Whole-exome sequencing and RNA sequencing analyses of acinic cell carcinomas of the breast

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Aims: Acinic cell carcinoma (ACC) of the breast is a rare histological form of triple-negative breast cancer (TNBC). Despite its unique histology, targeted sequencing analysis has failed to identify recurrent genetic alterations other than those found in common forms of TNBC. Here we subjected three breast ACCs to whole-exome and RNA sequencing to determine whether they would harbour a pathognomonic genetic alteration.

Methods and results: DNA and RNA samples from three breast ACCs were subjected to whole-exome sequencing and RNA-sequencing, respectively. Somatic mutations, copy number alterations, mutational signatures and fusion genes were determined with state-of-the-art bioinformatics methods. Our analyses revealed TP53 hotspot mutations associated with loss of heterozygosity of the wild-type allele in two cases. Mutations affecting homologous recombination DNA repair-related genes were found in two cases, and an MLH1 pathogenic germline variant was found in one case. In addition, copy number analysis revealed the presence of a somatic BRCA1 homozygous deletion and focal amplification of 12q14.3–12q21.1, encompassing MDM2, HMGA2, FRS2, and PTPRB. No oncogenic in-frame fusion transcript was identified in the three breast ACCs analysed.

Conclusions: No pathognomonic genetic alterations were detected in the breast ACCs analysed. These tumours have somatic genetic alterations similar to those of common forms of TNBC, and may show homologous recombination deficiency or microsatellite instability. These findings provide further insights into why breast ACCs, which are usually clinically indolent, may evolve into or in parallel with high-grade TNBC.

Keywords: acinic cell carcinoma, breast cancer, DNA damage repair, massively parallel sequencing

Introduction
Acinic cell carcinoma (ACC) of the breast is an exceedingly rare special histological type of breast cancer.1 Breast ACCs are morphologically similar to
their salivary gland counterparts, and are characterised by infiltrative microglandular or solid-nest structures composed of cells with diffuse serous differentiation, with abundant eosinophilic to amphophilic cytoplasm and coarse or fine granules resembling Paneth cells. Areas composed of clear cells with a hypernephroid appearance or non-specific glandular cells may be present. Despite their triple-negative phenotype, pure breast ACCs are low-grade carcinomas that usually show indolent clinical behaviour, but may, nonetheless, be associated with or progress to high-grade triple-negative breast cancer (TNBC). 

Previous studies from our group and others have revealed that breast ACCs, microglandular adenosis (MGA), and atypical MGA, which show a marked phenotypic overlap, show genetic alterations characteristic of common forms of TNBC, including complex patterns of copy number alterations (CNAs) and highly recurrent TP53 mutations. These observations suggest that these entities may represent a low-grade triple-negative breast neoplasia family with no or minimal metastatic potential even when not associated with high-grade TNBC.

Notwithstanding their unique phenotype and previous efforts to characterise them by copy number and targeted sequencing analyses, no pathognomonic genetic alterations have been identified underpinning breast ACCs. Therefore, we sought to investigate the repertoire of somatic genetic alterations of pure breast ACCs by using whole-exome sequencing (WES) and RNA sequencing. WES allowed the detection of genetic alterations in genes not surveyed in previous studies of breast ACCs employing targeted sequencing panels. Additionally, RNA sequencing analysis was performed to determine whether pure breast ACCs harbour a highly recurrent fusion gene.

Materials and methods

Subjects and samples

Following Institutional Review Board approval, formalin-fixed paraffin-embedded tissue blocks of pure breast ACCs were retrieved from the archives of the Department of Pathology of Nottingham University City Hospital (Nottingham, UK). Samples were anonymised, and reviewed by three pathologists (F.P., E.G.-R., and J.S.R.-F). Of the two pure breast ACCs previously subjected to targeted capture sequencing as reported in Guerini-Rocco et al., only ACC12 was included in the present study, owing to tissue and nucleic acid availability. ACC1 and ACC18 have not been previously reported and are unique to this study.

Results

All breast ACCs studied here featured an infiltrative microglandular growth pattern and cytoplasmic eosinophilic Paneth cell-like granules. DNA was extracted from microdissected representative tumour and normal breast tissue, as previously described, and subjected to WES at the Integrated Genomics Operations (IGO) of Memorial Sloan Kettering Cancer Center (MSKCC), as previously described. Tumour RNA samples were subjected to RNA sequencing at the IGO of the MSKCC, as previously described.

