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ORIGINAL ARTICLE

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Loss of CCAAT-enhancer-binding protein alpha (CEBPA) is linked to poor prognosis in *PTEN* deleted and *TMPRSS2:ERG* fusion type prostate cancers

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Background: The transcription factor CCAAT-enhancer-binding protein alpha (CEBPA) is a crucial regulator of cell proliferation and differentiation. Expression levels of CEBPA have been suggested to be prognostic in various tumor types.

Methods: Here, we analyzed the immunohistochemical expression of CEBPA in a tissue microarray containing more than 17 000 prostate cancer specimens with annotated clinical and molecular data including for example *TMPRSS2:ERG* fusion and *PTEN* deletion status.

Results: Normal prostate glands showed moderate to strong CEBPA staining, while CEBPA expression was frequently reduced (40%) or lost (30%) in prostate cancers. Absence of detectable CEBPA expression was markedly more frequent in ERG negative (45%) as compared to ERG positive cancers (20%, $P < 0.0001$). Reduced CEBPA expression was linked to unfavorable phenotype ($P < 0.0001$) and poor prognosis ($P = 0.0008$). Subgroup analyses revealed, that the prognostic value of CEBPA loss was entirely driven by tumors carrying both *TMPRSS2:ERG* fusions and *PTEN* deletions. In this subgroup, CEBPA loss was tightly linked to advanced tumor

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stage ($P < 0.0001$), high Gleason grade ($P < 0.0001$), positive nodal stage (0.0003), and early biochemical recurrence ($P = 0.0007$), while these associations were absent or markedly diminished in tumors with normal *PTEN* copy numbers and/or absence of *ERG* fusion.

Conclusions: CEBPA is down regulated in about one third of prostate cancers, but the clinical impact of CEBPA loss is strictly limited to the subset of about 10% prostate cancers carrying both *ERG* fusion and deletions of the *PTEN* tumor suppressor. Our findings challenge the concept that prognostic molecular markers may be generally applicable to all prostate cancers.

KEYWORDS

CEBPA, deletion, prostate cancer, TMA

1 | INTRODUCTION

Prostate cancer is the most prevalent cancer in men in Western societies.[1] Despite a rather indolent clinical course of most prostate cancers, this disease still represents the third most common cause of cancer related death in men. A reliable distinction between the indolent and the aggressive forms of the disease is highly desirable to enhance therapeutic decisions. The only established pretreatment prognostic parameters currently include Gleason grade and tumor extent on biopsies, preoperative prostate-specific antigen (PSA), and clinical stage. These data are statistically powerful but not sufficient for optimal individual treatment decisions. It is 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.

CCAAT-enhancer-binding protein alpha (CEBPA) is one of six members of the leucine zipper family of transcription factors.[2,3] Emerging research has identified relevant roles of CEBPA in the regulation of cell proliferation[4] and terminal differentiation,[5,6] in maintenance of energy metabolism[7] and control of immune and inflammatory processes.[8,9] Interestingly, CEBPA has been identified as a repressor of TERT, the protein subunit of telomerase, and loss of CEBPA correlated with the activation of TERT expression during tumor genesis.[10] In addition to its role as a transcriptional activator, CEBPA mediates growth arrest through protein-protein interactions leading to stabilization of p21[6,11] and binding to cell cycle related proteins such as cyclin-dependent kinases 2 and 4[4] and the E2F complex.[12,13] CEBPA is expressed in a wide variety of normal tissues including liver, fat tissue, lung, small intestine, skin, mammary gland, adrenal gland, hematopoietic cells, ovaries, placenta, and prostate.[14,15] In cancers, CEBPA expression is often altered. However, there is no clear-cut pathogenic level of CEBPA, because it becomes up regulated in some cancer types[16–18] but down regulated in others.[19–21] For example, high CEBPA expression levels have been associated with poor prognosis in liver and ovarian cancer,[17,18] but with good prognosis in lung, head and neck, breast, and skin cancer.[19,22–24] CEBPA might be of particular interest in prostate cancer as it modulates transcription of androgen responsive genes.[25] In addition, alterations of CEBPA expression had been linked to high Gleason grade in two studies involving 21 and 105 cancers.[16,26] In addition, CEBPA was predicted to be one of the deregulated transcription factors that are responsible for up regulation of TERT based on a bioinformatical analysis using mixed integer linear programming based regulatory interaction predictor[27] of transcriptome data from the TCGA sequencing project.

