Correspondence

The *MECT1-MAML2* Gene Fusion and Benign Warthin's Tumor

Is the *MECT1-MAML2* Gene Fusion Specific to Mucoepidermoid Carcinoma?

To the Editor-in-Chief:

Recent studies have shown that mucoepidermoid carcinomas (MECs) of the salivary and bronchial glands are characterized by a recurrent t(11;19)(q21;p13) translocation, resulting in a *MECT1-MAML2* fusion in which the cAMP response element binding protein (CREB)-binding domain of the CREB-regulated transcriptional coactivator *MECT1* (also known as *CRTC1*, *WAMTP1*, or *TORC1*) is fused to the transactivation domain of the Notch coactivator *MAML2*.^{1,2} The molecular consequences of the fusion are still largely uncertain. Earlier studies have indicated that *MECT1-MAML2* can activate Notch signaling independent of ligand stimulation,^{1,2} and recent studies have demonstrated that *MECT1-MAML2* can activate transcription of multiple cAMP/CREB target genes *in vitro* and that the N-terminal CREB-binding domain is crucial for the transforming activity of the fusion protein.^{3,4} Collectively, these studies indicate that *MECT1-MAML2* is a multifunctional protein with effects on several signaling pathways.

Interestingly, recent studies have indicated that the *MECT1-MAML2* fusion may not exclusively occur in MEC but may also be found in other types of glandular tumors. Thus, we have identified the fusion in two apparently unrelated benign tumor types: Warthin's tumor (WAT) and clear cell hidradenoma of the skin.^{2,5} In this respect, *MECT1-MAML2* is reminiscent of the *ETV6-NTRK3* fusion that has been encountered in tumors originating from multiple cell lineages.⁶

We read with interest the study by Martins et al⁷ in *The Journal of Molecular Diagnostics* in which they studied the *MECT1-MAML2* fusion in 10 MECs and 7 WATs using fluorescence *in situ* hybridization (FISH) and/or reverse transcriptase-polymerase chain reaction. The fusion was detected in seven MECs but in none of the WATs. It should be noted that none of the classical WATs in this study had a t(11;19) translocation. However, one of their translocation- and fusion-positive MECs (case 10) was of particular interest because it was originally diagnosed and published as a benign WAT.⁸ This tumor was now reclassified as a MEC ex

WAT. As indicated by the authors, it is known that MECs in rare instances may arise from or coexist with WAT. Case 10 in their series was suggested to represent such a case. The authors conclude that the *MECT1-MAML2* fusion appears to be rare in WATs that do not contain concomitant evidence of MEC and that the fusion might be a useful diagnostic marker for MEC, especially in histologically challenging cases.

The observations by Martins et al prompted us to re-examine our previously published t(11;19)- and fusion-positive WAT^{2,9} both histologically and molecularly. Analysis of tissue sections from multiple levels of this tumor confirmed the original diagnosis of a benign WAT. Despite extensive sampling, we found no areas reminiscent of MEC in any of the sections examined.

To further characterize this tumor, we also performed FISH on paraffin sections using a dual-color break-apart rearrangement probe for *MAML2*. Rearrangements of *MAML2*, indicating a *MECT1-MAML2* fusion, were only detected in the morphologically benign tumor cells lining the duct-like structures and cysts of the tumor (Figure 1A). Split signals were not observed in the surrounding lymphoid cells, supporting the concept that these cells are non-neoplastic stromal cells.

Immunostaining of the tumor using a *MECT1-MAML2* polyclonal antibody¹⁰ revealed nuclear staining of the benign tumor cells lining the duct-like structures and cysts, in agreement with the FISH results (Figure 1B). The surrounding lymphoid cells were mainly negative. Taken together, our results show that the present tumor represents a benign WAT with a *MECT1-MAML2* gene fusion.

