Loss of the adhesion molecule CEACAM1 is associated with early biochemical recurrence in TMPRSS2:ERG fusion-positive prostate cancers

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Altered expression of the carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) has been linked to adverse tumor features in various cancer types. To better understand the role of CEACAM1 in prostate cancer, we analyzed a tissue microarray containing tumor spots from 17,747 prostate cancer patients by means of immunohistochemistry. Normal prostate glands showed intense membranous CEACAM1 positivity. Immunostaining was interpretable in 13,625 cancers and was considered high in 28%, low in 43% and absent in 29% of tumors. Low and lost CEACAM1 expression was strongly linked to adverse tumor features including high classical and quantitative Gleason grade, lymph node metastasis, advanced tumor stage, positive surgical margin, a high number of genomic deletions and early biochemical recurrence (p < 0.0001 each). Subset analysis of molecularly defined cancer subsets revealed that these associations were strongest in V-ets avian erythroblastosis virus E26 oncogene homolog (ERG) fusion-positive cancers and that CEACAM1 loss was prognostic even in tumors harboring genomic deletions of the phosphatase and tensin homolog tumor suppressor (p < 0.0001). Multivariate analysis suggested that CEACAM1 analysis can provide independent prognostic information beyond established prognosis parameters at the stage of the initial biopsy when therapy decisions must be taken. In conclusion, loss of CEACAM1 expression predicts poor prognosis in prostate cancer and might provide clinically useful prognostic information particularly in cancers harboring the TMPRSS2:ERG fusion.

Introduction

Prostate cancer is the most prevalent cancer in men in western societies. Despite a rather indolent clinical course of most cases, prostate cancers still represent 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. Nevertheless, there is hope that a deeper insight in disease biology will eventually identify clinically applicable molecular markers that enable a more reliable prediction of prostate cancer aggressiveness or might function as a target for a directed antitumor therapy.

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Additional Supporting Information may be found in the online version of this article.

Key words: CEACAM1, prognosis, prostate cancer, TMA

Abbreviations: CEACAM1: carcinoembryonic antigen-related cell adhesion molecule 1; FISH: fluorescence in situ hybridization; GEO: Gene Expression Omnibus; IHC: immunohistochemistry; IR: insulin receptor; Ki67LI: Ki67 labeling index; PTEN: phosphatase and tensin homolog; PSA: prostate-specific antigen; TMA: tissue microarray; ERG: V-ets avian erythroblastosis virus E26 oncogene homolog

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DOI: 10.1002/ijc.32957

History: Received 14 Nov 2019; Accepted 2 Mar 2020; Online 9 Mar 2020

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Int. J. Cancer: 147, 575–583 (2020) © 2020 The Authors. International Journal of Cancer published by John Wiley & Sons Ltd on behalf of IJCC
Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) is a member of the immunoglobulin superfamily. It is encoded by CEACAM1 gene that resides on Chromosome 19. Two major isoforms of CEACAM1 (L and S) exist that differ in their cytoplasmic domains and intracellular localization. CEACAM1 is physiologically expressed on leukocytes, epithelia and endothelia, where it mediates cell–cell adhesion by binding to itself or to related proteins of the carcinoembryonic antigen cell adhesion subfamily such as CEACAM-5, CEACAM-6 and CEACAM-8. In addition, CEACAM1 is an important corceptor for molecules governing cell activity and growth. On T cells and natural killer cells, CEACAM plays a role in the regulation of immune surveillance, immune evasion and inflammation as it acts as a coinhibitory receptor for the immune-inhibitor Tim3. On endothelial cells, CEACAM1 is the coreceptor of vascular epithelial growth factor receptor 2. CEACAM1 is also expressed on a wide range of epithelial cells including different types of glands and their ducts, where it contributes to signaling cascades effecting tissue homeostasis, architecture and metabolism. Numerous studies have implicated CEACAM1 alterations in tumor development and progression, but its role appears to be different depending on the tumor type. For example, CEACAM1 downregulation was reported for liver cancer, kidney cancer, urinary bladder cancer, endometrial cancer and...
breast cancer, while upregulation was described in thyroid cancer, pancreatic cancer, nonsmall cell lung cancer, malignant melanoma, colon and gastric cancer. Altered CEACAM1 protein expression levels were linked to tumor aggressiveness and/or poor outcome in some of these studies.