Detailed analytical methods are described in Data S1.

WE AND RNA SEQUENCING

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mutated in common forms of TNBC, associated with LOH in ACC1 (Figures 2A, S1; Table S4). No cancer gene other than TP53 was found to be mutated in more than one of the breast ACCs studied here.

Copy number analysis revealed that ACC1 and ACC18 had complex copy number profiles, with multiple gains and losses and focal high-level amplifications; in contrast, ACC12 showed a rather simple copy number profile (Figure 2B). CNAs present in both ACC1 and ACC18 included gains of 1q, 2q, and 8q, and losses of 3p, 12p, 12q, 14q, 17p, and 17q. In line with previous studies reporting breast ACCs arising in BRCA1 germline mutation carriers, we identified a somatic homozygous deletion in 17q21.31 encompassing BRCA1 in ACC18 (Figure 1B). In addition, we found that ACC18 harboured a focal amplification in 20p12.3 encompassing PCNA, which encodes proliferating cell nuclear antigen, a key promoter of processive DNA synthesis. ACC1

Figure 1. Histological characteristics of acinic cell carcinomas (ACCs) of the breast. A,B. Representative photomicrographs of haematoxylin and eosin (H&E)-stained breast ACCs from this study. A–C. ACC1 shows an infiltrative growth pattern (A) with microglandular features (B), and is composed of Paneth-like cells with coarse intracytoplasmic granules (C). D,E. ACC12 shows a microglandular growth pattern (D) with cells featuring amphophilic cytoplasm with fine granules (E). F–H. ACC18 shows microglandular areas (F) composed of eosinophilic cells with coarse granules (G) and hypernephroid areas composed of clear cells (H). Scale bars: 200 μm (A,D), 50 μm (B,E), 20 μm (C,G,H), and 100 μm (F).
Figure 2. Repertoire of somatic mutations and mutational signatures of the acinic cell carcinomas (ACCs) of the breast. A. Non-synonymous somatic mutations affecting cancer-related genes and mutations shared among cases identified in the breast ACCs (n = 3) subjected to whole-exome sequencing. Cases are shown in columns and genes are shown in rows. B. Copy number plots depicting segmented log₂ ratios (y-axis) plotted according to genomic position (x-axis). Chromosomes are demarcated by alternating blue and grey colours. C. Mutational signatures of all somatic single-nucleotide variants in breast ACCs (n = 3). Pie charts indicate the proportion of the different mutational signatures identified in each case. D. A representative haematoxylin and eosin micrograph of ACC12 arising in a patient with an MLH1 germline mutation (top) and a micrograph depicting loss of MLH1 expression in the tumour cells (*). Normal breast (right lower corner) shows retention of MLH1 expression. Scale bar: 50 μm. Sig, signature.
was found to harbour a high-level amplification of 12q14.3–12q21.1, which encompasses several cancer genes, such as MDM2, HMGA2, WIFI, FRS2, and PTPRB. In contrast, and consistent with its DNA mismatch repair (MMR) deficiency, ACC12 showed a simple genome without detectable CNAs.

We next sought to determine whether breast ACCs had genomic features suggestive of homologous recombination DNA repair deficiency (HRD) or other biological processes that would confer genomic instability. ACC1 was found to have a dominant signature 5, ascribed to ageing (Figure 2C). ACC18, which harboured a BRCA1 homozygous deletion, showed genomic features suggestive of HRD, including a dominant signature 3 (HRD-related), along with a high large-scale state transitions score (24), a high telomeric allelic imbalance (MtAI) score (23), and a high number of ‘small deletions’ of >5 bp (Figure 2C; Table S5). Although we did not identify somatic mutations in MLH1 or in other core MMR genes, ACC12, which harboured a pathogenic germline splice site mutation in MLH1 (c.790+2dupT), a key tumour suppressor of the MMR system (Table S4), had a dominant signature 6, ascribed to defective MMR (Table S5). Consistent with these findings, this case additionally showed loss of MLH1 protein expression in the tumour by immunohistochemistry (Figure 2D).

Discussion

Previous studies from our group and others have suggested that ACCs of the breast and MGA, which are entities with overlapping histological characteristics, form part of the spectrum of low-grade triple-negative disease, and harbour genomic features indistinguishable from those of common forms of TNBC. Further supporting this notion, our study revealed few recurrently mutated genes, such as TP53, and complex copy number profiles.

