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Are acinic cell carcinomas of the breast and salivary glands distinct diseases?

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Abstract

Aims—Acinic cell carcinomas (AcCC) of the breast have been reported to constitute the breast counterpart of salivary gland AcCCs, based on the similarities of their histological and immunohistochemical features. Breast AcCC is a vanishingly rare form of triple-negative breast cancer (TNBC). Recent studies have demonstrated that in TNBCs, the two driver genes most frequently mutated are *TP53* (82%) and *PIK3CA* (10%). We sought to define whether breast AcCCs would harbour *TP53* and *PIK3CA* somatic mutations, and if so, whether these would be present in salivary gland AcCCs.

Methods and results—Sanger sequencing of the entire coding region of *TP53* and of *PIK3CA* hotspot mutation sites of 10 breast and 20 salivary gland microdissected AcCCs revealed eight *TP53* (80%) and one *PIK3CA* (10%) somatic mutations in breast AcCCs. No somatic mutations affecting these genes were found in the 20 salivary gland AcCCs analysed.

Conclusions—Our findings demonstrate that breast AcCCs display *TP53* and *PIK3CA* mutations at frequencies similar to those of common types of TNBCs, whereas these genes appear not to be altered in salivary gland AcCCs, suggesting that despite their similar histological appearances, AcCCs of the breast and salivary glands probably constitute unrelated diseases.

Keywords

breast; *PIK3CA*; salivary gland; sequencing; *TP53*

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Supporting Information

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

Table S1. List of primers used to investigate the entire coding region of the *TP53* gene and the hotspot mutation sites of the *PIK3CA* gene.

Introduction

Salivary gland-type tumours of the breast constitute a heterogeneous group of rare breast neoplasms that display histological features similar to those of the homonymous tumours arising in the salivary glands.[1,2] Recent studies have demonstrated that the genetic abnormalities found in specific types of salivary gland tumours are also found in the homonymous types of breast cancers.[3–7] For instance, adenoid cystic carcinomas of the salivary glands have been shown to harbour a recurrent t(6;9) translocation that results in the formation of the *MYB–NFIB* fusion gene,[3] which is also found in approximately 90% of adenoid cystic carcinomas of the breast.[3,4,8] Similarly, secretory carcinomas of the breast harbour the *ETV6–NTRK3* fusion gene,[5] which has resulted in the identification of the mammary-analogue secretory carcinoma of the salivary glands.[9,10]

Acinic cell carcinomas (AcCCs) account for approximately 10% of all malignant salivary gland cancers,[11] and originate almost exclusively in the parotid. These tumours are characterized by serous acinar cell differentiation with zymogen-type cytoplasmic granules and immunohistochemical expression of amylase, lysozyme and alpha-1 anti-chymotrypsin.[1] In the breast, AcCCs were first described by Roncaroli *et al.* in 1996[12] as the breast counterpart of salivary gland tumours displaying similar histological and immunohistochemical characteristics. Some morphological features that have been reported frequently in AcCC of parotid gland, however, are not usually seen in the breast counterpart, including pushing borders, prominent intratumoural lymphoid infiltrate and variegated architectural growth patterns with solid and cystic areas.[1] Hence, it is unclear as to whether AcCCs of the salivary gland and breast would be driven by the same constellation of molecular aberrations.

AcCCs of the breast are extremely rare. Only 39 cases have been described in the English literature to date, usually as case reports or small case series.[13–32] In addition to immunoreactivity for serous differentiation markers, AcCCs of the breast have been reported to display a triple-negative phenotype;[14] interestingly, however, at variance with the common forms of triple-negative breast cancer (TNBC), breast AcCCs are reported to have indolent clinical behaviour.[13–32]

Recent massively parallel sequencing analyses of TNBCs have revealed that these tumours are characterized by a heterogeneous repertoire of somatic genetic aberrations, with a high mutational load per case.[33–35] The genes affected most frequently by somatic mutations in these cancers include *TP53* in 82% of cases and *PIK3CA* in approximately 10% of cases.[33] These genes have been shown to be the most frequently somatically mutated driver genes in TNBCs.[33,35]

Based on these observations, we posited that breast AcCCs would harbour recurrent somatic mutations of *TP53* and/or *PIK3CA* akin to other forms of TNBCs. In addition, we reasoned that if breast and salivary gland AcCCs were a single entity, they would have alterations affecting these genes at similar frequencies. To investigate these hypotheses, we subjected a

series of 10 breast AcCCs and 20 salivary gland AcCCs to Sanger sequencing analysis of all exons of *TP53* and of the hotspot mutation sites in exons 9 and 20 of *PIK3CA*.

