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Review

Heterogeneity of Genetic Landscapes in Salivary Gland Tumors and Their Critical Roles in Current Management

Tükürük Bezi Tümörlerinde Genetik Faktörlerin Heterojenliği ve Mevcut Yönetimdeki Kritik Rolleri

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ABSTRACT

Salivary gland neoplasms (SGNs) are rare and heterogeneous tumors in the head and neck region. Although progress has been recently made in revealing the molecular landscape of salivary glands tumors, it is limited and appears to be the tip of the iceberg. Some genetic aberrations include chromosomal translocations, such as CRTC1/3-MAML2 in mucoepidermoid carcinoma, g MYB-NFIB gene fusions in adenoid cystic carcinoma, and PLAG1-HMGA2 gene changes in pleomorphic adenoma and carcinoma ex pleomorphic adenoma. These chromosomal translocations provide fresh insights into the molecular etiology of diverse SGNs and aid in their classification and in approaching treatment. In future, these genetic variations may serve as critical tools for diagnosing salivary gland tumors and optimizing the management as well as prognosis of patients. This review presents the most recent advances in the molecular pathology of salivary gland cancers, with an emphasis on distinguishing molecular features that can be used for optimizing current patient management.

Keywords: Salivary glands tumor, chromosomal aberrations, benign tumor, malignant tumor, genetic mutations, hypermethylation

ÖZ

Tükürük bezi neoplazmaları (SGN'ler) baş ve boyun bölgesindeki nadir ve heterojen tümörlerdir. Tükürük bezi tümörlerinin moleküler yapısını ortaya çıkarmada son zamanlarda ilerleme kaydedilmiş olsa da, bu sınırlıdır ve buzdağının görünen kısmı gibi görünmektedir. Bazı genetik anormallikler, mukoepidermoid karsinomdaki CRTC1/3-MAML2, adenoid kistik karsinomdaki g MYB-NFIB gen füzyonları ve pleomorfik adenom ve karsinom eks pleomorfik adenomdaki PLAG1-HMGA2 gen değişiklikleri gibi kromozomal translokasyonları içerir. Bu kromozomal translokasyonlar, çeşitli SGN'lerin moleküler etiyolojisi hakkında yeni bilgiler sağlamaktadırlar ve sınıflandırılmalarına ve tedaviye yaklaşımlara yardımcı olmaktadırlar. Gelecekte, bu genetik varyasyonlar, tükürük bezi tümörlerinin teşhisinde ve hastaların prognozunun yanı sıra yönetimin optimize edilmesinde kritik araçlar olabilirler. Bu derleme, mevcut hasta yönetimini optimize etmek için kullanılabilecek moleküler özellikleri ayırt etmeye vurgu yaparak, tükürük bezi kanserlerinin moleküler patolojisindeki en son gelişmeleri sunmaktadır.

Anahtar kelimeler: Tükürük bezi tümörü, kromozomal anormallikler, iyi huylu tümör, kötü huylu tümör, genetik mutasyonlar, hipermetilasyon

INTRODUCTION

Salivary gland neoplasms (SGNs) comprise a diverse category of cancers and include 24 histologically distinct cancer subtypes[1]. These tumors are considered a subset of head and neck cancers owing to the low degree of variation in histologic subtypes, overlapping characteristics, and relative rarity[2]. Thus, heterogeneity in molecular pathways and aberrant genetics contribute to the formation of each unique tumor and may play a role in the diagnosis and treatment of these tumors. Irrespective of the diagnostic challenges, histomorphological examination is the most essential pathological finding of salivary gland malignancies.

