BMC Cancer

RESEARCH

Open Access

Potentially actionable molecular alterations in particular related to poor oncologic outcomes in salivary gland carcinomas

Julia Pikul[1†], Marcin M. Machnicki[3†], Anna Rzepakowska[1*†], Natalia Winiarska[2], Agnieszka Chudy[4], Albert Moskowicz[4], Kacper Król[2], Łukasz Fus[5], Grażyna Kostrzewa[6] and Tomasz Stokłosa[3†]

Abstract

Aim The study was designed to evaluate molecular alterations, relevant to the prognosis and personalized therapy of salivary gland cancers (SGCs).

Materials and methods DNA was extracted from archival tissue of 40 patients with various SGCs subtypes. A targeted next-generation sequencing (NGS) panel was used for the identification of small-scale mutations, focal and chromosomal arm-level copy number changes. The final analysis included selected genes with potential actionable aberrations for targeted therapies and outcome predictions in 37 tumours' samples.

Results The follow-up of the SGCs study cohort revealed disease recurrence or metastasis in 19 patients and indicated poor individual outcomes. The mean disease-free survival (DFS) within the poor outcome group was 2.4 years, and the overall survival (OS) was 5.4 years. The DFS and OS of the remaining 18 patients with favourable outcomes were 8.3 years. The genes most frequently affected with aberrations were *NF1* ($n=9$, 24%) and *TP53* ($n=8$, 22%), with increased occurrence observed in the poor outcome group: *NF1* ($n=6$, 32%) and *TP53* ($n=6$, 32%). *CDKN2A* biallelic deletion was the most common copy number variation ($n=5$), and was detected in 4 cases with identified disease relapse. *TERT* promoter mutation and amplification were found in myoepithelial carcinoma. A p.Ile35Thr mutation was discovered in *CTNNB1* in two cases of adenoid cystic carcinoma. ERBB2 alterations were remarkable for SDC ex PA. Furthermore, *TP53* mutation was established as a relevant negative prognostic factor for overall survival ($p=0{,}04$). The analysis revealed potentially actionable genes in detected alterations in: MECA 100% (1/1), SDC 100% (7/7), AD 92% (11/12), Ca ex PA 82% (18/22), MECA 65% (20/31), AdCC 64% (9/14) and AcCC 0% (0/1).

Conclusions SGCs are a heterogeneous group of malignancies with distinct molecular landscape that characterized by poor prognosis and inadequate treatment options. Nonstandard strategies might be beneficial for patients who suffer from salivary gland cancers. Wider utilization of NGS analysis may increase the opportunity for patients with those rare cancers to receive more precise, personalized therapy.

[†]Julia Pikul and Marcin M. Machnicki contributed equally to this work.

[†]Anna Rzepakowska and Tomasz Stokłosa contributed equally are jointly supervised this work.

*Correspondence:
Anna Rzepakowska
arzepakowska@wum.edu.pl
Full list of author information is available at the end of the article

Keywords Salivary gland cancer, Next-generation sequencing, Genetic analysis, Mutational landscape, Targeted therapy, Precision oncology

Introduction

According to the International Agency for Research on Cancer in 2020, more than 19 million new cancer cases were recognized worldwide, and nearly 10 million deaths were registered. Salivary gland cancers (SGCs) constituted 53 583 of all morbidity cases (0.3%) and the mortality was greater than 20 000 cases (0.2%) [1]. The data demonstrates an unfavourable prognosis in most SGCs patients.

Although SGCs constitute 8.5% of head and neck malignancies, they are characterised by considerable aggressiveness and mortality [2].

Distant metastases are observed in 20% of cases and are associated with high-grade pathological types, tumour size, vascular infiltration, perineural invasion and genetic mutations, resulting in poorer patient outcomes [3]. Recurrent or metastatic (R/M) salivary gland cancer patients have the median overall survival (OS) of 15 months, because there are no specific therapeutic options recognized [4].

An additional complicating factor is the histological heterogeneity of SGCs [5]. In compliance with the World Health Organization (WHO), more than 20 various types of salivary gland malignancies are distinguished; and the most common is mucoepidermoid carcinoma (MEC), followed by acinic cell carcinoma (AcCC), adenoid cystic carcinoma (AdCC), carcinoma ex-pleomorphic adenoma (Ca ex Pa), and adenocarcinoma (AC) [6, 7]. An accurate diagnosis could therefore be challenging, with a substantial risk of misdiagnosis and delayed treatment. Uncertain cases with morphologic or immunohistochemical overlap require molecular tests for definitive classification, and modern diagnostic methods are moving in that direction with increasing confidence and accuracy [7]. The current SGCs classification of the World Health Organization (WHO) includes molecular alterations in the differential diagnosis [7]. The standard therapy in SGCs is complete surgical excision of the pathology, with postoperative radiotherapy or chemoradiotherapy, depending on the tumour stage and histological features [8]. Current recommendations of the American Society of Clinical Oncology (ASCO) include postoperative radiation (RT) for patients with lymph node metastases, perineural or vascular invasion but in all AdCC cases [9]. The rare incidence prevents the possibility of randomized clinical trials in SGCs to compare the outcomes of surgery with or without postoperative RT [10]. Therefore, the risk of overtreatment in some cases is impossible to avoid. The indication for systemic therapy in SGCs is not clearly defined with the moderate strength of the recommendations [9].

SGCs patients are applied to standard systemic therapy, similar to other head and neck cancer patients. The situation is even worse for recurrent or metastatic SGCs, for which reliable evidence for optional regimen lacks. We still search for more specific therapies for this heterogeneous group of cancers and recognize the possibility to identify relevant molecular factors in order to optimize and individualize the protocols. Nowadays, genetic alterations are becoming essential not only in proper diagnosis but also creating personal precision medicine. At the present time, increasing evidence confirms the significance of mutations in the *NOTCH 1–4, MYB, VEGF,* and *EGFR* genes in AdCC as well as the expression of *HER2* and *AR* in SDC for extended and targeted treatment possibilities [11–13]. In parallel, ongoing clinical trials estimate immune checkpoint inhibitors in SGCs [11]. However, in rare solid tumors we observe an increasing number of basket trials, where therapeutic intervention is designed for patient's tumor specific aberration [14]. The procedure is especially promising for SGCs patients for whom other standard treatment options in the recurrent or metastatic disease stage are unavailable. The approach based on patient's specific genetic aberrations therapy, substitutes the phase III trials in the conventional drug registration route, which is of great difficulty in rare cancers.

Currently, a large number of genetic aberrations have been matched with specific therapies. Therefore, it is crucial to search for nucleotide and copy number variants in SGCs patients with poor prognosis.

Taking under consideration the above, we attempted to evaluate the molecular landscape of the most prevalent salivary gland malignancies. Our study was designed to compare the prevalence of DNA aberrations in SGCs patients with different oncologic outcomes after a standard treatment protocol. A DNA-based targeted next-generation sequencing (NGS) panel was used to detect single and multiple nucleotide variants and copy number variants. The literature review was performed to select genes that have been so far identified as potentially relevant in the diagnosis and prognosis of different types of SGCs. The remaining genes were selected depending on their involvement in commonly known signalling pathways [15].

The final analysis of our cohort included 79 genes with potential actionable aberrations for targeted therapies and others linked by common molecular signalling pathways, as well as those related to a worse prognosis and are collected in Table 1.

Materials and methods

The study was conducted in accordance with national guidelines and regulations and approved by the Bioethics Committee at Medical University of Warsaw (reference number: AKBE/175/2021).

SGCs samples collection

The medical records were searched to select patients with the primary diagnosis of SGCs treated surgically with the radical intent in the Otorhinolaryngology, Head and Neck Surgery Department of the Medical University of Warsaw between 2010 and 2017. The exclusion criteria involved: (1) histological types other than mucoepidermoid carcinoma (MEC), adenocarcinoma (AC), myoepithelial carcinoma (MECA), adenoid cystic carcinoma (AdCC), salivary duct carcinoma (SDC), acinic cell carcinoma (AcCC) and carcinoma ex pleomorphic adenoma (Ca ex PA); (2) history of radiation in head and neck region; (3) incomplete treatment after the operation with radiotherapy or chemoradiotherapy according to the protocol; and (4) inaccessible follow-up data until May 2023. The study was designed for NGS evaluation of 40 tumor samples, and an adequate number of formalin-fixed paraffin-embedded (FFPE) blocks were retrieved from the repository of the Pathology Department. The diagnosis of the tumor type, histopathological features and advancement was verified in each case by an experienced pathologist.

DNA next-generation sequencing

DNA was isolated from manually dissected tissue fragments from FFPE blocks, selected based on histopathological examination of hematoxylin and eosin-stained sections. Isolation was performed using QuickGene-Auto12S/24S nucleic acid extractor and AutoS DNA Extraction FFPE Tissue Kit (Kurabo), according to manufacturer's protocol.

For each sample 100–500 ng were converted to genomic libraries using Library Preparation Kit (Twist Biosciences). Libraries were then pooled and enriched using a Custom Panel (Twist Biosciences), capturing ~ 5 Mb of coding sequences of 1345 cancer-associated genes and selected non-coding regions. Enriched libraries were sequenced on NovaSeq 6000 (Illumina) instrument using 2 × 150 bp reads. Mean coverage was in range of 63.7–751.9 × (median 369.3 ×) and ge20 was in range 95.4–98.6% (median 98.3%) for all samples.

Raw sequencing data processing was done according to Broad Institute recommendations [16] and involved quality control of FASTQ files, read mapping to hg38 genome using BWA-MEM [17], duplication removal, quality recalibration using GATK and Picard and variant calling using HaplotypeCaller and Mutect2 [16].

