UNIVERSITÄTSKLINIKUM HAMBURG-EPPENDORF

Institut für Pathologie

Prof. Dr. Guido Sauter

Überexpression von Biglycan ist assoziiert mit einer schlechten Prognose und PTEN Deletionen bei Patienten mit Prostatakarzinomen

Dissertation

zur Erlangung des Grades eines Doktors der Zahnmedizin
an der Medizinischen Fakultät der Universität Hamburg.

vorgelegt von:

Juliane Viktoria Kraft
aus Kassel

Hamburg 2019

Angenommen von der Medizinischen Fakultät am: 22.01.2019

Veröffentlichung mit Genehmigung der Medizinischen Fakultät der Universität Hamburg

Prüfungsausschuss, der/die Vorsitzende: Prof. Dr. Guido Sauter

Prüfungsausschuss, 2. Gutachter/in: PD Dr. Sabine Windhorst

Inhaltsverzeichnis

NEOPLASIA
www.neoplasia.com

Volume 19 Number 9 September 2017 pp. 707–715 707

Up-regulation of Biglycan is Associated with Poor Prognosis and PTEN Deletion in Patients with Prostate Cancer

Frank Jacobsen[*,1], Juliane Kraft[*,1], Cornelia Schroeder[†], Claudia Hube-Magg[*], Martina Kluth[*], Dagmar S. Lang[*], Ronald Simon[*], Guido Sauter[*], Jakob R. Izbicki[†], Till S. Clauditz[*], Andreas M. Luebke[*], Andrea Hinsch[*], Waldemar Wilczak[*], Corinna Wittmer[*], Franziska Büscheck[*], Doris Höflmayer[*], Sarah Minner[*], Maria Christina Tsourlakis[*], Hartwig Huland[‡], Markus Graefen[‡], Lars Budäus[‡], Imke Thederan[‡], Georg Salomon[‡], Thorsten Schlomm[‡,§] and Nathaniel Melling[†]

[*]Institute of Pathology, University Medical Center Hamburg-Eppendorf, Germany; [†]General, Visceral and Thoracic Surgery Department and Clinic, University Medical Center Hamburg-Eppendorf, Germany; [‡]Martini-Clinic, Prostate Cancer Center, University Medical Center Hamburg-Eppendorf, Germany; [§]Department of Urology, Section for translational Prostate Cancer Research, University Medical Center Hamburg-Eppendorf, Germany

Abstract

Biglycan (BGN), a proteoglycan of the extracellular matrix, is included in mRNA signatures for prostate cancer aggressiveness. To understand the impact of BGN on prognosis and its relationship to molecularly defined subsets, we analyzed BGN expression by immunohistochemistry on a tissue microarray containing 12,427 prostate cancers. Seventy-eight percent of 11,050 interpretable cancers showed BGN expression, which was considered as low intensity in 47.7% and as high intensity in 31.1% of cancers. BGN protein expression rose with increasing pathological tumor stage, Gleason grade, lymph node metastasis and early PSA recurrence ($P < .0001$ each). Comparison with our molecular database attached to the TMA revealed that BGN expression was linked to presence of TMPRRS2:ERG fusion and PTEN deletion ($P < .0001$ each). In addition, BGN was strongly linked to androgen-receptor (AR) levels ($P < .0001$), suggesting a hormone-depending regulation of BGN. BGN up-regulation is a frequent feature of prostate cancer that parallels tumor progression and may be useful to estimate tumor aggressiveness particularly if combined with other molecular markers.

Neoplasia (2017) 19, 707–715

Abbreviations: BGN, Biglycan; CHD1, Chromodomain-helicase-DNA-binding protein 1; ERCC1, Excision repair cross-complementation; ERG, Erythroblast transformation-specific (ETS) related gene; FISH, Fluorescence in situ hybridization; FOXP1, Fork head box protein P1; Ki67LI, Ki67 labeling index; MAP3K7, Mitogen-activated protein kinase kinase kinase 7; PSA, Prostate specific antigen; PTEN, Phosphatase and tensin homolog; SLRP, Small leucine-rich proteoglycan; TGF-β, Transforming growth factor β; TMA, Tissue microarray; TMPRSS2, Trans membrane protease, serine 2.

Address all correspondence to: Ronald Simon, Institute of Pathology, University Medical Center Hamburg-Eppendorf, Martinistr. 52, 20246 Hamburg, Germany.
E-mail: R.Simon@uke.de

Disclosure/Conflict of interest: There are no proprietary interests and no financial support was received. No conflicts of interest regarding the article exist.
[1] These authors contributed equally to this work.
Received 21 March 2017; Revised 2 June 2017; Accepted 5 June 2017

1476-5586
http://dx.doi.org/10.1016/j.neo.2017.06.003

Introduction

Prostate cancer is the most prevalent cancer in men in Western societies. Although most prostate cancers have a rather indolent clinical course, this disease still represents the third most common cause of cancer related death in men [1]. A reliable distinction between indolent and aggressive forms of the disease is highly desirable to enhance the quality of therapeutic decisions. Despite recent advances, the only established pretreatment prognostic parameters currently remain Gleason grade and tumor extent on biopsies, preoperative prostate-specific antigen (PSA), and clinical stage. Because these data are statistically powerful but not sufficient for optimal individual treatment decisions, it can be hoped that a better understanding of disease biology will eventually lead to the identification of clinically applicable molecular markers that enable a more reliable prediction of prostate cancer aggressiveness.

Biglycan (BGN) is a member of the small leucine-rich repeat proteoglycans (SLRP) family characterized by a core protein with leucine-rich repeats attended by cysteine clusters [2]. BGN is normally secreted from extracellular matrix fibroblasts and facilitates assembly of collagen fibrils and bone matrix [3]. In addition, BGN up-regulation has been implicated in the response of inflammatory processes triggered by transforming growth factor β (TGF-ß) [4–6]. More importantly, BGN up-regulation has been reported from many malignant epithelial tumors, including a large variety of gastro-intestinal cancers [7–10] as well as gynecological tumors [11]. In some of these cancers, BGN up-regulation has been linked to advanced [8,12] and metastatic [11,13,14] cancers or adverse patient prognosis. That opposite observations have been made in other tumor types [12,15] may suggest tumor type specific roles of BGN expression. Earlier work has demonstrated that the prostate is an abundant secretor of glycoproteins, including many types of proteoglycans [16]. In prostate cancer, BGN has gained interest because it is part of a commercial RNA expression signature for estimating prostate cancer aggressiveness [17].

To understand the role of BGN for prostate cancer biology and is association to molecular features of the disease, we analyzed our large tissue microarray (TMA) resource including more than 12,400 prostate cancers for immunohistochemical BGN expression. The database attached to our TMA contains pathological and clinical follow-up data, as well molecular data of key molecular alterations of this disease.

Materials and Methods

Patients

Radical prostatectomy specimens were available from 12,427 patients, undergoing surgery between 1992 and 2012 at the Department of Urology and the Martini Clinics at the University Medical Center Hamburg-Eppendorf. The local ethics committee (Ethics commission Ärztekammer Hamburg, WF-049/09 and PV3652) approved the use of specimens and data for research. According to local laws (HmbKHG, §12,1), informed consent was not required for this study. Patient records/information was anonymized and de-identified prior to analysis. All work has been carried out in compliance with the Helsinki Declaration. Follow-up data were available for a total of 11,665 patients with a median follow-up of 50.0 months (range: 1 to 264 months; Table 1). Prostate specific antigen (PSA) values were measured following surgery and PSA recurrence was defined as a postoperative PSA of 0.2 ng/ml and increasing at first of appearance. All prostate specimens were analyzed according to a standard procedure, including a complete embedding of the entire prostate for histological analysis [18]. The TMA manufacturing process was described earlier in detail [19,20]. In short, one 0.6 mm core was taken from a representative tissue block from each patient. The tissues were distributed among 27 TMA blocks, each containing 144 to 522 tumor samples. For internal controls, each TMA block also contained various control tissues, including normal prostate tissue. The molecular database attached to this TMA contained results on ERG expression in 10,678 [21], ERG break apart fluorescence in situ hybridization (FISH) analysis in 7099 (expanded from [22]) and deletion status of 5q21 (CHD1) in 7932 (expanded from [23]), 6q15 (MAP3K7) in 6069 (expanded from [24]), PTEN (10q23) in 6704 (expanded from [25]) and 3p13 (FOXP1) in 7081 (expanded from [26]) cancers.

Table 1. Pathological and Clinical Data of the Arrayed Prostate Cancers

	No. of Patients (%)	
	Study Cohort on TMA(n = 12,427)	Biochemical Relapse Among Categories
Follow-up (mo.)		
n	11,665 (93.9%)	2769 (23.7%)
Mean	62.9	-
Median	50.0	-
Age (y)		
≤50	334 (2.7%)	81 (24.3%)
51–59	3061 (24.8%)	705 (23%)
60–69	7188 (58.2%)	1610 (22.4%)
≥70	1761 (14.3%)	370 (21%)
Pretreatment PSA (ng/ml)		
<4	1585 (12.9%)	242 (15.3%)
4–10	7480 (60.9%)	1355 (18.1%)
10–20	2412 (19.6%)	737 (30.6%)
>20	812 (6.6%)	397 (48.9%)
pT stage (AJCC 2002)		
pT2	8187 (66.2%)	1095 (13.4%)
pT3a	2660 (21.5%)	817 (30.7%)
pT3b	1465 (11.8%)	796 (54.3%)
pT4	63 (0.5%)	51 (81%)
Gleason grade		
≤3 + 3	2848 (22.9%)	234 (8.2%)
3 + 4	6679 (53.8%)	1240 (18.6%)
3 + 4 Tertiary 5	433 (3.5%)	115 (26.6%)
4 + 3	1210 (9.7%)	576 (47.6%)
4 + 3 Tertiary 5	646 (5.2%)	317 (49.1%)
≥4 + 4	596 (4.8%)	348 (58.4%)
pN stage		
pN0	6970 (91%)	1636 (23.5%)
pN+	693 (9%)	393 (56.7%)
Surgical margin		
Negative	9990 (81.9%)	1848 (18.5%)
Positive	2211 (18.1%)	853 (38.6%)

Percent in the column "Study cohort on TMA" refers to the fraction of samples across each category. Percent in column "Biochemical relapse among categories" refers to the fraction of samples with biochemical relapse within each parameter in the different categories. NOTE: Numbers do not always add up to 12,427 in different categories because of cases with missing data. Abbreviation: AJCC, American Joint Committee on Cancer.

Immunohistochemistry

Freshly cut TMA sections were stained in 1 day and in one experiment. Slides were deparaffinized and exposed to heat-induced antigen retrieval for 5 minutes in an autoclave at 121 °C in pH 7.8 Tris-EDTA-citrate buffer. Primary antibody specific for BGN (rabbit polyclonal antibody, Sigma-Aldrich, Germany; cat#HPA003157;

dilution 1:1350) was applied at 37 °C for 60 minutes. Bound antibody was then visualized using the EnVision Kit (Dako, Glostrup, Denmark) according to the manufacturer's directions. BGN protein expression was typically seen in the cytoplasm of all (100%) tumor cells. Accordingly, the staining intensity in prostate epithelial cells was recorded in three categories for each cancer, including negative (no to weak detectable staining), low (moderate staining) and high (strong staining).

