Impact of Amplified Oncogenes on Salivary Gland Carcinomas

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carcinomas (SGCs) demonstrate a variety of histogenetic types. They are characterized by a broad spectrum of chromosomal and gene alterations. In particular, amplifications in specific genes [human epidermal growth factor receptor 2 (HER2), human epidermal growth factor receptor 4 (HER4), epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), Mouse double minute 2 homolog (MDM2), androgen receptor (AR), programmed death (ligand 1 (PD-L1), neurogenic differentiation factor 2 (NEUROD2), phosphatidylinositol 3,4,5-trisphosphate-dependent RAC exchanger 1 protein (PREX1), cyclin-dependent kinase4/6 (CDK4/6), proline-rich acidic protein 1 (PRAP1), kell antigen system (KEL), glutamate receptor subunit epsilon 2 (GRIN2D), Ewing sarcoma RNA-binding protein 1 (EWSRI), MYC proto-oncogene (MYC)] combined or not with chromosomal numerical imbalances (aneuploidy/polysoy/monosomy) form different genetic signatures affecting the response to monoclonal antibody-based, oncologically targeted regimens. Different SGC histotypes demonstrate specific combinations of mutated/amplified genes that modify their clinicohistological features. In the current molecular review, we present the most important amplified oncogenes and their impact on the biological behavior of SGCS.
Gross chromosomal instability and numerical or structural gene modifications represent the main mechanisms of genetic alterations that are involved in epithelial neoplastic and progressively malignant transformation (1, 2). Gene mutations, rearrangements and translocations are described as structural changes, whereas gene amplification or gain is the result of an excessive production of gene copies leading to protein overexpression (3, 4). Gene amplification and mutations affect a broad spectrum of (proto-) oncogenes and suppressor genes respectively. Concerning the molecular mechanisms that lead to these alterations, viral-dependent carcinogenesis is an excellent model for understanding them (5, 6). Proto-oncogenes promote a variety of intra- or intercellular functions in normal epithelia (7). Cell growth, proliferation and apoptotic death that secure tissue homeostasis are regulated by them (8). Additionally, signaling transduction from the cell periphery (extracellular space) to the nucleus is controlled by proto-oncogenes that are present in specific molecular pathways (9, 10). Oncogenes represent the mutated or amplified forms of the corresponding proto-oncogenes during epithelial malignant transformation, as occurs in breast adenocarcinoma (11, 12).

Salivary gland carcinomas (SGCs) are malignancies that belong to the head and neck carcinoma super family of tumors. Mucoepidermoid (MEC), adenoid cystic (ADCC) and salivary duct (SDC) carcinomas are the prominent histotypes, according to the updated World Health Organization histogenetic categorization (13). MEC, ADCC and SDC are characterized by differences regarding their grade of differentiation, recurrence rates, and metastatic potential (14-16). Interestingly, polymorphous adenocarcinoma, sebaceous adenocarcinoma, acinic-cell carcinoma, basal cell adenocarcinoma, secretory carcinoma, mucinous adenocarcinoma, clear-cell carcinoma, cystadenocarcinoma, sebaceous lymphadenocarcinoma, and cystadenocarcinoma represent infrequent SGC histotypes (17, 18). Moreover, carcinosarcoma, epithelial–myoepithelial carcinoma, secretory carcinoma, squamous cell carcinoma, anaplastic small-cell carcinoma, undifferentiated carcinomas, and intraductal carcinoma are ‘exotic’ types, very rare but, in the majority of cases, aggressive forms of SGC (19). In the current review, we focused on the oncogenes that have been found to be amplified in SGCs, forming specific genetic signatures in them.

**Oncogenes Amplified in SGC Histotypes**

SGC histotypes harbor a variety of mutated, fused/translocated and amplified oncogenes (20, 21). This genetic landscape mainly includes amplification of human epidermal growth factor receptor 2 (HER2), human epidermal growth factor receptor 4 (HER4), epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), Mouse double minute 2 homolog (MDM2), androgen receptor (AR), programmed death ligand 1 (PD-L1), neurogenic differentiation factor 2 (NEUROD2), MYC proto-oncogene (MYC) genes at different frequencies (Table I).

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Table I. Genes detected as being amplified in salivary gland carcinoma (SGC) histotypes.

