

## Research Article

# A subset of plasmacytoid pleomorphic adenoma showing *CTNNB1::PLAG1* fusion

Nagla Salama<sup>1</sup>, PhD and Mahmoud Fathy<sup>1</sup>, PhD

## Abstract

**Background** This study aimed to explore the morphological, clinical, and molecular aspects of pleomorphic adenoma (PA) and myoepithelial salivary gland neoplasms, investigating their behavior and potential diagnostic markers.

**Objective** This article further explores and expands upon this spectrum of molecularly labeled subsets showing specific morphologic features.

**Method** Tissue samples from 25 cases of PA were examined, and RNA next-generation sequencing (NGS) was performed on paraffin-embedded sections using the Illumina TruSight RNA Pan-cancer panel. Cellular characteristics, clinical attributes, and molecular profiles were assessed to identify unique features and potential diagnostic indicators.

**Results** PA, a prevalent benign tumor, exhibited diverse morphologies and posed risks of recurrence and malignant transformation. Notably, specific metaplasias within PA cells hinted at microenvironment changes and potential behavioral variations. Furthermore, plasmacytoid myoepitheliomas, more aggressive than PA, posed challenges in accurate differentiation. In 12 out of 25 cases, the presence of the *CTNNB1:PLAG1* fusion gene alongside plasmacytoid cells confirmed the diagnosis of plasmacytoid pleomorphic adenoma.

**Advances in knowledge** The identification of specific metaplasias and fusion genes, notably *CTNNB1-PLAG1*, provided diagnostic insights, potentially influencing improved diagnostics and targeted treatments in the future.

**Keywords:** Salivary gland tumors, pleomorphic adenoma, plasmacytoid

## Introduction

Pleomorphic adenoma (PA) is a benign neoplasm known for its diverse morphologic features. PA is the most prevalent benign tumor affecting the salivary glands in both children and adults. Complete excision of the tumor significantly reduces the chances of recurrence[1-3]. However, tumoral capsular disruption and certain demographic parameters or multifocality can increase the risk of its return. PA has the potential to become malignant, spreading to nearby lymph nodes and distant areas or evolving into a carcinoma ex PA[4-6].

In PA, the presence of squamous, mucinous, osseous, or lipomatous metaplasias signals a transformation of cells into types not typically found[7-9]. These shifts hint at the tumor's the changing microenvironment. When endocrine cells appear, it may indicate deviations from the typical appearance of pleomorphic adenoma and hint at its behavior. Oncocytic changes reflect cell alterations resulting in increased pink staining due to abundant mitochondria[10-12]. Pertinently, they might signify metabolic shifts or cellular adaptations within the tumor cells. Conspicuously, when plasmacytoids dominate in pleomorphic adenomas, it often indicates a potential for aggressive behavior[13].

Myoepithelial cells, found in various salivary gland tumors, play several crucial roles. They aid in tumor cell differentiation, produce both basement-membrane and non-basement-membrane components, secrete significant amounts of the tumor-suppressor maspin and various proteinase inhibitors that accumulate in the surrounding extracellular matrix, and importantly, they hinder invasion and angiogenesis. Myoepithelial cells are either spindle, plasmacytoid (hyaline), epithelioid, and clear cells[14-16]. Plasmacytoid cells typically exhibit vimentin expression, occasionally keratin 19, and sometimes keratin 18. Although elongated cells with nuclei slightly off-center show positivity for  $\alpha$ -smooth muscle actin ( $\alpha$ SMA), possibly representing a transitional stage between plasmacytoid and spindle-shaped neoplastic myoepithelial cells, genuine plasmacytoid cells do not express  $\alpha$ SMA[16,17]. The distinct hyaline cytoplasm of plasmacytoid cells comprises randomly arranged filaments. Initially mistaken for actin microfilaments, these filaments were originally misinterpreted as neoplastic. Plasmacytoid myoepithelioma demonstrates higher aggressiveness compared to pleomorphic adenoma and poses challenges in differentiation from plasmacytoid pleomorphic adenoma[18-20].