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Biomarkers, such as immunohistochemistry markers, can also be used to increase diagnostic accuracy. Currently, it is also essential to delineate the molecular mechanism of malignancies for identifying distinct genetic variants unique to various tumor types[3]. Translocations, which are genetic changes, have been identified in several types of malignancies and can be utilized as essential diagnostic tools. Furthermore, there is a growing need to understand the diagnostic value and genetic changes in terms of predictive and prognostic significance. Promising breakthroughs in efficient and targeted therapy for various salivary gland malignancies can be expected in the future. Systemic therapy, which includes cisplatin-based anti-cancer medicines, is used to treat advanced-stage cancers and to achieve a limited response[4]. Nonetheless, the discovery and widespread application of novel genetic tools has simplified the understanding of the molecular characteristics of tumor types. In the majority of salivary gland carcinoma (SGC) subtypes, several recurrent chromosomal translocations involving a tumor-specific gene fusion network have been found. The molecular targets of these translocations are transcriptional co-activators, tyrosine kinase receptors, and transcriptional factors, which are important in growth factor signaling and cell-cycle control. These downstream targets and fusions (important biomarkers) are vital for the development of novel therapeutics for several SGCs[5]. This review reports on the current genetic landscape in the varied and distinct groups of cancers. The purpose of this study is to identify the genetic variants (molecular biomarkers) associated with each SGC, as well as oncogenes and tumor suppressor genes (TSGs) that play a role in cancer development. This study will serve as a foundation for future research into gene-targeted treatment and more specific diagnostic tools.

Genetic Abnormalities in Malignant and Benign Salivary Gland Neoplasms

Primary SGCs are a morphologically diverse group of cancers that are associated with considerable diagnostic challenges for pathologists as well as treatment conundrums for oncologists and surgeons[6]. The yearly incidence of benign and malignant carcinomas is fewer than five persons per 100,000. As approximately 80% of all tumors are benign, SGNs are exceedingly rare, with reported rates of just 2.5-3.0 instances per 100,000 and accounting for only approximately 5% of all head and neck malignancies[6,7]. Patients, both men women, are often beyond 40 years. However, it is important to highlight that some of the more common tumors, such as pleomorphic adenoma (PA), are more prevalent in females, with a male-to-female ratio of 1:1.48. Approximately 80% of all SGCs are benign, with PA accounting for 55% of large gland lesions and 50% of small gland lesions[8]. Parotid gland carcinomas account for approximately 70% of SGCs, the submandibular gland for approximately 10%, and the sublingual gland for <1%, culminating in a 20% involvement of the small glands. Although neoplasms are less common in small glands, approximately half of them are malignant (metastatic) compared with only approximately 20% in large glands. It is worth mentioning that sublingual gland neoplasms are almost always malignant. Of the 70% of tumors occurring in the parotid gland, 50-60% are PA, 20-30% are Warthin's tumors (WTs), and approximately 10% are mucoepidermoid carcinomas (MECs)[7,8].

Malignant Salivary Gland Tumors (SGTs)

1. Mucoepidermoid Carcinoma and Salivary Duct Carcinoma (SDC)

Karpinets et al.[9] studied shared genetic and type-specific alterations associated with the development and evolution of the most common SGCs. They demonstrated a significant genetic difference between myoepithelial, dual epithelial adenoid cystic carcinoma (ACC) and solely epithelial-(MEC), and SDC-cell derived carcinomas. In the case of dual epithelial/myoepithelial composition (e.g., ACC), cancers are caused by chromosomal deletions, whereas epithelium-derived subtypes are caused by chromosomal gains and gene amplifications. These findings are consistent with previous genomic studies on several individual tumors[10], and these studies provide pragmatic support for the segmental ductal origin hypothesis[11].

The suppressive nature of myoepithelial cells may explain the limited genetic variation and the delayed clinical manifestation of ACC. There exists intra-subtype genetic heterogeneity in canonical gene fusion-negative and -positive MECs and ACCs, as demonstrated by Karpinets et al.[9]. Mutations in the NOTCH pathway were found to be associated with canonical gene fusion-negative ACC, whereas deletions at the 12q12-13 region (containing keratin type I and II genes as well as the *ERBB3* gene) were associated with canonical gene fusion-positive ACC, indicating high stage and solid tumors[12].

