

Utility of Prostatic Markers in the Differential Diagnosis of Adenocarcinomas Not Otherwise Specified from metastatic Tumours of the Oral Cavity

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Abstract: *Objectives:* The aim of the present study was to evaluate the expression of prostate-specific antigen (PSA), prostatic acid phosphatase (PAP) and [alpha]-methylacyl-CoA racemase gene (P504S) in normal salivary glands and adenocarcinomas not otherwise specified (NOS).

Material & Methods: Tissue specimens of 18 normal salivary glands and 22 cases of adenocarcinomas NOS of the oral cavity, formalin-fixed, and paraffin-embedded were studied by using monoclonal antibodies for PSA, PAP and P504S.

Results: Positive staining for PSA was detected with one of the two used monoclonal antibodies in normal salivary glands (89%) and adenocarcinomas NOS (45.45%) whereas negative staining was observed for PAP and P504S.

Conclusions: Our results suggest that the detection of prostatic markers such as PAP, P504s with the use of monoclonal antibodies may offer an adjunctive aid in the differential diagnosis of adenocarcinomas NOS from metastatic carcinomas, when the origin of the primary tumours is unknown.

Keywords: Prostate-specific antigen (PSA), prostatic acid phosphatase (PAP), [alpha]-methylacyl-CoA racemase (AMACR gene), adenocarcinoma not otherwise specified (NOS), metastatic tumours.

INTRODUCTION

Prostate-specific antigen (PSA), a kallikrein-like serine protease (human kallikrein, hk3), is encoded by one of the three genes of the human kallikrein gene family mapped to long arms of chromosome 19 and therefore shares structural homology with tissue kallikrein (human kallikrein 1, hk1) and glandular kallikrein (human kallikrein 2, hk2) [1]. Prostatic acid phosphatase (PAP) is the prostatic isoenzyme of the widely distributed enzymatic acid phosphatase. Although PSA and PAP have become valuable markers for diagnosis of prostate carcinoma, their presence in a variety of normal, benign and malignant epithelial tissues [2] casts doubts about their specificity of their tissue expression. P504S, a prostate cancer specific gene, was recently discovered by using cDNA library subtraction with subsequent high throughput microarray screening of human prostate tissue. P504S, also referred to as AMACR gene, encodes [alpha]-methylacyl-coenzyme A racemase, a 382- amino acid protein involved in the beta-oxidation of branched-chain fatty acids. Although P504S was suggested as marker with specificity for prostate carcinoma its presence was documented in some normal tissues and carcinomas [3].

Adenocarcinoma not otherwise specified (NOS), is a salivary gland carcinoma with ductal or glandular differentiation that does not resemble any known types of salivary gland carcinomas. It is uncommon and 60% of cases occur in major salivary glands with the parotid to be the most frequently involved [4].

The demonstration of PSA and PAP in normal salivary glands and neoplasm with the use of polyclonal and monoclonal antibodies is controversial [5-9]. Examination of P504S in adenocarcinoma NOS has not been reported in the literature.

The aim of the present study was to evaluate the expression of PSA, PAP and P504S in normal salivary glands and adenocarcinomas NOS, with the use of monoclonal antibodies in order to examine their possible utility in the differential diagnosis of primary oral adenocarcinomas NOS and metastatic tumours to the oral cavity.

MATERIAL & METHODS

Twenty two cases of adenocarcinomas NOS, routinely processed, formalin-fixed, paraffin-embedded samples were retrieved from the files of diagnostic pathology of the Department of Oral Medicine and Oral Pathology and the Department of Histopathology of Theagenion Anticancer Hospital. Serial sections were cut and stained with hematoxylin and eosin and were reviewed for adequacy of tissue to study and for agreement with the original diagnosis by using estab-

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lished criteria [10]. Clinical features of all cases were recorded. The medical history of patients did not revealed any previous malignancy. Informed consent was obtained by all the patients, and the whole study was performed according to Helsinki II Declaration. Histologic grading of the tumours was performed according to previously described criteria [10]. Eighteen normal salivary glands (6 parotids, 6 submandibular glands and 6 labial) which were obtained from random biopsies or from non-neoplastic lesions were additionally used.

