**ORIGINAL ARTICLE**

# Dimorphic cells: a common feature throughout the low nuclear grade breast neoplasia spectrum

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## Abstract

Columnar cell lesions (CCLs) are recognized precursor lesions of the low nuclear grade breast neoplasia family. CCLs are cystic enlarged terminal duct lobular units with monotonous (monoclonal) columnar-type luminal cells. CCLs without atypia are regarded as benign and CCLs with atypia as true precursor lesions with clonal molecular changes, a certain progression risk, and an association with more advanced lesions. However, reproducibility of designating atypia in CCL is not optimal, and no objective markers of atypia have been identified, although 16q loss seems to be associated with atypical CCLs. Dimorphic ("pale") cell populations have been described in low nuclear grade ductal carcinoma in situ (DCIS) but not in CCLs and atypical ductal hyperplasia (ADH). Therefore, we searched for pale cells in CCL ($N = 60$), ADH ($N = 41$), and DCIS grade 1 ($N = 84$). Diagnostic criteria were derived from the WHO, and atypia was designated according to the Schnitt criteria. Pale cells occurred in 0% (0/30), 73% (22/30), 56% (23/41), and 76% (64/84) of CCLs without atypia, CCLs with atypia, ADH, and DCIS grade 1, respectively. Pale cells expressed ERα, E-cadherin and p120 and variably cyclin D1, and lacked expression of CK5 and p63. In conclusion, dimorphic "pale" cells occur throughout the low nuclear grade progression spectrum, increasing in frequency with progression. Interestingly, CCL lesions without atypia do not seem to bear showed pale cells, indicating that the presence of pale cells may serve as a diagnostic morphological feature of atypia in CCLs.

**Keywords** Breast cancer · Precursor lesions · Atypia · Columnar cells

## Introduction

Columnar cell lesions (CCLs) of the breast are cystically dilated enlarged terminal duct lobular units lined by columnar luminal cells with uniform, ovoid nuclei and often with apical cytoplasmic blebs or snouts presenting at the luminal surface. The lining is usually one or two cell layers (columnar cell change) although multiple cell layers may be present, usually denoted columnar cell hyperplasia. Intraluminal secretions and microcalcifications are commonly seen [1]. In columnar cell change with atypia, also denoted flat epithelial atypia (FEA), the columnar cells show nuclear atypia of relatively round to ovoid nuclei with or without prominent nucleoli and an increased nuclear/cytoplasmic ratio and/or disturbed nuclear orientation along the basement membrane. A complex architectural pattern (micropapillae, rigid cellular bridges, bars and arcades, or cribriform architecture) necessitates upgrading a CCL to atypical ductal hyperplasia (ADH) or low-grade ductal carcinoma in situ (DCIS) [2, 3].

The diagnosis of atypia in a CCL is of clinical importance. They are both recognized as low-grade preneoplasms of the breast with clonal molecular alterations [4, 5]. Nevertheless, the diagnosis CCL without atypia does not have consequences for treatment because of a low upgrade risk. In contrast, CCLs with atypia are considered true precursor lesions of the low nuclear grade breast cancer family [4–7], with upgrade rates of 5–9% [8, 9] and an association with more advanced lesions (ADH [10–16], DCIS grade 1 [1, 11, 14, 17, 18], lobular neoplasia [10, 12, 14, 16, 19–22], and tubular cancer [7, 17, 19, 20, 23, 24]) in about 20% of patients [25]. This necessitates a discussion about further follow-up and/or treatment in individual patients with atypical CCLs.

However, reproducibility of designating atypia in CCL is generally low. Although O'Malley achieved excellent agreement (multi-rater kappa value 0.83) in diagnosing

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atypical CCLs after a tutorial in a selected case set [26], other groups found substantially lower kappa values (0.27 and 0.41) [27–29]. Two recent meta-analyses described pooled upgrade rate of pure FEA diagnosed by CNB of 5% and 9% [8, 9]. The difference between these studies is that Wahab et al. also included imaging follow-up, whereas Ferre et al. analyzed only the results of surgical excision. In contrast to the results of these studies based on standardized second opinion, publications without this standard show upgrade rates at the surgical specimen between 0 and 30% for CNB-based diagnosed pure FEAs [30]. This also indicates that the reproducibility of diagnosis of atypia in CCL is not optimal in routine practice. So far, no phenotypical markers of atypia have unfortunately been identified.

