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Original Article

Diagnostic utility of E-cadherin and P120 catenin cocktail immunostain in distinguishing DCIS from LCIS

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Abstract: Background: Breast carcinoma in situ (CIS) is classified into ductal carcinoma in situ (DCIS) and lobular carcinoma in situ (LCIS). DCIS is treated with surgical excision while LCIS can be clinically followed with or without hormonal treatment. Thus, it is critical to distinguish DCIS from LCIS. Immunohistochemical (IHC) staining for E-cadherin is routinely used to differentiate DCIS from LCIS in diagnostically challenging cases. Circumferential diffuse membranous staining of E-cadherin is the typical pattern in DCIS, whereas LCIS lacks or shows decreased E-cadherin expression. Recent studies have shown that DCIS has membranous staining of P120 catenin and LCIS has diffuse cytoplasmic staining of P120 catenin. We developed a cocktail composed of E-cadherin and P120 catenin primary antibodies so that only one slide is needed for the double immunostains. Designs: Twenty-seven blocks of formalin-fixed paraffin-embedded tissue from 26 cases of DCIS or LCIS were retrieved from the archives of Houston Methodist Hospital. Four consecutive sections from the same blocks were used for H&E and immunohistochemical (IHC) stains. The E-cadherin antibody was a rabbit polyclonal antibody and the P120 catenin antibody was a mouse monoclonal antibody. The E-cadherin primary antibody was detected using a secondary antibody raised against rabbit antibody and was visualized with a brown color. The P120 catenin primary antibody was detected using a secondary antibody raised against mouse antibody and was visualized with a red color. Results: Using individual antibodies, 15 of 15 DCIS lesions had diffuse circumferential membranous E-cadherin staining (brown stain) or P120 catenin staining (red stain). All 12 LCIS cases showed cytoplasmic P120 red staining or loss of E-cadherin staining when the single P120 catenin or E-cadherin antibody was used. When stained with the antibody cocktail, all 15 DCIS samples showed diffuse red and brown membranous staining without cytoplasmic stain; all 12 LCIS samples showed diffuse cytoplasmic red staining for P120 catenin but no membranous staining for E-cadherin. Conclusions: 1. This antibody cocktail can be applied in daily practice on paraffin-embedded tissue and is especially useful in small biopsies with small foci of CIS lesions. 2. Immunohistochemical staining with the antibody cocktail showed 100% concordance with the traditional single antibody immunostaining using either E-cadherin or P120 catenin antibody. 3. Our antibody cocktail includes E-cadherin as a positive membranous stain for DCIS and P120 catenin as a positive cytoplasmic stain for LCIS, which may enhance accuracy and confidence in the differential diagnoses.

Keywords: DCIS, LCIS, E-cadherin, p120 catenin

Introduction

Women with breast mammary carcinoma in situ (CIS) are at higher risk for invasive carcinoma [1-3]. CIS in general is defined as clonal proliferation of epithelial cells without invading through basement membrane into surrounding stroma. Breast CIS is reported to originate from stem cells in the terminal duct lobular unit (TDLU). Breast CIS includes two main types: ductal carcinoma in situ (DCIS) and lobular carcinoma in situ (LCIS). DCIS is composed of monoclonal proliferation of cohesive epithelial cells, which usually cause mammary ductal expansion. Although the lesion is called DCIS, it has been shown that the tumor cells originate from TDLU but not from mammary duct [4, 5]. The DCIS tumor cells range from low to high grade and are cohesive with preservation of membranous E-cadherin expression [6]. The

