

PSMD9 expression correlates with recurrence after radiotherapy in patients with cervical cancer

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Abstract. In the current retrospective cohort study, the expression of the Proteasome 26S non-ATPase Subunit 9 (PSMD9) was investigated in 102 patients with cervical cancer. The rat homologue of PSMD9, Bridge-1, was identified as a binding protein of the transcription factors PDX-1 and E-12 via its PDZ-domain. The aim of the current study was to evaluate the prognostic or predictive value of PSMD9 expression as a biomarker for patients with cervical cancer. Tissue microarrays were constructed from formalin-fixed paraffin-embedded tissue specimens of cervical cancer and peritumoral stroma after hysterectomy and a Bridge-1 antibody was used to perform immunohistochemistry. The immunoreactions were analyzed using an immunoreactive score, which evaluated the number of positive cells as well as their intensity of PSMD9 expression. A misinterpretation of statistically significant results after multiple testing was controlled by the false discovery rate correction using the algorithm of Benjamini and Hochberg. All tumor tissues and almost all peritumoral stroma tissues expressed PSMD9. The PSMD9 expression in tumor tissues was significantly higher compared with the peritumoral stroma. PSMD9 expression correlated significantly with the expression of the proliferation marker MIB-1. Patients with stronger PSMD9 expression tended to exhibit a higher odds ratio for the recurrence of the disease in all patients (n=102) as well as in the subgroup of 47 patients having received a combined chemoradiotherapy following hysterectomy. In the group of 62 patients having that received radiotherapy following hysterectomy, which included the chemoradiotherapy patients, a higher PSMD9 expression

significantly increased the odds for a recurrence to 1.983-fold even after FDR correction (P=0.0304). In conclusion, PSMD9 was indicated to be overexpressed in tumor tissues and associated with tumor cell proliferation. Therefore, PSMD9 may be useful as a tumor marker. Furthermore, increased PSMD9 overexpression may be used to predict resistance against radiation.

Introduction

According to the World Health Organization, cervical cancer is the fourth most frequent cancer in women representing 6.6% of all female cancers worldwide. In 2018, there have been an estimated 570,000 new cases. Worldwide, approximately 266,000 women died of cervical cancer in 2012 (1). Approximately 75% of cervical carcinomas are squamous cell cancers. It is well established that an infection with human papilloma virus (HPV) is responsible for the formation of cervical cancer in more than 90% of all cancers (2). Up to date, there are more than 120 subtypes of HPV described and at least 14 are classified 'high risk', i.e. oncogenic for cervical cancer (1). HPV16 and HPV18 are most prominent among the sexually transmitted 'high risk' viruses causing more than 70% of cervical cancers (3). In high-income countries, a vaccination of adolescents protects against the infection with most high-risk subtypes of HPV and prevents the development of cervical cancer. Furthermore, in these countries a widely performed screening by PAP-testing provides an early diagnosis of pre-invasive cervical lesions and, under these circumstances, the therapeutic success increased during the last decades. However, the diagnosis of early-stage pre-cancerous neoplasia might be further improved by the identification of molecular markers for early diagnosis, prediction and prognosis as well as for the establishment of novel therapeutic targets in cervical carcinomas.

The treatment options for cervical cancer include the radical hysterectomy with pelvic and paraaortal lymphonodectomy or chemoradiotherapy according to the cancer stage. Following the guidelines of the European society for Medical Oncology from 2017 and the German S3 guideline from 2014, chemoradiotherapy following surgery should be restricted to cases of up-staging after surgery (4,5). A fertility-preserving surgery

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is possible for early detected cancers of small size or low risk. In severe cases with metastases, the therapy can include actual medications e.g., the anti-VEGF antibody Bevacizumab (6). Otherwise, the search for new molecular structures as possible target options is ongoing.

26S proteasome non-ATPase regulatory subunit 9 (PSMD9) is the human homolog of the protein Bridge-1 from rat. Thomas *et al* described the transcriptional co-activator Bridge-1 as a PDZ-domain containing protein that binds to E12-box DNA-binding protein and transcription factors PDX-1 and E47 and is functioning as a transcriptional co-regulator in the glucose homeostasis (7).

