MECT1-MAML2 Fusion Transcript Defines a Favorable Subset of Mucoepidermoid Carcinoma

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Abstract

Purpose: Mucoepidermoid carcinoma is the most common primary malignancy of the salivary gland. *Mucoepidermoid carcinoma translocated gene 1-mastermind-like gene family* (*MECT1-MAML2*) gene fusion was identified from a recurring t(11;19)(q21;p13) translocation, which is often the sole cytogenetic alteration in this disease. This fusion transcript has been frequently detected in mucoepidermoid carcinoma and shown to be involved in the transformation of epithelial cells. However, its clinicopathologic significance remains unclear.

Experimental Design: Seventy-one cases of mucoepidermoid carcinoma and 51 cases of nonmucoepidermoid carcinoma salivary gland tumors (including 26 Warthin tumor cases) were retrospectively analyzed. RNA was extracted from archival materials: histologic paraffin specimens in all cases and cytologic specimens in 10 mucoepidermoid carcinoma cases. The *MECT1-MAML2* fusion transcript was detected by a reverse transcription-PCR assay, which can be applied to both histologic and cytologic specimens. The presence of the fusion transcript was correlated with relevant clinicopathologic and survival data of the mucoepidermoid carcinoma patients.

Results: The *MECT1-MAML2* fusion transcript was detected in 27 of the 71 (38%) mucoepidermoid carcinoma cases but not in any case of nonmucoepidermoid carcinoma tumors. The reverse transcription-PCR results showed no difference between histologic and cytologic specimens. Detection of the *MECT1-MAML2* fusion transcript was associated with a less advanced clinical stage and a low-grade tumor histology. The presence of the transcript was associated with longer disease-free and overall survivals on univariate analysis and emerged as an independent prognostic factor for longer overall survival on multivariate analysis.

Conclusions: The *MECT1-MAML2* fusion transcript may be specific to mucoepidermoid carcinoma and associated with a distinct mucoepidermoid carcinoma subset that exhibits favorable clinicopathologic features and an indolent clinical course.

Mucoepidermoid carcinoma, presenting in 5% of all salivary gland tumors and 20% of the malignant forms, is the most frequent primary malignancy of the salivary gland in both adults and children. Approximately half of these tumors occur in the major salivary glands and the other half occur in the minor salivary glands (1). Mucoepidermoid carcinoma has been associated with a recurring chromosomal translocation, t(11;19)(q21;p13), which is often the sole cytogenetic alteration (2). Recently, molecular analysis of this translocation resulted in the identification of a fusion transcript resulting from the binding of exon 1 of a novel gene of unknown function, *mucoepidermoid carcinoma translocated gene 1* (*MECT1*), at 19p13 with exons 2 to 5 of a novel member of the *mastermind-like gene family* (*MAML2*) at 11q21 (3–6).

The *MECT1* gene (also called *TORC1* and *WAMTP1*) was shown to be a coactivator of cyclic AMP/cyclic AMP–responsive element–binding protein signaling in two independent screens using large-scale cDNA array methodology (7, 8). On the other hand, the *MAML2* gene is related to the *Drosophila* gene, *mastermind*, and to the mammalian mastermind-like gene, *MAML1*, and was shown to be an essential coactivator for NOTCH receptor transcriptional activation and signaling (3, 9). Recent data suggest that ectopic expression of the *MECT1-MAML2* fusion transcript induces the activation of either genes that are known cyclic AMP/cyclic AMP–responsive element–binding protein targets or genes that contain cyclic AMP–responsive element sequences near their transcriptional start

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Note: M. Okabe and S. Miyabe contributed equally to the study and should both be regarded as first authors.

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sites and may be previously unrecognized cyclic AMP–responsive element–binding protein–regulated targets (5, 6). The *MECT1-MAML2* fusion has been shown to efficiently transform epithelial cells *in vitro* (3, 5).

The various prognostic factors that have been reported for mucoepidermoid carcinoma include histologic grade, age, gender, clinical stage, extraglandular extension, mitotic activity, MIB1 expression, and p27 expression (10–14). However, there are no prognostically useful genetic factors at present. In this retrospective study, we analyzed 71 cases of primary salivary gland mucoepidermoid carcinoma and sought to evaluate the clinicopathologic significance of the *MECT1-MAML2* fusion transcript. We developed a reverse transcription-PCR (RT-PCR) assay for this transcript, which can be applied to histologic and cytologic archival specimens.