Evidence suggests that CEACAM1 expression might also be clinically relevant in prostate cancer. Four studies analyzing CEACAM1 expression by immunohistochemistry (IHC) in samples from 15 to 202 patients suggested that CEACAM1 is downregulated in prostate cancer and that loss of CEACAM1 staining may be linked to high Gleason grade. The analysis of mRNA expression data from 426 patients by using the Gene Expression Omnibus (GEO) database also proposed that reduced CEACAM1 expression might be related to metastatic spread. Based on these data, a tissue microarray (TMA) containing more than 17,000 cancer specimens was analyzed by IHC in our study to determine the potential prognostic relevance of CEACAM1 protein expression in comparison with the established parameters.

**Materials and Methods**

**Patients**

Radical prostatectomy specimens were available from 17,747 patients, undergoing surgery between 1992 and 2015 at the Department of Urology and the Martini Clinics at the University Medical Center Hamburg-Eppendorf. All prostate specimens were analyzed according to a standard procedure, including a complete embedding of the entire prostate for histological analysis. Histopathological data were retrieved from the patients’ records, 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. In brief, for every prostatectomy specimen, the percentages of Gleason 3, 4, and 5 patterns were recorded in cancerous tissues as part of the regular process of Gleason grading.

Gleason 3 + 4 and 4 + 3 cancers were subdivided according to their percentage of Gleason 4. For practical use, we subdivided the 3 + 4 and 4 + 3 cancers in seven 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%, and 4 + 3 >60% Gleason 4. Additional groups were defined by the presence of a tertiary Gleason 5 pattern, including 3 + 4 Tert. and 4 + 3 Tert. Follow-up data were available for a total of 14,667 patients with a median follow-up of 48 months (range: 1–275 months; Supporting Information Table S1). PSA values were measured after surgery, and PSA recurrence was defined as a postoperative PSA of ≥0.2 ng/ml or increasing PSA values in subsequent measurements. The TMA manufacturing process was described earlier in detail. In short, one 0.6-mm core was taken from a representative tissue block from each tumor. The attached molecular database included data on Ki67 labeling index (Ki67LI) from 5,492 tumors (expanded from, V-ets avian

| CEACAM1 | Parameter | n Evaluable | Negative (%) | Low (%) | High (%) | p-Value |
|---------|-----------|-------------|--------------|---------|----------|---------|
| All cancers | 13,625 | 28.7 | 43.1 | 28.2 | | |
| Tumor stage | | | | | | |
| pT2 | 8,682 | 24.7 | 44.1 | 31.2 | <0.0001 |
| pT3a | 3,032 | 31.9 | 42.5 | 25.7 | | |
| pT3b-pT4 | 1,856 | 41.8 | 39.5 | 18.8 | | |
| Gleason grade | | | | | | |
| ≤3 + 3 | 2,516 | 22.4 | 45.9 | 31.8 | <0.0001 |
| 3 + 4 | 7,261 | 25.0 | 44.3 | 30.6 | | |
| 3 + 4 Tert.5 | 649 | 27.3 | 42.1 | 30.7 | | |
| 4 + 3 | 1,337 | 39.8 | 39.4 | 20.8 | | |
| 4 + 3 Tert.5 | 949 | 39.0 | 41.1 | 19.9 | | |
| ≥4 + 4 | 801 | 51.1 | 33.5 | 15.5 | | |
| Lymph node metastasis | | | | | | |
| N0 | 8,198 | 29.4 | 43.0 | 27.6 | <0.0001 |
| N+ | 1,009 | 42.6 | 39.7 | 17.6 | | |
| Preop. PSA level (ng/ml) | | | | | | |
| ≤4 | 1,624 | 23.3 | 42.9 | 33.9 | <0.0001 |
| 4–10 | 8,067 | 25.7 | 44.1 | 30.2 | | |
| 10–20 | 2,850 | 33.2 | 43.1 | 23.8 | | |
| >20 | 1,000 | 47.4 | 36.2 | 16.4 | | |
| Surgical margin | | | | | | |
| Negative | 10,854 | 26.9 | 43.6 | 29.4 | <0.0001 |
| Positive | 2,722 | 35.5 | 41.0 | 23.5 | | |

