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Infiltrating epitheliosis of the breast: characterization of histologic features, immunophenotype and genomic profile

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Abstract

AIMS—Infiltrating epitheliosis is a rare complex sclerosing lesion of the breast, characterized by infiltrating ducts immersed in a scleroelastotic stroma and filled with cells having architectural and cytological patterns reminiscent of those of usual ductal hyperplasia. Here we sought to define the molecular characteristics of infiltrating epitheliosis.

METHODS AND RESULTS—Eight infiltrating epitheliosis, adjacent breast lesions (one usual ductal hyperplasia, one papilloma, one micropapillary ductal carcinoma *in situ* and one low-grade adenosquamous carcinoma), and corresponding normal breast tissue from each case were microdissected and subjected to massively parallel sequencing analysis targeting all coding regions of 254 genes recurrently mutated in breast cancer and/or related to DNA repair. Mutations in components of the PI3K pathway were found in all infiltrating epitheliosis samples, seven of which harbored *PIK3CA* hotspot mutations, while the remaining case displayed a *PIK3R1* somatic mutation.

CONCLUSIONS—Somatic mutations affecting PI3K pathway genes were found to be highly prevalent in infiltrating epitheliosis, suggesting that these lesions may be neoplastic rather than hyperplastic. The landscape of somatic genetic alterations found in infiltrating epitheliosis is

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SUPPORTING INFORMATION

Supplementary Figure 1: Validation of *PIK3CA* mutations identified by targeted capture massively parallel sequencing in samples of infiltrating epitheliosis by Sanger sequencing.

Supplementary Figure 2: Repertoire of somatic mutations identified in samples of infiltrating epitheliosis and adjacent lesions.

Supplementary Table 1: Summary of antibody sources, dilutions, pretreatment protocols and detection methods used.

Supplementary Table 2: List of 254 genes included in the targeted capture massively parallel sequencing platform.

Supplementary Table 3: List of primers used to investigate the hotspot regions of the *PIK3CA* gene by Sanger sequencing.

Supplementary Table 4: Somatic single nucleotide variants (SNVs) and insertion/deletions (indels) present in the cases of infiltrating epitheliosis and their adjacent breast lesions analyzed.

similar to that of radial scars/complex sclerosing lesions, suggesting that infiltrating epitheliosis may represent one end of this spectrum of lesions.

Keywords

infiltrating epitheliosis; complex sclerosing lesions; massively parallel sequencing; immunohistochemistry

INTRODUCTION

The term infiltrating epitheliosis (IE) was first used by John Azzopardi in 1979.¹ Though IE has been described as “sclerosing adenosis with pseudo-infiltration”² and “sclerosing papillary proliferation”,³ its classification is still controversial. To clarify its status, Eusebi and Millis described its histologic features as: 1) bulk of the lesion composed of florid epitheliosis, with frequent focal squamoid appearance; 2) scleroelastotic stromal changes seen throughout the lesion, adjacent to epithelial foci, rather than confined to a central scleroelastotic nidus as in radial scars (RSs) or complex sclerosing lesions (CSLs); and 3) the frequent presence of a desmoplastic stromal reaction and keloid-like fibrous bands.^{1,4} Unlike usual ductal hyperplasia (UDH), the involved ducts often have jagged or irregular edges and the proliferating epithelium often appears to “flow-out” into adjacent stroma. The overall appearance of IE is more infiltrative than UDH and differs from that of usual sclerosing lesions; therefore, invasive carcinoma is often considered in the differential diagnosis.

Despite these distinctive histologic features, most pathologists currently classify IE in the RS/CSL spectrum. Thus, its true incidence and association with carcinoma are unknown. The incidence of RS/CSL ranges from 4.7–8.2%,^{5–7} and long-term follow-up of women with RS/CSL indicates a 1.5–2 fold increase in subsequent breast cancer risk,^{5–7} which persists after adjusting for concurrent proliferative disease.⁸ Whether RS/CSLs are truly an independent risk factor for carcinoma and/or constitute non-obligate breast cancer precursors remains controversial. The presence of a coexisting carcinoma in RS/CSLs ranges from 3.6–32%^{9–11} and a rare association between CSL and low-grade metaplastic carcinomas has been described^{12,13}. From a genetic standpoint, RS/CSLs have been shown to harbor *PIK3CA* activating mutations in 63.6%,¹⁴ and these were more prevalent when epithelial atypia was superimposed (56.3% versus 83.3%).¹⁴ Activating *PIK3CA* mutations, the second most frequently mutated gene in breast cancer¹⁵, have also been documented in papillomas,¹⁶ columnar cell lesions¹⁷ and UDH,¹⁸ and it has been hypothesized that *PIK3CA* mutations may be more relevant for proliferation than for malignant transformation in breast epithelium.¹⁴

The pathologic and molecular features of IEs have yet to be fully characterized. Whether IEs are hyperplastic or neoplastic lesions and the relevance of splitting this lesion from more common sclerosing and proliferative lesions is uncertain. To address these questions, we sought to characterize the histologic, immunophenotypic and genomic profiles of IEs.

