Phenotypic and Functional Characterization of Ductal Carcinoma In Situ–Associated Myoepithelial Cells

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Abstract

This study was undertaken to assess the characteristics of ductal carcinoma in situ (DCIS)-associated myoepithelial cells. Phenotypic and functional markers of myoepithelial cells were studied in pure DCIS, the DCIS component of infiltrating duct carcinoma (IDC), and adjacent normal breast tissue. There was decreased expression of myoepithelial cell markers in both groups of DCIS compared with normal breast tissue myoepithelial cells, suggesting that DCIS–associated myoepithelial cells are phenotypically different from their normal counterparts.

Background: Ductal carcinoma in situ (DCIS) is contained by myoepithelial cells that are morphologically similar to normal breast tissue myoepithelial cells. However, phenotypic and functional characteristics of DCIS-associated myoepithelial cells are not known. In this study, we aimed to assess the characteristics of DCIS-associated myoepithelial cells. **Materials and Methods:** Immunophenotypic and functional characteristics of myoepithelial cells of pure DCIS, the DCIS component of infiltrating duct carcinoma (IDC), and the adjacent normal breast tissue of both groups (30 cases in each group) was assessed using phenotypic (CK5/6, CK14, p63, and calponin) and functional markers (maspin and CXCL14). **Results:** There was a decrease in expression of CK14, p63, and calponin in pure DCIS-associated myoepithelial cells compared with normal breast tissue myoepithelial cells (43.3% vs. 80.3%, 3.3% vs. 70%, 46.6 vs. 93.3%, respectively) and in the DCIS component of IDC compared with normal breast tissue myoepithelial cells (56.6% vs. 100%, 3.3% vs. 73.3%, 56.6% vs. 96.6%, respectively). CK5/6 expression was low to absent in myoepithelial cells of pure DCIS and the DCIS component of IDC as well as normal breast tissue myoepithelial cells. Maspin was expressed in all samples of normal breast tissue; however, 20% of pure DCIS and 26.6% of the DCIS component of IDC showed decreased expression. CXCL14 expression was greater in pure DCIS compared with adjacent normal breast tissue and the DCIS component of IDC. **Conclusion:** Decreased expression of myoepithelial cell markers in DCIS suggests that DCIS-associated myoepithelial cells are phenotypically different from their normal counterparts. Two or more markers, preferably p63 and calponin, should be used to distinguish in situ from invasive breast carcinomas.

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Introduction

Ductal carcinoma in situ (DCIS) is a malignant clonal proliferation of cells growing in the basement membrane and myoepithelial cell–bound structures of the breast, with no evidence of invasion into the surrounding stroma. DCIS constitutes approximately 5% of breast cancers in which mammographic breast screening is not performed, but within screening programs, it composes approximately 20% to 25% of these tumors.[1,2] There is general consensus that DCIS represents an intermediate step between normal breast tissue and invasive breast cancer, which, if untreated, develops into invasive carcinoma in a significant proportion of cases.[3-5]

Myoepithelial cells surround mammary ducts and lobular acini and have important roles in normal mammary gland development and physiology.[6,7] Myoepithelial cells have natural tumor suppressor functions, including maintenance of the basement membrane

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around ductal-lobular structures, providing a physical barrier between epithelial cells and the surrounding stroma, and maintenance of epithelial cell polarity.[1,8] The mechanisms by which myoepithelial cells may act to suppress tumor progression in vivo and how these functions are compromised during cancer development remain largely unknown. Myoepithelial cells exhibit many antitumorigenic properties such as inhibition of the growth of breast cancer cells by inducing a G_2/M cell cycle arrest, inhibiting tumor cell invasion, and reducing angiogenesis by paracrine regulation. Evolving experimental evidence indicates that myoepithelial cells naturally exhibit a tumor-suppressive phenotype.[6,9] Myoepithelial cells also express many extracellular matrix structural proteins and proteinase inhibitors and accumulate extracellular matrix rather than degrade it, which may explain in part why DCIS lesions are not invasive.[10]

