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Genetyczne uwarunkowania nowotworów złośliwych gruczołów ślinowych

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu

w dyscyplinie nauki medyczne

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Obrona rozprawy doktorskiej przed Radą Dyscypliny Nauk Medycznych

Warszawskiego Uniwersytetu Medycznego

Warszawa 2025 r.

Słowa kluczowe w języku polskim:

nowotwory złośliwe gruczołów ślinowych

gruczolak wielopostaciowy

mutacje genetyczne

analiza genetyczna

sekwencjonowanie następnej generacji

terapia celowana

onkologia personalizowana

Keywords:

salivary gland carcinomas

pleomorphic adenoma

mutational landscape

genetic analysis

next-generation sequencing

targeted therapy

precision oncology

Nazwa i numer projektu badawczego

Badanie markerów wpływających na przeżycie u chorych na nowotwory złośliwe gruczołów ślinowych opracowane w artykule „Potentially actionable molecular alterations in particular related to poor oncologic outcomes in salivary gland carcinomas" i wchodzące w skład cyklu publikacji przedstawionej pracy doktorskiej, zostało przeprowadzone dzięki środkom pozyskanym w ramach programu „Studenckie koła naukowe tworzą innowacje" Ministerstwa Nauki i Szkolnictwa Wyższego – numer grantu SKN/SP/534651/2022.

Podziękowania

Szczególne podziękowania kieruję do mojej Promotor, Pani dr hab. n. med. Anny Rzepakowskiej za nieustanne wsparcie, inspirację, motywację, wiarę w moje możliwości, a przede wszystkim za życzliwe i merytoryczne kierownictwo naukowe.

Podziękowania dla wszystkich zaangażowanych współautorów, w szczególności dla Pana dr n. med. Marcina Machnickiego oraz Pana prof. dr med. Tomasza Stokłosy za nieoceniony wkład w realizację prezentowanych badań oraz cenne uwagi merytoryczne.

Dziękuję Kierownikowi Kliniki Otorynolaryngologii Chirurgii Głowy i Szyi Warszawskiego Uniwersytetu Medycznego, Panu prof. dr hab. n. med. Kazimierzowi Niemczykowi za umożliwienie rozwoju naukowego oraz przeprowadzenia poniższych badań.

Pragnę podziękować również wszystkim zaangażowanym w proces diagnostyczno-terapeutyczny oraz sprawującym opiekę nad Pacjentami.

Rodzicom dziękuję za wszystko.

Wykaz publikacji stanowiących rozprawę doktorską:

1. **Pikul J**, Rzepakowska A. Molecular landscape of salivary gland malignancies. What is already known? Contemp Oncol (Pozn). 2024;28(3):201-216. doi:10.5114/wo.2024.144288

IF 2,9

MEiN 70

Q2

2. **Pikul J**, Rzepakowska A, Machnicki M, Stokłosa T. FGFR2 point mutation in 2 cases of pleomorphic adenoma progressing to myoepithelial carcinoma. Contemp Oncol (Pozn). 2023;27(3):211-216. doi:10.5114/wo.2023.133592

IF 2,9

MEiN 70

Q2

3. **Pikul J**, Machnicki MM, Rzepakowska A, Winiarska N, Chudy A, Moskowicz A, Król K, Fus Ł, Kostrzewa G, Stokłosa T. Potentially actionable molecular alterations in particular related to poor oncologic outcomes in salivary gland carcinomas. BMC Cancer. 2025;25(1):42. Published 2025 Jan 8. doi:10.1186/s12885-024-13421-0

IF 3,4

MEiN 100

Q2

Sumaryczna bibliometria cyklu publikacji:

Sumaryczna punktacja MNiSW 240 pkt

Sumaryczny Impact Factor 9,2

Spis treści

Wykaz stosowanych skrótów

AC – gruczolakorak (adenocarcinoma)

AcCC – rak zrazikowokomórkowy (acinic cell carcinoma)

AC NOS – gruczolakorak inaczej nieokreślony (adenocarcinoma not otherwise specified)

AdCC – rak gruczołowo-torbielowaty (adenoid cystic carcinoma)

ASCO – Amerykańskie Towarzystwo Onkologii Klinicznej (American Society of Clinical Oncology)

Ca ex PA – rak w gruczolaku wielopostaciowym (carcinoma ex pleomorphic adenoma)

CRT – chemioradioterapia (chemoradiotherapy)

CNVs – zmiana liczby kopii genów (copy number variations)

DNA – kwas deoksyrybonukleinowy (deoxyribonucleic acid)

EMC – rak nabłonkowo-mioepitelialny (epithelial-myoepithelial carcinoma)

FFPE – utrwalone w formalinie zatopione w parafinie (formalin – fixed paraffin embedded)

FNAB – biopsja aspiracyjna cienkoigłowa (fine – needle aspiration biopsy)

HNCs – nowotwory złośliwe głowy i szyi (Head and Neck Cancers)

IARC – Międzynarodowa Agencja Badań nad Rakiem (International Agency for Research on Cancer)

LOH – utrata heterozygotyczności (loss of heterozygosity)

MEC – rak śluzowo-naskórkowy (mucoepidermoid carcinoma)

MECA – rak mioepitelialny (myoepithelial carcinoma)

MECA ex PA – rak mioepitelialny w gruczolaku wielopostaciowym (myoepithelial carcinoma ex pleomorphic adenoma)

MRI – obrazowanie metodą rezonansu magnetycznego (Magnetic Resonance Imaging)

NGS – sekwencjonowanie następnej generacji (next – generation sequencing)

OS – przeżycie całkowite (overall survival)

PA – gruczolak wielopostaciowy (pleomorphic adenoma)

PET – pozytonowa tomografia emisyjna (Positron Emission Tomography)

R/M – nawrotowy lub przerzutowy (recurrent or metastatic)

SDC – rak przewodowy gruczołów ślinowych (salivary duct carcinoma)

SDC ex PA – rak przewodowy w gruczolaku postaciowym (salivary duct carcinoma ex pleomorphic adenoma)

SGCs – nowotwory złośliwe gruczołów ślinowych (salivary gland carcinomas)

VAF – częstotliwość występowania alleli (variant allele frequency)

Vs. – w stosunku do (z języka łacińskiego- versus)

WHO – Światowa Organizacja Zdrowia (World Health Organization)

Streszczenie w języku polskim

Genetyczne uwarunkowania nowotworów złośliwych gruczołów ślinowych

Nowotwory złośliwe gruczołów ślinowych (salivary gland carcinomas – SGCs) to heterogenna grupa patologii, różniących się nie tylko budową histologiczną, lecz również odmiennym obrazem klinicznym. Chociaż występują rzadko, cechują się nieprzewidywalnym przebiegiem choroby, licznymi nawrotami oraz dużym wskaźnikiem śmiertelności. Przerzuty odległe występują w 10-40% przypadków, w tym najczęściej do płuc. W przyszłości przewidywany jest globalnie znaczący wzrost zachorowalności, jak również śmiertelności związanej z SGCs. Według Światowej Organizacji Zdrowia (World Health Organization – WHO) wśród SGCs można wyróżnić ponad 20 typów histopatologicznych. Precyzyjna diagnoza SGCs na etapie przedoperacyjnym stwarza wiele trudności. W dostępnej literaturze opisano wiele przypadków fałszywie negatywnej diagnozy zmian złośliwych ślinianek. W diagnostyce różnicowej przydatne są unikalne zmiany genetyczne dla poszczególnych typów histopatologicznych. Obecnie wiedza na temat wartości prognostycznej czy predykcyjnej tych zmian jest niewielka. Stąd potrzeba dalszych badań w tym zakresie, dla uzyskania wiarygodnych danych w tych rzadkich nowotworach. Objawy klinicznie sugerujące złośliwy charakter zmiany w śliniance to w szczególności szybki wzrost zmiany, dolegliwości bólowe, objawy porażenia nerwu twarzowego czy naciekanie skóry i/ lub okolicznych struktur. Wymagają one wówczas diagnostyki z wykorzystaniem wieloparametrycznego obrazowania metodą rezonansu magnetycznego (Magnetic Resonance Imaging – MRI). Złotym standardem postępowania u chorych z SGCs jest radykalna resekcja chirurgiczna, z następową radioterapią lub chemioradioterapią w zależności od oceny histopatologicznej zaawansowania, typu i radykalności. Rzadkie występowanie SGCs powoduje, że wciąż brakuje optymalnych schematów postępowania opartych na dowodach naukowych, w szczególności w przypadkach wznowy oraz występowania zmian przerzutowych (recurrent or metastatic – R/M). Stale poszukiwane są bardziej specyficzne opcje terapeutyczne, a identyfikacja odpowiednich markerów, z uwzględnieniem aberracji genetycznych, jest niezbędna, aby zoptymalizować protokoły postepowania oraz poprawić dostęp pacjentów do bardziej spersonalizowanego leczenia. Niestety, rzadkie występowanie SGCs uniemożliwia przeprowadzanie typowych badań klinicznych w tej grupie nowotworów. Szansą dla pacjentów, szczególnie w przypadkach R/M stają się badania koszykowe (z ang. basket trials), w których działania terapeutyczne zaprojektowane są dla określonych aberracji genetycznych w danej grupie pacjentów. Dodatkowo, istnieje ciągła potrzeba określenia markerów prognostycznych w SGCs. Możliwości przeprowadzania analiz genetycznych, w tym sekwencjonowania następnej

generacji (next – generation sequencing – NGS) dla SGCs w praktyce klinicznej są nadal ograniczone. W przyszłości stosowanie NGS przyczyni się do lepszego przewidywania przebiegu choroby, doboru bardziej odpowiednich, spersonalizowanych metod leczenia oraz poprawy nadzoru onkologicznego w tej grupie pacjentów.

Celem niniejszej pracy doktorskiej była charakterystyka aberracji genetycznych występujących w najczęstszych typach histopatologicznych nowotworów złośliwych gruczołów ślinowych, w szczególności tych związanych z ryzykiem wznowy lub wystąpienia przerzutów odległych, a tym samym gorszym rokowaniem pacjentów.

Publikacja *Molecular landscape of salivary gland malignancies. What is already known?* jest przeglądem systematycznym. Na podstawie analizy literatury zebrano i przedstawiono aktualną wiedzę na temat zidentyfikowanych zmian genetycznych w najczęstszych typach histopatologicznych nowotworów złośliwych gruczołów ślinowych. Uwzględniono zmiany genetyczne w SGCs o charakterze fuzji genowych, mutacji somatycznych oraz zmiany liczby kopii genów (copy number variations – CNVs). Dodatkowo wskazano te aberracje, które mogą być związane z niekorzystnym rokowaniem pacjentów. W pracy przedstawiono również charakterystykę kliniczną poszczególnych typów SGCs.

W drugiej pracy pt. *FGFR2 point mutation in 2 cases of pleomorphic adenoma progressing to myoepithelial carcinoma* przedstawiono opis dwóch przypadków przemiany złośliwej gruczolaka wielopostaciowego w raka mioepitelialnego w gruczolaku wielopostaciowym (myoepithelial carcinoma ex pleomorphic adenoma – MECA ex PA). Dokonano również analizy genetycznej materiału z obu rodzajów nowotworu u każdej z pacjentek. W porównanych próbkach, zarówno w pierwotnym gruczolaku wielopostaciowym, jak i we wznowie – MECA ex PA, stwierdzono liczne CNVs, jak również mutacje w genie *FGFR2*. Biorąc pod uwagę dane z literatury, zmiany te uznano za istotne w procesie kancerogenezy gruczolaka wielopostaciowego, progresji choroby, jak również wpływające niekorzystnie na wyniki leczenia onkologicznego pacjentek.

Trzecia z prezentowanych publikacji pt. *Potentially actionable molecular alterations in particular related to poor oncologic outcomes in salivary gland carcinomas* jest pracą retrospektywną, oryginalną, w której przeprowadzona została analiza NGS najczęściej występujących typów histopatologicznych SGCs. Dodatkowym celem pracy było określenie

tych zmian genetycznych, które mogą być związane z niekorzystnym rokowaniem w badanej grupie pacjentów, czyli ze wznową lokoregionalną lub przerzutami. Materiał pochodził od 40 pacjentów hospitalizowanych w Klinice Otorynolaryngologii Chirurgii Głowy i Szyi Warszawskiego Uniwersytetu Medycznego, w latach 2010-2017, poddanych pierwotnej resekcji chirurgicznej nowotworu złośliwego ślinianki. Przedstawiono również dane kliniczno-patologiczne w tym: płeć, wiek rozpoznania, lokalizację guza, stopień zaawansowania, jak również rokowanie pacjentów. Najczęściej wykrywanymi zmianami genetycznymi w badanej kohorcie, jak również w grupie pacjentów o niekorzystnym rokowaniu były aberracje w genach: *NF1* (24% vs. 32%), *TP53* (22% vs. 32%) oraz *CDKN2A* (14% vs. 21%). Mutacje w genie *TP53* uznano za istotny negatywny czynnik prognostyczny dla przeżycia całkowitego (overall survival – OS (p=0,04)). Mutacje w promotorze *TERT* oraz amplifikacja *TERT*, mutacja p. Ile35Thr w genie *CTNNB1* zostały stwierdzone, odpowiednio, w raku mioepitelialnym (myoepithelial carcinoma – MECA) oraz w raku gruczołowo-torbielowatym (adenoid cystic carcinoma – AdCC). Aberracje *ERBB2* wykryto wyłącznie w raku przewodowym w gruczolaku wielopostaciowym (salivary duct carcinoma ex pleomorphic adenoma – SDC ex PA). Dodatkowo określono, że wykryte zmiany genetyczne stwarzają możliwość podjęcia prób leczenia celowanego w przyszłości, w szczególności w grupie chorych o niekorzystnym rokowaniu.

Podsumowując, nowotwory złośliwe gruczołów ślinowych należą do chorób rzadkich, które różnią się nie tylko klinicznym obrazem, lecz również spektrum molekularnym. W najbliższych latach przewidywany jest znaczący wzrost zachorowalności, jak również śmiertelności na SGCs. Wciąż brakuje prognostycznych biomarkerów oraz optymalnych protokołów postępowania opartych na dowodach naukowych, w szczególności w przypadkach R/M. Istotne dla poprawy rokowania chorych jest zastosowanie analizy NGS w praktyce klinicznej w nowotworach złośliwych gruczołów ślinowych w celu stworzenia pacjentom możliwości zastosowania spersonalizowanych metod leczenia. Konieczne są dalsze prospektywne wieloośrodkowe badania z odpowiednio dobraną kohortą. Wydaje się, że w najbliższej przyszłości, analiza NGS stanie się standardowym narzędziem w praktyce klinicznej w rzadkich nowotworach, co może przyczynić się do poprawy rokowania pacjentów.

Streszczenie w języku angielskim

Genetic determinants of Salivary Gland Carcinomas

Salivary gland carcinomas (SGCs) constitute a heterogeneous group of malignancies that are distinct both histopathologically and clinically. Although they occur rarely, they are characterized by an unpredictable disease course, numerous relapses and significant mortality. Distant metastases are presented in 10-40% of cases, with lungs being predominantly affected. Moreover, a significant increase in morbidity and mortality in SGCs is predicted in the near future. According to the World Health Organization (WHO), more than 20 histopathological types of SGCs are recognized. Thus, diagnostic accuracy might pose numerous difficulties. In the available literature, a number of cases of misdiagnosis of malignant tumours as benign have been shown. Nowadays, unique genetic alterations are considered in the differential diagnosis. However, studies have shown that exceptional lesions in particular type might not have a prognostic or predictive impact. Therefore, further comprehensive studies are required. Suspicious lesions, especially those with rapid growth, facial nerve palsy, ulceration or painful swelling, indicate malignancy and should be preferably investigated by multiparametric magnetic resonance imaging (MRI). Radical surgical excision is a standard of treatment, with further radiotherapy (RT) or chemoradiotherapy (CRT). Nevertheless, there is still a lack of reliable, evidence-based regimen methods, especially in recurrent or metastatic (R/M) cases. There are attempts to find more specific therapies in SGCs and to identify relevant markers, including molecular factors, therefore, it is imperative to optimize protocols and increase patients' access to more personalized therapies. However, the rarity of SGCs prevents clinical trials in this group, which creates a treatment gap. The chance for the patients is to join basket trials, where the therapeutic approach is tailored to the profile of specific molecular alterations. Additionally, there is an imperative need to determine prognostic markers in SGCs. However, the utilization of genetic analysis in clinical practice, including next-generation sequencing (NGS), is still limited. Wider usage of NGS contributes to better disease course predictions and more suitable and personalized treatment options. It also improves oncological supervision for SGCs patients.

The aim of this dissertation was to characterize genetic alterations in the most commonly occurring types of salivary gland carcinomas, especially those related to relapses or metastases, thus worse patients' oncological outcomes.

The study *Molecular landscape of salivary gland malignancies. What is already known?* is a comprehensive review that presents the genetic variety of selected histopathological SGCs types. The most frequently detected alterations in SGCs, including gene fusions, somatic mutations, and copy number variations (CNVs), were collected. Additionally, those that might be associated with unfavourable patients' outcomes were presented along with the clinical characteristics of SGCs.

The second study entitled *FGFR2 point mutation in 2 cases of pleomorphic adenoma progressing to myoepithelial carcinoma* is a case report with conducted genetic analysis of pleomorphic adenoma (PA) and myoepithelial carcinoma ex pleomorphic adenoma (MECA ex PA) tissues.. Comparison of tumour samples of both patients from benign and malignant lesions revealed various common CNVs and *FGFR2* point mutations. Taking into consideration the available literature, these genetic aberrations were established as related to carcinogenesis, quick disease progression and poor oncological outcomes.