MATERIALS AND METHODS

Cases

Eight cases, from seven patients, diagnosed as IE were retrieved from the consultation files of one of the authors (IOE) at University Nottingham Hospitals (Nottingham, UK). Samples were anonymized prior to analysis and the study was approved by the Institutional Review Board (IRB) at University Nottingham Hospitals. Informed consent was obtained where appropriate following the IRB approved protocol. Histologic evaluation was centrally performed by three pathologists (CAE, JSR-F, IOE), and the lesions were classified using diagnostic criteria described by Azzopardi¹ and Eusebi and Millis.⁴ Consensus among all three pathologists was required for case inclusion, which took place at the Memorial Sloan Kettering Cancer Center (MSKCC) Department of Pathology. The clinicopathologic features of IE are summarized in Table 1.

Immunohistochemistry

Immunohistochemistry was performed on representative 4µm-thick sections, using monoclonal antibodies against estrogen receptor (ER), cytokeratin (CK) 5/6, p63, smooth muscle myosin heavy chain (SMM-HC), C-KIT, CD34 and CD31. The antibody sources, dilutions, pre-treatment protocols, and detection methods are summarized in Supplementary Table 1. Positive and negative controls were included in each experiment. ER was scored according to the American Society of Clinical Oncology (ASCO) / College of American Pathologists (CAP) guidelines¹⁹. For CK5/6, any cytoplasmic staining in proliferating epithelial cells was recorded as positive. For C-KIT, only membranous staining of proliferating cells was considered specific, and any staining was reported as positive. All cases were reviewed by at least two pathologists (CAE, JSR-F and/ or IOE).

Microdissection and DNA extraction

Representative 8µm-thick sections from each sample were subjected to microdissection of tumor and matched normal tissue with a sterile needle under a stereomicroscope (Olympus SZ61, Center Valley, PA) to ensure >90% of tumor cells, as previously described.^{20–22} DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) according to manufacturer's guidelines²³, and quantified using the Qubit Fluorometer (Life Technologies, Carlsbad, CA). DNA was also extracted from microdissected adjacent UDH (case 2), low-grade adenosquamous carcinoma (LGASC, case 3), micropapillary DCIS (case 3) and a papilloma (case 7) and processed as above.

Targeted Capture massively parallel sequencing (MPS)

Tumor and germline DNA were subjected to targeted capture MPS using a custom oligonucleotide hybridization capture platform (NimblegenSeqCap) containing baits targeting all exons of 254 genes recurrently mutated in breast cancer or involved in DNA repair pathways (Supplementary Table 2).^{20,22,24} Barcoded sequence libraries were prepared, pooled into a single capture reaction and sequenced on a HiSeq2000 (Illumina, San Diego, CA).^{20,24,25} Sequence reads were aligned to the reference human genome GRCh37 using the Burrows-Wheeler Aligner.²⁶ Somatic point mutations were identified

using MuTect²⁷ and small insertions and deletions (indels) using VarScan²⁸ and Strelka.²⁹ Indels were reviewed manually using the Integrative Genomics Viewer.³⁰ Variants covered by <10 reads in the tumor or <5 reads in the germline were disregarded. Mutations supported by <5 reads, located outside of the target regions or for which the tumor variant allele fraction was <5 times that of the normal variant allele fraction were disregarded²¹. Variants found at >5% global minor allele frequency in dbSNP (Build 137) were disregarded.

Sequence data have been deposited to the Sequence Read Archive (accession SRP055006).

PCR amplification and Sanger sequencing

Somatic *PIK3CA* mutations identified by targeted capture MPS were investigated in the index cases and their corresponding adjacent lesions by Sanger sequencing. Sample 02-UDH, as the DNA extracted was insufficient for MPS, was screened for *PIK3CA* mutations in the frequently mutated exons 7, 9 and 20 (Supplementary Table 3).^{21,31} PCR conditions and Sanger sequencing were performed as previously described.²¹

RESULTS

Histopathologic analysis of infiltrating epitheliosis

All patients diagnosed with IE were female and the mean age at diagnosis was 63 years (range 54–80; Table 1). One patient had bilateral IE lesions (case 5). All IEs were tumor-forming lesions. Given that these lesions were received in consultation, limited clinical information and no follow-up data are available.

