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Down-Regulation of S100A8 is an Independent Predictor of PSA Recurrence in Prostate Cancer Treated by Radical Prostatectomy[1]

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Abstract

Dysregulation of S100A8 is described in many different human tumor types, but its role in prostate cancer is unknown. To evaluate the clinical relevance of S100A8 expression in prostate cancer, a tissue microarray containing 13,665 tumors was analyzed by immunohistochemistry. Cytoplasmic S100A8 staining was compared to prostate cancer phenotype, patient prognosis and molecular features including *TMPRSS2:ERG* fusion status and deletions of *PTEN*, 3p, 5q and 6q. S100A8 immunostaining was typically seen in normal prostate tissue but lost in 60% of 9786 interpretable prostate cancers. In the remaining tumors, S100A8 was considered weak in 17.9%, moderate in 17.8% and strong in 5.4% of cases. Loss of S100A8 expression was linked to advanced tumor stage, high Gleason grade, positive nodal status, positive surgical margin and high preoperative PSA ($P < .0001$ each). In addition, loss of S100A8 expression was associated with *TMPRSS2:ERG* fusions ($P < .0001$), deletions of *PTEN*, 3p, and 6q ($P < .005$), and a high number of genomic deletions per tumor ($P = .0009$). Absence of S100A8 immunostaining was also linked to an elevated risk for early PSA recurrence ($P < .0001$). In a multivariate analysis limited to features that are preoperatively available, the prognostic impact of S100A8 expression ($P < .0001$) was independent of clinical stage, Gleason grade, and serum PSA level ($P < .0001$). Taken together, the results of our study demonstrate that complete loss of S100A8 expression is linked to adverse tumor features and predicts early biochemical recurrence in prostate cancer. S100A8 measurement, either alone or in combination might be of clinical utility in prostate cancers.

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Abbreviations: CHD1, chromodomain-helicase-DNA-binding protein 1; ERG, erythroblast transformation-specific (ETS) related gene; ETS, erythroblast transformation-specific; FISH, fluorescence in situ hybridization; FOXP1, forkhead box protein P1; MAP3K7, mitogen-activated protein kinase kinase kinase 7; PSA, prostate specific antigen; PTEN, phosphatase and tensin homolog; S100A8, S100 calcium-binding protein A8; TMA, tissue microarray; TMPRSS2, transmembrane protease, serine 2

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Introduction

In Western societies prostate cancer is the most prevalent male cancer [1]. The majority of prostate cancers behave in an indolent manner. But a small subset is aggressive and needs extensive treatment [2,3]. The challenge is to predict these aggressive and potentially metastatic cancers. Currently established preoperative prognostic parameters are Gleason grade and tumor extent on biopsies, prostate-specific antigen (PSA), and clinical stage. While these data are statistically powerful in the cohort, they are suboptimal for individual treatment decisions. Thus it is hoped that novel molecular markers will improve prediction of prostate cancer aggressiveness.

The S100 protein family comprises more than 20 closely related calcium-binding proteins, which can be actively or passively secreted by the cells. S100 proteins regulate intracellular Ca^{2+} levels and influence numerous Ca^{2+} dependent signaling pathways contributing to proliferation, differentiation, apoptosis, energy metabolism and inflammation [4]. Genes encoding S100 proteins have been subdivided into 5 groups according to their genomic localization on chromosome 1 (S100A), chromosome X (S100G), chromosome 21 (S100B), chromosome 4 (S100P) and chromosome 5 (S100Z). The largest subgroup (S100A) consists of 16 family members (S100A1-S100A16), which are located into two tandem clusters within a 2 Mb region on chromosome 1q21.

Dysregulation of various S100 protein family members have been reported to occur in almost all human cancer types and typically involves their up-regulation [5]. For example, S100B is an established biomarker for malignant melanoma [6], and S100P may be helpful for pancreatic cancer detection [7]. Aberrant expression of several members of the S100A family have been linked to adverse outcome in multiple cancer types [5]. Among this subgroup, most data are available for the closely related S100A8 and S100A9 proteins, which can function as homodimers or heterodimers also known as calprotectin [8]. S100A8 and/or S100A9 up-regulation is described in many different cancer types like gastric cancer [9–11], cervical cancer [12], colon cancer [13–15], breast cancer [16–19], liver cancer [20], thyroid cancer [21], lung cancer [22] and renal cancer [23] and has been linked to poor patient prognosis in some of them [9,13,14,17,20,21]. In prostate cancer, studies on 75 and 167 patients suggested that S100A9 overexpression may be linked to advanced tumor stage and poor outcome [24,25], but the role of S100A8 is unknown.

In order to better understand the significance of S100A8 expression in prostate cancer, we took advantage of our existing prostate cancer tissue microarray (TMA) with attached database containing histological, clinical, and molecular data from more than 13,000 patients.

