

## Original Article

# Invasive ductal carcinoma with in situ pattern: how to avoid this diagnostic pitfall?

Narasimhamurthy Mohan<sup>1</sup>, Jennifer O Black<sup>2</sup>, Mary R Schwartz<sup>3</sup>, Qihui Jim Zhai<sup>4</sup>

<sup>1</sup>Department of Pathology, Faculty of Medicine, University of Botswana, Botswana; <sup>2</sup>Department of Pathology and Laboratory Medicine, University of Colorado Anschutz Medical Campus School of Medicine, Anschutz Medical Campus Aurora, Colorado, USA; <sup>3</sup>Department of Pathology and Laboratory Medicine, Baylor College of Medicine, Department of Pathology and Genomic Medicine, Houston Methodist Hospital, Houston, Texas, USA; <sup>4</sup>Department of Pathology and Laboratory Medicine, Mayo Clinic Jacksonville, Florida, USA

Received May 6, 2016; Accepted July 9, 2016; Epub August 15, 2016; Published August 30, 2016

**Abstract:** Although the microscopic features of invasion are usually readily recognized, occasionally invasive ductal carcinoma may mimic the pattern of comedo ductal carcinoma in situ (DCIS) by forming large cellular nests with circumscribed borders, but lacking a definitive myoepithelial cell layer. In these cases, the histologic pattern may appear deceptively noninvasive and the absence of a myoepithelial layer can be easily overlooked. We prospectively examined 10 cases of high grade DCIS. P63, smooth muscle actin, muscle specific actin and calponin immunohistochemical stains were used to identify the presence of myoepithelial cells. In our study, 20% of apparent high grade DCIS cases did not exhibit a myoepithelial layer surrounding large, solid nests with comedo necrosis. Since invasion is defined by the absence of a myoepithelial layer, these results suggest that a DCIS-like pattern may actually represent invasive disease in some cases. Immunohistochemical studies may be essential in making this distinction and in avoiding the potential diagnostic pitfall.

**Keywords:** Invasion, myoepithelial cells, ductal carcinoma in situ, invasive ductal carcinoma, immunohistochemical stain

## Introduction

Management and prognosis of breast carcinoma depend on many well established prognostic factors. Among them, invasion is the single most important prognostic determinant of breast cancer outcomes [1]. Microscopic features of invasion are usually easily recognized but there are reports of axillary lymph node metastasis in high grade ductal carcinoma in situ (DCIS) without recognizable evidence of invasion on light microscopy, suggesting that occult invasion occurs [2, 3]. We observed a case of presumed comedo DCIS that when stained with myoepithelial markers demonstrated absence of a myoepithelial cell layer, consistent with invasive ductal carcinoma. Given this finding, we have examined additional cases of comedo DCIS for the presence of a myoepithelial cell layer to understand the frequency of invasive ductal carcinoma among cases that are morphologically consistent with high grade DCIS.

## Material and methods

### Samples

We prospectively examined 10 cases of high-grade DCIS. Inclusion criteria included radiographic or gross presentation of a breast mass of at least 1.0 cm with microscopic features of confluent high grade DCIS with comedonecrosis, involving more than 15 ducts. Only lumpectomy or mastectomy specimens were included.

### Immunohistochemistry

Sections of the formalin-fixed, paraffin-embedded tissue were assembled and immunoperoxidase stains were applied to deparaffinized and rehydrated sections. Slides were incubated with primary antibodies against p63 (Labvision, AB14A4, 1:100), smooth muscle actin (Biogene, 1A4, 1:5), muscle-specific actin (Dako, HHF35, 1:400) and calponin (Dako, 1:400) as shown in the **Table 1**. A second incubation was per-

## Histopathology of invasive ductal carcinoma

**Table 1.** Immunohistochemical myoepithelial markers panel

Antibody	Myoepithelial cell component identified	Manufacturer	Dilution
SMA	Contractile element	Biogene	1:5
P63	Transcription factor, Nucleus	Labvision	1:100
Muscle specific actin (HHF35)	Contractile element	Dako	1:400
Calponin	Contractile element	Dako	1:400

SMA-Smooth muscle actin.

**Table 2.** Myoepithelial Immunohistochemical pattern

S.No	Diagnosis	SMA	P63	Calponin	HHF-35
1	DCIS, comedo	Negative	Negative	Negative	Negative
2	DCIS, comedo	Positive, Weak	Positive	Positive, weak	Negative
3	DCIS, comedo	Positive	Positive	Positive	Positive
4	DCIS, comedo	Positive	Positive	Positive	Positive
5	DCIS, comedo	Positive	Positive	Positive, focally	Negative
6	DCIS, comedo	Positive	Positive	Positive	Positive
7	DCIS, comedo	Negative	Negative	Negative	Negative
8	DCIS, comedo	Positive	Positive	Negative	Negative
9	DCIS, comedo	Positive	Positive, weak	Positive, focally	Positive, focally
10	DCIS, comedo	Positive	Positive	Positive	Positive, Weak

formed with secondary antibody to biotinylated mouse antihuman immunoglobulin G (Dako). The detection was executed using 3, 3'-diaminobenzidine as a chromogen, counterstained with hematoxylin.