*PLAG1* and *HMGA2* fusion genes have been the focus of PA and carcinoma ex PA[21-23]. Recently, specific subsets of PA with distinct morphologies correlating to particular molecular labeling, showing *HMGA2::WIF1* and canalicula-like features have been identified[24]. This article further explores and expands upon this spectrum of molecularly labeled subsets showing specific morphologic features.

## Method

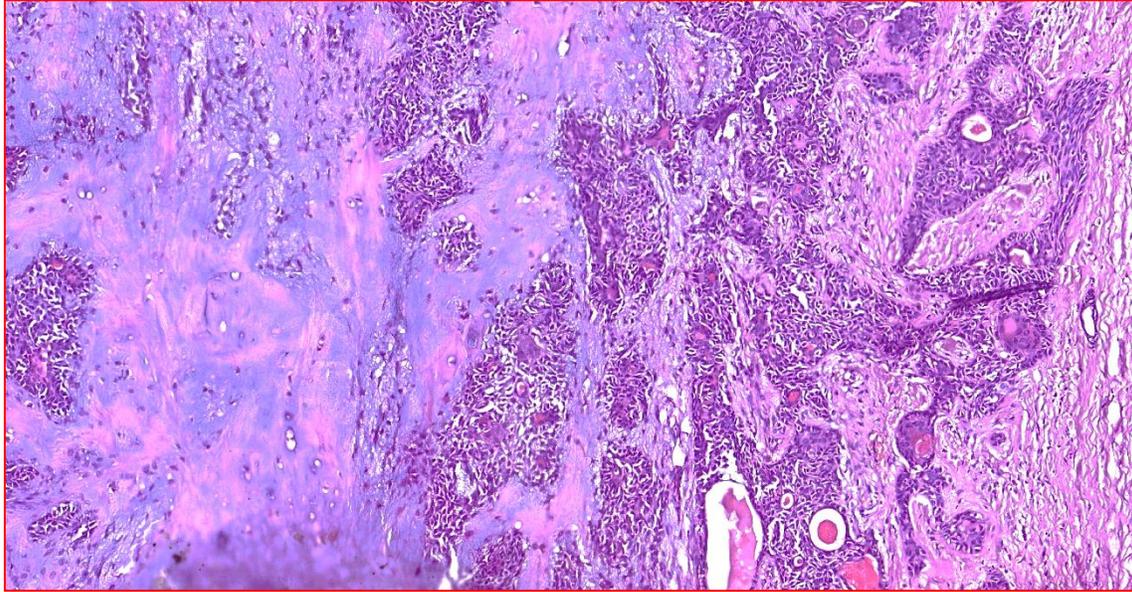
Paraffin blocks containing 25 unusual Pas retrieved from 2019 to 2022 were examined with ethical approval from the MU. These blocks held preserved tissue samples stored in paraffin wax. Visual inspection of selected paraffin blocks identified specific areas of plasmacytoid dominance within benign neoplasms showing myxocartilagenous stromas. The cases were visually assessed, and sectioned at 4 microns using a microtome. Conventional MECs, being extensively studied, served as a reliable reference point. Hematoxylin and eosin (H&E) staining of prepared slides confirmed the diagnoses.

Following a previously described method[25], tissue blocks containing the targeted cells were utilized for nucleic acid isolation. These blocks served as a source of cellular material for experimentation. Disruption of cells within the tissue blocks facilitated access to the nucleic acids enclosed. RNA isolation employed the TRIzol/phenol-chloroform method, known for effectively extracting high-quality RNA from biological samples by isolating it from other cellular components. RNA concentration and purity were evaluated post-extraction using a spectrophotometer.

