J Tokyo Wom Med Univ
84 (Extra 3) E417～E423 (2014)

Original

Assessment of Epithelial Mesenchymal Transition in Non-mass Image-forming Breast Cancer

Hiroko MIURA, Takako KAMIO and Shingo KAMEOKA

Department of Surgery II, Tokyo Women's Medical University

(Accepted September 16, 2014)

Purpose: Many breast cancers that are found in calcified lesions without mass and diagnosed by biopsy are ductal carcinoma in situ (DCIS). By contrast, some patients are diagnosed postoperatively found to have invasive lesion in the specimens. Epithelial-mesenchymal transition (EMT) acquisition has been implicated in the process of progression from DCIS to invasive ductal carcinoma (IDC); however, a full assessment has not been described. In this study, we compared the differences in marker staining between specimens with and without invasive lesions. **Materials and Methods:** The subjects were 43 patients with primary breast cancer who were diagnosed with DCIS and underwent surgery. Immunostaining was performed using E-cadherin (E-cad) as an epithelial marker and vimentin (Vim) as a mesenchymal marker. Twenty-four patients were assigned to the pure DCIS group and 19 patients were assigned to the DCIS with invasion component group. The latter group was divided into two subgroups, the invasive and non-invasive lesion subgroups. **Results:** E-cad was underexpressed ($p < 0.001$) and Vim was overexpressed ($p = 0.001$) in the invasive lesion subgroup compared with the pure DCIS group. Vim expression was showing a slightly higher tendency ($p = 0.079$) in the non-invasive lesion subgroup compared with the pure DCIS group. Although E-cad was underexpressed in the invasive lesion subgroups ($p = 0.001$), Vim expression was showing a slightly higher tendency ($p = 0.151$) compared with the non-invasive lesion subgroup. **Conclusion:** In this study, it was suggested that EMT was acquired in the invasive lesion subgroup. E-cad expression was the lowest in the invasive lesion group, followed by the non-invasive lesion subgroup and the pure-DCIS group. Vim expression was the highest in the invasive lesion subgroup. The possibility was suggested that specimens which were diagnosed as DCIS include invasive component when lesions were determined as being Vim-positive by immunostaining.

Key Words: ductal carcinoma in situ, invasive ductal carcinoma, epitherial-mesenchymal transition, E-cadherin, vimentin

Introduction

Patients with breast cancer in whom calcified lesions without mass are detected by mammography undergo stereotactic-guided vacuum-assisted core biopsy (S-VAB) for pathologic diagnosis. Patients who are pathologically diagnosed with ductal carcinoma in situ (DCIS) are typically less malignant and slowly progressive; therefore, they are usually completed with topical treatment. By contrast, some patients who have been diagnosed preoperatively with DCIS have been found to have more malignant lesion postoperatively following the detection of invasive lesions in the surgical specimens, and have required systemic treatment including adjuvant therapy. However, to date there is no established method to discriminate between them prior to surgery. Patients are currently determined by postoperative pathology. Therefore, it is expected that it would be useful to establish a procedure to detect DCIS with invasive lesions prior to surgery.

Previous studies have shown that epithelial-mesenchymal transition (EMT) is involved in the invasive and metastatic potential of cancer cells to the surrounding organs. EMT is a phenomenon pro-

posed by Hay et al in early 1980s by which epithelial cells can manifest morphological changes similar to mesenchymal cells[1)~4)].

EMT is a process by which epithelial cells lose their cell polarity and cell-cell adhesion, and gain migratory and invasive properties to become mesenchymal stem cells. EMT is divided into groups that include developmental processes, i.e., embryonic development, wound healing with fibrosis, and cancer invasion and metastasis[4)].

Cancer cells acquire mesenchymal cell phenotypes and promote invasion and metastasis[5)6)]. In breast cancer, similarly to other cancers, EMT acquisition is implicated in progression from DCIS to invasive ductal carcinoma (IDC). EMT acquisition in previous studies has been evaluated by immunostaining using E-cadherin (E-cad) as an epithelial marker for EMT acquisition, and vimentin (Vim), N-cadherin, smooth muscle actin and ostonectin as mesenchymal markers[7)]. Epithelial markers Snail, SIP1 and Twist, which are E-cad transcription factors, are also overexpressed in EMT acquisition[8)~10)].

