IMMUNOHISTOCHEMICAL ASPECTS IN PLEOMORPHIC ADENOMA, RELATED TO ITS HISTOGENESIS AND MALIGNIZATION

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ABSTRACT
Known and described since the nineteenth century as mixed tumour of salivary gland, and subsequently called pleomorphic adenoma, this tumour is a benign lesion with a complex structure, composed of epithelial type areas mixed with fibrous, mucoid or condroid stromal areas. The lesion raised many discussions related to its histogenesis and its possibility of malignancy, talks which currently continue. In order to argue for some of the theories, immunohistochemical reactions with anti-cytokeratin, anti-vimentin, anti-S100 protein, anti-p53 protein, anti-Ki67 protein, and anti-CD34 monoclonal antibodies were used. It was noted that all the cellular components of the lesion respond positively to cytokeratin and vimentin, more intensely in myoepithelial cells and periductular cells. All the proliferation markers were positive in all the cells, regardless of the morphological type and the intensity was increased in cases of malignancy. Thus, our research supports the hypothesis of the unique epithelial histogenesis and the possibility of malignization of pleomorphic adenoma, which differs from the primitive salivary gland carcinomas.
KEYWORDS: pleomorphic adenoma, histogenesis, malignization, immunohistochemistry.

INTRODUCTION
Pleomorphic adenoma (PA), originally called mixed tumour of the salivary glands, is the most common benign tumour (53%) of the major salivary glands, especially of the parotid gland.

Known and described since the classical histopathology period [1], as mentioned Bill Roth and Virchow 1850, the histogenesis of this injury and its evolution to malignant forms, are currently the subject of controversy and recent researches.

In order to bring some data on the histogenesis and malignization of the pleomorphic adenoma, we performed an immunohistochemical study on cases with clearly benign characters and also with malignant looking areas (MPA).

MATERIAL AND METHODS
From a retrospective histopathological study realized in OMF Surgery Clinic, Iasi, for a period of 10 years, which is the subject of other research, we selected 30 cases of PA and 7 cases of MPA, for an immune-histochemical study.

The following antibodies were used:
- anti-cytokeratin AE1/AE3 antibody
- anti-vimentin antibody
- anti S-100 protein antibody

- anti-p53 protein antibody
- anti-Ki-67 antibody
- anti-CD34 antibody.

We used the avidin-biotin-peroxidase technique, modified by Bussolati and Gugliotta.

In the evaluation and interpretation of results were used fragments of normal salivary gland.

For objectification of the immuno-histochemical reactions, we performed the assessment of percentage positivity, by counting 300 cells on different microscopic fields, selected randomly.

RESULTS

In all the cases of PA, the usual histological techniques showed a wide variety of aspects ranging from case to case and in the structure of the same case. The capsule was present, with variable thickness, without discontinuities and it clearly defined the areas of PA by those with microscopic aspects of normal salivary gland. Histopathologically, we observed areas of compact epithelial squamous-cells proliferation, with keratotic globules and stromal cells proliferation, in a muco-hyaline abundant matrix (Fig. 1); we also observed condroid areas, with rare cells such as chondrocytes, disposed in an Alcian + matrix.

In most cases, there were areas of epidermoid cells with obvious dyskaryotic and anaplastic changes (Fig. 2). We took into consideration changes in the connective tissue capsule, particularly through its infiltration of anaplastic cells.

Analysing immune-histochemical aspects, it was observed that cytokeratin is positive for both groups of epithelial cells and also in stellate or fusiform stromal cells of the PA cases (Fig. 3).

In the cases of MPA, epithelial cells appeared dispersed, polyhedral, strongly positive for cytokeratin in a myxoid stroma with stellate and fusiform cells which were weakly positive for this antibody (Fig. 4). But we observed also areas of stromal fusiform cells with cytokeratin positive reaction.

The reactions for vimentin were positive in all cellular types of the pleomorphic adenoma (Fig. 5).

The MPA cases showed an intensely positive reaction in stromal fusiform cells (Fig. 6) and also in epithelial cells diffusely dispersed in a homogeneous matrix.

In the PA cases, the fragments showed a positive reaction for the anti S-100 protein antibody in stromal areas, compared with areas of epithelial type (Fig. 7).

In the fragments collected from areas with malignancy, the nuclear reaction was enhanced in epithelial areas (Fig. 8).

The proliferation marker Ki67 presented a positive, intensely reaction, especially in the nuclei of the myxoid areas (Fig. 9).

In the MPA fragments, the Ki67 marker showed intense positive reaction in all cells (Fig. 10).

