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EXTENDED ABSTRACT OF DOCTORAL DISSERTATION

CLINICOPATHOLOGICAL, MORPHOLOGICAL, IMMUNOHISTOCHEMICAL, AND MOLECULAR BIOLOGICAL CHARACTERISTICS OF RARE SALIVARY GLAND TUMORS

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The dissertation was written during full time doctoral study programme P5145 Pathology at the Department of Pathology Faculty of Medicine in Pilsen, Charles University.

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ABSTRAKT

Disertační práce je výsledkem doktorandského studia Oleny Koshyk na Univerzitě Karlově v Praze, Lékařské fakultě v Plzni, v letech 2019 až 2023. Autorka zaměřila hlavní část svého výzkumu na vzácné nádory slinných žláz a také vzácné sinonazální nádory měkkých tkání.

Disertační práce obsahuje zdokumentované studie vzácných variant mukoepidermoidního a myoepiteliálního karcinomu s popisem morfologických a imunohistochemich charakteristik a molekulárních alterací. Zvláštní pozornost byla věnována diferenciální diagnostice. Studie myoepiteliálního karcinomu také pojednává o srovnání biologie myoepiteliálních nádorů slinné žlázy a měkkých tkání a kůže.

Studie karcinomu z acinárních buněk se zaměřila na genetické změny s rearanžemigenu NR4A2. Dále zkoumá imunohistochemické vlastnosti proteinů NR4A3 a NR4A2 v acinárním karcinomu jakožto ekonomicky dostupné a diagnosticky užitečné alternativy k molekulárně genetickému testování.

Ve skupině benigních nádorů se objektem zájmu stal nově reklasifikovaný novotvar nazývající se sklerotizující polycystický adenom slinné žlázy. Byla navržena diagnostická kritéria pro rozlišení dysplazie nízkého a vysokého stupně v solidních a kribriformních epiteliálních proliferacích u sklerotizujícího polycystického adenomu s molekulárním profilem. Byl zahrnut ojedinělý případ apokrinního intraduktálního karcinomu slinných žláz s transformací v karcinom slinných kanálků, vzniklý ze sklerotizujícího polycystického adenomu nesoucího mutaci v dráze PI3K/Akt ve všech složkách nádoru.

Druhá část dizertační práce představuje vzácné mezenchymální sinonazální maligní entity. Prvním je vzácný fenomén bifenotypickéhoo sinonazálního sarkomu s fúzí genů *PAX3::MAML3* s transformací v rhabdomyosarkom vysokého stupně malignity. Diskutována byla diferenciální diagnostika a povinné použití molekulárního testování. Poslední studie se týkala zcela nového agresivního polyfenotypického sarkomu s fúzí *EWSR::POU2AF3* s predilekčním výskytem v sinonazální lokalizaci. Poprvé byly popsány podskupiny s „low-grade“ a „high-grade“ morfologií s komplexními imunohistochemickými charakteristikami a genovými fúzemi *EWSR1::POU2AF3* a *FUS::POU2AF3*. Prokázané morfologické a genetické změny poskytly důkaz o tom, že se jedná o jednu nádorovou jednotku vykazující široké morfologické spektrum.

SUMMARY

The doctoral thesis is the result of Olena Koshyk's doctoral study at Charles University in Prague, Faculty of Medicine in Pilsen, from 2019 to 2023. The author focused the main part of her research on rare salivary gland tumors as well as rare sinonasal soft tissue tumors.

The dissertation contains documented studies of rare variants of mucoepidermoid carcinoma and myoepithelial carcinoma with comprehensive morphological, immunohistochemical characteristics, and molecular alterations. Special attention was paid to the differential diagnosis for accurate diagnosis. The myoepithelial carcinoma study also discusses a comparison of the biology of myoepithelial tumors of the salivary gland and soft tissues and skin.

The acinic cell carcinoma study focused on genetic alteration with rare *NR4A2* rearrangements as well as NR4A3 and NR4A2 immunostains as a cost-effective method for the accurate diagnosis of acinic cell carcinoma and surrogate markers of occurred rearrangements.