Here, we took advantage of our large tissue microarray (TMA) resource including more than 17 000 prostate cancers to study the role of CEBPA expression in this disease. The database attached to our TMA contains pathological and clinical follow-up data, as well as abundant molecular data of key molecular alterations of this disease.

2 | MATERIALS AND METHODS

2.1 | Patients

The 17 747 patients had radical prostatectomy (RPE) between 1992 and 2014 at the University Medical Center Hamburg-Eppendorf (Department of Urology and the Martini Clinics). The RPE specimens were analyzed as described before.[28] Histopathological data (tumor stage, Gleason grade, nodal stage, and stage of the resection margin) were retrieved from the patients' records. Follow-up was available for a total of 14 464 patients (median 48 months, range: 1 to 275 months; Table 1). Prostate specific antigen (PSA) values were controlled post-RPE and PSA recurrence was defined as a PSA of ≥0.2 ng/mL or increasing PSA values in subsequent measurements. The TMA manufacturing process was described earlier in detail.[29,30] Each TMA block contained various controls, including normal prostate tissue. The molecular database attached to this TMA contained results on ERG expression in 10 677,[31] deletion status of 10q23 (*PTEN*) in

TABLE 1 Pathological and clinical data of the arrayed prostate cancers

	Study cohort on TMA (n = 17 747)	Biochemical relapse among category
Follow-up	14 464	3612 (25%)
Mean/median (month)	56.3/48.0	-
Age (y)		
≤50	433	66 (15%)
51-59	4341	839 (19%)
60-69	9977	2073 (21%)
≥70	2936	634 (22%)
Pretreatment PSA (ng/mL)		
<4	2225	313 (14%)
4-10	10 520	1696 (16%)
10-20	3 662	1043 (29%)
>20	1231	545 (44%)
pT stage (AJCC 2002)		
pT2	11 518	1212 (11%)
pT3a	3842	1121 (29%)
pT3b	2233	1213 (54%)
pT4	85	63 (74%)
Gleason grade		
≤3+3	3570	264 (7%)
3+4	9336	1 436 (15%)
3+4 Tertiary 5	1697	165 (10%)
4+3	2903	683 (24%)
4+3 Tertiary 5	1187	487 (41%)
≥4+4	999	531 (53%)
Nodal (pN) stage		
pN0	10 636	2243 (21%)
pN+	1255	700 (56%)
Surgical margin (R) status		
Negative	14 297	2307 (16%)
Positive	3388	1304 (39%)

Numbers do not always add up to 17 747 in the different categories because of cases with missing data. AJCC, American Joint Committee on Cancer.

6704 cancers,[32] and SOX9 expression in 7565 cancers.[33] Archived diagnostic leftover tissues was pseudo-anonymized and used for research purposes without consent as approved by local laws (HmbKHG, §12a) and by the local ethics committee (Ethics commission Hamburg, WF-049/09). The work has been carried out in compliance with the Helsinki Declaration.

2.2 | Immunohistochemistry

Freshly cut TMA sections were immunostained on 1 day and in one experiment. Slides were deparaffinized and exposed to heat-induced antigen retrieval for 5 min in an autoclave at 121°C in pH 7.8 Tris-EDTA-Citrate buffer. Primary antibody HPA065037 specific for CEBPA (rabbit, polyclonal antibody; Sigma-Aldrich, Germany; cat#HPA065037; dilution 1:150) was applied at 37°C for 60 min. Bound antibody was then visualized using the EnVision Kit (Dako, Glostrup, Denmark) according to the manufacturer's directions. CEBPA staining was validated with positive and negative control tissues and was in line with data of the Human Protein Atlas (www.proteinatlas.org) {Uhlen, 2015 #35}. In glandular cells of the normal prostate (Figure 1a) and stomach (data not shown) the nucleus of cells was stained. Tumors with complete absence of staining were scored as "negative" (Figure 1b). A "low" score was given to cancers with a staining intensity of 1+, or 2+ in >30% and ≤70% of tumor cells, or 3+ in ≤30% of tumor cells (Figure 1c). The score was "high" if staining intensity was 2+ in >70% of tumor cells or 3+ in >30% of tumor cells (Figure 1d).

