Molecular Subtypes of Ductal Carcinoma In Situ (DCIS) Identified by Expression of ER, PR, HER2, and Ki-67

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ABSTRACT

Objective: The estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and Ki-67 are used to classify invasive breast cancer into various subtypes. In the case of ductal carcinoma in situ (DCIS), however, the information relating to subtypes using these markers is still limited.

Methods: The pathological specimens of 267 patients diagnosed with DCIS at Siriraj Hospital were analyzed. By using the expressions of ER, PR, HER2, and Ki-67, breast cancer patients were classified into the five molecular subtypes: luminal A, luminal B/HER2 negative, luminal B/HER2 positive, HER2 overexpression, and triple-negative. Based on the specific molecular subtypes, age, clinical presentation, tumor size, and tumor grade were analyzed separately using univariate analysis.

Results: 135 (50.6%), 1 (0.4%), 58 (21.7%), 59 (22.1%), and 14 (5.2%) DCIS were luminal A, luminal B/HER2 negative, luminal B/HER2 positive, HER2 overexpression, and triple-negative, respectively. Patients with luminal A DCIS significantly presented with mass, compared to the other subtypes that displayed with mass and microcalcification in combination ($p = 0.008$). The luminal A subtype was also associated with tumors of a smaller size (less than 2 cm; $p = 0.007$) and a lower nuclear grade ($p = 0.000$) than the HER2 overexpression subtype.

Conclusion: Although luminal A DCIS was the most common DCIS subtype of Thai women, HER2 overexpression DCIS was significantly correlated with poor clinicopathological features, including a large tumor size and a high nuclear grade, which are recognized prognostic factors for tumor recurrence.

Keywords: Clinicopathological features; ductal carcinoma in situ; molecular subtypes; prevalence (Siriraj Med J 2019; 71: 432-437)

INTRODUCTION

DNA microarray profiling of invasive breast cancer is used to classify breast cancers into distinct subtypes that are explicitly associated with oncological outcomes, and to identify therapeutic options.[1] In addition, various studies have confirmed that immunohistochemical staining of paraffin sections are well-correlated with, and are used as reliable surrogate markers for, molecular subtype classifications categorized by DNA microarrays.[1] The staining of immunohistochemistry (IHC) applied to

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pathological specimens includes antibodies for estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and Ki-67.[2]

Based on the protein expressions of these IHC markers, invasive breast cancer subtypes are categorized into luminal A, luminal B/HER2 negative, luminal B/HER2 positive, HER2 overexpression, and triple-negative.[2] Multiple previous studies have shown that luminal A has the best prognosis and the lowest rate of locoregional recurrence, whereas the HER2 overexpression and triple-negative subtypes are strongly associated with a high risk of regional recurrence and distant metastases.[3-5]

Ductal carcinoma in situ (DCIS), which accounts for 20%–25% of new breast cancer diagnoses, is the proliferation of malignant mammary ductal epithelial cells confined within the basement membrane.[6] Identification of the predictive and prognostic markers associated with the biology of DCIS would provide better therapeutic options and oncological outcomes.

However, the study of subtype classifications in DCIS has been limited to date. This study primarily aimed to establish the incidence of DCIS sorting by individual molecular subtypes. The secondary aim was to identify the relationship between each DCIS subtype and the associated clinicopathological features and outcomes in Thai women.

MATERIALS AND METHODS

Data collection

A retrospective review was performed of pathological specimens obtained from patients diagnosed with pure DCIS at the Department of Surgery, Faculty of Medicine Siriraj Hospital, between December 2006 and September 2012. A total of 267 pathological specimens were collected. Protein expressions of ER, PR, HER2, and Ki-67 were detected by IHC. Pathological specimens containing microinvasions, extensive intraductal components and nodal metastasis were excluded. Information on the patient and tumor characteristics (comprising age, clinical presentation, tumor size, and tumor grading) was collected. This study was approved by the Ethics Committee of the Siriraj Institutional Review Board, Faculty of Medicine Siriraj Hospital (Si 435/2010).

