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Atypical cystic lobules: an early stage in the formation of low-grade ductal carcinoma in situ

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Abstract Evidence from many studies has established the neoplastic potential of ductal carcinoma in situ, but the origin and the morphological characteristics of the early stages of this proliferation remain unidentified. Workers writing in the early twentieth century observed a cystic transformation of lobules and proposed that it represented one such early stage, and contemporary European and Japanese pathologists have reached the same conclusion. We describe the characteristics of this cystic transformation, which we call us "atypical cystic lobules," and present evidence to support the proposal that the alteration is a step in the formation of low-grade ductal carcinoma in situ. Atypical cystic lobules are a proliferation of luminal cells showing low-grade cytological atypia without architectural atypia. The study group comprised 21 cases of atypical cystic lobules from specimens also showing conventional low-grade ductal carcinoma in situ or lobular neoplasia. Immunohistochemical staining for hormone receptors, keratin 19, and cyclin D1 revealed that atypical cystic lobules demonstrated a consistent immunophenotype, which differs from the pattern shown by normal lobules and benign lesions and matches that of low-grade ductal carcinoma in situ. In about 40% of the cases, atypical cystic lobules merged with fully established micropapillary/cribriform ductal carcinoma in situ. The similarities in the cytological and immunohistochemical features and the proximity of the two types of proliferation suggest that atypical cystic lobules represent an early stage in the formation of certain types of low-grade ductal carcinoma in situ.

Key words Atypical ductal hyperplasia · Ductal carcinoma in situ · Estrogen receptor · Keratin 19 · Cyclin D1

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Introduction

In 1931, Sir Lenthal Cheatle proposed that invasive breast cancer arises from a malignant intraductal proliferation, which he called "epithelial neoplasia that is still confined within normal boundaries" [8]. One year later, Dr. Albert Broders suggested the term "carcinoma in situ" for this form of proliferation [5]. A large body of clinical and pathological studies carried out in the subsequent 65 years has validated Dr. Cheatle's proposal and established Dr. Broders' designation in common usage. The same half century has also seen the emergence of the belief that ductal carcinoma in situ originates from cells of the terminal duct lobular unit, which undergo a stepwise transformation from normal cells to carcinoma cells. The morphological features of the intermediate stages predicted by this theory remain undefined. Many eminent early twentieth century students of breast pathology recognized a distinctive cystic transformation of lobules in which the glandular cells became columnar and the acini dilated, and these observers regarded the change as an early step in the formation of ductal carcinoma in situ. In the contemporary literature references to this type of alteration are rare. European writers call it "well-differentiated clinging carcinoma of flat type" [1, 9–11], Americans, "atypical ductal hyperplasia" [12, 34], and Japanese authors, "atypical cystic duct" [36] and

"atypical lobule" [16]. In this report we present morphological and immunohistochemical evidence to support the hypothesis that this atypical proliferation, which we call "atypical cystic lobule," is a forerunner of certain common forms of low-grade ductal carcinoma in situ.

Materials and methods

The study cases were clinical specimens received by the pathology Department of the Massachusetts General Hospital between 1992 and 1997. We collected examples of three types of lesions: low-grade ductal carcinoma in situ (44 cases); lobular carcinoma in situ (31 cases), and fibrocystic changes (32 cases). By examining the original hematoxylin-eosin stained sections, we confirmed the original diagnosis, determined the presence of atypical cystic lobules and conventional ductal hyperplasia, and chose samples for immunohistochemical staining. The paraffin blocks contained sufficient material to stain atypical cystic lobules in 21 specimens (16 cases also harboring ductal carcinoma in situ and 5 cases also containing lobular neoplasia) and sufficient to stain foci of ductal carcinoma in situ in 13 cases. We studied the adjacent tissue to determine the properties of normal lobules (type 1 and type 2/3), fibrocystic changes (apocrine metaplasia, microcyst formation, and ductal-type epithelial hyperplasia), and blunt duct adenosis.

We applied conventional morphological criteria in making the diagnoses of low-grade ductal carcinoma in situ, lobular carcinoma in situ, and ductal-type epithelial hyperplasia [28]. The atypical cystic lobules that we studied each consisted of a distended terminal duct lobular unit lined by a single layer of epithelial cells showing low-grade cytological atypia. We excluded from our group of atypical cystic lobules any ductal proliferations with pleomorphic nuclei and any low-grade carcinomas forming micropapillary tufts, cribriform spaces, Roman arches, or trabecular bars. The atypical cystic lobules in our study group displayed the following characteristics. The affected terminal duct lobular unit appeared enlarged, often to a size of 1–2 mm (Fig. 1). Such enlargement of the lobule develops because of distention of acini rather than an increase in their number. In fact, in our specimens, the number of acini often seemed smaller than usual; a simple or pseudostratified epithelium lined the distended acini (Fig. 2). Neither true stratification nor the formation of papillary structures or cribriform spaces occurred; luminal cells constituted the predominant type of epithelial cell (Fig. 2). The basal (myoepithelial) cells that remained varied in number and distribution. They most commonly appeared to be fewer and more widely spaced than normal, especially in the most distended acini; however, portions of an affected lobule sometimes showed the usual number of basal cells arranged in a continuous layer. In a well-developed atypical lobule, basal cells amounted to less than 10% of the epithelial population.

