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RESEARCH ARTICLE

Columnar Cell Lesion and Apocrine Hyperplasia of the Breast: Is There a Common Origin? The Role of Prolactin-induced Protein

Amedeo Sciarra, MD, Gianluca Lopez, MD,* Chiara Corti, MD,* Letterio Runza, MD,* Giulia Ercoli, BS,* Arturo Bonometti, MD,* Luca Despini, MD,† Concetta Blundo, MD,† Donatella Gambini, MD,‡ and Nicola Fusco, MD*§*

Abstract: Noninvasive breast lesions encompass a heterogeneous group of risk indicators and nonobligate precursors of breast cancer, such as apocrine hyperplasia (AH) and columnar cell lesions (CCLs). Given the different expression of ER and ER-regulated genes in AH and CCL, these 2 alterations are currently considered discrete conditions. However, whether they share early biologic changes is not clear to date. Here, we sought to define the clinicopathologic and immunohistochemical features of a prospective series of combined lesions made up by CCLs and AH forming a continuum within single TDLUs. The study group included 19 cases, whereas 25 cases of synchronous contiguous CCLs and AH served as control group. The different components of each case were subjected to immunohistochemical analysis for ER, PR, AR, HER2, BCL2, CCND1, MUC1, and PIP. Although CCLs and AHs arising in continuity showed opposite patterns of ER expression, the PIP-positive apocrine signature was consistently present in both components. In conclusion, apocrine changes are highly recurrent in CCLs growing within foci of AH, regardless of the ER activation. Our results suggest that PIP-positive and PIP-negative CCLs are likely to represent biologically distinct conditions and that apocrine changes might occur earlier than ER activation in the natural history of breast precursor lesions.

Key Words: breast, fibrocystic changes, apocrine hyperplasia, columnar cell lesion, immunohistochemistry, PIP, GCDFP15

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Breast cancer risk indicators, precursors, and nonobligate precursors are part of a complex and heterogeneous group of lesions, which represents a matter of remarkable interest from both clinical and biological standpoints.[1] These frequent alterations of the breast range from benign metaplastic changes to high-grade ductal carcinoma in situ (DCIS) and are associated with an increased probability of finding neighboring malignancies.[1,2] Their histologic subclassification is usually straightforward and morphology-based.[3] However, in a subset of cases, particularly in bioptic samples, the diagnosis might be challenging, given their tight dimensions and overlapping morphologies.[2,4,5]

On the basis of the activation of the estrogen receptor (ER) and ER-regulated genes, a multistep model of breast cancer evolution, encompassing most of the precursors and nonobligate precursors of breast cancer, has been proposed.[1] To date, high-throughput sequencing studies are validating this hypothesis.[5–8] Regrettably, the individual risk assessment of breast noninvasive lesions is far from being achieved. Among all, subclassification of columnar cell lesions (CCLs) is particularly challenging.[9] The alterations belonging to this group are morphologically characterized by dilated acinar structures lined by a single layer of ER-positive, HER2-negative bland columnar cells with apical snouts, showing different degrees of cytological atypia.[3,9,10] Molecular studies showed that the majority of CCLs, together with atypical ductal hyperplasia (ADH) and low-grade DCIS, are clonal and nonobligate precursors of invasive breast cancer, belonging to the so-called "low-grade breast neoplasia family."[2] CCLs are often seen in association with a wide spectrum of other breast alterations, including apocrine hyperplasia (AH).[10] This common condition is histologically defined by the presence of cells with abundant eosinophilic cytoplasm, containing small, glycolipid granules, and large round-to-oval monomorphic nuclei with prominent nucleoli.[7,11] AH immunophenotype shows substantial differences compared with CCLs, given the lack of ER expression and the diffuse expression of the prolactin-induced protein (PIP), also known as gross cystic disease fluid protein 15.[7,12,13] At present, the role of apocrine changes in breast cancer tumorigenesis is a subject of debate among pathologists, with several observational and genetic studies suggesting that AH is a bona fide risk indicator for breast cancer development.[1] Furthermore, based on the identification of allelic alterations in apocrine

