THE IMMUNOHISTOCHEMICAL PROFILE OF LUMINAL EPITHELIAL NEOPLASTIC COMPONENT FROM PLEOMORPHIC ADENOMAS OF SALIVARY GLANDS

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Summary. The immunohistochemistry study made on 30 cases of salivary glands pleomorphic adenomas aims to establish the antigen profile of luminal epithelial neoplastic component proliferates into these tumors. We ascertain that luminal epithelial neoplastic cell were almost exclusively positive to antibody peculiar to epithelial differentiation (AE1/AE3, MNF 116, CK 8, CK 19, CK20, EMA) and occasional to S-100 protein and negative to vimentin, α-smooth muscle actin, calponin, Glial Fibrillary Acidic Protein (GFAP). This kind of immunoreactivity was similar to ductal cells from striated channels of salivary glands, suggesting the origin of such pleomorphic adenomas from this place.

Key words: immunochemistry, epithelial component, pleomorphic adenoma, salivary gland.

INTRODUCTION

The salivary gland pleomorphic adenoma is a benign epithelial neoplasm, histologically characterized by a great diversity of morphological aspects. Its structural pleomorphism is given both by the epithelial component, as a result of the cytological differentiations and the growing patterns, and by the stromal component because of its rich morphological and quantitative diversity [1, 2]. Two distinctive cellular populations have represented the basic cellular components of this kind of tumor: a luminal one (represented especially by the ductal luminal cell) and an abluminal one (represented by the myoepithelial cell and the myoepithelial-like cells derived from it).

The luminal cell from normal salivary glands has the following antigen profile:

– the acinar one is: intense positive to cytokeratins with low molecular weight, weak positive for cytokeratins with high molecular weight, intense positive to Amylase, weak positive for Lactoferrin, Lysosym, Carcinoembryonic Antigen

(CEA) and Secretory Component (SC), negative to Epithelial Membrane Antigen (EMA), Vimentin, Actin, Myosin, S-100, Alkaline Phosphatase (AP) and ATP-ase;

– the luminal cell of intercalated ducts is: intense positive to cytokeratins with high molecular weight, negative to cytokeratins with low molecular weight, intense positive to EMA, Lactoferrin, Lysosym, weak positive for CEA and SC, and negative for Amylase, Vimentin, Actin, Myosin, S-100, Alkaline Phosphatase (AP) and ATP-ase;

– the luminal cell of striated ducts is: intense positive to cytokeratins with high molecular weight, negative to cytokeratins with low molecular weight, moderate positive for S-100, weak positive to SC and negative for Lactoferrin, Lysosym, CEA, EMA, Amylase, Vimentin, Actin, Myosin, Alkaline Phosphatase (AP) and ATP-ase;

– the luminal cell of excretory ducts is: intense positive to cytokeratins with high molecular weight, negative to cytokeratins with low molecular weight, moderate positive for EMA, weak positive to SC and negative to Lactoferrin, Lysosym, CEA, Amylase, Vimentin, Actin, Myosin, S-100, Alkaline Phosphatase (AP) and ATP-ase [3, 4, 5, 6, 7, 8, 9].

The literature data designate for the luminal neoplastic cell from pleomorphic adenoma the following antigen profile: positive to cytokeratins (constantly for ECK, PKK1, AE1-AE3, MNF-116, CK 19, CK 18, versatile to CK 10, CK 11, CK 13, CK 14 and CK 16), EMA, CEA, Lysosym, Lactoferrin, Gross Cystic Disease Fluid Protein-15 (GCDFP-15), α1-Antitrypsin, α1-Antichymotrypsin, Prostate Acid Phosphatase (PAP), Prostate-Specific Antigen (PSA), cartilage-derived retinoic acid-sensitive protein (CD-RAP), versatile for S-100, and negative to Vimentin, Myosin, Desmin, Fibronectin, Calponin, Caldesmon and GFAP [1, 2, 8, 10–18].

MATERIAL AND METHODS

There were selected thirty cases of pleomorphic adenomas of salivary glands. The surgical specimens were provided by the Oral Maxilla Facial Surgery Department of the Clinical County Hospital from Craiova, mostly from 15–65 year-old females (20 cases), with prevalent parotid localization (18 cases). The parotidectomy pieces were routinely fixed with 10 per cent buffered formalin and sent to the Laboratory of Cytology and Pathology of the same hospital. They were processed by the classical histopathological technique with paraffin embedding and stained with Haematoxylin-Eosin (H-E), Trichromic Masson and Periodic Acid Schiff-Blue Alcian (PAS-AA). The immunohistochemical processing was made in Laboratory of histological, histopathological and immunohistochemical techniques from U.M.F. Timişoara and in the Laboratory of histological, histopathological and immunohistochemical techniques from U.M.F. of Craiova.

The paraffin blocks acquired by histopathological processing were sectioned at microtome resulting sections of 5 μ in thickness mounted on microscopic slides cover with polylysin. Subsequently, the sections were deparaffinized (three successive baths of benzene, 1 hour at thermostat at 58°C for first bath and 10 minutes at room temperature for the remainder baths) and rehydrated (three successive alcohol baths with decrease concentrations 96%, 80% and 70%, 10 minutes per each bath, followed by a bath with distillated water were the section where hold for 10 minutes). As working methods we utilized DAKO the LSAB® 2 System, HRP (Universal DAKO Labelled Streptavidin-Biotin® 2 System, Horseradish Peroxidase) technique and rade to use antibodies from DAKO (indicated in Table 1, with their main characteristics).

Table 1
The list of antibodies used in immunohistochemical investigation of pleomorphic adenomas

Antibodies (RTU)	Clone	Class / subclass	Code
Monoclonal Mouse Anti-Human Cytokeratin AE1/AE3	AE1 şi AE3	AE1: IgG_1, kappa; AE3: IgG_1, kappa	N1590
Monoclonal Mouse Anti-Human Cytokeratin	MNF 116	IgG_1, kappa	N1523
Monoclonal Mouse Anti-Human Cytokeratin 8	35βH11	IgM, kappa;	N1560
Monoclonal Mouse Anti-Human Cytokeratin 19	RCK108	IgG_1, kappa	M0888
Monoclonal Mouse Anti-Human Cytokeratin 20	K_s20.8	IG_{2a}, kappa	N1627
Monoclonal Mouse Anti-Human Epithelial Membrane Antigen (EMA)	E 29	IG_{2a}, kappa	N1504
Monoclonal Mouse Anti-Human Vimentin (VIM)	V9	IgG_1, kappa	N1521
Polyclonal rabbit Anti-S-100			N1573
Mouse Anti-Human Alpha-smooth Muscle Actin (α-SMP)	1A4	IG_{2a}, kappa	N1584
Monoclonal Mouse Anti-Human Calponin	CALP	IgG_1, kappa	M 3556
Polyclonal rabbit Anti-Glial Fibrillary Acidic Protein (GFAP)			N1506

For calponin and CK 19 we used the ABC/HRP (Avidin complexed with peroxidase biotinylated) technique. For calponin we used 1:50 dilution, pre-enzymatic digestion with trypsin 1% (in distillated water) for 5 minutes, washing about 5 minutes in distillated water and antigen unmask at microwave in 10 mM citrate buffer for 40 minutes. For CK 19 we used 1:50 dilution and heat treatment in 10 mM citrate buffer at microwave for 15 minutes. We mention that for each utilized antibody we made both positive external tissue control and negative external tissue control by using of the same DAKO LSAB® 2 System, HRP technique. The tumors were diagnosed according to WHO classification [19].

