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CRTC1–MAML2 fusion in mucoepidermoid carcinoma of the breast

Gregory R Bean[1], Gregor Krings,[2] Christopher N Otis,[3] David A Solomon,[2] Joaquín J García,[4] Annemieke van Zante,[2] Sandra Camelo-Piragua,[5] Jessica van Ziffle[2] & Yunn-Yi Chen[2]

[1]*Department of Pathology, Stanford University School of Medicine, Stanford, CA,* [2]*Department of Pathology, University of California San Francisco (UCSF), San Francisco, CA,* [3]*Department of Pathology, Baystate Medical Center (University of Massachusetts Medical School-Baystate), Springfield, MA,* [4]*Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, and* [5]*Department of Pathology, University of Michigan, Ann Arbor, MI, USA*

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Aims: Mucoepidermoid carcinomas (MEC) are the most common malignant neoplasms of salivary glands, but are uncommon in other sites. Salivary gland MEC are most frequently associated with *CRTC1–MAML2* translocations. Exceedingly rare MEC of the breast demonstrate a basal-like and often triple (oestrogen and progesterone receptor, HER2)-negative immunophenotype, with a single case previously reported to show *MAML2* rearrangement, although the fusion partner was not known. Comprehensive genomic studies of breast MEC are lacking. In this study, we analysed the immunophenotype and molecular landscape of two breast MEC to elucidate the pathogenesis of these rare tumours.

Methods and results: Two breast MEC were subjected to capture-based next-generation DNA sequencing of 479 cancer-related genes. The presence of the *CRTC1–MAML2* fusion transcript was interrogated by reverse transcriptase–polymerase chain reaction. In addition, the immunoprofiles of breast MEC were compared to salivary gland MEC. Both breast MEC harboured *CRTC1–MAML2* fusions. In contrast to most triple-negative breast carcinomas of no special type, the mutational burden of MEC was very low, with one case demonstrating only an inactivating *SETD2* mutation, and the other harbouring no somatic variants in genes on the panel. No copy number alterations were identified. The immunoprofiles of breast and salivary gland MEC were overlapping, but not identical.

Conclusions: The findings highlight MEC as a breast cancer subtype more closely related to its salivary gland counterpart than to basal-like/triple-negative breast cancers of no special type.

Keywords: breast cancer, cancer genetics, *CRTC1–MAML2*, mucoepidermoid carcinoma, salivary gland

Introduction

Mucoepidermoid carcinomas (MEC) are the most common malignant neoplasms of the salivary gland, but also arise infrequently at other sites. It is an exceedingly rare diagnosis in the breast; although the estimated incidence is 0.2–0.3% of all mammary tumours, it appears even more rare in practice.[1,2] Fewer than 40 cases of MEC of the breast have been reported in the literature to date.[2–5] Primary MEC belong to an uncommon group of salivary gland-like neoplasms of the breast, which also

Address for correspondence: Y-Y Chen, Department of Pathology, University of California, San Francisco (UCSF), 1825 4th Street, M2355, San Francisco, CA 94143, USA.
e-mail: yunn-yi.chen@ucsf.edu

includes secretory carcinoma, adenoid cystic carcinoma, acinic cell carcinoma and adenomyoepithelioma, among others. Although most lack oestrogen receptor (ER) and progesterone receptor (PR) expression and human epidermal growth factor receptor 2 (HER2) overexpression, prognosis is better than expected in comparison to conventional triple-negative breast cancers of no special type.[4,6,7]

MEC have also been reported in the lacrimal gland, oesophagus, lung, pleura, thymus, thyroid, pancreas, penis, tonsils and skin.[8–17] Morphological features are similar regardless of site, with tumours characterised by variably sized circumscribed cystic to solid nests and nodules of multiple cell populations and prominent extracellular mucin. Intermediate/basaloid, epidermoid/squamoid and mucinous cells are present in varying proportions, although some authors differentiate these into four cell types.[2,3,5,18] Mucinous or mucin-producing cells are usually located at the luminal aspect of the glands and nests and may be tall columnar or goblet-shaped with obvious cytoplasmic mucin or be more subtle, requiring special stains to highlight the mucin. Intermediate cells are most concentrated at the tumour periphery and are small with high nuclear-to-cytoplasmic ratio and oval hyperchromatic nuclei. Epidermoid cells are polygonal, with well-defined borders and abundant eosinophilic cytoplasm.[19] Focal intercellular bridges may be observed in rare cases, but squamous pearls or individual cell keratinisation should be absent. Cytological features may also include clear cell or oncocytic change. A prominent lymphocytic infiltrate is often seen around tumour nodules, which in the salivary gland is also referred to as tumour-associated lymphoid proliferation (TALP). Reports about *in-situ* components vary.[5,20] The most commonly used grading systems for MEC in the salivary gland are the Brandwein and Armed Forces Institute of Pathology (AFIP) methods. These three-tiered and point-based systems incorporate the relative proportion of cystic components and the presence of neural invasion, necrosis, mitotic rate and nuclear anaplasia (as well as vascular and bone invasion in Brandwein) to equate to low, intermediate or high grade.[21,22] In breast MEC, the AFIP system and the modified Scarff–Bloom–Richardson (SBR) system appear largely interchangeable and yield similar prognostic data.[19]

