Original Article

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P-cadherin and CD10 Expression to Distinguish between Ductal Carcinoma in Situ and Invasive Ductal Carcinoma of the Breast

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Abstract:

Objective: To use placental cadherin (P-cadherin) and cluster of differentiation 10 (CD10) immunohisto chemical staining, to separate ductal carcinoma in situ (DCIS) from invasive ductal carcinoma (IDC).

Material and Methods: DCIS (n=48), equivocal (n=18), and IDC grade 1 (n=17) cases were evaluated by using immuno-histo chemical staining, with P-cadherin and CD10 for identifying the myoepithelial cells.

Results: P-cadherin is positive in myoepithelial cells in almost all cases of DCIS (79.0%), and equivocal groups (61.0%). CD10 also shows a positive result in most cases of DCIS (98.0%) along with equivocal groups (72.0%). Both, P-cadherin and CD10 are negative in all cases of IDC grade 1. P-cadherin shows a high percentage of positivity in luminal cell in DCIS (83.0%), equivocal group (100.0%) and IDC grade 1 (88.0%). CD10 shows a low positive in the luminal cell of most cases of DCIS (13.0%), equivocal group (6.0%) and IDC grade 1 (0.0%). CD10 is positive in myofibro-blastic cells in approximately 30.0% of all cases, but P-cadherin shows all negative staining.

Conclusion: P-cadherin and CD10 show high sensitivity for detecting the myoepithelial cells, but P-cadherin has a lower specificity, due to it having more luminal cells expression. Therefore, P-cadherin may be helpful for diagnosis in some cases that have a high expression of CD10 in myofibroblastic cells.

Keywords: CD10, ductal carcinoma in situ, invasive ductal carcinoma, myoepithelial cell, P-cadherin

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Introduction

Breast cancer is the second most common cancer in the world, and the most common cancer in women.[1] More than 95.0% of breast cancers are invasive ductal carcinoma (IDC). This malignancy has proliferation of the dysplastic ductal epithelial cells, that penetrates through the basement membrane into the stroma, causing loss of the myoepithelial cells around it. The pre-invasive lesion of IDC is ductal carcinoma in situ (DCIS). The diagnosis between DCIS and IDC is importance because, they have both a different prognosis and treatment, however sometimes it is difficult to detect in small biopsy specimens.[2-5] The use of immunohisto chemical staining, such as; smooth muscle actin (SMA), calponin, transformation related protein 63 (p63) and cluster of differentiation 10 (CD10), for detection of myoepithelial cells is a very useful tool in the determination of a correct diagnosis.[6] However, these antibodies have varying specificities, and show reactivity in myofibroblasts.[7]

CD10 is a 90–110 kilodaltons cell surface, zinc-dependent metalloprotease that inactivates the peptide molecules. In breast tissue, CD10 expresses in myoepithelial cells, and also positive in myofibroblasts, but the degree of cross-reactivity is less than that seen with SMA.[8-10] Placental cadherin (P-cadherin) is a 118 kilodaltons calcium dependent cell-cell adhesion glycoprotein in the cadherins family. It expresses in the stratified epithelial basal layer of the skin, prostate and myoepithelial cells of breast. The functions of P-cadherin are to control the intercellular connection, cellular differentiation, cellular growth and migration.[11,12] Previous studies of P-cadherin in both, benign and malignant breast tumors show that P-cadherin expresses in myoepithelial cells and, rarely expresses in normal luminal cells or malignancy cells.[13-20]

There are limited studies of P-cadherin expression in DCIS and IDC, when compared with other myoepithelial markers. Hence, the objective of this study is to use P-cadherin and CD10 immunostaining to distinguishing between DCIS and IDC of the breast.

Material and Methods

Study population

The cases were retrospectively selected from Songklanagarind Hospital, between; January 2010 and December 2014. The clinicopathological data were recorded (patient age, surgical operation, tumor site, tumor size, tumor grade and stage). The tumors were staged following the American Joint Committee on Cancer (AJCC) staging system 7th edition. All cases had paraffin-embedded tissue from a breast biopsy, wide excision or mastectomy. All patients did not receive neoadjuvant chemotherapy. The cases that did not have complete clinical data, or did not have an adequate specimen were excluded.