Materials and methods

CASES

Ten cases of AcCCs of the breast were retrieved from the consultation files of one of the authors (I.O.E.) at Nottingham University Hospital NHS Trust, Nottingham, UK ($n = 9$) or from the pathology archives of the Vall d'Hebron University Hospital, Barcelona, Spain ($n = 1$). The criteria employed for the diagnosis of breast AcCCs followed those outlined by Roncaroli *et al.*[12] and Damiani *et al.*[14] In brief, cases were considered to be AcCCs if neoplastic cells displayed Panneth cell-like granular eosinophilic cytoplasm, often admixed with cells harbouring basophilic granular cytoplasm. The neoplastic cells were arranged in rounded microglandular or microacinar infiltrative growth patterns, lacking myoepithelial cells and basement membrane, and eliciting minimal or no stromal response. This typical pattern often merged with areas displaying more solid tumour growth. Focal microcystic and papillary cystic areas were not uncommonly found, as were cells with clear cytoplasm admixed with the cells displaying serous differentiation. As part of the diagnostic workup, all cases were subjected to immunohistochemical analysis and in each case the AcCC component was uniformly oestrogen receptor (ER)-, progesterone receptor (PR)- and human epidermal growth factor receptor 2 (HER2)-negative, and expressed lysozyme, epithelial membrane antigen and S100 protein (data not shown). Cases were considered to constitute pure AcCCs if >90% of the tumour was composed of typical AcCC areas and mixed if ≥50% but ≤90% of the tumour was composed of typical AcCC areas.[36] In cases of mixed AcCC, the non-AcCC component was typed according to the World Health Organization (WHO) criteria.[36] The Nottingham grading system was employed for both the AcCC and the non-AcCC components of the mixed cases.[37] As part of the diagnostic work-up, all breast cancers were subjected to fluorescence *in-situ* hybridization analysis for the presence of the *ETV6–NTRK3* fusion gene, as described previously.[23] All cases displayed an intact *ETV6* locus, ruling out a diagnosis of secretory carcinoma. Twenty consecutive salivary gland AcCCs of classic morphology[11] were collected from the pathology files of the Department of Pathology, Cleveland Clinic, Cleveland, OH, USA and Memorial Sloan Kettering Cancer Center, New York, NY, USA. Haematoxylin and eosin-stained sections of each case were reviewed independently by six pathologists (Z.H., N.K., E.A.R., I.O.E., B.P.R. and J.R.S.-F.) and the diagnosis was confirmed in all cases. Tumour growth pattern and histological grade were defined following WHO guidelines[38] and according to the criteria defined by Gomez *et al.*,[39] respectively. The samples were anonymized prior to the analysis and approval by the local ethics committees was obtained. Available clinicopathological features of the cases are summarized in Table 1.

MICRODISSECTION AND DNA EXTRACTION

Representative 8-μm-thick sections of formalin-fixed paraffin-embedded (FFPE) blocks of tumour and adjacent normal tissue were stained with nuclear fast red and microdissected using a sterile needle under a stereomicroscope (Olympus SZ61; Center Valley, PA, USA), to ensure >80% of tumour cell content and that the normal tissue was devoid of any

potentially neoplastic cells, as described previously.[40] Genomic DNA was extracted from the AcCC component of each tumour and matched normal tissue using a DNA extraction kit (DNeasy Blood and Tissue Kit; Qiagen, Valencia, CA, USA) and quantified using the Qubit Fluorometer assay (Life Technologies, Norwalk, CT, USA), following the manufacturers' protocols.[41] All samples had sufficient quantity and quality of DNA for Sanger sequencing analysis.

IMMUNOHISTOCHEMISTRY

The immunohistochemical profile of the 10 breast AcCCs was assessed on 4-μm-thick sections, using antibodies against ER, PR and HER2, as described previously.[42] Positive and negative controls were included in each experiment. ER, PR and HER2 status was evaluated by three pathologists (E.G.-R., N.K. and H.Y.W.) according to the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines.[43,44]

POLYMERASE CHAIN REACTION (PCR) AMPLIFICATION AND SANGER SEQUENCING

We employed primer sets that amplify the entire coding region of *TP53* and the hotspot mutation sites in exons 9 and 20 of the *PIK3CA* gene. The selection of these two genes was based on the observation that in large whole exome sequencing studies of TNBCs, *TP53* and *PIK3CA* were found to be the most frequently mutated genes in these tumours.[33,35] We designed the primer pairs as described previously[45] (for the primer sets used, please see Table S1). PCR amplification of 10 ng of genomic DNA was performed using the AmpliTaq 360 Master Mix Kit (Life Technologies) on a Veriti Thermal Cycler (Life Technologies). The thermocycling protocol consisted of an initial incubation step of 95°C for 5 min and then 35 cycles of 95°C for 30 s, 56°C for 30 s, 72°C for 30 s and one final extension step of 72°C for 10 min. PCR fragments were purified with ExoSAP-IT (Affymetrix, Santa Clara, CA, USA), and the sequencing reactions were performed on an ABI 3730 capillary sequencer using ABI BigDye Terminator chemistry (version 3.1; Life Technologies), according to the manufacturer's instructions. Sequences of the forward and reverse strands were analysed using MacVector software (MacVector, Inc., Cary, NC, USA).[45] All analyses were performed in duplicate. Insertions and deletion were annotated manually.

STATISTICAL ANALYSIS

The chi-square test and Fisher's exact test were employed for the analysis of categorical variables. A two-tailed P-value <0.05 was considered to be statistically significant. All statistical comparisons were performed using SPSS statistics version 20 (IBM, Armonk, NY, USA).