Materials and Methods

Case selection. Seventy-three cases of mucoepidermoid carcinoma originating in the major and minor glands were retrieved from the pathology files of Nagoya City University Medical School, Aichi Cancer Center Central Hospital, and Okayama University Dental School. Those of the lung or other sites were not included in this series. All cases were carefully reviewed by two independent pathologists (H.I. and T.E.) according to the criteria of the WHO Classification for Pathology and Genetics of Head and Neck Tumors (1), and two cases with no evidence of epithelial mucin production by special stains were excluded. Finally, 71 mucoepidermoid carcinoma cases were included in this study. Formalin-fixed, paraffin-embedded histologic specimens of the resected tumors were obtained from all cases. In addition to the mucoepidermoid carcinoma cases, we also collected 26 cases of Warthin tumor, 19 cases of pleomorphic adenoma, and 6 cases of adenoid cystic carcinoma. In 10 of the 71 mucoepidermoid carcinoma cases, cytologic specimens obtained by fine-needle aspiration were also available. The study was conducted in accordance with the Declaration of Helsinki.

Clinicopathologic data. The following clinicopathologic factors were analyzed: age, sex, primary tumor site, tumor size, metastasis to regional lymph nodes, clinical stage (15), histologic grade, treatment, and follow-up. Mucoepidermoid carcinomas were histologically classified according to a three-grade system (1, 10) that has been widely used for mucoepidermoid carcinoma cases involving the major and minor salivary glands. In this system, the tumor grade is determined from the sum of the point values assigned to each of five histologic factors: cystic component, neural invasion, necrosis, mitosis, and anaplasia (Table 1).

Table 1. Variables for grading mucoepidermoid carcinoma and point values for each grade

Histopathologic features	Point value
Cystic component <20%	2
Neural invasion present	2
Necrosis present	3
Four or more mitoses per 10 high-power field	3
Anaplasia	4

Grade	Total point score
Low	0-4
Intermediate	5-6
High	≥7

RNA extraction from histologic and cytologic specimens. Total RNA was extracted from histologic and cytologic archival specimens as described previously (16). For RNA extraction from the histologic specimens, deparaffinized sections of formalin-fixed, paraffin-embedded tissue were scraped off with knife and collected in a tube and then incubated at 56°C overnight in protease K digestion buffer. RNA was extracted using concentrated phenol/guanidine isothiocyanate (Trizol, Life Technologies, Friendswood, TX) followed by DNase I treatment (Takara, Otsu, Japan). For RNA extraction from the cytologic specimens, the coverslips were removed in warm xylene, and phenol/guanidine isothiocyanate was directly spread on an area where tumor cells were present. After several pipettings on the slide surface, the extraction reagent was collected in a tube, and total RNA was extracted and treated with DNase I. The slides were dipped in ethanol, air-dried, restained, and remounted.

RT-PCR assay for the* MECT1-MAML2 *fusion transcript. One-tube RT-PCR followed by a nested PCR was done as described previously (17). Extracted RNA (5 μL) were heated to 70°C and then placed on ice. The RT-PCR mixture was then added, and the final mixture of 25 μL/tube contained 10 units RNase inhibitor (Toyobo, Osaka, Japan), 50 units reverse transcriptase (ReverTra Ace, Toyobo), 20 pmol/L of the primers, 200 μmol/L of each of four deoxynucleotides, 1.25 units TaqGOLD DNA polymerase (Applied Biosystems, Foster City CA), 1× Taq buffer containing 1.5 mmol/L $MgCl_2$, and template RNA. The thermocycler was programmed for an initial incubation of 30 minutes at 45°C and for 10 minutes at 95°C for inactivation of reverse transcriptase and activation of DNA polymerase. Then, 35 cycles of PCR at 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds were carried out. The primers used for the one-tube RT-PCR were as follows: MECT1A 5′-AAGATCGCGCTGCACAATCA-3′ and MAML2A 5′-GGTCGCTTGCTGTTGGCAGG-3′. The one-tube RT-PCR products were diluted with water to 1:50 and subjected to the nested PCR using TaqGOLD DNA polymerase and 1.5 mmol/L $MgCl_2$. The amplification conditions consisted of 35 cycles at 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, and primer sequences were as follows: MECT1B 5′-GGAGGAGACGGCGGCCTTCG-3′ and MAML2B 5′-TTGCTGTTGGCAGGAGATAG-3′. The band size of the final PCR product was 117 bp. In all positive cases, the breakpoints were confirmed by direct sequencing. The PCR fragments obtained in the nested PCR were separated and purified. They were then directly sequenced by cycle sequencing with dye-labeled terminators (BigDye Terminators, Applied Biosystems) and analyzed on a DNA sequencer (model 310, Applied Biosystems). As an internal control for RNA quality, the ubiquitously expressed β-actin mRNA fragment (190 bp) was amplified as described previously (17).