Statistics

For statistical analysis, the JMP 12.0 software (SAS Institute Inc., NC, USA) was used. Contingency tables were calculated to study association between BGN expression categories and clinicopathological variables, and the chi-square (Likelihood) test was used to find significant relationships. Kaplan–Meier curves were generated using biochemical (PSA) recurrence as the clinical endpoint. The log-rank test was applied to test the significance of differences between stratified survival functions. Cox proportional hazards regression analysis was performed to test the statistical independence and significance between pathological, molecular, and clinical variables.

Results

Technical Issues

A total of 11,050 (89.0%) tumor samples were interpretable in our TMA analysis. Reasons for non-informative cases (1377 spots; 11.0%) included lack of tissue samples or absence of unequivocal cancer cells in the TMA spot.

BGN Expression in Normal and Cancerous Prostate Epithelium

Normal prostate epithelium did not show detectable BGN staining under the selected experimental conditions. In cancers, BGN staining was localized in the cytoplasm. Positive BGN staining was seen in 8701 of our 11,050 (78.7%) interpretable prostate cancers and was considered as low intensity in 47.7% and as high intensity in 31.1% of cancers. Representative images of negative and positive BGN staining are given in Figure 1.

Association with TMPRSS2:ERG Fusion Status, ERG Protein Expression and PTEN Deletion

Data on TMPRSS2:ERG fusion status obtained by FISH were available from 6462 and by immunohistochemistry from 9686 tumors with evaluable BGN staining. Data on both ERG FISH and IHC were available from 6201 cancers, and an identical result (ERG IHC-positive and break by FISH or ERG IHC-negative and missing break by FISH) was found in 5919 of 6201 (95.5%) cancers. High-level BGN staining was linked to TMPRSS2:ERG rearrangement and ERG positivity in prostate cancers. BGN staining was seen in 87.3% and 86.8% of cancers with TMPRSS2:ERG fusion detected by IHC and FISH, but found in only 73.8% of cancers without ERG staining and 76.7% of cancers without ERG rearrangements detected by FISH ($P < .0001$ each). High-level BGN staining was also significantly linked to PTEN deletion (Figure 2). The effect was additive and of similar size as the ERG fusion effect.

Associations with Tumor Phenotype

High grade BGN expression was significantly linked to advanced pT stage, high Gleason grade, lymph node metastases, high preoperative PSA-levels ($P < .0001$ each) and surgical margin positivity ($P = .0007$, Table 2). Subgroup analysis revealed that these associations held also true in subsets of cancers with and without ERG fusion (Supplementary Tables 1 and 2).

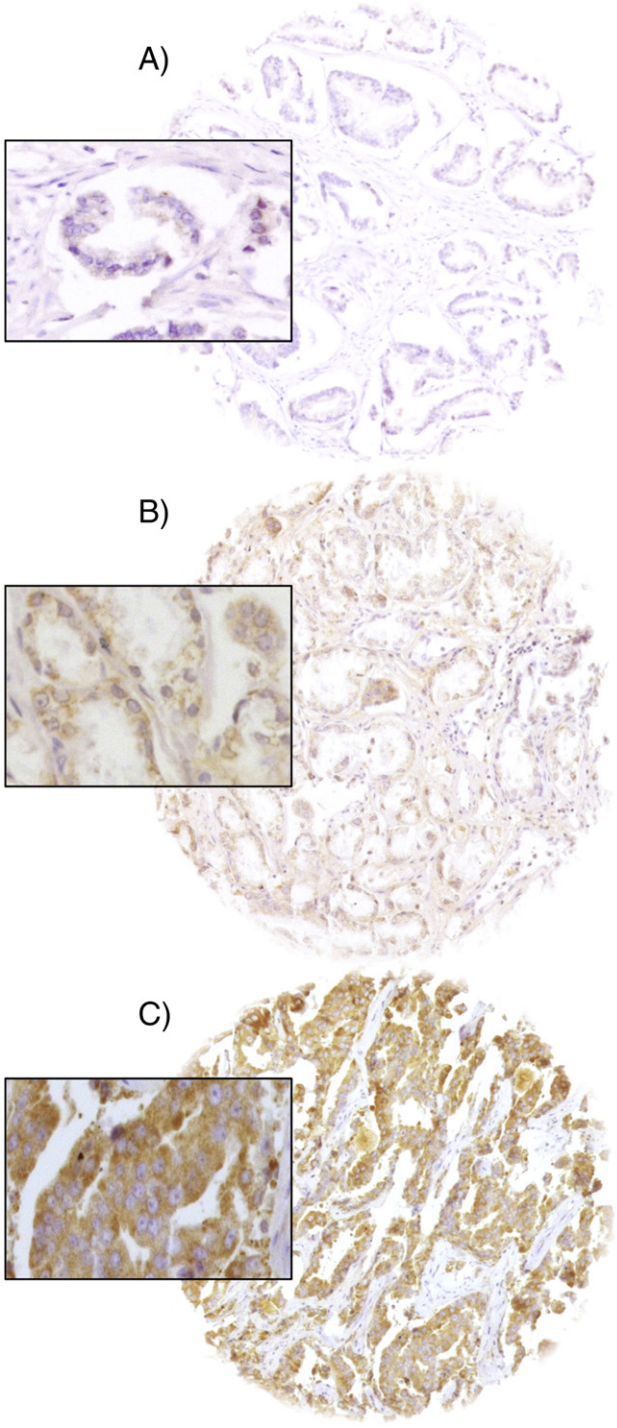

Figure 1. Representative pictures of BGN staining in prostate cancer with negative (A), low (B), and high-intensity (C) staining at 100×, insets 400× magnification and spot size of 600 μm.

Association with Other Key Genomic Deletions

To study whether BGN expression might be particularly associated with genomic deletions, BGN data were compared to preexisting findings on PTEN (10q23), 3p13 (FOXP1), 6q15 (MAP3K7) and 5q21 (CHD1) deletion. This analysis revealed that high BGN

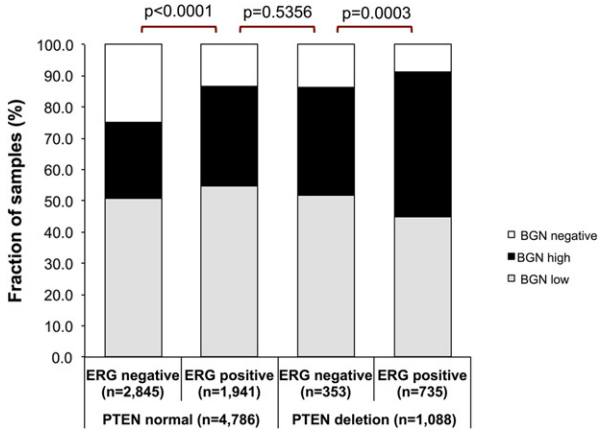

Figure 2. Association between BGN staining, ERG status determined by immunohistochemistry and PTEN deletion by fluorescence in situ hybridization.

expression was significantly linked to all of these deletions (Figure 3*A*) and that the association was most striking for PTEN deletions. Subset analysis of cancers with and without ERG fusion (Figure 3, *B* and *C*) revealed that only PTEN deletion was strongly linked to high BGN expression in both subgroups. For the other deletions, the association with high level BGN expression was largely limited to the subset of ERG-negative cancers.

Association with Tumor Cell Proliferation

High-level BGN staining was significantly linked to high cell proliferation as measured by Ki67 labeling index (LI). The average Ki67LI increased from 2.1 ± 0.07 in cancers lacking BGN expression to 2.9 ± 0.05 in cancers with low and to 3.0 ± 0.06 in cancers with high BGN levels ($P < .0001$). This association held true in all tumor subsets with identical Gleason score (≤3 + 3: $P < .001$, 3 + 4: $P < .0001$, 4 + 3: $P = .0085$, ≥4 + 4: $P = .0137$; data not shown).

Table 2. Association between BGN Staining Results and Prostate Cancer Phenotype

	BGN IHC Result (%)				
Parameter	n Evaluable	Negative	Low	High	*P* Value
All cancers	11,050	21.3	47.7	31.1	
Tumor stage					
pT2	7182	23.8	49.0	27.2	
pT3a	2430	17.6	47.1	35.3	<0.0001
pT3b-pT4	1395	14.6	42.2	43.3	
Gleason grade					
≤3 + 3	2460	27.0	48.6	24.4	
3 + 4	5663	21.4	48.6	29.9	
3 + 4 Tert.5	410	16.8	46.1	37.1	<0.0001
4 + 3	949	18.8	46.6	34.7	
4 + 3 Tert.5	580	14.1	48.4	37.4	
≥4 + 4	474	15.8	42.0	42.2	
Lymph node metastasis					
N0	6271	19.5	47.7	32.8	<0.0001
N+	634	13.7	41.3	45.0	
Preoperative PSA level (ng/ml)					
<4	1366	20.8	50.2	29.0	
4–10	6618	22.1	49.1	28.7	<0.0001
10–20	2186	20.1	43.6	36.2	
>20	759	17.8	42.6	39.7	
Surgical margin					
Negative	8801	21.7	48.1	30.2	0.0007
Positive	2047	19.5	46.1	34.4	

Association with Androgen Receptor

Androgen receptor (AR) expression is a characteristic feature of neoplastic and non-neoplastic prostate epithelial cells. Immunohistochemical data on AR expression were obtained from a previous study [21]. AR expression was strongly correlated to BGN staining. For example, high BGN expression was found in 16.9% of cancers without detectable AR expression, but in 36.8% of tumors with strong AR expression ($P < .0001$ each). These associations held also true in subsets of cancers with and without ERG fusion ($P < .0001$ each, Figure 4).

Association with PSA Recurrence

Follow-up data were available for 10,359 patients with interpretable BGN staining on the TMA. The prognostic impact of pT stage, traditional Gleason grade, and quantitative Gleason grade are shown in Figure 5, *A–C*). High-level BGN expression was significantly associated with early PSA recurrence in all tumors and also in subgroup analyses limited to the subsets of ERG-negative and ERG-positive cancers ($P < .0001$ each, Figure 5, *D–F*). To better understand the prognostic power of BGN, we performed further subset analyses in cancers with identical classical and quantitative Gleason scores. Here, BGN staining did not provide prognostic information beyond the Gleason score, neither in any subsets defined by the classical Gleason score (Supplementary Figure 1a) nor by the quantitative Gleason score (Supplementary Figure 1b–h).

Multivariate Analysis

Four different types of multivariate analyses were performed evaluating the clinical relevance of BGN expression in different scenarios (Supplementary Table 3). Scenario 1 evaluated all postoperatively available parameters including pathological tumor stage, pathological lymph node status (pN), surgical margin status, preoperative PSA value and pathological Gleason grade obtained after the morphological evaluation of the entire resected prostate. In scenario 2, all postoperatively available parameters with exception of nodal status were included. The rational for this approach was that the indication and extent of lymph node dissection is not standardized in the surgical therapy of prostate cancer and that excluding pN in multivariate analysis can markedly increase case numbers. Two additional scenarios had the purpose to model the preoperative situation as much as possible. Scenario 3 included BGN expression, preoperative PSA, clinical tumor stage (cT stage) and Gleason grade obtained on the prostatectomy specimen. Since postoperative determination of a tumors Gleason grade is "better" than the preoperatively determined Gleason grade (subjected to sampling errors and consequently under-grading in more than one third of cases [27]), another multivariate analysis was added. In scenario 4, the preoperative Gleason grade obtained on the original biopsy was combined with preoperative PSA, cT stage and BGN expression. Overall, these data show, that BGN expression did not provide independent prognostic information, neither in all cancers nor in the subsets of ERG-positive and -negative subgroups.