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From the *Division of Pathology; †Breast Surgery Unit; ‡Oncology Unit, Fondazione IRCCS Ca' Granda—Ospedale Maggiore Policlinico, via Francesco Sforza 35 20122, Milan, Italy; and §Department of Biomedical, Surgical, and Dental Sciences, University of Milan, Milan, Italy.
The authors declare no conflict of interest.
Reprints: Nicola Fusco, MD, Division of Pathology, Fondazione IRCCS Ca' Granda—Ospedale Maggiore Policlinico, Via Francesco Sforza, 35, 20122 Milan, Italy (e-mail: nicola.fusco@unimi.it).

lesions, it has recently been hypothesized that at least a subset of them are clonal.[7]

Although CCLs and AH display distinct repertoires of morphologic, immunophenotypic, and molecular features, their coexistence is frequently observed.[3,14] However, the biologic relationship between these 2 lesions has yet to be elucidated. The aim of the current study was to define the clinicopathologic and immunohistochemical features of a prospective series of combined lesions made up by CCLs and AH arising in continuity within single terminal duct-lobular units (TDLUs).

MATERIALS AND METHODS

Patients and Tissue Specimens

Nineteen cases of breast fibrocystic changes made up by CCLs forming a continuum with AH inside of the same TDLU were included in this study. Specifically, 17 cases were prospectively collected between January 2015 and May 2016 in the Division of Pathology, Fondazione IRCCS Ca' Granda—Ospedale Maggiore Policlinico, Milan, Italy, whereas 2 additional surgical samples were retrieved from our institutional database. Follow-up time ranged from 13 to 58 months. As a control group, 25 breast bioptic or surgical samples in which both CCLs and AH coexisted in contiguity but not in continuity were retrospectively retrieved. Clinical and follow-up data were recorded for all patients. The samples were anonymized before analysis, and the study was fully compliant with the local ethical guidelines. All cases were reviewed independently by 2 breast pathologists (L.R. and N.F.) and classified according to standard criteria.[2,3]

Immunohistochemistry

Representative 4 μm-thick sections of all cases were subjected to immunohistochemical analyses using prediluted antibodies against ER, PR, androgen receptor (AR), HER2, B-cell lymphoma 2 (Bcl2), cyclin D1 (CCND1), MUC1, and PIP. For each case, the expression of all markers was evaluated separately in the morphologically distinct components. Briefly, the protocol uses the Dako automated staining platform (Dako Omnis) and antihuman prediluted antibodies.[15,16] Protein expression was analyzed separately in the discrete components. The methods and scoring systems employed followed previously reported criteria[17–21] and guidelines,[22,23] as detailed in Table 1.

RESULTS

All patients included in the study group except one were female (18/19, 94%), with a mean age at diagnosis of 53 years (range, 32 to 72). Bioptic procedures, either core or vacuum-assisted biopsy, were performed in 17 of 19 (89%) cases, whereas in the remaining 2 cases the tissue was obtained after surgical excision. All cases showed foci of fibrocystic changes in which a single TDLU displayed the abnormal presence of single-layered, pseudostratified bland columnar cells with apical snouts, and an adjacent hyperplastic proliferation of eosinophilic cells with abundant cytoplasm, monomorphic nuclei, containing glycolipid granules, consistent with a CCLs growing within foci of AHs (Fig. 1). Adjacent breast carcinoma of no special type was present only in the 2 surgically resected specimens and in 1 bioptic specimen (3/19, 16%). Interestingly, only 1 patient developed an invasive carcinoma during the follow-up time. In this case, CCLs and AH were associated with LCIS at the time of diagnosis. Other patients without carcinoma at the diagnosis presented an indolent behavior. Clinicopathologic features of the study group are detailed in Table 2.