Results

For the 10 breast AcCCs-affected female patients, at diagnosis the median age was 43.5 years (range 34–70 years) and the median tumour size was 1.9 cm (range 1.1–5 cm). Three and seven cases were classified as pure and mixed AcCCs, respectively (Figure 1 and Table 1). In the mixed cases, the non-AcCC components were of histological grades 2 and 3 in one and six cases, respectively. All but one non-AcCC components were classified as invasive ductal carcinoma of no special type; the remaining case was a high-grade, metaplastic breast

carcinoma (Table 1). The 20 salivary gland AcCCs were of the classic type,[11] affecting six female and 14 male patients, whose median age at diagnosis was 56.5 years (range 21–76 years, Figure 1 and Table 1), and median tumour size was 2.4 cm (range 1.2–4.5 cm, Table 1). No significant differences in age at diagnosis and size of the lesions were found between the two series (Mann–Whitney *U*-test, $P < 0.05$).

Sanger sequencing analysis of the entire *TP53* coding region revealed *TP53* somatic mutations in eight of 10 breast AcCCs (80%; six missense and two frameshift mutations), including three mutations affecting the hotspot amino acid residue R273 (Table 2 and Figure 2). By contrast, none of the salivary gland AcCCs analysed harboured *TP53* somatic mutations (Table 2). In fact, *TP53* somatic mutations were found significantly more frequently in AcCCs of the breast than in AcCCs of the salivary glands (eight of 10 versus none of 20, $P < 0.0001$, Fisher's exact test; Table 2). A comparative analysis of the frequency of *TP53* somatic mutations in breast AcCCs and TNBCs included in The Cancer Genome Atlas (TCGA)[33] analysis of breast cancers[33] revealed no significant differences (80% in breast AcCCs versus 82% in TNBCs, Fisher's exact test $P > 0.1$, www.cbioportal.org,[46] accessed 26 December 2014). No histological differences were observed between the breast AcCCs with and without *TP53* somatic mutations.

Analysis of the hotspot mutation sites in exons 9 and 20 of the *PIK3CA* gene (i.e. at amino acid residues E542, E545 and H1047)[33,46] revealed an E542K *PIK3CA* somatic mutation affecting one of the AcCCs of the breast (Figure 2); no *PIK3CA* hotspot mutations were detected in the salivary gland AcCCs analysed (Table 2). The frequency of *PIK3CA* mutations found in breast AcCCs was similar to that reported in TNBCs from TCGA[33] (10% in breast AcCCs versus 10% in TNBCs, Fisher's exact test $P > 0.1$, www.cbioportal.org,[46] accessed 26 December 2014). The breast AcCC harbouring the E542K *PIK3CA* somatic mutation displayed histological features similar to the other cases included in this study.

Taken together, our results demonstrate that driver genes mutated frequently in common forms of TNBCs are also mutated in breast AcCCs, providing evidence to suggest that these cancers are part of the spectrum of TNBCs. Furthermore, our findings are consistent with the notion that salivary gland AcCCs are distinct from breast AcCCs given that, at variance with their breast counterpart, salivary gland AcCCs lack *TP53* and *PIK3CA* somatic mutations.

Discussion

Here we demonstrate that unlike AcCCs of the breast, AcCCs of the salivary gland do not harbour mutations in *TP53* and *PIK3CA*, suggesting that despite some overlapping histological features, breast and salivary gland AcCCs probably constitute distinct entities. Conversely, the frequencies of *TP53* and *PIK3CA* somatic mutations in breast AcCCs were found to be similar to those of TNBCs of no special type (i.e. 82%),[33] consistent with the notion that breast AcCCs probably constitute part of the spectrum of TNBCs.[6,7]

AcCC of the breast were first identified as the mammary counterpart of AcCC of the salivary glands. It should be noted, however, that despite some overlapping histological and

immunohistochemical features, breast and salivary gland AcCCs differ in their morphological characteristics.[1,14,16] First, although both breast and salivary gland AcCCs display features of serous differentiation, these lesions differ in that several histological features observed in salivary gland AcCCs are not usually found in AcCCs of the breast, including pushing borders, prominent intratumoural lymphoid infiltrate, and variable growth patterns, including alternating solid and cystic areas.[1] Secondly, the infiltrative microglandular growth pattern is exceptionally rare in salivary gland AcCCs,[1] whereas it represents the most frequent pattern reported in breast AcCCs. Thirdly, the tinctorial properties of the secretory granules in breast and salivary gland AcCCs are distinct; while in the former, pink, eosinophilic granules are a common denominator, in the latter the granules are predominantly basophilic (Figure 1).[11] Our findings provide additional evidence to demonstrate that breast and salivary gland AcCCs are probably unrelated lesions, given that while breast AcCCs harbour recurrent *TP53* somatic mutations, these mutations were not identified in 20 bona fide salivary gland AcCCs. Taken together, one could argue that breast and salivary gland AcCCs are distinct tumour types that merely happen to share histological, histochemical and immunohistochemical features of serous differentiation, but that are driven by distinct repertoires of somatic driver genetic alterations. The lack of *TP53* somatic mutations in salivary gland AcCCs documented in the present study is consistent with reports demonstrating that aberrant p53 expression is not a frequent phenomenon in typical AcCCs of the salivary glands.[47–51] Our findings warrant further studies to investigate the repertoire of driver genetic alterations in salivary gland AcCCs.