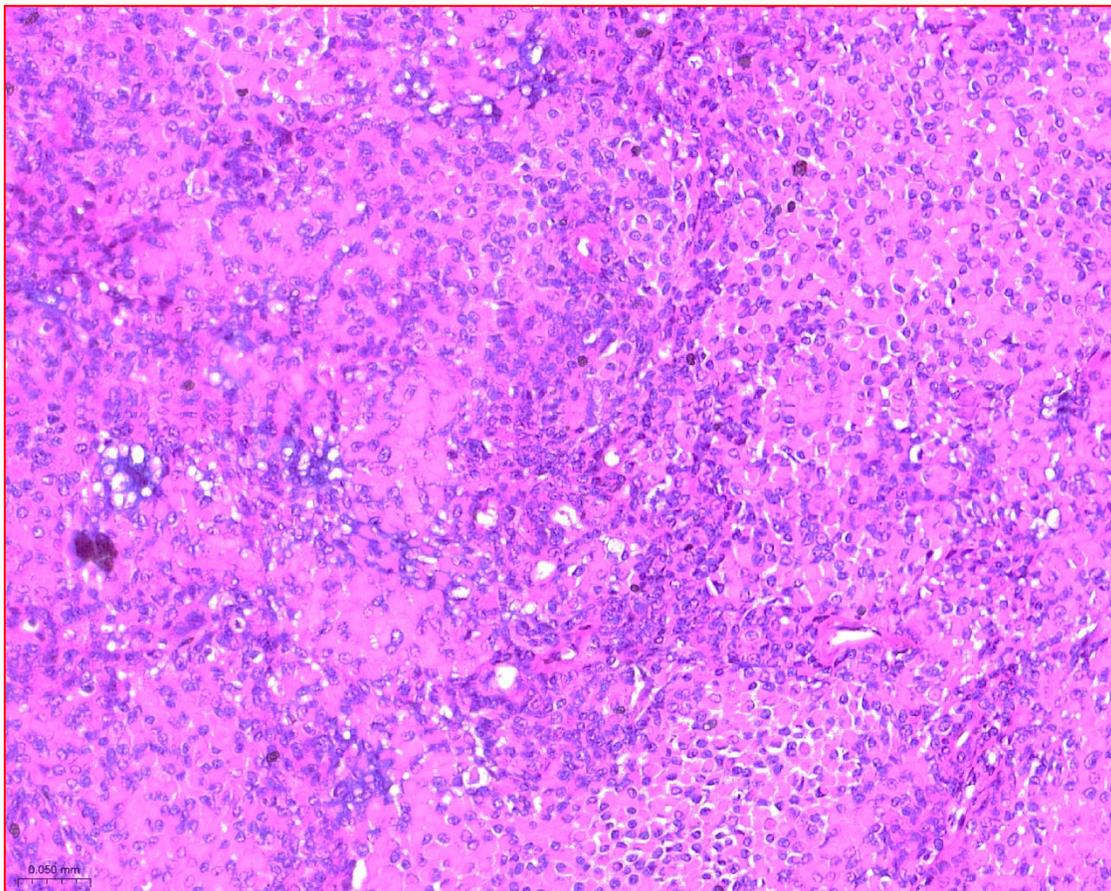
During cDNA synthesis, 500 ng of RNA was used following established protocols to ensure accuracy and reproducibility. This standardized input aimed to generate consistent and reliable cDNA samples for subsequent analysis. Initial RNA-seq was conducted using the Illumina TruSight RNA Pan-cancer panel to identify the fusion gene.