In a group of benign tumors, the newly reclassified neoplasm sclerosing polycystic adenoma of the salivary gland became an object of interest. For the first time diagnostic criteria for low-grade and high-grade dysplasia in the solid and cribriform epithelial proliferations in sclerosing polycystic adenoma with molecular profile were presented. A unique case of a salivary gland apocrine intraductal carcinoma with transformation to salivary duct carcinoma, arising from sclerosing polycystic adenoma harboring a mutation in the PI3K/Akt pathway in all tumor components was included.

The second part represents rare mesenchymal sinonasal malignant entities. First is the rare phenomenon of biphenotypic sinonasal sarcoma with *PAX3::MAML3* fusion with transformation into high-grade rhabdomyosarcoma. The differential diagnosis and the mandatory use of molecular testing were discussed. The last study covered a completely new aggressive polyphenotypic sarcoma with *EWSR::POU2AF3* fusion with sinonasal predilection. The subgroups with low and high-grade morphology with comprehensive immunohistochemical characteristics and *EWSR1::POU2AF3* and *FUS::POU2AF3* fusions were described for the first time. The morphological features and genetic alterations of the tumors provided evidence of a single entity with a wide morphological spectrum.

CONTENTS

INTRODUCTION

Salivary gland tumors comprise approximately 3–6% of all head and neck tumors. The incidence rate is 0.4 to 13.5 cases per 100,000 population, most salivary gland tumors are benign neoplasms [1]. The current 2022 World Health Organization salivary gland tumor classification comprises mostly benign and malignant epithelial entities, tumors of the hematolymphoid and soft tissue have a very low incidence [2].

The histologically salivary gland consists of a variety of cells including serous and mucous acinar, myoepithelial, and ductal cells (intercalated, striated, and interlobular ducts). So, the tumors can originate from one to several cell types leading to overlapping different entities. Besides, immunohistochemical and molecular heterogeneity of salivary gland tumors makes this group of neoplasms challenging and may cause diagnostic problems. Numerous immunohistochemical markers use routinely now in salivary gland pathology for diagnostic purposes. But in some neoplasms, the role of IHC is limited. For such cases, further genetic testing may be helpful for diagnosis and biology understanding.

Recently, various molecular techniques have also been integrated into routine diagnostic practice. In salivary gland pathology, molecular alterations were included in the definition of mucoepidermoid carcinoma, adenoid cystic carcinoma, secretory carcinoma, polymorphous adenocarcinoma, hyalinizing clear cell carcinoma, mucinous adenocarcinoma, and microsecretory adenocarcinoma [2]. Genetic findings help to understand the biology of lesions and correctly classify them. Some genetic findings have served as the basis for the creation of a new targeted treatment. Thus, the finding of *NTRK* gene fusion that encodes tropomyosin-related kinase transmembrane proteins TRKA, TRKB, and TRKC in secretory carcinoma and leads to the activation of cell growth, makes it possible to treat such tumors with NTRK inhibitors [3].

Sinonasal malignancies compose approximately 5% of all head and neck neoplasms, with an incidence of 0.556 per 100,000 individuals [4]. The new 2022 World Health Organization sinonasal tumor classification includes a few entities, the diagnosis of which is based on genetic events (e.g., SWI/SNF complex-deficient sinonasal carcinomas, NUT and *DEK*::*AFF2* carcinoma, *IDH*-mutant sinonasal malignancies, etc.). Some genetic alterations have correlated with specific morphological features, e.g., SWI/SNF complex-deficient sinonasal carcinomas with monomorphic structure and sometimes with rhabdoid

phenotype, NUT carcinoma with monotonous undifferentiated cells and abrupt keratinization focuses.

Immunohistochemistry plays an important role in the differential diagnosis of sinonasal neoplastic lesions, especially in poorly differentiated cases. Sometimes, IHC is also used as a surrogate cost-effective method for the identification of genetic rearrangements (e.g., NUT protein as a marker of *NUTM1* gene rearrangement in NUT carcinoma, SMARCB1 or SMARCA4 proteins as markers of inactivating SMARCB1 or SMARCA4 mutations in SWI/SNF-deficient sinonasal carcinomas, etc).

