ORIGINAL ARTICLE

Molecular phenotypes of ductal carcinoma-*in-situ* and invasive ductal carcinoma: A comparative study

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ABSTRACT

Aims and Objectives: This study was aimed at analyzing the prevalence of molecular phenotypes in invasive ductal carcinoma (IDC) and coexisting ductal carcinoma-*in-situ* (DCIS) and to correlate with clinicopathological features. **Materials and Methods:** In this study, 75 cases of IDC with coexisting DCIS were included. Molecular phenotype was determined using expression of estrogen receptor, progesterone receptor, HER2/neu, and cytokeratin 5/6. Statistical analysis was performed for correlation between molecular phenotypes and clinicopathologic parameters. **Results:** Of the 75 cases, the invasive component in all cases was IDC—not otherwise specified. About one-third of our patients were post-menopausal. The most common molecular phenotype was luminal A (45.3%) followed by HER2-expressing type (24%). In all cases, the molecular phenotype was identical in DCIS and the invasive component. HER2-expressing tumors were found to be larger in size with frequent nodal involvement. On statistical analysis, tumor size and grade were found to correlate with the molecular phenotype. **Conclusion:** In conclusion, the molecular phenotype in DCIS correlates well with that of coexisting IDC, suggesting that DCIS is a precursor lesion in these tumors. This correlation of molecular phenotype can be utilized in prediction of phenotype of the invasive component in a case with in-situ carcinoma.

KEY WORDS: Breast carcinoma, cytokeratin 5/6, estrogen receptor, HER2/neu, molecular phenotypes, progesterone receptor

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INTRODUCTION

Carcinoma of the breast is one of the most common neoplasms affecting predominantly female patients and has a wide range of pathologic appearances and clinical outcome. Recent gene expression studies have suggested classification according to the expression of hormone markers and cytokeratin subtypes. This classification includes luminal A, luminal B, HER2-expressing, basal-like, and unclassified.[1,2] These molecular phenotypes have been shown to correlate with clinical prognostic indicators.[3,4] Some investigators have suggested that the genetic profiles of breast carcinomas are fixed at the inception stage of the tumor.[5]

Ductal carcinoma-in-situ (DCIS), a group of pre-invasive breast tumors, is currently classified on the basis of cytoarchitectural features.[6-8] The molecular phenotyping has been attempted in DCIS in only a few studies.[9-12] Some of these previous studies have shown a difference in prevalence of the molecular phenotypes between DCIS and IDC. However, these studies have included pure DCIS and invasive carcinomas for evaluation.[12] A study by Steinman *et al.*[11] evaluated coexisting DCIS and IDC. They found a high concurrence in the expression of ER, PR, HER2/neu, and cytokeratin in DCIS and its coexisting invasive component.[11]

The present study aimed at evaluating the immunohistochemical expression of ER, PR, HER2/neu, and CK5/6 in invasive ductal carcinoma (IDC) and coexisting carcinoma-in-situ (DCIS) in an attempt to assess the concordance of molecular phenotype in DCIS and IDC. The molecular phenotype was also correlated with the clinicopathologic parameters.

MATERIALS AND METHODS

This retrospective study included cases of IDC of breast showing a coexisting component of DCIS diagnosed over a period of four years (2006-2009). Relevant clinical information, including age of the patient and menopausal status, was recorded. The histological sections were reviewed [Figure 1a,b] and pathologic data including tumor size, tumor grade (low/intermediate and high), and lymph node status were evaluated.

Immunohistochemistry in all the cases was performed for estrogen receptor (ER), progesterone receptor (PR), HER2/neu, and basal-type cytokeratin (CK5/6) using

streptavidin-biotin-peroxidase technique with 3',3'-diamino benzidine hydrochloride as the chromogen. The percentage (0-100%) and intensity of staining (0-negative, 1-weak, 2-intermediate, and 3-strong) were recorded. ER [Figure 1c] and PR results were reported as "H-score" giving the sum of percentage staining multiplied by intensity of staining (score ranging from 0 to 300).[13] HER2/neu was assessed for strong membranous staining in the tumor cells [Figure 1d], equivalent to 3+ positivity in HercepTest guidelines.[14] CK5/6 demonstrated cytoplasmic staining in the tumor cells. The staining for all the four markers was assessed in both the DCIS and invasive components, independent of each other.

The molecular phenotypes were classified as suggested in previous studies: Luminal A (ER+ and/or PR+, HER2-, any CK5/6); Luminal B (ER+ and/or PR+, HER2+, any CK5/6); HER2-expressing (ER-, PR-, HER2+, any CK5/6); basal-like (ER-, PR-, HER2-, CK5/6+), and unclassified (negative for all markers).[4]

Appropriate statistical methods were employed to assess the significance of difference between molecular phenotypes and the clinicopathologic parameters. The correlation of molecular phenotypes between DCIS and the invasive component was also evaluated. The study was approved by the institutional ethics committee.

