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Review Article

The Molecular Biology of MEC Salivary Gland Tumors

Elizabeth Gensterblum-Miller[1,2], Molly E. Heft Neal[1,4] and Chad Brenner J[1-4]*

[1]*Department of Otolaryngology – Head and Neck Surgery, University of Michigan, USA*
[2]*Cellular and Molecular Biology Program, University of Michigan, USA*
[3]*Department of Pharmacology, University of Michigan, USA*
[4]*Rogel Cancer Center, University of Michigan, USA*

***Corresponding author**

Chad Brenner J, Department of Otolaryngology – Head and Neck Surgery, University of Michigan, Ann Arbor, MI, USA, Tel: 734-763-2761.

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Abstract

Mucoepidermoid carcinoma (MEC) is one of the most common salivary gland cancers, comprising 30-40% of all salivary malignancies. Importantly, a common family of translocations, *CRTC(1/3)-MAML2* rearrangements, have been well established as highly recurrent genomic events driving disease progression in half of all cases. Until recently, however, the molecular basis of this disease beyond these rearrangements was largely unknown. While understanding the molecular etiology of MEC tumors is complicated by significant intra- and inter-tumor heterogeneity, several recent studies have made significant strides characterizing this disease. This review focuses on synthesizing the data from the current literature to further elucidate the role of additional somatic genomic alterations, including *TERT* translocations, chr9p21.3 and 8q24.3 copy number alterations, and NOTCH pathway mutations, that drive the molecular biology of this disease. These discoveries have been crucial to understanding MEC molecular etiology.

ABBREVIATIONS

MEC: Mucoepidermoid Carcinoma; DFS: Disease-Free Survival; CRE: Camp Response Element; HCCC: Hyalinizing Clear Cell Carcinoma; SNV: Single Nucleotide Variant; CNA: Copy Number Alteration; Acgh: Array Custom Genomic Hybridization; WES: Whole Exome Sequencing; WGS: Whole Genome Sequencing; CGP: Comprehensive Genomic Profiling; FISH: Fluorescent *In-Situ* Hybridization; CISH: Chromogenic *In Situ* Hybridization; Msigdb: Molecular Signatures Database

INTRODUCTION

Mucoepidermoid carcinoma (MEC) is one of the most common salivary malignancies, comprising 30%-40% of all salivary gland cancers, and 2-7% of all head and neck cancers [1,2]. The majority of these tumors occur in the major salivary glands, with 50-80% in the parotid gland [3,4], and survival outcomes correlate strongly with tumor stage and grade. In fact, five-year disease-free survival (DFS) of patients with low-grade, intermediate-grade, and high-grade MEC tumors is 92-100%, 90-100%, and 37-67%, respectively [5-7]. Meanwhile, DFS ranges from 91.7% for T1 stage patients to 12.5% for T4 patients [5]. MEC does not share risk factors common in other head and neck cancers, such as human papillomavirus infection or history of smoking [8-10], though some studies have suggest an association with environmental carcinogens, such as ionizing radiation [11,12].

The standard of care for non-metastatic MEC is surgery, including removal of the affected salivary gland and possible neck dissection depending on tumor grade and stage. Indications for post-operative radiotherapy are extrapolated from other head and neck malignancies, and include positive margins, extranodal extension, node positivity, high T stage, high grade, lymphovascular space invasion, and/or perineural invasion. Evidence for a role for cytotoxic chemotherapies or targeted therapies is currently limited [13]. Therefore, there is an active need for the development of novel interventional approaches for MEC patients, especially for those with high-grade tumors.

MEC INTRA-TUMOR HETEROGENEITY

Mucoepidermoid carcinoma is histologically characterized by the presence of mucinous, epidermoid, and intermediate cells [14,15]. Several studies have shown cell type-specific expression of cancer-relevant proteins in each cell type, though the roles of heterogeneous cell types in MEC etiology are currently unknown [16,18]. All three cell types are thought to differentiate from cancer stem

cells, though the specific mechanism of differentiation has not been described [19]. It has been postulated that intermediate cells are a progenitor cell, differentiating into mucinous and epidermoid cells, though evidence for this mechanism is sparse [19]. Cancer stem cells have been detected in MEC tumors and tumor-derived cell lines, marked with a $CD44^{high}ALDH^{high}$ phenotype and increased tumorigenic potential [20]. CD44 expression is associated with high-grade tumors, characterized by aggressive growth, while increased ALDH protein levels are associated with higher TNM staging (AJCC 7^{th} edition) [21,23]. These results support the hypothesis that a small proportion of cancer stem cells maintain tumor growth and survival through self-renewal and differentiation [20]. This stem cell tumorigenic activity may be regulated by mTOR signaling and epigenetic regulation [24,25]. Taken together, these data suggest that MEC growth is mediated by progenitor cells capable of differentiating into mucinous, epidermoid, and intermediate cells, leading to a tumor comprised of three histologically and molecularly different cell types.

SOMATIC ALTERATIONS AND MEC TUMOR BIOLOGY

Somatic mutation formation is a hallmark characteristic of cancer [26]. In many cancers, driver mutations induce the development of cancer phenotypes, such as growth and metastasis. Characterizing these commonly altered driver genes and pathways is key to understanding tumor etiology and identifying new therapeutic targets.

CRTC1/3-MAML2 TRANSLOCATIONS

In MEC, the most well described and recurrent genetic alteration is a translocation between chromosome 11 and chromosome 19, resulting in formation of the CRTC1-MAML2 fusion protein [27,28]. The CRTC1-MAML2 fusion is found in 38-88% of all MEC tumors depending on the cohort [29,35]. Fusion-positive tumors are associated with a lower tumor grade and improved overall survival [30,35]. The CRTC1-MAML2 fusion contains the first exon of *CRTC1*, containing a CREB binding domain, and exons 2-5 of *MAML2*, containing a transcription activation domain [28,36]. While wild-type CRTC1 and MAML2 are both transcription factors, the CRTC1-MAML2 fusion protein gains transcription regulation function unique from both constituents. Initial studies demonstrated that transduction with CRTC1-MAML2, but not CRTC1 or MAML2 alone, is sufficient to induce neoplastic transformation in an immortalized cell line [28,37]. This transformation is thought to be due to transcriptional regulation of several cancer-associated pathways.

Functionally, CRTC1-MAML2 directly activates CREB via the CRTC1 CREB binding domain leading to constitutive activation of CREB-dependent gene regulation [28,38]. Unlike CRTC1, CRTC1-MAML2 is localized to the nucleus, where the fusion constitutively activates CREB. CREB activation has been associated with several different tumor types, and plays a role in uncontrolled cellular division, suppression of apoptosis, and metastatic potential [39]. In addition, CRTC1-MAML2-mediated malignant transformation is decreased by CREB inhibition, suggesting that CRTC1-MAML2 plays a driver role in MEC tumorigenesis [40].

CRTC1-MAML2 also regulates transcription via interactions with other transcription factors beyond CREB. For example, Amelio *et al*., first discovered activation of MYC regulated genes by CRTC1-MAML2, but not CRTC1 or MAML2 [41]. CRTC1-MAML2 directly binds with MYC, activating MYC-regulated genes. Importantly, they also demonstrate that the previously described CRTC1-MAML2-mediated transformed colony formation is abrogated by transduction with MYC with a loss-of-function mutation.

Chen J *et al.,* is the first study to measure differential gene expression in fusion-positive MEC-derived cell lines [36]. By comparing RNA expression of fusion-positive to fusion-knockdown MEC-derived cell lines, the team identifies a CRTC1-MAML2-associated gene signature. The genes inhibited by *CRTC1-MAML2* knockdown include genes that are activated by previously associated transcription factors, such as CREB and MYC, but also previously unassociated transcription factors, such as NFkB and HIF1A. These genes are also involved in regulating cancer phenotypes; this gene signature is enriched in pathways such as cell growth, cell survival, and cell-to-cell signaling. *C6orf176*, encoding LINC00473, is the gene most highly repressed by *CRTC1-MAML2* knockdown. In a subsequent study, the group determines that LINC00473 is necessary for CRTC1-MAML2-mediated growth [42]. CRTC1-MAML2 directly induces LINC00473 expression in a CREB-dependent manner. In addition, LINC00473 knockdown leads to reduced growth and increased apoptosis in both a fusion-positive cell line and xenograft, as well as downregulation of genes associated with cell survival and growth. LINC00473 directly interacts with the NONO protein, a coactivator necessary for CREB activation in non-cancerous cells. Therefore, the authors propose that LINC00473 enhances CRTC1-MAML2-mediated CREB activation by interacting with the cAMP response element (CRE) site in a feed-forward loop.

By performing RNA sequencing on MEC fusion-positive and fusion negative tumors, Musicant *et al.,* reveal a fusion-

specific gene expression signature, which is enriched for IGF-1 signaling-associated genes [43]. While the *IGF1* promoter lacks a CRE site, the authors demonstrate that CRTC1-MAML2 instead induces expression of the splice variant PGC-1a4, which coactivates PPARγ, which directly induces IGF-1 expression.

Through the transcriptional regulation of multiple pathways, CRTC2-MAML2 is thought to be the primary driver alteration in fusion-positive MEC tumors, as demonstrated by Chen *et al* [40]. The authors report that *CRTC1-MAML2* knockdown is sufficient to fully stop MEC xenograft growth. Furthermore, they develop a salivary gland *CRTC1-MAML2* transgenic mouse model by crossing mice with a Cre-*CRTC1-MAML2* transgene with a MMTV-Cre transgenic line, which selectively expresses Cre in salivary ductal cells. Induction of salivary gland-specific CRTC1-MAML2 fusion expression is sufficient to generate murine MEC tumors, which are phenotypically similar to human MEC tumors, containing mucinous, epidermoid, and intermediate cells. They then treat CRTC1-MAML2-expressing mice with CDK4/6 inhibitor and EGFR inhibitor combination therapy, which leads to decreased tumor size. This suggests a strong pre-clinical rationale for prospective evaluation of similar combinational therapies in human subjects.

Taken together, these data demonstrate that CRTC1-MAML2 is sufficient to cause tumorigenesis and neoplastic transformation. This fusion directly regulates large-scale changes in gene transcription within the MEC tumor, through coactivating with the transcription factors CREB, MYC, and PPARγ.