The most common translocation of salivary gland MEC fuses exon 1 of *CRTC1* at chromosome 19p13 to exons 2–5 of *MAML2* at chromosome 11q21. Alternative fusions with *CRTC3* have also been described.[23] Low-grade tumours are more likely to be fusion-positive than high-grade tumours. The extent to which the presence of the *CRTC1–MAML2* translocation influences clinical outcome in salivary gland tumours is unclear.[24–26] Molecular characterisation of breast MEC is scant. One case demonstrated an 11q21 deletion at the site of the *MAML2* gene, but a fusion partner was unknown.[5] Given the lack of genomic data or comparative genetic studies, the relatedness of MEC of the breast to analogue tumours arising in other sites, as well as conventional basal/triple-negative breast cancers, remains uncertain.

In this study we demonstrate for the first time, to our knowledge, the presence of the *CRTC1–MAML2* fusion transcript in MEC of the breast. In addition, we used capture-based next-generation sequencing of 479 cancer-related genes to more comprehensively characterise the genomics of two breast MEC. The findings shed light on our understanding of breast MEC biology and may help to explain the favourable clinical behaviour of these tumours.

Materials and methods

CASE SELECTION

This study was approved by the institutional review boards of the University of California San Francisco (UCSF) and the University of Massachusetts Medical School–Baystate. Two breast MEC were confirmed by experienced breast pathologists (Y.C. and C.N.O.) using a combination of routine haematoxylin and eosin (H&E)-stained sections, immunohistochemistry and genetic findings. Selected findings in case 2 were reported previously.[5]

TISSUE MICROARRAY CONSTRUCTION

Tissue microarrays (TMA) were created from low-grade MEC of salivary gland origin. Three 2-mm punch biopsy tissue cores, with each core containing the three cell types of MEC, were obtained from each tumour for analysis. Positive and negative on-slide controls consisted of normal breast, normal salivary gland and invasive ductal carcinoma, not otherwise specified.

IMMUNOHISTOCHEMISTRY

The following antibodies were used: cytokeratin (CK) 7 (OV-TL12/30, 1:100; Dako, Santa Clara, CA, USA),

Cam5.2 (1:100; Becton Dickinson, Franklin Lakes, NJ, USA), CK5/6 (D5/16B4, 1:200 with anti-background; Millipore, Billerica, MA, USA), mucin (MUC)4 (8G7, 1:500, Millipore), MUC5AC (MRQ-19, undiluted; Cell Marque, Rocklin, CA, USA), GATA binding protein 3 (GATA3) (L50-823, undiluted; Ventana, Tucson, AZ, USA), mammaglobin (304-1A5, 1:4; Dako), gross cystic disease fluid protein 15 (GCDFP-15; 23A3, undiluted; Covance, Dedham, MA, USA), p63 (4A4, undiluted; Ventana), smooth muscle myosin (SMM) (SMMS-1, 1:300; Dako), calponin (26A11, undiluted; Leica Biosystems, Buffalo Grove, IL, USA), smooth muscle actin (SMA) (alpha sm-1, undiluted; Leica Biosystems), ER (SP1, undiluted; Ventana), PR (1E2, undiluted; Ventana), HER2 (4B5, undiluted; Ventana) and androgen receptor (AR; SP107, undiluted; Cell Marque). Antigen retrieval was as follows: for SMA, none; for CK7, Cam5.2, CK5/6, MUC4, mammaglobin, GCDFP-15, p63 and SMM, Bond epitope retrieval solution 1 (Leica Biosystems); for calponin, Bond epitope retrieval solution 2 (Leica Biosystems); and for GATA3, MUC5AC, ER, PR, HER2 and AR, cell conditioning solution 1 (Ventana). For ER, PR and HER2, positive staining was defined according to ASCO/CAP guidelines.[27,28] For the two breast MEC, immunohistochemistry was performed and evaluated on standard sections, whereas TMA was applied for MEC of salivary gland origin.

MAML2 FLUORESCENCE *IN-SITU* HYBRIDISATION

Dual-colour fluorescence *in-situ* hybridisation (FISH) was performed using 3′*MAML2* DNA (clones CTD-2544I7, RP11-936C10, RP11-1123F20, CTD-252L1 and RP11-7D4) labelled with SpectrumGreen deoxyuridine triphosphate (dUTP) (Abbott Molecular/Vysis Products, Abbott Park, IL, USA) and 5′*MAML2* DNA (clones RP11-8N17, CTD-2325K3 and RP11-1056O10) labelled with SpectrumOrange dUTP (Abbott Molecular/Vysis Products). The probe set was applied to 5 μm-thick unstained formalin-fixed paraffin-embedded (FFPE) sections on glass slides, hybridised and washed as described previously.[26] Enumeration of the fusion and break-apart signals was conducted using an Applied Imaging Workstation (Foster City, CA, USA). In each case, 100 cells were analysed in the targeted region.

