Potential Utility of GATA3 and P63 Immunohistochemistry in Differentiating Urothelial Carcinoma from Prostate Adenocarcinoma

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Background: Prostatic adenocarcinoma and urothelial carcinoma of the urinary bladder are common cancers in men. High grade forms of these tumors may present ambiguous morphologic features that make distinguishing invasive high-grade urothelial carcinoma (UC) from high-grade prostatic adenocarcinoma difficult. The distinction between the two tumors has significant staging and therapeutic implications. Hence, an accurate diagnosis is essential.

Aim: To compare the expression of GATA3 and p63 in urothelial carcinomas and adenocarcinomas of prostate.

Materials and Methods: Immunohistochemistry (IHC) for GATA binding protein 3 (GATA3) and p63 was performed on a tissue sections of 30 cases of invasive high-grade UC and of 30 high-grade prostatic adenocarcinomas.

Results: GATA3 and p63 were positive in 25 (83.3%) and in 27 (90%) of high-grade UC, respectively. All GATA3 positive staining was diffuse, from which 22 (88%) cases demonstrated moderate-strong staining, and 3 (12%) cases demonstrated weak staining. Of the 5 cases that failed to express GATA3, 3 were positive for p63, while 2 cases were negative for both 2 markers. None of the prostatic adenocarcinomas expressed either GATA3 or p63.

Conclusion: GATA3 and p63 IHC are sensitive markers for UC and can be used to distinguish prostatic adenocarcinomas from urothelial carcinomas in difficult cases. They are also highly specific in excluding high-grade prostatic carcinoma.

Keywords: GATA3, p63, immunohistochemistry, prostatic adenocarcinoma, urothelial carcinoma

Introduction

Bladder cancer is the seventh most common cancer worldwide, with an estimated 260,000 new cases occurring each year in men. Approximately 98% of malignant tumors arising in the urinary bladder is of epithelial origin and of these, 90% are urothelial carcinomas (1).

Prostatic carcinoma is the most frequent malignancy and the second cause of death among men (2).
In Egypt, bladder cancer and prostatic carcinoma constitute 30.3% and 48% of all cancers, respectively (3,4).

Distinguishing poorly differentiated prostate cancer arising in the urinary bladder neck from high grade urothelial carcinoma with prostatic extension frequently can be a challenging task for surgical pathologist owing to overlapping morphologic characteristics and similar clinical manifestations in the two entities. This distinction has significant therapeutic and staging implications. The determination of the tumor stage, for its prognostication would require correct diagnosis as extension of bladder cancer into the prostate as well as prostate cancer into the bladder would signify pT4 disease (5,6).

GATA3 (GATA binding protein 3 to DNA sequence [A/T]GATA[A/G]) is 1 of 6 members of a zinc finger transcription factor family, and it plays an important role in promoting and directing cell proliferation, development, and differentiation in many tissues and cell types, including luminal glandular epithelial cells of the mammary gland, T lymphocytes thymocytes, adipose tissue, kidney, sympathetic nervous system, and hair follicles of the skin. (7,8,9). Recent studies have identified GATA3 immunohistochemistry (IHC) as a sensitive marker for ductal breast carcinoma, and transitional proliferations of the gynecological tract (10,11,12, 13).

P63 is a nuclear protein encoded by a gene on chromosome 3q27--29 with homology to p53 (a tumor suppressor gene), it has shown to regulate growth and development in epithelium of the skin, cervix, breast, and urogenital tract. Specific isotopes are expressed in basal cells of prostate, myoepithelial cells of breast, urothelium, and squamous epithelium. p63 has similar applications to those of high-molecular-weight cytokeratins in the diagnosis of prostatic adenocarcinoma, but with the advantages that p63 stains a subset of 34betaE12 negative basal cells; is less susceptible to the staining variability of 34betaE12 (particularly in transurethral resection of prostate (TURP) specimens with cautery artifact); and is easier to interpret because of its strong nuclear staining intensity and low background (1).

The current study evaluates the sensitivity of GATA3 and p63 IHC in distinguishing high-grade UC from high grade prostate adenocarcinoma.

