of prostate cancer that can develop into invasive cancer. In contrast, LGPIN is not believed to be associated with PCa risk and its presence on the prostate biopsy is not yet reported [4]. Since the relation inflammation-cancer was demonstrated as mechanism, contributing to carcinogenesis, another possible PCa precursor, linked to chronic inflammation-the Proliferative Inflammatory Atrophy (PIA), has been [5]. PIA includes changes predominantly found in the peripheral zone of the prostate [6] caused by many factors, leading to prostatic injury with subsequent PCa development [7, 8]. The atrophic epithelial cells in the merging HGPIN / PCa lesions demonstrate intermediate phenotype with a higher proliferative and low apoptosis rate, expressing specific proteins [9-11]. A critical point during prostate cancer progression is the loss of Basal Cell Membrane (BM), which is a hallmark of malignant progression. This mechanism is very intricate and includes multiple alterations in the cellular homeostasis. Among the alterations described critical point to malignant invasion take place aberrant

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The integrin β6 expression score in various prostatic lesions was scored under high power fields (>20) separately. Thirty ocular measuring fields have been chosen randomly for every section.

Expression of Integrin β6 in various prostatic lesions

The integrin β6 expression score in various prostatic lesions was scored under high power fields (>20) separately. Thirty ocular measuring fields have been chosen randomly for every section.

Statistical Analysis

Statistical analyses were performed with SPSS version 21 for Windows. The final scores (M) of αv, β6 and αvβ6 expression and their associations with the age, PSA, Gleason score and pathologic stage were analyzed with Mann-Whitney test. The comparison of αv, β6 and αvβ6 integrins expression was assessed using Kruskal-Wallis test. Chi-square test was performed to analyse the expression of Integrin β6 in various prostatic lesions and p < 0.05 was considered statistically significant.

Results

Demographic and clinical features

Patient characteristics are reported in Table 1. Mean age was 65 years (range: 52-81) and PSA level was 8.8 ng/ ml (range: 2.3-20). Significances were calculated compared among age, PSA, Gleason score and pathologic stage in expression final scores (M) of different integrins (αv, β6, αvβ6). For the statistical analysis in
our sample size tumours were categorized as low (Gleason score ≤ 7; n= 28), and high grade (Gleason score > 7; n= 20).

Various Expression of Integrin αν, β6 and ανβ6 in different prostatic lesions

We assessed the expression of Integrin αν, β6 and ανβ6 in the normal-appearing acini (n= 22), atrophy (n= 48), LGPIN (n = 29), HGPIN (n = 42) and PCa (n = 45). All samples contained atrophy component. The expression of these integrins is shown in Table 2. Scattered staining was found in macrophages. None of these Integrins were expressed in myofibrous stromal cells.

Increased integrin αν expression in high PCa grade and PCa bone metastasis

Integrin αν was negative or weakly stained in the normal-appearing acini (Mean ± SD, 0.2 ± 0.7); increased immunostaining was seen in the atrophy (1.0 ± 1.3), LGPIN (1.1 ± 1.3), and HGPIN (1.9±1.4), and in PCa (1.7 ± 1.7) as well. In the PCa samples, the expression of Integrin αν in relation to different Gleason grades was analyzed (Table 3). Integrin αν levels were increased in higher Gleason grade areas. In the PCa bone metastasis samples, Integrin αν showed highly intensive cytoplasm immunostaining (Mean ± SD, 0 ± 1.8), which was significantly higher than in any other GS lesions (Figure 1; Table 3, 4).