Due to the wide variety of salivary gland tumors, in the dissertation, the author focuses on the clinicopathological, morphological, immunohistochemical, and molecular biologic characteristics of selected benign and malignant rare salivary gland and sinonasal tumors.

AIMS AND HYPOTHESIS

The purpose of the study was to identify morphological features, and specific immunohistochemical markers that can aid in the differential diagnosis of rare salivary gland and sinonasal tumors, investigate the molecular biological characteristics, including genetic alterations, mutations, and molecular pathways involved in tumor development and progression, establish correlations between the clinical presentation, histopathology, and molecular profile to aid in their accurate diagnosis and clinical management.

In achieving these goals, we can improve the understanding diagnosis, and management of rare salivary gland and sinonasal tumors, ultimately leading to better patient outcomes.

MATHERIAL AND METHODS

For salivary glands studies the cases were obtained from Salivary Gland Tumor Registry in Pilsen. The cases of sinonasal sarcomas were the review of collective consultation cases.

Clinical and follow-up information was obtained from the medical databases or referring pathologists.

Except for the morphological method, wide panels of immunohistochemical markers were used for a more complete and broader investigation of all possible variants of expressions.

The range of molecular genetic testing including fluorescence in situ hybridization (FISH), *reverse transcription* polymerase chain reaction (RT-PCR), and DNA and RNA-based next-generation sequencing (NGS) were applied for enhancing understanding of tumor biology.

For NGS testing some of the cases used comprehensive (both DNA and RNA-based) genomic profiling with identification of all relevant variants implicated in various solid tumor types, including detection of homologous recombination repair genes (HRR) which made it possible to find all available genetic alterations.

RESULTS

Salivary gland researches

The oncocytic variant of mucoepidermoid carcinoma (OMEC) is a rare variant of mucoepidermoid carcinoma (MEC), but it poses diagnostic challenges due to its overlapping morphological features with benign and low-grade malignant salivary gland tumors with oncocytic morphology [5-7].

In our study, we reviewed 125 archived cases of tumors classified as "low-grade/uncertain oncocytic tumor," "oncocytoma," and "oncocytic carcinoma". As a result of our work, 22 cases were reclassified as OMEC.

The tumors showed nested and multinodular growth patterns composed of oncocytic cells, most tumors have invasive growth into the surrounding tissues. The oncocytic changes of neoplastic cells covered up to 100% of all tumor cells. In some cases, true mucocytes were completely absent.

We identified the immunohistochemical characteristics that could be useful in routine histological examinations, such as strong diffuse nuclear staining for p63, p40 proteins, and antimitochondrial antigen (MIA).

MECs are harboring *CRTC1::MAML2* or *CRTC3::MAML2* gene fusions in up to 80% of cases [8-13]. In our study, we found *CRTC1::MAML2* gene fusion in 59% and *CRTC3::MAML2* gene fusion in 4.5% of OMEC cases. Interestingly, the tumor harboring a *CRTC3::MAML2* fusion has S100 protein and SOX10 positivity. 23% of OMEC cases were negative on NGS but *MAML2* rearrangement was confirmed by FISH.

In conclusion, we recommend *MAML2* rearrangement testing for all patients with salivary gland tumors exhibiting oncocytic morphology to identify this rare variant of MEC achieving an accurate morphological diagnosis.

Myoepithelial carcinoma (MC) of salivary glands is a challenging entity with diverse morphology. One of the subtypes of MC is the clear cell variant (CCMC), which typically presents as a multi-nodular tumor. The nodes consist of a hypocellular myxoid central zone with occasional necrosis, hypercellular areas are present at the periphery. In most cases, clear cells dominate, although spindle and rhabdoid/plasmacytoid areas may also be observed [14,15].

In our study, a total of 94 cases of MC with clear cell morphological features were investigated. Notably, a wide spectrum of nuclear atypia, areas of necrosis, and a high mitotic index (mean of 42%) was observed.