The population was separated into 3 groups. The first group was DCIS. The second group was an equivocal group, consisting of; DCIS or IDC (grade 1 and 2), that required immunostaining for diagnosis. The last group was IDC grade 1. The IDC grade 1 was selected for representation of invasive cancer, because this type of cancer usually has histology of luminal formation which can mimic the benign lesions of DCIS. The total number of cases were 83, including; 48 cases of DCIS, 18 cases of equivocal group and 17 cases of IDC grade 1.

Immunhistochemistry

Tissue samples were fixed in 10.0% neutral buffer formalin, and embedded in paraffin. The tissue was stained by Hematoxylin and Eosin stain, as per usual methods for diagnostic purposes. The most representative areas of all cases were selected for this study. Formalin-fixed, paraffin embedded tissues were cut into 1-3 micron-thick sections, with a microtome, and then placed on a super-frost slide. The sections must be reversed in order to deparaffinize in xylene, and rehydrate in graded ethanol series.

Immunohisto chemical staining was performed on the selected slides, using mouse monoclonal P-cadherin antibody (dilution 1:100, clone 56C1, Abcam^{TM}), and mouse monoclonal CD10 antibody (dilution 1:250, clone 56C6,

Novocastra™). Staining was performed with the Leica BOND-MAX™ automated stainer. The slides were incubated with peroxidase-blocking reagent, followed by the primary antibody, then the visualization reagent using the bond polymer refine detection kit. After that, the slides were incubated with 3,3-diaminobenzidine, as a chromogen, and counterstained by Mayer hematoxylin.

Interpretation

The results of the immunostaining were evaluated by scoring the degrees of myoepithelial cell expression. The percentage of P-cadherin and CD10 staining in myoepithelial, and luminal cells were further recorded in positive cases. The staining intensity was scored as: negative (0), weak (1+), moderate (2+) and strong (3+). The membranous staining ≥ 1+ intensity, in more than 1.0% of myoepithelial cells, was considered positive for P-cadherin and CD10. The examples of positive and negative cases are shown (Figure 1).

Statistical analysis

Descriptive analysis was performed to evaluate the expression pattern of P-cadherin as well as CD10 immunohisto chemical staining. Fisher's exact test was used to analyze the difference of P-cadherin, and CD10 expression patterns in each group. The statistical significance was defined as: p-value<0.05. All analyses were calculated by using RStudio software version 0.99.473.

