CRTC1 rearrangements in the absence of t(11;19) in primary cutaneous mucoepidermoid carcinoma

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Accepted for publication
10 February 2009

Key words
MECT1, mucoepidermoid carcinoma, Notch coactivator

Conflicts of interest
None declared.

DOI 10.1111/j.1365-2133.2009.09200.x

Summary

Background Mucoepidermoid carcinoma (MEC) of the skin is an uncommon neoplasm with a remarkable resemblance to MEC of the salivary glands. The latter has been shown to harbour an oncogenic translocation resulting in a fusion gene consisting of exon 1 of *CRTC1/MECT1/TORC1* at 19p and exons 2–5 of *MAML2* at 11q.

Objectives While t(11;19) and rearrangements of the involved loci have been demonstrated in MEC of the salivary gland and other sites, it remains to be determined if morphological similarities in cutaneous MEC are reflected at the molecular level.

Methods Cases of cutaneous MEC were defined by three histopathological features: (i) cystic dermal nodule with (ii) overlying intact epidermis and (iii) presence of three cell types (squamoid, intermediate, mucinous), and characterized by reverse transcription-polymerase chain reaction (RT-PCR), interphase fluorescent *in situ* hybridization (FISH) and immunohistochemistry.

Results Eight primary cutaneous MECs were analysed. All informative cases showed *CRTC1* rearrangements; none of the cases had *MAML2* rearrangements or the presence of t(11;19) by RT-PCR. One case of primary MEC of the breast showed amplification of *MAML2* in the absence of *CRTC1* or t(11;19). Two MECs metastatic to the skin, histologically identical to primary cutaneous MEC, were included, one of which harboured the *CRTC1–MAML2* fusion gene by RT-PCR, verified by interphase FISH and sequencing.

Conclusions MEC of the skin harbours *CRTC1* rearrangements, a molecular finding that reflects morphological similarities between glandular and cutaneous MEC. The absence of oncogenic t(11;19) or *MAML2* aberrations in our series, which is the largest reported, may explain the innocuous clinical behaviour of this uncommon adnexal tumour.

Mucoepidermoid carcinoma (MEC) of the skin is an uncommon neoplasm with ~30 reported cases; whereas MEC accounts for 30% of malignancies arising in salivary glands.[1] Regardless of the primary site, these tumours share distinct histopathological features that allow for straightforward diagnosis in most cases.[2–4] The differential diagnosis includes metastatic MEC to the skin[1,3,5] and clear cell hidradenoma (CCH).

In addition, genetic aberrations characteristic of salivary gland MEC have also been demonstrated in CCH and various other tumours.[6,7] The classic alteration, t(11;19)(q21;p13), which creates a fusion gene between exon 1 of *CRTC1/MECT1/TORC1* at 19p13 and exons 2–5 of *MAML2* at 11q21,[8] is complemented by a related *CRTC3–MAML2* fusion as well as multiple recurring rearrangements of other chromosomal regions.[9]

The current study investigates whether cutaneous MEC shares a molecular linkage to the glandular counterpart. This report describes the presence of *CRTC1* rearrangement in the absence of t(11;19) in a series of cutaneous MECs.

Materials and methods

Cases were identified from our files and formalin-fixed and paraffin-embedded tissues were used. Haematoxylin and eosin, special stains and immunohistochemistry followed routine protocols. This study was approved by the Institutional Human Studies Committee.

Cutaneous MEC was defined by three histopathological features: (i) cystic dermal nodule with (ii) overlying intact epidermis and (iii) the presence of three cell types (squamoid,

intermediate, mucinous). Established histopathological criteria identical to the features of glandular MEC were applied.[2]

Molecular testing followed previously published protocols.[10] In brief, RNA extraction was performed using a modified guanidine isothiocyanate method. For reverse transcription-polymerase chain reaction (RT-PCR), samples were reverse transcribed using Mulv transcriptase and an RNA-PCR kit (Perkin-Elmer, Foster City, CA, U.S.A.). Nested PCR for *CRTC1–MAML2* transcripts followed published primers and controls; intact RNA and amplifiable cDNA was assessed via β_2-microglobulin. PCR products were cloned into vector pCR2.1 using the TA Cloning kit (Invitrogen, Carlsbad, CA, U.S.A.), followed by fluorescent DNA sequencing (373A; Applied Biosystems Inc., Foster City, CA, U.S.A.). Fluorescent *in situ* hybridization (FISH) experiments utilized a break-apart approach. At least 100 nuclei were assessed and > 15% of nuclei had to have split signals to be scored as 'positive for rearrangement'; cells were scored as 'positive for amplification' when either large clusters of overlapping signals (too numerous to count) or ≥ 10 distinct signals were found.