Integrin β6 expression in the prostatic atrophy and merging lesions

Compared to Integrin αν, integrin β6 was expressed in a lesser extent in the PCa tissues, especially in those with high Gleason grade (0.4 ± 0.8 in Gleason 4 area and 0 in Gleason 5) and metastasis cancer (0.6 ± 1.4), respectively. In contrast, its expression appeared stronger in atrophy (3.0 ± 1.8), LGPIN (4.5 ± 1.9), HGPIN (5.2 ± 2.2) and in the merging-lesions (Figure 2, Table 3). We next analysed the Integrin β6 expression in the atrophy-merging lesions (Table 4). Five to ten high power field (x20) per case were reviewed to assess the expression in the pure atrophic

Table 1: Demographic and clinical characteristics of the cohort

<table>
<thead>
<tr>
<th>Variables</th>
<th>αν</th>
<th>β6</th>
<th>ανβ6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>&lt; 65</td>
<td>2.3 ± 1.9</td>
<td>0.033</td>
</tr>
<tr>
<td></td>
<td>≥ 65</td>
<td>1.1 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>PSA</td>
<td>&lt; 8.8</td>
<td>1.8 ± 1.4</td>
<td>0.881</td>
</tr>
<tr>
<td></td>
<td>≥ 8.8</td>
<td>1.9 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>Gleason score</td>
<td>≤ 6</td>
<td>1.8 ± 2.0</td>
<td>0.554</td>
</tr>
<tr>
<td></td>
<td>≥ 7</td>
<td>1.5 ± 1.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥ 8</td>
<td>2.6 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>Pathologic stage</td>
<td>T2</td>
<td>2.0 ± 1.9</td>
<td>0.339</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>1.4 ± 1.4</td>
<td></td>
</tr>
</tbody>
</table>

SD: Standard Deviation; PSA: Prostate-Specific Antigen; *Mann-Whitney test

Figure 1: Integrin αν expression in human prostate cancer. (A) PCa, Gleason grade 4 (B) PCa bone metastasis

Table 2: Comparison of αν, β6 and ανβ6 antibodies expression in various prostatic lesions

<table>
<thead>
<tr>
<th>Prostate lesions</th>
<th>αν</th>
<th>β6</th>
<th>ανβ6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>22</td>
<td>0.2 ± 0.7</td>
<td>12 ± 1.2</td>
</tr>
<tr>
<td>Atrophy</td>
<td>48</td>
<td>1.0 ± 1.3</td>
<td>3.0 ± 1.8</td>
</tr>
<tr>
<td>Low-PIN</td>
<td>29</td>
<td>1.1 ± 1.3</td>
<td>29 ± 1.5</td>
</tr>
<tr>
<td>High-PIN</td>
<td>42</td>
<td>1.9 ± 1.4</td>
<td>52 ± 2.2</td>
</tr>
<tr>
<td>PCa</td>
<td>45</td>
<td>1.7 ± 1.7</td>
<td>15 ± 1.9</td>
</tr>
</tbody>
</table>

pb-value < 0.001 < 0.001 < 0.001

Table 3: Comparison of the integrins expression in Gleason 3, 4, 5 grades and bone metastasis lesions

<table>
<thead>
<tr>
<th>PCa</th>
<th>αν</th>
<th>β6</th>
<th>ανβ6</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG-3</td>
<td>36</td>
<td>1.3 ± 1.5</td>
<td>23 ± 2.2</td>
</tr>
<tr>
<td>GG-4</td>
<td>28</td>
<td>2.4 ± 2.0</td>
<td>0.4 ± 0.8</td>
</tr>
<tr>
<td>GG-5</td>
<td>2</td>
<td>4.5 ± 2.1</td>
<td>0 ± 2.0</td>
</tr>
<tr>
<td>Bone Metastasis</td>
<td>11</td>
<td>0.0 ± 1.8</td>
<td>0.6 ± 1.4</td>
</tr>
</tbody>
</table>

pb-value < 0.001 < 0.001 < 0.001

Integrin αν demonstrates increased reaction in PCa, especially in the higher Gleason areas. Integrin αν is overexpressed in all 11 bone metastasis samples, which is essentially higher than in any other GS cancers. SD: Standard Deviation; PCa: Prostate Cancer; GG: Gleason Grade; *Kruskal-Wallis test

and merging-LGPIN, -HGPIN and -PCA lesions. The Integrin β6 expression in the atrophic epithelial and merging- atypical cells was also scored. The atrophic component in atrophy-LGPIN and -HGPIN merging lesions showed positive integrin β6 staining 12.2% (11/90) in LGPIN and 4.8% (2/41) in HGPIN, respectively. Conversely, the atypical cells in these entities demonstrated positive immunostaining up to 88.9% (80/90) in LGPIN and 97.6% (40/41) in HGPIN (p < 0.001). However, in all 6 observed atrophy-PCa merging areas, the malignant elements showed complete negative signalling for integrin β6.