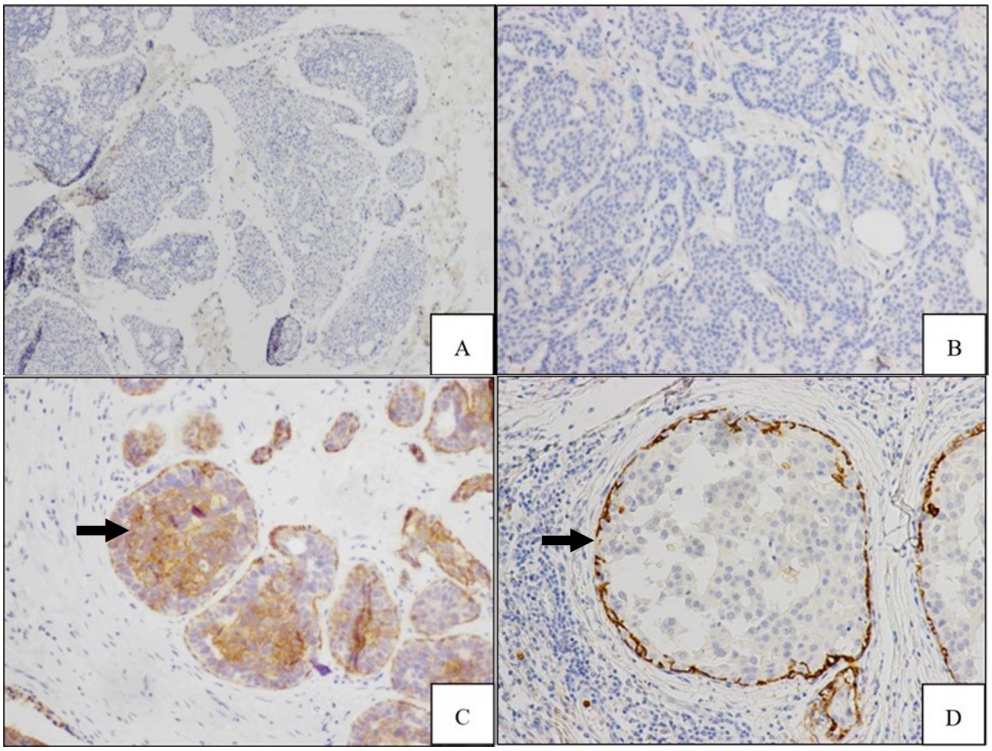

Figure 1 (A) The negative staining of P-cadherin in invasive ductal carcinoma. (B) The negative staining of cluster of differentiation 10 in invasive ductal carcinoma. (C) The positive staining of P-cadherin in myoepithelial cells of ductal carcinoma in situ (➞). (D) The positive staining of cluster of differentiation 10 in myoepithelial cells of ductal carcinoma in situ (➞).

Results

Demographic data

The age of the patients was between 27-78 years of age. Most tumors were in the right breast (39.0%), with the tumor sizes ranging between; 0.2-7.0 centimeters. Most tumors were high grade DCIS (36.0%). All tumors were in stage 0 in the DCIS group, and most invasive tumors were in stage 1 (47.0%) in the IDC group. The demographic data of patients is summarized (Table 1).

Immunohistochemistry

Myoepithelial cell expression

P-cadherin was positive in most cases of DCIS (79.0%), and in the equivocal group (61.0%). Fifteen cases (32.0%) of the DCIS group, and three cases (17.0%) within the equivocal group revealed strong intensity (3+) of P-cadherin expression. CD10 was positive in most cases of both the DCIS (98.0%) and the equivocal groups (72.0%). Forty-one cases (86.0%) within the DCIS group and ten cases (55.0%) in the equivocal group revealed strong intensity (3+) of CD10 expression. Both, P-cadherin and CD10 were negative in all cases of the IDC grade 1 group. The difference between myoepithelial cells expression of P-cadherin and CD10 in DCIS, and the equivocal group revealed no statistical significance (p-value=0.30 and 0.50, respectively).

Table 1 Demographic data of patients

Data	DCIS (n=48)	IDC grade 1 (n=17)	Equivocal group (n=18)
Age (years)			
Range	27-66	35-86	40-78
Mean	47	55	53
Tumor site			
Left breast	15 (31.0%)	7 (41.0%)	10 (56.0%)
Right breast	33 (69.0%)	10 (59.0%)	8 (44.0%)
Size (centimeters)			
Range	0.2-7.0	0.8-6.0	0.4-7.0
Mean	1.5	2.0	2.0
Tumor			
Low grade DCIS	7 (15.0%)	-	2 (15.0%)
Intermediate grade DCIS	11 (23.0%)	-	11 (85.0%)
High grade DCIS	30 (62.0%)	-	-
IDC grade 1	-	17 (100.0%)	3 (60.0%)
IDC grade 2	-	-	2 (40.0%)
Stage			
0 (DCIS)	48 (100.0%)	-	13 (72.2%)
1	-	8 (47.0%)	4 (22.2%)
2	-	6 (35.0%)	1 (5.6%)
3	-	3 (18.0%)	-

DCIS=ductal carcinoma in situ, IDC=invasive ductal carcinoma

Luminal cell expression

P-cadherin was positive in most of the cases within the DCIS group (83.0%), all of the cases in the equivocal groups (100.0%), and in most cases of the IDC grade1 group (88.0%). Sixteen cases (33.0%) of the DCIS group, four cases (22.0%) of the equivocal group and three cases (18.0%) of the IDC grade 1 group revealed strong intensity (3+) of P-cadherin expression. CD10 showed as a low positive in the luminal cell of most cases of the DCIS (13.0%), the equivocal group (6.0%) as well as the IDC grade 1 (0.0%). Six cases (12.0%) of the DCIS group, and one case (6.0%) of the equivocal group revealed strong intensity (3+) of CD10 expression. The difference between luminal cell expression of P-cadherin, and CD10 in both the DCIS and equivocal groups revealed to not be statistically significant (p-value= 0.09 and 1, respectively).

Myofibroblastic cell expression

CD10 expression in myofibroblasts of all three groups was positive in 30.0% of cases, while P-cadherin expression was negative.