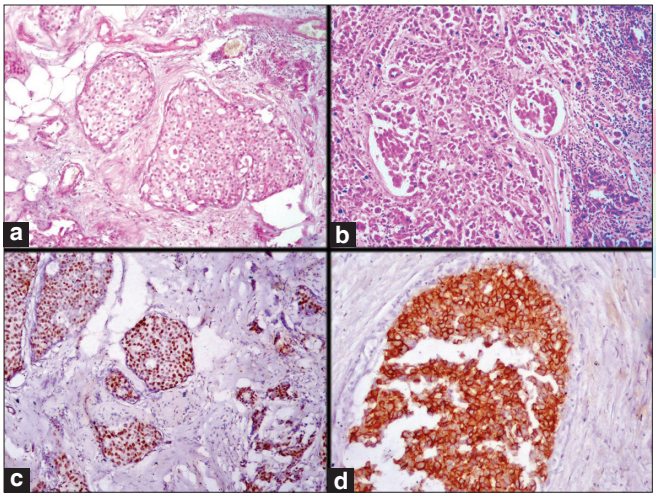

Figure 1: A panel of photomicrographs demonstrating solid-type ductal carcinoma-*in-situ* (a, H and E ×100) with invasive component as ductal carcinoma, not otherwise specified (b, H and E ×100). Immunohistochemistry displays nuclear positivity for estrogen receptor in the invasive as well as in-situ components (c, LSAB ×100). Another case shows strong membrane staining for HER2/neu in the in-situ component (d, LSAB ×200)

RESULTS

A total of 326 cases of breast carcinoma were diagnosed during the study period. Of these, 75 cases of invasive carcinoma with a coexisting DCIS component were included in this study. In all the 75 cases included, the invasive component was invasive duct carcinoma, not otherwise specified (IDC-NOS). The age of our patients ranged from 30 to 82 years, with a mean of 53 years (±11.8 years). Of the 75 patients, 24 (32%) were postmenopausal at the time of diagnosis of breast carcinoma. None of the patients gave a family history of breast cancer.

Histopathologically, the nuclear grade of the DCIS and coexisting invasive carcinoma was found to be concordant in 71 (94.67%) cases. Of these, 12 were low-grade, 45 cases had intermediate grade, and 14 showed high-grade features in both DCIS and invasive component. In the other four cases, DCIS demonstrated a high nuclear grade while the invasive component was low nuclear grade.

The molecular phenotype of DCIS in our study was as follows: luminal A – 34 cases (45.3%); luminal B – 16 cases (21.3%); HER2 type – 18 cases (24%); and unclassified – 7 (9.3%). There was no case of basal-like phenotype in our study. In all the included cases (100%), the molecular phenotype derived from immunohistochemical staining pattern was identical in DCIS and the invasive component.

Furthermore, the molecular phenotype was correlated with tumor size, grade, lymph node involvement, and postmenopausal status. The data of these parameters are tabulated in Table 1. As shown in the table, HER2 type tumors were larger in size with frequent lymph nodal involvement. On the other hand, unclassified tumors were smaller with frequent low nuclear grade and lower frequency of nodal metastasis. Luminal A type breast cancers were also node-negative in 100% of the cases. On statistical evaluation, tumor size ($P<0.001$) and tumor grade ($P<0.001$, Chi-square test) correlated with the molecular phenotype. Lymph nodal metastasis and menopausal status did not show any significant correlation with the phenotype.

Table 1: Correlation of clinicopathological features with molecular phenotype of breast carcinoma

		Luminal A (%)	*Luminal B (%)*	*HER2 (%)*	*Unclassified (%)*
Tumor size	<2.0 cm	22 (64.7)	9 (56.2)	0	5 (71.4)
	>2.0 cm	12 (35.3)	7 (43.7)	18 (100)	2 (28.6)
Tumor grade	Low/Intermediate	31 (91.1)	13 (81.2)	6 (33.3)	7 (100)
	High	3 (8.8)	3 (18.7)	12 (66.7)	0
Node status	Negative	18 (52.9)	10 (62.5)	6 (33.3)	5 (71.4)
	Positive	16 (47)	6 (37.5)	12 (66.7)	2 (28.6)
Menopausal status	Post-menopausal	8 (23.5)	8 (50)	6 (33.3)	2 (28.6)
	Pre-menopausal	26 (76.5)	8 (50)	12 (66.7)	5 (71.4)