This study has several limitations. First, only 10 breast AcCCs were analysed in this study; despite the relative small sample size, this is one of the largest series of AcCCs reported to date. Secondly, only somatic mutations affecting *TP53* and *PIK3CA* were investigated in this study. It should be noted, however, that by focusing on the driver genes most frequently mutated in TNBCs, our analysis revealed that breast AcCCs are similar to common forms of TNBCs and probably distinct from salivary gland AcCCs. Given that the breast AcCCs included in this study were obtained retrospectively, and from the consultation files of one of the authors (I.O.E.), an analysis of the outcomes of these patients was not possible. Further analyses of a larger series of breast AcCCs are required to define the impact of *TP53* mutations on the outcomes of patients with breast AcCC and to compare the outcome of breast AcCCs and common forms of TNBCs.

In conclusion, this study demonstrates that at variance with other tumour types that affect the breast and salivary glands and harbour similar driver genetic alterations (e.g. adenoid cystic carcinomas, secretory carcinomas),[3–8] breast and salivary gland AcCCs probably constitute distinct entities, despite displaying features of serous differentiation. While breast AcCCs have recurrent somatic mutations affecting *TP53* and *PIK3CA* akin to other forms of TNBCs, the driver genetic alterations of salivary gland AcCCs remain to be identified. Massively parallel whole genome, whole exome and/or RNA sequencing studies investigating the potential drivers of salivary gland AcCCs are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Pia-Foschini M, Reis-Filho JS, Eusebi V, Lakhani SR. Salivary gland-like tumours of the breast: surgical and molecular pathology. J. Clin. Pathol. 2003; 56:497–506. [PubMed: 12835294]
2. Foschini MP, Krausz T. Salivary gland-type tumors of the breast: a spectrum of benign and malignant tumors including ‘triple negative carcinomas’ of low malignant potential. Semin. Diagn. Pathol. 2010; 27:77–90. [PubMed: 20306833]
3. Persson M, Andren Y, Mark J, Horlings HM, Persson F, Stenman G. Recurrent fusion of MYB and NFIB transcription factor genes in carcinomas of the breast and head and neck. Proc. Natl Acad. Sci. USA. 2009; 106:18740–18744. [PubMed: 19841262]
4. Wetterskog D, Lopez-Garcia MA, Lambros MB, et al. Adenoid cystic carcinomas constitute a genomically distinct subgroup of triple-negative and basal-like breast cancers. J. Pathol. 2012; 226:84–96. [PubMed: 22015727]
5. Tognon C, Knezevich SR, Huntsman D, et al. Expression of the ETV6–NTRK3 gene fusion as a primary event in human secretory breast carcinoma. Cancer Cell. 2002; 2:367–376. [PubMed: 12450792]
6. Weigelt B, Reis-Filho JS. Histological and molecular types of breast cancer: is there a unifying taxonomy? Nat. Rev. Clin. Oncol. 2009; 6:718–730. [PubMed: 19942925]
7. Weigelt B, Geyer FC, Reis-Filho JS. Histological types of breast cancer: how special are they? Mol. Oncol. 2010; 4:192–208. [PubMed: 20452298]
8. Marchio C, Weigelt B, Reis-Filho JS. Adenoid cystic carcinomas of the breast and salivary glands (or ‘the strange case of Dr Jekyll and Mr Hyde’ of exocrine gland carcinomas). J. Clin. Pathol. 2010; 63:220–228. [PubMed: 20203221]
9. Connor A, Perez-Ordonez B, Shago M, Skalova A, Weinreb I. Mammary analog secretory carcinoma of salivary gland origin with the ETV6 gene rearrangement by fish: expanded morphologic and immunohistochemical spectrum of a recently described entity. Am. J. Surg. Pathol. 2012; 36:27–34. [PubMed: 21989350]
10. Skalova A, Vanecek T, Sima R, et al. Mammary analogue secretory carcinoma of salivary glands, containing the ETV6–NTRK3 fusion gene: a hitherto undescribed salivary gland tumor entity. Am. J. Surg. Pathol. 2010; 34:599–608. [PubMed: 20410810]
11. Chiosea SI, Griffith C, Assaad A, Seethala RR. The profile of acinic cell carcinoma after recognition of mammary analog secretory carcinoma. Am. J. Surg. Pathol. 2012; 36:343–350. [PubMed: 22301503]
12. Roncaroli F, Lamovec J, Zidar A, Eusebi V. Acinic cell-like carcinoma of the breast. Virchows Arch. 1996; 429:69–74. [PubMed: 8865856]
13. Sakuma T, Mimura A, Tanigawa N, Takamizu R. Fine needle aspiration cytology of acinic cell carcinoma of the breast. Cytopathology. 2013; 24:403–405. [PubMed: 22734799]
14. Damiani S, Pasquinelli G, Lamovec J, Peterse JL, Eusebi V. Acinic cell carcinoma of the breast: an immunohistochemical and ultrastructural study. Virchows Arch. 2000; 437:74–81. [PubMed: 10963383]
15. Schmitt FC, Ribeiro CA, Alvarenga S, Lopes JM. Primary acinic cell-like carcinoma of the breast – a variant with good prognosis? Histopathology. 2000; 36:286–289. [PubMed: 10809602]
16. Coyne JD, Dervan PA. Primary acinic cell carcinoma of the breast. J. Clin. Pathol. 2002; 55:545–547. [PubMed: 12101208]
17. Elster EA, Markusic J, Ball R, et al. Primary acinic cell carcinoma of the breast. Am. Surg. 2002; 68:993–995. [PubMed: 12455793]
18. Hirokawa M, Sugihara K, Sai T, et al. Secretory carcinoma of the breast: a tumour analogous to salivary gland acinic cell carcinoma? Histopathology. 2002; 40:223–229. [PubMed: 11895487]

