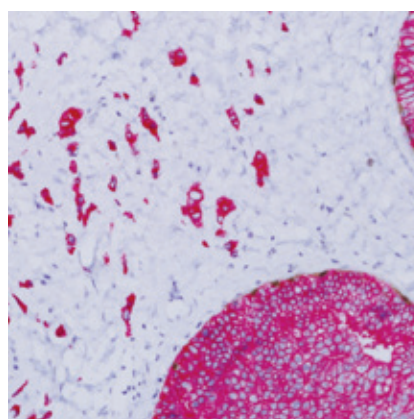
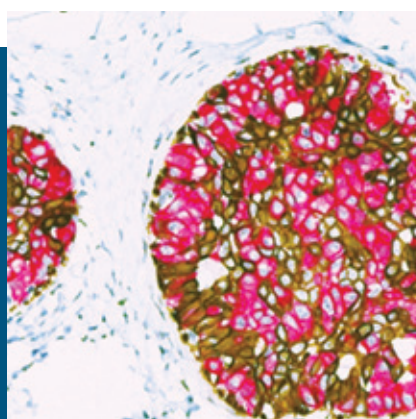
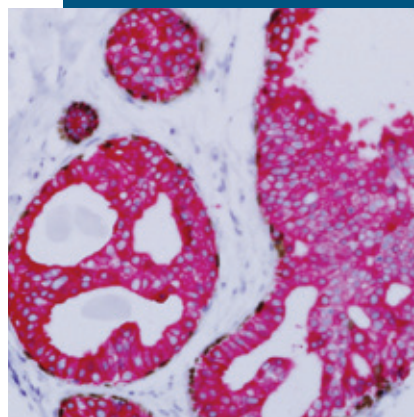
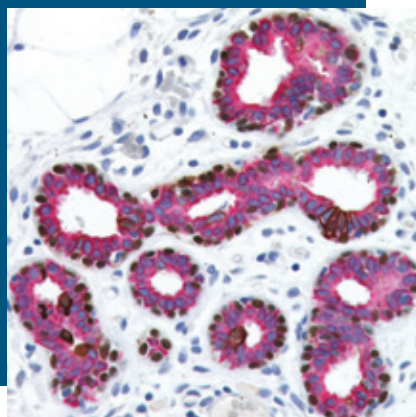
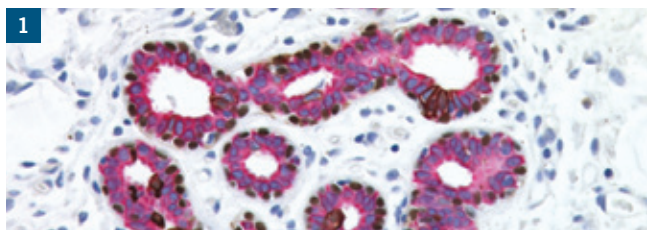


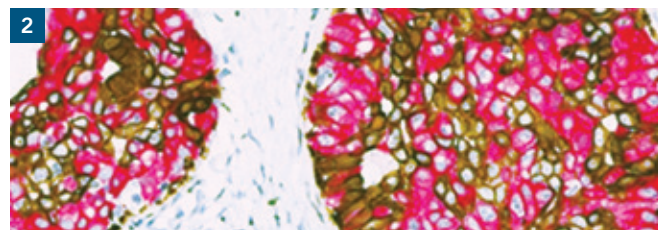
A Rapid Double Immunostaining Technique with a Single Cocktail of CK5, CK14, p63, CK7 and CK18 Distinguishes Between Hyperplasia of the Usual Type, Atypical Hyperplasia, Microinvasive and Basal Phenotype Breast Cancers

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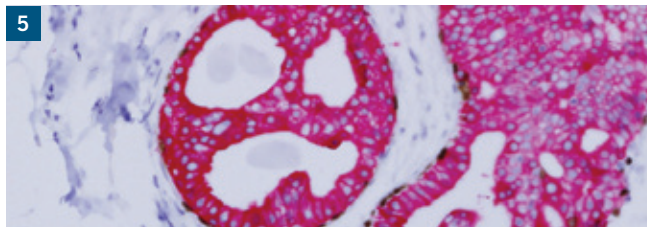




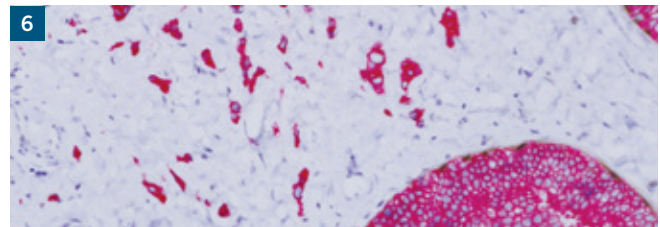
Normal breast tissue



Hyperplasia of the usual type; p63 staining basal myoepithelium cells (DAB); CK5/14 (DAB) and CK7/18 (FR) staining luminal epithelium