Results

Clinically, cutaneous MEC presented as a palpable, nontender mass (Fig. 1, Table 1).

Microscopically, all nodules were well circumscribed with an intact overlying epidermis without any dysplastic changes. These dermal-based neoplasms consisted of three distinct cell

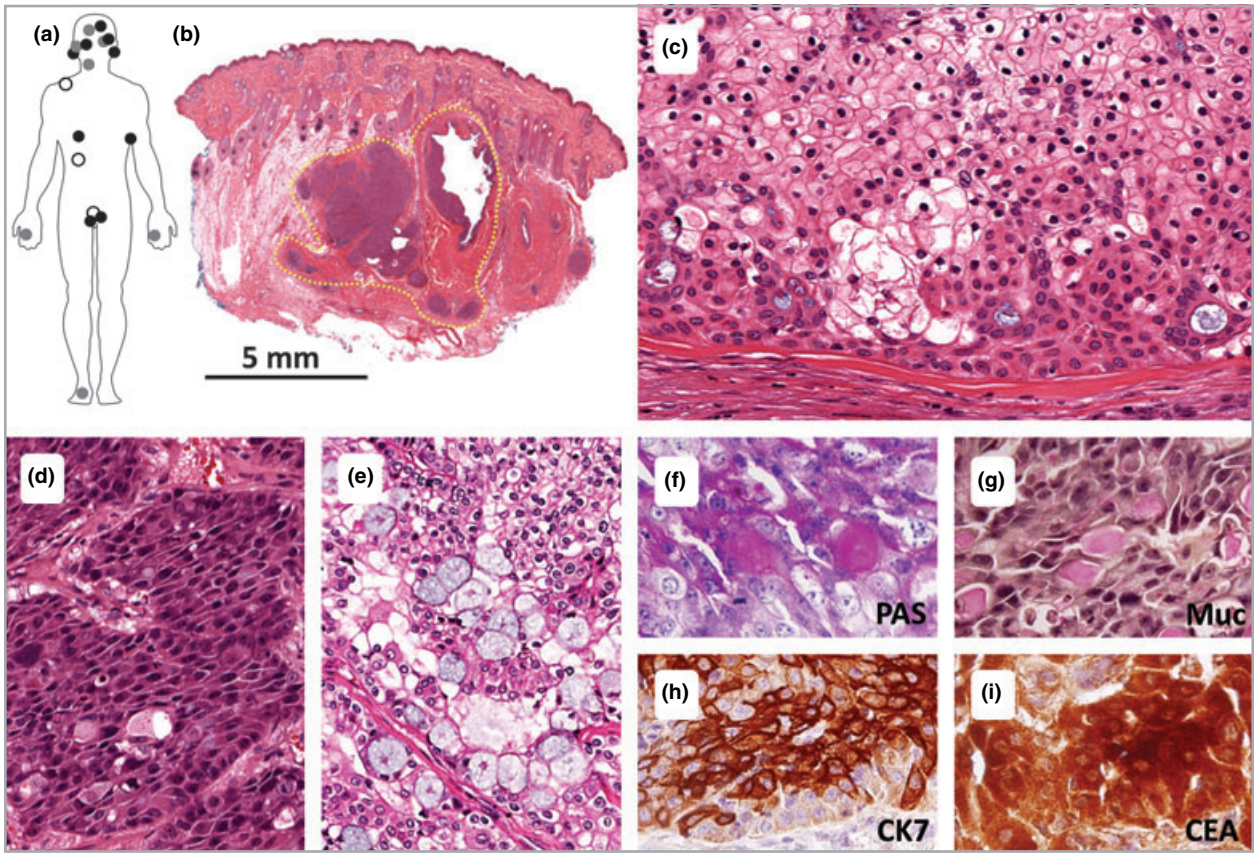

Fig 1. Cutaneous mucoepidermoid carcinoma (MEC). (a) Anatomical distribution of the current (black circles) and reported[1] (grey circles) cases illustrates occurrence predominantly in regions with specialized adnexal glands; note the high incidence in the head and neck region. Metastatic lesions (current study) are indicated by open circles. (b) 'Whole mount' of a recurrent MEC of the scalp (case 7) shows clusters and islands of cells that form a solid and cystic dermal nodule that is well circumscribed; the overlying epidermis is intact. (c) Solid area (case 1) with the three cell types and focal clear cell change. The epidermoid cells (top) contained abundant eosinophilic cytoplasm and rarely showed keratinization or intercellular bridges; these cells commonly formed solid and cystic architectural patterns. Mucinous cells (blue) were randomly interspersed with the other cell types or formed clusters (e) but did not form definitive glands; individual mucinous cells were not present in every case but a recognizable proportion (≥ 5%) of the tumour had to show cytoplasmic droplets/mucin cells for the diagnosis of MEC.[2] Intermediate cells were the most abundant cell type, ranging from small basal cells to oval or polygonal cells, found in clusters, sheets, or admixed with epidermoid and mucinous cells. (d) High-grade nuclear features (case 5). (e) Intracytoplasmic mucin globules (faint blue) without glandular differentiation (case 2). The presence of mucin in mucinous cells was confirmed by periodic acid–Schiff (PAS) (case 3) (f) and mucicarmine (Muc) (case 6) (g) stains. MEC of the skin showed an immunophenotype that is also characteristic of glandular MEC,[2] namely: cytokeratin (CK) 7+ (case 2) (h), carcinoembryonic antigen (CEA)+ (case 4) (i), CK20− (not shown) and epithelial membrane antigen+ (not shown).

Table 1 Demographic, clinicopathological, molecular and immunophenotypical features

Case	Age (years)/sex	Anatomical site and other history	Tumour size (cm)	Histological grade[a]	Treatment and course	t(11;19) by RT-PCR	*CRTC1* by FISH	*MAML2* by FISH	IHC	Status
Primary cutaneous MEC										