Mutations in CRTC1 fusion-negative MECs were confined to *MUC16*, *CIC*, and *LRFN1* genes, whereas mutations in CRTC1 fusion-positive MECs were found in *NEAT1*, *KCNQ1OT1*, *BAP1*, and *CCDC58* genes. These findings emphasize the connectivity of mutually incompatible genomic pathways in canonical gene fusion-negative and -positive MECs and ACCs, implying that cancer is induced by random genetic pathways of

equal differentiation. Karpinets et al.[9] discovered two new fusion genes, CACNA1B-NBPF[10], in three canonical gene fusion-negative MECs and ENOX1-TYRO3 in two canonical gene fusion-negative ACCs. However, the specificity and consistency of these fusion genes are still unclear, and additional testing is necessary. Compared with MECs and ACCs, SDCs exhibit a wide-ranging genetic aberration, lack of recurrent chromosomal abnormalities, and ERBB2 and TP53 variance. These are consistent with previous findings[13].

In human cancer, methylation, including transcriptional inactivation, has been found to be the most critical epigenetic change in TSGs. The maintenance of methylation patterns occurs through the family of DNA methyltransferases (DNMTs), consisting of three enzymes: DNMT1, DNMT3A, and DNMT3B. DNMTs such as DNMT3A and DNMT3B cannot help distinguishing methylated and unmethylated regions as they are in charge of (*de novo*) methylation. However, DNMT1 is responsible for maintaining methylation patterns after DNA duplication. Epigenetic processes were previously thought to be implicated in MECs. Magno Guimarães et al.[14], revealed that epigenetic control is important in defining the features of cancer cells, such as differentiation as well as self-renewal. The abnormal activity of DNMT promotes malignant transformation via site-specific methylation of TSGs. In contrast, inhibiting DNMT activity reduces the stemness of cancerous cells. Thus, DNA demethylation agents comprise a potential new class of cancer therapeutics. Moreover, 5-azacytidine, the first compound to exhibit DNA hypomethylation capabilities, has already been licensed by the Food and Drug Administration for myelodysplastic syndrome. It has been shown to possess demethylating activities that indirectly block DNMTs, activating pathways responsible for cellular differentiation, decreased cell proliferation, cell-cycle arrest, and cell death[14].

2. Adenoid Cystic Carcinoma

Various genetic abnormalities play a significant role in ACC. MYB expression is a distinguishing characteristic of SGTs and a useful diagnostic tool. Bell et al.[15] studied MYB expression in 156 patients with ACC. The overexpression of MYB was noted in 55% patients. However, the effect of downstream targets bcl2, cox, and c-kit, as well as individual *MYB* genes, on survival was not significant. Survival was higher in the *MYB*+/c-kit+/cox-2+ combination than in the *MYB*-/c-kit+/cox-2+ combination (p=0.01744). Thus, MYB may be a potential target for cancer management[15].

West et al.[16] studied 37 individuals and found that patients with balanced MYB-NFIB fusion had a greater probability of local relapse and perineural invasion. A further investigation of 409 cases of non-SGTs, 112 cases of other SGNs, and 37 cases of ACC revealed that balanced MYB-NFIB translocation was observed in approximately half of ACC patients (18/37, 49%). In few patients with ACC (6/37, 16%), an abnormal MYB-fluorescent *in situ* hybridization (FISH) pattern was identified, indicating an atypical translocation of MYB without the participation of NFIB. This might have happened as a result of MYB local duplication in human T-ALL cells. A considerable percentage of individuals with additional SGT translocations did not demonstrate any obvious MYB involvement, indicating an alternate pathway of tumorigenesis[16]. This finding is consistent with prior studies on chromosomal analysis, which suggested that only a subset of ACC had recurring chromosomal abnormalities[16]. Akrish et al.[17] assessed malignant salivary tumors and found negligible cox-2 staining in 6 ACC patients.