The results of the immunohistochemical examination of the thirty pleomorphic adenomas were compared with those obtained from the residual normal glandular parenchyma immunohistochemical observation (the fragments that were placed at a suitable distance away from the tumoral tissue in 15 from the thirty investigated tumors). The external tissue control for the performed immunohistochemical reactions was attained by using fragments of sublingual salivary parenchyma, removed during necropsy of three patients older than 50 years. The number of immunoreactive cells from each investigated case was semi quantitative evaluated as follow: +++ >75% positive cells, ++ 75–25% positive cells, + <25% positive cells, – negative cells.

For the appreciation of the intensity of immunostaining reaction we use the interpretation criteria from literature data [20], consigned like below:

(+++), when immunostaining reaction is intense positive or with peculiar "all over", obvious at small magnification;

(++), when immunostaining reaction is focal or with moderate intensity, evident on average magnification;

(+), when immunostaining reaction is weak or very focal, visible only at strong magnification;

(±), when immunostaining reaction is very reduce, at limit;

(–), when immunostaining reaction is negative.

Were excluding from interpretation the microscopic fields with folds, necrosis and hemorrhages.

RESULTS

EPIDEMIOLOGICAL ASPECTS

There were selected thirty pleomorphic adenomas cases from fifty-six salivary gland tumors, diagnosed during 1997–2002. 15–65 year-old patients provided the immunohistochemical specimens. The pick incidence occurred in the fifth decade of life (10 cases, representing about 33.33% of our casuistry). The patients were mostly females (20 cases, representing 66.66%), with a male to female ratio of 2 to 1. The tumors developed especially in the major salivary glands (18 cases in the parotid, 7 cases in the submandibular glands and 2 cases in the sublingual gland, all representing about 90% of our casuistry). The parotid was the most frequently involved gland (60%). The minor salivary glands were involved only in three cases: two the level of the lower lip and one at the palatal level (representing about 10% of our casuistry).

THE RESIDUAL FRAGMENTS OF THE NORMAL SALIVARY GLAND PARENCHYMA

On the remainder major salivary gland parenchyma we notice the presence of all excretory system components: intralobular (intercalated ducts of Boll and

striated ducts of Pfluger) şi extralobular ducts. The intercalated ducts lumina were outline by a single layer of flatten epithelium, positive to cytokeratins (AE1-AE3, MNF 116, CK 19, CK 20, CK 8), EMA and negative to Vimentin, S-100, α-Smooth Muscle Actin, Calponin and GFAP. The epithelial lining of striated ducts was simple columnar and was positive to cytokeratins (AE1-AE3, MNF 116, CK 19, CK 20, CK 8), EMA, versatile to S-100 and negative to Vimentin, α-Smooth Muscle Actin, Calponin and GFAP. The extralobular ducts were lined by a bilayered cuboidal epithelium, positive to cytokeratins (AE1-AE3, MNF 116, CK 19, CK 20, CK 8), EMA and negative to Vimentin, S-100, α-Smooth Muscle Actin, Calponin and GFAP (Figure 1, a-f).

The myoepithelial cells bounded the acini, the intercalated ducts and occasionally the striated ducts of the major salivary glands. These cells were constantly positive to AE1/AE3, MNF 116, α-Smooth Muscle Actin, Calponin, S-100, GFAP; versatile positive for Vimentin, and negative to CK8 and EMA.

The acinar cells were positive to AE1/AE3, weak positive for CK 20, negative to MNF 116, CK 8, EMA, vimentin, α-Smooth Muscle Actin, Calponin, S-100, GFAP. Moreover, we notice the positivity for α-Smooth Muscle Actin of the pericytes and smooth muscle cells from wall of blood vessels (internal positive control). Also, vimentin was positive in stromal fibroblasts (internal positive control) and vascular endothelial cells.

As well, we constantly observe S-100 positivity of the adipocytes present in interlobular stroma of the major salivary glands and for the nervous fibers. Furthermore the Schwann cells were positive to GFAP.

HISTOPATHOLOGICAL ASPECTS OF PLEOMORPHIC ADENOMA

According to Seifert's [23], classification, based on the relative proportion of the stromal and epithelial component, the thirty cases of pleomorphic adenomas were represented by: pleomorphic adenomas with stromal predominance (18 cases), pleomorphic adenomas with well-balanced stromal / parenchyma proportion (8 cases) and pleomorphic adenomas with parenchyma predominance (4 cases). In the cases of pleomorphic adenomas with stromal predominance the epithelial component was reduced (about 20% of the tumoral mass) and was represented by neoplastic epithelial proliferations with trabecular, tubular and insular patterns. These small cellular groups were often disposed at the periphery of the myoid stromal component or inside it. The predominant stromal component was mainly of myxoid type. The pleomorphic adenomas with well balanced stromal / parenchyma proportion presented tubular and solid-insular neoplastic proliferations as dominant growing pattern. The stromal component had mainly myxoid, chondro-myxoid and chondroid differentiations, more often associated.

In the structure of the pleomorphic adenomas with parenchyma predominance the most encountered patterns were the tubular and the insular ones. The stromal component was reduced (no more than 20–30% of the tumoral mass) and mostly of

myxoid type. Histopathologically, we have found in the structure of these tumors a lot of cellular differentiation, such as: luminal cells, abluminal cells, "mixoid" cells, "myoid" cells and chondroid cells.

IMMUNOHISTOCHEMICAL ASPECTS OF PLEOMORPHIC ADENOMA

The quantitative and qualitative investigation of immunostaining for AE1/AE3 of the 30 typical pleomorphic adenomas is reproducing on Table 2 from below.

Table 2
Quantitative and qualitative results of AE1/AE3 investigation of the typical pleomorphic adenomas

Antibodies type	**The intensity of immunostaining**					
Cytokeratin	*Luminal epithelial cell*				*Abluminal epithelial cell*	*Stromal cell*
AE1/AE3	+++	++	+	±	++→ –	–
No. of cases	4	8	18	0	21	0

From the data presented in Table 2 results that maximum of intensity is acquire in the cytoplasm of luminal cells. Positive reaction for AE1/AE3 of these cells was present in all cases of pleomorphic adenomas because the tumoral proliferations with tubular pattern were identifying in all investigated typical pleomorphic adenomas. The immunostaining of abluminal cells varies from moderate to absence of immunomarker. The absence of immunomarker for abluminal cells present in seven cases is explains by presence of small epithelial tumoral component (under 10% from tumoral mass), which in the most part has a trabecular or cystic pattern. The stromal cells are negative to immunostaining for AE1/AE3.