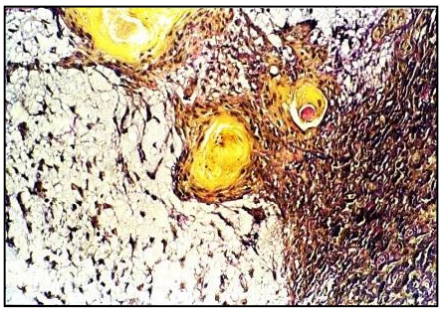

Fig. 1. Pleomorphic adenoma: epidermoid area with keratotic globules and myxoid stromal zone, van Gieson stain, magnification 400x

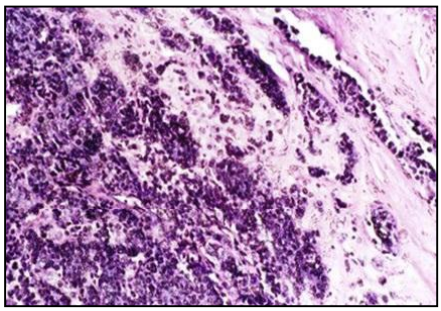

Fig. 2. Malignant pleomorphic adenoma: infiltrative aspect of stroma and capsule, H.E. stain, magnification 200x

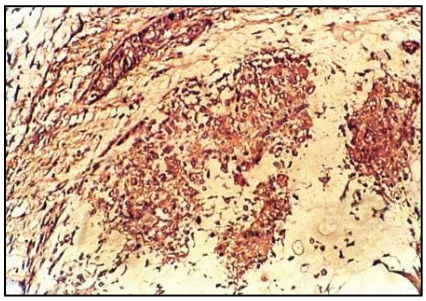

Fig. 3. Pleomorphic adenoma: Positive IHC expression for Cytokeratin in the epidermoid areas and myxoid stroma cells, magnification 200x

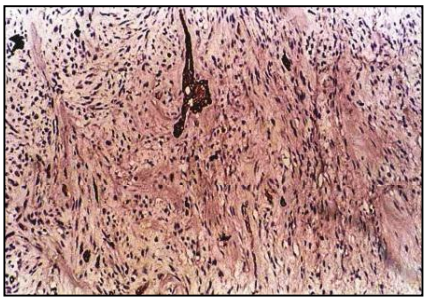

Fig. 4. Malignant pleomorphic adenoma: Intense positive IHC expression for Cytokeratin in the grouped epithelial cells and stromal fusiform cells, magnification 200x

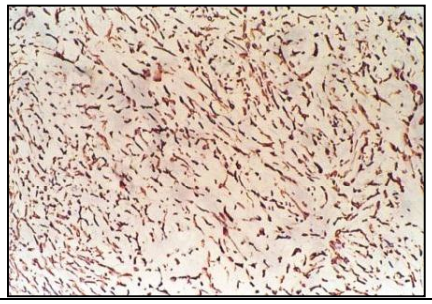

Fig. 5. Pleomorphic adenoma: Stromal positive IHC expression for Vimentin, magnification 100x

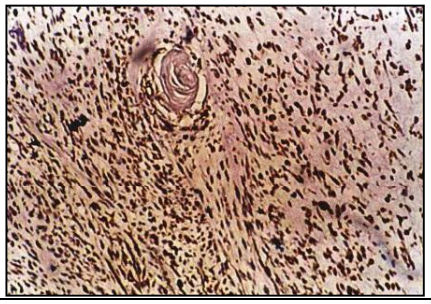

Fig. 6. Malignant pleomorphic adenoma: Intense positive IHC expression for Vimentin in the stromal cells, magnification 200x

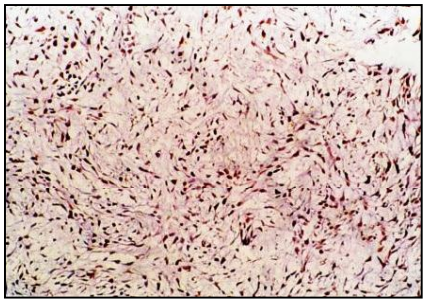

Fig. 7. Pleomorphic adenoma: Positive IHC expression for S-100 protein in the stromal cells, magnification 200x

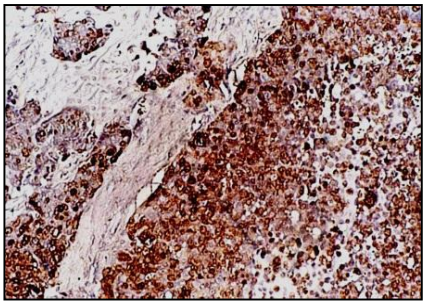

Fig. 8. Malignant pleomorphic adenoma: Intense positive IHC expression for S-100 protein in the groups of epithelial cells, magnification 200x

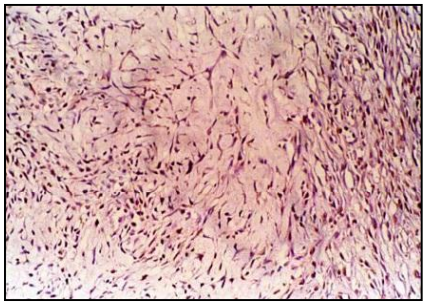

Fig. 9. Pleomorphic adenoma: Intense positive IHC expression for ki-67 in the nuclei of stromal cells, magnification 200x

