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The Importance of Prostate-Specific Membrane Antigen Expression in Salivary Gland Tumors

Original Investigation

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Objective: Prostate-specific membrane antigen (PSMA) is a cell membrane protein expressed by prostate tissues. It is not prostate specific and is also expressed by some non-prostatic solid neoplasms. Our study aimed to investigate the potential role of PSMA in salivary gland tumors.

Methods: The present study was designed to retrospectively analyze our cases that presented with salivary gland tumors. The files of 105 patients were reviewed and their paraffin embedded blocks were retrieved from the pathology department. Immunohistochemical examination and staining were done using PSMA antibody. Tumor tissue PSMA immunohistochemical staining was scored semi-quantitatively with the modified quartile approach. Negative staining was scored 0, >0% and ≤25% tissue expression was considered weak (score 1), >25% and ≤50% tissue expression was considered mild (score 2), >50% and ≤75% tissue expression was considered moderate (score 3), and >75% tissue expression was considered strong (score 4).

Results: Eighty-eight patients (55 males, 33 females) were included in the study. Forty-eight patients had pleomorphic adenoma (PA), 35 had Warthin's tumor (WT), two had mucoepidermoid carcinoma, two had adenoid cystic carcinoma, and one had squamous cell carcinoma. There was statistically significant difference in terms of PSMA expression between PA and WT (p=0.003). PSMA expression was high in PA and absent in WT.

Conclusion: PSMA is a potential source of inspiration for future studies on the development of novel diagnostic and theranostic investigations of salivary gland tumors. Prospective studies targeting intratumoral PSMA in salivary gland tumors should be planned.

Keywords: Prostate-specific membrane antigen, immunohistochemistry, salivary glands, pleomorphic adenoma

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Introduction

Prostate-specific membrane antigen (PSMA) is a cell membrane protein expressed by prostate cells. However, PSMA can also be physiologically expressed by other cells such as the small intestinal, renal tubular, salivary glands, and astrocytes (1, 2). PSMA is significantly overexpressed in prostate cancer but is also expressed in the neo-vasculature of certain non-prostatic solid tumors (1, 3). Klein Nulent et al. (4) reported that adenoid cystic carcinoma (AdCC) of the head and neck salivary gland showed PSMA expression on immunohistochemistry. PSMA overexpression may have important implications for PSMA targeted imaging and new potential for non-invasive therapy strategies.

The primary treatment for salivary gland tumors is surgery. However, some benign and malignant salivary gland tumors have the potential for local recurrence. Treatment of these recurrent neoplasms are challenging. Especially, there is no standard treatment for the local recurrence of pleomorphic adenoma (PA). Another challenge in surgical treatment is that the operation can be risky due to senility and comorbid diseases in some patients (5).

Expressions of new molecules can pave the way to new imaging and/or treatment modalities. In this way, PSMA seems to be a promising and distinctive target for this purpose. In our study, we analyzed PSMA staining pattern in salivary gland tumors.

Methods

One-hundred-and-five patients that were operated on for salivary gland tumors in our clinic between January 2010 and January 2021 were analyzed. The paraffin blocks of 88 out of the 105 patients were suitable for PSMA staining and immunohistochemical examination. The Clinical Research Ethics Committee of the Süleyman Demirel University Faculty of Medicine approved our study protocol (decision no: 18/254, approval date: 11.09.2020). Informed consent was not obtained from the individuals since the study was designed retrospectively.

After the records of all patients' files were reviewed and the paraffin embedded blocks were acquired from the pathology department, immunohistochemical examination and staining were done using PSMA antibody.

Tissue Samples

All formalin-fixed paraffin blocks were sectioned in 4-5 µm and Hematoxylin-Eosin-stained slides obtained from the archive were re-read to visualize gross morphology. Appropriate blocks were selected as the tissue block most representative of the tumor. All cases were signed out according to the 4th Edition of the World Health Organization Classification of Head and Neck Tumours (5). Reporting was performed according to the protocol for the examination of specimens from patients with carcinomas of the major salivary glands of The College of American Pathologists which comprises the 8th edition TNM Classification System of the American Joint Committee on Cancer (6).