Material and Methods

Patients

Radical prostatectomy specimens were available from 13,665 patients, undergoing surgery between 1992 and 2014 at the Department of Urology and the Martini Clinics at the University Medical Center Hamburg-Eppendorf. Histopathological data were retrieved from the patient files, including tumor stage, Gleason grade, nodal stage and stage of the resection margin. In addition to the classical Gleason categories, "quantitative" Gleason grading was performed as described before [26]. In brief, for every prostatectomy specimen, the percentages of Gleason 4 patterns in cancerous tissues were estimated during the regular process of pathologic interpretation. Gleason 3 + 4 and 4 + 3 cancers were subdivided according to their percentage of Gleason 4 in 8 subgroups: 3 + 4 ≤ 5% Gleason 4, 3 + 4 6–10%, 3 + 4 11–20%, 3 + 4 21–30%, 3 + 4 31–49%, 4 + 3 50–60%, 4 + 3 61–80% and 4 + 3 > 80% Gleason 4. Furthermore, separate groups were defined by the presence of a tertiary Gleason 5 pattern, including 3 + 4 Tert. 5 and 4 + 3 Tert. 5. Follow-up was available for a total of 13,433 patients (median 64.4 months, range 1 to 241 months; Table 1). Prostate specific antigen (PSA) level were measured following surgery and PSA recurrence was defined when postoperative PSA was at least 0.2 ng/ml and increasing at subsequent measurements. Specimens were analyzed according to a standard procedure [27]. The TMA was manufactured as described earlier and included normal prostate tissue for control [28]. The TMA was annotated with results on ERG expression, ERG break apart FISH analysis [29]) and deletion status of 5q21 (CHD1) [30]), 6q15 (MAP3K7) [31]), *PTEN* (10q23) [32]) and 3p13 (FOXP1) [33]. Anonymized diagnostic left-over tissues were used approved by local laws (HmbKHG, §12a) and by the local ethics committee (Ethics commission Hamburg, WF-049/09). All work has been carried out in compliance with the Helsinki Declaration.

Table 1. Pathological and clinical data of the arrayed prostate cancers

	No. of patients (%)	
	Study cohort on TMA	Biochemical relapse among categories
Follow-up		
n	13,433	2759 (20.5%)
Mean/median (month)	63.7/64.4	-
Age (years)		
≤50	310	54 (17.4%)
51–59	3278	656 (20%)
60–69	7539	1693 (22.5%)
≥70	2251	501 (22.3%)
Pretreatment PSA (ng/ml)		
<4	1659	242 (14.6%)
4–10	7942	1355 (17.1%)
10–20	2807	737 (26.3%)
>20	940	397 (42.2%)
pT stage (AJCC 2002)		
pT2	8646	1095 (12.7%)
pT3a	2904	817 (28.1%)
pT3b	1765	796 (45.1%)
pT4	68	51 (75%)
Gleason grade		
≤3 + 3	2638	264 (10%)
3 + 4	7172	1436 (20%)
3 + 4 Tertiary 5	645	165 (25.6%)
4 + 3	1224	683 (55.8%)
4 + 3 Tertiary 5	987	487 (49.3%)
≥4 + 4	756	531 (70.2%)
pN stage		
pN0	7899	1821 (23.1%)
pN+	855	546 (63.9%)
Surgical margin		
Negative	10,768	1833 (17%)
Positive	2613	1059 (40.5%)

Numbers do not always add up to 13,665 in the different categories because of cases with missing data.
Abbreviation: AJCC, American Joint Committee on Cancer.

Immunohistochemistry

Freshly cut TMA sections were immunostained in one experiment. Slides were deparaffinized and exposed to antigen retrieval at 121 °C

in pH 7,8 Tris-EDTA-citrate buffer for 5 minutes. Mouse monoclonal antibody clone 8-5C2 specific for S100A8 (Dianova GmbH, Hamburg, Germany, dilution 1:1350) was applied at 37 °C for 60 minutes. Bound antibody was visualized with the EnVision Kit (Dako, Glostrup, Denmark) according to the manufacturer's directions. S100A8 clone 8-5C2 detects the epitope DVWFKE corresponding to amino acids 52 to 57 of the S100A8 protein (uniprot ID P05109). A protein–protein search using the basic local alignment search tool (BLAST) of the National Center for Biotechnology Information (NCBI) indicates that this epitope is unique to the S100A8 protein including its isoforms. S100A8 staining was found in the cytoplasm and nucleus of stained cancer cells, with identical intensity. Staining was typically found in all (100%) cancer cells of S100A8 positive tumors. Accordingly, the cytoplasmic and nuclear staining intensity was not separately analyzed but recorded in a four-step scale (0, 1+, 2+, and 3+) for each tissue spot.

Statistics

Statistical calculations were performed with JMP® 10 software (SAS Institute Inc., NC, USA). Contingency tables and the chi^2-test were performed to search for associations between molecular parameters and tumor phenotype. Survival curves were calculated according to

