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Review

Diagnostic and therapeutic implications of new molecular biomarkers in salivary gland cancers

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SUMMARY

Salivary gland carcinomas (SGCs) are uncommon tumors, constituting approximately 5% of all cancers of the head and neck. They are a heterogeneous group of diseases that pose significant diagnostic and therapeutic challenges. The treatment of patients with SGCs is mainly restricted to surgery and/or radiation therapy and there is only limited data available on the role of conventional systemic and targeted therapies in the management of patients with advanced disease. There is thus a great need to develop new molecular biomarkers to improve the diagnosis, prognostication, and therapeutic options for these patients. In this review, we will discuss the most recent developments in this field, with focus on pathognomonic gene fusions and other driver mutations of clinical significance. Comprehensive cytogenetic and molecular genetic analyses of SGCs have revealed a translocation-generated network of fusion oncogenes. The molecular targets of these fusions are transcription factors, transcriptional coactivators, and tyrosine kinase receptors. Prominent examples of clinically significant fusions are the *MYB–NFIB* fusion in adenoid cystic carcinoma and the *CRTC1–MAML2* fusion in mucoepidermoid carcinoma. The fusions are key events in the molecular pathogenesis of these tumor types and contribute as new diagnostic, prognostic, and therapeutic biomarkers. Moreover, next-generation sequencing analysis of SGCs have revealed new druggable driver mutations, pinpointing alternative therapeutic options for subsets of patients. Continued molecular characterization of these fusions and their down-stream targets will ultimately lead to the identification of novel driver genes in SGCs and will form the basis for development of new therapeutic strategies for these patients.

Introduction

Salivary gland carcinomas (SGCs) are uncommon neoplasms, accounting for up to 5% of all cancers of the head and neck. They include a wide spectrum of histologic entities with variable biological behavior and responsiveness to therapy [1–3]. The diagnosis of SGCs remains challenging, mainly because of the diversity of histologic subtypes and the often overlapping morphological patterns seen in many of these lesions. The primary treatment for patients with localized disease is surgery and/or radiation therapy. A number of recent studies have indicated that radiation plays an important role in local control in both the postoperative setting and in the definitive setting for unresectable cancers ([3] and references therein). However, the response rates to radiation vary between different histologic subtypes and tumor grades. Chemotherapy is employed almost exclusively with a palliative aim in patients with metastatic and/or recurrent disease. Targeted therapies are currently recommended only for patients in clinical trials. New treatment strategies are therefore needed for the majority of patients with SGCs.

Important advances have recently been made in the understanding of the molecular pathogenesis of SGCs. Thus, several recurrent chromosome translocations have been identified and shown to generate a tumor-type specific gene fusion network involving the most common subtypes of SGCs ([4–6] and references therein). The molecular targets of these translocations are tyrosine kinase receptors, transcriptional coactivators, and transcription factors involved in cell cycle regulation and growth factor signaling. The fusions and their downstream targets are new important biomarkers for molecular diagnosis and most importantly also for development of new therapeutic strategies

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for SGCs [3]. Moreover, recent studies using next generation sequencing and genomic and expression profiling methods have identified several additional biomarkers of potential clinical significance [7,8]. The aim of this review is to discuss the clinical implications of these new biomarkers with focus on their diagnostic and therapeutic significance in SGCs. A summary of these molecular biomarkers and their potential therapeutic targets is presented in Table 1.

Mucoepidermoid carcinoma (MEC)

MEC is the most common histological subtype of SGC, and includes a wide spectrum of lesions ranging from aggressive high-grade cancers to mainly non-aggressive, low-grade tumors [1]. We have previously shown that MECs, irrespective of histological grade, are characterized by a recurrent chromosome translocation t(11;19)(q21–22;p13) [4,5,9]. The translocations, which are found in a high frequency of MECs originating from diverse anatomical locations, result in fusions involving the *MAML2* and *CRTC1,* or more rarely *CRTC3*, genes [10–13] (Fig. 1). *CRTC1*, encodes a CREB (cAMP response element-binding protein) coactivator [14] and *MAML2* a Mastermind-like coactivator for Notch receptors [15]. As a consequence of the fusion, the Notch-binding domain of MAML2 is replaced by the CREB-binding domain of CRTC1/3 linked to the transactivation domain of MAML2 [10,11]. The molecular consequences of the fusion are still obscure. However, recent studies have shown that the EGFR-ligand AREG (Amphiregulin) is a downstream target of the fusion and that upregulation of AREG leads to activation of EGFR-signaling in an autocrine manner and increased cell growth and survival of MEC-cells (Fig. 2) [16,17]. In line with this observation, CRTC1–MAML2 positive MEC-cells were shown to be highly sensitive to inhibition of EGFR-signaling in a xenograft model, suggesting that targeting this pathway with small molecule EGFR-inhibitors may offer a new approach to systemic treatment of patients with advanced, unresectable fusion-positive MECs (Fig. 2) [3,17].