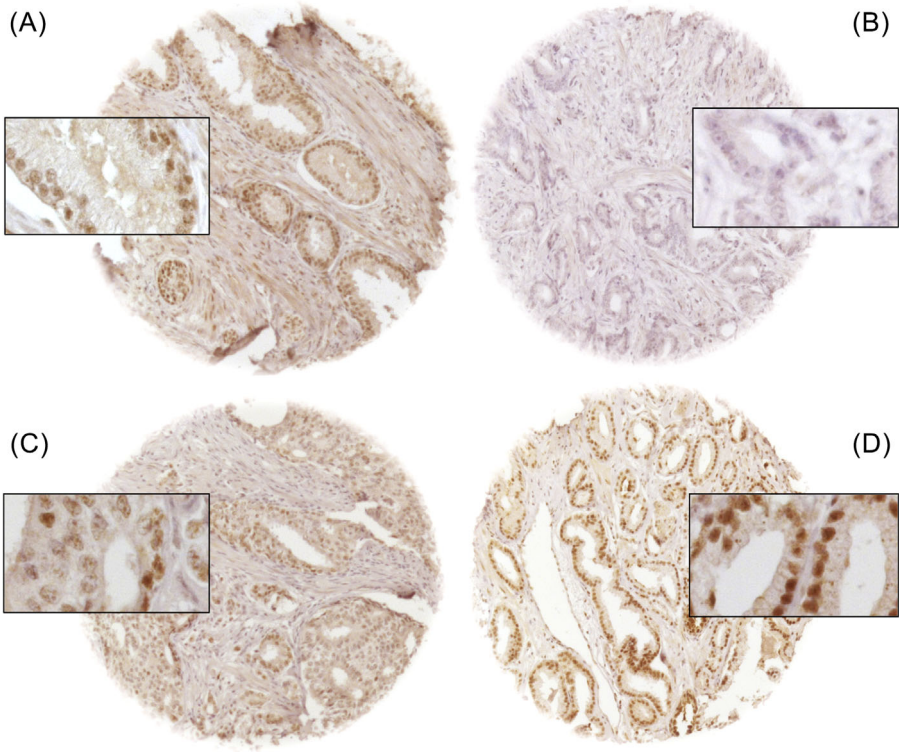

FIGURE 1 Representative pictures of CEBPA immunostaining in (A) normal prostate and prostate cancer with (B) negative, (C) low, and (D) high CEBPA staining; magnification 100×/400×, spot size 600 µm. [Color figure can be viewed at wileyonlinelibrary.com]

2.3 | Statistics

For statistical analysis, JMP 12.0 (SAS Institute Inc., NC) was used. Contingency tables were calculated to study association between CEBPA expression and clinico-pathological variables, and the chi-square test was used to find significant relationships. Kaplan-Meier curves were generated using 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.

3 | RESULTS

A total of 12 692 (72%) of tumor samples were interpretable in our TMA analysis. Reason for non-informative cases (5055 spots; 28%) included lack of tissue samples or absence of unequivocal cancer tissue in the TMA spot. Normal prostate epithelial tissues typically showed intense (2+ to 3+) nuclear staining in basal and luminal cells under the selected experimental conditions. In cancers, detectable nuclear CEBPA immunostaining was seen in 8824 of our 12 692 (69.5%) tumors and was considered low in 41% and high in 28.6% of cancers. The remaining 3868 (30.5%) tumor containing tissue spots were negative for CEBPA. Representative images of CEBPA immunostainings are given in Figure 1.

3.1 | Association with ERG fusion and PTEN deletion

There was a strong association between CEBPA and the ERG and/or PTEN status or their combinations (Figure 2; $P < 0.0001$ each). CEBPA expression was strongly up regulated in ERG positive as compared to ERG negative cancers. For example, high CEBPA expression was found in 33% of ERG positive, but only in 19% of ERG negative cancers (Figure 2a). The PTEN status had less impact on CEBPA levels (Figure 2a). However, combined analysis with ERG revealed that the impact of PTEN status on CEBPA depended on the ERG status (Figure 2b): PTEN deletion was associated with higher CEBPA expression in ERG negative cancers (29% strongly CEBPA positive in PTEN deleted cancers vs 20% in PTEN normal cancers, $P = 0.0018$) but with lower CEBPA expression in ERG positive cancers (29% strongly CEBPA positive in PTEN deleted cancers vs 36% in PTEN normal cancers, $P = 0.0004$).

