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Diagnostic Value of GATA3 and Uroplakin 3 in Differentiating Urothelial Carcinoma from Prostatic and Colorectal Carcinoma

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Abstract

BACKGROUND: Prostate involvement by urothelial carcinoma (UC) can occur from direct invasion. Involvement of the urinary bladder by prostate adenocarcinoma (PAC) may similarly occur, representing a common diagnostic problem especially in high-grade tumors. Furthermore, colorectal carcinoma (CRC) invades the urinary bladder which represents another challenging task for the pathologists, a problem not only in the urinary bladder but also in lymph node metastasis. Uroplakin 3 has been used for differentiation but unfortunately, its sensitivity is not so high especially in high-grade cases. Recently, GATA3 was recognized as being involved in development of urothelium.

AIM: Few studies elaborated its expression in high-grade UC; therefore, this study aimed at evaluating GATA3 and comparing it to uroplakin 3 expression in differentiating the three tumors from each other.

MATERIALS AND METHODS: Sixty paraffin blocks collected and distributed as 20 cases of invasive UC, 20 cases of PAC, and 20 cases of CRC, all high grade, to be studied immunohistochemically against GATA3 and uroplakin 3.

RESULTS: GATA3 expression was positive in 80% of UC compared to none of the cases of PAC or CRC. The sensitivity of GATA3 in diagnosing UC was 80% whereas the specificity was 100%. Uroplakin 3 was positive in 50% of UC compared to none of the cases of PAC or CRC. The sensitivity of uroplakin 3 in diagnosing UC was 50% whereas the specificity was 100%. GATA3 and uroplakin 3 showed statistically significant inverse relation with lymphovascular and muscle invasion.

CONCLUSION: GATA3 is more sensitive than uroplakin 3 for UC that can be effectively used to exclude PAC and CRC. Moreover, GATA3 and uroplakin 3 expression significantly decreases with lymphovascular invasion as well as muscle proper invasion which emphasizes their good prognostic role.

Introduction

Prostate involvement by urothelial carcinoma (UC) can occur either from direct invasion into prostatic stroma or from intraductal extension of the UC even without stromal invasion of the prostate [1]. Involvement of the urinary bladder by prostate adenocarcinoma (PAC) may also occur by direct extension [2]. A common diagnostic problem in this situation is to differentiate between both tumors especially high grade, when both tumors can demonstrate poorly differentiated morphologic features with loss of characteristic histologic features, such as cellular atypia, hyperchromasia, pleomorphism, prominent nucleoli, and bizarre infiltrating cells [3].

In fact, this particular problem of distinguishing between these two tumors is crucial because of the different treatment strategies for both tumors. Advanced UC is usually managed with chemotherapy, while advanced PAC is often managed with anti-androgen hormone therapy [1]. Moreover, focal expression of prostate-specific antigen (PSA) can occasionally be detected in some invasive UC which urges the need for exploring other immunohistochemical markers to be used with PSA as a panel for more accurate diagnosis [4].

Another problem faced is that colorectal carcinomas (CRCs) sometimes invade adjacent structures. Among these adjacent organs, tumors of the sigmoid colon or upper rectum most commonly invade the urinary bladder which represents another challenging task for the pathologists especially when it is difficult to determine whether the tumor is just adhering to the urinary bladder or actually invading it, so patients undergo *en bloc* resection to ensure a clear surgical margin [5].

The difficulty in accurately separating UC, PAC, and CRC from each other does not only lie in the urinary bladder site but also in lymph node metastasis [3] as PAC, UC, and CRC in inferior part of rectum metastasize to the same internal iliac lymph nodes [6].

Uroplakin 3 has been the marker of choice in differentiating these tumors from each other, but unfortunately, its sensitivity is not high with a considerable number of false negative cases; moreover, its sensitivity decreases with high-grade tumors [7]. GATA3 is a

promising urothelial marker used in diagnostic surgical pathology practice. However, the expression status of GATA3 in high-grade UC and its prognostic significance has not been thoroughly investigated. It is only recently being recognized as being involved in luminal differentiation of breast epithelium, development of collecting/urothelium and trophoblastic differentiation. There have been few studies done to study its expression in high-grade UC [8].

