Received: 9 August 2018 | Accepted: 9 October 2018
DOI: 10.1002/pros.23734

ORIGINAL ARTICLE

WILEY The Prostate

Urotensin II receptor expression in prostate cancer patients: A new possible marker

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Background: Urotensin II receptor has been poorly studied in prostate cancer. To evaluate the expression of urotensin II receptor (UII-R) in patients undergoing radical prostatectomy.

Methods: Overall, we identified 140 patients treated with retropubic radical prostatectomy (RP) in one center. UII-R was evaluated in prostate biopsies with immunohistochemical staining, resulting in a granular cytoplasmic positivity, through automated system using the kit Urotensin II Receptor Detection System provided by Pharmabullet srl. Immunostained slides were independently and blindly evaluated by ten uro-pathologists. To evaluate UTII-R expression three different parameters were considered: localization, granules dimensions and intensity of expression. A score from 0 to 3 was applied to each parameter to obtain a score from 0 to 9. Each parameter and the total score were evaluated as predictors of high grade disease on surgical pathology and of advanced stage disease. Accuracy of total score for the prediction of upgrading and upstaging was analyzed using receiver operator characteristics curve and decision curve analysis (DCA).

Results: On radical prostatectomy 92/140 (66%) presented high grade disease on surgical pathology. Patients with high grade disease presented an apical distribution of the receptor, larger granules and a more intense expression when compared to patients with low grade disease. A well they presented a higher total score. Subscores and total scores were found to be predictors of upgrading and upstaging. On ROC analysis total score presented an AUC of 0.72 and 0.70, respectively, for the prediction of upgrading and upstaging. On DCA total score showed a clinical benefit in the prediction of adverse pathological outcomes.

Conclusion: Urotensin II receptor is a potential marker of adverse pathological outcomes. Further studies should confirm our data and evaluate its role as a prognostic marker.

KEYWORDS
accuracy, upgrading, upstaging, urotensin

1 | INTRODUCTION

Prostate cancer (PCa) shows an extremely heterogeneous clinical course, ranging from indolent and organ-confined to aggressive and metastatic disease. An accurate assessment of the tumor characteristics is crucial so that the appropriate treatment options can be considered.[1]

Stratification into risk groups based on preoperative prostate-specific antigen (PSA) levels, clinical stage (cT), and biopsy Gleason score (bGS) was initially introduced by D'Amico et al. Unfortunately, current methods of clinically assessing PCa grade and stage are not accurate, and upgrading or upstaging is still a major clinical problem in one out of three patients.[2–4]

In the recent years, several studies are searching novel prostate cancer specific biomarkers that can identify patients with clinically significant prostate cancer, to improve the prediction of tumor aggressiveness as an adjunct to Gleason score. Urotensin II and Urotensin-II receptors are important molecular factors that regulate vasoconstriction and all the diseases that are linked to abnormalities in blood pressure regulation (ie, hypertension, kidney diseases, cirrhosis etc.). Recently, Urotensin II and its receptor have also been involved in metabolic syndrome, diabetes, and schizophrenia.[5–8] Recent strong findings suggest that Urotensin II and its receptor are involved in the onset and development of different epithelial cancers. Indeed, it was reported that cell growth, motility and invasion in human breast, bladder, prostate, colorectal and glioblastoma cancer cells were regulated by Urotensin II and Urotensin-II receptor axis. However, only few studies have evaluated the role of Urotensin II receptor in prostate cancer.[7,9] More specifically only one small clinical study is available on the subject. Considering the low level of evidence available on the subject, aim of our study was to describe the expression of UIIR in patients with prostate cancer undergoing radical prostatectomy.

2 | MATERIALS AND METHODS

2.1 | Patient selection

After obtaining the institutional review board approval, a retrospective analysis of a prospectively maintained database of consecutive men who underwent radical prostatectomy (RP) between January 2016 and January 2018 in one center (Villa Tiberia, Rome Italy) was carried out. All patients signed an informed consent, all the procedures were approved by the local ethics committee and the study was carried out according to the principles of the Declaration of Helsinki. We included patients with a histologically confirmed prostate biopsy diagnosis of PCa scheduled for surgery within 3 months. Patients who received neo-adjuvant hormonal therapies or presented incomplete data were excluded from the study.

