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NR4A3 Expression is Consistently Absent in Acinic Cell Carcinomas of the Breast: A Potential Nosologic Shift

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Abstract

Acinic cell carcinoma (AciCC) is a tumor that is recognized in both the breast and salivary glands. Recently, the recurrent genomic rearrangement, t(4;9)(q13;q31) was identified in salivary AciCC that results in constitutive upregulation of the nuclear transcription factor NR4A3, which can be detected by immunohistochemistry (IHC). Here we sought to evaluate NR4A3 expression in breast AciCC using IHC. Strong and diffuse nuclear staining was considered a positive result. Sixteen AciCC were studied including 8 pure AciCC and 8 AciCC admixed with other types (invasive carcinoma of no special type in 5 cases and metaplastic carcinoma in 3 cases). All 16 AciCC were negative for NR4A3 expression. Four cases with available material were evaluated for rearrangements of the *NR4A3* gene by fluorescence *in situ* hybridization (FISH) and no rearrangements were observed. Whole-genome sequencing of one AciCC revealed a *TP53* splice-site mutation, high levels of genomic instability, and genomic features of homologous recombination DNA repair defects; structural variant analysis of this case did not reveal the presence of a t(4;9) rearrangement. We conclude that 1) breast AciCC consistently lack NR4A3 rearrangement or overexpression, unlike the vast majority of salivary AciCC, and 2) consistent with prior results, breast AciCC is associated with genomic alterations more similar to those seen in triple-negative breast carcinomas (TNBC) than salivary gland AciCC. These results suggest that unlike other salivary gland-like tumors that occur in the breast, the molecular underpinnings of salivary gland and breast AciCC are different and that salivary gland and breast AciCC likely represent distinct entities.

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Author Contributions

E.T.R., B.W., J.S.R-F., V.Y.J., and S.J.S. developed the study concept and design; E.T.R., P.S., F.P., P.D.C., E.H., B.W., J.S.R-F., J.L.H., V.Y.J., and S.J.S. acquired, analyzed, and interpreted the data, and wrote the manuscript. All authors read and approved the final paper.

Keywords

Breast; acinic cell carcinoma; NR4A3; molecular characterization; homologous recombination DNA repair

Introduction

Acinic cell carcinoma (AciCC) of the breast is a rare subtype of breast carcinoma first described in 1996 and characterized by morphologic similarities to AciCC of the salivary gland[1]. It is categorized as a special type of estrogen receptor (ER)/progesterone receptor (PR)/HER2-negative (triple-negative) breast carcinoma in the current World Health Organization (WHO) classification of tumors of the breast[2].

Breast AciCC is characterized by an infiltrative growth pattern with glandular and solid components, eosinophilic luminal secretions in the glands, prominent eosinophilic cytoplasmic fine or coarse ("Paneth cell-like") granules, and in most cases low-to-intermediate grade nuclear atypia[3]. AciCCs of the breast may show a microglandular pattern, making distinction from microglandular adenosis (MGA) challenging in some cases[4]. Other AciCCs are found in association with high-grade TNBC, and in cases with mixed histologic patterns of AciCC and other types of breast carcinoma, shared genomic alterations have been identified in each component, suggesting clonal relatedness and the possibility of high-grade transformation[5]. In its pure form AciCC generally has a favorable prognosis but a more aggressive clinical course has been reported in those associated with high-grade TNBC[6].

We have previously demonstrated a similar spectrum of mutations in breast AciCC and other TNBCs, including frequent mutations in *TP53* and *PIK3CA*, as well as additional mutations in known breast carcinoma-associated genes such as *BRCA1*, *ERBB3*, *ERBB4*, *CTNNB1, INPP4B*, and *FGFR2*, and complex patterns of chromosomal copy number gains and losses[5,7,8].

In contrast, in AciCC of the salivary gland, mutations in *TP53* and other oncogenes are rare[9], and the most common recurrent genetic alteration is the t(4;9)(q13;q31) translocation involving *NR4A3* and the secretory Ca-binding phosphoprotein gene cluster, leading to overexpression of NR4A3 in tumor cells[10]. Other rearrangements have been described in salivary gland AciCC, including *HTN3*::*MSANTD3* and *PRB3*::*ZNF217* fusion genes[11,12]. Salivary gland AciCC also characteristically expresses DOG-1, a calcium-activated chloride channel and marker of serous acinar differentiation[13].