Available data do, however, indicate that the fusion presumably is rare in WAT.⁷ In addition to the present case, there is only one previously reported WAT with a t(11;19)(q21;p13) translocation and one case with a possible variant translocation t(11;16)(q13-14;q23).^{11,12} The fact that the *MECT1-MAML2* fusion may also infrequently occur in WAT is important to consider when using the fusion as a diagnostic marker for MEC. This is particularly important in metaplastic variants of WAT with atypia and mitotic activity, which may simulate MEC.¹³

Our findings of the *MECT1-MAML2* fusion in two unrelated benign tumor types are of particular interest in relation to recent data showing that *MECT1-MAML2* preferentially occurs in low-grade MECs and that fusion-

Figure 1. A: FISH analysis of a paraffin section of the WAT demonstrating split *MAML2* signals (separation of one green and one red signal) in the tumor cells (TC) lining the cystic spaces. In contrast, no rearrangements of *MAML2* are detected in the lymphoid cells (LC). **B:** Immunostaining of the *MECT1-MAML2* fusion protein. Note the predominant nuclear staining of the tumor cells lining the cystic spaces of the tumor. The surrounding lymphoid stroma is mainly negative.

positive patients have a significantly lower risk of local recurrence, metastasis, or tumor-related death compared with fusion-negative patients.¹⁰ When considering tumor-related deaths only, the estimated median survival for fusion-positive patients was found to be >10 years compared with 1.6 years for fusion-negative patients. Taken together, these observations suggest that the *MECT1-MAML2* fusion is preferentially associated with benign and low-grade malignant tumors of glandular origin and that the fusion may be a useful marker in predicting the biological behavior of MECs.¹⁰ Additional studies of larger series of tumors are, however, needed to further substantiate these observations.

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A Unifying Proposal for *MECT1-MAML2* Tumorigenesis

Authors' Reply:

Winnes et al have previously reported the detection of *MECT1-MAML2* expression in a case of Warthin's tumor (WAT),¹ and in the present correspondence they have restated their confidence in the histological diagnosis of this case and propose that the *MECT1-MAML2* fusion onco-

gene is not specific to mucoepidermoid-like (MEC-like) tumors. Although we cannot comment on the diagnosis of an isolated case of WAT based on a single photomicrograph, we would like to make two points to clarify what we believe to be the best interpretation of the emerging biology of MECT1-MAML2-associated tumorigenesis.

First, we would like to emphasize that malignancies with a MEC-like histological pattern have been detected as primary lesions in a wide range of anatomical locations, including 1) each of the major and minor salivary glands, 2) the upper and lower respiratory tract, and 3) rare cases involving mandible, thyroid, breast, esophagus, stomach, pancreas, skin, and other sites.²⁻⁹ Although the majority of these cases of MEC-like tumors have not yet been tested for the presence of *MECT1-MAML2* gene expression, it is quite remarkable that identical *MECT1-MAML2*-positive cases have already been reported in tumors arising from parotid, submaxillary, oral, and many different thoracic sites within the bronchopulmonary tree.¹⁰⁻¹² In addition, we and others have noted the histological similarities between salivary MEC and certain cutaneous MEC-like tumors, which can include some cases of clear cell hidradenomas or eccrine acrospiroma.^{9,13} Therefore, the detection of the identical *MECT1-MAML2* transcript in a cutaneous clear cell hidradenomas¹⁴ may suggest a common histological, genetic, or biological origin that serves to unify the concept of a MECT1-MAML2-associated tumorigenesis pathway.

Second, we would like to suggest that the otherwise histogenetically distinct entity called WAT is not associated with *MECT1-MAML2* gene expression. We base this conclusion on the absence of *MECT1-MAML2* expression in seven different WATs cases¹¹ and on the absence of *MECT1-MAML2* in 26 different WATs in a separate study by Okabe et al.¹⁵ Although it is partly semantic to argue the fine distinction between the reality of "no occurrence" versus "a very rare occurrence," we prefer the interpretation that an outlier sample of a *MECT1-MAML2*-positive non-MEC salivary gland tumor is more likely a case of misdiagnosis. This is particularly likely given the coincident presence of MEC and WAT within the same tumor samples.¹⁶

Finally, we agree that the ETV6-NTRK3 (EN) chimera represents an interesting example where the same EN fusion can result in either pediatric mesenchymal tumors or secretory breast cancer of adults and children.¹⁷ Accordingly, it is possible that MECT1-MAML2 could also be associated with histologically dissimilar tumors. Although much remains to be learned about the biology of MECT1-MAML2 tumorigenesis, the emerging data to date suggest that MECT1-MAML2 is etiologically linked with a subset of MEC-like tumors and not with WAT.

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