The analysis of data from Table 2 shows that the maximum intensity for AE1/AE3 immunostaining was achieve in those four cases of pleomorphic adenoma with parenchymatous predominance, in which dominate the tubular proliferations with an intense cytoplasmic reaction (+++) at the level of ductal luminal cells (Figure 2, a and b). The abluminal cells present a moderate (++) reaction. The most intense reaction was observed especially in tumoral zones with squamous differentiation. In the solid zones, cells with histological features of myoepithelial cells had a positive reaction with variable intensity from moderate to the absence of cytoplasm reaction (Figure 2, c and d). On restricted stromal areas with myxoid character, the immunostaining was weak and isolated to same cells with stellate morphology. The fibrous and fibro-hyaline stromal zones were negative to AE1/AE3 immunostaining. In the eight cases of pleomorphic adenomas with well-balanced stromal/parenchyma ratio the immunostaining to AE1/AE3 on global was moderate (++). In the composition of these tumors are present in balanced proportion stromal and epithelial proliferations. The epithelial proliferate areas were representing by zones with tubular and insular patterns which had a

similar immunostaining reaction to those from pleomorphic adenomas with stromal predominance.

The stromal zones represent by myxoid and chondro-myxoid differentiations was that which impose the moderate immunostaining reaction to these groups of tumor; isolated cells from these myxoid areas had a weak immunostaining positively (+) to AE1/AE3 and the chondroid and chondromyxoid zones were negative. Intensity of immunostaining in 18 cases of pleomorphic adenomas with stromal predominance was weak (+), these tumors were predominating make from myxoid, chondroid and chondromyxoid areas. As such, I show proceeding the chondroid and chondromyxoid are negative to AE1/AE3 and myxoid zones had weak and focal immunoreactions (+). The epithelial proliferate zones were reduce and generally had a trabecular and insular pattern in which the immunostaining was reduce. The epithelial areas with tubular pattern being rare, but intense positive, on ensembles, participate very few at qualitative appreciation of immunostaining to this tumoral type.

The results of immunostaining investigation for MNF 116 of the 30 typical pleomorphic adenomas are reproducing on Table 3 from below. As could we can observe from the data presented in Table 3, intensity of immunostaining to MNF 116 is maximum to the luminal cells level. Although the immunostaining of these cells is variable, it was present in all cases of pleomorphic adenomas. The abluminal cells present an immunostaining, which varies from weak to negative according to the degree of cellular differentiation. In 20 cases the immunostaining of abluminal cells was negative, the epithelial component in these cases being reduce. Tumoral stromal cells were negative to MNF 116 immunostaining.

Table 3

Quantitative and qualitative results of MNF 116 investigations of the typical pleomorphic adenomas

Antibodies type	**The intensity of immunostaining**						
Cytokeratin	*Luminal epithelial cell*					*Abluminal epithelial cell*	*Stromal cell*
MNF 116	+++	++	+	±	–	+→ –	–
No. of cases	2	5	15	5	3	10	0

The analysis of the data from the preceding table denotes the following:

– an intense positive reaction only in two cases and these were represented by pleomorphic adenomas with parenchymatous predominance in which the predominant proliferate pattern was tubular with an intense (+++) cytoplasmic immunostaining of the luminal cells (Figure 2d). The abluminal cells and the epithelial proliferate zones with solid pattern had a weak immunostaining that varies until to the absence of this reaction. The restricted myxoid stromal zones were also negative to these immunostaining.

– a moderate positive reaction (++) was present in only five cases, represented by two pleomorphic adenomas with parenchymatous predominance and three pleomorphic adenomas with balanced stroma / parenchymatous

component ratio. In all cases the moderate positive reaction to MNF 116 was given by the presence in reduced proportion of proliferate areas with tubular pattern.

– most often immunostaining to MNF 116 was very weak. In those eight cases of pleomorphic adenomas with balanced stromal / parenchymatous ratio and 15 pleomorphic adenomas with stromal predominance, detected on restricted areas epithelial proliferate zones with tubular pattern positive to MNF 116. In those 23 cases predominated epithelial proliferate zones with insular and trabecular pattern (whose immunostaining varies from weak to negative) beside myxoid, chondroid, chondromyxoid and fibro-hyaline stromal areas (negative to MNF 116).

– a very weak immunostaining at the limit (±) was noticed in three cases of pleomorphic adenoma with stromal predominance. In such tumors a stromal component predominates, which is negative for this immunoreactions and the epithelial component, which is reduced, had an insular and trabecular pattern and so was also negative to MNF 116. On limited areas were present neoplastic epithelial structures with tubular pattern that are positive to MNF 116 immunostaining.

– only in three cases, the immunostaining was negative. These was represented by pleomorphic adenoma with stromal predominance in which the neoplastic epithelial proliferations with tubular pattern (which are positive to MNF 116) were extreme rare. In such tumors predominate myxoid and chondromyxoid stromal areas which are negative at this immunoreactions; beside that appear reduce epithelial proliferate zones with predominate trabecular pattern and rare nest of epithelial cells (negative to immunostaining with MNF 116).

The results of immunostaining investigation for cytokeratins 19 and 20 (Figure 2f) of the 30 typical pleomorphic adenomas were overlapping quantitative and qualitative with those achieve in the MNF 116 investigation. The results of immunostaining investigation for cytokeratins 8 of the 30 typical pleomorphic adenomas are illustrating below (Table 4).

Table 4

Quantitative and qualitative results of CK 8 investigation of the typical pleomorphic adenomas

Antibodies type	**The intensity of immunostaining**					
Cytokeratin	*Luminal epithelial cell*				*Abluminal epithelial cell*	*Stromal cell*
CK 8	+++	++	+	±	+→ –	–
No. of cases	3	4	20	3	7	0

Immunoreactions to CK 8 of the luminal cells is present in all cases of pleomorphic adenoma, but the intensity of the immunostaining varies from intense to (±) from tumor to tumor. Immunostaining of the abluminal cells was obvious only in seven cases and the immunoreactions vary from weak to the absence of marker. In the great majority of the cases immunoreactions to Ck 8 of the abluminal cells was negative. The stromal cells were negative to such immunostaining.

The analysis of the data presented in the preceding table reveals the following aspects:

– a strong immunoreactions (+++) present at three from four cases of pleomorphic adenomas with epithelial predominance. In the composition of such tumors predominates the epithelial proliferate areas with tubular pattern in which the luminal cells presents a strong cytoplasmic reaction for CK 8 (Figure 3a). The abluminal cells presents a variable immunostaining and in general with focal character. The same type of immunoreactions was also present in the tumoral solid zones (Figure 3b). The stromal areas were negative;

– a moderate immunoreactions (++) was found in four cases, three being pleomorphic adenomas with balanced stromal / parenchymatous ratio and one cases with epithelial predominance. In such cases the proliferate epithelial zones with tubular pattern were rare, the predominant one being that with solid and trabecular pattern in which immunoreactions to CK 8 was weak and focal. The myxoid, chondroid and chondromyxoid stromal areas were negative to CK 8;

– a weak immunoreactions (+) was identified in five cases of pleomorphic adenoma with balanced stroma / parenchyma proportion and in 15 cases of pleomorphic adenoma with stromal predominance. In these tumors prevail stromal areas, which were negative to such immunostaining and the parenchymatous component presents an insular or trabecular predominant pattern in which the immunoreactions was focal and weak;

– immunoreactions to limit (±) were present in three cases of pleomorphic adenoma with stromal predominance. In this tumors predominate stroma with myxoid and chondromyxoid character (negative to such immunoreactions) and the parenchymatous component was represent in generally by epithelial structure with insular and trabecular pattern (zones with variable positive and focal immunoreactions).