Dimorphic cells have been described in the literature in three original publications so far [31–33] Altogether, they have been described in 70 cases (Lefkowitz (1994), 20 cases; Ueno (2018), 50 cases; Koerner (2010), not specified), predominantly in papillary carcinomas [31, 33] and besides in 40 invasive NST carcinomas but also in 10 DCIS cases [32]. The frequency of dimorphic cells in DCIS is however not well-established. Koerner described that careful scrutiny reveals frequent cellular dimorphism in DCIS [33]. Others describe a dimorphic type DCIS as an unusual variant [34, 35].

The cells are characterized by clear cytoplasm-simulating myoepithelial cells but with nuclei similar to those in the adjacent malignant cells, rounded cell borders, and clear cytoplasm in the H&E stain. Several of these articles indicate that dimorphic cell populations are especially seen in low nuclear grade DCIS, which makes it plausible that these "pale cells" would also occur in earlier precursor lesions of the low nuclear grade family. Indeed, our impression was that we regularly encounter pale cells in our practice in low-grade precursor lesions, but dimorphic differentiation has to the best of our knowledge not been described in CCLs and ADH before. This prompted us to systematically retrospectively evaluate the presence of pale cells in a group of ADH and CCL lesions to cover the earliest spectrum of the low nuclear grade precursor lesions, in search of further morphological features of CCLs, especially with regard to the designation "atypia."

## Material and methods

Slides from 185 formalin-fixed, paraffin-embedded breast tissue samples (biopsies or resections) with CCLs ($N = 60$), ADH ($N = 41$), and DCIS grade 1 ($N = 84$) were collected from the Department of Pathology of the University Medical Center Utrecht between July 2017 and July 2018. CCLs were graded according to the classification described by Schnitt and Vincent-Salomon [3] as CCLs without atypia ($N = 30$) and CCLs with atypia ($N = 30$). Designation of DCIS grade 1 and atypical ductal hyperplasia was assessed by two experienced observers, according to the World Health Organization classification [36, 37]. The presence of co-existing lobular neoplasia (LN) was noted, confirmed by E-cadherin immunohistochemistry when deemed necessary.

A dimorphic cell population was defined as epithelial cells with clear cytoplasm with nuclei similar to those in the adjacent clonal cells, rounded cell borders, and clear cytoplasm in the H&E stain, often located between the luminal and myoepithelial layers, simulating pagetoid spread of LN. The CCLs, ADH, and DCIS lesions were screened for the presence of these pale cells. This was not done in pure LN since pale cells resemble the cells of LN. Routinely performed immunohistochemical stains were screened to identify the expression patterns of pale cells. Since pale cells are often scattered as single cells throughout lesions, E-cadherin stains were especially scrutinized for adjacent pale cells and pale cell groups to pinpoint membrane expression or lack thereof.

## Results

Table 1 shows the frequency of pale cells in the various low nuclear grade breast precursor lesions studied. Pale cells occurred in 0% (0/30), 73% (22/30), 56% (23/41), and 76% (64/84) of CCLs without atypia, CCLs with atypia, ADH, and DCIS grade 1, respectively. Figure 1 shows examples of pale cells in CCLS with atypia, ADH, and DCIS grade 1. In some ADH and DCIS lesions, clusters of pale cells were observed ("clonal expansion") that rarely formed tubular structures (Fig. 1).

Pale cells turned out to be expressing ERα, PR, E-cadherin, AR and p120 and variably cyclin D1, and lacked expression of

**Table 1** Frequency of dimorphic ("pale") cells in different lesions throughout the spectrum of the low nuclear grade breast neoplasia family (*CCL*, columnar cell lesion; *ADH*, atypical ductal hyperplasia; *DCIS*, ductal carcinoma in situ)

| Diagnosis | # | # patients | Mean age (range) | # biopsy | # resection | # with pale cells (%) |
|---|---|---|---|---|---|---|
| CCL without atypia | 30 | 29 | 49.7 (37–70) | 25 | 5 | 0 (0%) |
| CCL with atypia | 30 | 27 | 51.3 (39–71) | 21 | 9 | 22 (73%) |
| ADH | 41 | 41 | 44.5 (40–76) | 32 | 9 | 23 (56%) |
| DCIS grade 1 | 84 | 67 | 61.6 (35–84) | 35 | 49 | 64 (76%) |

