Primary mucoepidermoid carcinoma of the breast: a case report with immunohistochemical analysis and comparison with salivary gland mucoepidermoid carcinomas

Maria Heloisa Rached Palermo[1], Michelli Bárbara Pinto[2], Juliana Silva Zanetti[3], Alfredo Ribeiro-Silva[3]

[1]Pathology Service, Hospital Regional de Franca, Franca, São Paulo, Brazil
[2]Pathology Service, Santa Casa de Passos, Passos, Minas Gerais, Brazil
[3]Department of Pathology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

Mucoepidermoid carcinomas in mammary glands represent 0.3% of all breast tumors. Features of salivary gland mucoepidermoid carcinoma have been used in studies concerning mucoepidermoid carcinomas of the breast because both share similar morphologic and molecular features. We report a case of primary mucoepidermoid carcinoma of the breast with an immunohistochemistry staining panel. We verified that MUC5AC occurs in more than 50% of high-grade tumors, and MUC1 correlates with shorter disease-free survival. The comparative analysis of mucin profiles may provide further insights into the clinical behavior of these tumors.

Key words: breast carcinoma, mucoepidermoid, immunohistochemistry, mucins, hormonal receptors.

Introduction

Mucoepidermoid carcinoma of the breast was first described in 1979 [1]. It is a rare form of cancer, representing 0.2 to 0.3% of all breast carcinomas [2]. There are some authors who believe the true incidence is higher than that described in the literature because some cases could be misclassified as carcinomas with squamous metaplasia or intracystic carcinomas [3]. Salivary gland-type neoplasms of the breast are uncommon and comprise numerous entities analogous to that more commonly seen in salivary glands. The clinicopathologic spectrum ranges from benign to malignant but there are important differences as compared with those of their salivary counterpart [4]. This possibility must be taken into consideration during the differential diagnosis.

There are reports of low- [3] and high-grade [5] mucoepidermoid carcinomas of the breast. Both of them have intermediate, epidermoid and glandular cells. Low-grade mucoepidermoid tumors have epithelial cells that show keratinization and small lumen-forming glandular cells. High-grade mucoepidermoid tumors have epidermoid and mucinous cells present as isolated elements with focal necrosis [6]. Reports about *in situ* components found in these tumors are contradictory [2, 7, 8]. In mucoepidermoid carcinomas of the breast, the Elston and Ellis [9] histologic classification, which is widely used to classify invasive breast carcinomas, correlates with the Ellis and Auclair [10] classification used for salivary gland mucoepidermoid carcinomas [12].

There are few descriptions of mucoepidermoid breast tumors using immunohistochemical analysis that specifically emphasize cytokeratin 14 (CK14) expressed by basaloid cells surrounding nests and cysts and expression of cytokeratin 7 (CK7) by mucinous cells near cystic spaces. Further, immunohistochemical analyses of hormonal receptor expression have yielded conflicting results [2, 5, 8].

Here, we report a primary mucoepidermoid breast carcinoma with emphasis on morphologic features along with a discussion of an immunohistochemistry panel that includes CK7, p63, estrogen receptor (ER), and progesterone receptor (PR), in addition to mucins MUC1, MUC2, MUC5AC and MUC6. Our results were also compared to those described in the literature for salivary gland tumors.

Case presentation

The protocol used in this study conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the local Ethics Committee. An 80-year-old female patient sought medical attention in her hometown (Passos, MG), complaining of a nodule in the right breast. A biopsy showed histological features compatible with an invasive neoplasm in the breast. A subsequent surgical specimen was noted as being a whitish, firm lump with a diameter of 4 cm and was diagnosed as well-differentiated mucoepidermoid carcinoma of the breast. No imaging exams were done before the biopsy procedure.