19. Kahn R, Holtveg H, Nissen F, Holck S. Are acinic cell carcinoma and microglandular carcinoma of the breast related lesions? Histopathology. 2003; 42:195–196. [PubMed: 12558752]
20. Peintinger F, Leibl S, Reitsamer R, Moinfar F. Primary acinic cell carcinoma of the breast: a case report with long-term follow-up and review of the literature. Histopathology. 2004; 45:645–648. [PubMed: 15569060]
21. Kinkor Z, Skalova A. Acinic cell-like differentiation in invasive ductal carcinoma and in ductal hyperplasia of the breast – report of two cases. Cesk. Patol. 2005; 41:29–33. [PubMed: 15816121]
22. Tanahashi C, Yabuki S, Akamine N, Yatabe Y, Ichihara S. Pure acinic cell carcinoma of the breast in an 80-year-old Japanese woman. Pathol. Int. 2007; 57:43–46. [PubMed: 17199742]
23. Reis-Filho JS, Natrajan R, Vatcheva R, et al. Is acinic cell carcinoma a variant of secretory carcinoma? A fish study using ETV6 'split apart' probes. Histopathology. 2008; 52:840–846. [PubMed: 18462362]
24. Huo L, Bell D, Qiu H, Sahin A, Wu Y, Sneige N. Paneth cell-like eosinophilic cytoplasmic granules in breast carcinoma. Ann. Diagn. Pathol. 2011; 15:84–92. [PubMed: 21163678]
25. Chang ED, Lee EJ, Lee AW, Kim JS, Kang CS. Primary acinic cell carcinoma of the breast: a case report with an immunohistochemical and ultrastructural studies. J. Breast Cancer. 2011; 14:160–164. [PubMed: 21847414]
26. Zhao Y, Li W, Lang R, et al. Primary acinic cell carcinoma of the breast: a case report and review of the literature. Int. J. Surg. Pathol. 2014; 22:177–181. [PubMed: 23564703]
27. Shingu K, Ito T, Kaneko G, Itoh N. Primary acinic cell carcinoma of the breast: a clinicopathological and immunohistochemical study. Case Rep. Oncol. Med. 2013; 2013:372947. [PubMed: 24191209]
28. Osako T, Takeuchi K, Horii R, Iwase T, Akiyama F. Secretory carcinoma of the breast and its histopathological mimics: value of markers for differential diagnosis. Histopathology. 2013; 63:509–519. [PubMed: 23944930]
29. Winkler N, Morrell G, Factor RE. Invasive carcinoma with acinic cell-like features of the breast. Breast J. 2013; 19:334–335. [PubMed: 23600952]
30. Ripamonti CB, Colombo M, Mondini P, et al. First description of an acinic cell carcinoma of the breast in a brca1 mutation carrier: a case report. BMC Cancer. 2013; 13:46. [PubMed: 23374397]
31. Falleti J, Coletti G, Rispoli E, et al. Acinic cell carcinoma of the breast arising in microglandular adenosis. Case Rep. Pathol. 2013; 2013:736048. [PubMed: 24369519]
32. Limite G, Di Micco R, Esposito E, et al. The first case of acinic cell carcinoma of the breast within a fibroadenoma: case report. Int. J. Surg. 2014; 12(Suppl 1):S232–S235. [PubMed: 24859396]
33. The Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. Nature. 2012; 490:61–70. [PubMed: 23000897]
34. Banerji S, Cibulskis K, Rangel-Escareno C, et al. Sequence analysis of mutations and translocations across breast cancer subtypes. Nature. 2012; 486:405–409. [PubMed: 22722202]
35. Shah SP, Roth A, Goya R, et al. The clonal and mutational evolution spectrum of primary triple-negative breast cancers. Nature. 2012; 486:395–399. [PubMed: 22495314]
36. Lakhani, SR. WHO classification of tumours of the breast. 4th. Lyon: IARC Press; 2012. International Agency for Research on Cancer, World Health Organization.
37. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology. 1991; 19:403–410. [PubMed: 1757079]
38. Ellis, G.; Simpson, RHW. Acinic cell carcinoma. In: Barnes, L.; Eveson, JW.; Reichart, P.; Sidransky, D., editors. Pathology and genetics of head and neck tumours. Lyon: IARC Press; 2005. p. 216-218.
39. Gomez DR, Katabi N, Zhung J, et al. Clinical and pathologic prognostic features in acinic cell carcinoma of the parotid gland. Cancer. 2009; 115:2128–2137. [PubMed: 19309749]
40. 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]