MC reported a high prevalence of oncogenic gene fusions, mainly involving the *PLAG1* gene [16]. Additionally, an EWSR1 gene rearrangement in a subset of MCs of salivary glands, predominantly composed of clear cells was described [17]. Because of morphological similarities between CCMC and myoepithelial tumors of soft tissues and skin and hyalinizing clear cell carcinoma, to understand the biological nature of CCMC, a molecular-genetic study was conducted.

Overall, *PLAG1* gene rearrangements were detected in 26% of the tested cases. Fusion transcripts were found in 40% of cases, such as *LIFR::PLAG1, CTNNB1::PLAG1, CHCHD7::PLAG1*, and *EWSR1::ATF1*. The case with *EWSR1::ATF1* fusion was reviewed and reclassified as clear cell carcinoma (CCC). The study revealed that 27.6% of the tumors showed split signals for *EWSR1* by FISH, but none of these tumors exhibited an *EWSR1* fusion transcript.

The novel findings from our study suggest that CCMC is a gene fusion-driven disease with PLAG1 rearrangement involving fusion partners *CTNNB1, LIFR,* and *CHCHD7,* as well as EWSR1-FISH positivity. The presence of EWSR1-FISH abnormality in CCMCs without identifiable gene fusion may represent a passenger mutation.

The study also highlighted genetic differences between salivary gland myoepithelial carcinomas and myoepithelial carcinomas of soft tissues and skin, despite the morphological, immunohistochemical similarities and common *EWSR1* rearrangement. The authors believe that the existence of *PLAG1* rearrangements reflects pleomorphic adenoma-like features in MCs, while *EWSR1* fusions may be associated with the clear cell phenotype.

Acinic cell carcinoma (AciCC) constitutes approximately 10% of malignant salivary gland tumors [2]. Classic low-grade AciCC has a favorable prognosis, but some cases undergo a high-grade transformation, leading to an aggressive clinical course [18-20].

The authors conducted a study with 128 archival cases of AciCC, including 85 cases of low-grade AciCC, 7 cases of high-grade AciCC, and 36 cases with high-grade transformation (HGT). Low-grade AciCCs displayed a typical solid-microcystic pattern with abundant

lymphoid stroma. High-grade AciCCs and cases with HGT exhibited moderate to high-grade cytologic atypia, high mitotic activity, and comedo-type necrosis in the majority of cases.

We revealed DOG1 and SOX10-positivity by IHC in 98% and 99% of cases respectively. NR4A3 was at least focally positive in 82% of cases, with comparable proportions in low-grade, HGT, and high-grade AciCC groups. All 6 NR4A3-immunonegative cases displayed NR4A2-positivity, while none of the NR4A3-positive ones showed NR4A2 immunopositivity.

The majority of AciCC cases are driven by t(4;9)(q13;q31) translocation, which leads to the transposition of active enhancer regions from the secretory calcium-binding phosphoprotein cluster of genes to the proximity of the *NR4A3* gene [21]. Rare cases may show upregulation of the *NR4A2* gene located at chromosome band 2q24.1 [22].

In our study, *NR4A3* rearrangement was confirmed in 82% of NR4A3-immunopositive and only 25% of NR4A3-immunonegative analyzable cases. The *NR4A2* break-apart was confirmed in 25% of analyzable cases, while 75% of cases were negative for aberration. Overall, 17 cases were negative for both NR4A3 and NR4A2 by IHC.

The author recommends using NR4A3, NR4A2, DOG1, and SOX10 immunostains as a cost-effective method for AciCC diagnostics. From our point of view, both NR4A3 and NR4A2-immunostains are especially useful in small biopsy cases and challenging cases with unusual morphology.

We also suggest conducting a FISH analysis of *NR4A3* rearrangement in all NR4A3 and NR4A2-immunonegative cases. We believe that a negative result of molecular testing does not exclude the possibility of translocation. As reported by Haller et al. [22], some cases with breakpoints of chromosome 9 located higher upstream from the *NR4A3* gene might show normal, non-translocated signals by FISH. qRT-PCR might be employed to detect the *NR4A3* rearrangement for such cases. Further research is needed to explore potential different genetic aberrations in a very minor group of AciCC cases.

