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More microinvasive foci in larger tumours of breast ductal carcinoma *in situ*

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ABSTRACT

Introduction: Microinvasion (Mi) is often thought to be an interim stage between ductal carcinoma *in situ* (DCIS) and invasive ductal carcinoma. This study aimed to investigate the potential influence of Mi on survival and assess its correlations with clinicopathological parameters, prognosis and molecular markers.

Methods: The number of Mi foci in a cohort of 66 DCIS-Mi cases was assessed from haematoxylin and eosin–stained sections. Disease-free survival, clinicopathological parameters and biomarker expression were correlated with the number of Mi foci.

Results: Higher numbers of Mi foci were found in larger tumours ($p = 0.031$).

Conclusion: Greater extent of DCIS is associated with multifocal Mi.

Keywords: DCIS, ipsilateral invasive recurrence, microinvasion, microinvasive foci, progression

INTRODUCTION

There has been a global increase in the detection of breast malignancies, with about 20%–25% identified as breast ductal carcinoma *in situ* (DCIS)[1] after the introduction of mammographic screening. DCIS is often referred to as a preinvasive, pathologically and biologically heterogenous malignant tumour.[2,3] In DCIS, neoplastic epithelial cells are enclosed within the breast ducts and lobules, without breaching the basement membrane.[4]

The World Health Organization[2] and the American Joint Committee on Cancer[5] define the presence of microinvasion (Mi), which measures up to 1 mm in size and often observed with DCIS, as microinvasive carcinoma or 'T1mi' in the Tumour, Nodes, Metastasis (TNM) staging system. About 1% of all breast cancers are microinvasive[5] and up to 5%–10% of DCIS cases are associated with Mi.[6] Most DCIS cases with Mi are usually associated with higher nuclear grade, comedo-like morphology and larger tumour extent.[6,7]

Microinvasive carcinoma is rarely observed without adjacent DCIS.[7] Despite DCIS being a nonobligate precursor of invasive ductal carcinoma (IDC), DCIS-Mi is often regarded as a transitional step from DCIS to IDC.[8]

Although there is a significant body of research on DCIS-Mi, current histopathological and clinical findings are still inadequate to understand the biological behaviour and patient survival outcomes.[8-13] This study aimed to assess the potential correlation of Mi foci with molecular markers, clinicopathological parameters, prognosis and patient survival.

METHODS

A total of 66 DCIS-Mi cases, consisting of 27 cases with unifocal Mi and 39 cases with multifocal Mi, were diagnosed at the Department of Anatomical Pathology, Singapore General Hospital, Singapore, from 1994 to 2010. Clinicopathological parameters, such as ethnicity, age,

Mi, nuclear grade, calcification, necrosis and tumour extent, were recorded. This study was approved by the Institutional Review Board (CIRB Ref No. 2016/2393).

Tissue sections of 4 µm thickness were cut from formalin-fixed paraffin-embedded blocks with Leica RM2125 Compact microtome (Leica Biosystems Inc, Wetzlar, Germany) and adhered onto microscope slides (Thermo Fisher Scientific Inc, Waltham, MA, USA). The slides were heated at 80°C for three minutes before haematoxylin and eosin (H&E) staining with Autostainer XL (Leica Biosystems Inc). The slides were subsequently scanned in IntelliSite Ultra Fast Scanner 1.6 (Koninklijke Philips NV, Amsterdam, Netherlands).

Table I shows the details of antibodies, antigen retrieval methods and cut-off values for immunoreactivity. Immunostaining for oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) was performed on the Dako Autostainer Plus (Dako, Santa Clara, CA, USA). DCIS sections of 4-µm thickness were first deparaffinised and subjected to antigen retrieval before being quenched with hydrogen peroxide. The samples were then incubated with their respective primary antibodies, followed by secondary antibodies conjugated to horseradish peroxidase. Bound antibodies were detected using the Dako REAL™ EnVision™ Detection System (Dako). Haematoxylin was used as a counterstain. Subsequently, the samples were mounted with DPX mountant (CellPath Ltd, Wales, UK) and covered with glass coverslips after dehydration. Appropriate controls were used for every batch of slides. According to the American Society of Clinical Oncology/College of American Pathologists clinical practice guidelines, a sample is considered ER or PR positive if ≥ 1% of the tumour cell nuclei are immunoreactive,[14] and HER2 positive if > 10% of the tumour cell membranes are strongly immunoreactive with an immunohistochemistry result of 3+.[15]

Table I. Details of antibodies.