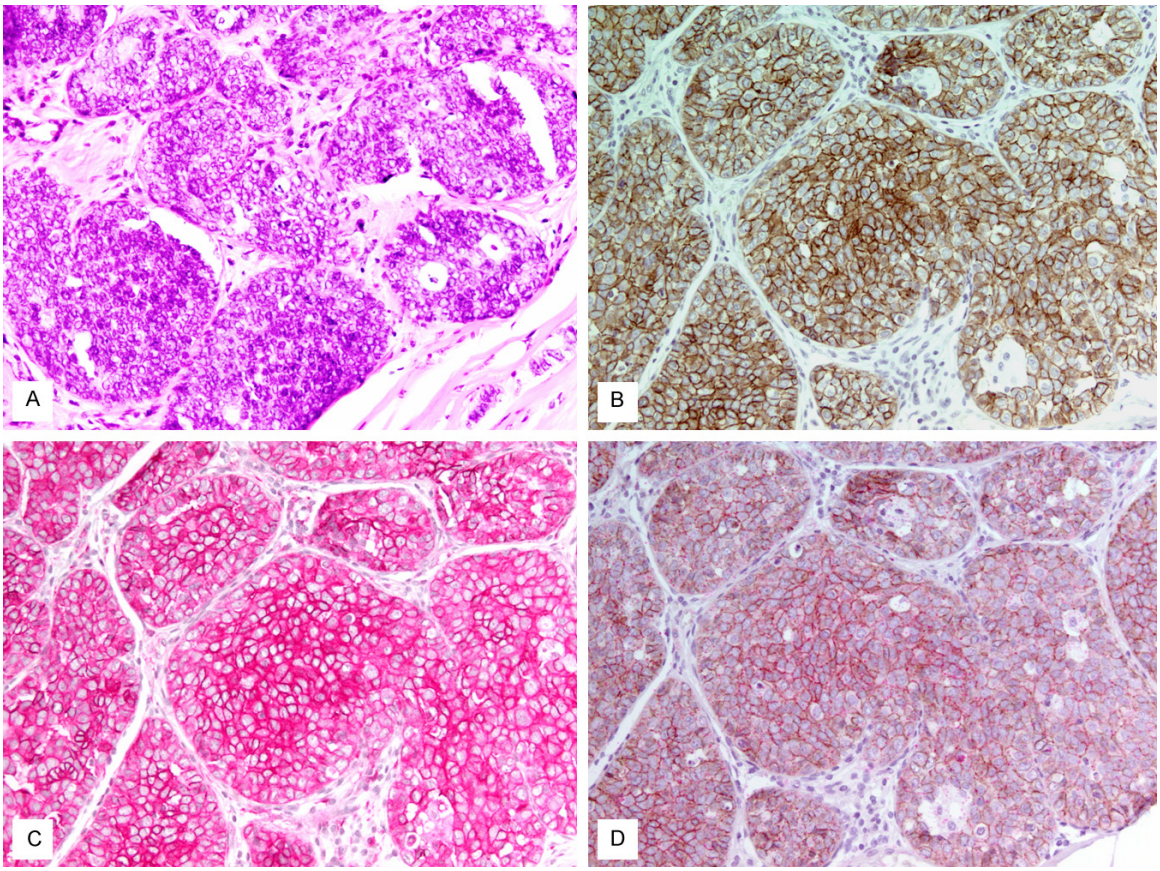

Figure 1. Immunoprofile of E-cadherin, P120 catenin and their cocktail in ductal carcinoma in situ. A. H&E section illustrates typical histology; B. E-cadherin staining shows a diffuse strong membranous pattern; C. P120 catenin staining shows a diffuse strong membranous pattern; D. Cocktail of E-cadherin and P120 shows a strong membranous pattern in a mixture color of brown and red.

preservation of E-cadherin is thought to be responsible for the cohesive appearance of DCIS.

LCIS is a monoclonal proliferation of cells with dyscohesive appearance. The LCIS cells are usually uniform and discohesive with low nuclear grade and scant cytoplasm; although pleomorphic LCIS can show high nuclear features with abundant cytoplasm [7, 8]. Both typical and pleomorphic LCIS, lack membranous E-cadherin immuno-positivity. Differentiating LCIS from DCIS is critical in clinical management of the patient. Generally, only E-cadherin immunostain is used to differentiate DCIS from LCIS. A strong diffuse membranous stain supports the diagnosis of DCIS and negative result supports LCIS. Although the characteristics of immunohistochemical (IHC) stain of E-cadherin have been well established, diagnosis based on a single negative stain may not be reliable, especially on small core biopsies. Thus a positive stain for LCIS is desirable and P120 catenin can serve as such positive stain for LCIS.

Recent studies show that P120 catenin is a valuable positive stain for LCIS [9-11]. DCIS, when stained with P120 catenin, shows strong membranous stain as E-cadherin, whereas LCIS shows redistribution of P120 catenin from membrane to cytoplasm [9-11].

