Role of Estrogen Receptors Protein Expression in Pleomorphic Adenoma and Mucoepidermoid Carcinoma of Salivary Glands

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Abstract: Estrogen receptors (ERs) expression has been studied in salivary gland tumors, however, there are conflicting results regarding its expression in the literatures. The aim of this study was to investigate the expression of estrogen receptors protein (ERs) in pleomorphic adenoma (PA) and mucoepidermoid carcinoma (MEC) of salivary glands using immunohistochemistry. 35 cases of intraoral minor salivary glands tumors including 20 cases of MEC and 15 cases of (PA) were examined by a light microscope and immunohistochemistry for (ERs) expression. Five cases of normal salivary glands or of normal salivary gland tissues adjacent to the tumor were also used as control. A positive brownish staining of ERs was observed in ductal cells of normal salivary gland tissues. In MEC the expression of ERs was detected in tumor cells in 10 cases out of 20 (50%). The staining was either nuclear or cytoplasmic. The positive staining was strong (+++) in 4 cases (20%), moderate (++) in 4 cases (20%) and weak (+) in 2 cases (10%). Negative staining of ERs was detected in 10 cases (50%) of MEC. All cases of PA showed negative staining for ERs. This result indicated that ERs is not frequently expressed in salivary gland tumors and it may have a role in pathogenesis of MEC, but it does not play any significant role in tumorgenises of salivary gland PA.

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1. Introduction

Steroid hormones including estrogen and progesterone are hydrophobic molecules that bind to intracellular receptor proteins localized within the cytoplasm and the nuclear membrane. These hormones regulate the transcription of specific genes depending on the metabolic condition of the cell (Greene et al, 1986, Beato 1989). Estrogen stimulates cell proliferation of breast epithelial cells, and the close relationship between the expression of estrogen receptor (ERs) and the prognosis of breast cancer has been well characterized (Ma et al. 2009). It was also reported that estrogen stimulate the proliferation and maturation of gingival connective tissue, epithelium and salivary glands (Parkar et al. 1982, Välimaa et al. 2004). ERs have been identified in a variety of human tumors rather than breast carcinomas using histochemical, immunohistochemical, and molecular biology techniques. Moreover, the expression of sex hormone receptors in certain tumors suggests a role for these receptors in tumor pathogenesis, progression and therapy (Ciocca et al. 1989, Campbell-Thompson et al. 2001, Radzikowska et al. 2002). Salivary gland tumors comprise no more than 1% of all tumors and 3% of all the head and neck malignancies. The tumor of minor salivary glands accounting for 14-22 % of all salivary gland carcinomas (Eveson and Cawson 1985). Benign tumors are more common than malignant growths, constituting about 75% of parotid tumors but accounting for <50% of the tumors of the other salivary glands (Neville et al., 2002). PA is the most common benign tumor of salivary glands; it accounts 60% of cases. The majority of tumors arising from the minor salivary glands are malignant and Mucoepidermoid carcinoma (MEC) is the most common malignant salivary gland tumor, accounting for about 3 to 15% of all salivary gland tumors and 12 to 40% of salivary malignancies (Pons-Vicente O et al., 2008). Some reports suggested that the similarities of MECs from both mammary and salivary glands in morphological features and a common cytogenetic alteration could have similar treatment strategies (Camelo-Piragua et al., 2009).

The expression of ERs in various salivary gland tumors were studied but with variable and conflicting results (Jeannon et al., 1999, Lamey et al., 1987, Barnes et al. 1994, Dimery et al., 1987 , Ozono et al., 1995). There is a prognostic significance of estrogen antagonist treatment of patients with ERs positive breast carcinomas and androgen receptor (AR)-positive prostate carcinomas ((EBCTCG) 2005, Berthelet et al., 2005).

Trials of hormone therapy have been suggested as adjunctive protocols in salivary duct carcinoma and adenoid cystic carcinoma unresponsive to conventional therapeutic strategies with variable

response (Elkin et al. 2008, Van der Hulst et al., 1994, Locati et al., 2008). The aim of this study was to evaluate the expression of ERs protein in MEC and PA of intraoral minor salivary gland.

