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Original Article

Molecular alterations differentiate microinvasive carcinoma from ductal carcinoma in situ and invasive breast carcinoma: retrospective analysis of a large single-center series

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Abstract: Microinvasive carcinoma (MIC) of the breast is a rare lesion. The clinicopathologic features and biologic behavior of MIC are unclear. Whether MIC is a distinct entity or an interim stage in the progression from ductal carcinoma in situ (DCIS) to invasive breast carcinoma (IBC) remains to be determined. A retrospective review of clinicopathologic features and analysis of the expression of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER-2), and Ki-67 in patients with MIC (90 cases), DCIS (268 cases) and IBC (1504 cases) was performed. Most MICs (93.3%) exhibited an intermediate to high nuclear grade, and this proportion was larger than that of DCIS (62.7%, $P < 0.001$) or IBC (85.4%, $P = 0.036$). The incidence of sentinel lymph node metastasis in MIC (12.5%) was higher than that of DCIS (1.6%, $P < 0.001$), but much lower than that of IBC (39.7%, $P < 0.001$). MICs had higher expression of HER-2 and lower expression of ER and PR compared to DCIS and IBC; and MIC was more likely to present with a HER-2+ subtype. Furthermore, DCIS exhibited greater HER-2 overexpression or gene amplification ($P < 0.001$) levels and lower proliferation index of Ki-67 ($P < 0.001$) compared to IBC. Our results suggest that the clinicopathologic and molecular phenotype of MIC are different from DCIS and IBC. Thus, MIC may be a distinct entity rather than an interim stage in the progression from DCIS to IBC. The prognosis of MIC and the biologic behavior of this uncommon subset need to be further explored.

Keywords: Microinvasive carcinoma, breast carcinoma, molecular subtype

Introduction

Since the introduction of mammographic screening, an increasing number of patients with early-stage breast cancer are now being identified, including both ductal carcinoma in situ (DCIS) and microinvasive carcinoma (MIC) [1]. The term "microinvasive" was first applied to breast cancer in 1982 [2]. Subsequently, many different definitions have been used. According to the World Health Organization Classification of Tumours of the Breast [3], MIC of the breast is a lesion characterized by one or more clearly separate microscopic foci of tumor cells infiltrating into the mammary stroma in a background of DCIS, and a microscopic focus of invasion is defined as ≤ 1 mm in greatest dimension. The vast majority of microinvasive lesions are found in association with DCIS. Although the presence of DCIS is not mandatory for MIC diagnosis, MIC is rarely seen in the absence of any in situ background. MIC is often described as "DCIS with microinvasion" in many publications [4-6]. To date, the diagnosis of MIC is still major challenge in pathology.

Immunohistochemical staining of the basement membrane and myoepithelial cells is used to confirm microinvasion.

MIC is a rare lesion representing 0.7% to 3.4% of all breast carcinomas [7] and occurs in 10-20% of patients with DCIS [8]. Since the incidence of MIC is low and the size of the invasive foci is very small, the prognosis of this cancer subtype and treatment strategies for it are not well established. Currently, MIC is explicitly defined by the American Joint Committee on Cancer (AJCC) Staging Manual 8th Edition as a microinvasive pathologic T1 tumor (pT1mi) [9],

and is recommended to be treated in the same way as small invasive carcinomas (< 20 mm) by the National Comprehensive Cancer Network (NCCN) [10].

Previous studies of MIC are based on a small number of cases, and available data for this special breast cancer subtype are varied and controversial. Some studies have revealed that MIC with its potential for invasion and metastasis might represent a distinct entity, with features different from pure DCIS [11, 12]. The results in a recent large series implied that the clinical behavior of MIC is similar to that of DCIS, and that there is limited benefit for routine node sampling in MIC, suggesting MIC should be managed in a similar manner to DCIS [13].

The aim of this study was to compare the clinicopathologic features and molecular phenotype among patients with MIC, pure DCIS, and invasive carcinoma of the breast to further explore characteristics of MIC and to understand the natural causes of progression from DCIS to invasive breast cancer (IBC).

Materials and methods

Patients

All samples were anonymously coded prior to the analyses and the study was approved by the Research Ethics Committee of the Guangdong Women and Children Hospital. A total of 1862 patients with pathologically confirmed breast carcinoma between January 2012 and September 2019 at the Guangdong Women and Children Hospital were enrolled in this study. The histopathologic classification of tumors was performed according the World Health Organization classification criteria for the breast tumors [3]. All patients were Chinese women who had not received any local or systemic anticancer therapies before the surgery. Tissue samples were obtained from patients undergoing lumpectomy or mastectomy.