Table 1. Association between CEACAM1 staining and prostate cancer phenotype in all prostate cancers
erythroblastosis virus E26 oncogene homolog [ERG] protein expression from 13,089 and ERG rearrangement analysis by fluorescence in situ hybridization (FISH) from 7,225 tumors, as well as deletion status of 3p13 (FOXP1) from 5,503 tumors, 5q21 (CHD1) from 6,145 tumors, 6q15 (MAP3K7) from 4,663 tumors, 8p21 from 5,556 tumors, 10q23 (phosphatase and tensin homolog [PTEN]) from 5,158 tumors, 12p13 (CDKN1B) from 4,887 tumors, 12q24 from 5,721 tumors, 13q14 (FOXO1, RB1) from 5,915 tumors, 16q24 from 4,413 tumors, 17p13 (TP53) from 6,437 tumors and 18q21 from 5,578 tumors.

Ethics approval and consent to participate

The use of anonymized archived diagnostic leftover tissues and data without informed patient consent for manufacturing of tissue microarrays and their analysis for research purposes has been approved by local laws (HmbKHG, §12,1) and by the local ethics committee (Ethics commission Hamburg, WF-049/09). All work has been carried out in compliance with the Helsinki Declaration.

Immunohistochemistry

Freshly cut TMA sections were immunostained on one 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 Tris–EDTA–citrate buffer of pH 7.8. Primary antibody specific for CEACAM1 (mouse monoclonal, Clone 283324, R&D Systems, Minneapolis, MN; cat# MAR22441; 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. CEACAM1 staining was membranous and cytoplasmic. Membranous staining was often limited to the apical cell pole. Tumors with complete absence of staining were scored as “negative.” Tumors with 1+ staining in ≤70% of tumor cells or 2+ staining in ≤30% of tumor cells were considered “low,” and tumors with 1+ staining in >70% of tumor cells or 2+ staining in >30% of tumor cells, or 3+ staining were considered “high.”

Statistics

For statistical analysis, the JMP 12.0 software (SAS Institute Inc., Cary, NC) was used. Contingency tables were calculated to study association between CEACAM1 expression and clinicopathological variables, and the chi-square (likelihood) test was used to find significant relationships. Analysis of variance and F-test were applied to find associations between CEACAM1 expression and tumor cell proliferation as measured by the Ki67LI. 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.

Data availability

Data will be made available upon reasonable request.

Results

Technical issues

A total of 13,625 (77%) of tumor samples were interpretable in our TMA analysis. Reasons for noninformative cases (4,122 spots; 23%) included lack of tissue samples or absence of unequivocal cancer tissue in the TMA spots.

Figure 3. Association between CEACAM1 immunostaining and numbers of deletions in all prostate cancers.

Int. J. Cancer: 147, 575–583 (2020) © 2020 The Authors. International Journal of Cancer published by John Wiley & Sons Ltd on behalf of UICC
CEACAM1 expression in normal and cancerous prostate tissue

Normal prostate glands showed intense membranous staining of the luminal cells that was typically limited to the apical cell pole. Basal cells were negative. Membranous staining was accompanied by cytoplasmic staining in a small fraction (<5%) of cancers, and it was often not so strictly limited to the apical cell pole as in normal prostate glands. Purely cytoplasmic staining without membranous staining was very rare (<0.5%) (Supporting Information Fig. S1). However, major variations were found in the staining intensity. Positive staining was found in 9,720 of 13,625 (71.3%) interpretable tumors and was considered low in 43.1% and high in 28.2% of cancers. Representative images of CEACAM1 staining intensity are given in Figure 1.

CEACAM1 and tumor phenotype

Loss of CEACAM1 immunostaining was significantly linked to advanced tumor stage, high Gleason grade, lymph node metastasis, high preoperative PSA level and tumor-positive resection margin

![Figure 4. Kaplan–Meier plot of prostate specific antigen (PSA) recurrence-free survival after radical prostatectomy and immunostaining of CEACAM1 in (a) all cancers, (b) the ERG fusion-negative cancers and (c) the ERG fusion-positive cancers as well as (d) the 10q23 (PTEN) normal and (e) 10q23 (PTEN) deleted subset.](image-url)
when all cancers were jointly analyzed ($p < 0.0001$, Table 1). These associations held also true in subset analyses of ERG fusion-negative or ERG fusion-positive cancers (Supporting Information Tables S2 and S3).