All cases demonstrated infiltrating ducts filled with a florid UDH-like proliferation with some epithelial foci having irregular or jagged edges (Figure 1A–C) and at least some areas where the proliferating cells appeared to “flow-out” or infiltrate into adjacent stroma (Figure 1B). At high power, the solid epithelial proliferation was reminiscent of UDH, with frequent intranuclear inclusions (Figure 1D). The surrounding stromal changes varied from desmoplastic to scleroelastotic to myxoid; six cases had areas of stromal desmoplasia (Figure 1A–C) and five cases, at least focally, displayed keloid-like fibrous bands (Figure 1A). Focal squamoid appearance was observed in cases 2 and 3 (Figure 1B).

An underlying papillary architecture was observed in four cases (cases 1, 5, 7 and 9; Figures 2A and 2B). Three cases were associated with other proliferative lesions including classic UDH (case 2), low-grade micropapillary DCIS (case 3), and papilloma (case 7). The classic UDH in case 2 (Figure 2C) retained a peripheral myoepithelial cell (MEC) layer as demonstrated by p63 immunostaining, while the adjacent IE showed a complete lack of p63-positive MECs at the periphery (Figure 2D). Case 3 contained in addition to the IE a DCIS (Figure 2E), which displayed the expected p63-positive MEC layer (Figure 2F), and a 1.2 cm LGASC characterized by glands with bland nuclear features and varying degrees of squamous differentiation, haphazardly arranged in an infiltrative spindle cell stroma (Figure 3A). Case 7 contained IE and a papilloma, which were distinct from but adjacent to each other, producing a single mass-forming tumor (Figure 3B).

Immunophenotypic characterization of infiltrating epitheliosis

Similar to classic UDH, IE displayed a heterogeneous epithelial phenotype, composed of an admixture of CK5/6-positive basal/intermediate-type cells and both ER-positive and ER-negative/C-KIT-positive luminal-type cells (Table 2 and Figure 4). MECs were found to be attenuated or absent in IEs. In contrast to classic UDH and most sclerosing lesions, using p63 and SMM-HC, peripheral MECs were absent or discontinuous around involved ducts in all but one case (Table 2, Figure 4). In case 9, p63-positive MECs formed a continuous layer around the duct-like structures, but SMM-HC was discontinuous (Table 2). In case 2, IE showed a CK-pattern of staining (Figures 4A–D) similar to the adjacent classic UDH, but a MEC layer was absent (Figure 2D). Although the DCIS in case 3 had diagnostic morphologic features, including long and slender malignant micropapillae composed of monotonous cells with low nuclear grade and retention of a MEC layer around the periphery of the lesion, the neoplastic cells displayed an unusual immunophenotype (ER-negative and CK5/6-positive; data not shown) for a low-grade DCIS. As previously reported for this special type of carcinoma,³² the LGASC of case 3 was ER-negative, showed glandular luminal p63-staining in 10% of cells with no staining of stromal cells, and displayed occasional/scattered CK5/6 expression in stromal/spindle cells, with discontinuous and absent circumferential staining of p63 and SMM-HC, respectively (Table 2).

Comparison of cases with Eusebi and Millis' criteria⁴ for IE

In agreement with the Eusebi and Millis criteria,⁴ two of eight cases were completely ER-negative, whereas six cases displayed heterogeneous ER expression (5–30% positive cells), as expected for an UDH-like proliferation (Table 3). Furthermore, Eusebi and Millis reported that the majority of IE cells express a combination of high molecular-weight keratins and p63.⁴ We partly confirmed this observation with all cases showing heterogeneous expression of CK5/6; however, only two cases harbored p63-positive proliferating cells. Eusebi and Millis also reported that MECs are present around IE; using two MEC markers, p63 and SMM-HC, we confirmed that MECs are present, but discontinuous in six of eight IEs using at least one of these markers. In two cases, however, MECs were not observed after thorough histologic and immunohistochemical assessment.

Infiltrating epitheliosis is characterized by frequent mutations affecting the PI3K pathway

To characterize the repertoire of somatic mutations in the genes most frequently mutated in breast cancer or associated with DNA repair, we performed targeted capture MPS from microdissected IEs, adjacent breast lesions and matched normal tissue to a median read depth of 586× (range 207×–1237×). We identified somatic non-synonymous mutations in the phosphatidylinositol 3-kinase (PI3K) family in all IEs studied (*PIK3CA*, n=7 and *PIK3R1*, n=1; Figure 5 and Supplementary Table 4), which were validated by Sanger sequencing in the corresponding IEs and adjacent lesions (Supplementary Figure 1). Specifically, of the seven IEs with *PIK3CA* mutations, six and one harbored the hotspot H1047R and E542K mutations, respectively. The only IE lacking a *PIK3CA* mutation (case 9) harbored a *PIK3R1* (L380del) small deletion.

