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Expression of unusual immunohistochemical markers in mucinous breast carcinoma

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ABSTRACT

Background: Mucinous breast carcinoma is characterized by the production of variable amounts of mucin. Some studies have addressed immunohistochemical characterization of mucinous breast carcinoma using a limited set of antibodies. However, the purpose of the present study was to investigate a larger panel of markers not widely used in daily practice and to determine their pathological implications.
Methods: Forty patients diagnosed with mucinous breast carcinoma were enrolled. An immunohistochemical study was performed on whole sections of paraffin embedded tissue, using antibodies for the following markers: estrogen receptor alpha and beta, progesterone receptor, androgen receptor, HER2, EGFR, Ki-67, E-cadherin, β-catenin, p53, chromogranin, synaptophysin, GCDFP15, mammaglobin, and CDX2.
Results: The pure mucinous type was more prevalent in older patients and more frequently expressed GCDFP15. Capella type B presented more frequently with a high Ki-67 index and neuroendocrine differentiation. Although there was a lower frequency of vascular invasion and lymph node metastases in the pure type, the difference was not statistically significant. No case expressed CDX2 (a marker for gastrointestinal tumors), while 85% of the cases expressed at least one of the two typical breast markers (GCDFP15 and mammaglobin), suggesting that these markers may be reliably used for differential diagnosis. Expression of estrogen receptor beta was related to the presence of mucin cell producing lymph node metastasis, with potential prognostic and predictive value.
Conclusion: our findings support the immunohistochemical homogeneity of mucinous breast carcinomas because only minor differences were found when subgrouping them into Capella types A and B or into types pure and mixed.

1. Introduction

Mucinous breast carcinoma, a rare entity with special histological features, is characterized by the production of variable amounts of mucin. Despite the rarity and the assumed homogeneity of this histological subtype, mucinous breast carcinoma is subclassified using two different systems: pure or mixed mucinous breast carcinoma, and Capella type A, B, or AB mucinous breast carcinoma (Capella et al., 1980; Lakhani, 2012).

Pure mucinous breast carcinoma, which accounts for around 2% of all invasive breast carcinomas, is characterized by tumor cells forming clusters floating in extracellular mucin; the extracellular mucin makes up at least 90% of the tumor tissue (Lakhani, 2012). It generally affects older women and is usually associated with a good clinical outcome (Barkley et al., 2008; Li et al., 2005; Vo et al., 2007). Mixed mucinous breast carcinoma is defined as a tumor with 50% to 90% mucinous components, admixed with another histological subtype, usually invasive ductal or lobular carcinoma (Lakhani, 2012). The mixed type has a worse outcome compared with the pure form (Barkley et al., 2008; Komaki et al., 1988; Li et al., 2005; Toikkanen and Kujari, 1989; Vo et al., 2007).

Capella et al. reported that pure mucinous breast carcinoma consists of more than a single homogeneous entity. They described:

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Capella type A tumors, characterized as the "classical" type, which contain large amounts of extracellular mucin (60% to 90%) with hypocellularity; Capella type B tumors, described as containing small quantities of extracellular mucin (33% to 75%) and tumor hypercellularity showing, in most cases, neuroendocrine differentiation and argyrophilia, which affects 10–60% of tumor cells. Capella also described type AB tumors, however this type of tumors is found in a minority of cases, present features found in both types A and B, and they are exclusion diagnoses (Capella et al., 1980). As far as it is currently known, this subclassification does not seem to have any prognostic significance, although some studies have shown that neuroendocrine differentiation (expression of synaptophysin and/or chromogranin A) in mucinous breast carcinomas could be associated with favorable prognostic (Tse et al., 2004).

Gene expression profiling has improved our comprehension and characterization of breast carcinoma taxonomy by identifying five major molecular subtypes: luminal A, luminal B, HER2 overexpression, triple-negative, and normal breast-like (Perou et al., 2000). This molecular classification has shown relevance in prognostication, from a good to a poor prognosis in this sequence of types (Hu et al., 2006; Sorlie et al., 2003). It has been demonstrated that mucinous breast carcinoma is consistently identified as the luminal molecular subtype, reinforcing its good prognosis (Weigelt et al., 2008). However, other molecular characteristics support the separation of mucinous breast carcinomas from others meeting the luminal profile (Horlings et al., 2013; Weigelt et al., 2009).

Recently, researchers advocate a short immunohistochemical panel for clinical therapeutic decision making, including estrogen receptor alpha (ERα), progesterone receptor (PR), human epidermal growth factor receptor type 2 (HER2), and the proliferation marker (Ki-67), to be used for all breast cancers, including mucinous breast carcinomas (Voduc et al., 2010). In pathological practice most cases have a consistent pattern of histological features, such as Nottingham grade and immunohistochemical-based subtyping, which are in agreement with other features indicating a favorable histological prognosis. However, some cases display discordance between pathological and clinical characteristics, since they exhibit favorable pathological characteristics (e.g., low histological and nuclear grade, expression of ER and PR, low counts of Ki-67), and yet present vascular invasion, lymph node infiltration, and distant metastases. This might be due to the fact that such routinely evaluated markers are not sufficiently sensitive to detect tumor heterogeneity.