41. Natrajan R, Wilkerson PM, Marchiò 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]

42. Piscuoglio S, Ng CK, Martelotto LG, et al. Integrative genomic and transcriptomic characterization of papillary carcinomas of the breast. Mol. Oncol. 2014; 8:1588–1602. [PubMed: 25041824]

43. 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]

44. Wolff AC, Hammond ME, Hicks DG, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J. Clin. Oncol. 2013; 31:3997–4013. [PubMed: 24101045]

45. 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]

46. Cerami E, Gao J, Dogrusoz U, et al. The CBIO cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012; 2:401–404. [PubMed: 22588877]

47. Wen S, Tsuji T, Li X, Mimura Y, Sasaki K, Shinozaki F. Immunohistochemical detection of p53 protein and proliferating cell nuclear antigen (PCNA) in salivary-gland tumors. Oncol. Rep. 1995; 2:233–236. [PubMed: 21597718]

48. Doi R, Kuratate I, Okamoto E, Ryoke K, Ito H. Expression of p53 oncoprotein increases intratumoral microvessel formation in human salivary gland carcinomas. J. Oral Pathol. Med. 1999; 28:259–263. [PubMed: 10426198]

49. Skálová A, Sima R, Vanecek T, et al. Acinic cell carcinoma with high-grade transformation: a report of 9 cases with immunohistochemical study and analysis of tp53 and her-2/*neu* genes. Am. J. Surg. Pathol. 2009; 33:1137–1145. [PubMed: 19461506]

50. Triantafillidou K, Iordanidis F, Psomaderis K, Kalimeras E. Acinic cell carcinoma of minor salivary glands: a clinical and immunohistochemical study. J. Oral Maxillofac. Surg. 2010; 68:2489–2496. [PubMed: 20678839]

51. Nordkvist A, Röijer E, Bang G, et al. Expression and mutation patterns of p53 in benign and malignant salivary gland tumors. Int. J. Oncol. 2000; 16:477–483. [PubMed: 10675478]

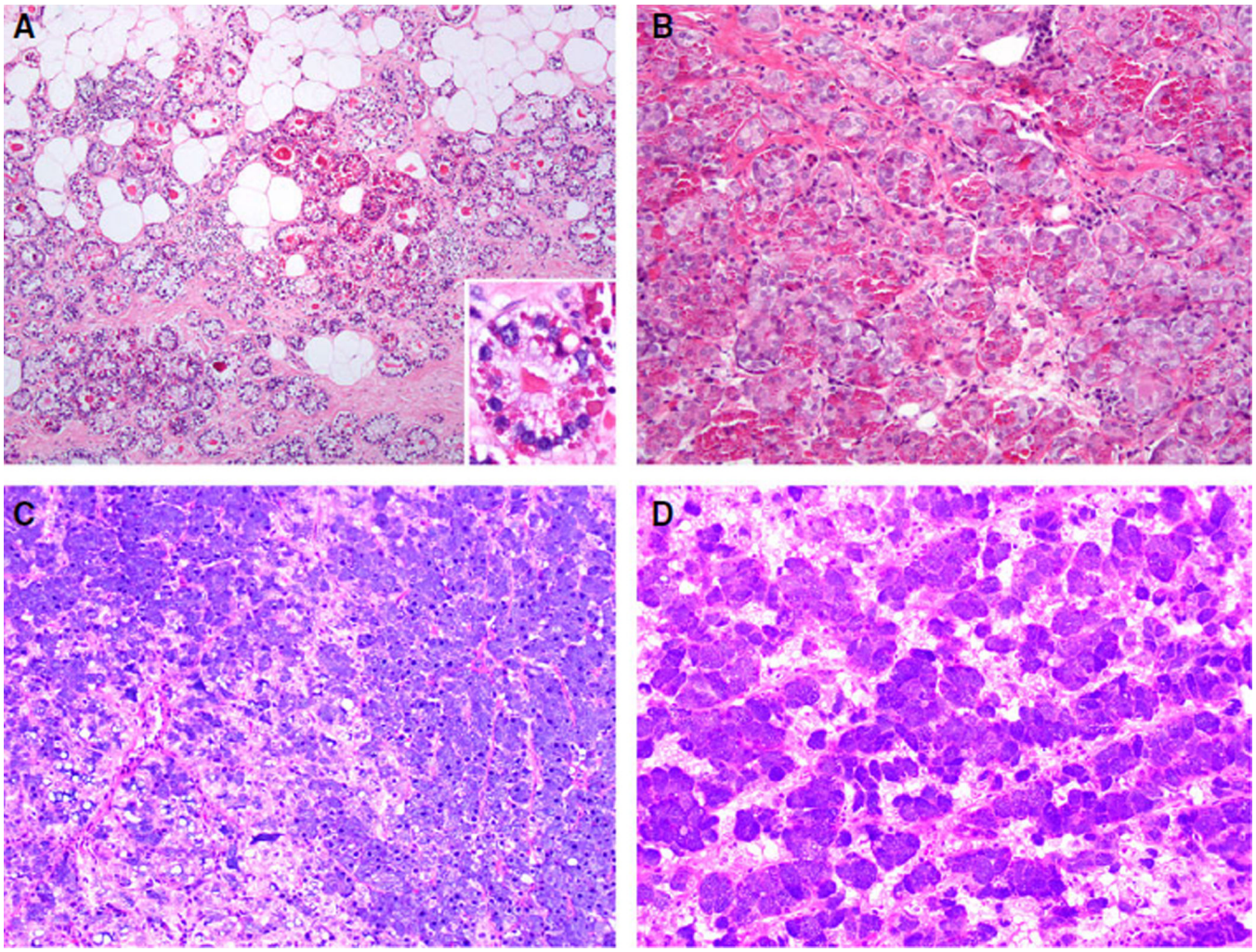

Figure 1.