For the immunohistochemical detection of antigens, endogenous peroxidase was quenched with 3% H₂O₂ for 10 min at room temperature. Sections that intended for the detection of P504S were pretreated for antigen retrieval, whereas for the rest of the antibodies was omitted according to the recommendations of the product's company (Table 1). Then the sections were rinsed with phosphate buffer saline (PBS) and further blocking was performed by using normal goat serum at a 1: 20 solution for 20 min at room temperature. After being washed with PBS the sections were incubated with monoclonal antibodies (Table 1). Tissue sections incubated for the same time with normal goat serum were used as negative controls and sections from a prostate carcinoma as positive controls. The procedures were performed using a TechMate 1000 (Ventana Med Systems, Tuscon, AZ, USA) automated immunostainer. The avidin-biotin-complex method was performed for the detection of PSA and PAP with the primary antibody supplied from Biogenex (San Ramon, CA, USA) and streptavidin-biotin-complex method for the detection of PSA and P504S with the primary antibodies supplied from Novocastra (Newcastle upon Tyne, UK) and Biocare (Carmino Diablo, CA, USA) respectively. The reaction was developed using diaminobenzidine and hematoxylin was used as counterstain. Owing to the high level of endogenous biotin, especially in striated and excretory ductal cells, a biotin blocking system (XO590 DakoCytomation, Glostrup, Denmark) was applied to both immunohistochemical methods. The staining intensity was assessed as weak, moderate and strong and the pattern of immunostaining as focal, multifocal and diffuse. Sections were examined by two of the authors (E.A and K.A) independently of each other. The slides then were reviewed by the examiners as a group and discussion was occasionally necessary to establish uniformity.

RESULTS

Normal salivary glands and adenocarcinoma NOS expressed PSA with the use of one monoclonal antibody (Biogenex) but not with the other (Novocastra). Positive immunostaining for PSA was found in 16/18 (89%) of cases of

normal salivary glands (6/6 parotids, 6/6 submandibular and 4/6 labial glands) in 8 males and 8 females. The cells of striated and excretory ducts of parotid and submandibular glands expressed PSA with strong intensity whereas acini and intercalated ducts were negative (Fig. 1). In normal labial salivary glands the intralobular ductal cells expressed PSA with strong intensity whereas acinar cells were negative (Fig. 2). In 2 cases of adenocarcinomas NOS which were PSA positive, the adjacent normal minor salivary glands showed results similar to labial glands.

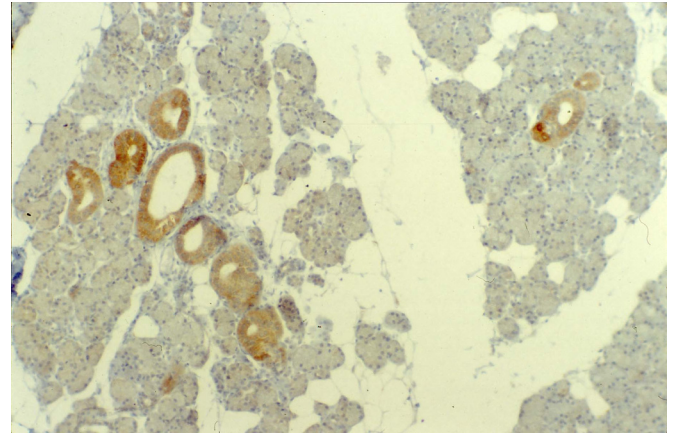


Fig. (1). Normal parotid gland. Strong expression of prostate-specific antigen in the cells of striated and excretory ducts. (Avidin-Biotin-Complex method. Original magnification X 33).

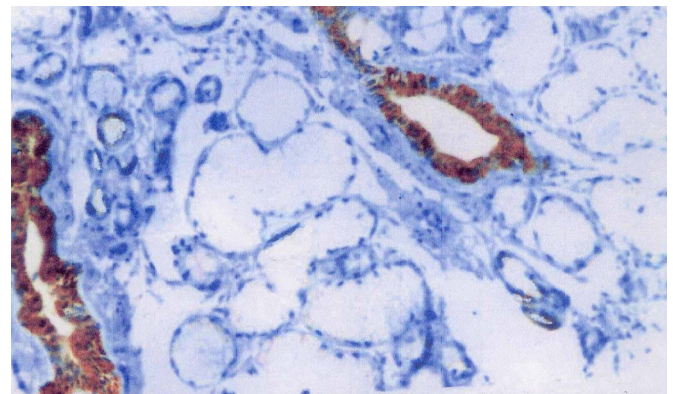


Fig. (2). Normal labial salivary glands. The cells of excretory ducts are strongly immunoreactive for prostate specific antigen. (Avidin-Biotin-Complex method. Original magnification X 66).