PSMD9 has already been investigated in tumors. In a cohort of 157 patients with breast cancer, Langlands *et al* used a PSMD9 antibody for an immunohistochemical analysis, following the idea that a lack of proteasome function could be linked to the sensitivity of breast cancer cells for radiotherapy (8). Indeed, they found that low expression of PSMD9 in the tumor was associated with less local recurrences in patients treated with radiotherapy. Banz-Jansen *et al* detected PSMD9 protein and mRNA in tumor tissues of breast cancer patients (9). Furthermore, they could show that PSMD9 expression is regulated by activin A, an inhibitor of breast carcinoma cell proliferation. Vice versa, the same group showed that downregulation of PSMD9 in MCF-7 breast cancer cells resulted in a decrease of the activin A signal transduction proteins Smad-2, -3 and -4 (10). This indicates that PSMD9 could be involved in the signaling cascade of activin A and might be critical for the growth regulation of breast cancer cells or cancer cells in general.

This study evaluated the expression of PSMD9 on tissue samples from patients with cervical cancer by immunohistochemistry.

Materials and methods

Patients. A total of 102 patients with squamous cell cancer of the cervix were included into the retrospective immunohistological analysis of PSMD9 expression in formalin fixed, paraffin embedded (FFPE)-tissue samples. All patients gave their written informed consent for the use of their tissues and the publication of results. The local ethics committee at the University of Lübeck approved this study with the number 15-134 on June 9, 2015.

The patients had undergone hysterectomy including lymph node excision in the Department of Gynecology and Obstetrics at the University Medical Center Schleswig-Holstein, Campus Lübeck between 2003 and 2012. Patients with carcinoma *in situ* (preinvasive), adenocarcinoma and those having received neoadjuvant radio- or chemotherapy were excluded from the study.

Formaldehyde fixation and paraffin embedding was performed immediately after surgery. The patients' data and disease specific information were taken from medical records and pathologists' reports. The immunohistochemical data for the expression of the proliferation marker MIB-1 were taken from a previous study using the same tissue microarrays (TMAs) (11).

Tissue micro arrays. Tissue microarrays (TMA) were prepared from FFPE-tissue samples using a semi-automated arrayer (TMArrayer; Pathology Devices, Inc.). The arrays were made

as described (12,13). In brief, with a hollow stainless-steel needle, one tumor containing tissue cylinder and another one from peritumoral stroma were taken for each patient. The tumor areas had previously been evaluated on hematoxylin stained 4 μ m sections of entire FFPE-samples. After assembly, the TMA-blocks were hardened first at 42°C for 2 h and then at room temperature over-night. Sections of 4 μ m thickness were cut with a microtome and spread onto glass slides.

For the immunohistochemistry of the TMAs a monoclonal murine anti-Bridge-1 antibody (Clone 30, cat. no. 612458; BD Biosciences) was used in a 1:50 dilution in Bond-Polymer-Antibody-Diluent (Leica Biosystems). Previously, the slides were deparaffinized and pretreated for antigen retrieval in Epitope Retrieval Solution (Leica Biosystems) for 20 min. The tissues were submerged with the primary antibody for 15 min and washed. Antibody reactions were detected with a horseradish peroxidase linked secondary antibody using the Bond-Polymer-Refine-Detection-Kit (Leica Biosystems) including DAB-chromogen staining and a hematoxylin counterstain. The tissues were dehydrated and mounted with Cytoseal-60 (Thermo Fischer Scientific). The PSMD9 expression was evaluated using the immune reactive score (IRS) by Remmele and Stegner (14). The IRS (0-12) multiplies scores for the percent of positive cells (PP; 0-4) and for staining intensity (SI; 0-3). An IRS \geq 3 was classified as positive expression.

Statistics. To compare the PSMD9 expression between tumor tissues and peritumoral stromata the Wilcoxon matched-pairs signed rank test was used. The age of patients and data from the patients' clinical reports with polytomous variables were correlated with the IRS of PSMD9 expression by the Spearman correlation coefficient (r_s). Patients' data with dichotomous outcome were used to calculate odds ratios with simple logistic regression. Statistical significance was defined at $P \leq 0.05$. Multiple testing of the same cohort might lead to a false discovery of significant results. Therefore, the false discovery rate (FDR) was corrected using the algorithm by Benjamini & Hochberg in a web-based calculator (15).