Targeted capture MPS analysis of the DNA samples obtained from lesions adjacent to IEs revealed that in case 3, the IE, DCIS and LGASC harbored identical *PIK3CA* (H1047R) and *SF3B1* (K700E) hotspot mutations, suggesting that these lesions are clonally related, and that IE and DCIS may have constituted the substrate for the development of the LGASC. In case 7, both the IE and the papilloma contained an identical *PIK3CA* H1047R mutation with an additional *ERBB3* mutation found in one of the two IE samples (Supplementary Table 4, Supplementary Figure 2). The UDH component of case 2 did not harbor the *PIK3CA* H1047R mutation found in the IE; instead a *PIK3CA* C420K mutation was found in this lesion (Supplementary Figure 1), suggesting that the respective IE and UDH were unlikely to be clonally related and providing an example of a convergent phenotype in the development of these lesions, given that the PI3K pathway was activated through distinct somatic genetic alterations.

DISCUSSION

IE is currently not routinely recognized in breast pathology practice, and cases of IE are often diagnosed as RS/CSLs. Here, we confirm the observation from Eusebi and Millis⁴ in that IE has a more infiltrative appearance than usual sclerosing lesions, with scleroelastotic stromal changes present throughout the lesion rather than confined to a central nidus as in RS/CSLs. In fact, none of our cases contained a central scleroelastotic nidus. Additionally, while epithelial proliferation varies in RS/CSLs and is usually restricted to the periphery, florid epitheliosis comprises the bulk of IE. Finally, and possibly most importantly, thorough histologic analysis coupled with p63 and SMM-HC immunohistochemistry revealed an absent-to-discontinuous peripheral MEC layer in most IEs. While rare in classic UDH, this may occur in RS/CSLs, as reported by Hilson *et al.*³³, where 17.4% and 27.3% of RS/CSLs, respectively, have reduced staining of myoepithelial cells with p63 and SMM-HC antibodies. One may argue with more sensitive MEC markers, such as smooth muscle actin, a continuous MEC layer may be highlighted in our IE cases.³³ Nevertheless, our data demonstrate that the MEC layer is, at least, immunophenotypically abnormal in the majority of IEs.

Using targeted capture MPS, *bona fide* oncogenic *PIK3CA* or *PIK3R1* mutations were identified in all IEs, suggesting that these lesions are neoplastic rather than hyperplastic and that the PI3K pathway is involved in IE pathogenesis. This finding is consistent with the study by Wolters *et al.* where >60% of RSs contained *PIK3CA* activating mutations,¹⁴ and supports the contention that RS and IE are related, where IE may represent the most proliferative end of this spectrum of lesions. The PI3K pathway, which plays pivotal roles in cell survival, proliferation, and migration,³⁴ is altered in 25–50% of breast carcinomas, in particular low-grade, ER-positive carcinomas^{16,35–43} and their precursors.¹⁷ However, unlike the typical ER-positive/CK5/6-negative immunophenotype of low-grade breast neoplasms, IEs were CK5/6-positive and heterogeneous or even negative for ER. Therefore, though our data suggest that IE is a clonal neoplastic lesion with PI3K activation, IEs do not seem to follow the same evolutionary pathway as traditional ER-positive breast cancer precursors.

Targeted capture MPS revealed that in one patient, the ER-negative/CK5/6-positive IE, DCIS and LGASC harbored identical hotspot mutations affecting *PIK3CA* and *SF3B1*.

These observations are consistent with the hypothesis that the IE may have constituted the substrate from which the DCIS and LGASC originated. Although the occurrence of low-grade ER-negative metaplastic carcinomas in sclerosing lesions has been documented,^{12,13} here we provide evidence that the UDH-like proliferation in sclerosing lesions may be clonally related to the carcinoma. Further studies investigating why a subset of these lesions may be prone to progress to invasive cancer are warranted.