Immunohistochemistry (IHC)

Blocks of representative formalin-fixed and paraffin-embedded (FFPE) tumor tissues were cut into 4-micrometer thick sections for subsequent immunohistochemical analysis. Detection was performed on an automatic staining system using primary antibodies (Ventana Medical Systems) to estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER-2), and Ki-67. The details of the antibodies used for these four markers have been previously described [14]. Nuclear staining was assessed for ER, PR, and Ki-67. Membranous staining was assessed for HER-2. Tumors were considered positive for ER and PR if ≥ 1% tumor cell nuclei were immunoreactive in the sample [15]. The proliferation index was considered high if IHC staining for Ki-67 was seen in ≥ 14% of tumor cell nuclei [16]. The resulting HER-2 score determined by IHC was from 0, 1+, 2+, or 3+. A score of 3+ was considered as HER-2 positivity, whereas tumors with score 0 or 1+ were regarded as negative. Tumors with a score of 2+ were recommended for additional analysis by a reference laboratory [17]. IHC staining for cytokeratin 19 (Ventana Medical Systems) was performed on sentinel lymph node biopsy (SLNB) tissue that were initially read as negative by hematoxylin-eosin (H&E) staining.

Fluorescence in situ hybridization (FISH)

For tumors with an IHC HER-2 score of 2+, HER-2 gene amplification was assessed by dual-probe FISH using a FISH kit for the detection of HER-2 amplification (GP Medical Technologies, Beijing, China) according to the manufacturer's instructions. The interpretation of FISH assays was performed according to the 2013 ASCO/CAP guidelines [17].

Statistical analysis

Statistical analysis was performed using SPSS16.0 statistical software (SPSS Inc, Chicago, IL). Continuous variables were expressed as the mean ± SD, and an independent-samples *t*-test was used to determine the significance of differences between two groups. The chi-square test or Fisher's exact test (the expected value in any cell was < 5) were used as appropriate for categorical variables. All tests were 2-sided, and P value < 0.05 was considered significant.

Results

Patients and clinicopathologic characteristics

The clinicopathologic features of all patients enrolled in this study are summarized in **Table 1**. All patients were women, 90 were diagnos-

Table 1. Expression of ER, PR, HER-2 and Ki-67 in MIC, DCIS and IBC

Clinicopathologic finding	MIC (n = 90)	DCIS (n = 268)		IBC (n = 1504)		DCIS vs. IBC
	n (%)	n (%)	P^a Value	n (%)	P^b Value	P^c Value
Mean age, years (range)	46.0 ± 9.4 (range, 24-69)	46.6 ± 9.8 (range, 21-85)	0.600	47.5 ± 10.0 (range, 22-86)	0.166	0.185
Histopathologic grade						
Low grade	6 (6.7%)	100 (37.3%)		216 (14.6%)		
Intermediate to high grade	84 (93.3%)	168 (62.7%)	< 0.001	1262 (85.4%)	0.036	< 0.001
ER						
Negative	48 (53.3%)	72 (26.9%)		469 (31.2%)		
Positive	42 (46.7%)	196 (73.1%)	< 0.001	1035 (68.8%)	< 0.001	0.157
PR						
Negative	59 (65.6%)	95 (35.4%)		631 (42.0%)		
Positive	31 (34.4%)	173 (64.5%)	< 0.001	873 (58.0%)	< 0.001	0.046
HER-2						
Negative	29 (32.2%)	169 (63.1%)		1074 (71.4%)		
Positive	61 (67.8%)	99 (36.9%)	< 0.001	430 (28.6%)	< 0.001	0.006
Ki-67						
Low expression	30 (33.3%)	135 (50.4%)		319 (21.2%)		
High expression	60 (66.7%)	133 (49.6%)	0.005	1185 (78.8%)	0.007	< 0.001
Molecular subtype						
Luminal A	12 (13.3%)	112 (41.8%)		272 (18.1%)		
Luminal B	31 (34.5%)	88 (32.8%)		777 (51.7%)		
HER-2+	43 (47.8%)	61 (22.8%)		219 (14.5%)		
Triple negative	4 (4.4%)	7 (2.6%)	< 0.001	236 (15.7%)	< 0.001	< 0.001
Lymph node status at time of primary diagnosis						
Metastasis	9 (12.5%)	3 (1.6%)		491 (39.7%)		
No metastasis	63 (87.5%)	182 (98.4%)	< 0.001	745 (60.3%)	< 0.001	< 0.001

p^a comparisons between MIC and DCIS, p^b comparisons between MIC and IBC, p^c comparisons between DCIS and IBC.