**Fig. 1** Representative examples of **A**. Single pale cells in CCL with atypia (**B**). Single pale cell in ADH, as well as examples of clonal expansion of pale cells, linear in CCL with atypia (**C**), and forming tubular structures (**D**) in DCIS grade 1
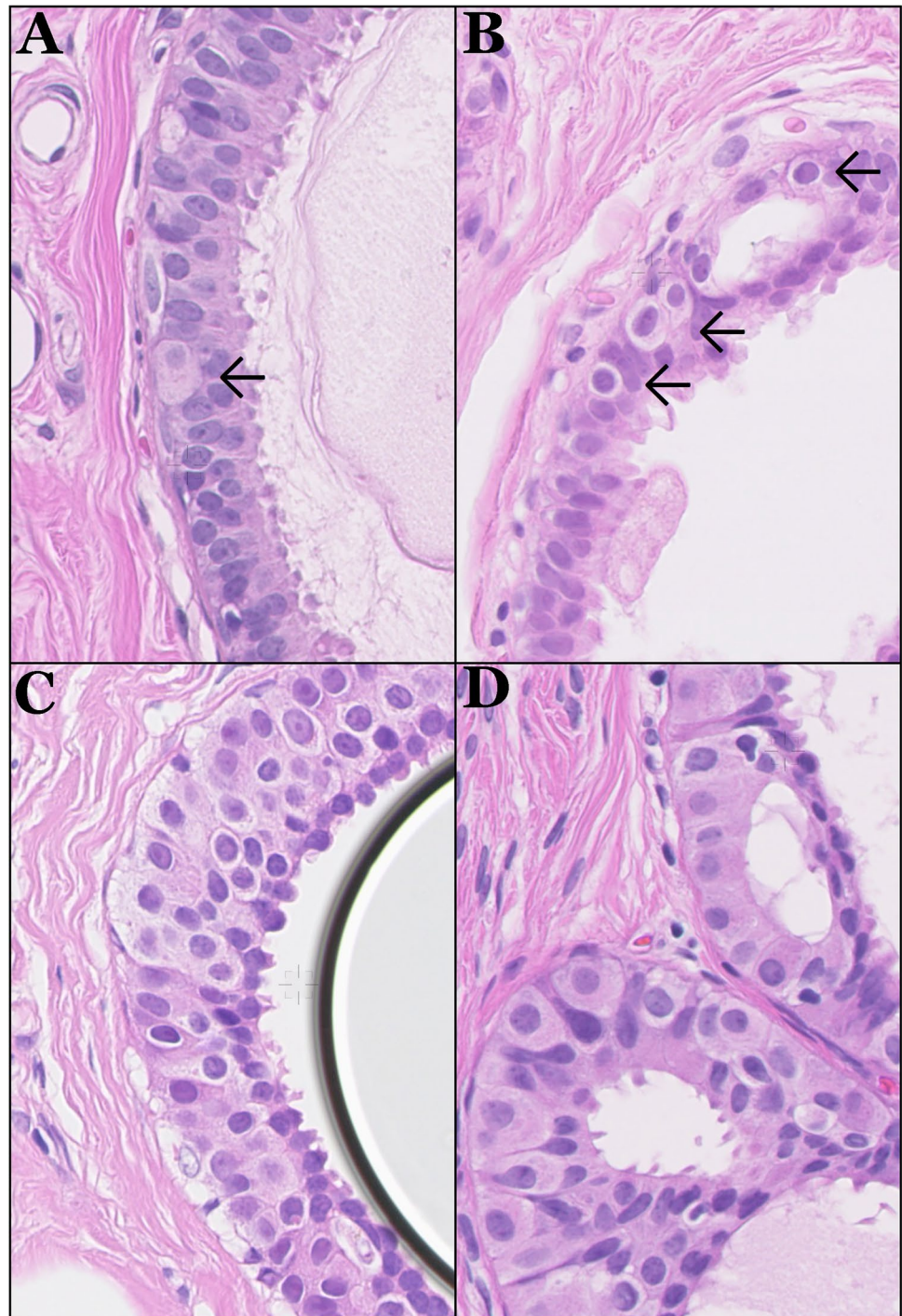


CK5 and p40 (Fig. 2 and Fig. 3). Figure 2 also shows a comparison of the immunophenotype of pale cells and its mimics. Pagetoid spread of LN below the pre-existent luminal epithelium clonally expressed ERα while lacking CK5, p40 expression, and E-cadherin. Prominent myoepithelium in blunt duct adenosis expressed CK5 and p40 while lacking ERα expression, and clusters of ductal hyperplastic cells below the pre-existent luminal epithelium expressed CK5 and ERα while lacking p40.

