Estrogen Receptor, Progesterone Receptor, and HER-2 Expression in Recurrent Pleomorphic Adenoma

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ABSTRACT: Pleomorphic adenoma (PA) is the most common salivary gland neoplasm and, although mostly benign, recurrences, being called recurrent pleomorphic adenoma (RPA) and malignant transformation to carcinoma ex pleomorphic adenoma (CXPA), do occur. Recently, attention has been focused on molecular targeted cancer therapy in various tumors, including salivary gland tumors. The aim of this study was to investigate the role of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER-2) in PA, RPA, and CXPA. In total, 20 cases of PA, 18 of RPA, and 7 cases of CXPA were immunohistochemically studied for ER, PR, and HER-2. For evaluation of ER and PR, only nuclear expression and greater than 10% positive cells were regarded as cutoff criteria. HER-2 was evaluated semiquantitatively and graded from 0 to 3+. HER-2 amplification was assessed by chromogenic in situ hybridization (CISH). Tumors were negative for ER, PR, and HER-2 in all cases of PA and RPA. A case of CXPA showed moderate and complete membranous staining, and 6 cases were negative. HER-2 amplification was not observed in any case. In conclusion, the lack of ER, PR, and HER-2 expression in PA, RPA, and CXPA suggests that these proteins are not involved in progression, recurrence, or malignant transformation of PA.

KEYWORDS: Pleomorphic adenoma, recurrence, estrogen, progesterone, HER-2

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Introduction

Pleomorphic adenoma (PA) is the most common neoplasm of salivary glands.[1] PA is classified as a benign tumor; however, the incidence of recurrence (RPA) after initial operative treatment is significant and varies largely because of differences in surgical technique.[2,3] Recurrences have been associated with an increased risk of malignant transformation to carcinoma ex pleomorphic adenoma (CXPA).[4,5]

Some factors are related to the increase in the recurrence, such as multinodularity and pseudopodia, the age of the patients, and incomplete surgical excision.[6,7] Recurrence has also been associated with cell biological and molecular changes.[7-12]

In previous studies from our group, several aspects of RPA have been performed and important results were obtained. Hamada et al[7] and Soares et al[10] observed that Mucin 1 (MUC 1), a glycoprotein that plays a role in homeostasis and carcinogenesis, is related to recurrence and malignant transformation of PA. Similar results were observed with fibroblast growth factor and platelet-derived growth factor (PDGF), in which the RPA presented a higher immunohistochemical expression of these factors when compared with PA.[8] The p16, cyclin D1, and E2F proteins, which compound the retinoblastoma pathway that controls cell cycle phases, also showed strong expression in RPA. These results show that cell cycle-related changes in RPA are similar to changes in CXPA.[12]

Estrogen and progesterone are steroid hormones responsible for many biological processes with the potential to cause specific changes in anatomy and physiology throughout human development.[13] Estrogen and progesterone function through the interaction between hormones and their respective receptor proteins, namely estrogen (ER) and progesterone (PR) receptors, respectively. Regarding ER, there are 2 described receptors, named ERα and ERβ, where the ERα is the most common isoform studied.[14] ER and PR are commonly implicated in cell growth by hormone-induced cell proliferation.[15] Human epidermal growth factor receptor-2 (HER-2) is a proto-oncogene present on chromosome 17q and it is overexpressed in a variety of malignancies.[16] HER-2 is associated with increased vessel permeability, endothelial cell growth, proliferation, migration, and differentiation.[17,18]

The participation of ER, PR, and HER-2 in the pathogenesis and development of tumors is widely documented, especially in breast, endometrium, and prostate tumors.[19-21] In breast tumors, ER, PR, and HER-2 expression assists in the choice of treatment. In general, hormone-dependent lesions have better prognosis compared with nonhormone-dependent tumors.[22] The involvement of ER, PR, and HER-2 is also described in benign and malignant salivary gland tumors.[15,23-34] A few studies on hormone and HER-2 therapy to treat malignant tumors of the salivary gland have been published.[35-38]

Table 1. Details of the antibodies used for immunohistochemistry.