Common variants were filtered out using public (gnomAD) and internal databases [18]. The remaining, rare variants were classified with the aid of bioinformatics predictors and databases (REVEL [19], PrimateAI [20], SpliceAI [21], dbNSFP [22], ClinVar [23], COSMIC [24], cBioPortal [25]), internal (Genebe.net) [26] and external (Varsome.com) [27] implementations of ACMG classification and published data.

Copy-number variations (CNVs) were identified with CNVkit 0.9.5 [28] and copy-neutral losses-of-heterozygosity were identified using an in-house script. Python

Table 1 The genes analysed in SGCs and their represented signalling pathways. Bolded are genes with ongoing clinical trials according to the OncoKB™ website platform and literature

Signaling pathway	Genes
Cell cycle	***CDKN2A***, *CCND2*, ***CDK4***, *CCNC*
DNA damage response	*MDM1*, ***MDM2***, *MDM4*, ***TP53***
DNA mismatch repair	***MLH1***, ***MSH2, MSH6, PMS1-2***
Epigenetic regulation	***KDM6A***, *KMT2C, KMT2D, NSD1*
Homologous recombination in DNA repair	***ATM, BRCA1-2, BRIP1, CHEK2, ERCC2***
NOTCH	***FBXW7***, ***NOTCH 1–4***
PI3K/AKT/mTOR	***AKT1, PIK3CA, PTEN, TSC2, RICTOR***
RTK-Ras-ERK	***ALK, BRAF, ERBB2***, *ERBB3*, ***EGFR, FGFR1-3***, ***FGFR4***, ***HRAS, KRAS***, ***RAF1, MET, NF1, NRAS***
SWI/SNF complex	***ARID1***, *ARID1B, ARID4B,* ***SMARCA2,*** *SMARCA4,* ***SMARCB1***, *SMARCC1*
WNT- beta- catenin	*AJUBA, APC, AXIN1, AXIN2, CDH1,* ***CTNNB1***, *FAT1*
Others	*AR, ETV6, MYB, MAPK1, IGF1, NFKB1,* ***NTRK1-3,*** *PRKD2, PTPN11, RELN,* ***TERT***, *FRS2,* ***EZH2***, *PBRM1*

and R packages Pandas 2.1.3 and Maftools 2.18.0 were used for data handling and visualization [29].

Statistical analysis of associations between genetic variants and clinical data was done using Maftools.

Maftools function *SomaticInteractions*, which performs Pair-wise Fisher's Exact test, was used to detect mutual exclusivity or co-occurrence of mutational events (small-scale mutations and CNVs). *MafSurvival* function was used to draw Kaplan–Meier curves, hazard ratios and unadjusted *p*-values and analyze patient survival (OS and RFS) with regard to small-scale mutations and CNVs. We analyzed the prognostic impact of pre-defined groups of genes, selected on the basis of their involvement in signalling pathways (Tables 1 and 3), as well as of single genes and concurrently mutated pairs of genes. The significance of the latter two was estimated using a similar *maftools* function, *SurvGroup*; we limited this analysis to the genes mutated in at least 3 patients.

The analysis of genetic data included only pathogenic or likely pathogenic variants and CNVs.

Descriptive statistics were used to summarize the clinical data, which were analyzed using SPSS version 25.0.

Potentially actionable genes, were highlighted in Table 1, based on the OncoKB™ website platform [30, 31], ongoing clinical trials, and the available literature [32–38].

Results

Patient characteristics

A total of 40 patients were initially included in this study, but the NGS data of reliable quality were acquired for 37 patients, who constituted the final study cohort. One patient was excluded from the study after histopathological re-evaluation (SG15). Two patients (SG8 and SG26) were excluded due to sequencing failure resulting from poor-quality DNA. In four patients (SG20, SG32, SG33, and SG37), some CNV results were manually curated due to high noise levels in CNV calling. However, it is unlikely that the ability to identify high-level amplifications or deep deletions was substantially affected.

The evaluated SGCs types included: 7 MEC, 7 AC, 6 MECA, 6 AdCC, 4 SDC, 2 AcCC and 5 CA ex PA (3 SDC ex PA (sample number: 36, 37, 39); 2 AC NOS (sample number: 35, 38)). The median age at the diagnosis was 59.7 years (range 21–87), and 62% were female. The primary tumor was located in the parotid gland in 31 cases (84%), and in the submandibular gland in the other 6 cases (16%). The pathological TNM staging revealed 25 patients (67.6%) with T1 and T2 advancement, and 27 (73%) without nodal involvement. Three patients (1 with AdCC and 2 with SDC) had suspicious lung nodules that were potentially metastatic on chest CT. All patients were treated surgically with the curative intent; however, the final histology revealed nonradical resection (R1) in 6 patients, in all cases due to very close margins. Perineural invasion was identified in 6 patients, and vascular infiltration was even rarer– 2 patients. The postoperative radiotherapy (RT) was applied in 28 patients, and systemic treatment with RT was applied in 4 patients. 19 SGCs patients in the study cohort developed recurrent/metastatic disease during follow-up and were identified as poor outcomes patients. The mean disease-free survival (DFS) for this subgroup was 2.4 years, and the overall survival (OS) was 5.4 years. The other 18 patients were disease free during the follow-up of at least 5 years and were considered favourable outcomes patients with the mean DFS and OS of 8.3 years. The group with poor survival rates was older, with a mean age of 63.5 years, compared with 55.7 years in the favourable outcome subgroup. The sex distribution was similar. The histopathology type of MEC yielded the most favourable outcomes with recurrent disease in only one patient. Whereas all patients with SDC had the disease progression ($n=4$). The outcome distribution in other cancer types was comparable. The subgroup with a poor prognosis had more advanced tumor size (8 patients with T3 and T4) and the higher rate of nodal involvement (9 patients with N+). The three patients with lung metastasis experienced disease progression during follow-up.

The clinical data was collected in Table 2.

Only pathogenic or likely pathogenic mutations were considered further. In our cohort, we found genetic abnormalities in 73% of the patients (27/37). Moreover, 96% of patients with identified gene mutation had at least one mutation in potentially actionable genes (26/27).

Among the 79 analysed genes, 49 of them were potentially targetable (62%). In 55% of this group at least one change was detected. Furthermore, 70% of the study cohort showed mutations in those genes, including 89% of the patients with poor outcomes. In all patients with poor outcome and SDC the mutations in potentially targetable genes were identified 100% in SDC (7/7). In other histological types the rates were also high with 92% in AD (11/12), 82% of Ca ex PA (18/22), 65% of MECA (20/31), 64% of AdCC (9/14) and 100% MEC (1/1).

Somatic mutations

In our cohort, we identified *NF1* ($n=8$) and *TP53* ($n=8$) genes as the most frequently mutated. These alterations were predominantly harboured by patients with poor outcomes (6 patients for each gene: MEC, AC, MECA, SDC, 2 Ca ex PA (SDC ex PA, AC NOS ex PA) and AC, 2 SDC and 3 Ca ex PA (2 AC NOS ex PA, 1 SDC ex PA), respectively). *TP53* mutation was observed in 3 patients with Ca ex PA (50%) with poor outcome. This mutation was also found in 50% SDC, whereas *NF1* abnormalities

Table 2 Clinical characteristics of the study cohort

	All patients	Disease relapse	Disease free survivals
Age (years)	59.7	63.5	55.7
Male/Female	14/23	8/11	6/12
Location (No)			
Parotid gland	31	17	14
Submandibular gland	6	2	4
Histopathological types (No)	37	19	18
Mucoepidermoid cancer	7	1	6
Adenocarcinoma	7	4	3
Myoepithelial carcinoma	6	3	3
Adenoid cystic carcinoma	6	3	3
Salivary duct carcinoma	4	4	0
Acinic cell carcinoma	2	1	1
Carcinoma ex pleomorphic adenoma	5	3	2
TNM staging (Tumor)			
T1	9	5	4
T2	16	6	10
T3	8	6	2
T4	4	2	2
TNM staging (Nodules)			
N0	27	9	16
N1	6	3	3
N2	7	6	1
TNM staging (Metastases)			
M0	34	15	19
M1	3	3	0
Perineural invasion	6	4	2
Perivascular invasion	2	2	0
Radical dissection			
R0	31	14	17
R1	6	4	2
Type of surgery:			
Superficial parotidectomy	5	3	2
Total parotidectomy	19	9	10
Radical parotidectomy	7	4	3
Submandibular gland resection	6	2	4
Selective neck dissection	19	9	10
Adjuvant therapy			
None	5	0	5
RT	28	16	12
RT with CT	4	3	1
Disease free survival (years)	5.1	2.4	8.3
Overall survival (years)	6.4	5.4	8.3

RT radiotherapy

CT chemotherapy

were detected in half of the MECA patients (2 with favourable and 1 with poor outcome). Furthermore, the only one MEC patient who developed recurrence, harboured multiple mutations in *NF1* (two missense and single splice-site).

Co-mutation of these genes was detected in 4 patients with the disease relapse (2 patients with Ca ex PA, AC and SDC).

The genetic changes that were confirmed solely in patients with unfavorable outcomes included: *ARID1A* ($n=3$; AC, MECA, SDC), *ERCC2* ($n=2$; AC, AdCC), *NSD1* ($n=2$; AdCC, Ca ex PA), *ARID1B* (AdCC*), FGFR2* (MECA*), FGFR4* (Ca ex PA), *KMT2C* (AC), *NOTCH1*(AdCC), *PTEN (Ca ex PA), SMARCB1(AC)* and *TSC2* (Ca ex PA*)* (each in a single case).