Acinic cell carcinomas of the breast and salivary glands. Representative micrographs of breast acinic cell carcinomas (**A**, case 9; **B**, case 17) and salivary gland acinic cell carcinomas (**C**, case 2; **D**, case 3). Note the tinctorial differences of the intracytoplasmic granules in breast (**A**, inset, and **B**) and salivary gland acinic cell carcinomas (**C, D**).

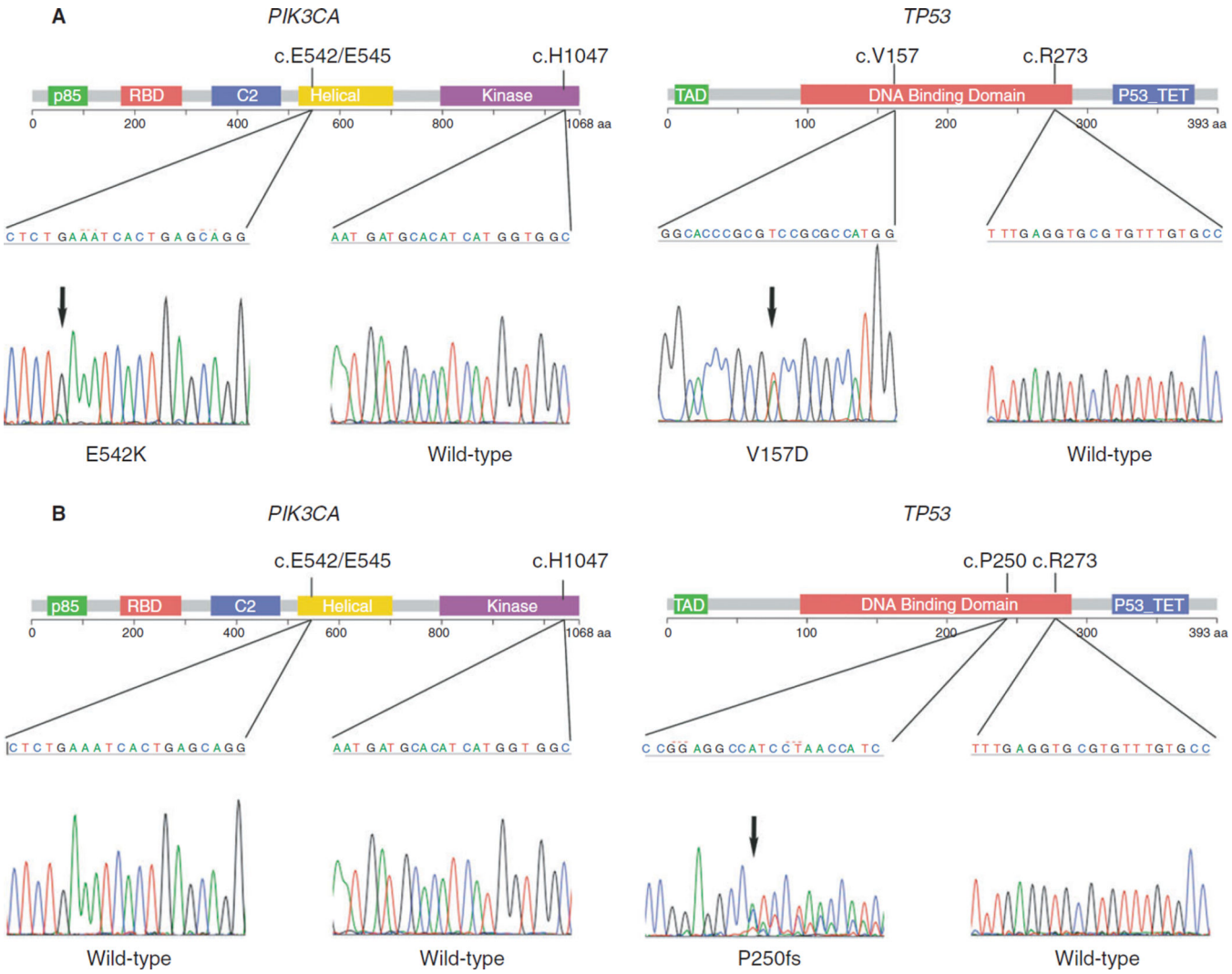

Figure 2.

Breast acinic cell carcinomas harbour *TP53* and/or *PIK3CA* somatic mutations. Sanger sequencing traces and protein domains of *PIK3CA* and *TP53* (**A**, case 16; **B**, case 17). Schematic plots were adapted from www.cbioportal.org[46] and protein domains were annotated according to UniProt (http://www.uniprot.org). The somatic mutations in *TP53* and *PIK3CA* are highlighted by arrows.