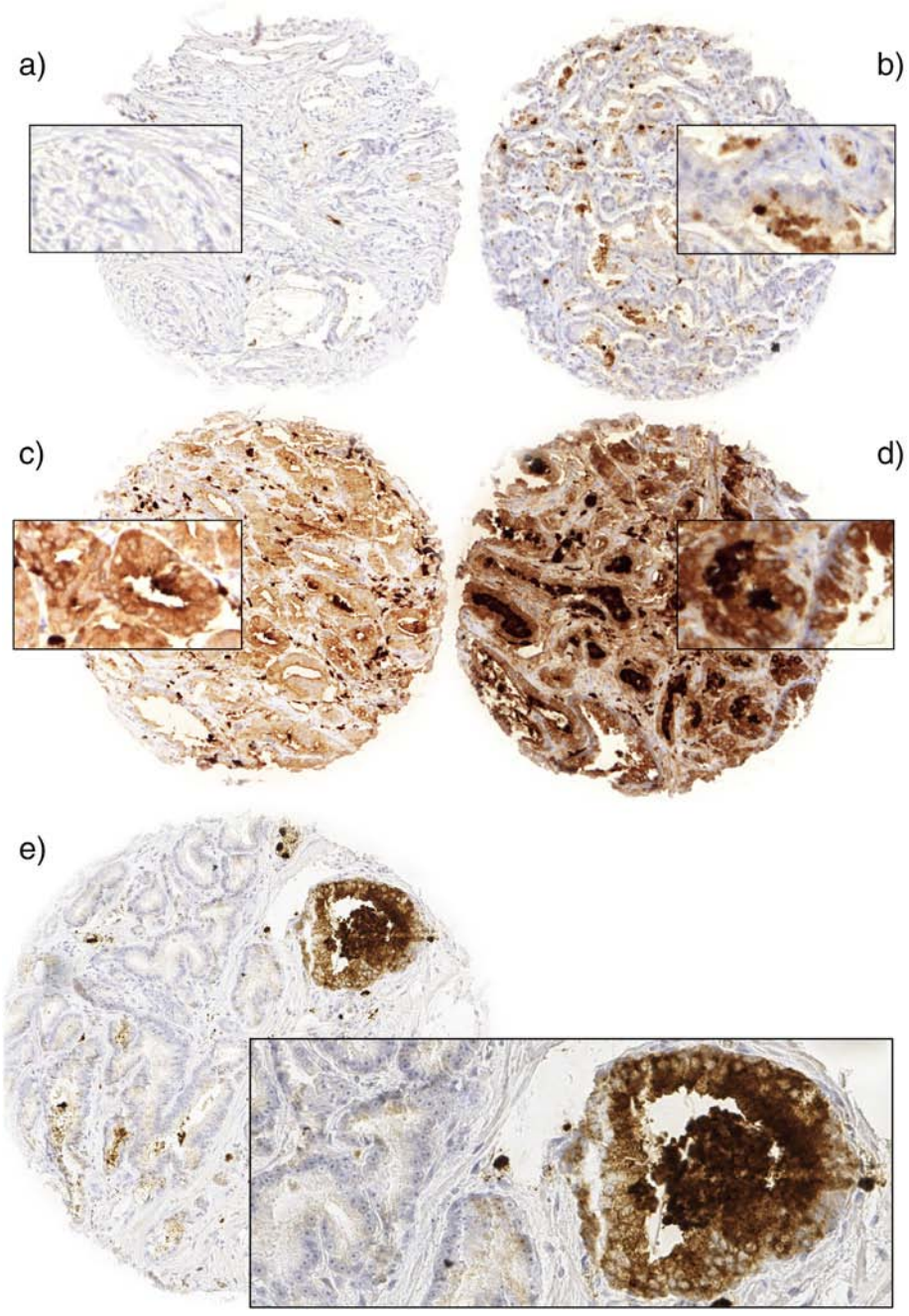

Figure 1. Examples of S100A8 immunostainings. a-d) Cancer spots with a) lack of staining b), weak staining, c) moderate staining and d) strong staining. e) Comparison of S100A8 staining in normal and cancerous prostate glands of the same TMA spot. Magnification is 100x/400x of originals with a spot size of 600 μm.

Kaplan–Meier. The log-rank test was applied to detect significant differences between groups. Cox proportional hazards regression analysis was performed to test the statistical independence and significance between pathological, molecular and clinical variables. Separate analyses were performed using different sets of parameters available either before or after prostatectomy.

Results

Technical Issues

A total of 9786 (71.6%) of tumor samples were interpretable in our TMA analysis. Reasons for non-informative cases (3879, 28.4%) included lack of tissue samples or absence of unequivocal cancer tissue in the TMA spot.

S100A8 Expression in Normal and Cancerous Prostate Tissues

S100A8 immunostaining was typically localized in the cytoplasm and the nucleus of positive cells. Normal prostate tissue showed variable (typically weak to moderate) staining levels in luminal and basal epithelial cells. In contrast, S100A8 immunostaining was more often negative in cancers. Here, a complete lack of S100A8 Immunostaining was seen in 5846 of our 9786 (59.7%) interpretable prostate cancers. In the remaining tumors, S100A8 was considered weak in 17.9%, moderate in 17.8% and strong in 5.4% of cases. Examples of S100A8 negative cancers next to S100A8 positive normal tissues, as well as of S100A8 positive cancers are given in Figure 1.

Associations with Tumor Phenotype

There was a strong inverse association between the level of S100A8 staining and tumor stage, classical and quantitative Gleason grade, nodal status, surgical margin status and preoperative PSA ($P < .0001$; Table 2). For example, lack of S100A8 staining was found in 77% of Gleason ≥4 + 4 cancers but only in 48% of Gleason ≤3 + 3 cancers. The associations between S100A8 staining and tumor phenotype held also true for the ERG negative and positive subsets (data not shown).

TMPRSS2:ERG Fusion Status and ERG Protein Expression

Data on both ERG FISH and IHC were available from 5003 cancers, and an identical result (or ERG IHC negative and missing break by FISH or ERG IHC positive and break by FISH) was found in 95.9% and 95.6% of the examined cancers. Lack of S100A8 immunostaining was weakly linked to *TMPRSS2:ERG* fusion and ERG expression in our study: negative S100A8 staining was seen in 58.7% of ERG IHC negative and in 63.4% of ERG IHC positive cancers ($P < .0001$; Figure 2).