The results of immunostaining investigation for EMA of the 30 typical pleomorphic adenomas are illustrating in Table 5, from below. Immunostaining of the luminal cells to EMA was generally intense positive in all the 30 typical pleomorphic adenomas immunohistochemical investigated. The abluminal cells present an immunoreaction with variable intensity from weak to negative from tumor to tumor. The stromal cells from all investigated cases do not express epitopes for this immunostaining.

Table 5

Quantitative and qualitative results of EMA investigation of the typical pleomorphic adenomas

Antibodies type	**The intensity of immunostaining**						
EMA	*Luminal epithelial cell*					*Abluminal epithelial cell*	*Stromal cell*
	+++	++	+	±	–	+ → –	–
No. of cases	2	5	15	5	3	5	0

The analysis of the data from Table 5 reveal the presence of an intense immunoreaction (+++) in only two cases of pleomorphic adenoma with

parenchymatous predominance. In these, we noticed the presence on broad areas of a predominant tubular proliferate pattern (Figure 3c). At the level of these structures the luminal cells present a intense immunoreaction to the apical cytoplasm, which linear outline the lumen of the neoplastic ductal proliferations; the abluminal cells was generally weak positive (intensity of the immunoreaction varies from the + → –) and with focal character (Figure 3d). The rest of the epithelial proliferations together with stromal areas indifferent of the differential type are negative to immunoreaction with EMA.

A moderate immunostaining (++) to EMA was encounter in five cases, two of them were pleomorphic adenomas with epithelial predominance and three were pleomorphic adenomas with balanced stroma / parenchyma proportion. In such tumors we noticed alongside epithelial proliferations with insular and trabecular pattern with negative immunoreaction (the maximum intensity was present in those two cases with epithelial predominance). At the tumoral stroma areas the immunostaining was negative. In five cases of pleomorphic adenoma with balanced stroma / parenchyma proportion and in 15 cases of pleomorphic adenoma with stromal predominance the immunoreaction was weak (+). In these tumors the tubular epithelial neoplastic proliferations were very few, the prevalent epithelial proliferates pattern are insular and trabecular.

In five cases of pleomorphic adenomas with stromal predominance the immunostaining was at limit (±). In these cases were identified rare epithelial tubular neoplastic proliferations widespread among tumoral stroma, which are negative to EMA. In only three cases the immunostaining was interpreted as negative (–). In these cases of pleomorphic adenomas with stromal predominance, the tubular epithelial proliferations are extremely rare and with small lumina. The rest of the epithelial proliferations had a predominant insular and trabecular pattern, so the immunoreaction in these cases can be considered as negative. The results of immunostaining investigation for vimentin of the 30 typical pleomorphic adenomas are illustrated in Table 6, from below.

Table 6

Quantitative and qualitative results of vimentin investigation of the typical pleomorphic adenomas

Antibodies type	**The intensity of immunostaining**					
Vimentin	*Stromal cell*				*Abluminal epithelial cell*	*Luminal epithelial cell*
	+++	++	+	±	+→ –	–
No. of cases	3	7	15	5	10	0

The analysis of the data from the previous table reveals a selective immunoreaction of the stromal cells with antibody anti-vimentin. Intensity of the immunostaining varies from very intense (generally characteristic to mesenchymal high-specialized cells, *e.g.*, chondrocyte) to weak positive for young mesenchymal cells from myxoid tumoral areas. The abluminal cells present an immunoreaction,

which varies from negative to weak positive from tumor to tumor. The luminal cells were negative to immunostaining with vimentin.

Analyse of the results from Table 6 reveal that only in three cases we founded an intense immunostaining (+++). These were represented by pleomorphic adenomas with stromal predominance, in which the most tumoral part was made from areas with chondroid and chondromyxoid zones (Figure 3e). Although the immunostaining of the cells from these tumoral zones was cytoplasmic moderate per global, comparative with the immunostaining results of the other cases it was considerate as intense positive. In the limited epithelial areas the immunoreaction was negative. In seven cases, the immunoreaction to vimentin was moderate. These were pleomorphic adenomas with stromal predominance in which stromal areas had chondroid, chondromyxoid and myxoid differentiations. At myxoid areas, the cells with stellate morphology have a moderate to weak cytoplasm immunoreaction. Moreover, a weak vimentin immunostaining was evident at the periphery of epithelial areas.

In the majority-investigated cases, immunoreaction to vimentin was very weak positive. These were represented by eight cases of pleomorphic adenomas with stromal predominance and seven cases of pleomorphic adenoma with well-balanced stroma / parenchyma proportion. In the last cases, the cells with stellate morphology from myxoid areas were weak positive and we noticed a rare positively of the abluminal cells from solid epithelial proliferate areas. In five cases, one represented by a pleomorphic adenoma with well-balanced proportion between stroma and parenchyma and all the other four cases by the pleomorphic adenoma with epithelial predominance the immunoreaction to vimentin, was at limit.

These tumors had in their composition numerous neoplastic epithelial structures that where generally negative to this immunostaining. The immunoreaction was net negative, especially in epithelial areas with tubular pattern. At the periphery of these zones, in the transition epithelial-mesenchymal areas and in limited isolated areas from solid epithelial proliferations we noticed a weak cytoplasmic immunoreaction.

In the only case with well-balanced stroma / parenchymatous ratio beside these weak positive areas, we marked out a preponderant fibro-myxoid stroma, which was negative to vimentin. The results of immunostaining investigation for S-100 protein of the 30 typical pleomorphic adenomas are illustrated below in Table 7.

Table 7

Quantitative and qualitative results of S-100 investigation of the typical pleomorphic adenomas

Antibodies type	**The intensity of immunostaining**					
S-100 protein	*Abluminal epithelial cell*				*Stromal cell*	*Luminal epithelial cell*
	+++	++	+	±	++→ –	±
No. of cases	6	15	6	3	25	2

The analysis of the data from Table 7 shows the absence of immunostaining to S-100 of the luminal cells and presence of an immunoreaction with variable intensity of the abluminal cells in all 30 investigated cases of typical pleomorphic adenomas. Immunostaining intensity of the abluminal cells varies from strong positive for the abluminal cells with myoepithelial morphology to moderate in the cases of pure epithelial cells and weak positive for the modified myoepithelial cells (which are engaged on transdifferentiation lines to chondrocyte) and even to a limit immunoreaction at the level of complete transdifferentiated cells to non-chondrocyte mesenchymal cells or to squamous, plasmacytoid, oncocyte, mucinous, clear, etc. metaplastic cells.

The young mesenchymal cells and the fibroblast-like cells are negative to S-100 immunostaining. This jigsaw puzzle of mesenchymal cells make that according to predominance of one from the others the intensity of this immunoreaction can varies from moderate positive to negative. In those five cases in which the immunostaining of the stromal cells was negative predominates the fibroblast cell type and young mesenchymal cells; these cases were represented by pleomorphic adenoma with epithelial predominance and respective in only one cases was about a pleomorphic adenoma with well-balanced stroma / parenchyma ratio, but in which predominates myxoid stromal areas rich in fibroblast cells and areas with fibrous and sclerohyalin stroma.

