Alpha-dystroglycan staining pattern and mortality in patients undergoing radical prostatectomy with lymph node positive prostate cancer

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ALZUBAIDI AN, NEPPLE KG, VOLLSTEDT A, DAHMOUSH L, MA D, HENRY MD, BROWN JA. Alpha-dystroglycan staining pattern and mortality in patients undergoing radical prostatectomy with lymph node positive prostate cancer. *Can J Urol* 2019;26(6):10054-10060.

Introduction: *Dystroglycan (DG) is a cell surface receptor for extracellular matrix proteins involved in tissue mechanical stability and matrix organization. Initial work has demonstrated that alpha-DG expression is decreased in many types of adenocarcinoma, including prostate, and potentially associated with the development of metastatic disease. However, the consistency between prostate and lymph node alpha-DG staining has not been previously reported. In addition, identification of an immunohistochemical marker associated with prostate cancer grade, stage, need for adjuvant or salvage therapy and mortality would have potential clinical value.*

Materials and methods: *Node positive, margin negative radical prostatectomy specimens at a single institution from 1982 to 2012 were reviewed and identified 35 prostate specimens, including 26 patients with available tissue from both the primary prostatectomy and lymph node specimens. The expression levels of the alpha-DG subunit were analyzed using immunohistochemistry and graded from 0 to 4. Survival was compared in different staining pattern groups.*

Results: *Strength of alpha-DG staining was found to be consistent between prostate and lymph node specimens ($p < 0.004$). The median overall survival was shorter in those without alpha-DG staining in the prostate compared to those with positive staining, but this difference was not statistically significant (13.2 years versus 19.4 years, $p = 0.21$). In addition, negative staining was associated with higher mean PSA, pathologic T stage, Gleason grade and the need for adjuvant or salvage therapy compared to positive group but none reached statistical significance (16.06 ng/mL versus 11.67 ng/mL, $p = 0.79$; 89% versus 68%, $p = 0.38$; 33.3% versus 23.1%, $p = 0.66$; 88.9% versus 76.9%, $p = 0.44$).*

Conclusions: *DG expression by immunohistochemistry staining was consistent between prostate and metastatic lymph node specimens. In a small cohort of prostate cancer patients with margin negative but node positive disease, DG staining was not associated with Gleason grade or with overall mortality.*

Key Words: prostate cancer, dystroglycan, adenocarcinoma, immunohistochemistry, prognosis

Accepted for publication September 2019

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Introduction

Prostate cancer is the second-most common cause of cancer death in men in the United States, accounting for 9% of cancer deaths in 2018.[1] Despite this mortality

risk, the ability to predict aggressive disease and the optimal sequence and timing of therapy remains limited.[2-5] The most important clinical prognostic factors used for prostate cancer are serum PSA ,pathologic stage, and Gleason score.[6,7] These factors, however, are limited in their ability to predict mortality, and extreme-risk groups have not been universally defined.[8] In patients undergoing radical prostatectomy, patients with positive regional lymph nodes have a poor prognosis compared to node negative prostate cancer patients; however, treatment with adjuvant androgen deprivation therapy or radiation therapy remains controversial.[9] Additional prognostic information beyond standard pathologic assessment would be of value in patient counseling and decision making.

Dystroglycan (DG) is a cell surface receptor for extracellular matrix proteins that is involved with the mechanical stability of tissue and matrix organization. DG is composed of two subunits: the glycosylated alpha subunit, which is covalently bonded to the transmembrane beta subunit. It has been shown that alpha-DG expression and glycosylation is decreased in many adenocarcinomas including prostate adenocarcinoma.[10-17] Alpha-DG expression is frequently assessed by staining with the monoclonal antibody IIH6, which detects glycosylation of alpha-DG that is essential to the function of DG and its binding to extracellular matrix proteins.[18-21] Loss of αpha-DG expression in metastatic disease suggests a potential role for DG and other matrix protein organization in the disease process outside of the primary organ site,[11] with the hypothesis that DG is an important physical or biochemical barrier in the maintenance of epithelial cell integrity and the prevention of metastasis. To date, decreased alpha-DG expression has been associated with increased mortality in renal, gastric, and pancreatic adenocarcinomas;[14,17,22] however, its impact on survival in patients with prostate cancer has not been definitively evaluated.