Atypical ductal hyperplasia; CK5/14 and p63 staining basal myoepithelium (DAB), CK7/18 staining luminal epithelium (FR)



Microinvasion; CK7/18 only (FR); loss of CK5/14 and p63 staining

Abstract

The aim of this study is to examine immunohistochemical staining characteristics in one hundred fifty benign proliferative breast disease, noninvasive breast malignancies and invasive breast cancers. In breast cancer, immunohistochemical classification of hyperplasia of the usual type versus atypical hyperplasia can be difficult. This is especially the case on core biopsies which are subject to tissue artifacts, such as crush and retraction. CK7, CK8 and CK18 have been shown to stain normal breast and provide excellent luminal staining for breast cancer.^{1,2,3} Recently, progenitor and/or adult stem cells have been identified which express CK5 and CK14, as do myoepithelial cells.

A 4-step rapid multiplex IHC technique has been developed to characterize a spectrum of intraductal epithelial proliferations, including ductal hyperplasia of usual type, atypical intraductal hyperplasia, ductal carcinoma in situ and micro-invasive carcinoma. A cocktail of CK5 + CK14 + p63 + CK7 + CK18 was developed and evaluated to simultaneously highlight progenitor and myoepithelial (CK5/14 and/or p63, DAB) and luminal cells (CK7/CK18, Fast Red). This cocktail was determined to be useful for distinguishing hyperplasia of the usual type (mixture of DAB and Fast Red staining cells) versus atypical hyperplasia (Fast Red staining cells). It easily identified micro-invasive breast carcinomas and aided in the identification of tumors with a basal phenotype, which were negative for ER/PR and c-erbB-2, and showed expression of CK5/p63 and/or CK14.

Background

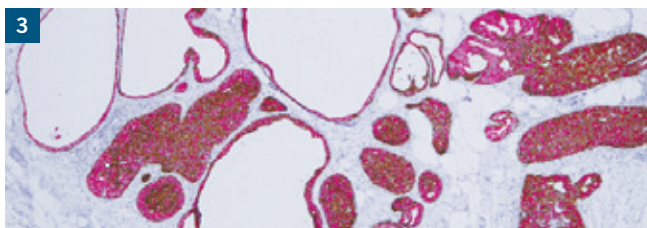
In breast cancer, ADH and usual hyperplasia are challenging diagnostic problems. Further, is microinvasion a commonly misdiagnosed cancer phenotype. Recently, cytokeratins (CK) CK5 and CK14 were characterized in normal breast tissue. These cells were shown to represent progenitor or adult stem cells that give rise to the glandular and myoepithelial cell lineage^{4,5,6}. CK7, CK8 and CK18 have all been shown to stain glandular epithelium in normal breast tissue and breast cancer. A rapid 4-step multiplex IHC technique was developed to characterize a spectrum of intraductal epithelial proliferations,

namely benign usual ductal hyperplasia, atypical ductal hyperplasia, microinvasion, ductal carcinoma in situ and basal phenotype, all of which are thought to represent a gradual sequence in developmental breast cancer. A cocktail of CK5 + CK14 + p63 + CK7 + CK18 was developed. The five antibody cocktail was designed to determine if we could distinguish invasive from non-invasive breast lesions with the presence or absence of myoepithelium (CK5/14 and/or p63, DAB) and glandular staining of breast cancer (CK7/CK18, Fast Red).

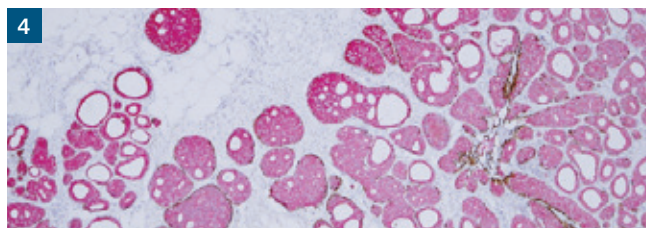
Methods and Materials

Monoclonal antibodies CK5, CK14, p63 and rabbit monoclonal antibodies CK7 and CK18 were individually titrated on breast cancer TMA tissues and then validated for specificity and staining intensity. All five antibodies were then multiplexed with a single antibody diluent and applied to formalin-fixed paraffin-embedded specimens. A biotin-free multistain detection reagent composed of a cocktail of goat-anti-mouse-HRP and goat anti-rabbit-AP was then applied. DAB and Fast Red chromogens were applied sequentially. Tissues were counterstained with hematoxylin. One hundred fifty breast cancer tissues including tissue microarrays and individual breast biopsies were used for analysis. A full spectrum of breast cancer tissues were analyzed.