3.2 | Association with PSA recurrence

The analysis of cancer subsets characterized by ERG and PTEN (Figure 3), the most important known aberrations in prostate cancer revealed, that the prognostic impact of reduced CEBPA expression was more prominent in ERG positive (Figure 3a $P = 0.0008$) than in ERG negative (Figure 3b, $P = 0.0273$) and in PTEN deleted (Figure 3c, $P = 0.0011$) than in PTEN undeleted cancers (Figure 3d, $P = 0.7$). The

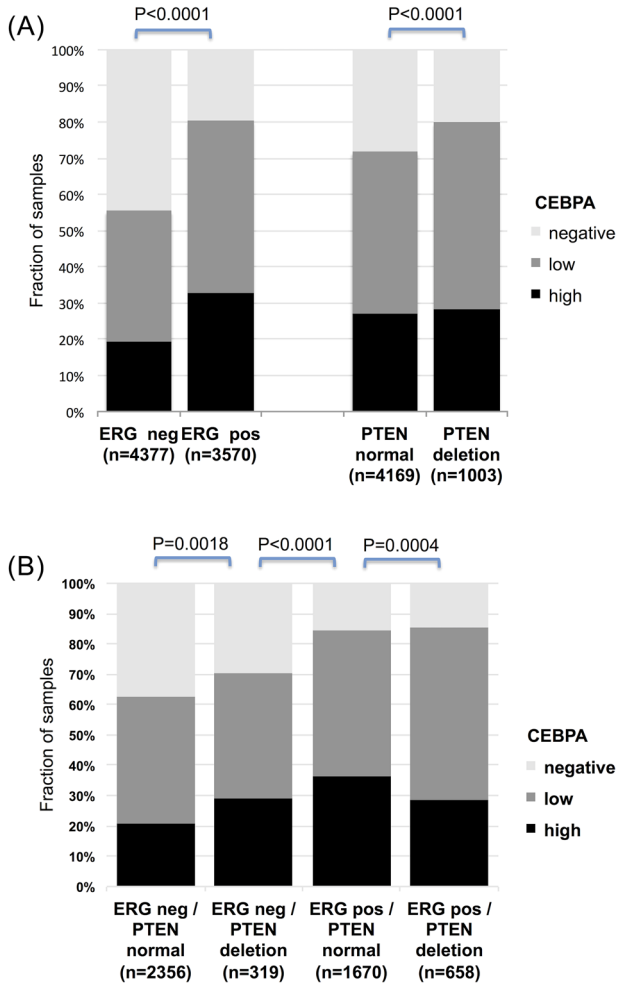

FIGURE 2 Association between different levels of CEBPA immunostaining and (A) immunohistochemical ERG expression and *PTEN* deletion as measured by fluorescence in-situ hybridization analysis and (B) subsets of cancers with identical ERG/*PTEN* status. [Color figure can be viewed at wileyonlinelibrary.com]

combination of ERG and PTEN for subgroup definition revealed that reduced CEBPA expression had a striking prognostic impact in 611 cancers with both alterations (Figure 3e, $P = 0.0007$) but no significant influence on outcome in cancers having none of these alterations (Figure 3f, $P = 0.5539$), ERG positivity alone (Figure 3g, $P = 0.6636$), or PTEN deletion alone (Figure 3h, $P = 0.0578$).

3.3 | Association with tumor phenotype

Because the prognostic impact of CEBPA was strongest in ERG positive cancers harboring PTEN deletions, we separated our dataset into cancers with ERG fusion and PTEN deletion and all remaining cancers (including ERG pos./PTEN normal cancers and ERG negative cancers with or without PTEN deletion) to search for associations between CEBPA expression and tumor phenotype. It showed that loss of CEBPA expression was significantly associated with advanced tumor stage ($P < 0.0001$), high Gleason grade ($P < 0.0001$), positive nodal stage ($P = 0.0003$), and high preoperative PSA level ($P = 0.0066$) in the subset of ERG positive and PTEN deleted cancers. Strikingly, most of these associations were lost or at least markedly diminished in the subset of cancers with normal PTEN copy numbers. All data are summarized in Table 2.