Results

In 96 patients PSMD9 expression could be evaluated by immunohistochemistry in both, tumor and peritumoral stroma tissues (Fig. 1). An expression of PSMD9, defined as positive with an IRS \geq 3, could be detected in all tumor tissues and in 90% of the peritumoral stromata. According to Table I and Fig. 2, PSMD9 was detected statistically significantly higher ($P \leq 0.0001$) even after controlling the FDR ($P = 0.0012$) in tumor tissues (IRS=8.48 \pm 1.9) compared to the surrounding peritumoral stroma (IRS=3.18 \pm 0.81).

The IRS of PSMD9 significantly correlated with the expression of the proliferation marker MIB-1 with a fair correlation (r_s : 0.2866; CI: 0.0928-0.4595; $P = 0.0017$; FDR: $P = 0.0102$; Table I, Fig. 3A).

According to Table I, all included patients ($n = 102$) had a mean age \pm standard deviation (mean \pm SD) of 53.28 \pm 12.36 years ranging from 27 to 79 years. The patients' age did not correlate with the expression of PSMD9 (r_s : 0.3926; CI: -0.1611-0.2365; $P = 0.3469$; Fig. 3B). The patients' tumor classifications

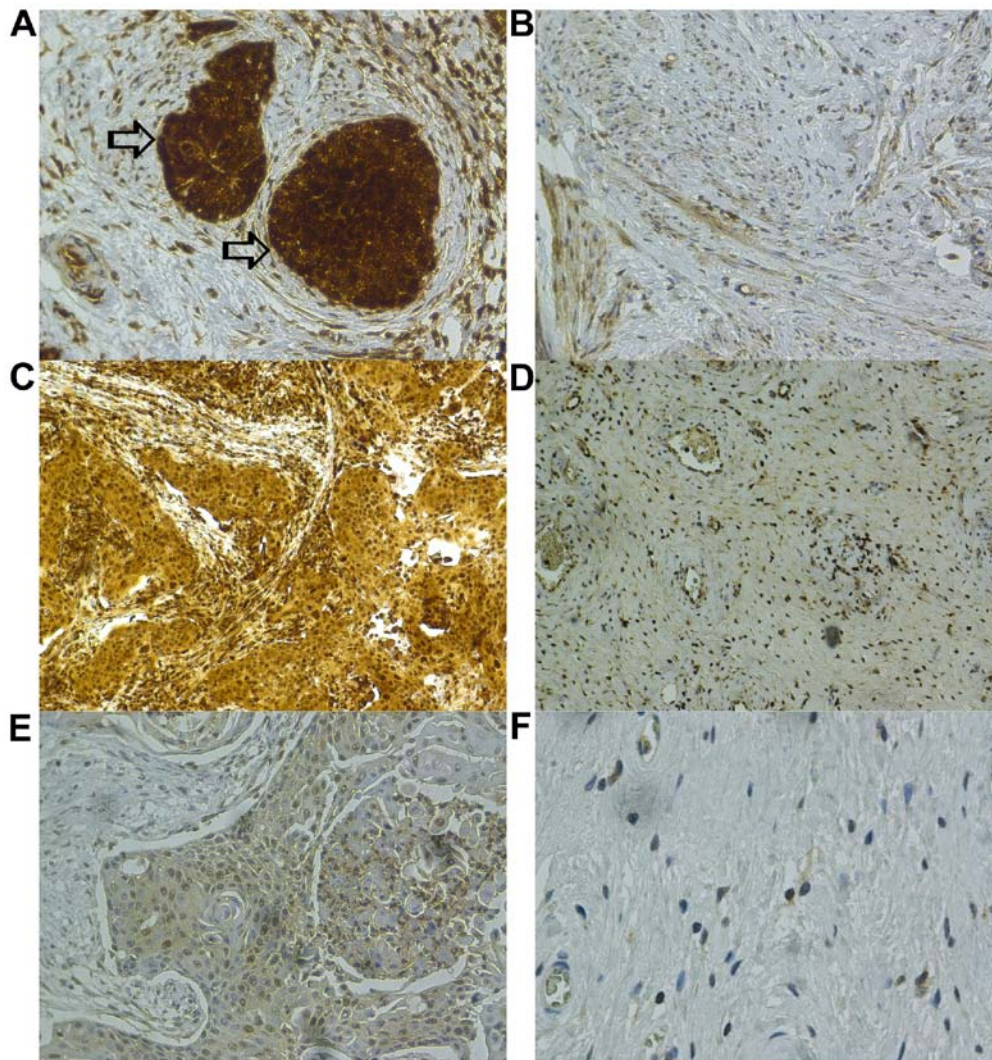


Figure 1. Immunohistochemistry of PSMD9 in cervical cancer and peritumoral stroma tissues with varying IRS. Antibody reactions were visualized using DAB-chromogen staining and a hematoxylin counterstain was used. (A) Tumor tissues indicated by the arrows: IRS 12 (PP 4, SI 3); magnification, x20. (B) Peritumoral stroma: IRS 2 (PP 2, SI 1); magnification, x20. (C) Tumor tissue: IRS 8 (PP 4, SI 2); magnification, x10. (D) Peritumoral stroma: IRS 3 (PP 3, SI 1); magnification, x10. (E) Tumor tissue: IRS 4 (PP 4, SI 1); magnification, x20. (F) Peritumoral stroma: IRS 1 (PP 1, SI 1); magnification, x40. PSMD9, Proteasome 26S non-ATPase Subunit 9; IRS, immunoreactive score; PP, percentage positive cells; SI, staining intensity.

concerning tumor size (r_s : -0.1584; CI: -0.3483-0.0441; $P=0.0568$; Fig. 3C), tumor cell grading (r_s : -0.0158; CI: -0.2182-0.1879; $P=0.4384$; Fig. 3D) and FIGO classifications (r_s : -0.1336; CI: -0.325-0.0683; $P=0.0904$; Fig. 3E) did not correlate with the expression of PSMD9 in tumor tissues. The graphs in Fig. 3C-E have a different