In this study, we performed immunostaining using E-cad and Vim, as epithelial and mesenchymal markers, respectively, in postoperative specimens from patients who were diagnosed with DCIS by S-VAB. We compared the differences in marker staining in the postoperative specimens, as indicators of EMT acquisition, between the group with and without invasive lesions.

Materials and Methods

The study was approved by the institutional review board (IRB) of Tokyo Women's Medical University Hospital. Of patients in whom calcified lesions without mass were detected by mammography, 318 underwent S-VAB in the Tokyo Women's Medical University Hospital from January 2010 to December 2013. Sixty-seven patients were diagnosed with DCIS and 43 of them underwent surgery in our hospital. The surgical cases included 19 DCIS with an invasive component (DCIS invasive group) and 24 cases of pure DCIS. The characteristics of the cases are summarized in Table 1. Surgical procedures of mastectomy and partial mastectomy were performed in 12 patients each in the pure DCIS group, whereas mastectomy was performed in 14 patients and partial mastectomy in 5 patients in the DCIS invasive group. Specimens in partial mastectomy were collected by holoblastic cleavage of 5 mm of tissue sliced vertically from the line connecting the tumor and nipple.

Clinicopathologic information was obtained by reviewing medical records and from Hematoxylin and Eosin-stained (H&E-stained) sections evaluated by pathologists for the presence of invasive lesions and for selection of specimens appropriate for immunostaining. The following histopathologic variables in the pure DCIS and DCIS invasive groups were analyzed: tumor size of invasion, expanse of DCIS component, DCIS grade, histopathologic invasion, lymphovascular invasion, histological characteristics of invasion component and biomarkers. Hormone receptors were determined to be positive if the rate of positive cells was at least 10% in the invasive component using J-score. HER2 (human epidermal growth factor receptor 2) was defined as positive (2+) based on HER2/neu protein overexpression in FISH (fluorescence in situ hybridization) and positive (3+) if the rate of positive cells was at least 30% in the invasive component using Immunohistochemistry.

E-cad and Vim staining was evaluated in samples from the pure DCIS group and in non-invasive and invasive lesions in samples from the DCIS invasive group. Immunostaining of formalin-fixed and paraffin-embedded surgical specimens was performed with anti-E-cadherin antibody (clone 36, mouse, monoclonal; Ventana, Tucson, AZ, USA) and anti-vimentin antibody (V9, mouse, monoclonal; Ventana) using an automated immunostaining apparatus (Ventana NX20 Roche Diagnostics K.K.).

No definitive description of E-cad downregulation was found in previous studies. In the pure DCIS group and in non-invasive lesions in the DCIS invasive group, E-cad expression is almost always positive. Therefore in this study, E-cad downregulation was defined as $<90\%$ positive cells based on visual evaluation. Vim expression $\geq 10\%$ was defined as positive using visual evaluation, based on a previous

Table 1 Characteristics of specimens

	The pure DCIS group (number of cases)	The DCIS with invasion component group (number of cases)
Cases	24	19
Age (years)	42 to 82	41 to 74
Mean	62	57.5
Tumor size of invasion (mm)		
T1mic (≤1 mm)	0	6
T1a (≤5 mm)	0	5
T1b (5<T≤10 mm)	0	6
T1c (10<T≤20 mm)	0	2
An expanse of DCIS component (mm)	2 to 80	3 to 75
Mean	41	39
DCIS grade		
Low grade	12	8
Intermediate grade	5	4
High grade	7	7
Histopathologic invasion		
g	24	8
f	0	11
Lymphovascular invasion		
ly (−)	24	15
ly (+)	0	4
v (−)	24	19
v (+)	0	0
Histological characteristics of invasion component		
Papillotubular	-	10
Scirrhous	-	6
Solid-tubular	-	1
Special type (Apocrine)	-	1
Special type (Mucinous)	-	1
Biomarkers (ER, PgR, HER2)		
(+), (+), 0/1+	15	11
(+), (−), 0/1+	3	2
(+), (+), 3+	2	2
(−), (−), 3+	2	3
(−), (−), 0/1+	2	1

DCIS: ductal carcinoma in situ, g: gland, f: fat, ly: lymphatic invasion, v: vessel invasion, ER: estrogen receptor, PgR: progesterone receptor, HER2: human epidermal growth factor receptor 2.

report[5] (Fig. 1).

