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COMPARATIVE MOLECULAR CHARACTERIZATION OF PLEOMORPHIC ADENOMA AND CARCINOMA EX-PLEOMORPHIC ADENOMA

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Article Info

Abstract

Introduction: Salivary gland tumours can be categorized as either benign or malignant based on histopathology. Benign tumors comprise about two-thirds of all neoplasms of the salivary glands. The most common type is pleomorphic adenoma. Malignant tumors are less common but biologically different and clinically significant. A malignant epithelial tumour recognized as carcinoma ex pleomorphic adenoma (Ca ex PA) develops from an underlying pleomorphic adenoma (PA), signifying a change from benign to malignant neoplasia in the salivary glands. The diagnosis of these tumours have been completely transformed by molecular techniques.

Objective: The objective of this study was to characterize the specific genetic alterations that differentiate pleomorphic adenoma (PA) from carcinoma ex-pleomorphic adenoma (Ca ex PA) using NGS.

Material and Methods: This study was conducted at Institute of Pathology and diagnostic Medicine KMU, Shaukat Khanum Memorial Cancer Hospital Lahore and Lab Genetics Lahore. Four patients of both genders were selected through Non-probability convenience sampling technique. All patients included were diagnosed with Pleomorphic Adenoma and Carcinoma-ex-Pleomorphic Adenoma of salivary gland. Bioinformatics analysis were performed on a Linux computer cluster.

Results: In this study 4 cases of salivary gland tumors (2 benign pleomorphic adenomas and 2 malignant carcinoma ex-pleomorphic adenoma, Ca ex PA) were analyzed using clinical data and next-generation sequencing (NGS) to investigate the molecular drivers of benign and malignant transformation. Recurrent PLAG1 activation emerged as a central oncogenic driver across four salivary gland tumors, involving PLAG1–CTNNB1 and PLAG1–LIFR fusions in pleomorphic adenomas, each leading to constitutive PLAG1 overexpression. In contrast, carcinoma ex-pleomorphic adenomas demonstrated greater genomic complexity, including PLAG1 rearrangements or PLAG1–TCEA1 fusion accompanied by HMG2 copy-number gains. These findings suggest that while PLAG1-driven fusions initiate tumorigenesis, secondary alterations such as HMG2 amplification promote malignant transformation and increased tumor aggressiveness.

Conclusions: PLAG1 gene fusions (with CTNNB1, LIFR) are key molecular events leading to pleomorphic adenoma formation. Progression to carcinoma ex-pleomorphic adenoma occurs when PLAG1 or LIFR fusions are accompanied by HMG2 gene gain, indicating dual oncogenic activation.

Keywords:
Pleomorphic adenoma (PA), carcinoma ex-pleomorphic adenoma (Ca ex PA)

INTRODUCTION

Salivary gland tumours can be categorized as either benign or malignant based on histopathology. Pleomorphic adenoma is the most prevalent one among benign neoplasms, followed by Warthin's tumour, basal cell adenoma, and canalicular adenoma (1). Mucoepidermoid carcinoma, adenoid cystic carcinoma, acinic cell carcinoma, secretory carcinoma, and carcinoma ex pleomorphic adenoma are the various types of malignant salivary gland tumours (1). Current molecular profiling has shown distinctive genetic changes that improve diagnostic precision, such as the MYB–NFIB fusion in adenoid cystic carcinoma and the CRTC1–MAML2 fusion in mucoepidermoid carcinoma (2).

About 60–70% of all benign salivary gland tumours are pleomorphic adenomas (PA), also known as benign mixed tumours. PA is the supreme dominant neoplasm of the salivary glands (3). A biphasic cellular structure, including both epithelial and myoepithelial cells organized within an adjustable stromal background that may look myxoid, chondroid, or fibrous, is histologically revealing of pleomorphic adenoma (4). Warthin's tumor (papillary cystadenoma lymphomatosum) is typically seen in older male patients, the second most frequent benign lesion is frequently bilateral in the parotid gland (5).

The pathophysiology of pleomorphic adenoma has been well silent thanks to fresh molecular and genetic investigation. Growth-regulatory transcription issues are overexpressed as a result of regular chromosomal rearrangements involving PLAG1 (8q12) and HMGA2 (12q14–15), which are now recognized as significant oncogenic drivers (2). These molecular changes are extremely exact and serve as diagnostic signs that differentiate pleomorphic adenoma after additional morphologically like salivary gland tumors (6).