**Integrin αvβ6 showed high expression in prostatic atrophy**

We assessed the integrin αvβ6 expression in different prostatic lesions (Figure 3, Table 2). The highest expression levels of αvβ6 were found in the atrophic changes (Mean ± SD; 3.2 ± 2.7), compared to normal (0.2 ± 0.7), LGPIN (1.2 ± 1.9), HGPIN (1.3 ± 1.5), and PCA (0.6 ± 1.1) (p < 0.001). Further αvβ6 analysis in the primary PCa and bone metastasis samples demonstrated its highest score in the bone specimens (5.1 ± 3.1), opposite to lower scores in the primary PCa (p < 0.001).

**Discussion**

The integrins, including αv, β6, and αvβ6, play a vital role in the cross-talk between the cell and extracellular matrix, enhancing the growth, migration, invasion and metastasis of cancer cells [18,19], their role in the normal prostate, as well as the PCa development has been demonstrated in many studies. Studies have shown that the normal prostate expresses integrins, such as αvβ1 and β4. Among the β subunits, β1c and β1a are expressed in normal prostatic epithelium.

A critical point in prostate cancer progression is the loss of basal cell membrane, which is strongly associated with changes in the integrin levels and their abnormal expression is a one of the characteristic features of the prostatic carcinoma. In contrast to normal prostate, the expression of integrins in PCa demonstrates some specific features. For example, higher Gleason score was correlated with low or negative expression of subunit α3. Similarly, the expression of subunit α6 decreases with the increase of the histologic grade of PCa [12]. α2, α4, α5

**Table 4: Expression of Integrin β6 in atrophy, PIN and merging-lesions**

<table>
<thead>
<tr>
<th>Prostatic lesions</th>
<th>Atrophic epithelium</th>
<th>Atypical component</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrophy</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Atrophy merging with LGPIN</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Atrophy merging with HGPIN</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Atrophy merging with PCA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Integrin β6 is weakly expressed in the PCa tissues, especially in those with high Gleason grade, and metastasis cancer. However, its expression is much higher in the atrophy, LGPIN, HGPIN, and merging lesions. LGPIN: Low-Grade Prostatic Intraepithelial Neoplasia; HGPIN: High-Grade Prostatic Intraepithelial Neoplasia; PCA: prostate cancer and αv-subunits are down-regulated in prostatic carcinoma and some subunits such αvβ6 are specifically expressed in PCa but not in normal tissue [12,17,18]. β1c that is normally expressed in normal prostate, has been found also in the PCa. In addition, studies have shown that expression of β1A can promote cell proliferation [12-19]. Up regulation of β3 and β6 subunits was described in PCa, as well as the specific expression of αvβ3 and αvβ6 in PCa, compared to normal prostate, where they are not detected [12]. As unique expression feature was shown also the α2 subunit, which is up regulated in lymph nodes metastasis, compared with primary lesions, where it is not

Here, we assessed the expression of αv, β6 and αvβ6 in different prostatic tissues as well as in the bone metastasis samples.


**Figure 2:** Integrin β6 expression various prostatic lesions. Strong cytoplasm immunostaining in high grade PIN (A and B) and the HGPIN components in HGPIN-merging lesion (C and D). Cytoplasm immunostaining in low grade PIN (E and F) and the LGPIN-merging lesions (G and H). To note, PCa cells show negative/weak positive immunostaining (I and J).