2. Material and Methods

Thirty-five cases of minor salivary gland tumors including 20 cases of MEC and 15 cases of PA were selected from archival pathology files of department of oral pathology, Faculty of Dentistry, Tanta University. Their diagnosis was based on the clinical and histopathological examination.

The clinical recorded data were collected from the patients' file. Five cases of normal salivary gland tissues or from normal tissues adjacent to tumor were also included in this study. The tissue specimens were all surgical materials and were fixed in 10% neutral buffered formalin, routinely processed and embedded in paraffin. Serial sections were cut at 5µm thickness, and one of each set of sections was stained with haematoxylin and eosin (HE). Another set was used for immunohistochemical staining for ERs proteins.

Immunohistochemistry:

Immunohistochemical staining was performed using a peroxidase labeled streptavidin biotin complex. Five µm thick sections of paraffin-embedded tissues were deparaffinized in xylene and routinely processed through ascending hydrated alcohol. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol. Prior to immunostaining, the sections were pretreated with microwave in citrate buffer for antigen retrieval and then incubated with 10 % normal goat serum for 30 minutes to block non-specific binding. Primary mouse monoclonal antibodies for estrogen receptors proteins (ERs) clone NCL-ER-6F11, Novocastra Laboratories, UK (1:50 dilutions) in phosphate-buffered saline (PBS) were applied directly to the slides and incubated at 4º C overnight.

The sections were treated with secondary antibodies and then detected by using streptavidin biotin conjugates (Vectastain ABC kit, Vector Laboratories, Burlingame, CA). The sections were incubated for 30 minutes at room temperature for both steps. Visualization of the reaction products was developed with 0.02% 3, 3'-diaminobenzidine in 0.05 M Tris-HCl (pH 7.4) containing 0.005% hydrogen peroxide (DAB substrate kit, Vector Lab). The sections were counterstained with Meyer's hematoxylin. Positive controls were used from sections associated with the kit. For negative control studies, the primary antibodies were replaced with normal mouse or rabbit IgGs.

Analysis of the staining:

The protein expression was analyzed according to the staining intensity using a semiquantitative validated scoring method. Five microscopic fields at magnification (x 400) were randomly selected in each case. The following scoring system was used: Score 0 (no staining); when positive cells <10%, Score 1+ (weak staining); when positive cells are >10% <20%, Score 2 (++) (moderate staining); when positive cells are >20% < 50% and Score 3 (+++) (strong staining); when positive cells are >50%. The results were tabulated and statistically analyzed using statistical package for social science (SPSS for Windows, release 15.0; SPSS, Inc., Chicago, IL).

3. Results

Clinical data:

The clinical data for the patients revealed that the mean age of PA was 48.2 year in males and 26 year in female. The mean age of MEC cases was 23.7 year in male and 34.3 years in female. There was a slight female sex predilection with a ratio of 1:1.4. The incidence of the tumors is different between male and female according to the type of the tumor, females were more affected than males regarding to MEC while male are more affected regarding to PA (table 1). The common site for the tumors were in the palate (20 cases) 57.1 %, followed by the maxilla (9 cases) 25.7 %, followed by the mandible (3 cases) 8.6 %, the upper lip (2 cases) 5.7 % and the sublingual area (1 case) 2.9 % (table 2).

Table 1: Clinical data of cases of salivary gland tumors; **M**; male, **F**; female

Diagnosis	No.	Sex		Mean Age		
		M	F	M	F	Total
PA	15	9	6	48.2	26	38.6
MEC	20	11	9	23.7	34.3	28.1
Total	35	20	15	39.4	32.6	36.8

Table 2: Number and percentage of studied cases in different sites

Site	PA	MEC	Total	%
Palate	8	12	20	57.1
Maxilla	4	5	9	25.7
Mandible	1	2	3	8.6
Upper lip	1	1	2	5.7
Sublingual	1	0	1	2.9
Total	15	20	35	100

