Molecular advances in salivary gland pathology and their practical application

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Abstract

The review summarizes the new findings in salivary gland pathology with particular reference to molecular genetic developments. In particular, newly recognized entities and specific chromosomal translocations associated with salivary gland carcinomas are discussed. Firstly, there are three types of salivary gland carcinomas which harbour important oncogenic translocations: mucoepidermoid carcinoma with the translocation t(11; 19)(q21; p13) *CRTC1-MAML2* (as well as several other less frequent ones), adenoid cystic carcinoma with the translocation t(6; 9)(q22–23; p23–24) *MYB-NFIB*, and the recently described entity of mammary analogue secretory carcinoma (MASC) characterized by the translocation t(12; 15)(p13; q25) *ETV6-NTRK3*. Secondly, sclerosing polycystic adenosis was described in 1996 as possibly a salivary counterpart to benign fibrocystic disease of the breast, but recent molecular evidence of clonality suggests it is neoplastic in nature. Finally, new molecular developments in salivary duct carcinoma and molecular mechanisms responsible for high grade transformation and tumour progression in other neoplasms will be addressed.

Keywords adenoid cystic carcinoma; *CRTC1-MAML2*; *ETV6-NTRK3*; mammary analogue secretory carcinoma; mucoepidermoid carcinoma; *MYB-NFIB* translocation; salivary gland

Introduction

In recent years, molecular testing has become a standard method in the diagnosis and consequent treatment decisions in many fields of pathology including cancer of the head and neck. The spectrum of tumours of major and minor salivary glands is wide,[1] and includes several newly recognized entities.[2–4] They are often diagnostically challenging with interesting and controversial morphological features often overlapping between different entities. Although morphology in combination with immunohistochemical findings still provide the most important clues for diagnosis, recent advances in molecular pathology offer new potential avenues in investigating both differential diagnosis and prognosis.[5]

This review will address several unique salivary gland tumours with oncogenic translocations, in particular mucoepidermoid carcinoma (MEC) with the translocation t(11; 19)(q21; p13) *CRTC1-MAML2* – several other chromosomal rearrangements may also be found in this tumour[6]; adenoid cystic carcinoma (AdCC) with t(6; 9)(q22–23; p23–24) *MYB-NFIB*[7]; and the recently described salivary gland tumour entity, mammary analogue secretory carcinoma (MASC) characterized by a recurrent balanced chromosomal translocation t(12; 15)(p13; q25) *ETV6-NTRK3*.[2] In all three examples, the translocated genes encode novel fusion proteins, which participate in development and progression of the carcinomas.[2,8,9]

This review will also discuss recent molecular evidence of clonality in the entity of sclerosing polycystic adenosis, which suggests that its nature is neoplastic rather than reactive or inflammatory.[10] Finally, new molecular developments in salivary duct carcinoma[11] and molecular mechanisms responsible for high grade transformation[12] and tumour progression will be considered.[13,14]

Oncogenic translocations in salivary gland carcinomas

Mucoepidermoid carcinoma

Mucoepidermoid carcinoma (MEC) represents about 5% of all salivary gland tumours and 20% of salivary malignancies.[15] In a significant number of cases, a recurring t(11; 19)(q21; p13) *CRTC1-MAML2*(*known also as MECT1-MAML2*) translocation is present (Figure 1 a, b, d).[6] This alteration results in a novel fusion oncogene, in which the CREB-binding domain of the CREB regulated transcription coactivator CRTC1 (also known as MECT1, TORC1 or WAMTP1) is fused to the transactivation domain of the Notch coactivator MAML2. The fusion protein influences expression of cAMP/CREB (FLT1) and Notch (HES1 and HES5) target genes, and is then involved in the transformation of the neoplastic cells.[6,16]

Clinical follow-up studies revealed that patients with fusion-positive MECs had a significantly lower risk of local recurrence, metastases, or tumour-related death compared to fusion-negative patients. When considering tumour-related deaths only, the estimated median survival for fusion-positive patients was greater than 10 years compared to 1.6 years for fusion-negative patients.[6] These findings suggest that classifying MECs on the basis of CRTC1-MAML2 fusion is clinically relevant.[6] Subsequently, Seethala et al.[17] analyzed a large cohort of MECs for the presence of the CRTC1/MAML2 translocation by fluorescent in-situ hybridization (FISH) and real-time RT-PCR. Overall, CRTC1/MAML2 translocation was present in 66% of MECs whereas all other salivary gland tumours were negative for it. Low or intermediate-grade MECs had a higher frequency of the