## Results

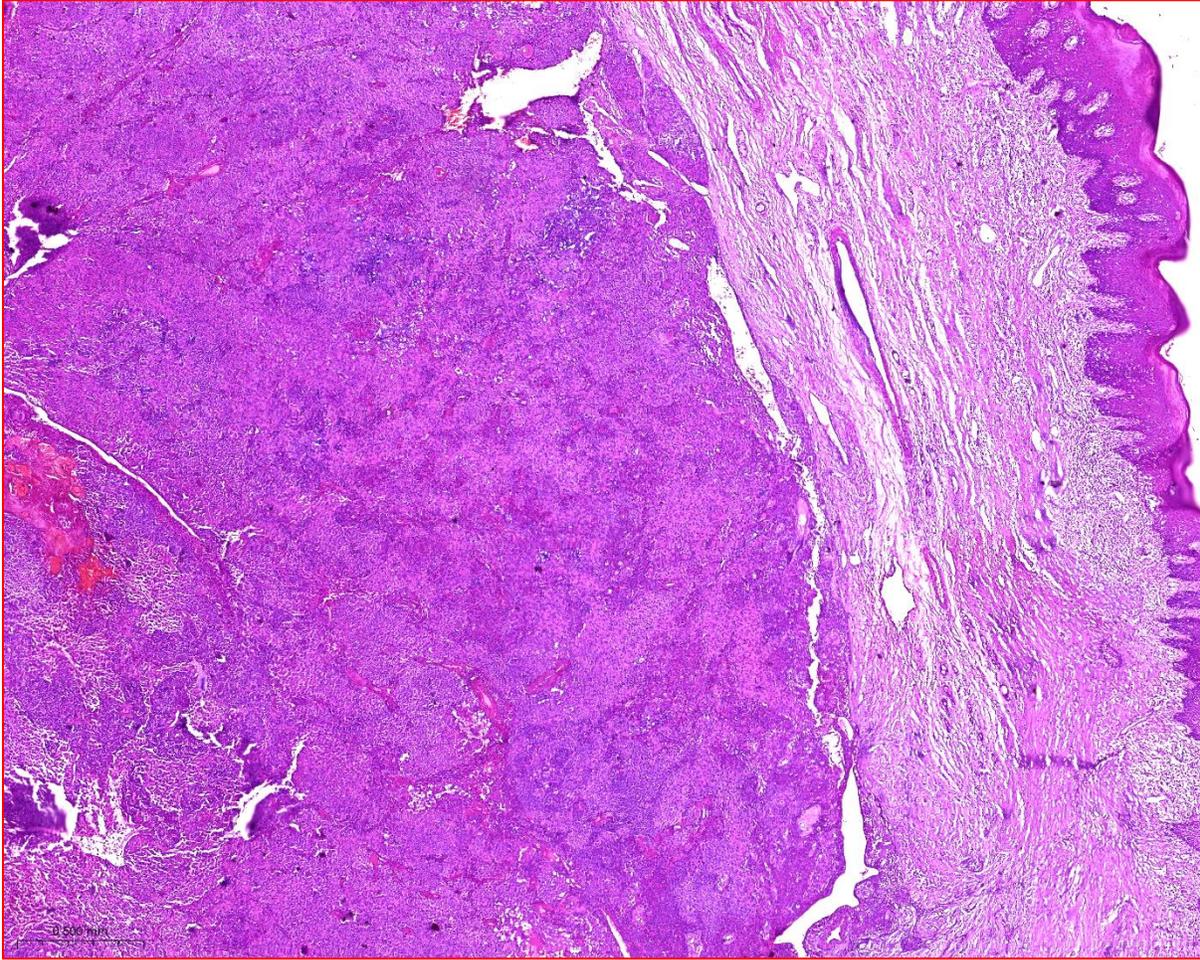
Our findings reveal that primary nodules typically measured around 3.1 cm in size, with tumor cellularity. Most cases displayed satellite nodules connected to the main tumor, infiltration into nearby tissues, and a tissue environment with characteristics like myxoid stroma(Figure 1). Additionally, fifteen cases exhibited changes in myoepithelial cells with plasmacytoid predominance (Figures 2-3) of which 12 were molecularly labeled



**Figure 1.** PA showing characteristics chondromyxoid stroma



**Figure 2.** PA showing characteristics plasmacytoid predominance.



**Figure 3.** PA with plasmacytoid predominance showing thin fragmented capsule .

Regarding recurrence rates, the overall rate after the first occurrence was 16.4%, occurring at a median interval of 46 months. For those with a second recurrence, the median interval between recurrences was 97 months. Remarkably, cases showing a transformation into malignancy were not found. HMGA2 immunohistochemistry consistently displayed diffuse positive staining in all cases, including those negative for fusion.

