Genomics of Mucoepidermoid and Adenoid Cystic Carcinomas

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Objective: To report on the current state of the literature on the genetics of mucoepidermoid and adenoid cystic carcinomas of the salivary glands with a focus on genomic screens and recently discovered genetic translocations.

Methods: A PubMed based literature review was performed to query for genetics related basic science and preclinical studies about mucoepidermoid and adenoid cystic carcinomas of the salivary glands.

Results and conclusions: Genetic translocations between CRTC1 and MAML2 in mucoepidermoid carcinoma and between MYB and NFIB in adenoid cystic carcinoma have been recently discovered and have therapeutic implications. Key signaling pathways such as the EGFR pathway in mucoepidermoid carcinoma and the Notch pathway, chromatin regulation, and c-kit mediated epithelial-mesenchymal transitions in adenoid cystic carcinoma have recently been elucidated, pointing to possible therapeutic targets in both cancers.

Key Words: adenoid cystic carcinoma, mucoepidermoid carcinoma, salivary gland, genomics, genetic translocations.

INTRODUCTION
The salivary glands are exocrine organs of the head and neck that produce and secrete saliva. There are three pairs of major salivary glands—parotid, submandibular, and sublingual—in addition to a number of minor salivary glands located throughout the upper aerodigestive tract. While rare, salivary gland tumors can arise from any major or minor salivary gland. While most salivary gland tumors are benign, over the last four decades there has been an increasing incidence of malignant salivary gland tumors that can lead to patient morbidity and mortality. There has also been a rise in tumors with regional and distant disease. The relative incidence of malignant versus benign salivary tumors is known to vary by anatomic site.

Malignant salivary gland cancers are staged based on tumor, node and metastasis (TNM) staging as identified by the American Joint Committee on Cancer (AJCC). Staging is based on clinical parameters including tumor size, extraparenchymal extension, and nodal and distant metastases. While TNM staging guidelines for oropharyngeal cancers have recently been modified based on p16 positivity, there are no specific genetic markers to guide staging of salivary gland tumors. The current mainstay of treatment of salivary gland carcinomas is surgical excision with consideration of postoperative radiation for high tumor stages, high grade, positive margins, or other concerning pathologic features. Chemotherapy has been considered for salivary gland carcinomas, but currently only plays a role in palliation.

Overall, salivary gland tumors represent a tremendously pathologically diverse set of tumors. A recent report from the World Health Organization (WHO) identified 24 subtypes of malignant epithelial salivary tumors and 10 subtypes of benign epithelial salivary tumors. Concurrent with pathologic diversity, there is remarkable genetic diversity in regards to the various genetic pathways and chromosomal rearrangements that govern each subtype. In mucoepidermoid carcinoma and adenoid cystic carcinoma, the two most common salivary malignancies in the major glands, specific chromosomal rearrangements have been identified. This review focuses on these two types of salivary gland cancers, surveying the recent literature on the genetics behind these two cancer types, to provide a summary to guide development of future targeted therapies.

METHODS
A comprehensive PubMed search of primary literature from 2000 to 2017 relating to the genetics of salivary gland cancers was reviewed. The search terms, “mucoepidermoid salivary gland genetics” yielded 241 results, and the search terms, “adenoid cystic salivary gland genetics” yielded 373 results. Additional articles were selected by review of references of the articles identified using the above search terms.

RESULTS AND DISCUSSION
Mucoepidermoid Carcinoma
Mucoepidermoid carcinoma is the most common malignancy in the major salivary glands. While half of these cancers present in the parotid gland, a smaller
percentage of these tumors present in the submandibular and sublingual glands.8,9 A recent large population-based cohort study found that 17% of malignant tumors in the submandibular gland were mucoepidermoid carcinomas.10 Mucoepidermoid carcinomas may also present in other sites such as the lungs.

There have been relatively few preclinical genetic studies in mucoepidermoid carcinoma. However, the discovery of a specific genetic translocation between chromosomes 11 and 19, t(11;19)(q14-21; p12-13).12 In 2003, Tonon and colleagues identified a novel chimeric gene product associated with this translocation.6 The gene product combines exon 1 of a novel gene on chromosome 19, mucoepidermoid carcinoma translocated 1 (MECT1), to exons 2–5 of a gene on chromosome 11 associated with the Notch signaling pathway, Mastermind-like 2 (MAML2).6 The Mect1 protein was later shown to activate cAMP-responsive element binding protein (CREB) signaling, and was renamed CREB-related transcrip
tional coactivator 1 (CRTC1).13,14

The CRTC1-MAML2 Fusion in Mucoepidermoid Carcinoma

Mucoepidermoid carcinoma is associated with a specific genetic translocation between chromosomes 11 and 19, t(11;19)(q14-21; p12-13).12 In 2003, Tonon and colleagues identified a novel chimeric gene product associated with this translocation.6 The gene product combines exon 1 of a novel gene on chromosome 19, mucoepidermoid carcinoma translocated 1 (MECT1), to exons 2–5 of a gene on chromosome 11 associated with the Notch signaling pathway, Mastermind-like 2 (MAML2).6 The Mect1 protein was later shown to activate cAMP-responsive element binding protein (CREB) signaling, and was renamed CREB-related transcriptional coactivator 1 (CRTC1).13,14 The fusion gene has been confirmed in a large scale genomic screen.11 This CRTC1-MAML2 protein product has been shown to confer tumorigenesis, and knockdown of the fusion product inhibits tumor growth.15

### TABLE I.
Summary of Pathways and Fusions in Adenoid Cystic Carcinoma and Mucoepidermoid Carcinoma Described in this Manuscript.

| Tumor Type                  | Pathway/Fusion         | References | Key Observations                                                                 |
|-----------------------------|------------------------|------------|----------------------------------------------------------------------------------|
| Mucoepidermoid Carcinoma    | CRTC1-MAML2 fusion     | Tonon, 2003 | Discovery paper. MAML2 protein product activates downstream Notch gene, HES1.   |
|                            |                        | Wu, 2005   | CRTC1 protein product activates CREB-related signaling                            |
|                            |                        | Coxon, 2005| CRTC1-MAML2 fusion correlated with improved prognosis                             |
|                            |                        | Okumura, 2011 | Knockdown of AREG halts growth of fusion positive cells                           |
|                            |                        | Tirado, 2007 | EGFR copy number alterations and overexpression in high grade tumors, irrespective of fusion status |
|                            | EGFR pathway           | Chen, 2014 | EGFR copy number alterations and overexpression in high grade tumors, irrespective of fusion status |
|                            |                        | Chen, 2015 | Knockdown of Notch genes (Notch 1, Notch2, Notch4) inhibit growth and invasion in adenoid cystic carcinoma |
|                            |                        | Shinomiya, 2016 | EGFR copy number alterations and overexpression in high grade tumors, irrespective of fusion status |
|                            |                        | Nakano, 2013 | EGFR copy number alterations and overexpression in high grade tumors, irrespective of fusion status |
|                            |                        | Lujan, 2010 | EGFR copy number alterations and overexpression in high grade tumors, irrespective of fusion status |
|                            | P53                    | Kang, 2017 | p53 often mutated in high grade mucoepidermoid carcinomas                         |
|                            | MYB-NFIB fusion        | Persson, 2009 | Discovery paper. Significance unknown                                              |
|                            | MYBL1-NFIB fusion      | Brayer, 2015 | Discovery papers. Tumors with MYB-NFIB fusions and MYBL1-NFIB fusions are associated with a similar gene expression profile, and negatively correlated with survival. |
|                            | Notch pathway          | Stephens, 2013 | Notch pathway genes are commonly altered in adenoid cystic carcinoma              |
|                            |                        | Rettig, 2016 | Knockdown of Notch genes (Notch 1, Notch2, Notch4) inhibit growth and invasion in adenoid cystic carcinoma |
|                            |                        | Ding, 2010  | Knockdown of Notch genes (Notch 1, Notch2, Notch4) inhibit growth and invasion in adenoid cystic carcinoma |
|                            |                        | Chen, 2013  | Knockdown of Notch genes (Notch 1, Notch2, Notch4) inhibit growth and invasion in adenoid cystic carcinoma |
|                            |                        | Su, 2014    | Knockdown of Notch genes (Notch 1, Notch2, Notch4) inhibit growth and invasion in adenoid cystic carcinoma |
|                            |                        | Chen, 2015  | Knockdown of Notch genes (Notch 1, Notch2, Notch4) inhibit growth and invasion in adenoid cystic carcinoma |
|                            |                        | Qu, 2016    | Knockdown of Notch genes (Notch 1, Notch2, Notch4) inhibit growth and invasion in adenoid cystic carcinoma |
|                            | SMARC family           | Ho, 2013    | SMARC family of chromatin regulators (SMARCA2, ARID1A) are commonly altered in adenoid cystic carcinoma |
|                            | DNA methylation        | Stephens, 2013 | SMARC family of chromatin regulators (SMARCA2, ARID1A) are commonly altered in adenoid cystic carcinoma |
|                            |                        | Rettig, 2016 | SMARC family of chromatin regulators (SMARCA2, ARID1A) are commonly altered in adenoid cystic carcinoma |
|                            | TGF-beta               | Dong, 2013  | TGF-beta is differentially expressed in adenoid cystic carcinoma lung metastases |
|                            |                        | C-kit       | Overexpression of C-kit induces TGF-beta and mesenchymal markers                  |
|                            |                        | Jain, 2016  | Overexpression of C-kit induces TGF-beta and mesenchymal markers                  |
|                            |                        | Tang, 2014  | Overexpression of C-kit induces TGF-beta and mesenchymal markers                  |

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The CRT1-MAML2 fusion product can be detected readily by fluorescence in-situ hybridization (FISH), and is specific to malignant mucoepidermoid carcinomas. This gene product is present in anywhere from 34 to 81% of mucoepidermoid carcinomas of the salivary glands. Fusion-positive tumors tend to be highly differentiated, occurring in clinically smaller tumors and in younger patients. Overall, multiple authors have shown that the presence of the fusion product confers an improved prognosis, with improved disease-free survival and fewer distant metastases. Rare exceptions to this rule—for instance, there are fusion-positive high grade mucoepidermoid carcinomas with poor prognoses that have multiple additional genetic insults such as mutations in the gene CDKN2A.

In efforts toward designing personalized therapies, several studies have attempted to clarify the mechanism by which the CRT1-MAML2 fusion product leads to tumor formation. It was initially thought that the fusion protein caused tumor growth by constitutive activation of Notch signaling via the MAML2 portion of the gene. The fusion product is able to activate the transcription of the downstream Notch gene HES1, and gene expression studies have demonstrated differential expression of Notch genes in tumors with the fusion gene. However, more recent studies have demonstrated a significant role of the CRT1 portion of the fusion product. The CRT1 gene is expressed in early submandibular gland development, disappears with maturation of the gland and reappears with early tumorigenesis. In addition, expression of the fusion product in HeLa cells activates CREB-related signaling but not Notch signaling.

EGFR Signaling in Mucoepidermoid Carcinoma

In mucoepidermoid carcinomas with the CRT1-MAML2 fusion, knockdown studies have revealed a specific downstream target affected by CRT1 called AREG, which is an EGFR ligand. AREG expression is positively correlated with CRT1-MAML2 expression in tumor samples. In addition, knockdown of AREG leads to a decrease in the growth of fusion positive cells. Therefore, EGFR signaling could represent the mechanism of action by which the fusion gene promotes carcinogenesis.

Mutations in the EGFR receptor itself are rare in salivary gland carcinomas. The other hand, copy number alterations in EGFR are frequently found in high-grade mucoepidermoid carcinomas, regardless of fusion gene positivity. In addition, EGFR overexpression is present in 73% of high grade tumors and is associated with poor prognosis. These observations suggest an overall role of EGFR in the pathogenesis of mucoepidermoid carcinoma and implicate the pathway as a possible therapeutic target.

P53 Mutations in Mucoepidermoid Carcinoma

A recent genomic screen in mucoepidermoid carcinoma identified p53 as the most frequently mutated gene in mucoepidermoid carcinoma of the salivary gland. This gene was also identified in another genomic screen that examined mucoepidermoid carcinomas in multiple tissue types. Interestingly, p53 mutations were only found in intermediate and high-grade tumors. These findings are corroborated by immunostaining studies that found correlations between p53 expression and the proliferation marker Ki-67. These observations suggest that mutations in p53 might represent a genetic switch that converts a tumor from a low-grade to a high-grade phenotype.

Adenoid Cystic Carcinoma

Adenoid cystic carcinoma is another malignancy that can present in both major and minor salivary glands, but has a natural tendency to present in smaller glands. As such, it is the most common malignancy in the submandibular gland, representing 36% of malignancies in the gland. Adenoid cystic carcinoma has a prolonged, indolent course but has a propensity toward perineural invasion and distant metastatic spread, leading to death in many patients after 10–15 years. Similar to mucoepidermoid carcinoma, specific genetic translocations associated with fusion proteins have been identified in adenoid cystic carcinoma. Several whole genome and whole exome screens have been performed, reaffirming these genetic translocations while also implicating specific genetic pathways such as chromatin dysregulation and Notch signaling in adenoid cystic carcinoma. Numerous studies have also investigated the mechanisms by which adenoid cystic carcinoma develops the ability to seed metastases. These studies provide many avenues for therapies against adenoid cystic carcinoma.

MYB-NFIB and MYBL1-NFIB Fusions in Adenoid Cystic Carcinoma

Similar to mucoepidermoid carcinoma, adenoid cystic carcinoma is associated with a specific genetic translocation between chromosomes 6 and 9—t(6;9)(q22–23;p23–24). In 2009, Persson and colleagues identified a fusion product associated with this translocation between the myeloblastosis (MYB) oncogene and the transcription factor gene, Nuclear Factor 1B (NFIB). The translocation leads to overexpression of MYB and its downstream targets, including genes associated with cell cycle control, cell growth, apoptosis and cell adhesion.

The MYB-NFIB fusion and can be detected by FISH and is present in 28–60% of adenoid cystic carcinomas. The fusion is specific to adenoid cystic carcinomas and not present in pleomorphic adenomas or other salivary gland cancers. Whole genome sequencing studies confirm the presence of this genetic abnormality. However, unlike the CRT1-MAML2 fusion in mucoepidermoid carcinoma, the significance of the MYB-NFIB fusion remains unclear. A handful of studies suggest that the MYB-NFIB rearrangement is associated with improved survival. However, other studies show no survival benefit, while others still report a survival benefit.
disadvantage. Expression of Myb downstream targets also do not appear to be correlated with survival.

Recent studies also reported another novel fusion gene in adenoid cystic carcinoma associated with a translocation between chromosomes 8 and 9, generating a fusion product between MYBL1 and NFIB. One study found that tumors with MYB-NFIB and MYBL1-NFIB fusions have similar gene expression profiles, suggesting a common downstream mechanism. In this study, the two translocations were associated with poor prognosis.

**Notch Signaling in Adenoid Cystic Carcinoma**

The Notch signaling pathway is an intracellular pathway that is triggered by interaction of Notch ligands (Notch1 through Notch4) and Notch receptors on adjacent cells by cell–cell contact. Notch signaling has long been implicated in tumor biology across a number of different tumor types, and the pathway can have both oncogenic and tumor suppressive roles. In adenoid cystic carcinoma, mutations in Notch pathway genes such as NOTCH1, NOTCH2, SPEN, and FBXW7 were recurrently identified on whole genome screens as commonly altered genes, implying a possible role of this pathway as a whole.

In addition to these large genetic screens, isolated studies have implied a pro-tumorigenic role of Notch signaling in adenoid cystic carcinoma. Knockdown of Notch1 has been shown to suppress growth, migration, and proliferation of adenoid cystic carcinoma cells in both in vitro and in vivo models. Notch2 has been shown to play a similar role. Finally, inhibition of Notch4 with siRNAs blocks invasion in adenoid cystic carcinoma cell lines. Together, these studies identify Notch as a potentially targetable pathway in adenoid cystic carcinoma.

**Epigenetic Modifications in Adenoid Cystic Carcinoma**

Epigenetic signaling by way of chromatin regulation or DNA methylation changes play a large role in the pathogenesis of many cancers. The aforementioned genetic screens have specifically implicated chromatin deregulation as a major component of adenoid cystic carcinoma tumorigenesis. Specifically, aberrations in components of the SWIF/SNF-associated actin-dependent regulator of chromatin (SMARC) family, such as SMARCA2 and ARID1A, have turned up in multiple screens. There have been few studies examining the specific mechanisms by which chromatin deregulation relate to the carcinogenesis of adenoid cystic carcinoma, so the results revealed in these studies represent an area worthy of further investigation.

DNA methylation is another method by which epigenetic regulation can modify gene expression. In adenoid cystic carcinoma, several methylation screens have been performed. Daa and colleagues performed a screen of cyclin-dependent kinase inhibitors (CKIs) and found frequent methylations of CKI promoters such as p15, p18, p19, and p21. More recently, two global methylation screens have been performed. Shao and colleagues identified eight candidate oncogenes that were hypomethylated and overexpressed in adenoid cystic carcinoma. They focused on aquaporin1 (AQP1), which is able to promote cell proliferation and colony formation in adenoid cystic carcinoma cell line. Aquaporin1 was also demonstrated to be a poor prognostic marker in adenoid cystic carcinoma. Ling and colleagues performed a similar global methylation array, identifying over 3000 genes that were differentially methylated in adenoid cystic carcinoma cells. They validated their screen using one of their top candidate genes, HCN2, demonstrating that expression of HCN2 is associated with prognostic factors and survival.

**The Epithelial-Mesenchymal Transition (EMT) and Its Role in Adenoid Cystic Carcinoma Metastases**

Unlike mucoepidermoid carcinoma, adenoid cystic carcinoma has a specific propensity for late invasion and metastases eventually leading to patient mortality. Because of this, there has been a drive to identify the key players responsible for metastatic spread. The epithelial-mesenchymal transition (EMT) is a well-described phenomenon responsible for metastatic spread in other cancers such as breast and colon cancer, where cells transform from an epithelial-like to a mesenchymal-like morphology while gaining the capability to invade and metastasize. Recent studies have pointed to a similar transition in adenoid cystic carcinoma. Ishii and colleagues demonstrated that in an adenoid cystic carcinoma mouse model, cancer cells lose epithelial markers such as E-cadherin and gain the mesenchymal marker vimentin when undergoing metastases. Snail (SNA1) is a transcription factor that is known to repress E-cadherin expression and lead to EMT; its expression is closely linked to prognosis, perineural invasion, tumor recurrence and tumor metastases.

Further studies have examined the mechanism by which adenoid cystic carcinoma cells undergo EMT. TGF-beta, a growth factor that regulates EMT in other cancer types, is highly expressed in adenoid cystic carcinoma lung metastases, implying that it might play a role in EMT in adenoid cystic carcinoma. Specific to adenoid cystic carcinoma, studies have also pointed toward c-kit as a possible mediator. C-kit is a tyrosine kinase receptor that is frequently overexpressed in adenoid cystic carcinoma. C-kit was found to be closely associated with the transcription factor Slug, a known mediator of EMT. Furthermore, ectopic overexpression of c-kit induces TGF-beta and is sufficient to cause expression of mesenchymal markers in adenoid cystic carcinoma cells. These studies suggest that c-kit and TGF-beta might work together in enabling adenoid cystic carcinoma metastases.

**CONCLUSION**

The recent discovery of genetic translocations in mucoepidermoid carcinoma and adenoid cystic carcinoma
have implications in the treatment of these rare but significant salivary gland malignancies. Both the CRTC1-MAML2 fusion gene in mucoepidermoid carcinoma and the MYB-NFIB fusion gene in adenoid cystic carcinoma are detectable by FISH and therefore can serve roles as tumor markers. While studies are still ongoing, the CRTC1-MAML2 fusion appears to be linked to EGFR signaling in mucoepidermoid carcinoma. The mechanism of tumorigenesis behind the MYB-NFIB fusion is still unknown.

In mucoepidermoid carcinoma, the EGFR growth factor pathway has arisen as a possible targetable pathway, both downstream and in parallel to the CRTC1-MAML2 fusion gene. A recent genomic screen identified p53 as a player in high grade tumors. In adenoid cystic carcinoma, several key targetable pathways have been identified in large-scale genomic screens. Specifically, the Notch signaling pathway and chromatin regulators have appeared in multiple genetic screens, suggesting their roles in tumor pathogenesis. Meanwhile, other studies have implicated the c-kit receptor and the growth factor TGF-beta as drivers in adenoid cystic carcinoma metastases. These studies form the basis for possible future therapies for these harmful malignancies.

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