The third, retrospective, original research entitled *Potentially actionable molecular alterations in particular related to poor oncologic outcomes in salivary gland carcinomas* is the NGS analysis of the most common histopathological types of SGCs. The additional aim of the study was to evaluate the relation of genetic changes to relapse or metastasis. The analysed material was obtained from 40 patients with the primary diagnosis of SGCs, who were hospitalized in the Otorhinolaryngology, Head and Neck Surgery Department of the Medical University of Warsaw between 2010-2017 and for whom surgical excision of malignancy with radical intent was performed. The clinicopathological data, including sex, age of diagnosis, localization of the tumour, tumour stage, as well as patients' outcomes were described. The predominant genetic alterations in the study cohort as well as in the group of patients with unfavourable outcomes, were *NF1* (24% vs. 32%), *TP53* (22% vs. 32%) and *CDKN2A* (14% vs. 21%), respectively. Moreover, *TP53* mutation was established as a relevant negative prognostic factor for overall survival (p=0.04). *TERT* promoter mutation and *TERT* amplification, p. Ile35Thr mutation in *CTNNB1* were found in myoepithelial carcinoma (MECA), and adenoid cystic carcinoma (AdCC), respectively. *ERBB2* alterations were remarkable for salivary duct carcinoma ex PA (SDC ex PA). Moreover, abnormalities detected in the study presented the possibility of targeted treatment in the future, especially in patients with poor outcomes.

In summary, salivary gland carcinomas belong to rare entities with diverse clinical behaviour and molecular landscape. They are characterized by an unpredictable disease course and considerable mortality. The prognosis of increasing morbidity and mortality are predicted for

the following years. There is still a lack of prognostic biomarkers as well as optimal therapy protocols based on scientific evidence, in particular for patients with R/M SGCs. The utilization of the NGS analysis in clinical practice for SGCs is essential for improving patient prognosis and providing opportunities for more personalized and precise therapy. However, further prospective multicenter research with a suitable cohort study is needed. It is expected that, in the near future, NGS analysis will be a standard tool in clinical practice in rare cancers and will enhance patients' outcomes.

Genetyczne uwarunkowania nowotworów złośliwych gruczołów ślinowych

Wstęp

Wśród patologii gruczołów ślinowych można wyróżnić zarówno zmiany łagodne jak i złośliwe. Rozwijają się w dużych i małych gruczołach ślinowych, a ślinianka przyuszna stanowi najczęstszą lokalizację [1-3]. W 80% są to zmiany łagodne, w tym najczęściej stwierdzany jest gruczolak wielopostaciowy (pleomorphic adenoma – PA) i gruczolak limfatyczny (guz Warthina), rzadziej gruczolak podstawnokomórkowy czy mioepithelioma [3-5].

Gruczolak wielopostaciowy stanowi ponad 70% wszystkich zmian łagodnych, występuje najczęściej w śliniance przyusznej u kobiet w średnim wieku [4, 6]. Pacjenci przeważnie zgłaszają się z wolno rosnącą, niebolesną masą. Postępowaniem z wyboru jest chirurgiczna resekcja guza [7]. W niewielkim odsetku przypadków dochodzi do nawrotu choroby, a wśród czynników ryzyka wyróżnić można niekompletną resekcję oraz młodszy wiek w chwili rozpoznania [6]. Opisywane są przypadki przerzutowego PA o niekorzystnym rokowaniu [8, 9]. Szacuje się, że transformacji złośliwej ulega niewielki odsetek PA, a ryzyko zezłośliwienia zwiększa się wraz z czasem trwania choroby [10-12].

Rak w gruczolaku wielopostaciowym (Carcinoma ex Pleomorphic Adenoma – Ca ex PA) stanowi 5-15% wszystkich nowotworów złośliwych gruczołów ślinowych. Zawiera jednocześnie łagodną oraz złośliwą komponentę i rozwija się w pierwotnym lub nawrotowym PA [10, 13, 14]. Postawienie prawidłowego rozpoznania może okazać się wyzwaniem [15]. W dostępnej literaturze opisano przypadki, gdzie początkowo Ca ex PA błędnie został zdiagnozowany jako zmiana łagodna [12, 14, 16, 17]. Komponentę złośliwą może stanowić każdy z wielu podtypów histopatologicznych, jednak najczęściej stwierdzany jest gruczolakorak NOS (adenocarcinoma not otherwise specified – AC NOS), a następnie rak mioepitelialny (myoepithelial carcinoma – MECA) oraz rak przewodowy gruczołów ślinowych (salivary duct carcinoma – SDC) [11, 18]. Ca ex PA cechuje się agresywnym przebiegiem, częstymi wznowami jak również przerzutami odległymi, a 5-letnie przeżycie całkowite (OS – overall survival) jest niekorzystne i szacowane między 25-76% [2, 11, 12, 14].

W przebiegu transformacji złośliwej zidentyfikowano liczne aberracje molekularne, w tym zmiany liczby kopii genów (copy number variations – CNVs) [12, 19]. Utrata heterozygotyczności (loss of heterozygosity – LOH) w obrębie ramion chromosomu 8q (*PLAG1*, *MYC*, *GDAP1*), 12q (*HMGA2*, *MDM2*, *MDM1*, *FRS2*) oraz 17p (*TP53*) należą do

najczęściej wykrywanych [12, 20]. (W nawiasach podano wybrane geny mające loci w obrębie podanych ramion chromosomów).

Rearanżacje *PLAG1*/*HMGA2* jak również zmiany w genach *TP53*, *ERBB2 PIK3CA*, *HRAS* są opisywane jako najczęstsze w Ca ex PA (w zależności od typu histopatologicznego) [19, 21-23]. Co więcej, ich obecność wiąże się potencjalnie z możliwością leczenia celowanego [24, 25]. Nie zostało jednoznacznie określone, czy Ca ex PA, czy jego odpowiednik *de novo* cechuje się większą złośliwością [26, 27]. Warto podkreślić, że analiza genetyczna umożliwia odróżnienie Ca ex PA od jego odpowiednika *de novo* [19].

Nowotwory złośliwe gruczołów ślinowych (salivary gland cancers – SGCs) stanowią grupę heterogenną zarówno pod względem przebiegu klinicznego jak również obrazu molekularnego. Powstają zarówno w małych jak i dużych gruczołach ślinowych, a najczęstszą lokalizacją jest ślinianka przyuszna. Stanowią około 5-8,5% wszystkich nowotworów złośliwych głowy i szyi (Head and Neck Cancers – HNCs). Chociaż nowotwory te występują rzadko, cechują się agresywnością, nieprzewidywalnym przebiegiem oraz znaczną śmiertelnością, sięgającą blisko 40% [2, 10, 28]. W ponad 50% przypadków dochodzi do wznowy choroby, pomimo zastosowanego leczenia [29]. Przerzuty odległe występują w 10-40% przypadków. Najczęściej stwierdzane są w: płucach (>50%), kościach (40%) oraz wątrobie (20%). Rozwój przerzutów jest związany nie tylko z wysokim stopniem złośliwości typów SGCs, rozmiarem guza, naciekiem wzdłuż naczyń, lecz również inwazją okołonerwową. Proces ten jest uwarunkowany zmianami genetycznymi oraz czynnikami immunoonkologicznymi predysponującymi do inwazyjnego przebiegu choroby [30-33]. Przykładowo, mutacje w genie *TP53* zostały stwierdzone u pacjentów z nawrotowym lub przerzutowym AC NOS (recurrent or metastatic – R/M) [34], a aberracje w genie *CDKN2A/B* zostały stwierdzone z wysoką częstością w przypadku R/M raka zrazikowokomórkowego (acinic cell carcinoma – AcCC) [34, 35]. Mediana OS u pacjentów z R/M rakiem gruczołów ślinowych wynosi 15 miesięcy, ponieważ wciąż brakuje specyficznych opcji leczenia [32]. W najbliższej perspektywie Międzynarodowa Agencja Badań nad Rakiem (International Agency for Research on Cancer – IARC) przewiduje istotny wzrost zapadalności oraz śmiertelności na SGCs [36].

Badania pokazują, że 5-letnie względne przeżycie pacjentów chorujących na nowotwory rzadkie jest istotnie niższe w porównaniu do grupy pacjentów z często występującymi nowotworami (49% vs. 63% w Europie; 55% vs. 75% w grupie mężczyzn, 60% vs. 74% w grupie kobiet w USA) [37, 38]. Uważa się, że dysproporcje te spowodowane są między innymi: biologią nowotworów, niedoborem odpowiedniej diagnostyki i leczenia (w tym leczenia

celowanego), trudnościami w prowadzeniu badań klinicznych, spowodowanymi w szczególności zbyt małą liczbą pacjentów. Protokoły dotyczące postępowania oraz nowe metody leczenia oparte na dowodach naukowych w nowotworach rzadkich są wyjątkami [37-39].

Według Światowej Organizacji Zdrowia (the World Health Organization – WHO) wyróżnić można ponad 20 typów histopatologicznych nowotworów złośliwych gruczołów ślinowych, co stanowi nie tylko wyzwanie diagnostyczne, lecz może prowadzić do błędnego rozpoznania, a tym samym opóźnienia wdrożenia odpowiedniego leczenia [40]. W dostępnej literaturze opisano liczne przypadki, w których zmiany złośliwe błędnie diagnozowano jako łagodne [16, 17]. Rak śluzowo-naskórkowy (mucoepidermoid carcinoma – MEC) jest najczęściej rozpoznawanym typem histopatologicznym, cechującym się stopniowym wzrostem oraz korzystnym rokowaniem. Coraz częściej opisywane są jednak przypadki MEC o agresywnym przebiegu [41]. Anzick i wsp. wykazali, że delecja *CDKN2A* ma związek ze złym rokowaniem u pacjentów z MEC, u których stwierdzono również najczęściej występującą w tym typie nowotworu fuzję *CRTC1::MAML2* [42]. Chociaż, SDC, MECA oraz Ca ex PA stwierdzane są z mniejszą częstotliwością, przebieg choroby jest dynamiczny, a rokowania pacjentów niekorzystne [10, 18]. Biorąc pod uwagę powyższe, niepewne przypadki z nakładaniem się cech morfologicznych lub immunohistochemicznych, wymagają badań molekularnych w celu ostatecznej klasyfikacji, a nowoczesne metody diagnostyczne zwiększają możliwości w tym zakresie z coraz większą pewnością oraz dokładnością. Aktualna klasyfikacja SGCs według WHO zawiera mutacje genetyczne, jako istotny element w diagnostyce różnicowej [40].

Występowanie SGCs jest częstsze u mężczyzn, a ryzyko rozwoju wzrasta wraz z wiekiem. Wcześniejsza ekspozycja na radioterapię w okolicy głowy i szyi jest udowodnionym czynnikiem ryzyka rozwoju SGCs. Dodatkowo, choroba nowotworowa w wywiadzie, w tym w rejonie głowy i szyi, zwiększają ryzyko zachorowania [2, 43, 44]. W dostępnej literaturze opisywane są liczne czynniki, które mogą predysponować do rozwoju SGCs, jednak w większości przypadków badania są ograniczone, a wnioski niejednoznaczne [2, 43-46]. W przeciwieństwie do HNCs ani palenie tytoniu, ani spożywanie alkoholu nie zwiększa jednoznacznie ryzyka rozwoju SGCs [43, 47]. Gwałtowny wzrost guza, z towarzyszącym bolesnym obrzękiem, owrzodzenie, porażenie nerwu twarzowego, sugerują rozwój zmiany złośliwej i wymagają poszerzenia diagnostyki w trybie pilnym [10]. Jednakże, przy braku powyższych objawów nie można wykluczyć rozrostu nowotworowego. W badaniu przeprowadzonym przez Zbären i wsp. jedynie u 21% chorych objawy sugerowały nowotwór złośliwy [14].

Do metod diagnostycznych zalicza się badania obrazowe, w tym: badanie ultrasonograficzne, tomografię komputerową, obrazowanie metodą rezonansu magnetycznego (Magnetic Resonance Imaging – MRI) jak również pozytonową tomografię emisyjną (Positron Emission Tomography – PET), przy czym w celu oceny zmian w gruczołach ślinowych preferowane jest wykonanie wieloparametrowego MRI [48, 49]. Przedoperacyjna biopsja aspiracyjna cienkoigłowa (fine – needle aspiration biopsy – FNAB) również znajduje zastosowanie w przedoperacyjnej diagnostyce różnicowej [48, 49].

Złotym standardem w leczeniu jest radykalna, chirurgiczna resekcja, która może stanowić wyzwanie ze względu na złożoność regionu anatomicznego, w tym bliskość istotnych struktur: nerwowych, naczyniowych, jak również mięśniowych [50, 51]. W zależności od stopnia zaawansowania choroby, jak również rozpoznania histopatologicznego, stosowane jest leczenie uzupełniające: radioterapia lub chemioradioterapia [48, 49]. Aktualne rekomendacje Amerykańskiego Towarzystwa Onkologii Klinicznej (American Society of Clinical Oncology – ASCO) zalecają zastosowanie radioterapii uzupełniającej w przypadku: nowotworów o wyższym stopniu złośliwości, pozytywnych marginesach, przerzutach w węzłach chłonnych, inwazji naczyń, guzów w stopniach zaawansowania T3-T4 oraz w każdym przypadku raka gruczołowo-torbielowatego (adenoid cystic carcinoma – AdCC) [48]. Jednakże, ze względu na rzadkość występowania SGCs, niemożliwe jest przeprowadzenie badań klinicznych z randomizacją w celu porównania wyników leczenia operacyjnego z oraz bez radioterapii adjuwantowej [49]. Dlatego w niektórych przypadkach uniknięcie zjawiska overtreatment (z ang.) może być niemożliwe. Co więcej, wskazania do zastosowania terapii systemowej nie są jasno zdefiniowane, a siła tych zaleceń jest umiarkowana [48]. Pacjenci z SGC otrzymują standardową terapię systemową, podobnie jak inni pacjenci z rakami płaskonabłonkowymi w rejonie głowy i szyi. Sytuacja jest jeszcze gorsza w przypadku R/M SGCs, dla których brakuje wiarygodnych dowodów na opcjonalne metody leczenia.

Wciąż poszukiwane są bardziej specyficzne metody terapii dla tej heterogennej grupy nowotworów. Identyfikacja odpowiednich czynników molekularnych w celu optymalizacji i personalizacji protokołów wydaje się być niezbędna w tym zakresie. Aktualnie trwają badania nad możliwością zastosowania leczenia celowanego dla nowotworów z mutacjami w genach *NOTCH 1-4*, *MYB*, *VEGF* i *EGFR* w AdCC, a także z ekspresją *HER2* i *AR* w SDC [52-56]. Równolegle, trwają badania kliniczne nad zastosowaniem inhibitorów punktów kontrolnych w SGCs [52]. Dodatkowo, w przypadku rzadkich guzów litych obserwowana jest wzrastająca liczba prób koszykowych (z ang. basket trials), w których interwencja terapeutyczna jest

możliwa, pod warunkiem wykrycia specyficznych aberracji w danym nowotworze [57]. Procedura ta jest w szczególności obiecująca dla pacjentów z SGCs, dla których nie są dostępne inne standardowe opcje leczenia, w szczególności w fazie nawrotu lub przerzutów. Podejście oparte na specyficznych dla danego pacjenta zmianach genetycznych zastępuje badania III fazy w konwencjonalnej drodze rejestracji leku. Tradycyjne badania kliniczne w rzadkich nowotworach są znacznie utrudnione. Aktualnie dużą liczbę aberracji genetycznych w nowotworach powiązano z konkretnymi terapiami. Dlatego, tak istotne jest poszukiwanie mutacji oraz zmian liczby kopii w SGCs, zwłaszcza w tych związanych z niekorzystnym rokowaniem.

W ciągu ostatnich kilkudziesięciu lat nastąpił znaczący postęp wiedzy o biologii nowotworów. Wśród dostępnych narzędzi badawczych, sekwencjonowanie następnej generacji (next-generation sequencing – NGS) odgrywa istotną rolę w kompleksowej analizie genetycznej oraz personalizacji terapii. W porównaniu do wcześniejszych metod sekwencjonowania, NGS pozwala na masowe, równoległe sekwencjonowanie pojedynczych cząsteczek DNA (kwas deoksyrybonukleinowy, deoxyribonucleic acid – DNA) [58-60]. Badania genetyczne, umożliwiają nie tylko wykrycie mutacji, biorących udział w rozwoju i progresji nowotworów, lecz również pozwalają na dopasowanie leczenia celowanego, co pozwala zwiększyć efektywność terapii oraz minimalizuje jej skutki uboczne. Dzięki temu, możliwe staje się określenie predykcyjnych oraz prognostycznych biomarkerów. Dysponując takimi narzędziami jesteśmy w stanie nie tylko przewidzieć przebieg choroby czy rokowanie, lecz również określić odpowiedź na leczenie [59-61]. Niestety dostęp do badań molekularnych, w tym NGS, jest nadal znacznie utrudniony w codziennej praktyce klinicznej w przypadku HNCs, a w szczególności SGCs i ograniczony w dużej mierze jedynie do badań klinicznych [62]. Wciąż brakuje dogłębnych analiz genetycznych w SGCs, w szczególności uwzględniających charakterystykę kliniczną oraz rokowanie pacjentów.