Another gene mutated in multiple cases was *HRAS* ($n=4$), mostly within the subgroup with disease relapse ($n=3$; 2 AC, 1 MECA). In various types of SGCs, mutations in *KMT2D (*AdCC, 3 cases of Ca ex PA*), PIK3CA* (AC, 2 cases of Ca ex PA) and *SMARCA2* (AC, 2 cases of SDC) were quite commonly detected. The *TERT* gene promoter (*pTERT*) was mutated in cases of AdCC and MECA with favorable outcomes. A hotspot mutation in *CTNNB1*(3:41224616-T > C, p.(Ile35Thr)) was found in two cases of AdCC. *ERBB2* mutation was unique to SDC ex PA.

The characteristics of the pathogenic genetic alterations in the cohort of salivary gland cancers are presented in Fig. 1.

Copy number variations

Significant copy number variations were detected in 9 patients (24%), and in 55% of cases they were related to unfavorable disease outcomes. *CDKN2A* biallelic deletion was identified as the most common change ($n=5$ cases:1 AC, 2 MECA, 1 AdCC, 1 SDC), and in all cases except AdCC, it was connected with the disease relapse. The most frequent amplifications of the *MDM1, MDM2* and *FRS2* genes coexisted in 3 cases of MECA (2 favourable and 1 unfavourable outcome). One of those patients (sample number 25) harbored the highest level of molecular changes, with additional *KRAS, TERT, RICTOR, CCND2, ETV6, ERBB3* and *CDK4* amplifications. Finally, amplification of *ERBB2* was observed purely in two samples of SDC ex PA. Figure 2 presents the co-occurrence of the gene mutations and copy number changes co-occurrence in the studied cohort.

Mucoepidermoid carcinoma

In the present study, 19% of patients suffered from MEC. In one sample, *NF1* mutation was detected. This case was related to an unfavourable outcome, which is a rare event in this subtype.

Adenocarcinoma

In 80% ($n=4$) of patients with this subtype, worse prognoses were reported. In that group, alterations in *ARID1A*, *ERCC2*, *FBXW7*, *KMT2C*, NF1 and *PIK3CA* among others, were found. Additionally, *HRAS* mutations were harboured in 2 patients with 2- and 3-year OS. Mutations in *TP53* gene were discovered in both patients with and without relapse.

Myoepithelial carcinoma

The MECA subtype was the most abundant in different genetic alternations. The sample number 25 was the most changed one. *HRAS, ARID1A,* and *NF1* mutations, and amplifications of the *CCND2, CDK4, CHEK2, ERBB3, ETV6, FRS2, KRAS, MDM1, MDM2, RICTOR, TERT*, as well as *CDKN2A* deletion, were found in patients with unfavourable outcomes. Whereas, in the counterpart group mutations in *FGFR2, NF1, TERT, TP53, CHEK2, PTPN11*, deletion of the *CDKN2A*, as well as amplification of the *FGFR1, FRS2, IGFR1, MDM1,* and *MDM2* were observed.

Adenoid cyctic carcinoma

64% of all changed genes constituted those with potential actionability. The same p.Ile35Thr mutation was discovered in *CTNNB1* in two cases in this subtype. In one sample, it was exclusively genetic change and the patient outcome was established as poor. Moreover, in that group mutations in *ARID1B, KMT2D, NOTCH1, NSD1* and *ERCC2* were found. In patients with favourable outcomes, we observed alterations in *HRAS, FGFR3, PRKD2, SMARCA2, TERT, CCNC* and *CDKN2A* as well.

Salivary duct carcinoma

All alternations detected in SDC were targetable ones. Outcomes in that subtype were established as poor in all cases. Alterations in *ARID1A, SMARCA2, NF1, TP53*, and *CDKN2A* were found.

Acinic cell carcinoma

The *ETV6* mutation was the only one that was found in AcCC in a 21-year-old male patient.

Carcinoma ex pleomorphic adenoma

82% of detected alternations were potentially actionable. *ERBB2* aberrations were exclusive for SDC ex PA. *TP53* mutations were found in this subtype purely in patients with poor outcomes. In that group: *KMT2D, NF1,*

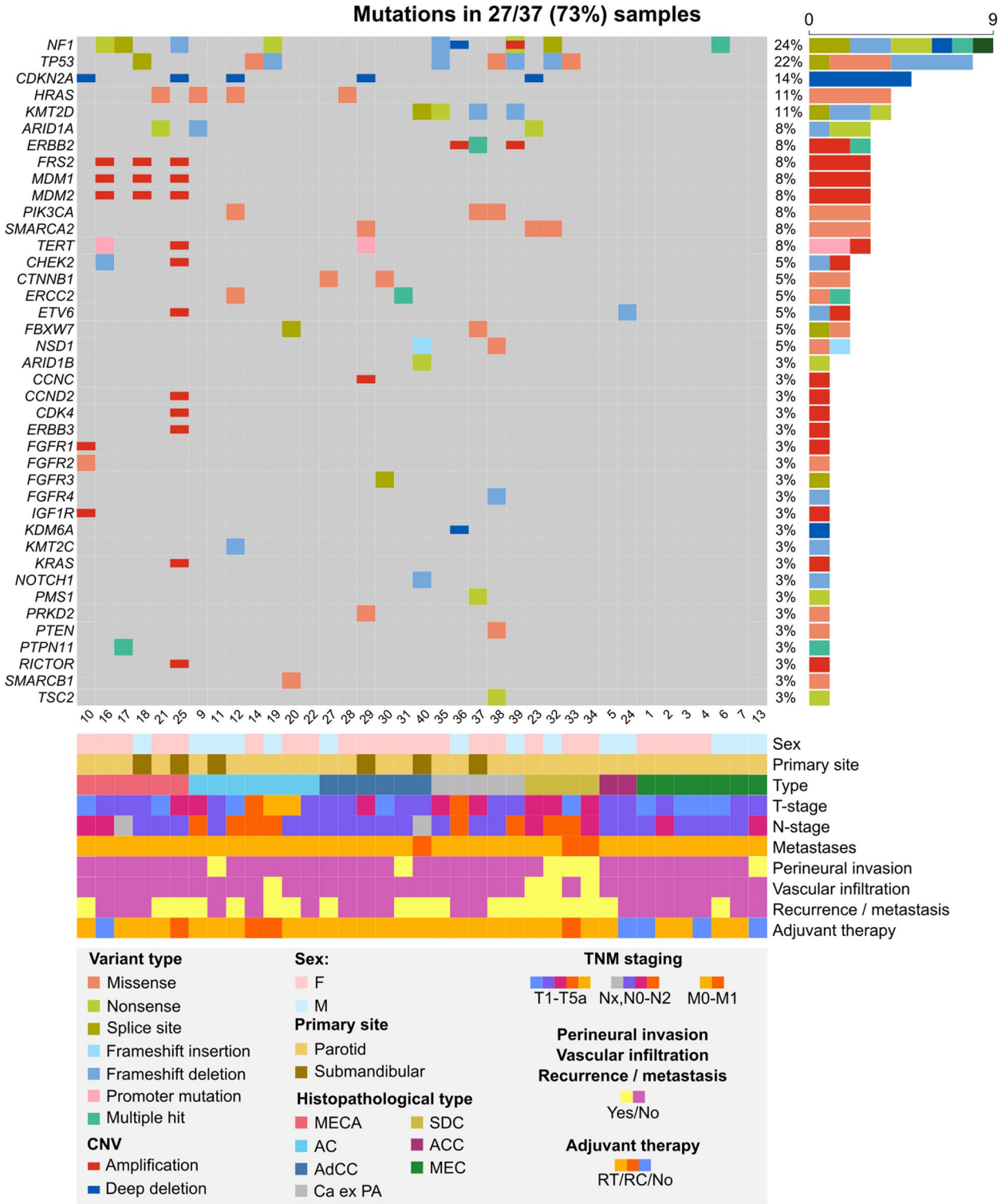

Fig. 1 The characteristics of the pathogenic genetic alterations in the cohort of salivary gland cancers

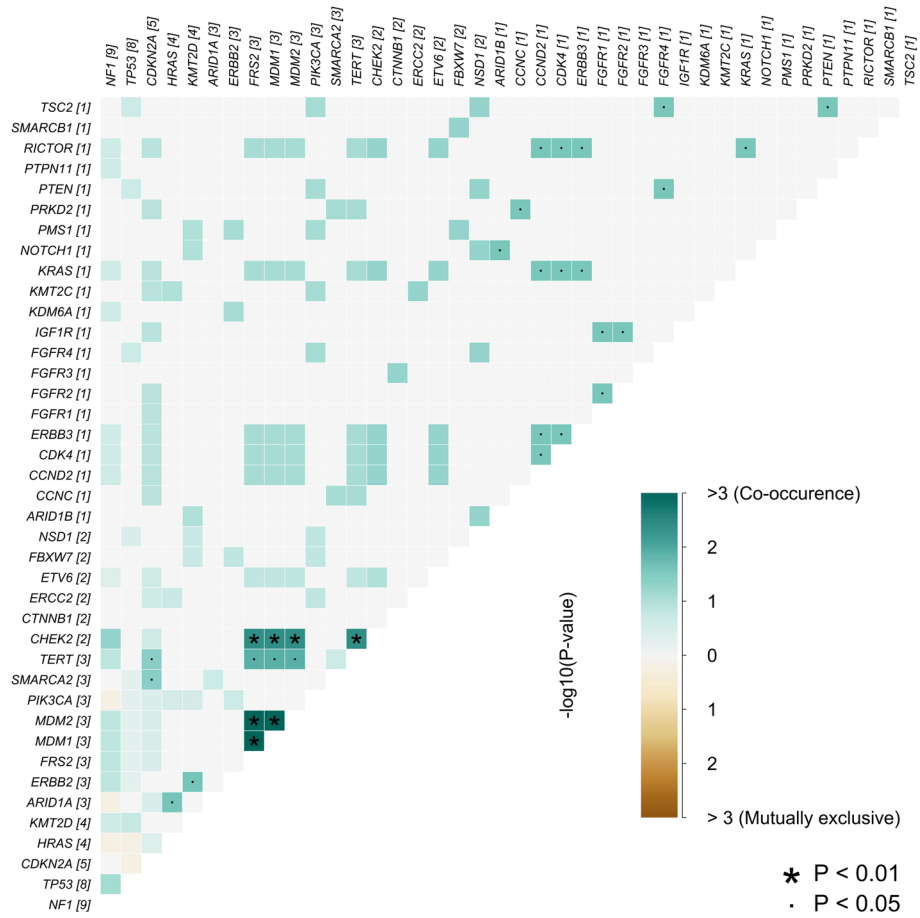

Fig. 2 The co-occurrence of the gene mutations and copy number changes in the studied cohort of salivary gland cancers

FGFR4, NSD1, PIK3CA, PTEN, and *TCS2* alterations were also revealed.