Sclerosing polycystic adenoma (SPA) is a rare salivary gland neoplasm that shows characteristic histologic features, which are reminiscent of sclerosing adenosis and various intraductal epithelial proliferations in the breast [2]. The later data assumed that it is a genuine neoplasm [23-27].

In our 36 case series, all cases showed lobulated well-circumscribed growth patterns, the presence of large abnormal acinar cells with brightly eosinophilic intracytoplasmic granules, variably sized and shaped ducts lined by flattened, cylindrical, or apocrine epithelium. Foci of intraluminal solid and cribriform intercalated duct-like epithelial proliferation with variable degrees of dysplasia was observed in every case. In our work, we present for the first time diagnostic criteria for low and high-grade dysplasia in the solid and cribriform epithelial proliferations in SPA.

By IHC, ductal epithelial proliferations were positive for S100 protein, SOX10, and mammaglobin and have intact layers of myoepithelial cells on the periphery (IDC-type) in 58% of cases. They showed an ummunophenotype of intercalated ducts and were negative for AR and GATA3. 39% of cases had focal intraductal cribriform and micropapillary apocrine-type dysplastic epithelial structures composed of AR-positive and S100/SOX10 negative cells (apocrine type). All cases were negative for DOG1, PLAG1, and NOR1.

The results of molecular testing were comparable with the observations of Bishop et al.[28] and Hernandez-Prera et al. [27]. We found that 92% of SPA cases had *PIK3CA* gene mutations. Morphologically all tumors with *PIK3CA* mutation are represented by focal solid and cribriform proliferation of epithelium with IDC-like phenotype and mild to moderate dysplasia. In contrast, the cases with detected *HRAS* and *AKT1* mutations displayed extensive apocrine solid and cribriform dysplastic components with severe atypia.

Our index case of apocrine IDC with invasive SDC component arising from SPA showed an identical mutation in the *PIK3CA* gene in benign and both malignant components. Moreover, we found an additional *HRAS* mutation only in the malignant component. None of the *HRAS* and *AKT1* gene mutations has been documented in SPA previously. We didn't also find any gene fusions in our case series.

Our findings suggest a close relationship between SPA, apocrine IDC, and invasive SDC. SPA may represent a precursor lesion for the apocrine IDC and sometimes invasive SDC.

Mesenchymal sinonasal tumors researches

Biphenotypic sinonasal sarcoma (BSNS) is a low-grade sarcoma of the sinonasal tract with neural and myogenic features [29]. Clinically, these tumors usually are indolent with frequent recurrences but no metastases[29-31].

We present a unique case of aggressive high-grade rhabdomyosarcoma (RMS) originating from a typical BSNS of the right ethmoid, maxillary, and frontal sinuses. Partly, the tumor consisted of infiltrative hypercellular fascicles of uniform spindle cells, with pale eosinophilic cytoplasm, ovoid nuclei, and small basophilic nucleoli. The morphology was typical for BSNS. Partly it was represented by a high-grade sarcoma with rhabdoid features, high mitotic activity, and necrosis.

Immunohistochemically, the conventional BSNS areas showed diffuse strong positivity with PAX7, S100, and patchy expression of MyoD1, SMA, while desmin and myogenin were completely negative. The high-grade areas were completely negative for S100 protein and SMA, and patchy positive with PAX7, whereas the diffuse positivity of desmin, MyoD1, and myogenin were observed.

NGS revealed *PAX3*(exon7)*:: MAML3*(exon2) fusion which was confirmed by FISH, using *MAML3* (4q31.1) and *PAX3* (2q36.1) break-apart probes in both the conventional and high-grade components.

During development, the *PAX3* gene is required for melanocytic, neuronal, and skeletal muscle differentiation and regulates normal myogenesis and muscular regeneration [32-34], while the *MAML3* gene has been shown as a transactivator of *PAX3* response elements [35]. *PAX3::FOXO1* and *PAX3::NCOA1* fusions have also been described in rare cases of alveolar RMS [32]. BSNS with rhabdomyoblastic differentiation and *RREB1::MRTFB* fusion showed histological features and molecular alteration potentially overlapping with ectomesenchymal chondromyxoid tumors (EMCMT) [36,37].