### Evaluation of staining results

Immunohistochemically stained slides were evaluated for the presence of a positive reaction, cellular localization (nuclear or cytoplasmic) and pattern of staining (focal or diffuse), and intensity of reaction in individual tumor cells (strong or weak). Any positive nuclear reaction for p63, irrespective of the percentage of reactive cells, was recorded as positive. In other words, there was no arbitrary percentage cutoff point used in this study. The intensity of positive nuclear reactions was evaluated against the reaction in respective internal control samples (whenever available) or the known positive external control sample.

### Results

The staining pattern and intensity of myoepithelial cells using four myoepithelial cell markers are shown in the **Table 2**. Of 10 cases of apparent high grade DCIS as diagnosed on routinely stained sections, 2 (20%) demonstrated

complete absence of a myoepithelial layer using all four immunohistochemical markers of myoepithelial cells. In general, p63 and smooth muscle actin were the strongest, most consistent markers of myoepithelial cells and muscle-specific actin and calponin were more often weak or negative in cases of DCIS with a myoepithelial layer confirmed by another stain.

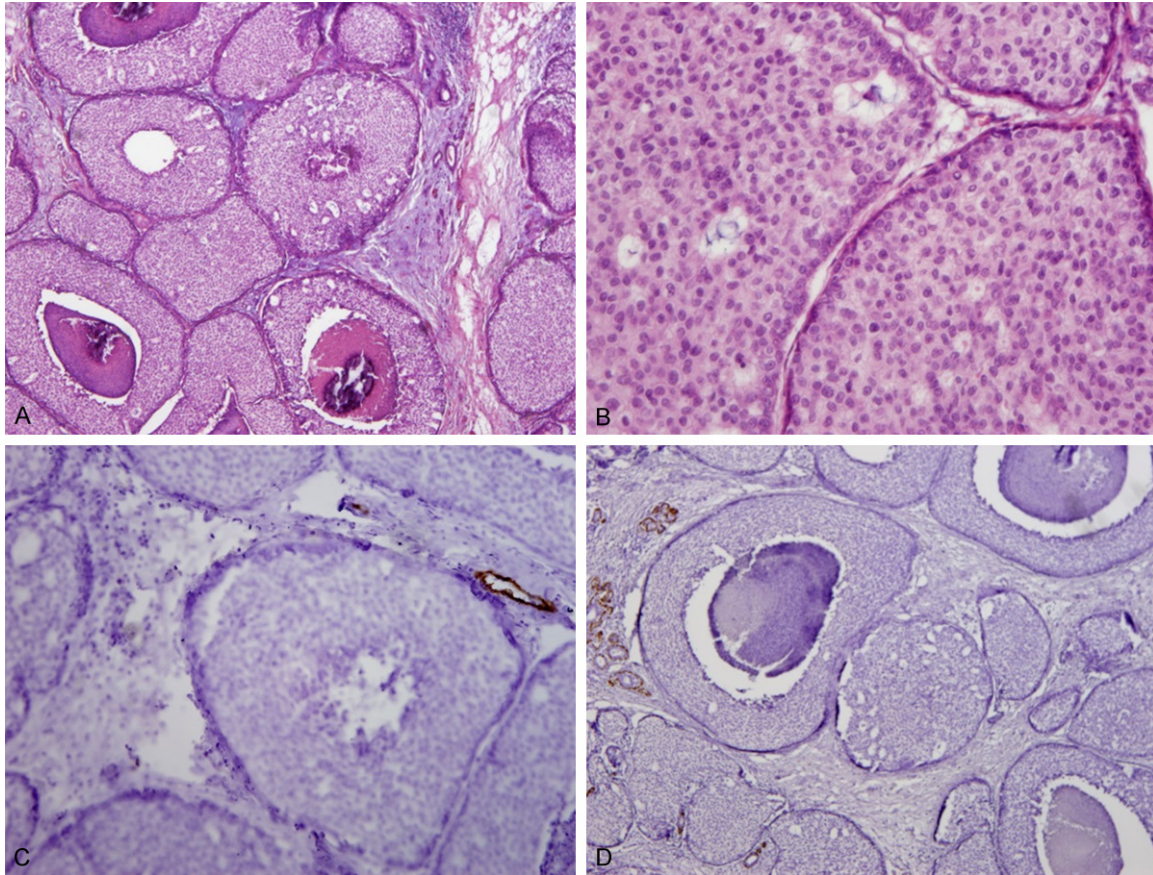
### Discussion

DCIS represents 20 percent of newly diagnosed breast carcinoma cases [3, 4]. Breast carcinoma is life threatening when it becomes invasive, at which point it carries potential for metastasis. Therefore, it is critical to distinguish invasive breast carcinomas (IBC) from DCIS.