Immunohistochemistry:

In the negative controls, no staining was seen for ERs. In normal salivary gland tissues the expression and immunolocalization of ERs was weak positive brownish staining observed in nuclei of some ductal cells, acinar cells, and inflammatory cells (Fig 1). In MEC, positive nuclear and cytoplasmic staining of ERs was observed in the tumor cells ranged from weak to strong expression. The expression of ERs was immunolocalized in cytoplasm of tumor cells in low-grade malignancy (LG) and intermediate-grade malignancy (IG) of MEC (Fig 2 A, B). The expression was mainly nuclear in high-grade malignancy (HG) of

MEC (Fig 3). The expression was observed in 10 cases out of 20 (50%) ranging from strong positive (+++) in 4 cases in 2 male and 2 female, moderately positive (++) in 2 male and 2 female cases, and weakly positive (+) in 2 female cases (table 3, 4) (fig 4). All cases of PA were stained negative (-).

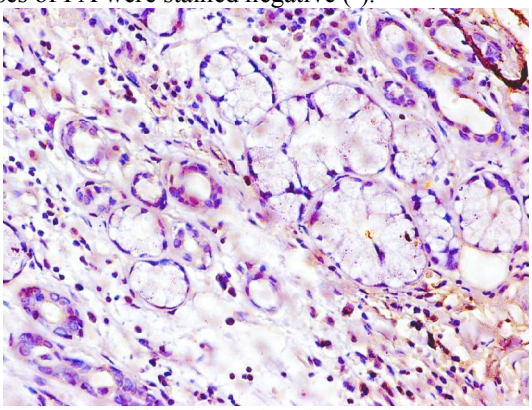

Fig 1: Immunohistochemical staining of ERs in normal salivary gland tissue (NSG), hematoxylene counterstain: positive weak nuclear and cytoplasmic staining of some duct cells, acinar cells and inflammatory cells X200

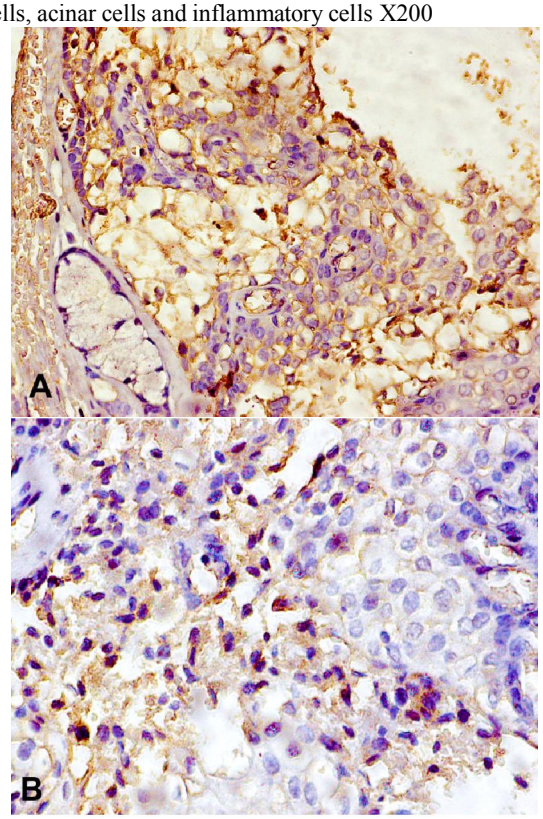

Fig. 2: Immunohistochemical staining of ERs in MEC. A) low grade malignancy (LG-MEC); B) intermediate grade malignancy (IG-MEC); C) high grade malignancy (HG-MEC), hematoxylene counterstain. Positive strong cytoplasmic staining in tumor cells of LG-MEC (A) cytoplasmic and nuclear staining of tumor cells in IG- MEC (B). X200