Discussion

The results of our study identify high BGN expression as a weak prognostic feature in prostate cancer, which is also linked to PTEN deleted cancers. Our immunohistochemical analysis revealed cytoplasmic BGN staining in about 80.0% of 11,050 of the interpretable prostate cancers. The lack of BGN staining in normal prostate

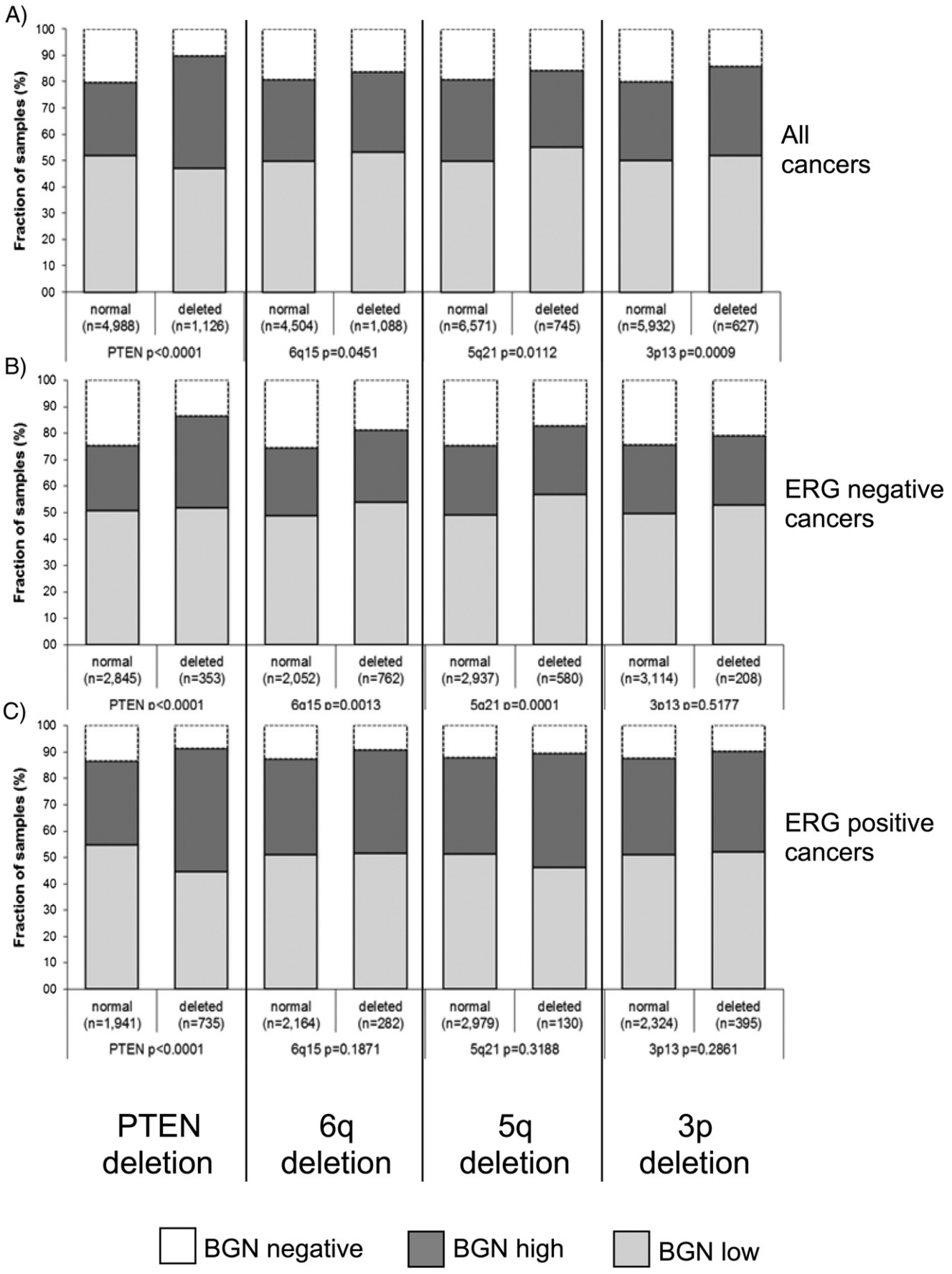

Figure 3. Association between positive BGN staining and 10q23 (PTEN), 5q21 (CHD1), 6q15 (MAP3K7), 3p13 (FOXP1) deletions in all cancers (A), in ERG-negative (B), and ERG-positive cancers (C).

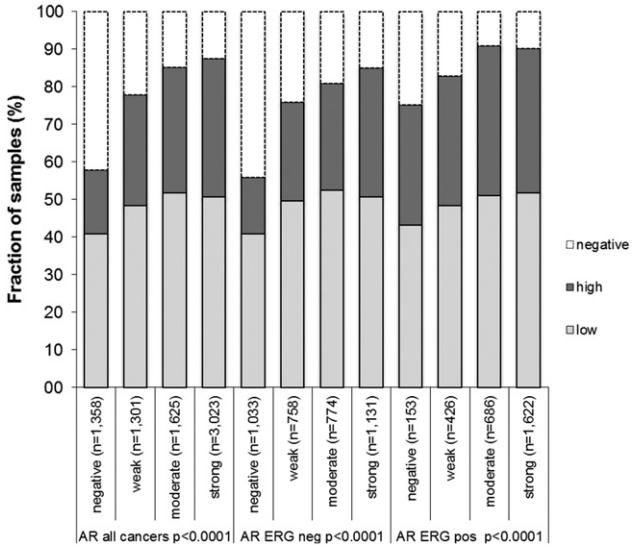

Figure 4. Association between BGN staining results and androgen receptor (AR) in all cancers, ERG-negative tumors, and ERG-positive tumors.

epithelium indicates that BGN becomes unregulated during prostate cancer development in a large fraction of patients. Comparable data on immunohistochemical BGN expression in prostate cancer are currently lacking in the literature. However, tumor associated up-regulation of BGN has been also reported from esophageal squamous cell carcinoma [9], pancreatic [7,15], gastric [10], colorectal [8], odontogenic [28], endometrial [11,29] and ovarian cancer [30]. BGN overexpression was significantly associated with an unfavorable tumor phenotype in our study, including advanced pT stage, high Gleason grade, accelerated cell proliferation, lymph node metastases and early biochemical recurrence ($P < .0001$ each). These findings are in line with data from studies on various other cancer types. For example, BGN overexpression has been linked to tumor aggressiveness and shortened patient survival in pancreatic, gastric and endometrial cancer [7,10,29]. Reasons for the tumor-associated up-regulation may include the role of BGN as a downstream target of various growth and signal transduction pathways like TGFb, Wnt and Akt signaling [31–33], activation of which is frequently found in malignant tumors [34–37]. The general importance of these pathways in prostate cancer [38–41] may not only explain the high fraction of BGN expressing cancers but also its comparatively low prognostic impact. That BGN up-regulation has also been linked to reduce cell proliferation in some cell lines from cancers and fibroblasts [12,15,42] is consistent with the cell-type dependent effects of BGN expression. The molecular database attached to our TMA allowed us to draw conclusions on some molecular mechanisms associated with BGN up-regulation. It is well known that about 50% of all prostate cancers carry a gene fusion linking the androgen-regulated serine protease TMPRSS2 with the ETS-transcription factor ERG resulting in an androgen-related overexpression of ERG with subsequent transcriptional deregulation of more than 1600 ERG target genes [21,43,44]. Others and we have shown that activation of the TGF-ß signaling pathway is one important consequence of ERG fusion in prostate cancer [44–46]. That the extracellular matrix modulator TGF-β1 is an important stimulator of BGN transcriptional and post translational modifications [47,48] provides a mechanistic explanation for the higher fraction of cancers with BGN up-regulation in ERG-positive as compared to ERG-negative cancers. In addition, earlier work involving computational promoter analyses revealed a binding site for ETS transcription factors [49], suggesting that activated ERG might also directly contribute to the up-regulation of BGN in prostate cancer. Another interesting finding was the strong association of BGN overexpression with AR levels, which suggests an androgen-dependent regulation of BGN in prostate cancer. This is supported by studies reporting a role of androgen in regulating collagen and proteoglycan organization in the cervix [50] as well as an androgen depending synthesis of BGN in vascular smooth muscle cells [51]. Given that increased AR signaling is a hallmark of prostate cancer [52], it is tempting to speculate that androgen-dependent up-regulation of BGN may contribute to changes in the extracellular matrix causing the increased tissue stiffness of cancer areas that is often felt during digital rectal examination. The reaction of the prostate to cancer cell invasion is thought to resemble processes involved in wound healing, including increased cellular density, elevated micro vascularity, and increased collagen deposition in the stroma [53,54]. In fact, also other markers of collagen synthesis such as serine proteases, matrix metalloproteases and propeptides of type I collagen have been found to be significantly increased not only in prostate cancer cells but also in surrounding unaffected tissues [55].

Recurrent deletions including PTEN, 6q15, 5q21, 3p13, are another hallmark of prostate cancer [56,57]. PTEN inactivation results in hyperactive AKT signaling and is associated with tumor growth, progression and poor clinical outcome [58]. That BGN overexpression was particularly linked to PTEN deletions is of interest with respect to previous reports linking class I proteoglycans – including BGN — to several growth pathways including AKT signaling [2,31]). For example, earlier work demonstrates that excess extracellular matrix concentrations of soluble BGN can induce cell growth and survival via activation of multiple growth factor receptors including the AKT-upstream receptor EGFR [31,59]. That activated AKT is also required for BGN core protein synthesis [33] is seen as further support for a role of BGN in signal regulation networks [2], and might also provide an explanation for the marked up-regulation of BGN in PTEN-deleted and AKT-hyperactivated cancers. Recently Li et al. reported a role of PTEN in induction of type I interferon linking the tumor suppressor with the innate immune system [60]. Since BGN is a prototype endogenous ligand to Toll-like receptors stimulating innate immunity [61], it is tempting to speculate that up-regulation of BGN in the progress to aggressive prostate cancer may be counteracted by PTEN loss. Although BGN expression was a prognostic factor in univariate calculations, its prognostic impact was lost in most multivariate analyses including established morphological parameters. The power of morphological methods competing with biomarkers for predicting prostate cancer aggressiveness is best demonstrated by the separate analysis of tumors with comparable morphology. Already within traditional grade groups, the prognostic impact of BGN expression was lost. Based on the large cohort of prostate cancers available at our institution, we had recently shown that prognostic Gleason Grade information can be further refined by using the percentage of Gleason 4 grades as a continuous variable (quantitative Gleason Grade) [62]. Both in biopsies and in prostatectomy samples, prostate cancer prognosis continuously deteriorates with increasing percentage of Gleason 4 pattern. That BGN expression lacks any prognostic impact in all subgroups defined by a comparable