The luminal cells usually had a cuboidal or columnar shape and sometimes looked extremely tall and closely crowded (Fig. 2). The nuclei were sited at the base of the cells, and cytoplasm collected near the lumen to form an abundant apical cytoplasmic compartment. When the crowding was extreme, the nuclei occupied varying positions within the cells, creating a pseudostratified pattern. These nuclei exhibited cytological characteristics of low-grade atypia (Fig. 2). The nuclear shapes varied from round to long, slender ovals, and the nuclei had smooth contours. The chromatin appeared finely dispersed and slightly hyperchromatic. Nucleoli were difficult to see. The specialized stroma of the terminal duct lobular unit (fibroblasts and myxoid ground substance) did not appear to be noticeably increased. The stroma consists of fibroblasts and myxoid ground substances. The intralobular capillaries appeared more numerous and larger than usual in a few cases, and small, round lymphocytes occupied the specialized stroma in others.

Since the majority of the cells of low-grade ductal carcinoma in situ stain for estrogen receptors [6, 39], progesterone receptors [39], keratin 19 [2], and cyclin D1 [13], we chose these proteins to characterize the immunohistochemical profiles of lesions we studied.

The antibodies used and their dilutions are as follows: monoclonal anti-cyclin D1, 1:100, (clone 5D4 was kindly supplied by Dr. M. Seto, Aichi Cancer Center, Japan); monoclonal anti-keratin 19, 1:100 (clone RCK 108; DAKO, Carpenteria, Calif.); anti-estrogen receptor, 1:10, (clone 1D5; DAKO); anti-progesterone receptor, 1:5, (clone PGR-1A6; Biogenex, San Ramon, Calif.). The specificity of the anti-cyclin D1 antibody has been described and confirmed previously [19, 24], and we have published data summarizing the sensitivity and specificity of the staining for estrogen and progesterone receptors in our laboratory [14, 18].

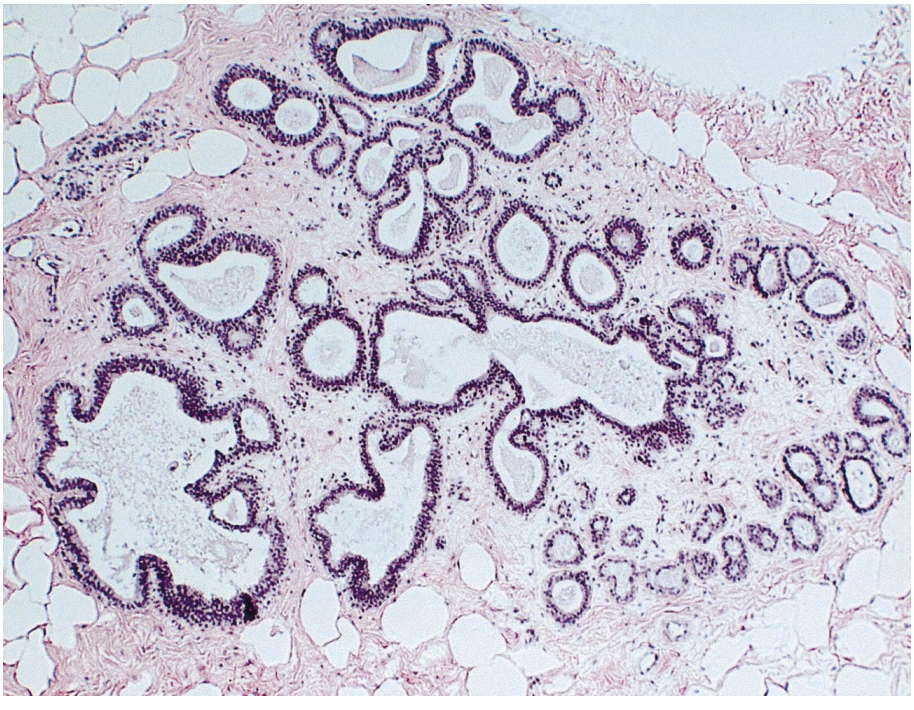


Fig. 1 Atypical cystic lobule (medium magnification). The acini of this lobule appear enlarged and lined by a single layer of epithelial cells. ×125

Immunohistochemical analyses were performed on formalin-fixed, paraffin-embedded tissue sections using slight modifications of the avidin–biotin peroxidase complex method [17]. The sections were deparaffinized, endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol, and the sections were washed with phosphate-buffered saline. The sections were then treated with one of several antigen retrieval techniques. Staining for cyclin D1 required heating in citrate-buffered saline (10 mM, pH 6.0) to 90°C for 7 min. For keratin 19 staining, the sections were digested with pepsin for 15 min at 37°C. Sections for estrogen and progesterone receptor detection were heated in citrate-buffered saline to 90°C for 20 min. After antigen retrieval, the usual ABC method was followed. Diaminobenzidine was used as the chromagen and hematoxylin as the counterstain.

Staining of the appropriate cellular component constituted a positive result. We used the following semiquantitative scores to estimate the number of positive cells in each section: 0–5% negative; 5–50% 1+; and over 50% 2+.

Results

Observations of our 16 cases harboring both atypical cystic lobules and low-grade ductal carcinomas in situ revealed that the cells of the atypical lobules possessed the same cytological characteristics as those of the fully developed carcinomas in situ present in the same specimen (Fig. 3).