Nuclear expression of NR4A3 detected by immunohistochemistry (IHC) has been shown to be a highly sensitive and specific marker of AciCC in the salivary gland[14,15]. Nuclear NR4A3 protein expression is detected in AciCCs harboring the t(4;9) translocation involving *NR4A3*, and NR4A3 overexpression is found in tumors associated with the *HTN3*::*MSANTD3* fusion, indicating that NR4A3 overexpression is a critical pathogenetic mechanism for salivary AciCC[14]. Overexpression of a related nuclear receptor, NR4A2, has been observed in rare salivary AciCC lacking NR4A3 overexpression, suggesting that this

pathway and transcription factor family is important for development of this type of salivary gland carcinoma[16]. Given prior findings that raise questions about the relationship between breast and salivary gland AciCC, we sought to define the expression of NR4A3 protein and the presence of *NR4A3* rearrangements in breast AciCC to further assess whether there these are truly related to salivary AciCC. We also sought to perform a novel characterization of breast AciCC by means of deep whole-genome sequencing.

Materials and Methods

Study Materials and Case Selection

AciCCs of the breast diagnosed by subspecialty breast pathologists between 2015 and 2021 using the WHO criteria[2] were identified from the surgical pathology consultation archives of the authors' institutions. Eighteen specimens from sixteen unique patients with AciCC of the breast were identified, including four cases which have been published previously[5], and are further characterized in this study. Clinical characteristics for the patients were obtained from the original institutional and consultation pathology reports. Hematoxylin and eosin (H&E)-stained slides and immunostained slides of all cases were reviewed by two of the authors (E.T.R. and S.J.S.) to confirm the diagnosis of AciCC and document any additional pathologic findings prior to including the cases in this study. Freshly prepared whole tissue sections were obtained from the original archival formalin-fixed, paraffin-embedded (FFPE) tissue blocks wherever possible.

Immunohistochemical Analysis

Unstained slides were subjected to standard immunohistochemical techniques in the Immunohistochemistry Laboratory of Brigham and Women's Hospital, as described in a previous study[15]. Briefly, 4-micron FFPE whole-tissue sections were subjected to antigen retrieval in pH 6.1 citrate buffer using a pressure cooker (Target Retrieval Solution, Dako, Carpinteria, CA, USA). The NR4A3 (NOR-1) mouse monoclonal primary antibody (clone H-7 at 1:50 dilution; Santa Cruz Biotechnology, Dallas, TX, USA) and DOG-1 mouse monoclonal primary antibody (clone K9 at 1:30 dilution; Leica Biosystems, Buffalo Grove, IL, USA) were applied followed by detection with the Novolink Polymer Detection System (Leica) for NR4A3 and EnVision+ (Dako) for DOG-1, according to the manufacturer's instructions.

For AciCCs with mixed histologic patterns, areas of AciCC morphology were evaluated separately. Nuclear NR4A3 expression and membranous or cytoplasmic DOG-1 expression were considered positive. External positive control slides were tested and evaluated alongside the cases and showed appropriate immunoreactivity (the NR4A3 external positive control was an established case of AciCC of the salivary gland by morphologic and standard diagnostic IHC characterization, and the DOG-1 external positive control was a known gastrointestinal stromal tumor).

Fluorescence In Situ Hybridization Detection and Analysis

Interphase FISH was performed according to standard methods, as described in a previous study[15]. Briefly, FISH was attempted on at least one specimen from each unique patient

with available material. A total of nine specimens were analyzed by FISH for *NR4A3*. Customized dual-color break-apart probes were developed for the 5' and 3' regions of *NR4A3* at 9q22, with signal separation of >3 signal diameters considered as split signals and >4% split signals considered a positive result for rearrangement (this cut-off was determined by internal laboratory validation studies for the *NR4A3* probe). At least 50 nuclei with hybridization signals were needed for an evaluable result. A known case of extracellular myxoid chondrosarcoma, which is characterized by *NR4A3* rearrangement involving various fusion partners[17,18], was used as a positive external control for FISH and demonstrated *NR4A3* rearrangement in 92% of tumor cell nuclei.