Correlation of gene alterations and outcomes

The analysis confirmed that disease-free survival was influenced by the presence of *ARID1A* mutation ($p=0.005$). A significant decrease in DFS was also noted for patients with mutations in at least one of the "chromatin remodeling genes" (*ARID1A, ARID1B, SMARCA2, SMARCB1, p=0.02*) with simultaneous mutations of *TP53* and *NF1* ($p=0.02$). *TP53* mutation was also confirmed as a significant negative prognostic factor for overall survival in the study group ($p=0.04$). A significant impact on OS was also demonstrated when at least one gene from the following groups was affected:

- *MDM1, MDM2, TP53 (p=0.006)*
- *PIK3CA, PTEN, TSC2 (p=0.02)*
- *ERBB2, ERBB3, FGFR1, FGFR2, FGFR3, FGFR4, HRAS, KRAS, NF1, PTPN11 (p=0.006)*
- *HRAS, KRAS, NF1, PTPN11 (0.03)*
- *ERBB2, ERBB3, FGFR1, FGFR2, FGFR3,FGFR4 (0.03)*

The results of regression analysis of the influence of gene mutations on DFS and OS are presented in Table 3.

Figure 3 presents the Kaplan -Meier curves for DFS and OS in the studied cohort in relation to the identified genetic alterations.

Discussion

Estimates predict an increase in the incidence of new SGCs cases over the next 20 years in Asia, Northern America and Europe, with rates expected to rise by 50%, 40% and 20%, respectively [39]. To prevent the consequent increase in morbidity, there is a need for more reliable prognostic markers, well-defined predictive factors and targeted treatment options. Therefore, delineating the genetic landscape of salivary gland cancers has become imperative to enable the most precise care in the near future. In the present study, we comprehensively investigated approximately 80 genes for potentially

Table 3 The regression analysis of gene mutations influence on the disease free survival (DFS) and overall survival (OS)

	Mutated genes + CNV	*p*-value	HR	WT	Mutant
DFS	*CDKN2A*	0.06	2.80	32	5
DFS	*TP53*	0.08	2.36	29	8
DFS	*NF1*	0.16	1.96	28	9
DFS	*ARID1A*	**0.005**	6.16	34	3
DFS	Chromatin remodelling *ARID1A, ARID1B, SMARCA2, SMARCB1*	**0.02**	3.15	30	7
DFS	*NF1,TP53*[a]	**0.02**	3.82	33	4
OS	*TP53*	**0.04**	2.15	29	8
OS	*NF1*	0.14	2.15	28	9
OS	*CDKN2A*	0.22	2.23	32	5
OS	TP53 pathway *MDM1, MDM2, TP53*	**0.006**	3.75	27	10
OS	PI3K/AKT/mTOR pathway *PIK3CA, PTEN, TSC2*	**0.02**	3.84	33	4
OS	RTK-RAS-MAPK pathway *ERBB2, ERBB3, FGFR1, FGFR2, FGFR3, FGFR4, HRAS, KRAS, NF1, PTPN11*	**0.006**	4.37	20	17
OS	MAPK pathway *HRAS, KRAS, NF1, PTPN11*	**0.03**	2.92	23	14
OS	RTKs *ERBB2, ERBB3, FGFR1, FGFR2, FGFR3, FGFR4*	**0.03**	3.05	30	7

WT wild-type / no mutation

[a] simultaneous mutations

actionable and clinically relevant aberrations, particularly those related to poor outcomes in different subtypes of SGCs. In our cohort, 70% of SGCs patients presented with genetic aberrations with potential actionability, and in the subgroup with disease relapse the rate was 89%. According to the literature, the proportion of patients with actionable genetic aberrations varies among subtypes, ranging from 28.3% in AdCC to 81.8% in SDC [40].

Most frequently identified gene mutations

Mutations in *TP53* are frequently observed in various sporadic cancers including 40% of head and neck cancers (HNC) [41], and are associated with unfavourable patients outcomes and chemoresistance [41–43]. We detected *TP53* mutations in 22% of the SGCs patients ($n=8$; 3 Ca ex PA, 2 SDC, 2 AC, 1 MECA), and the majority ($n=6$, 75%; 3 Ca ex PA, 2 SDC, 1 AC) of the identified alterations were associated with cases exhibiting unfavorable outcomes (32%), with a significant negative impact on overall survival (Fig. 3).

Similarly, *TP53* mutation is found to be one of the most commonly occurring mutations in various subtypes of SGCs. In our previous study, which provided a comprehensive literature review of the molecular landscape of SGCs, *TP53* abnormalities were described in: 55–100% of Ca ex PA, greater than 80% in mucinous adenocarcinoma (MAC), 39–60% of SDC and 21–42% of MEC [44]. Furthermore, Ross et al. found a high occurrence of *TP53* mutation in R/M cases of adenocarcinoma NOS [45]. Interestingly, this alteration is uncommon in AdCC, including those with recurrence and metastasis [32, 46, 47].

Nowadays, numerous attempts are being made to affect p53, including *MDM2* inhibition [48]. Promising results have been obtained in a phase Ia/Ib trial of the MDM2–p53 antagonist brigimadlin in patients with advanced or metastatic solid tumours [49]. Furthermore, persistent concerns regarding making *TP53* targetable have led to advanced research development. Preclinical trials presented approximately 80% tumour regression in mice

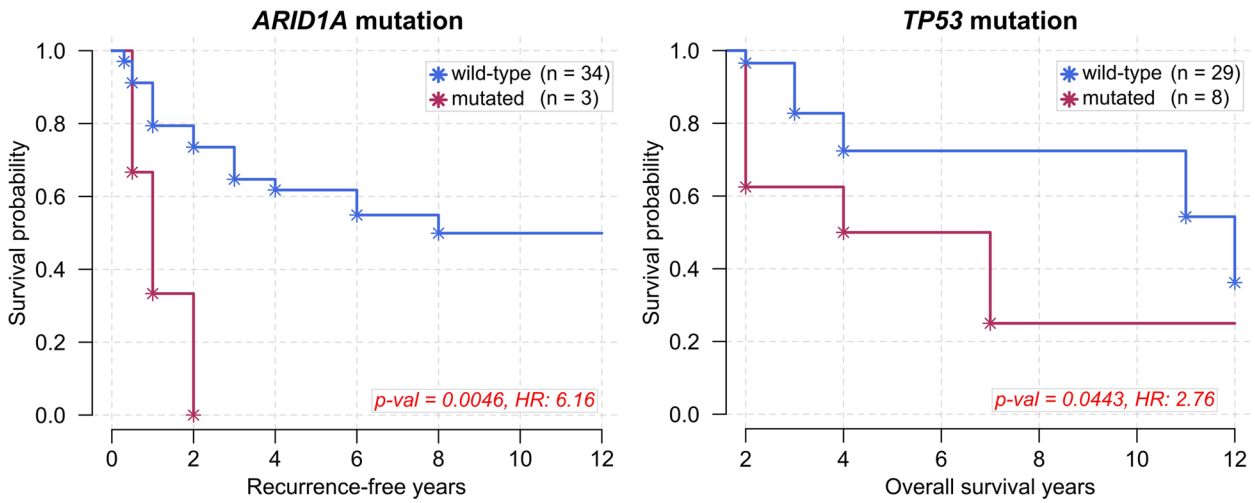

Fig. 3 The Kaplan -Meier curves for disease free survival and overall survival in the studied cohort in relation to the identified genetic alterations

that received orally p53 protein-selective reactivator [50]. At the present time, phase a 1/2 of the clinical trial NCT04585750 is ongoing. The trial has evaluated the efficacy of PC14586 (rezatapopt), the first oral, small molecule p53 reactivator, in monotherapy and in combination with pembrolizumab in participants with advanced solid tumors harbouring a *TP53* Y220C mutation (PYNNACLE) [51].