Histological slides of the tumor showed a cystic and solid mammary tumor, with cystic areas represented by papillary mucinous carcinoma (Fig. 1A) and solid areas consisting of intraductal and mucoepidermoid invasive carcinoma (Fig. 1B and 1D). The *in situ* com-

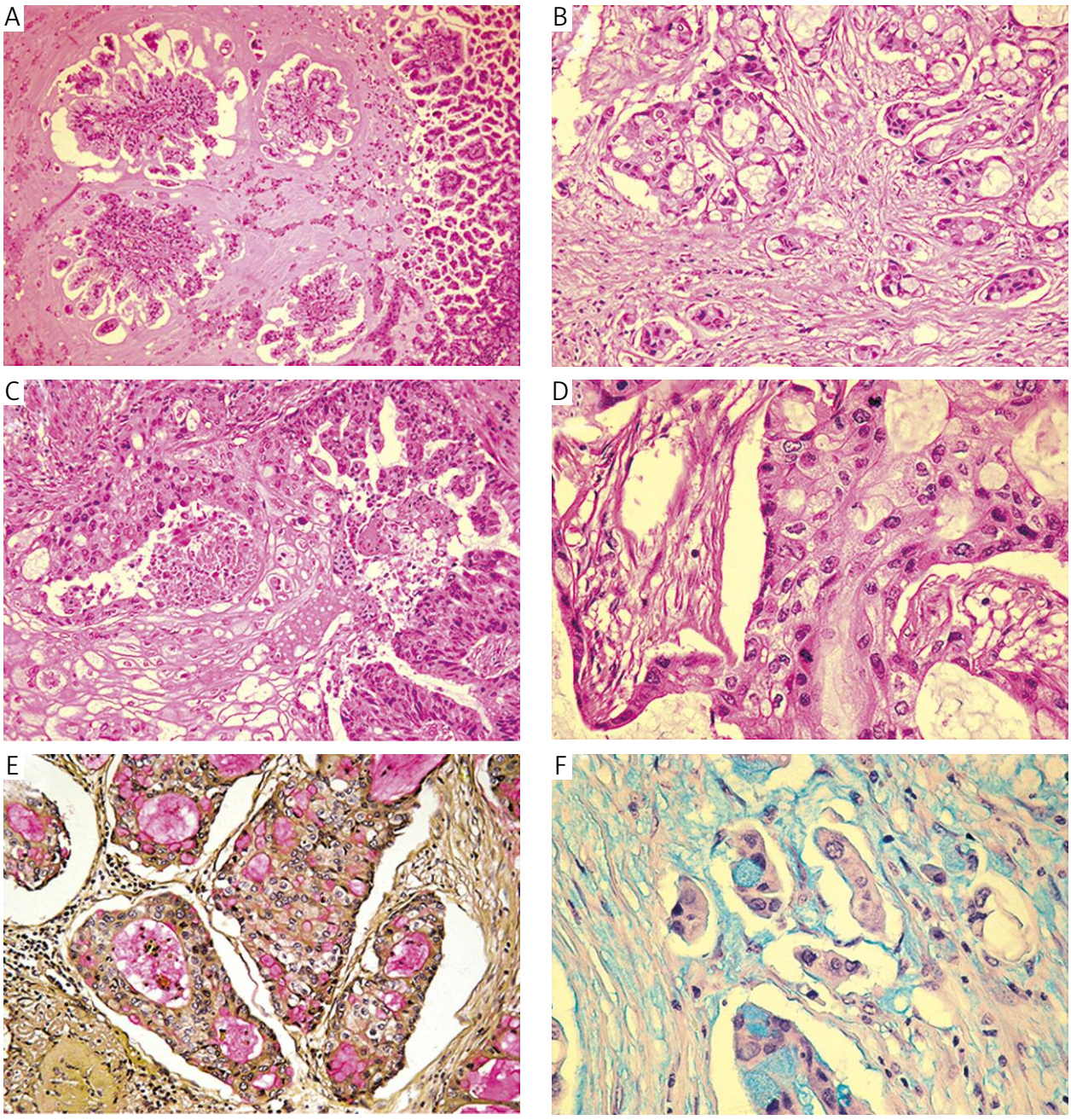

Fig. 1. Breast primary mucoepidermoid histopathology (A, B, C, D – hematoxylin/eosin, E – Mayer mucicarmine, F – Alcian blue stains). Magnification 200×

Table I. Parameters for histological classification of mucoepidermoid carcinomas

PARAMETER	PARAMETER POINT VALUE
intracystic component < 20%	+2
neural invasion present	+2
necrosis present	+3
four or more mitoses per 10 HPF[a]	+3
anaplasia	+4
grade	total point score[b]
low grade	0-4
intermediate grade	5-6
high grade	7 or more