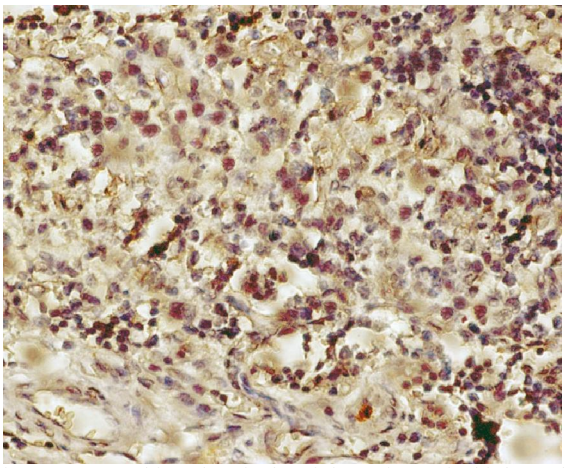

Fig. 3: Immunohistochemical staining of ERs in high-grade MEC (HG-MEC), hematoxylene counterstain showing positive staining mainly nuclear. X200

Table 3: Staining intensity of ERs, number of cases and percentages in MEC of salivary gland

ERs score intensity	MEC n= 20		Total
	Male	Female	
0	7 (35%)	3 (15%)	10
+	0 (0 %)	2 (10%)	2
++	2 (10%)	2 (10%)	4
+++	2 (10%)	2 (10%)	4
Total	11	9	20

Table 4: results of immunostaining in ERs positive cases of MEC

Case #	Sex/ Age	Site	ERs Score	Grade
1	F/ 56	Palate	+++	HG
2	M/ 35	Palate	+++	HG
3	M/30	Mandible	+++	HG
4	F/ 30	Maxilla	+++	HG
5	F/ 12	Palate	++	IG
6	F/ 50	Maxilla	++	IG
7	M/ 22	Upper lip	++	IG
8	M/	Palate	++	LG
9	F/	Maxilla	+	LG
10	F/	Palate	+	LG

HG; high-grade malignancy, IG; intermediate- grade malignancy, LG; low-grade malignancy

Table 5: Summary of the expression of ERs in MEC of salivary gland in previous reports

Auther	year	No. of cases	ERs + (%)	Ref #
Dimery *et al*	1987	2	1 (50)	20
lamey *et al*	1987	1	0	17
Wilson *et al*	1993	1	0	33
Gaffney *et al*	1995	6	0	36
Jeannon *et al*	1999	10	3 (30)	15
Nasser *et al*	2003	10	1 (10)	30
Pires *et al*	2004	136	0	31
Ito *et al*	2009	30	0	32
Kolude *et al*	2013	8	1 (12.5)	34
Present study	2014	20	10 (50)	-

4. Discussions

In this study, the expression of ERs in MEC and PA of salivary gland using immunohistochemistry technique was studied. Positive expression of ERs was detected in 50% of MEC cases but it was negative in PA. This results support the findings of previous reports that the expression of ERs is variable in salivary gland tumors (Jeannon et al., 1999, Lamey et al., 1987, Barnes et al. 1994, Dimery et al., 1987, Ozono et al., 1995).

Expression of ERs was demonstrated in experimentally induced epidermoid carcinoma of the submandibular salivary gland in rats supporting its participation in tumorigenesis of salivary gland tumors and the possibility to use the hormone therapy in salivary gland tumors (Ozono et al., 1995).

Comparable studies, either biochemically or immunohistochemically, revealed contradictory data regarding the positive expression of ERs in salivary gland tumors. The positive ERs expression in MEC exhibited a wide range varying from 10 % to 50 % of studied positive cases.