OTHER SOMATIC ALTERATIONS IN MEC TUMORS

In addition to *MAML2* translocations, other somatic alterations contribute to cancer etiology, in both *MAML2* translocation-positive and *MAML2* translocation-negative tumors. A summary of the genes affected by these alterations can be found in **Table 1**. A variety of high-throughput methods have been used to successfully detect structural variations, copy number alterations (CNAs) and single nucleotide variations (SNVs) throughout the MEC tumor genome.

STRUCTURAL VARIATIONS

Structural variants, including translocations forming the CRTC1-MAML2 fusion, play an important role in MEC tumor etiology. While the t(11;19) translocation, leading to the *CRTC1-MAML2* fusion gene, is the best studied driver alteration in MEC, other somatic alterations have also been identified, which may either contribute to cancer phenotypes in CRTC1-MAML2 fusion-positive MEC tumors or contribute to pathogenesis in fusion-negative tumors. In addition to *CRTC1-MAML2, CRTC3-MAML2* fusions, though less common, have also been detected [44,46]. CRTC1-MAML2 and CRTC3-MAML2 positive MEC tumors are histologically similar with similar clinicopathologic features, but the fusions are mutually exclusive [44,46].

Recent increased adoption of genome wide techniques, including whole genome sequencing (WGS) and long read sequencing, are powerful tools to identify novel structural variants driving MEC tumorigenesis. For example, one such study uses WGS to sequence 18 primary MEC tumors, as part of a common salivary gland cancer cohort [47]. Unlike other previously used methods, such as WES and capture sequencing, WGS allows for detection of intronic and intergenic variants [47]. First, the authors identified missense SNVs in *BAP1, LRFN1, CIC, MUC16, ATN1, CARD154, CCDC58*, and *TTN*. In addition to these findings, the authors apply a variety of bioinformatics tools to understand more genetic information from these tumors. For example, they use a mutational signature analysis to determine the relative contribution of thirty well-defined patterns of single nucleotide changes [47]. Several of these signatures are associated with known mutational processes, suggesting potential mutagenic mechanisms for MEC tumor SNV formation. Specifically, MEC tumors have broad enrichment for multiple signatures associated with DNA repair pathways: most notably signatures 3 (homologous recombination), 6, 15, 20 (mismatch repair), and 24 (nucleotide excision repair) [47]. In addition, the CRTC1-MAML2 fusion could be detected in 50% of patients, using bioinformatics-based analysis of WGS data, rather than the orthogonal and time-consuming methods of previous studies, such as fluorescence *in-situ* hybridization (FISH). This method is then used to identify additional fusions, including HFM1-RYR2, CACNA1B-NBPF10, EWSR1-ATF1, and FSIP1-BAZ2A. However, these fusions were not verified by secondary methods, such as rtPCR, and the way they affect cancer phenotypes is not currently known. Interestingly, the EWSR1-ATF1 is a hallmark fusion of hyalinizing clear cell carcinoma, a histologically similar salivary gland cancer [48]. Nevertheless, the identification of these fusions represents an exciting new frontier in MEC genetic research.

COPY NUMBER ALTERATIONS

Single nucleotide variants and copy number alterations (SNVs and CNAs) in MEC have been previously identified using microarray techniques. For example, using a genotyping microarray covering 261,563 single nucleotide polymorphisms (SNPs), one study uses the relative signal

Table 1: Summary of genes that are recurrently altered in MEC tumors. Genes are sorted based on their association with featured pathways or biological processes. Gene set membership is curated by the Molecular Signatures Database (MSigDB)

Gene Set	Genes with CNAs	Genes with SNVs	Gene fusions
GO BP apoptotic process	*LYN, SMAD4, CDKN2A, APEX1, DCC*	*PTEN, EGFR, NF1, HRAS, CCND1, TP53, ATM, PIK3CA, KRAS, BRAF, MYC, NOTCH1, TERT, BRCA1, BRCA2, ERBB2, FGFR1, RB1, CDKN2A, MAP3K9, NOTCH2, PALB2, TSC2, ATN1, BAP1*	*RYR2, TERT, SGK3, PPP2R1B*
GO BP positive regulation of cell population proliferation	*LYN, SMAD4, MAPT, MOS*	*PTEN, EGFR, NF1, HRAS, TP53, ATM, PIK3CA, KRAS, BRAF, MYC, NOTCH1, BRCA1, BRCA2, ERBB2, FGFR1, NOTCH2, TSC2, KIT, IRAK1, OBSCN, CTBP2*	*TERT*
GO BP regulation of intracellular signal transduction	*LYN, SMAD4, MAPT, PLAG1*	*PTEN, EGFR, NF1, HRAS, CCND1, PIK3CA, KRAS, BRAF, MYC, NOTCH1, TERT, ERBB2, FGFR1, NOTCH2, KIT, IRAK1, CDK4*	*SGK3*
Hallmark PI3K AKT mTOR signaling		*HRAS, CDK4, PTEN, EGFR, TSC2*	*ATF1, PPP2R1B*
Hallmark p53 pathway	*CDKN2A*	*HRAS, NOTCH1, CDKN2A, TP53, RB1, CDKN2B, IRAK1*	
Hallmark G2M checkpoint		*CDK4, NOTCH2, CCND1, MYC, BRCA2*	*EWSR1*
KEGG pathways in cancer	*CDKN2A, SMAD4*	*TP53, CCND1, CDK4, RB1, KRAS, HRAS, EGFR, ERBB2, PIK3CA, PTEN, FGFR1, CDKN2A, BRAF, BRCA2, CDKN2B, MYC, KIT, DCC, MET, CTBP2*	
Altered genes not involved in featured pathways	*GALR1, MAFA, OR4N4, POTEB, TP53TG3, MAML2*	*TTN, KMT2C, ARID1A, MTAP, CIC, ITGAL, MUC16, OTOGL, CCDC58, LRFN1, MUC12, MUC3A, MUC4, MUC5B, PCLO, PRB1, PRB4,PRSS3, TTC28, WDR33*	*CRTC1, BAZ2A, CRTC3, MAML2, CACNA1B, HFM1, NBPF10, FSIP1, MYBL1*

Abbreviations: CNA: copy number alteration. SNV: single nucleotide variant. G2M: Growth 2 phase to mitosis checkpoint. GO BP: Gene Ontology: Biological Process. KEGG: Kyoto Encyclopedia of Genes and Genomes. MSigDB: Molecular Signatures Database

of these SNPs to identify copy number variants [49]. The most reproducible CNA mutations overlap with *MAPT* (50% patients with copy number gain/40% copy number loss), *APEX1* (35% gain/45% loss) *POTEB* (45%/25%), *OR4N4* (40%/25%), and *TP53TG3* (5%/25%). Interestingly, CRTC1-MAML2 positive tumors have a lower rate of copy number alterations, compared to fusion-negative tumors [49,51]. Another study uses an array custom genomic hybridization (aCGH), a microarray that can detect copy number imbalances in 99,000 human sequences [52]. They detect a chr9p21.3 (*CDKN2A*) deletion in 5/15 patients. Four patients have both a CRTC1-MAML2 translocation and a chr9p21.3 deletion, and notably, all four are confirmed to have died from metastatic MEC [52]. This *CDKN2A* loss was reproduced using a similar aCGH array-based assay to measure copy number alterations relative to noncancerous patient-matched control tissue in 28 MEC tumors, 7 of which were found to contain a 9p21.3 loss [50]. This aCGH assay was also used to identify copy number losses in 18q12.2-qter (*DCC, SMAD4, GALR1*) and copy number gains in 8q24.3 (*MAFA*) and 8q11.1-q12.2 (*LYN, MOS, PLAG1*) [50]. One recent study has shown that loss of p16 protein, which is produced from *CDKN2A,* is associated with increased tumor size, CRTC1-MAML2 negativity, and a worse prognosis, further suggesting that the effects of *CDKN2A* mutations on cancer phenotypes is a promising area for future research [53].

SINGLE NUCLEOTIDE VARIANTS

The first WES study of MEC tumors, including 18 tumors plus matched normal controls, identifies 43 ± 63.5 mutations per tumor [29]. Specifically, these alterations include somatic SNVs in *TP53, IRAK1, MAP3K9, ITGAL, ERGG4, OTOGL, KMT2C* and *OBSCN* (≥2/18 tumors for all genes listed). This study also bioinformatically analyzes CNAs in these 18 tumors, 32/33 of which were tumor specific. A second study performed WES on 26 MEC tumors, identifying recurrent SNVs in *BAP1* (15%), *CDKN2A* (8%), *MET* (8%), and *TP53* (8%) [54]. Coupling these data with RNAseq in 27 cases, they successfully identify gene fusions. These fusions include 11 samples with CRTC1-MAML2 fusions and 16 tumor-specific fusions. 2 samples contain EWSR1 fusions (EWSR1-ATF1, and EWSR1-CREM, respectively), which are hallmark fusions of salivary gland cancers, including hyalinizing clear cell carcinoma [48]. They then analyze differential gene expression between 7 recurrent/metastatic and 18 progression-free MEC tumors, finding 193 genes, including 20 transcription factors, differentially expressed in patients with disease progression. These genes are enriched for processes, including epithelial mesenchymal transition, TNFA signaling via NFkB, and Myc targets.

Our group recently performed WES of 11 MEC tumors, identifying recurrent SNVs in several genes, including *MUC12*, *MUC3A*, *MUC4*, *MUC5B*, and *TERT* [55]. Interestingly, however, recurrent *NOTCH2* alterations were found in 6/11 tumors. These alterations include one missense mutation predicted to be function-altering, one 5'-UTR deletion, two frameshift alterations, and two single copy deletions. Moreover, these alterations are found in both CRTC1-MAML2 positive and negative tumors. This suggests a CRTC1-MAML2-independent role for NOTCH pathway in MEC tumor formation.

FINDINGS FROM TARGETED GENETIC METHODS

Several targeted methods have been used to attempt to discover driving genomic alterations in MEC tumors.

For example, comprehensive genomic profiling (CGP) is a capture sequencing-based technology and is an FDA-approved test for identifying cancer driver mutations [56]. In one study, CGP covering 315 cancer-related genes is used to detect SNVs, but not copy number alterations or translocation events, in a pan-salivary gland cancer cohort, including 57 MEC tumors [57]. The authors detected a tumor mutational burden of 4 mutations per tumor, most commonly affecting *CDKN2A*, *TP53*, *CDKN2B*, *BAP1*, *PIK3CA*, *ERBB2*, *BRCA2*, *HRAS*, and *FGFR1* [57,58]. Other targeted sequencing on pan-salivary gland cancer cohorts have been published, though they include too few MEC tumors (N = 5, 8 respectively) to identify genes that are mutated in more than one patient, except for *TP53* [59,60].

Other genetic research has directly genotyped genes with well-studied effects on cancer phenotypes. For example, using Sanger sequencing and chromogenic *in-situ* hybridization (CISH), Nakano *et al.,* detect *MAML2* fusions (15/31 tumors), *HER2* amplification (4/31 tumors), and *EGFR* copy number increase (4/31 tumors) in MEC tumors [61]. HER2 protein overexpression was highly correlated with gene amplification; however, EGFR protein overexpression occurs in the epidermoid and intermediate cells of 18 of 31 tumors, often unrelated to copy number status. Another strategy used a custom 123-SNV microarray panel targeting 19 commonly mutated genes to detect a *KRAS* A59T mutation in 1 of 30 tumors, but no other variants [62].

These low-throughput techniques have the potential to successfully identify mutations in genes with available targeted therapies, with the end goal of identifying potential therapeutic targets. Multiple clinical trials have tested the efficacy of therapeutics that inhibit HER2 or EGFR in general salivary gland cancer cohorts, though these studies are limited due to low MEC patient enrollment (reviewed by Sama *et al* [13]). Therefore, these genotyping studies inform future experiments to identify treatments for MEC patients.

In summary, genome-wide and targeted studies have identified many SNV, copy number, and translocation mutations in MEC tumors. While some of these mutations, such as CRTC1-MAML2, CRTC3-MAML2, and *TP53* SNVs, are recurrent to many tumors, several tumor-specific variants have also been described. Further study is required to determine the recurrence rate of less commonly mutated genes, and to identify relationships between alterations and tumor progression.

SEQUENCING ADVANCEMENT AND NOVEL STRUCTURAL REARRANGEMENTS

Given the low rate of both recurrent somatic mutations and copy number variant loci in MEC, we have recently hypothesized that, similar to the CRTC1-MAML2 translocation, additional highly recurrent genomic rearrangements served as common genetic drivers of this disease. Critically, long read sequencing technologies provide a method to more easily characterize translocation sites by genome-wide direct sequencing. To that end, a recent study by our group utilizes two sequencing methods to identify MEC tumor structural variants [55]. These include long read sequencing, which facilitates genome-wide profiling of structural variation. Long read sequencing on 4 MEC-derived cell lines identifies recurrent *TERT* promoter translocations, as well as the *CRTC1-MAML2* fusion. We then identify *TERT* translocations in 2 additional cohorts. *TERT* alterations are detected in 36/39 primary MEC tumors (*TERT* break apart FISH) and translocation breakpoints could be directly sequenced in 13/36 MEC tumors (custom targeted sequencing). Moreover, in a cell line containing a *TERT* translocation, *TERT* knockdown by shRNA is sufficient to impair clonogenic cell survival, demonstrating how structural variant identification is critical to the molecular biology of MEC.

Our study highlights the importance of using novel techniques to probe MEC genomes. Indeed, as genomic and bioinformatics techniques, including advances in long-read sequencing, improve, this will facilitate more accurate and direct detection of structural variants, including gene fusions [63,64]. With additional repetition and validation, direct tumor sequencing coupled with bioinformatics analysis strategies has the potential to identify novel driver mutations.

CONCLUSIONS

Mucoepidermoid carcinoma is a rare disease with extremely variable prognoses. In MEC, the best studied driver alteration is the CRTC1-MAML2 gene fusion, though several other tumorigenic genetic changes have recently been described, including the highly recurrent *TERT* promoter rearrangements discovered by our group. These exciting new discoveries open future research into understanding how these newly described and recurrent somatic alterations affect tumor etiology, especially in CRTC1-MAML2 negative patients and patients with deadly outcomes. This improved understanding of MEC biology will likely be key to identifying companion targeted therapies. Indeed, these new discoveries in the MEC field open exciting new avenues for research, so of which may have the long-term potential to positively impact outcomes for patients with aggressive forms of the disease.

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REFERENCES

1. McHugh JB, Visscher DW, Barnes EL. Update on selected salivary gland neoplasms. Arch Pathol Lab Med. 2009; 133: 1763-1774.
2. Spiro RH. Salivary neoplasms: overview of a 35-year experience with 2,807 patients. Head Neck Surg. 1986; 8: 177-184.
3. Peraza A, Gomez R, Beltran J, Amarista FJ. Mucoepidermoid carcinoma. An update and review of the literature. J Stomatol Oral Maxillofac Surg. 2020; 121: 713-720.
4. Goode RK, Auclair PL, Ellis GL. Mucoepidermoid carcinoma of the major salivary glands: clinical and histopathologic analysis of 234 cases with evaluation of grading criteria. Cancer. 1998; 82: 1217-1224.
5. McHugh CH, Roberts DB, El-Naggar AK, Hanna EY, Garden AS, Kies MS, et al. Prognostic factors in mucoepidermoid carcinoma of the salivary glands. Cancer. 2012; 118: 3928-3936.
6. Chen MM, Roman SA, Sosa JA, Judson BL. Histologic grade as prognostic indicator for mucoepidermoid carcinoma: a population-level analysis of 2400 patients. Head Neck. 2014; 36: 158-163.
7. Ali S, Sarhan M, Palmer FL, Whitcher M, Shah JP, Patel SG, et al. Cause-specific mortality in patients with mucoepidermoid carcinoma of the major salivary glands. Ann Surg Oncol. 2013; 20: 2396-2404.
8. Haeggblom L, Ursu RG, Mirzaie L, Attoff T, Gahm C, Nordenvall LH, et al. No evidence for human papillomavirus having a causal role in salivary gland tumors. Diagn Pathol. 2018; 13: 44.
9. Sawabe M, Ito H, Takahara T, Oze I, Kawakita D, Yatabe Y, et al. Heterogeneous impact of smoking on major salivary gland cancer according to histopathological subtype: A case-control study. Cancer. 2018; 124: 118-124.
10. Gu W, Bhangale A, Heft Neal ME, Smith JD, Brummel C, McHugh JB, et al. Analysis of Human Papilloma Virus Content and Integration in Mucoepidermoid Carcinoma. Viruses. 2022;14: 2353.
11. Saku T, Hayashi Y, Takahara O, Matsuura H, Tokunaga M, Tokunaga M, et al. Salivary gland tumors among atomic bomb survivors, 1950-1987. Cancer. 1997; 79: 1465-1475.
12. Leopard D, El-Hitti E, Puttasiddaiah P, McLeod R, Owens D. Twenty-seven years of primary salivary gland carcinoma in Wales: an analysis of histological subtype and associated risk factors. J Laryngol Otol. 2022; 136: 167-172.
13. Sama S, Komiya T, Guddati AK. Advances in the Treatment of Mucoepidermoid Carcinoma. World J Oncol. 2022; 13: 1-7.
14. Stewart FW, Foote FW, Becker WF. Muco-Epidermoid Tumors of Salivary Glands. Ann Surg. 1945; 122: 820-844.
15. Coca-Pelaz A, Rodrigo JP, Triantafyllou A, Hunt JL, Rinaldo A, Strojan P, et al. Salivary mucoepidermoid carcinoma revisited. Eur Arch Otorhinolaryngol. 2015; 272: 799-819.
16. Robinson L, van Heerden MB, Ker-Fox JG, Hunter KD, van Heerden WFP. Expression of Mucins in Salivary Gland Mucoepidermoid Carcinoma. Head Neck Pathol. 2021; 15: 491-502.
17. Alos L, Lujan B, Castillo M, Nadal A, Carreras M, Caballero M, et al. Expression of membrane-bound mucins (MUC1 and MUC4) and secreted mucins (MUC2, MUC5AC, MUC5B, MUC6 and MUC7) in mucoepidermoid carcinomas of salivary glands. Am J Surg Pathol. 2005; 29: 806-813.
18. Arruda CFJ, Coutinho-Camillo CM, Marques MM, Nagano CP, Bologna SB, Bettim BB, et al. Claudin expression is maintained in mucoepidermoid carcinoma of the salivary gland. Pathol Res Pract. 2020; 216: 153161.
19. Adams A, Warner K, Nor JE. Salivary gland cancer stem cells. Oral Oncol. 2013; 49: 845-853.
20. Adams A, Warner K, Pearson AT, Zhang Z, Kim HS, Mochizuki D, et al. ALDH/CD44 identifies uniquely tumorigenic cancer stem cells in salivary gland mucoepidermoid carcinomas. Oncotarget. 2015; 6: 26633-26650.
21. Binmadi N, Elsissi A, Elsissi N. Expression of cell adhesion molecule CD44 in mucoepidermoid carcinoma and its association with the tumor behavior. Head Face Med. 2016; 12: 8.
22. Irani S, Jafari B. Expression of vimentin and CD44 in mucoepidermoid carcinoma: A role in tumor growth. Indian J Dent Res. 2018; 29: 333-340.
23. da Silva LP, Lopes M, Sarmento ASC, de Albuquerque Borges M, de Moura SRS, Sobral APV, et al. Increased expression of ALDH-1 is associated with clinical parameters of salivary glands neoplasms. Exp Mol Pathol. 2020; 117: 104552.
24. Andrade NP, Warner KA, Zhang Z, Pearson AT, Mantesso A, Guimaraes DM, et al. Survival of salivary gland cancer stem cells requires mTOR signaling. Cell Death Dis. 2021; 12: 108.
25. Guimaraes DM, Almeida LO, Martins MD, Warner KA, Silva AR, Vargas PA, et al. Sensitizing mucoepidermoid carcinomas to chemotherapy by targeted disruption of cancer stem cells. Oncotarget. 2016;7: 42447-42460.
26. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011; 144: 646-674.
27. Dahlenfors R, Lindahl L, Mark J. A fourth minor salivary gland mucoepidermoid carcinoma with 11q14-21 and 19p12 rearrangements. Hereditas. 1994; 120: 287-288.
28. Wu L, Liu J, Gao P, Nakamura M, Cao Y, Shen H, et al. Transforming activity of MECT1-MAML2 fusion oncoprotein is mediated by constitutive CREB activation. EMBO J. 2005; 24: 2391-2402.
29. Kang H, Tan M, Bishop JA, Jones S, Sausen M, Ha PK, et al. Whole-Exome Sequencing of Salivary Gland Mucoepidermoid Carcinoma. Clin Cancer Res. 2017; 23: 283-288.
30. Saade RE, Bell D, Garcia J, Roberts D, Weber R. Role of CRTC1/MAML2 Translocation in the Prognosis and Clinical Outcomes of Mucoepidermoid Carcinoma. JAMA Otolaryngol Head Neck Surg. 2016; 142: 234-240.
31. Luk PP, Wykes J, Selinger CI, Ekmejian R, Tay J, Gao K, et al. Diagnostic and prognostic utility of Mastermind-like 2 (MAML2) gene rearrangement detection by fluorescent in situ hybridization (FISH) in mucoepidermoid carcinoma of the salivary glands. Oral Surg Oral Med Oral Pathol Oral Radiol. 2016; 121: 530-541.
32. Seethala RR, Dacic S, Cieply K, Kelly LM, Nikiforova MN. A reappraisal of the MECT1/MAML2 translocation in salivary mucoepidermoid carcinomas. Am J Surg Pathol. 2010; 34: 1106-1121.
33. Behboudi A, Enlund F, Winnes M, Andren Y, Nordkvist A, Leivo I, et al. Molecular classification of mucoepidermoid carcinomas-prognostic significance of the MECT1-MAML2 fusion oncogene. Genes Chromosomes Cancer. 2006; 45: 470-481.
34. Okabe M, Miyabe S, Nagatsuka H, Terada A, Hanai N, Yokoi M, et al. MECT1-MAML2 fusion transcript defines a favorable subset of mucoepidermoid carcinoma. Clin Cancer Res. 2006; 12: 3902-3907.
35. Okumura Y, Miyabe S, Nakayama T, Fujiyoshi Y, Hattori H, Shimozato K,