Artykuły wchodzące w skład rozprawy doktorskiej

Pierwsza praca prezentowanego cyklu, *Molecular landscape of salivary gland malignancies. What is already known?,* jest przeglądem systematycznym, stanowiącym wstęp do analizy zmian genetycznych w nowotworach ślinianek na podstawie dotychczasowych badań. W publikacji scharakteryzowano oraz pogrupowano najczęściej występujące zmiany genetyczne w dominujących typach histopatologicznych SGCs. Dodatkowo, podjęto próbę ustalenia, które z typów aberracji genetycznych mogą być odpowiedzialne między innymi za: progresję choroby, liczne nawroty choroby, wystąpienie przerzutów odległych lub znaczną śmiertelność, a tym samym wpływających na gorsze rokowanie pacjentów. Określono również możliwość zastosowania terapii celowanej w poszczególnych przypadkach aberracji genetycznych. Praca uwzględnia charakterystykę kliniczno-patologiczną dla poszczególnych typów histopatologicznych SGCs.

Jest to jedna z nielicznych prac przeglądowych, w której nie tylko podsumowano najczęściej występujące zmiany genetyczne w różnych typach SGCs, lecz również omówiono ich użyteczność kliniczną jako czynniki prognostyczne lub predykcyjne.

Druga praca prezentowanego cyklu, *FGFR2 point mutation in 2 cases of pleomorphic adenoma progressing to myoepithelial carcinoma,* jest to opis przypadku z analizą genetyczną materiału z tkanki guza pierwotnego i wznowy, pobranych od dwóch pacjentek, poddanych radykalnej resekcji PA, u których w okresie krótszym niż rok od pierwotnego leczenia zmian łagodnych, doszło do gwałtownego rozwoju nowotworów złośliwych (MECA ex PA). Wykryte zmiany uznano za biorące udział w transformacji złośliwej zmian łagodnych. Co więcej, mutacja w genie *FGFR2* stwarza możliwości zastosowania leczenia celowanego z wykorzystaniem zarejestrowanych inhibitorów FGFR2.

Powyższe badanie, chociaż obejmuje jedynie analizę dwóch przypadków, to wraz z danymi z literatury wskazuje, że wykryte zmiany genetyczne mogą być odpowiedzialne za transformację nowotworową, progresję choroby i niekorzystne rokowanie. Przedstawiona w badaniu analiza molekularna jest jednym z niewielu dotychczas opublikowanych badań, dotyczących transformacji PA w MECA ex PA.

Trzecia praca prezentowanego cyklu, *Potentially actionable molecular alterations in particular related to poor oncologic outcomes in salivary gland carcinomas,* jest to praca oryginalna

retrospektywna, w której przeprowadzono badanie genetyczne z uwzględnieniem klinicznego przebiegu choroby oraz analizę potencjalnych możliwości zastosowania leczenia celowanego u pacjentów z najczęściej występującymi, pierwotnymi nowotworami złośliwymi gruczołów ślinowych (MEC, AC, MECA, AdCC, SDC, AcCC, Ca ex PA). Badanie wykonano u chorych z SGCs leczonych pierwotnie metodą chirurgii w Klinice Otorynolaryngologii Chirurgii Głowy i Szyi Warszawskiego Uniwersytetu Medycznego w latach 2010-2017.

Powyższe badanie, pomimo ograniczonej liczby analizowanych próbek, jest jedną z nielicznych kompleksowych analiz genetycznych, przeprowadzonych za pomocą NGS w SGCs. W pracy zaprezentowano dodatkowo charakterystykę kliniczno-histopatologiczną, w tym rokowania pacjentów po minimum 5 letniej obserwacji. Podjęto próbę określenia zmian genetycznych, które mogą być związane z niekorzystnym rokowaniem u chorych z wybranymi typami SGCs oraz przedstawiono potencjalne możliwości zastosowania leczenia celowanego.

Założenia i cel pracy

1. Analiza aktualnej wiedzy na temat zmian genetycznych w najczęstszych typach nowotworów złośliwych gruczołów ślinowych.
2. Porównanie częstości występowania aberracji DNA u pacjentów z najczęściej występującymi typami histopatologicznymi SGCs.
3. Określenie zmian genetycznych związanych z nawrotem choroby, wystąpieniem przerzutów i gorszym rokowaniem w wybranych typach SGCs oraz predysponujących do złośliwej transformacji zmian łagodnych ślinianek.
4. Analiza występowania wśród chorych z określonymi typami SGCs aberracji genetycznych potencjalnie umożliwiających zastosowanie terapii celowanej.

Kopie opublikowanych prac

1. Molecular landscape of salivary gland malignancies. What is already known?

Salivary gland carcinomas (SGCs) are highly heterogeneous histopathological entities that arise in either the major or minor salivary glands. Although uncommon, these tumours exhibit considerable aggressiveness, unpredictable progression, and significant mortality. The fifth edition of the World Health Organisation classification of head and neck tumours distinguishes between 24 salivary gland malignancies. This may lead to difficulties in terms of diagnostic accuracy and suitable therapeutic selection. Mucoepidermoid carcinoma occurs most frequently and is characterised by gradual disease progression. Although salivary duct carcinoma, myoepithelial carcinoma, and carcinoma ex pleomorphic adenoma are rarely detected, they contribute to poor patient outcomes. Currently, attempts have been made to establish molecular characterisation of SGCs to improve differential diagnosis and create targeted treatments. This study aimed to summarise current knowledge regarding genetic variations in the most common salivary gland malignancies.

Key words: salivary gland carcinoma, molecular landscape, genetic alterations, NGS.

Contemp Oncol (Pozn) 2024; 28 (3): 201–216
DOI: https://doi.org/10.5114/wo.2024.144288

Review paper

Molecular landscape of salivary gland malignancies. What is already known?

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Introduction

Malignancies of the salivary glands are rare and account for approximately 5–8.5% of all head and neck cancers (HNC) [1–3]. Their occurrence is rare, with an annual incidence of 0.69 cases per 100,000 [4, 5]; however, the mortality rate is 40% [1]. Moreover, an increase of approximately 50% in both morbidity and mortality is predicted in the near future [6]. Salivary gland cancers (SGCs) are characterised by miscellaneous disease courses and clinical behaviours that contribute to unfavourable patient outcomes [2]. Among SGCs, more than 20 histopathological varieties have been classified by the World Health Organisation. Mucoepidermoid carcinoma (MEC) is the most common type of cancer, followed by acinic cell carcinoma (AcCC), adenoid cystic carcinoma (AdCC), carcinoma ex-pleomorphic adenoma (Ca *ex* PA), and adenocarcinoma (AC) [2, 7, 8]. The number of histopathological features interfering with benign lesions might also contribute to misdiagnosis and inappropriate management [9–11]. The incidence of these tumours is greater in males, and the risk of development increases with age. Former exposure to radiotherapy is also a well-known risk factor [3, 12–15]. A history of other cancers, including HNC, and occupational hazards are also associated with SGC incidence [3, 13]. In contrast to HNC risk factors, neither alcohol consumption nor tobacco use increases the risk of salivary gland malignancies [12, 13]. Numerous other causative factors have been proposed; however, studies are limited, and the results are inconclusive. Suspicious lesions, especially those with rapid growth, associated painful swelling, facial nerve palsy, or ulceration, indicate malignancy and should be investigated by imaging methods, preferably multiparametric magnetic resonance imaging.

Preoperative fine-needle aspiration enables the differentiation between benign and malignant tumours as well [2, 3, 16]. Radical surgical excision is the standard management option. Owing to tumour advancement and histopathological features, patients must receive further adjuvant radiotherapy or chemoradiotherapy [5, 16]. Park *et al.* reported disease recurrence in more than 50% of SGCs, despite radical primary treatment [17]. Distant metastases (DMs) occur in 10–40% of cases, frequently in the lungs (more than 50%), bones (40%), and liver (20%). Metastasis development is related not only to tumour type and stage but also to genetic alterations in tumour cells. These factors are therefore responsible for poor patient outcomes despite radical treatment [18–20].

Currently, the value of genetic analysis with next-generation sequencing (NGS) is particularly highlighted in SGCs. This will not only improve the knowledge about the molecular background of the pathologies but also enable the introduction of targeted therapies, especially for recurrent diseases, advanced stages, and drug-resistant cases [16, 21–24]. Additionally, it might be a pivotal tool in differential diagnosis, especially in ambiguous cases [25]. A summary of the clinical characteristics of SGCs with respect to incidence, histological subtype, predominant location, and survival is presented in Table 1. The most common genetic rearrangements in SGCs are listed in Table 2. The purpose of this paper was to review genetic variations,

Table 1. Clinicopathological features of salivary gland malignancies

Parameters	Mucoepidermoid carcinoma	Adenoid cystic carcinoma	Acinic cell carcinoma	Salivary duct carcinoma	Myoepithelial carcinoma	Epithelial-myoepithelial carcinoma	Secretory carcinoma	Carcinoma ex-pleomorphic adenoma	Clear cell carcinoma	Intraductal carcinoma	Adenocarcinoma	Polymorphous adenocarcinoma	Microsecretory adenocarcinoma
Histopathological variant/growth pattern	Oncocytic Clear-cell Sclerosing Low-grade Intermediate-grade High-grade	Cribriform Tubular Solid	Solid papillary-cystic Follicular Microcystic Low-grade Intermediate-grade High-grade	Cribriform Solid Cystic Papillary	Solid Trabecular Reticular	Sebaceous Oncocytic Double-clear	Microcystic Tubular Solid	Myoepithelial carcinoma Salivary duct carcinoma Adenoid Epithelial-myoepithelial carcinoma	Single cells Nested Solid Sheet-like Cords Trabeculae	Intercalated duct type Apocrine Hybrid Oncocytic Low-grade Intermediate-grade High-grade	Variety of growth patterns	Lobular Trabecular Microcystic Cribriform Papillary	Variety of growth patterns
Incidence per 1,000,000 (% of all SGC)	0.62–1.80 (9–30)	0.41–1.72 (6–25)	0.41–1.73 (6–17)	0.27–0.69 (4–10)	0.14–0.83 (2–12)	0.34 (< 5)	0.14–0.27 (2–4)	0.20–1.10 (3–16)	< 300 cases were described	< 200 cases were described	0.14–1.24 (2–18)	~1%	A few cases
Predominant location Other location	Major salivary glands (90% in the parotid glands)	Submandibular glands or minor salivary glands Lung, breast	Major salivary glands (87% in the parotid glands)	Major salivary glands	Major salivary glands	Major salivary glands	Major salivary glands	Major salivary glands	Intraoral minor salivary glands	Major salivary glands	Major salivary glands	Minor salivary glands	Minor salivary glands
5-year survival (%)	37.5–100	60–90	33–96	20–50	50–64	80–96	~95	25–96	No data	No data	43–81	75–100	No data
References	[2, 26–28]	[2, 51, 185, 186]	[2, 73, 185, 186]	[2, 185]	[88, 185, 187]	[143, 185]	[152, 154, 183, 185]	[2, 69, 185, 186]	[168]	[176, 185]	[69, 185]	[2, 105, 185, 188]	[189]

SGCs – salivary gland carcinomas

Table 2. The most frequent genetic alterations in salivary gland carcinomas

Histopathological type	Fusions	Other genetic changes	References
Mucoepidermoid carcinoma (MEC)	***CRTC1-MAML2,*** 56–88%	***TP53,*** 21–42% ***CDKN2A,*** 42–56% ***CDKN2B,*** 31% ***BAP1,*** < 21% ***PIK3CA,*** 17–21% ***HRAS,*** < 14%	Saade *et al.* [31] Kang *et al.* [34] Seethala *et al.* [35] Zerdan *et al.* [47] Wang *et al.* [48] Morita *et al.* [49]
Acinic cell carcinoma (AcCC)	***SCPP* gene cluster – *NR4A3,*** > 80%	***CDKN2A/B*** high percentage in high-grade tumours and metastases cases ***ATM,*** 7–14% ***PTEN,*** 10–12%	Haller *et al.* [75] Dogan *et al.*[78] Ross *et al.* [69]
Adenoid cystic carcinoma (AdCC)	***MYB-NFIB,*** 60–80% *MYBL1-NFIB,* *MYBL1-YTHDF3*	***NOTCH*** signalling pathway, ~ 40% (***NOTCH1,*** 26%) R/M primary tumours, ~ 13 (***NOTCH1,*** 8.5) ***KDM6A,*** ~ 15 ***BCOR,*** 13–17 ***ARID1A,*** 7–14	Wagner *et al.* [61] Ho *et al.* [59] Lee *et al.* [66] Ross *et al.* [69] Wang *et al.* [68]
Adenocarcinoma (AC)			
Polymorphous adenocarcinoma (PAC)		***PRKD1*** hotspot mutation, 50–73%	Andreasen *et al.* [108] Weinreb *et al.* [107]
Cribriform adenocarcinoma (CA)	***PRKD1-3*** fusions, > 80%		Weinreb *et al.* [115]
Microsecretory adenocarcinoma (MiAC)	***MEF2C-SS18,*** ~ 90%		Skálová *et al.* [39]
Basal cell adenocarcinoma (BCAC)		***CYLD*** mutation, 29%	Rito *et al.* [190]
Mucinous adenocarcinoma (MAC)		***AKT1 E17K*** mutation, 100% ***TP53*** mutation, 88%	Rito *et al.* [190] Rooper *et al.* [191]
Salivary duct carcinoma (SDC)		***TP53,*** 39–60% ***HRAS,*** 11–49% ***ERBB2,*** 10–100% ***NF1,*** 7–20% ***PIK3CA,*** 19–47% ***PTEN,*** 6–13.5% ***AR*** overexpression	Dalin *et al.* [126] Ku *et al.* [140] Kohsaka *et al.* [136] Dogan *et al.* [127] Mueller *et al.* [123]
Myoepithelial carcinoma (MECA) *de novo* MECA ex PA	*TGFBR3-PLAG1,* 25% ***FGFR1-PLAG1,*** 29%	Various copy number alternations	Dalin *et al.* [88]
Epithelial-myoepithelial carcinoma (EMC)		***HRAS,*** 27–87% ***PIK3CA,*** 22–40% ***AKT1,*** 6.5–20%	Urano *et al.* [146] Grünewald *et al.* [148] Chiosea *et al.* [149] Nakaguro *et al.* [150]
Secretory carcinoma (SC)	***ETV6-NTRK3,*** > 95%		Baněčková et al. [192]
Carcinoma ex-pleomorphic adenoma (CA ex PA)	***PLAG1/HMGA2*** rearrangements	***TP53,*** 55–100% ***ERBB2,*** 39–57% ***PIK3CA,*** 8–42% ***HRAS,*** 4–23%	Stenman *et al.* [90] Dalin *et al.* [88] Chiosea *et al.* [128] Grünewald *et al.* [141] Dogan *et al.* [127] Kohsaka *et al.* [136]
Clear cell carcinoma (CCC)	***EWSR1-ATF1,*** > 90%		Antonescu *et al.* [170]
Intraductal carcinoma (IC)	***RET* rearrangements,** ~ 45% ***NCOA4-RET*** (mainly in intercalated subtype) ***MYO18A-ALK***	***HRAS*** ***PIK3CA*** High percentage (only in apocrine subtype)	Skálová *et al.* [179] Weinreb *et al.* [180] Hsieh *et al.* [182] Majewska *et al.* [183]

including novel findings, in the most known histopathological types of SGCs.

A comprehensive literature search was performed in the PubMed database. We analysed the full texts of the articles published in English in the period 1984–2024. The exclusion criteria were as follows: languages other than English, only abstracts available, papers concerning HNC holistically without specific analysis of SGCs, and analysis

of malignant transformation of benign lesions, e.g. pleomorphic adenoma.

The search was performed with the following keywords: "salivary gland carcinoma", "genetic alterations", "molecular abnormalities", "NGS", "targeted therapy", "precision therapy", "mucoepidermoid carcinoma", "acinic cell carcinoma", "adenoid cystic carcinoma", "carcinoma ex-pleomorphic adenoma", "Ca *ex* PA", "adenocarcinoma", "salivary duct carcinoma", "myoepithelial carcinoma", "epithelial-myoepithelial carcinoma", "secretory carcinoma", "polymorphous adenocarcinoma", "cribriform adenocarcinoma", "microsecretory adenocarcinoma", "basal cell adenocarcinoma", "mucinous adenocarcinoma", "clear cell carcinoma", and "intraductal carcinoma".

The results of the search are presented in relation to the histopathological types of SGCs.

Mucoepidermoid carcinoma

Mucoepidermoid carcinoma is the predominant salivary gland neoplasm and is detected in more than 30% of all salivary malignancies [26]. Generally, it is characterised by gradual growth, rare recurrence, and favourable patient outcomes. However, this type of cancer can be highly heterogeneous and can present as low-, intermediate-, or high-grade cancer, with the latter being associated with poor outcomes. Additionally, the mean age at diagnosis is lower than that of other subtypes and ranges from 45 to 49 years [2, 26–29].