#### *Molecular labeling*

Twenty-five cases were identified as myoepithelial salivary gland neoplasms, leaning towards pleomorphic adenoma. Paraffin-embedded sections from the cell block were subjected to RNA next-generation sequencing (NGS). This Illumina TruSight RNA Pan-cancer panel detected the presence of the *CTNNB1-PLAG1* fusion gene. The existence of the *PLAG1* fusion gene, coupled with the presence of plasmacytoid cells, confirmed a diagnosis of plasmacytoid pleomorphic adenoma, alongside scattered areas displaying chondromyxoid and collagenous stroma.

## Discussion

Precaution must be taken with diagnosing PA, especially with FNA. Schwannoma-like PA[15,26,27], keratinizing PA[28–30], myxolipomatous PA, oncocytic PA, and mucinous PA have been reported, with the latter mimicking mucoepidermoid carcinoma[28]. Mucin production can be observed in mucinous PA, mucinous adenocarcinomas, adenosquamous carcinoma and other mucin-rich carcinomas[31]. In the literature, some aggressive cases of PA showed strong expression for b-catenin and the corresponding molecular gene of *CTNNB1* [1,32]

The fusion partner was *WIF1* in most cases, with a few instances involving *RPSAP52* and *HELB*. Separate testing of the components in a tumor showed *HMGA2::WIF1* fusion.. In PAs, *PLAG1* collaborates with a range of chromosomal proteins, forming specific chimeric oncoproteins, which significantly influence the tumor's growth. Notably, these fusions include *CTNNB1::PLAG1*, *LIFR::PLAG1*, *CHCHD7::PLAG1*, and *TCEA1::PLAG1*[33–38]. Among these fusions, the *CTNNB1::PLAG1* connection occurs most frequently. These interactions are pivotal in shaping the mechanisms that drive the formation and advancement of pleomorphic adenomas. This study confine this *CTNNB1::PLAG1* fusion to a subset of with plasmacytoid predominance that is associated with remarkable clinical aggression.

## Conclusion

The study investigated pleomorphic adenoma (PA) exploring their various morphologies, behavior, and molecular traits. PA, a common benign tumor, carries risks of recurrence and potential malignancy. Unique cell transformations within PA hint at changes in the tumor's environment and behavior. Additionally, plasmacytoid myoepitheliomas, more aggressive than PA, pose challenges in accurate identification.

Recently, fifteen PAs showing exclusively canalicular/trabecular while 13 had intermingled or well-demarcated conventional (chondromyxoid) component comprising 5 to more than 50% of the tumor. The monomorphic areas expressed uniformly CK7, vimentin, S100, SOX10 and variably p63 and mammaglobin but were negative with p40, smooth muscle actin, and MUC4. Targeted RNA sequencing revealed *CTNNB1::PLAG1* fusions in 12 out of 25 PA cases, the presence of the *CTNNB1::PLAG1* alongside plasmacytoid cells. Clinically, primary nodules exhibited specific traits, like size and recurrence rates, and notably, no malignant transformations were observed. These findings expand our understanding and could influence improved diagnostics and targeted treatments in the future.