All the atypical cystic lobules that we studied exhibited identical immunohistochemical characteristics. The majority of the cells stained for keratin 19 (Fig. 4), estrogen receptors, and progesterone receptors (Fig. 5), and between 5% and 50% of the cells stained for cyclin D1 (Fig. 6). The 13 examples of ductal carcinomas in situ showed the same immunophenotype as the atypical lobules. The immunohistochemical profiles of normal terminal duct lobular units and those altered by blunt duct adenosis and fibrocystic changes differed from the pattern of atypical lobules. Conventional apocrine cells failed to stain for either hormone receptor, but most cells stained for keratin 19 and about half the cases contain a few cyclin D1-positive cells. Mature (type 2/3) lobules contained a few cells harboring estrogen receptors, progesterone receptors, and keratin 19. Simple (type 1) lobules and those altered by blunt duct adenosis showed staining of most of the luminal cells for estrogen and progesterone receptors and keratin 19. We found rare cyclin D1-positive cells in one primitive (type 1) lobule; other normal lobules and the foci of blunt duct adenosis did not stain. [In our preliminary studies of cyclin D1 staining of normal breast tissue, we observed positive cells only in scattered lobules in specimens resected during the luteal phase (data not shown).] Table 1 list these results.

In summary, low-grade ductal carcinoma in situ and atypical cystic lobules consist of a population of cytologically atypical cells that stain for estrogen receptor, progesterone receptor, keratin 19, and cyclin D1. The expression of estrogen receptor, progesterone receptor, and keratin 19 by all the cells of atypical cystic lobules distinguishes this lesion from normal complex (type 2/3) lobules and apocrine metaplasia, and the presence of cyclin D1 differentiates the atypical lobules from type 1 lobules and blunt duct adenosis.

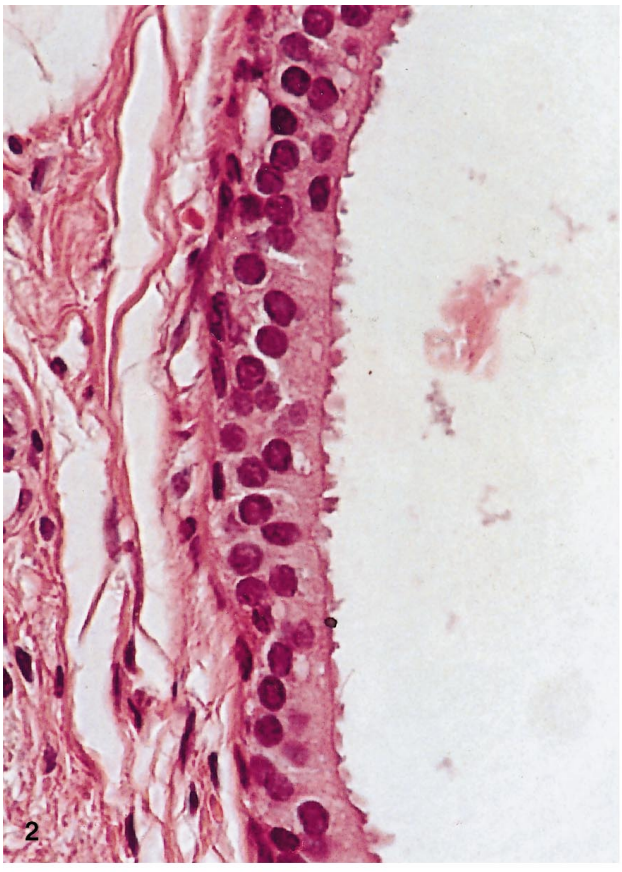
We discovered atypical cystic lobules in 36% of specimens containing ductal carcinoma in situ and 29% of cases showing lobular neoplasia. In contrast, only 3% of samples showing fibrocystic changes also contained atypical cystic lobules (Table 2). The three groups of cases have similar frequencies of conventional ductal hyperplasia, and we therefore believe that vagaries of case selection and specimen sectioning do not account for the different frequencies of atypical cystic lobules. Seven of the 16 cases (44%) harboring both low-grade ductal carcinoma in situ and atypical cystic lobules showed merging of the two lesions. Figure 7 illustrates one example of this phenomenon.

Table 1 Immunohistochemical staining results in cases of atypical cystic lobules (*ACL*), ductal carcinoma in situ (*DCIS*), and benign lesions (*BDA* blunt duct adenosis, *Lob 1* type 1 lobule, *Lob 2/3* type 2/3 lobule, *Ap Met* apocrine metaplasia)

	Keratin 19				Cyclin D1				Progesterone receptor				Estrogen receptor			
Lesion	2+	1+	Neg	Total	2+	1+	Neg	Total	2+	1+	Neg	Total	2+	1+	Neg	Total
ACL	21	0	0	21	7	14	0	21	20	1	0	21	20	1	0	21
DCIS	12	0	0	12	7	6	0	13	12	1	0	13	13	0	0	13
BDA	3	0	0	3	0	0	3	3	3	0	0	3	3	0	0	3
Lob 1	10	1	0	11	0	1	9	10	9	2	0	11	7	3	1	11
Lob 2/3	2	9	0	11	0	0	11	11	1	10	0	11	1	8	0	9
Ap Met	5	0	1	6	0	3	4	7	1	1	5	7	0	0	7	7

Table 2 Frequency of atypical cystic lobules and conventional hyperplasia in three lesions