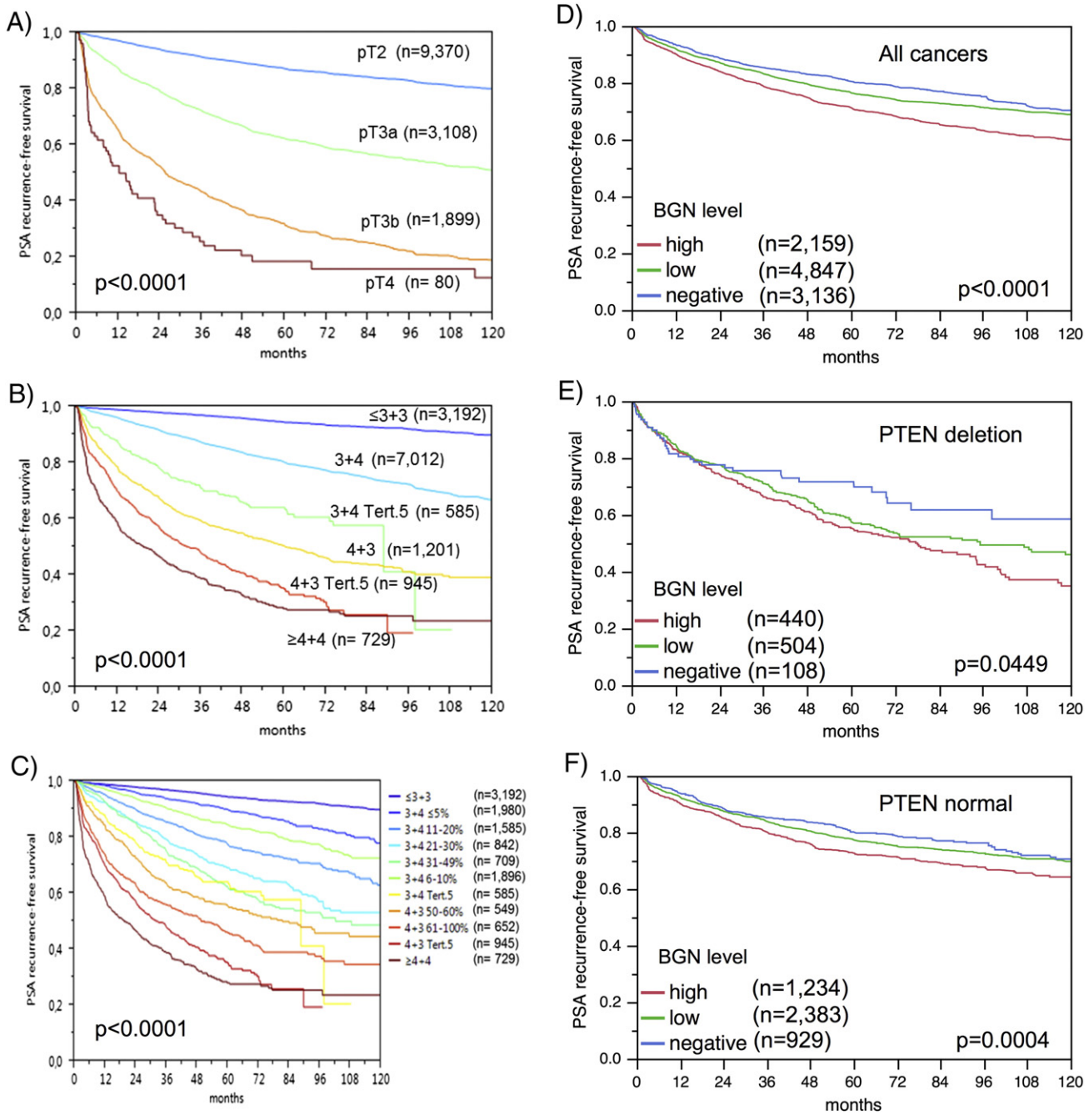

Figure 5. Kaplan–Meier plots of prostate specific antigen (PSA) recurrence after radical prostatectomy and pathological tumor stage (A), classical Gleason score (B), quantitative Gleason score (C), BGN expression in all cancers (D), in PTEN deletion (E), and PTEN normal subset (F).

quantitative Gleason grade demonstrates how difficult it is for biomarkers to outperform morphological malignancy parameters in prostate cancer. However, it is our anticipation that prognostic gene sets will assist routine clinical decision-making in prostate cancer in the future. It appears likely that combining molecular markers will enable a better and reproducible prognosis prediction than single markers.

In summary, BGN overexpression is a frequent feature of prostate cancer, which parallels tumor progression and is particularly linked to PTEN deleted cancers. Although BGN mRNA measurement is a part of a commercial gene signature estimating prostate cancer aggressiveness the prognostic power of BGN protein measurement is weak and may have clinical use only if combined with other molecular markers.

Acknowledgements

The authors appreciate the excellent technical support of Christina Koop, Sylvia Schnöger and Sasha Eghtessadi.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.neo.2017.06.003.

References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, and Jemal A (2015). Global cancer statistics, 2012. *CA Cancer J Clin* **65**(2), 87–108.

[2] Schaefer L and Iozzo RV (2008). Biological functions of the small leucine-rich proteoglycans: from genetics to signal transduction. *J Biol Chem* **283**(31), 21305–21309.

[3] Xing X, Gu X, Ma T, and Ye H (2015). Biglycan up-regulated vascular endothelial growth factor (VEGF) expression and promoted angiogenesis in colon cancer. *Tumour Biol* **36**(3), 1773–1780.

[4] Nastase MV, Young MF, and Schaefer L (2012). Biglycan: a multivalent proteoglycan providing structure and signals. *J Histochem Cytochem* **60**(12), 963–975.

[5] Hildebrand A, Romaris M, Rasmussen LM, Heinegard D, Twardzik DR, Border WA, and Ruoslahti E (1994). Interaction of the small interstitial proteoglycans biglycan, decorin and fibromodulin with transforming growth factor beta. *Biochem J* **302**(Pt 2), 527–534.

[6] Schaefer L, Babelova A, Kiss E, Hausser HJ, Baliova M, Krzyzankova M, Marsche G, Young MF, Mihalik D, and Gotte M, et al (2005). The matrix component biglycan is proinflammatory and signals through Toll-like receptors 4 and 2 in macrophages. *J Clin Invest* **115**(8), 2223–2233.

[7] Aprile G, Avellini C, Reni M, Mazzer M, Foltran L, Rossi D, Cereda S, Iaiza E, Fasola G, and Piga A (2013). Biglycan expression and clinical outcome in patients with pancreatic adenocarcinoma. *Tumour Biol* **34**(1), 131–137.

[8] Gu X, Ma Y, Xiao J, Zheng H, Song C, Gong Y, and Xing X (2012). Up-regulated biglycan expression correlates with the malignancy in human colorectal cancers. *Clin Exp Med* **12**(3), 195–199.

[9] Zhu YH, Yang F, Zhang SS, Zeng TT, Xie X, and Guan XY (2013). High expression of biglycan is associated with poor prognosis in patients with esophageal squamous cell carcinoma. *Int J Clin Exp Pathol* **6**(11), 2497–2505.

[10] Wang B, Li GX, Zhang SG, Wang Q, Wen YG, Tang HM, Zhou CZ, Xing AY, Fan JW, and Yan DW, et al (2011). Biglycan expression correlates with aggressiveness and poor prognosis of gastric cancer. *Exp Biol Med (Maywood)* **236**(11), 1247–1253.

[11] Sun H, Wang X, Zhang Y, Che X, Liu Z, Zhang L, Qiu C, Lv Q, and Jiang J (2016). Biglycan enhances the ability of migration and invasion in endometrial cancer. *Arch Gynecol Obstet* **293**(2), 429–438.

[12] Niedworok C, Rock K, Kretschmer I, Freudenberger T, Nagy N, Szarvas T, Vom Dorp F, Reis H, Rubben H, and Fischer JW (2013). Inhibitory role of the small leucine-rich proteoglycan biglycan in bladder cancer. *PLoS One* **8**(11), e80084.

[13] Maishi N, Ohba Y, Akiyama K, Ohga N, Hamada J, Nagao-Kitamoto H, Alam MT, Yamamoto K, Kawamoto T, and Inoue N, et al (2016). Tumour endothelial cells in high metastatic tumours promote metastasis via epigenetic dysregulation of biglycan. *Sci Rep* **6**, 28039.

[14] Hu L, Duan YT, Li JF, Su LP, Yan M, Zhu ZG, Liu BY, and Yang QM (2014). Biglycan enhances gastric cancer invasion by activating FAK signaling pathway. *Oncotarget* **5**(7), 1885–1896.

[15] Weber CK, Sommer G, Michl P, Fensterer H, Weimer M, Gansauge F, Leder G, Adler G, and Gress TM (2001). Biglycan is overexpressed in pancreatic cancer and induces G1-arrest in pancreatic cancer cell lines. *Gastroenterology* **121**(3), 657–667.

[16] Munkley J, Mills IG, and Elliott DJ (2016). The role of glycans in the development and progression of prostate cancer. *Nat Rev Urol* **13**(6), 324–333.

[17] Knezevic D, Goddard AD, Natraj N, Cherbavaz DB, Clark-Langone KM, Snable J, Watson D, Falzarano SM, Magi-Galluzzi C, and Klein EA, et al (2013). Analytical validation of the Oncotype DX prostate cancer assay - a clinical RT-PCR assay optimized for prostate needle biopsies. *BMC Genomics* **14**, 690–702.

[18] Erbersdobler A, Hammerer P, Huland H, and Henke RP (1997). Numerical chromosomal aberrations in transition-zone carcinomas of the prostate. *J Urol* **158**(4), 1594–1598.

[19] Mirlacher M and Simon R (2010). Recipient block TMA technique. *Methods Mol Biol* **664**, 37–44.

[20] Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, Leighton S, Torhorst J, Mihatsch MJ, Sauter G, and Kallioniemi OP (1998). Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nat Med* **4**(7), 844–847.

[21] Weischenfeldt J, Simon R, Feuerbach L, Schlangen K, Weichenhan D, Minner S, Wuttig D, Warnatz HJ, Stehr H, and Rausch T, et al (2013). Integrative genomic analyses reveal an androgen-driven somatic alteration landscape in early-onset prostate cancer. *Cancer Cell* **23**(2), 159–170.

[22] Minner S, Enodien M, Sirma H, Luebke AM, Krohn A, Mayer PS, Simon R, Tennstedt P, Muller J, and Scholz L, et al (2011). ERG status is unrelated to PSA recurrence in radically operated prostate cancer in the absence of antihormonal therapy. *Clin Cancer Res* **17**(18), 5878–5888.

[23] Burkhardt L, Fuchs S, Krohn A, Masser S, Mader M, Kluth M, Bachmann F, Huland H, Steuber T, and Graefen M, et al (2013). CHD1 is a 5q21 tumor suppressor required for ERG rearrangement in prostate cancer. *Cancer Res* **73**(9), 2795–2805.

[24] Kluth M, Hesse J, Heinl A, Krohn A, Steurer S, Sirma H, Simon R, Mayer PS, Schumacher U, and Grupp K, et al (2013). Genomic deletion of MAP3K7 at 6q12-22 is associated with early PSA recurrence in prostate cancer and absence of TMPRSS2:ERG fusions. *Mod Pathol* **26**(7), 975–983.