The purpose of our study was to examine the expression of DG in patients who underwent radical prostatectomy and were identified to have lymph node positive prostate cancer. The study hypotheses were that DG expression on immunohistochemistry would be consistent between prostate specimens and lymph node specimens, and that decreased DG expression would be associated with greater architectural distortion and more aggressive disease – thus an elevated preoperative serum PSA, greater pathologic T stage, higher Gleason score, increased use of adjuvant/salvage therapy and mortality.

Materials and methods

Study cohort

Institutional Review Board approval for the study was obtained. An institutional pathology database was examined to identify 74 patients from 1982 to 2012 who underwent radical prostatectomy, either open or robotic, with lymph node positive disease and available pathologic specimens. The impact of local recurrence from a positive surgical margin is a potentially confounding factor when evaluating the outcomes of patients with lymph node positive disease; therefore, the study cohort was limited to patients with negative surgical margins, which created a final study cohort of 35 prostatectomy patients. Twenty-six of 35 prostatectomy specimens had lymphadenectomy specimens available.

Retrospective chart review was performed to identify patient and disease characteristics. Pathological staging was performed according to the TNM classification of the 7th edition of the American Joint Committee on Cancer Staging Manual.[23] Low (Gleason score ≤ 7) grade and high(> 7) grade Gleason score was assigned according to the International Society of Urological Pathology consensus classification.[24]

Dystroglycan immunohistochemistry

Expression of alpha-DG was judged by intensity of immunohistochemical staining. Immunohistochemical analysis was performed with anti-alpha-DG monoclonal antibody IIH6 (1:100, Santa Cruz Biotechnology) on routinely processed, formalin-fixed, paraffin-embedded tissues emptying an avidin-biotin complex immunoperoxidase technique. A total of 7 αm sections were cut from biospecimens and dewaxed then rehydrated in a microwave with 0.3 hydrogen peroxide in methanol for 30 minutes. Slides were then reviewed by a genitourinary pathologist who was blinded to the clinical characteristics (PSA) and outcomes. Prostate specimen alpha-DG scoring was done by a single genitourinary pathologist according to a system whereby 0 is negative and 1 to 4 is positive with higher numbers corresponding to more DG expression on immunohistochemistry. For lymph node metastases, samples were scored as either positive or negative for membranous staining with the antibody. In several cases, the metastatic foci were no longer present in the deeper levels for immunostaining. In patients with multiple positive lymph nodes, only one node with the largest metastatic focus was stained.

Study outcomes

Patients were followed with PSA testing during

regularly scheduled post-prostatectomy clinic visits. Node positive patients were treated with various postoperative regimens including initial surveillance (77.1%) or androgen deprivation therapy (22.9%) with (14.3%) or without (8.6%) radiation therapy in the adjuvant setting (within 12 weeks of surgery). During follow up, 20 additional patients (57.1%) received androgen deprivation therapy with (28.6%) or without (28.6%) radiation therapy. Overall, 28/35 (80%) of our cohort received additional therapy in the adjuvant or salvage setting. The primary outcome was overall survival, which was assessed by chart review and use of an institutional oncology registry. The latter Oncology Registry at the Holden Comprehensive Cancer Center has been in place throughout the study period and annually reviews patients for vital status via a combination of chart review, patient contact, review of death index data, and newspaper obituary review. Overall survival was defined as the time from surgery until date of last follow up or death with censoring of patients who were alive on the date of last follow up. The median and mean follow-up time for our cohort after radical prostatectomy patients was 10.3 years and 9.96 years respectively.

The number of patients managed with surveillance alone was not adequate to evaluate the outcome of biochemical recurrence. In addition, some patients followed locally after surgery so the outcome of metastasis could not be reliably assessed in all patients.

Statistical analysis

Associations between the two staining groups and their clinical characteristics were compared using the Fisher's exact test, ANOVA, and chi-square test. Consistency between DG expression on immunohistochemistry on prostate and lymph node staining was calculated using Fisher's exact test. Survival probabilities by the DG staining group were estimated and plotted using the Kaplan-Meier method. Differences between survival curves were compared using the log-rank test. All statistical testing was two-sided and assessed for significance at the 5% level using SAS v9.4 (SAS Institute, Cary, NC, USA).