Chromosomal translocation t(11;19)(q14-21; p12-13) is unique for MEC and results in CREB regulator transcriptional coactivator (*CRTC1*) (also known as *MECT1*)-mastermind-like transcriptional coactivator 2 (*MAML2*) oncogene fusion. It has been detected in more than 80% of patients with this cancer subtype. This alteration leads to cell proliferation and survival through autocrine amphiregulin (AREG)/epidermal growth factor receptor (EGFR) signalling [30–35]. Chen *et al*. revealed that aberrantly activated AREG-EGFR signalling in CRTC1-MAML2-positive MEC cells made them highly sensitive to EGFR inhibition, suggesting benefit from EGFR-targeted therapies, e.g. cetuximab [36]. However, the results of further studies were unsatisfactory, and Ni *et al.* proposed simultaneous therapy with erlotinib-EGFR inhibitors and Notch inhibitors as more effective [32]. Since *MAML2* is involved in NOTCH signalling pathway activation [33, 37, 38], this drug combination becomes more target specific. The other translocation, t(11;19)(q21;q26), results in a *CRTC3-MAML2* fusion product that is detected in 6% of cases [30, 39, 40]. Another rare change is the translocation t(6;22)(p21;q12), which promotes *ESWR1 POU5F1* fusion [40]. Previously, the *CRTC1-MAML2* fusion product was considered a positive prognostic factor [41–43]. However, further research did not reveal significant differences in survival between patients with and without the translocation [31, 44, 45]. In contrast, Anzick *et al.* revealed that adverse outcomes in patients with translocations might be related to other genetic alterations, such as *CDKN2A* deletion [46]. However, copy number variations (CNVs) and somatic mutations associated with this alteration have not been frequently analysed in MEC. Zerdan *et al*. performed NGS analysis of 118 MEC tumours and reported *CDKN2A* abnormalities in 53% of the cohort. Other frequent changes included those in *TP53* (41%), *CDKN2B* (31%), *BAP1* (19%), *PIK3CA* (17%), *TERT* (15%), and *HRAS* (14,5%) [47]. Similar observations regarding the most common variations were reported by Wang *et al.* [48]. In contrast, the analysis of comparable sample sizes by Morita *et al.* revealed that *HRAS* mutations are rarely detected [49]. On the other hand, Kang *et al.* reported whole-exome sequencing results for 18 MEC tumours, and the second most frequent variation after *TP53* was the *POU6F2* gene (17%) [34]. In addition, alterations in *BRCA2* and *ERBB2* are quite common in MEC (17% and 13%, respectively) [30]. Although *NF1* alterations are not frequently detected, Kato *et al.* reported *NF1* and *TP53* commutation [47, 50]. However, the significance of these findings remains unclear. Further studies are needed to obtain a more in-depth molecular inquiry into MEC molecular pathogenesis, especially in cases with poor outcomes.

Adenoid cystic carcinoma

Adenoid cystic carcinoma frequently arises in the submandibular or minor salivary glands. Its occurrence in the parotid gland is rare. Although AdCC is known as a histopathological type with indolent growth, it tends to recur, with perineural invasion and DM, especially to the lungs [51–54]. Cases of relapse and metastasis (R/M) are frequently incurable because of a lack of effective systemic therapies, despite ongoing clinical trials. Therefore, there is an urgent need to verify the possibility of using targeted treatment.

The activating neurogenic locus notch homologue protein 1 (*NOTCH1*) mutation and v-myb avian myeloblastosis viral oncogene homologue (*MYB*) overexpression are related to AdCC development, progression, perineural invasion, and even chemoresistance, which predisposes patients to unfavourable outcomes [30, 55–58]. In contrast, Ho *et al.* did not find a correlation between mutational *MYBs* and either R/M or survival [59]. In approximately 80% of cases, *MYB* alternations present as the t(6;9)(q22-23;p23-24) translocation, which involves the *MYB* proto-oncogene and the nuclear factor 1B gene (*NFIB*) transcription factor, leading to overexpression of the fusion product and worsening the prognosis [30, 60, 61]. *MYB NFIB* translocation is associated with high MYB expression. This translocation disrupts the MYB 3′ UTR, a microRNA regulatory site responsible for downregulating *MYB*. The existence of additional mechanisms for *MYB* overexpression in AdCC was investigated, revealing alternate rearrangements that translocate super-enhancers in the *NFIB* and *TGFBR3* loci to the *MYB* locus. The MYB protein binds these super-enhancers, which in turn physically interact with the *MYB* promoter, drive its overexpression, and establish a positive feedback loop [62].

To emphasise the importance of *MYB* gene activity, it coordinates the upregulation of pivotal targetable genes involved in several functions related to carcinogenesis, such as apoptosis (*API5, BCL2, BIRC3, HSPA8,* and *SET*),

cell cycle control (*CCNB1, CDC2,* and *MAD1L1*), cell growth and angiogenesis (*MYC, KIT, VEGFA, FGF2,* and *CD53*), and cell adhesion (CD34) [63, 64]. Notably, in 35% of *MYB-NFIB* fusion-negative tumours, *MYBL1* alterations were identified [65]. Interestingly, *MYB/MYBL1* rearrangements were not very common in R/M AdCCs (22%). In contrast, NOTCH signalling pathway alterations were noted in approximately 40% of R/M cases (with NOTCH1 mutations observed in 26% of these), while only 13% of primary tumours demonstrate increased signalling in the pathway (NOTCH1 mutations in 8.5%) [59, 66].

Notably, Ho *et al.* also reported frequent alterations in R/M AdCC among genes involved in chromatin remodelling: *KDM6A, KMT2C/MLL3, ARID1A, ARID1B, BCOR, MLL2/KMT2D*, and *CREBBP,* with increased frequency compared with primary tumours. *TERT* promoter mutations were found in > 10% of the R/M patients. Interestingly, *NOTCH1* and *MYB/MYBL1* fusions are practically undetectable in these lesions [59]. In parallel, Stephens *et al.*, in addition to significant *MYB* activation, reported *SPEN* gene alterations (negative NOTCH signalling regulators) in more than 20% of the study cohort [67]. Similar findings regarding *NOTCH1, KDM6A, ARID1A, BCOR, CREEB,* and *TERT* have been previously reported. Less frequently detected alterations were in *MLL2, RUNX1, PTEN, BAP1, PIK3CA, CDKN2A, ACTB, MGA, CTNNB1, FOXD1, IGFR1, MUC5B, OBSCN, PIK3R1, SPHKAP, TTN FGFR2,* and *BRAF* [68, 69]. In contrast, *TP53* mutations are rarely found in AdCCs, including R/M cases. Compared with tumours with favourable outcomes, recurrent and metastatic tumours harbour notably greater loads of mutations. Thus, the options of targeted therapies are quite extensive for verifying their efficiency in advanced stages [56, 70, 71].

Acinic cell carcinoma

The characteristics of AcCC are generally similar to those of MEC. However, some cases of aggressive metastatic AcCC have been reported recently [72–74]. Current knowledge regarding the molecular alterations in AcCC has not yet been properly established.

Haller *et al.* detected rearrangement t(4;9)(q13;q31), which results in secretory Ca-binding phosphoprotein (*SCPP*) gene cluster (*STATH, HTN1, HTN3, ODAM, FDCSP*, and *MUC7*) and nuclear receptor subfamily 4 group A member 3 (*NR4A3*) fusion in most tumours of the analysed cohort (more than 80%). The former translocation is unique to AcCC and allows for differentiation of AcCC from mammary analogue secretory carcinoma (MASC), particularly in cases with high-grade transformation. Moreover, the resulting fusion gene acts as an oncogenic driver, with the NR4A3 transcription factor being upregulated due to the translocation of active enhancers from the SCPP gene cluster (which is highly expressed in salivary glands) to the region upstream of NR4A3 [75, 76]. The second most common fusion involves the histatin 3 and Myb/SANT-like DNA-binding domain containing 3 genes (*HTN3-MSANTD3*) (t(4;9)(q13.3;q31.1)), which have been described in a few cases (4–8%) [75–77]. According to the authors, the former translocation is exceptional for AcCC and provides an effective differential diagnosis of MASC, especially in cases with high-grade transformation. Moreover, NR4A3 might be considered an oncogenic driver through enhancer hijacking, whereby *NR4A3* is upregulated [75, 77]. In a recent study, Ross *et al.* reported *CDKN2A* and *CDKN2B* alterations in 76% and 45% of patients with relapses or metastases, respectively [69]. Simultaneously, Dogan *et al.* performed a genetic analysis and reported that the *CDKN2A/B* gene changed solely in high-grade tumours (58% of this group), whereas in the disease course with distant metastasis, these rearrangements were found in nearly 90% of the patients [78], confirming them as a negative prognostic factor. Notably, for tumours with identified negative markers, there are targetable treatment options based on CDK4/6 inhibitors, immunotherapy, or DNA methyltransferase inhibitors [79, 80]. Moreover, in advanced AcCC, other genetic changes have also been observed [78]. The most common rearrangements were related to *ATM* (7–14%), *PTEN* (10–12%), *FBXW7*, and *TP53* rearrangements, whereas alterations in *BRAF*, *NF1*, *HRAS*, *NOTCH1*, *TERT*, *ARID2*, *BIRC3*, *MTAP*, and *FAT1* were less common [69, 78]. Importantly, some of these alterations may provide opportunities for utilising precision therapy.

Carcinoma ex-pleomorphic adenoma

Carcinoma *ex* PA is a rare primary SGC arising from a preexisting PA. It is estimated that 5–15% of benign pleomorphic adenomas undergo malignant transformation to carcinoma (Ca *ex* PA) [81, 82]. Thus, the detection of the benign part of the tumour might lead to a final misdiagnosis, but rapid growth and other symptoms should indicate suspicion of malignancy [83]. Although salivary duct carcinoma, myoepithelial carcinoma (MECA), and adenocarcinoma not otherwise specified (NOS) are considered the most commonly detected malignant components of Ca *ex* PA, other types of SGC histopathology have also been described [84–89]. The pleomorphic adenoma gene 1 (*PLAG1*) and the high-mobility group AT-hook 2 (*HMGA2*) genes are most frequently altered in both PAs and Ca *ex* PAs [90], but not typical for primary salivary duct carcinoma (SDC), MECA, or AC. Katabi *et al.* presumed that rearrangements in these genes were specific to both PA or Ca *ex* PA and could distinguish Ca *ex* PA from its *de novo* counterparts [91]. Nonetheless, further investigations have shown their occurrence in *de novo* lesions [88]. Carcinoma *ex* PA tumours have abundant copy number alterations (CNAs) that are suspected to be involved in the malignant transformation from benign lesions. The most common loss of heterozygosity is the amplification of 12q genes (*HMGA2*, *MDM2*), deletions of 5q, gains of 8q12.1 (*PLAG1*) and 8q22.1-q24.1 (*MYC*), and amplification of 17 chromosomes (*ERBB2*) [88, 92–94]. Table 3 lists the most commonly detected genetic alterations, including fusions and histopathological subtypes of Ca *ex* PA, reported in the literature.

Myoepithelial carcinoma

The incidence of MECA is estimated to be very low, at 2% among all SGCs. Nonetheless, because of the difficulty of proper diagnosis, the actual number of cases is predicted

Table 3. Malignant component of carcinoma ex pleomorphic adenoma as reported in respective studies

Gene	Identified malignant component in Ca *ex* PA	References
Genes fusions		
CTNNB1-PLAG1	MECA, SDC ~ 30%	Asahina *et al*. [193], Skálová *et al*. [194], Dalin *et al*. [126]
FBXO32-PLAG1	ND	Bubola *et al*. [195]
FGFR1-PLAG1	MECA, SDC, ND	Dalin *et al*. [88], Chiosea *et al*. [128], Skálová *et al*. [194], Bubola *et al*. [195]
LIFR-PLAG1	MECA, SDC	Skálová *et al*. [194], Dalin *et al*. [126]
MEG3-PLAG1	ND	Bubola *et al*. [195]
ND4-PLAG1	MECA	Dalin *et al*. [88]
PLAG1-NFIB	ND	Bubola *et al*. [195]
TGFBR3-PLAG1	MECA	Dalin *et al*. [88], Rupp *et al*. [196]
HMGA2-CNOT2	ND	Bubola *et al*. [195]
HMGA2-NFIB	ND	Bubola *et al*. [195]
HMGA2 fusions	MECA	Dalin *et al*. [88]
Oher PLAG1 fusions	MECA	Dalin *et al*. [88]
HMGA2-WIF1	ND, Adenoid cystic carcinoma with sarcomatoid transformation, MECA	Persson *et al*. [92] Katabi *et al*. [197]
ETV6-RET	SC	Smith *et al*. [198]
ZCCHC7-NTRK2	ND (recurrence and metastatic case)	Pircher *et al*. [199]
Somatic gene mutations		
TP53	SDC, MECA	Chiosea *et al*. [128], Grünewald *et al*. [141], Dogan *et al*. [127], Rupp *et al*. [196], Dalin *et al*. [126], Kohsaka *et al*. [136], Mueller *et al*. [123]
PIK3CA	SDC, MECA, EMC	Chiosea *et al*. [128], Dogan *et al*. [127], Dalin *et al*. [88], Hallani *et al*. [144], Dalin *et al*. [126], Kohsaka *et al*. [136], Mueller *et al*. [123]
HRAS	SDC, MECA, EMC	Chiosea *et al*. [128], Dogan *et al*. [127], Dalin *et al*. [88], Hallani *et al*. [144], Dalin *et al*. [126]
ERBB2	SDC (gain/amp)	Chiosea *et al*. [128], Dogan *et al*. [127], Dalin *et al*. [126], Kohsaka *et al*. [136], Mueller *et al*. [123]
AKT1	SDC	Dalin *et al*. [126]
ALK	SDC	Mueller *et al*. [123]
APC	SDC	Dogan *et al*. [127], Mueller *et al*. [123]
AR	SDC	Dogan *et al*. [127]
ARID1A	SDC	Kohsaka *et al*. [136]
ASXL1	SDC	Dogan *et al*. [127]
ATM	SDC, MECA	Chiosea *et al*. [128], Dalin *et al*. [88], Mueller *et al*. [123]
ATR	MECA	Dalin *et al*. [88]
AURKA	SDC	Dogan *et al*. [127]
BAP1	SDC	Dogan *et al*. [127]
BRAF	SDC	Chiosea *et al*. [128], Kohsaka *et al*. [136]
BRCA1	MECA	Dalin *et al* [88]
BRCA2	SDC	Dogan *et al*. [127], Kohsaka *et al*. [136]
BTK	SDC	Dogan *et al*. [127]
CCNE1	SDC	Dogan *et al*. [127], Mueller *et al*. [123]
CCND3	SDC	Mueller *et al*. [123]
CDH1	SDC	Dogan *et al*. [127]
CDK4	SDC	Grünewald *et al*. [141], Mueller *et al*. [123]
CDK6	SDC	Mueller *et al*. [123]
CDK12	SDC	Dogan *et al*. [127]
CDKN1B	SDC	Dogan *et al*. [127]
CDKN2A	SDC	Chiosea *et al*. [128], Mueller *et al*. [123]

Table 3. Cont.

Gene	Identified malignant component in Ca *ex* PA	References
CHEK2	SDC	Mueller *et al*. [123]
CREBBP	MECA, SDC	Dalin *et al*. [88], Mueller *et al*. [123]
CTCF	SDC	Dogan *et al*. [127]
DNMT1, DNMT3A, NMT3B	SDC	Dogan *et al*. [127]
DOCK7	SDC	Dalin *et al*. [126]
EGFR	SDC	Dogan *et al*. [127]
EP300	SDC	Mueller *et al*. [123]
ERBB3	SDC	Dogan *et al*. [127]
EWSR1	MECA (clear cell)	Skálová *et al*. [194]
FANCA, FANCC	SDC	Dogan *et al*. [127]
FASN	SDC	Dalin *et al*. [126]
FAT1	SDC, MECA	Dogan *et al*. [127], Dalin *et al*. [88]
FAT4	MECA	Dalin *et al* [88]
FBXW7	SDC	Dogan *et al*. [127], Mueller *et al*. [123]
FGFR1	MECA, SDC	Dalin *et al*. [88], Dalin *et al*. [126], Mueller *et al*. [123]
FGFR2	MECA	Dalin *et al*. [88]
FGFR3	SDC	Chiosea *et al*. [128]
FGFR4	SDC	Mueller *et al*. [123]
FH	SDC	Dogan *et al*. [127]
FLCN	SDC	Dogan *et al*. [127]
FOXA1	SDC	Dalin *et al*. [126], Kohsaka *et al*. [136]
GATA2	SDC	Dogan *et al*. [127]
HMGA2	ND	Persson *et al*. [92]
HNF1A	SDC	Dogan *et al*. [127]
JUN	SDC	Dogan *et al*. [127]
KDR	SDC	Dalin *et al*. [126]
KIT	SDC	Mueller *et al*. [123]
KMT2A	SDC	Dogan *et al*. [127], Kohsaka *et al*. [136]
KMT2B	SDC	Dalin *et al*. [126]
KMT2C	SDC	Dogan *et al*. [127], Dalin *et al*. [126], Kohsaka *et al*. [136]
KMT2D	SDC	Kohsaka *et al*. [136]
LIFR	MECA	Dalin *et al*. [88]
MAP2K2	SDC	Kohsaka *et al*. [136]
MAP3K1	SDC	Dogan *et al*. [127]
MDM2	ND, SDC	Persson *et al*. [92], Mueller *et al*. [123]
MET	MECA	Dalin *et al*. [88]
MLH3	SDC	Dalin *et al*. [126]
MML2	MECA	Dalin *et al*. [88]
MN1	MECA	Dalin *et al*. [88]
MSH5	SDC	Dalin *et al*. [126]
MTOR	SDC	Dalin *et al*. [126]
MYC	SDC	Dogan *et al*. [127]
NCOA1, NCOA2	MECA	Dalin *et al*. [88]
NCOR1	SDC	Dogan *et al*. [127], Dalin *et al*. [126]
NF1	SDC	Dogan *et al*. [127], Dalin *et al*. [126], Kohsaka *et al*. [136], Mueller *et al*. [123]
NOTCH1	MECA, SDC	Dalin *et al*. [88], Mueller *et al*. [123]
NOTCH2-3	SDC	Mueller *et al*. [123]

Table 3. Cont.