Antibody	Clone	Source	Dilution	Antigen retrieval	Cellular localisation	Cut-off value
ER	SP1	Lab Vision RM-9101-R7	1:50	0.01 M Tris–EDTA pH 9.0, 98°C, 15 min	Nucleus	1%
PR	SP2	Lab Vision RM-9102-S	1:200	0.01 M Tris–EDTA pH 9.0, 98°C, 15 min	Nucleus	1%
HER2	SP3	Lab Vision RM-9103-R7	1:200	0.01 M Tris–EDTA pH 9.0, 98°C, 15 min	Cytoplasmic membrane	3+ >10%

Follow-up data was obtained from case records. The mean and median follow-up periods were 7.6 years and 7.2 years, respectively, with a maximum of 18.8 years. Disease-free survival was defined as time from the date of diagnosis to the date of ipsilateral invasive recurrence or last follow-up.

Findings were analysed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp, Armonk, NY, USA). The expression of molecular markers and their relationship with clinicopathological parameters were analysed using chi-square, Fisher's exact and Mann-Whitney U-tests. Survival outcome was estimated using the Kaplan-Meier method and compared between groups using the log-rank test. A p-value < 0.05 was considered to be statistically significant.

RESULTS

The clinicopathological characteristics of 66 patients harbouring DCIS with 1 Mi focus and > 1 Mi foci are shown in Table II. The cohort consisted of women with a median age of 51 (range 38–90) years and median tumour extent of 27 (range 2–100) mm. The tumour extent was arbitrarily stratified into two different size categories, following the T1 and T2 stages for IDC in the TNM staging system.[5] We acknowledge that for DCIS, there is no difference in staging

with respect to size, with all cases of DCIS staged as pTis regardless of the tumour extent. 27 and 39 cases were diagnosed as DCIS with 1 Mi focus and > 1 Mi foci, respectively, by histopathological assessment. Fig. 1 shows a histological illustration of DCIS-Mi stained with H&E. More Mi foci were significantly associated with larger tumours (p = 0.031). However, no significant associations were found between the number of Mi foci and the nuclear grade (p = 0.253), presence of necrosis (p = 0.226) and DCIS morphology (p = 0.313) (Table II). There was also no significant association between the number of Mi foci in DCIS and biomarker expression (Table II). On the basis of Kaplan-Meier analyses, no significant associations were found between the number of Mi foci and ipsilateral invasive (p = 0.119) or ipsilateral *in situ* (p = 0.686) recurrences.

Table II. Association between number of microinvasion (Mi) foci in ductal carcinoma *in situ* (DCIS) and clinicopathological parameters/biomarkers.

Clinicopathological parameter	No. (%)		p-value
	DCIS with 1 Mi focus (n = 27)	DCIS with > 1 Mi foci (n = 39)	
Age (yr)			0.782
< 50	12 (42.9)	16 (57.1)	
≥ 50	15 (39.5)	23 (60.5)	
Ethnicity			0.893
Chinese	22 (41.5)	31 (58.5)	
Malay	1 (50.0)	1 (50.0)	
Indian	2 (28.6)	5 (71.4)	
Others	2 (50.0)	2 (50.0)	
Laterality			0.131
Left	9 (31.0)	20 (69.0)	
Right	17 (47.2.0)	19 (52.8)	
Bilateral	1 (100.0)	0 (0.0)	
DCIS extent (mm)			0.031*
≤ 20	15 (57.7)	11 (42.3)	
> 20	12 (30.8)	27 (69.2)	
Nuclear grade			0.253
Low	0 (0.0)	3 (100.0)	
Intermediate	9 (52.9)	8 (47.1)	
High	18 (39.1)	28 (60.9)	
Necrosis			0.226
Absent	1 (14.3)	6 (85.7)	
Present	26 (44.1)	33 (55.9)	
Calcification			0.392

Absent	7 (33.3)	14 (66.7)	
Present	20 (44.4)	25 (55.6)	
DCIS morphology			0.313
Comedo	7 (43.8)	9 (56.3)	
Cribriform	3 (60.0)	2 (40.0)	
Papillary	1 (50.0)	1 (50.0)	
Solid	2 (100.0)	0 (0.0)	
Mixed	14 (34.1)	27 (65.9)	
Margin status† (mm)			0.371
Negative (≥ 2)	19 (37.3)	32 (62.7)	
Positive (< 2)	8 (53.3)	7 (46.7)	
Ipsilateral invasive recurrence			0.164
No	25 (39.1)	39 (60.9)	
Yes	2 (100.0)	0 (0.0)	
ER			0.911
Negative	12 (41.4)	17 (58.6)	
Positive	14 (40.0)	21 (60.0)	
PR			0.611
Negative	12 (37.5)	20 (62.5)	
Positive	14 (43.8)	18 (56.2)	
HER2			0.418
Negative	17 (44.7)	21 (55.3)	
Positive	9 (34.6)	17 (65.4)	
Triple negativity			0.705
No	22 (39.3)	34 (60.7)	
Yes	4 (50.0)	4 (50.0)	