EMT marker expression was compared by chi-square test and Fisher exact test among three groups (pure DCIS, non-invasive lesions, and invasive lesions) using IBM SPSS 22.0. All reported p values are two-sided. $p<0.05$ was considered to be statistically significant.

Results

In the pure DCIS group, E-cad expression was positive in 22 of 24 subjects and no Vim expression was observed. In the invasive lesion group, E-cad was underexpressed in 13 (68.4%) of 19 subjects, while Vim was overexpressed in 8 (42.1%) of 19 subjects. Six of 19 subjects exhibited negative E-cad and positive Vim expression. In the non-invasive lesion group, E-cad was underexpressed in 2 (10.5%) of 19 subjects, while Vim was overexpressed in 3 (15.8%) of 19 subjects (Table 2).

E-cad was significantly underexpressed and Vim was significantly overexpressed in the invasive lesion group compared to the pure DCIS group ($p<0.001$, $p=0.001$). In contrast, the pure DCIS group and non-invasive lesion group showed no significant difference in E-cad expression ($p=0.602$) or Vim expression ($p=0.079$); with no expression of Vim observed in the pure DCIS group and slightly higher

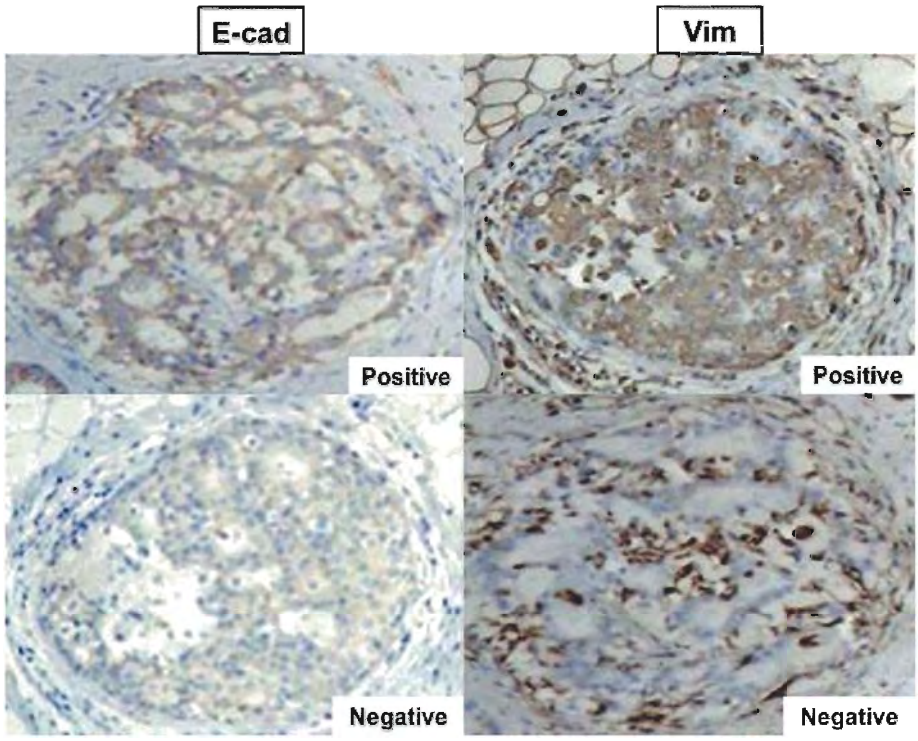

Fig. 1 An appraisal standard of E-cad/Vim
Immunohistochemical staining was performed using anti-E-cad and anti-Vim antibodies in formalin fixed and paraffin-embedded surgical specimens. Specimens that were E-cad positive in ≥90% cells were defined as E-cad positive. Specimens that were Vim positive in ≥10% cells were defined as Vim positive.
E-cad: E-cadherin, Vim: vimentin.