[25] Krohn A, Diedler T, Burkhardt L, Mayer PS, De Silva C, Meyer-Kornblum M, Kotschau D, Tennstedt P, Huang J, and Gerhauser C, et al (2012). Genomic deletion of PTEN is associated with tumor progression and early PSA recurrence in ERG fusion-positive and fusion-negative prostate cancer. *Am J Pathol* **181**(2), 401–412.

[26] Krohn A, Seidel A, Burkhardt L, Bachmann F, Mader M, Grupp K, Eichenauer T, Becker A, Adam M, and Graefen M, et al (2013). Recurrent deletion of 3p13 targets multiple tumour suppressor genes and defines a distinct subgroup of aggressive ERG fusion-positive prostate cancers. *J Pathol* **231**(1), 130–141.

[27] Epstein JI, Feng Z, Trock BJ, and Pierorazio PM (2012). Upgrading and downgrading of prostate cancer from biopsy to radical prostatectomy: incidence and predictive factors using the modified Gleason grading system and factoring in tertiary grades. *Eur Urol* **61**(5), 1019–1024.

[28] Modolo F, Biz MT, Martins MT, Machado de Sousa SO, and de Araujo NS (2010). Expression of extracellular matrix proteins in adenomatoid odontogenic tumor. *J Oral Pathol Med* **39**(3), 230–235.

[29] Liu Y, Li W, Li X, Tai Y, Lu Q, Yang N, and Jiang J (2014). Expression and significance of biglycan in endometrial cancer. *Arch Gynecol Obstet* **289**(3), 649–655.

[30] Pan S, Cheng L, White JT, Lu W, Utleg AG, Yan X, Urban ND, Drescher CW, Hood L, and Lin B (2009). Quantitative proteomics analysis integrated with microarray data reveals that extracellular matrix proteins, catenins, and p53 binding protein 1 are important for chemotherapy response in ovarian cancers. *OMICS* **13**(4), 345–354.

[31] Iacob S and Cs-Szabo G (2010). Biglycan regulates the expression of EGF receptors through EGF signaling pathways in human articular chondrocytes. *Connect Tissue Res* **51**(5), 347–358.

[32] Wang H, Sun W, Ma J, Pan Y, Wang L, and Zhang WB (2015). Biglycan mediates suture expansion osteogenesis via potentiation of Wnt/beta-catenin signaling. *J Biomech* **48**(3), 432–440.

[33] Osman N, Getachew R, Burch M, Lancaster G, Wang R, Wang H, Zheng W, and Little PJ (2011). TGF-beta stimulates biglycan core protein synthesis but not glycosaminoglycan chain elongation via Akt phosphorylation in vascular smooth muscle. *Growth Factors* **29**(5), 203–210.

[34] Zhao Y, Scott A, Zhang P, Hao Y, Feng X, Somasundaram S, Khalil AM, Willis J, and Wang Z (2016). Regulation of paxillin-p130-PI3K-AKT signaling axis by Src and PTPRT impacts colon tumorigenesis. *Oncotarget*.

[35] Duchartre Y, Kim YM, and Kahn M (2016). The Wnt signaling pathway in cancer. *Crit Rev Oncol Hematol* **99**, 141–149.

[36] Martin GS (2003). Cell signaling and cancer. *Cancer Cell* **4**(3), 167–174.

[37] Padua D, Zhang XH, Wang Q, Nadal C, Gerald WL, Gomis RR, and Massague J (2008). TGFbeta primes breast tumors for lung metastasis seeding through angiopoietin-like 4. *Cell* **133**(1), 66–77.

[38] Ma F, Ye H, He HH, Gerrin SJ, Chen S, Tanenbaum BA, Cai C, Sowalsky AG, He L, and Wang H, et al (2016). SOX9 drives WNT pathway activation in prostate cancer. *J Clin Invest* **126**(5), 1745–1758.

[39] Iwasa K, Hayashi S, Fujishiro T, Kanzaki N, Hashimoto S, Sakata S, Chinzei N, Nishiyama T, Kuroda R, and Kurosaka M (2014). PTEN regulates matrix synthesis in adult human chondrocytes under oxidative stress. *J Orthop Res* **32**(2), 231–237.

[40] Doersch KM, Moses KA, and Zimmer WE (2016). Synergistic immunologic targets for the treatment of prostate cancer. *Exp Biol Med (Maywood)*.

[41] Fang F, Qin Y, Hao F, Li Q, Zhang W, Zhao C, Chen S, Zhao L, Wang L, and Cai J (2016). CD147 modulates androgen receptor activity through the Akt/Gsk-3beta/beta-catenin/AR pathway in prostate cancer cells. *Oncol Lett* **12**(2), 1124–1128.

[42] Recktenwald CV, Leisz S, Steven A, Mimura K, Muller A, Wulfanger J, Kiessling R, and Seliger B (2012). HER-2/neu-mediated down-regulation of biglycan associated with altered growth properties. *J Biol Chem* **287**(29), 24320–24329.

[43] Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, Varambally S, Cao X, Tchinda J, and Kuefer R, et al (2005). Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science* **310**(5748), 644–648.

[44] Brase JC, Johannes M, Mannsperger H, Falth M, Metzger J, Kacprzyk LA, Andrasiuk T, Gade S, Meister M, and Sirma H, et al (2011). TMPRSS2-ERG -specific transcriptional modulation is associated with prostate cancer biomarkers and TGF-beta signaling. *BMC Cancer* **11**, 507–515.

[45] Fang J, Xu H, Yang C, Kayarthodi S, Matthews R, Rao VN, and Reddy ES (2014). Molecular Mechanism of Activation of Transforming Growth Factor Beta/Smads Signaling Pathway in Ets Related Gene-Positive Prostate Cancers. *J Pharm Sci Pharmacol* **1**(1), 82–85.

[46] Fang J, Xu H, Yang C, Morsalin S, Kayarthodi S, Rungsrisuriyachai K, Gunnal U, McKenzie B, Rao VN, and Reddy ES (2014). Ets Related Gene and Smad3 Proteins Collaborate to Activate Transforming Growth Factor-Beta Mediated Signaling Pathway in ETS Related Gene-Positive Prostate Cancer Cells. *J Pharm Sci Pharmacol* **1**(3), 175–181.

[47] Tiede K, Melchior-Becker A, and Fischer JW (2010). Transcriptional and posttranscriptional regulators of biglycan in cardiac fibroblasts. *Basic Res Cardiol* **105**(1), 99–108.

[48] Heegaard AM, Xie Z, Young MF, and Nielsen KL (2004). Transforming growth factor beta stimulation of biglycan gene expression is potentially mediated by sp1 binding factors. *J Cell Biochem* **93**(3), 463–475.

[49] Schmitz B, Salomon A, Rotrige A, Ritter M, Ringelstein EB, Fischer JW, Paul M, Brand E, and Brand SM (2013). Interindividual transcriptional regulation of the human biglycan gene involves three common molecular haplotypes. *Arterioscler Thromb Vasc Biol* **33**(4), 871–880.

[50] Ji H, Dailey TL, Long V, and Chien EK (2008). Androgen-regulated cervical ripening: a structural, biomechanical, and molecular analysis. *Am J Obstet Gynecol* **198**(5) 543 e541-549.

[51] Hashimura K, Sudhir K, Nigro J, Ling S, Williams MR, Komesaroff PA, and Little PJ (2005). Androgens stimulate human vascular smooth muscle cell proteoglycan biosynthesis and increase lipoprotein binding. *Endocrinology* **146**(4), 2085–2090.

[52] Tan MH, Li J, Xu HE, Melcher K, and Yong EL (2015). Androgen receptor: structure, role in prostate cancer and drug discovery. *Acta Pharmacol Sin* **36**(1), 3–23.

[53] Bigler SA, Deering RE, and Brawer MK (1993). Comparison of microscopic vascularity in benign and malignant prostate tissue. *Hum Pathol* **24**(2), 220–226.

[54] Tuxhorn JA, Ayala GE, Smith MJ, Smith VC, Dang TD, and Rowley DR (2002). Reactive stroma in human prostate cancer: induction of myofibroblast phenotype and extracellular matrix remodeling. *Clin Cancer Res* **8**(9), 2912–2923.

[55] Burns-Cox N, Avery NC, Gingell JC, and Bailey AJ (2001). Changes in collagen metabolism in prostate cancer: a host response that may alter progression. *J Urol* **166**(5), 1698–1701.

[56] Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, Arora VK, Kaushik P, Cerami E, and Reva B, et al (2010). Integrative genomic profiling of human prostate cancer. *Cancer Cell* **18**(1), 11–22.

[57] Sun J, Liu W, Adams TS, Sun J, Li X, Turner AR, Chang B, Kim JW, Zheng SL, and Isaacs WB, et al (2007). DNA copy number alterations in prostate cancers: a combined analysis of published CGH studies. *Prostate* **67**(7), 692–700.

[58] Wang Y, He X, Ngeow J, and Eng C (2012). GATA2 negatively regulates PTEN by preventing nuclear translocation of androgen receptor and by androgen-independent suppression of PTEN transcription in breast cancer. *Hum Mol Genet* **21**(3), 569–576.

[59] Gaspar R, Pipicz M, Hawchar F, Kovacs D, Djirackor L, Gorbe A, Varga ZV, Kiricsi M, Petrovski G, and Gacser A, et al (2016). The cytoprotective effect of biglycan core protein involves Toll-like receptor 4 signaling in cardiomyocytes. *J Mol Cell Cardiol* **99**, 138–150.

[60] Li S, Zhu M, Pan R, Fang T, Cao YY, Chen S, Zhao X, Lei CQ, Guo L, and Chen Y, et al (2016). The tumor suppressor PTEN has a critical role in antiviral innate immunity. *Nat Immunol* **17**(3), 241–249.

[61] Frey H, Schroeder N, Manon-Jensen T, Iozzo RV, and Schaefer L (2013). Biological interplay between proteoglycans and their innate immune receptors in inflammation. *FEBS J* **280**(10), 2165–2179.

[62] Sauter G, Steurer S, Clauditz TS, Krech T, Wittmer C, Lutz F, Lennartz M, Janssen T, Hakimi N, and Simon R, et al (2016). Clinical Utility of Quantitative Gleason Grading in Prostate Biopsies and Prostatectomy Specimens. *Eur Urol* **69**(4), 592–598.

2. Darstellung der Publikation

2.1 Einleitung:

Prostatakrebs ist die häufigste Krebserkrankung und dritthäufigste tumorbedingte Todesursache bei Männern in Deutschland[1]. Patienten mit einer positiven Familienanamnese haben ein erhöhtes Risiko an Prostatakrebs zu erkranken[2,3]. Das Prostatakarzinom hat eine sehr langsame Wachstumsrate und bleibt aufgrund häufig fehlender Symptome in vielen Fällen ein postmortaler Zufallsbefund in der Autopsie[4]. Das Prostatakarzinom manifestiert sich klinisch fast ausschließlich in einem fortgeschrittenen Stadium und geht für die Patienten oft nur mit geringen Beschwerden einher. Durch Vorsorgeuntersuchung können Tumore oft früh erkannt werden[1,5]. Viele der durch Vorsorgeuntersuchung entdeckten Karzinome würden wahrscheinlich auch ohne Therapie nicht klinisch relevant werden[6]. Durch die stetige Forschung hat sich die Prognose für die Patienten mit Prostatakarzinomen im Laufe der Jahre immer weiter verbessert[1,7]. Der Erfolg einer Therapie hängt von vielen Faktoren ab. Das Alter des Patienten bei der Diagnose, das Tumorstadium, die persönlichen Lebensumstände und eventuelle Begleiterkrankungen spielen eine wichtige Rolle[1,6]. Man unterscheidet 4 Tumorstadien. Im ersten Stadium liegt der Tumor innerhalb der Prostata (pT2). Im zweiten Stadium infiltriert der Tumor die Kapsel (pT3a). Im nächsten Stadium infiltriert der Tumor die Samenblase (pT3b) und im letzten Stadium infiltriert der Tumor die weitere Umgebung (pT4)[8]. Je nachdem welches Stadium der Tumor erreicht hat und welche Voraussetzungen der Patient mitbringt, gibt es verschiedene Behandlungsmöglichkeiten: Die radikale Prostatektomie, die perkutane Strahlentherapie, die Brachytherapie, das abwartende Beobachten („Watchful Waiting“) oder die aktive Überwachung („Active Surveillance“)[9-12].