## Discussion

Dimorphic "pale" cell populations were first described in papillary DCIS as epithelial cells with clear cytoplasm-simulating myoepithelial cells, but with nuclei similar to those in the adjacent malignant cells, rounded cell borders, and clear cytoplasm in the H&E stain [29], later designated as a feature of low nuclear grade DCIS. We here show that

**Fig. 2** Immunophenotype of pale cells versus its mimics. Column **A**, pale cells in micropapillary ductal carcinoma in situ (column **A**) clonally expressing ERα while lacking CK5 and p40 expression, with normal membrane expression of E-cadherin. Column **B**, pagetoid spread below the pre-existent luminal epithelium of lobular neoplasia cells clonally expressing ERα while lacking CK5 and p40 expression as well as lacking membrane expression of E-cadherin. Column **C**, blunt duct adenosis with prominent myoepithelium that expresses CK5 and p40 while lacking ERα expression. Column **D**, clusters of ductal hyperplastic cells below the pre-existent luminal epithelium expressing CK5 and ERα while lacking p40
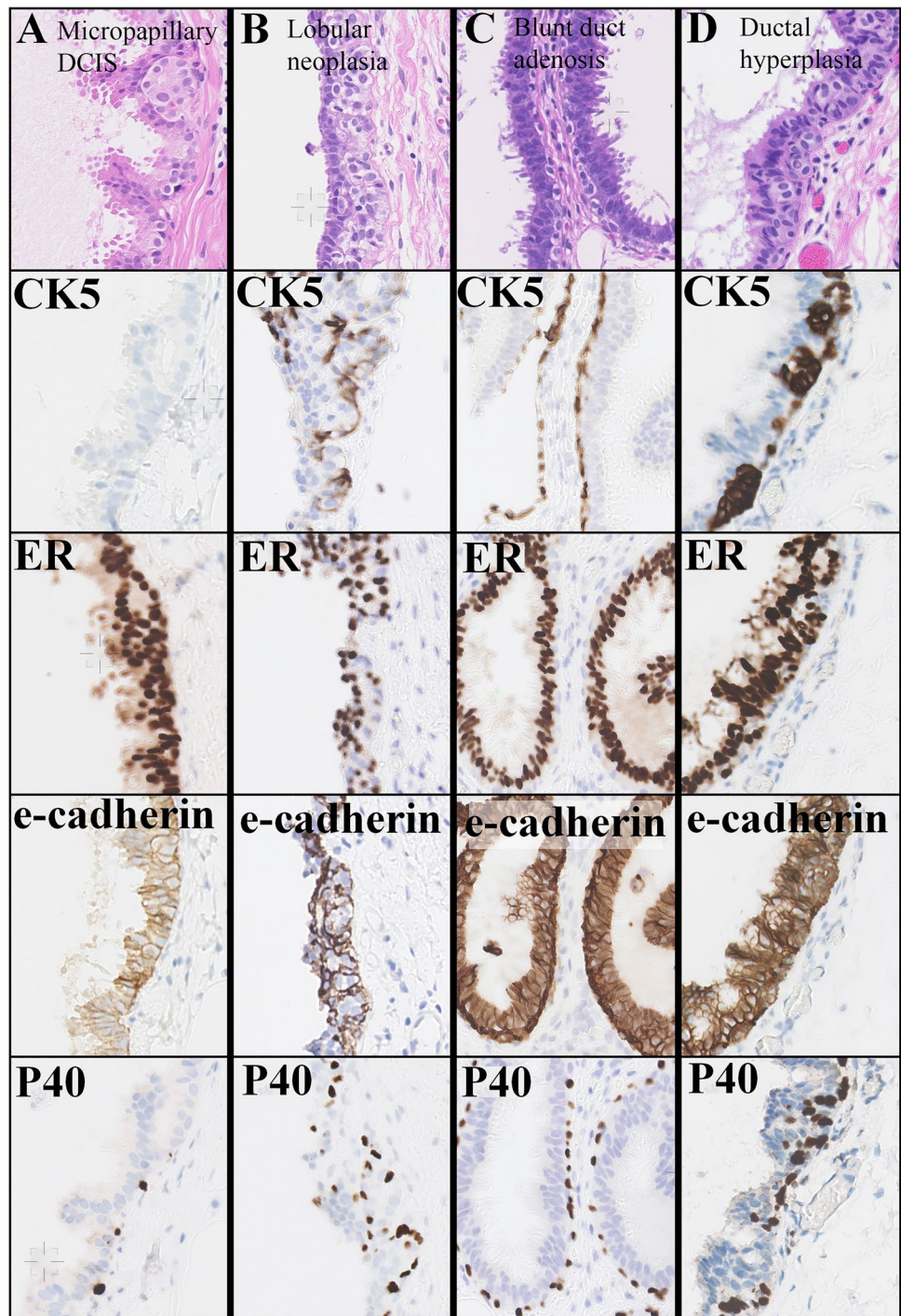


pale cells frequently occur in true low-grade nuclear breast precursor lesions, in 109 of 155 precursor lesions (CCL with atypia 22/30, ADH 23/41, DCIS grade 1 64/84) while being absent in 30 CCL lesions without atypia. This indicates that the presence of pale cells may serve as a diagnostic feature of atypia in CCLs. Pale cells expressed ERα, E-cadherin and p120 and variably cyclin D1, and lacked expression of CK5 and p40.