**Material and methods:**

A retrospective, controlled study comprised formalin-fixed, paraffin-embedded blocks of tissues from male patients with urothelial carcinoma and prostate adenocarcinoma were retrieved from the archives of the Departments of
Pathology, National Cancer Institute (NCI), Cairo University and Benha Faculty of Medicine, Benha University. All patients had undergone surgical removal of the lesions between the years 2002 and 2013. Hematoxylin and eosin (H and E) stained slides were reviewed in all cases to verify the histologic findings. Representative formalin--fixed, paraffin--embedded tissue blocks were selected for GATA3 and p63 immunohistochemical staining. Cases studied containing 30 invasive high-grade UCs and 30 high-grade prostatic adenocarcinomas [Gleason score 8 (n=4), Gleason score 9 (n=12), Gleason score 10 (n=14)].

Of the high-grade prostatic adenocarcinomas, Gleason pattern 4 was represented by poorly formed glands. Of the UCs examined, none showed squamous differentiation. IHC was performed on 4 μm sections taken from formalin fixed paraffin embedded tissue blocks.

Sections were prepared on positive charged slides and kept overnight at 50°C. Deparaffinization and rehybridation were carried out, followed by antigen retrieval in the microwave using citrate buffer at 9.0 pH. Then peroxidase block and protein block were carried out. Incubation of the slides with the primary antibodies at 4°C overnight was carried out using anti-P63 to the human P63 antigen (a mouse monoclonal antibody, clone 4A4, 7 ml ready to use, Cat.No.CMA442R, Cell Marque, USA) and with mouse monoclonal anti-GATA3 antibodies (catalogue No. GATA3 [HG3-31];sc-268; Santa Cruz Biotech, Santa Cruz, CA) (1:25 dilution, EDTA antigen retrieval, and 45-minute incubation for primary antibody). This was followed by the secondary biotin-conjugated antibody for 1 h and finally peroxidase-conjugated streptavidin for another hour. Diaminobenzidine tetrachloride (freshly prepared) was added for 25 min, and then counter staining was performed in Harris hematoxylin, followed by dehydration, clearing, and mounting.

The positive control for P63 was the basal cells of the benign prostatic glands present in the slides examined (positive internal control). And that for GATA3 is normal breast tissue.

**Staining Interpretation:**

Nuclear staining for GATA3 was graded as weak, moderate, or strong, and focal (20% of cells) or non-focal (>20% of cells) (6).

For P63, The staining results of the tumor were expressed as the product of the brown nuclear staining intensity and the percentage of positive cells. The whole section was scanned at low power in order to assess the general
level of intensity throughout. The average intensity of the staining corresponds to the presence of negative, weak, moderate, and strong staining. The percentage of tumor cells was scored as follows: 0 = no reactivity; 1 = less than 10% of cancer cell nuclei positive; 2 = 10-25% positive; 3 = 25-50% positive, 4 = 50-75% positive; and 5 = >75% of tumor cell nuclei positive (1).

**Statistical analysis**

Statistical analysis was performed using the SPSS (statistical program for social science) software version 15.0 (SPSS Inc., Chicago, Illinois, USA).

**Results:**

The age of the all patients ranged from 40 years to 81 years with a median age of 62 years.
The age of the patients of urothelial carcinoma ranged from 40 to 78 years with a median age of 59.5 years.
The age of the patients of prostatic adenocarcinomas ranged from 46 to 81 years with a median age of 69 years.

GATA3 Immunohistochemical results for UC cases are shown in Table (1). GATA3 was positive in 25 (83.3%) cases of invasive high-grade UCs (Fig.1). Of the GATA3 positive cases, 22 (88%) demonstrated moderate-strong staining and 3 (12%) had weak staining. All positive cases showed non-focal diffuse staining. None of the 30 high-grade prostatic adenocarcinomas were GATA3 positive, however weak GATA3 staining was present in occasional basal cells of benign prostate glands, in a few benign atrophic glands, and in glands with urothelial metaplasia (Fig 2). GATA3 positivity was observed in 83.3% of urothelial carcinomas and none of prostatic adenocarcinomas with a P value of 0.001 (Table 2).