With the wide use of mammographic screening and other radiographic imaging techniques, many breast cancers are being detected at an early stage. These lesions can be small in core needle biopsies, which make diagnosis difficult. Although most of the cases can be confidently diagnosed by morphology, a good proportion of cases may be diagnostically challenging. In those cases, IHC stains may be very helpful in arriving at the correct diagnosis.

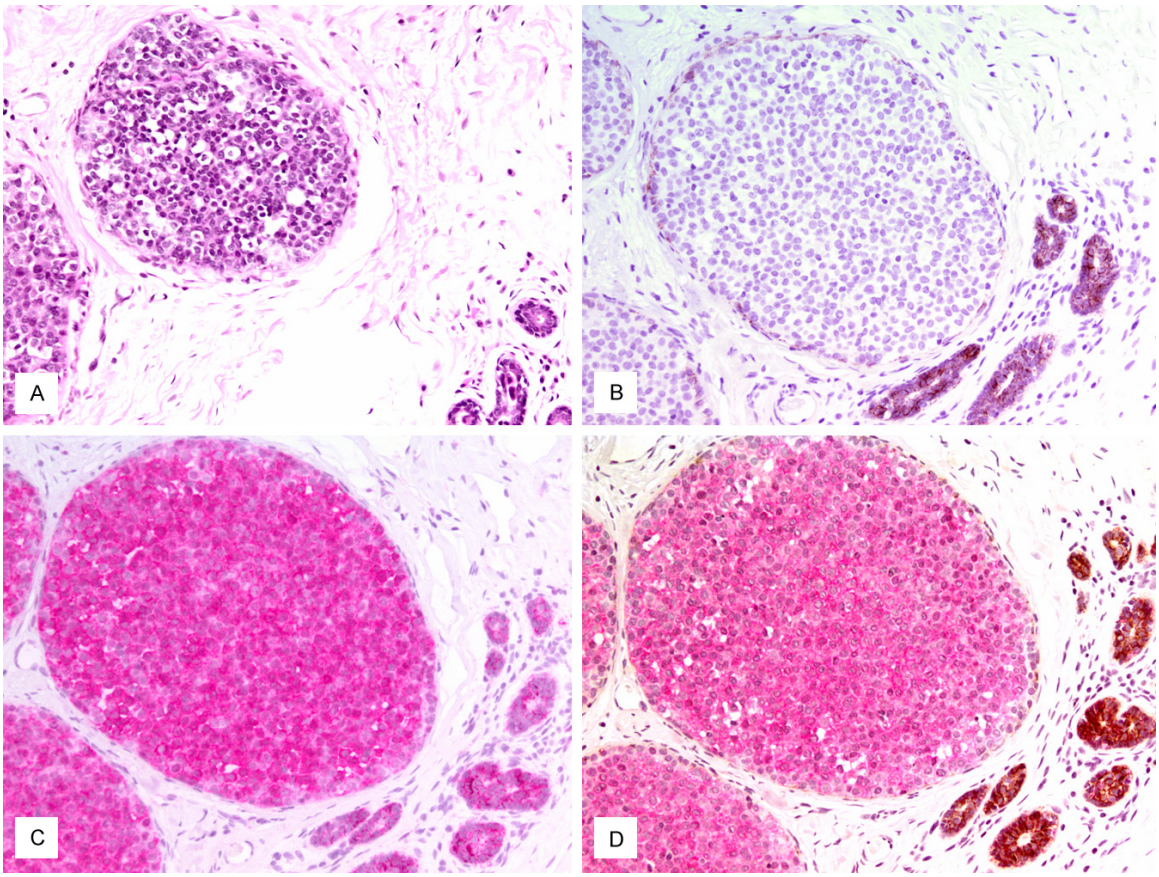

Figure 2. Immunoprofile of E-cadherin, P120 catenin and their cocktail in lobular carcinoma in situ. A. H&E section illustrates typical histology; B. Negative staining of E-cadherin; C. P120 catenin staining shows a diffuse strong cytoplasmic pattern; D. Cocktail of E-cadherin and P120: the LCIS component shows a diffuse cytoplasmic pattern, while the adjacent benign ducts serve as an internal control with a strong membranous pattern with E-cadherin.