appearance to Fig. 3A and B, because in categorical data most values shared the same points on the line of each category. The odds ratios (OR) for lymph node evasion (OR: 0.92; CI: 0.6987-1.191; $P=0.5293$; Fig. 3), distant metastases (OR: 0.8537; CI: 0.5323-1.367; $P=0.5186$; Fig. 3), and the appearance of lymphangiosis carcinomatosa (OR: 1.065; CI: 0.794-1.426; $P=0.6697$; Fig. 3) were not significantly changed with the expression of PSMD9 in tumor tissues.

Follow-up data from 102 patients were collected between 2 and 160 months after surgery with a mean \pm SD of 65.14 \pm 39.4 months. According to Table II, 18 patients experienced a recurrence of the disease and 84 were recurrence free. The mean ages (mean \pm SD) of patients with recurrence (52.89 \pm 13.89 years; range: 34-75 years) and recurrence free patients (53.37 \pm 12.1 years; range: 27-79 years) were not different

($P=0.9013$). PSMD9 expression (mean \pm SD) was stronger in patients with a recurrence (9.33 \pm 1.94) compared to those without a recurrence (8.36 \pm 1.87). The odds for a recurrence were estimated 1.304 fold (CI: 1.0-1.706; $P=0.0497$) when the IRS for PSMD9 was 1 point higher. After controlling the FDR, the odds ratio was not significant anymore (FDR: $P=0.1136$).

Two subgroups were established, one with patients having received radiotherapy ($n=62$) and within this subgroup another one including patients having received combined chemoradiotherapy ($n=47$; Table II). The IRS (mean \pm SD) of PSMD9 expression was higher in patients after receiving radiotherapy with a recurrence (8.92 \pm 1.75; $n=13$) compared to recurrence-free patients (7.84 \pm 1.07; $n=49$). The estimated odds of a recurrence for patients with a radiotherapy was 1.974 (CI: 1.186-4.239; $P=0.0076$) and the result was still significant after controlling the FDR ($P=0.0304$). In the subgroup of patients having received chemoradiotherapy, who are included in the radiotherapy subgroup, PSMD9 had an odds ratio of 1.983 for a recurrence (CI: 1.101-4.369; $P=0.0222$) which was not significant anymore after FDR control ($P=0.0666$). These

Table I. Patients' data and results of the PSMD9 expression.