ed with MIC, 268 were confirmed to have pure DCIS, and 1504 had IBC. Paget's disease of the nipple was present in 2 (2.2%) patients with MIC, 4 (1.5%) patients with DCIS, and 20 (1.3%) patients with IBC. The mean age of patients with MIC (46.0 ± 9.4 years) was lower than the mean age of patients with DCIS (46.6 ± 9.8) and that of patients with IBC (47.5 ± 10.0 years), but the difference was not significant.

There was no significant difference in the gross appearance among DCIS, MIC and IBC. Some tumors could be visualized as an evident mass with gritty texture. Some tumors could not be detected visually. The tumor edge was usually well-defined in DCIS, while it was usually moderately or poorly defined and lacking sharp circumscription in IBC. MIC had the gross features of DCIS. The histologic features and immunohistochemical staining of ER, PR, HER-2, and Ki-67 are shown in **Figure 1**.

Most MICs (93.3%) exhibited intermediate to high nuclear grade, and this proportion was larger than that of DCIS (62.7%, $P < 0.001$) and IBC (85.4%, $P = 0.036$). IBC tended to have higher nuclear grade than DCIS ($P < 0.001$). SLNB and/or axillary lymph node dissection (ALND) was performed in 72 (80.0%) of the 90 patients with MIC, 185 (69.0%) of the 268 patients with DCIS, and 1236 (84.0%) of the 1504 patients with IBC. Among the 72 patients with MIC, two were sentinel lymph node (SLN)-positive for macrometastasis and ALND-negative, five were SLN-positive for micrometastasis, and two were confirmed to have isolated tumor cells (ITCs). Three of the 185 patients with DCIS were SLN-positive for micrometastasis or had ITCs confirmed by immunohistochemistry. The incidence of lymph node metastasis in MIC was 7.8 times higher than that of DCIS (12.5% versus 1.6%, $P < 0.001$), while it was much lower than that of IBC (12.5% versus 39.7%, $P < 0.001$, **Table 1**).

Status of ER, PR, HER-2, and Ki-67 in MIC, DCIS, and IBC

Of the MICs, 46.7% (42/90) were ER-positive. This proportion was significantly lower than that of DCIS (73.1%, $P < 0.001$) and IBC (68.8%, $P < 0.001$); however, there was no significant difference between the latter two groups ($P = 0.157$). Similarly, 34.4% (31/90) of MICs were PR-positive, and this proportion was significantly lower compared to that of DCIS (64.5%, $P < 0.001$) and IBC (58.0%, $P < 0.001$). HER-2 overexpression or gene amplification was demonstrated in 67.8% of MIC. This proportion was significantly higher than that of DCIS (36.9%, $P < 0.001$) and IBC (28.6%, $P < 0.001$). Compared to IBC, DCIS demonstrated increased expression of PR ($P = 0.046$) and HER-2 ($P = 0.006$). A high expression of Ki-67 was seen in 66.7% of MIC tumors, which was higher than that of DCIS tumors (49.6%, $P = 0.005$) and lower than that of IBC tumors (78.8%, $P = 0.007$). All tumors qualified for molecular subtyping according to immunohistochemical features [18, 19]. The HER-2+ subtype was more often present in MIC, while patients with DCIS were more likely to have the Luminal A subtype and patients with IBC were more likely to have Luminal B and triple-negative subtype tumors (all $P < 0.001$).

Most MICs presented with intermediate to high nuclear grade (84/90, 93.3%). Thus, a nuclear grade-matched analysis was performed in patients with MIC, DCIS, and IBC.

In the patients with intermediate to high nuclear grade, ER negative, PR negative, and HER-2 positive status was more frequent in MIC tumors than in DCIS and IBC tumors (all $P < 0.05$). In addition, the HER-2+ subtype was more often present in MIC than in DCIS ($P = 0.010$) and IBC ($P < 0.001$). The proliferation index of Ki-67 was lower in MIC than that in IBC ($P = 0.001$). No difference in Ki-67 expression was observed between MIC and DCIS ($P = 0.632$). Compared to IBC, DCIS exhibited greater HER-2 overexpression or gene amplification ($P < 0.001$) and a lower Ki-67 proliferation index ($P < 0.001$). Expression of ER and PR in DCIS were not significantly different from those of IBC ($P > 0.05$, **Table 2**).