Because of the small tissue volume, and even smaller volume of lesions, the number of IHC stains which can run a biopsy may be limited. To solve this problem, we developed a cocktail immunostaining composed of two primary antibodies raised against E-cadherin and P120 catenin from rabbit and mouse, respectively. The two primary antibodies were then detected using secondary antibodies conjugated with different colors of chromogen. Thus only one slide is required for the double staining with E-cadherin and P120 catenin. In this study, we validated the reproducibility of the antibody cocktail compared with conventional single IHC staining with E-cadherin or P120.

Materials and methods

Case selection

Twenty seven formalin fixed paraffin embedded tissue blocks from twenty six cases were retrieved from the archival files of the Department of Pathology and Genomic Medicine at Houston Methodist Hospital. All tissues were from either lumpectomy or mastectomy procedures. All tissues had been previously diagnosed as either DCIS or LCIS.

Immunohistochemistry

Four consecutive sections from the same paraffin embedded blocks were used for H&E and IHC stains. The immunohistochemistry protocol for individual antibody has been described previously (12). The cocktail immunostains were performed with Benchmark XT (Ventana Medical Systems, Inc, Tucson, Arizona). Briefly, the tissues were subjected to heat-induced epitope antigen retrieval for 30 minutes at 90 degrees using Solution Cell Conditioning 1. Then the sections were incubated with the cocktail composed of primary rabbit antibody for E-cadherin

Table 1. Expression pattern of E-cadherin, P120 catenin and their cocktail in DCIS and LCIS

Lesions (# of cases)	E-cadherin single antibody (# of cases)	P120 catenin single antibody (# of cases)	cocktail antibody (# of cases)	concordance
DCIS (15)	Membranous (15)	Membranous (15)	Membranous (15)	100%
LCIS (12)	Negative (12)	Cytoplasmic (12)	Cytoplasmic P120 catenin staining (12)	100%

(1:100, from Invitrogen, Camarillo, CA) and the primary mouse monoclonal antibody for P120 catenin (1:100, from BD Transduction Laboratories, location). The expression of E-cadherin was detected using the Ultraview Detection Kit and the expression of P120 was detected using the Ultraview Red Detection Kit (Ventana Medical Systems, Inc, Tucson, Arizona). 3, 3'-diaminobenzidine hydrochloride (DAB) was used as the chromogen for E-cadherin signal (brown color) and fast red was used to visualize the P120 signal (red). Then sections were counter-stained with hematoxylin before being mounted and examined by light microscopy.

Evaluation of immunohistochemistry

The positivity of E-cadherin stain using a single antibody was defined as diffuse strong membranous stain with a brown color. Focal weak E-cadherin stain was considered negative. The positivity of P120 catenin stain using a single antibody was defined as either strong diffuse membranous stain or strong diffuse cytoplasmic stain. Expected staining pattern for the antibody cocktail was double membranous staining for DCIS or cytoplasmic staining of P120 catenin for LCIS. Concordance of the antibody cocktail with each single antibody staining was evaluated.

Results

Staining patterns of ductal carcinoma in situ

Of the 27 tissues with breast CIS, 15 cases were DCIS. All cases of DCIS had positive membranous staining for E-cadherin (**Figure 1B**) or p120 catenin (**Figure 1C**) when a single antibody was used. When the 15 DCIS lesions were stained using the antibody cocktail of E-cadherin and P120, all demonstrated diffuse strong membranous staining in a mixed color of brown and red (**Figure 1D**), indicating positive membranous staining of both E-cadherin and P120 catenin. No cytoplasmic P120 catenin staining was identified using either single P120 antibody or the cocktail.

Staining patterns of lobular carcinoma in situ

Of the 12 LCIS cases, all were negative for E-cadherin staining (**Figure 2B**) and all showed strong diffuse cytoplasmic P120 catenin staining (**Figure 2C**) when single antibody was used. When these tissues were stained with the antibody cocktail, they showed diffuse strong cytoplasmic stain with a red color and no brown membranous staining was identified (**Figure 2D**). No membranous P120 catenin staining was identified in all the 12 LCIS cases. The staining patterns of E-cadherin, P120 catenin and the antibody cocktail in DCIS and LCIS are summarized in **Table 1**.