At immunohistochemical analysis, as expected, CCLs showed a diffuse ER, PR, BCL2, expression, while lacking AR, HER2, CCND1, and MUC1. Conversely, the

TABLE 1. List of Antibodies, Clones, Dilutions, Antigen Retrieval Methods, and Scoring Systems Adopted for Immunohistochemical Analyses

Marker	Clone	Dilution	Antigen Retrieval	Company	Scoring
ER	EP1	RTU	EnVision FLEX, high pH, 20′	Dako	ASCO/CAP guidelines; positive if ≥1% of tumor cell nuclei are immunoreactive[22]
PR	PgR 636	1:100	EnVision FLEX, high pH, 30′	Dako	ASCO/CAP guidelines; positive if ≥1% of tumor cell nuclei are immunoreactive[22]
AR	AR441	1:50	EnVision FLEX, high pH, 20′	Dako	Positive if ≥1% cells showed a faint to strong nuclear staining[17]
HER2	Polyclonal	1:400	EnVision FLEX, low pH, 30′	Dako	ASCO/CAP guidelines; positive if ≥1% complete, intense circumferential membrane staining[23]
Bcl2	124	RTU	EnVision FLEX, high pH, 30′	Dako	Positive if ≥1% cells showed strong nuclear staining[18]
Cyclin D1	EP12	RTU	EnVision FLEX, high pH, 30′	Dako	Semiquantitative scoring according to the Allred method[19]
MUC1	E29	RTU	EnVision FLEX, high pH, 30′	Dako	Positive if ≥1% showed any cytoplasmic staining[20]
PIP	23A3	RTU	EnVision FLEX, high pH, 30′	Dako	Positive if cells showed strong and diffuse cytoplasmic staining[21]

AR indicates androgen receptor; Bcl2, B-cell lymphoma 2; ER, estrogen receptor alpha; MUC1, mucin 1 cell surface associated (aka EMA, epithelial membrane antigen); PIP, prolactin inducible protein (aka GCDFP15, gross cystic disease fluid protein 15); PR, progesterone receptor; RTU, ready to use.