In ACCs, cyclin-dependent kinase inhibitors (CKIs) are commonly methylated. These proteins act as tumor suppressors as well as cell-cycle regulators, and their involvement in cancer formation is well understood. In ACC carcinogenesis, hypermethylation of p27 (a particular CKI) may result in alteration of cell cycle as well as its downregulation. Thus, alteration in the synthesis of this protein by abnormal DNA methylation results in dysregulation of the cell cycle (G0 stage), thus leading to abnormal neoplastic cell development. The authors propose that p27 downregulation may play a role in ACC carcinogenesis. The participation of this protein in ACC carcinogenesis is an important epigenetic event that warrants more exploration[18].

3. Acinic Cell Carcinoma (AciCC)

In a study conducted by Haller et al.[19], nuclear NR4A3 immunostaining was introduced as an innovative biomarker, which is highly sensitive and specific for AciCC. Moderate-to-strong immunostaining of nuclear NR4A3 was observed in 98% of 64 patients with AciCCs; however, no nuclear NR4A3 immunostaining was identified in any of the other 70 patients with SGCs, 29 mammary analog secretory carcinoma (MASC), and normal parotid gland tissues. Irrespective of the occurrence of high-grade transformation identified in 17% cases of AciCCs, immunostaining of NR4A3 was constantly reported during metastasis, during recurrences, and in primary tumors. NR4A3 upregulation is found to be the genetic driver in AciCCs, which is also possessed by high-grade

transformed cases. NR4A3 immunostaining has been used as a unique diagnostic tool owing to its high sensitivity and specificity for the differential diagnosis of SGCs, particularly in "zymogen granule"-poor intercalated duct type within differential diagnostic range of MASCs and AciCCs[20].

Most SGCs, involving recurrent translocations, are characterized by mutations within a coding gene that result in the creation of an oncogenic fusion gene. The frequent genomic translocation t(4;9) (q13;q31) does not result in the development of a chimeric fusion gene in AciCCs. This mutation, in contrast, introduces highly active chromatin regions from the secretory Ca-binding phosphoprotein (SCPP) gene cluster. This gene cluster encodes a number of overexpressed salivary gland genes close to the NR4A3 locus, allowing whole coding area of the NR4A3 gene to be upregulated[21].

Human retinoic acid receptor beta 2 (RARβ2) and RASSF1 were shown to be commonly methylated in SDCs as well as AciCCs. RARβ2 is a component of the nuclear receptor superfamily that plays an important role in controlling the ramifications of retinoic acid on cell proliferation as well as differentiation. In addition, reduction of RARβ2 expression has been associated with the emergence of mammary ductal carcinoma[18].

In a recent study, researchers discovered a new gene fusion combining the histatin 3 (HTN3) and MYB/SANT-like DNA binding domain containing protein 3 *(MSANTD3)* genes. The incidence of this fusion was determined to be 4.4 percent in 273 cases of AciCCs, and no histomorphological or clinical differences were detected between HTN3-MSANTD3 fusion-positive and fusion-negative AciCCs. Haller et al.[19] reported four (8 percent) cases of AciCCs, with positivity for HTN3-MSANTD3 gene fusion and nuclear NR4A3 immunohistochemistry.

The existence of a *SCPP* gene cluster with significant active enhancer elements on chromosome 4q13 is linked to the *HTN3* gene locus. This association was revealed by H3K27ac chromatin immunoprecipitation followed by sequencing of AciCC tumor tissues with the normal parotid gland. However, further research is needed to clarify the role of the HTN3-MSANTD3 fusion in AciCC carcinogenesis[21].

4. Myoepithelial Carcinoma (MECA) and Carcinoma ex-PA (CA ex-PA)

Changes in PLAG1 and HMGA2 have been found in histologically distinct subtypes of CA ex-PA and their de novo counterparts[22], according to Katabi et al.[23] CA ex-PA develops in conjunction with a coexisting PA component or at the same site of previously excised PA. In rare cases, pre-existing benign PA may be totally eradicated by the carcinoma component. Only hyalinized nodules with epithelial components can be observed in certain cases, increasing the likelihood of preceding PA section, although pathologists are cautious when accepting this as adequate for CA ex-PA diagnosis. CA ex-PA is more aggressive and has a worse prognosis in instances of MECA than its de novo counterparts[24].