DETECTION OF *CRTC1–MAML2* FUSION BY RT–PCR

Total RNA was extracted from 10-μm-thick unstained FFPE sections using the RNeasy FFPE Isolation Kit (Qiagen, Hilden, Germany), followed by reverse transcription–polymerase chain reaction (RT-PCR) using the SuperScript III One-Step RT–PCR System (ThermoFisher Scientific, Foster City, CA, USA) with previously reported primers CRTC1 5′-TCGCGCTGCA-CAATCAGAAG-3′ and MAML2 5′-GGTCGCTTGCTG-TTGGCAGG-3′.[29,30] These products were diluted 1:50 and subjected to a nested PCR with inner primers CRTC1 5′-GAGGTCATGAAGGACCTGAG-3′and MAML2 5′-TTGCTGTTGGCAGGAGATAG-3′.[29,30] Products were resolved by agarose gel electrophoresis and Sanger sequenced using BigDye terminator chemistry (Thermo Fisher Scientific) following standard techniques.

CAPTURE-BASED NEXT-GENERATION DNA SEQUENCING

Capture-based next-generation sequencing was performed at the UCSF Clinical Cancer Genomics Laboratory, using an assay (UCSF500 panel) that targets the coding regions of 479 cancer-related genes, selected introns from 41 genes (not including *CRTC1* or *MAML2*) and the *TERT* promoter, with a total sequencing footprint of 2.8 Mb (Supporting information, Table S1). Sequencing libraries were prepared from genomic DNA of tumour and matched normal FFPE tissue extracted from macrodissected unstained sections. Target enrichment was performed by hybrid capture using a custom oligonucleotide library. Sequencing was performed on a HiSeq 2500 (Illumina, San Diego, CA, USA). Duplicate sequencing reads were removed computationally to allow for accurate allele frequency determination and copy number calling. The analysis was based on the human reference sequence UCSC build hg19 (NCBI build 37), using the following software packages: BWA: 0.7.10-r789; Samtools: 1.1 (using htslib 1.1), Picard tools: 1.97 (1504), GATK: 2014.4-3.3.0-0-ga3711, CNVkit: 0.3.3, Pindel: 0.2.5a7, SATK: 2013.1-10- gd6fa6c3, Annovar: v2015Mar22, Freebayes: 0.9.20 and Delly: 0.5.9.[31–40] Only insertions/deletions (indels) up to 100 base pairs (bp) in length were included in the mutational analysis. Somatic single nucleotide variants and indels were visualised and verified using Integrated Genome Viewer. Genomewide copy number analysis based on on-target and off-target reads was performed by CNVkit and Nexus Copy Number (Biodiscovery, Hawthorne, CA, USA).

Results

CLINICAL DATA

Case 1

A 53-year-old woman presented with a circumscribed nodule in the left breast on screening mammogram. Diagnostic mammogram revealed a mildly lobulated 1-cm nodule at 12 o'clock, 2 cm from the nipple. Ultrasound demonstrated a 0.9 cm heterogeneous mass with a mildly nodular contour. Needle core biopsy was performed; the initial diagnosis was reported as 'carcinoma with mucin secretion and squamoid features'. FISH was subsequently performed and was positive for *MAML2* and negative for *ETV6* gene rearrangements. An addendum diagnosis of 'low-grade mucoepidermoid carcinoma' was issued. The patient underwent subsequent wire-localised partial mastectomy and sentinel node lymphadenectomy; histological examination confirmed the diagnosis, revealing a 1.6-cm tumour with negative margins and lymph nodes. The tumour was low grade by the AFIP grading system and grade 1 by the modified SBR system. Biomarker testing was negative for ER, PR and HER2. She was treated with partial breast radiation and was alive and well at 16 months after surgery.

Case 2

The clinical data of case 2 were reported previously.[5] Briefly, a 49-year-old woman presented with a palpable right breast mass. Mammogram demonstrated a 1.5-cm round mass at 12 o'clock, as well as a 3-cm area of increased density in the posterior medial right breast. The patient underwent modified radical mastectomy with sentinel node lymphadenectomy and axillary dissection. Histological examination demonstrated mucoepidermoid carcinoma of at least 5 cm with one positive sentinel lymph node. The tumour was intermediate-grade by the AFIP system and grade 2 by the modified SBR system. Biomarker testing was negative for ER, PR and HER2. She was treated with chemotherapy and was alive and well at 1 year after diagnosis.

