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

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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.

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.

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The authors declare no conflict of interest.

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