DCIS is contained by myoepithelial cells, which appear morphologically similar to normal breast tissue myoepithelial cells. However, it is not known whether DCIS-associated myoepithelial cells are also functionally similar or have an altered phenotype compared with normal breast tissue myoepithelial cells that favors progression to invasive breast cancer. Until now, all studies on DCIS have focused on luminal epithelial cells, and myoepithelial cells have remained neglected. Recent molecular studies have indicated that DCIS-associated myoepithelial cells show differences from myoepithelial cells in normal breast tissue. Such alterations may influence the progression of DCIS to invasive cancer.[11] In this study, we aimed to assess the immunophenotypic and functional characteristics of DCIS-associated myoepithelial cells using antibodies to myoepithelial cell phenotypic markers (CK5/6, CK14, p63, and calponin) and functional markers (maspin and CXCL14).

Materials and Methods

This study included lumpectomy or mastectomy specimens from patients with a diagnosis of pure DCIS (n = 30) and invasive breast cancer with a DCIS component > 70% (n = 30), along with sections from adjacent normal-appearing breast tissue in both groups (n = 30 in each group). All cases were reviewed according to our Institutional Review Board Ethics Committee. All patients treated with chemotherapy before surgery and those with a previous history of lumpectomy followed by mastectomy for residual disease were excluded. All specimens were processed for paraffin sections for routine hematoxylin & eosin staining and immunohistochemical evaluation.

Light Microscopy

Detailed morphologic features, histologic type, grade, and necrosis were noted on light microscopic examination of the hematoxylin and eosin–stained sections. Histologic grading of the DCIS component of the tumor was done by using the Van Nuy classification and prognostic index.[12]

Immunohistochemical Analysis

All specimens of DCIS and adjacent normal breast tissue and the DCIS component of infiltrating duct carcinoma (IDC) and adjacent normal breast tissue were evaluated for the presence and functional assessment of myoepithelial cells. Antibodies used included myoepithelial cell phenotype markers (CK5/6, CK14, p63, and calponin) and functional markers (maspin and CXCL14). Details of antibodies used in the study are listed in Table 1. Adult breast tissue parenchyma was used as a positive control. Negative controls were obtained by omitting the primary antibodies.

Assessment of Immunostaining

For CK5/6, CK14, calponin, maspin, and CXCL14, cytoplasmic immunoreactivity in myoepithelial cells was defined as positive, and for p63, nuclear staining was defined as positive. Expression was assessed in a proportion of myoepithelial cells staining positive in the circumference of a duct. A proportion score was assigned from 0 to 3 (0, negative; 1, < 50%, interrupted; 2, > 50%, interrupted; 3, 100%, complete) representing the proportion of myoepithelial cells, and an intensity score was assigned from 0 to 3 (0, absent; 1, weak; 2, moderate; 3, strong) representing the average staining intensity of myoepithelial cells. Staining intensity for myoepithelial cells that was the same as cells surrounding normal ducts and lobular units was assigned a score of 3; cells with slightly decreased staining intensity were scored 2; cells with markedly decreased staining were scored 1; and cells with a complete absence of staining were scored 0. A total score was calculated by multiplying the proportion score and the intensity score (ranging from 0-9).

Statistical Analysis

Data were analyzed using SPSS Statistics, version 17.0 for Windows (SPSS, Chicago, IL). Correlations among categorical variables in the multivariate and bivariate analysis were determined using the Mann-Whitney test, Wilcoxon signed-rank test, and Pearson χ^2 test. Significance was assumed at $P < .05$.

Results

Morphologic Parameters: Van Nuys Classification

Pure DCIS. The various architectural patterns observed in pure DCIS specimens were solid, papillary, clinging, cribriform, comedo, and micropapillary. Most of the specimens had more than one architectural pattern. The comedo pattern was the most common

Table 1 Details of Antibodies Used in the Study

Antibody	Manufacturer	Clone	Dilution	Positivity Pattern
CK14	Novocastra	NCL-LL002	1:20	Cytoplasmic ± membranous
CK5/6	DAKO	D5/16B4	1:80	Cytoplasmic ± membranous
p63	Sigma-Aldrich	TP63	1:150	Nuclear
Calponin	DAKO	CALP	1:300	Cytoplasmic
Maspin	Sigma-Aldrich	SERPINB5	1:300	Cytoplasmic
CXCL14	Novocastra	Polyclonal	1:50	Cytoplasmic