1	51/F	Right cheek lesion	0·5	L	Excision	No	Rearranged	No evidence of rearrangement	NA	LW: 14 years
2	49/F	Adnexal malignancy, left lower eyelid	0·4	L	Block resection after LR	No	Rearranged	No evidence of rearrangement	CK7+ CK20−	LW: 4 years 2 months
3	71/F	Vulval mass	0·4	L	Radical hemivulvectomy	No	Rearranged	No evidence of rearrangement	NA	DU: 6 years 7 months
4	56/M	Recurrent adnexal carcinoma	0·9	L	Mohs surgery	No	Not informative	Not informative	CEA+ CK20−	LW: 19 years
5	77/F	Vulval mass; bone extension by imaging	2·2	H	Radiation therapy after LR	No	Rearranged	No evidence of rearrangement	NA	DOT: 2 months
6	39/M	Skin lesion behind right ear; mastoid process	0·7	L	Biopsy	NA	NA	NA	NA	LW: 2 years 7 months
7	9/M	Occipital mass, scalp[3]	5	L	Excision, LR × 2	No	Rearranged	No evidence of rearrangement	CK7+ CEA+ CK20−	LW: 2 years 10 months
8	79/F	Firm, painless, nonfixed mass, axilla[1]	3·7	L	Excision	NA	NA	NA	CK7+ CEA+ EMA+ CK20−	LW: 5 years 9 months
Noncutaneous MEC[b]										
9	46/F	'Breast cancer', right upper outer quadrant	1·6	NA	Lumpectomy	No	Not informative	Amplified	NA	LW: 7 years 10 months
Glandular MEC metastatic to the skin[c]										
10	58/M	Multiple bone lesions and left mediastinal mass; history of bronchial MEC	> 2	NA	Palliative care	Yes	Rearranged	Rearranged	NA	DOT: 9 months
11	50/F	Shoulder and liver masses; history or parotid MEC	NA	NA	Excision	No	Not informative	Not informative	NA	No FU

[a]Histological grade based on cytological features: high-grade tumours showed focal necrosis, brisk mitotic activity or anaplasia (Fig. 1). [b]The breast can be viewed as a specialized cutaneous appendage; we found *MAML2* alterations only in case 9. [c]MEC of the salivary glands can initially present as cutaneous metastasis with identical histological appearance and has been reported in the absence of a recognized head and neck primary tumour or after many years of disease-free interval.[5] We excluded a prior diagnosis of MEC in all cases of primary cutaneous MEC. RT-PCR, reverse transcription-polymerase chain reaction; FISH, fluorescent *in situ* hybridization; IHC, immunohistochemistry; MEC, mucoepidermoid carcinoma; H, high-grade; L, low-grade; LR, local recurrence; CK, cytokeratin; CEA, carcinoembryonic antigen; EMA, epithelial membrane antigen; LW, living and well; DU, dead, unrelated; DOT, died of tumour; FU, follow-up; NA, not applicable/not available for testing/not tested.