Whole-genome sequencing analysis

DNA extracted from a breast AciCC and matched normal FFPE tissue (case 14, which had sufficient material available for sequencing of paired tumor and normal tissue) was subjected to whole-genome sequencing at Memorial Sloan Kettering Cancer Center's (MSK's) Integrated Genomics Operation using validated protocols[19]. Whole-genome sequencing data were processed using validated bioinformatics methods[19]. In brief, sequence reads were aligned to the reference human genome GRCh37 using the Burrows-Wheeler Aligner (BWA v0.7.15)[20]. Somatic single nucleotide variants (SNVs) were detected with MuTect (v1.0)[21]. Insertion and deletions (indels) were detected using Strelka (v2.0.15)[22], VarScan2 (v2.3.7)[23], Platypus (v0.8.1)[24], Lancet (v1.0.0)[25], and Scalpel (v0.5.3)[26]. SNVs with <10% mutant allele fraction were removed to limit FFPE-derived artefactual mutations. Structural variants were detected using Manta in paired tumor-normal mode[27]. Copy number alterations and loss of heterozygosity (LOH) were determined using FACETS[28]. Based on the copy-number alterations, large-scale state transitions were defined as previously described[29,30]. Somatic mutations in tumor suppressor genes that were deleterious/loss-of-function or targeting a mutational hotspot in oncogenes were considered pathogenic. Mutations targeting hotspot loci were annotated according to Chang et al[31]. Cancer cell fractions (CCFs) for each mutation were computed with ABSOLUTE[32]. Mutational signatures (COSMIC v3.1) were computed with Signal[33]. MSISensor[34] was run to compute the level of microsatellite instability. HRDetect[35] was run using mutations, copy number and structural variant calls derived from whole-genome sequencing.

Results

Clinical characteristics

A total of 18 specimens of AciCC of the breast were analyzed from 16 unique patients. For three patients, paired core needle biopsy and surgical resection specimens were evaluated. All patients were female. The median patient age at AciCC diagnosis was 46 years (range, 29–74 years) and the median tumor size was 2.2 cm (range, 0.6–10.5 cm; tumor size information available for 14 of 16 patients). Of the 16 patients included in this study, 11 had axillary lymph node sampling; one patient with a mixed AciCC and invasive carcinoma of no special type (NST) had metastatic carcinoma involving 10 of 17 lymph nodes (case 6), one patient with a pure AciCC had isolated tumor cells (ITC) in one lymph node (regarded as node-negative according to the American Joint Committee on Cancer[36]; case 10), and the remaining patients with lymph node sampling were node-negative (Table 1).

Pathologic characteristics

All breast AciCCs displayed characteristic histopathologic features, including haphazardly distributed small glands and solid nests, eosinophilic luminal secretions in the glands, and cells with finely to coarsely granular eosinophilic cytoplasm (Figure 1). Mixed histologic patterns were present in 8 AciCCs (Table 1). Of these, 5 AciCCs were admixed with invasive carcinoma of NST and 3 AciCCs were admixed with metaplastic carcinoma (1 spindle cell, 2 chondroid). The tumors showed immunophenotypic features characteristic of breast AciCC, including expression of S100 protein and proteins such as lysozyme, alpha-1-antitrypsin, or alpha-1-antichymotrypsin, and lack of ER, PR, and HER2 expression (Table 1; some cases were evaluated with a subset of these markers). All AciCC of the breast were negative for NR4A3 expression (Figure 1). Expression of DOG-1, a marker of serous acinar differentiation that has been used to identify AciCC of the salivary gland[13], was detected focally only in 2 of 16 breast AciCCs tested; the remaining 14 breast AciCCs lacked any expression of this marker (Figure 1 and Table 1).

Analysis of NR4A3 rearrangements by FISH

To assess the presence of *NR4A3* rearrangements, nine AciCCs were subjected to FISH analysis. Areas of AciCC were marked on H&E-stained sections for correlation with FISH slides to ensure evaluation was limited to the neoplastic cells and areas with acinic cell morphology. Analysis of the four breast AciCCs yielding optimal hybridization and interpretable results revealed no rearrangements (Table 1). The remaining five cases were not evaluable due to technical issues despite the optimal results in the external controls, and FISH was attempted twice for each case, with no detectable signals.