The gene-expression profile of DCIS is quite similar to that of IBC and alterations in the neoplastic cells of DCIS that underlie the progression to IBC have not yet been elucidated [4]. Therefore, in recent years, attention has been focused on the role of myoepithelial cells in the progression of DCIS to IBC [5-10].

High-grade DCIS, in particular, has been associated with the breakdown of the myoepithelial cell layer and basement membrane surround-

## Histopathology of invasive ductal carcinoma



**Figure 1.** A. Low magnitude view of DCIS-like invasive ductal carcinoma. The smooth contour of the glandular architecture and central comedo necrosis is of typical appearance of a high grade DCIS. B. High magnitude view of DCIS-like invasive ductal carcinoma. Some flat cells make one think of myoepithelial cells. C. Immunostain of muscle specific actin (HHF35). The vessel wall served as the positive control, and the tumor glands are negative for HHF35 expression. D. p63 immunostain. The nuclear expression around the adjacent benign ducts served as a good positive control. The tumor glands are negative for p63 expression.

ing the ductal lumen. The assessment of myoepithelial lining is the most reliable method to distinguish IBC from DCIS [11, 12]. A number of immunohistochemical markers for myoepithelial cells have been utilized to help establish the presence (or absence) of stromal invasion [10, 13-16]. Use of immunohistochemical stains has been advocated in multiple publications highlighting axillary lymph node metastasis in various types of DCIS [1-4], which by definition, do not metastasize. In these unusual cases, obscure foci of invasion were not found despite exhaustive histologic examination of the entire breast tissue with routine staining. Alternatively, metastases may have developed in the absence of invasion demonstrable by means of light microscopic examination [2].

On light microscopy, nested infiltrative breast carcinoma with central necrosis and cribriform

carcinoma can mimic comedo DCIS or cribriform DCIS, respectively [17]. The most important aspect of this concept is the realization that a breast carcinoma may be partly or entirely DCIS like, yet invasive [18]. Hence, cases with morphologic features of confluent, high grade DCIS with comedonecrosis that present as a mass lesion must be carefully examined for evidence of invasion, including microinvasion and loss of the myoepithelial lining around large nests.

In our limited study, 2 cases (20%) of high grade DCIS cases did not exhibit any of the four myoepithelial cell markers surrounding large, solid nests with comedonecrosis (**Figure 1A-D**). In addition, Zhang and colleagues reported 2 cases of DCIS with morphologically identifiable myoepithelial cells but lacking the expression of nine corresponding immunophenotypic mark-

## Histopathology of invasive ductal carcinoma

ers. The underlying mechanism for the loss of myoepithelial markers in such cases is unknown [10]. However, it has experimentally been proven that the proteolytic enzymes produced by the neoplastic ductal epithelial cells alter the physical integrity of basement membrane and possibly altering the phenotypic features of myoepithelial cells [19-21]. In addition, exposure to certain chemical compounds such as lambda-carrageenan may specifically result in filament disassembly of myoepithelial cells [22]. Structural change in the filaments of the myoepithelial cells has significant impact on the ductal epithelial cells ability for invasion [23].

We also found that the sensitivity of some myoepithelial markers is lower in DCIS associated myoepithelial cells than in the normal myoepithelial cells, as has been previously reported [9]. In our experience, smooth muscle actin and p63 are more sensitive markers of myoepithelial cells when compared to muscle-specific actin or calponin, though muscle-specific actin is most specific for staining of myoepithelial cells. Additionally, the significance of the total loss or reduced expression of some of the myoepithelial markers remains to be understood, though our observations and previous studies show that such alterations may influence the progression of DCIS into invasive carcinoma.

In summary, our study demonstrated that, 20% of apparent high grade DCIS cases did not exhibit a myoepithelial layer surrounding large, solid nests with comedo necrosis. Because invasion is defined by the absence of a myoepithelial layer, these results suggest that a DCIS-like pattern may actually represent invasive disease and careful examination using immunohistochemical studies is warranted to distinguish in situ carcinoma from invasive carcinoma.

### Disclosure of conflict of interest

None.

**Address correspondence to:** Qihui Jim Zhai, Department of Pathology and Laboratory Medicine, Mayo Clinic, 4500 San Pablo Road, Jacksonville FL 32224 USA. Tel: 904-956-3318; Fax: 904-956-3336; E-mail: zhai.qihui@mayo.edu

### References

[1] Rosai J. Rosai and Ackerman's surgical pathology. Elsevier Health Sciences 2011.