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<thead>
<tr>
<th>SGC type</th>
<th>HER2</th>
<th>ALK</th>
<th>MDM2</th>
<th>MYC</th>
<th>AR</th>
<th>PD-L1</th>
<th>EGFR</th>
<th>NEUROD2</th>
<th>PREX</th>
<th>GRIN2D</th>
<th>CDK4/6</th>
<th>PRAP1</th>
<th>HER4</th>
<th>KEL</th>
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ADCC: Adenoid cystic carcinoma; CAMSG: cribriform adenocarcinoma of (minor) salivary gland; CCMC: clear-cell myoepithelial carcinomas; CexPA: carcinoma ex pleomorphic adenoma; IDC: intraductal carcinoma; MEC: mucoepidermoid carcinoma; OSGC: oncocytic salivary gland carcinoma; SDC: salivary duct carcinoma. Genes: ACTN4: Actinin-4; HER2: human epidermal growth factor receptor 2; HER4: human epidermal growth factor receptor 4; EGFR: epidermal growth factor receptor; ALK: anaplastic lymphoma kinase; MDM2: mouse double minute 2 homolog; AR: androgen receptor; PD-L1: programmed death-ligand 1; NEUROD2: neurogenic differentiation factor 2; PREX1: phosphatidylinositol 3,4,5-trisphosphate-dependent RAC exchanger 1 protein; CDK4/6: cyclin-dependent kinase 4/6; PRAP1: proline-rich acidic protein 1; KEL: kell antigen system; GRIN2D: glutamate receptor subunit epsilon 2; EWSR1: Ewing sarcoma RNA-binding protein 1; MYC: proto-oncogene MYC.
The \textit{HER2/neu} proto-oncogene is located on the long arm of chromosome 17 (gene locus: 17q21) and encodes for a transmembranous glycoprotein that acts as a tyrosine kinase receptor. It is a member of the epidermal growth factor receptor super-family that includes \textit{EGFR} (\textit{HER1}), \textit{HER2}, \textit{HER3} and \textit{HER4} genes (22). Signalling transduction is mediated by intracellular proteins following \textit{HER2} activation. \textit{HER2} protein overexpression is frequently detected in a variety of solid malignancies, including of the breast, lung, stomach and colon (23, 24). Concerning HNCs, especially SGCs, there is a variety of monoclonal antibodies, such as alpelisib, trastuzumab and lapatinib, that target \textit{HER2} protein on cancerous cell membranes (25). Furthermore, \textit{HER2/neu} overactivation seems to be correlated to an aggressive SGC phenotype, especially MEC (26, 27). A study group explored the role of the two main molecular techniques that are applied for detecting \textit{HER2} gene amplification, namely fluorescence \textit{in situ} hybridization, and next-generation sequencing (28). They reported a fine agreement comparing these two methods for the assessment of gene copy numbers in protein overexpressing cases. Interestingly, although amplification is the main mechanism of \textit{HER2} oncogenic activation, this gene is also mutated in a subset of SGCs. Another molecular analysis identified synchronous \textit{HER2} and tumor protein 53 (TP53) mutations in a series of oncocytic SGCs, which represent a distinct histogenetic variant (29). Based on the previously referred molecular data regarding the \textit{HER2} amplification /mutation status in SGCs, anti-\textit{HER2} targeted agents, including afatinib and trastuzumab, were used to inhibit the receptor, thereby preventing excessive signaling transduction (30, 31). Interestingly, a novel monoclonal antibody–drug conjugate, fam-trastuzumab deruxtecan, seems to be a very promising agent because it provides simultaneous inhibition of \textit{HER2} and topoisomerase I in selected breast adenocarcinoma and SGC cases. In one of them, a \textit{HER2}-positive metastatic parotid gland carcinoma, the malignancy demonstrated no recurrence as a result of a complete, well-tolerated response (32). Interestingly, increased \textit{HER2} copy numbers combined with PD-L1 overexpression have been detected in some SDC cases and correlated with a better prognosis and response rate to immunotherapeutic targeted anti-PD-L1 strategies (33).

Besides \textit{HER2} amplification, extensive gene-scanning analyses, mainly based on whole-genome sequencing platforms, have revealed focal amplifications on 8q21-q23 gene loci (\textit{MYC}/\textit{MYB} genes) in the majority of SGC subtypes, including MECs and ADCCs (34). Additionally, \textit{ALK} amplification was detected in a molecular study with combined fluorescence \textit{in situ} hybridization and next-generation sequencing analyses. This is an alternative to the \textit{ALK} rearrangement mechanism found in a very limited subgroup of patients with SDC (35). Interestingly, in subsets of clear-cell myoepithelial carcinomas, the \textit{EWSR1} gene has been found to be amplified, although rearrangement is the prominent mechanism of its alteration in SGCs (36). Concerning another gene, \textit{AR}, there is limited, but strong evidence that its protein overexpression in rare cases of SDCs is a result of its gene amplification (37). Finally, actin 4 (\textit{ACTN4}) gene has been found to be overexpressed due to amplification in solid malignancies of different histotypes including SGCs (38). In contrast to \textit{HER2} and \textit{EWSR1} genes, \textit{MDM2} and \textit{CDK4} oncogenes, which share the chromosomal region 12q13-15, demonstrate a low amplification rate in SDCs. A molecular study showed that although rare, the combination of \textit{MDM2} and \textit{CDK4} amplification is correlated to \textit{TP53} mutations and high-mobility group protein 2 (\textit{HMG2}) rearrangement/amplification in subgroups of ex-pleomorphic adenomas (39). Similarly, another study group analyzed a series of SDCs by implementing a next-generation sequencing assay (40). They reported only one \textit{MDM2}-amplified case compared to \textit{HER2}-amplified ones. The majority of the previous referred studies concluded that \textit{HER2} but not \textit{MDM2} amplification is correlated to an aggressive phenotype and poor survival rates in subgroups of patients. For this reason, they suggest that targeting the \textit{HER2}-mediated pathway by monoclonal antibodies is an optimal oncological approach that could provide benefits for these patients.

In conclusion, amplification affects specific genes (\textit{HER2}/\textit{HER4/EGFR, ALK, MDM2, AR, PD-L1, NEUROD2, PREX, CDK4/6, PRAP1, KEL, GRIN2D, EWSR1, MYC}) in SGCs, combined or not with numerical imbalances (aneuploidy/polysomy/monosomy) in the corresponding chromosomes. Amplification of these oncogenes is associated with an excessive expression of their products (oncoproteins). As with mutations in specific genes, these amplified genes lead to different genetic signatures and modify the rate of response to targeted, monoclonal antibody-based therapeutic strategies in the corresponding patients improving their survival potential.

\section*{Conflict of Interest}

The Authors have no conflicts of interest to declare.

\section*{Authors' Contributions}

ET, AC, and VP: Design of the study and article writing; DS, DP, VR, NM and EK: academic advisors; PP, AA, SP, DR, GP and SM: collection and management of references and published data. All Authors read and approved the final article.

\section*{References}


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