Für die Prognoseabschätzung gibt es mehrere etablierte Parameter: Das Gleason-Muster, das TNM-Stadium, der präoperative PSA und das klinische Stadium[6.] Derzeit reichen diese Parameter leider nicht aus, um Patienten vor einer Übertherapie zu schützen oder aber Hochrisikopatienten zu identifizieren. Mit den bislang in der klinischen Routine genutzten Prognoseparametern kann eine Unterscheidung dieser beiden Gruppen nicht sicher gewährleistet werden. Angesichts des stetig zunehmenden Wissens um die Genetik dieser Tumoren besteht die Hoffnung, dass durch molekulare Analysen eine Verbesserung der Prognosevorhersage und somit auch der Therapiestrategie ermöglicht wird.

Biglycan (BGN) gehört zu der Familie der kleinen leucinreichen Proteoglycane (SLRP), die durch ein Kernprotein mit Leucin-reichen Wiederholungen von Cystein Clustern charakterisiert sind[13]. BGN wird normalerweise von Fibroblasten in der extrazellulären Matrix sezerniert. Es erleichtert das Aggregieren von Kollagenfibrillen und Knochenmatrix[14]. BGN ist an der Antwort von Entzündungsprozessen beteiligt und wird auch durch tumorassozierte Signalwege wie dem Transforming growth factor ß (TGF-ß) Signalweg heraufreguliert [15-20]. Frühere Studien zeigten, dass die Prostata Glykoproteine aller Arten sezerniert, darunter auch Proteoglykane[18]. BGN könnte als prognostisch relevanter Marker bei Prostatakarzinomen in Frage kommen, da es Teil eines kommerziellen RNA-Expressionstests ist, mit welchem man die Aggressivität von

Prostakarzinomen einschätzen kann[19]. Zudem wurde auch bei anderen soliden Tumoren eine Prognoserelevanz der veränderten BGN Expression beschrieben[21-23]. Zum Beispiel wurde die Überexpression von BGN bei vielen malignen Epithelialkarzinomen einschließlich eines weiten Spektrums an gastrointestinal Karzinomen[20-23] und gynäkologischen Karzinomen[24] festgestellt. Bei einigen dieser Karzinomen war die BGN Überexpression mit einer erhöhten Metastatenrate[24-26] und einer ungünstigen Prognose[21,27] für den Patienten verbunden. Gegensätzliche Beobachtungen wurden bei anderen Tumortypen[27,28] gemacht, was vermuten lässt, dass die BGN Expression tumortyp-abhängig reguliert wird. Studien an Blasenkarzinomen haben gezeigt, dass Biglycan ein endogener Inhibitor der Blasenzellproliferation ist, welcher hochreguliert als Antwort auf antiproliferative Tyrosine Kinase Inhibitoren fungiert. Bei Blasenkarzinomen stand eine hohe Biglycan Expression im Zusammenhang mit einer günstigen Prognose[27].

Bei Prostatakarzinomen ist BGN sehr interessant, da es Teil eines kommerziellen RNA-Expressionstest ist, mit welchem man die Aggressivität von Prostakarzinomen einschätzen kann[19]. Um die Rolle von BGN bei Prostatakarzinomen aus biologischer Sicht und im Zusammenhang mit den molekularen Eigenschaften besser zu verstehen, wurden in dieser Doktorarbeit anhand eines Tissue Microarrays (TMA) mehr als 12.400 Prostatakarzinome immunhistochemisch auf die BGN Expression untersucht. Die Datenbank des verwendeten TMAs beinhaltet pathologische und klinische Daten.

2.2 Material und Methoden

Patientenkollektiv: Für die vorliegende Studie wurde ein Gewebemikroarray (TMA) mit Gewebeproben von 12.427 Patienten verwendet. Von jedem Patienten wurde ein repräsentativer Tumorgewebeblock ausgewählt und jeweils eine 0,6mm Stanze entnommen, die in den TMA eingebracht wurde. Alle Patienten wurden in der Martini-Klinik, des Universitätsklinikums Hamburg Eppendorf prostaektomiert. In der Datenbank zu dem TMA wurden klinisch-pathologische Parameter und zahlreiche molekulare Parameter erfasst. In dieser Studie wurden Immunhistochemie-Daten der ERG- und Androgenrezeptor-expression[29,30] sowie des Ki67 labeling index (LI) und Fluoreszenz in Situ Hybridisierungs- (FISH) Daten von *ERG*-Rearregement sowie *PTEN*-, 6q-, 5q- und 3p-Deletionen aus vorhergehenden Studien verwendet[31-34].

Immunhistochemie: Frisch angefertigte 4 µm-Schnitte des TMAs wurden immunhistochemisch am selben Tag und in einem Experiment gefärbt. Es wurde ein BGN spezifischer Primärantikörper (Kaninchen, polyklonal, Sigma-Aldrich, Deutschland; cat#HPA003157; Verdünnung 1:1350) verwendet. BGN spezifische Färbung im Zytoplasma war typischerweise in allen Tumorzellen (100%) positiver Tumore sichtbar. Die Färbeintensität wurde in drei Stufen (negativ, gering, hoch) eingeteilt.

2.3 Ergebnisse:

Die wichtigsten Ergebnisse unserer Studie sind:

- Unter den gewählten experimentellen Bedingungen zeigte normales Prostataepithel keine BGN Immunofärbung. Im Tumorgewebe hingegen war die Färbung im Zytoplasma lokalisiert. Positive BGN Immunofärbung konnte in 8.701 (78,7%) interpretierbaren Prostatakarzinomen gezeigt werden, davon 47,7% mit geringer Intensität und 31,1% mit starker Intensität.
- Eine starke BGN Expression ist mit dem Vorliegen einer *TMPRSS2:ERG* Fusion hochsignifikant assoziiert.
- Eine starke BGN Expression ist assoziiert mit einem fortgeschrittenen pT Stadium, einem hohen Gleason-Grad, Lymphknotenmetastasen, hohen präoperativen PSA-Werten und positiven chirurgischem Rand.
- Eine starke BGN Expression ist signifikant mit allen Deletionen in allen Tumoren assoziiert. Für *PTEN*-Deletionen konnte eine hohe Signifikanz zusätzlich in den Tumorsubgruppen (ERG positiv, ERG negativ) gezeigt werden.
- Eine starke BGN Expression war signifikant mit einer hohen Zellproliferationsrate (Ki67LI) assoziiert, dies konnte auch bei identischen Gleason-Grad Subgruppen gezeigt werden.
- Die Androgenrezeptor (AR) Expression korreliert signifikant mit der BGN Expression. Eine starke BGN Expression wurde bei 16,9% der Tumoren ohne AR Expression gemessen. Diese stieg auf 36,8% bei einer starken AR Expression an.
- Die BGN Expression war signifikant mit einem frühen PSA Rezidiv assoziiert.
- Multivariante Analysen ergaben, dass die BGN Expression keine unabhängige prognostische Information lieferte.

2.4 Diskussion:

Die Ergebnisse unserer Studie zeigen, dass eine erhöhte BGN Expression bei Prostata Karzinomen ein schwacher Prognosemarker ist. Die immunhistochemische Analyse ergab eine zytoplasmatische BGN Färbung in 80,0% der 11.050 interpretierbaren Prostatakarzinome. Das Fehlen einer BGN Färbung im normalen Prostataepithelium lässt darauf schließen, dass es während der Tumorgenese des Prostatakarzinoms zu einer Hochregulation der BGN Expression kommt. Immunohistchemische BGN Expressionsdaten bei Prostatakarzinomen gibt es in der Literatur bisher nicht. Eine tumorassoziierte Hochregulierung von BGN wurde aber zum Beispiel beim Ösophagus-[22], Pankreas-[20,28], Magen-[23], Blasen-[27], Colorektal-[21], Odontogenen-[35], Endometrial-[36] und Ovarialkarzinom[37] gefunden.

Unsere Studie konnte eine signifikante Assoziation der BGN Überexpression mit einem ungünstigen Phänotyp, fortgeschrittenen pT Stadium, hohen Gleason-Grad, hoher

Zellproliferationsrate, Lymphknotenmetastasen und frühem biochemischen Rezidiv zeigen. Diese Ergebnisse stimmen mit Studien an anderen Tumortypen überein. Bei Pankreas-, Magen- und Endometriumkarzinomen konnte ebenfalls gezeigt werden, dass eine BGN Überexpression mit einer hohen Tumoraggressivität und einem kurzen Patientenüberleben assoziiert ist[20,23,36].

Ursachen für die tumorassoziierte Hochregulierung von BGN könnten durch die Funktion von BGN begründet sein. BGN ist ein nachgeschaltetes Zielprotein in verschiedenen Wachstums- und Signaltransduktionswegen, wie zum Beispiel den TGF-ß, Wnt- und Akt-Signalwegen[37-40]. Deren Aktivierung wurde von verschiedenen Autoren bei zahlreichen Tumorentypen[41-44] einschließlich dem Prostatakarzinom[45-48] nachgewiesen. Die generelle Bedeutung dieser Signaltransduktionswege bei Tumoren könnte daher nicht nur den hohen Anteil an BGN expremierenden Tumoren erklären, sondern auch die vergleichsweise geringe prognostische Bedeutung von BGN. Die Hochregulierung der BGN Expression scheint auch im Zusammenhang mit der Reduzierung der Zellproliferation in einigen Tumor- und Fibroblasten-Zelllinien[27,28,49] zu stehen. Dies lässt auf Zelltyp-abhängigen Effekte der BGN Expression schließen.