Statistical analysis. Statistical evaluation of data from two groups was done using the Fisher's exact test and Student's *t* test. All analyses were two-tailed. To identify the variables significantly associated with disease-free and overall survivals, the survival rate was calculated by the Kaplan-Meier method and the statistical difference was estimated using Cox's proportional hazard model. Multivariate proportional hazards survival analysis was done by entering the variables significant in the univariate analysis. $P < 0.05$ for each test was regarded as statistically significant. All of the analyses were done using the statistical package JMP v5 (SAS Institute, Inc., Cary, NC).

Results

Detection of the* MECT1-MAML2 *fusion transcript in histologic and cytologic specimens. We carried out a preliminary examination of the extent of RNA preservation in all cases by RT-PCR amplification of β-actin mRNA, and all specimens (both histologic and cytologic) were shown to possess RNA of satisfactory quality. Using RNA extracted from histologic specimens of the 71 cases of mucoepidermoid carcinoma, the *MECT1-MAML2* fusion transcript was detected in 27 (38%) cases (Fig. 1). All

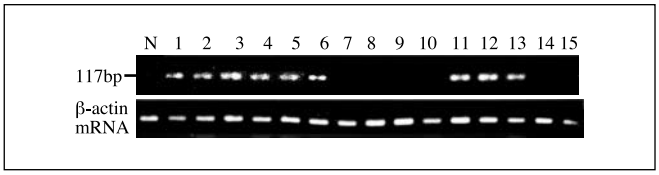

Fig. 1. Detection of the *MECT1-MAML2* fusion transcript by RT-PCR. Mucoepidermoid carcinoma cases positive (*lanes 1-6* and *11-13*) and negative (*lanes 7-10, 14*, and *15*) for the fusion transcript. Total RNA was extracted from histologic (*lanes 1-10*) and cytologic (*lanes 11-15*) specimens. *N,* normal parotid gland (negative control).

fusion transcripts were fused in-frame, and none of the positive cases showed an atypical transcript, such as an insertion or deletion, as confirmed by direct sequencing. Cytologic tumor specimens were obtained from 10 of 71 mucoepidermoid carcinoma cases; 3 were positive for the fusion transcript, whereas the remaining 7 were negative (Fig. 1). This finding was identical with that obtained using histologic specimens as a source of RNA. We also tested 51 nonmucoepidermoid carcinoma cases of Warthin tumors, pleomorphic adenomas, and adenoid cystic carcinomas, but none was positive for the *MECT1-MAML2* fusion transcript.

Clinical characteristics of the patients with mucoepidermoid carcinoma. The group of mucoepidermoid carcinoma patients consisted of 35 males and 36 females with ages ranging from 10 to 89 years (mean, 55.1 years; median, 57 years). The sites of primary tumors were the major salivary glands in 30 cases (the parotid gland in 25 cases, submandibular gland in 3, and sublingual gland in 2) and minor salivary glands in 41 (the palate in 16 cases, retromolar areas in 8, tongue in 5, and other sites in 12). Forty-three tumors were >2 cm in diameter and 14 tumors showed metastasis to the regional cervical lymph nodes. Patients with distant metastasis at diagnosis were not included in this study. Twenty-eight cases were classified as clinical stage I, 22 as stage II, 14 as stage III, and 7 as stage IV. Histologically, 31 cases had cystic component of >20%, 7 had neural invasion, 10 showed necrosis, 12 had an increased number of mitotic figures, and 20 had evidence of anaplasia. Consequently, 46 mucoepidermoid carcinoma cases were histologically classified as low grade, 10 cases as intermediate grade, and 15 cases as high grade. All patients were surgically treated with curative intent. Five patients received additional chemotherapy, 12 received additional radiation therapy, and 5 received both. The follow-up period ranged from 7 to 284 months with a median of 42 months. At the last follow-up, 42 patients were alive with no evidence of disease, 13 patients were alive with disease, 14 patients died of the disease, and 2 died of other causes.