Discussion

The prevalence of breast carcinoma in situ has dramatically increased due in part to the wide spread use of screening examinations [13, 14]. DCIS has a 30-50% increased risk of developing into an invasive carcinoma if left untreated [12]. Unfortunately, it cannot be predicted which DCIS will progress to invasive carcinoma. Thus DCIS is surgically resected followed by radiation therapy in most cases of lumpectomy and hormonal therapy, if indicated [15, 16]. On the other hand, LCIS is usually low grade and a significant number of cases of LCIS do not cause breast mass, skin retraction or nipple retraction. The majority of LCIS cannot be detected by imaging studies [17, 18]. These lesions are often incidental findings. Because of the controversial role of LCIS as a precursor lesion or a risk marker, the management of LCIS varies from institution to institution. Some authors have advocated surgical removal of LCIS diagnosed on core biopsies as they found that a significant number of LCIS lesions were upstaged in subsequent excisions [18-21]. Others have failed to detect such a correlation and thus have suggested close follow-up and consideration of chemoprevention for LCIS [18, 19, 22, 23]. Because the clinical management of DCIS and LCIS can be dramatically different,

it is imperative to differentiate DCIS from LCIS on core biopsies.

The tumor cells in DCIS are cohesive with distinct cell boundary. The morphology of classic type LCIS is generally monotonous proliferation of low grade cells with discohesive features. Correct diagnosis based on morphology can be rendered for majority of cases. However, for challenging cases with overlapping histologic features, IHC stains are needed. Pleomorphic LCIS is a relatively recently defined entity and shows more pleomorphic nuclear features and morphologically mimicking DCIS [7, 8]. The distinction of pleomorphic LCIS from DCIS is mainly based on E-cadherin expression. Because of the limited number of reported cases and lack of long term follow ups, the biological behavior of pleomorphic LCIS is not clear. Surgical excision is recommended to treat pleomorphic LCIS.

Immunohistochemically, DCIS is positive for E-cadherin membranous stain, and LCIS is negative for E-cadherin stain. The membranous E-cadherin expression corresponds with the cohesive morphology of DCIS. Recent studies have shown that DCIS also shows the membranous staining of P120 catenin, while LCIS shows redistribution of P120 catenin with diffuse cytoplasmic staining [9, 10].

The transmembrane protein E-cadherin forms complex with α-, β-, γ-, and P120 catenins, which anchor E-cadherin to cytoplasmic actin filaments [24]. In normal breast tissue and DCIS, the E-cadherin is located on the membrane and the catenins are located at the inner membrane area. Thus DCIS shows diffuse and strong membranous stain for both E-cadherin and P120 catenin. LCIS, on the other hand, loses membranous E-cadherin and the inner membranous distribution of P120 catenin, which gives the discohesive morphology of LCIS. The loss of membranous distribution of both E-cadherin and P120 catenin are concurrent. The loss of E-cadherin causes redistribution of P120 from membranous to cytoplasmic in LCIS.

It is of special importance to differentiate DCIS from LCIS in tissue from fine needle aspirations or core needle biopsies. Traditionally, the diagnosis of CIS is based on morphology and if needed, E-cadherin immunopositivity. DCIS has positive membranous staining for E-cadherin, whereas LCIS is negative. However, diagnosis based purely on a single negative IHC stain could potentially be problematic. A negative IHC stain may reflect absence of the targeted antigen or may be false negative due to a variety of factors, including the quality of antibody, detection kit, procedural errors as well as many other technical and human errors. The concurrent use of the P120 catenin stain can address this issue and reduce the likelihood of false negative diagnoses.

The samples from core needle biopsies are usually small. The lesions in these samples can be even smaller. Extra levels of the blocks will increase the risk of depleting the lesion of interest. Our antibody cocktail will be very useful in these scenarios. This antibody cocktail allows two concurrent immunostains on the same slide. The results of our cocktail immunostains are 100% concordant with results of the single antibodies (**Table 1**).

Antibody cocktails have been used in the diagnosis of prostate cancers [25-28]. The published data indicate that the usage of antibody cocktails increases the sensitivity of detecting tumors. The same rationale may apply to our antibody cocktail.

In conclusion, our results indicate that our antibody cocktail composed of E-cadherin and P120 shows 100% concordance when compared with individual antibody stains of E-cadherin and P120 respectively. This antibody cocktail can be used to differentiate DCIS from LCIS, and may be especially valuable when handling small biopsies.

Disclosure of conflict of interest

None.

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