Gene	Identified malignant component in Ca *ex* PA	References
NSD1	SDC	Dalin *et al*. [126]
PIK3R1	SDC	Dogan *et al*. [127]
PTEN	SDC	Chiosea *et al*. [128], Dogan *et al*. [127], Kohsaka *et al*. [136]
PTPN11	SDC	Dogan *et al*. [127]
PTPRS	SDC	Dogan *et al*. [127]
RAD51C	SDC	Dogan *et al*. [127]
RET	SDC	Dalin *et al*. [126]
RICTOR	SDC	Mueller *et al*. [123]
ROS1	SDC	Mueller *et al*. [123]
RTEL1	SDC	Dogan *et al*. [127]
SF3B1	SDC	Dalin *et al*. [126]
SMAD4	SDC	Dalin *et al*. [126]
SMARCA4	MECA, SDC	Dalin *et al*. [88], Dalin *et al*. [126]
TSC2	SDC	Mueller *et al*. [123]
ZFHX3	SDC	Kohsaka *et al*. [136]

EMC – epithelial-myoepithelial carcinoma, MECA – myoepithelial carcinoma, ND – no data available, SDC – salivary duct carcinoma

to be greater [10, 95]. The tumour might occur as a *de novo* lesion or arise from the malignant transformation of a PA or myoepithelioma [96]. These data suggest that MECA *ex* PAs are more frequently detected than *de novo* lesions [88, 97]. However, the conclusion regarding which component is characterised by more aggressive behaviour or poorer patient outcomes remains debatable [95, 97–100]. In most cases, this subtype of cancer is associated with adverse patient results, including early local and DM [10, 88, 95]. Myoepithelial carcinoma is one of the most commonly confirmed components of Ca *ex* PAs [89, 101].

Salivary gland MECA rarely occurs; therefore, few genetic studies of this type are available. Dalin *et al.* analysed 40 tumours with divisions on either the MECA *de novo* or the MECA *ex* PA, as well as cases with and without recurrence. In MECA *ex* PA, more genetic alterations, including fusions, somatic mutations, and CNVs, were found. According to the authors, CNVs are responsible for the malignant transformation of the PA into the MECA *ex* PA and are also associated with a worse prognosis. *FGFR1-PLAG1* fusion was the most commonly (18%) identified in the MECA *ex* PA, followed by *TGFBR3-PLAG1* but with no evidence of their prognostic value. Furthermore, *EWSR1-ATF1* was described only in the MECA *de novo,* with or without recurrence [88]. In contrast to the research conducted by Skálová *et al.*, *EWSR1* rearrangements were found frequently in the clear cell component of MECA both in *de novo* cases and those arising from the PA, but the fusion partner genes were not identified [102]. In the aforementioned study, *PIK3CA* was present only in patients without relapse, whereas *FGFR2* mutations were found in patients with recurrence [88]. The findings are summarised in Table 4. *FGFR2* mutations were also described in 2 patients after radical PA excision, in which the MECA rapidly developed. In both PAs and MECAs ex PAs, FGFR2 point mutations were confirmed, which might be indicative of an aggressive disease course [103]. Recently, Gandhi *et al.* reported a novel *CTCF-NCOA2* fusion in a single MECA patient [104]. Furthermore, Cormier *et al.* presented a novel *TERT* promoter mutation in metastatic MECA *ex* PA (the tumour was previously misdiagnosed as PA) [9].

Adenocarcinoma

Polymorphous adenocarcinoma

Polymorphous adenocarcinoma (PAC) is a rare, slow-growing malignant tumour. It mainly arises from the minor salivary glands (second most common histopathological type), particularly those localised on the hard palate. There is a higher prevalence in women than in men, and patient outcomes are defined as one of the most favourable outcomes among SGCs [105, 106].

Weinreb *et al.* revealed a *PRKD1* p.E710 hotspot mutation in nearly 73% of tumours, and these observations were not identified in other SGCs. Thus, this alteration is unique to PAC and may be useful for differentiating it from its mimics [107, 108]. Notably, in cribriform adenocarcinoma (CA), *PRKD1-3* fusions are the most common. CA is classified as an aggressive variant of PAC with a high predisposition to metastasis [109–112]. Among the fusion partners *ARID1A, ATL2, DDX3X, PPP2R2A, PRKAR2A, SNX9,* and *STRN3* (cases with high-grade transformation) should be mentioned [113–116]. However, the type of genomic alteration is not specific for any AC subtype, and occasionally, either *PRKD1-3* fusions or *PRKD1* rearrangements are found in PAC and CA, respectively [109]. Therefore, differentiation between these 2 variants with various behaviours might be challenging.

Adenocarcinoma not otherwise specified

Tumours with a histopathological diagnosis of adenocarcinoma NOS constitute a heterogeneous group that has not

Table 4. Genetic rearrangements in the MECA *de novo* and the MECA *ex* PA presented in the study by Dalin *et al.* in relation to oncological outcomes

MECA *de novo*		MECA *ex* PA	
No recurrence	**Recurrence**	**No recurrence**	**Recurrence**
TGFBR3-PLAG1	*HMGA2* fusions	*TGFBR3-PLAG1*	*FGFR1-PLAG1*
Other *PLAG1* fusions	*EWSR1-ATF1*	*FGFR1-PLAG1*	Other *PLAG1* fusions
EWSR1-ATF1	*FGFR2*	Other *PLAG1* fusions	*HMGA2* fusions
MSN-ALK		*HMGA2* fusions	*FGFR2*
PIK3CA		*HRAS*	*MAML2*
MAML2		*PIK3CA*	*NOTCH1*
NOTCH1		*FGFR1*	*ATM*
ATM		*LIFR*	*ATR*
KMT2C		*MET*	*BRCA1*
SETD2		*MAML2*	*MN1*
PCM1		*ATR*	*COL2A1*
TRIP11		*CREBBP*	*SMARCA4*
		NCOA1	
		NCOA2	
		FAT1	
		FAT4	

MECA – myoepithelial carcinoma, PA – pleomorphic adenoma

yet been well characterised. For example, *NTRK2-ZCCHC7* and *SS18-ZBTB7A* fusions have been described [116, 117]. In R/M cases, *TP53* (55%), *PIK3CA*, *HRAS*, *CDKN2A*, *ERBB2*, *PTEN*, *NF1*, and *ARID1A* alterations were observed with considerable frequency [69].

On the basis of genetic pattern analysis, microsecretory adenocarcinoma has been distinguished from NOS. Microsecretory adenocarcinoma harbours *MEF2C-SS18* fusion in approximately 90% of cases [39, 118].

The most common alterations in basal cell adenocarcinoma and mucinous adenocarcinoma are shown in Table 1.

Salivary duct carcinoma

Salivary duct carcinoma is one of the most aggressive SGCs, with either early relapse or frequent DM. It is also associated with significant mortality. Predilection in elderly males with a smoking history is usually combined with advanced-stage presentation and parotid gland localisation [119–123]. The estimated morbidity is 5.5–12% [124, 125]. Moreover, SDCs *ex* PAs have also been detected [122, 126–128]. Table 2 provides genetic information for this subtype.

In addition to the microscopic structure resembling high-grade ductal carcinoma of the breast, SDC is also characterised by the overexpression of human epidermal growth factor receptor 2 (*HER2*). Instead of oestrogen and progesterone receptor positivity, androgen receptor (AR) expression is detected in 75–98% of cases [122, 126, 129, 130]. Notably, *AR* is seldom detectable in other SGCs [131]. However, studies are inconclusive regarding the prognostic value of the AR [129, 131, 132]. Nevertheless, Kawakita *et al.* showed in a retrospective study that the utilisation of HER2-targeted therapy and androgen deprivation therapy significantly improved patients results compared with conventional therapy management [133]. The anti-HER2 therapies that induce improvement in clinical responses in SDC patients use trastuzumab in combination with chemotherapy (i.e. taxanes, capecitabine, carboplatin, eribulin) or with another anti-HER2 targeted agent (i.e. pertuzumab). Further expectations and therapeutic advances are related to novel anti-HER2 drugs such as antibody-drug conjugates (i.e. trastuzumab emtansine, trastuzumab deruxtecan) introduced in this setting [134].

In recent years, genetic knowledge about SDC has increased profoundly, but it still has not been comprehensively investigated. The tumour mutation burden is extremely high in most SDC cases, in contrast to other SGCs. Vos *et al.* evaluated therapy with nivolumab (anti-PD-1) and ipilimumab (anti-CTLA-4) in patients with metastatic SGC. Although the efficacy was limited in AdCC, with infrequent responses, they found it promising for non-AdCC SGCs, particularly salivary duct carcinomas [135]. Genetic fusions are not recurrent events in this subtype, whereas somatic mutations as well as CNVs are considerably more common [123, 126, 136]. Moreover, most of them provide opportunities for the utilisation of targeted treatment for this unpredictable cancer [30, 127, 137–139]. *TP53, HRAS, PIK3CA,* and *ERBB2* (*HER*) rearrangements are the most common, and some of them are related to poor outcomes [123, 126–128, 136, 140, 141]. Interestingly, although *HRAS* mutations constitute the majority of *de novo* lesions, they are rare in SDC *ex* PAs [123, 126, 127, 136]. Data regarding the molecular landscape of SDCs are presented in Table 5.

Epithelial-myoepithelial carcinoma

Epithelial-myoepithelial carcinoma (EMC) is rarely detected, and it was first reported by Donath *et al.* in 1972. Previously, it appeared under other terminology of adenomyoepithelioma or clear cell adenoma. The tumour consists of a dual cell population that forms a double layer: inner ductal cells and outer myoepithelial cells [142–144]. Notably, various histological subtypes of EMCs exist, including sebaceous, oncocytic, and double-clear subtypes.

Table 5. The genetic pathways most commonly affected in salivary duct carcinoma

Pathway	Genes	References
DNA damage	*TP53* (39–60%), *ATM, BRCA2, CHEK2, MDM2, MDM4, MLH3, MLH5*	[123, 126, 127, 136, 140, 141]
MAPK	*HRAS* (11–49%), *NF1* (7–20%), *BRAF, KRAS, NRAS*	[123, 125, 126, 127, 128, 136, 137, 140]
RTK	*ERBB2* (10–100%), *ALK, EGFR, ERBB3-4, FGFR1-2, FGFR4, FLT3, JAK2, KDR, KIT, MET, NTRK2, PDGFRA, RET*	[123, 126, 127, 136, 137, 140]
PI3K/AKT/mTOR	PIK3CA (19–47%), *PTEN* (6–13.5%), *AKT1-3, PIK3R1, RICTOR, RPTOR, TSC2*	[123, 125, 126, 127, 128, 136, 137, 140]
Androgen signalling	*AR, FASN, FOXA1*	[126, 136]
Histone modification	*KDM6A, KMT2A, KMT2C, KMT2D, KMT2E, NSD1*	[126, 127, 136, 140]
Cell cycle	*CDK4, CDK6, CDK12, CDKN1A, CDKN1B, CDKN2A, CCNE1, CCND1-3, RB1*	[123, 126, 127, 136, 140, 141]
NOTCH	*CREBBP, EP300, FBXW7, NOTCH1-3*	[123, 140]
SWI/SNF complex	*ARID1A, SMARCA4, SMARCB1*	[123, 126, 127, 136]
WNT-β-catenin	*APC, CDH1, CTNNB1, FAT1*	[123, 126, 140]
Other	*ABL1, AURKA, BCOR, CCND1, CCNE1, FLCN, GNAS, HMGA2, IDH1-2, IGFR1, IKBKE, KLF5, AMP, MAP2K1, MAP2K4, MITF, MPL, MYC, PRDM1, SMAD4, SMO, STK11, TNIK, VHL, ZFHX3*	[123, 126, 127, 136, 140, 141]
Fusions	*ETV6-NTRK3, ABL1-PPP2R2C, BCL6-TRADD, HNRNPH3-ALK, EML4-ALK, RAPGEF6-ACSL6*	[126, 127, 195]

Thus, the differential diagnosis could pose difficulties [145, 146]. Morbidity predominates in females more than males. Most commonly, the parotid gland is affected, and the tumour is characterised by a high overall survival rate. Although DM rarely occur, relapses are common [143, 147].

HRAS (27–87%) was described as the most frequently mutated gene in EMC [146, 148–150]. In the studies conducted by Urano *et al.* and Nakaguro *et al.,* these findings were not detected in EMCs *ex* PAs [146, 150]. In parallel, Hallani *et al.* did not prove *HRAS* alterations for *de novo* EMC [144]. *PIK3CA* and *AKT1* have been reported quite commonly in EMC (22–40% and 6.5–20%, respectively) [146, 148]. *CTNNB1, FBXW7,* and *TP53* rearrangements and *SMARCB1* deletions have been reported in single cases (the last 3 in high-grade tumours) [144, 148]. Mäkelä *et al.* described rare metastatic EMC in a 36-year-old woman, where in addition to *HRAS* mutation, *ARID1B, ATR, CDK12, ERBB4, MAPK1, NANOG, NOTCH2, PIK3R1,* and *RPTOR* alterations were detected [151].

Secretory carcinoma

Secretory carcinoma (SC) (previously known as mammary analogue secretory carcinoma) is a novel salivary gland tumour that was described by Skálová *et al.* in 2010 [152]. Most of these tumours were previously classified as AcCC [153]. The age at diagnosis is relatively low (mean 45 years), including paediatric patients. There is a greater predilection in men, and the disease course is indolent, with favourable patient outcomes [154, 155].

Secretory carcinoma has a significant histological and molecular resemblance to breast secretory carcinoma. It is characterised by harbouring the same translocation t(12;15)(p13;q25), resulting in the *ETV6-NTRK3* fusion gene encoding a chimeric oncoprotein-tyrosine kinase (unlike AcCC) [152, 155, 156]. Other *ETV6* fusion partners have also been discovered, including *ETV6-MAML3* [157], *ETV6-MET* [158], and *ETV6-RET* [157, 159]. Notably, some of these genes remain unknown (*ETV6-X*) [160]. Recently, other novel fusions, such as *VIM-RET* [161], *CTNNA1-ALK* [162], and dual fusion, *ETV6-RET* and *EGFR-SEPT14,* were identified in an 18-year-old male [159]. *ETV6-NTRK3* and *MYB-SMR3B* fusions were found in recurrent high-grade submandibular tumours [161]. Only a few studies have analysed genetic rearrangements other than fusions. Na *et al.* identified pathogenic *PRSS1* mutations, mainly in patients with an aggressive disease course and recurrence, whereas other findings were classified as likely pathogenic or of uncertain significance [163]. In contrast, Skálová *et al.* analysed 3 tumours with high-grade transformation and did not detect the most commonly occurring genetic alterations associated with poor outcomes (*TP53, CTNNB1, EGFR, CCND1*) [164].

Testing for *ETV6-NTRK3* gene rearrangements is critical for SC patients care since entrectinib, an inhibitor of tropomyosin receptor kinase (TRKs), has been reported to be effective and safe in treating solid tumours with NTRK fusion genes. In an integrated analysis of phase 1–2 trials (STARTRK-1, STARTRK-2, and ALKA-372-001) of solid tumours with the NTRK fusion gene, the response rate to the TRK inhibitor entrectinib was 57%, and the median progression-free survival was 11.2 months [165]. Another TRK inhibitor, larotrectinib, is also effective in the treatment of solid tumours with the NTRK fusion gene [166]. Other potential therapies for SC patients with identified oncogenic RET fusions, namely ETV6-RET , are selpercatinib and pralsetinib selective RET inhibitors, currently under preclinical and clinical testing [167].

Clear cell carcinoma

Clear cell carcinoma (CCC) (previously known as hyalinising clear cell carcinoma) is an indolent low-grade tumour that typically arises from the intraoral minor salivary glands. There is a higher prevalence in females, whereas relapses and metastases are rare [168].

Considering the occurrence of clear cells in other SGCs, differential diagnosis may be a challenge [169]. Antonescu *et al.* first described genetic rearrangement in the CCC-*EWSR1-ATF1* fusion *t*(12;22)(q13;q12). It occurs in more than 90% of cases, and, being unique for CCC, it is therefore a helpful differentiation tool [170]. *EWSR1-CREB1, EWSR1-CREM,* and *SMARCA2-CREM* fusions have been reported in single cases thus far [171–173].

Intraductal carcinoma

Intraductal carcinoma (IC) is a rare salivary gland tumour that affects mainly the parotid gland, with features similar to mammary atypical ductal hyperplasia or ductal carcinoma *in situ* of the breast [174, 175]. Recent studies have classified 4 distinctive subtypes: intercalated duct type, apocrine, hybrid, and oncocytic [176].

RET rearrangements, including recurrent *NCOA4-RET* (intercalated, oncocytic, seldom hybrid), *TRIM27-RET* (hybrid, apocrine), and *TRIM33-RET* (oncocytic) rearrangements, have been detected [177–179]. In contrast, *RET* gene alterations have not yet been confirmed in the apocrine subtype [180].

The relationship between IC and SDC remains controversial, even though they are considered diverse entities. Intraductal carcinoma, especially invasive apocrine IC, is a precursor for more aggressive cancers, such as SDC [174, 176, 180]. Nevertheless, this issue requires further investigation. Molecular evidence of resemblance to SDC revealed a high occurrence of *HRAS* and *PIK3CA* hotspot mutations in apocrine IC [174, 180–182]. Additionally, *ATM, SPEN*, and *TP53* mutations and either *DFFA-ARID1A* or *KIF13B-EPB41L4B* fusions were found in this subtype [174,180]. In parallel, *BRAF* V600E mutations in the oncocytic subtype and novel fusions of *TUT1-ETV5* and *KIAA1217-RET* in intercalated duct variants and hybrid intercalated duct tumours with invasive growth have also been identified [178, 179].