[a]HPF – high-power field
[b]Example – a tumor whose entire area is estimated to be 40 percent cystic (0 points) but shows anaplasia (4 points) and 6 mitoses per 10 HPF (3 points) receives a total score of 7 points and is, therefore, considered a high-grade tumor. Adapted from Tekade et al. 2010 {11}

ponent had micropapillary and cribriform patterns with high nuclear grade, frequent comedonecrosis and ectatic ducts. The *in situ* component represented approximately 50% of the neoplasm and was distributed peripherally in relation to the invasive component. The invasive component was represented by cohesive groups of predominantly intermediate and mucinous cells (Fig. 1B and 1D) and, less frequently, by well-differentiated epidermoid cells (Fig. 1C). Rare, focal stromal invasion by isolated cells was observed, and there was focal tumor necrosis (Fig. 1C). The mitotic index was 10 mitoses per 10 high-magnification fields, and included atypical mitoses. No vascular or perineural invasion was observed.

Histochemistry with Mayer mucicarmine and Alcian blue stains showed numerous mucinous cells in the invasive component (Fig. 1E and 1F). Furthermore, focal or extensive mucinous metaplasia, represented by goblet cells with intracytoplasmic mucin, was observed in the intraductal component.

Immunohistochemical assays were performed on formalin-fixed, paraffin embedded tissues. The following antibodies were used: anti-Estrogen Receptor, anti-Progesterone Receptor, anti-p63, anti-MUC1, anti-MUC2, anti-MUC5AC, anti-MUC6 and anti-cytokeratin 7 (Table I). The assays followed the protocols previously established and validated in our laboratory [13, 14]. We observed diffuse expression of cytokeratin 7 in tumor epithelial cells, except in well-differentiated epidermoid foci (Fig. 2A). The cells in these foci expressed p63 (Fig. 2B), but did not express hormone receptors.

Staining for membrane-bound epithelial mucin (MUC1) revealed intense and widespread expression in all cell types in the invasive component. MUC1 expression was located on either the apical side or on the entire membrane (Fig. 1C). Stains for MUC2, MUC5AC and MUC6 (secreted gel-forming) showed no MUC2 expression (Fig. 2D); however, intense expression of MUC5AC in the invasive component of glandular mucinous cells was observed (Fig. 2E). Intermediate cells did not express MUC5AC (Fig. 1F). In the invasive component, both mucinous and intermediate cells were extensively stained by MUC6 (Fig. 2G) but the well-differentiated epidermoid component did not show positive staining (Fig. 2H).

Widespread and intense expression of MUC1 and MUC6, and lack of expression of MUC2 and MUC5AC were observed *in situ*. In non-neoplastic mammary glands, hormone receptors and CK7 were expressed in ductal cells, and expression of p63 was observed in myoepithelial cells, which was expected. MUC1 was observed only in ductal epithelial cells, and normal mammary cells did not stain for MUC2, MUC5AC or MUC6. All of these results are shown in Table III.

Discussion

The term "mucoepidermoid" was initially used for in salivary gland carcinomas and was adopted in earlier reports of this type of cancer because these carcinomas have both an epidermoid component and a mucus-secreting component [13, 16]. Over a period of 15 years, Stewart *et al*. (1945) found 45 cases of mucoepidermoid carcinomas among 700 major and minor cases of salivary gland neoplasms. They were ranked from malignant to benign and were described as "relatively favorable" or "highly unfavorable", according to histological characteristics and biological behavior. As a potential mechanism governing the development of these tumors, the authors proposed a process of progressive squamous metaplasia of stem cell ductal basal cells that acquired epidermoid characteristics. They observed a number of cells with intermediate characteristics of squamous differentiation, supporting this hypothesis. Mucoepidermoid carcinoma of breast can be classified as an adenosquamous variant of a metaplastic tumor [16], as verified in salivary gland tumors.

Because salivary and mammary glands are both tubuloacinar exocrine glands, some of their tumors, including mucoepidermoid carcinomas, share morphological, immunohistochemical and molecular features [2, 7]. However, they have different incidences and clinical behaviors when they develop as primary salivary gland or primary breast tumors.