**Notes:** None

**Acknowledgements:** None

**Funding resources:** None

**Conflict of interest:** The authors declare no conflict of interest.

## References

- [1] Jo VY, Sholl LM, Krane JF. Distinctive Patterns of *CTNNB1* ( $\beta$ -Catenin) Alterations in Salivary Gland Basal Cell Adenoma and Basal Cell Adenocarcinoma 2016;40:1143–50. <https://doi.org/10.1097/PAS.0000000000000669>.
- [2] Miliauskas JR, Hunt JL. Primary unilateral multifocal pleomorphic adenoma of the parotid gland: Molecular assessment and literature review. *Head Neck Pathol* 2008;2:339–42. <https://doi.org/10.1007/s12105-008-0081-2>.
- [3] Papadogeorgakis N, Kalfarentzos EF, Petsinis V, Parara E, Kopaka M-E. Multinodular neck recurrence of parotid gland pleomorphic adenoma: A case report. *Oral Maxillofac Surg* 2012. <https://doi.org/10.1007/s10006-011-0279-4>.
- [4] Di Palma S, Skálová A, Vanìèek T, Simpson RHW, Stárek I, Leivo I. Non-invasive (intracapsular) carcinoma ex pleomorphic adenoma: Recognition of focal carcinoma by HER-2/neu and MIB1 immunohistochemistry. *Histopathology* 2005;46:144–52. <https://doi.org/10.1111/j.1365-2559.2005.02058.x>.
- [5] Antony J, Gopalan V, Smith RA, Lam AKY. Carcinoma ex Pleomorphic Adenoma: A Comprehensive Review of Clinical, Pathological and Molecular Data 2012;6:1–9. <https://doi.org/10.1007/s12105-011-0281-z>.
- [6] Valstar MH, Mast H, ten Hove I, Moonen LR, Balm AJM, Smeele LE, et al. Malignant transformation of salivary gland pleomorphic adenoma: proof of principle. *J Pathol Clin Res* 2021;7. <https://doi.org/10.1002/cjp2.216>.
- [7] Dos Santos JLM, Gonçalo RIC, Santos JWM, Silveira MLM, Queiroz LMG. Pleomorphic adenoma mimicking oral lipoma . *Rev Cubana Estomatol* 2021.
- [8] Musayev J, Önal B, Hasanov A, Farzaliyev I. Lipomatous pleomorphic adenoma in the hard palate: Report of a rare case with cyto-histo correlation and review. *J Cytol* 2014. <https://doi.org/10.4103/0970-9371.130689>.
- [9] Agaimy A, Ihrler S, Märkl B, Lell M, Zenk J, Hartmann A, et al. Lipomatous salivary gland tumors: A series of 31 cases spanning their morphologic spectrum with emphasis on sialolipoma and oncocytic lipoadenoma. *Am J Surg Pathol* 2013;37:128–37. <https://doi.org/10.1097/PAS.0b013e31826731e0>.
- [10] Seethala RR, Barnes EL. Rare Malignant and Benign Salivary Gland Epithelial Tumors. *Surg Pathol Clin* 2011;4:1217–72. <https://doi.org/10.1016/j.path.2011.07.006>.
- [11] Triantafyllou A, Ruggles N. Lysosomal and cytoskeletal events in epithelial salivary tumours as assessed by imunohistochemistry for CD63 and HSP27. *Pathol Res Pract* 2022;229. <https://doi.org/10.1016/j.prp.2021.153691>.
- [12] Etit D, Tan A, Bayol U, Altinel D, Cumurcu S, Cukurova I. Oncocytic carcinoma ex pleomorphic adenoma. *Head Neck Pathol* 2010. <https://doi.org/10.1007/s12105-009-0156-8>.
- [13] Alzumaili B, Xu B, Saliba M, Abubashem A, Ganly I, Ghossein R, et al. Clinicopathologic characteristics and prognostic factors of primary and recurrent pleomorphic adenoma a single institution retrospective study of 705 cases. *Am J Surg Pathol* 2021.