MICROSCOPIC FEATURES OF BREAST MUCOEPIDERMOID CARCINOMAS

H&E sections of both breast MEC showed histological features characteristic of MEC. Both tumours had broad pushing margins associated with a peripheral dense lymphoplasmacytic infiltrate (Figure 1A). The neoplastic cells were variably arranged in macrocystic or microcystic/cribriform structures and solid nests, with basophilic and/or eosinophilic secretions filling the cystic spaces (Figure 1B,C). Intermediate and epidermoid cells were well represented (Figure 1D); mucinous cells were morphologically more subtle but could be highlighted with a mucicarmine stain (Figure 1E). In the partial mastectomy specimen of case 1, the central portion of the tumour was predominantly composed of large irregular cysts, with smaller irregular nodules scattered at the periphery (Figure 1C). Case 2 demonstrated extensive ductal carcinoma *in-situ* (DCIS), with expanded lobules and ducts lined by variable numbers of intermediate, epidermoid and mucinous cells and forming fenestrations filled with basophilic secretions (Figure 1F). There were two foci of microinvasion consisting of small irregular nests with a desmoplastic response (Figure 1 in ref. 5). Lymphovascular invasion was not identified in either case.

IMMUNOHISTOCHEMICAL FEATURES OF BREAST AND SALIVARY GLAND MUCOEPIDERMOID CARCINOMAS

Both breast MEC expressed low molecular weight cytokeratins (LMWCK: CK7, CAM5.2), high molecular weight cytokeratins (HMWCK: CK5/6) and p63, with immunohistochemistry differentially highlighting the various cell populations and their distribution. Intermediate cells were positive for p63 and HMWCK and negative for LMWCK; epidermoid cells reacted with both LMWCK and HMWCK; and mucinous cells preferentially expressed LMWCK (Figure 2A,B). The peripheral p63 staining of intermediate cells could be difficult to distinguish from myoepithelial cell staining (Figure 2C); however, the invasive tumour was negative for other myoepithelial cell markers, including SMM (Figure 2D), calponin and SMA. The tumour in case 2 consists predominantly of DCIS with foci of microinvasion; the DCIS and invasive components show similar cytomorphology. This scenario illustrates the limited utility of p63 alone as a myoepithelial cell marker in this context. The staining of peripheral myoepithelial cells by p63 may be difficult to discern from the prominent co-staining of intermediate cells in DCIS, but an intact myoepithelial layer can be highlighted by positive SMM (Figure 2E) and calponin staining.

The immunoprofiles of the two breast MEC were compared to seven salivary gland MEC analysed by TMA (Table 1 and Figure 2F–H). Both breast and all seven salivary gland MEC were negative for ER, PR and HER2 by ASCO/CAP guidelines. Breast and