Whole-genome sequencing of a breast AciCC

To define the mutational signatures and their causative DNA repair defects and potential carcinogenic insults, we sought to subject breast AciCCs to whole-genome sequencing. Of the cases with matched normal tissue availability, only one had sufficient material and DNA of sufficient quantity and quality for sequencing of paired tumor and normal tissue (AciCC case 14). Whole-genome sequencing was subsequently performed on the matched tumor and normal tissue samples from AciCC case 14, which arose in a patient known to carry germline pathogenic variants affecting *BRCA1* and *MLH1* (Figure 2). Structural variant analysis did not reveal the presence of the *HTN3*::*MSANTD3* fusion or the t(4,9)(q13;q31) rearrangement resulting in NR4A3 overexpression that are seen in salivary gland AciCC. A total of 4,549 somatic mutations were detected in AciCC case 14, including a clonal *TP53* splice-site mutation coupled with copy number loss of the wild-type allele. Allelic-specific copy number analysis revealed LOH of the wild-type allele of *BRCA1* and retention of the wild-type allele of *MLH1*, supportive of causative role for *BRCA1* in the development of this carcinoma. Consistent with this notion, this breast AciCC displayed a low tumor mutation burden and a low MSISensor score (3.38), ruling out microsatellite instability. This case, however, displayed high levels of chromosomal instability, high fraction of the genome altered (67.6%), as well as genomic features of homologous recombination (HR) deficiency[29,30], including a high large-scale transition score (LST) of 22, a dominant HR deficiency-related signature (Signature 3, 27% and Signature 8, 31%), and a high HRDetect

score (0.99), consistent with a role of *BRCA1* loss of function in the oncogenesis of this breast AciCC.

Discussion

This study demonstrates that AciCCs of the breast consistently lack detectable immunohistochemical expression of NR4A3 and that *NR4A3* rearrangements are absent. These findings stand in sharp contrast to the frequent NR4A3 overexpression driven by translocation and enhancer hijacking seen in AciCC of the salivary gland[10,14], results that have potentially important biologic and nosologic implications.

We have previously reported on the genetic differences between AciCC of the breast and of the salivary gland. We observed through targeted, whole-exome and RNA-sequencing that breast AciCCs harbor recurrent *TP53* hotspot mutations (>80%), *PIK3CA* mutations (10%), and mutations in DNA repair genes, including pathogenic *BRCA1* and *MLH1* alterations, however no oncogenic in-frame fusion transcripts were identified[5,7,8,37]. In contrast, no *TP53* and *PIK3CA* mutations were identified in salivary gland AciCC[37]. In both studies, the pattern and frequency of alterations in breast AciCC were similar to those seen in TNBC NST, and no specific pathognomonic genomic alterations were identified. The whole-genome sequencing analysis performed on breast AciCC case 14 in this current study further supports and expands on our earlier findings. This case harbored not only a *TP53* splice site mutation and high levels of genomic instability, but also displayed genomic features of HR DNA repair deficiency, including high large-scale state transitions and HR deficiency-related mutational signatures, akin to a subset of common-type TNBCs. This patient was known to have germline *BRCA1* and *MLH1* pathogenic alterations; our whole-genome sequencing findings support the notion that this patient developed a *BRCA1*-related breast AciCC rather than a Lynch Syndrome cancer, given the presence of genomic features of HR deficiency, including a high HRDetect score, and LOH of the *BRCA1* wild-type allele, while retaining the wild-type allele of *MLH1.*

Another study has previously reported a breast AciCC occurring in a patient with a germline *BRCA1* mutation; this case demonstrated *BRCA1* LOH and a somatic *TP53* mutation as well[38]. The alterations seen in our AciCC case 14 lend further support to the notion that breast AciCC is characterized by *TP53* alterations and associated genomic instability, similar to other TNBCs, and distinct from salivary gland AciCC. It should be noted however, that AciCC case 14 harbored both *BRCA1* and *MLH1* pathogenic germline mutations. While *BRCA1* and *MLH1* alterations have been reported in AciCCs previously, further studies are warranted to assess the prevalence and causative role of germline *BRCA1* alterations in patients with breast AciCC. These results, in combination with the lack of *NR4A3* rearrangements by FISH and absence of both NR4A3 and DOG-1 protein expression by IHC provide strong evidence that breast AciCC and salivary gland AciCC are distinct entities with different underlying molecular pathogenesis.

Breast AciCC should be contrasted with other salivary gland-like breast carcinomas which have the same translocations as their counterparts in the salivary gland and are associated with a relatively favorable prognosis, including adenoid cystic carcinoma

(*MYB*::*NFIB, MYBL1* rearrangements)[39–41], secretory carcinoma (*ETV6*::*NTRK3*)[42], and mucoepidermoid carcinoma (*CRTC1*::*MAML2*)[43]. Notably, NR4A3 IHC is more sensitive than FISH (which is limited given the variable breakpoints on 9q31) as it detects overexpression in salivary AciCC regardless of underlying genetic alteration[14].

Despite some overlapping morphologic features with salivary AciCC, breast AciCC also shares morphologic and molecular genetic features with MGA[7,44,45], has similar driver mutations to TNBC NST and metaplastic carcinomas, and lacks NR4A3 and DOG-1 expression, whereas salivary gland AciCC is a translocation-associated carcinoma with a simple genome and consistently demonstrates NR4A3 overexpression, which has been associated with its tumorigenesis[10]. Our data and prior work[7] have demonstrated relatively frequent co-occurrence of breast AciCC morphology with other histologic types of breast carcinoma, with similar molecular alterations in both compartments, suggesting these entities are clonally related to each other.

While this cohort of breast AciCC is the largest studied to date, it is still limited by its small sample size, and our negative results may be related to a sampling bias. Further studies are needed to ascertain whether a subset of breast AciCCs are associated with NR4A3 alterations or overexpression, as well as to define the molecular pathogenesis of this rare special type of breast carcinoma and any molecular correlates for high-grade transformation and metastasis.

Despite these limitations, our data demonstrate that breast AciCCs lack the cardinal molecular features of salivary AciCC, including *NR4A3* rearrangements and protein overexpression, suggesting a distinct molecular pathogenesis. These data also contribute to growing evidence that AciCC of the breast exists on a morphologic spectrum with MGA and can co-occur with other types of TNBC; MGA and AciCC may represent a “low-grade triple-negative neoplasia” pathway with the potential for progression to high-grade TNBC[7,44,45], with accompanying risk for metastasis and mortality.

In conclusion, available data suggest that a nosologic shift may be in order for tumors that fulfill the current WHO criteria for breast AciCC[2]. It is becoming evident from the growing body of literature that AciCC in the breast is distinct from and unrelated to AciCC in the salivary gland and is a unique tumor type amongst the breast tumors with recognized analogous salivary gland counterparts. This is not entirely surprising given that tumors designated as AciCC of the breast have several morphologic differences from salivary AciCC. How to best classify these breast carcinomas is uncertain; however, a change in diagnostic terminology should be considered to better reflect their histopathologic and molecular features and lack of relationship to salivary AciCC. Rosen has suggested that “so-called mammary acinic cell carcinoma is in fact invasive carcinoma with acinic cell differentiation arising in microglandular adenosis”[46]. Given the morphologic, immunophenotypic and genetic overlap between breast AciCC and MGA, the concept of including tumors currently categorized as AciCC within the spectrum of MGA/atypical MGA/carcinoma arising in MGA merits consideration.

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Conflicts of Interest

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Hornick: Consultant to Aadi Bioscience and TRACON Pharmaceuticals, outside the scope of this work.

Jo: Spouse is an employee of Merck and Company, outside the scope of this work.

Schnitt: Member of the scientific advisory boards of PathAI, Ibex Medical Analytics, and PreciseDx, outside the scope of this work.

Ethics Approval

This study was approved by the Institutional Review Board of Brigham and Women's Hospital under waiver of consent (protocol number 2017P000536). This work was performed in accordance with the Declaration of Helsinki.

Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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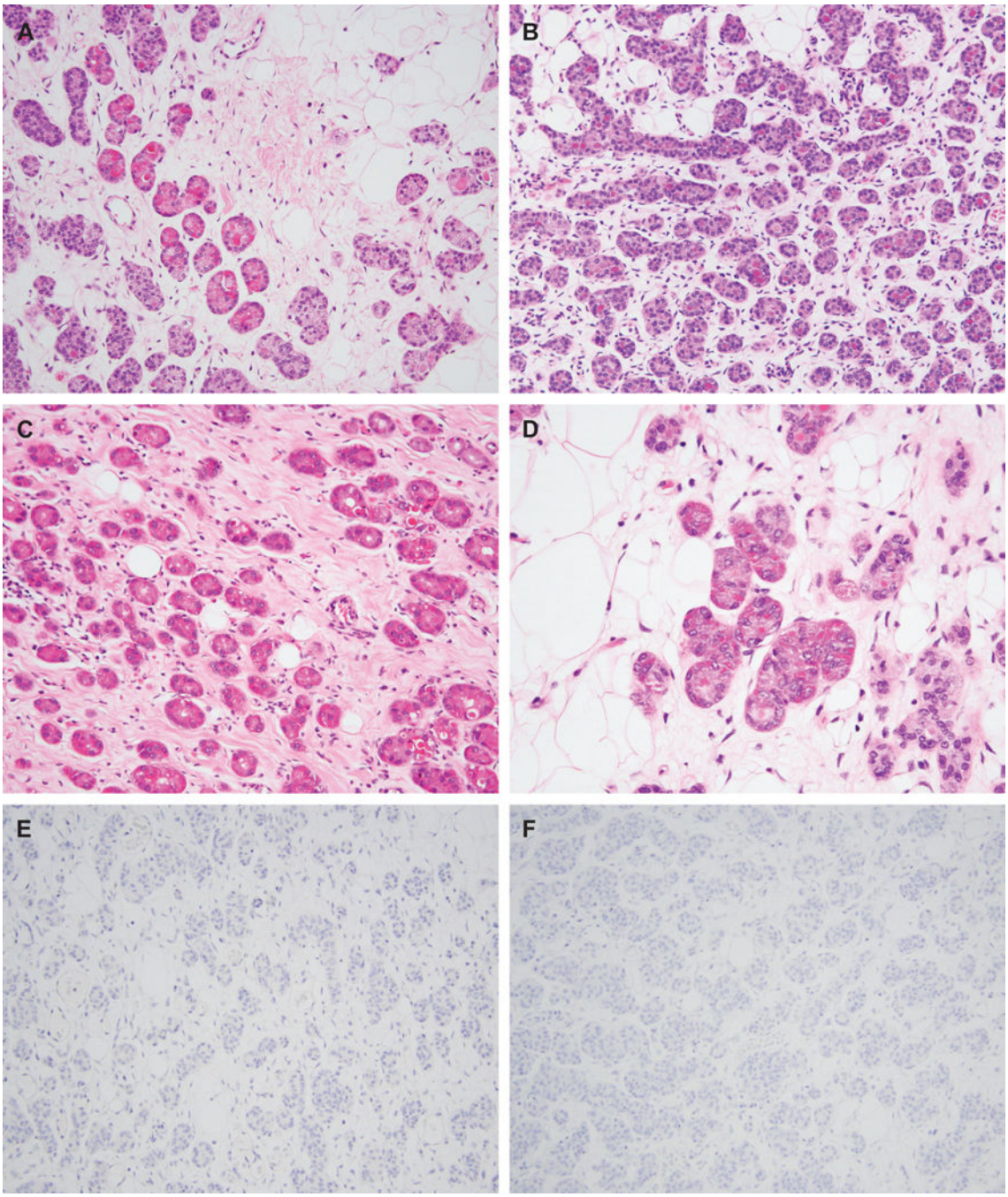

Figure 1.

Morphology of pure breast AciCC and immunohistochemistry results for NR4A3 and DOG-1. A-D. Pure breast AciCCs, showing a haphazard proliferation of small glands with eosinophilic luminal secretions and eosinophilic cytoplasmic granules, with variation in the prominence and proportion of the small glandular proliferation demonstrating the eosinophilic granules. E. Representative IHC for NR4A3 in breast AciCC, showing no nuclear staining. F. Representative IHC for DOG-1 in breast AciCC, showing no staining.