- <https://doi.org/10.1097/PAS.0000000000001837>.
- [14] Nagao T, Sugano I, Ishida Y, Tajima Y, Matsuzaki O, Konno A, et al. Salivary gland malignant myoepithelioma: A clinicopathologic and immunohistochemical study of ten cases 1998;83:1292–9. [https://doi.org/10.1002/\(SICI\)1097-0142\(19981001\)83:7<1292::AID-CNCR4>3.0.CO;2-L](https://doi.org/10.1002/(SICI)1097-0142(19981001)83:7<1292::AID-CNCR4>3.0.CO;2-L).
- [15] Triantafyllou A. Degenerative nuclear atypia in pleomorphic adenoma of salivary glands: Histological and immunohistochemical observations. *Pathol Res Pract* 2021;224. <https://doi.org/10.1016/j.prp.2021.153482>.
- [16] Chitturi RT, Veeravarmal V, Madhavan Nirmal R, Venkatramana Reddy B. Myoepithelial cells (MEC) of the salivary glands in health and tumours. *J Clin Diagnostic Res* 2015. <https://doi.org/10.7860/JCDR/2015/11372.5707>.
- [17] Ogawa Y, Kishino M, Atsumi Y, Kimoto M, Fukuda Y, Ishida T, et al. Plasmacytoid cells in salivary-gland pleomorphic adenomas: Evidence of luminal cell differentiation. *Virchows Arch* 2003;443:625–34. <https://doi.org/10.1007/s00428-003-0890-3>.
- [18] Cuadra Zelaya F, Quezada Rivera D, Tapia Vazquez JL, Paez Valencia C, Gaitán Cepeda LA. Plasmacytoid myoepithelioma of the palate. Report of one case and review of the literature. *Med Oral Patol Oral Cir Bucal* 2007.
- [19] Souliou CE, Tzermpos F, Argyris P, Tosios K. Plasmacytoid myoepithelioma of the hard palate: Case report . *Acta Stomatol Croat* 2017. <https://doi.org/10.15644/asc51/2/9>.
- [20] Soberanis-Piña PD, Fernández-Ferreira R, Buerba-Vieregge HH, Varela-Santoyo E, Rodriguez-Cid JR, MacAri-Jorge A, et al. Myoepithelial Carcinoma Arising in a Plasmacytoid Myoepithelioma of the Parotid Gland Synchronized with Melanoma: A Case Report and Review of the Literature. *Case Rep Oncol* 2021. <https://doi.org/10.1159/000510937>.
- [21] Toper MH, Sarioglu S. Molecular Pathology of Salivary Gland Neoplasms: Diagnostic, Prognostic, and Predictive Perspective. *Adv Anat Pathol* 2021;28:81–93. <https://doi.org/10.1097/PAP.0000000000000291>.
- [22] M. R, I. F. Salivary Gland Neoplasms: Does Morphological Diversity Reflect Tumor Heterogeneity. *Pathobiology* 2018;85.
- [23] Katabi N, Ghossein R, Ho A, Dogan S, Zhang L, Sung Y-S, et al. Consistent PLAG1 and HMGA2 abnormalities distinguish carcinoma ex-pleomorphic adenoma from its de novo counterparts 2015;46:26–33. <https://doi.org/10.1016/j.humpath.2014.08.017>.
- [24] Agaimy A, Ihrler S, Baněčková M, Martineau VC, Mantsopoulos K, Hartmann A, et al. HMGA2-WIF1 Rearrangements Characterize a Distinctive Subset of Salivary Pleomorphic Adenomas With Prominent Trabecular (Canalicular Adenoma-like) Morphology. *Am J Surg Pathol* 2022;46. <https://doi.org/10.1097/PAS.0000000000001783>.
- [25] El-Nagdy SY, Shetty P, Abdullahab B, Sabetid S. YAP1 Rearrangement in Parotid Sclerosing Mucoepidermoid Carcinoma. *J Calif Dent Assoc* 2023. <https://doi.org/10.1080/19424396.2023.2283225>.
- [26] Lombardi M, Socciarelli F, Fini G, Leonardi A, Bartolazzi A. Schwannoma-Like