Furthermore, Majewska *et al.* reported an *MYO18A-ALK* fusion in intercalated duct-type IC in elderly patients after radical excision and no disease relapse during follow-up [183].

Recently, Watanabe *et al.* presented a case of a 59-year-old male with high-grade intercalated-type IC and DM. Despite radical excision and postoperative radiotherapy, the patient developed multiple DM. Genetic analysis revealed a *CTNNA1-ALK* fusion and *TP53* mutation. Despite further ALK-TKI therapy, the patient's condition declined, and NGS analysis of the blood samples revealed a novel *PIK3CA* mutation (*ALK* fusion was not detected). The importance of this shift remains uncertain. Nevertheless, treatment failure might be related to novel alterations and the predominance of other abnormalities in recurrent tumour tissue [184].

Conclusions

Salivary gland carcinomas are rare entities with unpredictable disease courses. The diversity of both the histological architecture and molecular alterations is distinct among individual subtypes, which leads to diagnostic difficulties. Moreover, because of the rare incidence of SGCs, multicentre clinical trials are urgently needed to provide targeted therapeutic options. Currently, the value of genetic analysis has been highlighted, particularly in terms of the possibilities of precision therapies and in light of the insufficient effectiveness of standard treatment options. Knowledge of the molecular landscape of SGC, especially related to outcome predictors, will provide novel and precise methods for diagnosis and therapy in the future.

Disclosures

1. Institutional review board statement: Not applicable.
2. Assistance with the article: None.
3. Financial support and sponsorship: None.
4. Conflicts of interest: None.

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Submitted: 04.07.2024
Accepted: 20.09.2024

2. FGFR2 point mutation in 2 cases of pleomorphic adenoma progressing to myoepithelial carcinoma

Introduction: Salivary gland tumours are rare neoplasms. Pleomorphic adenoma (PA) is the most frequent benign lesion. Myoepithelial carcinoma (MECA) is rarely recognized malignancy, but the prognosis is unfavourable. The aim of this study was to identify genetic rearrangements that might be responsible for dynamic MECA progression in patients with primary radical PA excision.

Material and methods: Next-generation sequencing (NGS) of 1500 gene coding sequences was performed in primary and recurrent tumour tissue collected from 2 patients, in whom PA was initially diagnosed and within one year multifocal MECA was detected. Formalin-fixed paraffin-embedded blocks with tumour tissues were subject to NGS analysis, involving small-scale mutations, as well as focal and chromosomal arm-level copy number changes.

Results: This study showed mutations in the FGFR2 gene in PA and MECA tissues, obtained from both patients. One of them, pathogenic mutation p.Pro253Arg, was associated with sensitivity to registered drug inhibitors. Additionally, FGFR1, EGFR, and CDK4/CDK6 amplification, as well as CDKN2A/B deletion, were detected in one case. Furthermore, mutations in suppressor gene APC2 and PIK3C2A were detected, but only in MECA tissue. The analysis also identified the following chromosomal copy alterations: 4q12-q13.3, 9p21.3, 5q23.1-q34, del8p23.3-p12, and del13q21.31-q31.1.

Conclusions: Rearrangement of the FGFR2 gene, identified in primary PA and MECA ex PA samples of both our patients, may be responsible for the malignant transformation and the disease progression. Further studies are encouraged to confirm the relevance of the findings. The therapy option with FGFR2 inhibitors may be considered in advanced or metastatic MECA ex PA with confirmed FGFR2 mutations.

Key words: salivary gland, pleomorphic adenoma, myoepithelial carcinoma, malignant transformation, next-generation sequencing, FGFR2 mutation.

Contemp Oncol (Pozn) 2023; 27 (3): 211–216
DOI: https://doi.org/10.5114/wo.2023.133592

Case report

FGFR2 point mutation in 2 cases of pleomorphic adenoma progressing to myoepithelial carcinoma

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Introduction

Salivary glands tumours are a histologically heterogeneous group of lesions [1]. Pleomorphic adenoma (PA) is the most common benign salivary gland tumour. It occurs slightly more often in women between 40 and 50 years of age. Most cases are recognised in parotid glands. Among risk factors, radiation exposure seems most significant [2–5]. Superficial or total parotidectomy with facial nerve preservation are the best treatment options [6]. The risk of recurrence of PA amounts to nearly 3% and may be associated with margin-positive resection and younger age. Approximately 5–15% of pleomorphic adenomas may transform to carcinoma ex-pleomorphic adenomas (Ca *ex* PA), an aggressive malignancy [5, 7]. The malignant component of Ca *ex* PA is most frequently adenocarcinoma not otherwise specified [8], followed by myoepithelial carcinoma (MECA) [9]. In the study by Zbären *et al.* only 21% of malignant salivary neoplasms led to clinical symptoms [10]. Therefore, the differentiation between recurrent PA and malignancy can be a huge challenge and lead to misdiagnosis. In the available literature, many cases with false diagnosis of Ca *ex* PA as PA were recognized [8, 11–15]. Xu *et al.* reported the misdiagnosis of MECA *ex* PA with the benign myoepithelioma [12].

Myoepithelial carcinoma occurs very infrequently. It is estimated that less than 2% of all cases are confirmed. Apparently, the number is higher because of the difficulty in proper diagnosis [12, 16]. It has been proven that no predilection occurs in sexes [16–18]. The prognosis for patients with MECA is poor and related to early local and distant metastases [12, 16, 19].

Some researchers proved that MECA *de novo* is characterized by worse outcomes than MECA *ex* PA [18, 20]. At the same time, other studies suggest that MECA *ex* PA is characterized by higher aggressiveness than *de novo* lesions, even though it is intracapsular or of minimal invasiveness [16, 21, 22]. Additionally, MECA *ex* PA are detected more commonly than *de novo* lesions [22]. The major issue is a proper diagnosis because MECA may mimic other lesions, especially PA. This leads to frequent misdiagnoses and delays in appropriate treatment and recovery [12].

Though salivary gland cancers occur very rarely, they are characterized by considerable aggressiveness and mortality. Nowadays, we are facing a continual lack of prognostic as well as predictive markers that would enable more personalized treatment and improve the outcomes.

The aim of this study was to identify genetic rearrangements that might be responsible for dynamic MECA progression in patients with primary radical PA excision.

Material and methods

The study was conducted in accordance with national guidelines and regulations. The Bioethics Committee at the Medical University of Warsaw

approved the protocol of the study (No. AKBE/175/2021). The tissue material was collected from 2 patients treated in the tertiary Otorhinolaryngology, Head and Neck Surgery Department. The material consisted of 4 formalin-fixed, paraffin-embedded blocks with primary and recurrent tumour tissues. The next-generation sequencing (NGS) was performed in both retrieved PA and MECA samples. DNA was isolated with E.Z.N.A. FFPE DNA Kit (Omega Bio-Tek), and for each sample 100 ng were converted to genomic libraries using KAPA HyperPlus Kit (Roche). Libraries were then enriched using SeqCap EZ probes (Roche), capturing 8.4 Mb and ~1500 cancer-associated genes and sequenced on Illumina HiSeq1500 instrument using 2 × 100 bp reads. Mean coverage was in the range 174–215×, and ge20 was > 91 for all samples. Raw sequencing data processing was done according to Broad Institute recommendations [23] and involved quality control of FASTQ files, adapter trimming and low-quality read removal using Trimmomatic [24], read mapping to hg19 genome using BWA-MEM [25], duplication removal, local realignment and quality recalibration using GATK and Picard, and variant calling using HaplotypeCaller and Mutect2. Downstream analysis involved identification of small-scale mutations, as well as focal and chromosomal arm-level copy number changes and was conducted as described previously [26]. Briefly, common variants were filtered out using public and internal databases, and the remaining, rare variants were classified with the aid of bioinformatics predictors, public databases, and published data. Finally, copy-number variations (CNV) were identified with CNVkit 0.9.5 [27] and copy-neutral losses-of-heterozygosity were identified using an in-house script.

Ethical approval

The study was approved by the Bioethics Committee at the Medical University of Warsaw with the reference number AKBE/175/2021. Due to retrospective and anonymized character of the study, the Ethics Committee waived the requirement of written informed consent.

Case reports

Case 1 concerned an 84-year-old woman, who had 30 years history of right submandibular gland tumour. Case 2 was a 63-year-old female, who had a tumour in the deep part of the left parotid gland, progressing for 10 years. Initially, the radical surgical resection was performed in both cases and the PA was confirmed. Unfortunately, both patients after 6 and 9 months, respectively, had the regrowth of the lesion and the PA recurrences were suspected. However, after the revision surgery and resection, histopathological examination showed multifocal MECA *ex* PA. Histopathological re-assessments of primary lesions were performed to exclude the possibility of misdiagnosis. The re-analysis, however, did not reveal any malignancy in the primary tumour. The presence of PA cells was confirmed. A rapid progression of malignancies after PA excision encouraged us to analyse both PAs' genetic materials and the secondary malignancy to detect genetic patterns that may be responsible for the development of multifocal myoepithelial carcinomas. Additional radiotherapy was administered in the first patient, and chemoradiotherapy in the second case. The overall survival of the first patient was 3 years. The second patient died after one year, due to disease progression. The comprehensive description of both patients' clinical symptoms, treatment, and histopathology analysis was previously presented by Szablewska *et al.* [28]. A summary of patients' data is collected in Table 1.

Results

Copy number variation

Analysis of tumour samples revealed multiple CNVs on focal and chromosomal-arm levels is presented in Table 2. The patterns were different for each patient, but aberrations remained mostly stable in PA and MECA tissues. Specifically, in Patient 1, FGFR1 and CDKN2A were affected by amplification and homozygous deletion, respectively.

Table 1. Patients' characteristics

Parameters	Patient 1	Patient 2
Age at primary resection (years)	84	63
Tumour location	Submandibular gland	Deep part of parotid gland
Time of development of primary PA (years)	30	10
Recurrent Ca ex PA size [mm]	18 multifocal	60
TNM classification of Ca ex PA	T1N1M0	T3N0M0
Perineural invasion (on histology)	Not identified	Present
Facial nerve function (House-Brackmann scale)		
Preoperatively	2	1
Postoperatively	2	1
Radicality of the primary surgery	Complete	Complete
Adjuvant therapy	Radiotherapy	Chemoradiotherapy
Overall survival (months)	36	12

Ca ex PA – carcinoma ex pleomorphic adenoma, PA – pleomorphic adenoma

Table 2. Copy number alterations in patients' samples

Patient 1			Patient 2		
Chromosomal region	**Type of alteration**	**Selected genes in region**	**Chromosomal region**	**Type of alteration**	**Selected genes in region**
1q	Gain		del 3p22.1-p13	Loss	CTNNB1
Chr2	Gain		amp 5p	Gain	
Chr3	CN-LOH		amp 5q11.1-q23.1	Gain	
4p	Loss		del 5q23.1-q34	Loss	
4q	CN-LOH		amp 5q34-q35.3	Gain	
4q12-q13.3	Loss		-6q	Loss	
Chr5	Gain		del 8p23.3-p12	Loss	
Chr6	CN-LOH		del 13q21.31-q31.1	Loss	
7p	Gain	EGFR			
7q11.21-q34	Gain	MET, CDK6, PIK3CG			
7q34-q36.3	Gain	BRAF			
8p	Amplification	FGFR1			
8q11.1-q12.1	Amplification	LYN, PLAG1			
8q	CN-LOH				
Chr9	CN-LOH				
Chr10	CN-LOH				
9p21.3	Deep deletion	CDKN2A/B			
10q21.2-q21.3	Amplification				
Chr11	Gain				
Chr12	Gain	ERBB3, CDK4			
14q	CN-LOH				
15q	CN-LOH				
15q26.3	Amplification	IGF1R			
-16q	Loss				
Chr17	Gain	ERBB2			
Chr18	CN-LOH				
Chr19	Gain				
Chr20	Gain				
22q	Gain				

Deep deletion – 0, loss – 1, gain – 3–4, amplification – 5
CN-LOH – copy-neutral loss of heterozygosity (duplication) provided boundaries for CN-LOH are approximate

Somatic mutations

Among notable genetic aberrations, *FGFR2* mutation was discovered in both cases. In Patient 1's PA and MECA samples the variant allele frequency (VAF) of pathogenic p. Pro253Arg/c.758 C > G variant was nearly 100% and was related to copy-neutral duplication of chromosome 10. This mutation was accompanied by *FGFR1* and *IGF1R* amplifications and elevated copy numbers of *EGFR, MET, ERBB2*, and *ERBB3*, suggesting dependence of cancer cells to receptor tyrosine kinase signalling. Furthermore, a variant of unknown significance in the *APC* gene was identified in both samples F while somatic mutations of *KDM6A* and *ZFHX3* were associated only with PA. In Patient 2, VAF of pathogenic p.Leu550Phe/c.1648C > T variant in *FGFR2* was over 45% in the samples of PA and MECA. Selected variants identified by NGS in our study are collected in Table 3.

Discussion

Due to the histological heterogeneity of salivary gland tumours and inconclusive data concerning prognostic factors, current research focuses on specific genetic alterations. It is believed that a better understanding of carcinogenesis in these tumours may contribute to the improvement and more individual approach to treatment.

The most commonly occurring genetic changes in benign PA are associated with the PA gene 1(*PLAG1*) and the high-mobility group AT-hook 2 (*HMGA2)* genes [29]. The fusions of *PLAG1* and *HMGA2* constitute diagnostic biomarkers, enabling differentiation of PA from other salivary lesions. These are also important markers to identify whether Ca *ex* PA developed from PA or *de novo*. However, translocations in these genes were described also in MECA *de novo* [30]. According to researchers, *TGFBR3-PLAG1* fusion is unique to MECA. *EWSR1-ATF1* and *MSN-ALK* were

Table 3. Selected variants identified by next-generation sequencing

Case	Gene variant	Gene	Mutation	VAF (%)	
				PA	MECA
1	Chr10:123279674-G > C	FGFR2	NM_000141.4:p.Pro253Arg/c.758C > G	94	92
	Chr5:112179729-C > A	APC	NM_000038.5:p.Thr2813Lys/c.8438C > A	22	24
	ChrX:044938480-G > T	KDM6A	NM_021140.3:p.Glu1010*/c.3028G > T	10	0
	Chr16:072832557-C > A	ZFHX3	NM_006885.3:p.Gly1342*/c.4024G > T	6	0
	Chr19:001460220-T > G	APC2	NM_005883.2:p.Tyr448*/c.1344T > G	0	14
2	Chr10:123258033-G > A	FGFR2	NM_000141.4:p.Leu550Phe/c.1648C > T	49	46
	Chr11:017191063-T > G	PIK3C2A	NM_002645.2:p.Met76Leu/c.226A > C	0	10

MECA – myoepithelial carcinoma, PA – pleomorphic adenoma, VAF – variant allele frequency

detected only in *de novo* lesions. The *FGFR1-PLAG-1* was primarily considered characteristic only for MECA *ex* Pa [19]. However, Freiberger *et al.* confirmed this fusion also in PA, Ca *ex* PA, and MECA *de novo* [31]. The most commonly described genetic rearrangements in MECA are *EWSR*, *PIK3CA*, and *HRAS* mutations [30].

Our knowledge about genetic changes in salivary gland tumours is evolving rapidly, but the results are not conclusive. The genetic alterations that were identified unique for benign lesions have been confirmed also in malignant tissue. Therefore, there is still a need for reliable differential indicators for the improvement of the diagnosis and the optimal therapy.

In the available literature, there are not many studies about genetic sporadic mutations in salivary gland tumours, especially in PA and MECA. Cormier *et al*. described the history of a patient, in whom metastatic MECA *ex* PA developed in a short period after superficial parotidectomy performed due to PA. The re-histopathological examination showed MECA misdiagnosed as PA. The genetic analysis confirmed *TERT* promoter mutation [11]. Currently, the meaning of this finding remains unknown. The instance proves the ongoing difficulty in differentiation in salivary gland tumours. In line with our research, Dalin *et al.* discovered *FGFR2* mutation in a patient who developed MECA *ex* PA. Additionally, they also identified this alteration in the case of MECA *de novo*. Both tumours (MECA *ex* PA and MECA *de novo)* were associated with local recurrence and poor patients' outcomes [19]. These findings suggest a potential association of the *FGFR2* mutation with tumour development and progression. Fibroblast growth factors (FGFs) through their receptors (FGFRs) regulate proliferation, migration, differentiation, and survival in normal cells. In cancer progression, FGFs are involved in invasion and angiogenesis [32–35]. The family of FGFR is engaged in the development of a wide range of cancers, unfortunately in most cases related with poor prognosis [36, 37]. Currently, FGFR2 inhibitors are applied in the therapy in advanced cancer stages, or to patients with contraindications to surgery, and when standard systemic regiments have failed [38, 39]. Erdafitinib and Pemigatinib have been registered for urothelial cancer and cholangiocarcinoma, respectively [37, 40].

Our results are consistent with the findings of Dalin *et al*. and indicate that the FGFR2 mutation may be related to MECA *ex* PA salivary gland development and progression. These data highlight the importance of further analysis of other cases to confirm the accuracy and propose optional treatment to improve patients' outcomes.

Other genetic aberrations of interest and with the potential for further exploration were identified in a single sample of MECA *ex* PA. The *PIK3C2A* gene and encoded proteins play a major role in the autophagy process [41]. The *CDKN2A* gene is located on chromosome 9p21 and encodes p14 and p16 suppressor genes, involved in the activation of p53 and Rb. Both proteins are engaged in regulation of the cell's cycle. In human cancers with high frequency of genetic and epigenetic alterations in the *CDKN2A* gene, the strategies of modulation of the alteration for prevention or therapy are promising. Another identified suppressor gene, *APC2,* is involved in WNT- catenin pathways and therefore in cell adhesion. Mutations of *APC* gene are mostly associated with colorectal cancer and familial adenomatous polyposis but occur also in other types of cancers [42]. The encoded protein prevents the uncontrolled growth of cells and controls the epithelial-mesenchymal transition.