**CEACAM1 and Tmprss2:ERG fusion status**
Among patients with interpretable CEACAM1 staining, data on Tmprss2:ERG fusion status obtained by FISH were available from 5,702 and by immunohistochemistry from 8,425 patients. Data on both ERG FISH and IHC were available from 5,500 cancers with evaluable CEACAM1 staining result, and an identical result (ERG IHC positive and break by FISH or ERG IHC negative and missing break by FISH) was found in 5,256 of 5,500 (95.6%) cancers. Loss of CEACAM1 immunostaining was more frequent in the subset of ERG-negative cancers: Negative CEACAM1 immunostaining was seen in 37.0% of cancers without ERG staining and 33.9% of cancers without ERG rearrangements, but only in 22.3% and 20.9% of cancers with Tmprss2:ERG fusion detected by IHC and FISH ($p < 0.0001$ each; Supporting Information Fig. S2).

**CEACAM1 and genomic deletions**
Loss of CEACAM1 staining was associated with six of 11 analyzed deletions when all cancers were jointly analyzed (Fig. 2). Because most of these deletions are either linked to the subset of ERG-negative (5q, 6q, 13q, 18q) or ERG-positive cancers (3p, 8p, PTEN, 12q, 16q, 17q), further subset analysis were performed. Within ERG-negative cancers, there were six deletions (5q21, 6q15, 8p21, 12q24, 16q24, 18q21) (Supporting Information Fig. 3a) and within ERG-positive cancers there were four deletions (6q15, 8p21, 16q24, 17p13) (Supporting Information Fig. 3b), which were significantly linked to reduced CEACAM1 expression. Loss of CEACAM1 was also associated with high numbers of deletions present in a cancer (Fig. 3).

**CEACAM1 and tumor cell proliferation (Ki67LI)**
Loss of CEACAM1 expression was linked to cell proliferation as measured by Ki67LI. However, the differences were relatively small. The average Ki67LI slightly changed from 2.91 ± 0.07 in cancers with high CEACAM1 expression to 2.60 ± 0.06 in cancers negative for CEACAM1 ($p < 0.0001$). This association held true in tumor subsets with identical Gleason score of ≤3 + 3 ($p = 0.0003$), 3 + 4 ($p < 0.0001$) and 4 + 3 ($p = 0.0022$) (Supporting Information Table S4).

**CEACAM1 and PSA recurrence**
Loss of CEACAM1 staining was significantly associated with early PSA recurrence ($p < 0.0001$, Fig. 4a). This association was mainly caused by the subset of ERG-positive cancers ($p < 0.0001$, Fig. 4b) while outcome differences were only minimal (although statistically highly significant) in ERG-negative cancers ($p < 0.0001$, Fig. 4c). Extended analyses in subsets of cancers defined by PTEN alterations or identical classical and quantitative Gleason scores further revealed that CEACAM1 provided additional prognostic information in PTEN-deleted cancers ($p < 0.0001$, Fig. 4e) and in Gleason 3 + 4 cancers ($p = 0.0189$, Supporting Information Fig. 4a), but not in any subsets defined by the quantitative Gleason score (Supporting Information Figs. 4b–4h).

**Multivariate analyses**
Four different multivariate analyses were performed to evaluate the clinical relevance of CEACAM1 expression in different scenarios (Table 2). Scenario 1 evaluated all postoperatively available parameters including pT, pN, surgical margin status, preoperative PSA value and Gleason grade obtained on the prostatectomy specimen. In Scenario 2, all postoperatively available parameters except pN were included. The rationale for this approach was that the indication and extent of lymph

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Table 2. Multivariable analysis including established prognostic parameters and CEACAM1 immunostaining (for definition of the scenarios, see “Statistics” section)