Table 1

Clinicopathological features of acinic cell carcinomas analysed in this study

	Age (years)	Gender	Tumour size (cm)	Location	Growth pattern	Grade	Pure or mixed	Type and grade non-AcCC component
AcCC of the breast								
Case 1	49	F	1.5	Breast	Microglandular	2	Pure	IDC-NST, grade 3
Case 3	45	F	2.1	Breast	Microglandular	1	Mixed	IDC-NST, grade 2
Case 7	36	F	5	Breast	Clear cell	1	Mixed	IDC-NST, grade 3
Case 9	55	F	1.9	Breast	Microglandular	1	Mixed	IDC-NST, grade 3
Case 10	34	F	NA	Breast	Microglandular	1	Mixed	IDC-NST, grade NA
Case 12	42	F	1.1	Breast	Microglandular	1	Pure	IDC-NST, grade 3
Case 14	34	F	3.6	Breast	Microglandular	1	Mixed	IDC-NST, grade 3
Case 15	48	F	2	Breast	Microglandular	1	Mixed	Metaplastic, grade 3
Case 16	70	F	1.4	Breast	Microglandular	1	Mixed	IDC-NST, grade NA
Case 17	35	F	1.8	Breast	Microglandular	1	Pure	IDC-NST, grade 3
AcCC of the salivary glands								
Case 1	65	M	2.7	Parotid	Microcystic	LG	Pure	NA
Case 2	68	M	1.8	Parotid	Microcystic and solid	LG	Pure	NA
Case 3	66	M	3	Parotid	Microcystic and solid	LG	Pure	NA
Case 4	43	F	3	Parotid	Solid and microcystic	LG	Pure	NA
Case 5	57	M	3.5	Parotid	Microcystic	LG	Pure	NA
Case 6	57	F	1.7	Parotid	Microcystic and solid	IG	Pure	NA
Case 7	36	F	2.5	Parotid	Microcystic and solid	LG	Pure	NA
Case 8	74	M	4.5	Parotid	Solid and microcystic	HG	Pure	NA
Case 9	34	M	1.7	Parotid	Microcystic	LG	Pure	NA
Case 10	31	M	3.1	Parotid	Solid and microcystic	LG	Pure	NA
Case 11	66	M	3.5	Parotid	Microcystic	LG	Pure	NA
Case 12	66	M	3.4	Parotid	Solid and microcystic	LG	Pure	NA
Case 13	65	F	1.8	Parotid	Solid and microcystic	LG	Pure	NA
Case 14	56	M	2	Parotid	Microcystic	LG	Pure	NA

	Age (years)	Gender	Tumour size (cm)	Location	Growth pattern	Grade	Pure or mixed	Type and grade non-AcCC component
Case 15	45	M	1.5	Parotid	Solid and microcystic	LG	Pure	NA
Case 16	48	M	3	Parotid	Microcystic	LG	Pure	NA
Case 17	76	F	2.3	Parotid	Solid	LG	Pure	NA
Case 18	21	M	1.5	Parotid	Microcystic	LG	Pure	NA
Case 19	36	M	2	Parotid	Solid	LG	Pure	NA
Case 20	50	F	1.2	Parotid	Solid	LG	Pure	NA

AcCC, Acinic cell carcinoma; F, Female; IDC-NST, Invasive ductal carcinoma of no special type; HG, High grade; IG, Intermediate grade; LG, Low grade; M, Male; NA, Not available.

Table 2

TP53 and *PIK3CA* mutations identified in acinic cell carcinomas of the breast and salivary glands

	***TP53* mutation (amino acid)**	***PIK3CA* hotspot mutation (amino acid)**
AcCC of the breast		
Case 1	M237I	Wild-type
Case 3	Wild-type	Wild-type
Case 7	R273C	Wild-type
Case 9	M237I	Wild-type
Case 10	R273H	Wild-type
Case 12	Wild-type	Wild-type
Case 14	R273C	Wild-type
Case 15	S303fs	Wild-type
Case 16	V157D	E542K
Case 17	P250fs	Wild-type
AcCC of the salivary glands		
Case 1	Wild-type	Wild-type
Case 2	Wild-type	Wild-type
Case 3	Wild-type	Wild-type
Case 4	Wild-type	Wild-type
Case 5	Wild-type	Wild-type
Case 6	Wild-type	Wild-type
Case 7	Wild-type	Wild-type
Case 8	Wild-type	Wild-type
Case 9	Wild-type	Wild-type
Case 10	Wild-type	Wild-type
Case 11	Wild-type	Wild-type
Case 12	Wild-type	Wild-type
Case 13	Wild-type	Wild-type
Case 14	Wild-type	Wild-type
Case 15	Wild-type	Wild-type
Case 16	Wild-type	Wild-type
Case 17	Wild-type	Wild-type
Case 18	Wild-type	Wild-type
Case 19	Wild-type	Wild-type
Case 20	Wild-type	Wild-type

AcCC, Acinic cell carcinoma. Sanger sequencing was employed to assess the entire coding region of *TP53*, and of the hotspot mutation sites in exons 9 and 20 of *PIK3CA*.