The current direction of the research promotes the role of gene copy alterations to be responsible for malignant transformation of PA [19]. Additionally, the changes are usually associated with poor prognosis of MECA *ex* PA and development of metastasis. Consistently, we also detected 5p, as well as 8p and 8q amplification, not only in MECA, but also in primary PA tissue in our patients. In our study, we also found deletion of 3p22.1-p13 in both PA and MECA, but in a single patient. On this locus *CTNNB1* gene is encoded, crucial for synthesis of β-catenin [43, 44]. The protein is activated in WNT pathways, involved in the regulation of cell migration, polarity, differentiation, and function. Molecular abnormalities in *CTNNB1* have so far been confirmed in different types of cancers, such as colon, hepatocellular, and breast cancer [45]. In salivary gland tumour, the loss of 3p22.2–p14.3 was described by Mariano *et al.* in a patient who suffered from metastatic PA [46]. The most intriguing, however, are the findings by Persson *et al.*, who suggested contribution of deletions of 5q23.2-q31.2, gains of 8q12.1 (*PLAG1*), and amplification of 17 chromosome, which encodes the *ERBB2* gene to malignant transformation of PA to carcinoma [47]. Most of these genetic alterations were also detected in samples from our patients.

Identification of specific molecular patterns in salivary gland lesions can pose considerable diagnostic and treatment improvement. Genetic rearrangement appears to be very useful for proper diagnosis. Further studies are needed to reveal genetic patterns in the development and progression of salivary gland tumours.

Our research included the material from only 2 patients and therefore does not allow us to draw strong conclusions. However, the development of MECA in a short time after radical PA excision is quite extraordinary and may be related to some biological conditions. It is possible that mutations in *FGFR2* could accelerate the tumour transformation and progression. We believe that our results may contribute to the most accurate detection of genetic alterations in salivary gland tumours and improvement of the diagnosis and treatment in the future. Additionally, identification of specific mutations in benign salivary gland lesions predisposing to malignant transformation will improve patients' oncological supervision and prognosis.

Conclusions

Aberrations of the *FGFR2* gene, identified in primary PA and MECA *ex* PA samples of both our patients, may be responsible for the malignant transformation and disease progression. Further studies are encouraged to confirm the relevance of these findings. The therapy option with FGFR2 inhibitors may be considered in advanced or metastatic MECA *ex* PA with confirmed *FGFR2* mutation.

Next-generation sequencing analysis contributes to improving knowledge on the development and progression of salivary gland tumours. Identification of reliable markers for diagnosis, prognosis, and individual treatment is urgently needed in salivary gland tumours to improve outcomes.

The authors declare no conflict of interest.

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Submitted: 06.09.2023
Accepted: 13.11.2023

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

Pikul *et al. BMC Cancer* (2025) 25:42
https://doi.org/10.1186/s12885-024-13421-0

BMC Cancer

RESEARCH Open Access

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

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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.

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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,*

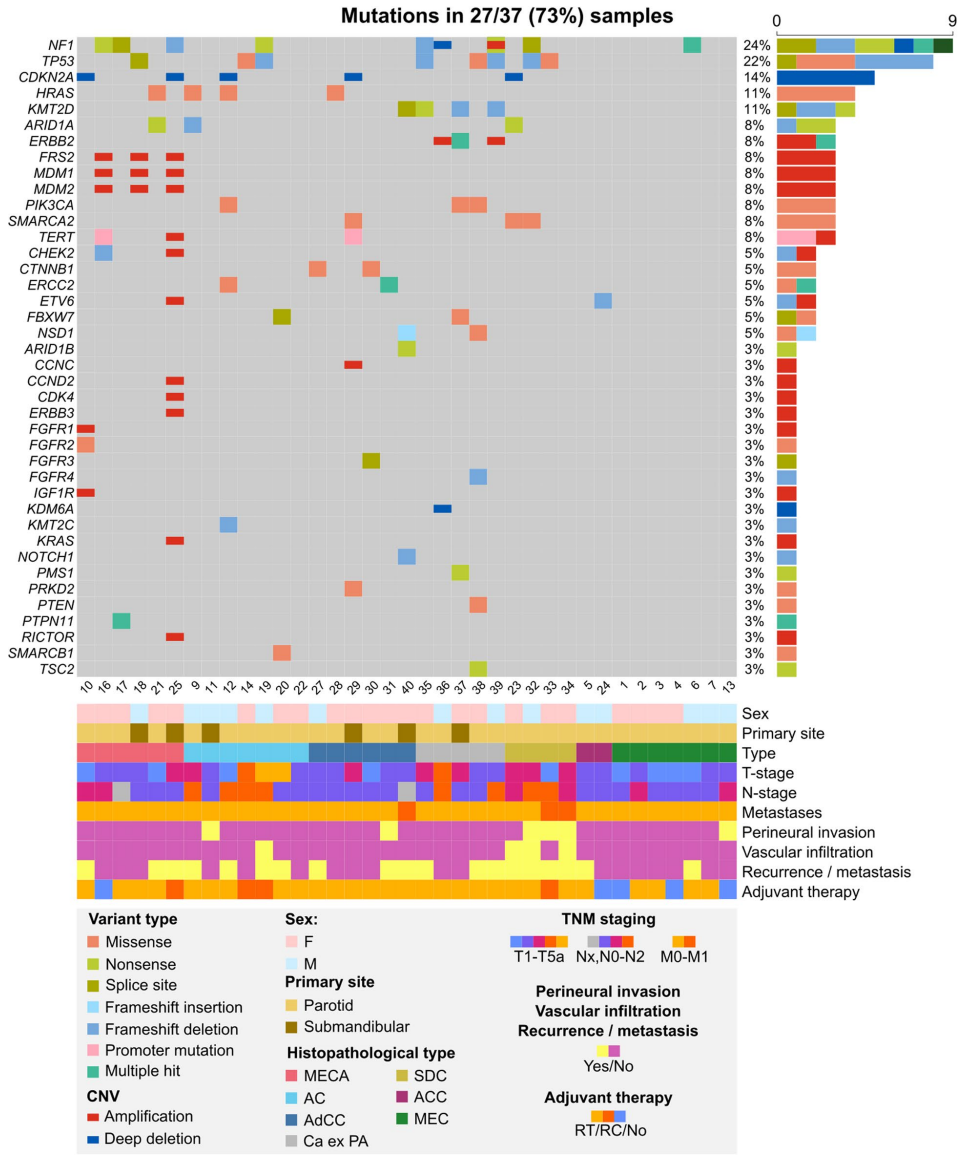

Fig. 1 The characteristics of the pathogenic genetic alterations in the cohort of salivary gland cancers

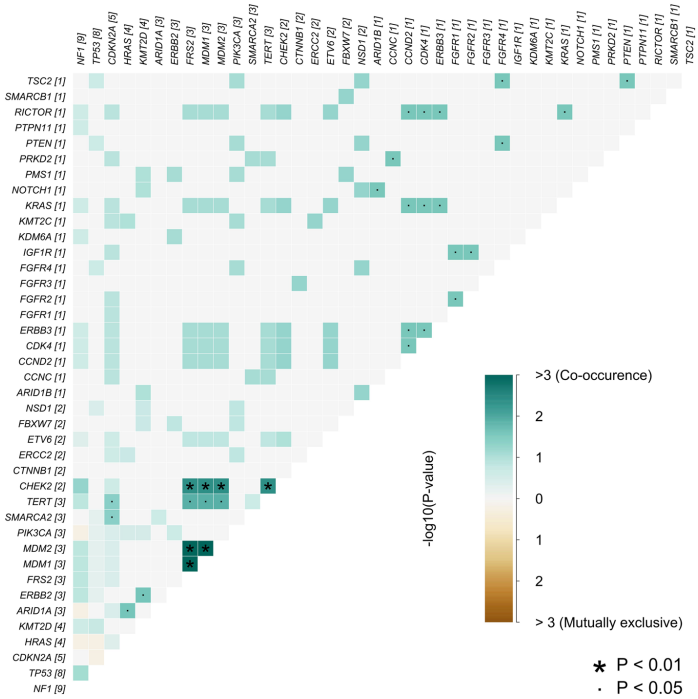

Fig. 2 The co-occurrence of the gene mutations and copy number changes in the studied cohort of salivary gland cancers

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

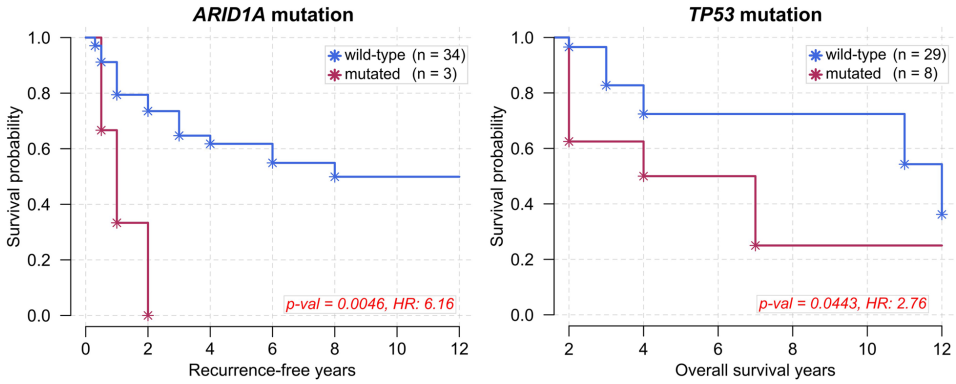

Fig. 3 The Kaplan -Meier curves for disease free survival and overall survival in the studied cohort in relation to the identified genetic alterations

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.

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

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Publisher's Note

Podsumowanie

Przedstawiony w rozprawie doktorskiej cykl publikacji ukazuje zróżnicowanie kliniczno-histopatologiczne i molekularne najczęściej występujących nowotworów złośliwych gruczołów ślinowych. Wśród omawianych zmian molekularnych wyróżnić można zarówno fuzje genowe, mutacje somatyczne jak również zmiany liczby kopii genów. Aktualny stan wiedzy dotyczący zmian genetycznych w SGCs przedstawiono w pracy *Molecular landscape of salivary gland malignancies. What is already known?* która stanowiła przegląd dostępnych danych, analizę kierunków badań molekularnych w SGCs i wstęp do opracowania metodologii kolejnych projektów z wykorzystaniem materiału klinicznego. Najczęściej występujące aberracje genetyczne w wybranych typach SGCs przedstawiono w pracy w formie tabel w celu przejrzystości i dogodniejszego wykorzystania praktycznego opracowanych zestawień. Na podstawie analizy danych z publikacji wskazano te zmiany genetyczne, które mogą być związane z ryzykiem wznowy, wystąpieniem przerzutów oraz niekorzystnym przebiegiem choroby. Na podstawie aktualnej wiedzy dla większość z tych zmian istnieją obecnie metody terapii celowanych.

Celem badania przedstawionego w pracy pt. *FGFR2 point mutation in 2 cases of pleomorphic adenoma progressing to myoepithelial carcinoma* była identyfikacja aberracji genetycznych, które mogły być związane z dynamicznym rozwojem raka u chorych po radykalnej resekcji gruczolaka wielopostaciowego. Analiza genetyczna zmian pierwotnych jak również MECA ex PA została przeprowadzona za pomocą NGS. Analiza obejmowała mutacje małej skali, a także ogniskowe i chromosomalne zmiany liczby kopii. W obu próbkach zarówno PA jak i MECA ex PA stwierdzono liczne, wspólne CNVs. Dodatkowo, zarówno w PA jak i MECA ex PA u obu pacjentek stwierdzono mutacje w genie *FGFR2* (przypadek 1 - p. Pro253Arg; przypadek 2- p. Leu550Phe) z wysoką częstotliwością występowania alleli (variant allele frequency - VAF). Mutacja zidentyfikowana w pierwszym przypadku stwarza możliwość zastosowania leczenia celowanego w wykorzystaniem zarejestrowanych inhibitorów FGFR2. Na podstawie uzyskanych danych oraz analizy materiału z dostępnej z literatury zidentyfikowane mutacje w genie *FGFR2* wskazano jako istotne w procesie transformacji złośliwej PA oraz związane z niekorzystnym rokowaniem.

W pracy oryginalnej, retrospektywnej *Potentially actionable molecular alterations in particular related to poor oncologic outcomes in salivary gland carcinomas* przeprowadzono analizę genetyczną najczęściej występujących typów histopatologicznych SGCs. Przedstawiono: obraz kliniczny, aberracje genetyczne związane z niekorzystnym rokowaniem

oraz potencjalne możliwości zastosowania leczenia celowanego. Analizowany materiał pochodził od 37 pacjentów hospitalizowanych w Klinice Otorynolaryngologii Chirurgii Głowy i Szyi Warszawskiego Uniwersytetu Medycznego w latach 2010-2017, poddanych leczeniu chirurgicznemu. Mediana wieku w chwili rozpoznania nowotworu wynosiła 59.7 lat (zakres 21-87), a 62% badanej kohorty stanowiły kobiety. W 84% przypadków zmiany stwierdzone zostały w śliniance przyusznej. Za niekorzystne rokowanie uznano nawrót choroby lub wystąpienie przerzutów w czasie obserwacji, które stwierdzono u 51% badanej kohorty. W tej grupie pacjentów stwierdzono istotnie niższe DFS oraz OS w porównaniu do grupy pacjentów, u których nie doszło do progresji choroby (odpowiednio 2.4 lat, 5.4 lat vs. 8.3 lat, 8.3 lat). Materiał DNA został odpowiednio wyizolowany z próbek utrwalonych w formalinie zatopionych w parafinie (formalin – fixed paraffin embedded – FFPE). Następnie po uzyskaniu bibliotek genomowych przystąpiono do ich sekwencjonowania. Przeprowadzona została kompleksowa analiza genetyczna z użyciem NGS. Wyselekcjonowane zostały jedynie patogenne lub prawdopodobnie patogenne warianty oraz CNVs (blisko 80 genów). Najczęstsze aberracje genetyczne stwierdzono w genach: *NF1*, *TP53* oraz *CDKN2A*. Odsetek stwierdzonych mutacji dla powyższych genów był większy w grupie chorych z niekorzystnym rokowaniem, w porównaniu do chorych bez progresji choroby (odpowiednio: 32% vs. 24%, 32% vs. 22% oraz 21% vs. 14%). Ponadto, komutację genów *NF1* oraz *TP53* stwierdzono u 21% pacjentów z niekorzystnym rokowaniem (AC, Ca ex PA, SDC). Wśród tej grupy chorych odnotowano również częste występowanie mutacji w następujących genach: *HRAS* (16%), *ARID1A* (16%), *ERCC2 (11%), NSD1* (11%).

Analizując wykryte mutacje w kontekście poszczególnych typów histopatologicznych, najmniej zmian stwierdzono w jedynym przypadku MEC o niekorzystnym rokowaniu (liczne mutacje w genie *NF1* dotyczące zmiany sensu oraz splicingu) oraz AcCC o korzystnym przebiegu (*ETV6*). Pozostałe typy histopatologiczne wykazywały większą ilość zmienionych genów. W przypadku MECA zaobserwowano największe zróżnicowanie krajobrazu molekularnego. W tabeli zestawiono zidentyfikowane mutacje w genach dla analizowanych typów histopatologicznych SGCs.

	Zmutowane geny	
Typ histopatologiczny	Rokowanie korzystne	Rokowanie niekorzystne
Rak gruczołowy (AC)	*TP53*	*ARID1A, CDKN2A, ERCC2, FBXW7, HRAS, KMT2C, NF1, PIK3CA, SMARCB1, TP53*
Rak zrazikowokomórkowy (AcCC)	*ETV6*	
Rak gruczołowo-torbielowaty (AdCC)	*CCNC, CDKN2A, CTNNB1, FGFR3, HRAS, PRKD2, SMARCA2, TERT*	*ARID1B, CTNNB1, ERCC2, KMT2D, NOTCH1, NSD1*
Rak w gruczolaku wielopostaciowym (Ca ex PA)	*ERBB2* (SDC ex PA), *FBXW7* (SDC ex PA), *KDM6A* (SDC ex PA, *KMT2D* (SDC ex PA), *NF1* (SDC ex PA), *PIK3CA* (SDC ex PA), *PMS1 (SDC ex PA)*	*ERBB2* (SDC ex PA), *FGFR4* (AC NOS ex PA), *KMT2D* (SDC ex PA, AC ex PA), *NF1* (SDC ex PA, AC ex PA), *NSD1* (AC NOS ex PA), *PIK3CA* (AC NOS ex PA), *PTEN (AC NOS ex PA), TP53* (AC ex PA, AC NOS ex PA, SDC ex PA), *TCS2* (AC NOS ex PA)
Rak śluzowo-naskórkowy (MEC)		*NF1*
Rak mioepithelialny (MECA)	*CHEK2, FRS2, MDM1, MDM2, NF1, PTPN11, TERT, TP53*	*ARID1A, CCND2, CDK4, CDKN2A, CHEK2, ERBB3, ETV6, FGFR1, FGFR2, HRAS, FRS2, IGFR1, KRAS, MDM1, MDM2, NF1, RICTOR, TERT*
Rak przewodowy (SDC)		*ARID1A, CDKN2A, NF1, SMARCA2, TP53*

Mutację w genie *ERBB2* uznano za wyłączną dla SDC ex PA. Dodatkowo została wykryta mutacja promotora genu *TERT* w AdCC oraz MECA z korzystnym rokowaniem. Mutacja hotspot w genie *CTNNB1* (3:41224616-T>C, p. (Ile35Thr)) została wykryta w 1/3 wszystkich przypadków AdCC. Koamplifikację genów *MDM1*, *MDM2* oraz *FRS2* wykazano w 50% MECA. Mutacje w genie *TP53* uznano za istotny negatywny czynnik prognostyczny dla OS (p=0,04) w badanej grupie chorych. W analizowanej kohorcie u 70% pacjentów stwierdzono aberracje genetyczne z możliwością zastosowania terapii celowanej, przy czym u osób z R/M SGCs odsetek ten wynosił 89%. W poniższej tabeli przedstawiono wybrane geny wraz z potencjalnymi możliwościami zastosowania terapii celowanej. Większość z podanych leków pozostaje obecnie w fazie badań, jednak wyniki onkologiczne ich zastosowania są obiecujące. Wyniki badań molekularnych oraz ich zastosowanie w kontekście klinicznym omówiono szczegółowo w dyskusji powyższej pracy.