Association of* MECT1-MAML2 *fusion with clinicopathologic characteristics of patients with mucoepidermoid carcinomas. Table 2 shows the correlation of the *MECT1-MAML2* fusion transcript with clinicopathologic factors of the mucoepidermoid carcinoma patients. Clinically, the *MECT1-MAML2* fusion-positive cases were associated with a less advanced clinical stage ($P = 0.0082$). Age, sex, tumor site, tumor size, and regional lymph node metastasis did not reveal any significant difference between fusion-positive and fusion-negative tumors. Histologically, the fusion-positive cases were associated with a lower histologic grade ($P < 0.0001$). Of the five factors constituting the histologic grade, four factors (i.e., >20% cystic component, infrequent mitotic figures, absence of necrosis, and a lesser degree of anaplasia) correlated significantly with the presence of the fusion transcript, but neural invasion failed to show any statistically significant association.

Effect of* MECT1-MAML2 *fusion on disease-free and overall survivals. The disease-free 5-year survival rate of the mucoepidermoid carcinoma patients was 58% and the overall 5-year survival rate was 73% (Table 3). None of the patients with fusion-positive mucoepidermoid carcinomas died of the tumor. To identify factors useful for predicting disease-free and overall survivals, univariate and multivariate analyses were done entering the following factors as variables: age, sex, tumor site,

Table 2. Clinicopathologic characteristics of 71 patients for *MECT1-MAML2* fusion transcript

Variables	*MECT1-MAML2* fusion transcript		*P*
	Positive (*n* = 27)	Negative (*n* = 44)	
Clinical findings			
Age (y)			
Mean	52.8	56.5	NS
>60	11	22	
≤60	16	22	
Sex			
Male	11	24	NS
Female	16	20	
Tumor site			
Major	15	15	NS
Minor	12	29	
Tumor size (cm)			
≥2	14	29	NS
<2	13	15	
Nodal status			
Positive	2	12	NS
Negative	25	32	
Clinical stage			
I, II	24	26	0.0082
III, IV	3	18	
Histologic findings			
Histologic grade			
Low	26	20	<0.0001
Intermediate	1	9	
High	0	15	
Cystic component (%)			
≥20	21	10	<0.0001
<20	6	34	
Neural invasion			
Positive	1	6	NS
Negative	26	38	
Necrosis			
Positive	0	10	0.01
Negative	27	34	
Mitotic figures (per 10 high-power field)			
≥4	1	11	0.023
<4	26	33	
Anaplasia			
Positive	1	19	0.0003
Negative	26	25	

Table 3. Prognostic factors affecting disease-free and overall survivals

	Disease-free survival (*P*)		Overall survival (*P*)	
	Univariate	Multivariate	Univariate	Multivariate
Age (y)				
>60	0.012	NS	0.044	NS
≤60				
Sex				
Male	NS		NS	
Female				
Tumor site				
Major	NS		NS	
Minor				
Tumor size (cm)				
≥2	0.0075	NS	0.0023	0.034
<2				
Nodal status				
Positive	0.0014	NS	0.014	NS
Negative				
Histologic grade				
Low + intermediate	0.0001	0.032	0.0002	NS
High				
MECT1-MAML2 fusion				
Positive	0.028	NS	0.0002	0.0049
Negative				
Clinical stage				
I, II	0.0003*		0.017†	
III, IV				

*When clinical stage and histologic grade are analyzed in the multivariate analysis, both are selected as independent factors ($P = 0.0064$ for the former and $P = 0.0027$ for the latter).

†When clinical stage and *MECT1-MAML2* fusion are studied in the multivariate analysis, the latter is selected as an independent factor ($P = 0.0007$) and the former fails to achieve statistical significance ($P = 0.093$).

tumor size, regional lymph node metastasis, histologic grade, and *MECT1-MAML2* fusion. For disease-free survival, advanced age, large tumor size, lymph node metastasis, high histologic grade, and absence of the *MECT1-MAML2* fusion (Fig. 2A) were selected as risk factors in the univariate analysis. In multivariate analysis using these significant factors, a high histologic grade remained as an independent risk factor. Because the histologic grade was determined from five different factors, correlation with each of these factors was further examined. The mitotic figures were the only factor that correlated significantly with disease-free survival ($P = 0.015$). In the univariate analysis, the clinical stage was a risk factor for disease-free survival. When the clinical stage and histologic grade were analyzed by multivariate analysis, both were selected as independent risk factors for disease-free survival ($P = 0.0064$ for the former and $P = 0.0027$ for the latter).