ANTIBODIES	CLONE	DILUTION	SOURCE	BUFFER (AR)
ER	1D5	1:150	Dako[a]	EDTA
PR	PgR 636	1:300	Dako[a]	EDTA
HercepTest			Dako[a]	

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; EDTA: ethylenediaminetetraacetic acid.
[a]Dako Corporation, Glostrup, Denmark.

The management of RPA is challenging due to the multiple nodules that may add up to as many as 130 in a single patient,[9] which hinders complete excision of the lesion thus favoring future recurrences and malignant transformation. In such scenarios, one wonders whether hormone therapy could be a useful approach in the management of RPA. Therefore, the aim of this study was to investigate the role of ER, PR, and HER-2 in PA, RPA, and CXPA.

Materials and Methods

This study examined 20 cases of PA without recurrence, 18 cases of RPA, and 7 cases of CXPA. The PA group included 12 women and 8 men. The tumors were located in the parotid gland in 17 cases and in the submandibular gland in 3 cases. The mean age was 43 years. The RPA group included 8 women and 10 men, the mean age was 42 years, and the tumors were located in the parotid glands in 17 cases and in the submandibular gland in 1 case. Information was not available for 1 case of RPA. The CXPA group included 4 men and 2 women, and the mean age was 64 years. The tumors were situated in the parotid gland in 4 cases and in the minor salivary glands in 2 cases. Information was not available for 1 case of CXPA.

Four cases of CXPA were recurrences of previous PA, and in 3 cases, this information was not available. The diagnosis of CXPA was based on histopathologic characteristics and classified as noninvasive and invasive. All studied cases showed malignant transformation of the PA luminal cells. Five cases of CXPA were noninvasive and 2 cases were invasive.

The median time interval between initial operation and recurrence (RPA) was 10.6 years (range of 1-25 years), with 2 (11.11%) cases with a first recurrence within 5 years, 2 (11.11%) after 5 years, and 9 (50%) after more than 10 years. Information was not available for 5 (27.78%) patients.

Immunohistochemistry

Three-μm sections were taken from each block and mounted on aminopropyltriethoxysilane-coated slides. Following deparaffinization in xylene and rehydration through decreasing concentrations of ethanol, endogenous peroxidases were inhibited using 3% H_2O_2 in methanol at room temperature. Immunohistochemistry for ERα and PR was subsequently performed using the antibodies and detection methods as specified in Table 1. Immunoreactivity was observed with 3.3′-diaminobenzidine tetrahydrochloride (DAB, 5 minutes at 37°C), and the slides are counterstained with Mayer hematoxylin. Immunohistochemistry staining for HER-2 protein was performed using the HercepTest Kits as recommended by the manufacturer (DakoCytomation).

Evaluation of immunohistochemistry staining

ER, PR, and HER-2 immunohistochemical reactions were interpreted by 2 authors. Only nuclear immunoreactivity was considered positive to ER and PR. Cases were considered positive for ER and PR according to standardized guidelines using a cutoff of >10% stained tumor nuclei.[39,40] Only membranous staining was considered positive to HER-2, and the staining was scored semiquantitatively using the HercepTest protocol (Dako, Carpinteria, CA, USA) where the grades are 0 to 3+. Score 0 = no staining or staining in <10% of tumor cells; score 1, faint and partial staining in ⩾10% of tumor cells; score 2, weak-to-moderate complete membrane staining in ⩾10% of tumor cells; and score 3, strong complete membrane staining in ⩾10% of tumor cells.[41] Breast carcinomas cases were used as positive control in all reactions.