3.4 | Multivariate analysis

To estimate the prognostic power of CEBPA relative to the established clinical-pathological prognostic parameters, we selected the subset of PTEN deleted and ERG positive cancers where CEBPA best predicted prognosis and calculated four different multivariate models that resemble typical clinical scenarios (Table 3). No 1 was utilizing 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 and CEBPA expression. Scenario 2 was utilizing CEBPA expression and all postoperatively available parameters with exception of nodal status. 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 were added to model the preoperative situation. Scenario 3 included CEBPA expression, preoperative PSA, clinical tumor stage (cT stage), and Gleason grade obtained on the prostatectomy specimens. Since postoperative determination of a tumor's Gleason grade is generally more precise 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. In scenario 4, the preoperative Gleason grade obtained on the original biopsy was combined with preoperative PSA, cT stage, and CEBPA expression. It turned out that CEBPA was inferior to these parameters in all scenarios.

3.5 | Combination with SOX9

In an earlier study using our TMA,[33] we found that the prognostic value of SOX9 expression loss was limited to the same set of ERG positive and *PTEN* deleted cancers as CEBPA. This prompted us to study whether a combination of CEBPA and SOX9 data would allow for a better prediction of prognosis than the individual markers alone. To reduce data complexity, we binned all cancers with data on both CEBPA and SOX9 into three groups, including (1) cancers showing any level of positive immunostaining for both markers (both positive); (2) cancers that were positive for CEBPA but negative for SOX9 or vice versa (one negative, one positive); and (3) cancers without detectable staining of both markers (both negative). The result of a Kaplan-Meier analysis in the subset of ERG positive and PTEN-deleted cancers is shown in Figure 4. Patient prognosis worsened with decreasing CEBPA and SOX9 expression: The best prognosis was found for patients with tumors expressing both proteins, an intermediate prognosis for cancers lacking detectable expression of one protein ($P = 0.0025$), and the worst prognosis for cancers lacking both proteins ($P = 0.0473$).