Discussion

Microinvasive carcinoma (MIC) appears to be a rare lesion identified in 10-20% of patients with DCIS, and is significantly less common than IBC. Several definitions have been used for MIC for many years. It is not surprising the data on this uncommon entity are limited and with discordant results. Although several researchers have reported on the histopathologic findings and prognosis of MIC [12, 20, 21], the biologic behavior of this cancer subtype is not

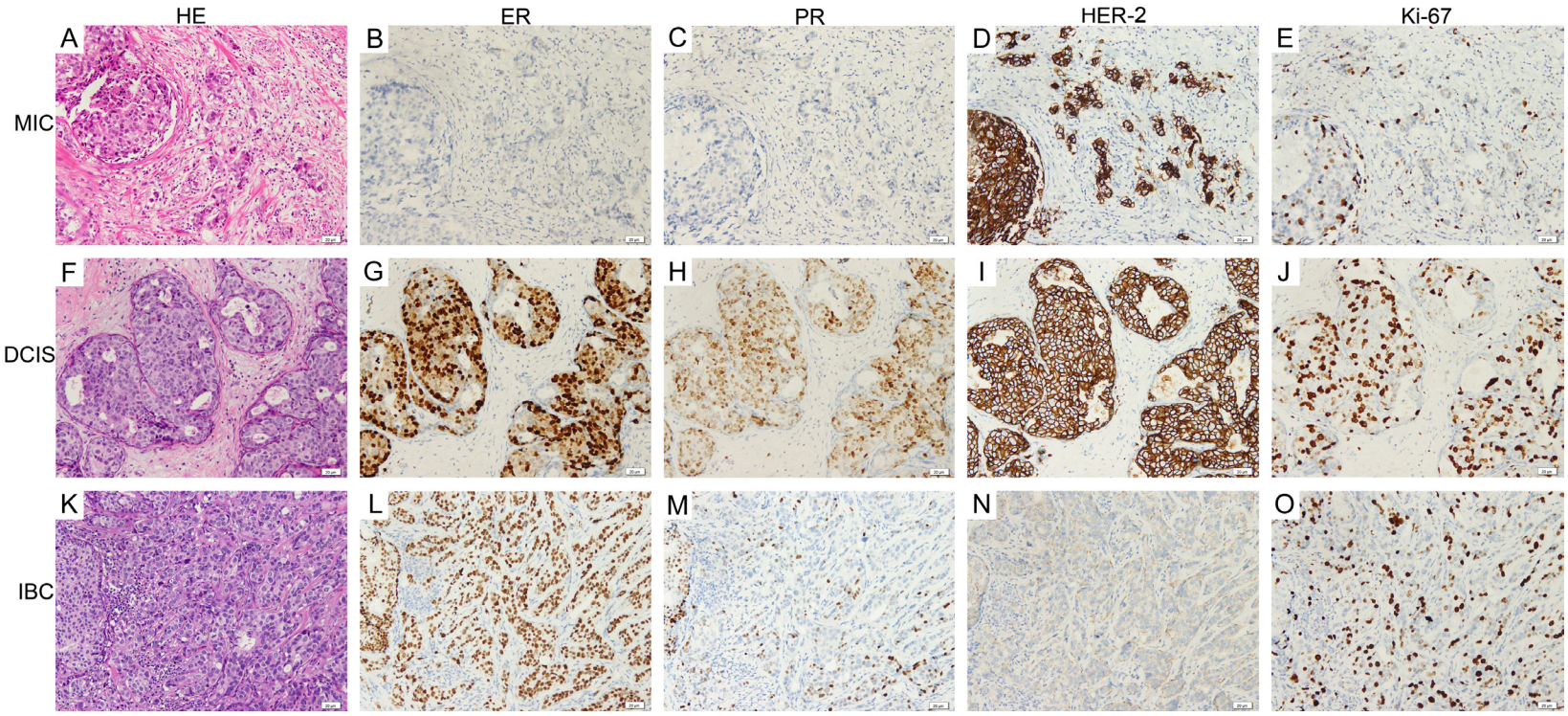

Figure 1. Hematoxylin-eosin and immunohistochemical staining of tissues, ×200. (A-E) Hematoxylin-eosin staining (A) and Immunohistochemical staining of ER (-), PR (-), HER-2 (3+), and Ki-67 (25%+) in MIC (B-E); (F-J) Hematoxylin-eosin staining (F) and Immunohistochemical staining of ER (85%+), PR (85%+), HER-2 (3+), and Ki-67 (70%+) in DCIS (G-J); (K-O) Hematoxylin-eosin staining (K) and Immunohistochemical staining of ER (95%+), PR (30%+), HER-2 (2+), and Ki-67 (60%+) in MIC (L-O).