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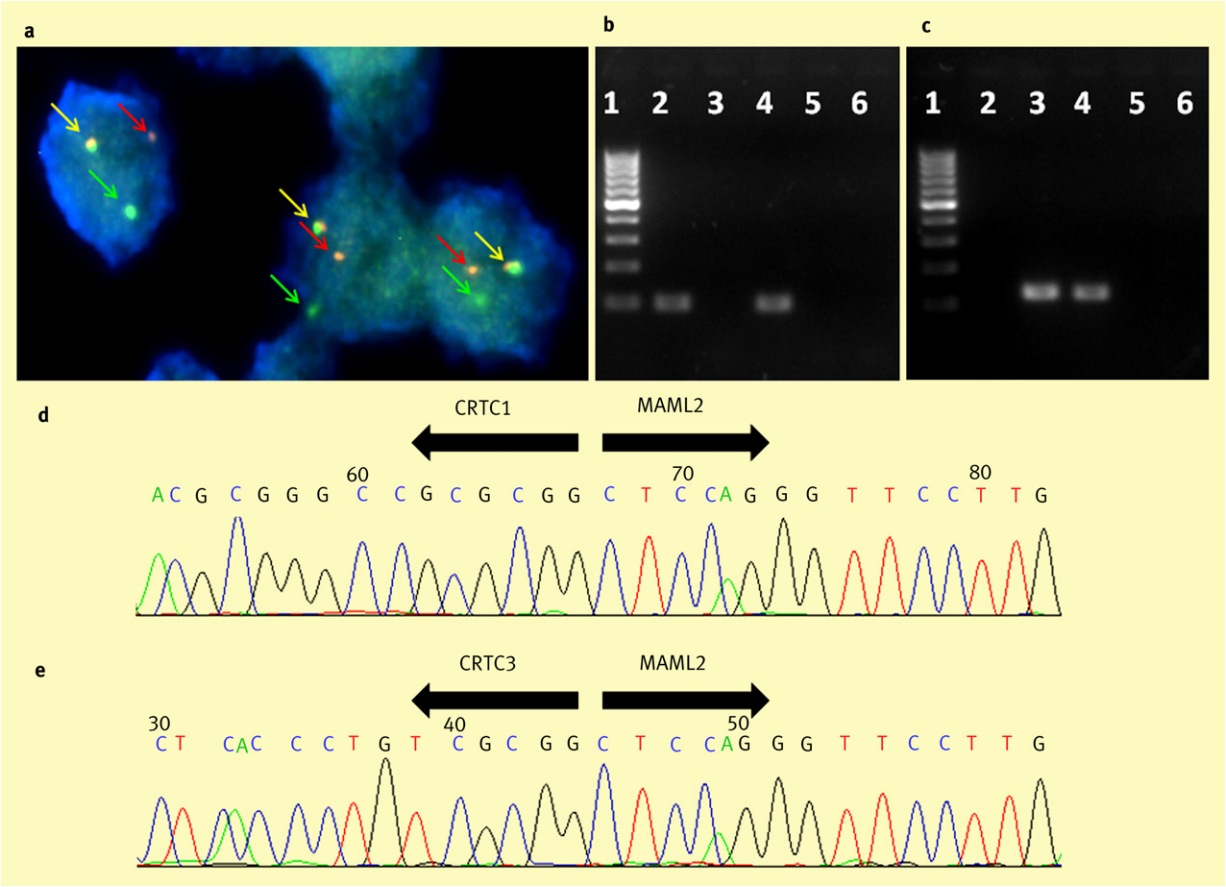

Figure 1 Analysis of CRTC1-MAML2 and CRTC3-MAML2 fusion genes in MEC. (**a**) Fluorescence *in-situ* hybridization (FISH) using ZytoLight SPEC MAML2 dual colour, break apart probe (ZytoVision). Green and red arrows show split signals indicating break of MAML2 gene. Yellow arrows show nonaltered chromosome. (**b**) Expression of CRTC1-MAML2 fusion transcript detected by RT-PCR. 1 — Marker, 2 — CRTC1-MAML2 positive MEC sample, 3 — CRTC1-MAML2 negative MEC sample, 4 — positive amplification control, 5 — negative amplification kontrol, 5 — non-template control. (**c**). Expression of CRTC3-MAML2 fusion transcript detected by RT-PCR. 1 — Marker, 2 — CRTC3-MAML2 negative MEC sample, 3 — CRTC3-MAML2 positive MEC sample, 4 — positive amplification control, 5 — negative amplification control, 5 — non-template control. (**d**) Sequence analysis of CRTC1-MAML2 fusion transcript. Arrows show the translocation breakpoint. (**e**) Sequence analysis of CRTC3-MAML2 fusion transcript.

translocation (75%) than high grade MECs (46%). In addition, a novel fusion partner of MAML2 from the CRTC family, namely CRTC3 localized on chromosome 15q26.1, was recently found to be present in a subset of mucoepidermoid carcinomas (Figure 1c, e).[18] Separately, it has been shown that the CRTC3-MAML2 fusion may be associated with favourable clinicopathological features and also, patients may be younger than those with CRTC1-MAML2 fusion or those with no detectable gene fusion.[19] When positive for the fusions CRTC1—MAML2 or CRTC3—MAML2, even 'high-risk' patients, including those with a higher histological grade or an advanced clinical stage, showed an excellent prognosis.[20] Thus, it has been proposed that fusion-positive MECs should be regarded as representing a distinctive entity separate from fusion-negative cases.[20] Recently, the *EWSR1-POU5F1* fusion gene was documented in mucoepidermoid carcinomas of salivary gland as well as in hidradenoma of skin, providing further evidence for a genetic link between these two tumour types.[21] In contrast, this fusion gene appears to be more common in less well differentiated examples of mucoepidermoid carcinoma, suggesting that it too may have prognostic significance.[21]

Practice points

- *CRTC1-MAML2* translocation is identified in a high proportion of mucoepidermoid carcinomas and imparts a better prognosis
- *CRTC3-MAML2* fusion is associated with favourable clinicopathological features and may be seen in younger patients
- Fusion-positive MECs even in 'high-risk' patients, with a high histological grade or an advanced clinical stage, generally have a prognosis

Adenoid cystic carcinoma

Adenoid cystic carcinoma (AdCC) is a common malignancy that can occur in both major and minor salivary glands and usually pursues a relentless clinical course, typically characterized by late recurrences and distant metastatic disease.[22] The characteristic molecular feature of this lesion appears to be recurrent chromosomal translocation t(6; 9)(q22—23; p23—24), which generates a fusion transcript involving the gene for transcription factors *MYB* and *NFIB*. The *MYB* is highly expressed in immature