Mucoepidermoid carcinomas are the most frequently occurring salivary gland tumors [10]. The classic morphological description correlates with staging and immunohistochemical findings [10]. The majority of mucoepidermoid carcinomas show a predominance of papillary or cystic components, typically containing mucin-secreting epithelial cells surrounded by mucinous intermediate or epidermoid cells. The neoplastic cells are represented by mucinous cells, which

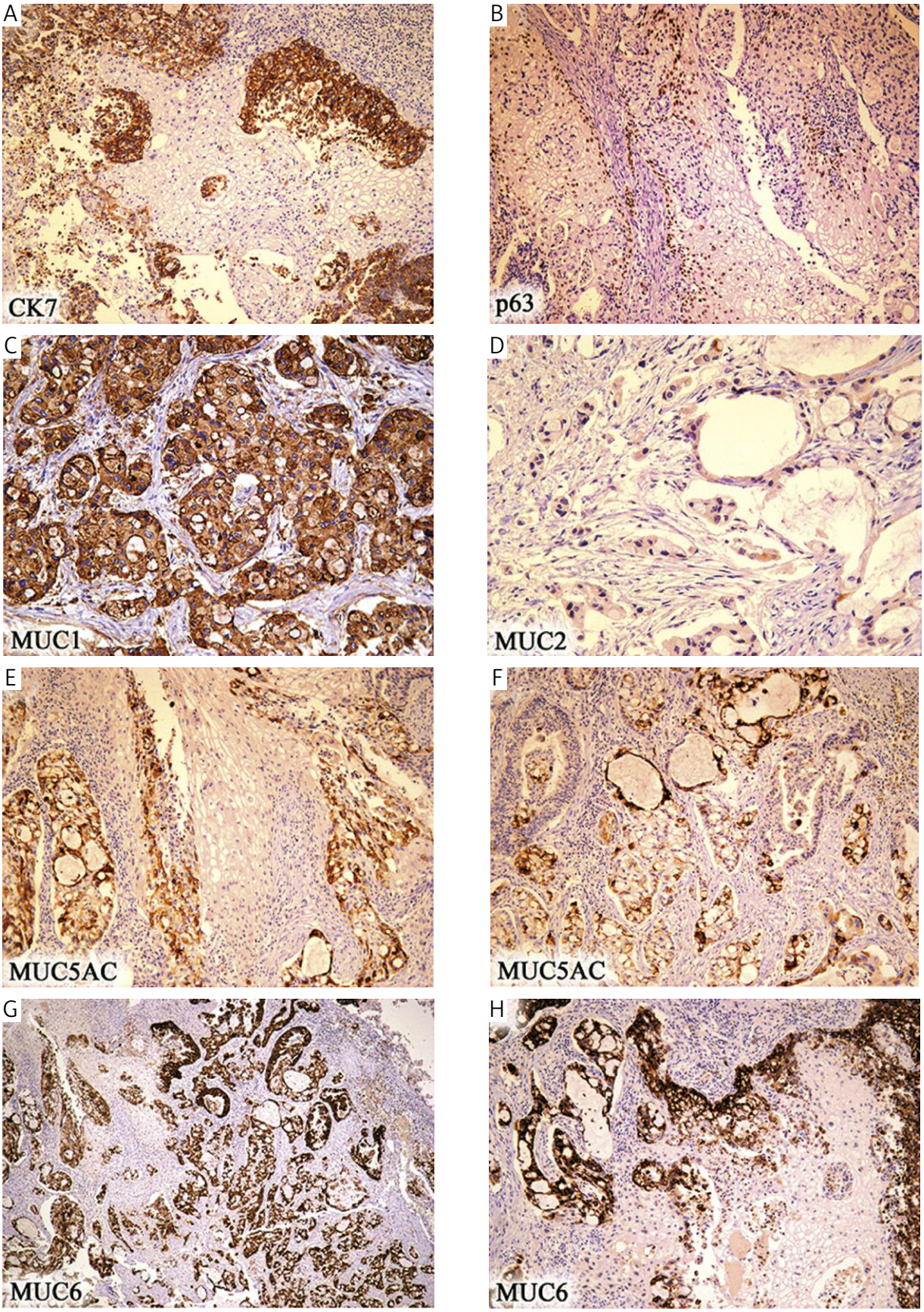

Fig. 2. Immunoexpression of CK7, p63, MUC1, MUC2, MUC5AC and MUC6 in breast invasive mucoepidermoid carcinoma. Immunohistochemistry reactions; magnification 200×