Index lesion	Total cases	Mean age (years)	Cases with atypical cystic lobules	Cases with conventional hyperplasia
Fibrocystic changes	32	50.7	1 (3%)	10 (31%)
Lobular neoplasia	31	52.9	9 (29%)	9 (29%)
Ductal carcinoma in situ	44	62.3	16 (36%)	11 (25%)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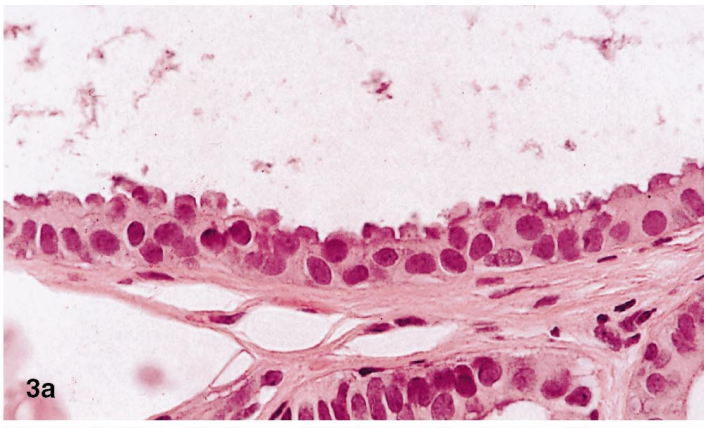
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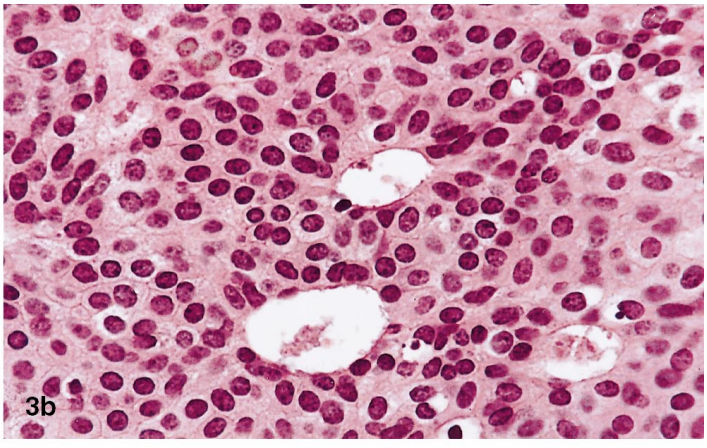
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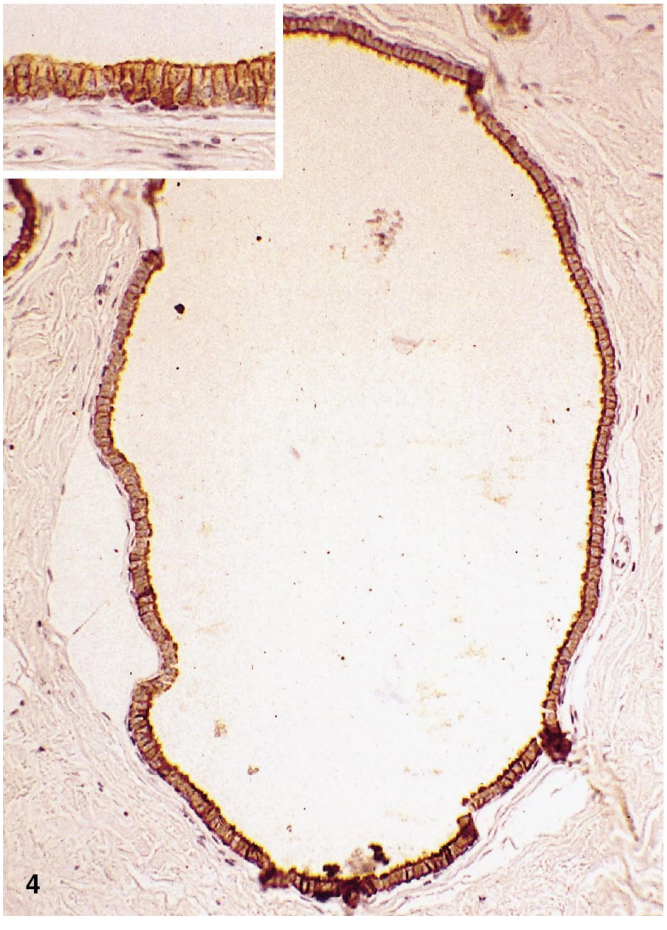
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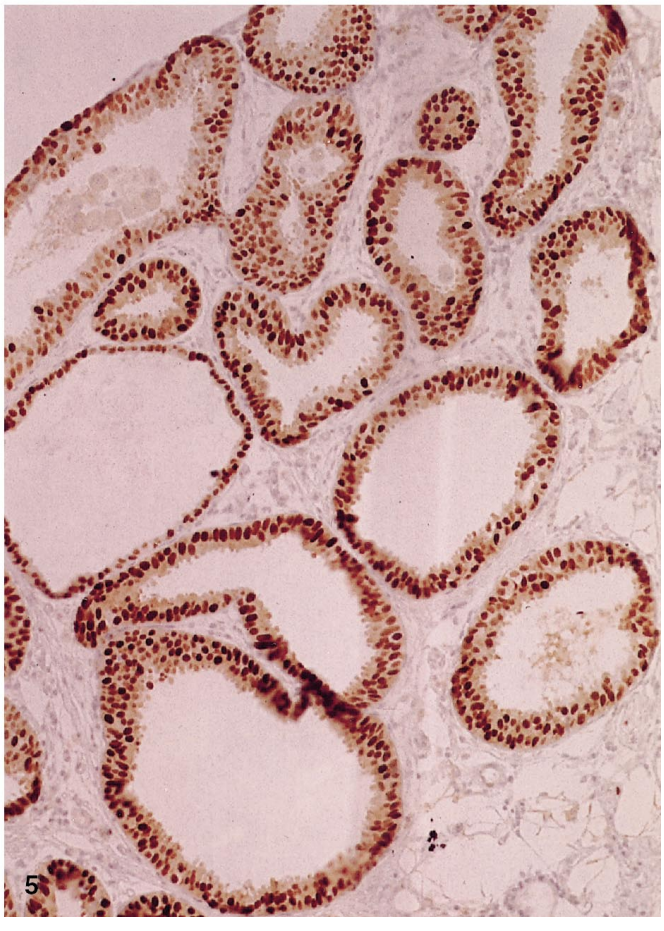
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Discussion