Variable	Patients (n)	Value	P-value	FDR P-value
Age	102			
r_s (95% confidence interval)		0.03926 (-0.1611-0.2365)	0.3469	0.5204
Tissues ^d	96	IRS (mean \pm SD)		
Cervical carcinoma		8.48 \pm 1.89		
Peritumoral stroma		3.18 \pm 0.81	\leq 0.0001 ^c	0.0012 ^b
MIB-1 expression	102			
r_s (95% confidence interval)		0.2866 (0.0928-0.4595)	0.0017 ^b	0.0102 ^a
Tumor size	101	IRS (mean \pm SD)		
T1	62	8.71 \pm 2.01		
T2	34	8.18 \pm 1.42		
T3	3	9.33 \pm 2.31		
T4	2	6.00 \pm 2.83		
r_s (95% confidence interval)		-0.1584 (-0.3483-0.0441)	0.0568	0.1136
Nodal status	93	IRS (mean \pm SD)		
N =0	69	8.43 \pm 1.94		
N \geq 1	24	8.17 \pm 1.44		
Odds ratio (95% confidence interval)		0.92 (0.6987-1.191)	0.5293	0.5774
Metastasis	102	IRS (mean \pm SD)		
M =0	97	8.56 \pm 1.95		
M \geq 1	5	8.00		
Odds ratio (95% confidence interval)		0.8537 (0.5323-1.367)	0.5186	0.5774
Lymphangiosis carcinomatosa	58	IRS (mean \pm SD)		
L0	38	8.58 \pm 1.98		
L1	20	8.8 \pm 2.09		
Odds ratio (95% confidence interval)		1.065 (0.794-1.426)	0.6697	0.6697
Grading (UICC)	99			
G1	2	8		
G2	47	8.6 \pm 1.86		
G3	50	8.48 \pm 1.96		
r_s (95% confidence interval)		-0.0158 (-0.2182-0.1879)	0.4384	0.5774
FIGO staging	102	IRS (mean \pm SD)		
Stage I	62	8.71 \pm 2.02		
Stage II	34	8.29 \pm 1.57		
Stage III	3	9.33 \pm 2.31		
Stage IV	3	6.67 \pm 2.31		
r_s (95% confidence interval)		-0.1336 (-0.325-0.0683)	0.0904	0.155

IRS, immunoreactive score; SD, standard deviation; r_s , Spearman correlation coefficient; FDR, false discovery rate; N, nodal status; M, metastasis; statistical significance, ^aP<0.05; ^bP<0.01; ^cP<0.001. ^dWilcoxin signed rank test.

patients expressed PSMD9 with higher IRS (mean \pm SD; 9.14 \pm 1.95; n=7) compared to recurrence-free patients (7.95 \pm 1.29; n=40). The mean ages (mean \pm SD) within the subgroup of patients who received radiotherapy after surgery were not different (P=0.4382) between patients with a recurrence (54.77 \pm 14.6 years; range: 34-75 years) and recurrence free patients (51.39 \pm 11.24 years; range: 27-71 years). The same was true for patients with chemoradiotherapy after surgery. Patients with a recurrence (55.71 \pm 15.03 years; range: 36-73 years) were not different in age (mean \pm SD)

from recurrence free patients (50.86 \pm 10.91 years; range: 27-69 years; P=0.3976).