In conclusion, we demonstrated that IE displays a heterogeneous epithelial phenotype similar to classic UDH. In contrast to UDH and other proliferative and sclerosing lesions, IE has other features including an infiltrative appearance coupled with absence or discontinuity of a MEC layer, which may pose difficulties for the differential diagnosis with invasive carcinoma. Targeted capture MPS revealed recurrent mutations affecting the PI3K pathway in all IEs; together with the histologic characteristics of these lesions, these genetic findings support the contention that these lesions are clonal and neoplastic. As a group, IEs appear to be related to RS/CSLs and may represent the most proliferative end of the spectrum of these lesions. Although at present the management of IEs should not differ from that of other complex sclerosing lesions, recognition of this rare lesion is not a mere academic exercise, as further studies are essential to catalogue its clinical behavior and to define the most appropriate surgical treatment. This should be possible through the recognition of the distinctive features of IEs, including its infiltrative appearance and the attenuation of the MEC layer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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IOE and JSR-F conceived the study. IOE and EAR provided the samples. RAS cut the slides for immunohistochemical analysis. CAE, EAR, ME and JSR-F performed the pathologic review and evaluated the immunohistochemical results. CAE, SP, CKYN, FCG and BW carried out experiments and analyzed data. CAE wrote the first draft of the manuscript that was revised by SP, EAR, CKYN, FCG, BW, JSR-F and IOE. All authors reviewed and approved the final version of the manuscript.

REFERENCES

1. Azzopardi, JG. Overdiagnosis of Malignancy. London, UK: W.B. Saunders Company, Ltd; 1979.
2. McDivitt, RW.; Stewart, RE.; Berg, JW. Tumors of the Breast. Washington, DC: Armed Forces Institute of Pathology; 1968.
3. Fenoglio C, Lattes R. Sclerosing papillary proliferations in the female breast. A benign lesion often mistaken for carcinoma. Cancer. 1974; 33:691–700. [PubMed: 4815573]
4. Eusebi V, Millis RR. Epitheliosis, infiltrating epitheliosis, and radial scar. Semin Diagn Pathol. 2010; 27:5–12. [PubMed: 20306826]
5. Jacobs TW, Byrne C, Colditz G, Connolly JL, Schnitt SJ. Radial scars in benign breast-biopsy specimens and the risk of breast cancer. N Engl J Med. 1999; 340:430–436. [PubMed: 9971867]

6. Sanders ME, Page DL, Simpson JF, et al. Interdependence of radial scar and proliferative disease with respect to invasive breast carcinoma risk in patients with benign breast biopsies. *Cancer*. 2006; 106:1453–1461. [PubMed: 16502407]
7. Berg JC, Visscher DW, Vierkant RA, et al. Breast cancer risk in women with radial scars in benign breast biopsies. *Breast Cancer Res Treat*. 2008; 108:167–174. [PubMed: 18297395]
8. Aroner SA, Collins LC, Connolly JL, et al. Radial scars and subsequent breast cancer risk: results from the Nurses' Health Studies. *Breast Cancer Res Treat*. 2013; 139:277–285. [PubMed: 23609472]
9. Wellings SR, Alpers CE. Subgross pathologic features and incidence of radial scars in the breast. *Hum Pathol*. 1984; 15:475–479. [PubMed: 6724567]
10. Sloane JP, Mayers MM. Carcinoma and atypical hyperplasia in radial scars and complex sclerosing lesions: importance of lesion size and patient age. *Histopathology*. 1993; 23:225–231. [PubMed: 8225240]
11. Manfrin E, Remo A, Falsirollo F, Reghellin D, Bonetti F. Risk of neoplastic transformation in asymptomatic radial scar. Analysis of 117 cases. *Breast Cancer Res Treat*. 2008; 107:371–377. [PubMed: 17457669]
12. Denley H, Pinder SE, Tan PH, et al. Metaplastic carcinoma of the breast arising within complex sclerosing lesion: a report of five cases. *Histopathology*. 2000; 36:203–209. [PubMed: 10692021]
13. Gobbi H, Simpson JF, Jensen RA, Olson SJ, Page DL. Metaplastic spindle cell breast tumors arising within papillomas, complex sclerosing lesions, and nipple adenomas. *Mod Pathol*. 2003; 16:893–901. [PubMed: 13679453]
14. Wolters KL, Ang D, Warrick A, et al. Frequent PIK3CA mutations in radial scars. *Diagn Mol Pathol*. 2013; 22:210–214. [PubMed: 24193002]
15. Ng CK, Schultheis AM, Bidard FC, Weigelt B, Reis-Filho JS. Breast cancer genomics from microarrays to massively parallel sequencing: paradigms and new insights. *J Natl Cancer Inst*. 2015; 107:djv015. [PubMed: 25713166]
16. Troxell ML, Levine J, Beadling C, et al. High prevalence of PIK3CA/AKT pathway mutations in papillary neoplasms of the breast. *Mod Pathol*. 2010; 23:27–37. [PubMed: 19898424]
17. Troxell ML, Brunner AL, Neff T, et al. Phosphatidylinositol-3-kinase pathway mutations are common in breast columnar cell lesions. *Mod Pathol*. 2012; 25:930–937. [PubMed: 22460814]
18. Ang DC, Warrick AL, Shilling A, et al. Frequent phosphatidylinositol-3-kinase mutations in proliferative breast lesions. *Mod Pathol*. 2014; 27:740–750. [PubMed: 24186142]
19. Hammond ME, Hayes DF, Dowsett M, et al. American Society of Clinical Oncology/College Of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *J Clin Oncol*. 2010; 28:2784–2795. [PubMed: 20404251]
20. Natrajan R, Wilkerson PM, Marchio C, et al. Characterization of the genomic features and expressed fusion genes in micropapillary carcinomas of the breast. *J Pathol*. 2014; 232:553–565. [PubMed: 24395524]
21. Weinreb I, Piscuoglio S, Martelotto LG, et al. Hotspot activating PRKD1 somatic mutations in polymorphous low-grade adenocarcinomas of the salivary glands. *Nat Genet*. 2014; 46:1166–1169. [PubMed: 25240283]
22. Ng CK, Martelotto LG, Gauthier A, et al. Intra-tumor genetic heterogeneity and alternative driver genetic alterations in breast cancers with heterogeneous HER2 gene amplification. *Genome Biol*. 2015; 16:107. [PubMed: 25994018]
23. Marchio C, Iravani M, Natrajan R, et al. Genomic and immunophenotypical characterization of pure micropapillary carcinomas of the breast. *J Pathol*. 2008; 215:398–410. [PubMed: 18484683]
24. Guerini-Rocco E, Hodi Z, Piscuoglio S, et al. The repertoire of somatic genetic alterations of acinic cell carcinomas of the breast: an exploratory, hypothesis-generating study. *J Pathol*. 2015; 237:166–178. [PubMed: 26011570]
25. Martelotto LG, De Filippo MR, Ng CK, et al. Genomic landscape of adenoid cystic carcinoma of the breast. *J Pathol*. 2015; 237:179–189. [PubMed: 26095796]
26. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*. 2009; 25:1754–1760. [PubMed: 19451168]