Furthermore, CA ex-PA and, to a lesser extent, PA have a wide range of histologic features, and differentiation from (histologic mimics) basal cell adenoma (BCA)/adenocarcinoma, polymorphous low-grade adenocarcinoma, and epithelial-MECA (EMC) may be difficult on the basis of morphology alone. The FISH auxiliary test was found to be an effective tool for genetic variant-based diagnosis. *PLAG1* and *HMGA2* gene rearrangement, amplification, and genetic alterations have previously been identified in PA[25]. These genetic changes have also been identified in CA ex-PA[26] and soft tissue tumors with comparable histology[27].

In a study by Bahrami et al.[27], one of three patients with CA ex-PA tested positive for the HMGA2 mutation and 12 of 19 patients (63%) tested positive for PLAG1 translocation using FISH. The findings of a 2015 study by Katabi et al.[22] was consistent with those of a study by Bahrami et al.[27], who discovered PLGA1 and/or HMGA2 mutations in 19 of 22 (86%) patients with CA ex-PA. However, Bahrami et al.[27] assessed a broad histologic variety of CA ex-PA types, such as carcinosarcoma, squamous cell carcinoma, EMC, MECA, SDC, and not otherwise specified adenocarcinoma. In addition, the results of Katabi et al.[22] were in contrast with those of Bahrami et al.[27], in which they focused on SDC and MEC, the two most common histologic types of CA ex-PA.

Reportedly, p16 is the most commonly methylated gene in CA ex-PA as compared to benign lesions. This occurrence may be linked to the tumor development in this carcinoma. Furthermore, *RASSF1* gene was shown to be hypermethylated in both CA ex-PA as well as PA. RASSF1 appears to be a TSG, required for DNA repair; however, its true function in biological mechanisms is unknown. However, it has been discovered to be frequently hypermethylated in numerous malignancies[18].

PLAG1/HMGA2 negative, Katabi et al.[22] reported three instances of CA ex-PA, demonstrating apparent benign PA component of SDC, suggesting either alternate events for carcinogenesis or undetected PLAG1 aberration using FISH investigation. Furthermore, they reported two cases with PLAG1 translocations in both benign and malignant

sections in one case and HMGA2 translocations in just the malignant component in the other case[22]. These findings were consistent with the implications of Bahrami et al.[28]. Katabi et al.[22] reported three cases of CA ex-PA with hyalinized nodules and PLAG1 translocations. These findings have diagnostic implications in the surgical pathology of salivary glands. Kas et al.[29] reported that the t(3;8)(p21;q12) mutation causes promoter switching between PLAG1, a new developmentally controlled zinc finger gene at 8q12, and CTNNB1, a ubiquitously expressed protein interface involved in the WG/WNT signaling pathway and cell fate specification during embryogenesis.

Table 1 lists some of the most common mutations seen in malignant SGTs.

Benign Salivary Gland Tumors

1. Pleomorphic Adenoma

PA is the most prevalent benign SGT, accounting for more than half of all occurrences. Some tumors are aggressive in nature. Even after surgery, there is a significant recurrence rate, and the malignant transformation rate for PA is believed to be 6%. The mechanism underlying PA's high recurrence rate and malignant change is unclear. One cause for the variations in SGCs is myoepithelial differentiation. The myoepithelial cell is thought to be the major proliferative cell in PA[31]. The histologic variation in malignant PA is a result of various cellular changes in the myoepithelium. Desmosomes between neighboring cells, intermediate size filaments, endocytic vesicles, and microfilaments indicate a dual epithelial and smooth muscle phenotype in salivary gland myoepithelial cells, which are linked with acini and intercalated ducts and exhibit a dual epithelial and smooth muscle phenotype. Changes in gene regulation have also been related to tumor characteristics such as invasiveness, recurrence, and metastasis. Identifying the mechanism underlying these features in PA will help predict a better treatment outcome. Myoepithelial neoplasms are intriguing low-grade tumors that have a large amount of extracellular matrix[32].