Mouse double minute 2 (*MDM2*) is an oncogene responsible for the negative regulation of *TP53*, with some evidence suggesting a rare tumour suppressor function. Increasingly, the *TP53*-independent role of *MDM2* in tumorigenesis is highlighted, particularly as it impacts the cell cycle (ubiquitination and degradation of cell-cycle regulators, such as Rb, p21, and Fox3A) and suppresses apoptosis. *MDM2* also contributes to metastasis because it participates in epithelial-mesenchymal transition (EMT) through the regulation of E-cadherin [52–55]. *MDM2* amplification is particularly frequent in soft tissue tumours, whereas it occurs seldom in other types of cancers [56]. Among SGCs, MDM2 amplification has rarely been detected in SDCs. Few studies have reported *MDM2* amplification in SDC and Ca ex PA as well [57–59]. Moreover, Persson et al. proved that *MDM2* amplification is one of the factors responsible for the malignant transformation of benign pleomorphic adenoma (PA) [60]. Our study revealed *MDM2* amplification exclusively to MECA (3 cases), including one patient with an unfavourable outcome. Interestingly, the *MDM2* amplifications detected in our patients were accompanied by coamplification of *MDM1* and the fibroblast growth factor receptor substrate 2 gene (*FRS2*). Such *MDM2* and *FRS2* alterations were described as frequent in soft tissue malignancies [61–63]. However, the role of these findings in SGCs have not been established yet.

Aberrations of *MDM2* were also shown to affect cancer therapy (apart from the above affecting p53), yet the mechanisms in detail have not been established. Firstly, in HER2 positive breast cancer, resistance to the HER2 inhibitor-lapatinib might occur in MDM2-amplified tumors [64]. Secondly, radioresistance and poor disease-free survival rates were observed in patients with MDM2-amplified oral squamous cell carcinoma. Moreover, attempts are being made to determine whether *MDM2* could become both a diagnostic and prognostic biomarker [53].

Alterations of the RAS- mitogen-activated protein kinases (MAPK) signalling pathway, which regulates, among others, cellular growth, proliferation and apoptosis, are commonly described changes in human cancers [65, 66]. In our cohort, we identified *NF1* gene as the most frequently mutated (8 patients with small-scale mutations; 3 MECA, 2 Ca ex PA, 1 MEC, 1 AC, 1 SDC, one with concurrent amplification in SDC ex PA and one with deep deletion in SDC ex PA) and particularly occurring in the subgroup with poor outcomes (in 1: MEC, AC, SDC, MECA and 2 cases of Ca ex PA). Neurofibromin is an *NF1* tumour suppressor gene product, which downregulates RAS. Loss of *NF1* causes elevated activation of RAS-MAPK pathway by increasing RAS-GTP levels, and consequently leads to uncontrolled cell growth. Additionally, cells are prevented from apoptosis due to elevated phosphoinositide-3 kinase PI3K/AKT/mTOR signalling pathway stimulation [15]. *NF1* germline variants cause a well-known hereditary cancer syndrome, neurofibromatosis type 1 (NF1), while somatic mutations are frequently found in sporadic cancers [15]. Interestingly, *NF1*-mutated tumors are characterized by aggressiveness, metastasis, radio- and chemoresistance (including to cisplatin), hence the patient's adverse outcomes [15, 67–70].

Among SGCs, these alternations have been described mainly in SDC in 7–20%, of cases as well as other histopathological subtypes such as SDC ex PA, AC, MEC or AcCC [44]. Moreover, Kato et al. proved significant dependence of the cooccurrence of *NF1* and *TP53* gene mutations in SGCs in univariate analysis in 75% of *NF1*-mutated cases [71]. In this study, *NF1* and *TP53* comutations were observed with increased frequency, exclusively in patients with unfavourable outcomes; in AC, SDC and 2 Ca ex PA, and were significantly associated with decreased DFS.

Currently, clinical trials are ongoing for sporadic cancers with *NF1* alterations. Researchers focus particularly on inhibition of two above-mentioned signalling pathways, utilizing the MEK inhibitors, or inhibitors of the PI3K-AKT-mTOR pathway as well as immunotherapy [69].

Mutations of another member of RAS-MAPK, *HRAS,* are relatively common in HNC. The meta-analysis by Novoplansky et al. on prevalence of *RAS* mutations in HNC confirmed the highest rate for *HRAS* (7%) and found it more prevalent in oral cavity and salivary gland tumours [72]. In available literature these alterations were described in high occurrence in: EMC (27–87%), SDC (11–49%), Ca ex PA (4–23%), MEC (~ 10%) and apocrine subtype of intraductal carcinoma (IC), as well [44]. Interestingly, *HRAS* mutation is known as one of the most common in EMC. The study conducted by Urano et al. and Nakaguro et al. maintained that *HRAS* mutation has not been reported before in SGCs histopathological types that resembled EMC [73, 74]. Nevertheless, in our study, we confirmed *HRAS* mutation in 4 cases (11% of the study cohort): two adenocarcinoma (AC), myoepithelial carcinoma (MECA), adenoid cystic carcinoma (AdCC), which include the entities manifesting EMC-like features.

Moreover, the overall survival of two adenocarcinoma patients with *HRAS* constituted only 2 and 3 years, with early disease recurrence. The *HRAS*-mutated MECA had also poor survival outcome, contrary to *HRAS*-mutated AdCC. Moreover, in two *HRAS*-mutated cases (AC and MECA) with poor outcomes, the AT-rich interaction domain 1A (*ARID1A)* comutations were found. Similar genetic coincidence was described by Rupp et al. in a 70-year-old female with parotid epithelial-myoepithelial carcinoma (EMC) [75], however, the outcome of the patient remained unknown.

Currently, tipifarnib is being evaluated in clinical trials as a promising, selective inhibitor of farnesyltransferase in *HRAS* mutated HNC [76, 77]. Moreover, evaluation of tipifarnib efficiency among R/M HRAS-mutated SGCs has shown relatively promising results, including the median OS constituted 18 months (95% CI, 9.6–22.4 months) [78]. However, further clinical trials with suitable numbers of participants are required.

ARID1A gene is the subunit of SWITCH/Sucrose Non-Fermentable (SWI/SNF)- subfamily of ATP- dependent chromatin remodelling complexes. The loss of *ARID1A* function is related to cancer progression, aggressiveness and poor prognosis. *ARID1A* alterations occur quite frequently in various solid tumours, however are described rarely in SGC, mainly in AdCC and SDC [79–81]. Our analysis revealed *ARID1A* genetic alterations solely in patients with disease failure ($n=3$; AC, MECA, SDC) and, more generally, that SWI/SNF components' mutations were associated with recurrence. Changes in *ARID1B*, *SMARCA2*, *SMARCB1* were found as follows: AC, AdCC and SDC [82]. *ARID1A* variations may be related to cisplatin resistance, an essential agent in standard chemotherapy in HNC [79]. Utilization of Poly(ADP-ribose) polymerase (PARP) inhibitors and ATR inhibitors yield propitious results in *ARID1A*-mutated cancers [79, 82–84].

Finally, our investigation revealed several *CDKN2A* losses ($n=5$, 14%; 2 MECA, 1 AC, 1 AdCC and 1 SDC). These tumour suppressors encode p16 (INK4A) and p14 (ARF) proteins that are responsible for cell cycle regulation and are commonly lost in many cancers, including HNCs and SGCs as well.

Studies conducted by Wang et al. and Zerdan et al. described *CDKN2A* loss as one of the most commonly detected in MEC (~45%) [85, 86]. The first of them found these abnormalities exclusively in intermediate and high grade tumours. Nevertheless, clinical data in detail, particularly regarding the patients outcomes were not included in the above studies. MEC is characterized by *CRTC1-MAML2* fusions, while CNVs in MEC have not been frequently analysed. There are numerous studies with different conclusions regarding this fusion as a outcome predictors. In parallel, Anzick et al. revealed that other genetic alterations including *CDKN2A* in patients with *CRTC1-MAML2* fusion may lead to a deterioration of the patient outcome [87].

Moreover, *CDKN2A* alterations were detected in SDC de novo as well as in Ca ex PA [88, 89]. CDKN2A/B alterations were also found with high prevalence in AcCC, mainly in high-grade tumors and R/M cases [45, 90].

Cipriani et al. described *CDKN2A/B* loss beside recurrent *ETV6-NTRK3* fusion and *APC* mutation in rare case of high-grade transformation in secretory carcinoma (SC). The authors described the case of a 44-year-old male with a buccal tumour, who despite surgical excision distant metastases rapidly developed. Despite, further chemotherapy, the disease progressed quickly and doctors noted patient death in no time [91]. The authors link *CDKN2A/B* abnormalities to worse outcomes, which is uncommon in this SGCs subtype.

Treatment strategies tested in CDKN2A/B-deficient cancers include CDK4/6 inhibitors, immunotherapy as well as DNA methyltransferase inhibitors [92, 93]. Interestingly, an attempt of application of CDK4/6 inhibitors in combination with HER-2 inhibitor may come as a new potential druggable target, especially due to described poor response to HER-2 inhibition with simultaneous p16 loss [94].

Other alterations

The WNT pathway is a well-known signalling cascade involved in embryonic development, adult tissue homeostasis and regeneration [95]. Since its initial discovery, the WNT pathway has been associated with cancerogenesis. Its regulation is complicated and multilevel and aberrant activation can be triggered by mutations in *CTNNB1* gene, which encodes beta-catenin [35, 96]. In the present study, we identified recurrent *CTNNB1* p.(Ile35Thr) mutation in 5% of SGCs, solely in AdCC. In the available literature, this variant of mutation has been described primarily in salivary gland lesions, either benign basal cell adenomas or malignancies such as basal cell adenocarcinoma, AdCC and EMC as well. Moreover, *CTNNB1* alternations were also described in SDC [97–103]. Furthermore, a very rare case of MECA with a *CTNNB1* mutation in a 7-year-old female was described by Thompson et al. During 16 years of follow-up, nine recurrences and also numerous distant metastases, among others; to the liver, temporal bone as well as neck lymph nodes were observed. The very aggressive, atypical occurrence of the disease at a young age was probably related to *CTNNB1* mutation [104]. Standard therapy, including surgery, followed by RT or chemotherapy for nonresectable tumour, proves ineffective in such cases.