27. Cibulskis K, Lawrence MS, Carter SL, et al. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. *Nat Biotechnol.* 2013; 31:213–219. [PubMed: 23396013]
28. Koboldt DC, Zhang Q, Larson DE, et al. VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. *Genome Res.* 2012; 22:568–576. [PubMed: 22300766]
29. Saunders CT, Wong WS, Swamy S, et al. Strelka: accurate somatic small-variant calling from sequenced tumor-normal sample pairs. *Bioinformatics.* 2012; 28:1811–1817. [PubMed: 22581179]
30. Robinson JT, Thorvaldsdottir H, Winckler W, et al. Integrative genomics viewer. *Nat Biotechnol.* 2011; 29:24–26. [PubMed: 21221095]
31. Weigelt B, Warne PH, Downward J. PIK3CA mutation, but not PTEN loss of function, determines the sensitivity of breast cancer cells to mTOR inhibitory drugs. *Oncogene.* 2011; 30:3222–3233. [PubMed: 21358673]
32. Lauer S, Oprea-Ilie G, Cohen C, Adsay V, Adams AL. Acidophilic nuclear inclusions are specific for florid ductal hyperplasia among proliferative breast lesions. *Arch Pathol Lab Med.* 2011; 135:766–769. [PubMed: 21631270]
33. Hilson JB, Schnitt SJ, Collins LC. Phenotypic alterations in myoepithelial cells associated with benign sclerosing lesions of the breast. *Am J Surg Pathol.* 2010; 34:896–900. [PubMed: 20463570]
34. Courtney KD, Corcoran RB, Engelman JA. The PI3K pathway as drug target in human cancer. *J Clin Oncol.* 2010; 28:1075–1083. [PubMed: 20085938]
35. Hernandez L, Wilkerson PM, Lambros MB, et al. Genomic and mutational profiling of ductal carcinomas in situ and matched adjacent invasive breast cancers reveals intra-tumour genetic heterogeneity and clonal selection. *J Pathol.* 2012; 227:42–52. [PubMed: 22252965]
36. Cancer Genome Atlas N. Comprehensive molecular portraits of human breast tumours. *Nature.* 2012; 490:61–70. [PubMed: 23000897]
37. Stemke-Hale K, Gonzalez-Angulo AM, Lluch A, et al. An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. *Cancer Res.* 2008; 68:6084–6091. [PubMed: 18676830]
38. Fedele CG, Ooms LM, Ho M, et al. Inositol polyphosphate 4-phosphatase II regulates PI3K/Akt signaling and is lost in human basal-like breast cancers. *Proc Natl Acad Sci U S A.* 2010; 107:22231–22236. [PubMed: 21127264]
39. Gewinner C, Wang ZC, Richardson A, et al. Evidence that inositol polyphosphate 4-phosphatase type II is a tumor suppressor that inhibits PI3K signaling. *Cancer Cell.* 2009; 16:115–125. [PubMed: 19647222]
40. Dunlap J, Le C, Shukla A, et al. Phosphatidylinositol-3-kinase and AKT1 mutations occur early in breast carcinoma. *Breast Cancer Res Treat.* 2010; 120:409–418. [PubMed: 19418217]
41. Miron A, Varadi M, Carrasco D, et al. PIK3CA mutations in in situ and invasive breast carcinomas. *Cancer Res.* 2010; 70:5674–5678. [PubMed: 20551053]
42. Kalinsky K, Heguy A, Bhanot UK, Patil S, Moynahan ME. PIK3CA mutations rarely demonstrate genotypic intratumoral heterogeneity and are selected for in breast cancer progression. *Breast Cancer Res Treat.* 2011; 129:635–643. [PubMed: 21617917]
43. Sakr RA, Weigelt B, Chandarlapaty S, et al. PI3K pathway activation in high-grade ductal carcinoma in situ--implications for progression to invasive breast carcinoma. *Clin Cancer Res.* 2014; 20:2326–2337. [PubMed: 24634376]