The clinical features, histologic grade and immunostaining for PSA in adenocarcinomas NOS, are summarized in Table 2. The age of patients ranged from 28 to 80 years with an average value 59.8 years. The tumour most often (54.5%)

Table 1. Clone, Source and Pretreatment for Antigen Retrieval, Dilutions and Incubation Time of Primary Antibodies

Antibody	Source	Clone	Pretreatment	Dilution	Incubation
PSA	Biogenex, San Ramon, CA, USA	Er-Pr-8	No	1:80	30 min
PSA	Novocastra Lab. Newcastle upon, Tyne, UK	28/A4	No	1:30	30 min
PAP	Biogenex, San Ramon, CA, USA	BGX013A	No	1:150	30 min
P504S	Biocare Med. Carmino Diablo, CA, USA	P504S	Microwave; citrate buffer 0.01M; pH 7, 95°C; 15 min	Prediluted	30 min

PSA, Prostate-specific antigen; PAP, Prostatic acid phosphatase; P504S, [alpha]-methylacyl-conenzyme A racemase.

Table 2. Clinical Features, Histologic Grade and Immunostaining of Prostate-Specific Antigen (PSA) in Adenocarcinomas NOS

Case	Gender	Age	Location	Histologic Grade	Immunostaining Pattern	Intensity of Staining
1	F	64	Palate	II	Diffuse	Moderate
2	F	28	Buccal mucosa	I	Multifocal	Moderate
3	M	63	Labial mucosa	II	Negative	-
4	M	74	Labial mucosa	II	Focal	Weak
5	F	50	Palate	I	Negative	-
6	F	64	Palate	I	Negative	-
7	F	55	Buccal mucosa	I	Negative	-
8	M	65	Palate	II	Negative	-
9	F	39	Buccal mucosa	I	Diffuse	Weak
10	M	63	Palate	I	Negative	-
11	M	65	Palate	I	Diffuse	Moderate
12	F	49	Buccal mucosa	I	Negative	-
13	F	51	Buccal mucosa	I	Multifocal	Moderate
14	F	64	Palate	I	Negative	-
15	F	56	Buccal mucosa	I	Diffuse	Weak
16	M	68	Palate	II	Negative	-
17	M	80	Palate	II	Negative	-
18	F	67	Palate	I	Diffuse	Moderate
19	F	63	Palate	I	Negative	-
20	M	66	Palate	I	Diffuse	Moderate
21	F	57	Palate	I	Negative	-
22	M	65	Labial Mucosa	II	Focal	Weak

occurred in the 6th decade of life and located in palate (59%). Histologically 68% of cases were low-grade (grade I) and 32% intermediate grade (grade II) (Fig. 3). Expression of PSA was observed in 10/22 (45.45%) of cases independently of patient's sex and histologic grade. The staining intensity ranged from weak to moderate (Fig. 4).

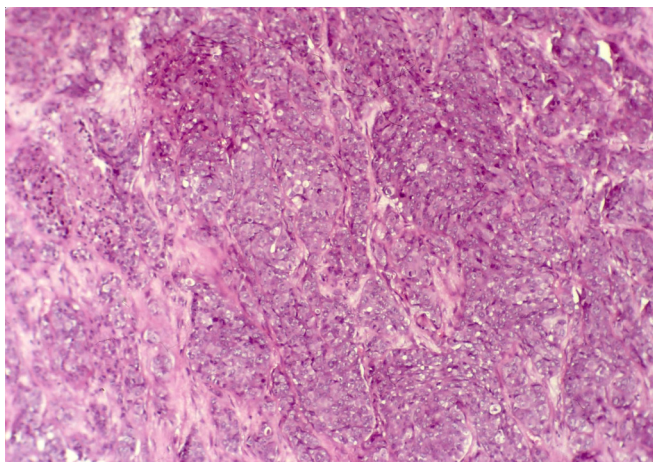


Fig. (3). Low grade (I) adenocarcinoma, not otherwise specified. Glandular and ductal structures. (Hematoxylin and eosin. Original magnification X 33).

Normal salivary glands and adenocarcinomas NOS did not express PAP and P504S.