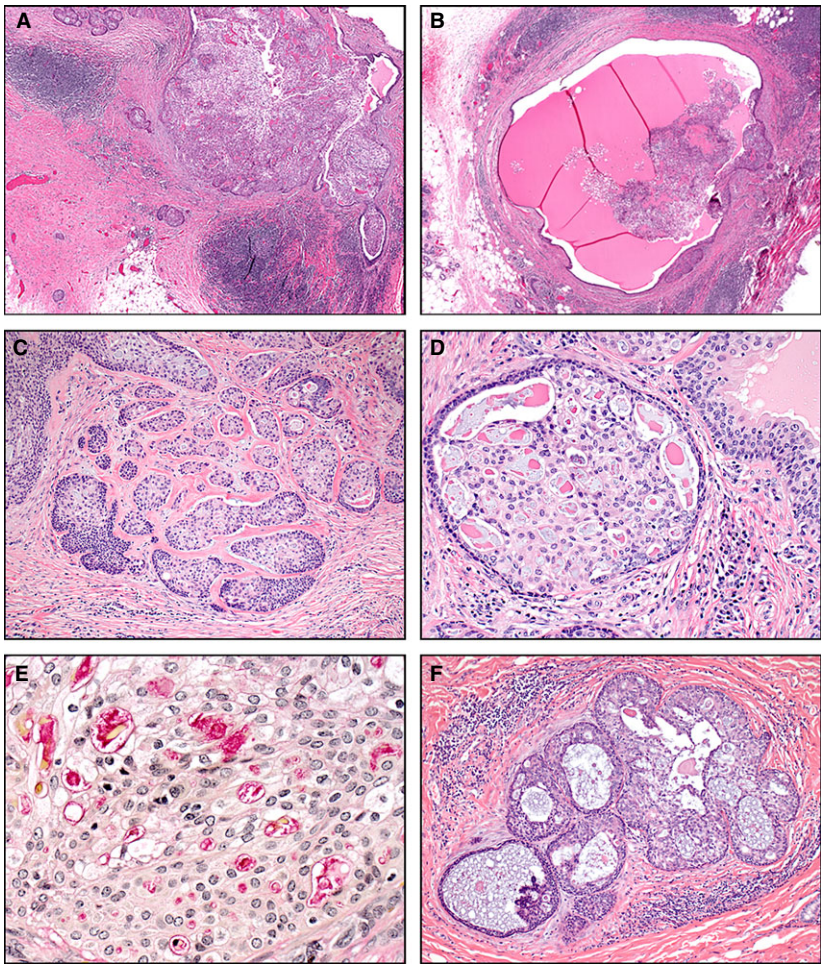

Figure 1. Morphological features of breast mucoepidermoid carcinomas. **A,** Low-power view of case 1 demonstrates variably sized cystic nodules and nests with a prominent lymphoid infiltrate at the edge of the tumour. **B,** Cystic architecture is prominent in the low-grade lesion. **C,** Small irregular solid nests are noted focally. **D,** Intermediate, epidermoid and mucinous cells are present in variable proportions with basophilic and eosinophilic intraluminal secretions. **E,** Mucicarmine stain highlights mucinous cells with cytoplasmic mucin vacuoles. **F,** *In-situ* component shows similar cytomorphology to invasive tumour as well as a periductal lymphoid infiltrate.

salivary gland MEC expressed CK5/6 and MUC4 and showed no to minimal expression of GCDFP-15 and AR (0–5% staining). Both breast MEC showed patchy or diffuse GATA3 and mammaglobin staining, in contrast to salivary gland MEC, in which staining for these markers was absent or focal (≤10%) in most cases (seven of seven and six of seven, respectively). In contrast, five of seven salivary gland MEC but neither of the breast MEC showed patchy MUC5AC expression. Of note, while cytokeratins and p63 demonstrated differential expression depending on the tumour cell types, other markers did not exhibit this staining pattern.

IDENTIFICATION OF *CRTC1–MAML2* TRANSLOCATION IN BREAST MUCOEPIDERMOID CARCINOMAS

FISH was positive for *MAML2* gene rearrangement in both breast MEC (Figure 3A–C). Both tumours demonstrated one green/orange fusion signal, reflecting a normal *MAML2* locus. Case 1 additionally revealed one separate green and one separate orange signal (Figure 3B), indicative of a translocation event involving one *MAML2* gene. In case 2, one green signal was accompanied by loss of the orange signal (Figure 3C), indicating deletion of the 5′ portion of the *MAML2* locus. RT–PCR analysis revealed *CRTC1–MAML2* fusion transcripts in both cases (Figure 4A), which were confirmed by Sanger sequencing (Figure 4B).

NEXT-GENERATION DNA SEQUENCING OF BREAST MUCOEPIDERMOID CARCINOMAS

Both breast MEC were subjected to targeted sequencing of 479 cancer-related genes. The mean target sequencing coverage was 724 and 507 unique reads per target interval in cases 1 and 2, respectively. No non-silent single nucleotide variants or indels were detected in case 1. Case 2 demonstrated a solitary pathogenic nonsense mutation in *SETD2* (p.S543*).