Prostate biopsies were conducted through a transrectal-ultrasound (TRUS) guided approach. During the sampling, 12 cores were taken from all the patients, regardless the size of the gland. Surgery was performed by experienced surgeons, utilizing open technique. Extended lymph node dissection was carried out according to European Association of Urology guidelines.[10]

2.2 | Data collected

Evaluated variables included age, body mass index (BMI), calculated as weight in kilograms divided by height in meters, squared (kg/m^2), prebiopsy Prostate Specific Antigen (PSA), clinical and pathological stage, needle biopsy and pathologic prognostic grade group (PGG). Biopsy and RP Gleason scores were assigned according to the new 2014 ISUP described by Epstein et al.[11] All the histological specimens were reviewed by a single expert pathologist. High grade cancer is defined as: PGG ≥3.

2.3 | Urotensin receptor evaluation

The immunohistochemical staining, resulting in a granular cytoplasmic positivity, was performed through automated system using the kit Urotensin II Receptor Detection System provided by Pharmabullet srl. Immunostained slides were independently and blindly evaluated by ten uro-pathologists. In order to evaluate UTII-R expression three different parameters were considered: localization, granules dimensions and intensity of expression. A new score was used to classify the UTII-R expression intensity. UTII-R color intensity was defined as mild, medium, high; intracytoplasmic location of UTII-R in neoplastic cells as basal, medium\apical, apical\apocrine and dimension of UTII-R granules as small, medium, large. For each parameter a partial score was attributed (range 0-3). A total score was then calculated summing the three sub-scores. The score was developed after the consensus of the ten pathologists involved (Figure 1).

2.4 | Statistical analysis

Statistical analysis was performed using the SPSS 24.0 software. Evaluation of data distribution showed a non-normal distribution of the study data set. Differences between groups of patients were

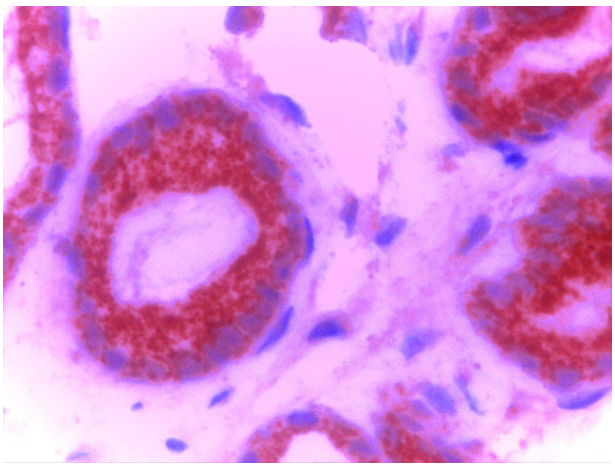

FIGURE 1 UTII-R corpuscles gain larger size in neoplastic cells

evaluated with the Kruskal-Wallis one-way analysis for quantitative variables and the chi-square test for categorical variables. Using multiple logistic regression with the enter method, the statistical significant variables as assessed in the univariate analysis were entered and investigated as predictors of high grade disease, upgrading and upstaging. Single sub-scores were not included in the multivariate analysis to avoid the risk of multicollinearity. Upgrading was defined as an increase from ≤2 to ≥3 and from 3 to ≥4 in PGG system. Upstaging was defined as pT ≥3a. Receiver operator characteristic curves (ROC) were produced to evaluate the area under the curve (AUC) and the diagnostic performance of the total score. Decision curves were generated to evaluate the net benefit of the total score. A *P*-value of 5% was considered as the threshold for significance. Data are presented as median (range), mean ± standard deviation, and median with interquartile range (IQR).

3 | RESULTS

Overall 140 patients were enrolled. General characteristics of the enrolled population are listed in Table 1.