Table 2. Expression of ER, PR, HER-2 and Ki-67 in intermediate to high grade MIC, DCIS, and IBC

Clinicopathologic finding	MIC (n = 84)	DCIS (n = 168)		IBC (n = 1262)		DCIS vs. IBC
	n (%)	n (%)	P^a Value	n (%)	P^b Value	P^c Value
Mean age, years (range)	45.9 ± 9.5 (range, 24-69)	46.1 ± 8.5 (range, 25-85)	0.825	47.5 ± 10.1 (range, 22-86)	0.150	0.057
ER						
Negative	48 (57.1%)	68 (40.5%)		441 (34.9%)		
Positive	36 (42.9%)	100 (59.5%)	0.012	821 (65.1%)	< 0.001	0.159
PR						
Negative	59 (70.2%)	83 (49.4%)		576 (45.6%)		
Positive	25 (29.8%)	85 (50.6%)	0.002	686 (54.4%)	< 0.001	0.358
HER-2						
Negative	23 (27.4%)	79 (47.0%)		868 (68.8%)		
Positive	61 (72.6%)	89 (53.0%)	0.003	394 (31.2%)	< 0.001	< 0.001
Ki-67						
Low expression	25 (29.8%)	55 (32.7%)		201 (15.9%)		
High expression	59 (70.2%)	113 (67.3%)	0.632	1061 (84.1%)	0.001	< 0.001
Molecular subtype						
Luminal A	7 (8.3%)	40 (23.8%)		162 (12.8%)		
Luminal B	30 (35.7%)	64 (38.1%)		672 (53.2%)		
HER-2+	43 (51.2%)	58 (34.5%)		204 (16.2%)		
Triple negative	4 (4.8%)	6 (3.6%)	0.010	224 (17.8%)	< 0.001	< 0.001
Lymph node status at the time of primary diagnosis						
Metastasis	9 (12.9%)	3 (2.4%)		433 (41.7%)		
No metastasis	61 (87.1%)	124 (97.6%)	0.003	606 (58.3%)	< 0.001	< 0.001

p^a comparisons between MIC and DCIS, p^b comparisons between MIC and IBC, p^c comparisons between DCIS and IBC.

well understood. MICs have a wide variety of pathologic features. Some previously reported data describing the clinicopathologic features and lymph node metastasis capacity of MIC suggest that the natural progression of MIC is similar to that of DCIS [21, 22]. Patients with MIC are generally considered to have a worse prognosis than those with pure DCIS, but better than those with IBC [12]. Thus, MIC has been considered an intermediate stage in the progression from DCIS to IBC. Recently, several researchers have suggested that MIC may be a distinct entity from pure DCIS [23, 24]. Here, we compared the pathologic features and molecular phenotypes of MIC to DCIS and IBC. We found that patients with MIC had higher-grade tumors than patients with DCIS or IBC. Similar results were reported in a recent SEER database review of 134,569 women, which also suggested that patients with MIC had higher-grade tumors than patients with invasive disease [4].

It is well-known that the status of axillary lymph nodes (ALN) remains the most important prognostic factor in breast carcinoma. Therefore, pathologic evaluation of ALNs may affect the choice of subsequent systemic therapy suggested for each patient. The advent of SLNB and its minimally invasive procedure prompted interest in its use for patients with DCIS and MIC. NCCN guidelines recommend SLNB for patients with DCIS undergoing mastectomy. In the setting of breast conserving surgery (BCS) for DCIS patients, SLNB is performed on those with invasive lesions. Unlike with DCIS, SLNB for MIC is more controversial and the prevalence of metastases in MIC patients varies greatly. Previously published findings have demonstrated that SLN metastases are present in 0-20% of patients with MIC [25-29]. The reasons for this variation in the positive SLN rate may include the varying definition of MIC and/or selection bias in the choice of patients receiving SLNB. At our institution, SLNBs were performed in patients who received preoperative core needle biopsy (CNB) or had an intraoperative pathologic diagnosis of DCIS, patients undergoing mastectomy, and all patients with IBC. IHC staining was performed in SLNBs initially read as negative by H&E staining. We found that the prevalence of metastases in the SLN was very low in the patients with DCIS, and these metastases were micrometastases or isolated tumor cells (ITCs) confirmed by immunohistochemistry. However, SLN metastases were identified in 12.5% of the patients with MIC. This proportion was higher than that of DCIS patients, but much lower than that of IBC patients. The probability of SLN involvement in MIC was similar to that reported recently by other authors [27, 29] and fell within the range described in the literature [7].