Immunohistochemistry Applications

A clinically validated PSMA antibody was applied to the blocks representing tumor morphology. PSMA antibody was prepared according to the instructions of the manufacturer, as shown in the datasheet. We used the automatized sample preparation and staining system Dako Omnis. The tissue samples were cut from the formalin-fixed paraffin-embedded blocks and transferred to 4-µm thickness adhesive-coated slides that were assembled with prostate tissue samples as the control positive antibody. For antigen retrieval, tissues were incubated with Envision-FLEX (Carpinteria, CA, USA), high PH solution for 30 minutes at 97 °C and then rinsed with wash buffer for two minutes. After the antigen retrieval step, PSMA antibody incubation was performed for 20 minutes. Then the slides were rinsed with wash buffer for two minutes. Subsequently, Envision-FLEX peroxidase-blocking solution was applied for three minutes; then the slides were again rinsed for two minutes. Before the 20-minute Envision-FLEX/HRP incubation step, slides were incubated with Envision-FLEX mouse linker for crisped staining for 10 minutes, followed by two times two minutes washing steps. Envision substrate working solution was incubated as chromogen for five minutes, and the 2-minute washing cycle was done two times. Finally, hematoxylin was applied for counterstaining for three minutes.

Immunohistochemical Staining

The slides were blindly interpreted by one pathologist experienced in surgical pathology. The slides were examined under a light microscope, and the case was accepted as positive for any cytoplasmic staining and membrane staining. PSMA immunohistochemical stained slides were scored for the percentage of positive tumor cells (4).

The non-tumoral tissue around the tumor showed a diffusely positive and faint reaction therefore these areas (expression between 1-10%) were scored as negative (score 0). Tumor tissue PSMA immunohistochemical staining was scored semi-quantitatively with the modified quartile approach (7, 8). Negative staining was scored 0, >0% and ≤25% tissue expression was considered weak (score 1), >25 and ≤50% tissue expression was considered mild (score 2), >50 and ≤75% tissue expression was considered moderate (score 3), and >75% tissue expression was considered strong (score 4). The immunohistochemical staining intensity of tumor epithelium was gradually increased from score 1 to score 4 (Figure 1).

Statistical Analysis

The data were transferred to SPSS v.23.0 (IBM Corp., Armonk, NY, USA) for statistical analysis. Prior to analysis, controls were made to see whether there were any data entry errors and whether the parameters were within the expected range. Normality assumptions of continuous variables were examined with the Shapiro-Wilk test. Mean and standard deviation were given in the descriptive statistics of continuous variables, and frequency (n) and percentage (%) values were given in the definition of categorical variables. In the comparisons of three or more groups, the Kruskal-Wallis test was used when the data did not show normal distribution. Relationships between categorical variables were examined with the chi-square test, and $p<0.05$ was accepted as the level of significance in all analyses.

Results

Eighty-eight patients (55 males, 33 females) were included in this study. The ages of patients ranged from 13 to 82 years (mean, 49.50±16.77 years). Forty-eight patients had PA, 35 had Warthin's tumor (WT), two had mucoepidermoid carcinoma (MEC), two had AdCC, and one had squamous cell carcinoma (SCC). Gender, tumor side, type of surgery, and local recurrence data of the patients are shown in Table 1. PSMA expression in tumor epithelium is shown in Table 2. There was statistically significant difference in PSMA expression between PA and WT (p=0.003). We observed that PSMA expression was higher in PA than in other tumors (Table 1). There was no PSMA expression in the tumor epithelium of WT. Local recurrence was detected in three patients with PA and in one patient with AdCC. PSMA expression was score 1 in one of the three patients with recurrent PA and score 2 in the other two. There were no differences between the PSMA expression of these three patients and the non-recurrent 45 patients. There was no statistically significant relationship between PSMA and local recurrence (p=0.193).