- Pleomorphic Adenoma: A Case Report with Review of the Literature. *Head Neck Pathol* 2014. <https://doi.org/10.1007/s12105-013-0473-9>.
- [27] Tille JC, Reychler H, Hamoir M, Schmitz S, Weynand B. Schwannoma-like pleomorphic adenoma of the parotid. *Rare Tumors* 2011;3. <https://doi.org/10.4081/rt.2011.e40>.
- [28] Tandon A, Jaiswal R, Siddiqui S, Bordoloi B. Keratinizing pleomorphic adenoma: An unusual case report. *J Oral Maxillofac Pathol* 2018;22:S69-72. [https://doi.org/10.4103/jomfp.JOMFP\\_200\\_17](https://doi.org/10.4103/jomfp.JOMFP_200_17).
- [29] Leena Sankari S, Dhanavelu P, Sangeetha Priya P, Jimson S. Keratinizing pleomorphic adenoma: A rare variant in an unusual location. *J Oral Maxillofac Pathol* 2021;25. [https://doi.org/10.4103/jomfp.jomfp\\_362\\_21](https://doi.org/10.4103/jomfp.jomfp_362_21).
- [30] Guo SP, Cheuk W, Chan JKC. Pleomorphic adenoma with mucinous and squamous differentiation: A mimicker of mucoepidermoid carcinoma. *Int J Surg Pathol* 2009;17. <https://doi.org/10.1177/1066896909335509>.
- [31] Khalele B, Laforga JB, Kajo K, Macháleková KK. Adenosquamous Carcinomas and Mucinous Adenocarcinoma of the Minor Salivary Glands: Immunohistochemical and Molecular Insights. *J Mol Pathol* 2022;3:273-85. <https://doi.org/10.3390/jmp3040023>.
- [32] Schneider S, Thurnher D, Seemann R, Brunner M, Kadletz L, Ghanim B, et al. The prognostic significance of  $\beta$ -catenin, cyclin D1 and PIN1 in minor salivary gland carcinoma:  $\beta$ -catenin predicts overall survival. *Eur Arch Oto-Rhino-Laryngology* 2016;273. <https://doi.org/10.1007/s00405-015-3609-6>.
- [33] Shen S, Lu H, Liu S, Yang W, Liu L, Xu W. Integrative analysis of long non-coding RNAs and mRNAs associated with tumorigenesis of salivary gland pleomorphic adenoma. *Arch Oral Biol* 2022;133. <https://doi.org/10.1016/j.archoralbio.2021.105303>.
- [34] Asahina M, Hayashi T, Takamochi K, Saito T, Onagi H, Kishi M, et al. Identification of CTNNB1-PLAG1 gene rearrangement in a patient with pulmonary pleomorphic adenoma. *Virchows Arch* 2020. <https://doi.org/10.1007/s00428-020-02810-y>.
- [35] Bubola J, MacMillan CM, Demicco EG, Chami RA, Chung CTS, Leong I, et al. Targeted RNA sequencing in the routine clinical detection of fusion genes in salivary gland tumors. *Genes Chromosom Cancer* 2021;60:695-708. <https://doi.org/10.1002/gcc.22979>.
- [36] Asahina M, Saito T, Hayashi T, Fukumura Y, Mitani K, Yao T. Clinicopathological effect of PLAG1 fusion genes in pleomorphic adenoma and carcinoma ex pleomorphic adenoma with special emphasis on histological features. *Histopathology* 2019. <https://doi.org/10.1111/his.13759>.
- [37] Iida Y, Serizawa M, Mukaigawa T, Kamijo T, Nakajima T, Asakura K, et al. Molecular profile of a pleomorphic adenoma of the hard palate: A case report. *Medicine (Baltimore)* 2020;99:e21207. <https://doi.org/10.1097/MD.00000000000021207>.
- [38] Rupp NJ, Höller S, Brada M, Vital D, Morand GB, Broglie MA, et al. Expanding the clinicopathological spectrum of *TGFBR3-PLAG1* rearranged salivary gland neoplasms with myoepithelial differentiation including evidence of high-grade transformation. *Genes Chromosom Cancer* 2022;61:94-104. <https://doi.org/10.1002/gcc.23009>.