Table 2 The expression of EMT markers between three groups

	Epitherial marker		Mesenchymal marker		Total
	E-cad positive (n)	E-cad negative (n)	Vim positive (n)	Vim negative (n)	
The pure DCIS group	22	2	0	24	24
The non-invasive lesion subgroup	17	2	3	16	19
The invasive lesion subgroup	6	13	8	11	19

EMT: epithelial-mesenchymal transition, DCIS: ductal carcinoma in situ, E-cad: E-cadherin, Vim: vimentin.

expression in the non-invasive lesion group. E-cad was significantly underexpressed in the invasive lesion group compared to the non-invasive lesion group ($p = 0.001$), while Vim expression did not differ significantly between these two groups ($p = 0.151$) (Fig. 2).

Discussion

The results of this study showed that 37.2% of patients with a preoperative diagnosis of DCIS had invasive lesions in postoperative specimens with significant changes in EMT marker staining [11)12)]. Nineteen-30% of patients who were diagnosed as DCIS preoperatively are diagnosed with invasive ductal carcinoma postoperatively.

We examined the presence of EMT acquisition using immunostaining of E-cad, an epithelial marker, and Vim, a mesenchymal marker. E-cad is a typical adhesion molecule in epithelial cells involved in cell-cell adhesion. E-cad expression has an inverse correlation with cancer malignancy and is suppressed by EMT acquisition; therefore, E-cad is a common marker for EMT. Analyses of E-cad transcriptional regulation have shown that E-cad is pathologically positive in epithelial cells and negative

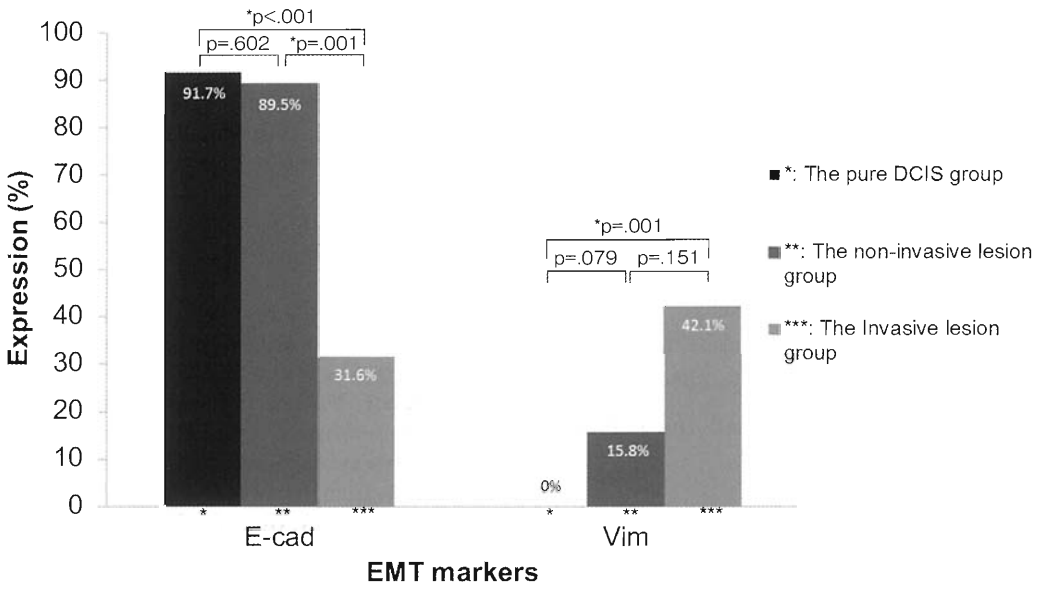

Fig. 2 The expression of EMT markaers between three groups
E-cad was underexpressed ($p<0.001$) and Vim was overexpressed ($p=0.001$) in the invasive lesion subgroup compared with the pure DCIS group. Vim expression was showing a slightly higher tendency ($p=0.079$) in the non-invasive lesion subgroup compared with the pure DCIS group. Although E-cad was underexpressed in the invasive lesion subgroups ($p=0.001$), Vim expression was showing a slightly higher tendency ($p=0.151$) compared with the non-invasive lesion subgroup.

in lobular carcinoma, a type of breast cancer. In this study, patients with lobular carcinoma were excluded[8)~10)]. Vim is a cytoskeletal protein that is distributed in mesenchymal cells. It is a pathological marker that is used for epithelial and nonepithelial discrimination, and is expressed by cancer cells that acquire mesenchymal characteristics from EMT[13)14)].