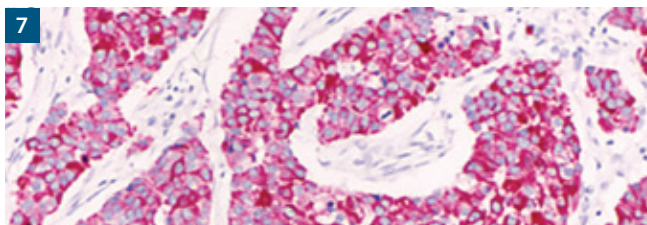
Antibody	Chromogen	Cell Type
CK5	DAB Brown	Progenitor cells Myoepithelial / luminal Basal phenotype
CK14	DAB Brown	Progenitor cells Myoepithelial / luminal Basal phenotype
p63	DAB Brown	Basal Myoepithelium Basal phenotype
CK7	FR Red	Normal breast cells Glandular epithelium Luminal epithelium
CK18	FR Red	Normal breast cells Glandular epithelium Luminal epithelium



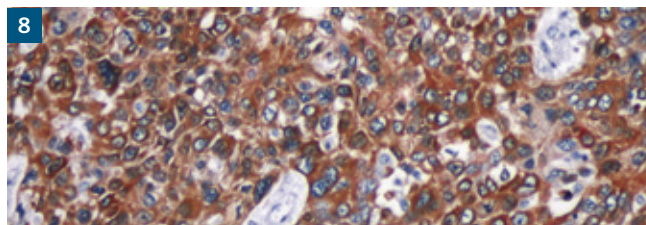
Hyperplasia of the usual type



Atypical hyperplasia



Invasive breast carcinoma; CK7/18 staining only



Basal phenotype; luminal expression CK5/14 (DAB)

Results

Staining patterns distinguished invasive from non-invasive breast lesions with the presence or absence of myoepithelium (CK5/14 and/or p63, DAB) and glandular staining of breast cancer (CK7/CK18, Fast Red). The cocktail was useful for the interpretation of normal tissue (photo 1) versus usual hyperplasia versus atypical hyperplasia. Usual ductal hyperplasia displays a luminal staining pattern with expression of both CK5/14 and CK7/18. Residual p63 was observed in the nuclei of the myoepithelium (photos 2/3).

This is in contrast to atypical ductal hyperplasia or ductal carcinoma in situ, which display the differentiated glandular immunophenotype (CK7/CK18 positive, fuschia to pink), but are CK5/14-negative (photos 4/5) except for the myoepithelium.

Microinvasive breast cancer was easily identified as invasive tumor cells were CK7/CK18 positive (Fast Red) (photo 6).

Invasive ductal carcinoma was stained with CK7/18 in a variety of staining intensities associated with histological grade (photo 7). A decrease in CK7/18 staining was observed with increased histologic grade.

Finally, in breast cancers that were typically ER/PR and c-erbB-2 negative (triple negative), staining with CK5/14 was observed. Basal phenotypes were mostly high-grade and consistently showed expression of CK5/14 (DAB) and some p63 (DAB) (photo 8).

Discussion

The use of cytokeratins for diagnosis of usual hyperplasia and atypical ductal hyperplasia has been shown using fluorescent techniques in the past, 6 however, use of a rapid multiplex IHC application employing a five-antibody cocktail has not been demonstrated. In this study, we were able to obtain a limited number of cases of atypical hyperplasia and microinvasion; despite limited tissue, the staining patterns for these phenotypes were consistent, thus, this rapid 4-step multiplex stain shows great promise. The application of this multiplex stain is

intriguing because of the many phenotypes that can be diagnosed on a single tissue section which is particularly significant in cases where limited tissue is an issue. The CK7 and CK18 have been shown to stain a wide spectrum of breast cancers. 3

Interestingly, CK5/14 and/or p63 have been shown to correlate with true basal phenotype which is highly correlated with BRCA1 tumors in invasive breast carcinomas, giving further evidence for the pathogenesis of the basal phenotype of breast cancer. 7,8

Conclusion

- This 5 antibody cocktail has great utility and may be used for interpretation of breast cancer on a single slide.
- This cocktail differentiates between benign usual ductal hyperplasia, atypical ductal hyperplasia, microinvasion, invasive ductal carcinoma and true basal phenotypes.
- The five stages outlined above are thought to represent a gradual (developmental) sequence in breast cancer.
- This new technology can be applied manually and on some automated stainers.

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