Results

Descriptive statistics for patients with prostate staining

A total of 35 prostatectomy specimens were reviewed. Patient characteristics are summarized in Table 1. Mean age of diagnosis was 61 years (range 50-74), and mean PSA was 12.8 ng/mL. Nine of the 35 patients were pathologic stage T2, 25 were stage T3 and 1 was unknown. Gleason score ranged from 7 to 9 with the majority being Gleason 7 (26/35) with some patients with Gleason score 8 (3/35) and Gleason score 9 (7/36).

Expression of alpha-DG was evaluated in all 35 specimens. Twenty-six of 35 (74%) specimens stained positive, and 9 of 35 (26%) stained negative. The alpha-DG negative staining cohort was of numerically greater mean PSA, pT3 stage, and high (> 7) Gleason score, but none reached statistical significance, ($p > 0.05$, Table 1).

Descriptive statistics for patients with lymph node staining

Expression of alpha-DG subunit in the lymph nodes was evaluated using immunohistochemistry staining for all 26 available specimens, Table 2. Sixteen of

TABLE 1. **Descriptive statistics for all patients with node-positive margin-negative prostate cancer (n = 35)**

Characteristics	Negative staining (n = 9)	Positive staining (n = 26)	p value
Mean age (years)	61.8	60.57	0.52
Mean PSA (ng/mL)	16.06	11.67	0.79
Stage			
pT2	1	8	0.39
pT3	8	17	
Gleason grade, %			
Low (≤ 7)	66.6	76.9	0.66
High(> 7)	33.3	23.1	
Additional therapy,%			
Yes	88.9	76.9	0.44
No	11.1	23.1	

TABLE 2. **Descriptive statistics for all patients with node-positive margin-negative prostate cancer and lymph node specimens (n = 26)**

Characteristics	Negative staining (n = 10)	Positive staining (n = 16)	p value
Mean age (years)	63.6	58.57	0.07
Mean PSA (ng/mL)	13.44	11.88	1.00
Stage			
pT2	2	3	1.00
pT3	8	12	
Gleason grade, %			
Low	70	81	0.64
High	30	19	

26 (62%) specimens stained positive, and 10 of 26 (38%) stained negative. Comparison of both groups found no statistically significant difference in age, preoperative PSA, pathologic stage, Gleason score and adjuvant or salvage therapy ($p > 0.05$ for all, Table 2), although Gleason grade and pathologic T stage were numerically greater in the dystroglycan negative staining groups.

Consistency between prostate and lymph node dystroglycan staining

For the group that had both prostate and pelvic lymph node specimens available (n = 26), DG staining was concordant in 21 of 26 (81%), with positive in both (n = 16) or negative in both (n = 5). When prostate staining was negative (n = 5), the lymph node staining was always negative. However, when prostate staining was positive (n = 21), 16 of 21 (76%) lymph node specimens also stained positive while 5 of 21 (24%) samples had negative lymph node staining. This consistency between the primary tumor and its respective lymph node was confirmed to be statistically significant using Fisher's exact test (Table 3, $p < 0.004$). Immunohistological concordance demonstrated in Figure 1.

Survival outcomes

Association of prostate alpha-DG staining with mortality

Overall, 13 out of the 35 total patients died during follow up. The median survival was shorter in the negative staining group compared to the positive staining group, but the difference was not statistically significant (Figure 2, 13.2 years versus 19.4 years, $p = 0.21$). Adjusting grouping of DG groups (0-1 versus 2-4) did not alter conclusions (data not shown).

Association of lymph node alpha-DG staining with mortality

Overall, 11 out of the 26 patients with lymph node tissue available died during follow up. The median survival was similar between the positive and negative staining lymph node groups (Figure 3, 13.2 years versus 12.0 years, $p = 0.90$).