Discussion

PSMA is a potential and promising molecule that can be used in the imaging and staging of some malignant tumors, the molecular characterization of tumors, the molecular

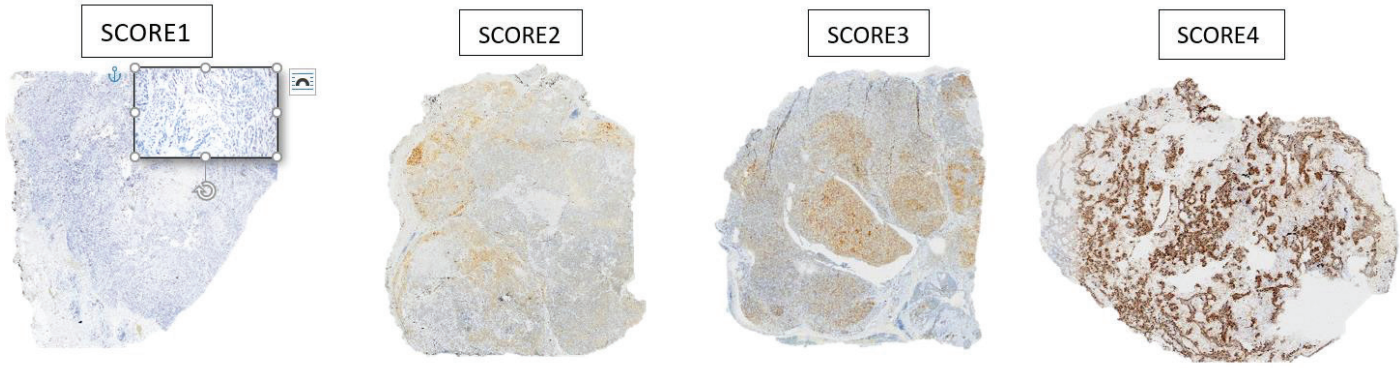

Figure 1. Tissue PSMA expression pattern as depicted
PSMA: Prostate-specific membrane antigen

Table 1. Gender, tumor side, type of surgery, and local recurrence data of the patients

		PA	WT	MEC	SCC	AdCC	
				n (%)			p-value
Gender	Male	23 (26.1)	31 (35.2)	0 (0.0)	0 (0.0)	1 (1.1)	**<0.001**
	Female	25 (28.4)	4 (4.5)	2 (2.3)	1 (1.1)	1 (1.1)	
Side	Right	28 (31.8)	13 (14.8)	2 (2.3)	1 (1.1)	0 (0.0)	0.150
	Left	20 (22.7)	21 (23.9)	0 (0.0)	0 (0.0)	2 (2.3)	
	Bilateral	0 (0.0)	1 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	
Surgery	Superficial	42 (47.7)	35 (39.8)	0 (0.0)	0 (0.0)	0 (0.0)	**<0.001**
	Total	4 (4.5)	0 (0.0)	2 (2.3)	1 (1.1)	2 (2.3)	
	Submandibular gland excision	2 (2.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Recurrence	No	45 (51.1)	35 (39.8)	2 (2.3)	1 (1.1)	1 (1.1)	0.12
	Yes	3 (3.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.1)	

AdCC: Adenoid cystic carcinoma, MEC: Mucoepidermoid carcinoma, n: Number of patients, PA: Pleomorphic adenoma, SCC: Squamous cell carcinoma, WT: Warthin's tumor

Table 2. PSMA expression scores of the patients in tumor epithelium

		PA	WT	MEC	SCC	AdCC	
				n (%)			p-value
Expression	Negative	7 (8.0)	35 (39.8)	0 (0.0)	0 (0.0)	0 (0.0)	0.003*
	Score 1	10 (11.4)	0 (0.0)	2 (2.3)	1 (1.1)	1 (1.1)	
	Score 2	17 (19.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
	Score 3	13 (14.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
	Score 4	1 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.1)	

