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Original contribution

Myoepithelial cells in lobular carcinoma in situ: distribution and immunophenotype☆,☆☆

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Summary Myoepithelial cells have important physical and paracrine roles in breast tissue development, maintenance, and tumor suppression. Recent molecular and immunohistochemical studies have demonstrated phenotypic alterations in ductal carcinoma in situ–associated myoepithelial cells. Although the relationship of lobular carcinoma in situ (LCIS) and myoepithelial cells was described in 1980, further characterization of LCIS-associated myoepithelial cells is lacking. We stained 27 breast specimens harboring abundant LCIS with antibodies to smooth muscle myosin heavy chain, smooth muscle actin, and calponin. Dual stains for E-cadherin/smooth muscle myosin heavy chain and CK7/p63 were also performed. In each case, the intensity and distribution of staining in LCIS-associated myoepithelial cells were compared with normal breast tissue on the same slide. In 78% of the cases, LCIS-associated myoepithelial cells demonstrated decreased staining intensity for one or more myoepithelial markers. The normal localization of myoepithelial cells (flat against the basement membrane, pattern N) was seen in 96% of LCIS, yet 85% of cases had areas with myoepithelial cell cytoplasm oriented perpendicular to the basement membrane (pattern P), and in 30% of cases, myoepithelial cells appeared focally admixed with LCIS cells (pattern C). This study characterizes detailed architectural and immunophenotypic alterations of LCIS-associated myoepithelial cells. The finding of variably diminished staining favors application of several myoepithelial immunostains in clinical practice. The interaction of LCIS with myoepithelial cells, especially in light of the perpendicular and central architectural arrangements, deserves further mechanistic investigation.

1. Introduction

In normal breast ducts and lobules, myoepithelial cells are situated between the luminal (secretory) epithelium, and the basement membrane. Classic ultrastructural studies demonstrate that they are flattened against the basement membrane with circumferentially oriented nuclei [1]. Furthermore, myoepithelial cells connect with one another through intermediate or gap junctions, to the epithelial cells through

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desmosomes, and to the basement membrane through hemidesmosomes [2,3]. Myoepithelial cells have important developmental functions, maintain the basement membrane and epithelial cell polarity, and, during lactation, have a contractile function in the process of milk secretion [2–5]. In recent years, the assessment of the presence or absence of myoepithelium using specific immunohistochemical markers has been widely used as a convenient ancillary method to assist in diagnosis of in situ or invasive breast carcinoma, respectively [6].

In 1980, Bussolati et al [1,7] elegantly characterized the relationship of lobular carcinoma in situ (LCIS) and myoepithelial cells using anti-actin immunohistochemistry and electron microscopy. In addition to the commonly recognized "flattened" myoepithelial architecture ("basket-like" pattern A), they also described myoepithelial cells perpendicular to the basement membrane ("offsite disarrangement" pattern B), or myoepithelial cells intermingled with LCIS ("nest-like" pattern C) [7].

Furthermore, recent molecular and immunohistochemical studies have demonstrated that ductal carcinoma in situ (DCIS)–associated myoepithelial cells are different from myoepithelial cells in normal breast and have suggested both physical and paracrine functions in tumor inhibition (or promotion) [2,4,8–12]. Nevertheless, the phenotypic characterization of LCIS-associated myoepithelium remains largely unclear. In this study, we sought to revisit the distribution and characterize the phenotypic features of LCIS-associated myoepithelial cells in a larger series of cases, using a panel of contemporary immunohistochemical markers, including nuclear, cytoplasmic, and dual stains.

2. Materials and methods

2.1. Patients and LCIS specimens

With institutional review board approval, the pathology files of Oregon Health & Science University (2012-2015) were searched for surgical breast resection specimens containing LCIS. Slides were reviewed to select cases with abundant LCIS, many including pagetoid LCIS, and one representative block containing LCIS and normal breast tissue was used for further study. The final study group included 27 specimens from 25 women (2 patients with bilateral LCIS), including 21 classic LCIS and 6 pleomorphic LCIS.

2.2. Immunohistochemical staining

Representative blocks were stained for E-cadherin/smooth muscle myosin heavy chain (SMMHC) dual stain, CK7/p63 dual stain, SMMHC alone, smooth muscle actin (SMA), or calponin using standard methods (Supplementary Table 1) on Benchmark XT or Ultra automated stainers (both Ventana, Tucson, AZ). The Supplementary Data also describe 10 cases immunofluorescently stained for calponin, SMA, and p63.

2.3. Immunohistochemical scoring

In each case, the intensity and distribution of staining in LCIS-associated myoepithelial cells were visually compared with the myoepithelial cells surrounding normal breast tissue on the same slide using the following criteria for all markers: score 1, staining intensity of the myoepithelial cells in LCIS weaker than the normal; score 2, staining intensity in LCIS equals myoepithelial cells in normal breast; and score 3, staining intensity in LCIS stronger than the normal, based on the dominant (most abundant) pattern in LCIS. In addition, architectural patterns were scored, for cytoplasmic markers, pattern N represents normal, flattened peripherally against basement membrane; pattern P, perpendicular to the basement membrane, or ''net-like''; and pattern C, central or nest-like. Architectural criteria, for p63, are as follows: pattern N, normal, against basement membrane; pattern P, above basement membrane, especially with 1 layer of epithelial nuclei between p63+ and basement membrane; and pattern C, more than 1 cell layer above the basement membrane. Slides were scored independently by one pathologist and one trainee (M. L. T., Y. W.), and discrepancies were resolved by consensus review.

3. Results

3.1. Patients and LCIS specimens

We stained representative blocks from 27 breast resection specimens containing LCIS, from 25 patients (2 bilateral) with a panel of contemporary myoepithelial markers, including dual stains. Cases included 21 classic LCIS and 6 pleomorphic LCIS. Many patients had invasive lobular (19) or ductal (5) carcinoma

Table 1 Staining intensity of LCIS-associated myoepithelial cells compared with myoepithelial cells in accompanying normal breast

Stain	LCIS weaker (pattern 1)	LCIS equal (pattern 2)	LCIS stronger (pattern 3)
Calponin	18/27 (67%)	8/27 (29%)	1/27 (4%)
P63	1/27 (4%)	26/27 (96%)	0
SMA	7/27 (26%)	20/27 (74%)	0
SMMHC	14/27 (52%)	13/27 (48%)	0
SMMHC in dual stain [a]	17/26 (65%)	9/26 (35%)	0

NOTE. LCIS showed variable staining intensity; the dominant (most abundant) pattern was tallied.

[a] As compared with SMMHC stained singly, the SMMHC in dual stain was weaker in 7 (28%) of 25, equal in 15 (60%) of 25, and stronger in 3 (12%) of 25.