The analysis of the results indicates an intense (+++) immunoreaction in six cases, all these cases being represented by pleomorphic adenomas with stromal predominance. These cases were predominantly made from stromal areas with chondroid, chondromyxoid and on limited areas myxoid differentiations. Cells from these chondroid and chondromyxoid stromal areas present an intense both cytoplasm and nuclear S-100 positive immunoreaction. Cells from myxoid areas present a moderate immunostaining and generally with cytoplasm pattern. In the few epithelial areas the immunostaining was reduced to limit. The immunoassayed cells from these areas were especially the abluminal cells and those from the periphery of the solid neoplastic areas.

In three cases, all represented by pleomorphic adenoma with parenchymatous predominance, the immunoreaction was at limit and was own to positively of the cells from stromal myxoid areas, from the periphery of the epithelial-mesenchymal transition areas and from the solid or trabecular epithelial proliferating zones. Only in two cases we noticed a weak positive reaction in the cytoplasm of luminal cells from tubular neoplastic areas (Figure 3f).

In the majority of cases [15], represented by 12 cases of pleomorphic adenoma with stromal predominance and three cases with well-balanced stroma / parenchyma ratio, the immunostaining was moderate (++). In the composition of these tumors, stromal areas with chondromyxoid and myxoid differentiations were prevalent. The epithelial component had an important contribution by solid (Figure 4a) and trabecular areas, in which were present numerous myoepithelial

cells immunoassayed both cytoplasmic and nuclear. In six cases, the immunostaining to S-100 was weak positive. Five from these cases were represented by pleomorphic adenomas with well-balanced stroma / parenchyma ratio, at which the immunoreaction was reduced and were made in principal by mesenchymal cells from myxoid tumoral zones and very few by the cells from the periphery of the solid and trabecular epithelial areas. In only one case of pleomorphic adenoma with epithelial predominance, the positively to S-100 was reduce because small areas of myxoid and chondromyxoid stromal areas (that are weak positive) were present, also participate the cells from transition epithelial-mesenchymal zones (also weak positive) and cells with myoepithelial morphology from solid proliferate areas. The last one had a weak and focal cytoplasm immunoreaction. In three cases, all represented by pleomorphic adenoma with parenchymatous predominance, the immunoreaction was at limit and was own to positively of the cells from stromal myxoid areas, from the periphery of the epithelial-mesenchymal transition areas and from the solid or trabecular epithelial proliferating zones. The immunoreaction of the myoepithelial abluminal cells from the tubular zones was negative. The results of immunostaining investigation for α-Smooth Muscle Actin of the 30 typical pleomorphic adenomas are illustrated in Table 8, from below.

Table 8
Quantitative and qualitative results of α smooth muscle actin investigation of the typical pleomorphic adenomas

Antibodies type	**The intensity of immunostaining**						
A-smooth muscle actin	*Abluminal epithelial cell*					*Stromal cell*	*Luminal epithelial cell*
	+++	++	+	±	–	–	–
No. of cases	4	8	8	5	5	0	0

As such, we observe from the data presented in Table 8, the immunostaining to α-Smooth Muscle Actin was present in all investigated cases but only at the level of abluminal cells. The immunoreaction intensity of these cells varies from intense positive to limit (±) from tumor to tumor. The luminal epithelial cells and the stromal cells were negative to α-Smooth Muscle Actin. The analysis of the data presented in Table 8 indicates an intense immunoreaction to α-Smooth Muscle Actin in four cases, all represented by pleomorphic adenomas with epithelial predominance. At the level of these tumors predominate the proliferations with tubular pattern, in which the abluminal cells were intense (+++) cytoplasmic positive (Figure 4b).

Together with these tubular structures we also encounter compact-insular epithelial proliferations, in which cells with myoepithelial morphology were also intense cytoplasmic positive. In rare myxoid tumoral areas, scattered trough epithelial proliferations, the immunostaining was very weak (+) and with focal character, limited only to same cells with stellate morphology (Figure 4c).

In all eight cases of pleomorphic adenomas with well-balanced stroma / parenchyma ratio, the immunostaining to α-Smooth Muscle Actin was moderate (++). In the composition of these tumors we found few neoplastic epithelial structure and the stromal areas were more well represented than in the previous cases. These stromal areas were represented by myxoid zones with weak immunoreaction and by chondromyxoid and chondroid areas, which are negative to α-Smooth Muscle Actin immunoreaction.

In other eight cases represented by pleomorphic adenomas with stromal predominance, the α-Smooth Muscle Actin immunoreaction was very weak. In the composition of these tumors, the epithelial proliferations were very weak, represented by some tubular structures and clusters of cells with myoepithelial morphology. The great majority of the tumoral mass was occupied by tumoral stroma, which predominantly had a myxoid feature (in which we noticed the presence of a weak and focal immunoreaction of some cells with stellate morphology).

In five cases the immunoreaction was at limit (±). These results were noticed in pleomorphic adenoma with stromal predominance in which the epithelial areas were small and with insular pattern (weak positive to α-Smooth Muscle Actin), but the stromal component morphological takes more frequent chondromyxoid and chondroid features (negative to immunoreaction with α-Smooth Muscle Actin) and much rare pure myxoid aspect (weak positive and with focal character).

In five cases, the immunoreaction to α-Smooth Muscle Actin was negative. In these cases was about pleomorphic adenoma with stromal predominance in which rarely were observed epithelial proliferations; these one appearing like small cellular clusters weak positive at this immunoreaction. The stromal component was represented especially by chondroid, chondromyxoid and fibrohyalin areas, which are negative to α-Smooth Muscle Actin. The results of immunostaining investigation for calponin of the 30 typical pleomorphic adenomas were overlapping quantitative and qualitative with those achieved in the α-Smooth Muscle Actin investigation (Figure 4d). The GFAP reaction was present only in the cytoplasm of abluminal cells (Figure 4, e and f). The immunostaining intensity of these varies from moderate to at limit (±) from tumor to tumor. The luminal cells and the stromal one were negative to GFAP immunostaining.

DISCUSSIONS

The majority of the cellular neoplastic population of this kind of tumor is represented by luminal cells and abluminal cells, which proliferates in a great variety of patterns (tubular, insular, trabecular, fascicular, cystic, pseudo-angiomatous, cribriform, etc.), more often associated. The most frequent cytoarchitectonics model is the tubular one. This was present in all cases, but in

variable proportion from case to case. The neoplastic tubules are line to lumen by a single row of ductal epithelial cell and at exterior by a sheet with variable thickness of myoepithelial cells, which are disposed with the long axis perpendicular on tubule axis and scatters radial into the surrounding stroma. Immunohistochemically the luminal cells were positive to AE1/AE3, MNF 116, CK 8, CK 19, CK20, EMA, versatile for S-100 and negative to VIM, α-SMA, CALP, GFAP.

The analysis of the immunostaining to AE1/AE3 of the typical pleomorphic adenoma cells reveals the existence of an immunoreaction with variable intensity from tumor to tumor. The variability of the immunostaining of these cells from intense to limit it is explained by sensibility of the specific epitopes for AE1/AE3 antibody to formol fixation. Other factor that influenced the immunostaining sensitivity it is the degree of tubular cell differentiation (the intensity is maximum at complete mature cells). The variability of the abluminal cell immunostaining from moderate to the absence of the immunoreaction is explained by the tendency of myoepithelial cell to transdifferentiation to mesenchymal cell line.