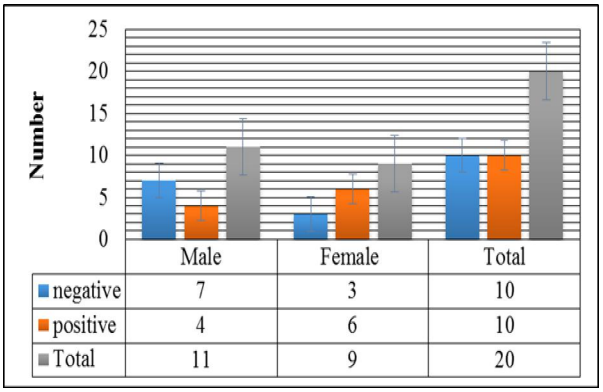

Fig 4: Representative graph of negative and positive ERs expression in MEC

Although, several studies reported negative expression of ERs in MEC (Dimery et al., 1987, lamey et al., 1987, Wilson et al., 1993, Gaffney et al., 1995, Jeannon et al., 1999, Nasser et al., 2003, Pires et al 2004, Ito et al., 2009, Kolude et al., 2013) (table 5).

As regard to PA the expressions of ERs were also controversial as it is reported to be ranged between 7% and 40 % of studied cases (15, 29, 34) some of them supporting the use of hormone therapy in positive cases. On the other hand, Previous studies and the present study reported a negative ERs expression in PA (lamey et al., 1987, Jeannon et al., 1999, Glas et al., 2002 Nasser et al., 2003, Teymoortash et al., 2003, Ito et al., 2009, Kolude et al., 2013) (table 6).

Table 6: Summary of the expression of ERs in PA of salivary gland in previous reports

Auther	year	No. of cases	ERs + (%)	Ref #
lamey *et al*	1987	4	0	17
Jeannon *et al*	1999	10	4 (40)	15
Glas *et al*	2002	69	13 (19)	29
Nasser *et al*	2003	10	0	30
Teymoortash *et al*	2001	5	0	35
Ito *et al*	2009	41	0	32
Kolude *et al*	2013	13	1 (7)	34
Present study	2014	15	0	-

Many authors suggested that the marked conflicting results of ERs expression could be related to several factors such as differences in tissue fixation, the sensitivity and specificity of the antibodies used, the methods used by each group, or even the criteria adopted for judging a tumor positive for the marker. In addition, some of the differences might be related to the relatively small number of cases studied (Glas et al., 2002 Nasser et al., 2003, Pires et al 2004, Ito et al., 2009). Therefore, it is significant to standardize protocols for evaluation of salivary gland tumors similar to that described for the analysis of breast cancer tissues. Moreover, larger studies that take into account the aforementioned factors may be necessary for a more definitive assessment of ERs expression in salivary gland tumors (Gaffney et al., 1995).

MEC of the salivary glands have a biological resemblance to MEC of the breast, they share a common cytogenetic alteration in the form of a reciprocal translocation t(11;19)(q21;p13) (MAML2:MECT) (Tonon et al., 2003). This translocation creates a fusion product (MAML2:MECT1) that activates transcription of cAMP/CREB target genes (Tonon et al., 2003, 2004). The expression of the protein fusion gene was associated with a significantly lower risk of death compared to those without the fusion protein MAML2:MECT1 (Behboudi et al., 2006). Accordingly, therapeutic strategies used in MEC of the breast can be used for MEC of salivary glands positive for ERs. Hormonal therapy has been successfully used as adjunctive treatment in some cancers such as breast and prostate carcinoma (Ma CX et al., 2009, Berthelet et al., 2005, (EBCTCG), 2005).

The hormone receptor status in breast cancer has been pivotal in determining the likelihood of response to hormonal manipulation. Tumors which are both estrogen and progesterone receptor positive are much more likely to respond to anti-hormone therapy than negative tumors (Gaffney et al., 1995).

The effects of hormonal therapy of salivary gland tumors has not been widely studied. Adjuvant hormonal therapy of salivary gland tumors was suggested for in MECs with positive expression of ERs (Pires et al 2004).

Nevertheless, hormonal therapy was suggested as adjunctive treatment in salivary duct carcinoma and adenoid cystic carcinoma that are resistant to conventional therapeutic strategies even though it is negative to ERs, in view of their aggressive behavior (Elkin and Jacobs, 2008). Added to that, partial and complete remission of cancers of parotid gland were reported in the previous studies (Van der Hulst et al., 1994, Locati et al., 2003).