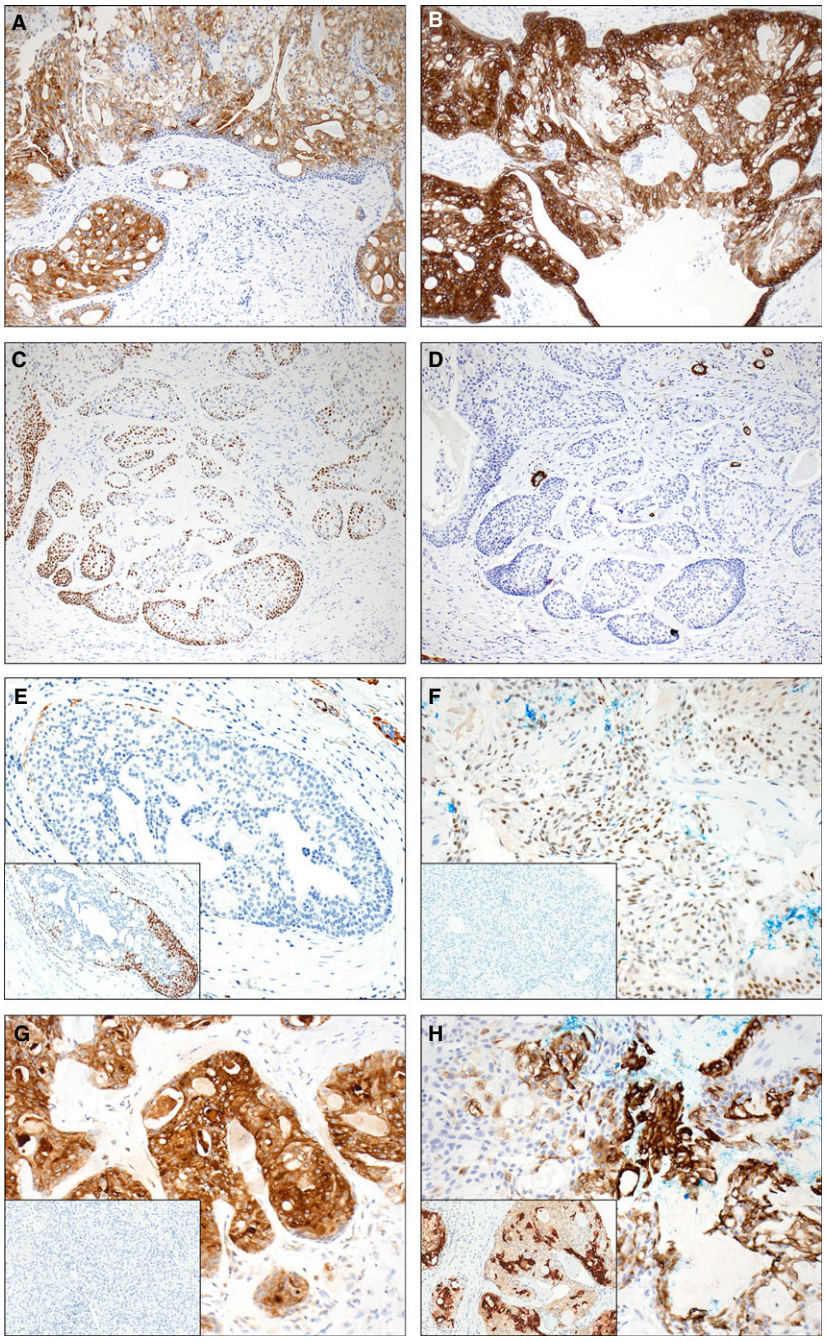

Figure 2. Immunohistochemical profile of mucoepidermoid carcinomas (MEC) of the breast and salivary gland. Immunohistochemical stains for cytokeratins differentially highlight the cell types of breast MEC, often in a zoning pattern with their spatial distribution. **A,** Low molecular weight cytokeratins (LMWCK) such as CAM5.2 preferentially stains mucinous and epidermoid cells. **B,** High molecular weight cytokeratins (HMWCK) such as cytokeratin (CK)5/6 highlights intermediate and epidermoid cells. **C,** p63 also shows a zoning pattern with staining of peripherally situated intermediate cells, which can be confused with myoepithelial cell staining. **D,** Other myoepithelial markers such as smooth muscle myosin (SMM) are negative. **E,** In contrast, *in-situ* carcinoma shows peripheral SMM staining, while p63 (inset) highlights both intermediate and myoepithelial cell populations. **F,G,** Mammary-specific markers are positive in breast MEC, compared to MEC in the salivary gland (insets). **F,** GATA binding protein 3 (GATA3) shows patchy to diffuse staining in breast MEC, but not salivary gland. **G,** Mammaglobin staining is also more diffuse in breast MEC than in salivary gland. **H,** Mucin 4 (MUC4) demonstrates positive staining in both.

Table 1. Immunophenotypes of breast and salivary gland mucoepidermoid carcinomas

	Breast MEC		Salivary gland MEC						
Immunostain*	1	2	1	2	3	4	5	6	7
GATA3	90	50	<1	5	0	0	1	0	<1
Mammaglobin	60	40	0	5	<1	10	15	<1	10
GCDFP15	0	1	0	<1	0	0	<1	0	0
ER	<1	<1	0	0	0	0	0	0	0
PR	0	0	0	0	0	0	0	0	0
HER2	0	0	0	0	1	0	0	0	0
AR	1	2	<1	1	0	0	1	5	<1
CK5/6	90	90	70	90	40	90	90	50	70
MUC4	20	80	80	100	60	70	80	90	80
MUC5AC	0	<1	20	1	70	5	50	50	70

*Values are percentages of positive-staining tumour cell, except for HER2 where values represent staining intensity as defined by the ASCO/CAP guidelines.

No copy number alterations were identified in either case. No pathogenic germline variants associated with increased cancer risk were identified in either patient.

Discussion

In this study, we describe the morphological features, immunophenotype and detailed genetic landscape of two breast MEC. The characteristic histological features of MEC are distinctive in the breast. However, as pathologists may not be familiar with this rare subtype of breast cancer, MEC can be confused with various benign and malignant conditions when assessing limited material on core biopsy or fine needle aspiration, depending on the architectural pattern and dominant cell type (Table 2). Partially sampled MEC with macrocystic architecture could easily be misinterpreted as simple cysts. Microcystic-predominant architecture may raise consideration of cribriform pattern DCIS. Conversely, the heterogeneous cell populations with bland cytology and irregular fenestrations can mimic usual ductal hyperplasia, which may be further confounded by positive CK5/6 staining. Epidermoid cells and the immunophenotype (positive CK5/6 and p63 and triple-negative) may also be confused with squamous metaplasia or a squamous metaplastic carcinoma. Lastly, low-grade MEC could be mistaken for secretory carcinoma, another rare salivary gland-type carcinoma.[41] Both tumours can have a prominent cystic component, abundant PAS-D-positive secretory material and cytologically bland cells which may have eosinophilic, clear or vacuolated cytoplasm. In addition, both are immunohistochemically positive for mammaglobin, MUC4 and CK5/6 and negative for ER, PR and HER2. However, in contrast to secretory carcinoma, MEC is consistently positive for p63 and is usually negative or only focally positive for S100. Distinction can be definitively established by FISH, using *MAML2* and *ETV6* break-apart probes, as in case 1.