Since a high-grade tumor present in the urinary bladder showing loss of characteristic histologic features may carry the possibility of being a primary UC, metastatic PAC, and even metastatic CRC, same applies to a metastatic internal iliac lymph node, and since the therapeutic approach of the three tumors is completely different making the differentiation between them that is of utmost importance; therefore, this study aimed at using immunohistochemical technique to evaluate the use of GATA3 in differentiating the three tumors from each other and whether it is better than uroplakin 3 in this purpose.

Materials and Methods

Sixty paraffin blocks were collected and distributed as 20 cases of invasive high-grade UC, 20 cases of prostatic adenocarcinoma Gleason score ≥8, and 20 cases of high-grade colorectal adenocarcinoma. Specimens were collected in the form of incisional biopsy (colonoscopic biopsy, TUR, and core biopsy). Serial sections were cut at 3–4 micron thickness from each paraffin block for histopathological evaluation, as well as immunohistochemical staining.

Immunohistochemical staining

For the assessment of immunohistochemical expression, representative slides from each paraffin block were stained using antibodies (Anti-GATA-3 Antibody (HG3-31), sc-268 is a mouse monoclonal immunoglobulin (Ig) G_1 (kappa light chain) provided at 200 μg/ml, SANTA CRUZ BIOTECHNOLOGY, OR., USA) and (Anti-uroplakin 3 antibody (C-6) is a mouse monoclonal IgG at 200 μg/ml SANTA CRUZ BIOTECHNOLOGY, OR., USA).

Immunohistochemical evaluation

Slides were examined at 400× magnification.

GATA3

Only nuclear GATA3 immunostaining was considered positive. The percentage of positively stained tumor cells was scored as: Score 0: No tumor cells stained, Score 1: 1–10%, Score 2: 11–50%, Score 3: 51–80%, and Score 4: 81–100%.

The staining intensity of positive tumor cells was also scored as 1: Weak, 2: Moderate, and 3: Strong. Finally, an immunoreactivity score was calculated for GATA3 expression by multiplying the number representing the percentage of immunoreactive cells by the number representing their staining intensity then the cases were categorized into:

- Negative: Scores 0–1
- Weak: Scores 2–4
- Moderate: Scores 5–8
- Strong: Scores 9–12 [8].

Uroplakin 3

Cytoplasmic and/or membranous staining was assessed simultaneously and reported as:

- 0 (negative staining)
- 1+ (focal and weakly positive)
- 2+ (moderately positive)
- 3+ (strong and diffusely positive) [9].

Statistical analysis

Data were statistically described in terms of means and standard deviation or medians and ranges as well as percentages. Comparison between the groups was done by Chi-square and Wilcoxon signed rank tests. All statistical calculations were done using computer program Statistical Package for the Social Science (SPSS); SPSS Inc., Chicago, IL, USA) version 25. $P < 0.05$ was considered statistically significant. All steps are approved by the Ethical Committee.

Results

The present study was conducted on 60 cases equally distributed between invasive high-grade UC, high-grade colorectal adenocarcinoma, and prostatic adenocarcinoma Gleason score ≥8 (20 cases each, 33.3%). Immunohistochemical results of GATA3 expression revealed positive expression in 80% (16 cases) of UC compared to none of the cases of prostatic adenocarcinoma or CRC. Further study of GATA3 expression in UC revealed weak expression in 4 cases (20%) as shown in Figure 1, moderate expression in 4 cases (20%) as shown in Figure 2, and strong expression in 8 cases (40%) as shown in Figure 3. The sensitivity of GATA3 in diagnosing UC was 80% whereas the specificity was 100%.