Table II. Immunohistochemistry markers

Antibody	Clone	Dilution	Buffer
cytokeratin 7	Novocastra, clone DV-TL 12/30	1/200	Citrate pH 6.0
p63	Dako, clone 4A4	1/1000	Citrate pH 6.0
estrogen receptor	Dako, clone 1D5	1/300	Tris-EDTA pH 9.0
progesterone receptor	Dako, clone PgR-636	1/100	Citrate pH 6.0
MUC1	Cell Marque, Clone MRQ-17	1/250	Tris-EDTA pH 9.0
MUC2	Cell Marque, Clone MRQ-18	1/100	Tris-EDTA pH 9.0
MUC5AC	Cell Marque, Clone MRQ-19	1/250	Tris-EDTA pH 9.0
MUC6	Cell Marque, Clone MRQ-20	1/250	Tris-EDTA pH 9.0

Table III. Results of immunohistochemical staining in neoplastic and non-neoplastic glands

Antibody	Intraductal component	Invasive component	Non-neoplastic gland
CK7	positive	positive mucin cells and intermediary cells	positive
p63	positive myoepithelial cells	positive epidermoid cells	positive myoepithelial cells
estrogen receptor	negative	negative	positive
progesterone receptor	negative	negative	positive
MUC1	positive	positive	positive
MUC2	negative	negative	negative
MUC5AC	negative	positive mucin cells	negative
MUC6	positive	positive mucin cells and intermediary cells	positive

contain epithelial mucin. In many tumors, these cells are shown only by Alcian blue or Mayer mucicarmine [10] staining. In addition to the mucinous intermediate and epidermoid cells, clear and columnar cells are also observed.

Histological classification is determined according to the criteria established by Ellis and Auclair (adapted from Takade *et al*. 2010) [10, 11] (Table I). This system shows a good correlation with survival in mouth salivary gland and parotid mucoepidermoid carcinomas. Submandibular gland tumors, however, show a significant potential for metastasis independent of histological grade. Some authors have observed that the Ellis and Auclair [10] classifications subclassify the mucoepidermoid carcinomas of salivary glands and suggested a modified system of classification based on parameters related to lymphovascular invasion, bone invasion and invasion in the form of small nests or islets. This modified system increases reproducibility and predictability and allows for stratification of patients into more uniform groups with different prognoses.

Immunohistochemical analysis is not required for the diagnosis of mucoepidermoid tumors of salivary glands, but it can provide valuable information regarding biological behavior. MUC1 expression is associated with higher tumor grade and shorter disease-free survival, indicating poor prognosis. MUC5AC expression is observed in more than 50% of high-grade tumors, and its expression in these tumors helps in differential diagnosis between high-grade mucoepidermoid carcinoma and squamous cell carcinomas. Further, some studies report different mucin expression in normal salivary glands and mucoepidermoid carcinomas.

In the present case, the histopathology shows characteristics of high histological grade in the form of numerous mitoses, and necrosis and cellular anaplasia according to the criteria of Ellis and Auclair [10] for salivary gland tumors. The absence of metastasis in axillary lymph nodes is of little help in determining prognosis; there are reports of small mucoepidermoid tumors of the breast with high histological grade but no lymph node metastases that are found to be clinically aggressive during a 25-month follow-up and are unresponsive to any treatment modality [5].

The absence of expression of hormonal receptors (estrogen and progesterone) is in accordance with the literature for most metaplastic carcinomas, including mucoepidermoid carcinoma [5]. CK7 expression was similar to previously described results, and the expression of p63, highlighting the well-differentiated epidermoid component, also correlates with reports in the literature. The use of immunohistochemistry to stain mucins

was the most significant contribution of our case and was motivated by a similar study of mucoepidermoid tumors of salivary glands. We have observed differential expression of MUC5AC and MUC6 in mucoepidermoid neoplasms and non-neoplastic mammary glands. This differential expression has also been observed in salivary glands. Positive staining for MUC5AC and MUC6 is also found in invasive neoplasms. MUC6 was observed on the intraductal component and was absent in non-neoplastic mammary glands.