**Figure 3:** Integrin αvβ6 expression in prostate atrophic lesions. Integrin αvβ6 show cytoplasm staining. To note, prostate cancer shows negative Immunostaining.
Integrin αv

In our study we stated an increased trend of the integrin αv expression from PIA to HGPIN, with significant peak in the bone metastases samples; its relation to Gleason grade also was shown. Our results are consistent with previous studies, focused on the αv role in promotion and development of PCa bone metastasis [16-19]. αv integrins also play an important role in developmental angiogenesis. Before dissemination, cancer cells become motile and detach from the primary tumor, which is accompanied by αv elevation in the primary tumor and overexpression in bone metastases [19-23]. In a study performed with primary PCa and lymph node metastasis samples, the integrin αv was abnormal in the primary neoplasms, whereas in the lymph node metastases its levels were significantly reduced. The global down regulation of αv was demonstrated as a sign of advanced disease and poor prognostic factor [20-24]. Similarly, the unregulated integrin αv expression was found in higher grade tumors with lymph node metastases [24-26], demonstrating its various expression in different types of neoplasms.

PIA and LGPIN as potential PCa precursors

PCa develops from lesions, preceding its development by many years. There is no consensus on possible precursors and presently only HGPIN is considered as a PCa driver. There are several criteria to consider a prostatic lesion as premalignant [4-28]. PIA is suggested as another precursor, contributing to PCa [6-30], which is frequently found in the peripheral zone, adjacent to HGPIN and cancer areas [25,26]. Genetic pathway in-between PIA, HGPIN, and PCa also has been demonstrated [6-31]. Unlike HGPIN, LGPIN is believed do not contribute to PCa and is no longer reported as finding [29-34]. However, the likely outcome of isolated LGPIN lesions in prostate biopsies remains unclear. In a study performed with younger men, PIN changes were seen even in their 20’s and 30’s. Most foci were LGPIN, with increasing frequency of HGPIN with advancing age [32-34]. Another analysis demonstrated 30% risk of PCa on repeat sextant biopsy in the LGPIN cohort [31]. Albeit HGPIN is the only known currently accepted PCa precursor, in this study there was at least a comparable percentage of patients who had cancer on repeat biopsies [31]. This finding is highlighted as worrying since LGPIN is not considered as a risk factor. The authors showed the predictive value for PCa to be equal with both, isolated LGPIN and HGPIN at biopsies and LGPIN was demonstrated as a risk factor for PCa progression. The conclusion made is that chemoprevention can be beneficial not only in HGPIN, but in LGPIN.

Integrin β6 subunit

In the normal prostate β6 levels are absent or expressed in a lesser extent, but increased in cases of BPH [24] and significantly elevated in PCa [32-37]. In our study the β6 levels in the normal prostate were insignificant, and then they gradually increased in PIA and reached a peak in both, LGPIN and HGPIN, and declined in PCa. The β6 expression in atrophic and merging lesions indicates the plausible mechanism of PCa transformation from its precursors. Key finding is the significant expression of β6 in LGPIN, suggesting its malignant potential in PCa development. Limited data shows the possible outcome of LGPIN on prostate biopsy and our results cautiously suggest the likely malignant potential of LGPIN, suggesting the need for closer follow-up in patients with LGPIN at the initial biopsy. PIN lesions don’t affect PSA levels and are detectable only by prostate biopsies, frequently associated with a risk of infections, requiring hospitalization and antibiotic treatment. However, studies have demonstrated a link between PIN grades and the level of disruption of the basal cell layer, accompanied by expression of functional molecules, specific to every stage of tumor progression [5]. Therefore, our data can contribute to development of new markers, useful for the selection of patients at risk that may need closer follow-up and re-biopsy with those, in whom this intervention can be safely skipped.