**Significant at p < 0.05. †Edge of tissue resected during surgery; negative margin defined as ≥ 2 mm and positive margin defined as < 2 mm from DCIS.*

DISCUSSION

DCIS is frequently defined as noninvasive or preinvasive mammary carcinoma.[4] Although patients who experience a recurrent invasive episode have an elevated risk for breast cancer-related mortality, those diagnosed with DCIS tend to have good prognoses. Despite nuclear grading not being a perfect predictor of invasion, the assessment is still generally accepted and performed to determine the likelihood of progression.[16] Currently, there is a lack of consensus regarding parameters that can stratify DCIS in terms of progression to IDC. DCIS does not invariably progress to IDC, with DCIS-Mi regarded as an interim stage.[8] However, elements of this progression are still uncertain. Cancer staging systems recognise Mi,[5] suggesting potential clinical implications in its definition from pure DCIS. This study explored the

possible influence of Mi on survival and attempted to assess any relationship with clinicopathological parameters, prognosis and molecular markers.

Mi is more frequently observed in DCIS with high nuclear grade,[17] necrosis, and comedo-like architecture.[6,7] However, similar to the study by Kim et al,[18] we did not find any statistical significance in the association between the number of Mi foci and the mentioned clinicopathological parameters, such as nuclear grade, presence of necrosis and DCIS morphology.

On the basis of the traditional linear progression of breast cancer and various observational studies on tumour size and metastatic dissemination,[19,20] metastatic capacity may be estimated based on primary tumour size. Accordingly, DCIS-Mi has a higher probability of progression compared to pure DCIS. Although the number of Mi foci was significantly associated with the tumour extent of DCIS in this study, no differential effect on survival was observed.

In conclusion, although the cohort size was small, the results from this study showed that the tumour extent of DCIS is a predictor of the number of Mi foci in DCIS. Further characterisation studies on a larger cohort have to be performed to elucidate the biological role and implication of DCIS-Mi as an interim stage between DCIS and IDC, and its potential as a predictor of the progression of DCIS to established invasive disease.

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FIGURE

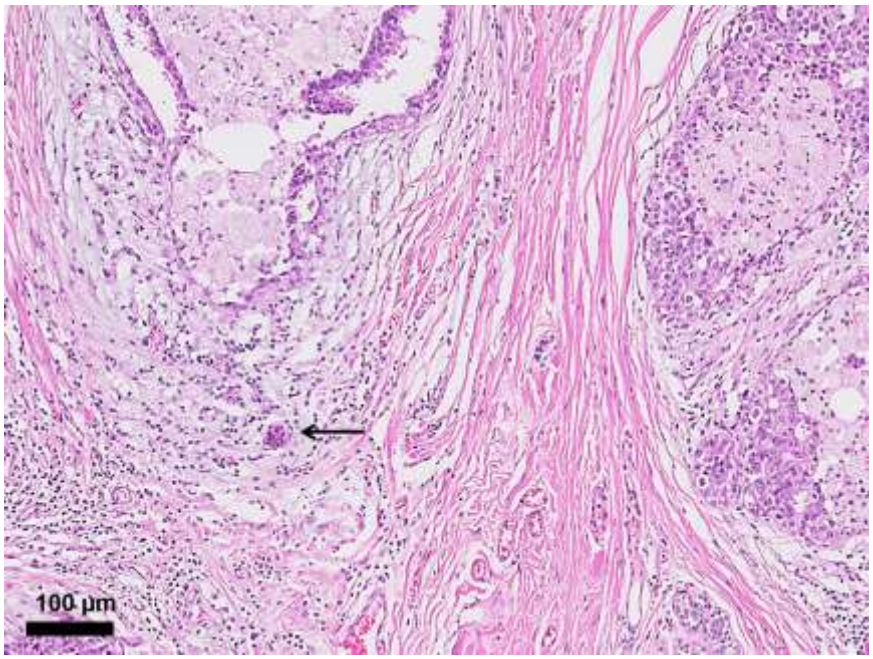

Fig. 1 Photomicrograph shows a microinvasive focus (arrow) comprising a tiny cluster of tumour cells within oedematous inflamed stroma (H&E, 100×). Adjacent intermediate nuclear grade ductal carcinoma *in situ* is present.

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