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The Importance of Immunohistochemical Examinations in Salivary Tumors

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Abstract: Immunohistochemical studies play a crucial role in diagnosing and developing treatment strategies for salivary gland tumors. This method allows for the determination of the morphological and molecular characteristics of salivary gland tumors, as well as the assessment of their biological behavior and prognosis. Immunohistochemical tests are used to differentiate types of salivary gland tumors and establish a differential diagnosis. The significance of immunohistochemical studies lies in their ability to provide essential information for clinical practice, including accurate diagnosis, treatment planning, and prognosis evaluation. These methods also enable a deeper understanding of the molecular mechanisms of salivary gland tumors and facilitate the development of targeted therapies.

Objective of the study: To identify immunohistochemical changes in patients with salivary gland tumors.

Conclusion: Immunohistochemical studies are a necessary and effective tool in the diagnosis and treatment of salivary gland tumors. They ensure accuracy and reliability in clinical practice, making the study of this topic an urgent scientific task.

Keywords: salivary glands, tumor, immunohistochemistry, cytokeratin, diagnosis.

Introduction. The parotid gland is one of the largest and most complex alveolar glands in the human body, weighing about 25–30 grams. According to the type of secretion, this gland is classified as a serous gland, as it produces saliva rich in proteins. Anatomically, the parotid gland is divided into superficial (external) and deep (internal) parts. Although the gland is surrounded by a capsule, due to its low density, some areas may be covered by soft tissues. Many pathologies occur in the salivary gland, especially inflammation of the salivary gland, the formation of stones, defects, and neoplasia. Salivary gland neoplasms account for approximately 1–2% of all human tumors. They include precancerous lesions, primary benign and malignant neoplasms. The disease occurs mainly in people aged 40–60 years, with an almost equal incidence in men and women. The most commonly affected glands are the parotid and submandibular glands, with tumors of the sublingual and minor salivary glands being relatively rare. Low-grade salivary gland tumors metastasize to the lymph nodes and can spread through the blood to the bones, lungs, liver, and brain. Modern diagnostic methods are used to detect salivary gland tumors. The most popular methods include: radiographic examinations (CT and MRI with and without contrast, scintigraphic studies), ultrasound diagnostics (ultrasonography), various types of biopsy (incisional, excisional, precision, puncture), as well as immunological, histological and immunohistochemical analyses [4,7].

Each of these methods is used to detect a variety of human neoplasms, including salivary gland tumors. Salivary gland tumors account for 1 to 5% of all human neoplasms [6]. Among the most common benign salivary gland tumors, pleomorphic adenoma stands out, accounting for 61% to 90% of cases [5]. It is also possible to identify tumors and make a differential diagnosis using histological and immunohistochemical analyses. The study presents analyzes of immunohistochemical examination methods in the diagnosis of salivary gland tumors. Analysis of immunohistochemical markers in salivary gland tumors. Immunohistochemical analyses play an important role in salivary gland tumors. In pleomorphic adenomas, markers such as PLAG1, HMGA2, S-100, CK7, p40, p63, and SOX10 are widely used. PLAG1 is detected in 100% of pleomorphic adenomas and is an important marker for