Thus, it can be concluded that ERs staining was observed in 10 cases out of 20 tumor samples of MEC but it was negative in all cases of PA. It seems that ERs probably has a role in tumorigenesis, prognosis of MEC and the hormonal therapy of MEC in particular high-grade malignancy positive for ERs may be effective. On the other hand, the role of ERs in salivary gland tumorgenises of PA was not supported in the present study.

Further studies of more cases, using the immunohistochemical method and other diagnostic methods such as *in situ* hybridization or RT-PCR, are recommended in order to clarify the exact role of ERs in pathogenesis salivary gland tumors especially MEC.

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References

1. Greene GL, Gilna P, Waterfiled M, Baker A, Hort Y, Shine J. Sequence and expression of human estrogen receptor complementary DNA. Science 1986; 231: 1150–4.
2. Beato M. Gene regulations by steroid hormones. Cell 1989; 56: 335–44.
3. Ma CX, Sanchez CG, Ellis MJ. Predicting endocrine therapy responsiveness in breast cancer. Oncology 2009: 23: 133–42.
4. Parkar MH, Newman HN, Olsen I. Polymerase chain reaction analysis of oestrogen androgen receptor expression in human ginigival and periodontal tissue. Archs oral Biol 1982; 41: 979-83.
5. Välimaa H, Savolainen S, Soukka T, Silvoniemi P, Mäkelä S, Kujari H, Gustafsson JA, Laine M. Estrogen receptor-β is the predominant estrogen receptor subtype in human oral epithelium and salivary glands. J Endocrinol 2004; 180: 55–62.
6. Ciocca DR, Puy LA, Fasoli LC. Study of estrogen receptor, progesterone receptor, and the estrogen-regulated Mr 24,000 protein in patients with carcinomas of the endometrium and cervix. Cancer Res 1989;49:4298-304.
7. Campbell-Thompson M, Lynch IJ, Bhardwaj B. Expression of estrogen receptor (ER) subtypes and ER-beta isoforms in colon cancer. Cancer Res 2001;61:632-40.
8. Radzikowska E, Langfort R, Giedronowicz D. Estrogen and progesterone receptors in non small cell lung cancer patients. Ann Thorac Cardiovasc Surg 2002; 8: 69–73.
9. Eveson JW, Cawson RA. Salivary gland tumours. A review of 2410 cases with particular reference to histological types, site, age and sex distribution. J Pathol 1985; 146: 51-8.
10. Neville. BW, Damm DD, Allen CM, Bouquot JE. Pathology of salivary glands. In: Oral & Maxillofacial Pathology. 2nd edition. Philadelphia, W.B. Saunders, 2002. pp. 373-88.
11. Pons-Vicente O, Almendros-Marqués N, Berini-Aytés L, Gay-Escoda C. Minor salivary gland tumors: A clinicopathological study of 18 cases. Med Oral Patol Oral Cir Bucal 2008; 13: 582-8.
12. Camelo-Piragua SI, Habib C, Kanumuri P, Lago CE, Mason HS, Otis CN. Mucoepidermoid carcinoma of the breast shares cytogenetic abnormality with mucoepidermoid carcinoma of the salivary gland: a case report with molecular analysis and review of the literature. Hum Pathol 2009; 40: 887–92.
13. Jeannon JP, Soames JV, Bell H, et al. Immunohistochemical detection of oestrogen and progesterone receptors in salivary tumours. Clin Otolaryngol. 1999;24:52-4.
14. Lamey PJ, Leake RE, Cowan SK, et al. Steroid hormone receptors in human salivary gland tumours. J Clin Pathol. 1987;40:532-4.
15. Barnes L, Rao U, Contis L, et al. Salivary duct carcinoma, II: immunohistochemical evaluation of 13 cases for estrogen and progesterone receptors, cathepsin D, and c-erbB-2 protein. Oral Surg Oral Med Oral Pathol. 1994;78:74-80.
16. Dimery IW, Jones LA, Verjan RP, Raymond AK, Goepfert H, Hong WK.. Estrogen receptors in normal salivary gland and salivary gland carcinoma. Arch Otolaryngol Head Neck Surg 1987;113:1082-5.
17. Ozono S, Sato K, Ito Y, et al. Immunohistochemical evidence that tumors elicit the synthesis of estrogen receptors in the submandibular gland of female rats. Experientia 1995: 51;220-2.
18. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet. 2005;365:1687-717.