AdCC: Adenoid cystic carcinoma, MEC: Mucoepidermoid carcinoma, n: Number of patients, PA: Pleomorphic adenoma, SCC: Squamous cell carcinoma, WT: Warthin's tumor, PSMA: Prostate-specific membrane antigen

*Statistically significant difference between Warthin's tumor and Pleomorfic adenoma.

characterization of neovascularization and PSMA-targeted radioligand therapy (2). Positron emission tomography (PET) imaging and targeted radioligand therapies with PSMA ligands are widely used in prostate cancer. However, PSMA is not specific to the prostate tissue or prostate cancer. PSMA overexpression has also been detected in some malignancies other than prostate cancer (1, 3). This entity gives PSMA a promising and important role for targeted imaging and new treatment strategies for other malignancies.

Klein Nulent et al. (4) analyzed the value of PSMA-11-PET/computed tomography (CT) for AdCC of the salivary gland in nine patients. They concluded that PSMA PET/CT was able to detect and visualize local recurrent and distant metastatic AdCC. PSMA imaging of neoplasms other than prostate cancer generally originates from the neovascular tissues, but the authors observed that AdCC cells expressed PSMA. They also found that both PSMA PET/CT and immunohistochemistry were positive in all patients' tumor tissues (4).

Although PSMA expression is detected in some non-prostatic tumors and salivary glands (physiologically), there are few studies that investigated PSMA in salivary gland tumors other than AdCC (1, 3). In our study we investigated 88 patients with different salivary gland tumors. Forty-eight patients had PA, 35 had WT, two had MEC, two had AdCC, and one had SCC; and the difference in PSMA expression was statistically significant among the tumor types. We observed that PSMA expression was higher in PA than in other tumors. WT had no PSMA expression in tumor cells. Grade 1 expression was detected in all MEC and SCC specimens. One specimen had Grade 1 and one had Grade 4 PSMA expression in AdCC (Table 1).

Salivary gland tumors are rare neoplasms of the head and neck and are commonly found in the parotid gland. The primary treatment for these tumors is surgery. However, some benign and malignant salivary gland neoplasms have the potential for local recurrence. Treatment of these recurrent salivary gland tumors is also very challenging. Another problem for surgical treatment is that the operation can be risky due to senility and comorbid diseases in some patients. In PSMA-PET using current 68Ga- and 18F-tracers, salivary glands are the second organ with the strongest tracer accumulation (9). Reliable with the comparable distribution of therapeutic PSMA radioligands as 177Lu-PSMA-617, salivary glands are exposed to the most elevated dosages and are considered the basic organs in PSMA radionuclide treatment (10). Exposure to high dose makes salivary gland tumors a very suitable potential treatment target for radioligand therapy. It can be predicted that this targeted therapy will be even more effective in tumors with high PSMA expression. In most tumors PSMA is found within the tumor-associated endothelium, and not on cancer cell membrane. This may lead to an enlarged interval between the radiation emitter and the tumor cell that would likely reduce the possibility of an emitted electron to influence a neoplastic cell (11). But we observed that in PA, MEC, AdCC and SCC PSMA was detected on the cell membrane. Therefore, the possibility of reduced influence of radiation due to enlarged interval between the radiation emitter and the tumor cell did not exist in PSMA positive tumors in our study. We believe that, because of these properties, salivary gland tumors would be quite suitable for PSMA-targeted radioligand therapies.

PA is the most common salivary gland neoplasm. Although a benign neoplasm, it has a potential for malignant transformation, and its rates for transformation were reported between one and 23% (12). PA has a tendency for local recurrence, with rates ranging from two to 45% (13). There is no standard treatment for recurrent PA. Treatment options are re-excision and radiotherapy. They both have disadvantages and side effects. With repeated excision, further recurrence rates have increased up to 50% and the risk of morbidity, especially facial nerve dysfunction, is also increased (14). In addition, the scar tissue that develops with repeated surgeries makes the dissection of the tumor more difficult. Thus, the possibility of facial nerve injury increases. Radiotherapy may have negative impact on the recovery of facial nerve injury and increase the risk of malignant transformation and secondary malignancies (15). In our study, we observed that PSMA expression was high in PA. We believe that this may open a window of opportunity for a new treatment strategy (radioligand therapy) for PA.