et al. Impact of CRTC1/3-MAML2 fusions on histological classification and prognosis of mucoepidermoid carcinoma. Histopathology. 2011; 59: 90-97.

36. Chen J, Li JL, Chen Z, Griffin JD, Wu L. Gene expression profiling analysis of CRTC1-MAML2 fusion oncogene-induced transcriptional program in human mucoepidermoid carcinoma cells. BMC Cancer. 2015; 15: 803.

37. Tonon G, Modi S, Wu L, Kubo A, Coxon AB, Komiya T, et al. t(11;19)(q21;p13) translocation in mucoepidermoid carcinoma creates a novel fusion product that disrupts a Notch signaling pathway. Nat Genet. 2003; 33: 208-213.

38. Coxon A, Rozenblum E, Park YS, Joshi N, Tsurutani J, Dennis PA, et al. Mect1-Maml2 fusion oncogene linked to the aberrant activation of cyclic AMP/CREB regulated genes. Cancer Res. 2005; 65: 7137-7144.

39. Sapio L, Salzillo A, Ragone A, Illiano M, Spina A, Naviglio S. Targeting CREB in Cancer Therapy: A Key Candidate or One of Many? An Update. Cancers (Basel). 2020; 12: 3166.

40. Chen Z, Ni W, Li JL, Lin S, Zhou X, Sun Y, et al. The CRTC1-MAML2 fusion is the major oncogenic driver in mucoepidermoid carcinoma. JCI Insight. 2021; 6: e139497.

41. Amelio AL, Fallahi M, Schaub FX, Zhang M, Lawani MB, Alperstein AS, et al. CRTC1/MAML2 gain-of-function interactions with MYC create a gene signature predictive of cancers with CREB-MYC involvement. Proc Natl Acad Sci U S A. 2014; 111: E3260-3268.

42. Chen Z, Lin S, Li JL, Ni W, Guo R, Lu J, et al. CRTC1-MAML2 fusion-induced lncRNA LINC00473 expression maintains the growth and survival of human mucoepidermoid carcinoma cells. Oncogene. 2018; 37: 1885-1895.

43. Musicant AM, Parag-Sharma K, Gong W, Sengupta M, Chatterjee A, Henry EC, et al. CRTC1/MAML2 directs a PGC-1alpha-IGF-1 circuit that confers vulnerability to PPARgamma inhibition. Cell Rep. 2021; 34: 108768.

44. Fehr A, Roser K, Heidorn K, Hallas C, Loning T, Bullerdiek J. A new type of MAML2 fusion in mucoepidermoid carcinoma. Genes Chromosomes Cancer. 2008; 47: 203-206.

45. Birkeland AC, Foltin SK, Michmerhuizen NL, Hoesli RC, Rosko AJ, Byrd S, et al. Correlation of Crtc1/3-Maml2 fusion status, grade and survival in mucoepidermoid carcinoma. Oral Oncol. 2017; 68: 5-8.

46. Nakayama T, Miyabe S, Okabe M, Sakuma H, Ijichi K, Hasegawa Y, et al. Clinicopathological significance of the CRTC3-MAML2 fusion transcript in mucoepidermoid carcinoma. Mod Pathol. 2009; 22: 1575-1581.