Discussion

In our collective of cervical cancer patients, PSMD9 was overexpressed in all cancer tissues compared to peritumoral stroma. Thus, PSMD9 could play a role as a tumor marker in cervical cancer. An upregulation of PSMD9 in tumor cells could be reasonable due to its role in the proteasome. Rapidly

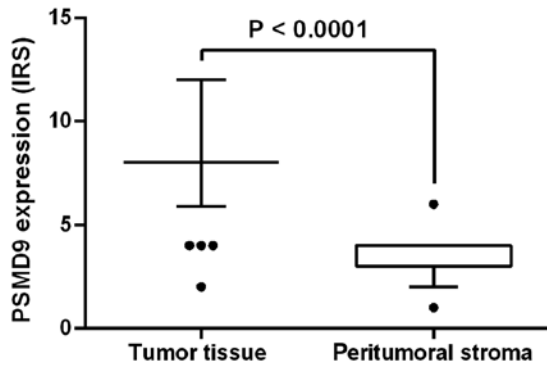


Figure 2. PSMD9 expression in cervical tumor tissue compared with peritumoral stroma using the Wilcoxon signed-rank test. The box plot shows the different PSMD9 expression for 96 patients with the mean IRS of 8.479 and 3.177 for tumor tissues and peritumoral stroma, respectively. PSMD9, Proteasome 26S non-ATPase Subunit 9; IRS, immunoreactive score.

dividing cells require an increased protein turnover and therefore, the proteins assembling the proteasome are upregulated (16). Moreover, PSMD9 expression correlated with the expression of the proliferation marker MIB-1. The MIB-1 antibody recognizes the cell cycle progression marker Ki-67. Together with another tumor marker, p16^{INK4a}, Ki-67 is a useful diagnostic tool for the classification of precancerous cervical intraepithelial neoplasia (CIN) (17,18). One could hypothesize that PSMD9 as a tumor and proliferation marker in cervical tumor tissue might also be associated with higher TNM- or FIGO-stages of cervical cancer. Here, the study that included only patients who underwent hysterectomy was not able to point onto this question. An explanation might be the fact that patients with neoadjuvant chemoradiotherapy and thereby, most of the severe cases, were excluded from this study, because patients with higher FIGO stages who did not undergo surgery according to present guidelines were excluded. According to the existing guidelines, FIGO stages 3 and 4 were treated with neoadjuvant chemoradiotherapy. In our patient collective only three patients each were included with FIGO stages III and IV, respectively. These patients underwent hysterectomy with clinical up-staging following surgery.

In the subgroups of patients who received radiotherapy or combined chemoradiotherapy following surgery, stronger PSMD9 expression resulted in significantly higher odds for the appearance of a recurrence. The patients with chemoradiotherapy were included in the radiotherapy-subgroup. After controlling the FDR, only the radiotherapy-subgroup was still significant. As a conclusion from this result, PSMD9 might be a candidate protein for the prediction of radiation sensitivity in cervical carcinoma patients. However, the present patient collective with recurrence following radiotherapy was small with only 14 patients. Overall, within the patient collective, only 18 of 102 patients showed recurrence. This might be caused by the exclusion of higher cancer stages. With regard to the small number of patients, these findings, though statistically significant, must be interpreted with care and further studies would be advisable. Otherwise, Langlands *et al* received a similar result in a study with breast cancer patients. They found an association of PSMD9 expression with a shorter time to recurrence in a subgroup of 110 patients who had radiotherapy (8).