Although we believe that our study represents the first publication devoted primarily to the morphological characteristics of atypical cystic lobules, we acknowledge that others have recognized the lesion. For example, drawings in the works of Sasse [31] and Schimmelbusch [32] depict alterations consistent with that diagnosis. In an address to the American Medical Association in 1905, Warren discussed the classification of benign tumors of the breast to illustrate the need for close communication between pathologists and surgeons. His illustration of a lesion designated "abnormal involution" shows an atypical lobule in continuity with an atypical ductal proliferation (Fig. 10 in [37]). Bloodgood's depiction of the "adenocystic stage of senile parenchymatous hypertrophy" (Fig. 22 in [3]) seems to show an atypical cystic lobule merging with micropapillary ductal carcinoma in situ. MacCarty shows two atypical lobules (Figs. 22, 24 in [22]), which he considers examples of the lesion described by Warren. Cheatle's discussion of the origin and clinical significance of cysts includes the clearest early description of atypical cystic lobules: "[The cyst's wall is] lined by columnar epithelium, beautiful in appearance, with feathery, well-defined cells." [7]. Among modern workers, Wellings and Jensen [38] illustrate a family of alterations called "atypical lobules, type A," which clearly includes the lesion that we discuss. Azzopardi described in detail a form of ductal carcinoma in situ that he called "clinging carcinoma." His example of low-grade "clinging carcinoma" (Fig. 10-6 in [1]) appears identical to our atypical cystic lobules, and so does the lesion depicted in Eusebi et al.'s Fig. 4 [11]. The distended duct illustrated by Rosen and Oberman (Fig 223 in [28]) and the ectatic ducts pictured by Goldstein and O'Malley [15] share similarities with atypical cystic lobules. We believe that the "atypical cystic duct" of Tsuchiya [36] represents the lesion we report. Finally, a group of lesions called "columnar alteration with prominent apical snouts and secretions" [12] includes examples that we would classify as atypical cystic lobules.

We did not undertake our study so as to add yet another name to this lengthy list of overlapping but not synonymous terms. The designation "atypical cystic lobule" seems to characterize the lesion without making unwarranted implications about its pathogenesis or clinical behavior. The goal of our work was centered rather on defining the neoplastic nature of the lesion, and we wish to draw attention to the two ways in which our findings support this hypothesis.

First, our findings demonstrate that atypical cystic lobules represent a distinct lesion with a consistent immunophenotype. Although atypical lobules have characteristics that overlap with those of certain normal lobules or lobules altered by blunt duct adenosis or apocrine metaplasia, we find that one can readily distinguish these lesions by observing the following points of differential diagnosis. Terminal duct lobular units become more complex during the reproductive years [30]. Prior to the first pregnancy, simple (type 1) lobules predominate. They develop more fully during gestation and remain as type 2 and type 3 lobules when lactation ceases (Fig. 8). These complex lobules (type 2/3) do not look like atypical cystic lobules in any way, but type 1 lobules superficially resemble early atypical lobules. Type 1 lobules consist of coiled, simply branching ductules set in a modest amount of specialized stroma. The glandular lumens appear only slightly larger than those of normal acini. These early lobules have a prominent layer of basal cells, most of which do not contain hormone receptor proteins. Atypical cystic lobules, on the other hand, consist of a cluster of large cystic acini surrounded by sparse intralobular stroma. Luminal cells with atypical cytological characteristics constitute the predominant epithelial cell type, whereas basal cells appear diminished in number.

The dilated ductules seen in one type of blunt duct adenosis [26, 33] (Fig. 9) can also resemble the acini of atypical cystic lobules, but the following four features help to differentiate the two lesions. First, the epithelium of blunt duct adenosis consists of both luminal and basal cells. The latter look especially prominent, and they form a continuous layer around the luminal cells. Atypical cystic lobules, on the other hand, lack the prominent basal cell layer seen in blunt duct adenosis. Second, the luminal cells in blunt duct adenosis possess less cytoplasm than the cells of atypical cystic lobules. The nuclei of the former look completely conventional, whereas those of the latter appear atypical. Third, the glands of blunt duct adenosis often appear flattened, and they branch in a simple, staghorn pattern, whereas those of atypical cystic lobules exhibit less flattening and almost no branching. Finally, the specialized stroma of blunt duct adenosis often appears abundant and sometimes extends into the surrounding parenchyma in a pseudoinfiltrative pattern. Atypical cystic lobules usually contain smaller amounts of specialized stroma, which appears compact and well-defined.