When studying GATA3 expression in comparison to the different clinicopathologic parameters as shown

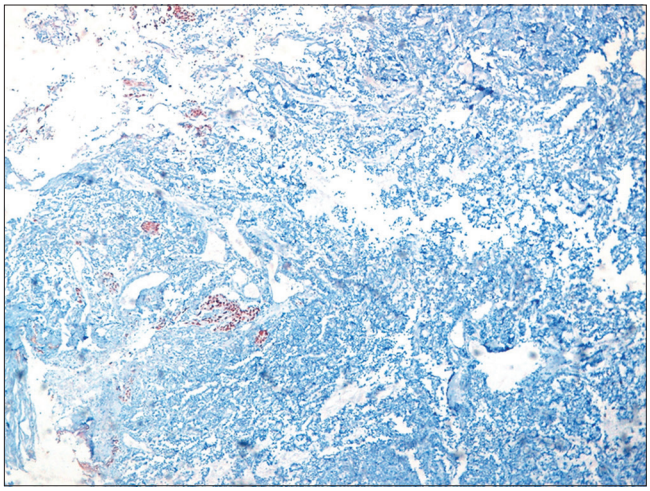

Figure 1: A case of high-grade urothelial carcinoma showing positive moderate intensity expression against GATA III in less than 10% of tumor cells representing weak expression (×100 original power)

in Table 1, we observed a highly statistically significant relation between GATA3 expression and lymphovascular invasion (LVI) as all cases showing moderate and strong expression were of the tumors lacking LVI ($p < 0.001$).

As for muscle invasion, another statistically significant relation was observed between GATA3 expression and muscle invasion as 75% of cases showing negative and weak expression showed muscle invasion in contrast to 25% only of cases showing strong and moderate expression ($p < 0.005$). As for age and sex, in UC cases, the patients' age ranged from 40 up to 85 years with mean 60.4. Male: female ratio was 1.24. No statistically significant correlation was noted between GATA3 expression and these two parameters (0.179 and 0.19, respectively).

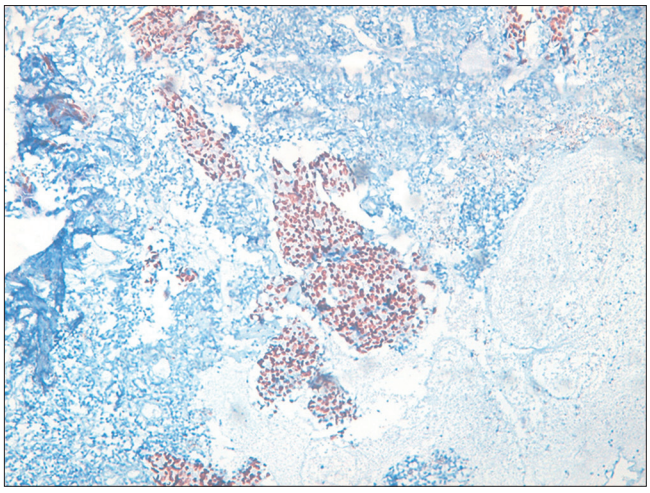

Figure 2: A case of high-grade urothelial carcinoma showing positive moderate intensity expression against GATA III in about 60% of tumor cells representing moderate expression (×200 original power)

Concerning uroplakin 3, positive expression was found in 50% (10 cases) of UC compared to none of the cases of prostatic adenocarcinoma or CRC. All cases showed weak +1 intensity as shown in Figure 4. The sensitivity of uroplakin 3 in diagnosing UC was only 50% whereas the specificity was also 100%. When studying uroplakin 3 expression in comparison

Table 1: Correlation between GATA3 expression and the available clinicopathological parameters in UC

Clinicopathological parameters	Negative n = 4 (%)	Weak n = 4 (%)	Moderate n = 4 (%)	Strong n = 8 (%)	p-value
Age					
>60	75	75	75	100	0.179
≤60	25	25	25	0	
Sex					
Male	25	25	50	87.5	0.19
Female	75	75	50	12.5	
LVI					
No	0	0	100	100	<0.001
Yes	100	100	0	0	
Muscle proper invasion					
No	25	25	75	75	<0.005
Yes	75	75	25	25	

LVI: Lymphovascular invasion, UC: Urothelial carcinoma.

to the different clinicopathologic parameters, we observed a highly statistically significant relation between uroplakin 3 expression and LVI as all positively stained cases were of the tumors lacking LVI ($p < 0.001$). Concerning muscle invasion, another statistically significant relation was observed between uroplakin 3 expression and muscle invasion as all positively stained cases showed no muscle invasion ($p < 0.005$). No statistically significant correlation was noted between uroplakin 3 expression and either patients' age or sex.