On prostate biopsies 75/140 (54%) presented high grade disease (PGG ≥ 3). Patients with high grade disease were older, with higher PSA levels and presented higher stage of disease. In terms of UTII-R expression when comparing low versus high grade disease, no statistically significant differences were noted in terms of localization, dimensions of the granules and intensity of expression (Figure 2).

On radical prostatectomy 92/140 (66%) presented high grade disease on surgical pathology. These patients were older, with higher PSA and worst pathological stage. When looking at the biopsy histological findings in terms of UTII-R expression patients with high grade disease presented an apical distribution of the receptor, larger granules and a more intense expression when compared to patients with low grade disease. Apical distribution of the receptor, larger granules, a more intense expression and total score were predictors of high grade disease on univariate analysis. Total score, in age and PSA adjusted multivariate analysis, was found to be a predictor of high grade disease on radical prostatectomy (Table 2).

Clinically significant upgrading was recorded in 45/140 (32%) patients. Patients with clinically significant upgrading were older, presented higher PSA levels and higher stage of disease on surgical pathology. In terms of UTII-R expression on prostate biopsies these patients presented a more intense expression with larger granules and an apical distribution. On univariate analysis apical localization, big granules and high intensity were risk factors for upgrading. Total score considering these three factors was significantly higher in patients with upgrading when compared to patients without significant upgrading. On multivariate analysis, adjusted for age and PSA, total score was an independent predictor of upgrading (Table 2). On ROC analysis area under the curve for the prediction of upgrading was 0.73 (95%CI: 0.64-0.82; $P = 0.001$) for total score. As well, UTII-R score presented a net benefit in the prediction of upgrading in the 15-60% range of probabilities (Figure 3).

TABLE 1 General characteristics of the cohort

	Median (Interquartile range)
Age	66 (61/68)
PSA	5.2 (4.3/7.8)
Biopsy prognostic grade group	
1	20/140 (14%)
2	45/140 (32%)
3	63/140 (45%)
4	8/140 (6%)
5	4/140 (3%)
Pathological prognostic grade group	
1	17/140 (12%)
2	31/140 (22%)
3	64/140 (46%)
4	24/140 (17%)
5	4/140 (3%)
pT ≥3a	72/140 (51%)
pN	
x	70 (50%)
0	63 (40%)
1	7 (10%)
Localization	
0: Absent	2/140 (1%)
1: Basal	41/140 (29%)
2: Medium-Apical	54/140 (39%)
3 Apical	43/140 (31%)
Granules dimension	
0 Absent	6/140 (4%)
1: Small	61/140 (44%)
2: Medium	57/140 (41%)
3: Large	16/140 (11%)
Intensity	
0: Absent	10/140 (7%)
1: Mild	36/140 (26%)
2: Medium	56/140 (40%)
3: Strong	38/140 (27%)

Upstaging was recorded in 72/140 (53%) patients. Patients with upstaging were older and with higher PSA levels. In terms of UTII-R expression on prostate biopsies these patients presented a more intense expression with larger granules and an apical distribution. On univariate analysis apical localization, big granules and high intensity were risk factors for upgrading. Total score considering these three factors was significantly higher in patients with upstaging when compared to patients without significant upstaging. On multivariate analysis adjusted for age and PSA total score was an independent predictor of upstaging (Table 2). On ROC analysis area under the curve for total score was 0.70 (95%CI: 0.61-0.79; $P = 0.001$). As well, UTII-R

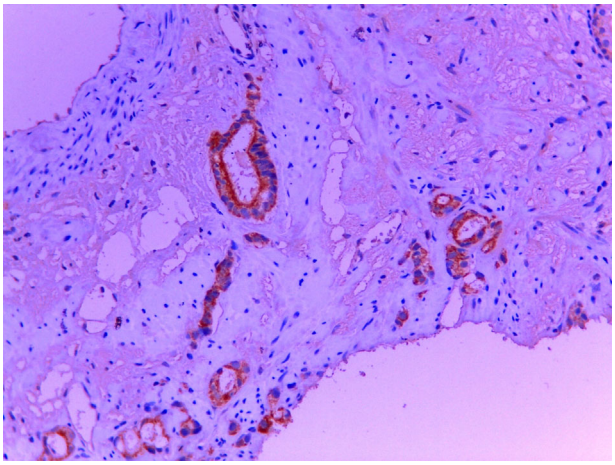

FIGURE 2 UTII-R corpuscles in neoplastic cells are placed in more apical position

score presented a net benefit in the prediction of upgrading in the 30-65% range of probabilities (Figure 3).