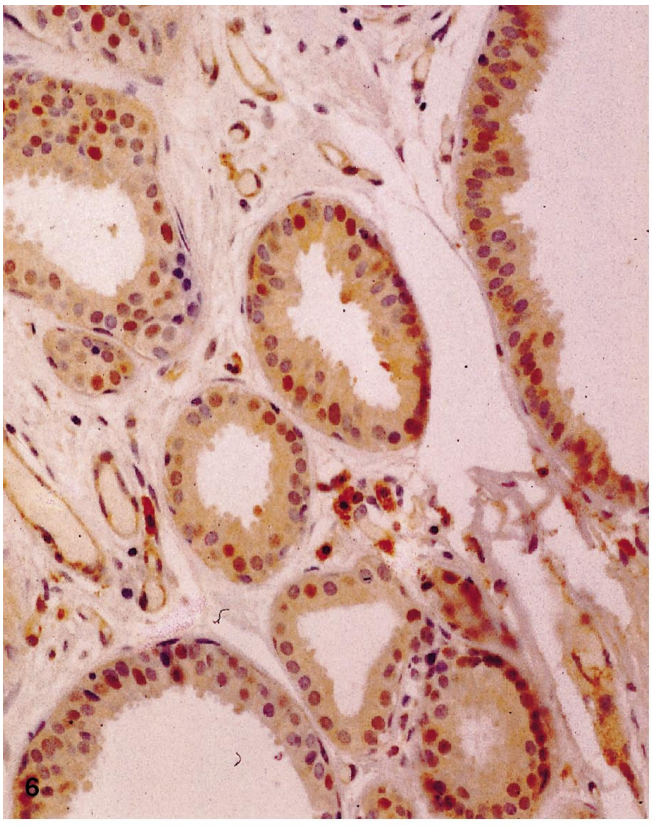
Acini showing apocrine metaplasia superficially resemble atypical cystic lobules because the cells in both conditions possess finely granular, eosinophilic cytoplasm. Apocrine cells have much more abundant cytoplasm than the cells of atypical cystic lobules, however, and the nucleoli of the former appear much more prominent than the latter. Furthermore, apocrine cells virtually always lack the estrogen and progesterone receptors that characterize the cells of atypical cystic lobules.

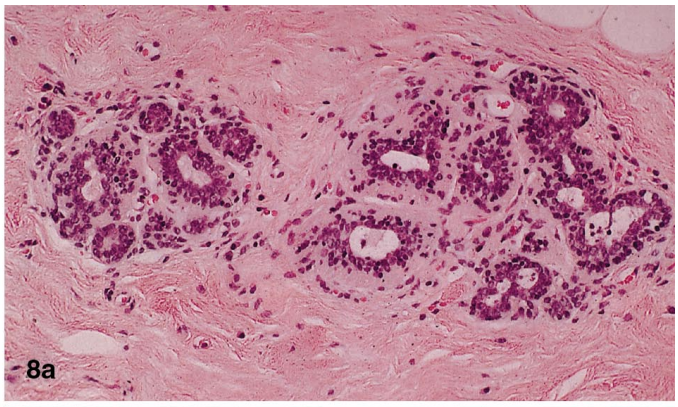
◀ **Fig. 2** Atypical cystic lobule (high magnification). The epithelial cells contain abundant apical cytoplasm and atypical nuclei. Myoepithelial cells are inconspicuous. ×640

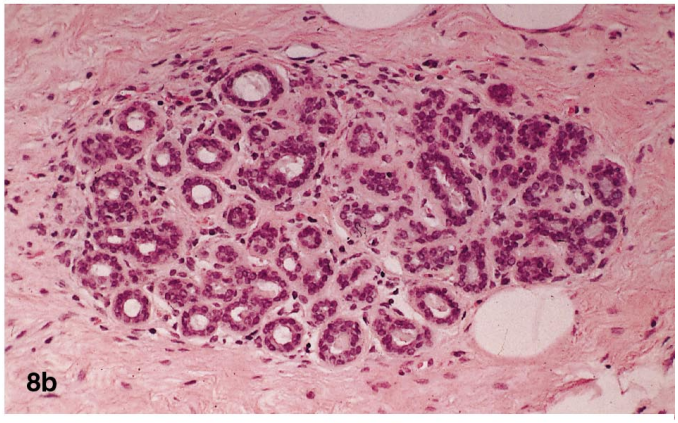
Fig. 3 a Atypical cystic lobule and **b** ductal carcinoma in situ. The cells appear identical in both lesions. ×400

Fig. 4 Atypical cystic lobule stained with antibody to keratin nineteen. All cells show cytoplasmic staining with a diffuse pattern. ×100; *Inset* ×400

Fig. 5 Atypical cystic lobule stained with antibody to progesterone receptor. All cells show nuclear staining. ×100







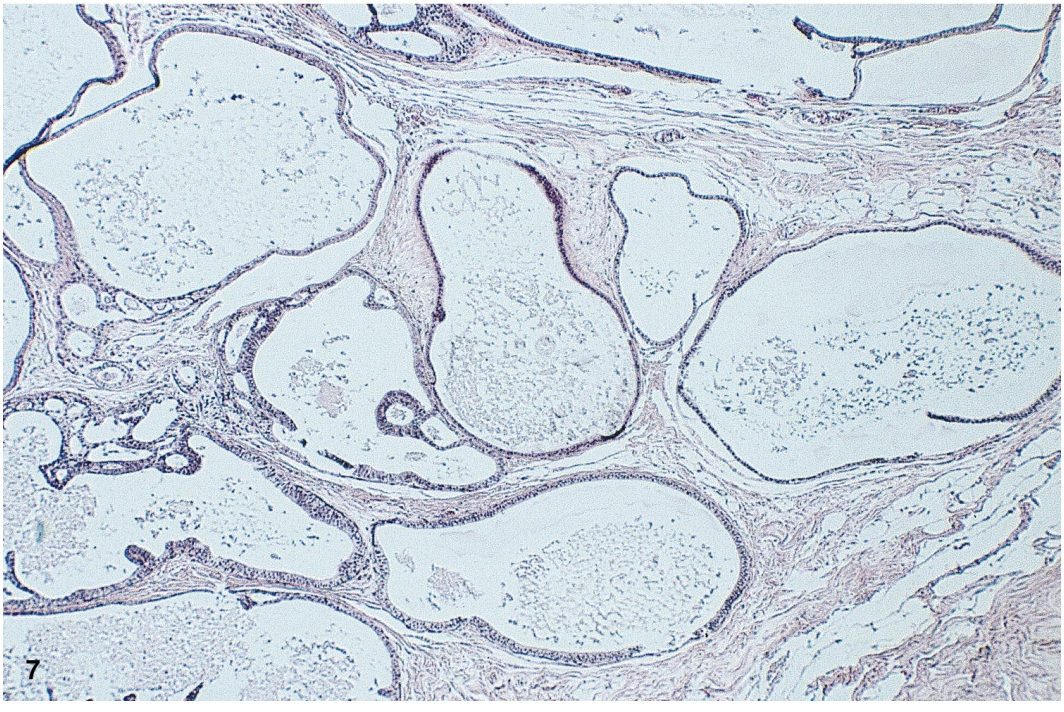


Fig. 6 Atypical cystic lobule stained with antibody to cyclin D1. Many cells show nuclear staining. ×250

Fig. 7 Atypical cystic lobule merging with low-grade ductal carcinoma in situ. Most of the epithelium grows in a single layer, but a few micropapillary/cribriform areas are present. ×40

Fig. 8a, b Normal terminal duct-lobular units. Two type 1 lobules (**a**) consist of coiled ductules. A type 2/3 lobule (**b**) contains acini. ×100

The results of our study also help to establish the neoplastic nature of atypical cystic lobules by providing morphological evidence that links them to low-grade micropapillary/cribriform ductal carcinoma in situ. The evidence takes three forms. First, atypical cystic lobules and low-grade ductal carcinoma in situ display similar cytological characteristics; both exhibit nuclear atypia and cellular polarization. So closely do the cells of the two

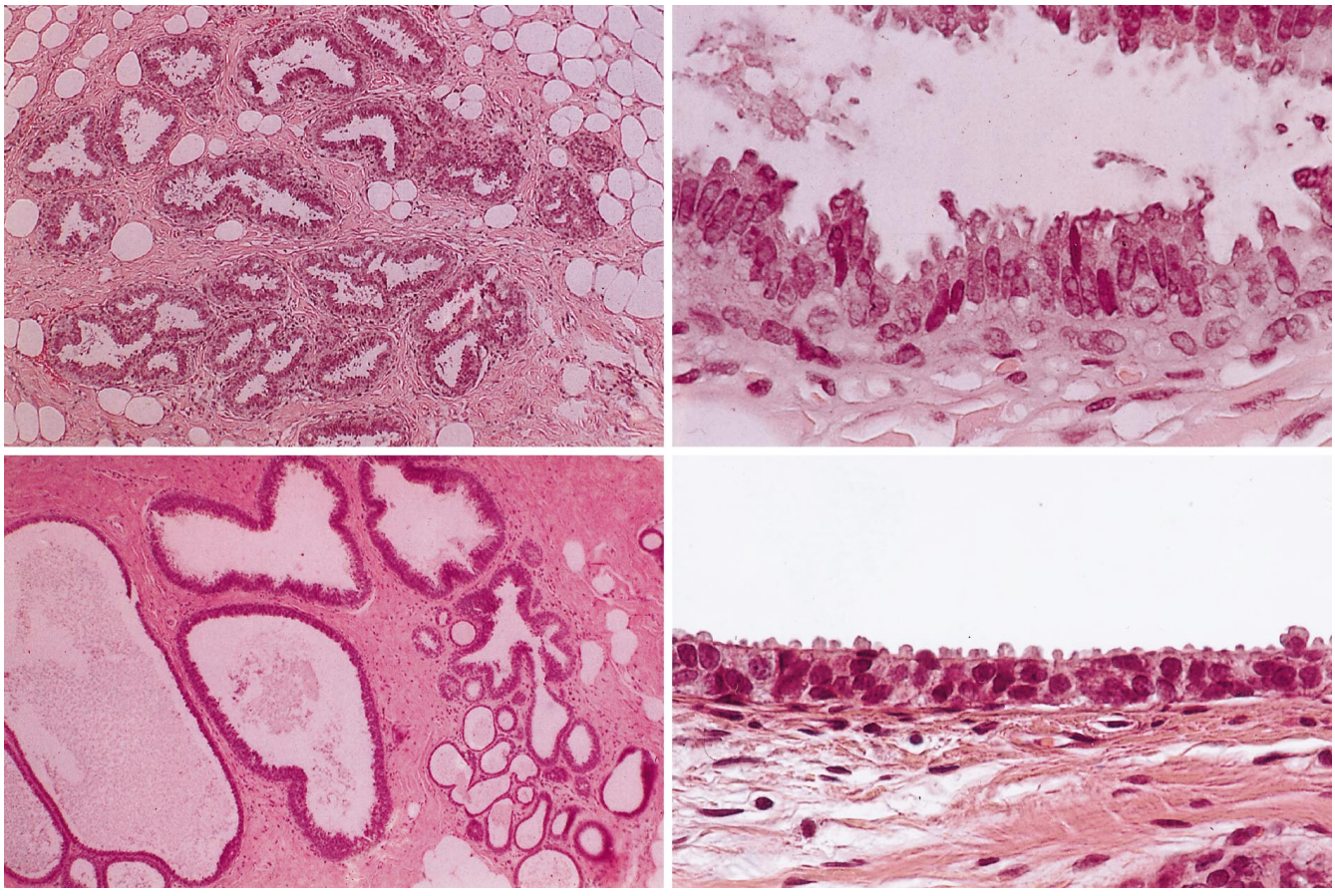


Fig. 9 Blunt duct adenosis (*upper panels*) and atypical cystic lobule (*lower panels*) differ in the shape of the glands, the appearance of the specialized stroma, and the prominence of the myoepithelial cells. *Left panels* ×40, *right panels* ×400)