There is still no approved precision therapy targeting the WNT/beta-catenin pathway, mainly due to complex and not thoroughly understood network of interactions in the healthy and pathological tissue. Currently, the promising perspective is that the DKK1- neutralizing monoclonal antibody DKN-01 is under investigation in patients with hepatocellular cancer (NCT03645980). However, the antagonist mechanism of DKK1 on WNT/b-catenin signalling and cancer promotion is still unknown [105].

Telomerase has a fundamental role in tumorigenesis. The telomerase reverse transcriptase promoter (*pTERT*) is responsible for both telomerase activity and the regulation of telomere length. Abnormalities in *pTERT* are very common in different malignancies [38, 106, 107]. Interestingly, two *pTERT* genetic alterations were found in the MECA of our cohort. According to the current state of the knowledge, this genetic rearrangement is very rare in SGCs. Our research is the first to identify *pTERT* alterations in the de novo MECA subtype. Previously, Cormier et al. described a *pTERT* (c.−124C > T) mutation in a 76 -year-old female with advanced MECA ex PA [108], whereas Zare-Mirzaie et al. identified a *pTERT* mutation (c.−146 C > T) in an 82-year-old male with AdCC [109]. We also identified *pTERT* mutation in a female patient with AdCC without disease progression. Ho et al. study confirmed *pTERT* mutation in 13% of recurrent or metastatic AdCC of the salivary glands [32]. At the present time, *pTERT* mutation is related to advanced stage, relapse, or metastasis in many malignancies. Nevertheless, the results are inconclusive, and further studies are needed to establish the significance of TERT promoter mutations in outcome prediction in diverse types of cancers [106]. The potential treatments for *pTERT*-mutated tumors include immunotherapy, direct or indirect telomerase inhibitors, and nucleoside analogues, nonetheless, an effective strategy is still needed [38].

Fibroblast growth factors (FGFs) through their receptors (FGFRs), regulate the proliferation, migration, differentiation, and survival of normal cells [110–113]. Mutation of *FGFR,* which occurs in fewer than 10% of malignancies, is related to the development of numerous cancers in different tissues and is associated with an unfavourable prognosis [36, 114, 115]. In the present study, we identified *FGFR2* variation only in a MECA patient with unfavorable outcome, with coexisting *CDKN2A* deletion. In our previous study, *FGFR2* mutations were also found in two patients after radical PA excision, where the MECA quickly arose. In either PA or MECA (without a PA component), *FGFR2* point mutations were detected, which might be a factor that was responsible for the aggressiveness of the disease course [116]. In parallel, Dalin et al. in a comprehensive genetic analysis of MECA tumors found *FGFR2* mutations in both de novo and MECA ex PA lesions [117]. Moreover, the patients outcomes were poor due to recurrences. Other *FGFR* alternations were found also in single cases in: SDC and Ca ex PA (malignant component of the MECA and SDC) [117, 118].

The United States Food and Drug Administration (FDA) approved erdafitinib, infigratinib, derazantinib or futibatinib, among other specific inhibitors of FGFRs,—in urothelial carcinoma and cholangiocarcinoma therapy after confirmation of their clinical efficacy and durable responses [36]. Thus, increased molecular profiling, especially in SGCs patients with either advanced-stage or metastasis, may provide future opportunities for precision therapy.

Current recommendations of the ESMO—European Reference Network on Rare Adult Solid Cancers (EURACAN) propose genetic analysis in salivary gland cancers for possible targeted treatment of genes, which are commonly identified as mutated in other solid cancers, including *PIK3CA, BRAF* and *MET* [10]. In the light of results from the literature and presented findings, the recommendations for SGCs therapy can change in the near future. Therefore, the emerging role of in-depth molecular analysis of the widest possible cohort of SGCs to maximize the precision is still an open task for the next few years.

Our presented study has several important limitations, namely, its retrospective nature with a limited number of patients and the absence of gene fusion analysis, which may be of increasing importance in this type of cancers.

Conclusions

Salivary gland carcinoma is a rare entity, distinction of both histopathological recognition and mutational landscape, prevents from the implementation of clinical trials. In this study, the most frequent alterations were: *NF1* (24%), *TP53* (22%) and *CDKN2A* deletions (14%), in that majority of cases, poor patient prognoses were noted. Genetic aberrations with potential actionability were identified in in 70% of the SGCs patients and 89% of the recurrent or metastatic patients. Increased NGS analysis utilization holds the potential to play a substantial role in comprehensive molecular landscape recognition in SGCs. Thus, the designation of outcome predictors ensures suitable oncological supervision. Moreover, we believe in increasing SGCs patients' access to the personalized therapy in the near future.

Abbreviations

SGCs	Salivary gland carcinomas
MEC	Mucoepidermoid carcinoma
AC	Adenocarcinoma
NOS	Adenocarcinoma not otherwise specified
MECA	Myoepithelial carcinoma

AdCC	Adenoid Cystic Carcinoma
SDC	Salivary duct carcinoma
AcCC	Acinic Cell Carcinoma
PA	Pleomorphic adenoma
Ca ex Pa	Carcinoma ex Pleomorphic adenoma
EMC	Epithelial-myoepithelial carcinoma
MAC	Mucinous adenocarcinoma
IC	Intraducal carcinoma
SC	Secretory carcinoma
NGS	Next-generation sequencing
HNC	Head and Neck Cancers
EMT	Epithelial-mesenchymal transition
RT	Radiotherapy
CT	Chemotherapy
DFS	Disease free survival
OS	Overall survival

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-024-13421-0.

Supplementary Material 1.

Acknowledgements
We would like to thank Dr. Piotr Stawiński for bioinformatics and all those involved in patients' care.

Institutional review board statement
The study was conducted in accordance with the Declaration of Helsinki. and approved by the Bioethics Committee at Medical University of Warsaw (No. AKBE/175/2021).

Informed consent statement
Patient consent was waived by the Bioethics Committee due to retrospective nature of the study.

Authors' contributions
Conceptualization. AR; data curation. JP, AR, MMM, NW, AC, AM, KK, GK, ŁF; formal analysis MMM, TS; founding acquisition AR; investigation. JP, AR, MMM, NW, AC, AM, KK, ŁF; methodology AR, MMM, JP, TS, AC, AM, GK.; project administration AR; resources MMM, GK, AR, TS; software MMM; writing—original draft preparation. JP; writing—review and editing. MMM, AR., TS ŁF; visualization MMM, J.P; supervision. AR, TS. All authors have read and agreed to the published version of the manuscript.

Funding
This research was funded by Polish Ministry of Education and Science, grant number SKN/SP/534651/2022.

Data availability
The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.

Declarations

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Otorhinolaryngology, Head and Neck Surgery, Medical University of Warsaw, Warsaw, Poland. [2]Student Scientific Research Group at Otorhinolaryngology Department, Head and Neck Surgery, Medical University of Warsaw, Warsaw, Poland. [3]Department of Tumor Biology and Genetics, Medical University of Warsaw, Warsaw, Poland. [4]Laboratory of Genetics, University Clinical Hospital, Medical University of Warsaw, Warsaw, Poland. [5]Department of Pathology Department, Medical University of Warsaw, Warsaw, Poland. [6]Department of Medical Genetics, Medical University of Warsaw, Warsaw, Poland.

Received: 10 August 2024 Accepted: 31 December 2024
Published online: 08 January 2025

References

1. Sung H, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209–49.
2. Gatta G, et al. Major and minor salivary gland tumours. Crit Rev Oncol Hematol. 2020;152: 102959.
3. Schwentner I, et al. Distant metastasis of parotid gland tumors. Acta Otolaryngol. 2006;126(4):340–5.
4. Nam SJ, et al. Risk Factors and Survival Associated with Distant Metastasis in Patients with Carcinoma of the Salivary Gland. Ann Surg Oncol. 2016;23(13):4376–83.
5. Carlson ER, Schlieve T. Salivary Gland Malignancies. Oral Maxillofac Surg Clin North Am. 2019;31(1):125–44.
6. Young A, Okuyemi OT. Malignant Salivary Gland Tumors, in StatPearls. 2022, StatPearls Publishing Copyright ©. Treasure Island (FL): StatPearls Publishing LLC. 2022.
7. WHO Classiffcation of Tumours Editorial Board. Head and Neck tumours. WHO classifcation of tumours series. 5th ed. Lyon: IARC; 2022.
8. Bensadoun RJ, et al. Malignant tumours of the salivary glands. Br J Cancer. 2001;84 Suppl 2(Suppl 2):42–8.
9. Geiger JL, et al. Management of Salivary Gland Malignancy: ASCO Guideline. J Clin Oncol. 2021;39(17):1909–41.
10. van Herpen C, et al. Salivary gland cancer: ESMO-European Reference Network on Rare Adult Solid Cancers (EURACAN) Clinical Practice Guideline for diagnosis, treatment and follow-up. ESMO Open. 2022;7(6): 100602.
11. Vathiotis IA, Johnson JM, Argiris A. New Systemic Therapies in Salivary Gland Cancer. In: Critical Issues in Head and Neck Oncology. Cham: Springer International Publishing. 2023.
12. Ferrarotto R, et al. Activating NOTCH1 Mutations Define a Distinct Subgroup of Patients With Adenoid Cystic Carcinoma Who Have Poor Prognosis, Propensity to Bone and Liver Metastasis, and Potential Responsiveness to Notch1 Inhibitors. J Clin Oncol. 2017;35(3):352–60.
13. Sahara S, Herzog AE, Nör JE. Systemic therapies for salivary gland adenoid cystic carcinoma. Am J Cancer Res. 2021;11(9):4092–110.
14. Park JJH, et al. An overview of precision oncology basket and umbrella trials for clinicians. CA Cancer J Clin. 2020;70(2):125–37.
15. Philpott C, et al. The NF1 somatic mutational landscape in sporadic human cancers. Hum Genomics. 2017;11(1):13.
16. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30(15):2114–20.
17. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009;25(14):1754–60.
18. Chen S, et al. A genomic mutational constraint map using variation in 76,156 human genomes. Nature. 2024;625(7993):92–100.
19. Ioannidis NM, et al. REVEL: An Ensemble Method for Predicting the Pathogenicity of Rare Missense Variants. Am J Hum Genet. 2016;99(4):877–85.
20. Sundaram L, et al. Predicting the clinical impact of human mutation with deep neural networks. Nat Genet. 2018;50(8):1161–70.
21. Jaganathan K, et al. Predicting Splicing from Primary Sequence with Deep Learning. Cell. 2019;176(3):535-548.e24.
22. Liu X, et al. dbNSFP v4: a comprehensive database of transcript-specific functional predictions and annotations for human nonsynonymous and splice-site SNVs. Genome Med. 2020;12(1):103.
23. Landrum MJ, et al. ClinVar: public archive of relationships among sequence variation and human phenotype. Nucleic Acids Res. 2014;42(Database issue):D980-5.
24. Tate JG, et al. COSMIC: the Catalogue Of Somatic Mutations In Cancer. Nucleic Acids Res. 2019;47(D1):D941-d947.
25. Cerami E, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012;2(5):401–4.