4 | DISCUSSION

UTII-R expression is here evaluated in a cohort of patients undergoing radical prostatectomy. Given the lack of data on UTII-R and prostate cancer we decided to describe localization, size of the granules and intensity of expression in biopsy specimens and compare patients with low and high-grade disease. When looking only at biopsy specimens no statistically significant differences were noted in terms of UTII-R expression when comparing low versus high grade disease. Conversely when looking at RP specimens patients with high grade disease presented a more apical distribution, in larger granules and with a higher intensity of expression. We therefore looked at upgrading and upstaging and found that these patients presented a more apical distribution, in larger granules and with a higher intensity of expression. Moreover, we confirmed that the total score including the three factors was a predictor of upgrading and of upstaging and demonstrated a clinical benefit on decision curve analysis. This is the first attempt in the literature to accurately describe the distribution of the UTII-R in detail using the new 2014 prognostic grading group classification. Standing to our results UTII-R pattern of expression is associated with adverse pathological outcomes.

UTII-R has been extensively investigated in different tumors as a possible prognostic marker. UII and its receptor are widely expressed and UII represents a potent endogenous vasoconstrictor with physiological mechanisms similar to other potent mediators.[5,8] In literature, there are several evidences that associate the altered expression of UTR in many cell lines and tumor tissues. Wei and colleagues recently showed how high UTII-R expression is associated with worse prognostic outcomes in patients with hepatocellular

TABLE 2 Univariate and multivariate analysis for the risk of high-grade disease, upgrading and upstaging

	Univariate	*P*	Multivariate	*P*
Odds Ratio and 95%CI for the risk of high grade disease				
Age	1.04 (1.01-1.06)	0.030	1.02 (0.98-1.04)	0.335
PSA	1.06 (1.02-1.10)	0.001	1.05 (1.00-1.07)	0.035
Apical distribution	3.01 (1.14-6.38)	0.004		
Large granules	2.54 (1.24-5.23)	0.011		
Intensity	6.09 (2.79-13.28)	0.001		
Total score	1.52 (1.25-1.86)	0.001	1.65 (1.19-1.899	0.001
Odds ratio and 95%CI for the risk of Upgrading (PGG ≥3)				
Age	0.99 (0.941.05)	0.816	0.99 (0.96-1.03)	0.656
PSA	1.09 (1.03-1.22)	0.001	1.05 (1.35-2.13)	0.001
Apical distribution	5.19 (1.89-14.36)	0.002		
Large granules	4.35 (1.97-9.64)	0.001		
Intensity	2.56 (1.57-4.19)	0.001		
Total score	1.70 (1.36-2.13)	0.001	1.60 (1.35-2.13)	0.001
Odds ratio and 95%CI for the risk of Upstaging (pT ≥3a)				
Age	1.00 (0.95-1.06)	0.754	1.01 (0.94-1.07)	0.454
PSA	1.10 (1.02-1.18)	0.025	1.08 (1.04-1.13)	0.015
Apical distribution	4.26 (1.92-9.43)	0.001		
Large granules	2.69 (1.34-5.40)	0.005		
Intensity	6.82 (2.97-15.7)	0.001		
Total score	1.45 (1.21-1.76)	0.001	1.35 (1.17-1.55)	0.001

CI, confidence Interval; PGG, prognostic grade group.