Figure 1. Histologic features of infiltrating epitheliosis

(A) Florid UDH-like proliferation with some epithelial foci having irregular or jagged edges (Case 2, 40 $\times$); keloid-like fibrous bands can also be seen within adjacent stroma. (B) Focal areas where proliferating cells appear to “flow-out” into adjacent stroma and have a squamoid appearance (Case 2, 100 $\times$). (C) and (D) Infiltrating ducts and duct-like structures filled with cells reminiscent of those seen in UDH (Case 5, 40 $\times$ and 200 $\times$, respectively). Figures 1A–D all demonstrate stromal desmoplasia adjacent to foci of epithelial proliferations.

Figure 2. Infiltrating epitheliosis, underlying architecture, and adjacent epithelial proliferative lesions

Underlying papillary architecture was observed in four cases of infiltrating epitheliosis (IE). (A) Representative micrograph of a hematoxylin-and-eosin stained IE demonstrating fibrovascular cores surrounded by a UDH-like epithelial proliferation (Case 1, 40 $\times$). (B) CD34 expression highlighting the underlying vasculature (Case 1, 40 $\times$). (C) Representative micrograph of a hematoxylin-and-eosin stained IE and adjacent classic UDH (Case 2, 100 $\times$). (D) p63 nuclear expression highlights the presence of a myoepithelial layer around the periphery of the classic UDH, but the complete absence of p63-positive myoepithelial cells

around the periphery of IE (Case 2, 100×). (E) Representative micrograph of a hematoxylin-and-eosin stained micropapillary DCIS (Case 3, 40×). (F) p63 expression highlighting the presence of a myoepithelial layer around the periphery of the DCIS (Case 3, 40×).

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Figure 3. Infiltrating epitheliosis and adjacent lesions

(A) Representative micrograph of a hematoxylin-and-eosin stained low-grade adenosquamous carcinoma which was found adjacent to the infiltrating epitheliosis of Case 3 (40×). (B) Infiltrating epitheliosis (bottom) and adjacent papillary lesion (top, case 7, 20×).

Figure 4. Immunohistochemical profile of infiltrating epitheliosis

Immunohistochemical analysis of infiltrating epitheliosis revealed heterogeneous expression of (A) estrogen receptor (ER, 100 $\times$) and (B) cytokeratin (CK) 5/6 (Case 2, 40 $\times$). Note the absence of (C) p63-positive and (D) smooth muscle myosin heavy chain (SMM-HC)-positive myoepithelial cells around the involved ducts, but their presence around uninvolved ducts (Case 2, C, 40 $\times$ and D, 100 $\times$). Immunohistochemical analysis of (E) ER, (F) CK 5/6 and (G) C-KIT revealed that the infiltrating epitheliosis displayed a heterogeneous epithelial phenotype, akin to that of usual ductal hyperplasia (Case 5, 40 $\times$). (H) p63-positive myoepithelial cells formed a discontinuous layer around the ducts affected by infiltrating epitheliosis (E–H, 40 $\times$).