The biological background of these pale cells is not clear. Theoretically, they could be luminal epithelial cells with a slightly different morphology, scattered apocrine cells, scattered LN cells, neuroendocrine cells, or myoepithelial cells. Since pale cells express ERα and PR, an apocrine origin is unlikely, and the expression of E-cadherin largely rules out LN. The expression of ERα and the lack of CK5 and p40 expression rule out myoepithelial origin [38]. We therefore

**Fig. 3** Immunophenotype of pale cells. Case 1, extensive pale cells in atypical ductal hyperplasia clonally expressing PR and AR. Case 2, pagetoid pale cells in micropapillary ductal carcinoma in situ, forming small cribriform structures, clonally expressing PR and AR
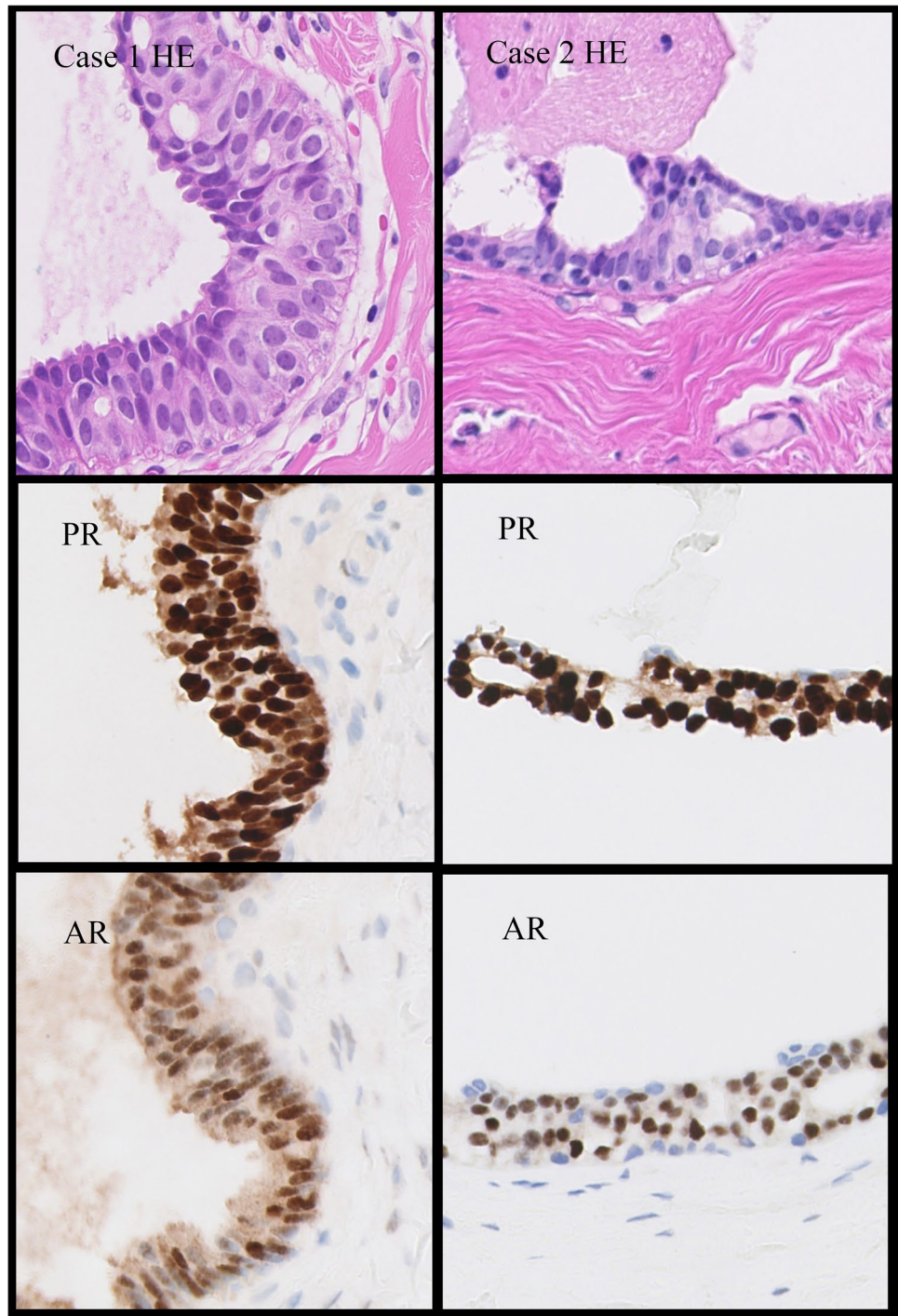