In a study by Nishida et al. (16) on the immunohistochemical reactivity of PSMA in salivary gland tumors the researchers performed immunohistochemistry for PSMA in 55 salivary gland tumors comprising 10 PAs, 10 WTs, nine basal cell adenomas, nine AdCCs, nine MECs, and eight salivary duct carcinomas. They found that 87% of the tumors were PSMA-positive. The positive ratios for PA and WT were the highest (100%) followed by those for basal cell adenoma, AdCC, and MEC (89%, 89%, and 78%, respectively). They observed that PA showed multifocal positive staining, while all WTs showed a diffusely positive, faint reaction (16). These results may seem different from our results, especially in immunohistochemical reactivity of WT; however, this difference is due to the use of different PSMA immunohistochemical staining scoring. Nishida et al. (16) used intensity scoring, population scoring and the product of the intensity score and population score. We used a scoring system positive for any cytoplasmic staining and membrane staining. In our study, the non-tumoral tissue around the tumor showed a diffusely positive and faint reaction therefore these areas were scored as negative (score 0). WT and normal peritumoral salivary gland tissue differentiation was weak. Therefore, PSMA expression in the tumor epithelium of WT was considered as score 0. This finding is in fact consistent with the finding reported by Nishida et al. (16) that WT showed a diffusely positive, faint reaction. While the number of benign tumor cases were much higher in our study, the number of malignant tumor cases were slightly higher in the referred study. For this reason, we believe that our study will provide additional and complementary contributions to the literature.

The limitations of our study are its small number of patients, especially in malignant tumors, and its retrospective design. Another important limitation is that our patients did not have PSMA-PET results that we could compare with our immunohistochemical results. Further studies are needed to resolve these limitations.

Tan et al. (17) concluded in their review that there was a potential role of PSMA-PET as a diagnostic tool, especially when conventional imaging is inconclusive or does not correlate with clinical findings. They also indicated that the use of PSMA ligand had the potential to be a form of theranostics whereby it can be used as a diagnostic and therapeutic tool in the management of salivary gland tumors (17). These comments are quite consistent with our results and conclusions.

Conclusion

PSMA expression in tumor epithelium was detected in PA, MEC, AdCC and SCC. Prospective studies should be planned targeting intratumoral PSMA in benign and malign salivary gland tumors. PSMA-targeted modalities may be future diagnostic and therapeutic methods.

Ethics Committee Approval: The Clinical Research Ethics Committee of the Süleyman Demirel University Faculty of Medicine approved our study protocol (decision no: 18/254, approval date: 11.09.2020).

Informed Consent: Informed consent was not obtained from the individuals since the study was designed retrospectively.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: M.E.S., H.Y., Design: M.E.S., H.Y., Data Collection and/or Processing: M.E.S., Y.Ç.K., O.E., S.S., Analysis and/or Interpretation: Y.Ç.K., O.E., Literature Search: M.E.S., H.Y., Y.Ç.K., O.E., S.S., Writing: M.E.S., H.Y.

Conflict of Interest: The authors have no conflicts of interest to declare.

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References

Main Points

- Prostate-specific membrane antigen (PSMA) is significantly overexpressed in prostate cancer but is also expressed in the neo-vasculature of certain non-prostatic solid tumors.
- There are few studies investigating PSMA in salivary gland tumors other than adenoid cystic carcinoma.
- PSMA expression in tumor epithelium was identified in pleomorphic adenoma, mucoepidermoid carcinoma, adenoid cystic carcinoma and squamous cell carcinoma.
- We observed that PSMA expression was higher in pleomorphic adenoma than in other tumors and there was no PSMA expression in the tumor epithelium of Warthin's tumor.
- PSMA-targeted approaches may become important diagnostic and therapeutic modalities in the future, especially for recurrent pleomorphic adenoma.

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