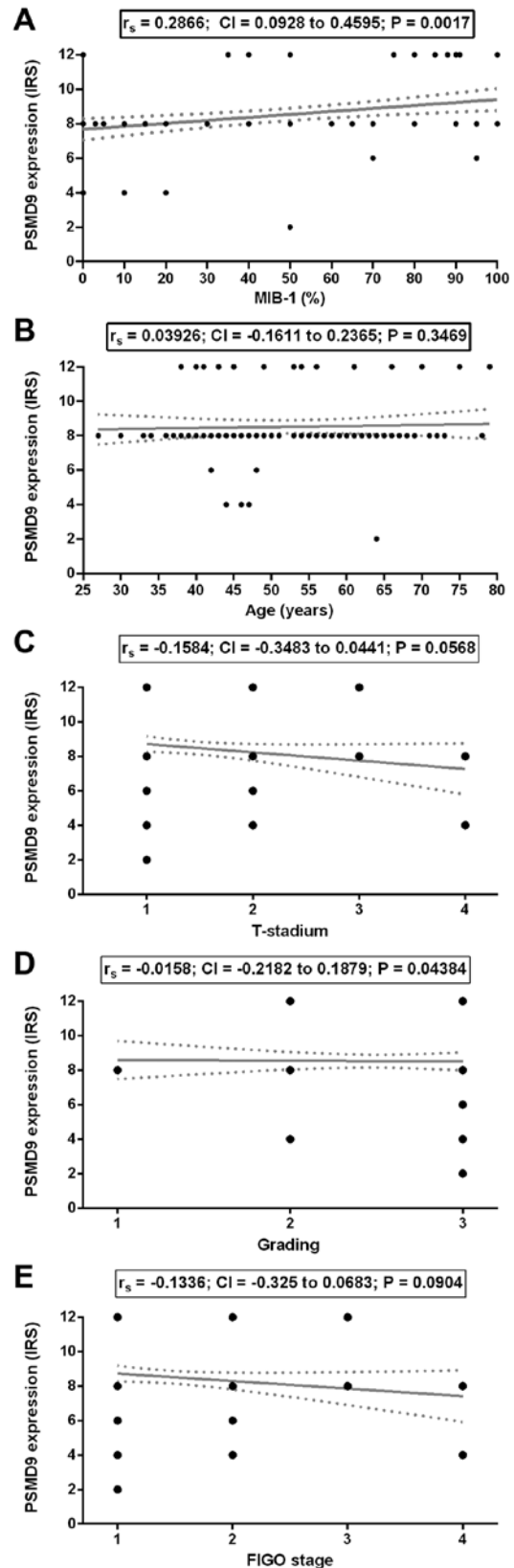


Figure 3. Tumor classifications and PSMD9 expression correlations with the Spearman's rank correlation coefficient. The scatterplots include a trendline (gray) with the 95% confidence interval (CI; gray dashed line). (A) The expression of the proliferation marker MIB-1 (antibody against Ki-67; MKI67) in correlation with PSMD9 expression. (B) The correlation of patients' age with the expression of PSMD9. (C) The expression of PSMD9 proliferation in correlation with the tumor size (T-stadium). (D) The tumor cell grading in correlation with PSMD9 expression. (E) The correlation of PSMD9 expression with the FIGO-stages. PSMD9, Proteasome 26S non-ATPase Subunit 9; CI, confidence interval; IRS, immunoreactive score.

Table II. Cancer recurrences and results of the PSMD9 expression.

Cancer recurrence	Patients (n)	IRS (mean ± SD)	P-value	FDR P-value
All patients	102	IRS (mean ± SD)		
Recurrence	18	9.33 ± 1.94		
Recurrence free	84	8.36 ± 1.87		
Odds ratio (95% confidence interval)		1.304 (1.0 to 1.706)	0.0497 ^a	0.1136
Radiation following surgery	62	IRS (mean ± SD)		
Recurrence	13	8.92 ± 1.75		
Recurrence free	49	7.84 ± 1.07		
Odds ratio (95% confidence interval)		1.974 (1.186 to 4.239)	0.0076 ^b	0.0304 ^a
Chemoradiotherapy following surgery	47	IRS (mean ± SD)		
Recurrence	7	9.14 ± 1.95		
Recurrence free	40	7.95 ± 1.29		
Odds ratio (95% confidence interval)		1.983 (1.101 to 4.369)	0.0222 ^a	0.0666

IRS, immunoreactive score; SD, standard deviation; FDR, false discovery rate; statistical significance, ^aP<0.05; ^bP<0.01.

The same publication describes that breast cancer cell lines are sensitized to radiotherapy *in vitro* after siRNA mediated downregulation of PSMD9.

It is widely accepted that a HPV infection is a prerequisite of cervical cancer. Furthermore, an HPV infection might influence the sensitivity to radiation therapy. Sabeena and colleagues collected follow-up data from ten published studies containing quantitative data from cervical cancer patients with radiation therapy and HPV infection status before or after treatment. In single publications significant prognostic values for testing HPV were described and the overall outcome showed 3 times the odds to develop a recurrence for patients with positive HPV after radiation, but statistical significance could not be reached (19). There seems to be higher prognostic value in HPV testings after therapy than before. Badaracco and colleagues tested HPV subtypes in a follow-up of 18 cervical cancer patients and found more recurrences when the HPV was still persistent after the chemoradiotherapy (20). Song and colleagues found the same in a bigger cohort of cervical cancer patients of 156 patients (21). Yu and colleagues advise to perform HPV tests after cervical cancer treatment, since they found a significant higher risk for recurrence in patients with high-risk HPV infection after therapy (22). Deng and colleagues tested the viral load in 246 patients before cervical cancer therapy with radical hysterectomy. High viral load was associated with the depth of lymphovascular invasion and with the recurrence of the disease (23).