The analysis of the immunostaining to MNF 116 of typical pleomorphic adenomas cells shows an intense positive reaction at luminal cell level. The variability of the luminal cell immunostaining is explained like in the case of AE1/AE3 immunostaining by sensitivity of MNF specific epitopes to formol tissue fixation and by the degree of cellular maturity. The variability of abluminal cells immunostaining is in accord with the degree of cellular differentiation. The absence of immunostaining in those 20 cases of abluminal cells is explained by the reduced contribution of epithelial component in such tumors and by the presence of a reduced number of mature myoepithelial cells positive to MNF 116.

This marker being specific to simple and squamous epithelia do not stains stromal cells. Different by AE1/AE3 reaction the immunostaining of epithelial cells to MNF 116 is less specific, fact that is explained by a confine of antigen spectrum, which can be revealed with such antibody against AE1/AE3 (5, 6, 8, 17, and 19 for MNF 116, and respectively 10, 13, 14, 15, 16 and 19 for AE1/AE3).

The analysis of CK 8 immunostaining of typical pleomorphic adenomas cells reveals presence of a reaction with maximum intensity to the level of luminal cells. The variability of luminal cells immunoreaction is own by the degree of cellular differentiation. CK8 is a specific cytoplasmic marker of the cells from simple epithelia; the tumoral abluminal cells were most negative to this immunostaining. The positive reaction of some abluminal cells from typical pleomorphic adenoma is explained by the possibility of these cells to metaplasia towards luminal cells. The CK 8 sensitivity of luminal cells is bigger than those to AE1/AE3 or MNF116.

We can conclude that the positivity of neoplastic luminal cells to cytokeratins markers is dependent to the degree of cellular differentiations, that means the degree of canalicular architectonics maintenance of the epithelial proliferations; the intensity of this immunoreaction decrease by the reduction of cellular differentiations and by the looseness of tubular disposition in detriment to insular

and trabecular patterns. According to data from literature, the luminal cells are positive to most antibody anti-cytokeratins, the cytoplasm immunostaining of these are more intense to CK of the simple epithelia (7, 8, 18, 19, and 20). The abluminal cells with myoepithelial morphology presents a weak immunostaining to these kind of cytokeratins, but more intense for CK of stratified epithelia (5 and 14) [21–24]. The most intense immunostaining of abluminal cells is present at CK 14 reaction, but this one can be partial, in some tumoral areas can be absent and in these areas, the immunostaining to CK 14 can be more intense to abluminal cells [14]. At solid tumoral areas in epithelial areas with solid pattern, the immunostaining is present especially to CK 14. In myxoid zones of pleomorphic adenomas, the CK 14 immunoreaction has a sporadic character, limited to some tumoral cells with stellate morphology [14].

In areas with squamous differentiation the tumoral cells are intensely positive to pancytokeratin (AE1/AE3, MNF 116 and CAM5.2) and inconstant to CK 14 [14, 22]. Zones with oncocytoid differentiation and those with clear cells are usually positive to pancytokeratins (AE1/AE3 and CAM5.2) [25], while tumoral zones with plasmacytoid differentiation are negative to CK 14, CK 19 [14].

The analysis of EMA immunostaining of the typical pleomorphic adenomas cells shows the presence of an intense immunoreaction especially at luminal cells in all investigated typical pleomorphic adenomas. The variability of immunoreaction intensity of luminal cells from strong to weak is explained by the degree of differentiation of these cells. Because it is a membrane immunomarker of the epithelial cells, the stromal cells from all cases of typical pleomorphic adenomas were negative to such immunoreaction. The review data shows that EMA immunoreaction evidences only the tubular neoplastic proliferations from pleomorphic adenomas, the rest of neoplastic proliferations being negative to such immunoreaction [22]. The myoepithelial cells and the modify myoepithelial cells from the proliferating areas from such kind of tumor are negative to EMA or CEA (carcinoembryonic antigen) [22, 23, 25].

The analysis of the vimentin immunostaining of the typical pleomorphic adenomas cells shows that the immunoreaction was nearly exclusive to stromal cells. The immunostaining variability of these cells is according to the degree of mesenchymal differentiations (chondrocytes had the most intense reaction and the youngest mesenchymal cells from myxoid areas of these tumors had a weak or at limit immunostaining).

This variability is own to different concentration of the vimentin intermediary filaments of various mesenchymal cell populations from the stroma of such tumors. The variable immunoreaction of the abluminal cells is own to the degree of transdifferentiation of myoepithelial cell to mesenchymal cellular type. The results are in correspondence with the literature data. Thus, is specified that the positive cells to such immunoreaction are the abluminal cells from ductal proliferating areas, the myoepithelial cells from the solid proliferating areas and the modified

myoepithelial cells and stromal mesenchymal cells [22–24]. The most intense immunostaining is present especially at stromal mesenchymal cells [18].

The analysis of S-100 immunoreaction of typical pleomorphic adenoma cells mark out the presence of a variable immunostaining at abluminal cells in all 30 investigated cases and the negative reaction of the luminal cells. The variability of abluminal cell immunostaining is explained by the degree of transdifferentiation of these cells to mesenchymal cells and by the degree of metaplastic change into squamous cells, plasmacytoid cells, oncocytoid cells, mucinous cells, clear cells etc. For the stromal immunostaining evaluation, we must keep count of the reactivity to this antibody of the chondroid cells derived from myoepithelial cells by transdifferentiation, of histiocytes, free unmyelinated nerve endings and adipocytes that can be present in the stroma of such tumors.

These results are in correspondence with the literature data. Thomas Aigner (1998) shows that myoepithelial cells from tubular and solid-insular proliferations areas and the so-called modified myoepithelial cells, having a moderate cytoplasmic S-100 positive reaction. The luminal cells are generally negative to S-100, but occasionally they can be focally positive [22]. The cells from transitional tubulo-myxoid zones have a cytoplasmic and nuclear positive immunostaining [18]. The stromal cells are intense cytoplasmic and nuclear positive to S-100 [24].

According to Dardick *et al.* (1991), the S-100 positivity of the myoepithelial cells from salivary gland tumors must be regarded with circumspection because these tumors have a rich network of free unmyelinated nerve endings scattered between epithelial neoplastic proliferations, which normally are S-100 positive. The α-Smooth Muscle Actin immunoreaction of the cells from typical pleomorphic adenomas set off a variable intensity of this reaction in all investigated cases, but only to the abluminal cells. The intensity of this immunoreaction depends on the degree of mesenchymal transdifferentiation and metaplastic transformation to other cellular lines (oncocytoid, plasmacytoid, clear cell, squamous cell etc.) of these cells. These results correspond with literature data. Thus, according to Brennan *et al.* (2000) the α-Smooth Muscle Actin in pleomorphic adenoma is expressed only by normal myoepithelial cells and by so-called modified myoepithelial cells. The weak positive immunoreaction of some cells from myxoid areas is explained by the possible origin of these cells from myofibroblasts [14].

Although, calponin was supposed until now to be the most specifically marker of myoepithelial proliferations, the maximum intensity being at the abluminal cells, at present it is proved that h1-calponin is expressed also by keratinocytes and nervous fibres [26, 27]. Moreover, they observe an h1-calponin immunoreaction of some luminal cells and at myxoid, chondroid and squamous and plasmacytoid tumoral areas. In their study, a greater specificity to myoepithelial immunodetaction seems to have h1-caldesmon [26]. In the literature data are quoted immunohistochemical studies which reveals the positivity of

luminal cells from pleomorphic adenomas to Carcinoembryonic Antigen (CEA), lysosym, alpha-1-antitrypsin, alpha-1-antichymotrypsin, lactoferrin, GCDFP-15 (Gross Cystic Disease Fluid Protein-15), interleukin-6 and steroid C-21 hydrolases [13, 28–34]. Usually the amylase is absent [23].