Out of the 30 urothelial carcinomas 27 stained with p63 (90%) (Fig 4 and 5). Overall, 60% of cases showed strong nuclear staining in >50% of tumor cells (Table3).

None of the 30 prostatic adenocarcinomas expressed p63. Basal cells of benign glands of prostate were taken as positive internal controls (Fig 3). p63 positivity was observed in 90% of urothelial carcinomas and none of prostatic adenocarcinomas with a P value of 0.001 (Table 4).

Of the 5 cases that failed to express GATA3, 3 cases were positive for p63, while 2 cases were negative for both 2 markers.
Table (1): GATA3 staining in urothelial carcinomas.

<table>
<thead>
<tr>
<th>GATA3 staining</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>5</td>
<td>16.67%</td>
</tr>
<tr>
<td>Weak</td>
<td>3</td>
<td>10%</td>
</tr>
<tr>
<td>Moderate</td>
<td>10</td>
<td>33.33%</td>
</tr>
<tr>
<td>Strong</td>
<td>12</td>
<td>40%</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100%</td>
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</table>

Table (2): Comparison between GATA3 expression in urothelial and prostatic carcinoma.

<table>
<thead>
<tr>
<th>GATA3 staining</th>
<th>Urothelial carcinoma</th>
<th>Prostatic adenocarcinoma</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>25 (83.33%)</td>
<td>0</td>
<td>0.001</td>
</tr>
<tr>
<td>Negative</td>
<td>5 (16.67%)</td>
<td>30 (100%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>30</td>
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</table>

Table (3): p63 Staining in urothelial carcinomas.

<table>
<thead>
<tr>
<th>P63 staining</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>3</td>
<td>10%</td>
</tr>
<tr>
<td>Positive&lt;10%</td>
<td>2</td>
<td>6.67%</td>
</tr>
<tr>
<td>Positive10-25%</td>
<td>2</td>
<td>6.67%</td>
</tr>
<tr>
<td>Positive 25-50%</td>
<td>5</td>
<td>16.67%</td>
</tr>
<tr>
<td>Positive 50-75%</td>
<td>8</td>
<td>26.67%</td>
</tr>
<tr>
<td>Positive &gt;75%</td>
<td>10</td>
<td>33.3%</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100%</td>
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</tbody>
</table>

Table (4): Comparison between P63 expression in urothelial and prostatic carcinoma.

<table>
<thead>
<tr>
<th>P63 staining</th>
<th>Urothelial carcinoma</th>
<th>Prostatic adenocarcinoma</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>27 (90%)</td>
<td>0</td>
<td>0.001</td>
</tr>
<tr>
<td>Negative</td>
<td>3 (10%)</td>
<td>30 (100%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>30</td>
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</table>
Figure (1): High grade urothelial carcinoma showing strong GATA3 nuclear staining of the malignant cells (streptavidin/biotin DAB, ×400).

Figure (2): High grade prostatic adenocarcinoma, Gleason's score 8 with entrapped benign gland showing absence of GATA3 staining in prostatic adenocarcinoma and weak positivity of basal cells of entrapped benign gland (streptavidin/biotin DAB, ×400)
Figure (3): Prostatic adenocarcinoma, Gleason's score 8 with normal benign glands showing absence of P63 staining in prostatic adenocarcinoma and positivity of basal cells of benign gland (streptavidin/biotin DAB, ×400).

Figure (4): Moderate nuclear p63 staining in <50% of malignant cells of high grade urothelial carcinoma (streptavidin/biotin DAB, x200)
Figure (5): High grade urothelial carcinoma showing strong nuclear p63 staining in >50% of malignant cells (streptavidin/biotin DAB x200).