CISH assessment of HER-2 status

Chromogenic in situ hybridization (CISH) to HER-2 was realized according to the manufacturer's protocol (Dako Her2 CISH pharmDx kit). Areas containing the highest HER-2 counts were identified by counting HER-2 and centromeric probe 17 (CEP17) in at least 20 nuclei. Only nonoverlapping nuclei with distinct nuclear borders were considered. The ratio between HER-2 and CEP17 was calculated, and the HER-2 gene was considered amplified when the ratio of gene-specific HER-2 to CEP17 signals was 2.0 or more or when an HER-2 signal cluster was observed. At least 1 CISH-positive spot was needed to assign a case to the HER-2-amplified category. When assessing intratumor variability, the results of CISH were considered separately for each core.

Statistical analysis

For comparison between the different tumor types, the Mann-Whitney test was used. Results with $P < 0.05$ were considered significant.

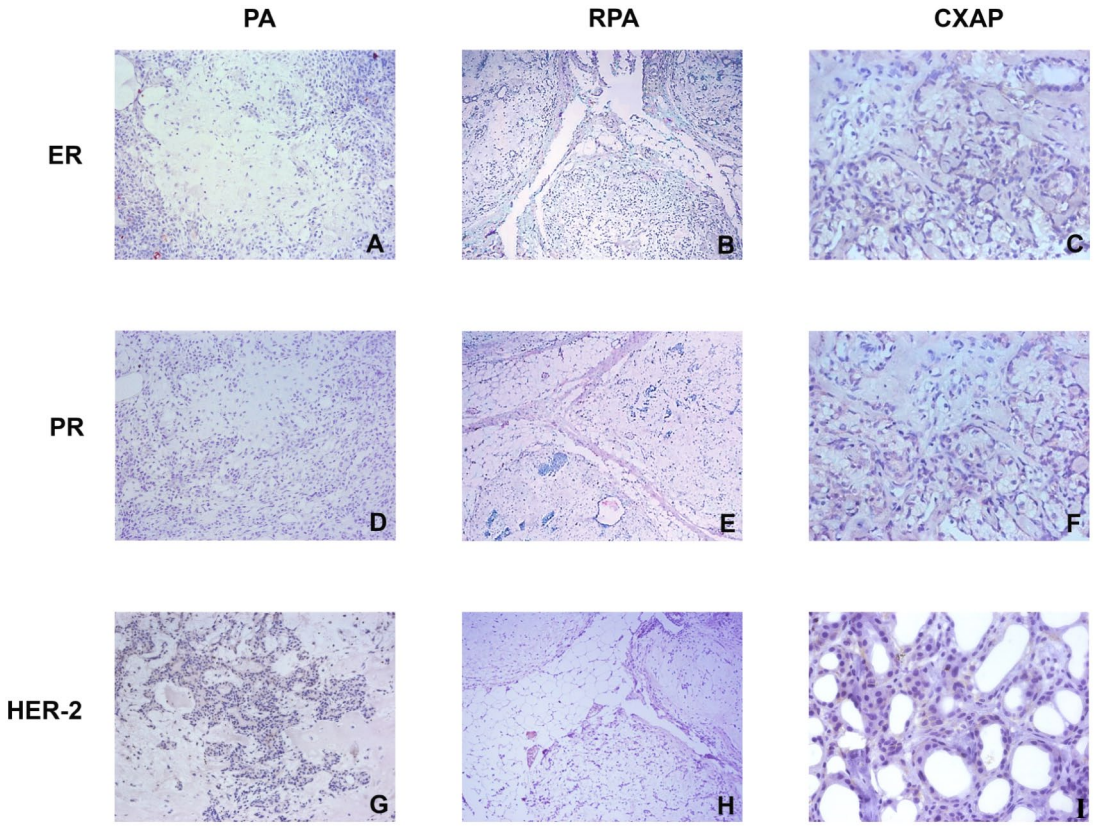

Figure 1. ER negative expression in PA (A), RPA (B), and CXPA (C). PR negative expression in PA (D), RPA (E), and CXPA (F). HER-2 negative expression in PA (G), RPA (H), and CXPA (I). PA indicates pleomorphic adenoma; RPA, recurrent pleomorphic adenoma; CXPA, carcinoma ex pleomorphic adenoma; ER, estrogen receptor; PR, progesterone receptor.

