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Original contribution

Molecular phenotypes of matched in situ and invasive components of breast carcinomas☆

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Summary The current system of pathologic classification of human breast cancers does not take into account the biologic determinants of prognosis, nor is there a consensus regarding the progression from in situ to invasive carcinoma. The present study compared the molecular phenotypes of in situ and invasive components of breast cancer in the same sample. We built a series of 189 in situ and invasive carcinomas using tissue microarrays and classified them according to their immunoprofiles regarding estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2, epidermal growth factor receptor, cytokeratin 5, P-cadherin, and the antigen Ki-67 into luminal A and B, human epidermal growth factor receptor 2 overexpressing, and basal-like carcinomas. We also correlated the subgroups of carcinomas with some of the classical prognostic factors such as histologic grade, tumor size, and lymph node metastasis, as well as with the age of the patient at diagnosis. The overall concordance on the molecular phenotypes between in situ and invasive components was 94%. For the in situ component, 63% of the cases were luminal A; 15%, luminal B; 12%, human epidermal growth factor receptor 2 overexpressing; and 7%, basal-like. Regarding the invasive component, 61% of the cases were luminal A; 16%, luminal B; 12%, human epidermal growth factor receptor 2 overexpressing; and 8%, basal-like. The present study allowed the identification of different immunoprofiles of in situ and invasive breast carcinomas using a specific panel of biomarkers and showed that in most cases, there is a concordance between in situ and invasive component profiles, supporting the theory of parallel disease in breast tumorigenesis.

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1. Introduction

Breast cancer is the most common cancer in women, with more than 1 million cases occurring worldwide annually [1]. Despite significant diagnostic and therapeutic innovations, the effect on the mortality rate has been modest. One of the factors contributing to this limited success is the relative lack of understanding of the natural history of this disease [2]. For example, the transition from in situ to invasive carcinoma is still a poorly understood event [3].

Nowadays, it is widely stated that the natural history of breast cancer involves progression through clinical and pathologic stages [3,4] from premalignant hyperplastic breast lesions, with or without atypia, to carcinoma in situ and then invasive carcinoma [5-7]. On the basis of molecular, epidemiologic, and pathologic studies, ductal carcinoma in situ (DCIS) is thought to be a precursor of invasive ductal carcinoma [4]. Although this model is supported by clinical and molecular research [8-11], it is only a starting point to understand breast tumorigenesis, as the relation between preinvasive lesions and invasive carcinoma remains unclear [12]. From the available data, 2 models have been proposed recently to explain the transition from DCIS to invasive breast carcinoma (IBC). The first one, the *theory of linear progression* [5,7,13], suggests that low-grade DCIS progresses to high-grade DCIS and then to invasive ductal breast carcinoma. This model implies that tumor progression follows a linear pattern. However, there is evidence that some in situ carcinomas never progress to invasion and that some DCIS have more genetic alterations than some invasive carcinomas [14], a finding which does not fit in this multistep model. Consequently, a second model of breast cancer tumorigenesis has been proposed: the *theory of the parallel disease*, wherein low-grade DCIS tends to progress to low-grade invasive ductal breast cancer, whereas high-grade DCIS tends to progress to high-grade invasive breast cancer [12]. In this model, a specific subtype of DCIS matches a specific subtype of invasive breast cancer.

Gene expression profiling is known to be a powerful tool for identifying tumor molecular profiles and for correlating gene expression profiles with outcome in breast cancer [14]. In addition, it has been an important tool to explore the transcriptional program that leads to invasion, comparing in situ and invasive carcinomas. Recently, Dalgin et al [15] studied 36 breast cancer patients with different pathologic stages of disease and revealed a hierarchical portrait of breast cancer progression, identifying genes and pathways for each stage, grade, and molecular subtype. These authors suggested that the heterogeneity of the disease across molecular subtypes is higher than the heterogeneity of disease progression within a subtype, suggesting that tumors with different molecular profiles are in fact distinct diseases.

Several studies have concentrated on the identification of specific biomarkers that could define the subtypes of in situ and IBCs [16-18]. Our group and others demonstrated that it is possible to translate the molecular classification, using immunohistochemistry (IHC) and tissue microarrays (TMAs) [18], where estrogen and progesterone receptors (ER and PgR) and human epidermal growth factor receptor 2 (HER-2) expression identify luminal A and B and HER-2 overexpression subtypes, whereas tumor protein 63 (p63), cytokeratin 5 (CK5), and P-cadherin (P-cad) allow the identification of basal-like tumors [17]. Recently, Paredes et al [18] also demonstrated the importance of P-cad and CK5 as useful adjunct markers to distinguish the basal-like subtype among the in situ carcinomas.

However, it was never determined whether the in situ and invasive carcinomas that develop in a particular breast cancer patient belong to the same molecular subtype or are different entities belonging to different molecular profiles.

In this study, our aim was to compare the molecular phenotypes of in situ and invasive components of breast cancer in the same sample, using IHC and TMAs and a specific panel of biomarkers, previously described by our group [17,18].

2. Materials and methods

2.1. Tumor specimens

One hundred eighty-nine formalin-fixed, paraffin-embedded samples harboring in situ and IBCs in the same block were collected from the archives of the Pathology Institute of Araçatuba, São Paulo, Brazil (1996-2006). All cases were classified from hematoxylin and eosin (H&E)–stained sections. The DCIS samples were subdivided into 3 groups: low, intermediate, and high grade, according to the nuclear grade and the extent of necrosis, as previously published by our group [19]. Briefly, tumors harboring nuclear grade 3 were all considered high grade, whereas tumors with nuclear grade 1 or 2 with necrosis were considered intermediate grade, and those of nuclear grades 1 and 2 without necrosis were considered low grade. Invasive breast cancers were classified as grade I, II, or III according to the method described by Elston and Ellis [20]. Some relevant data were available for analysis, including age and clinicopathologic features such as tumor size and lymph node metastasis.

2.2. TMAs construction

Representative areas of the in situ and IBCs were selected on H&E-stained sections and marked on the corresponding paraffin blocks. Two 2-mm tissue cores were obtained from each selected specimen (donor block) and deposited in a paraffin (receptor) block using a TMA workstation (TMA Builder ab1802; Abcam, Cambridge, UK). Twenty-two TMA blocks were constructed, each containing 24 tissue cores (4 × 6). In each TMA block, nonneoplastic breast and liver tissue cores were included as a control and a TMA guide, respectively. After the construction, 2-μm tissue sections were cut and attached to Superfrost Plus glass slides.