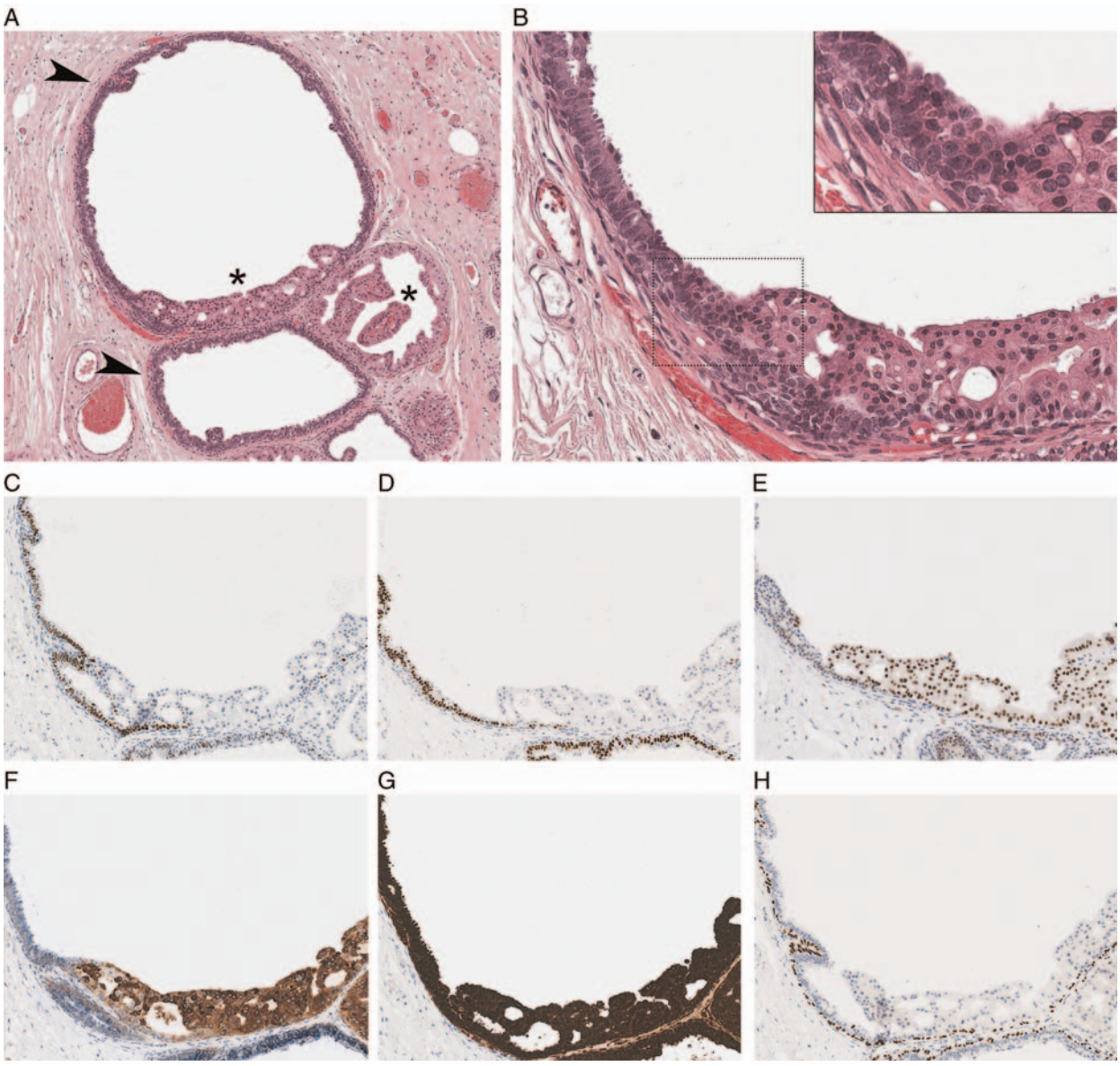

FIGURE 1. Representative micrographs of a columnar cell lesion and associated apocrine hyperplasia involving the same terminal duct. In this case, the columnar cell lesion (arrowheads) formed a continuum with a focus apocrine hyperplasia (stars) (A, hematoxylin and eosin, original magnification ×50) in the context of fibrocystic changes (lower part of the micrograph). At higher magnification (B, hematoxylin and eosin, original magnification ×200), the so-called "hybrid cells" were observed in the transition area between the 2 components, bearing intermediate cytological features, as shown in the inset (hematoxylin and eosin, original magnification ×400). The columnar cell lesion displayed ER (C) and PR (D) coexpression, whereas the adjacent apocrine hyperplasia showed strong positivity for AR (E) and cyclin D1 (F). Intriguingly, both the columnar cell lesion and apocrine hyperplasia shared an apocrine immunophenotype, as demonstrated by the strong and diffuse PIP overexpression in both components (G). The intact layer of myoepithelial cells surrounding the duct was highlighted by expression of p63 (H). ER indicates estrogen receptor; PR, progesterone receptor; AR, androgen receptor; PIP, prolactin-induced peptide.

areas of AH showed diffuse AR, CCND1, and MUC1 expression, while lacking ER, PR, and BCL2 expression. Intriguingly, despite these well-known divergent immunoprofiles, both components showed strong and diffuse PIP overexpression in 100% of the cases belonging to the study group (Fig. 1). Among the control group, only the AH showed a PIP-positive apocrine immunoprofile, whereas the contiguous CCL was consistently PIP-negative (Fig. 2). The results of the immunohistochemical analysis are summarized in Table 3.

DISCUSSION

In this study, we have characterized at morphologic and immunohistochemical levels a consecutive series of CCLs forming a continuum with foci of typical AH in the

TABLE 2. Clinicopathologic Features of 19 Columnar Cell Lesions Forming a Continuum With Apocrine Hyperplasia