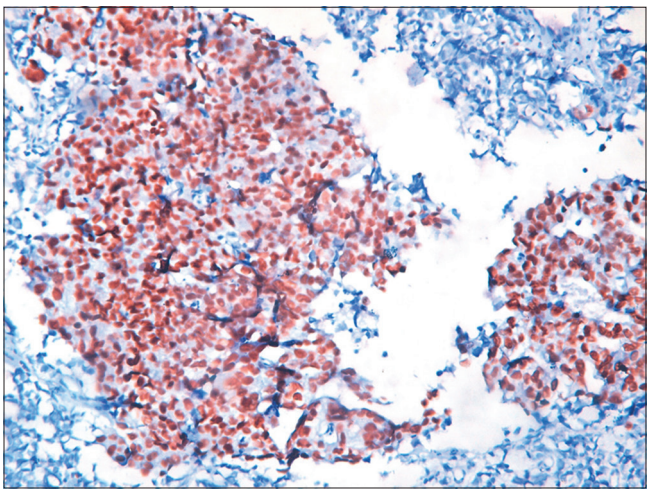

Figure 3: A case of high-grade urothelial carcinoma showing positive strong intensity expression against GATA III in more than 80% of tumor cells representing strong expression (×400 original power)

Discussion

Although the pathologic differentiation between UC, PAC, and CRC using hematoxylin and eosin staining is not difficult in most of cases, some cases may be challenging because the histologic appearance of high grade of these tumors can be very similar to each other. Since the findings with routine hematoxylin and eosin staining may overlap, immunohistochemical staining may help solve this diagnostic dilemma, particularly in metastatic sites or in carcinomas involving bladder and prostate or bladder and rectosigmoid [2].

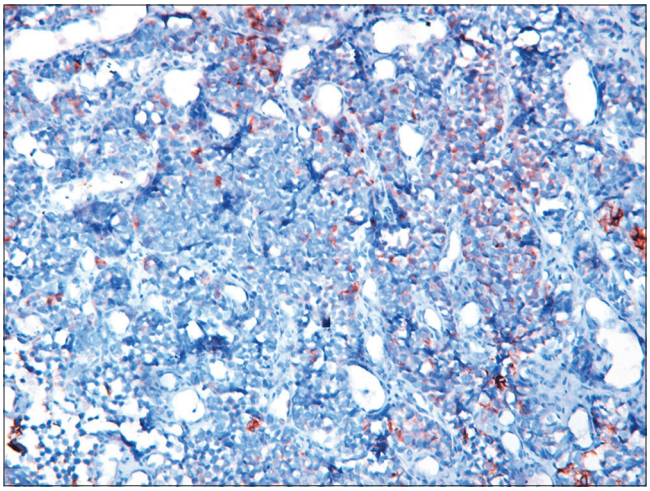

Figure 4: A case of high-grade urothelial carcinoma showing focal weak positive expression against UROPLAKIN III (×200 original power)

In the present study, we collected the paraffin blocks of 20 cases of each of invasive high-grade UC, high-grade CRC, and PAC Gleason score ≥8 to evaluate the use of GATA3 and uroplakin 3 expression in the differentiation between them. We found positive GATA3 expression in 80% of high-grade UC in contrast to none of PAC or CRC cases. Further study of GATA3 expression in UC revealed prevalence of strong expression (8/16), which emphasizes the useful role of GATA3 in diagnosing UC with 80% sensitivity and 100% specificity.