The data of P-cadherin and CD10 staining positivity in myoepithelial cells, and luminal cells of all three groups are summarized (Table 2). P-cadherin and CD10 expression in DCIS cases and CD10 expression in myofibroblasts are demonstrated (Figure 2).

The summarized combination using P-cadherin and CD10 to detect myoepithelial cell is demonstrated (Table 3).

Table 2 Placental cadherin, and cluster of differentiation 10 positivity in myoepithelial cells, and luminal cells of ductal carcinoma in situ, equivocal and invasive ductal carcinoma grade 1 group

IHC	DCIS (n=48)		Equivocal (n=18)		IDC grade 1 (n=17)	
	+ (%)	- (%)	+ (%)	- (%)	+ (%)	- (%)
Myoepithelial cell						
P-cadherin	38 (79.0)	10 (21.0)	11 (61.0)	7 (39.0)	0 (0.0)	17 (100.0)
CD10	47 (98.0)	1 (2.0)	13 (72.0)	5 (28.0)	0 (0.0)	17 (100.0)
Luminal cell						
P-cadherin	40 (83.0)	8 (17.0)	18 (100.0)	0	15 (88.0)	2 (12.0)
CD10	6 (13.0)	42 (87.0)	1 (6.0)	17 (94.0)	0 (0.0)	17 (100.0)
Myofibroblast						
P-cadherin	0 (0.0)	48 (100.0)	0 (0.0)	18 (100.0)	0 (0.0)	17 (100.0)
CD10	12 (25.0)	36 (75.0)	8 (44.4)	10 (55.6)	6 (35.2)	11 (64.8)

IHC=immunohisto chemical staining, DCIS=ductal carcinoma in situ, IDC=invasive ductal carcinoma, CD10=cluster of differentiation 10

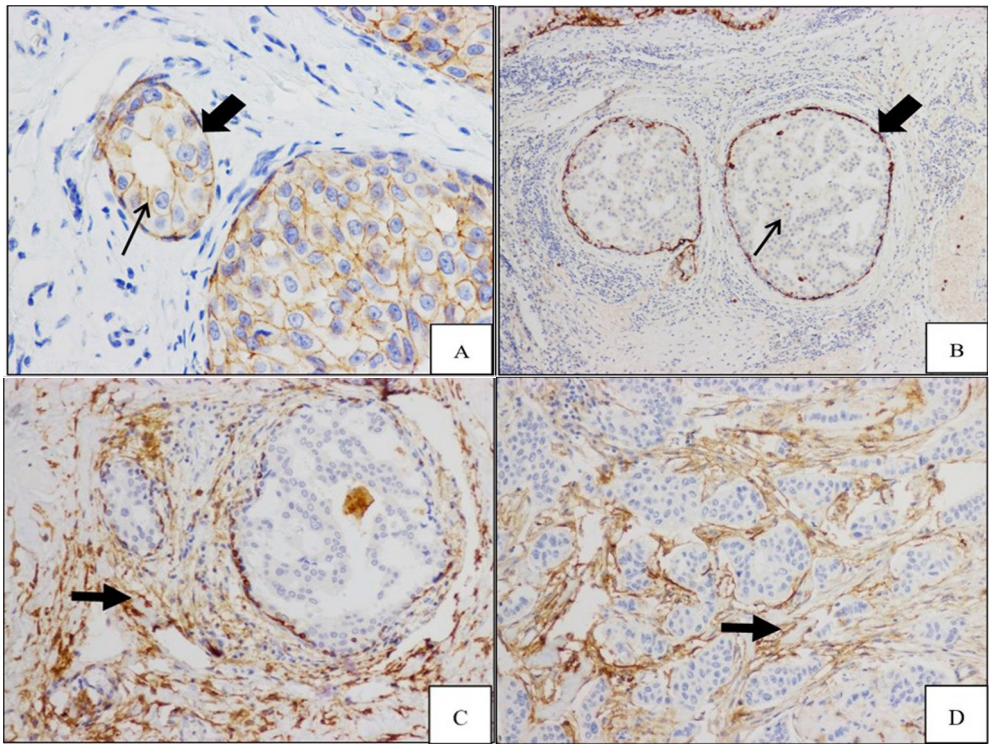

Figure 2 (A) P-cadherin shows positive staining in myoepithelial cells (➞) and luminal cells (→) in ductal carcinoma in situ. (B) cluster of differentiation 10 shows expression in myoepithelial cells (➞), but do not express in luminal cells (→) in ductal carcinoma in situ. (C) The expression of cluster of differentiation 10 in stromal cells of ductal carcinoma in situ (➞). (D) The expression of cluster of differentiation 10 in stromal cells of invasive ductal carcinoma (➞).