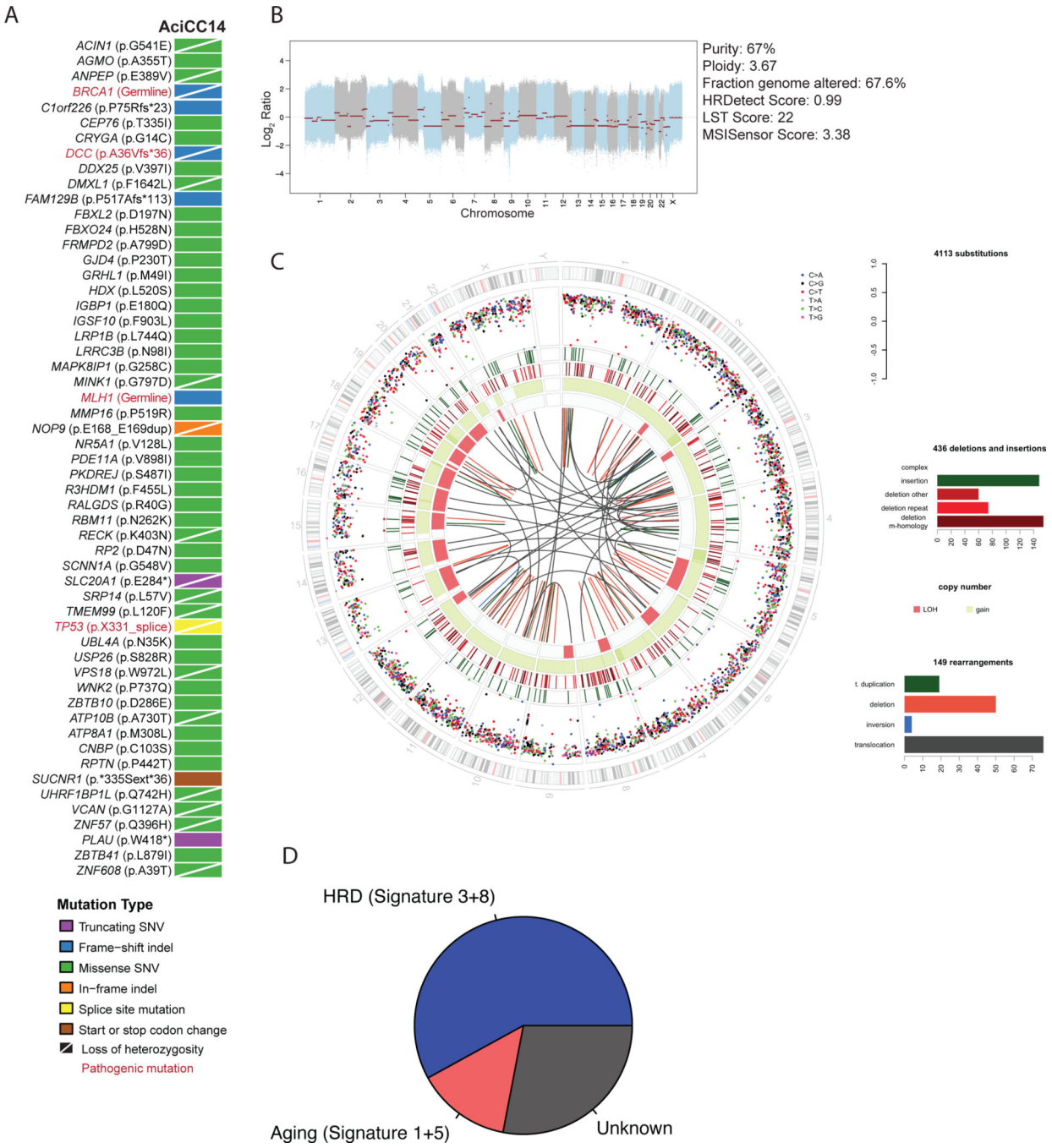

Figure 2.

Genomic analysis of whole-genome sequencing of pure breast AciCC. A. Exonic mutations detected in whole-genome sequencing with pathogenic mutations (including germline *BRCA1* and *MLH1* variants) annotated in red. B. Copy number profile computed from FACETS showing genome-wide segmentation of log-ratios. Genomic information such as purity, ploidy, fraction of genome altered, HRDetect score, LST score, and MSIsensor score are shown to the right of the plot. C. A circos plot displaying (from outside to inside) inter-variant distance and substitution type of SNVs, deletions, insertions, copy number, and

structural variants. D. Mutational signatures computed using Signal showing the proportion of mutations ascribed to each signature.

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Table 1.

Clinicopathologic characteristics of patients with acinic cell carcinoma of the breast in this cohort.