| Tumor subset | Scenario | n Analyzable | pT stage | Gleason-grade prostatectomy | pN stage | CEACAM1 expression |
|--------------|----------|--------------|----------|----------------------------|----------|-------------------|
| All cancers  | 1        | 7,127        | 0.0001   | 0.0001                      | 0.0001   | 0.0001            |
|              | 2        | 10,926       | 0.0001   | 0.0001                      | 0.0001   | 0.0001            |
|              | 3        | 10,764       |          | 0.0001                      | 0.0001   | 0.0001            |
|              | 4        | 8,480        |          | 0.0001                      | 0.0001   | 0.0001            |
| ERG-negative | 1        | 2,767        | 0.0034   | 0.0001                      | 0.0001   | 0.0001            |
| cancers      | 2        | 4,328        | 0.0001   | 0.0001                      | 0.0001   | 0.0001            |
|              | 3        | 4,292        |          | 0.0001                      | 0.0001   | 0.0001            |
|              | 4        | 4,233        |          | 0.0001                      | 0.0001   | 0.0001            |
| ERG-positive | 1        | 2,133        | 0.0007   | 0.0001                      | 0.0001   | 0.0001            |
| cancers      | 2        | 3,368        | 0.0001   | 0.0001                      | 0.0001   | 0.0001            |
|              | 3        | 3,314        |          | 0.0001                      | 0.0001   | 0.0001            |
|              | 4        | 3,258        |          | 0.0001                      | 0.0001   | 0.0001            |
node dissection is not standardized in the surgical therapy of prostate cancer and may introduce a bias towards high-grade cancers. Two additional scenarios were to model the preoperative situation as much as possible. Scenario 3 included CEACAM1 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 superior to the preoperatively determined Gleason grade (subjected to sampling errors and consequently under grading in more than one third of cases), this parameter was replaced by the preoperative Gleason grade obtained from the original biopsy in Scenario 4. The analysis identified CEACAM1 expression as an independent prognostic parameter only in Scenario 4.

Discussion
The results of our study suggest that CEACAM1 testing may have clinical value particularly in the subset of prostate cancers harboring TMPRSS2:ERG fusions.

In our study, CEACAM1 was found to be strongly expressed at the apical pole of normal prostate glandular cells. Reduced CEACAM1 expression was seen in more than two-thirds of cancers. Our findings fit well to those of earlier studies analyzing 15–202 prostate cancers. In those studies, the authors reported reduced or lost CEACAM1 staining in more than 90% of cancer samples as compared to normal prostatic tissues or to adjacent normal prostatic glands in the cancer samples. A strong association of CEACAM1 protein expression loss with unfavorable tumor phenotype and adverse clinical outcome was found in our cohort of more than 13,000 patients with available CEACAM1 IHC staining. This fits with results from one earlier IHC study analyzing 64 prostate cancers and describing an association of reduced CEACAM1 staining with high Gleason grade. Moreover, a meta-analysis using mRNA expression data from the GEO database on 426 patients had also found a significant association between reduced CEACAM1 expression and systemic tumor progression after radical prostatectomy. Increased cancer aggressiveness in case of reduced CEACAM1 expression has also been reported for colorectal, breast, lung and endometrial cancers as well as for malignant melanomas. The molecular mechanisms leading to CEACAM1 expression loss remain not fully understood, but recent evidence suggests that epigenetic chromatin modification might contribute to the loss of CEACAM1 expression in cancer cells. Interestingly, CEACAM1 may exist in two isoforms (S and L), with the L isoform being linked to expansion of the apical staining to the cell’s lateral surfaces and progression to aggressive disease in colorectal cancers and melanomas. Absence of such clear-cut lateral staining patterns in our tumors suggests that the L isoform does not play a major role in primary prostate cancers. However, we found cytoplasmic staining in rare cases of dedifferentiated cancers, suggesting that alterations affecting the apical predominance of CEACAM1 may also occur in a small fraction of prostate cancers. A general link between reduced CEACAM1 expression and poor prognosis fits well with the known role of CEACAM1 as a co-receptor for growth-inhibitory pathways. For example, CEACAM1 inhibits growth factor-induced DNA synthesis in densely growing cells through down-regulation of the ERK1/2 MAP kinase pathway and upregulation of the p27/KIP1 cyclin-dependent kinase inhibitor, and it interacts with β-catenin and the insulin receptor (IR) in order to control cell growth in response to Wnt and IR signaling. In addition, many more growth-related receptors, including endothelial growth factor receptor, vascular endothelial growth factor receptor, Toll-like and granulocyte-colony stimulating factor, interact with or are functionally modulated through CEACAM1.