S100A8 Expression and Genomic Deletions

A strong link of *PTEN* and 3p13 deletions to ERG positivity and of 5q21 and 6q15 deletions to ERG negativity has been described [30–33]. Here we saw a lack of S100A8 staining linked to *all of these* deletions. This was independent of the ERG status, although not all associations reached statistical significance in the subset analyses (Figure 3, *A–C*). Within the subset of 3023 cancers for which deletion data were available for all 4 loci, loss of S100A8 expression was significantly linked to a high number of deletions per tumor ($P = .0009$, Figure 4).

Table 2. Association between S100A8 immunostaining results and prostate cancer phenotype in all prostate cancers

Parameter	N	S100A8 (%)				*P*
		Negative	Weak	Moderate	Strong	
All cancers	9932	58.9	17.9	17.8	5.4	
Tumor stage						<.0001
pT2	6172	52.3	19.9	21.3	6.5	
pT3a	2346	65.1	16.7	13.8	4.3	
pT3b-pT4	1386	77.3	11.2	9.2	2.4	
Gleason grade						<.0001
≤3 + 3	1917	48.2	19.8	24.1	7.9	
3 + 4	5207	61.6	22.2	12.8	3.4	
3 + 4 Tertiary 5	434	72.1	13.5	11.4	2.9	
4 + 3	996	72.1	13.5	11.4	2.9	
4 + 3 Tertiary 5	661	72.2	12.3	11.4	4.1	
≥4 + 4	562	76.6	11.3	9.9	2.1	
Quantitative Gleason grade						<.0001
≤3 + 3	1922	48.2	19.8	24.1	7.9	
3 + 4 ≤ 5%	437	61.6	22.2	12.8	3.4	
3 + 4 6–10%	436	68.6	13.5	14.7	3.2	
3 + 4 11–20%	384	74.0	14.1	8.9	3.1	
3 + 4 21–30%	92	71.7	14.1	9.8	4.3	
3 + 4 31–49%	691	72.2	12.3	11.4	4.1	
3 + 4 Tertiary 5	514	75.7	11.9	10.3	2.1	
4 + 3 50–60%	436	68.6	13.5	14.7	3.2	
4 + 3 61–100%	384	74.0	14.1	8.9	3.1	
4 + 3 Tertiary 5	92	71.7	14.1	9.8	4.3	
≥4 + 4	691	72.2	12.3	11.4	4.1	
Lymph node metastasis						<.0001
N0	5840	60.9	17.9	16.4	4.7	
N+	695	76.3	10.6	10.2	2.9	
Preoperative PSA level (ng/ml)						<.0001
<4	1141	57.0	17.0	19.8	6.2	
4–10	5895	56.0	19.1	18.7	6.2	
10–20	2101	63.2	17.0	15.8	4.1	
>20	736	71.6	12.8	13.7	1.9	
Surgical margin						<.0001
Negative	7784	57.0	18.3	18.8	5.9	
Positive	2118	65.5	16.6	14.2	3.7	

Associations with PSA Recurrence

Follow-up data were available for 8578 patients with interpretable S100A8 immunostaining. Tumors lacking S100A8 staining had a significantly worse prognosis as compared to cancers with positive (weak, moderate or strong) staining ($P < .0001$, Figure 5). The S100A8 staining level did not provide substantial further prognostic information. The strong association between loss of S100A8 staining and poor outcome was also found in subsets of tumors with and

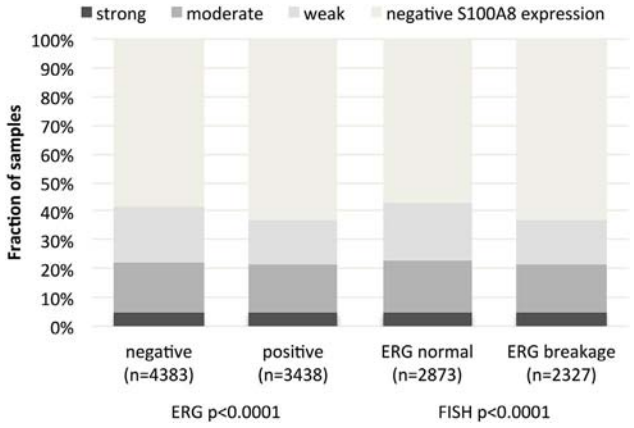

Figure 2. Association between positive S100A8 immunostaining and ERG status (IHC/FISH).