The extracellular matrix protein MFAP4[33] regulates cell-to-cell and cell-to-matrix interactions. During inflammation, it repairs collectins in the extracellular compartment. As a result, it is believed that stromal myoepithelial extracellular matrix production, which may be linked to MFAP4, is critical for the progression and development of PA[34]. MFAP4 has also been linked to stromal tumors and the *ex vivo* production of

Table 1. Frequent molecular mutations observed in malignant tumor types of salivary glands.

	Malignant tumor types	Frequent mutations	Reference
1.	Mucoepidermoid carcinoma	CRTC1-MAML2 and CRTC3-MAML2 fusions EWSR1-POU5F1fusion	Toper and Sarioglu[30]
2.	Adenoid cystic carcinoma	MYB-NFIB and MYBL1-NFIB fusion MYB-PDCD1LG2, MYB-EFR3A, MYBL1-RAD51B, MYBL1-YTHDF3, NFIB-AIG1 fusions	Toper and Sarioglu[30]
3.	Acinic cell carcinoma	*SCPP* gene cluster*-NR4A3 fusions HTN3-MSANTD3 fusion	Toper and Sarioglu[30]
4.	Salivary duct carcinoma	*AR* gene alterations ERBB2 amplification TP53, PIK3CA, H-RAS, KIT, EGFR, BRAF, N-RAS, AKT1, FBXW7, ATM, NF1 mutations Loss of heterozygosity of CDKN2A/p16 and PTEN ETV6-NTRK3 BCL6-TRADD HNRNPH3-ALK EML4-ALK ABL1-PPP2R2C fusions	Toper and Sarioglu[30]
5.	Myoepithelial carcinoma	EWSR1 rearrangements PIK3CA and HRAS mutations	Toper and Sarioglu[30]
6.	Carcinoma ex pleomorphic adenoma	PLAG1 rearrangements	Toper and Sarioglu[30]

SCPP: Secretory Ca-binding phosphoprotein

hematopoietic stem cells. Reportedly[33], MFAP4 may be linked to tumor development and fibrosis.

Intriguing research of 84 cases of SGTs (42 benign and 42 malignant) found that benign tumors are associated with particularly significant hypoacetylation of (lys9) histone H3, as compared to the malignant SGTs which involve hypoacetylation and results in chromatin condensation. Furthermore, it has been discovered that cancerous cells with greater level of acetylation, proliferate at a lesser rate. As a result, unlike other malignancies such as breast cancer and pancreatic adenocarcinoma, H3 acetylation has an inversely proportionate influence on proliferation in SGTs. This is most likely because of multiple processes involved in neoplasm formation in various tissues as well as functioning in various ways in tissues and the fact that these processes may be damaging to proliferation[18].

The *KCTD15* gene encodes the potassium channel tetramerization domain 15, which has been linked to obesity, but its effect in cancer is unknown. KCTD15 was formerly thought to have a negative association with the Wnt/beta-catenin pathway. The Wnt signaling pathway is required for adhesion strength, replication, maturation, and epithelial-mesenchymal transition. In PLAG1 transgenic mice, higher expression of the Wnt/beta-catenin pathway was found. However, PA was found to be associated with the overexpression of KCTD15, which might block the Wnt/beta-catenin pathway. More research into the KCTD15 and (Wnt/beta-catenin) signaling pathways is required to identify the role of KCTD15 in PA carcinogenesis[35].

2. Basal Cell Adenoma/Myoepithelioma

Jo et al.[36] demonstrated that the majority of patients with BCA (82%) showed positivity for nuclear beta-catenin, as well as for CTNNB1 change in (4/5) beta-catenin positive tumors. Despite its low sensitivity, nuclear beta-catenin reactivity has a high specificity of 96% for distinguishing BCA from its morphologic imitators such as PA, ACC, and EMC[36]. Nuclear beta-catenin expression has been discovered to be multifocal, metastatic, and be moderate-to-strong, with reactivity that is more prominent in BSA and predominant in the stroma.