Discussion

Distinction of poorly differentiated prostatic cancer from a high grade urothelial carcinoma in transurethral resection specimens is a relatively common problem, with the difficulty compounded by the relatively common occurrence of glandular differentiation in the latter and the frequently raised serum prostate-specific antigen in cases of urothelial carcinoma extending into the prostate gland. The presence of coexisting low grade papillary urothelial tumor or urothelial carcinoma in situ would favor high grade urothelial carcinoma, but the possibility of synchronous prostate cancer would have to be excluded as both tumor types occur in elderly men (1).

Invasive high-grade UC can be difficult to differentiate from high-grade prostatic adenocarcinoma as the morphology of high-grade UC is not always specific. High-grade prostate adenocarcinoma is composed of atypical but uniform cells with prominent nucleoli typically growing in sheets, cords, and/or as individual cells. UC is composed of atypical pleomorphic cells that tend to form nests. Cribriform architecture is characteristic of prostate adenocarcinoma and not a feature of UC. Confounding factors include gland-like lumina and true glandular differentiation in UC mimicking cribriform architecture. A minority of high-grade prostatic adenocarcinomas may have nest formation similar to UC (14).
Because of this morphologic overlap of high-grade UC with high-grade prostatic adenocarcinoma IHC may have a greater role. Although several markers have been analyzed to determine the prostatic or urothelial origin of poorly differentiated tumors, no marker to date has been sufficiently sensitive and/or specific. Prostate specific antigen and prostate-specific acid phosphatase traditionally have been used to confirm a prostatic origin; however, they are not expressed uniformly in poorly differentiated prostatic carcinoma and might be negative in up to 27% and 19% of cases, respectively (15,16). alpha--methyl--acyl--coenzyme A racemase (AMACR), may be useful in the diagnosis of prostatic carcinoma (17). Therefore, the lack of immunoreactivity to prostate specific markers in a poorly differentiated tumor within the prostate or bladder, does not exclude the diagnosis of a poorly differentiated prostatic adenocarcinoma.

Cytokeratin (CK) 7, CK20, and high-molecular-weight cytokeratin (HMWCK) and THROMBO have been studied as potential urothelial markers (10,18). Although they are useful in certain situations, they are not entirely specific for urothelial carcinoma. The reported sensitivities of THROMBO, and HMWCK for UC are 61%–91% and 90%, respectively (16,19). These stains are variably reliable when excluding a high-grade prostatic adenocarcinoma. Recently, uroplakin, a membranous glycoprotein, has emerged as a highly specific marker of urothelial carcinoma. However it is only moderately sensitive and is expressed only in 50% to 60% of urothelial carcinomas, typically in well--differentiated tumors (1).

When differentiating UC and high-grade prostate adenocarcinoma, HMWCK is the least specific marker as it is expressed in a small percentage of cells in almost 10% of high-grade prostatic adenocarcinomas (16,20).

In the current study, GATA3 and p63 IHC were used for differentiating high grade urothelial carcinoma and prostatic adenocarcinoma.

Eighty three percent of the cases of UC examined were GATA3 positive. All positive cases demonstrated non-focal staining and most showed moderate to strong staining intensity. None of the 30 high-grade prostatic adenocarcinomas positively stained with GATA3, so it is highly specific when differentiating high-grade UC from high-grade prostatic adenocarcinoma.

In accordance to these results Chang et al., (6) observed that GATA3 were positive in 80% of the cases of UC examined and none of the prostate adenocarcinomas cases were GATA3 positive, however. Higgins et al., (10) observed that 67% of UC were GATA3 positive with most exhibiting intense
non-focal staining and none of the prostate adenocarcinomas cases were GATA3 positive.

The difference accounted for the higher rate of GATA3 positivity in UC observed in the current study (83.3% versus 67%) may be due to difference in the antibody used.

p63, a basal cell marker for which usefulness in the diagnosis of prostatic carcinoma is supported by its lack of staining in atypical glands (21, 22).

Like the results of Kunju et al. (14) and Din et al. (1), we found p63 positivity in 90% of high grade urothelial carcinomas. None of the prostatic carcinoma cases were p63 positive. So, p63 could be a fairly sensitive and highly specific marker of urothelial carcinoma.