hypothesize that these pale cells are neoplastic luminal epithelial cells, compatible with the observed expression of ERα and the lack of CK5 expression. We have however no explanation why they morphologically stand out. This requires further molecular studies, e.g., applying single-cell sequencing on microdissected pale cells, but this is yet technically challenging on paraffin-embedded tissue. Perhaps, they are a subclone, as we sometimes see clonal expansion of pale cell-forming groups that start to take over precursor lesions (Fig. 1). This may also explain the previously described dimorphic lesions [31–35]. Further, when lesions are fully comprised of pale cells, they may be hard to designate as "pale," indicating that the frequency of pale cell lesions reported here may be underestimated. Pale cells have also been described to express AR and BRST2 [32], compatible with their luminal breast origin.

In conclusion, we here describe that dimorphic "pale" cells frequently occur throughout the low nuclear grade breast progression spectrum (CCL with atypia, ADH, DCIS grade 1). Interestingly, CCLs without atypia did not show pale cells, indicating that the presence of pale cells may serve as a diagnostic morphological feature of atypia in CCLs.

**Author contribution** Both authors participated in the conception and writing of the manuscript and reviewed the final version.

## Declarations

**Ethics approval** The use of archival tissue and data was done according to the General Data Protection Regulation and Dutch law.

**Conflict of interest** The authors declare no competing interests.

## References

1. Fraser JL, Raza S, Chorny K, Connolly JL, Schnitt SJ (1998) Columnar alteration with prominent apical snouts and secretions: a spectrum of changes frequently present in breast biopsies performed for microcalcifications. Am J Surg Pathol 22(12):1521–1527. https://doi.org/10.1097/00000478-199812000-00009

2. Schnitt S (2003) The diagnosis and management of pre-invasive breast disease: flat epithelial atypia—classification, pathologic features and clinical significance. Breast Cancer Res 5(5):263–268. https://doi.org/10.1186/bcr625

3. Schnitt S, Vincent Salomon A (2003) Columnar cell lesions of the breast. Adv Anat Pathol 10(3):113–124. https://doi.org/10.1097/00125480-200305000-00001

4. Simpson P, Gale T, Reis Filho J, Jones C, Parry S, Sloane J, Hanby A, Pinder S, Lee AHS, Humphreys S, Ellis I, Lakhani S (2005) Columnar cell lesions of the breast: the missing link in breast cancer progression? A morphological and molecular analysis. Am J Surg Pathol 29(6):734–746. https://doi.org/10.1097/01.pas.0000157295.93914.3b

5. de Boer M, Verschuur-Maes AHJ, Buerger H, Moelans CB, Steenkamer M, Savola S, van Diest PJ (2018) Role of columnar cell lesions in breast carcinogenesis: analysis of chromosome 16 copy number changes by multiplex ligation-dependent probe amplification. Mod Pathol 31:1816–1833. https://doi.org/10.1038/s41379-018-0099-2

6. Abdel-Fatah TMA, Powe D, Hodi Z, Reis Filho J, Lee AHS, Ellis I (2008) Morphologic and molecular evolutionary pathways of low nuclear grade invasive breast cancers and their putative precursor lesions: further evidence to support the concept of low nuclear grade breast neoplasia family. Am J Surg Pathol 32(4):513–523. https://doi.org/10.1097/PAS.0b013e318161d1a5

7. Moinfar F, Man YG, Bratthauer GL, Ratschek M, Tavassoli FA (2000) Genetic abnormalities in mammary ductal intraepithelial neoplasia-flat type ("clinging ductal carcinoma in situ"): a simulator of normal mammary epithelium. Cancer 88(9):2072–2081

8. Ferre R, Kuzmiak CM (2022) Upgrade rate of percutaneously diagnosed pure flat epithelial atypia: systematic review and meta-analysis of 1,924 lesions J Osteopathic Med 122:253–262. https://doi.org/10.1515/jom-2021-0206