Molecular subtype classification

Based on expressions of ER, PR, HER2, and Ki-67, the tumors were classified into the following subtypes:

- luminal A: ER positive, PR positive, HER2 negative, and Ki-67 < 14%;
- luminal B/HER2 negative: ER positive, PR positive, HER2 negative, and Ki-67 > 14%;
- luminal B/HER2 positive: ER positive, any PR, HER2 positive, and any Ki-67;
- HER2 overexpression: ER negative, PR negative, and HER2 positive; and
- triple-negative: all ER, PR, and HER2 negative.

Immunohistochemistry (IHC)

IHC was performed on 3-μm formalin-fixed, paraffin-embedded, pathological specimens. The expression of each marker was detected by IHC using rabbit anti-ER monoclonal antibody (ready-to-use; clone SP1, Ventana Laboratories, Tucson, AZ); rabbit anti-PR monoclonal antibody (ready-to-use; clone 1E2, Ventana Laboratories, Tucson, AZ); rabbit anti-HER2 monoclonal antibody (ready-to-use; clone 4B5, Ventana Laboratories, Tucson, AZ); and rabbit anti-Ki-67 monoclonal antibody (1:300 dilution; clone MIB-1, DAKO Laboratories, Carpinteria, CA).

Interpretation of staining

According to the American Society of Clinical Oncology and the College of American Pathologists, ER and PR assays are considered positive if there is at least 1% positive tumor nuclei staining in the samples.[7] HER2 assays are considered positive if there is IHC staining of 3+ (uniform, with intense membrane staining of > 30% of the tumor cells), but it is considered negative if there is IHC staining of 0 (no immunostaining) or 1+ (weak immunostaining, with < 30% of the tumor cells stained). However, IHC staining of 2+ (complete membrane staining, with either uniform or weak staining in at least 10% of the cells) is considered equivocal.[8] In this study, we did not perform in situ hybridization in all equivocal HER2 (2+); instead, we considered equivocal HER2 as the HER2 negative subtype. In accordance with the invasive breast cancer subtype classification from St. Gallen in 2011, Ki-67 was used to classify patients into 2 subtypes: low Ki-67 < 14% as luminal A, and high Ki-67 ≥ 14% as luminal B/HER2 negative.[9]

Statistical analysis

A chi-squared test was used for the binary variables and an analysis of the variance of the continuous variables in order to compare the clinicopathological characteristics of the four subtypes. Univariate logistic regression was used to determine any associations between each molecular subtype and age, clinical presentation, tumor size, and tumor grade. A p-value < 0.05 was considered to be statistically significant. All statistical analyses were performed by SPSS Statistics for Windows, version 15.0 (SPSS Inc., Chicago, Ill., USA).

RESULTS

The population baseline characteristics are described in Table 1. Of 267 patients with DCIS, the majority (86.5%) were over 40 years of age. The DCIS patients more commonly presented with a combination of mass and microcalcification (36.7%), a tumor size of generally less than 2 cm (60.7%), and a high nuclear-grade tumor (52.4%). The results of the IHC staining are shown in Table 2. The number of positive specimens for ER, PR, and HER2 was 189 (70.8%), 181 (67.8 %), and 117 (43.8%), respectively (Table 2). After Ki-67 staining was performed on the 136 patients with ER/PR positive and HER2 negative, only 1 patient (0.7%) had high Ki-67 (≥ 14%). According to the ER, PR, HER2, and Ki-67 statuses, the DCIS was divided into 4 subtypes (Table 3), with around half of the patients (50.6%) classified as luminal A. Table 4 illustrates the correlations between each tumor subtype and the clinicopathological characteristics determined by a univariate analysis. In terms of age, there were no differences between each molecular subtype. Moreover, the patients diagnosed with the luminal A subtype significantly exhibited mass, in contrast to the other subtypes, which presented with mass combined with microcalcification ($p = 0.008$). The tumor sizes of the luminal A and B groups were significantly smaller than those of DCIS carrying HER2 overexpression ($p = 0.007$). In addition, approximately three quarters (72.6%) of the luminal A DCIS had a significantly low nuclear grade, whereas the clear majority of the pathological specimens with the other subtypes (91.5% of those with the HER2 overexpression, 85.7% of the triple-negative, 100% of the luminal B/HER2 negative, and 63.8% of the luminal B/HER2 positive) displayed a high nuclear tumor grade ($p < 0.000$).