The present study validates the results of Kaufmann et al., (23) who found p63 positivity in 87% of urothelial carcinomas and in 2% of prostatic adenocarcinomas. Wu and Kunju (24) reported a case of prostatic adenocarcinoma that showed diffuse aberrant p63 expression. These carcinomas had unusual morphologic features such as atrophic cytoplasm and basaloid morphology.

This study also serves to corroborate the results of Langner et al., (25) in which p63 were positive in (92%) of high grade urothelial carcinomas. The negative staining of p63 staining in prostatic adenocarcinoma in the present study supports the results of Din et al., (1) and Signoretti et al., (5) that found p63 negativity in 97% of cases and 3% of cases showed p63 positivity in <1% of tumor cells. Also Srinivasan and Parwani (26) did not observe nuclear p63 positivity in any of their PCs. Thus, p63 appears to be a useful marker in distinguishing between UC and PC due to its high specificity for UC.

We conclude that GATA3 IHC is a sensitive marker for UC. GATA3 is also highly specific in excluding high-grade prostate adenocarcinoma. All of the prostatic adenocarcinomas are p63 negative and most of the urothelial carcinomas are p63 positive. So GATA3 and p63 are reliable markers of urothelial differentiation and can be used together with other prostatic markers in morphologically difficult cases when the differential diagnosis is between poorly differentiated adenocarcinoma of prostate and high grade urothelial carcinoma of urinary bladder.

References:

1-Din NU, Qureshi A, Mansoor S. Utility of p63 immunohistochemical stain in differentiating urothelial carcinomas from adenocarcinomas of


أهمية الاستدلال بالاندلاع: GATA3, P63 و سرطان البروستاتا باستخدام الصيغات المناعية الهستوكيميائية

أحلام على عبد المصطفى، عادل زكي الصعيدى

يعتبر سرطانات المثانة والبروستاتا من أكثر أنواع السرطان انتشارًا في الرجال ومن الامور التي تساعب تشخيص هذه الأنواع من السرطانات مكان حدوثهما واحتمال انتشار كل منها إلى العضو الآخر لقرب البروستاتا الشديد من المثانة البولية ومن الأمور التي تزيد الامر صعبة في تشخيص المكان الأولي لكل منها إذا كان السرطان من الدرجة العالية في عدم التمايز حيث كان هذا البحث لدراسة مدى قدرة الاستدلال لكل من الدلالات GATA3, P63 وتشخيص كلا من سرطان المثانة البولية وسرطان البروستاتا وخصوصا تلك عالية درجة عدم التمايز.

تمت الدراسة على 60 عينة، 30 عينة لسرطان البروستاتا و30 عينة لسرطان المثانة وكل الحالات المختارة كانت من الدرجة العالية في عدم التمايز وتم الحصول على هذه العينات من قسم الباثولوجى بكلية الطب جامعه بنها، وقد صبخت تلك العينات بصبغة الهيماتوكسيلين- ايوسين لتأكيد التشخيص وتحديد نوع ودرجة الورم، وكذلك باستخدام كيمياء المناعة على الأنسجة لاختبار وكذا تقييم وجود بروتينات P63 في سرطان البروستاتا والمثانة البولية، وفحص وتحليل النتائج وجد أن 25 حالة (38.3%) من حالات سرطان المثانة كانت موجبة بالنسبة للبروتين P63؛ F3، ونسبة للدالة GATA3، F3، فقد ظهر في 27 حالة (90%) من حالات سرطان المثانة وعلى النقيض لم تظهر حالات سرطان المثانة ايجابية لكل من GATA3, P63.

وعلى ذلك فإن التعديل عن كل من P63, GATA3، F3 باستخدام كيمياء المناعة GATA3, P63 يعتبر من الدلائل الحساسة في تشخيص التفريق بين سرطان المثانة وسرطان البروستاتا عالية درجة عدم التمايز فوجودهما بصورة إيجابية في هذه النوعية من السرطانات يستبعد تشخيص سرطان البروستاتا ويوافق تشخيص سرطان المثانة.