19. Berthelet E, Pickles T, Lee KW, Liu M, Truong PT. Long-term androgen deprivation therapy improves survival in prostate cancer patients presenting with prostate-specific antigen levels > 20 ng/mL. Int J Radiat Oncol Biol Phys. 2005;63:781-7.
20. Elkin AD, Jacobs CD. Tamoxifen for salivary gland adenoid cystic carcinoma: a report of two cases. J Cancer Res Clin Oncol 2008;134:1151–3.
21. Van der Hulst RW, Van Krieken JH, Van der Kwast TH, et al. Partial remission of parotid gland carcinoma after goserelin. Lancet 1994; 344:817.
22. Locati LD, Quattrone P, Bossi P, et al. A complete remission with androgen-deprivation therapy in a recurrent androgen receptor-expressing adenocarcinoma of the parotid gland. Ann Oncol 2003;14:1327–8.
23. Glas AS, Hollema H, Nap RE, Plukker JT. Expression of estrogen receptor, progesterone receptor, and insulin-like growth factor receptor-1 and of MIB-1 in patients with recurrent pleomorphic adenoma of the parotid gland. Cancer. 2002;94:2211-6.
24. Nasser SM, Faquin WC, Dayal Y. Expression of androgen, estrogen, and progesterone receptors in salivary gland tumors. Frequent expression of androgen receptor in a subset of malignant salivary gland tumors. Am J Clin Pathol. 2003;119:801-6.
25. Pires FR, da Cruz Perez DE, de Almeida OP, Kowalski LP. Estrogen receptor expression in salivary gland mucoepidermoid carcinoma and adenoid cystic carcinoma. Pathol Oncol Res 2004;10:166-8.
26. Ito FA, Ito K, Vargas PA, de Almeida OP, Lopes MA. Salivary gland tumors in a Brazilian population: a retrospective study of 496 cases. Int J Oral Maxillofac Surg 2005;34:533-6.
27. Wilson JA, Rogers MJC, Hawkins RA, et al. Epidermal growth factor receptors and oestrogen receptors in the head and neck. Clin Otolaryngol 1993;18: 66-8.
28. Kolude B, Adisa A, Adeyemi B, Lawal A. Immunohistochemical expression of oestrogen receptor-α and progesterone receptor in salivary gland tumours. J Oral Pathol Med 2013;42: 716-9.
29. Teymoortash A, Lippert BM, Werner JA. Steroid hormone receptors in parotid gland cystadenolymphoma. Clin Otolaryngol Allied Sci 2001;26:411-6.
30. Murase R1, Sumida T, Ishikawa A, Murase R, McAllister SD, Hamakawa H, Desprez PY. Novel therapeutic strategies for malignant salivary gland tumors: lessons learned from breast cancer. Int J Otolaryngol 2011; 2011:187623.
31. Gaffney EV, Pinkston JA, Eidson JJ. Estrogen receptors in parotid tumors. Endocr Res 1995;21:635-43.
32. Tonon G, Modi S, Wu L, et al. t (11;19) (q21;p13) translocation in mucoepidermoid carcinoma creates a novel fusion product that disrupts a Notch signaling pathway. Nature Genetics 2003; 33: 208–13.
33. Tonon G, Gehlhaus KS, Yonescu R, Kaye FJ, Kirsch IR. Multiple reciprocal translocations in salivary gland mucoepidermoid carcinomas. Cancer Genetics and Cytogenetics 2004;152:15–22.
34. Behboudi A, Enlund F, Winnes M, et al. Molecular classification of mucoepidermoid carcinomas –Prognostic significance of the MECT1-MAML2 fusion oncogene. Genes Chromosomes and Cancer 2006;45: 470–81.

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