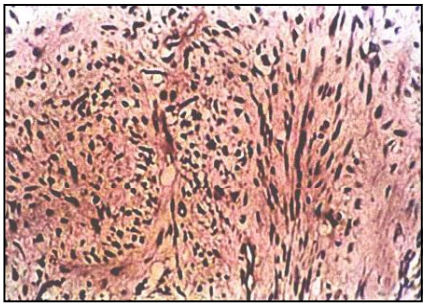

Fig. 10. Malignant pleomorphic adenoma: Intense positive IHC expression for ki-67 in the nuclei of stromal cells, magnification 400x

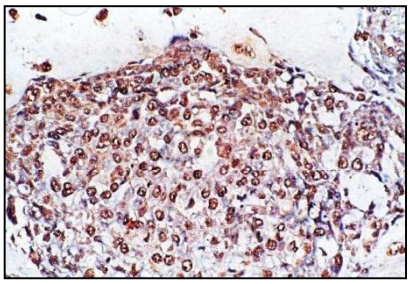

Fig. 11. Pleomorphic adenoma:Intense positive IHC expression for p-53 protein in the nuclei of epithelial cells and some stromal cells, 400x

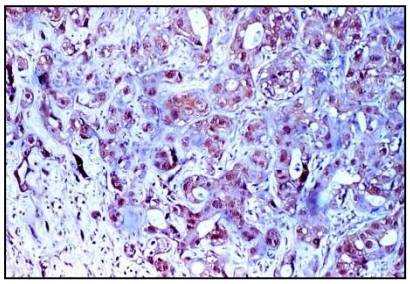

Fig. 12. Malignant pleomorphic adenoma: Ac anti-protein p53. Intense positive reaction in the nuclei of stromal cells. magnification 400x

P53 protein was positive in the nuclei of both epithelial areas and stromal areas (Fig. 11).

The reaction intensity was more increased in the cases of MPA (Fig. 12).

The CD34 marker revealed no significant differences between the PA and MPA.

DISCUSSIONS

The analysis of the aspects obtained by the performed immunohistochemical reactions, allowed some observations related to the PA histogenesis and its malignancy (described in the English literature as carcinoma ex pleomorphic adenoma) [2].

PA is considered a benign tumour, although metastases have been described in the literature [3]. The histogenesis of PA was and is widely debated in the literature [4, 5].

According to some recent data from the literature, the reactions performed in our research showed the presence of cytokeratin, a specific intermediate filament of the epithelium and vimentin, a specific intermediate filament of mesenchymal cells. In the normal salivary glands, both types are found in myoepitelial cells and periductular cells.

It is also noted that mesenchyme of cephalic extremity originates in the neural crest, unlike the mesenchyme of the rest of the body.

This different origin could explain the greater plasticity of the cellular elements and the histological heterogeneity who led to the initial name of pleomorphic adenoma as a mixed tumour.

In order to a better defining of the myoepitelial cells involved in PA and MPA, contractile filaments (actin, myosin) were studied, in parallel with intermediate filaments. It was observed the lack of cytokeratin in normal myoepitelial cells, but its presence along with vimentin in the neoplastic cells [6, 7].

It was demonstrated otherwise the regenerative role and the capacity of multiplication of periductular cells (intermediate channel). The proliferative activity of PA and MPA was explored using antibodies that mark the S100 proteins, p53 and Ki67 (markers of cell proliferation).

S100 protein was described first in nervous tissue and considered a marker of glial cells; then it was described in melanocytes and later in myoepitelial cells [8].

In our research, S100 protein was positive in myoepitelial cells of the PA and in the epithelial cells of MPA. The results are consistent with some literature data, which consider the S100 protein as a marker for diagnosis of initial stages of malignization [9, 10].

Ki67 marker showed a positive reaction especially in myxoid areas and in the myoepitelial cells present in the periphery of epidermoid areas. Monoclonal antibody Ki67 recognizes a nuclear antigen in cellular cycle phases, except G0 phase; this nuclear marker is correlated with mitotic activity. In our cases, the intensity of reactions was higher in

the MPA, results according with literature data [11, 12].

In all our cases, we can consider that Ki67 marker is useful in assessing the intensity of proliferation in the PA and gives indications on the risk of malignancy.

The p53 marker demonstrated a nuclear positivity in both epithelial areas and in the stroma of the PA. This marker showed also a more intense reaction in myoepitelial cells grouped or scattered into stromal tissue.

The literature indicates that p53 protein accumulation rate varies with the tumour type; that shows nuclear positivity and that the percentage of positive cells can vary from a few to all cells present in the lesion.

We found in our study a variation between different cases and even within the same case, which argues for different rates of proliferation.

Under physiological conditions, p53 gene is responsible for the integrity of DNA during cell division. In conditions of impaired DNA, p53 blocks the replication, promoting repair activity of the genome. It is considered that some alterations in the activity of p53 are involved in the development of PA and its evolution to malignancy [13, 14, 15].

The CD34 marker did not provide significant elements in relation to histogenesis and malignancy of pleomorphic adenoma.

CONCLUSION

- Immunohistochemical aspects plead for the epithelial origin of PA, mostly due to myoepitelial and periductular cells.
- PA should be considered as a purely benign epithelial tumour.
- PA malignancy is a reality.
- The proliferation markers P53, Ki67 and S100 protein, particularly in combination, are useful in the diagnosis and assessing the risk of malignancy.

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