All CTNNB1 variants have I35T variations. The positivity for beta-catenin in BCA was more variable, with just one patient showing alterations in transcriptional regulation as well as genes involved in the NF-kB and P13K pathways, but no CTNNB1 variation was observed despite the beta-catenin reactivity[36]. do Prado et al.[37] discovered nuclear beta-catenin expression in two patients with BCA for the first time in 2007.

The *CTNNB1* gene, which is located on chromosome 3p21, encodes beta-catenin. All BCA mutations were 135T (located at the amino terminus in exon 3), with CTNNB1 alterations being observed in most instances (four/five). Change in codon 41 and 45 have been observed in sporadic (desmoid type) fibromatosis, and these modifications are major CTNNB1 variations[38]. Changes in beta-catenin result in increased transcription of target genes (Wnt pathway) involved in cell proliferation as well as in intranuclear buildup of beta-catenin, which prevents degradation and promotes enhanced transcription. All tumor types with CTNNB1 mutations have rare changes in codon 35[39].

CA ex-PA, PA, and EMC all lack CTNNB1 mutations. Although it is uncertain whether there is a link between BCA and basal cell adenocarcinoma, many specialists believe that basal cell adenocarcinomas proliferate on their own. More study is needed to determine if the PIK3 and NF-kB pathways, as well as transcriptional regulator genes, have a role in basal cell adenocarcinoma. Despite varying beta-catenin expression, previous research suggests that basal cell adenocarcinomas are pathogenetically distinct from BCAs[40].

3. Warthin's Tumor

Wemmert et al.[41] studied 30 WTs with multiple chromosomal abnormalities using comparative genomic hybridization (CGH). Only one study using CGH in 15 cases of WTs reported previously identified chromosomal aberrations as well as novel locations of interest[41]. Giefing et al.[42] demonstrated that chromosomal 12q, 17p, and 22 deletions were the most consistent anomalies in a sample of 15 patients with WTs (47%, 53%, and 73%, respectively). The most prevalent chromosomal increases were seen on the chromosomes 2q, 6q, 4q, and 13q (27%, 33%, 60%, and 67%, respectively). The current analysis supports these regularly seen losses and gains, although at a roughly one-third lower incidence of impacted tumours than Giefing et al.[42]. Giefing et al.[42] and Wemmert et al.[41] investigated deletions in the terminal region of 9q in 13% of neoplasms. This region contains TSC1 on 9q34.13 and GAS1 on 9q21.33. In line with Giefing et al.[42], Wemmert et al.[41] reported further deletions on chromosomes 16, 17, and 22, with the least amount of overlap on 16p12p13.1, 17p13, and 22q12.

Giefing et al.[42] and Wemmert et al.[41] revealed that additions on 6q were a regular occurrence in WTs. The FYN oncogene is located on 6q21 in the designated consensus area, which was found to be impacted in 33% of the neoplasms investigated by Wemmert et al.[41]. Wemmert et al.[41] reported recurrent changes on

9p (23%) and 8p (33%), respectively, in WTs as unique discoveries. 8p23.1pter variants include genes involved in carcinogenesis as well as DNA damage response, such as TSGs (PINX1, ANGPT2, and MCPH1). Two genes, *CDKN2B* and *CDKN2A* (located on 9p21), were found to be involved in cell-cycle control. These genes were not detected in previous studies on CGH or karyotyping analysis[43] and therefore demand special attention in future studies. Wemmert et al.[41] investigated a set of WTs and found gains on chromosome 22 as well as losses on 22q12. TSGs such as TIMP3 and CHEK2 located on 22q12 have been linked to a higher chance of developing pancreatic endocrine carcinoma and prostate tumor. Furthermore, the growth factor PDGFB[44] regulates cell survival, migration, and proliferation. The total number of variants and specified copy number variations (8p deletions) have been linked to the development of prostate cancer, head and neck squamous cell neoplasia, and MEC[45]. These should be studied further to establish their possible function in WT tumorigenesis[41]. Table 2 lists selected mutations that are common in benign SGTs.