26. Stawiński P, Płoski R. Genebe.net: Implementation and validation of an automatic ACMG variant pathogenicity criteria assignment. Clin Genet. 2024;106(2):119–26. https://doi.org/10.1111/cge.14516.
27. Kopanos C, et al. VarSome: the human genomic variant search engine. Bioinformatics. 2019;35(11):1978–80.
28. Talevich E, et al. CNVkit: Genome-Wide Copy Number Detection and Visualization from Targeted DNA Sequencing. PLoS Comput Biol. 2016;12(4): e1004873.
29. Mayakonda A, et al. Maftools: efficient and comprehensive analysis of somatic variants in cancer. Genome Res. 2018;28(11):1747–56.
30. Suehnholz SP, et al. Quantifying the Expanding Landscape of Clinical Actionability for Patients with Cancer. Cancer Discov. 2024;14(1):49–65.
31. Chakravarty D, et al. OncoKB: A Precision Oncology Knowledge Base. JCO Precis Oncol. 2017;1:1–16.
32. Ho AS, et al. Genetic hallmarks of recurrent/metastatic adenoid cystic carcinoma. J Clin Invest. 2019;129(10):4276–89.
33. Even C, et al. Safety and clinical activity of the Notch inhibitor, crenigacestat (LY3039478), in an open-label phase I trial expansion cohort of advanced or metastatic adenoid cystic carcinoma. Invest New Drugs. 2020;38(2):402–9.
34. Le DT, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science. 2017;357(6349):409–13.
35. Neiheisel A, et al. Wnt pathway modulators in cancer therapeutics: An update on completed and ongoing clinical trials. Int J Cancer. 2022;150(5):727–40.
36. Kommalapati A, et al. FGFR Inhibitors in Oncology: Insight on the Management of Toxicities in Clinical Practice. Cancers (Basel). 2021;13(12):2968. https://doi.org/10.3390/cancers13122968.
37. Kim ST, et al. Rapamycin-insensitive companion of mTOR (RICTOR) amplification defines a subset of advanced gastric cancer and is sensitive to AZD2014-mediated mTORC1/2 inhibition. Ann Oncol. 2017;28(3):547–54.
38. Guterres AN, Villanueva J. Targeting telomerase for cancer therapy. Oncogene. 2020;39(36):5811–24.
39. Global Cancer Observatory. International Agency for Research on Cancer. Cancer Tomorrow. Available from: https://gco.iarc.fr/tomorrow/en.
40. Lassche G, et al. Advances and challenges in precision medicine in salivary gland cancer. Cancer Treat Rev. 2019;80: 101906.
41. Olivier M, Hollstein M, Hainaut P. TP53 mutations in human cancers: origins, consequences, and clinical use. Cold Spring Harb Perspect Biol. 2010;2(1): a001008.
42. Choi M, Kipps T, Kurzrock R. ATM Mutations in Cancer: Therapeutic Implications. Mol Cancer Ther. 2016;15(8):1781–91.
43. Hernández Borrero LJ, El-Deiry WS. Tumor suppressor p53: Biology, signaling pathways, and therapeutic targeting. Biochim Biophys Acta Rev Cancer. 2021;1876(1):188556.
44. Pikul J, Rzepakowska A. Molecular landscape of salivary gland malignancies. What is already known? Contemporary Oncology/Współczesna Onkologia. 2024;28(3):201–16.
45. Ross JS, 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(10):2539–46.
46. Wang S, et al. Whole-exome sequencing reveals genetic underpinnings of salivary adenoid cystic carcinoma in the Chinese population. J Genet Genomics. 2020;47(7):397–401.
47. Ho AS, et al. The mutational landscape of adenoid cystic carcinoma. Nat Genet. 2013;45(7):791–8.
48. Wang H, et al. Targeting p53 pathways: mechanisms, structures, and advances in therapy. Signal Transduct Target Ther. 2023;8(1):92.
49. LoRusso P, et al. The MDM2-p53 Antagonist Brigimadlin (BI 907828) in Patients with Advanced or Metastatic Solid Tumors: Results of a Phase Ia, First-in-Human. Dose-Escalation Study Cancer Discov. 2023;13(8):1802–13.
50. Dumble M, et al. Abstract LB006: PC14586: The first orally bioavailable small molecule reactivator of Y220C mutant p53 in clinical development. Cancer Res. 2021;81(13_Supplement):LB006t.
51. A Phase 1/2 Open-label, Multicenter Study to Assess the Safety, Tolerability, Pharmacokinetics, Pharmacodynamics, and Efficacy of PC14586 in Patients With Locally Advanced or Metastatic Solid Tumors Harboring a TP53 Y220C Mutation (PYNNACLE). 2020.
52. Klein AM, et al. The roles and regulation of MDM2 and MDMX: it is not just about p53. Genes Dev. 2021;35(9–10):575–601.
53. Karni-Schmidt O, Lokshin M, Prives C. The Roles of MDM2 and MDMX in Cancer. Annu Rev Pathol. 2016;11:617–44.
54. Manfredi JJ. The Mdm2-p53 relationship evolves: Mdm2 swings both ways as an oncogene and a tumor suppressor. Genes Dev. 2010;24(15):1580–9.
55. Zhao Y, Yu H, Hu W. The regulation of MDM2 oncogene and its impact on human cancers. Acta Biochim Biophys Sin (Shanghai). 2014;46(3):180–9.
56. Wade M, Li YC, Wahl GM. MDM2, MDMX and p53 in oncogenesis and cancer therapy. Nat Rev Cancer. 2013;13(2):83–96.
57. Sequeiros-Santiago G, et al. Oncogene amplification pattern in adenoid cystic carcinoma of the salivary glands. Oncol Rep. 2009;21(5):1215–22.
58. Kishi M, et al. Genetic and epigenetic alteration profiles for multiple genes in salivary gland carcinomas. Oral Oncol. 2005;41(2):161–9.
59. Grünewald I, et al. MDM2 and CDK4 amplifications are rare events in salivary duct carcinomas. Oncotarget. 2016;7(46):75261–72.
60. Persson F, et al. High-resolution genomic profiling of adenomas and carcinomas of the salivary glands reveals amplification, rearrangement, and fusion of HMGA2. Genes Chromosomes Cancer. 2009;48(1):69–82.
61. Amoroso L, et al. Genomic coamplification of CDK4/MDM2/FRS2 is associated with very poor prognosis and atypical clinical features in neuroblastoma patients. Genes Chromosomes Cancer. 2020;59(5):277–85.
62. He X, et al. Consistent Amplification of FRS2 and MDM2 in Low-grade Osteosarcoma: A Genetic Study of 22 Cases With Clinicopathologic Analysis. Am J Surg Pathol. 2018;42(9):1143–55.
63. Zhang K, et al. Amplification of FRS2 and activation of FGFR/FRS2 signaling pathway in high-grade liposarcoma. Cancer Res. 2013;73(4):1298–307.
64. Kurokawa M, et al. A network of substrates of the E3 ubiquitin ligases MDM2 and HUWE1 control apoptosis independently of p53. Sci Signal. 2013;6(274):ra32.
65. Santarpia L, Lippman SM, El-Naggar AK. Targeting the MAPK-RAS-RAF signaling pathway in cancer therapy. Expert Opin Ther Targets. 2012;16(1):103–19.
66. McCubrey JA, et al. Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. Biochim Biophys Acta. 2007;1773(8):1263–84.
67. Sabova L, Kretova M, Luciakova K. New insights into the role of NF1 in cancer. Neoplasma. 2013;60(3):233–9.
68. Yap YS, et al. The NF1 gene revisited - from bench to bedside. Oncotarget. 2014;5(15):5873–92.
69. Giraud JS, et al. NF1 alterations in cancers: therapeutic implications in precision medicine. Expert Opin Investig Drugs. 2023;32(10):941–57.
70. de Bruin EC, et al. Reduced NF1 expression confers resistance to EGFR inhibition in lung cancer. Cancer Discov. 2014;4(5):606–19.
71. Kato S, et al. Genomic landscape of salivary gland tumors. Oncotarget. 2015;6(28):25631–45.
72. Novoplansky O, et al. Worldwide Prevalence and Clinical Characteristics of RAS Mutations in Head and Neck Cancer: A Systematic Review and Meta-Analysis. Front Oncol. 2022;12: 838911.
73. Urano M, et al. Diagnostic Significance of HRAS Mutations in Epithelial-Myoepithelial Carcinomas Exhibiting a Broad Histopathologic Spectrum. Am J Surg Pathol. 2019;43(7):984–94.
74. Nakaguro M, et al. The Diagnostic Utility of RAS Q61R Mutation-specific Immunohistochemistry in Epithelial-Myoepithelial Carcinoma. Am J Surg Pathol. 2021;45(7):885–94.
75. Rupp NJ, et al. New Insights into Tumor Heterogeneity: A Case of Solid-Oncocytic Epithelial-Myoepithelial Carcinoma of the Parotid Gland Harboring a HRAS and Heterogeneous Terminating ARID1A Mutation. Head Neck Pathol. 2020;14(2):554–8.
76. Desilets A, Ho AL. Targeting HRAS in Head and Neck Cancer: Lessons From the Past and Future Promise. Cancer J. 2022;28(5):363–8.
77. Gilardi M, et al. Tipifarnib as a Precision Therapy for HRAS-Mutant Head and Neck Squamous Cell Carcinomas. Mol Cancer Ther. 2020;19(9):1784–96.