Breast MEC and its salivary gland counterpart share histopathological features and have an overlapping immunophenotype. However, although our study is limited by the small number of cases of these rare tumours, we note subtle differences in the morphology and immunohistochemical profile of tumours from these two sites. Mucinous cells in the two breast MEC lacked tall columnar or goblet cytology, which is often observed in salivary gland MEC and has been noted in a previous report.[19] Although MEC from both sites demonstrate a basal-like immunophenotype

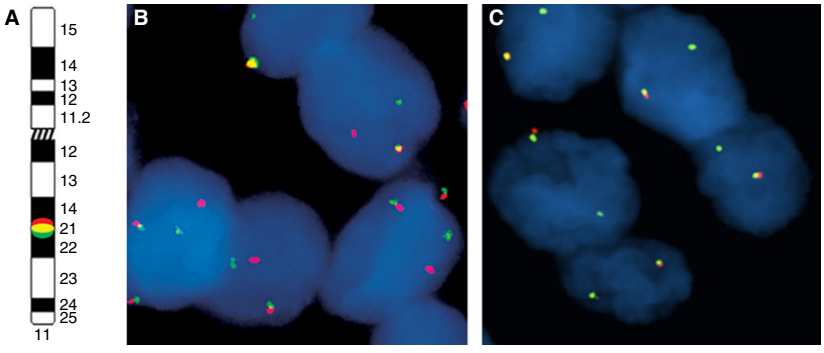

Figure 3. *MAML2* translocation in breast mucoepidermoid carcinomas. Fluorescence *in-situ* hybridisation with **A**, a *MAML2* break apart probe demonstrating **B**, one separate orange and one separate green signal in case 1 and **C**, one green signal and loss of the orange signal in case 2.

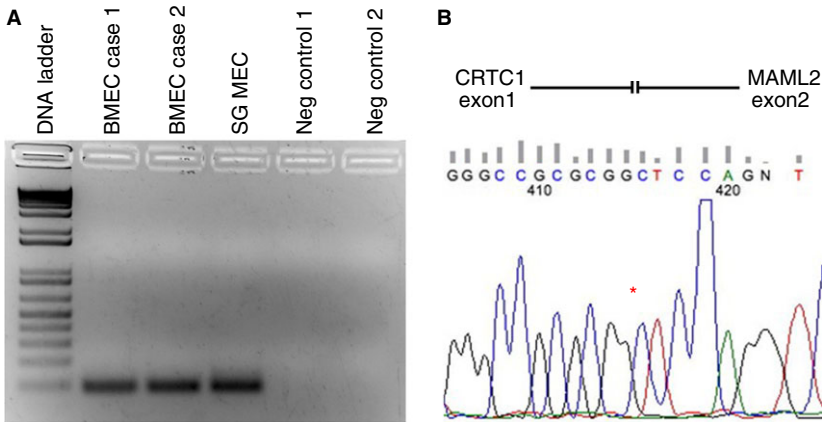

Figure 4. *CRTC1–MAML2* fusion transcript in breast mucoepidermoid carcinomas. **A,** Amplified reverse transcription–polymerase chain reaction (RT–PCR) products resolved by agarose gel electrophoresis, with 100 base pairs (bp) DNA ladder (lane 1), breast MEC case 1 (lane 2), breast MEC case 2 (lane 3), salivary gland MEC-positive control (lane 4) and water negative controls (lanes 5–6). The expected RT–PCR product is 95 bp. **B,** Direct (Sanger) sequencing of amplified RT–PCR product confirms the presence of *CRTC1–MAML2* fusion.

(ER, PR and HER2-negative and CK5/6-positive) and express MUC4, breast MEC show strong GATA3 and mammaglobin expression, whereas the salivary gland tumours are negative or only minimally positive for these latter markers. In contrast, MUC5AC expression, which has been previously reported in salivary gland MEC,[42] is absent or scant in breast MEC. In this context, it is interesting to note that a prior study of lung MEC found lack of expression of the pulmonary markers TTF-1 and napsin A in these tumours,[8] suggesting that MEC arising in some but not all sites may retain tissue-specific expression patterns, despite otherwise similar morphological and genetic features. Analysis of more cases is necessary to confirm these observations.