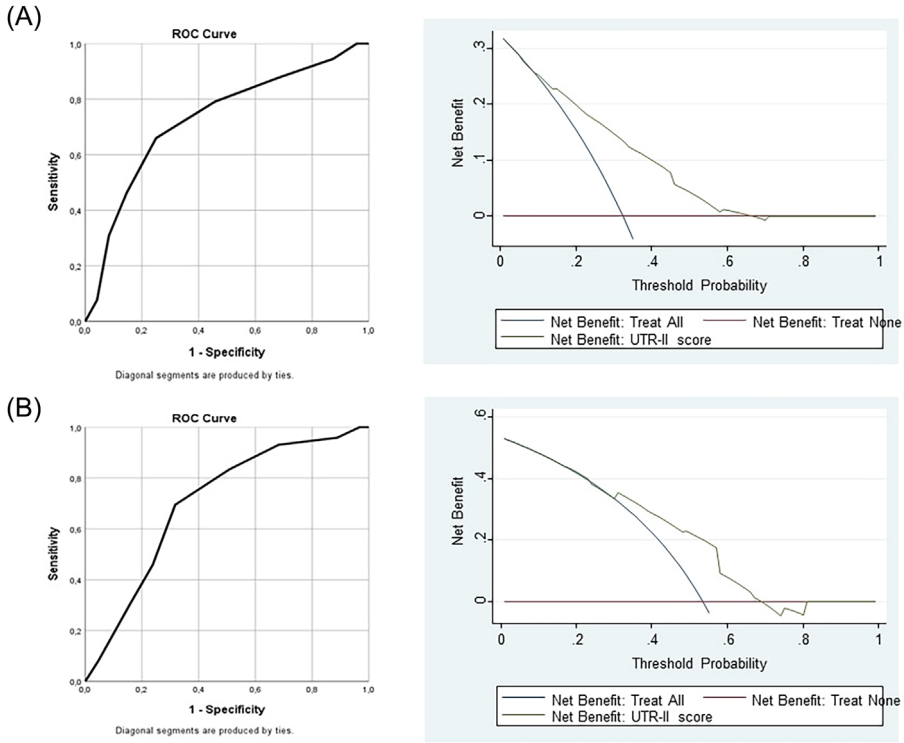

FIGURE 3 ROC curve analysis and decision curve analysis for upgrading (A) and upstaging (B)

carcinoma. More specifically both recurrence free (RR = 3.72; $P = 0.001$) and overall survival (RR = 4.58; $P = 0.038$) were significantly worse in patients with high UTII-R expression.[12] Conversely, in bladder cancer the evaluation of UTR expression can discriminate between NMIBC at high and low risk of relapse. Moreover, our data suggest that UTR is involved in the regulation of motility, invasion and proliferation of bladder cancer cells. High UTR expression is an independent prognostic factor of good prognosis for NMIBC regulating motility and invasion of bladder cancer cells.[6] In adrenal tumors Morimoto et al showed that both UII and UT-R were expressed in the adrenal tumors and attached non-neoplastic adrenal tissues and suggests possible roles of UII and UT-R in tumor growth and/or secretory activities of these tumors. Standing to the available evidence the role of UTII-R may be different depending on the tumor considered.

Evidence of UTII-R expression and prostate cancer is scarce.[7,9] When looking at the literature, the data on UTII-R and prostate cancer comes from one group. In their first paper they evaluated the correlation between UTR expression and prognosis of human prostate adenocarcinoma and the involvement of this receptor in the regulation of biological properties on both in vivo and in vitro models.[13] According to their results. UTR was always expressed at low intensity in hyperplastic tissues and at high intensity in well-differentiated carcinomas (Gleason 2-3). Moreover, they evaluated the effects of an antagonist of UTR, urantide on migration and invasion of LNCaP cells. Urantide induced a dose-dependent decrease of motility and invasion of LNCaP cells whose characteristic ameboid movement seems to be advantageous for their malignancy. They concluded that UTR can be considered a prognostic marker in human prostate adenocarcinoma patients. Latter on they evaluated UTR expression and upgrading in patients undergoing radical prostatectomy and found that UTII-R expression on biopsy was associated with Gleason upgrading and pathology upstaging in prostate cancer patients.[9] These data are completely against the present findings; however, it takes always more than one study to prove a hypothesis.