DISCUSSION

Ductal carcinoma in situ, a heterogenous disease, has similar molecular profiles to invasive breast cancer by genetic profiling.[10] IHC staining has replaced microarray genetic testing to classify breast cancer into 4 subtypes, based on the expressions of ER, PR, HER2, and Ki-67. These surrogate markers can be used to establish the treatment and prognosis of breast cancer.

The present study demonstrated that luminal A DCIS was the most common subtype (50.6%), followed by HER2 overexpression (22.1%), luminal B/HER2 positive (21.7%), triple-negative (5.2%), and luminal B/HER2 negative (0.4%). The DCIS incidences in our study were similar to those found by two previous studies, with luminal A DCIS being ranked first.[11,12] However, the current study found that the HER2 overexpression subgroup had a

TABLE 1. Population characteristics of the 256 patients included in this study.

Characteristics	Number (%)
Age, n (%)	
≤ 40 years	36 (13.5)
> 40 years	231 (86.5)
Clinical presentation	
Mass	76 (28.5)
Microcalcification	93 (34.8)
Combination	98 (36.7)
Tumor size	
≤ 2 cm	162 (60.7)
> 2 cm to ≤ 5 cm	83 (31.1)
> 5 cm	22 (8.2)
Tumor grading	
Low grade	38 (14.2)
Intermediate grade	88 (33.0)
High grade	141 (52.8)

TABLE 2. Results of immunohistochemistry staining of all 267 patients in this study.

IHC study	Number (%)
ER	
Positive	189 (70.8)
Negative	78 (29.2)
PR	
Positive	181 (67.8)
Negative	86 (32.2)
HER2	
Positive	117 (43.8)
Negative	150 (56.2)
Ki-67 (total 136)	
Low (< 14%)	135 (99.3)
High	1 (0.7)

Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor 2; PR, progesterone receptor

TABLE 3. Tumors classified according to molecular subtype.

Subtype	Number (%)
Luminal A	135 (50.6)
Luminal B/HER2 negative	1 (0.4)
Luminal B/HER2 positive	58 (21.7)
HER2 overexpression	59 (22.1)
Triple-negative	14 (5.2)
Total	267

TABLE 4. Univariate analysis to determine the associations between the clinicopathological features and subtypes.

Clinicopathological Variables	Luminal A (n = 135) (%)	Luminal B/HER2 negative (n = 1) (%)	Luminal B/HER2 positive (n = 58) (%)	HER2 overexpression (n = 59) (%)	Triple-negative (n = 14) (%)	P-value*
Age (n%)						
≤ 40 years	16 (11.9)	0 (0)	10 (17.2)	9 (15.3)	1 (7.1)	0.669
> 40 years	119 (88.1)	1 (100)	48 (82.8)	50 (84.7)	13 (92.9)	
Clinical presentation (n%)						0.008
Mass	47 (34.8)	0 (0)	15 (25.9)	11 (18.6)	3 (21.4)	
Microcalcification	54 (40.0)	0 (0)	18 (31.0)	18 (30.5)	3 (21.4)	
Combination	34 (25.2)	1 (100)	25 (43.1)	30 (50.8)	8 (57.1)	
Tumor size (n%)						0.007
≤ 2 cm	93 (68.9)	1 (100)	36 (62.1)	25 (42.4)	7 (50.0)	
> 2 cm to ≤ 5 cm	34 (25.2)	0 (0)	18 (31.0)	24 (40.7)	7 (50.0)	
> 5 cm	8 (5.9)	0 (0)	4 (6.9)	10 (16.9)	0 (0)	
Tumor grade (n%)						
Low grade	35 (25.9)	0 (0)	2 (3.4)	1 (1.7)	0 (0)	<0.001
Intermediate grade	63 (46.7)	0 (0)	19 (32.8)	4 (6.8)	2 (14.3)	
High grade	37 (27.4)	1 (100)	37 (63.8)	54 (91.5)	12 (85.7)	

* $p<0.05$ = significant

slightly higher proportion than the corresponding figures reported by those other publications (13% and 16%).