Ours is the first study to demonstrate the presence of *CRTC1–MAML2* fusion typical of salivary gland MEC in breast MEC and to analyse the genomics of these rare tumours using next-generation sequencing of a large panel of cancer-related genes. Both our cases harboured *MAML2* rearrangement by FISH and demonstrated the *CRTC1–MAML2* fusion

Table 2. Morphological mimics of breast mucoepidermoid carcinoma

Differential diagnosis	Features that may overlap with MEC	Features helpful in differential diagnosis
Simple cysts	Macrocystic architecture with one to few cell layers; mucoid material alone on limited sampling	Radiological–pathological correlation against simple cysts in MEC; presence of mucinous cells in MEC; negative SMM/calponin around cysts of MEC
UDH	Heterogeneous cell population with bland cytology and irregular microcystic spaces; strong positive CK5/6	Presence of mucinous and epidermoid cells in MEC; ER patchy positive in UDH and ER negative in MEC
DCIS, cribriform pattern	Rounded ductal contours with microcystic/cribriform architecture; well-defined cellular borders	Lack of nuclear polarisation around spaces in MEC; positive CK5/6 and negative ER in MEC; negative CK5/6 and diffuse strong ER in DCIS
Metaplastic SCC	Squamoid tumour cells with overlapping immunophenotype (triple negative, positive CK5/6 and p63)	Circumscribed nodules of MEC versus infiltrative growth of SCC; multiple cell types in MEC; lack of true keratinisation in MEC
Secretory carcinoma	Prominent cystic components; PASD-positive secretory material; cytologically bland tumour cells with overlapping immunophenotype (triple negative, positive mammaglobin and MUC4)	Positive p63 in MEC; positive S100 in secretory carcinoma; positive *MAML2* break-apart FISH in MEC; positive *ETV6* break-apart FISH in secretory carcinoma

MEC, mucoepidermoid carcinoma; UDH, usual ductal hyperplasia; DCIS, ductal carcinoma *in situ*; SCC, squamous cell carcinoma; PASD, periodic acid-Schiff, diastase; FISH, fluorescence *in-situ* hybridisation.

transcript by RT–PCR. Both tumours showed a simple genome with no copy number alterations and demonstrated a very low mutational burden of genes on the panel, with an inactivating *SETD2* mutation in one case as the only identified somatic non-synonymous variant. *SETD2* is a tumour suppressor gene that encodes a histone methyltransferase responsible for trimethylation of lysine 36 of histone H3 (H3K36me3). Most prevalent in clear cell renal cell carcinoma, inactivating mutations have been described in multiple cancers.[43,44] *SETD2* mutations have been rarely reported in breast tumours (at most ~3% of cases), notably only in luminal A cancers and phyllodes tumours but not in triple-negative breast carcinomas.[45–50] No *SETD2* alterations have been reported to date in MEC of the salivary gland,[51] and the significance of this isolated finding in one breast MEC is uncertain.

Comprehensive molecular studies of triple-negative breast carcinomas have shown a heterogeneous mutational milieu with frequent *TP53* and *PIK3CA* alterations.[45–49,52–56] However, these studies have generally not included triple-negative salivary gland-like tumours that can arise in the breast, including secretory carcinomas, acinic cell carcinomas and adenoid cystic carcinomas, as well as MEC and even more rare types.[1,5,7,19,57–59] Recent studies suggest that secretory carcinomas and adenoid cystic carcinomas of the breast are genetically more similar to their respective salivary gland counterparts than they are to other triple-negative carcinomas.[57,60] In contrast, acinic cell carcinomas arising in the breast harbour frequent *TP53* mutations and complex patterns of copy number alterations which are not present in salivary gland acinic cell carcinomas, suggesting that these two tumours are not related despite their histological similarity.[61,62] The molecular landscape of salivary gland MEC beyond *CRTC1–MAML2* rearrangement has only been explored recently.[51,63] Wang *et al.* utilised a panel of 315 cancer-related genes to interrogate 48 salivary gland MEC, including seven low-grade tumours. Alterations in common oncogenic drivers such as *TP53*, *PIK3CA*, *CDKN2A/B*, *BAP1*, *ERBB2* and *BRCA1/2* were frequently detected in intermediate- or high-grade MEC, but were absent or each present in only single cases of low-grade tumours.[63] Kang *et al.* performed whole-exome sequencing on 18 salivary gland MEC, including nine low-grade tumours. Although *TP53* was frequently mutated in intermediate- and high-grade MEC, none of the low-grade tumours had *TP53* mutations, with the only recurrent mutation in these tumours being *POU6F2* (in three tumours). In addition, most tumours had a low mutational burden with many (six of nine) showing no copy number alterations.[51] The breast MEC in our study lacked *TP53* or *PIK3CA* mutations or complex copy number profiles typical of high-grade triple-negative carcinomas of no special type, and indeed showed no or only isolated genetic abnormalities aside from *CRTC1–MAML2* fusions. *POU6F2* is not included on the UCSF500 panel and could not be analysed in our study. Our findings suggest that breast MEC, like other fusion gene-driven special breast cancer subtypes, are genetically more similar to their counterparts in the salivary gland than they are to other primary breast cancers.

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Conflicts of Interest

The authors have no conflicts of interest to disclose.

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Supporting Information

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

Table S1. UCSF500 gene list.