Application of Genetic Markers for SGC Management

There are a number of possible genetic markers that can be utilized to improve the treatment of SGTs.

1. Diagnosis and Prognostic Markers for MEC

Because MAML2 translocation is common and unique to MECs, FISH is an excellent diagnostic technique for MAML2. MAML2 rearrangements can be discovered using FISH and reverse transcription polymerase chain reaction (RT-PCR). These approaches can detect the histologic overlap of MEC with AciCC, oncocytoma, oncocytic cystadenoma, lymphadenoma, and WT. Indeed, identifying gene relocation is critical[46].

2. Diagnosis and Prognostic Markers for ACC

MYB/MYBL1 rearrangement is considered to be highly specific for ACC among SGTs. As a result, FISH (diagnostic marker) analysis is utilized to assess MYB and distinguish ACC from other forms of SGTs. FISH analysis for MYB is the most often used approach for identifying variations. RT-PCR[47] can also be used to detect fusion variants. This allows for an earlier identification of ACC and more successful therapy for these patients.

3. Diagnosis and Prognostic Markers for AciCC

A rearrangement of the *SCPP* gene cluster (NR4A3) has only been seen in AciCC. Immunohistochemical staining of NR4A3 has been described as a supplemental test to diagnose AciCC (recurrent, metastatic, and high-grade cancer), with a sensitivity of more than 90% and specificity of 100%. For AciCCs diagnosis[48], immunoexpression of NR4A3 was shown to be more accurate than FISH examinations. This genetic marker holds considerable potential for tailoring specialized treatment based on correct diagnosis for this patient population.

4. Diagnosis and Prognostic Markers for SDC

AR immunohistochemistry expression was reported in 70% of SDCs and apocrine-variant SDCs. In the majority of studies, the frequency of AR positive surpassed 100%. AR immunoexpression is thought to be a predictive and diagnostic biomarker in SDCs. Immunohistochemistry is used to detect increased ERBB2 expression in heterogeneous SDCs[49].

5. Diagnosis and Prognostic Markers for PA and CA ex-PA

FISH is highly useful in identifying HMGA2 and PLAG1 fusions. Immunohistochemistry can detect HMGA2 and PLAG1 relocations, which result in increased expression of HMGA2 and PLAG1 proteins. In terms of PA diagnosis, PLAG1 immunoexpression was found to be sensitive but less specific compared with FISH analysis. Notably, AR and HER2 overexpression or amplification can serve as therapeutic targets[50]. This is essential since the surgical strategy for PA differs greatly from that for CA ex-PA.

CONCLUSIONS

Our understanding of the molecular pathologic backdrop of SGTs has developed in recent decades but is still insufficient for a wide range of SGTs, both benign and malignant. Nonetheless, by acquiring additional

Table 2. Frequent molecular mutations in benign salivary gland tumors.

	Benign tumor type	Frequent mutations	Reference
1.	Pleomorphic adenoma	PLAG1 alterations HMGA2 alterations	Toper and Sarioglu[30]
2.	Warthin's tumor	Deletions of the short arm of chromosome 8, followed by deletions on 9p	Wemmert et al.[41]
3.	Basal cell adenoma/ myoepithelioma	CTNNB1 mutation	Toper and Sarioglu[30]

molecular data, we may better identify these disorders early in the process and provide more accurate diagnosis and successful therapy methods. As a consequence, by selecting a proper and an optimal treatment regimen and employing new therapy targets, we can enhance treatment results in this subset of patients. We may expect more scientific understanding about the genetic origin of these atypical cancers in the future to provide better therapeutic options to patients and enhance patient prognosis and survival.

Ethics

Peer-review: Externally peer-reviewed.

Author Contributions

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