Figure 5. Repertoire of somatic mutations in infiltrating epitheliosis
Heatmap shows the repertoire of somatic mutations identified in the 254 genes sequenced, ordered from top to bottom based on the number of alterations found in a given gene. The colors indicate the different types of mutations found. See color key.

Table 1

Clinicopathologic characteristics of infiltrating epitheliosis and adjacent breast lesions

Case	Lesion sample	Lesion components	Age (years)	Laterality	Size (cm)	Sequencing depth
1	01-IE	Infiltrating epitheliosis	63	Left	2.1	513x
2	02-IE	Infiltrating epitheliosis	59	Right	2.1	204x
	02-UDH	Usual ductal hyperplasia			0.3	NP
3	03-IE	Infiltrating epitheliosis	80	Right	0.6	823x
	03-DCIS	Micropapillary DCIS			0.5	900x
	03-LGASC	Low-grade adenocarcinoma			1.2	844x
5 (R) *	05-IE-R	Infiltrating epitheliosis	54	Right	0.8	550x
5 (L) *	05-IE-L	Infiltrating epitheliosis		Left	0.8	864x
7#	07-IE-1	Infiltrating epitheliosis (block 1)	63	Left	0.3	1363x
	07-IE-2	Infiltrating epitheliosis (block 2)			0.3	785x
	07-PAP-1	Papilloma (block 1)			0.6	1039x
	07-PAP-2	Papilloma (block 2)			0.3	361x
8 ⁺	08-IE-p63pos	Infiltrating epitheliosis (p63-positive)	66	NA	0.8	944x
	08-IE-p63neg	Infiltrating epitheliosis (p63-negative)				527x
9	09-IE	Infiltrating epitheliosis	55	Right	0.7	1311x

[^] Cases 5 (R) and 5 (L): Bilateral lesions in the same patient.

[#] Case 7: Two blocks, both containing the same IE lesion, were available for evaluation.

⁺ Case 8: 08-IE-p63pos identifies the main IE lesion (p63 expression at periphery) and 08-IE-p63neg identifies an area of the lesion lacking p63 expression at the periphery of the IE-ducts. DCIS, ductal carcinoma *in situ*; IE, infiltrating epitheliosis; NA, not available; NP, not performed, due to insufficient DNA.

Table 2

Immunohistochemical features of infiltrating epitheliosis

Case	ER (% cells ⁺)	CK5/6 (% cells ⁺)	C-KIT (% cells ⁺)	p63, within lesion (% cells ⁺)	p63-positive myoepithelial cells	SMM-HC-positive myoepithelial cells
1	0	80	10	0	Absent	Absent
2	20	40	<5	0	Absent	Absent
3	0	80	<5	10	Discontinuous	Absent
5 (R) [*]	25	70	5–60 (40)	0	Discontinuous	Discontinuous
5 (L) [*]	25	70	20	0	Discontinuous	Discontinuous
7 ^{**}	5–20 (15)	25–40 (35)	<1	1–5 (<5)	Discontinuous	Absent
8	30	75	30	0	Discontinuous	Discontinuous
9	5	90	70	0	Continuous	Discontinuous

^{*}Cases 5 (L) and 5 (R): Separate lesions in the same patient, one in each breast^{**}Case 7: Two blocks, both containing the same IE lesion were available for evaluation; therefore, a range of values for immunohistochemical stains is reported.⁺, positive; L, left; R, right; SMM-HC, smooth muscle myosin, heavy chain.

Table 3

Comparison of characteristics between current cases and Eusebi and Millis' infiltrating epitheliosis criteria.⁴

Eusebi and Millis, 2010 ⁴	Current infiltrating epitheliosis (n=8)
Florid epitheliosis	8/8
Some foci with irregular edges	8/8
Proliferating cells:	
“Flow out” into adjacent stroma	8/8
Some foci with squamoid appearance	2/8
Mostly ER negative	2/8 ER-negative 6/8 ER-positive (range 5–30%)
Majority expressing a combination of HMWK (CK14 & CK5/6) and p63	8/8 CK5/6-positive (range 35–90%) 2/8 p63-positive (range <5–10%)
Peripheral MECs present	5/8 MECs discontinuous [*] 1/8 MECs discontinuous ^{**} 2/8 MECs absent [*]
Desmoplastic and/ or scleroelastotic stroma	8/8 with scleroelastotic stroma 6/8 with areas of desmoplasia
Keloid-like fibrous bands may be present	5/8

^{*} Both p63 and SMM-HC

^{**} SMM-HC, but not p63.

HMWK, high-molecular weight cytokeratin; MECs, myoepithelial cells; SMM-HC, smooth muscle myosin, heavy chain.