types with interlacing solid squamoid to intermediate cellular nests with glandular and duct-like structures and occasional cysts lined by a mucinous type epithelium. Two additional similarities to glandular MEC were clear cell foci and an identical immunophenotype (Table 1).[1,2]

RT-PCR testing showed absence of *CRTC1–MAML2* fusion transcripts in all of the primary MECs of the skin. Only the cutaneous metastasis (case 10) showed the fusion, confirmed via DNA sequence analysis (Fig. 2) and interphase FISH.

Interphase FISH testing (Table 1) showed rearrangements of *CRTC1* in six cases. The cutaneous metastasis (case 10) showed *MAML2* rearrangement confirming the t(11;19) fusion detected by RT-PCR. All five informative cases of primary cutaneous MEC showed *CRCT1* rearrangements but none showed *MAML2* rearrangements. The MEC of the breast showed amplification of *MAML2* in the absence of rearrangements of either the *CRTC1* or *MAML2* loci. Case 4 was not informative despite repeated hybridization attempts. No polysomy for either locus was observed.

Discussion

Although included in the current World Health Organization classification of cutaneous adnexal neoplasms, some controversy has existed in the literature about the precise definition of this neoplasm and its differentiation from adenosquamous carcinoma.[1,4,11] Utilizing currently available histopathological criteria largely predicated on the similarities with its counterpart in the salivary glands,[2] this study of eight primary cutaneous MECs examined their molecular signature with respect to *CRTC1* and *MAML2*. The similarities at the morphological level between glandular and cutaneous MEC were reflected at a molecular level. In cutaneous MEC we demonstrate *CRTC1* rearrangements in the absence of the classical *CRTC1–MAML2* translocation characteristic of MEC arising in the salivary gland; this finding implies that cutaneous MEC is related but not identical to glandular MEC in a molecular-genetic sense.

All informative cases showed rearrangement of *CRTC1* in the absence of rearrangements or amplification of *MAML2*. The presence of genetic heterogeneity at the *CRTC1* locus in MEC is reminiscent of the genetic heterogeneity found in glandular MEC.[8] In previous studies, the proportion of glandular MECs lacking the *CRTC1–MAML2* translocation that nonetheless harbour rearrangement of either the *CRTC1* or *MAML2* locus has not been evaluated. However, a related *CRTC3–MAML2* fusion has been described indicating the existence of other fusion partners.[9] Recurrent complex rearrangements and variant translocations of 11q in the absence of 19p have been reported although the identity of the relevant genes remains unknown.[8–10] *CRTC1* rearrangements without *MAML2* alterations in cutaneous MEC suggest that other oncogenic fusion partners exist but they have yet to be defined.

The protein encoded by *CRTC1* is a potent coactivator of genes regulated by cAMP response elements known as CREs.[12]