Integrin αvβ6

Was strongly expressed in our atrophic and bone metastasis samples αvβ6 was shown to be implicated in progression of number of cancers, promoting the invasion and metastasis [43]. However, in the PCa and its precursors αvβ6 has been investigated in a lesser extent. Studies showed the αvβ6 expression in basal cells in the HGPIN, normal tissue adjacent to tumors [38], as well as in the areas of inflammation and proliferative inflammatory atrophy [37]. Once the expression of this integrin was associated with inflammation and its β6 subunit is increased in PCa, the αvβ6 has also been implicated in controlling PCa growth in conjunction with the androgen receptor. The elevation of αvβ6 indicates advanced disease, associated with poor prognosis. Similarly to β6, αvβ6 levels are inducible in PCa and bone metastasis and absent or insignificant in the normal prostatic tissues [39-42]. The association between αvβ6 and the inflammation, as well as a link between chronic inflammation and PCa initiation has been shown [35, 36]. TGF-β up regulates expression of the integrins and there is an extensive cross-talk between TGF-β and the large repertoire of these integrins, including αvβ6 [29-36]. TGF also regulates EMT, where cells acquire the invasive phenotype that is required for metastasis [37-40]. EMT is associated with increased αv expression. In cancer, alongside with the other integrins, αvβ6 has been shown to be important in allowing EMT to take place in the metastatic process and αvβ6 is also involved in this process [41-43]. The role of integrin αvβ6 in the relation inflammation-cancer was demonstrated in the POET study [44], where αvβ6 is expressed in both, inflammation and cancer and not in normal tissue, thus suggesting not only the link between inflammation and PCa [43]. Furthermore, besides the αvβ6 expression, induced by the inflammation in this study, this integrin has been proposed to support metastasis by activating TGF-β1 that is associated with metastasis [42]. In our study αvβ6 is mainly expressed in atrophic lesions, supporting the hypothesis of the role of atrophy as precursor lesion but also in the bone metastasis samples.

Studies about the role of integrins in PCa developing, led to conduction of several ongoing clinical trials, evaluating the efficacy of integrin antagonists as prostate cancer therapeutics [12]. Thus, the role of Cilengitide, a cyclic Arg-Gly-Asp peptide that inhibits αvβ3 and αvβ5, has been demonstrated. CNTO 95, as well as MEDI-522, monodonal antibodies against αv integrin
also have been suggested as promising therapeutic agents [12]. Therefore, our findings can also have far-reaching implications for the management and chemoprevention of PCa, inviting future studies to explain the malignant transformation. The early identification of PCa risk patients will help to tailor the best therapeutic approach [45] and designing novel therapeutic approaches based on inhibiting integrin expression may be a promising strategy.

We consider as a major limitation of this study the relatively small sample size and the clinical significance of data requires further investigations to validate recent results.

Conclusions

In summary, the present data identify the integrins αν, β6 and ανβ6 action in prostate cancer precursors. Prostate cancer is a chronic, multi-step disease that arises from its premalignant precursors and to date; HGPIN is the only accepted PCa precursor. The expression of integrins αν, β6 and ανβ6 reveals new evidence of malignant PCa initiation and transformation. In addition, the elevation of β6-subunit in the LGPIN may suggest more malignant potential than previously believed. Our findings can give new directions in the personalized therapy of PCa and may contribute to the therapeutic optimization of existing trials for PCa. This is a novel study for the integrins role in PCa precursors and molecular pathways by which integrins contribute to PCa initiation and progression need to be further elucidated.

Disclosure

This study was supported by ALF – Västerbotten, Lion Cancer Fond, and Umeå University. The study received institutional review board approval by the Ethical Board at the Norrland's University Hospital, Umeå, Sweden under the protocol Dnr 2010/366-31M

Acknowledgments

This investigation was supported by grants from the Cancer Research Foundation in Northern Sweden) Cancerforskningsfonden Norrland/Lions Cancerforskningsfond- Umeå, Sweden, LP 15-2096, 2015.

We also would like to thank the Departments of Pathology at the Norrland's University Hospital, Umeå, Sweden and Sahlgrenska University Hospital, Gothenburg, Sweden

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