benign tumors. Immunohistochemical analysis of pleomorphic adenomas: Immunohistochemical analysis of etiological and diagnostic criteria for pleomorphic adenomas of the salivary gland revealed the presence of five markers: HPV type 16, EBV, estrogen, progesterone, and PLAG1/2. Among pleomorphic adenomas, the highest immunohistochemical expression was observed for PLAG1 (100%) and HPV type 16 (80%) [1]. HMGA2 and PLAG1 in pleomorphic adenoma: PLAG1 and HMGA2 mutations were detected in 60% of salivary gland pleomorphic adenomas. HMGA2 showed positive expression in 48% of cases, indicating its widespread distribution in these tumors. HRAS Q61R mutation in epithelial-myoepithelial carcinoma (EMC): The HRAS Q61R mutation is specific for EMC and has been detected in 65% of cases by immunohistochemistry. HMGA2 is positive in 60% of cases and is an indicator of tumor recurrence. This method has shown high sensitivity and specificity in the diagnosis of EMC, especially in myoepithelial cells, making it an effective tool for the detection of this tumor [14]. Similar results in this study showed that the PLAGL2 gene plays an important role in the resistance of breast cancer cells to adriamycin (ADR). Deletion of PLAGL2 increased the sensitivity of cells to ADR and reduced their proliferation, migration, and invasiveness. PLAGL2 binds to the Wnt6 promoter region and activates its expression, which contributes to ADR resistance. Activation of the Wnt signaling pathway by BML-284 partially restores sensitivity to ADR in resistant cells. Thus, PLAGL2 has been shown to increase the aggressiveness and resistance of breast cancer cells to ADR by activating the Wnt pathway [16]. A diagnosis of mucoepidermoid carcinoma (MEC) arising from a pleomorphic adenoma was made, which is an extremely rare condition. The tumor had been located at the junction of the hard and soft palate for 12 months. Cytology and incisional biopsy were inconclusive and showed a benign tumor. After resection with a 5 mm clear margin, histology revealed a glioma with a high-grade malignant melanoma and 13 mm extracapsular invasion. The tumor cells were positive for cytokeratin and S100. Due to the high-grade malignancy and positive posterior margin after surgery, a course of radiotherapy was prescribed. This situation emphasizes the need for wide resection to account for rare tumors and prevent recurrence [10]. Pleomorphic adenoma may recur or transform into a malignant form (CXPA). The results of the study of Toll-like receptor (TLR) expression in pleomorphic adenoma, recurrent pleomorphic adenoma, and CXPA indicate that the expression of TLR-5 and TLR-9 is significantly higher in CXPA than in good-quality pleomorphic adenoma. In this study, TLR expression was analyzed in 25 pleomorphic adenomas, 34 recurrent pleomorphic adenomas, and 15 CXPA samples. Viruses such as herpes, papillomavirus, and parvovirus did not significantly affect TLR expression. TLR expression reflects tumor characteristics but is not associated with viral infection [11]. The Ki-67 index is used to assess cell proliferation activity, which helps to detect malignant changes early, even when morphological signs of mitosis are not yet clearly visible. Therefore, this index is important in differentiating benign and malignant tumors. In the study of salivary gland tumors, if Ki-67 is higher than 10%, this may indicate that the tumor has a high proliferative potential. According to data, recurrence of benign pleomorphic adenomas is detected in 50% of cases in the first two years, and in 80% in five years.

In such cases, additional investigations, such as re-aspiration biopsy or urgent cytological analysis during surgery, are recommended [2]. In addition, markers such as GFAP, PLAG1, SOX10, S-100, CK7, p40, p63, HMGA2 (+) are used to confirm the diagnosis [21]. Glial fibrillary acidic protein (GFAP) expression was assessed in 99 salivary gland tumors, including 54 pleomorphic adenomas. GFAP was detected in 94% of PAs and was mainly observed in myoepithelial cells. Strong and diffuse GFAP expression was also noted in some malignant tumors, such as epithelial-myoepithelial carcinomas (EMCCs) and myoepithelial carcinomas. GFAP is expressed in 100% of pleomorphic adenoma and basal cell adenoma biopsies. Rare expression of GFAP is characteristic of low-grade tumors that mimic pleomorphic adenoma. Strong and diffuse GFAP expression may indicate a benign tumor, especially pleomorphic adenoma [8]. In salivary gland tumors, p63 has been shown to promote differentiation in biphasic tumors. Recent studies have shown that the p63 gene plays an important role in the transformation of stem cells into squamous cell differentiation. Experimental models have shown that the expression of this gene during embryogenesis is necessary for the normal formation of the epidermis and the development of various structures such as teeth, hair, salivary glands, mammary

glands, prostate, sweat and tear glands. During the process of stem cell differentiation, undifferentiated stem cells transform into tissue-specific basal stem cells, forming normal epithelium [9].