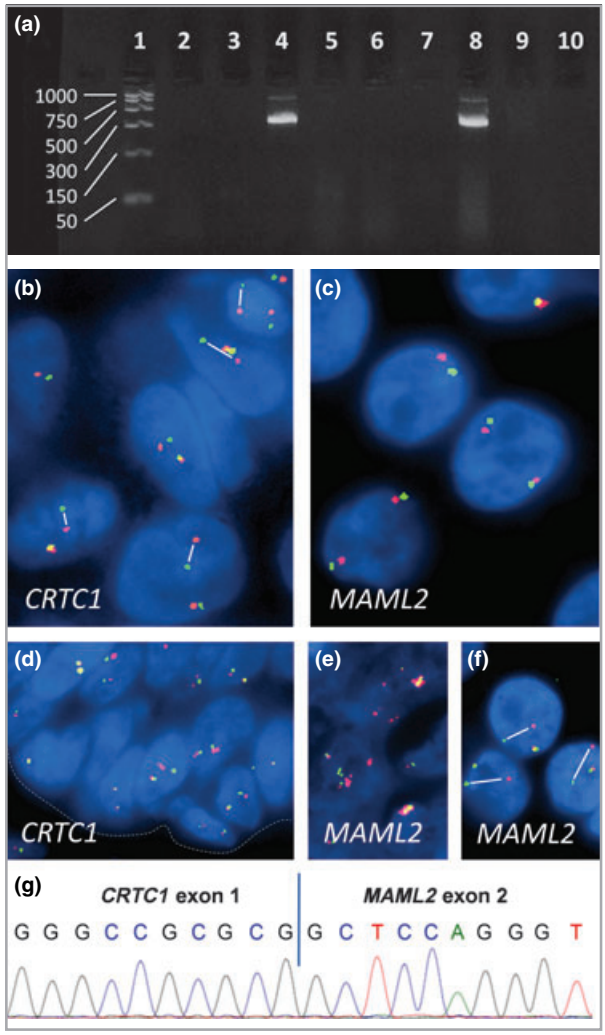

Fig 2. Molecular analysis. (a) Reverse transcription-polymerase chain reaction (RT-PCR). Identification of the *CRTC1–MAML2* fusion transcript in a case of metastatic mucoepidermoid carcinoma (MEC) to the skin (case 10). Primary MEC of the skin did not harbour the translocation. Lane 1, molecular weight markers in kDa; lane 2, case 4; lane 3, case 7; lane 4, case 10; lane 5, case 3; lane 6, case 1; lane 7, case 2; lane 8, salivary gland MEC known to harbour t(11;19)(q21;p13), positive control; lane 9, no input DNA, negative control; lane 10, sham RT treatment, negative control. (b–f) Dual-colour break-apart interphase fluorescent *in situ* hybridization. Probe splitting was defined as signals further than three signal diameters apart (indicated by white lines). Cutaneous MEC (case 1) demonstrates *CRTC1* rearrangement (b) in the absence of rearrangement of *MAML2* (c); the overlying epidermis shows no *CRTC1* rearrangement (d) as indicated by yellow fusion signals or minimal red-green signal separation (broken line: basement membrane). (e) MEC of the breast (case 9) showed amplification of *MAML2*. (f) Glandular MEC [same case as shown as positive control in (a), lane 8] showed *MAML2* rearrangement with probe sets separated by more than three probe signals (indicated by lines). (g) Sequence of the *CRTC1–MAML2* fusion transcript, Identity of RT-PCR product in case 10 [(a), lane 4] was confirmed by DNA sequence analysis that demonstrated an in-frame fusion between exon 1 of *CRTC1* and exon 2 of *MAML2*; vertical line indicates fusion boundary.

The transforming activity of constitutive CREB activation as a result of CRTC1 deregulation has been demonstrated.[12] Our findings of *CRTC1* rearrangements in cutaneous MEC suggest that alterations in CREB activation may be involved in the development of this tumour. Pharmaceutical agents that target CRTC1 and CREB alterations may provide an option for rare cases that are metastatic, locally aggressive, or nonresectable.[13]

In our series, the single MEC of the breast (case 9) showed amplification of *MAML2*, a transcriptional coactivator of Notch signalling involved in the regulation of many cellular processes including proliferation, apoptosis and differentiation.[14] The presence of *MAML2* alterations in tumours with higher metastatic potential, increased incidence of recurrence and worse prognosis suggests the absence of *MAML2* alterations in cutaneous MEC is related to its indolent clinical behaviour.[8,10,15]

Overall, the concept that the molecular signature of MEC reflects the morphological similarities of the tumour, regardless of the primary site, is appealing. It is necessary to point out that a subset of Warthin's tumours[6] shares aberrations of *CRTC1* and/or *MAML2*, a finding that suggests other molecular/genetic modifiers are involved as others have suggested.[8–10] In this context, it is interesting to note that the finding of *MAML2* aberrations in MEC of the cervix,[10] a tissue of Müllerian origin, contradicts the prevailing conjecture that the morphological resemblances at different anatomical sites are due to ectodermal derivation.[1,4,11] It may be the shared presence of rearrangements of *CRTC1* that is the common denominator in terms of the morphological features rather than the tissue of origin.

Acknowledgments

We thank Dr Wes Warren of the Washington University Genome Sequencing Center for generously supplying the BAC clones used for FISH. We extend our thanks to Dr Steven P. Nuernberger (Department of Pathology, Anderson Hospital, Maryville, IL, U.S.A.) for consult material and Julie Branson, Diane Robirds, Ruma Banerjee, Dr Xiaopei Zhu, Pros Amargo, as well as Walter Clermont for expert technical assistance. The administrative help of Jeannie Doerr is also greatly appreciated.

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