DISCUSSION

Breast cancer is a heterogeneous disease encompassing a wide range of pathologic entities with variable clinical behavior. Recent studies using gene expression profiling have provided newer insights into the classification of invasive breast cancer into two ER-positive and three ER-negative subtypes. The ER-positive subtypes include luminal A tumors, characterized by positive ER and/or PR and negative HER2 with favorable clinical features, and luminal B subtype that expresses HER2 in addition to ER/PR and has a less favorable clinical outcome. ER-negative tumors include the following three subtypes: HER2-expressing which is negative for ER/PR; basal-like tumors characterized by expression of CK5/6 and CK17; and an unclassified type.[1,2] Basal-like and HER2-expressing groups have been associated with poor clinical features and survival. A small number of studies have utilized selected immunohistochemical stains with similar stratification of tumors.[3,4]

Few studies have suggested that the molecular profiles of breast tumors are usually fixed at inception stage.[5] Hence, risk factors for developing breast cancer might be related to the molecular profiles that later affect the clinical behavior of the tumors. These molecular subtypes of breast cancer have been evaluated in only occasional population-based studies.[4,15] In the study by Carey *et al.*[4], the prevalence of basal-like and luminal A breast cancer was influenced by race (basal-like in African-American patients) and menopausal status (highest prevalence of basal-like among premenopausal women). The HER2-expressing (ER-negative) subtype did not vary with the race or menopausal status. The tumor-specific survival was lowest in HER2+/ER- and basal-like subtypes.[4] Yang *et al.*[15] reported similar results of unfavorable clinical features in HER2-expressing and basal-like tumors. In addition, risk factors like increasing body mass index, age at menarche, and family history were evaluated for the molecular subtypes.

Though information concerning the molecular heterogeneity of invasive breast cancer is gradually accumulating, the same is not true for DCIS. DCIS of the breast is a heterogeneous group of pre-invasive breast tumors with variable malignant potential. Hence, it is still challenging to accurately determine the risk of progression to invasive cancer. This is limited by the lack of information about molecular alterations in DCIS. The current classification of DCIS employs cytoarchitectural features with some, though not complete, correlation with clinical behavior.[6-8] Only few studies have attempted to evaluate whether the same molecular subtypes, as identified in invasive breast cancer, are also seen in DCIS.[9-12] Earlier studies have shown that DCIS can also be classified into the five molecular phenotypes that were initially described for invasive breast carcinoma. However, these studies showed a difference in prevalence of the molecular phenotypes between DCIS and IDC. An increased prevalence of luminal B and HER2 molecular subtypes was noted in DCIS compared with invasive carcinomas.[10,12] This difference has been suggested to indicate a higher prevalence of HER2/neu protein over-expression and gene amplification in DCIS, though the explanation for this over-expression remains unresolved.[12,16,17] In the present study, however, the frequency of various molecular phenotypes was similar in DCIS and IDC. This variance from previous studies in the literature is due to the type of cases included. The previous studies, like Tamimi *et al.*,[12] included cases of DCIS with invasive component as invasive carcinoma, while the category of DCIS included only pure in-situ carcinomas. In contrast, we studied cases of IDC with a significant DCIS component to evaluate the correlation of molecular phenotype, which was found to be 100%. This includes HER2 expression in DCIS and IDC, which was found to correlate across the grade of the tumor. Our results are in concordance with the study by Steinman *et al.*[11] who evaluated the phenotypes in pure DCIS and DCIS with an invasive component. The authors found a high rate of co-expression of ER, PR, HER2/neu, and cytokeratin markers in DCIS and its coexisting IDC. They thus suggested that DCIS is the most likely precursor lesion for its coexisting IDC.[11]

Previous studies evaluating invasive carcinoma have shown that high-grade tumors were more likely to be HER2 type and basal-like. Also, HER2 and basal-like tumors were more likely to be associated with adverse clinical features like larger tumor size and lymph nodal involvement.[4] We have demonstrated a similar relationship in our study. We found that HER2 type tumors were frequently larger than 2 cm in size and had lymph node metastasis. We did not have any case of basal-like carcinoma in our study.

One of the limitations of our study is that pure DCIS (without invasive component) was not included for comparison of the prevalence of molecular phenotypes. This may be attributed to the lack of breast cancer screening program with mammography in our region, resulting in low detection of early lesions. Also, immunohistochemical classification of molecular phenotypes of breast cancer, though arguably the most practical approach, does not correlate perfectly with the gene-based classification.[11,12] However, gene-based classification is not possible at all centers and hence, immunohistochemistry provides a good alternative for such classification of cases.

In conclusion, DCIS can also be sub-classified into the five molecular phenotypes similar to invasive carcinomas. Since the phenotypes of DCIS and its coexisting IDC are usually concordant, the molecular type of IDC can possibly be predicted in a lesion diagnosed as DCIS. This can aid in prediction of tumor behavior, progression, and response to therapy. Our results, however, need to be confirmed in further prospective studies.

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