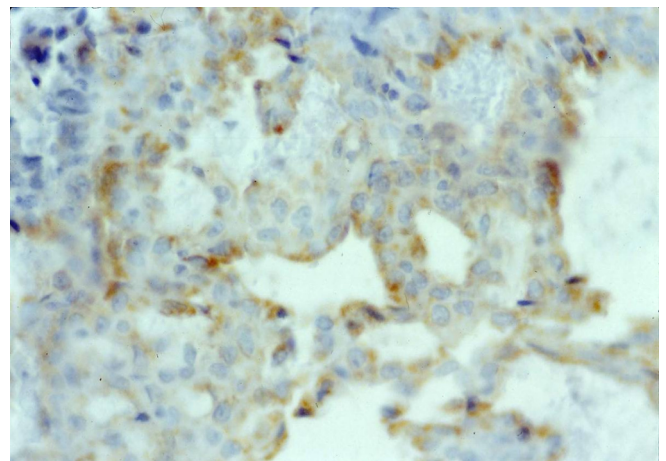


Fig. (4). Adenocarcinoma, not otherwise specified. Diffuse expression of prostate specific antigen. Moderate staining intensity of luminal and non-luminal tumor cells in ductal-like structures. (Avidin-Biotin-Complex method. Original magnification X 132).

DISCUSSION

Comparison of our results in normal salivary glands and immunohistochemical procedures with previous reports are

presented in Table 3. Our results for PSA in parotid gland are in concordance with previous studies where polyclonal [5,7,9] and monoclonal antibodies [6, 8] were used. On the contrary to our results in the studies of Fan *et al.* [8] and Tazawa *et al.* [9] expression of PSA was not demonstrated by using monoclonal antibodies. Our results in the submandibular gland agree with previous study [6] where the expression of PSA was detected by using two monoclonal antibodies. Also partial agreement exists with the study of Tazawa *et al.*

[9] where the expression of PSA was reported by using a polyclonal antibody but not by a monoclonal one. As concern our results in minor salivary glands discrepancy exists with the study of Tazawa *et al.* [9] where polyclonal and monoclonal antibodies failed to demonstrate the expression of PSA.

As concern the presence of PAP, the results of the current study agree with the reports of Van Krieken [5] and Tazawa

Table 3. Comparative Profile of Present Study and Previous Reports in Normal Salivary Glands

Authors	Anatomic Location Immunostaining	Source	Type of Antibody	Dilution	Antigen Retrieval
Van Krieken [5]	Parotid Positive	Dakopatts, Glostrup, Denmark	Polyclonal PSA	1: 200	NO
	Parotid Negative	Dakopatts, Glostrup, Denmark	Polyclonal PAP	1: 300	NO
Elgamal <i>et al.</i> [6]	Parotid Submandibular Positive	Dako Carpinteria, CA, USA	Monoclonal PSA	1: 20	NO
	Parotid Submandibular Positive	Zymed, Laboratories, CA, USA	Monoclonal PSA	1: 25	NO
	Parotid Submandibular Positive	Dako Carpinteria, CA, USA	Monoclonal PAP	1: 50	NO
Alanen <i>et al.</i> [7]	Parotid Negative	Biogenex, San Ramon, CA, USA	Monoclonal PSA	1: 10	NO
	Parotid Positive	Biogenex, San Ramon, CA, USA	Polyclonal PSA	1: 10	NO
	Parotid Positive	Dakopatts, Glostrup, Denmark	Monoclonal PSA	1: 1000	Microwave processing
	Parotid Positive	Dakopatts, Glostrup, Denmark	Polyclonal PSA	1: 1000	Pepsin Pretreatment
Fan <i>et al.</i> [8]	Parotid Negative	Signet	Monoclonal PSA	1: 100	NO
	Parotid Positive	Dako	Monoclonal PAP	Prediluted	NO
Tazawa <i>et al.</i> [9]	Parotid Submandibular Positive Minor Negative	Dako Kyoto, Japan	Polyclonal PSA	1: 100	NO
	Parotid Submandibular Minor Negative	Dako Kyoto, Japan	Monoclonal PSA	1: 20	Microwave processing
	Parotid Submandibular Minor Negative	Dako Kyoto, Japan	Monoclonal PAP	1: 400	Microwave processing
Present study	Parotid Submandibular Labial Positive	Biogenex, San Ramon, CA, USA	Monoclonal PSA	1: 80	NO
	Parotid Submandibular Labial Negative	Novocastra, Newcastle upon Tyne, UK	Monoclonal PSA	1: 30	NO
	Parotid Submandibular Labial Negative	Biogenex, San Ramon, CA, USA	Monoclonal PAP	1: 150	NO

et al. [9] where polyclonal and monoclonal antibodies were used respectively. On the contrary Elgamal *et al.* [6] and Fan *et al.* [8] detected expression of PAP with the use of monoclonal antibody.