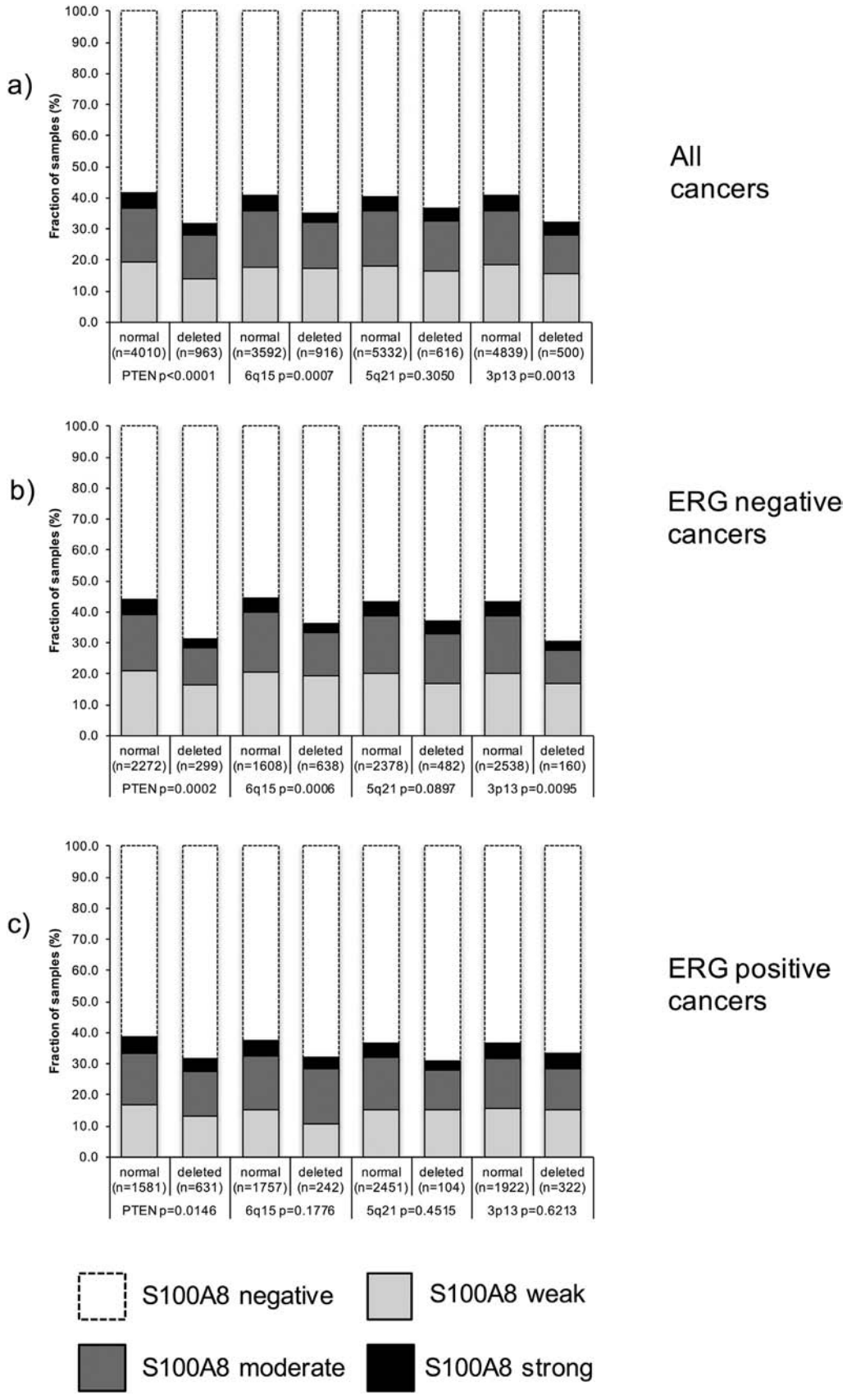

Figure 3. Association between positive S100A8 immunostaining and 10q23 (PTEN), 5q21 (CHD1), 6q15 (MAP3K7), 3p13 (*FOXP1*) deletions in all prostate cancers (a), in ERG negative cancers (b) and ERG positive cancers (c).

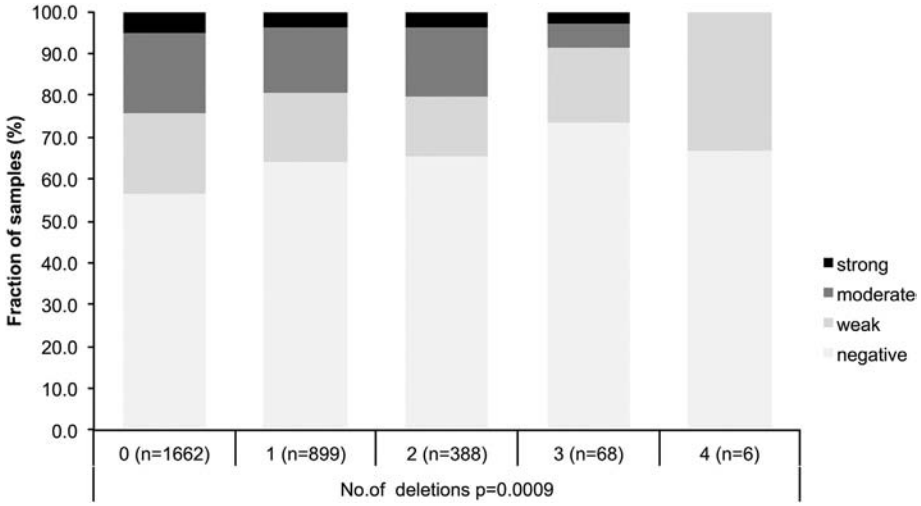

Figure 4. Association between S100A8 expression and number of deletions.