9. Wahab RA, Lee S-J, Mulligan ME, Zhang B, Mahoney MC (2021) Upgrade rate of pure flat epithelial atypia diagnosed at core needle biopsy: a systematic review and meta-analysis radiology. Imaging Cancer 3:e200116–e200116. https://doi.org/10.1148/rycan.2021200116

10. Boulos F, Dupont W, Simpson J, Schuyler P, Sanders M, Freudenthal M, Page D (2008) Histologic associations and long-term cancer risk in columnar cell lesions of the breast: a retrospective cohort and a nested case-control study. Cancer 113(9):2415–2421. https://doi.org/10.1002/cncr.23873

11. Chivukula M, Bhargava R, Tseng G, Dabbs D (2009) Clinicopathologic implications of "flat epithelial atypia" in core needle biopsy specimens of the breast. Am J Clin Pathol 131(6):802–808. https://doi.org/10.1309/AJCPLDG6TT7VAHPH

12. Collins L, Achacoso N, Nekhlyudov L, Fletcher S, Haque R, Quesenberry C, Alshak N, Puligandla B, Brodsky G, Schnitt S, Habel L (2007) Clinical and pathologic features of ductal carcinoma in situ associated with the presence of flat epithelial atypia: an analysis of 543 patients. Mod Pathol 20(11):1149–1155. https://doi.org/10.1038/modpathol.3800949

13. Kunju L, Kleer C (2007) Significance of flat epithelial atypia on mammotome core needle biopsy: should it be excised? Hum Pathol 38(1):35–41. https://doi.org/10.1016/j.humpath.2006.08.008

14. Lavoué V, Roger C, Poilblanc M, Proust N, Monghal Verge C, Sagan C, Tas P, Mesbah H, Porée P, Gay C, Body G, Levêque J (2011) Pure flat epithelial atypia (DIN 1a) on core needle biopsy: study of 60 biopsies with follow-up surgical excision. Breast Cancer Res Treat 125(1):121–126. https://doi.org/10.1007/s10549-010-1208-1

15. Lee T, Macintosh R, Rayson D, Barnes P (2010) Flat epithelial atypia on breast needle core biopsy: a retrospective study with clinical-pathological correlation. Breast J 16(4):377–383. https://doi.org/10.1111/j.1524-4741.2010.00934.x

16. Senetta R, Campanino P, Mariscotti G, Garberoglio S, Daniele L, Pennecchi F, Macrì L, Bosco M, Gandini G, Sapino A (2009) Columnar cell lesions associated with breast calcifications on vacuum-assisted core biopsies: clinical, radiographic, and histological correlations. Mod Pathol 22(6):762–769. https://doi.org/10.1038/modpathol.2009.21

17. Goldstein NS, O'Malley BA (1997) Cancerization of small ectatic ducts of the breast by ductal carcinoma in situ cells with apocrine snouts: a lesion associated with tubular carcinoma. Am J Clin Pathol 107(5):561–566. https://doi.org/10.1093/ajcp/107.5.561

18. Oyama T, Iijima K, Takei H, Horiguchi J, Iino Y, Nakajima T, Koerner F (2000) Atypical cystic lobule of the breast: an early stage of low-grade ductal carcinoma in-situ. Breast Cancer 7(4):326–331. https://doi.org/10.1007/BF02966399

19. Abdel-Fatah TMA, Powe D, Hodi Z, Lee AHS, Reis Filho J, Ellis I (2007) High frequency of coexistence of columnar cell lesions, lobular neoplasia, and low grade ductal carcinoma in situ with invasive tubular carcinoma and invasive lobular carcinoma. Am J Surg Pathol 31(3):417–426. https://doi.org/10.1097/01.pas.0000213368.41251.b9

20. Sahoo S, Recant W (2005) Triad of columnar cell alteration, lobular carcinoma in situ, and tubular carcinoma of the breast. Breast J 11(2):140–142. https://doi.org/10.1111/j.1075-122X.2005.21616.x

21. Dessauvagie B, Zhao W, Heel Miller K, Harvey J, Bentel J (2007) Characterization of columnar cell lesions of the breast: immunophenotypic analysis of columnar alteration of lobules with prominent apical snouts and secretions. Hum Pathol 38(2):284–292. https://doi.org/10.1016/j.humpath.2006.08.003

22. Kunju L, Ding Y, Kleer C (2008) Tubular carcinoma and grade 1 (well-differentiated) invasive ductal carcinoma: comparison of flat epithelial atypia and other intra-epithelial lesions. Pathol Int 58(10):620–625. https://doi.org/10.1111/j.1440-1827.2008.02280.x