The differences in the DCIS subtypes might relate to the inequity of their prognoses; likewise, the differences in the molecular subtypes might predict the prognoses of invasive breast cancers. Although the present study could not find any relationships between the DCIS subtypes and prognoses due to this not being included in our objectives, many studies, such as that by Williams et al., have demonstrated that luminal A DCIS had a lower recurrence either locoregionally or in regard to distance than the other subgroups (7.6% vs 15.5%–36.1%).[13] Moreover, HER2 overexpression in DCIS has been found to be an independent prognostic factor for invasive recurrence compared to the luminal A subtype (HR 17.8; 95% CI 2.14–148; p = 0.008).[13,14]

The correlations between the clinicopathological features and DCIS differ for the various subtypes.[15,16] In our study, despite mass associated with microcalcification being a major complaint of DCIS patients, the luminal A DCIS subgroup significantly presented with a solitary, smaller mass. This phenomenon implies that the aggressiveness of the disease is related to microcalcification. However, this study could not identify the characteristics of microcalcification, such as comedonecrosis, which is used to determine DCIS prognoses. In addition, tumor size was an important factor, with approximately 65% of the luminal DCIS patients having a smaller-sized tumor than the patients in the other subgroups (< 2 cm). This finding suggests that the less invasive properties of luminal DCIS, with its smaller tumor size, present a lower risk of recurrence.[15]

In addition, we found that a high nuclear grade was mostly associated with the luminal B, triple-negative, and in particular, HER2 overexpression DCIS subgroups. To our knowledge, nuclear grade is an important factor, with a higher grade correlating with a higher risk of recurrence and thus being associated with poor molecular

subtypes.[17] Recent publications have reported that a high nuclear grade was significantly found in non-luminal A subtypes, and it was considered to be an independent predictor for overall recurrence.[15,16]

In this study, we could prove a relationship between the molecular subtypes and the clinicopathological backgrounds. All of these findings could predict the prognosis of DCIS; for instance, luminal A DCIS tended to have better clinicopathological factors. We acknowledge that patient age, tumor size, comedonecrosis, nuclear grade, surgical margin, and adjuvant treatment are mandatory factors for recurrence.[18] The goal of treatment should therefore be to decrease the opportunities for disease relapse.

However, the most suitable DCIS treatment approach has been long debated, particular in the case of breast conserving surgery (BCS).[19] Heretofore, there has been strong evidence of the benefit of using radiation to decrease local recurrence, regardless of the DCIS subgroup.[20,21] On the other hand, recent reports have challenged this standard treatment by omitting radiation from the treatment given to some patients in light of the growing knowledge of the distinct differences in the risk of recurrence of each DCIS clinicopathological subgroup.[22] Unfortunately, healthcare professional teams give little consideration to the DCIS molecular subtypes when making treatment decisions, even though breast cancer treatment is tending to become more personalized.

Furthermore, in the near future, decision-making about the treatment approach for DCIS patients will not only depend on the clinicopathological parameters or molecular subtypes, but will also include multigene assays, which are more specific to individuals. As an example, *Oncotype Dx* can estimate the 10-year risk of locoregional recurrence based on an analysis of 12 genes.[23] This can be useful for escalating or de-escalating the treatment for DCIS patients.

This study has several limitations. Firstly, its retrospective design presented inherent limitations, such as missing data. In addition, the positivity of HER2 depended only on IHC; HER2 2+ on IHC was not further confirmed with in situ hybridization, and we used the early definition of Ki-67 score which have altered from present recommendation, however, it was slightly change in DCIS incidence but not effect to other study results. Lastly, as there were no available data on recurrence, the effects of the DCIS molecular subtypes on the risks of locoregional recurrence could not be evaluated.

CONCLUSION

In our study, luminal A DCIS was the main DCIS molecular subtype and was related to less aggressive clinicopathological features. Moreover, the HER2 overexpression subtype was mostly found concomitant with a larger tumor size and a higher nuclear grade. The use of the ER, PR, HER2, and Ki-67 statuses on IHC may suggest the need for further aggressive therapy for some particular subtypes of DCIS.

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