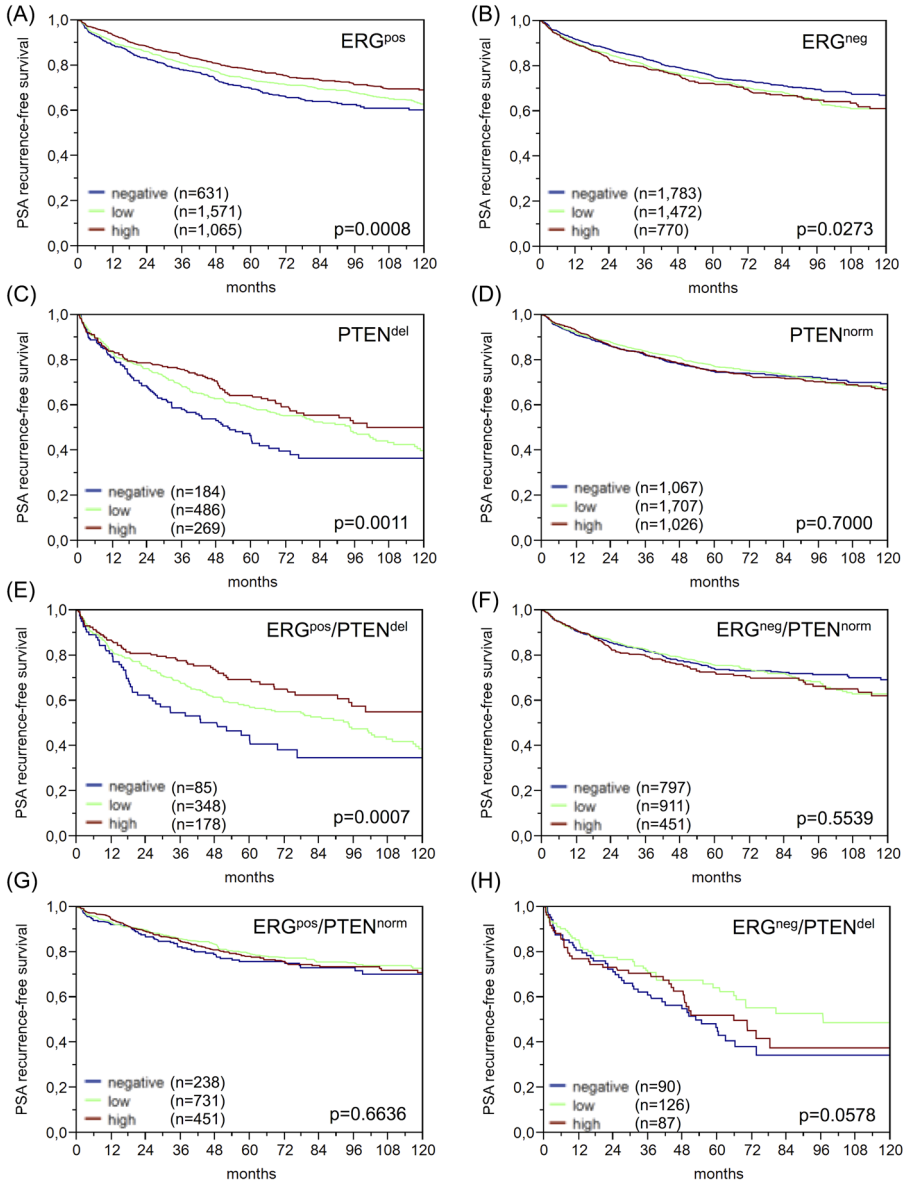

FIGURE 3 Association between CEBPA expression and biochemical recurrence in (A) ERG positive cancers, (B) ERG negative cancer, (C) *PTEN* deleted cancers, (D) *PTEN* non-deleted cancers, (E) ERG positive and *PTEN* deleted cancers, (F) ERG negative and *PTEN* non-deleted cancers, (G) ERG positive and *PTEN* non-deleted cancers, and (h) ERG negative and *PTEN* deleted cancers. [Color figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

The results of our study show that loss of CEBPA expression occurs in one third of prostate cancers and that the clinical impact of this molecular alteration is largely limited to the subset of ERG positive cancers harboring *PTEN* deletions.

Normal prostate glands showed moderate to strong nuclear CEBPA staining in our analysis. As compared to normal epithelium, nuclear CEBPA expression was frequently reduced (40%) or lost (30%) in tumors, suggesting that CEBPA down regulation might play a role for the development and/or progression of a large fraction of prostate cancers. Only two earlier studies, both performed by the group of Yin et al., employed immunohistochemistry for the analysis of CEBPA protein in prostate cancer.[16,26] The authors observed purely cytoplasmic staining and reported a shift from the basal to the apical cancer cell pole with increasing Gleason score. [26] We did not observe such cytoplasmic staining in our study. It cannot be excluded that the different staining patterns were related to the different antibodies used in these studies. For example, Yin et al. used an antibody from Santa Cruz (sc-61), which in the meantime is no longer available from the manufacturer. The rabbit polyclonal antibody HPA065037 applied in our study had been validated by the Human Protein Atlas project.[35] In addition, the nuclear staining here is clearly more consistent with the proposed function of CEBPA as a transcription factor than cytoplasmic staining.

TABLE 2 Association between CEBPA staining and prostate cancer phenotype depending on the ERG/*PTEN* status

	CEBPA (%) ERG-positive and PTEN deleted subset					CEBPA (%) in all other cancers				
Parameter	***n***	**Negative**	**Low**	**High**	***P***	***n***	**Negative**	**Low**	**High**	***P***
All cancers	658	14.7	56.7	28.6		4335	28.4	44.2	27.4	
Tumor stage										
pT2	250	10.0	53.2	36.8	**<0.0001**	**2827**	28.6	43.9	27.5	**0.416**
pT3a	236	12.7	60.2	27.1		**968**	27.2	43.9	28.9	
pT3b-pT4	170	24.1	57.1	18.8		**537**	29.6	46.0	24.4	
Gleason grade										
≤3+3	62	11.3	64.5	24.2	**<0.0001**	814	30.1	45.3	24.6	**0.018**
3+4	336	10.1	54.5	35.4		**2453**	27.5	42.8	29.8	
3+4 Tertiary 5	20	20.0	65.0	15.0		179	25.1	50.3	24.6	
4+3	119	18.5	56.3	25.2		407	28.0	48.7	23.3	
4+3 Tertiary 5	67	19.4	55.2	25.4		269	30.5	44.6	24.9	
≥4+4	54	31.5	61.1	7.4		220	33.6	42.3	24.1	
Lymph node metastasis										
N0	404	13.9	57.2	29.0	**0.0003**	**2470**	28.2	42.5	29.4	**0.034**
N+	95	24.2	56.8	19.0		**256**	30.9	49.2	19.9	
Preoperative PSA level (ng/mL)										
<4	81	9.9	50.6	39.5	**0.0066**	**482**	21.0	45.6	33.4	**<0.0001**
4-10	365	14.8	55.1	30.1		**2641**	26.6	44.4	29.0	
11-20	138	12.3	60.9	26.8		899	34.3	42.7	23.0	
>20	66	22.7	65.2	12.1		307	38.4	44.3	17.3	
Surgical margin										
Negative	469	13.9	55.2	30.9	**0.1137**	**3424**	27.8	44.3	27.9	**0.203**
Positive	187	16.6	60.4	23.0		**909**	30.5	43.9	25.6	

