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An immunohistochemical study of androgen receptor in carcinoma arising in pleomorphic salivary adenoma

Bassel Tarakji [1], Omar Kujan [2]

[1] Head of Department of Oral Pathology Faculty of Dentistry Aleppo University, Aleppo, Syria
[2] Head Department of Oral Pathology Faculty of Dentistry Al-Baath University, Hama, Syria

Correspondence:
Department of Oral Pathology
Faculty of Dentistry
Aleppo University
Aleppo, Syria
denpol@yahoo.co.uk

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Abstract

Summary: Carcinoma originating within a pre-existing in pleomorphic adenoma is well-known phenomena and is occasionally debated in the field of surgical pathology. The use of the hormone treatment in salivary gland cancers is controversial. The management of patients who show positive reactivity to androgen receptor in prostate carcinomas has guided the researchers to assess the expression of this receptor in a variety of other tumors, including those arising in the salivary glands and particularly in carcinoma ex pleomorphic adenoma.

Objective: Our study aimed to characterize alteration in the immunohistochemical expression of androgen receptor in the tumor cells of carcinoma arising in pleomorphic adenoma. Study design: 20 cases of carcinoma arising in pleomorphic adenoma (undifferentiated and adenocarcinoma types) were examined. Results: The results showed that 10 (50 %) of 20 cases had negative nuclear staining, whereas 10 (50%) of 20 cases had positive nuclear staining for androgen receptor. Conclusion: Our data suggest that carcinoma arising in pleomorphic adenoma may be dependent on endocrine function.

Key words: *Androgen receptor, salivary gland cancer.*

Introduction

Salivary gland cancers account for 0.4 % of all cancers and 5 % of all head and neck malignancies (1). The use of molecular biology technique has aided in studying such these tumors (2). The use of antagonist hormones for patients with prostate cancer positive to androgen receptor (AR) has shown reduction of the recurrences and improvement of the survival rates (3,4).

Also, the hormonal receptors facilitated the identification of breast cancer patients who can get the benefit from hormone therapy (5). Immunocytochemistry of estrogens receptor has been shown to predict response of breast cancer to endocrine therapy (6,7). Some studies (8-12) reported that some tumors other than breast cancer have hormone receptors e.g melanoma, carcinoid tumors, pancreatic, and renal cancers. There is limited number of published studies concerning the existence of androgen receptor in salivary gland cancers. It is unknown whether carcinoma ex pleomorphic adenoma is dependent or independent on the hormone function. The purpose of this study was to determine, with the use of immunohistochemistry, if androgen receptor can be

identified in formalin- fixed paraffin embedded tumor cells of carcinoma arising in pleomorphic adenoma.

Materials and Methods

The proposed criteria for defining carcinoma ex-pleomorphic adenoma by Nagao et al, (13), were used to select and reclassify our cases of carcinoma ex-pleomorphic adenoma.

The use of strict pathological criteria may underestimate the frequency of carcinoma ex pleomorphic adenoma because the malignant cells in some cases may obliterate the original pleomorphic adenoma.

-*Inclusion criteria for* carcinoma ex-pleomorphic adenoma

• Major gland primary lesion (parotid or submandibular)

• Co-existent benign and malignant elements

-*Benign* element can be:

• Pleomorphic adenoma within the tumor mass

• Biopsy proven history of previous PSA (pleomorphic salivary adenoma) indicated that it was in the same location as the subsequent carcinoma.

-*Malignant* elements can be:

• Undifferentiated carcinoma

• Adenocarcinoma

• Multiple patterns of differentiation including undifferentiated or adenocarcinoma patterns

-*Exclusion criteria for* carcinoma ex-pleomorphic adenoma includes any other type of tumor.

Twenty cases of carcinoma arising in pleomorphic adenoma were included in this study (table1). Immunostaining techniques were applied to localize the androgen receptor in the tissues.

Nuclear staining of androgen receptor was considered only as a positive result indicating presence of receptor protein. Two independent examiners scored the sections for the presence of androgen receptor. Following examination the entire section, five random areas were chosen from each slide. The scoring criteria was considered two categories: positive or negative nuclear staining. Androgen receptor positive nuclei was assessed by two independent observers and scored as: as negative is <75% staining and positive as greater than 75% of cells. Cytoplasmic staining was neglected. Positive and negative controls were included in all reactions. The Research Ethics Committee at Aleppo University provided a favorable ethical opinion.