The differential diagnosis of BSNS with rhabdomyosarcomatous component should be differentiated with embryonal and RMS, malignant Triton-tumor, sarcomas with *EWSR1::POU2AF3*-rearrangement, ectomesenchymal chondromyxoid tumors.

In small biopsies when the conventional BSNS component is absent, we consider a molecular genetic study for accurate diagnosis. It will be especially helpful in cases with evident rhabdomyoblastic differentiation. Moreover, the investigation of more cases is mandatory to delineate the morphological spectrum and the biological behavior of this rare entity.

Within the group of sarcomas with small round cell and spindle cell morphology affecting the head and neck, there are various tumors, including rhabdomyosarcoma, Ewing's sarcoma, sarcomas with *CIC* and *BCOR*-rearrangements, small cell osteosarcoma, etc.

EWSR1::POU2AF3 (*COLCA2*) sarcomas are a recently identified group of undifferentiated round/spindle cell neoplasms with a predilection for the head and neck region. This type of sarcoma has only been described in two studies, where 11 cases of small round cell/spindle cell sarcoma with a novel *POU2AF3* rearrangement were presented [38,39].

We analyzed 8 cases collected by us and combined them with 11 from previous studies. The presence of the *POU2AF3* rearrangement was observed in all cases. Although all tumors with *POU2AF3* rearrangement consisted of relatively uniform spindled to round cells, we found two distinct morphologic subgroups.

The first one had low-grade morphology and consisted of relatively bland, spindled/stellate cells in a fibromyxoid stroma, with mild to moderate nuclear atypia, and low mitotic activity.

The second, larger subgroup consisted predominantly of highly cellular sheets and fascicles of malignant-appearing spindled and round cells, with somewhat biphasic architecture. Mitotic activity was high and small areas of necrosis were present. The most distinctive feature of this second subgroup was the presence of nests or loose dispersed sheets of cells with “neuroendocrine” morphology. True epithelial glands and foci of osteogenic and rhabdomyoblastic differentiation were noted in single cases.

Polyphenotypic immunoprofile (variable expression of epithelial, neuroendocrine, and neurogenic markers) was identified. The molecular genetic data included fusions with genomic breakpoints in exons 9, 10, 14, 15, or 16 of *EWSR1,* and only breakpoints in exon 2 of the *POU2AF3* gene were detected. Only two cases harbored *FUS* rearrangement has been found in our cohort.

The nosology of these neoplasms has yet to be clarified. Since most of these tumors show an aggressive clinical behavior the use of molecular methods is strongly recommended to achieve early detection, enabling optimal treatment**.**

CONCLUSION

The Ph.D. thesis concludes postgraduate studies in pathology Olena Koshyk, MD. During the studies, the objectives were fulfilled over the study. With the coauthors, the author has documented the clinicopathological, morphological, immunohistochemical, and molecular characteristics of rare salivary gland and sinonasal tumors.

The result of the four-year study is one first-author work and 5 co-authored publications. The results of all papers presented in the doctoral thesis were published in various American and European journals with impact factors from 3,5 to 8,209.

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LIST OF OWN PUBLICATIONS

1. **Olena Koshyk**, Carina A. Dehner, Mari F.C.M. van den Hout, Isabelle Vanden Bempt, Raf Sciot , Hsuan-Ying Huang , Abbas Agaimy , Nasir Ud Din , Natálie Klubíčková, Elaheh Mosaieby, Alena Skálová, Květoslava Michalová, Patrick Schöffski, Andre M. Oliveira, Kevin C Halling, Sounak Gupta, John M. Gross, Johanna W.M. Nin, Michal Michal, Andrew L. Folpe, Kemal Kosemehmetoglu, Jorge Torres- Mora, Michael Michal. EWSR1::POU2AF3(COLCA2) Sarcoma: An aggressive, polyphenotypic sarcoma with a head and neck predilection **IF 8,209**