Breast carcinoma is a heterogeneous disease with variation in pathologic features and immunotypes. ER, PR, HER-2, and Ki-67 are the oldest biomarkers of breast cancer and were the first ones recommended for routine clinical use to predict prognosis. In our previous study, the results indicated that MIC was different from pure DCIS based on clinicopathologic characteristics and molecular alterations. Compared to DCIS, MIC had a higher nuclear grade, higher expression of HER-2, and lower expression of hormone receptor (HR) [14]. In theory, if MIC is an interim stage in the progression from DCIS to IBC, the expression alterations of these routine biomarkers in this group of tumors would be expected to be intermediate between DCIS and IBC, or at least to be similar to either of the two lesions. However, this was not the case in our present study. We analyzed the molecular phenotypes in a large series of patients with MIC, pure DCIS, and IBC. The results indicated that the molecular phenotype of MIC was significantly different from that of DCIS or IBC. MIC exhibited greater HER-2 overexpression or gene amplification and lower expression of ER and PR compared to that in DCIS. Similarly, compared to IBC, MIC was more commonly HER-2 positive (67.8% vs. 28.6%, $P < 0.001$), ER negative (53.3% vs. 31.2%, $P < 0.001$) and PR negative (65.6% vs. 42.0%, $P < 0.001$). In addition, MIC was more likely to have HER-2+ subtype, while patients with DCIS were more likely to have the Luminal A, and patients with IBC were more likely to have the Luminal B and triple-negative subtypes. Similar results were found in a recent large SEER database analysis by Champion [4] and Wang [30]. Furthermore, the results of a larger single-institution retrospective review of 563 patients also suggested that the ER negative-HER-2 positive subtype was found more frequently in MIC than in DCIS and small invasive breast cancers (T1a) [31]. MIC is most commonly seen in the background of high-grade DCIS, and HER-2 positivity is more

common in DCIS with a higher grade than in that with a lower grade. Therefore, we analyzed the difference in molecular phenotypes in a cohort of patients classified with intermediate to high grade tumors. These results also suggested that the HR-negative and HER-2 positive subtype was more often present in MIC than in DCIS and IBC. Interestingly, HER-2 expression was found in a greater proportion of DCIS tumors than in IBC tumors ($P < 0.001$). This is consistent with the results of previous studies. The rate of HER-2 overexpression in DCIS (range, 20%-55%) is higher than that of IBC (range, 12%-30%) [32, 33]. It was postulated that the expression of HER-2 might decrease or be lost as DCIS progresses to invasive disease, or that most IBCs might develop from DCIS tumors with low HER-2 expression [33, 34]. In the present study, the rate of HER-2-positive, ER-negative, and PR-negative tumors in the MIC group was much higher than that of DCIS and IBC groups. This result does not support the premise that MIC is an intermediate stage in the progression from DCIS to IBC, suggesting that MIC is a distinct entity of breast cancer. HER-2 is a known common oncogene and its overexpression is an unfavorable prognostic factor in IBC. The high incidence of HER-2 overexpression in MIC implies that this oncogene may play an important role in MIC. Whether or not this uncommon subset exhibits aggressive biologic behavior needs to be investigated with additional molecular studies.

In conclusion, this study was a single-center retrospective investigation in a large cohort of patients. We analyzed the clinicopathologic features and biomarker alteration in patients with MIC and compared them to the characteristics of patients with DCIS and IBC. The results demonstrated that MIC displayed a higher nuclear grade, a higher expression of HER-2, and a lower expression of HR compared to DCIS and IBC, and MIC tumors were more likely to be of HER-2+ subtype. The proportion of SLN metastases and the proliferation index in MIC were higher than that of DCIS, but much lower than that of IBC. These findings indicate that MIC might be a different entity rather than a transition stage in the progression from DCIS to IBC. Therefore, it is critical to learn more about MIC and its clinical diagnosis. Further study of the prognosis and biologic behavior of this uncommon subtype of breast cancer may help clinicians to optimize management strategy.

Disclosure of conflict of interest

None.

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