The most important finding of our study was that the clinical impact of CEBPA in prostate cancer strongly depended on the molecular environment. The molecular database attached to our TMA included key molecular alterations of prostate cancer such as *PTEN* deletion and the *TMPRSS2 ERG* fusion. *PTEN* deletion is one of the strongest prognostic parameters in prostate cancer, and closely associated to hyperactive PI3 K/AKT signaling and accelerated tumor growth.[36] Although we did not find an unequivocal association between CEBPA expression and *PTEN* loss, the results of our study strikingly demonstrate that the clinical effect of CEBPA in prostate

TABLE 3 Hazard ratios (95% confidence intervals) for biochemical relapse after prostatectomy for established risk factors and CEBPA expression in ERG positive and *PTEN* deleted cancers

Model		**Scenario 4**	**Scenario 3**	**Scenario 2**	**Scenario 1**
Variable	**Analyzable (N)**	**591**	**602**	**613**	**468**
Gleason grade biopsy	≥4+4 vs ≤3+3	3.16 (2.12–4.71)***			
cT stage	T2c vs T1c	1.93 (1.05–3.31)*	1.71 (0.96–2.85)		
Preoperative PSA level	≥20 vs <4	3.81 (2.21–6.79)***	3.78 (2.19–6.75)***	2.59 (1.51–4.60)**	2.85 (1.54–5.49)**
CEPBA expression	Negative vs High	1.50 (0.99–2.24)	1.15 (0.76–1.73)	1.15 (0.75–1.74)	1.01 (0.64–1.58)
Gleason grade prostatectomy	≥4+4 vs ≤3+3		8.15 (4.30–16.3)***	4.41 (2.25–9.08)***	5.71 (2.42–15.2)***
pT stage	T4 vs T2			2.63 (1.77–3.93)***	2.67 (1.69–4.29)***
Resection margin status	R1 vs R0			1.40 (1.06–1.84)*	1.34 (0.98–1.83)***
Nodal stage	N+ vs N0				1.01 (0.69–1.46)

*$P \le 0.05$, **$P \le 0.001$, ***$P \le 0.0001$.

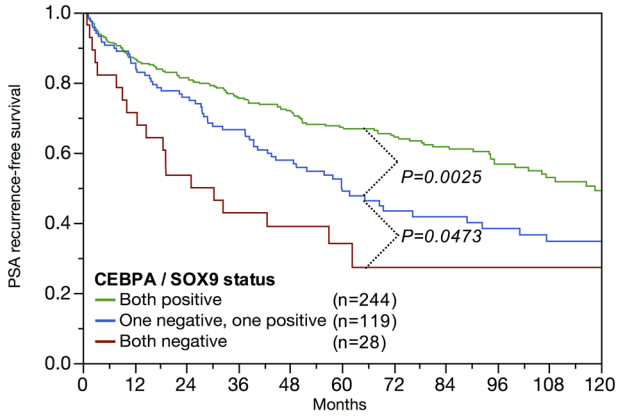

FIGURE 4 Prognostic relevance of combining immunohistochemical data of CEBPA and SOX9; "positive" refers to detectable staining of any level, "negative" to lack of any staining. [Color figure can be viewed at wileyonlinelibrary.com]

cancer depends on the presence of *PTEN* deletion and probably also *ERG* fusion. The phenotype and clinical course of tumors with both *PTEN* deletion and *ERG* fusions gradually worsened with decreasing levels of CEBPA staining, with worst prognosis in cancers without detectable CEBPA staining. This was in sharp contrast to cancers with normal *PTEN* copy numbers or negative ERG status, where the CEBPA levels lacked prognostic impact. Our findings are remarkable given that *PTEN* deletion is one of the strongest known prognostic features in prostate cancer. The Kaplan-Meier curves of patients with and without CEBPA expression differed by more than 20 percent points specifically in the subgroup of ERG positive and *PTEN* deleted cancers strongly. This finding suggests that a functional interaction of PTEN and CEBPA is present in double-inactivated cancers. Interactions between these two molecules are indeed supported by mice and zebra fish in vivo models indicating that CEBPA functions downstream of PTEN in a PI3 K dependent manner.[37,38] Relevant effectors may include p21 and cyclin-dependent kinases (CDK2 and CDK4), known binding partners of CEBPA that act downstream of PI3 K signaling.[4,12,39]