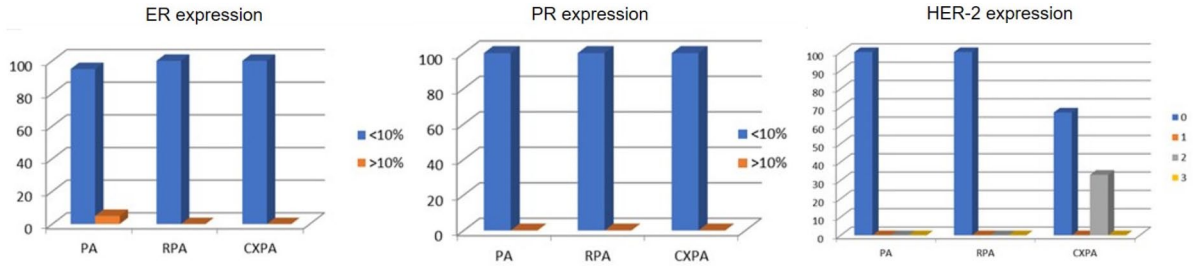

Figure 2. Graphics illustrating the expression of ER, PR, and HER-2 in PA, RPA, and CXPA, respectively. ER indicates estrogen receptor; PR, progesterone receptor; PA, pleomorphic adenoma; RPA, recurrent pleomorphic adenoma; CXPA, carcinoma ex pleomorphic adenoma.

Results

Estrogen receptor

In all tumor groups, ER immunoreactivity was observed only in a few myoepithelial and ductal cells, though most cells were negative. In PA, ER was negative in 19 cases, whereas 1 case showed more than 10% positive cells. For RPA, 18 cases were negative (Figures 1 and 2). The difference between PA and RPA was not statistically significant (Mann-Whitney test; $P = 1.000$).

All cases of CXPA were also negative for ER (Figure 1), though in 1 case of the remaining RPA, greater than 10% positivity was observed focally. In the normal glandular tissue, no staining was observed in ductal and acinar cells.

Progesterone receptor

All cases of PA and RPA showed less than 10% positivity for PR (Figure 1). The Mann-Whitney test revealed no significant difference for PR expression ($P = 1.000$) between PA and RPA (Figure 2). All CXPA cases were also negative for PR (Figure 1), though greater than 10% positivity was observed focally in 1 case of the remaining RPA. In the normal glandular tissue, a subtle staining was observed in acinar cells.

Human epidermal growth factor receptor-2

In tumors from groups PA and RPA, HER-2 protein immunoreactivity was negative (grade 0) (Figures 1 and 2). The

Mann-Whitney test revealed no significant difference for HER-2 expression ($P = 1.000$) between PA and RPA. In CXPA, HER-2 was "grade 0" in 6 cases and 1 case was "grade 2" (Figures 1 and 2). The case with labeling grade 2 was classified as invasive CXPA.

Chromogenic in situ hybridization

HER-2 amplification was not seen in none of the PA, RPA, and CXPA cases.

Discussion

RPA is lesion with peculiar clinical and biological features. Although classified as a benign lesion, its clinical behavior could be aggressive, which increases the risk of recurrence and malignant transformation. In previous studies from our group, we have demonstrated that some biological features of RPA, such as tumor vascularization, cellular proliferation index, extracellular matrix, and some growth factors are similar to those of PA.[8,9] However, features such as overexpression of proteins of the retinoblastoma cell cycle pathway, MUC 1, PDGF-A, PDGF-B, PDGF-Rα, FGF-2, Bek, and Flg are similar to those observed in CXPA.[7-12]