78. Hanna GJ, et al. Tipifarnib in recurrent, metastatic HRAS-mutant salivary gland cancer. Cancer. 2020;126(17):3972–81.
79. Mullen J, et al. Targeting ARID1A mutations in cancer. Cancer Treat Rev. 2021;100: 102287.
80. Fontana B, et al. ARID1A in cancer: Friend or foe? Front Oncol. 2023;13:1136248.
81. Zehir A, et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. Nat Med. 2017;23(6):703–13.
82. Mandal J, et al. Treating ARID1A mutated cancers by harnessing synthetic lethality and DNA damage response. J Biomed Sci. 2022;29(1):71.
83. Shen J, et al. ARID1A Deficiency Impairs the DNA Damage Checkpoint and Sensitizes Cells to PARP Inhibitors. Cancer Discov. 2015;5(7):752–67.
84. Yakovlev VA, et al. PARP inhibitors in the treatment of ARID1A mutant ovarian clear cell cancer: PI3K/Akt1-dependent mechanism of synthetic lethality. Front Oncol. 2023;13:1124147.
85. Bou Zerdan M, et al. Molecular Targets in Salivary Gland Cancers: A Comprehensive Genomic Analysis of 118 Mucoepidermoid Carcinoma Tumors. Biomedicines. 2023;11(2):519. https://doi.org/10.3390/biomedicines11020519.
86. Wang K, et al. Comprehensive genomic profiling of salivary mucoepidermoid carcinomas reveals frequent BAP1, PIK3CA, and other actionable genomic alterations. Ann Oncol. 2017;28(4):748–53.
87. Anzick SL, et al. Unfavorable prognosis of CRTC1-MAML2 positive mucoepidermoid tumors with CDKN2A deletions. Genes Chromosomes Cancer. 2010;49(1):59–69.
88. Mueller SA, et al. Molecular patterns in salivary duct carcinoma identify prognostic subgroups. Mod Pathol. 2020;33(10):1896–909.
89. Chiosea SI, et al. Subsets of salivary duct carcinoma defined by morphologic evidence of pleomorphic adenoma, PLAG1 or HMGA2 rearrangements, and common genetic alterations. Cancer. 2016;122(20):3136–44.
90. Dogan S, et al. Loss of CDKN2A/B is a Molecular Marker of High-grade Histology and is Associated with Aggressive Behavior in Acinic Cell Carcinoma. Mod Pathol. 2023;36(7): 100150.
91. Cipriani NA, et al. Salivary Gland Secretory Carcinoma With High-Grade Transformation, CDKN2A/B Loss, Distant Metastasis, and Lack of Sustained Response to Crizotinib. Int J Surg Pathol. 2017;25(7):613–8.
92. Kreuger IZM, et al. Therapeutic Strategies for Targeting CDKN2A Loss in Melanoma. J Invest Dermatol. 2023;143(1):18-25.e1.
93. Zhao R, et al. Implications of Genetic and Epigenetic Alterations of CDKN2A (p16(INK4a)) in Cancer. EBioMedicine. 2016;8:30–9.
94. Ni J, et al. p16(INK4A)-deficiency predicts response to combined HER2 and CDK4/6 inhibition in HER2+ breast cancer brain metastases. Nat Commun. 2022;13(1):1473.
95. Gajos-Michniewicz A, Czyz M. WNT/β-catenin signaling in hepatocellular carcinoma: The aberrant activation, pathogenic roles, and therapeutic opportunities. Genes Dis. 2024;11(2):727–46.
96. Duchartre Y, Kim YM, Kahn M. The Wnt signaling pathway in cancer. Crit Rev Oncol Hematol. 2016;99:141–9.
97. Oyama Y, et al. Difference in transducin-like enhancer of split 1 protein expression between basal cell adenomas and basal cell adenocarcinomas - an immunohistochemical study. Diagn Pathol. 2018;13(1):48.
98. Sato M, et al. Wnt/β-catenin signal alteration and its diagnostic utility in basal cell adenoma and histologically similar tumors of the salivary gland. Pathol Res Pract. 2018;214(4):586–92.
99. Tesdahl BA, et al. Epithelial-Mesenchymal Transition Protein Expression in Basal Cell Adenomas and Basal Cell Adenocarcinomas. Head Neck Pathol. 2016;10(2):176–81.
100. Sondka Z, et al. COSMIC: a curated database of somatic variants and clinical data for cancer. Nucleic Acids Res. 2024;52(D1):D1210-d1217.
101. Grünewald I, et al. Targeted next generation sequencing of parotid gland cancer uncovers genetic heterogeneity. Oncotarget. 2015;6(20):18224–37.
102. Daa T, et al. Mutations in components of the Wnt signaling pathway in adenoid cystic carcinoma. Mod Pathol. 2004;17(12):1475–82.
103. Ku BM, et al. High-throughput profiling identifies clinically actionable mutations in salivary duct carcinoma. J Transl Med. 2014;12:299.
104. Bravo Thompson H, et al. Parotid Myoepithelial Carcinoma in a Pediatric Patient with Multiple Recurrences: Case Report. Case Rep Oncol. 2021;14(2):989–97.
105. Kagey MH, He X. Rationale for targeting the Wnt signalling modulator Dickkopf-1 for oncology. Br J Pharmacol. 2017;174(24):4637–50.
106. Liu T, Yuan X, Xu D. Cancer-Specific Telomerase Reverse Transcriptase (TERT) Promoter Mutations: Biological and Clinical Implications. Genes (Basel). 2016;7(7):38. https://doi.org/10.3390/genes7070038.
107. Yuan X, Larsson C, Xu D. Mechanisms underlying the activation of TERT transcription and telomerase activity in human cancer: old actors and new players. Oncogene. 2019;38(34):6172–83.
108. Cormier C, Agarwal S. Myoepithelial Carcinoma Ex-Pleomorphic Adenoma: A Rare Pathology Misdiagnosed as Pleomorphic Adenoma; With a Novel TERT Promoter Mutation and High PD-L1 Expression. Head Neck Pathol. 2022;16(1):322–30.
109. Zare-Mirzaie A, et al. TERT Promoter Mutation in Benign and Malignant Salivary Gland Tumors. A Cross-Sectional Study Iran J Pathol. 2023;18(1):64–74.
110. Turner N, Grose R. Fibroblast growth factor signalling: from development to cancer. Nat Rev Cancer. 2010;10(2):116–29.
111. Ishiwata T. Role of fibroblast growth factor receptor-2 splicing in normal and cancer cells. Front Biosci (Landmark Ed). 2018;23(4):626–39.
112. Brooks AN, Kilgour E, Smith PD. Molecular pathways: fibroblast growth factor signaling: a new therapeutic opportunity in cancer. Clin Cancer Res. 2012;18(7):1855–62.
113. Ornitz DM, Itoh N. The Fibroblast Growth Factor signaling pathway. Wiley Interdiscip Rev Dev Biol. 2015;4(3):215–66.
114. Liu G, et al. Prognostic role of fibroblast growth factor receptor 2 in human solid tumors: A systematic review and meta-analysis. Tumour Biol. 2017;39(6):1010428317707424.
115. Weaver A, Bossaer JB. Fibroblast growth factor receptor (FGFR) inhibitors: A review of a novel therapeutic class. J Oncol Pharm Pract. 2021;27(3):702–10.
116. Pikul J, et al. FGFR2 point mutation in 2 cases of pleomorphic adenoma progressing to myoepithelial carcinoma. Contemp Oncol (Pozn). 2023;27(3):211–6.
117. Dalin MG, et al. Multi-dimensional genomic analysis of myoepithelial carcinoma identifies prevalent oncogenic gene fusions. Nat Commun. 2017;8(1):1197.
118. Dalin MG, et al. Comprehensive Molecular Characterization of Salivary Duct Carcinoma Reveals Actionable Targets and Similarity to Apocrine Breast Cancer. Clin Cancer Res. 2016;22(18):4623–33.

Publisher's Note