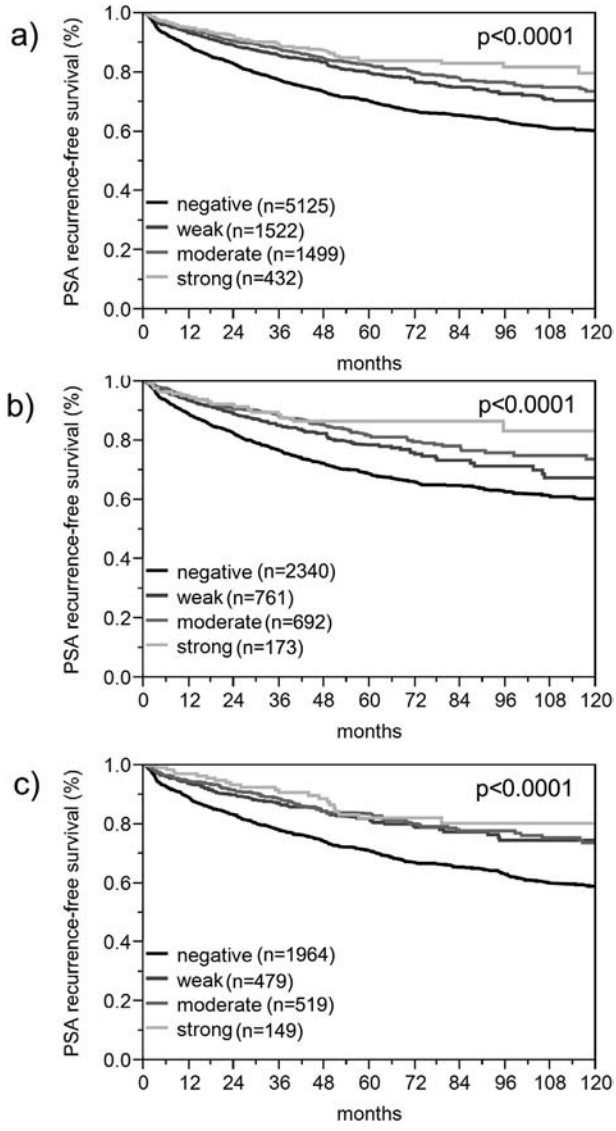

Figure 5. Association between S100A8 expression and biochemical recurrence in a) all cancers, b) ERG fusion negative cancers, c) ERG fusion positive cancers.

without ERG fusion (P < .0001 each, Figure 5 b) and c)). Further stratification of the analysis to subsets of cancers with identical Gleason grade revealed that the prognostic value of S100A8 expression was only retained in Gleason grade 3 + 4 tumors (P < .0001; Figure 6*A*). No prognostic significance was found in subgroups defined by comparable quantitative Gleason grading (Figure 6, B–H).

Multivariate Analysis

We tested four different models representing pre- and postoperative clinical scenarios (Table 3). Scenario 1 evaluated the postoperatively available parameters (pathological tumor stage (pT), 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, the nodal status was dropped. This can markedly increase case numbers. The mixed scenario 3 included S100A8 expression, preoperative PSA, clinical tumor stage (cT stage) and Gleason grade obtained on the prostatectomy specimen. Since postoperative determination of a tumor's 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 [34]), another multivariate analysis was added. The preoperative scenario 4 included the preoperative Gleason grade obtained on the original biopsy at various different pathology institutes with preoperative PSA, cT stage and S100A8 expression. S100A8 expression provided significant prognostic information beyond the established parameters in the scenario 3 and 4 ($P < .0001$). This also held true for the subgroups of ERG positive and negative cancers ($P < .05$).

Discussion

The results of our study demonstrate that loss of S100A8 expression is linked to prostate cancer aggressiveness and early biochemical recurrence.

In our analysis, S100A8 staining was typically positive in normal prostate cells but lost in a relevant fraction (60%) of prostate cancers, suggesting that S100A8 down-regulation often parallels prostate cancer development. These findings are in agreement with one earlier