47. Karpinets TV, Mitani Y, Liu B, Zhang J, Pytynia KB, Sellen LD, et al. Whole-Genome Sequencing of Common Salivary Gland Carcinomas: Subtype-Restricted and Shared Genetic Alterations. Clin Cancer Res. 2021; 27: 3960-3969.

48. Heft Neal ME, Gensterblum-Miller E, Bhangale AD, Kulkarni A, Zhai J, Smith J, et al. Integrative sequencing discovers an ATF1-motif enriched molecular signature that differentiates hyalinizing clear cell carcinoma from mucoepidemoid carcinoma. Oral Oncol. 2021; 117: 105270.

49. Zhang L, Mitani Y, Caulin C, Rao PH, Kies MS, Saintigny P, et al. Detailed genome-wide SNP analysis of major salivary carcinomas localizes subtype-specific chromosome sites and oncogenes of potential clinical significance. Am J Pathol. 2013; 182: 2048-2057.

50. Jee KJ, Persson M, Heikinheimo K, Passador-Santos F, Aro K, Knuutila S, et al. Genomic profiles and CRTC1-MAML2 fusion distinguish different subtypes of mucoepidermoid carcinoma. Mod Pathol. 2013; 26: 213-222.

51. Matse JH, Veerman ECI, Bolscher JGM, Leemans CR, Ylstra B, Bloemena E. High number of chromosomal copy number aberrations inversely relates to t(11;19)(q21;p13) translocation status in mucoepidermoid carcinoma of the salivary glands. Oncotarget. 2017; 8: 69456-69464.

52. Anzick SL, Chen WD, Park Y, Meltzer P, Bell D, El-Naggar AK, et al. Unfavorable prognosis of CRTC1-MAML2 positive mucoepidermoid tumors with CDKN2A deletions. Genes Chromosomes Cancer. 2010; 49: 59-69.

53. Kim Y, Song JS, Choi SH, Nam SY, Kim SY, Cho KJ. P16 expression and presence of lymphoid stroma are correlated with good prognosis in mucoepidermoid carcinoma of the head and neck. Pathol Res Pract. 2023; 251: 154874.

54. Wang X, Bai J, Yan J, Li B. The clinical outcome, pathologic spectrum, and genomic landscape for 454 cases of salivary mucoepidermoid carcinoma. NPJ Precis Oncol. 2024; 8: 238.

55. Gensterblum-Miller E, Bhangale A, Majid DA, Pienkowski VM, Rydzanicz M, Janiszewska J, et al. Long read sequencing identifies complex structural variant landscape and recurrent TERT rearrangements in mucoepidermoid carcinoma. Oral Oncol. 2024; 159: 107108.

56. Suh JH, Johnson A, Albacker L, Wang K, Chmielecki J, Frampton G, et al. Comprehensive Genomic Profiling Facilitates Implementation of the National Comprehensive Cancer Network Guidelines for Lung Cancer Biomarker Testing and Identifies Patients Who May Benefit From Enrollment in Mechanism-Driven Clinical Trials. Oncologist. 2016; 21: 684-691.

57. Ross JS, Gay LM, Wang K, Vergilio JA, Suh J, Ramkissoon S, et al. Comprehensive genomic profiles of metastatic and relapsed salivary gland carcinomas are associated with tumor type and reveal new routes to targeted therapies. Ann Oncol. 2017; 28: 2539-2546.

58. Wang K, McDermott JD, Schrock AB, Elvin JA, Gay L, Karam SD, et al. Comprehensive genomic profiling of salivary mucoepidermoid carcinomas reveals frequent BAP1, PIK3CA, and other actionable genomic alterations. Ann Oncol. 2017; 28: 748-753.

59. Kato S, Elkin SK, Schwaederle M, Tomson BN, Helsten T, Carter JL, et al. Genomic landscape of salivary gland tumors. Oncotarget. 2015;6: 25631-25645.

60. Grunewald I, Vollbrecht C, Meinrath J, Meyer MF, Heukamp LC, Drebber U, et al. Targeted next generation sequencing of parotid gland cancer uncovers genetic heterogeneity. Oncotarget. 2015; 6: 18224-18237.

61. Nakano T, Yamamoto H, Hashimoto K, Tamiya S, Shiratsuchi H, Nakashima T, et al. HER2 and EGFR gene copy number alterations are predominant in high-grade salivary mucoepidermoid carcinoma irrespective of MAML2 fusion status. Histopathology. 2013; 63: 378-392.

62. Cros J, Sbidian E, Hans S, Roussel H, Scotte F, Tartour E, et al. Expression and mutational status of treatment-relevant targets and key oncogenes in 123 malignant salivary gland tumours. Ann Oncol. 2013; 24: 2624-2629.

63. Pendleton M, Sebra R, Pang AW, Ummat A, Franzen O, Rausch T, et al. Assembly and diploid architecture of an individual human genome via single-molecule technologies. Nat Methods. 2015; 12: 780-786.

64. Tham CY, Tirado-Magallanes R, Goh Y, Fullwood MJ, Koh BTH, Wang W, et al. NanoVar: accurate characterization of patients' genomic structural variants using low-depth nanopore sequencing. Genome Biol. 2020; 21: 56.