Die molekulare Datenbank, die zu diesem TMA gehört, ermöglicht Zusammenhänge mit häufigen genetischen Veränderungen zu erfassen. Über 50 % der Prostatakarzinome weisen eine Genfusion der androgen-regulierten Serin-Protease TMPRSS2 und dem ETS-Transkriptionsfaktor ERG auf. Durch diese Fusion kommt es zu einer androgenvermittelten Überexpression von ERG, die letztlich zu einer transkriptionalen Dysregulation von mehr als 1600 ERG Zielgenen führt [29,50,51]. Mehrere Arbeitsgruppen konnten zeigen, dass die Aktivierung des TGF-ß-Signalweges eine typische Konsequenz aus der ERG Fusion in Prostatakarzinomen ist [50,52,53]. TGF-ß1 ist ein wichtiger Aktivator der transkriptionalen und posttranslationalen Modifikationen von BGN und somit ein zentraler Modulator der extrazelluläre Matrix [54,55]. Dies erklärt möglicherweise den hohen Anteil an Tumoren mit einer vermehrten BGN Expression in den ERG positiven Karzinomen im Vergleich zu den ERG negativen Karzinomen. In früheren Arbeiten konnte mittels digitaler Promotoranalyse eine Bindungsstelle für den ETS/ERG Transkriptionsfaktor am BGN Promoter nachgewiesen werden[56]. Dies lässt vermuten, dass aktiviertes ERG einen direkten Einfluss auf die Hochregulierung der BGN Expression in Prostatatumorzellen haben könnte. Die Assoziation der BGN Überexpression mit der AR Expressionsstärke lässt zudem auf eine androgenabhängige BGN Regulation bei Prostatakarzinomen schließen. Dies wird durch eine Studie an der Cervix gestützt[57]. Dort wird gezeigt, dass Androgen eine Rolle bei der zielgerichteten Kollagen- und Proteoglycan Organisation spielt. Des Weiteren konnte eine Androgen abhängige Synthese von BGN in glatten Gefäßmuskelzellen gezeigt werden[58]. Zusammengenommen kann spekuliert werden, dass durch ein erhöhtes AR Signal eine androgen-induzierte Hochregulierung der BGN Expression in Prostatatumoren zu einer Veränderung der extrazellulären Matrix führt. Prostatagewebe reagiert auf die Tumorzellinvasion mit Wundheilung. Diese ruft eine erhöhte Zelldichte, vermehrte Durchblutung und Kollagen-Ablagerungen in dem Stroma hervor[59,60]. Auch andere Marker der Kollagensynthese, wie Serineproteasen, Matrix Metalloproteasen und Bestandteile von Typ I Kollagen wurden signifikant erhöht in

Prostatatumorzellen und im umliegenden Gewebe[61] gefunden.

Häufige Deletionen wie PTEN, 6q15, 5q21 und 3p13 sind weitere Merkmale von Prostatakarzinomen[62,63]. Aus der PTEN Deletion resultiert eine Aktivierung des AKT Signalweges, die mit Tumorwachstum, Progression und einem schlechten klinischen Verlauf assoziert ist[64]. Von allen untersuchten Deletionen ist die PTEN Deletion mit der BGN Überexpression am stärksten assoziiert. Dies lässt sich durch einen Zusammenhang zwischen BGN mit dem AKT Signalweg erklären[13,38]: Eine frühere Arbeit konnte zeigen, dass eine stark erhöhte Konzentration an löslichem BGN in der extrazellulären Matrix ein Zellwachstum sowie Zellüberleben induzieren kann. Die Aktvierung des AKT-vorgeschalteten EGF-Rezeptors erfolgt durch lösliches BGN[38,65]. Ein aktivierter AKT-Signalweg ist wiederum für die BGN Protein-Biosynthese notwendig[40]. Ein solcher Regelkreis könnte eine Erklärung für die deutliche Hochregulierung der BGN-Expression in PTEN-deletierten und somit AKT-hyperaktiven Tumoren sein. Eine weitere Funktion des BGNs konnte kürzlich Li et al zeigen. Sie beschreiben eine entscheidende Rolle von PTEN bei der Induktion der angeborenen Immunität[66]. PTEN induziert den Signalweg über das Alpha-Interferon. Da BGN ein endogener Ligand der Toll-like Rezeptoren ist, die das angeborenen Immunsystem[67]aktivieren, kann man vermuten, dass ein Verlust von PTEN, die BGN Hochregulierung während der Entwicklung von aggressiven Prostatakarzinomen noch verstärkt.

Die BGN Expression zeigt in der univariaten Analyse eine prognostische Bedeutung, während diese in den multivariaten Analysen mit etablierten morphologischen Parametern nicht bestätigt werden kann. Der Vergleich von morphologischen Parametern mit konkurrierenden Biomarkern zur Vorhersage der Aggressivität der Prostatakarzinome lässt sich am besten durch Analysen innerhalb einer gleichen morphologischen Gruppe beurteilen. Bei traditionellen Gleason Gruppen hatte die BGN Expression keine zusätzliche Aussagekraft auf die Prognose. Basierend auf einer großen Kohorte Prostatakarzinome an unserem Institut, konnte die UKE Arbeitsgruppe kürzlich zeigen, dass die prognostische Information des Gleason Grades verfeinert werden kann, in dem man den Anteil der Gleason Grad 4 als laufende Variable festlegt (quantitativer Gleason Grad)[66]. Sowohl bei den Biopsien als auch bei den Prostataektomie Proben verschlechtert sich die Prognose kontinuierlich mit einer Zunahme des prozentualen Gleason Grad 4 Anteils. Der Verlust der prognostischen Aussagekraft der BGN Expression in allen Subgruppen des quantitativen Gleason-Musters ist ein Beispiel für die Problematik eines Biomarkers gegenüber morphologischen Malignitätsparameter in Prostatakarzinomen. Eine Kombination mehrerer molekularer Marker könnte im Vergleich zu einem einzelnen molekularen Marker möglicherweise eine deutlich bessere Prognosevorhersage ermöglichen.

Zusammenfassend lässt sich sagen, dass eine BGN Überexpression ein häufiges Merkmal von Prostatakarzinomen ist, die im Zusammenhang mit der Tumor-Progression und einer *PTEN* Deletionen steht.

2.5 Zusammenfassung:

Biglycan (BGN) ist ein Proteoglykan der extrazellulären Matrix und Bestandteil einer mRNA Signatur zur Abschätzung der Aggressivität von Prostatakarzinomen. Um die prognostische Aussagekraft von BGN und seine Beziehung zu definierten molekularen Untergruppen besser zu verstehen, haben wir die BGN Expression immunhistochemisch auf TMAs mit 12.427 Prostatakarzinomproben untersucht. 78% der 11.050 interpretierbaren Karzinomen zeigten eine BGN Expression. Davon wiesen 47,7 % eine geringe und 31,1 % eine starke Intensität auf. Die BGN Proteinexpression stieg mit einem erhöhten pathologischen Tumorstadium, Gleason-Grad, Lymphknotenmetastasen und einer erhöhten PSA-Rezidiv-Wahrscheinlichkeit an. Bei einem Vergleich der zu den TMAs gehörigen molekularen Datenbank mit der BGN Expression war eine Assoziation mit einer *TMPRSS2-ERG* Fusion und PTEN Deletion erkennbar (P<.0001 jeweils). Zudem war die BGN Expression stark mit dem Androgen-Rezeptor Expression assoziiert, was auf eine hormon-abhängige Regulation von BGN schließen lässt. Die Hochregulierung von BGN ist ein häufiges Merkmal bei Prostatakarzinomen, welches mit der Tumor Progression in Verbindung steht. Dies kann dabei helfen, die Tumoraggressivität in Verbindung mit anderen molekularen Markern besser einzuschätzen.

2.6 Abstract

Biglycan (BGN), a proteoglycan of the extracellular matrix, is included in mRNA signatures for prostate cancer aggressiveness. To understand the impact of BGN on prognosis and its relationship to molecularly defined subsets, we analyzed BGN expression by immunohistochemistry on a tissue microarray containing 12,427 prostate cancers. Seventy-eight percent of 11,050 interpretable cancers showed BGN expression, which was considered as low intensity in 47.7% and as high intensity in 31.1% of cancers. BGN protein expression rose with increasing pathological tumor stage, Gleason grade, lymph node metastasis and early PSA recurrence (P<.0001 each). Comparison with our molecular database attached to the TMA revealed that BGN expression was linked to presence of *TMPRRS2:ERG* fusion and PTEN deletion (P<.0001 each). In addition, BGN was strongly linked to androgen-receptor (AR) levels (P<.0001), suggesting a hormone-depending regulation of BGN. BGN up-regulation is a frequent feature of prostate cancer that parallels tumor progression and may be useful to estimate tumor aggressiveness particularly if combined with other molecular markers.

3. Literaturverzeichnis

1 Robert Koch Institut (RKI) Gesellschaft der epidmiologischen Krebsregister in Deutschland (GEKID) Krebs in Deutschland 2013/2014: 11 Ausgabe, Berlin: RKI, Stand 06.12.17

2 Jansson, K.F., et al. Concordance of tumor differentiation among brothers with prostate cancer. Eur Urol, 2012. 62: 656. 1 Haas, G.P., et al. The worldwide epidemiology of prostate cancer: perspectives from autopsy studies. Can J Urol, 2008. 15: 3866.

3 Johns LE, Houlston RS. A systematic review and meta-analysis of familial prostate cancer risk. BJU Int 2003;91(9):789-94

4 Haas, G.P., et al. The worldwide epidemiology of prostate cancer: perspectives from autopsy studies. Can J Urol, 2008. 15: 3866.

5 Haas GP et al. The worldwide epidemiology of prostate cancer: perspectives from autopsy studies. Can J Urol 2008;15(1):3866-71

6 Interdisziplinäre Leitlinie der Qualität S3 zur Früherkennung, Diagnose und Therapie der verschiedenen Stadien des Prostatakarzinoms. Langversion 3.1 2014 AWMF-Register-Nummer 043/022OL

7 Schröder FH et al. Prostate-cancer mortality at 11 years of follow-up. N Engl J Med 2012;366(11):981-90

8 Epstein, J.I., et al. The 2005 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma. Am J Surg Pathol, 2005. 29: 1228.

9 Ip S et al. An evidence review of active surveillance in men with localized prostate cancer. Evid Rep Technol Assess (Full Rep) 2011;(204):1-341

10 Klotz L et al. Clinical results of long-term follow-up of a large, active surveillance cohort with localized prostate cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2010;28(1):126-31

11 Bill-Axelson et al. Radical prostatectomy versus watchful waiting in early prostate cancer. The New England journal of medicine 2005;352(19):1977-84

12 Nilsson S, Norlen BJ, Widmark A. A systematic overview of radiation therapy effects in prostate cancer. Acta Oncol 2004;43(4):316-81

13 Schaefer, L and Iozzo, RV. Biological functions of the small leucine-rich proteoglycans: from genetics to signal transduction. J Biol Chem. 2008; 283: 21305–21309

14 Xing, X, Gu, X, Ma, T, and Ye, H. Biglycan up-regulated vascular endothelial growth

factor (VEGF) expression and promoted angiogenesis in colon cancer. Tumour Biol. 2015; 36: 1773–1780

15 Nastase, MV, Young, MF, and Schaefer, L. Biglycan: a multivalent proteoglycan providing structure and signals. J Histochem Cytochem. 2012; 60: 963–975

16 Hildebrand et al. Interaction of the small interstitial proteoglycans biglycan, decorin and fibromodulin with transforming growth factor beta. Biochem J. 1994; 302: 527–534

17 Schaefer et al. The matrix component biglycan is proinflammatory and signals through Toll-like receptors 4 and 2 in macrophages. J Clin Invest. 2005; 115: 2223–2233

18 Munkley, J, Mills, IG, and Elliott, DJ. The role of glycans in the development and progression of prostate cancer. Nat Rev Urol. 2016; 13: 324–333

19 Knezevic et al. Analytical validation of the Oncotype DX prostate cancer assay - a clinical RT-PCR assay optimized for prostate needle biopsies. BMC Genomics. 2013; 14: 690–702

20 Aprile et al. Biglycan expression and clinical outcome in patients with pancreatic adenocarcinoma. Tumour Biol. 2013; 34: 131–137

21 Gu et al. Up-regulated biglycan expression correlates with the malignancy in human colorectal cancers. Clin Exp Med. 2012; 12: 195–199

22 Zhu et al. High expression of biglycan is associated with poor prognosis in patients with esophageal squamous cell carcinoma. Int J Clin Exp Pathol. 2013; 6: 2497–2505

23 Wang et al. Biglycan expression correlates with aggressiveness and poor prognosis of gastric cancer. Exp Biol Med (Maywood). 2011; 236: 1247–1253

24 Sun et al. Biglycan enhances the ability of migration and invasion in endometrial cancer. Arch Gynecol Obstet. 2016; 293: 429–438

25 Maishi et al. Tumour endothelial cells in high metastatic tumours promote metastasis via epigenetic dysregulation of biglycan. Sci Rep. 2016; 6: 28039

26 Hu et al. Biglycan enhances gastric cancer invasion by activating FAK signaling pathway. Oncotarget. 2014; 5: 1885–1896

27 Niedworok et al. Inhibitory role of the small leucine-rich proteoglycan biglycan in bladder cancer. PLoS One. 2013; 8: e80084

28 Weber et al. Biglycan is overexpressed in pancreatic cancer and induces G1-arrest in pancreatic cancer cell lines. Gastroenterology. 2001; 121: 657–667

29 Weischenfeldt J et al. Integrative genomic analyses reveal an androgen-driven somatic alteration landscape in early-onset prostate cancer. Cancer Cell. 2013;23(2):159–170.