lesions resemble each other that we cannot distinguish them by means of cytological criteria alone. Second, the immunohistochemical characteristics of the atypical cells of atypical cystic lobules match those of the malignant cells of ductal carcinomas in situ. Both lesions consist of glandular cells containing ovarian steroid hormone receptors, keratin 19, and cyclin D1. Not only does this immunohistochemical profile link the two types of proliferation, but it also distinguishes them from normal lobules and benign alterations. Finally, atypical cystic lobules often merge with foci of conventional micropapillary/cribriform ductal carcinoma in situ. In fact, we found atypical lobules in direct continuity with foci of ductal carcinoma in situ in 44% of the specimens harboring both lesions. Although we interpret this close spatial relationship as evidence of a pathogenetic link, others might regard atypical lobules as the advancing edge of the established carcinoma in situ rather than an early stage in the formation of the carcinoma. Such an alternative hypothesis predicts that atypical cystic lobules should occur only in the immediate vicinity of obvious ductal carcinomas. Contrary to this prediction, we found atypical lobules in 10 of 62 study cases in which neither the specimen nor the patient showed any of evidence ductal carcinoma in situ. Similarly, about one-half of the cases of low-grade clinging carcinoma of flat type reported by Eusebi et al. [11] did not contain conventional micropapillary/cribriform ductal carcinoma in situ. We continue to encounter clinical specimens containing atypical cystic lobules without ductal carcinomas. Our experience leads us to reject the alternative hypothesis and to interpret atypical cystic lobules as a forerunner of more common forms of ductal carcinoma rather than its advancing edge.

The theory that atypical cystic lobules represent an early stage in the formation of low-grade ductal carcinoma in situ conflicts with certain contemporary theories regarding the evolution of ductal carcinoma. The most commonly held hypothesis places conventional ductal hyperplasia as the first step in the progression to carcinoma and atypical ductal hyperplasia, as the second [25]. As an intermediate lesion, atypical ductal hyperplasia exhibits certain features of conventional ductal hyperplasia and others of ductal carcinoma in situ. Although the proliferative nature of these putative precursor lesions, their statistical association with the development of invasive cancer [4, 21, 23, 27, 35], and the presence of genetic aberrations [20, 25, 29] support the conventional thinking, two other considerations argue against it. First, hyperplastic cells differ in their fundamental properties from carcinoma cells. The former cells lack nuclear atypia and cellular polarization and they stick together tightly, whereas the latter ones appear atypical and polar-

ized and they tend to separate from their neighbors. Moreover, most hyperplastic cells lack the estrogen and progesterone receptors and the keratin 19 found in virtually all low-grade ductal carcinoma cells. Second, one does not commonly find conventional ductal hyperplasia in continuity with ductal carcinoma in situ. Dr. Azzopardi provides the clearest discussion of this point [1]:

Thus it is that the 'early' carcinoma rarely has its origin in solid areas of epitheliosis or other types of benign epithelial hyperplasia and hypertrophy. Rather does it arise by cytological changes in the epithelial cells of the TDLU that do not show the typical features of any type of adenosis....This concept of the usual development of carcinoma from normal or near-normal structures negates the notion of a continuous spectrum of grades of hyperplasia in the lobule terminating in malignancy. ... *The erroneous concept of a continuous spectrum would lead pathologists to search for 'early' malignancy in the more solid forms of benign proliferation*. But, on the contrary, the earliest changes usually take place de novo in epithelium which is not affected by previous solid benign hyperplasia.

The theory that regards atypical cystic lobules as an early stage in the formation of low-grade ductal carcinoma in situ avoids both weaknesses of the conventional hypothesis. Unlike hyperplastic cells, the cells of atypical cystic lobules show the same atypia, polarization, dishesion, and immunohistochemical properties as the cells of ductal carcinoma in situ. Moreover, atypical cystic lobules frequently merge with established ductal carcinoma in situ.

A rigorous evaluation of the theory of the neoplastic nature of atypical cystic lobules requires more than morphological evidence, however. For example, the theory predicts that atypical cystic lobules should occur more commonly in women who develop carcinomas than in women who do not do so and that the frequency of atypical cystic lobules in the population at large should begin to rise before the age at which one sees an increase in the frequency of ductal carcinoma in situ. The design of our study does not permit us to address either of these predictions, and we know of only one publication that includes this type of clinical information. In a retrospective review of about 9500 specimens originally considered benign, Eusebi et al. [11] discovered 25 cases (0.263%) of low-grade clinging carcinoma of flat type (atypical cystic lobules) and 15 cases of ductal carcinoma in situ with a mixture of flat and micropapillary/cribriform patterns. The 25 women in the former group had an average age of 44 years, whereas the age of the latter 15 women averaged 51 years. During an average follow-up interval of about 19 years, none of the 25 women with low-grade clinging carcinoma of flat type developed invasive carcinoma and only 1 suffered a recurrence, which also consisted of low-grade clinging carcinoma of flat type. These results suggest that the flat type of proliferation can evolve into a conventional micropapillary/cribriform carcinoma but that this evolution does not occur frequently. It will require the collection of more epidemiological data of this type and its integration with histopathological observations like the ones we report to define the neoplastic potential of atypical cystic lobules (low-grade clinging carcinoma of flat type) in a thorough way.

In summary, atypical cystic lobules exhibit the cytological and immunohistochemical characteristics of low-grade ductal carcinoma in situ while maintaining a near-normal pattern of growth. The morphological similarities between atypical cystic lobules and ductal carcinoma in situ and the proximity of the two proliferations in many cases suggest that some low-grade ductal carcinomas in situ develop in a setting of atypical cystic lobules.

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