23. Aulmann S, Elsawaf Z, Penzel R, Schirmacher P, Sinn H (2009) Invasive tubular carcinoma of the breast frequently is clonally related to flat epithelial atypia and low-grade ductal carcinoma in situ. Am J Surg Pathol 33(11):1646–1653. https://doi.org/10.1097/PAS.0b013e3181adfdcf

24. Rosen PP (1999) Columnar cell hyperplasia is associated with lobular carcinoma in situ and tubular carcinoma. Am J Surg Pathol 12:1561. https://doi.org/10.1097/00000478-199912000-00017

25. Verschuur-Maes AH, van Deurzen CH, Monninkhof EM, van Diest PJ (2012) Columnar cell lesions on breast needle biopsies: is surgical excision necessary? A systematic review. Ann Surg 255:259–265. https://doi.org/10.1097/SLA.0b013e318233523f

26. O'Malley FP, Mohsin SK, Badve S, Bose S, Collins LC, Ennis M, Kleer CG, Pinder SE, Schnitt SJ (2006) Interobserver reproducibility in the diagnosis of flat epithelial atypia of the breast. Mod Pathol 19:172–179. https://doi.org/10.1038/modpathol.3800514

27. Gomes DS, Porto SS, Balabram D, Gobbi H (2014) Inter-observer variability between general pathologists and a specialist in breast pathology in the diagnosis of lobular neoplasia, columnar cell lesions, atypical ductal hyperplasia and ductal carcinoma in situ of the breast. Diagn Pathol 9:121. https://doi.org/10.1186/1746-1596-9-121

28. Tan PH, Ho BC, Selvarajan S, Yap WM, Hanby A (2005) Pathological diagnosis of columnar cell lesions of the breast: are there issues of reproducibility? J Clin Pathol 58(7):705–709. https://doi.org/10.1136/jcp.2004.025239

29. Haupt B, Schwartz MR, Xu Q, Ro JY (2010) Columnar cell lesions: a consensus study among pathology trainees. Hum Pathol 41(6):895–901. https://doi.org/10.1016/j.humpath.2009.12.003

30. DiPasquale A, Silverman S, Farag E, Peiris L (2020) Flat epithelial atypia: are we being too aggressive? Breast Cancer Res Treat 179:511–517. https://doi.org/10.1007/s10549-019-05481-9

31. Lefkowitz M, Lefkowitz W, Wargotz ES (1994) Intraductal (intracystic) papillary carcinoma of the breast and its variants: a clinicopathological study of 77 cases. Hum Pathol 25:802–809. https://doi.org/10.1016/0046-8177(94)90250-X

32. Ueno N, Kuroda H, Kurosumi M, Kozuka Y, Ito J, Kato H, Kubota K (2018) Imai Y (2010) Clinicopathological study of a dimorphic variant of breast carcinoma. Breast Cancer 25:151–158. https://doi.org/10.1007/s12282-017-0804-x

33. Koerner F (2010) Papilloma and papillary carcinoma. Semin Diagn Pathol 27:13–30. https://doi.org/10.1053/j.semdp.2009.12.004

34. Rakha E, Ellis I.(2016) Ductal carcinoma in situ. In: Dabbs DJ. Breast Pathology 2nd Edition. Elsevier, pp 471–488.

35. Hoda SA. (2020) Ductal carcinoma in situ. In: Hoda SA, Brogi E, Koerner FC, Rosen PP. Rosen's breast pathology. Fifth edition, Wolters-Kluwer pp363–451.

36. Tse GMK, Tan P, Lui PCW, Gilks CB, Poon CSP, Ma TKF, Lam Law BKB, WWM, (2007) The role of immunohistochemistry for smooth-muscle actin, p63, CD10 and cytokeratin 14 in the differential diagnosis of papillary lesions of the breast. J Clin Pathol 60(3):315–20. https://doi.org/10.1136/jcp.2006.036830

37. Cardenosa G, Eklund GW (1991) Benign papillary neoplasms of the breast: mammographic findings. Radiology 181(3):751–755. https://doi.org/10.1148/radiology.181.3.1947092

38. Sailer V, Lüders C, Kuhn W, Pelzer V, Kristiansen G (2015) Immunostaining of ΔNp63 (using the p40 antibody) is equal to that of p63 and CK5/6 in high-grade ductal carcinoma in situ of the breast. Virchows Arch 467(1):67–70. https://doi.org/10.1007/s00428-015-1766-z