30 Minner S et al. ERG status is unrelated to PSA recurrence in radically operated prostate cancer in the absence of antihormonal therapy. Clin Cancer Res. 2011;17(18):5878–5888.

31 Burkhardt L et al. CHD1 is a 5q21 tumor suppressor required for ERG rearrangement in prostate cancer. Cancer Res. 2013;73(9):2795–2805.

32 Kluth M et al. Genomic deletion of MAP3K7 at 6q12-22 is associated with early PSA recurrence in prostate cancer and absence of TMPRSS2:ERG fusions. Mod Pathol. 2013;26(7):975–983.

33 Krohn A et al. Genomic deletion of PTEN is associated with tumor progression and early PSA recurrence in ERG fusion-positive and fusion-negative prostate cancer. Am J Pathol. 2012;181(2):401–412.

34 Krohn A et al. Recurrent deletion of 3p13 targets multiple tumour suppressor genes and defines a distinct subgroup of aggressive ERG fusion-positive prostate cancers. J Pathol. 2013;231(1):130–141.

35 Modolo F, Biz MT, Martins MT, Machado de Sousa SO, de Araujo NS. Expression of extracellular matrix proteins in adenomatoid odontogenic tumor. J Oral Pathol Med. 2010;39(3):230–235.

36 Liu Y et al. Expression and significance of biglycan in endometrial cancer. Arch Gynecol Obstet. 2014;289(3):649–655.

37 Pan S et al. Quantitative proteomics analysis integrated with microarray data reveals that extracellular matrix proteins, catenins, and p53 binding protein 1 are important for chemotherapy response in ovarian cancers. OMICS. 2009;13(4):345–354.

38 Iacob S, Cs-Szabo G. Biglycan regulates the expression of EGF receptors through EGF signaling pathways in human articular chondrocytes. Connect Tissue Res. 2010;51(5):347–358.

39 Wang H et al. Biglycan mediates suture expansion osteogenesis via potentiation of Wnt/beta-catenin signaling. J Biomech. 2015;48(3):432–440.

40 Osman N et al. TGF-beta stimulates biglycan core protein synthesis but not glycosaminoglycan chain elongation via Akt phosphorylation in vascular smooth muscle. Growth Factors. 2011;29(5):203–210.

41 Zhao Y et al. Regulation of paxillin-p130-PI3K-AKT signaling axis by Src and PTPRT impacts colon tumorigenesis. Oncotarget. 2016

42 Duchartre Y, Kim YM, Kahn M. The Wnt signaling pathway in cancer. Crit Rev Oncol Hematol. 2016;99:141–149.

43 Martin GS. Cell signaling and cancer. Cancer Cell. 2003;4(3):167–174.

44 Padua D et al. TGFbeta primes breast tumors for lung metastasis seeding through angiopoietin-like 4. Cell. 2008;133(1):66–77.

45 Ma F et al. SOX9 drives WNT pathway activation in prostate cancer. J Clin Invest. 2016;126(5):1745–1758.

46 Iwasa K et al. PTEN regulates matrix synthesis in adult human chondrocytes under oxidative stress. J Orthop Res. 2014;32(2):231–237.

47 Doersch KM, Moses KA, Zimmer WE. Synergistic immunologic targets for the treatment of prostate cancer. Exp Biol Med (Maywood) 2016

48 Fang F et al. CD147 modulates androgen receptor activity through the Akt/Gsk-3beta/beta-catenin/AR pathway in prostate cancer cells. Oncol Lett. 2016;12(2):1124–1128.

49 Recktenwald CV et al. HER-2/neu-mediated down-regulation of biglycan associated with altered growth properties. J Biol Chem. 2012;287(29):24320–24329.

50 Brase JC et al. TMPRSS2-ERG -specific transcriptional modulation is associated with prostate cancer biomarkers and TGF-beta signaling. BMC Cancer. 2011;11:507–515.

51 Tomlins SA et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science. 2005;310(5748):644–648.

52 Fang J et al. Molecular Mechanism of Activation of Transforming Growth Factor Beta/Smads Signaling Pathway in Ets Related Gene-Positive Prostate Cancers. J Pharm Sci Pharmacol. 2014;1(1):82–85.

53 Fang J et al. Ets Related Gene and Smad3 Proteins Collaborate to Activate Transforming Growth Factor-Beta Mediated Signaling Pathway in ETS Related Gene-Positive Prostate Cancer Cells. J Pharm Sci Pharmacol. 2014;1(3):175–181.

54 Tiede K, Melchior-Becker A, Fischer JW. Transcriptional and posttranscriptional regulators of biglycan in cardiac fibroblasts. Basic Res Cardiol. 2010;105(1):99–108.

55 Heegaard AM, Xie Z, Young MF, Nielsen KL. Transforming growth factor beta stimulation of biglycan gene expression is potentially mediated by sp1 binding factors. J Cell Biochem. 2004;93(3):463–475.

56 Schmitz B et al. Interindividual transcriptional regulation of the human biglycan gene involves three common molecular haplotypes. Arterioscler Thromb Vasc Biol. 2013;33(4):871–880.

57 Ji H, Dailey TL, Long V, Chien EK. Androgen-regulated cervical ripening: a structural, biomechanical, and molecular analysis. Am J Obstet Gynecol. 2008;198(5) 543 e541-549.

58 Hashimura K et al. Androgens stimulate human vascular smooth muscle cell proteoglycan biosynthesis and increase lipoprotein binding. Endocrinology.

2005;146(4):2085–2090.

59 Bigler SA, Deering RE, Brawer MK. Comparison of microscopic vascularity in benign and malignant prostate tissue. Hum Pathol. 1993;24(2):220–226.

60 Tuxhorn JA et al. Reactive stroma in human prostate cancer: induction of myofibroblast phenotype and extracellular matrix remodeling. Clin Cancer Res. 2002;8(9):2912–2923.

61 Burns-Cox N, Avery NC, Gingell JC, Bailey AJ. Changes in collagen metabolism in prostate cancer: a host response that may alter progression. J Urol. 2001;166(5):1698–1701.

62 Taylor BS et al. Integrative genomic profiling of human prostate cancer. Cancer Cell. 2010;18(1):11–22.

63 Sun J et al. DNA copy number alterations in prostate cancers: a combined analysis of published CGH studies. Prostate. 2007;67(7):692–700.

64 Wang Y, He X, Ngeow J, Eng C. GATA2 negatively regulates PTEN by preventing nuclear translocation of androgen receptor and by androgen-independent suppression of PTEN transcription in breast cancer. Hum Mol Genet. 2012;21(3):569–576.

65 Gaspar R et al. The cytoprotective effect of biglycan core protein involves Toll-like receptor 4 signaling in cardiomyocytes. J Mol Cell Cardiol. 2016;99:138–150.

66 Li S et al. The tumor suppressor PTEN has a critical role in antiviral innate immunity. Nat Immunol. 2016;17(3):241–249.

4. Eigenanteil:

- Herstellung des TMAs
- Auswahl des Proteins BGN für die Studie
- Literaturrecherche zu BGN bei Tumoren, genetischen und molekularen Veränderungen, Prognose-Parametern beim Prostatakarzinomen sowie zu molekularen Prognosetests für die Einschätzung von Prostatakarzinomen
- Auswertung der immunhistochemischen Färbung unter Anleitung
- Statistische Auswertung der Ergebnisse unter Anleitung
- Erstellen der ersten Version der Publikation mit Abbildungen und Tabellen

Anteil der Co-Autoren:

- Pathologische Beurteilung und IHC-Analyse: Frank Jacobsen, Cornelia Schroeder, Andrea Hinsch, Andreas Luebke, Sarah Minner, Till Clauditz
- Statistische Auswertung, Datenakquisitation und Datenbankgenerierung: Claudia Hube-Magg, Martina Kluth, Dagmar Lang, Jakob Izbicki, Hartwig Huland, Markus Graefen, Lars Budäus, Imke Thederan, Georg Salomon
- Erstellung des Manuskriptes: Frank Jacobsen, Ronald Simon, Doris Höfelmayer, Maria Christina Tsourlakis, Corinna Wittmer, Waldemar Wilczak, Franziska Büscheck
- Studiendesign, Studienkoordination und fachliche Revision: Nathaniel Melling, Ronald Simon, Thorsten Schlomm und Guido Sauter

5. Danksagung

An dieser Stelle möchte ich mich bei allen bedanken, die am Zustandekommen dieser Arbeit beteiligt waren. Mein besonderer Dank geht an meinen Doktorvater Herrn Prof. Dr. med. Guido Sauter, der es mir ermöglicht hat am Institut für Pathologie zu promovieren und mir mit seinem umfassenden Wissen immer zur Seite stand.

Ich möchte mich auch beim TMA-Labor bedanken, vor allem bei Christiana Möller-Koop, die mich mit den Geräten und dem Material vertraut gemacht hat und mich vom ersten Tag an unterstützte. Bei PD Dr. Ronald Simon und Dr. Claudia Hube-Magg für die gute Zusammenarbeit, ihre konstruktive Kritik und Hilfestellungen. Mein Dank gilt auch den anderen Doktoranten für die kollegiale Zusammenarbeit.

Vielen Dank an meine Eltern, die mich stets motiviert haben und immer für mich da sind.

6. Lebenslauf

Lebenslauf entfällt aus datenschutzrechtlichen Gründen

7. Eidesstattliche Versicherung

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe. Ferner versichere ich, dass ich die Dissertation bisher nicht einem Fachvertreter an einer anderen Hochschule zur Überprüfung vorgelegt oder mich anderweitig um Zulassung zur Promotion beworben habe. Ich erkläre mich einverstanden, dass meine Dissertation vom Dekanat der Medizinischen Fakultät mit einer gängigen Software zur Erkennung von Plagiaten überprüft werden kann.

Unterschrift: