

# Upregulation of centromere protein F is linked to aggressive prostate cancers

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**Background:** Centromere protein F (CENPF) is a key component of the kinetochore complex and plays a crucial role in chromosome segregation and cell cycle progression. Recent work suggests that CENPF upregulation is linked to aggressive tumor features in a variety of malignancies including prostate cancer.

**Materials and methods:** Using a highly annotated tissue microarray, we analyzed CENPF protein expression from a cohort of 8,298 prostatectomized patients by immunohistochemistry to study its effect on prostate-specific antigen recurrence-free survival.

**Results:** CENPF overexpression was found in 53% of cancers, and was linked to higher Gleason grade, advanced pathological tumor stage, accelerated cell proliferation, and lymph node metastasis ( $p < 0.0001$ , each). A comparison with other key molecular features accessible through the microarray revealed strong associations between CENPF overexpression and presence of erythroblast transformation-specific (ETS)-related gene (*ERG*) fusion as well as phosphatase and tensin homolog deletion ( $p < 0.0001$ , each). CENPF overexpression was linked to early biochemical recurrence. A subset analysis revealed that this was driven by the ERG-negative subset ( $p < 0.0001$ ). This was independent of established preoperative and postoperative prognostic parameters in multivariate analyses.

**Conclusion:** The results of our study identify CENPF overexpression as an important mechanism and a potential biomarker for prostate cancer aggressiveness.

**Keywords:** CENPF, ERG, deletion, prostate cancer, tissue microarray, prognosis

## Introduction

In Western societies, the most prevalent cancer in men is prostate cancer.<sup>1</sup> The majority of prostate cancers are indolent. Only a small subset is highly aggressive and requires treatment.<sup>2,3</sup> Gleason grade and tumor extent on biopsies, prostate-specific antigen (PSA) levels, and clinical stage are established preoperative prognostic parameters. These parameters are statistically powerful in retrospective analysis but insufficient for optimal individual treatment decisions. The hope is that a further understanding of disease biology will identify additional clinically applicable molecular markers for more reliable predictions of prostate cancer aggressiveness.

Centromere protein F (CENPF, also known as mitotin) is a microtubule-associated protein involved in mitosis and cell differentiation.<sup>4</sup> CENPF becomes upregulated during the G2/M phase and accumulates to the kinetochore complex, facilitating microtubule attachment and chromosome segregation.<sup>5,6</sup> In addition, CENPF is essential – and probably rate limiting – for cell proliferation as it interacts with key

cell cycle checkpoint proteins, such as the retinoblastoma protein<sup>4</sup> and the Bub1 kinase,<sup>7</sup> or late telophase proteins including syntaxin 4<sup>8</sup> and SNAP25.<sup>9</sup> CENPF upregulation has been observed in breast cancer,<sup>11</sup> nasopharyngeal cancer,<sup>12</sup> hepatocellular carcinoma,<sup>13</sup> esophageal squamous cell carcinoma,<sup>14</sup> gastrointestinal stromal tumors,<sup>15</sup> and non-Hodgkin's lymphoma,<sup>16</sup> and in some cases it is associated with aggressive tumor phenotype and poor survival.<sup>11,12,14</sup>

Aytes et al showed that in prostate cancer cosilencing of the fork head box protein M1 (FOXM1) and CENPF abrogates tumor cell growth.<sup>10</sup> Zhuo et al described higher CENPF expression in 99 cancer samples compared with normal tissue and suggested an association with unfavorable tumor phenotype and poor prognosis.<sup>17</sup> Similar results were mentioned for the outcome of 821 patients with prostate cancers.<sup>10</sup> These promising data encouraged us to interrogate the prognostic impact of CENPF protein expression in a large set of prostate cancers. Therefore, we performed immunohistochemical analysis of CENPF protein expression on a panel of >11,000 prostate cancer specimens associated with follow-up information and an attached molecular database.

## Materials and methods

### Patients

The 12,427 radical prostatectomy specimens were from patients who had surgery between 1992 and 2014 at the Department of Urology and the Martini Clinic at the University Medical Center Hamburg-Eppendorf. The specimens were analyzed according to a standard procedure.<sup>18</sup> Tumor stage, Gleason grade, nodal status, and status of the resection margin were retrieved from the patients' records. Quantitative Gleason grading was performed using the percentage of Gleason 4 patterns.<sup>19</sup> Follow-up data were available for 11,665 patients with a median follow-up of 50 months (range: 1–264 months; Table 1). PSA values were measured following surgery, and PSA recurrence was defined as a postoperative PSA of 0.2 ng/mL and increasing in subsequent measurements. The tissue microarray manufacturing process was described in detail earlier.<sup>20</sup> In short, a single 0.6 mm core was taken from a representative tissue block. For internal control, various control tissues and normal prostate tissue were included. The molecular database attached to this tissue microarray contains results on erythroblast transformation-specific (ETS)-related gene (ERG) protein expression,<sup>21</sup> ERG rearrangement analysis by fluorescence in situ hybridization (FISH)<sup>22</sup> and deletion

**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
<b>Follow-up (month)</b>		
n	11,665	2,769 (23.7%)
Mean (median)	62.9 (50.0)	–
<b>Age (years)</b>		
≤50	334	81 (24.3%)
51–59	3,061	705 (23%)
60–69	7,188	1,610 (22.4%)
≥70	1,761	370 (21.0%)
<b>Pretreatment PSA (ng/mL)</b>		
<4	1,585	242 (15.3%)
4–10	7,480	1,355 (18.1%)
10–20	2,412	737 (30.6%)
>20	812	397 (48.9%)
<b>pT stage (AJCC 2002)</b>		
pT2	8,187	1,095 (13.4%)
pT3a	2,660	817 (30.7%)
pT3b	1,465	796 (54.3%)
pT4	63	51 (81.0%)
<b>Gleason grade</b>		
≤3+3	2,848	234 (8.2%)
3+4	6,679	1,240 (18.6%)
3+4 Tert.5	433	115 (26.6%)
4+3	1,210	576 (47.6%)
4+3 Tert.5	646	317 (49.1%)
≥4+4	596	348 (58.4%)
<b>Lymph node stage</b>		
pN0	6,970	1,636 (23.5%)
pN+	693	393 (56.7%)
<b>Surgical margin</b>		
Negative	9,990	1,848 (18.5%)
Positive	2,211	853 (38.6%)

**Notes:** In the column "Study cohort on TMA", numbers do not always add up to 12,427 in different categories because of cases with missing data. Percent in column "Biochemical relapse among categories" refers to the fraction of samples with biochemical relapse within each parameter in the different categories.

**Abbreviations:** PSA, prostate-specific antigen; AJCC, American Joint Committee on Cancer; TMA, tissue microarray.

status of 5q21 (chromodomain-helicase-DNA-binding protein 1 [*CHD1*]),<sup>23</sup> 6q15 (mitogen-activated protein kinase kinase kinase 7 [*MAP3K7*]),<sup>24</sup> 10q23 (phosphatase and tensin homolog [*PTEN*]),<sup>25</sup> and 3p13 (fork head box protein P1 [*FOXPI*])<sup>26</sup> cancers. The use of leftover archived diagnostic tissues for the manufacturing of tissue microarrays and their analysis in conjunction with anonymized patient data for research purposes was approved by local laws (HmbKHG, §12,1) and by the local ethics committee (Ethics commission Hamburg, WF-049/09 and PV3652). All work was carried out in compliance with the Helsinki Declaration.

## Immunohistochemistry (IHC)

Freshly cut tissue microarray sections were stained the same day and in a single run. Slides were deparaffinized and exposed to heat-induced antigen retrieval for 5 minutes at 121°C in pH 9.0 antigen retrieval buffer. Primary antibody specific for CENPF (mouse monoclonal antibody ab90, dilution 1:1350; Abcam, Cambridge, UK) was applied at 37°C for 60 minutes. Bound antibody was visualized with the EnVision Kit (Dako, Glostrup, Denmark). Since CENPF typically shows nuclear and cytoplasmic staining in 100% of the tumor cells of a tissue spot, we recorded only the staining intensity as negative, weak, moderate, and strong staining.

## Statistics

JMP 12.0 software (SAS Institute Inc., NC, USA) was used. Contingency tables were calculated to study the association between CENPF expression and other clinicopathological variables, and the likelihood test was used to find significant relationships. Analysis of variance and *F* test were applied to find associations between CENPF expression and tumor cell proliferation as measured by the Ki67-labeling index. Kaplan–Meier curves were generated using biochemical (PSA) recurrence as the clinical endpoint. The log-rank test

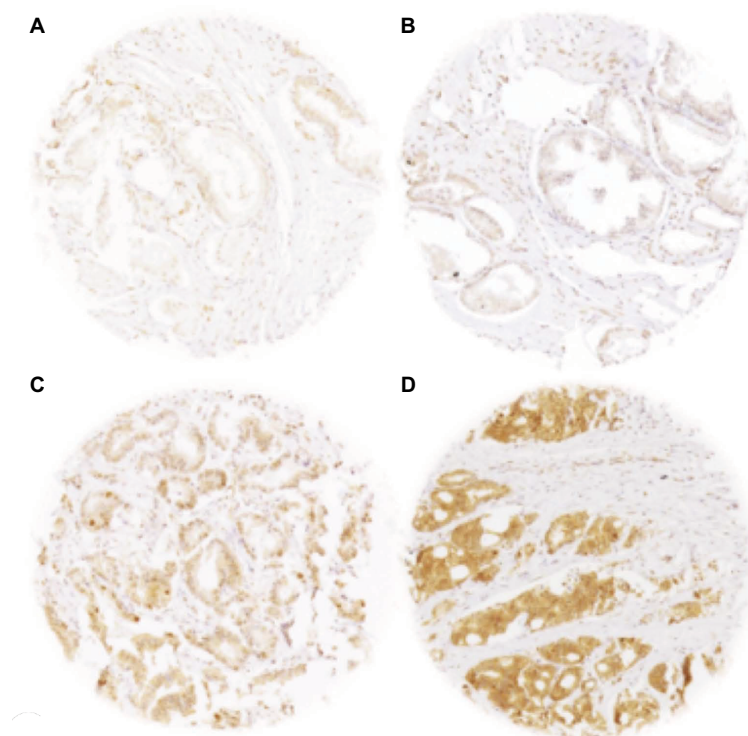
was applied to test the significance of differences between stratified survival functions.

## Results

A total of 9,055 (73%) of the 12,427 arrayed tumor samples displayed interpretable CENPF staining. Noninformative cases (27%) either had no tissue or an absence of unequivocal cancer tissue in the microarray spot. Normal prostatic glands showed negative to weak cytoplasmic CENPF staining in luminal and basal cells. Comparable staining was also found in stromal cells. In cancer cells, CENPF-positive staining was seen in 8,066 of our 9,055 (89%) interpretable prostate cancers and was considered weak in 36.6%, moderate in 34.9%, and strong in 17.6% of cancers. Representative images of CENPF staining in normal and cancerous prostate samples are given in Figure 1.

### Association with TMPRSS2:ERG fusion status and ERG protein expression

To evaluate whether CENPF expression is associated with ERG status in prostate cancers, we used data from previous studies.<sup>21,22</sup> Data on transmembrane protease, serine 2 (*TMPRSS2*):*ERG* fusion status obtained by FISH were



**Figure 1** Representative pictures of CENPF staining in (A) normal prostate glands (negative/weak) and in prostate cancer with (B) negative/weak, (C) moderate, and (D) strong staining intensity. Spot size 600  $\mu$ m, 100 $\times$  magnification.

**Abbreviation:** CENPF, centromere protein F.

available from 5,365 tumors and by IHC from 8,073 tumors with evaluable CENPF staining. Data on both ERG-FISH and IHC were available from 5,198 cancers, and an identical result (ERG-IHC positive and break by FISH or ERG-IHC negative and no break by FISH) was found in 4,975 of 5,198 (95.7%) cancers. Increased (ie, moderate to strong) CENPF staining was more frequent in *TMPRSS2:ERG* rearranged (72.05%) and ERG-positive prostate cancers (73.46%) than in ERG-negative tumors by FISH (39.11%) or IHC (38.3%,  $p<0.0001$  each, Figure 2). It is of note that ERG expression was not associated with the prognosis as indicated by identical Kaplan–Meier curves for PSA recurrence-free survival in the ERG-negative and ERG-positive subset (Figure S1).

### Association with tumor phenotype

Increasing CENPF staining was linked to advanced pathological tumor stage, higher Gleason grade, and lymph node positivity ( $p<0.0001$  each, Figure 3). Subset analysis of ERG-negative and ERG-positive cancers revealed that these associations were driven by the subset of ERG-negative cancers and much less by ERG-positive cancers (Tables S1 and S2).

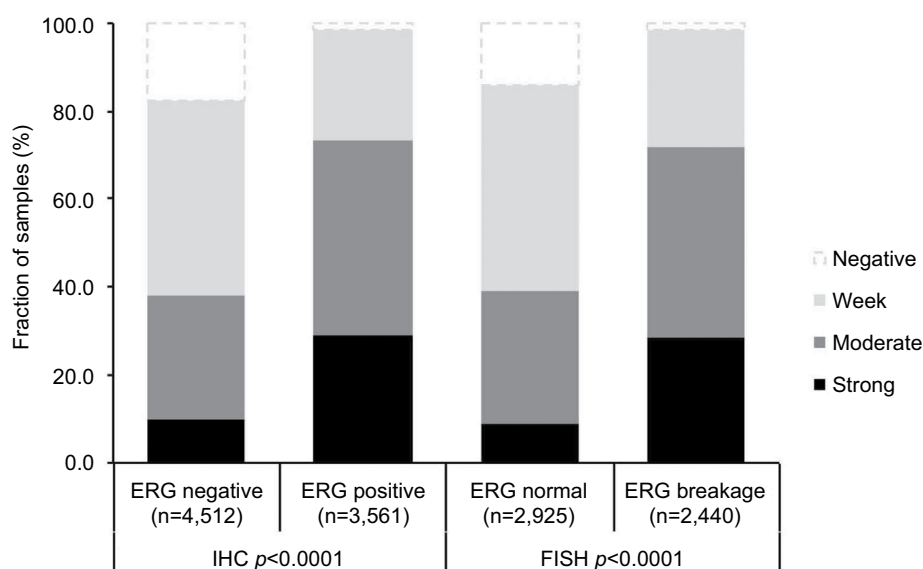
### Association with other key genomic deletions

Earlier studies have provided evidence for distinct molecular subgroups of prostate cancers defined by *TMPRSS2:ERG* fusion and several genomic deletions. This includes a strong association of *PTEN* and 3p13 deletions with ERG positivity

and of 5q21 and 6q15 deletions with ERG negativity.<sup>23–26</sup> To study whether CENPF expression might be particularly associated with one of these genomic deletions, CENPF data were compared with preexisting findings on 10q23 (*PTEN*), 3p13 (*FOXP1*), 6q15 (*MAP3K7*), and 5q21 (*CHD1*) deletions. For tumors that were jointly analyzed, there were positive associations between CENPF overexpression and deletions of *PTEN* or 3p13 ( $p<0.0001$  each), as well as negative associations between CENPF overexpression and deletions of 5q or 6q ( $p=0.027$  and  $p<0.0001$ , respectively, Figure 4A). These associations make intuitive sense due to the known link between these deletions and a positive ERG status (*PTEN*, 3p) or a negative ERG status (5q, 6q). However, subset analysis of ERG-negative and ERG-positive cancers demonstrated that CENPF overexpression was linked to *PTEN* deletions independent of the ERG status ( $p<0.0001$  each, Figure 4B and C).

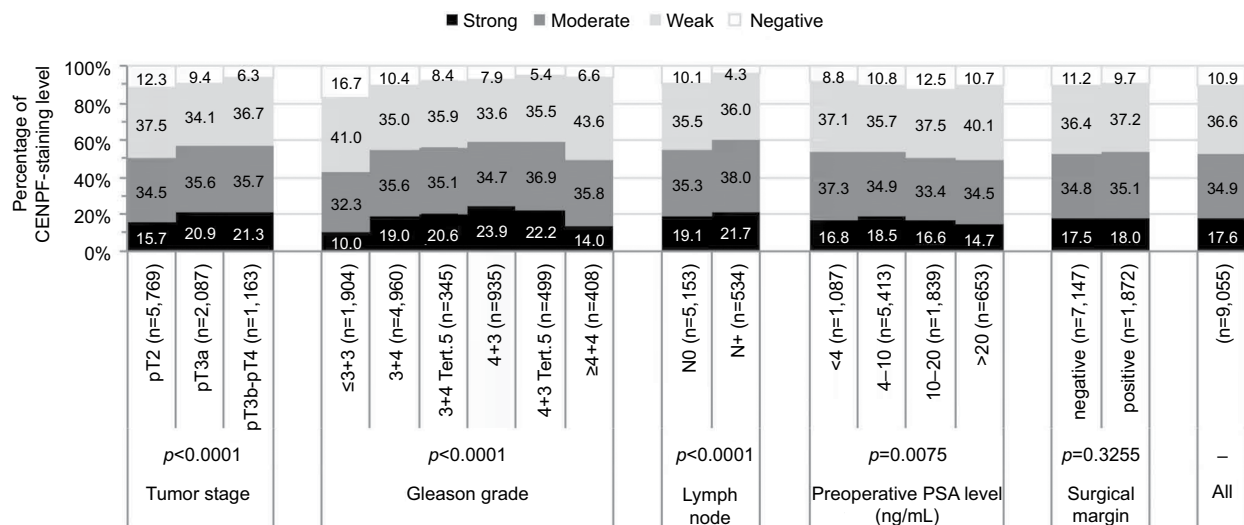
### Association with tumor cell proliferation

High-level CENPF staining was linked to accelerated cell proliferation as measured by the Ki67-labeling index (Ki67LI) obtained from an earlier study.<sup>27</sup> The average Ki67LI increased from 1.41 in cancers lacking CENPF expression to 3.35 in cancers with strong CENPF levels ( $p<0.0001$ , Figure 5). This association was also found both in ERG-negative ( $p<0.0001$ ) and ERG-positive cancers ( $p=0.0061$ ; data not shown). It was independent from the Gleason score as it held true in all tumor subsets with identical Gleason score (Figure 5).



**Figure 2** Association between CENPF staining level and clinical tumor phenotype.

**Abbreviations:** CENPF, centromere protein F; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry.



**Figure 3** Association between CENPF staining and *ERG* expression by immunohistochemistry or *TMPRSS2:ERG* fusion by FISH.

**Abbreviations:** CENPF, centromere protein F; *ERG*, erythroblast transformation-specific (ETS)-related gene; *TMPRSS2*, transmembrane protease, serine 2; FISH, fluorescence in situ hybridization.

## Association with PSA recurrence

Follow-up data were available for 8,298 patients with interpretable CENPF staining on the tissue microarray. A highly significant association between strong CENPF expression and early PSA recurrence was seen in all tumors ( $p < 0.0001$ , Figure 6A) and in the subset of *ERG* fusion-negative cancers ( $p < 0.0001$ , Figure 6B). The prognostic impact of CENPF was less significant in *ERG* fusion-positive cancers ( $p = 0.0188$ , Figures 6C). To better rate the prognostic power of CENPF, we performed further subset analyses in cancers with identical classical and quantitative Gleason scores. Here, CENPF staining did not provide clear-cut prognostic information beyond the Gleason score, neither in any subsets defined by the classical Gleason score (Figure S2A) nor in any subsets defined by the quantitative Gleason score (Figure S2B–H).

## Multivariate analyses

To evaluate whether CENPF expression can serve as a prognostic biomarker independent of the established prognostic factors (Gleason grade at biopsy, clinical stage, preoperative PSA level, Gleason grade at radical prostatectomy, pathological stage, surgical margin status, and nodal status), multivariate analyses of PSA recurrence-free survival were performed in 3 different scenarios (Table 2). CENPF proved to be a significant independent prognostic parameter in all 3 scenarios. The HR for PSA recurrence after prostatectomy peaked at 1.23 in the preoperative scenario 1. This held true

for the *ERG*-negative subset with the exception of scenario 3 and the *ERG*-positive subset with the exception of scenario 2.

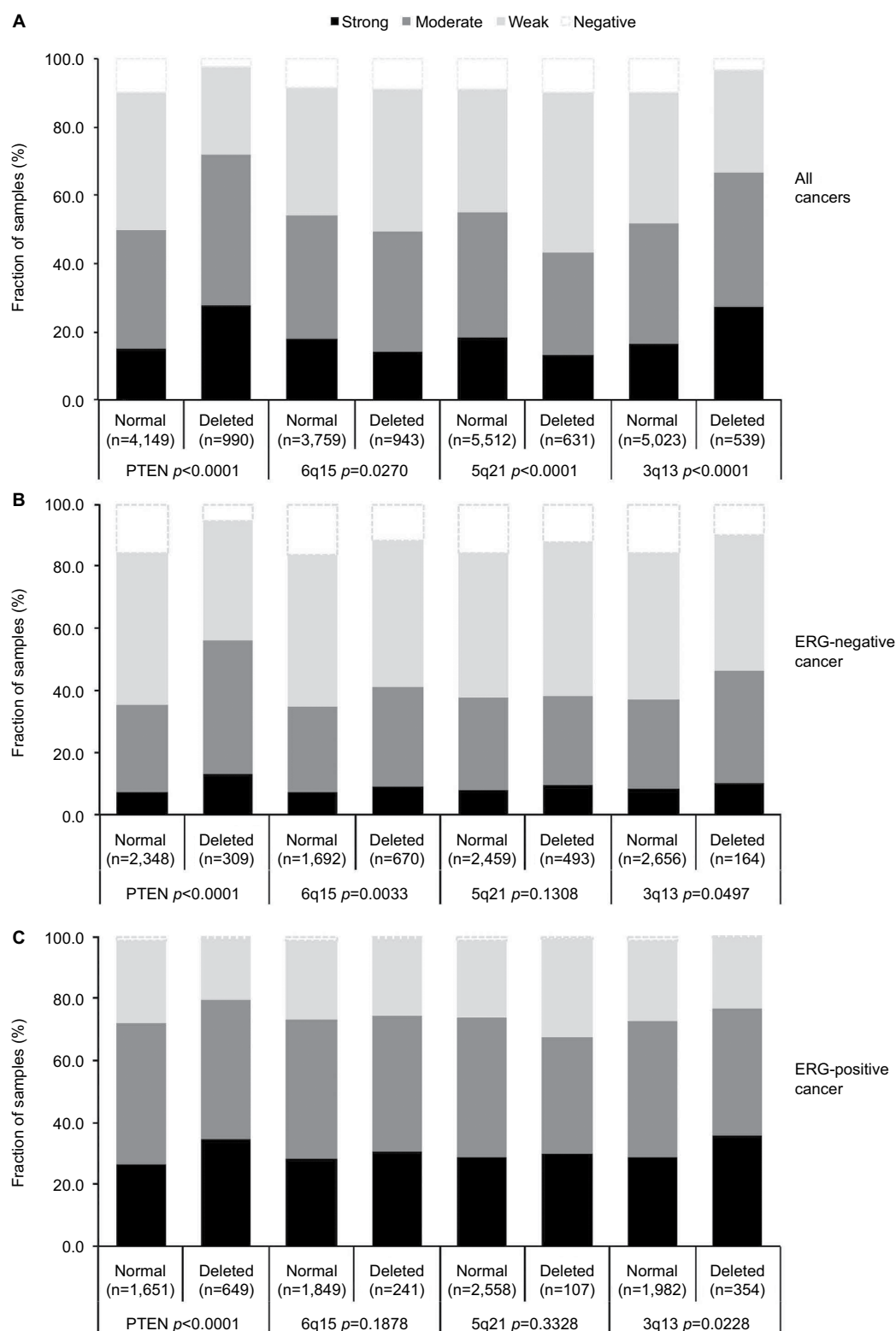
## Discussion

The results of our study identify CENPF as a strong and independent predictor of patient prognosis.

Successful analysis of >9,000 prostate cancers by IHC revealed detectable CENPF staining in 90% of prostate cancers, including almost 40% of tumors with no more than weak staining. The fact that such low-level staining was also found in normal prostate glands as well as in stromal cells suggests that our IHC protocol was sufficiently sensitive to detect physiological expression of CENPF. However, increased CENPF expression in 50% of cancers indicates that CENPF becomes upregulated during malignant transformation in a high fraction of prostate cancers. Only one published study has previously evaluated CENPF protein expression by IHC in prostate cancer. In line with our findings, Zhuo et al reported significantly higher CENPF levels in 99 tumor samples compared with benign tissues.<sup>17</sup> Tumor-associated upregulation of CENPF was also found in other tumor types including esophageal cancer, hepatocellular carcinoma, non-Hodgkin's lymphoma, nasopharyngeal cancer, and breast cancer.<sup>11–17</sup>

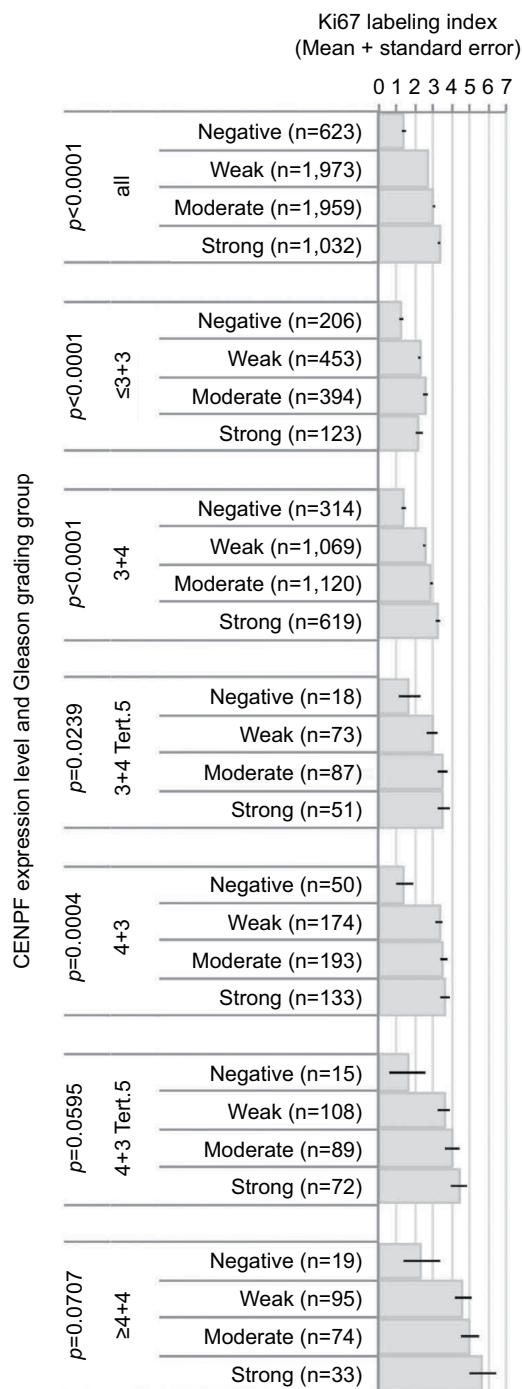
CENPF overexpression was linked to features of aggressive and prognostically unfavorable cancers in our study, including advanced tumor stage, higher Gleason grade, presence of lymph node metastasis, accelerated cell proliferation,





**Figure 4** Association between CENPF staining intensity and 10q23 (*PTEN*), 5q21 (*CHD1*), 6q15 (*MAP3K7*), and 3p13 (*FOXPI*) deletions in (A) all cancers, (B) the ERG-negative subset, and (C) the ERG-positive subset.

**Abbreviations:** CENPF, centromere protein F; *PTEN*, phosphatase and tensin homolog; *CHD1*, chromodomain-helicase-DNA-binding protein 1; *MAP3K7*, mitogen-activated protein kinase kinase kinase 7; *FOXPI*, fork head box protein PI.

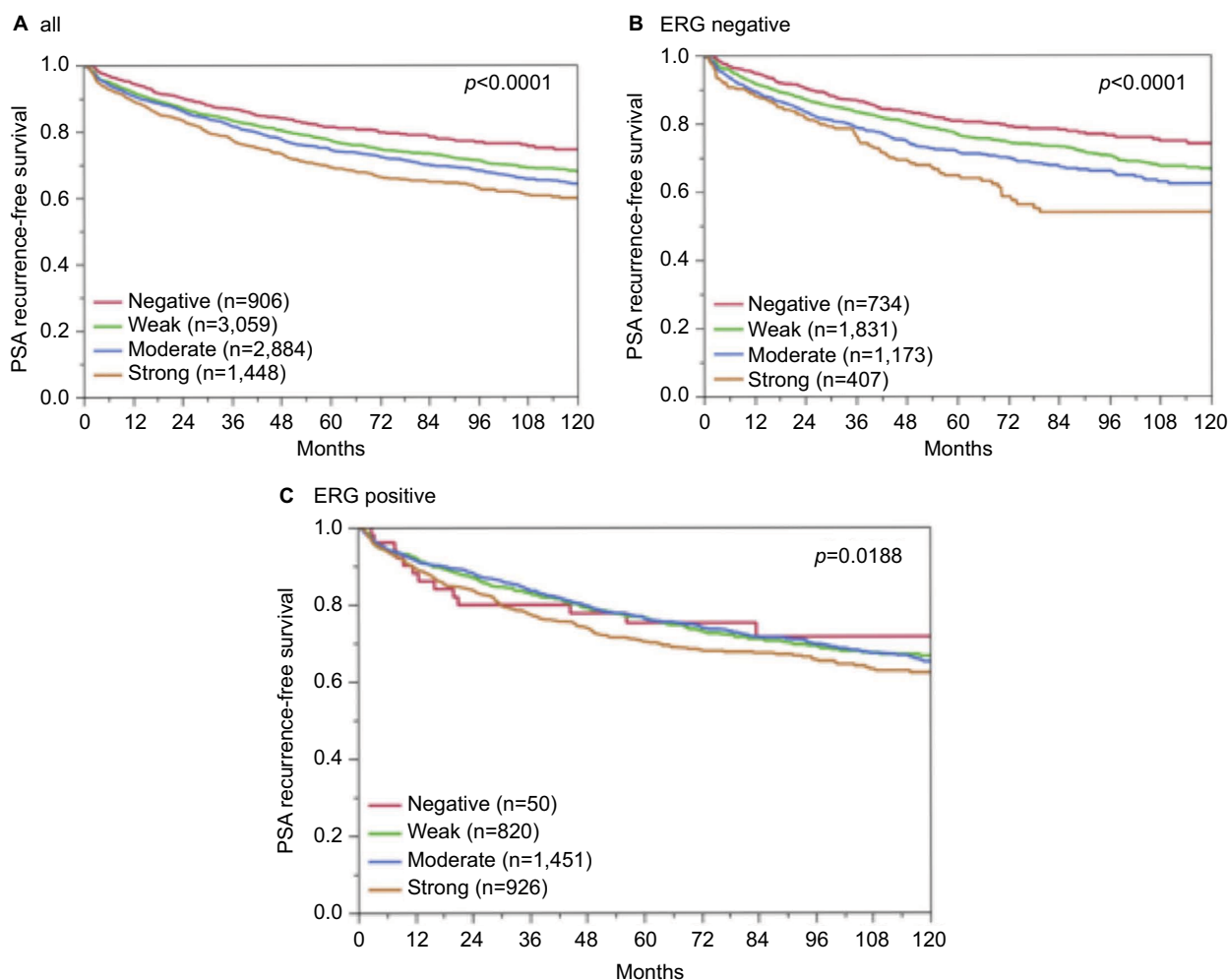


**Figure 5** Ki67 labeling index is independently associated with the CENPF expression level and the Gleason grade.  
Abbreviation: CENPF, centromere protein F.

and early biochemical recurrence ( $p < 0.0001$  each). Our results are in line with earlier reports demonstrating an important role of CENPF for tumor cell proliferation in general and prostate cancer biology. For example, Laoukili et al identified *CENPF* as a direct target gene of the master cell cycle regulator FOXM1, which controls expression of many G2-specific genes and the activity of PI3K/AKT and MAPK signaling pathways.<sup>28</sup> More recently, cross-species regulatory network analysis described a synergistic interaction between FOXM1 and CENPF in driving prostate cancer malignancy and suggested that patients with overexpression of CENPF and FOXM1 had a particularly poor outcome.<sup>10</sup> High CENPF expression has also been linked to increased tumor aggressiveness and poor survival in esophageal squamous cell carcinoma as well as nasopharyngeal and breast cancer.<sup>11,12,14</sup>

The molecular database attached to our tissue microarray allowed us to further study the impact of CENPF upregulation in molecularly defined subgroups of prostate cancers. 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*.<sup>21,29,30</sup> We found a massive upregulation of CENPF in a particularly high fraction of *ERG*-positive cancers. The reasons underlying this effect are not known. The *CENPF* promoter does not carry an *ERG* binding site according to the eukaryotic promoter database,<sup>31</sup> but it cannot be excluded that *CENPF* is an indirect transcriptional target of *ERG*. For example, global expression analyses indicate upregulation of other members of the CENP family, including CENPO, CENPL, and CENPV in *ERG*-positive as compared to *ERG*-negative prostate cancers.<sup>32</sup> Further evidence comes from recent work identifying a regulatory network comprising miR-101, CoupTFII, FOXM1, and CENPF,<sup>33</sup> which has been suggested to interfere with *ERG*-dependent transcription.<sup>34</sup>

Irrespective of the reasons leading to CENPF upregulation in *ERG*-positive cancers, our data demonstrate that the prognostic value of CENPF was reduced in *ERG*-positive cancers. In previous studies, using the same tissue microarray, we identified various proteins with higher expression levels in *ERG*-positive than in *ERG*-negative prostate cancers. With several of these, the prognostic impact was substantially stronger in *ERG*-negative than in *ERG*-positive cancers.<sup>35–37</sup> The present study demonstrates that CENPF belongs to this type of protein. Other biomarkers were only prognostic in *ERG*-positive cancers.<sup>38–40</sup> Overall, these data suggest that tumor-relevant functions of CENPF and other proteins can become modified in an *ERG*-positive molecular environment.



**Figure 6** Association between CENPF expression and PSA recurrence after prostatectomy in (A) all cancers, (B) the ERG-negative subset, and (C) the ERG-positive subset. **Abbreviations:** CENPF, centromere protein F; PSA, prostate-specific antigen; ERG, erythroblast transformation-specific (ETS)-related gene.

This is conceivable as ERG activation leads to substantial changes of the intracellular environment, affecting the expression of >1,600 genes.<sup>21,29,30</sup>

Next to *TMPRSS2:ERG* fusions, chromosomal deletions represent the second most frequent type of genomic aberration in prostate cancers, occurring at frequencies of up to 40%.<sup>32,41</sup> In particular, deletions of *PTEN* (20%), 6q (20%), 5q (10%), and 3p (10%) belong to the most prevalent genomic alterations in this disease. These are linked to either positive (*PTEN*, 3p) or negative ERG status (6q, 5q) and are associated with poor patient prognosis.<sup>23–26</sup> CENPF overexpression was unequivocally linked to deletions of *PTEN* only, suggesting a functional relationship between these two genes. *PTEN* deletion is the main cause for hyperactive PI3K/AKT signaling in prostate cancer and is associated with tumor growth, progression, and poor clinical outcome.<sup>42</sup> In fact, a relevant functional interaction between CENPF and PI3K/AKT signaling is supported by recent functional data demonstrating

that PI3K/AKT signaling was completely abrogated when CENPF was silenced, together with FOXM1, in DU145 prostate cancer cells.<sup>10</sup>

The results of our study identify CENPF as a promising candidate for a molecular test of prostate cancer aggressiveness. Our multivariate modeling suggests that a potential clinical application could be suitable to gain prognostic information not only at pretherapeutic stages, when only needle biopsies can be assessed, but also after radical prostatectomy when more comprehensive pathological data are available. It is of note that the Gleason grade is the strongest (and least expensive) prognostic feature in prostate cancer. In a recent analysis, we demonstrated that by using the percentage of unfavorable Gleason patterns, the Gleason grading could be transformed from a categorical into a continuous variable with an even finer discrimination of prognostic subgroups.<sup>19,43</sup> The prognostic impact of CENPF expression largely disappeared in groups defined by Gleason grade categories or by



Tumor subset	Scenario 1			Scenario 2			Scenario 3		
	Total	ERG negative	ERG positive	Total	ERG negative	ERG positive	Total	ERG negative	ERG positive
Analyzeable (N)	8,032	4,034	3,125	8,167	4,094	3,184	5,219	2,667	2,075
	Gleason grade biopsy								
	1.87 (1.67–2.09)***	1.67***	2.02***						
	1.74 (1.53–1.97)***	1.65***	1.75***						
	1.29 (1.13–1.48)**	1.26*	1.51**						
cT stage									
T2a vs. T1c	1.31 (1.16–1.47)***	1.30*	1.35**	1.28 (1.14–1.43)***	1.28*	1.33*			
T2b vs. T2a	1.44 (1.23–1.68)***	1.63***	1.24	1.33 (1.14–1.55)**	1.57**	1.08			
T2c vs. T2b	1.20 (0.94–1.51)	1.17	1.14	1.19 (0.94–1.51)	1.06	1.21			
T3a vs. T2c	0.68 (0.48–0.94)*	0.52*	0.89	0.79 (0.57–1.10)	0.67	1.02			
Preoperative PSA level									
4–10 vs. <4	1.33 (1.13–1.58)**	1.14	1.45*	1.32 (1.12–1.57)**	1.12	1.46*	1.10 (0.91–1.34)	1.00	1.18
10–20 vs. 4–10	1.54 (1.39–1.71)***	1.62***	1.45***	1.41 (1.27–1.57)***	1.49***	1.28*	1.20 (1.06–1.34)*	1.23*	1.11
>20 vs. 10–20	1.66 (1.44–1.90)***	1.48***	1.75***	1.49 (1.29–1.71)***	1.28*	1.75***	1.27 (1.09–1.46)*	1.18	1.30*
CENPF expression									
Weak vs. negative	1.12 (0.95–1.33)	1.11	1.20	1.03 (0.87–1.21)	1.01	1.15	1.12 (0.93–1.37)	1.09	1.16
Moderate vs. weak	1.14 (1.03–1.27)*	1.23*	1.04	1.17 (1.06–1.30)*	1.21*	1.05	1.06 (0.94–1.19)	1.25	0.94
Strong vs. moderate	1.23 (1.09–1.39)**	1.19	1.29*	1.17 (1.04–1.32)*	1.23	1.17	1.18 (1.03–1.34)*	1.18	1.20*
Gleason-grade RPE									
3+4 vs. ≤3+3				2.70 (2.31–3.17)***	2.78***	2.98***	2.09 (1.67–2.65)***	1.69**	2.69***
4+3 vs. 3+4				2.84 (2.56–3.14)***	2.33***	3.37***	2.02 (1.80–2.27)***	1.90***	2.03***
≥4+4 vs. 4+3				1.73 (1.49–2.01)***	1.71***	2.09***	1.15 (0.98–1.35)	1.14	1.41*
pT stage									
T3a vs. T2							2.00 (1.76–2.28)***	1.89***	2.23***
T3b–4 vs. T3a							1.57 (1.38–1.77)***	1.53***	1.67***
R status									
R1 vs. R0							1.25 (1.12–1.40)***	1.15	1.36**
N stage									
N1+ vs. N0							1.45 (1.26–1.67)***	1.43***	1.33*

**Abbreviations:** PSA, prostate-specific antigen; CENPF, centromere protein F; ERG, erythroblast transformation-specific (ETS)-related gene; cT, clinical tumor; RPE, radical prostatectomy; pT, pathological tumor.

comparable percentages of Gleason 4 patterns, demonstrating the power of morphological assessment of malignancy. These findings show that the threshold for a molecular test to significantly augment morphology in prognostication is rather high.

Limitations of the present study are that it was a retrospective study and that CENPF testing was done on prostatectomy specimens. To cope with the heterogeneity of prostate cancer, Kaplan–Meier analysis was done on 8,032 patients, which should guarantee a reliable result for the cohort. For individual decision-making, a prospective study is needed with at least 10 replicates of biopsy specimens for each proband.

Due to its critical role in cell proliferation and its frequent expression in cancer cells, CENPF gained interest as a potential molecular target for novel anticancer therapies. To date, no clinically applicable anti-CENPF drugs are available, but initial in vitro experiments are encouraging for the development of strategies for pharmacological inhibition. For example, zoledronic acid, a nitrogen-containing bisphosphonate usually applied in bone disease, is found to cause loss of CENPF from the kinetochore complex in breast cancer cell lines by inhibition of CENPF farnesylation/activation, subsequently leading to delayed cell cycle progression and inhibition of cell proliferation.<sup>44</sup> Moreover, due to the putative effect of CENPF on growth pathways, including PI3K/AKT and MAPK signaling,<sup>10</sup> CENPF inhibitors may provide an attractive means for inactivation of these signaling pathways.

## Conclusion

In summary, our study shows the prognostic value of CENPF. The strong association between CENPF upregulation and *PTEN* deletion supports the concept of a regulatory function of CENPF for major growth pathways, which makes it an attractive candidate for novel therapeutic approaches.

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## Author contributions

CS, AML, CHM, RS, and GS designed the study and drafted the manuscript. TS, HH, MG, and CÖ participated in study design. AH, EN, and FJ performed IHC analysis and scoring. MK, UM, DP, and RS participated in pathology data analysis. CS and RS

performed statistical analysis. GS, CMK, and MK participated in data interpretation and helped to draft the manuscript. All authors contributed toward data analysis, drafting and revising the paper, gave final approval of the version to be published and agree to be accountable for all aspects of the work.

## Disclosure

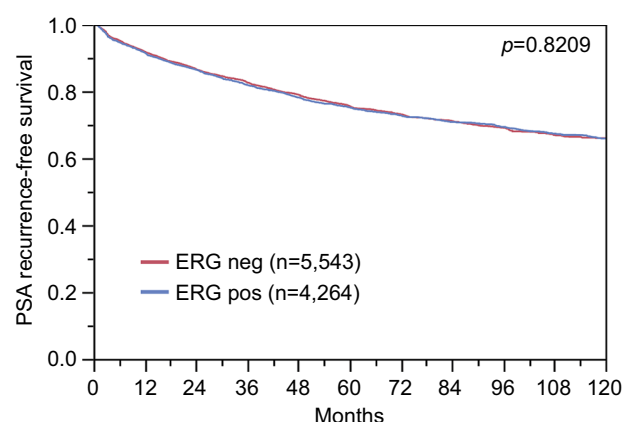
The authors report no conflicts of interest in this work.