Case	Age	Sex	Location	Specimen	Microcalcifications	Associated Lesions	EUSOMA Pathological Classification	Follow-up (mo)
CCLAH 1	47	Female	UOQ, right breast	Core biopsy	Yes	Fibrocystic changes	B2	NED (29)
CCLAH 2	56	Female	UIQ, right breast	Core biopsy	Yes	Fibrocystic changes	B2	NED (22)
CCLAH 3	72	Female	LOQ, right breast	Core biopsy, VAB	Yes	Fibrocystic changes, ADH	B3	~~Low grade~~ DCIS and fibrocystic changes at VAB (1); NED (21)
CCLAH 4	72	Female	UOQ, left breast	VAB	Yes	Fibrocystic changes	B2	NED (20)
CCLAH 5	62	Female	UOQ, right breast	Core biopsy	Yes	Fibrocystic changes, FEA	B3	NED (20)
CCLAH 6	58	Female	LIQ, right breast	Core biopsy	Yes	Fibrocystic changes	B2	NED (19)
CCLAH 7	70	Female	UOQ, right breast	Core biopsy	No	Fibrocystic changes, UDH	B2	NED (18)
CCLAH 8	53	Female	UOQ, left breast	Core biopsy	Yes	Fibrocystic changes	B2	FEA at core biopsy (12); FEA at core biopsy (1); FEA and fibrocystic changes at VAB (1); NED (17)
CCLAH 9	57	Female	UOQ, right breast	VAB	Yes	Fibrocystic changes, classic LCIS	B3	NED (3), NED (3), ILC (17)
CCLAH 10	59	Female	UOQ, right breast	VAB	Yes	Fibrocystic changes	B2	NED (16)
CCLAH 11	32	Male	LOQ, left breast	Core biopsy	No	Fibrocystic changes	B2	NED (16)
CCLAH 12	40	Female	UIQ, right breast	Core biopsy	Yes	Fibrocystic changes, FEA	B3	NED (1), NED (7), NED (15)
CCLAH 13	66	Female	UOQ, right breast	Core biopsy	Yes	Fibrocystic changes	B2	NED (14)
CCLAH 14	41	Female	LOQ, right breast	VAB	Yes	Fibrocystic changes, IC NST with apocrine differentiation	B5	Recurrence of IC NST (8)
CCLAH 15	53	Female	LIQ, right breast	Core biopsy	No	Fibrocystic changes	B2	NED (14)
CCLAH 16	44	Female	UOQ, right breast	Core biopsy	Yes	Fibrocystic changes	B2	NED (14)
CCLAH 17	39	Female	UOQ, left breast	Core biopsy	Yes	Fibrocystic changes	B2	NED (13)
BR_134	50	Female	UOQ, right breast	Quadrantectomy	No	Fibrocystic changes, IC NST	n/a	NED (58)
BR_205	47	Female	UOQ, right breast	Quadrantectomy	Yes	Fibrocystic changes, IC NST	n/a	NED (42)

ADH indicates atypical ductal hyperplasia; FEA, flat epithelial atypia; IC NST, invasive carcinoma of no special type; ILC, invasive lobular carcinoma; LIQ, lower-internal quadrant; LOQ, lower-outer quadrant; n/a, not applicable; NED no evidence of disease; UIQ, upper-internal quadrant; UOQ, upper-outer quadrant; VAB, vacuum-assisted biopsy.

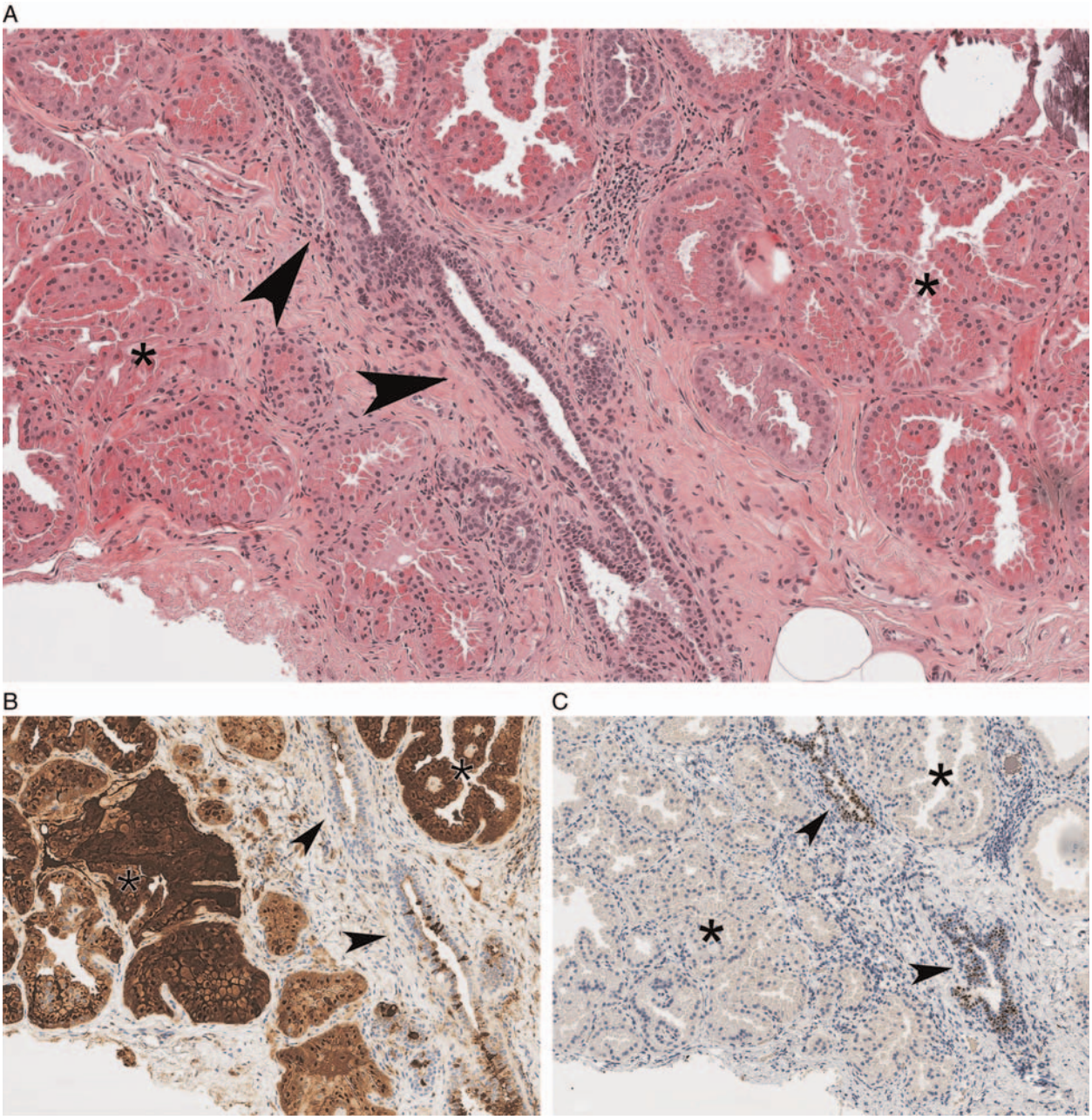

FIGURE 2. Representative micrographs of fibrocystic changes in which columnar cell lesion and apocrine hyperplasia are present in proximity but not in continuity. In this case, belonging to the control group, both columnar cell lesion (arrowheads) and apocrine hyperplasia (stars) were present within the same area of fibrocystic changes (A, hematoxylin and eosin, original magnification ×100) with associated numerous microcalcifications (upper right corner of the micrograph). The 2 lesions did not display any feature of morphologic conglomeration and PIP (B) and ER (C) proteins showed the characteristic patterns of expression of columnar cell changes and apocrine hyperplasia, respectively, in which the former is PIP-negative and ER-positive, whereas the latter is PIP-positive and ER-negative. ER indicates estrogen receptor; PIP, prolactin-induced peptide. full color online

context of the same TDLU and demonstrated that the apocrine immunophenotype is shared by the 2 contiguous components, regardless of their ER activation status.

The unprecedented comprehension that ER-positive and ER-negative lesions of the breast are clinically discrete conditions has designated the road to precision medicine in breast cancer patients.[1] However, based on the possible coexistence of these lesions, particularly at very early tumorigenic phases,[9,14,24] pathologists and oncologists are now expected to define the clinical implications of ~~the~~ interplay ~~between AH and CCLs as risk indicators and precursors of breast cancer~~. In this era of precision medicine, novel biomarkers that are able to predict the outcome related to noninvasive and preinvasive lesions at an individualized