Discussion

DG is a transmembrane adhesion glycoprotein expressed in a wide variety of tissues. Most of the data available about its role in epithelial cells relates to the process of

TABLE 3. **Correlation between prostate and lymph node alpha-DG immunohistochemistry staining in patients with node-positive margin-negative prostate cancer (n = 26)**

	Lymph node positive	Lymph node negative	Total
Prostate positive	16	5	21
Prostate negative	0	5	5
Total	16	10	26

Fisher's exact test, ($p < 0.004$)

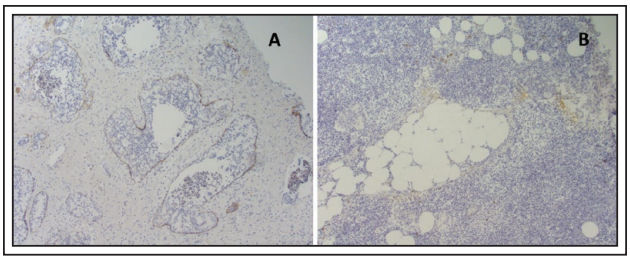

Figure 1. Concordance between prostate and matched lymph node using alpha-DG immunohistochemistry staining after radical prostatectomy in node-positive margin-negative prostate cancer.

tumor invasiveness and metastasis. It, therefore, may play an important role in tumor development by altering the interactions between cells and the surrounding matrix[25] and contribute to progression to metastatic disease. Loss of DG expression has been reported as a feature in many cancer types, including prostate, renal, breast, gastric, pancreatic, and colon cancer.[10,11,14-17] In addition, Increased mortality has been reported with loss of DG expression in renal, gastric, and pancreatic tumors[14,17,22] and decreased expression in prostate cancer was associated with higher grade disease,[11] but to our knowledge, the association between decreased DG expression and prostate cancer mortality had not been conclusively evaluated. We, therefore, evaluated DG in a cohort of men who had undergone radical prostatectomy with pelvic lymph node dissection and identified to have node positive disease. Immunohistochemical analyses of the alpha-DG subunit in this series of human primary prostate cancers demonstrated concordance between

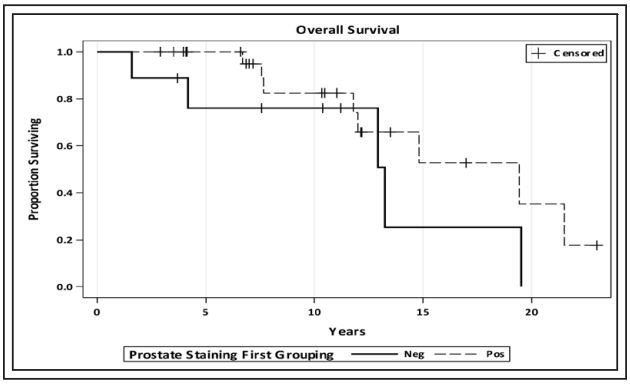

Figure 2. Overall survival after radical prostatectomy in node-positive margin-negative prostate cancer based on prostate αlpha-DG immunohistochemistry staining (positive vs. negative) (p = 0.21).

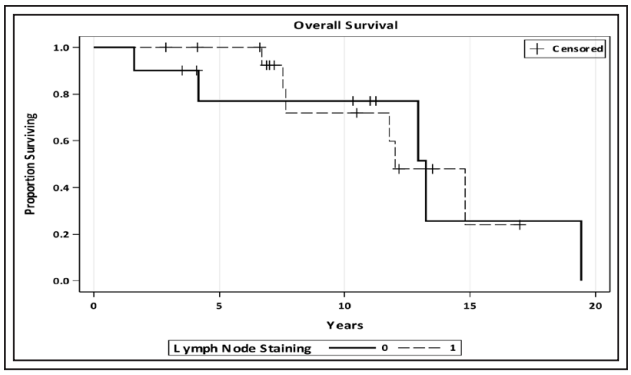

Figure 3. Overall survival after radical prostatectomy in node-positive margin-negative prostate cancer based on lymph node alpha-DG immunohistochemistry staining (positive vs. negative) (p = 0.90).

prostate specimens and metastatic lymph nodes and that detection of alpha-DG in the primary tumor is largely predictive of staining in the lymph nodes (100% and 76% correlation for alpha-DG negative and positive tumors, respectively).

Since immunohistochemistry staining is used routinely in both research and clinical medicine,[26-28] there would be value in a prostate cancer marker associated with survival. Patients with node positive prostate cancer are a specific treatment dilemma as there is substantial variation in outcomes and lack of high-quality evidence to guide management. As a result, NCCN guidelines include the options of observation or androgen deprivation therapy with or without external beam radiation therapy.[29] An immunohistochemical stain, or other biomarker, which could predict for response to radiation therapy, would be of clinical value.