Molecular data, which were available from earlier studies, enabled us to compare CEACAM1 expression with other parameters. For the purpose of our study, we were interested in the TMPRSS2:ERG fusion because this is the most common molecular alteration in prostate cancer, 11 different chromosomal deletions as these represent the next most common genomic changes in prostate cancer, as well as the tumor cell proliferation index (Ki67LI). About 50% of prostate cancers, predominantly from young patients, carry TMPRSS2:ERG fusions. These fusions lead to a constitutive overexpression of the transcription factor ERG. Although ERG overexpression lacks prognostic relevance in prostate cancer, it modulates the expression of more than 1,600 genes in prostate epithelial cells. The markedly higher rate of CEACAM1 positivity and the higher expression levels in ERG positive than in ERG negative cancers suggest that CEACAM1 belongs to the large group of genes that are either directly or indirectly regulated by ERG expression. Identifying the association between ERG and CEACAM1 expression by two independent methods (IHC/FISH) represents a strong validation of our experimental setup. This association with ERG expression could possibly be explained by the role of CEACAM1 as an inhibitor of Wnt-dependent epithelial–mesenchymal transition, which becomes activated as a consequence of the TMPRSS2:ERG fusion in prostate cancer. It is thus possible that CEACAM1 upregulation in ERG-positive tumors reflects a feedback mechanism to regain control on Wnt signaling activity. The much stronger prognostic effect of CEACAM1 expression loss in ERG positive than in ERG negative cancers further argues for a biologic relevance of the ERG/CEACAM1 interaction.

With the exception of the PTEN deletion, which is small and probably includes only one relevant gene, the functional effect of most of these deletions is enigmatic. Most likely, large deletions such as 6q and 16q contain numerous genes of which a reduced expression contributes to tumor development and progression. Most chromosomal deletions occurring in prostate cancer are linked to either positive (3p, 8p, PTEN, 12q, 16q, 17q) or negative ERG status (5q, 6q, 13q, 18q). The evaluation, whether a relationship exists between deletions and ERG-related proteins, must therefore be done in subgroups of ERG-positive and ERG-negative cancers. That CEACAM1 loss was significantly linked to 4–6 of 11 analyzed deletions in ERG-positive and ERG-negative cancers and also with the total number of deletions per
CEACAM1 expression loss was tightly linked to poor prognosis in the univariate analyses but failed to provide additional prognostic information in patient subsets defined by tumors with identical Gleason grades. This demonstrates the striking prognostic power of morphological prostate cancer assessment and shows how difficult it is for molecular prognostic markers to outperform the Gleason grade. The established clinicopathological features for prostate cancer prognosis assessment are statistically very powerful. However, they suffer from considerable limitations. Interobserver variability reaches almost 40% for the Gleason grading. Whether or not cancer positive lymph nodes are detected, largely depends on the extent of surgery and the methodological effort of pathologists. Both the nodal status and pT stage cannot be reliably determined prior to prostatectomy. For the future, we expect that prognostic assessment in prostate cancer patients will be complemented by molecular analysis of cancer tissue obtained at biopsy. Such future tests do not necessarily need to outperform currently established histopathological prognosticators but should be more reproducible. Most likely, future molecular testing will include panels of molecular features, perhaps applied through multiplex fluorescence immunohistochemistry. CEACAM1 may play a role in such panels, even though its overall prognostic significance was not very strong. This is particularly due to its independent prognostic role in preoperative scenarios in ERG-positive cancers and also by the remarkable prognostic impact within PTEN-deleted cancers. PTEN deletion belongs to the strongest prognostic parameters in prostate cancer. The limitation of the prognostic impact of CEACAM1 to a molecular subgroup such as ERG-positive cancers is not an exception. In earlier studies, we had found several prognostic markers that were applicable solely to ERG-negative cancers, for example, BCAR1 or ERG-positive cancers, for example, SOX9. This challenges the concept of developing a prognostic prostate cancer test that is applicable to all tumors.

In summary, these data demonstrate that reduced CEACAM1 expression is linked to poor disease outcome in ERG-positive prostate cancer. However, the prognostic impact of CEACAM1 expression found in the clinically aggressive subgroup of PTEN-deleted cancers argues for a clinical utility of CEACAM1 measurement at least for this tumor subset.

**Acknowledgements**

We thank Melanie Witt, Sunjé Seekamp and Inge Brandt for excellent technical assistance. Our study was supported by the German Federal Ministry of Education and Research (BMBF, Grant Number: 1KU1505B).

**Conflict of interest**

The authors have no conflict of interest to declare.

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