An underlying pleomorphic adenoma (PA) gives rise to a malignant epithelial tumor known as carcinoma ex pleomorphic adenoma (Ca ex PA), indicating a shift from benign to malignant neoplasia in the salivary glands. (7). The WHO 5th Edition (2022) classification of head and neck tumors recognizes Ca ex PA as a distinct entity, emphasizing the essential for both histologic and molecular confirmation. (8). Molecular lessons have recognized several genetic alterations fundamental malignant transformation. The benign component often harbors PLAG1 or HMGA2 rearrangements, while the malignant areas acquire additional mutations such as TP53, HRAS, and PIK3CA, contributing to oncogenic progression. (9).

Malignant tumors are less common but biologically different and clinically significant. The most common is mucoepidermoid carcinoma, composed of mucous, epidermoid, and intermediate cells, often associated with a CRTC1–MAML2 gene fusion (2). Adenoid cystic carcinoma is characterized by perineural invasion and a potential for distant metastasis, and is associated with MYB–NFIB translocation (2). Other malignant tumors include acinic cell carcinoma, polymorphous adenocarcinoma, secretory carcinoma (ETV6–NTRK3 fusion-positive), and carcinoma ex pleomorphic adenoma, which arises from malignant transformation of a pre-existing benign pleomorphic adenoma (10).

The diagnosis of these tumours have been completely transformed by molecular techniques, which makes it possible to better associate with genotype and morphology. Tumour classification, prognostication, and individualised treatment approaches have improved with the integration of histopathology with immunohistochemistry and genetic testing. (11, 12). The application of next-generation sequencing has

facilitated the identification of these driver mutations, allowing more accurate diagnosis and prognostication (13). Adjuvant radiotherapy is advised for high-grade or advanced malignancies, while surgical excision is the chief treatment option for the salivary gland tumours. Given the risk of distant metastasis, perineural invasion, and local recurrence, particularly in adenoid cystic carcinoma, long-term monitoring is important (14).

Material and Methods

This Comparative analytical Study was conducted for a period of six months from June 2025 to November 2025 at Institute of Pathology and diagnostic Medicine KMU, Shaukat Khanum Memorial Cancer Hospital Lahore and Lab Genetics Lahore. The study was approved by the ethical committee of Department of Pathology and Diagnostic Medicine, Khyber Medical University, Peshawar. Non-probability convenience sampling technique was used in this research study. All patients included were diagnosed with Pleomorphic Adenoma and Carcinoma-ex-Pleomorphic Adenoma of salivary gland. All salivary gland tumors except Pleomorphic Adenoma and Carcinoma-ex-Pleomorphic Adenoma or infection of salivary glands, sialolithiasis and diseases of salivary glands were excluded from the study. Before including any patient in the current study, their informed consent was obtained. This study analyzed 4 cases of salivary gland tumors (2 benign pleomorphic adenomas and 2 malignant carcinoma ex-pleomorphic adenoma, CA-ex-PA) using clinical data and next-generation sequencing (NGS) to investigate the molecular drivers of benign and malignant transformation. Bioinformatics analysis were done on a Linux compute cluster.

Results:

Table: 3.1: Demographic and Clinical Presentation of the Samples

Case	Diagnosis	Age (years)	Sex	Location
1	Pleomorphic Adenoma	57	F	Parotid
2	Pleomorphic Adenoma	45	M	Parotid
3	Carcinoma ex-pleomorphic adenoma	48	M	Parotid
4	Carcinoma ex-pleomorphic adenoma	60	F	Parotid

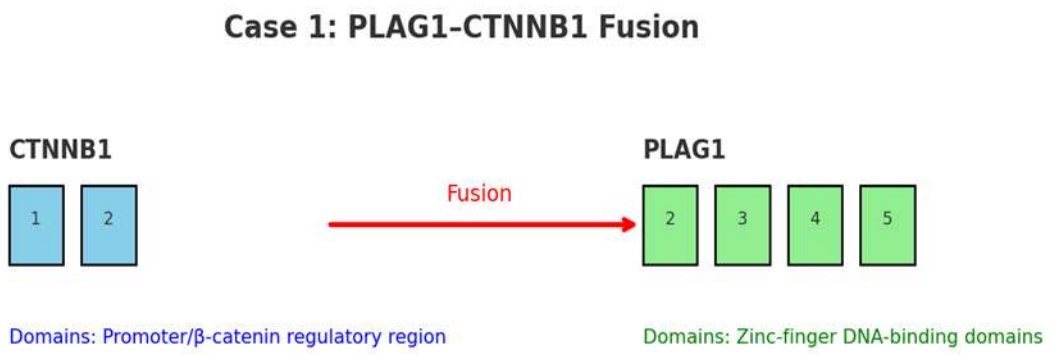
Table 3.1 demonstrate four patients with salivary gland tumors, all arising in the parotid gland. Two patients were diagnosed with pleomorphic adenoma (a 57-year-old female and a 45-year-old male), while the remaining two cases represented carcinoma ex-pleomorphic adenoma (a 48-year-old male and a 60-year-old female). This distribution reflects involvement across middle to older adulthood with an equal gender representation between benign and malignant categories