Van Krieken (1993) shows that salivary pleomorphic adenomas and some salivary carcinomas can react with prostate acidic phosphatase (PAP) and prostate-specific antigen (PSA). Therefore, neoplastic luminal cells represents an important cell population in pleomorphic adenomas, their morphological aspects and also the immunohistochemical profile remind those of ductal cells from normal salivary glands. The great majority of them take part of neoplastic proliferations with tubular pattern and much less to the solid-insular neoplastic areas. This tumoral pattern recalled the model of the normal intercalated ducts, suggesting the "abortive" tendency of the tumoral cells to achieve cytoarchitectonics structures similar those from the normal salivary gland parenchyma.

This supposition can be supported by ultrastructural arguments as Dardick and Erlandson proved [26, 35]. These authors reveal that seldom in to neoplastic proliferations can be notice tubular structures with intercalated duct morphology. However, the great majority of neoplastic proliferation with tubular pattern is made up from a single layer of cells with morphology of ductal epithelial cell and by a mantle of myoepithelial and myoepithelial-like cells with variable thickness. The cellular rows most closely to luminal layer have myoepithelial ultrastructural aspects. As well, in solid neoplastic areas, immunohistochemical and ultrastructural was notice a central group of cells with ductal epithelial morphology, surrounded by numerous myoepithelial cells.

Moreover, Batsakis issues a hypothesis according to which the salivary gland tumors and the salivary parenchyma regeneration recapitulate the embryonic stages of the salivary gland development [36, 37]. After an experiment on rats, Barka and Boshell achieved salivary gland hyperplasia, thus proving the regenerative potential of the acinar cells and implicitly of the myoepithelial cells [38, 39].

In an experiment made by Bockman regarding the pancreas carcinogenesis it was proved the existence of a gradual dedifferentiation process from the acinar units to tubular structures [40]. Moreover, the absence of the myoepithelial cells from the pancreas and their presence only in the salivary glands, the sweat glands and the breast, explained the pleomorphic adenoma development only in the latter structures and not in the pancreas. According to the latest investigations (using the double immunostaining procedure for Ki67 and α-actin or other subtypes of cytokeratins) the regenerative potential owns not only the myoepithelial cells but also the basal cells, intercalary ductal cells, acinar cells and oxyphil cells. The regeneration of the acinar and intercalary duct cells issues independently from those of myoepithelial cells or basal cells. The oxyphil regeneration and the majority of the epithelial metaplasia from the salivary glands derive from the basal cell of the striated ducts, which present a great capacity of pluripotential

morphogenetic differentiation [9]. Literature data shows that intercalated ductal cell is capable of oncocytoid, mucoid, squamous or sebaceous metaplasia [22].

According to Dardick *et al.*, the histogenetic model of these tumors is based on the myoepithelial cell capacity from the proliferating units of ductal-like type or solid-insular proliferating areas to progressive trans-differentiation, squamous metaplasia and detachment from these proliferating units as the extracellular matrix products accumulates. These processes take place simultaneously in different areas of the same tumor, fact that persuades the great histological variability of this tumor [41, 42].

Aigner *et al.* have proved that the pleomorphic adenomas represent the *in vivo* model epithelial-mesenchymal transdifferentiation process in adult [18]. In the pleomorphic adenomas case, the detection (by *in situ* hybridization technique) in the ductal cells of the genes involved in the aggregan core protein mRNA synthesis, has demonstrated the expression of the mesenchymal genes in the cells with epithelial origin [18].

Thus, the onset of the mesenchymal gene expression in the ductal cells leads to the secretion of an abundant extracellular matrix rich in glycosaminoglycan that generates the myxoid matrix formation. This fact suggests that the neoplastic ductal cells were involved in the genesis of the myxoid stromal areas from the pleomorphic adenomas [18].

More recently, Kusafuka *et al.* have reported that the genesis of the chondroid stromal areas is associated with the overexpression of the bone morphogenetic proteins (BMPs) by the neoplastic myoepithelial cells [43]. This protein belongs to the transforming growing factor (TGF)-β superfamily, which interferes with the regulation of the mesenchymal tissue formation. The TGF-β2 is expressed by the neoplastic ductal epithelial cells, while TGF-β3 is expressed by the neoplastic myoepithelial cells from the solid neoplastic epithelial proliferating areas, the metaplastic squamous cells and the luminal cells from the tubular neoplastic proliferating areas [43]. TGF-β is involved in the control of the luminal and myoepithelial neoplastic cell differentiation [48].

Moreover, Devlin and Sloan have showed that in only the ductal cells and not in the neoplastic myoepithelial cells there was expressed the cartilage-derived retinoic acid-sensitive protein (CD-RAP), a recently described immunomarker useful for cartilaginous cell detection involved in the morphogenesis and the development of the salivary glands [11].

Ogawa *et al.* demonstrated that the plasmacytoid cells from pleomorphic adenomas originate from luminal cells and not from myoepithelial cell (intense positive to vimentin, negative to α-Smooth Muscle Actin, versatile positive to calponin, CK 19, 18 and 14) [26]. Moreover, they showed that same of the abluminal cells could express cytokeratins 19, 18 and 8 (which are specifically to luminal differentiation), fact that impose the re-evaluation of the theory according to the fact that the non-luminal cells are modified myoepithelial cells (26).

CONCLUSIONS

The neoplastic luminal cell represents a major cell neoplastic population in pleomorphic adenomas. These cells have a key role in the genesis of these tumors, being capable of dedifferentiation, metaplasia and possible of transdifferentiation concurring thus to achievement of structural pleomorphism of this tumor. His immunohistochemical profile is an epithelial one; similar to ductal cells of striated ducts from normal salivary glands, suggesting that the pleomorphic adenomas origin at this level.