- [2] Rosen PP. Axillary lymph node metastases in patients with occult noninvasive breast carcinoma. *Cancer* 1980; 46: 1298-1306.
- [3] Lara JF, Young SM, Velilla RE, Santoro EJ and Templeton SF. The relevance of occult axillary micrometastasis in ductal carcinoma in situ. *Cancer* 2003; 98: 2105-2113.
- [4] Barth A, Brenner RJ and Giuliano AE. Current management of ductal carcinoma in situ. *West J Med* 1995; 163: 360-6.
- [5] Sternlicht M and Barsky S. The myoepithelial defense: a host defense against cancer. *Med Hypotheses* 1997; 48: 37-46.
- [6] Allinen M, Beroukhim R, Cai L, Brennan C, Lahti-Domenici J, Huang H, Porter D, Hu M, Chin L and Richardson A. Molecular characterization of the tumor microenvironment in breast cancer. *Cancer Cell* 2004; 6: 17-32.
- [7] Deugnier MA, Teulière J, Faraldo MM, Thiery JP and Glukhova MA. The importance of being a myoepithelial cell. *Breast Cancer Res* 2002; 4: 224-230.
- [8] Polyak K and Hu M. Do myoepithelial cells hold the key for breast tumor progression? *J Mammary Gland Biol Neoplasia* 2005; 10: 231-247.
- [9] Hilson JB, Schnitt SJ and Collins LC. Phenotypic alterations in ductal carcinoma in situ-associated myoepithelial cells: biologic and diagnostic implications. *Am J Surg Pathol* 2009; 33: 227-232.
- [10] Zhang RR, Man YG, Vang R, Saenger JS, Barner R, Wheeler DT, Liang CY, Vinh TN and Braithauer GL. A subset of morphologically distinct mammary myoepithelial cells lacks corresponding immunophenotypic markers. *Breast Cancer Res* 2003; 5: R151-R156.
- [11] Yaziji H, Gown AM and Sneige N. Detection of stromal invasion in breast cancer: the myoepithelial markers. *Adv Anat Pathol* 2000; 7: 100-109.
- [12] Werling RW, Hwang H, Yaziji H and Gown AM. Immunohistochemical distinction of invasive from noninvasive breast lesions: a comparative study of p63 versus calponin and smooth muscle myosin heavy chain. *Am J Surg Pathol* 2003; 27: 82-90.
- [13] Leonard GD and Swain SM. Ductal carcinoma in situ, complexities and challenges. *J Natl Cancer Inst* 2004; 96: 906-920.
- [14] Bhargava R and Dabbs DJ. Use of immunohistochemistry in diagnosis of breast epithelial lesions. *Adv Anat Pathol* 2007; 14: 93-107.
- [15] Yeh IT and Mies C. Application of immunohistochemistry to breast lesions. *Arch Pathol Lab Med* 2008; 132: 349-358.
- [16] Lerwill MF. Current practical applications of diagnostic immunohistochemistry in breast pathology. *Am J Surg Pathol* 2004; 28: 1076-1091.

## Histopathology of invasive ductal carcinoma

- [17] Venable JG, Schwartz AM and Silverberg SG. Infiltrating cribriform carcinoma of the breast: a distinctive clinicopathologic entity. *Hum Pathol* 1990; 21: 333-338.
- [18] Pervez S and Khan H. Infiltrating ductal carcinoma breast with central necrosis closely mimicking ductal carcinoma in situ (comedo type): a case series. *J Med Case Reports* 2007; 1: 83.
- [19] Goldfarb RH and Liotta LA. Proteolytic Enzymes in Cancer Invasion and Metastasis. *Semin Thromb Hemost* 1986; 12: 294-307.
- [20] Wang F, Weaver VM, Petersen OW, Larabell CA, Dedhar S, Briand P, Lupu R and Bissell MJ. Reciprocal interactions between  $\beta$ 1-integrin and epidermal growth factor receptor in three-dimensional basement membrane breast cultures: a different perspective in epithelial biology. *Proc Natl Acad Sci U S A* 1998; 95: 14821-14826.
- [21] Mignatti P, Robbins E and Rifkin DB. Tumor invasion through the human amniotic membrane: requirement for a proteinase cascade. *Cell* 1986; 47: 487-498.
- [22] Tobacman JK. Filament disassembly and loss of mammary myoepithelial cells after exposure to  $\lambda$ -carrageenan. *Cancer Res* 1997; 57: 2823-2826.
- [23] Gudjonsson T, Rønnev-Jessen L, Villadsen R, Rank F, Bissell MJ and Petersen OW. Normal and tumor-derived myoepithelial cells differ in their ability to interact with luminal breast epithelial cells for polarity and basement membrane deposition. *J Cell Sci* 2002; 115: 39-50.