Case Designation	Age at Diagnosis	Tumor size (cm)	Axillary lymph node status	Specimen Type	Histomorphology	IHC Results (for the AciCC component in cases with mixed histology) (+), positive; (−), negative	FISH Results
1	48	9.5	Negative (0 of 1)	Mastectomy	Pure AciCC	(+) S100, lysozyme, A1ACT (−) CK5/6, p63, ER, NR4A3, DOG-1	Testing attempted; no results obtained
2	45	10.5	Negative (0 of 2)	Excision; completion mastectomy	Pure AciCC	(+) S100, ER (20–30%, weak-to-moderate), lysozyme, A1ACT, A1AT (−) PR, HER2, NR4A3, DOG-1	Negative for *NR4A3* rearrangement; polysomy detected in 58% of nuclei
3	32	2.5	Negative (0 of 2)	Mastectomy	AciCC admixed with chondroid metaplastic carcinoma	(+) S100, lysozyme, A1ACT (−) ER, PR, HER2, NR4A3, DOG-1	Testing attempted; no results obtained
4	46	Unknown	Unknown	CNB	AciCC admixed with microinvasive carcinoma NST	(+) S100, EMA, PR (80%, moderate) (−) ER, HER2, p63, NR4A3, DOG-1	Negative for *NR4A3* rearrangement; polysomy detected in 56% of nuclei
5	58	0.6	Negative (0 of 2)	Mastectomy	Pure AciCC	(+) GATA3, CK7, S100, lysozyme, A1AT (−) ER, PR, HER2, synaptophysin, chromogranin, CK20, NR4A3, DOG-1	Negative for *NR4A3* rearrangement
6*	36	5.0	Positive (10 of 17)	Unknown	AciCC admixed with invasive carcinoma NST	(+) lysozyme (−) ER, PR, HER2, NR4A3, DOG-1	Not tested
7*	55	1.9	Negative (0 of 8)	Unknown	AciCC admixed with invasive carcinoma NST	(+) lysozyme (−) ER, PR, HER2, NR4A3, DOG-1	Not tested
8*	34	3.6	Negative (0 of 3)	Unknown	AciCC admixed with invasive carcinoma NST	(+) lysozyme (−) ER, PR, HER2, NR4A3, DOG-1	Not tested
9*	70	Unknown	Unknown	Unknown	AciCC admixed with chondroid metaplastic carcinoma	(+) lysozyme (−) ER, PR, HER2, NR4A3, DOG-1	Not tested
10	70	1.8	Negative (ITC in 1 of 4)	Excision	Pure AciCC	(+) S100, lysozyme, SOX10 (−) ER, PR, HER2, NR4A3, DOG-1	Not tested
11†	74	1.2	Unknown	CNB	Pure AciCC	(+) PR (1%, weak), S100, lysozyme, SOX10 (−) ER, NR4A3, DOG-1	Testing attempted; no results obtained
12	29	1.5	Negative (0 of 3)	CNB; excision	AciCC admixed with invasive carcinoma NST; multiple residual foci of pure AciCC invasion after neoadjuvant chemotherapy	(+) GATA3, GCDFP-15, S100, lysozyme, A1AT, PR (30%, moderate) (−) ER, HER2, NR4A3, DOG-1	Testing attempted; no results obtained
13†	43	Unknown	Unknown	CNB	Pure AciCC	(+) S100, EMA, lysozyme, A1AT (−) ER, PR, HER2, NR4A3, DOG-1	Testing attempted; no results obtained

Case Designation	Age at Diagnosis	Tumor size (cm)	Axillary lymph node status	Specimen Type	Histomorphology	IHC Results (for the AciCC component in cases with mixed histology) (+), positive; (−), negative	FISH Results
14	46	1.1	Negative (0 of 1)	Mastectomy	Dominant mass pure AciCC; separate focus of invasive carcinoma NST (0.55 cm)	(+) PR (30%, weak), GATA3, SOX10, S100, lysozyme, A1AT, DOG-1 (focal) (−) ER, HER2, BCL10, NR4A3	Negative for *NR4A3* rearrangement; polysomy detected in 48% of nuclei
15	38	2.9	Negative (0 of 1)	Mastectomy	Pure AciCC	(+) S100, EMA, GCDFP-15, SOX10, AR, lysozyme, A1AT, MUC4, DOG-1 (focal) (−) ER, PR, HER2, synaptophysin, mammaglobin, pan-TRK, NR4A3	Not tested
16	52	6.3	Unknown	Mastectomy	AciCC admixed with spindle cell metaplastic carcinoma	(+) pan-keratin MNF-116, AE1/AE3, Cam5.2, S100, lysozyme, A1AT, GCDFP-15 (−) ER, PR, HER2, CK5, 34βE12, p63, NR4A3, DOG-1	Not tested

Notes:

*Cases 6, 7, 8, and 9 in this current study have been published in a previous study[5] (corresponding to case designations 7, 9, 14, and 16 in that study), and are further characterized herein.

†For cases 11 and 13, only a core needle biopsy (CNB) specimen was available for analysis, each of which showed pure AciCC morphology.

Abbreviations: AciCC, acinic cell carcinoma; A1ACT, alpha-1-antichymotrypsin; A1AT, alpha-1-antitrypsin; AR, androgen receptor; CK, cytokeratin; ER, estrogen receptor; EMA, epithelial membrane antigen; GCDFP-15, gross cystic disease fluid protein 15; PR, progesterone receptor.