-*Immunhistochemistry*

Paraffin-embedded, 5-µm-thick tissue sections from all 20 specimens were cut. The sections were deparaffinized in xylene and rehydrated through graded alcohols. Sections were processed used streptavidin-biotin-peroxidase method. Briefly, the endogenous peroxidase was blocked by 3 % hydrogen peroxidase for 5 min followed by TBS wash. Non specific immunoreactivity was blocked by incubation with normal goat serum for 20 minutes. The sections were incubated with the following primary antibody: anti-Androgen receptor (clone AR441, Dako, USA) was diluted to 1: 25 (40 µL / ml) in tris buffer saline (TSA) containing 0.1 % bovine serum albumin for 10 minutes at the room temperature. All sections were washed by TBS for 5 minutes. Sections were incubated with the biotinylated secondary antibody reagent for 10 minutes followed by (TBS) wash for 5 minutes. Slides were incubated with streptavidin and horseradish peroxidase for 10 minutes followed by (TBS) tris buffer saline wash for 5 minutes. Incubate with a prepared chromogenic substrate solution (Diaminobenizidine) for 15 minutes. Sections were counterstained with 0.25 % methyl green in distilled water for 5 minutes. Sections were dehydrated and mounted in Depax. Tissue blocks of breast carcinoma of known positive and negative androgen receptor status were used as positive controls.

-*Statistical analysis*

The data were described using frequency distribution (descriptive data) .

Results

The results showed that 10 (50%) of 20 cases had negative nuclear staining for androgen receptor. 10 cases out of 20 (50%) showed positive nuclear staining for androgen receptor. The positive nuclear staining (positive control) from invasive breast cancer of the androgen receptor, and negative nuclear staining for androgen receptor in carcinoma arising in pleomorphic adenoma are shown in figure 1-3. The Clinical Hitsopathological and immunohistochemical characterization of androgen receptor in carcinomas ex-pleomorphic adenomas are shown in table 1.

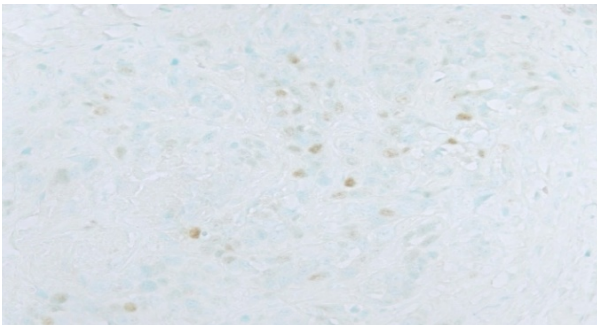

Fig. 1. Showing negative nuclear staining of Carcinoma ex pleomorphic adenoma for androgen receptor.

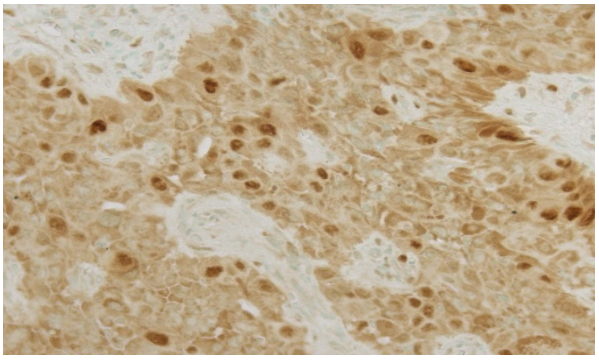

Fig. 2. Showing positive nuclear staining of Carcinoma ex pleomorphic adenoma for androgen receptor.

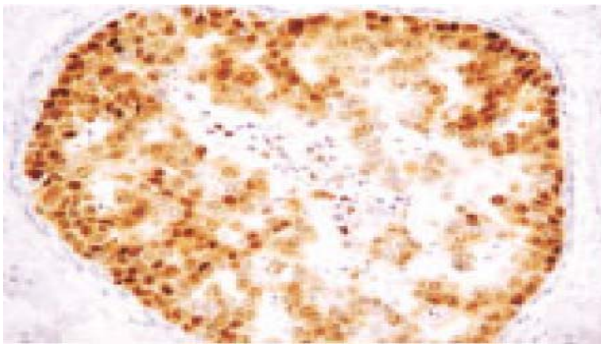

Fig. 3. Showing positive nuclear staining of androgen receptor in breast cancer.

Table 1. Shows the clinical, histophatological and immunohistochemical characterization of androgen receptor in carcinomas ex-pleomorphic adenomas

Case	Age	Gender	Gland	Histological subtype	Metastasis to lymph nodes*	Nuclear AR staining
1	60	M	Parotid	Adenocarcinoma	No	+
2	33	M	Parotid	Adenocarcinoma	No	+
3	66	F	Submandibular	Undifferentiated	Yes	+
4	47	F	Parotid	Adenocarcinoma	No	+
5	66	F	Parotid	Undifferentiated	No	+
6	78	F	Parotid	Undifferentiated	No	+
7	75	F	Parotid	Adenocarcinoma	No	+
8	57	F	Submandibular	Undifferentiated	Yes	+
9	61	F	Submandibular	Undifferentiated	Yes	+
10	50	M	Submandibular	Undifferentiated	No	+
11	48	M	Parotid	Undifferentiated	No	-
12	72	M	Parotid	Undifferentiated	No	-
13	54	M	Parotid	Undifferentiated	Yes	-
14	58	M	Parotid	Undifferentiated	Yes	-
15	48	M	Submandibular	Undifferentiated	No	-
16	38	F	Parotid	Undifferentiated	Yes	-
17	54	M	Parotid	Undifferentiated	No	-
18	49	F	Parotid	Undifferentiated	Yes	-
19	53	M	Parotid	Undifferentiated	Yes	-
20	62	F	Parotid	Undifferentiated	No	-

* Metastasis to lymph nodes at the time of tumour resection; AR : androgen receptor

Discussion

The assessment of the positive or negative nuclear staining cells is controversial. Many authors (14-16) used different criteria e.g (0=negative staining, 1= low, 2= moderate, 3= strong or 0-3= negative and 4= positive or 0-2= negative and 3-4=positive or negative and positive staining) so the results cannot be compared, therefore, we have used a strict criteria to evaluate the negative and positive staining to avoid any confusion in the interpretation of the results. The rational of 75% break point may provide a more complete assessment of protein expression and a clearer understanding of the roles played by potential tumour markers in predicting outcome. The existence of sex hormone receptors was investigated in some tumors such as carcinomas of the thyroid, renal cell carcinomas, and malignant melanoma (17-19), but the efficacy of the hormonal therapy in those tumors have not been confirmed yet. Androgen has an important role in the normal development and differentiation of a variety of cell and tissue types. This function is mediated by its binding to AR, a member of the family of steroid hormone receptors.

The presence of AR in a given cell type or organ system indicates a possible role for androgen in its growth and differentiation. AR mediates the effect of androgen by binding to specific DNA sequences and influences the transcription and translation of various genes (20,21). Fan et al, (22,23) suggested that AR may have a role in the pathogenesis of salivary duct carcinoma through the mediation of an epidermal growth factor receptor and transforming growth factor–α autocrine pathway similar to that seen in prostatic carcinoma. Nasser et al,(24) showed negative reactivity to androgen receptor in benign gland tumors, but positive reactivity was detected in two of ten mucoepidermoid carcinomas, two of ten adenoid cystic carcinomas, and most cases of salivary duct carcinoma. AR was demonstrated in all 14 cases of carcinoma ex pleomorphic adenoma. Androgen receptor showed positive staining in the tumour cells of the most salivary duct carcinomas (25, 26). Locati et al, (27) reported a complete remission with androgen-deprivation therapy in a recurrent adenocarcinoma of the parotid gland that expressed AR. Recently, Tarakji and nassani, (28) reported that p21 staining was positive in 14 (51.8%) cases out of 27 of carcinoma arising in pleomorphic adenoma. Tarakji et al, (29) reported that 27 (100 %) of 27 cases had negative nuclear staining for either estrogens or progesterone receptors in carcinoma arising in pleomorphic adenoma. Our study clearly demonstrated strong expression of AR in 50 % of carcinoma ex pleomorphic adenomas.

The difference in the results with the present study compared to others might be due to various factors:

1- Differences in tissue processing from study to study, especially with regard to the type of antibody and the application of antigen retrieval. Also there are several steps in tissue processing that may influence staining patterns and intensity. These include the duration of fixation, section thickness, antigen retrieval procedures, type and concentrations of primary, second and third step antibodies.

2- Interpretation of staining and presentation of the results are not standardised resulting in low intraobserver and interobserver reproducibility. Also the gender, age, specific tumour histology, immunohistochemical technique and the pathological interpretation of the staining due to a lack of strict evaluation criteria.

Conclusion

The results suggested that carcinomas arising in pleomorphic adenoma may be dependent on endocrine function. Our results indicates the necessary required to apply systematic evaluation of the role of AR in the pathogenesis and treatment of carcinoma arising in pleomorphic adenoma. This study included 20 cases of carcinoma arising in pleomorphic adenoma, which was a large sample compared with other studies. It is recommended that further work involves a large series of carcinoma arising in pleomorphic adenoma to determine if androgen receptor using sensitive and specific biochemical methods can be detected in those tumors.

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