Considering also data from other cancer types, it is currently unclear, whether CEBPA has a tumor promoting or a tumor suppressive role in cancer. Some earlier functional studies suggested a tumor promoting role in prostate,[16] liver,[17] and ovarian cancer,[18] while other authors reported a tumor suppressive function in lung cancer,[20,22] breast cancer,[19] head and neck cancer,[23] liver cancer,[40] acute myeloid leukemia,[41] and pancreatic cancer.[21] The striking dependency of the clinical impact of CEBPA on the *PTEN* status found in this set of data might suggest that the tumor promoting or tumor suppressive role of CEBPA may be substantially triggered by the cellular microenvironment. The inherent variability of the molecular findings between different cancer types may thus explain discrepant findings on the role of CEBPA expression in different tumor types. Furthermore, it is noted that CEBPA can both activate and repress transcription. On the one hand, its inhibition of Cdk2 and Cdk4[4] and of TERT[10] would counteract cell proliferation. At the same time, CEBPA has been reported to act as an oncogene in liver and ovarian cancer,[17,18] which might be related to its role in activating the antiapoptotic genes BCL2 and FLIP. C/EBPalpha or C/EBPalpha oncoproteins regulate the intrinsic and extrinsic apoptotic pathways by direct interaction with NF-kappaB p50 bound to the bcl-2 and FLIP gene promoters.[42] Accordingly, it is essential to evaluate the up- or down-regulation of CEBPA within the molecular context of specific tumor subgroups to rationalize its function as done here for the analysis of our prostate cancer sample set.

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 over-expression of ERG with subsequent transcriptional deregulation of more than 1600 ERG target genes.[18,43,44] The strong association between ERG and CEBPA suggests that CEBPA could be one of these genes. This is supported by earlier work identifying an ETS transcription factor-binding site in the human CEBPA enhancer region.[43] In our data set, about two thirds of all *PTEN* deleted cancers are ERG positive.[32] It is of note that the *PTEN* deletion was associated with higher CEBPA expression levels in ERG negative but with lower CEBPA expression in ERG positive cancers. It could thus be speculated that the effects of *PTEN* deletion could be mitigated by compensatory CEBPA up regulation in ERG negative cancers while such a mechanism cannot apply to ERG positive cancers that already have up regulated CEBPA.

Overall, these findings challenge the concept that prognostic molecular markers may be generally applicable to all prostate cancers, but rather highlight a crucial role of the molecular microenvironment on the diagnostic applicability of potential prognostic markers. Our data suggest that CEBPA expression levels have strong prognostic impact—but solely in *PTEN* deleted and ERG positive cancers.

The systematic testing of candidate prognostic molecular markers in our prostate cancer TMA had previously revealed another transcription factor—SOX9—as a prognostic marker that was solely valid in *PTEN* deleted and ERG positive cancers.[33] Interestingly, a combined analysis of CEBPA and SOX9 suggests that the expression levels of these two genes might have an additive effect on patient prognosis. It appears thus plausible, that combining selected prognostic markers depending on the molecular environment of a cancer might result in powerful clinical tests in the future. That CEBPA loss in *PTEN* deleted and ERG positive tumors was highly prognostic in univariate analysis but could not outperform classical histomorphological prognostic factors in a multivariate analysis demonstrates, how powerful established prognostic markers are and how challenging it is for molecular markers to compete with established classical prognosticators. Multivariate analysis was limited to these about 500 ERG positive cancers with *PTEN* deletion, because unequivocal prognostic significance of CEBPA loss was limited to this subgroup. It is possible, that the number of analyzed cancers was too low for performing multivariate analysis involving six parameters. That the analysis of initially 17 000 cancers resulted in a subgroup of interest that was less than 600 patients further illustrates that the evaluation of potential prognostic features in prostate cancer requires very large patient cohorts.

5 | CONCLUSIONS

In summary, loss of CEBPA expression is a frequent event in prostate cancer. The clinical impact of CEBPA loss strongly depends on the ERG fusion and *PTEN* deletion status of prostate cancers. Our findings challenge the concept that prognostic molecular markers may be generally applicable to all prostate cancers.

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CONFLICTS OF INTEREST

There are no conflicts of interest to declare.

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