ER and PR expression are well established in breast carcinoma, and their presence has been shown to confer a more favorable prognosis for the patients.[42] Several studies have analyzed the participation of ER and PR in the pathogenesis of salivary gland tumors.[23-25,28-33] In this study, PA cases presented predominantly negative markings for ER and PR. In the literature, in most of the studies, neither an ER nor a PR expression has been detected in a variety of benign tumors, such as PA, Warthin tumor, oncocytoma, basal cell adenoma, and myoepithelioma, demonstrating that these factors have no influence in the development tumors of the salivary glands.[26,30,31,43,44] Although some authors have considered that the ERβ expression may have contributed to the development of PA, this antibody was not researched in this study.[45]

In our study, ER and PR expression in all cases of RPA were negative, and the difference between the expression of ER in PA and RPA cases was not statistically significant. In the scientific literature, the knowledge about the role of ER and PR in RPA is scarce; only 1 study has been found. Glas et al[15] found no significant difference in ER expression in cases of PA and RPA. The authors postulated that the expression of ER in both groups was not sufficient to expect any influence of ER on tumor growth. Regarding PR, Glas et al[15] reported intense immunostaining in recurrent tumors. Of the cases examined only 2 were negative and about 27% of cases showed overexpression, with significant difference between cases of PA and RPA. The authors speculated that PR is a prognostic factor in the occurrence of recurrent pleomorphic adenoma. However, in our research, the difference between PR expression in PA and RPA was not significant, and we adopt that the hormone receptors not influence or predict recurrence of PA.

In all CXPA cases, the expression of ER and PR were negative, but curiously, 1 case showed moderate expression of both markers in the remaining PA area. Nasser et al[26] assessed ER and PR expression in cases of CXPA and not observed immunostaining in the tumors. The authors argue that larger studies take into account that the hormonal factors may be necessary for a more definitive assessment of hormonal receptors expression in salivary gland tumors.

HER-2 is a well-known epithelial tumor oncogene that encodes the epithelial growth factor receptor tyrosine kinase.[34] The expression of HER-2 has been identified in several types of human carcinoma, including breast, ovary, endometrial, and thyroid gland neoplasms, and has been associated with varied prognosis. In some salivary gland tumors, overexpression of the HER-2 protein has been shown with several outcomes.[17,27,34,46-50]

We found immunostaining for HER-2 negative in PA, which corroborates some studies.[27,34,51-53] Our results and literature reports suggest that HER-2 expression changes are not related to the development of PA. Regarding RPA, there is only 1 study regarding HER-2 expression,[54] and no expression was observed. In this study, all cases of RPA were negative for HER-2. Based on our results and on other one found in the literature, we can postulate that there is no correlation between the expression of HER-2 and recurrence of tumors analyzed.

Overexpression and amplification of HER-2 was reported in some malignant salivary gland tumors as salivary duct carcinoma, adenocarcinoma NOS, mucoepidermoid carcinoma, and CXPA. Hashimoto et al[34] demonstrated that HER-2 superexpression appears to begin at the intraductal phase of CXPA and was mostly retained during the progression from intraductal to extracapsular components in individual extracapsular CXPA. Di Palma et al[27] also reported HER-2 expression in cases of intracapsular CXPA, and the authors hypothesize that HER-2 amplification and expression is an early event in malignant transformation of CXPA. Freitas et al[47] assessed HER-2 expression in CXPA cases, and HER-2 reactivity was observed almost exclusively in malignant luminal-type cells. The authors speculated that HER-2 could be a useful marker of malignant transformation in PA. In this study, 1 case of invasive CXPA, classified as epithelial histologically, presented labeling grade 2 for HER-2, showing that HER-2 expression could be related to malignant transformation.

In view of the results herein and those from previous studies regarding HER-2, RPA still has an expression profile closer to PA than to CXPA. In conclusion, the lack of ER, PR, and HER-2 expression in PA, RPA, and CXPA suggests that these proteins are not involved in progression, recurrence, or malignant transformation of PA.

Author Contributions

All authors had the same contribution to the article.

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