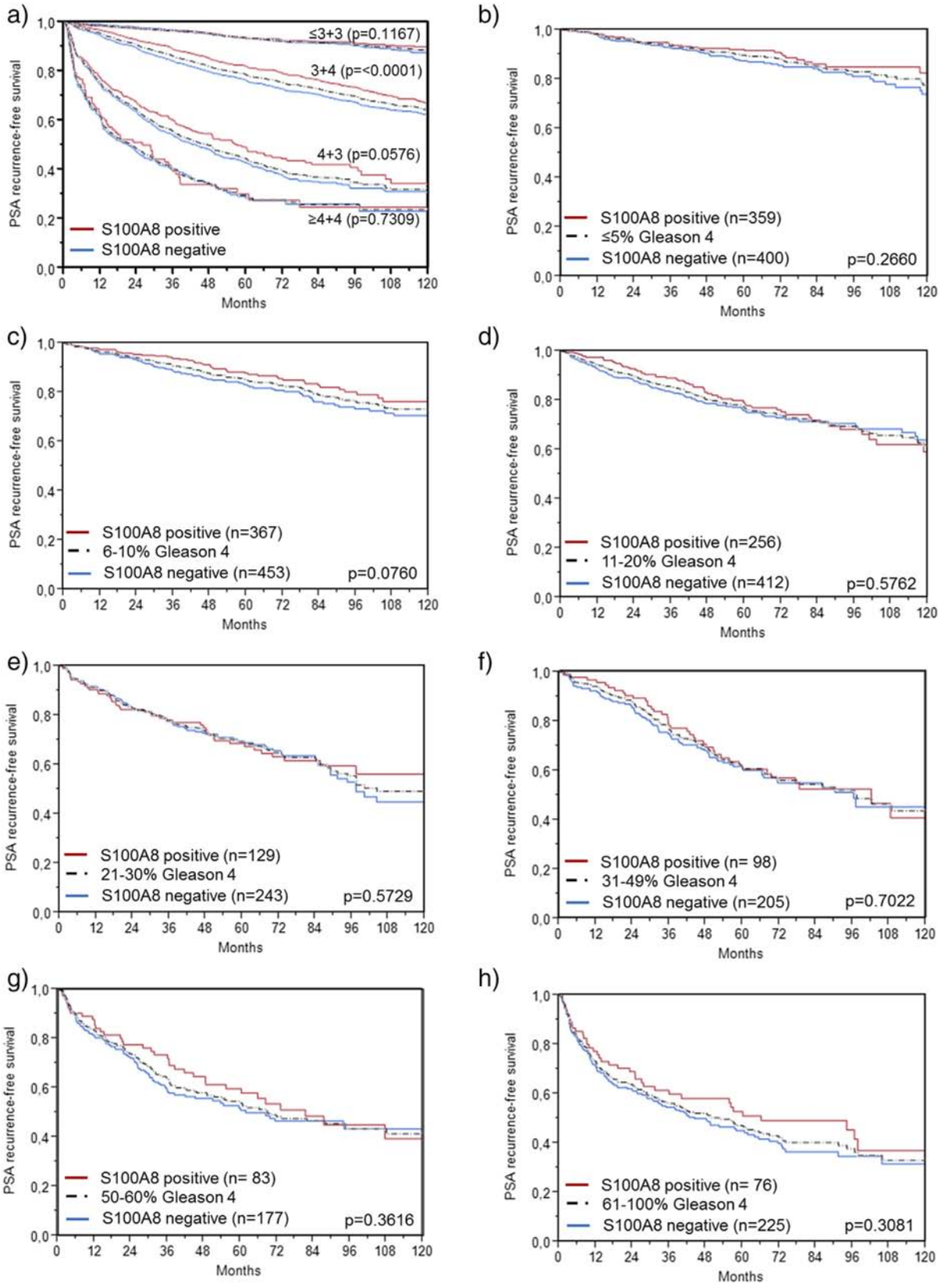

Figure 6. Prognostic impact of S100A8 expression in subsets of cancers defined by the Gleason score. a) Impact of negative (blue line) and strongly positive (red line) S100A8 expression as compared to the classical Gleason score categories (indicated by black dotted lines). b-h) Impact of negative (red line) and strongly positive (blue line) S100A8 expression as compared to the quantitative Gleason score categories (black dotted lines) defined by subsets of cancers with b) ≤5%, c) 6–10%, d) 11–20%, e) 21–30%, f) 31–49%, g) 50–60%, and h) ≥61% Gleason 4 patterns.

Table 3. Multivariate analysis including S100A8 expression in all cancers, ERG negative and ERG positive cancers

Subset	Scenario	n analyzable/n analyzable qGleason	*P* value							
			Preoperative PSA-Level/	pT Stage/	cT Stage/	Gleason grade prostatectomy/	Gleason grade biopsy/	pN Stage/	R Stage/	S100A8-Expression/
All	1	5421/4587	<.0001/	<.0001/	-	<.0001/	-	<.0001/.0007	.0558/.0409	.2184/.1761
	2	8370/7433	<.0001/	<.0001/	-	<.0001/	-	-	<.0001/	.0533/.0489
	3	8279/7355	<.0001/	-	<.0001/	<.0001/	-	-	-	.0001/.0005
	4	8173	<.0001	-	<.0001	-	<.0001	-	-	<.0001
ERG-negative	1	2514	.0102	<.0001	-	<.0001	-	.0009	.5515	.1913
	2	3873	.0002	<.0001	-	<.0001	-	-	.0343	.0733
	3	3834	<.0001	-	<.0001	<.0001	-	-	-	.0103
	4	3786	<.0001	-	<.0001	-	<.0001	-	-	<.0001
ERG-positive	1	1935	.0003	<.0001	-	<.0001	-	.0623	.0998	.2912
	2	3038	<.0001	<.0001	-	<.0001	-	-	.003	.1931
	3	2980	<.0001	-	<.0001	<.0001	-	-	-	.0018
	4	2934	<.0001	-	<.0001	-	<.0001	-	-	<.0001

Scenario 1 includes all postoperatively available parameters (pathological tumor (pT) stage, lymph node status (pN), surgical margin (R) status, preoperative PSA value and Gleason grade obtained after the morphological evaluation of the entire resected prostate. Scenario 2 excluded the nodal status from analysis. Scenario 3 included preoperative PSA, clinical tumor (cT) stage and Gleason grade obtained on the prostatectomy specimen. In scenario 4, the preoperative Gleason grade obtained on the original biopsy was combined with preoperative PSA, and cT stage.

Values for "quantitative" Gleason score.

Same *P* values as with conventional Gleason score.

study reporting significantly higher S100A8 protein expression in 20 non-cancerous glands than in 19 adenocarcinomas using an antibody from Abcam detecting the S100A8/A9 complex [35]. However, in another study on 48 benign and 75 cancerous prostate samples, a higher fraction of S100A8 positive tissues was described in cancerous (75%) than in normal glands (15%) [24] by applying a rabbit polyclonal antibody from Santa Cruz. It is possible that the use of different antibodies has contributed to the discrepant findings in this study.

Loss of S100A8 expression was strongly linked to adverse features of prostate cancer in our analysis. For example, there was a stepwise decrease of S100A8 expression from low to high-grade prostate cancer (51.8% in Gleason ≤3 + 3, 38.4% in Gleason 3 + 4, 27.9% in Gleason 4 + 3 and 23.4% in Gleason ≥4 + 4), and the fraction of cancers lacking S100A8 expression gradually increased with the proportion of Gleason 4 tumor glands. In addition, cancers lacking detectable S100A8 staining had the worst clinical outcome. Only two earlier studies have analyzed the prognostic impact of S100A8 before. Using the same antibody (clone 8-5C2) as in our study, Grebhard et al. could not find associations between S100A8 immunostaining and tumor phenotype or patient prognosis [25] in 167 prostate cancers. In contrast, Yun et al. reported that upregulation of S100A8 mRNA was linked to early biochemical recurrence in 132 prostate cancers [35]. It is possible that discrepant data as compared to our study were caused by cohort size or the use of RNA as an analyte including tumor cell content issues that are inherent to RNA analyses.

Overall, the available data suggest that the role of S100A8 varies between different tumor entities. Similar inverse associations as in prostate cancer linking S100A8 downregulation to cancer development [36–38] or adverse patient prognosis [39] have been reported from esophageal and head & neck cancers. That these studies came to similar results with different techniques, including mRNA expression analysis [36], 2D gel electrophoresis [37], and IHC (using different antibodies as in our study) [38,39], supports the validity of these findings. In the majority of cancer types, it is upregulation of S100A8 that is linked to tumor development and progression. For example, S100A8 upregulation has been linked to tumor development in gastric cancer [9–11], cervical cancer [12], colon cancer [13–15], breast cancer [16–19], liver cancer [20], thyroid cancer [21], lung cancer [22] and renal cancer [23].

More than half of all prostate cancers, particularly those of young patients, carry gene fusions linking the androgen-regulated *TMPRSS2* gene with the transcription factor *ERG* [40,41]. These genomic rearrangements result in an androgen-driven overexpression of ERG in affected cells [42] and, thus, altered expression of more than 1600 genes in prostate epithelial cells [43]. The weak but significant link between *TMPRSS2:ERG* fusions and reduced S100A8 expression suggests that ERG expression results – to some extent – in a deregulation of S100A8. The significant statistical association found between ERG expression and S100A8 loss is remarkable from a technical point of view as it represents an inverse IHC association. Such data are particularly certain to represent true associations because mild positive associations between immunohistochemically determined parameters can always be due to a fraction of samples that are generally non-reactive to immunohistochemistry resulting in "negative" staining results for all parameters measured. That all associations between S100A8 expression and prostate cancer phenotype and prognosis were independent from the ERG status indicates, however, that S100A8 acts largely independent from the molecular background governed by ERG and its downstream effectors. For various other proteins that were dependent on the ERG status, the prognostic impact differed largely between these subgroups [44–47].

Deletions of certain small and large chromosomal regions are another hallmark of prostate cancer. Data from next generation sequencing studies demonstrate that such deletions are more prevalent than any other mutations of specific coding genes and many of these deletions have that been linked to either ERG positive (i.e. *PTEN* and *3p13*) or ERG negative cancers (i.e. *6q15* and *5q23*). That loss of S100A8 was associated with all these individual deletions as well as the number of deletions per cancer suggests a link of S100A8 loss to mechanisms related to genetic instability. The known role of S100A8 as a scavenger of reactive oxygen species (ROS) may serve as a possible explanation for this observation [48]. A loss of S100A8 may thus contribute to an increase of free ROS. Elevated ROS has been earlier described as a major cause of DNA damage and genetic instability in eukaryotic cells [49].

The main purpose of our study was to determine, whether S100A8 protein expression analysis can serve as a suitable biomarker for

prognosis assessment. For this purpose, an optimal evaluation strategy would include the molecular analysis of the original needle biopsy of a patient and compare its prognostic value with preoperative Gleason grade obtained on the same biopsy as well as the preoperative PSA value. For practical purposes, this approach is not feasible because preoperative biopsies are typically distributed over many different centers and not available for studies. Moreover, even if available, these precious core needle biopsies would be exhausted after only few studies. A convoluted approach evaluating multiple different scenarios was thus utilized in this study. Overall, our multivariate modeling suggests a marked prognostic impact of S100A8 loss in prostate cancer that is independent of clinical and histopathological features available in the preoperative situation.

The Gleason Score is the strongest prognostic feature in prostate cancer [50]. Assessing the prognostic impact of S100A8 expression loss in tumors with identical Gleason grade revealed a prognostic role for S100A8 only in Gleason 3 + 4 cancers. This observation reflects the particularly high heterogeneity of this Gleason category. These cancers range from 3 + 4 carcinomas with only 5% Gleason 4 pattern representing borderline cases to Gleason 3 + 3 to Gleason 3 + 4 carcinomas with 45% Gleason 4 pattern. These latter tumors are clinically similar to Gleason 4 + 3 cancers [26]. That S100A8 expression levels lacked prognostic impact in cancers with identical quantitative Gleason grade demonstrates how difficult it is for molecular parameters to outperform optimal morphological malignancy assessment. Given the high interobserver variability of the Gleason grading reaching up 40%, and considering also that quantitative Gleason grading is not universally applied, these findings do not devaluate S100A8 as a potentially applicable prognostic biomarker.

Taken together, the results of our study demonstrate that complete loss of S100A8 expression is linked to particularly adverse tumor features and early biochemical recurrence in prostate cancer. S100A8 expression analysis may have prognostic utility either alone, or more likely, in combination with other biomarkers.

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