For overall survival, advanced age, large tumor size, lymph node metastasis, high histologic grade, and absence of the *MECT1-MAML2* fusion (Fig. 2B) emerged as significant risk factors on univariate analysis. With the multivariate analysis, large tumor size and negative *MECT1-MAML2* fusion transcript status were selected as independent factors for unfavorable overall survival. With univariate analysis, the clinical stage was selected as a risk factor. When clinical stage and *MECT1-MAML2* fusion status were analyzed by multivariate analysis, only the latter was selected as an independent prognostic factor ($P = 0.0007$) and the former did not achieve statistical significance ($P = 0.093$).

Discussion

We analyzed the clinicopathologic significance of the *MECT1-MAML2* fusion transcript in 71 cases of mucoepidermoid carcinoma of the salivary gland. For this study, we developed a detection assay consisting of a one-tube RT-PCR followed by a nested PCR. To date, there has been no report of an assay for the *MECT1-MAML2* fusion that can be applied to histologic and cytologic archival specimens, and the one described here should be useful not only for tissue-level investigations in combination with microdissection but also for retrospective and prospective large-scale studies or rare cases. Results of our RT-PCR assay using RNA of histologic specimens were identical with those obtained using RNA of cytologic specimens. Because cross-fixatives are not used for the preparation, cytologic specimens have been considered to be a much superior source of RNA compared with formalin-fixed histologic specimens (16). The perfect agreement in detection of the *MECT1-MAML2* fusion transcript between histologic and cytologic specimens indicates the high sensitivity and specificity of our RT-PCR assay. Insufficient frozen tumor tissue was

available to test whether genomic DNA alteration was present in our cases. The *MECT1-MAML2* fusion was detected in 38% (27 of 71 of mucoepidermoid carcinoma cases). This rate was lower than the 63% to 70% described previously in two small series investigations (4, 18). This discrepancy may be partly explained by case selection difference because mucoepidermoid carcinoma is marked by wide variation in histology ranging from typical low-grade cases to high-grade cases. The *MECT1-MAML2* fusion transcript is frequently detected in cases with a low-grade histology as shown in this study. Our series included 15 high-grade mucoepidermoid carcinoma cases, all of which were negative for the fusion transcript.

The most important finding of this study is that the presence of the *MECT1-MAML2* fusion transcript in mucoepidermoid carcinoma patients was associated with milder histopathologic features and a more favorable clinical outcome of mucoepidermoid carcinoma. The mucoepidermoid carcinoma cases positive for this fusion showed a uniformly low-grade histology (of 27 fusion-positive tumors, 26 were classified as low grade, 1 as intermediate grade, and 0 as high grade) and frequently had a more indolent clinical form of this carcinoma (24 of 27 cases were staged as I or II). In addition, the *MECT1-MAML2* fusion transcript was associated with longer disease-free and overall survivals and emerged as an independent factor for favorable overall survival. Although the patients with fusion-positive tumors showed 100% overall survival in this study, it should be noted that the fusion-positive tumors can be lethal because some of the patients had recurrent tumors and an advanced clinical stage. To date, some clinical prognostic factors have been reported for mucoepidermoid carcinoma (10–14). Because such factors do not address the underlying biology or pathogenesis of the tumor, search for the molecular indicators is necessary, which may be helpful in the design of specific therapies. The *MECT1-MAML2* fusion is expected to be useful in the development of novel molecular therapeutic strategies for mucoepidermoid carcinoma patients.