Choi et al compared EMT acquisition between IDC and DCIS and showed significant underexpression of E-cad and overexpression of Vim, and that EMT acquisition correlated with E-cad and Vim staining in IDC[5)]. A study of staining differences by subtype showed strong EMT acquisition in 'triple negative' breast cancer (negative for hormones and HER2). However, there has been no previous comparison of EMT acquisition in postoperative specimens of patients with early T1 cancer, such as DCIS preoperatively diagnosed by S-VAB, i.e., among the pure DCIS, and invasive and non-invasive lesion groups in this study.

The results of this study also suggested that EMT was acquired in the invasive lesion group because of significant underexpression of E-cad and overexpression of Vim in the invasive lesion group compared with the pure DCIS group. Furthermore, in patients with early cancer, including DCIS that was detected as calcified lesions without mass, as with the subjects of this study, E-cad and Vim expression in the invasive lesions differed from the non-invasive lesions, similar to IDC. Therefore, it is useful to evaluate E-cad and Vim staining for determining surgical procedures and adjuvant therapy.

E-cad expression was lowest in the invasive lesion group, followed by the non-invasive lesion group, and the pure DCIS group, respectively. Vim expression was not observed in the pure DCIS group, but was overexpressed to a greater degree in the invasive lesion group than in the non-invasive lesion group. In the non-invasive lesion group, the characteristics of H&E-stained sections were similar to those in the pure DCIS group. However, Vim was expressed similarly to that in the invasive lesion group; consequently, immunostaining showed EMT acquisition prior to characteristic changes in H&E-stained sections. These results suggest that lesions diagnosed as DCIS by S-VAB include invasive

lesions if EMT acquisition was determined by immunostaining. In future cases, examination of the presence or absence of EMT acquisition may provide useful information for selection of surgical procedures, including performance of sentinel lymph node biopsy (SLNB).

In this study, we investigated the possibility of discriminating between subjects with EMT acquisition and partial invasive lesions, despite the preoperative diagnosis of DCIS, based upon differences in E-cad and Vim staining, and showed the staining tendency of the three groups.

Conclusion

E-cad and Vim staining has potential in evaluation of the invasive potential of lesions prior to surgery, in addition to H & E-stained sections. This could provide very useful clinical information in deciding surgical procedures and adjuvant therapy.

Acknowledgement

Deepest appreciation is expressed to Dr. Toshiro Nishikawa for providing pathological slides of specimens in this study.

The authors declare that they have no conflict of interest.