In this study, decreased alpha-DG staining was not associated with a statistically significant decrease in survival. However, there was numerically shorter survival in patients with negative dystroglycan staining by 6.2 years, so it is possible a type 2 error (false negative) based on the sample size is present. However, our findings suggest that DG staining is unlikely to have a clinically significant impact in patient counseling or treatment decision making. Miller and colleagues, in a study performed at our institution, demonstrated within a cohort of 65 renal adenocarcinoma patients that survival was worse in patients with decreased alpha-DG expression.[22] Jiang XJ et al included 53 patients with pancreatic adenocarcinoma and showed poor survival in patients with lower αpha-DG expression.[17] Similarly, Shen et al evaluated survival in 20 patients with gastric adenocarcinoma and showed that decreased alpha-DG

expression was associated with poor survival.[14] Prostate cancer's more indolent nature, even in the setting of lymph node involvement, may necessitate a larger cohort with a longer follow up in order to demonstrate a significant difference, if present.

In addition, as we hypothesized, mean preoperative serum PSA, pathologic T stage, Gleason grade, adjuvant therapy use and mortality were numerically greater in the dystroglycan negative staining groups. However, these findings did not meet statistical significance, possibly due to limited numbers. In contrast, a previous study done at our institution with a cohort of 135 prostate cancer patients demonstrated a statistically significant inverse correlation between Gleason score and DG staining.[11] We believe that dystroglycan is not a perfect biomarker but may have some predictive value in identifying prostate cancer aggressiveness.

Our limited sample number and mortality events prohibits the ability to perform multivariate analysis, however on univariate analysis all variables analyzed were found to be similar between both groups. Additionally, sample size limits analysis of the full range of adjuvant or salvage therapy. A larger, likely multi-institutional, prospective study to more definitively evaluate the role for alpha-DG staining in prostate cancer, and specifically lymph node metastatic prostate cancer, would likely be of value. In addition, patients were treated based on standards of care at the time, and detailed data on metastasis and disease-specific survival are not available. We view this as an opportunistic evaluation of dystroglycan staining in an institutional cohort that identified consistent alpha-DG staining between prostate and lymph node but did not find a marked impact of alpha-DG on predicting overall survival or histology.

Conclusions

Alpha-DG expression by immunohistochemistry staining was consistent among the majority of prostate cancer specimens and their corresponding lymph node metastatic specimens. However, in this small cohort of prostate cancer patients with margin negative but node positive disease, DG staining was not associated with statistically significant worsening in prostate cancer grade, stage or overall mortality. □