In the present study we used monoclonal antibody for the detection of PSA on purpose to minimize cross-reactivity with human kallikrein 1 and 2 although theoretically cross-reactivity cannot be excluded. Also we used monoclonal antibody for the detection of PAP because the specificity of monoclonal antibodies is better than that of polyclonal antibodies [11]. The discrepancies of our results for PSA with the study of Fan *et al.* [8] and Tazawa *et al.* [9] as well as for PAP with those of Elgamal *et al.* [6] and Fan *et al.* [8], where monoclonal antibodies were used, may be due to different immunohistochemical procedures (Table 3). The limits of an immunohistochemical assay vary upon factors such as antigen retrieval procedure, the concentration of primary and secondary antibodies, the length of incubation with such antibodies and the sensitivity of enzymatic reaction. However, in the current study the expression of PSA in normal minor salivary glands was demonstrated and according to our knowledge a similar result has not previously reported in the literature.

In the present study expression of PSA was demonstrated in both sexes and this result is in accordance with the study of Elgamal *et al.* [6] where monoclonal antibodies were used. It has been suggested that PSA is expressed from salivary gland tumours in androgen dependent manner [1] and its presence in women is likely due to steroid hormones such as progestin or glyocorticoid that could derepress the gene in cells with the appropriate receptors [12]. The function of PSA in salivary glands is not clear. It has been proposed that may be involved in regulating insulin-like growth factor binding proteins 2 and 3 and insulin-like growth factor 1 [13].

The results of the present study showed positive expression for PSA (45.45%) in adenocarcinomas NOS, independently of the patient's sex and are similar to the study of Tazawa *et al.* [9] where positive results (41%) were found in both sexes with the use of polyclonal antibody but not with monoclonal one. Also they are similar to the results of Van Krieken [5] with the use of polyclonal antibody. Conclusively from the present and previous studies it seems that PSA may be demonstrated in adenocarcinomas NOS, with the use of both monoclonal and polyclonal antibodies whereas PAP only with polyclonal ones. In the current study the results for PSA confirm the existing doubts about the specificity of its tissue expression.

Metastatic adenocarcinomas occasionally simulate primary malignant neoplasms in major salivary glands and oral cavity and may present difficulties in separation from adenocarcinomas NOS, because of their relatively nonspecific histologic appearance [10]. Breast and lung carcinomas commonly metastasize in the oral cavity [14] whereas colorectal [15], hepatic [16], gastric [17, 18] and prostate carcinomas [19] are rare. Among these carcinomas, PSA expression has been demonstrated in breast [20-22] and lung carcinomas [23] with the use of polyclonal antibodies. Therefore pathologists in case of surgical material originating from males should use with caution polyclonal antibodies for the detection of PSA as an adjunctive aid for the differential diagnosis

between primary adenocarcinomas NOS, from metastatic lung and prostate carcinomas when the origin of the primary tumours is unknown. With regard to PAP, its expression has commonly been demonstrated in rectal carcinoid tumours [2] and prostate carcinoma [24] with the use of monoclonal antibody whereas its absence in adenocarcinomas NOS, was demonstrated in the current study and the report of Tazawa *et al.* [9]. These findings allow us to suggest that the detection of PAP with the use of monoclonal antibodies may offer an adjunctive aid in the differential diagnosis of metastatic rectal carcinoid tumours and prostate carcinoma from primary adenocarcinomas NOS.

In the current study P504S expression was not found in adenocarcinoma NOS whereas in prostate, hepatic and colorectal carcinomas its expression was reported in 92%, 81% and 80% of cases respectively [25]. Therefore the use of P504S may offer an adjunctive aid in the differential diagnosis of primary adenocarcinoma NOS from metastatic prostate, hepatic and colorectal carcinomas when the origin of the primary tumours is unknown.

In conclusion our results suggest that the immunohistochemical detection of PAP and P504s with the use of monoclonal antibodies, may offer an adjunctive aid in the differential diagnosis of primary oral adenocarcinomas NOS from metastatic carcinomas to the oral cavity, when the origin of the primary tumours is unknown.

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