Table 3.2: Among the four Patients Molecular Comparison of Pleomorphic Adenoma and Carcinoma Ex Pleomorphic Adenoma of Salivary Glands.

Case	Diagnosis	Key Variants Detected	Variant Type	Interpretation
1	Pleomorphic adenoma	PLAG1–CTNNB1 fusion	Gene fusion	Oncogenic driver PLAG1 activation via CTNNB1 promoter.
2	Pleomorphic adenoma	PLAG1–LIFR fusion	Gene fusion	Pathogenic constitutive PLAG1 activation.
3	Carcinoma ex-pleomorphic adenoma	PLAG1 rearrangement + HMG2 gain	Fusion + CNV	PLAG1 activation plus HMG2 amplification; malignant shift.
4	Carcinoma ex-pleomorphic adenoma	PLAG1–TCEA1 fusion + HMG2 gain	Fusion + CNV	PLAG1 fusion with HMG2 overexpression; aggressive tumor.

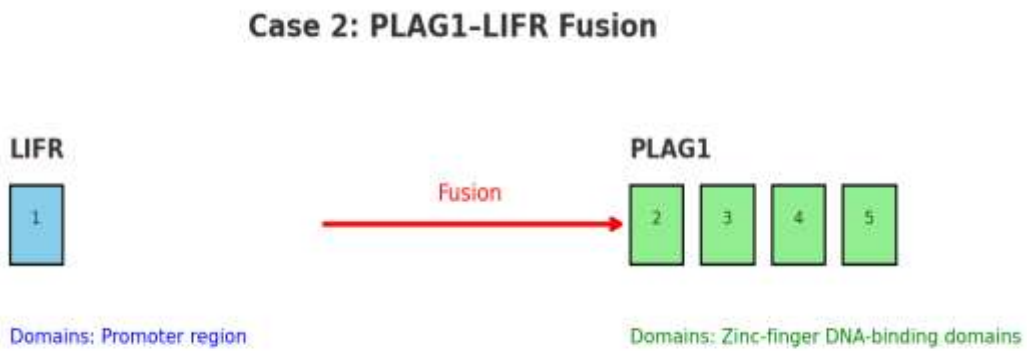
Table 3.2 demonstrate recurrent PLAG1 activation was identified by genetic profiling of four salivary gland tumors as a key oncogenic event in both benign and malignant lesions. PLAG1–CTNNB1 and PLAG1–LIFR fusions were present in two pleomorphic adenomas, each of which caused constitutive PLAG1 overexpression. The two carcinoma ex-pleomorphic adenoma cases, on the other hand, showed more genomic complexity, such as PLAG1 rearrangements or PLAG1–TCEA1 fusion events combined with HMG2 copy-number increases. These modifications imply that secondary alterations like HMG2 amplification contribute to malignant transformation and tumor aggressiveness, whereas PLAG1-driven fusions start carcinogenesis.

Figure 3.1.PLAG1–CTNNB1 fusion



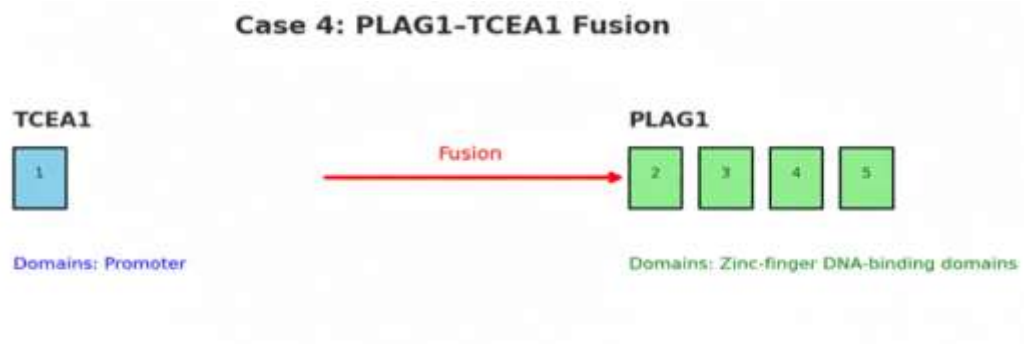
"This schematic shows the CTNNB1–PLAG1 fusion, where CTNNB1 exon 1–2 (promoter region) is fused upstream of PLAG1 exon 2. This promoter swapping places PLAG1 under the control of the strong CTNNB1 promoter, leading to overexpression of intact PLAG1, including its zinc-finger transcription factor domains. The resulting PLAG1 upregulation drives oncogenic activity in pleomorphic adenoma.