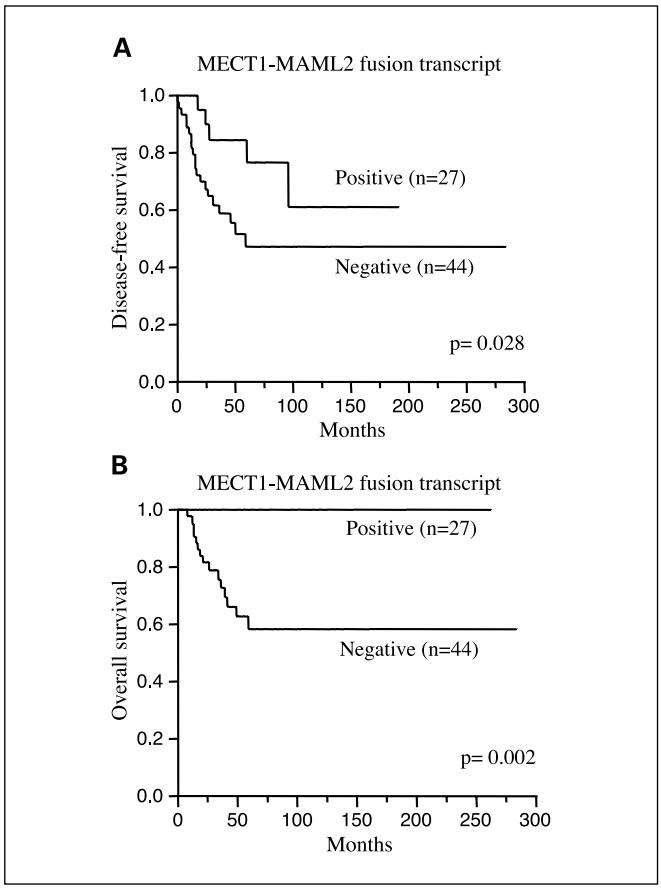

Fig. 2. Kaplan-Meier plots for disease-free and overall survivals of patients with mucoepidermoid carcinoma. *A,* disease-free survival for the *MECT1-MAML2* fusion transcript. *B,* overall survival for the *MECT1-MAML2* fusion transcript.

Another important finding is that this fusion was exclusively positive for mucoepidermoid carcinoma, whereas 51 cases of the nonmucoepidermoid carcinoma tumors were negative for the fusion. The involvement of the *MECT1-MAML2* fusion transcript in Warthin tumors has been controversial. The t(11;19)(q21;p13) and the *MECT1-MAML2* fusion have been reported in three cases (4, 19, 20). However, our group and another (18) were unable to detect the fusion transcript in 26 and 7 cases of Warthin tumor, respectively. In addition, a X chromosome–linked clonality assay has failed to show monoclonality in the epithelial component of Warthin tumors (21). We suggest that the *MECT1-MAML2* fusion has no specific association with Warthin tumors, and this should be further confirmed in large-scale studies.

Because of the risk of injury to the facial nerves and possible tumor cell dissemination, open biopsy of parotid and submandibular tumors has not been recommended, and diagnostic samples are often obtained by cytologic examination only. Fine-needle aspiration cytology has played a pivotal role in tumor diagnosis, and >70% of mucoepidermoid carcinoma cases have been accurately diagnosed (22, 23). As shown in this study, the *MECT1-MAML2* fusion and tumor size were selected in the multivariate analysis as independent prognostic factors for overall survival. Both factors could be accurately evaluated preoperatively with the RT-PCR assay using RNA from cytologic specimens and computed tomography or magnetic resonance imaging scanning, respectively, suggesting that the prognosis of mucoepidermoid carcinoma patients could be predicted accurately before surgical intervention. A precise preoperative estimation of the prognosis would be clinically useful and contribute greatly to therapeutic strategies for mucoepidermoid carcinoma patients. For example, tumor resection with good surgical margins followed by postoperative radiotherapy should be done for aggressive tumors. For indolent tumors, preservation of the facial nerve should be well considered. Radiotherapy alone might be a choice, especially when the patients' general conditions are poor (24).

Specific chromosomal rearrangements are commonly observed in hematopoietic and mesenchymal stromal tumors and define distinct clinicopathologic entities. However, <1% of all epithelial carcinomas show a recurrent, pathogenic chromosomal alteration (25). In the present study, we have shown that the *MECT1-MAML2* fusion may be specific to mucoepidermoid carcinoma and is associated with a low-grade histology and favorable clinical outcome. Similar to specific gene alterations in hematopoietic and mesenchymal stromal tumors, the *MECT1-MAML2* fusion may define a distinct clinicopathologic subset of mucoepidermoid carcinoma. Our study also suggests that mucoepidermoid carcinoma may be genetically heterogeneous. The clinicopathologic significance of this mucoepidermoid carcinoma–associated fusion should be further established by a large-scale prospective study with a long follow-up.

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