Most importantly, our findings provide further support for the association between breast ACC and HRD through BRCA1 inactivation. Our results demonstrate that ACC18 harboured a BRCA1 homozygous deletion (Figure 2C). In conjunction with previous reports by our group and others, loss-of-function alterations affecting BRCA1 concurrently with TP53 somatic mutations seem to be not uncommon in breast ACCs, even in those lacking a high-grade TNBC component. Our findings, however, suggest the tantalising possibility that both BRCA1 and TP53 loss of function may not be sufficient for a TNBC to show high-grade features, and that inactivation of these two genes may not be sufficient for the development of high-grade TNBC.

Here we also described an ACC (ACC12) that lacked mutations affecting TP53, and showed a simple copy number profile, high MSI levels, and a dominant signature 6 (MSI-related). This case arose in a patient carrier of a germline MLH1 splice-site mutation. Although we did not identify a somatic genetic alteration in MLH1, we cannot rule out that the possibility that the second MLH1 allele could have been inactivated by epigenetic silencing via promoter hypermethylation, as described in colorectal and endometrial carcinoma.

Our study has important limitations, including the small sample size, owing to the rarity of this entity, and the fact that only archival samples were available for analysis. We were unable to perform any methylation analyses to investigate epigenetic silencing as a mechanism of inactivation of the second MLH1 allele in ACC12, owing to the lack of residual DNA from this case. Despite these limitations, our data lend further support to the notion that breast ACCs are genetically heterogeneous and have genomic features overlapping with those of common forms of TNBC. These tumours appear not to be driven by a highly recurrent mutation or oncogenic fusion gene. Most importantly, our findings suggest that at least some breast ACCs may arise in the setting of HRD or MSI through distinct molecular mechanisms. Even though we could not establish a definitive causal link between BRCA1 mutations or MLH1 germline mutations and breast ACCs, our study demonstrates that HDR deficiency and high MSI levels occur in breast ACCs, and that the breast ACCs analysed showed high levels of genetic instability (either HDR defects or high MSI levels). Additional studies on the genetic or epigenetic basis of breast ACCs are warranted.

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Conflicts of interest

J. S. Reis-Filho reports receiving personal/consultancy fees from Goldman Sachs and REPAIR Therapeutics, membership of the scientific advisory boards of VolitionRx and Page. AI, and ad hoc membership of the scientific advisory boards of Roche Tissue Diagnostics, Ventana Medical Systems, Novartis and Genentech, outside the scope of this study. E. Guerini-Rocco has received honoraria from Thermo Fisher Scientific, Biocartis, Roche, MSD, AstraZeneca, and Novartis, outside the scope of this study. R. Gularte-Merida reports receiving personal/consultancy fees from Oxford Genetics, outside the scope of this study. All other authors declare no conflicts of interest.

Author contributions

F. Beca, F. Pareja, J. S. Reis-Filho and B. Weigelt conceived the study. E. Guerini-Rocco and F. Pareja coordinated the retrieval of samples. F. Pareja, E. Guerini-Rocco and J. S. Reis-Filho performed the pathology review. S. S. K. Lee, A. da Cruz Paula and P. Selenica performed experiments and the bioinformatic analysis. F. Beca, S. S. K. Lee, F. Pareja, L. Ferrando, R. Gularte-Merida, H. Y. Wen, H. Zhang, B. Weigelt and J. S. Reis-Filho analysed and interpreted the data. F. Beca, F. Pareja and J. S. Reis-Filho wrote the first draft of the manuscript, which was reviewed by all co-authors.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1 Supplementary methods.

Figure S1. Cancer cell fractions of non-synonymous somatic mutations affecting cancer-related genes identified in the acinic cell carcinomas (ACCs) of the breast by whole-exome sequencing.

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Table S1. Clinicopathological characteristics of the acinic cell carcinomas of the breast included in this study.

Table S2. Fusion genes identified by RNA sequencing analysis of acinic cell carcinomas of the breast.

Table S3. Whole-exome sequencing statistics.

Table S4. Non-synonymous somatic mutations identified in the acinic cell carcinomas of the breast by whole-exome sequencing.

Table S5. Genomic features of homologous recombination deficiency and microsatellite instability in the acinic cell carcinomas of the breast included in this study.