A major limitation of this study is the unavailability of HPV data in our patient collective, because HPV testing was only performed in approximately ten percent of the patients. It would be a great value to integrate data of HPV subtype infection and virus loads especially from periods following the therapy. According to the herein before mentioned literature, we thereby would have been able to show if the influence of HPV biased the results regarding radiation response. A further limitation of our study is the relatively small number of patients. Especially, the low rate of recurrences in our cohort could have diminished the statistical outcome. The low rate of recurrences was caused

by the including only patients with a surgery. Patients with higher FIGO-stages have therefore been excluded, because, following the guidelines, they must immediately be treated by chemoradiotherapy. At least, the accuracy of the evaluation of the PSMD9 expression by immunohistochemistry is limited. The staining as well as the evaluation by humans cannot reach perfectness. Otherwise, the IHC staining was performed in a routine lab and highly experienced pathologists controlled the tumor tissue selection as well as the visual IRS evaluation.

The question, whether the different PSMD9 expressions described here and elsewhere, relied on function of PSMD9 as the transcriptional cofactor or as a part of the small subunit of the proteasome, cannot be answered. Several groups have already investigated an influence of the proteasome activity on radiation sensitivity in cancer treatment. S. Kamer *et al* observed in the cervical cancer cell line SiHa that the proteasome function inhibitor bortezomid sensitized the cells for radiation (24). By contrast, in the cervical carcinoma cell line SiHa, Pajonk *et al* detected a resistance to radiation under hypoxic conditions when they inhibited the proteasome activity with MG-132 (25). The proteasome as a drug target is further discussed in a review by Crawford *et al* (26). The function of the proteasome itself is a probable player in tumor development. The proteasome is responsible for the degradation of proteins with important functions for the cell cycle regulation e.g., IκB, the inhibitor of transcription factor NFκB, or the cyclin dependent phosphatase p27^{KIP}. Therefore, proteasome function and its proteins might be a target option in cancer treatment. Recently, Harish and colleagues have pointed out the importance of PDZ-binding proteins e.g., PSMD9 for biological functions and their usefulness as therapeutic targets. In a binding complex with hnRNPA1 PSMD9 regulates NFκB signaling. The PDZ-domain was found to be the responsible region in PSMD9 that carries the functionality for regulation (27). Han *et al* described the role of TIP-1, another PDZ-domain containing protein, for the resistance against radiotherapy in malignant glioma cells. TIP-1 was overexpressed in malignant glioma patients with radiotherapy

resistance. Furthermore, TIP-1 was found as a binding partner in a protein complex that enforced the ubiquitination of p53 and its degradation. As a consequence, p53 was unable to direct the malignant glioma cells into apoptosis that would normally be induced by DNA damage after radiation (28).

To conclude, the overexpression of PSMD9 has been found to be associated with unfavorable tumor outcome and treatment resistance in this study and by others in various cancers. Further investigations about the role of PSMD9 in cancer development and treatment are advisable.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

FK evaluated the data and wrote the manuscript. LS evaluated the immunohistochemistry and prepared the data. JRI performed the immunohistochemical stainings and evaluated the histological sections. FH constructed the TMAs and revised the manuscript. KB contributed to the evaluation of the data and revised the manuscript. AR conducted the clinical evaluation of the study. CBJ conceived of and conducted the study, and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The local ethics committee at the University of Lübeck approved the study with the number 15-134 on June 9, 2015. All patients gave their written informed consent for the use of their tissues and data after pseudonymisation.

Patient consent for publication

All patients gave their written informed consent for the publication of the results including their data after pseudonymization.

Competing interests

The authors declare that they have no competing interests.

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