In our case, the expression of MUC1 and MUC5AC in mammary neoplasms compared to reports in salivary gland neoplasms would add an argument in favor of poor prognosis. As suggested by other authors, we also think it is important to emphasize that mucoepidermoid carcinoma must be considered in the differential diagnosis of invasive carcinomas with a large epidermoid component [3]. In these cases, the expression of MUC5AC enables the diagnosis of mucoepidermoid carcinoma, as noted by our findings.

Conclusions

In conclusion, we believe that this communication is relevant because of the rarity of primary mucoepidermoid carcinomas of the breast and the additional observations made due to the use of immunohistochemical staining of mucins.

The authors declare no conflict of interest.

References

1. Patchefski AS, Frauenhoffer CM, Krall RA, Kooper HS. Low-grade mucoepidermoid carcinoma of the breast. Arch Pathol Lab Med 1979; 103: 196-198.
2. Cayari R, McGuire L, Weinstein S, Lakhani S. Mucoepidermoid carcinoma of the breast: a case report and review of literature. Pathology 2010; 42: S-81.
3. Fisher ER, Palekar AS, Gregorio RM, Paulson JD. Mucoepidermoid and squamous cell carcinomas of breast with reference to squamous metaplasia and giant cell tumors. Am J Surg Pathol 1983; 7: 15-28.
4. Foschini MP, Krausz T. Salivary gland-type tumors of the breast: a spectrum of benign and malignant tumors including "triple negative carcinomas" of low malignant potential. Semin Diagn Pathol 2010; 27: 77-90.
5. Hastrup N, Sehested M. High-grade mucoepidermoid carcinoma of the breast. Histopathology 1985; 9: 887-892.
6. Hornychová H, Ryska A, Betlach J, et al. Mucoepidermoid carcinoma of the breast. Neoplasma 2007; 54: 168-172.
7. Camelo-Piragua SI, Habib C, Kanumuri P, et al. 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: 887-892.
8. Horii R, Akiyama F, Ikenaga M, et al. Muco-epidermoid carcinoma of the breast. Pathol Int 2006; 56: 549-553.
9. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer I. The value of histological grade in breast cancer: experience from a large study with long-term follow up. Histopathology 1991; 19: 403-410.
10. Ellis GL, Auclair PL. Tumors of the salivary glands. In: Atlas of tumor pathology. Ellis GL, Auclair PL (eds) 4th ed. Washington 2008, 173-192.
11. Tekade SA, Chaudhary MS, Gawande MN, Bagri K. Correlation between mucoepidermoid carcinoma grade and AgNOR count. J Oral Sci 2012; 52: 275-279.
12. Tommaso L, Foschini MP, Ragazzini T, et al. Mucoepidermoid carcinoma of the breast. Virchows Arch 2004; 444: 13-19.
13. Stewart FW, Foote FW, Becker WF. Muco-Epidermoid Tumors of Salivary Glands. Ann Surg 1945; 123: 22.1-22.2.
14. Tavassoli FA, Devilee P. Tumours of the breast and female genital organs. In: World Health Organization Classification. Tavassoli FA, Devilee P (eds.). IARCPress-WHO, Lyon 2003; 63-67.
15. Tavassoli FA, Eusebi V. AFIP. Atlas of tumor pathology: tumours of the mammary glands. In: Fourth Series fascicle 10. Tavassoli FA, Eusebi V (eds.). Armed Forces Institute of Pathology, Washington 2009; 265.
16. Foschini MP, Reis-Filho JS, Eusebi V, Lakhani SR. Salivary gland-like tumours of the breast: surgical and molecular pathology. J Clin Pathol 2003; 56: 497-506.

Address for correspondence

Alfredo Ribeiro-Silva
Department of Pathology
Ribeirão Preto Medical School
University of São Paulo
Avenida Bandeirantes 3900
Bairro Monte Alegre
14049-900 Ribeirão Preto, São Paulo, Brazil
tel. 55 16 3602 3172
fax 55 16 3633 1068
e-mail: arsilva@fmrp.usp.br