The data presented by the only group that has investigated the association between UTII-R and prostate cancer has several limitations. First of all, data is based on Gleason score classification and defines low grade disease as a Gleason score ≤6. According to the latest EAU guidelines PGG should be used to grade PCa and low grade disease should be defined as PGG ≤ 2.[14] Data from the in vitro and in vivo study is quite solid and seem promising, however, very often laboratory data do not correlate clinically. The clinical study presented has many pitfalls, first of all it is not adequately powered given the small sample size, the analysis is made only on 17 patients which presented a significant upgrading moreover the definition of high grade disease is outdated (Gleason score >6). In our study we were able to retrieve as much as 140 patients and graded them according to the new Epstein PGG.

It is important to underline the methodology behind our work. The primary objective of the study was to describe meticulously the expression of UTII-R in prostate cancer patients, to avoid the risk of upgrading and downgrading we selected only patients undergoing RP. In order to do so we described localization, granules dimension and intensity of UTII-R expression involving ten different uro-pathologists to reach a consensus. No real difference was initially noted when comparing low and high-grade disease defined on prostate biopsy.

However, when analyzing UTII-R expression and RP grading we found that patients with high grade disease present a more apical distribution, with larger granules and with a higher intensity of expression. At this stage we decided to investigate the possible role of UTII-R as a predictor of upgrading and upstaging although we have to acknowledge the study is not powered to prove the hypothesis. Notwithstanding the low numbers, an apical position, larger granules and intensity were all predictors of upgrading and upstaging. To avoid the risk of multicollinearity we created a total score and evaluated the predictive abilities of this score in predicting upgrading and upstaging and obtained good prediction abilities of the model. According to our results, if externally validated the present score could be useful as a predictor of upgrading and upstaging. Moreover, a prospective study is ongoing to evaluate the role of UTII-R as prognostic marker in terms of biochemical free survival and metastatic free survival.

Many studies have assessed clinical risk factors related to the risk of upgrading.[4] Age is associated with the risk of upgrading according to some authors, however the difference is no more than a year between patients with and without upgrading. Therefore, as pointed out by Epstein and De Nunzio, this small difference is probably the result of a large sample size effect and may explain the lack of association in our study and others.[15–17] As well, PSA is considered a risk factor only in very large series and the difference between patients with and without upgrading is no more than 1 ng/mL. Probably the best marker in terms of upgrading is PSA density which has been confirmed an accurate marker of upgrading. In Brassetti, study AUC for PSA density was as high as 0.89 confirming a role for PSA density in predicting upgrading.[18] Unfortunately, prostate volume was not available for all patients and therefore PSA density accuracy could not be calculated in this setting and is to be considered a limitation of our study. However, a prospective study including all predictors of upgrading and upstaging is ongoing, and results will be soon available. In terms of upstaging age, prostate volume and PSA levels are well established and already included in clinical nomograms for the prediction of upstaging.[19]

The present study has several limitations. First of all, this is a single center study, however to limit this possible bias all the specimens were evaluated and scored by ten independent pathologists. Moreover, the sample size is probably not adequate to evaluate upgrading and upstaging however the primary objective of the study was to describe the UTII-R expression and the latter analysis were just secondary outcomes. Lastly, the panorama of UTII-R seems very complex and therefore prospective multicenter studies are needed to clarify the exact role of this receptor in the pathogenesis and progression of PCa. Notwithstanding all these limitations the present study is the first available evidence on UTII-R expression using contemporary grading definitions and opens new insights in the molecular pathways of PCa development and progression.

5 | CONCLUSION

UTII-R expression is linked to PCa grade and stage. An apical expression of the receptor in a form of big granules and with high intensity is closely related to high grade disease and advanced stage. Further prospective studies should clarify the role of UTII-R as a prognostic marker in PCa patients.

ACKNOWLEDGMENT

None

CONFLICT OF INTEREST

The authors declare no conflict of interest

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How to cite this article: Giulianelli R, Nardoni S, Bruzzese D, et al. Urotensin II receptor expression in prostate cancer patients: A new possible marker. *The Prostate*. 2019;79:288–294.
https://doi.org/10.1002/pros.23734