Similarly, Chang *et al*. (2012) [10] reported that none of their studied 38 high-grade PAC was positive for GATA3. Another study done by Oh *et al*. (2016) [2] revealed GATA3 expression in 117/138 cases of UC compared to none of the 111 cases of PAC. Like our study Oh *et al*. (2016) [2] stated that GATA3 was 84.8% sensitive in diagnosing UC. Moreover, the study done by Agarwal *et al*. (2019) [8] observed GATA3 expression in 77% of UC cases but in none of PAC cases assuring our results, they also stated a statistically significant relation with the tumor grade, but this was not applicable in our study as we only included high grades. As regards CRC, close to our results was those done by Miettinen *et al*. (2014) [11] who observed GATA3 expression in >90% of UC in contrast to only <10% of CRC cases.

It seems that our results go with our previous colleagues' results but we thought that it was very important to extensively study GATA3 expression in high-grade tumors in particular as all previous researches included different grades of the tumor.

When studying GATA3 expression in comparison to the different clinicopathologic parameters, we observed a highly statistically significant inverse relation between GATA3 expression and LVI on one hand and muscle proper invasion on the other hand highlighting its good prognostic factor. Likewise, Agarwal *et al*. (2019) [8] concluded that a statistically significant inverse relation with muscle invasion as weaker or negative expression was seen in high-grade muscle invasive UC as compared to low-grade and non-invasive UC. Moreover, Wang *et al*. (2019) [12] proclaimed in their study that loss of GATA3 expression was statistically associated with poor clinicopathologic parameters, such as LVI, neural invasion, and lymph node metastasis. Correspondingly, Satochi *et al*. (2017) [13] who studied GATA3 expression in the upper urinary tract UC asserted its expression in 69.3% of their studied cases which statistically inversely correlated with muscle invasion. GATA3 good prognostic value of course is attributed to its being a member of a zinc finger transcription factor family that plays a key role in promoting and directing cell proliferation, differentiation, and development especially in breast epithelium and urothelium (Agarwal *et al*., 2019) [8].

As for uroplakin 3, we found positive expression in 50% of high-grade UC in contrast to none of PAC or CRC cases, all were of weak expression which is obviously less than cases stained positive for Gata3 with only 50% sensitivity but also 100% specificity. The moderate sensitivity of uroplakin 3 in diagnosing UC was also concluded by Matsumoto *et al*. (2008) [14] who studied 92 cases of UC of different grades and observed loss of uroplakin 3 expression with high-grade, muscle-invasive cancer. The study done by Kaufmann *et al*. (2000) [9] was not different as it revealed that uroplakin 3 positivity was seen in only 60% of their studied UC cases. Moderate sensitivity with high specificity was also declared by Naji *et al*. (2019) [15] who stated that uroplakin 3 expression sensitivity in their study was 44.3%, with higher percentage in low-grade versus high-grade tumors but without statistical significance.

The moderate sensitivity of uroplakin 3 in our study may be attributed to its being one of the urothelial plaques which are essential for differentiation of urothelium specifically superficial umbrella cell and subsequently its expression is known to be down regulated with high grades [15]. Therefore, negative or weak uroplakin 3 expression is associated with aggressive high-grade UC and since all our studied cases were high grade, we were not surprised to observe this moderate expression. This observation would appear to reduce the value of uroplakin 3 as a reliable marker for high-grade UC when there is a need to differentiate them from tumors metastatic to the bladder. This actually goes with our assumption that we need another marker for differentiating UC from other mimickers in high-grade cases.

Conclusion

From this study, it can be concluded that GATA3 is a reliable marker, with high sensitivity and specificity, for identifying UC which can be of great

value to exclude other malignancies that could show some similarities such as prostatic adenocarcinoma and CRC especially at metastatic site. Moreover, GATA3 expression significantly decreases with LVI as well as muscle proper invasion which also emphasizes its good prognostic role in UC. This makes GATA3 more superior than uroplakin 3 in differentiating UC in high-grade cases. Other researchers are welcome to further study GATA3 prognostic role to see if we can rely on this marker in determining LVI and muscle proper invasion in small endoscopic samples of urinary bladder, provided that they study larger sample size.

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