2. Skálová A; Agaimy A; Stanowska O; Baneckova M; Ptáková N; Ardighieri L; Nicolai P; Lombardi D; Durzynska M; Corcione L; Laco J; **Koshyk O**; Žalud R; Michal M; Vanecek T; Leivo I. Molecular Profiling of Salivary Oncocytic Mucoepidermoid Carcinomas Helps to Resolve Differential Diagnostic Dilemma With Low-grade Oncocytic Lesions. Am J Surg Pathol. 2020 Dec; 44(12):16121622, doi:10.1097/PAS.0000000000001590 IF **5,6**

3. Skálová A; Agaimy A; Vanecek T; Baněčková M; Laco J; Ptáková N; Šteiner P; Majewska H; Biernat W: Corcione L; Eis V: **Koshyk O**; Vondrák J: Michal M; Leivo I. Molecular Profiling of Clear Cell Myoepithelial Carcinoma of Salivary Glands With EWSR1 Rearrangement Identifies Frequent PLAG1 Gene Fusions but No EWSR1 Fusion Transcripts Am J Surg Pathol. 2021 Jan; 45(1):1-13 doi: 10.1097/PAS.0000000000001591 **IF 5,6**

4. Skálová A; Baněčková M; Laco, J; Di Palma S; Agaimy A; Ptáková N; Costes-Martineau V; Petersson B; van den Hout M; de Rezende G; Klubíčková N; Koblížek M; **Koshyk O**; Vaneček T; Leivo I. Sclerosing Polycystic Adenoma of salivary Glands: A Novel Neoplasm Characterized by PIK-AKT Pathway Alterations-NEW Insights Into a Challenging Entity. Am J Surg Pathol. 46(2):268-280, 01 Feb 2022 Am J Surg Pathol. 2022 Feb; 46(2):268-280 doi: 10.1097/PAS.0000000000001807 **IF 5,6**

5. Klubíčková N, Grossmann P, Šteiner P, Baněčková M, Mosaieby E, **Koshyk O**, Michal M, Leivo I, Skálová A. A minority of cases of acinic cell carcinoma of the salivary glands are driven by an NR4A2 rearrangement: the diagnostic utility of the assessment of NR4A2 and NR4A3 alterations in salivary gland tumors. Virchows Arch. 2023 Feb;482(2):339-345. doi: 10.1007/s00428-022-03464-8. Epub 2022 Dec 5. PMID: 36469101. **IF 3,5**

6. Meyer A, Klubíčková N, Mosaieby E, Grossmann P, Kalmykova A, **Koshyk O**, Michal M. Biphenotypic sinonasal sarcoma with PAX3::MAML3 fusion transforming into high-grade rhabdomyosarcoma: report of an emerging rare phenomenon. Virchows Arch. 2023 Apr;482(4):777-782. doi: 10.1007/s00428-023-03501-0. Epub 2023 Jan 31. PMID: 36719455; PMCID: PMC10067655. **IF 3,5**

PRESENTATION

1. POSTER- 109th ANNUAL MEETING USCAP, Los Angeles, California, USA, 2020. Novel Rearrangements in Salivary Gland Tumors Detected by Next Generation
2. POSTER- 109th ANNUAL MEETING USCAP, Los Angeles, California, USA, 2020. Report of 20 Cases of Seromucinous Hamartomas and Respiratory Epithelial Adenomatoid Hamartomas with Dysplastic and Malignant Features
3. POSTER- 34th EUROPEAN CONGRESS OF PATHOLOGY (ECP), Basel, Switzerland, 2022. Diagnostic Utility NR4A3 and NR4A2 Immunohistochemistry in salivary gland pathology: a single institution experience with 108 cases of acinic cell carcinoma
4. POSTER- 34th EUROPEAN CONGRESS OF PATHOLOGY (ECP), Basel, Switzerland, 2022. Molecular-genetic profile of sinonasal tumors: molecularly heterogeneous but histologically distinctive low-grade and high-grade tumors
5. POSTER- 34th EUROPEAN CONGRESS OF PATHOLOGY (ECP), Basel, Switzerland, 2022. Update to seromucinous hamartomas and respiratory epithelial adenomatoid hamartomas with dysplastic features and malignant transformation