Gen	Cel terapeutyczny	Przykłady testowanych leków	Typ nowotworu
ARID1A	inhibitory polimerazy poli (ADP-rybozy) (PARP)	olaparyb, niraparyb	R/M rak jajnika, rak endometrium [63-65]
	inhibitory ATR	M4344, M6620	guzy lite [64]
	inhibitory EZH2	tazemetostat, CPI-0209	guzy lite [64]
CDKN2A/B	inhibitory cdk4/6	palbocyklib, rybocyklib, abemacyklib	rak piersi, czerniak [66]
	immunoterapia- inhibitory punktów kontrolnych	anty-PD-1, anty-CTLA-4	R/M czerniak [66]
	inhibitory metylotranferazy DNA	decytabina	nowotwory hematologiczne [67]
CTNNB1	przeciwciało monoklonalne neutralizujące DKK1	DKN-01	R/M guzy lite [68]
ERBB2	rekombinowane humanizowane przeciwciało monoklonalne IgG1	trastuzumab	liczne guzy lite, w tym również trastuzumab + docetaxel w zaawansowanym lub R/M SDC [69, 70]

	inhibitory kinaz tyrozynowych	lapatynib, neratynib, pyrotynib	rak piersi [70]
FGFR	specyficzne inhibitory receptora czynnika wzrostu fibroblastów (FGFR)	erdafitynib, infigratynib, derazantynib, futibatynib	rak urotelialny, rak dróg żółciowych [71]
HRAS	inhibitor farnesylotransferazy	tipifarnib	R/M rak kolczystokomórkowy w obrębie głowy i szyi, R/M SGCs [72-74]
NF1	inhibitory MEK inhibitory PI3K-AKT-mTOR immunoterapia- inhibitory punktów kontrolnych	trametynib ewerolimus anty-PD-1, anty-PD-L1	R/M guzy lite, R/M czerniak guzy lite czerniak, rak płuca [75]
TP53	antagonista MDM2– p53 reaktywator p53	brigimadlin (NCT03449381) rezatapopt (PYNNACLE)	R/M guzy lite [76] R/M guzy lite [77]

Podsumowując, nowotwory złośliwe gruczołów ślinowych stanowią heterogenną grupę zmian, cechujących się nieprzewidywalnym przebiegiem choroby oraz w większości niekorzystnym rokowaniem. Zróżnicowanie histopatologiczne w tej grupie nowotworów może prowadzić do postawienia błędnego rozpoznania, a tym samym – opóźnienia leczenia. SGCs zaliczane są do nowotworów rzadkich, w przypadku których w dalszym ciągu brakuje odpowiednich standardów postępowania opartych na dowodach naukowych, w tym markerów predykcyjnych oraz prognostycznych. Analiza aberracji genetycznych staje się obecnie niezwykle istotna nie tylko w diagnostyce różnicowej, lecz również w opracowywaniu i stosowaniu metod leczenia personalizowanego. Jednakże, ze względu na rzadkie występowanie tych nowotworów, liczba badań obejmujących wyniki analiz genetycznych jest nadal ograniczona. Potrzebne są dalsze

badania molekularne, uwzględniające kliniczny przebieg choroby. Umożliwi to zapewnienie odpowiedniego nadzoru onkologicznego nad pacjentami z SGCs. Kompleksowe analizy genetyczne z wykorzystaniem NGS umożliwią w przyszłości szerszy dostęp do metod leczenia celowanego dla pacjentów z nowotworami rzadkimi, w tym SGCs, oraz przyczynią się do poprawy rokowania w tej grupie chorych.

Wnioski

1. Nowotwory złośliwe gruczołów ślinowych wykazują dużą heterogenność kliniczno-histopatologiczną i molekularną, co utrudnia ich klasyfikację, diagnostykę oraz dobór optymalnego leczenia. Aberracje, które uznawane są za charakterystyczne w diagnostyce różnicowej dla danego typu histopatologicznego, mogą nie mieć wartości prognostycznych.
2. W zależności od typu histopatologicznego, nowotwory złośliwe gruczołów ślinowych wykazują zróżnicowaną częstość występowania mutacji genetycznych. Największe zróżnicowanie molekularne w analizowanej kohorcie występuje w typie MECA. W typie MEC jest obecna z kolei jedynie mutacja w genie *NF1* u pacjenta z niekorzystnym rokowaniem. Najwięcej patognomonicznych mutacji obserwuje się w typie SDC ex PA (np. *ERBB2*), co może wspierać zastosowanie terapii celowanych opartych na profilu molekularnym nowotworu.
3. W grupie chorych, u których doszło do wznowy choroby lub wystąpienia przerzutów odległych, najczęstsze aberracje występują w genach: *NF1* (32%), *TP53* (32%) oraz *CDKN2A* (21%). Dodatkowo, komutację genów *NF1* oraz *TP53* identyfikuje się u 21% pacjentów z niekorzystnym rokowaniem. W tej grupie pacjentów również często występują mutacje w genach: *HRAS* (16%), *ARID1A* (16%), *ERCC2* (11%), *NSD1* (11%). Mutacje w genie *FGFR2* występują w przypadkach transformacji gruczolaka wielopostaciowego do myoepithelial carcinoma (MECA ex PA) mogą odgrywać kluczową rolę w procesie transformacji złośliwej i stanowią potencjalny cel terapii ukierunkowanej molekularnie.
4. W 70% analizowanych przypadków SGCs istnieją aberracje potencjalnie możliwe do wykorzystania terapeutycznego, co potwierdza rosnące znaczenie diagnostyki molekularnej w planowaniu leczenia personalizowanego. W grupie chorych z nawrotem lub przerzutami (R/M SGCs) odsetek ten wynosi aż 89%.
5. Ze względu na ograniczoną dostępność danych oraz rzadkość występowania SGCs, konieczne są dalsze wieloośrodkowe badania molekularne, które pozwolą na lepsze poznanie mechanizmów rozwoju, progresji i transformacji złośliwej w tej grupie nowotworów. Wykorzystanie analiz genetycznych w praktyce klinicznej w przypadku nowotworów rzadkich, mogłoby zwiększyć dostęp pacjentów do terapii celowanych, co w konsekwencji może przełożyć się na poprawę rokowania i efektywność leczenia onkologicznego.

Opinia Komisji Bioetycznej

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Warszawa, dnia 04 października 2021r.

AKBE/ 175 / 2021

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OŚWIADCZENIE

Niniejszym oświadczam, że Komisja Bioetyczna przy Warszawskim Uniwersytecie Medycznym w dniu 04 października 2021 r. przyjęła do wiadomości informację na temat badania pt. :"Wpływ dysregulacji genetycznych nowotworów złośliwych ślinianek na wyniki leczenia onkologicznego."Przedstawione badanie nie stanowi eksperymentu medycznego w rozumieniu art. 21 ust. 1 ustawy z dnia 5 grudnia 1996 r. o zawodach lekarza i lekarza dentysty(Dz.U. z 2018 r. poz. 617) i nie wymaga uzyskania opinii Komisji Bioetycznej przy Warszawskim Uniwersytecie Medycznym, o której mowa w art. 29 ust.1 ww. ustawy.

Przewodnicząca Komisji Bioetycznej

Prof. dr hab. n. med. Magdalena Kuźma –Kozakiewicz

Oświadczenia współautorów

Warszawa, 08.12.2024
(miejscowość, data)

Anna Rzepakowska
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „FGFR2 point mutation in 2 cases of pleomorphic adenoma progressing to myoepithelial carcinoma" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: przygotowanie koncepcji badania, projekt badania, pozyskiwanie danych, analiza oraz interpretacja danych, edycja i recenzja manuskryptu.

Mój udział procentowy w przygotowaniu publikacji określam jako 10%.

Wkład lek. Julii Pikul w powstawanie publikacji określam jako 70%,
obejmował on: przygotowanie koncepcji badania, pozyskiwanie danych, analiza oraz interpretacja danych, przygotowanie manuskryptu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Julii Pikul.

Anna Rzepakowska
(podpis oświadczającego)

Warszawa, 08.12.2024
(miejscowość, data)

Anna Rzepakowska

(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Molecular landscape of salivary gland malignancies. What is already known?" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: przygotowanie koncepcji oraz projektu pracy, pozyskiwanie danych, analiza oraz interpretacja danych, edycja i recenzja manuskryptu.

Mój udział procentowy w przygotowaniu publikacji określam jako 25%.

Wkład lek. Julii Pikul w powstawanie publikacji określam jako 75%,
obejmował on: przygotowanie koncepcji oraz projektu pracy, pozyskiwanie danych, analiza oraz interpretacja danych, przygotowanie manuskryptu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Julii Pikul.

.....

(podpis oświadczającego)

Warszawa, 08.12.2024
(miejscowość, data)

Anna Rzepakowska

(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Potentially actionable molecular alterations in particular related to poor oncologic outcomes in salivary gland carcinomas" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: przygotowanie koncepcji badania, projekt badania, pozyskiwanie danych, analiza oraz interpretacja danych, edycja i recenzja manuskryptu.

Mój udział procentowy w przygotowaniu publikacji określam jako 2%.

Wkład lek. Julii Pikul w powstawanie publikacji określam jako 60%,
obejmował on: przygotowanie koncepcji badania, pozyskiwanie danych, analiza oraz interpretacja danych, przygotowanie manuskryptu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Julii Pikul.

Anna Rzepakowska

.....

(podpis oświadczającego)

WARSZAWA 24.03.25

(miejscowość, data)

Dr n.med. Marcin Machnicki
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „FGFR2 point mutation in 2 cases of pleomorphic adenoma progressing to myoepithelial carcinoma" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: projekt pracy, pozyskiwanie danych, analiza oraz interpretacja danych, przygotowanie manuskryptu.

Mój udział procentowy w przygotowaniu publikacji określam jako 15%.

Wkład lek. Julii Pikul w powstawanie publikacji określam jako 70%,
obejmował on: przygotowanie koncepcji badania, pozyskiwanie danych, analiza oraz interpretacja danych, przygotowanie manuskryptu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Julii Pikul.

(podpis oświadczającego)

WARSZAWA 24.03.25
(miejscowość, data)

Dr n. med. Marcin Machnicki
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Potentially actionable molecular alterations in particular related to poor oncologic outcomes in salivary gland carcinomas" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: projekt pracy, pozyskiwanie danych, analiza oraz interpretacja danych, przygotowanie manuskryptu.

Mój udział procentowy w przygotowaniu publikacji określam jako 30%.

Wkład lek. Julii Pikul w powstawanie publikacji określam jako 60%,
obejmował on: przygotowanie koncepcji badania, pozyskiwanie danych, analiza oraz interpretacja danych, przygotowanie manuskryptu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Julii Pikul.

Machnicki

(podpis oświadczającego)

WARSZAWSKI
UNIWERSYTET
MEDYCZNY

ZAKŁAD BIOLOGII I GENETYKI NOWOTWORÓW
KATEDRA PATOMORFOLOGII

Warszawa, 10.12.2024

OŚWIADCZENIE

Jako współautor pracy pt. „FGFR2 point mutation in 2 cases of pleomorphic adenoma progressing to myoepithelial carcinoma" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: projekt badania, pozyskiwanie danych, analiza oraz interpretacja danych, edycja i recenzja manuskryptu.

Mój udział procentowy w przygotowaniu publikacji określam jako 5 %.

Wkład lek. Julii Pikul w powstawanie publikacji określam jako 70%,

obejmował on: przygotowanie koncepcji badania, pozyskiwanie danych, analiza oraz interpretacja danych, przygotowanie manuskryptu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Julii Pikul.

.....

(podpis oświadczającego)

Prof. dr med. Tomasz Stokłosa
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KATEDRA PATOMORFOLOGII

Warszawa, 10.12.2024

OŚWIADCZENIE

Jako współautor pracy pt. „Potentially actionable molecular alterations in particular related to poor oncologic outcomes in salivary gland carcinomas" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: projekt badania, pozyskiwanie danych, analiza oraz interpretacja danych, edycja i recenzja manuskryptu.

Mój udział procentowy w przygotowaniu publikacji określam jako 2%.

Wkład lek. Julii Pikul w powstawanie publikacji określam jako 60%,

obejmował on: przygotowanie koncepcji badania, pozyskiwanie danych, analiza oraz interpretacja danych, przygotowanie manuskryptu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Julii Pikul.

.....

(podpis oświadczającego)

Prof. dr med. Tomasz Stokłosa
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ŁUKASZ FUS
(imię i nazwisko)

Warszawa 10.12.24
(miejscowość, data)

OŚWIADCZENIE

Jako współautor pracy pt. „Potentially actionable molecular alterations in particular related to poor oncologic outcomes in salivary gland carcinomas" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: pozyskiwanie danych, analiza oraz interpretacja danych, edycja i recenzja manuskryptu.

Mój udział procentowy w przygotowaniu publikacji określam jako 1%.

Wkład lek. Julii Pikul w powstawanie publikacji określam jako 60%,
obejmował on: przygotowanie koncepcji badania, pozyskiwanie danych, analiza oraz interpretacja danych, przygotowanie manuskryptu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Julii Pikul.

Łukasz Fus
(podpis oświadczającego)

11.12.2024 Warszawa
(miejscowość, data)

Agnieszka Chudy
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Potentially actionable molecular alterations in particular related to poor oncologic outcomes in salivary gland carcinomas" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: izolacja materiałów z bloczków parafinowych oraz przygotowanie bibliotek NGS.

Mój udział procentowy w przygotowaniu publikacji określam jako 1%.

Wkład lek. Julii Pikul w powstawanie publikacji określam jako 60%,
obejmował on: przygotowanie koncepcji badania, pozyskiwanie danych, analiza oraz interpretacja danych, przygotowanie manuskryptu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Julii Pikul.

Chudy Agnieszka
(podpis oświadczającego)

Albert Mościcki
(imię i nazwisko)

Warszawa dn. 08-12-2024
(miejscowość, data)

OŚWIADCZENIE

Jako współautor pracy pt. „Potentially actionable molecular alterations in particular related to poor oncologic outcomes in salivary gland carcinomas" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: izolacja materiałów z bloczków parafinowych oraz przygotowanie bibliotek NGS.

Mój udział procentowy w przygotowaniu publikacji określam jako 1%.

Wkład lek. Julii Pikul w powstawanie publikacji określam jako 60%,
obejmował on: przygotowanie koncepcji badania, pozyskiwanie danych, analiza oraz interpretacja danych, przygotowanie manuskryptu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Julii Pikul.

Albert Mościcki
(podpis oświadczającego)

Katarzyna Kostueva
(imię i nazwisko)

Warszawa, 30.04.2025
(miejscowość, data)

OŚWIADCZENIE

Jako współautor pracy pt. „Potentially actionable molecular alterations in particular related to poor oncologic outcomes in salivary gland carcinomas" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: izolacja materiałów z bloczków parafinowych oraz przygotowanie bibliotek NGS.

Mój udział procentowy w przygotowaniu publikacji określam jako 1%.

Wkład lek. Julii Pikul w powstawanie publikacji określam jako 60%,
obejmował on: przygotowanie koncepcji badania, pozyskiwanie danych, analiza oraz interpretacja danych, przygotowanie manuskryptu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Julii Pikul.

Kostueva
(podpis oświadczającego)

Natalia Wiraska
(imię i nazwisko)

Warszawa 26.05.2025r.
(miejscowość, data)

OŚWIADCZENIE

Jako współautor pracy pt. „Potentially actionable molecular alterations in particular related to poor oncologic outcomes in salivary gland carcinomas" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: pozyskiwanie danych.

Mój udział procentowy w przygotowaniu publikacji określam jako 1%.

Wkład lek. Julii Pikul w powstawanie publikacji określam jako 60%,
obejmował on: przygotowanie koncepcji badania, pozyskiwanie danych, analiza oraz interpretacja danych, przygotowanie manuskryptu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Julii Pikul.

Natalia Wiraska
(podpis oświadczającego)

Warszawa, 28.04.2025
(miejscowość, data)

Kacper Król
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Potentially actionable molecular alterations in particular related to poor oncologic outcomes in salivary gland carcinomas" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: pozyskiwanie danych.

Mój udział procentowy w przygotowaniu publikacji określam jako 1%.

Wkład lek. Julii Pikul w powstawanie publikacji określam jako 60%,
obejmował on: przygotowanie koncepcji badania, pozyskiwanie danych, analiza oraz interpretacja danych, przygotowanie manuskryptu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Julii Pikul.

(podpis oświadczającego)

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