TABLE 3. Immunophenotypic Features of 19 Columnar Cell Lesions Forming a Continuum With Apocrine Hyperplasia (Study Group) and 25 Cases of Fibrocystic Changes Where Columnar Cell Lesions and Apocrine Hyperplasia Were Contiguous but not Continuous (Control Group)

	Study Group (19/19 Cases)		Control group (25/25 Cases)	
	Columnar Cell Lesion	Apocrine Hyperplasia	Columnar Cell Lesion	Apocrine Hyperplasia
ER	+	−	+	−
PR	+	−	+	−
AR	−	+	−	+
HER2	−	−	−	−
BCL2	+	−	+	−
CCND1	−	+	−	+
MUC1	−	+	−	+
PIP	+	+	−	+

In all cases where the 2 lesions involved the same terminal duct PIP was overexpressed in both components.

AR indicates androgen receptor; BCL2, B-cell lymphoma 2; CCMD1, cyclin D1; ER, estrogen receptor; PIP, prolactin-induced protein; PR, progesterone receptor.

level are warranted. In this respect, PIP (also known as gross cystic disease fluid protein 15, GCDFP15) is a marker of apocrine differentiation belonging to a family of glycoproteins that has been originally identified in the fluid of breast cysts of perimenopausal women.[25,26] This 17-kDa single protein is ubiquitously present in apocrine, lacrimal, salivary, ceruminous, Moll's, and eccrine glands but also in apocrine breast ductal cells and the so-called apocrine metaplasia.[26,27] Importantly, PIP is a well-known breast cancer associated polypeptide, given its overexpression in the majority of breast tumors.[28] It has a significant function in the biology of breast alterations, being involved in the mediation of cell invasion and regulation of signaling, particularly in ER-negative cases.[29,30] Antibodies against PIP have been shown to represent a useful tool for classifying breast carcinoma and its metastatic deposits.[27,31–33] It is of note, however, that this protein shows intratumor heterogeneous expression in the vast majority of breast cancers.[32] Our results showing that PIP overexpression can be irrespective of ER activation in the context of fibrocystic changes corroborate the notion that this protein is likely to play a key role in the multistep evolution of breast cancer, as previously hypothesized.[7,34,35]

Given that breast cancer cells have been shown to release PIP through increased androgen and prolactin levels, and that high estrogen levels are able to inhibit its release, this protein has been proposed as a biomarker to predict the nature of breast tumors.[28,32] The burgeoning interest in the multitude of noninvasive breast abnormalities may reflect their improved recognition by pathologists in everyday practice and the progress that are currently being made in the use of high-throughput sequencing methods. However, the risk assessment of each nonmalignant lesion has by no means fully clarified.[4,5,9,14,36,37] In particular, apocrine metaplasia and AH represent 2 distinct types of alterations within the fibrocystic changes spectrum and almost invariably display strong and diffuse PIP expression.[14,25] The role of this polypeptide, however, has never been explored in associated CCLs. Recurrent allelic imbalances and copy number alterations, as well as recurrent alterations in the epidermal growth factor receptor family genes, have been reported in CCLs and associated lesions, leading to the notion that these entities are likely to represent the earliest histologically detectable nonobligate precursors of low-grade breast cancer.[9,37] Despite CCLs and AH are currently considered biologically discrete conditions harboring distinct repertoires of molecular alterations, our results suggest that, under certain circumstances, they may exist along the same neoplastic spectrum, and not only as a mere association, as documented by the strong and diffuse immunohistochemical overexpression of PIP in both components.

In conclusion, the results of the current study provide novel insights in the subclassification of nonobligate precursors of breast cancers, where PIP-positive and PIP-negative CCLs can represent histologically distinct conditions. Our observations suggest that apocrine changes may constitute an early phenotypic connection between ER-positive and ER-negative noninvasive breast lesions. Whether apocrine changes could represent the trigger of a subset of ER-positive neoplastic processes, remains to be elucidated. In this respect, wide molecular studies are warranted to perform analyses on a large scale of nonmalignant lesions and their possible combinations. This would provide the substrate for broadening our understanding of risk indicators and nonobligate precursors to breast cancer, their biology, and the role of apocrine lesions in breast cancer tumorigenesis.

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