In the adult, p63 plays a key role in maintaining the stem cell population in stratified and squamous epithelia [15]. Active expression of p63 is observed in basal, squamous, buccal and alveolar cells, and this gene is also expressed in terminal bronchioles. In addition, p63 is also present in myoepithelial cells located in the submucosal layer of bronchial glands [11,15,20]. Studies investigating the role of p63 in pathological processes have shown that the transcription factor p63 is present in both normal and pathologically altered epithelial cells. Scientists suggest that the presence of this protein in healthy tissues may increase their susceptibility to malignant transformation. For example, in a study of patients with lung adenocarcinoma, cytoplasmic expression of p63 in tumor cells was associated with poor prognosis, and significant differences in survival were noted between groups of patients with and without cytoplasmic expression of p63 [13]. In another study, p63 and RASSF1A genes were evaluated as independent factors influencing the risk of tumor recurrence after surgery in stage 1–2 lung cancer with intact lymph nodes [19]. In a study of 84 pleomorphic adenomas of the salivary gland, rare cases of nuclear atypia were identified. In one case (a 26-year-old woman), eosinophilic cytoplasmic cells and large, irregularly shaped nuclei with condensed chromatin were observed, but there were no signs of mitosis. These cells did not cover the tracks and expressed the following specific markers: Cytokeratins 5/6, 7, 14, Actin, p63, S-100, WT1. The nuclear vacuoles were positive for cytokeratin and WT1, indicating the presence of cytoplasmic inclusions. Degenerative nuclear atypia in pleomorphic adenoma is not associated with malignant transformation and resembles similar changes observed in pleomorphic leiomyoma and schwannoma [11]. Immunohistochemical analysis revealed expression of cytokeratin and epithelial membrane antigen. S-100 protein was negative. Salivary gland tumors are a very heterogeneous group in terms of their histological structure. For the first time in 1986, Zarbo and co-authors found S100 expression in several salivary gland tumors. Later, in 1988, Domagala and co-authors detected keratin and vimentin expression. In 2004, Da Cruz Perez DE, Pires FR, and co-authors performed immunohistochemical analysis of histological preparations from 53 children treated at the Hospital do Cancer A.C. Camargo between 1953 and 1997 [3,17,18]. The studies evaluated the expression of p53, PCNA, Ki-67, c-erbB-2, bcl-2, and CEA. According to the results of the study, no correlation was found between the prognosis of the disease and the presence of these proteins. On the other hand, the co-authors studied the expression of Ki-67 in 30 patients with acinar cell carcinoma and obtained the following results. Thirteen patients with Ki-67 values above 5% developed disease recurrence, and three of them (with values of 56.2%, 16.6%, and 7.8%) died due to disease progression. However, 17 patients with Ki-67 values below 5% have been living without disease symptoms for 30 years [23]. The retinoblastoma family includes the nuclear phosphoproteins pRb/p105, as well as its analogs p107 and pRb2/p130, which are tumor suppressor proteins. Recent immunohistochemical studies of lung, endometrial, and choroidal melanomas have shown that there is an inverse relationship between histological grade and pRb2/p130 expression in the most aggressive tumors. These results prompted us to further investigate the role of pRb2/p130 in salivary gland tumors. In this study, we examined the expression of pRb2/p130, p107, E2F4, p27, and PCNA in a panel of salivary gland tumors by immunohistochemistry, and found a direct and statistically significant correlation between pRb2/p130 expression and tumor grade and the presence of metastases ($P < 0.001$).

Furthermore, increased cytoplasmic expression of pRb2/p130 was found to be significantly associated with decreased survival ($P < 0.001$). Interestingly, nuclear expression of p107 also showed a strong direct correlation with similar variables. When pRb2/p130 was compared with histological grade and PCNA expression in salivary gland malignancies, nuclear expression was found to be the most inversely correlated ($P < 0.0001$). E2F4 also showed a similar localization pattern to pRb2/p130. These data suggest that pRb2/p130 may play an important role in the pathogenesis and progression of some types of salivary gland cancers [21]. In the mammary gland and salivary gland, TRPS1 and GATA3 are expressed in adenoid cystic carcinoma and basaloid tumors. TRPS1 was highly expressed in basaloid triple-negative breast cancer (TNBC) and adenoid cystic carcinoma with solid components. GATA3

was negative in these tumors, while TRPS1 was positively expressed in 100% of mixed and solid adenoid cystic carcinoma cases.

These data suggest that these markers may be differentially expressed in mammary and salivary gland tumors [22]. The study assessed the importance of central pathology research in the diagnosis of adenoid-cystic carcinoma of the salivary glands. These data suggest that these markers may be differentially expressed in mammary and salivary gland tumors. The study assessed the importance of central pathology research in the diagnosis of adenoid-cystic carcinoma of the salivary glands. Retrospective reassessment resulted in a change in diagnosis in 9.6% of cases, including 2.7% of cases being reclassified as benign tumors (basal cell adenoma and pleomorphic adenoma) and 6.8% as malignant tumors (cribriform variant of basal cell adenoma and adenocarcinoma). This case shows the importance of distinguishing adenoid-cystic carcinoma from rare tumors in the diagnosis of salivary gland tumors [8].

Conclusions. Analysis of immunohistochemical markers is important for accurate diagnosis and effective treatment of salivary gland tumors. Immunohistochemical markers such as PLAG1, HMGA2, S-100, CK7, p40, p63, and SOX10 are important in the diagnosis of salivary gland tumors. PLAG1 is a reliable marker, being detected in 100% of pleomorphic adenomas, while HMGA2 is positive in 48–60% of cases. The HRAS Q61R mutation is found in 65% of cases in EMC, while in MEK, cytokeratin and S-100 expression are high, and radiotherapy is recommended as the main treatment method. Increased expression of TLR-5 and TLR-9 in CXPA indicates a predisposition to malignancy, while viral infections do not affect this process. A Ki-67 index of more than 10% indicates high tumor proliferation. Decreased expression of pRb2/p130 is associated with tumor aggressiveness and reduced survival, and E2F4 shows a similar localization. TRPS1 is positive in 100% of cases in TNBC and adenoid cystic carcinoma, while GATA3 is negative.

Modern diagnostic and targeted therapy methods play an important role in preventing tumor recurrence and malignancy and improving the quality of life of patients.

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