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## Supplementary materials



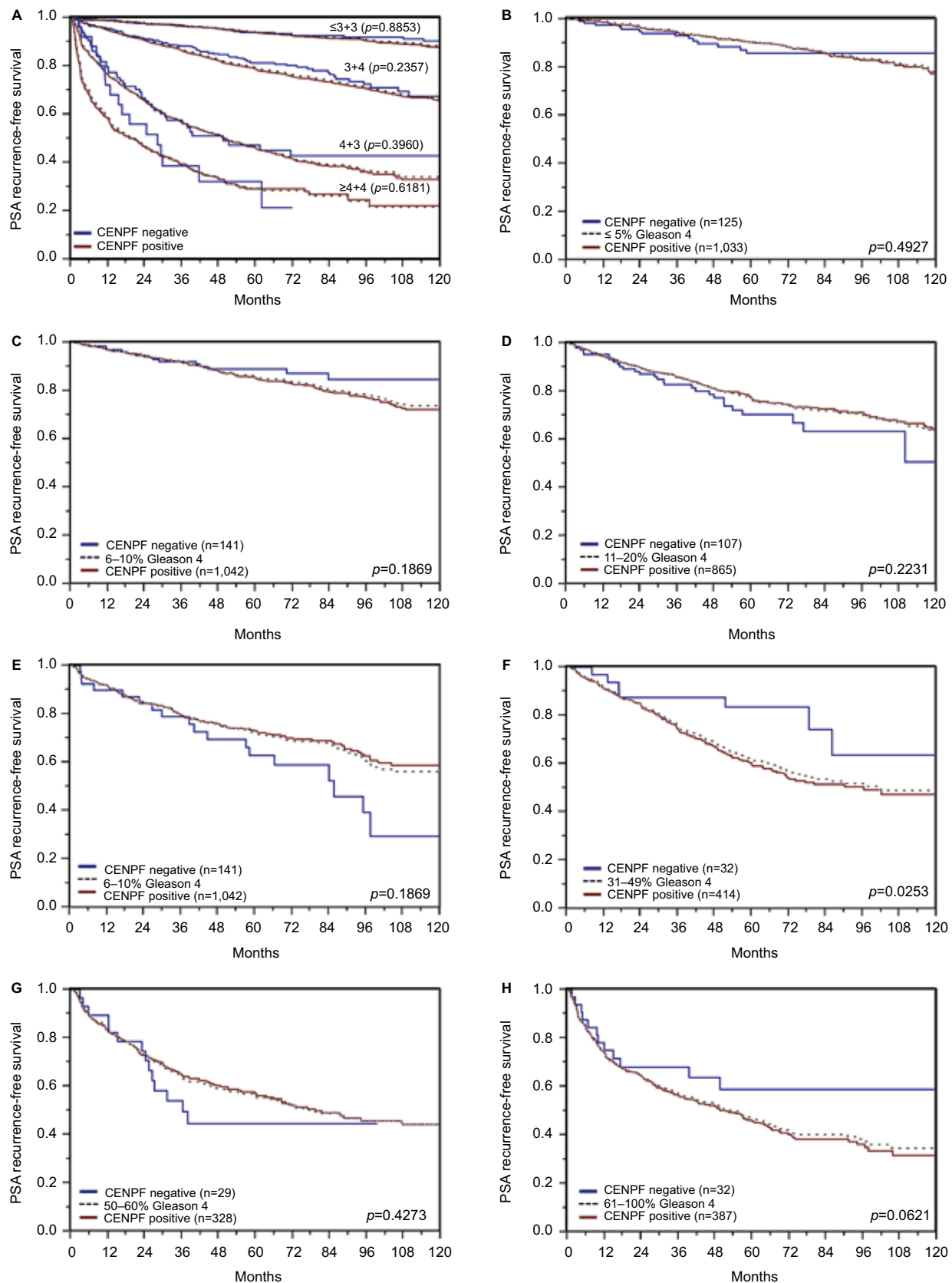
**Figure S1** Prognostic impact of ERG expression on biochemical relapse.

**Abbreviation:** ERG, erythroblast transformation-specific (ETS)-related gene.

**Table S1** Association between CENPF staining results and prostate cancer phenotype in ERG-negative tumors

Parameter	CENPF					p-value
	n evaluable	Negative (%)	Weak (%)	Moderate (%)	Strong (%)	
<b>All cancers</b>	4,512	17.8	43.9	28.5	9.8	
<b>Tumor stage</b>						
pT2	3,004	19.3	44.5	27.9	8.4	<0.0001
pT3a	936	17.0	42.1	29.7	11.2	
pT3b-pT4	559	10.6	44.4	29.9	15.2	
<b>Gleason grade</b>						
≤3+3	886	27.4	47.9	20.5	4.2	<0.0001
3+4	2,419	17.6	43.2	29.4	9.7	
3+4 Tert.5	210	11.4	42.9	29.0	16.7	
4+3	498	12.9	41.8	32.1	13.3	
4+3 Tert.5	260	8.8	41.5	31.9	17.7	
8–10	237	8.9	44.3	36.7	10.1	
<b>Lymph node metastasis</b>						
N0	2,646	16.3	42.8	29.7	11.1	0.0003
N+	254	7.9	41.7	34.3	16.1	
<b>Preop. PSA level(ng/ml)</b>						
<4	458	16.8	44.3	29.7	9.2	0.87
4–10	2,663	17.5	43.7	28.6	10.3	
10–20	990	19.3	43.6	27.6	9.5	
>20	378	16.1	46.3	28.8	8.7	
<b>Surgical margin</b>						
negative	3,582	18.0	43.7	28.2	10.2	0.2884
positive	917	16.7	45.0	29.8	8.5	

**Abbreviations:** CENPF, centromere protein F; ERG, erythroblast transformation-specific (ETS)-related gene; PSA, PSA, prostate-specific antigen.



**Figure S2** Prognostic impact of CENPF expression in subsets of cancers defined by the Gleason score.

**Notes:** (A) Impact of negative and positive CENPF expression compared with the classical Gleason score categories. (B–H) Impact of negative and positive CENPF expression compared with the quantitative Gleason score categories defined by subsets of cancers with (B)  $\leq 5\%$  Gleason 4 patterns, (C)  $6-10\%$  Gleason 4 patterns, (D)  $11-20\%$  Gleason 4 patterns, (E)  $21-30\%$  Gleason 4 patterns, (F)  $31-49\%$  Gleason 4 patterns, (G)  $50-60\%$  Gleason 4 patterns, (H)  $61-100\%$  Gleason 4 patterns.

**Abbreviation:** CENPF, centromere protein F.



**Table S2** Association between CENPF staining results and prostate cancer phenotype in ERG-positive tumors

Parameter	CENPF					p-value
	n evaluable	Negative (%)	Weak (%)	Moderate (%)	strong (%)	
<b>All cancers</b>	3,561	1.5	25.0	44.5	29.0	
<b>Tumor stage</b>						
pT2	2,075	1.4	25.1	45.7	27.9	0.5856
pT3a	976	1.7	24.8	42.4	31.0	
pT3b-pT4	496	1.6	25.2	43.5	29.6	
<b>Gleason grade</b>						
≤3+3	692	2.3	28.9	49.4	19.4	<0.0001
3+4	2,055	1.2	24.0	43.8	30.9	
3+4 Tert.5	114	2.6	21.1	49.1	27.2	
4+3	359	1.1	20.3	39.0	39.6	
4+3 Tert.5	206	1.5	25.7	45.1	27.7	
8–10	133	2.3	35.3	39.8	22.6	
<b>Lymph node metastasis</b>						
N0	2,040	1.8	23.9	43.4	31.0	0.5319
N+	234	0.9	26.9	42.7	29.5	
<b>Preop. PSA level (ng/ml)</b>						
<4	492	1.0	27.8	44.9	26.2	0.4383
4–10	2,159	1.4	24.4	44.2	30.0	
10–20	670	2.4	24.9	44.2	28.5	
>20	214	0.9	25.2	46.7	27.1	
<b>Surgical margin</b>						
Negative	2,773	1.6	24.9	44.8	28.7	0.8674
Positive	774	1.4	25.5	43.3	29.8	

**Abbreviations:** CENPF, centromere protein F; ERG, erythroblast transformation-specific (ETS)-related gene; PSA, prostate-specific antigen.

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