References

1) **Greenburg G, Hay ED**: Epithelia suspended in collagen gels can lose polarity and express characteristics of migrating mesenchymal cells. J Cell Biol **95** (1): 333–339, 1982
2) **Hay ED**: An overview of epithelio-mesenchymal transformation. Acta Anat (Basel) **154** (1): 8–20, 1995
3) **Hay ED**: The mesenchymal cell, its role in the embryo, and the remarkable signaling mechanisms that create it. Dev Dyn **233** (3): 706–720, 2005
4) **Kalluri R, Weinberg RA**: The basics of epithelial-mesenchymal transition. J Clin Invest **119** (6): 1420–1428, 2009
5) **Thiery JP**: Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer **2** (6): 442–454, 2002
6) **Thiery JP, Acloque H, Huang RY et al**: Epithelial-mesenchymal transitions in development and disease. Cell **139** (5): 871–890, 2009
7) **Choi Y, Lee HJ, Jang MH et al**: Epithelial-mesenchymal transition increases during the progression of in situ to invasive basal-like breast cancer. Hum Pathol **44** (11): 2581–2589, 2013
8) **Martin TA, Goyal A, Watkins G et al**: Expression of the transcription factors snail, slug, and twist and their clinical significance in human breast cancer. Ann Surg Oncol **12** (6): 488–496, 2005
9) **Nieto MA**: The snail superfamily of zinc-finger transcription factors. Nat Rev Mol Cell Biol **3** (3): 155–166, 2002
10) **Yang J, Mani SA, Donaher JL et al**: Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell **117** (7): 927–939, 2004
11) **Brennan ME, Turner RM, Ciatto S et al**: Ductal carcinoma in situ at core-needle biopsy: meta-analysis of underestimation and predictors of invasive breast cancer. Radiology **260** (1): 119–128, 2011
12) **Roses RE, Arun BK, Lari SA et al**: Ductal carcinoma-in-situ of the breast with subsequent distant metastasis and death. Ann Surg Oncol **18** (10): 2873–2878, 2011
13) **Blick T, Widodo E, Hugo H et al**: Epithelial mesenchymal transition traits in human breast cancer cell lines. Clin Exp Metastasis **25** (6): 629–642, 2008
14) **Micalizzi DS, Farabaugh SM, Ford HL**: Epithelial-mesenchymal transition in cancer: parallels between normal development and tumor progression. J Mammary Gland Biol Neoplasia **15** (2): 117–134, 2010

腫瘤非形成性乳癌における上皮間葉移行に関する検討

東京女子医科大学医学部外科学（第2）講座
三浦（ミウラ）　弘子（ヒロコ）・神尾（カミオ）　孝子（タカコ）・亀岡（カメオカ）　信悟（シンゴ）

〔背景〕乳癌において腫瘤非形成性石灰化病変で発見され，ステレオガイド下マンモトーム生検で診断された症例の多くは非浸潤性乳管癌であるが，術前診断が非浸潤性乳管癌でも術後検体で浸潤巣を含み，術後補助療法を要する悪性度の高い症例もみられる．術前に両者を鑑別する方法は確立されていない．癌細胞が浸潤能や転移能を獲得する際，上皮間葉移行の関与が知られ，乳癌においても非浸潤性乳管癌から浸潤性乳管癌へ進行する過程での関与が示唆されている．我々は，生検で非浸潤性乳管癌と診断された症例の術後検体を対象に，浸潤がみられなかった群と，浸潤がみられた群との間で上皮間葉移行獲得の指標となるマーカーの染色性に相違があるかを検討した．〔対象・方法〕生検で非浸潤性乳管癌と診断され手術を施行した43例の原発性乳癌患者を対象に，上皮系マーカーとしてE-cadherin（E-cad）を，間葉系マーカーとしてVimentin（Vim）を用いて免疫染色を施行した．浸潤がみられなかった群（非浸潤群）と一部に浸潤がみられた群とにわけ，さらに後者を非浸潤部分（浸潤群非浸潤部分）と浸潤部分（浸潤群浸潤部分）にわけて，三群における染色性の相違を検討した．〔結果〕浸潤群浸潤部分では，非浸潤群と比較してE-cadは発現が低下し（$p<0.001$），Vimは高発現していた（$p=0.001$）．浸潤群非浸潤部分では，非浸潤群と比較してVim発現が高い傾向にあった（$p=0.079$）．浸潤群浸潤部分と浸潤群非浸潤部分の比較では，E-cadは浸潤部分で発現が低下し（$p=0.001$），Vimは発現がやや高い傾向であった（$p=0.151$）．〔結論〕浸潤群浸潤部分では，有意にE-cad低発現・Vim高発現が認められたことから，上皮間葉移行を獲得していると考えられた．三群間において，E-cadは非浸潤群，浸潤群非浸潤部分，浸潤群浸潤部分の順に発現が低下する傾向がみられた．Vimは非浸潤群では発現がみられず，前述の順に発現が上昇する傾向がみられた．このことから，生検で非浸潤癌と診断された病変において，免疫染色によりVim陽性の傾向がみられた場合は，その病変が浸潤部分を含んでいる可能性が示唆された．