Table 3 The combination of placental cadherin, and cluster of differentiation 10 to detect myoepithelial cells in ductal carcinoma in situ, equivocal and invasive ductal carcinoma grade 1 group

Group	P-cad +/CD10 + (%)	P-cad +/CD10 − (%)	P-cad −/CD10 + (%)	P-cad −/CD10 − (%)
DCIS (n=48)	37 (77.1)	1 (2.1)	10 (20.8)	0 (0.0)
Equivocal group				
DCIS (n=13)	11 (84.6)	0 (0.0)	2 (15.4)	0 (0.0)
IDC (n=5)	0 (0.0)	0 (0.0)	0 (0.0)	5 (100.0)
IDC grade 1 (n=17)	0 (0.0)	0 (0.0)	0 (0.0)	17 (100.0)

DCIS=ductal carcinoma in situ, IDC=invasive ductal carcinoma, P-cadherin=placental cadherin, CD10=cluster of differentiation 10

Discussion

Many immunohisto, chemical markers for myo-epithelial cells are now commonly used to distinguish between DCIS and IDC, with various sensitivity and specificity. This study uses P-cadherin to evaluate its diagnostic value, comparing CD10 in three groups of cases that commonly have diagnostic difficulty.

For the expression in myoepithelial cells, this study found that most cases, in either the DCIS and equivocal groups, were positive for P-cadherin and CD10. The expression in luminal cells showed that most cases, in all three groups, were positive for P-cadherin, while they were negative for CD10. This result was different from a previous study, which showed a smaller proportion of P-cadherin luminal cells expression.[19] The discrepancy findings could be from the difference in clones, using anti-bodies, and the difference in the study population being smaller than this study. Although, the sensitivity as well as specificity of P-cadherin, for distinguishing between DCIS and IDC in the equivocal group, compared with CD10 were high, marked luminal cells expression may lead to false diagnosis such as; A P-cadherin expression in peripheral luminal cells of IDC looking similar to myoepithelial cells of DCIS.

The aberrant expression of P-cadherin in luminal cells has been explained by several hypotheses, although none have, as of yet, been proved valid. A previous study suggested that the expression of P-cadherin in the luminal tumor cell is responsible for epithelial cadherin down regulation, and the expression of P-cadherin will increase the ability of tumor cell proliferation, and increase the mitotic index for maintenance of the cancer cell nests.[14] The other explanation is that P-cadherin could be a member of an oncofetal protein family that, has high expression in embryogenesis and tumor cells, but focally expresses in normal, adult tissues.[21] Another study suggested that, the expression of P-cadherin in luminal cells could be related to histogenetic origins in cap cells, or an acquisition of a phenotype, similar to stem cells.[17]

However, the advantage of P-cadherin, that we found in this study; was the negativity for myofibroblasts comparing to CD10. Absence of P-cadherin expression in myofibroblasts resulted in clear delineation of the myo-epithelial cells. Myofibroblastic staining near the tumor nest in IDC cases may mimic myoepithelial cells. This can be misleading in the diagnosing of DCIS. The CD10 expression in myofibroblasts of our study was 30.0% of all cases. This result was quite similar to a previous study, which revealed CD10 stromal expression in 18.0% of all cases.[8]

From this study, we could suggest a diagnostic workflow of P-cadherin, and CD10 usage. If the morphology did not distinguish between DCIS and IDC, CD10 could be used as a first line myoepithelial cell marker. If CD10 is still problematic, especially due to the presence of myo-fibloblastic staining, P-cadherin could be a useful marker.

Conclusion

The results indicated that P-cadherin expression was specific for myoepithelial cells of the breast, however it also showed marked luminal cells expression. P-cadherin had less of a benefit for distinguishing between DCIS and IDC, when compared to CD10, but it may be a useful marker in some cases that have a high myofibroblastic expression of CD10.

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