The aims of this study were: (1) to evaluate the expression of non-usual immunohistochemical markers in mucinous breast carcinoma (estrogen receptor beta [ERβ], E-cadherin, β-catenin, human epidermal growth factor receptor type 1 [EGFR], androgen receptor [AR], p53 protein, mammaglobin, gross cystic disease fluid protein 15 [GCDFP15], and caudal type homeobox 2 [CDX2]); (2) to determine if their expression is homogeneous beyond mucinous breast carcinoma; and (3) to evaluate their implications in vascular invasion, lymph node mucin producing cells metastasis and differential diagnosis.

2. Materials and methods

2.1. Patients

All consecutive cases of breast carcinoma with a mucinous component were retrieved from the files of the Laboratory of Investigative Pathology of the Women's Hospital of the State University of Campinas, São Paulo, Brazil (LAPE-CAISM-UNICAMP) from January 2013 to September 2015. These cases were reviewed by two pathologists (GRP and JV) to assess pathological information. Initially, 40 cases were identified as mucinous breast carcinomas and classified according to the World Health Organization (WHO) criteria into pure (n = 25) and mixed (n = 15) mucinous breast carcinomas. All mixed carcinomas were associated with invasive and *in situ* ductal components. All cases were further categorized according to the criteria described by Capella et al. (Capella et al., 1980). Mixed mucinous breast carcinoma is not included in this classification; for the purposes of our study, only the mucinous component was individually considered. The Nottingham grading system was used to assess the histological grade of these tumors (Elston and Ellis, 1991). Only lymph nodes with mucin producing cells were considered as mucinous breast carcinoma metastasis. This study was approved by the institutional Ethics Committee.

2.2. Immunohistochemistry

Immunohistochemistry was performed as previously described (Serra et al., 2012) on 3 μm whole sections, using ERα, ERβ, PR, HER2, Ki-67, E-cadherin, β-catenin, EGFR, AR, synaptophysin, chromogranin A, p53, mammaglobin, GCDFP15, and CDX2 antibodies (Fig. 1). Briefly, sections were deparaffinized with xylene and dehydrated in alcohol; endogenous peroxidase was blocked with 3% hydrogen peroxide. Antigen retrieval was achieved by immersing slides in citrate buffer, pH 6.0 or EDTA buffer, pH 8.0 in a commercially available pressure cooker (Pascal, Dako, Carpenteria, CA, USA). The sections were incubated in a moist chamber, with the specific primary antibodies at 4 °C, during 17 h (Table 1). Then, slides were washed in PBS, pH 7.4. The ADVANCE™ HRP Detection System (Dako) was used according to manufacturer protocol. Finally, the slides were counterstained with Harris' hematoxylin for 30 s, dehydrated, and mounted in Entellan® (Merck, Darmstadt, Germany). Internal and external, positive and negative controls were used in order to validate the reactions. HER2 IQFISH pharmDx (Dako, Glostrup, Denmark) was used to verify *HER2* amplification in cases with protein HER2 evaluation considered 2+. Detailed information about the immunohistochemistry protocols, scoring systems, and cut-offs is provided on Table 1. The slides were independently interpreted by two pathologists (GRP and JV), who evaluated the expression of each marker in a subjective, semi-quantitative way, according to methods previously presented in the literature (Alvarenga et al., 2012; Hammond et al., 2010; Weigelt et al., 2008; Wolff et al., 2013; Yerushalmi et al., 2010). Only for Ki67 (cut-off 14%), at least 1000 neoplastic cells were counted in random fields and the percentage of positive cells was calculated. In discordant cases, the evaluation was reached by consensus under a co-observation microscope.

2.3. Statistical analysis

In the univariate statistical analysis, comparisons were performed by using χ^2 or Fisher's exact test for categorical data and the Mann-Whitney test for continuous data. The concordance of diagnosis was evaluated using the weighted Cohen κ coefficient. Significance of p-value was set at less than 0.05. *p*-values were corrected using Bonferroni correction to minimize α errors, and adjusted *p*-values less than 0.05 were considered significant. The R-3.1.1 statistical software (R team, Vienna, Austria) was used.

3. Results

3.1. Clinical and histopathological features

Patients' clinicopathological data are presented on Table 2, classifying the cases into pure and mixed, and on Table 3, using the Capella classification. Their median age was 63 years [interquartile range (IQR) = 50–75 years]. Pure and mixed mucinous breast carcinoma presented with significantly different ages

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