Although various grading systems have been introduced to improve the classification of MEC [18–22], their clinical usefulness have been limited because of subjective evaluation criteria and biological heterogeneity among different tumor grades. However, recent molecular genetic studies of large series of MECs have convincingly demonstrated that the fusion is a specific and clinically useful biomarker for this disease [22–26]. These studies have also shown that the highest incidence of the *CRTC1–MAML2* fusion is found in low- and intermediate-grade MECs with favorable prognosis. Although less frequently, the fusion may occur in high-grade MECs with a dismal prognosis [12,26,27]. Interestingly, the latter cases are often associated with deletion of the *CDKN2A* tumor suppressor gene [26,27]. However, most poorly differentiated MECs are fusion-negative, indicating that they may represent a misclassification of high-grade MEC-like adenocarcinomas not otherwise specified. A recent genome-wide arrayCGH study suggested that MECs may be subclassified in (1) fusion-positive, low- and intermediate-grade MECs with no or few genomic imbalances and favorable prognosis, (2) fusion-positive, high-grade MECs with or without *CDKN2A* deletions, multiple genomic imbalances, and poor prognosis, and (3) fusion-negative, high-grade MEC-like adenocarcinomas with multiple genomic imbalances and poor prognosis [3,26]. Taken together, available data demonstrate that *CRTC1–MAML2* may serve as a specific diagnostic and prognostic biomarker for MEC and that patients with histopathologically confirmed or suspected MECs should be screened by RT-PCR and/or FISH for the *CRTC1–MAML2* fusion before being enrolled in clinical trials using targeted therapies.

High-grade MEC-like tumors

Previous studies have shown that a subset of high-grade, *CRTC1–MAML2* negative tumors with a MEC-like phenotype has a t(6;22)(p21;q12) translocation resulting in an *EWSR1–POU5F1* gene fusion (Figs. 1 and 2) [28]. The resulting fusion protein is composed of the N-terminal domain of EWSR1 linked to the DNA-binding domain of POU5F1 (a. k. a. OCT4). POU5F1 is a transcription factor with a critical role in embryonic development and maintenance of pluripotency of embryonic stem cells. In agreement with the role of POU5F1 as a master regulator of cellular pluripotency, it should be noted that the *EWSR1–POU5F1* positive tumors were more immature compared to the *MAML2*-positive tumors. Of note, an identical *EWSR1–POU5F1* fusion has recently also been found in a subset of deep-seated soft tissue myoepithelial tumors of children and young adults [29].

Adenoid cystic carcinoma (ACC)

ACC is the second most common subtype of SGC but may also arise in other secretory glands, such as the breast, and in the sinonasal tract, tracheobronchial tree, skin, and vulva ([1,5] and references in [30,31]). ACC is a slow growing but aggressive cancer with a poor long-term prognosis mainly due to metastatic disease [1]. Eighty to 90% of patients with head and neck ACC die of disease in 10–15 years. The primary treatment of ACC is surgery and/or

Table 1
Molecular biomarkers and potential therapeutic targets in salivary gland cancers.

Tumor type	Diagnostic biomarkers	Other biomarkers	Activated oncogenes/ pathways	Potential therapeutic agents
ACC	*MYB–NFIB*	del(1p), del(6q)	*MYB–NFIB, EGFR, KIT, BRAF, HRAS, TRKC, FGFR1*	Cetuximab (EGFR), vemurafenib (BRAF), dacomitinib (panEGFR), imatinib (KIT), AZD7451 (NTRK3/TRKC), dovitinib (FGFR1)
MEC	*CRTC1–MAML2*	del(9p) *CDKN2A*	*CRTC1–MAML2, AREG*	Cetuximab (EGFR), erlotinib (EGFR)
MEC (high-grade)	*EWSR1–POU5F1*		*EWSR1–POU5F1*	
MASC	*ETV6–NTRK3*		*ETV6–NTRK3, IGF1R*	AZD7451 (NTRK3/TRKC), BMS-754807 (IGF1R, IR)
HCCC	*EWSR1–ATF1*		*EWSR1–ATF1*	
Ca-ex-Pa	*PLAG1*-fusions *HMGA2*-fusions *HER2* amplification	t(8q12) t(12q14–15) *MDM2/HMGA2* amplification *TP53* mutation	*PLAG1, HMGA2, HER2, MDM2*	Trastuzumab (HER2), nutlin-3 analogs (MDM2-*TP53*)
SDC	*HER2* amplification	AR+	*HER2, BRAF,* androgen receptor, PI3K	Trastuzumab (HER2), bicalutamide (androgen receptor), vemurafenib (BRAF), temsirolimus (mTOR)
AciCC		Inactivation of *PTEN* and *APC*	mTOR	Rapamycin, sirolimus (mTOR)
EMC	*HRAS*		MEK	Trametinib (MEK)