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin* 2018;68(1):7-30.
2. Zincke H, Lau W, Bergstralh E, Blute ML. Role of early adjuvant hormonal therapy after radical prostatectomy for prostate cancer. *J Urol* 2001;166(6):2208-2215.
3. Schroder FH, Kurth KH, Fossa SD et al. Early versus delayed endocrine treatment of pN1-3 M0 prostate cancer without local treatment of the primary tumor: results of European Organisation for the Research and Treatment of Cancer 30846--a phase III study. *J Urol* 2004;172(3):923-927.
4. Cheng L, Zincke H, Blute ML, Bergstralh EJ, Scherer B, Bostwick DG. Risk of prostate carcinoma death in patients with lymph node metastasis. *Cancer* 2001;91(1):66-73.
5. Van den Ouden D, Schroder FH. Management of locally advanced prostate cancer. 1. Staging, natural history, and results of radical surgery. *World J Urol* 2000;18(3):194-203.
6. Smith RA, Cokkinides V, Eyre HJ. Cancer screening in the United States, 2007: a review of current guidelines, practices, and prospects. *CA Cancer J Clin* 2007;57(2):90-104.
7. Sutcliffe P, Hummel S, Simpson E et al. Use of classical and novel biomarkers as prognostic risk factors for localised prostate cancer: a systematic review. *Health Technol Assess* 2009;13(5):111-122.
8. Gomez-Iturriaga A, Cabeza A, Pastor J et al. The number of risk factors is the strongest predictor of prostate cancer mortality: multi-institutional outcomes of an extreme-risk prostate cancer cohort. *Clin Transl Oncol* 2016;18(10):1026-1033.
9. Cai T, Nesi G, Tinacci G et al. Clinical importance of lymph node density in predicting outcome of prostate cancer patients. *J Surg Res* 2011;167(2):267-272.
10. Henry MD, Cohen MB, Campbell KP. Reduced expression of dystroglycan in breast and prostate cancer. *Hum Pathol* 2001;32(8):791-795.
11. Esser AK, Miller MR, Huang Q et al. Loss of LARGE2 disrupts functional glycosylation of alpha-dystroglycan in prostate cancer. *J Biol Chem* 2013;288(4):2132-2142.
12. Sgambato A, De Paola B, Migaldi M et al. Dystroglycan expression is reduced during prostate tumorigenesis and is regulated by androgens in prostate cancer cells. *J Cell Physiol* 2007;213(2):528-539.
13. Shimojo H, Kobayashi M, Kamigaito T, Shimojo Y, Fukuda M, Nakayama J. Reduced glycosylation of α-dystroglycans on carcinoma cells contributes to formation of highly infiltrative histological patterns in prostate cancer. *Prostate* 2011;71(11):1151-1157.
14. Shen JG, Xu CY, Li X et al. Dystroglycan is associated with tumor progression and patient survival in gastric cancer. *Pathol Oncol Res* 2012;18(1):79-84.
15. Sgambato A, Camerini A, Amoroso D et al. Expression of dystroglycan correlates with tumor grade and predicts survival in renal cell carcinoma. *Cancer Biol Ther* 2007;6(12):1840-1846.
16. Sgambato A, Migaldi M, Montanari M et al. Dystroglycan expression is frequently reduced in human breast and colon cancers and is associated with tumor progression. *Am J Pathol* 2003; 162(3):849-860.
17. Jiang X, Rieder S, Giese NA, Friess H, Michalski CW, Kleeff J. Reduced alpha-dystroglycan expression correlates with shortened patient survival in pancreatic cancer. *J Surg Res* 2011;171(1):120-126.
18. Hara Y, Kanagawa M, Kunz S et al. Like-acetylglucosaminyltransferase (LARGE)-dependent modification of dystroglycan at Thr-317/319 is required for laminin binding and arenavirus infection. *Proc Natl Acad Sci U S A* 2011;108(42):17426-17431.
19. Kanagawa M, Michele DE, Satz JS et al. Disruption of perlecan binding and matrix assembly by post-translational or genetic disruption of dystroglycan function. *FEBS Lett* 2005;579(21): 4792-4796.
20. Kanagawa M, Saito F, Kunz S et al. Molecular recognition by LARGE is essential for expression of functional dystroglycan. *Cell* 2004;117(7):953-964.
21. Michele DE, Barresi R, Kanagawa M et al. Post-translational disruption of dystroglycan-ligand interactions in congenital muscular dystrophies. *Nature* 2002;418(6896):417-422.

22. Miller MR, Ma D, Schappet J et al. Downregulation of dystroglycan glycosyltransferases LARGE2 and ISPD associate with increased mortality in clear cell renal cell carcinoma. *Mol Cancer* 2015;14:141.
23. Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. *Ann Surg Oncol* 2010;17(6):1471-1474.
24. Epstein JI, Allsbrook WC, Amin MB et al. The 2005 International Society of Urological Pathology (ISUP) consensus conference on Gleason grading of prostatic carcinoma. *Am J Surg Pathol* 2005;29(9):1228-1242.
25. Schwock J, Dhani N, Hedley DW. Targeting focal adhesion kinase signaling in tumor growth and metastasis. *Expert Opin Ther Targets* 2010;14(1):77-94.
26. de Matos LL, Trufelli DC, de Matos MGL, Pinhal MAS. Immunohistochemistry as an important tool in biomarkers detection and clinical practice. *Biomark Insights* 2010;5:9-20.
27. Kaliyappan K, Palanisamy M, Duraiyan J, Govindarajan R. Applications of immunohistochemistry. *J Pharm Bioallied Sci* 2012;4(Suppl 2):S307-S309.
28. Makki JS. Diagnostic implication and clinical relevance of ancillary techniques in clinical pathology practice. *Clin Med Insights Pathol* 2016;9:5-11.
29. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) Prostate Cancer. Version 4. 2018, August 15, 2018.