Figure 3.2 PLAG1–LIFR fusion



"The figure 3.2 illustrates the LIFR–PLAG1 fusion, where LIFR exon 1 (promoter region) joins PLAG1 exon 2 at the breakpoint (red arrow). This promoter substitution leads to constitutive PLAG1 overexpression, while its zinc-finger transcription factor domains remain intact. The fusion is a recognized oncogenic driver in pleomorphic adenoma."

Figure 3.3 shows case 3 and 4 PLAG1–TCEA1 fusion



In case (3) of carcinoma ex-pleomorphic adenoma shows a PLAG1 rearrangement combined with HMGA2 gain. The tumor exhibits a PLAG1 fusion with copy-number variation (CNV), leading to PLAG1 activation plus HMGA2 amplification, which likely contributes to the malignant transformation.

In case (4) carcinoma ex-pleomorphic adenoma demonstrates a PLAG1–TCEA1 fusion along with HMGA2 gain. The presence of a PLAG1 fusion with CNV results in PLAG1 overexpression together with HMGA2 upregulation, correlating with a more aggressive tumor phenotype.

Discussion:

Pleomorphic adenoma (PA) is the most common benign tumor of salivary glands and has historically been respect as a tumor with indolent clinical behavior, yet its biological significance lies in its potential to undergo malignant transformation into carcinoma ex-pleomorphic adenoma (CXPA), a rare but aggressive malignancy with poor prognosis. (15). The transition from PA to CXPA represents one of the best natural models of multistep tumorigenesis in head and neck oncology, and recently, molecular studies have begun

to unravel the precise genetic alterations that underlie both the initiation of PA and its malignant progression (16).

The initiating molecular events in PA are now well established to involve chromosomal translocations and rearrangements that result in deregulated expression of proto-oncogenes (17). The two most common genes implicated are PLAG1 (pleomorphic adenoma gene 1, located at 8q12) and HMGA2 (high mobility group AT-hook 2, located at 12q14-15). These rearrangements typically occur via promoter swapping, in which regulatory elements of highly expressed partner genes such as CTNNB1, LIFR, or HMGA2 (18).

Examination employing fluorescence in situ hybridization (FISH), immunohistochemistry, and RNA-based sequencing consistently detect PLAG1 or HMGA2 rearrangements in a large proportion of PAs, and the same alterations are retained in the benign remnants of CXPA, supporting a linear progression model. While PLAG1 rearrangements are slightly more than prevalent overall, HMGA2 rearrangements appear to carry distinct biological implications. 87. Molecular investigation of salivary gland tumors has important diagnostic utility, particularly in challenging cases where morphology alone is insufficient. Manifestation of PLAG1 or HMGA2 rearrangements can confirm PA lineage in ambiguous tumors, especially in small biopsies or tumors with unusual morphology. (15). Surgery remains the cornerstone of treatment, integration of molecular data is increasingly critical in expanding therapeutic options for patients with advanced or recurrent CXPA (19).

Conclusion:

It is concluded that pleomorphic adenoma and carcinoma ex-pleomorphic adenoma exemplify the multistep model of salivary gland oncogenesis in which initiating events, typically PLAG1 or HMGA2 rearrangements establish a benign neoplastic clone that persists over time. Malignant transformation is driven by accumulation of additional oncogenic alterations, including TP53 mutations, and widespread copy number alteration. Recognition of differences between PLAG1- and HMGA2-driven tumors further refines our understanding of tumor biology and patient risk stratification. Although current evidence is limited by rarity, small cohort size, and methodological variability, ongoing advances in molecular pathology and genomics hold promise for improved patient care through more accurate diagnosis, better prognostic models, and targeted therapeutic strategies. Thus, the molecular compression of PA and CXPA not only provides insight into fundamental oncogenic processes but also directly informs clinical practice in the management of salivary gland tumors.

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