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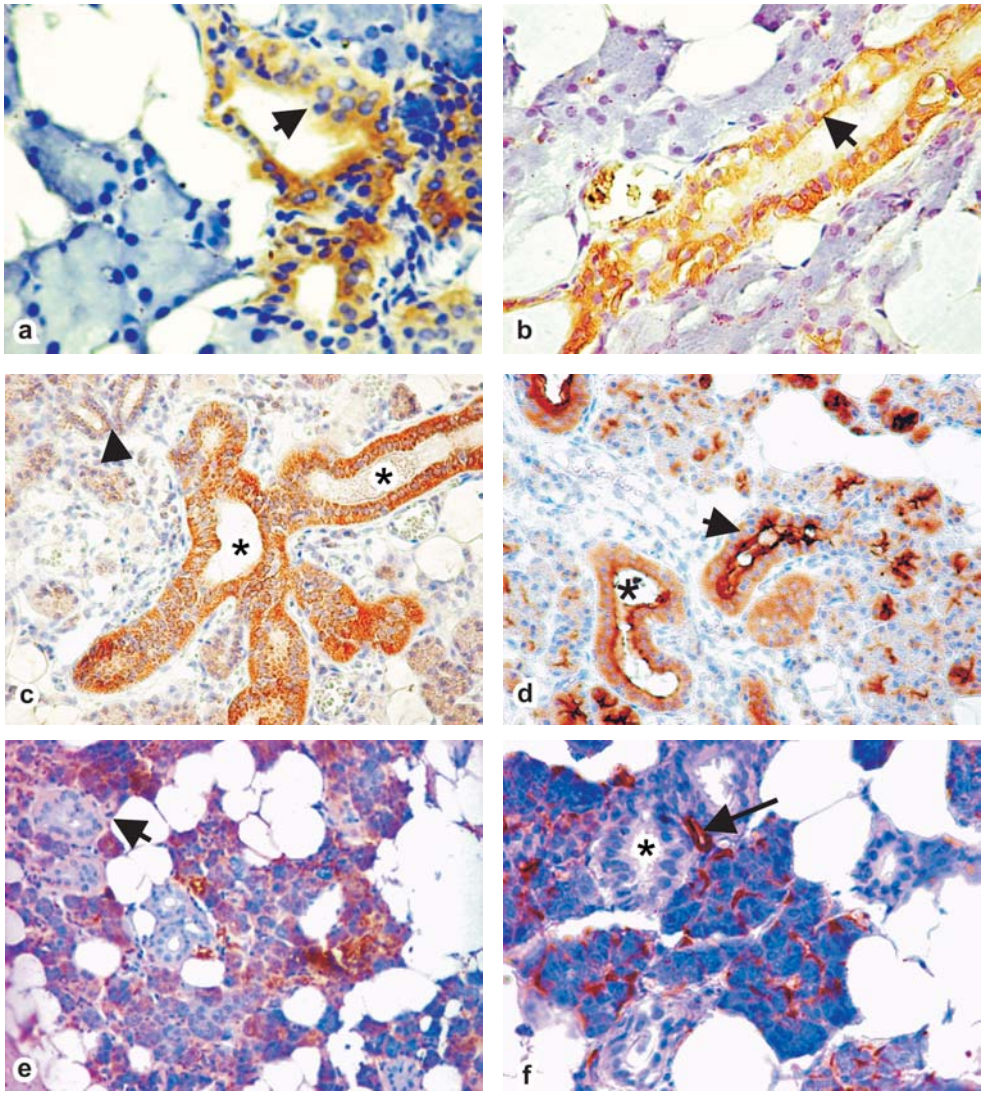

Figure 1 – Immunohistochemical aspects of the remainder parotid parenchyma: a) AE1-AE3 positive reaction at the level of intercalated ducts (asterisk) and extralobular ducts (arrrow), ×200; b) MNF positive reaction of extralobular duct (arrow), ×100; c) CK20 positive reaction in intralobular (arrows) and extralobular ducts (asterisk), ×100; d) EMA positive reaction at luminal slope of intralobular (arrows) and extralobular ducts (asterisk), ×100; e) S-100 positive reaction at the level of myoepithelial cells that borders intercalated ducts (arrow), ×200; f) SMA positive reaction in the cytoplasm of myoepithelial cells that borders intercalated ducts (arrows), ×200

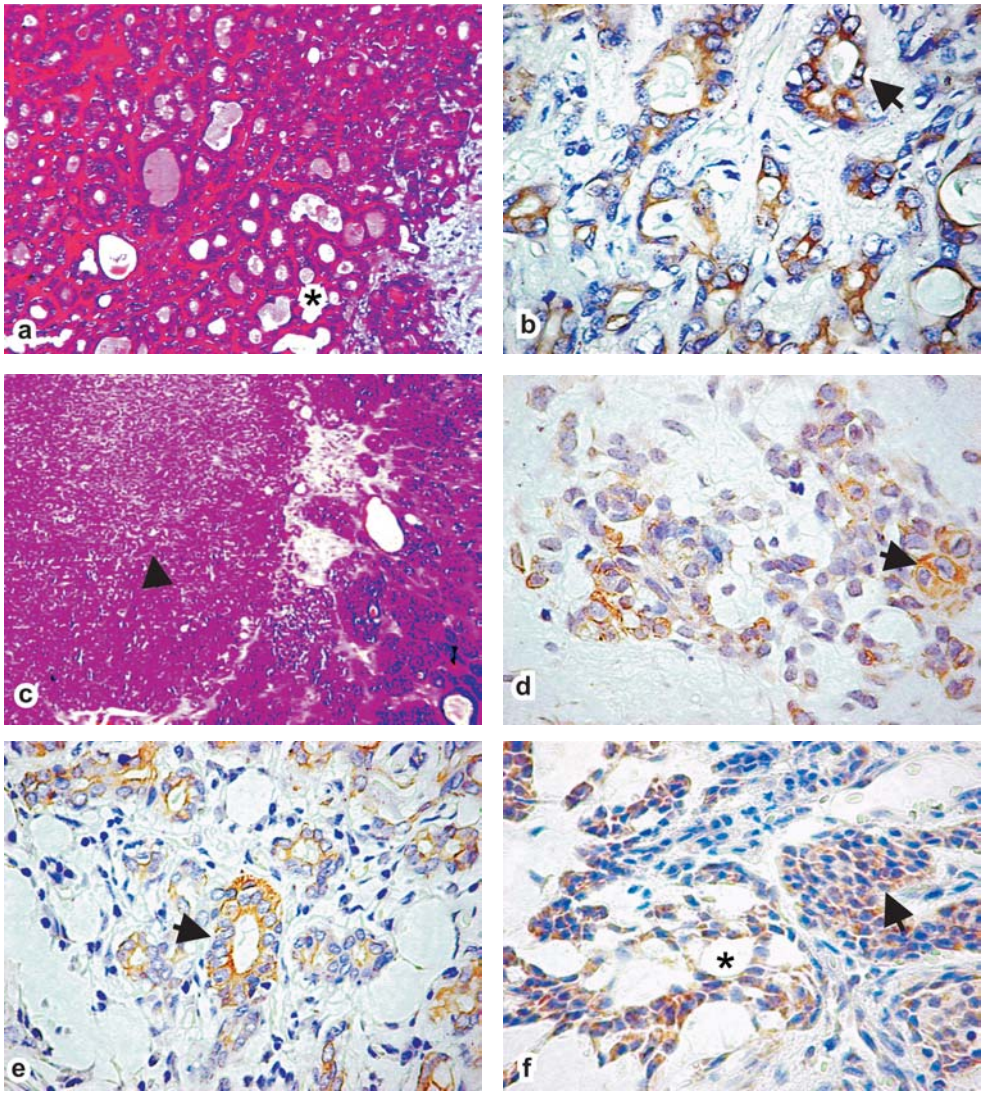

Figure 2 – a) Pleomorphic adenoma tubular pattern (asterisk), HE, ×100; b) AE1-AE3 positive reaction in luminal and abluminal cell of neoplastic tubular proliferations (arrow), ×200; c) Pleomorphic adenoma solid pattern (arrows), HE, ×100; d) AE1-AE3 positive reaction in abluminal cell of neoplastic insular proliferations (arrow), ×200; e) MNF positive reaction in the cytoplasm of luminal cells of neoplastic tubular proliferations (arrow), ×200; f) CK20 positive reaction in luminal and abluminal cell of neoplastic tubular (asterisk) and solid proliferations (arrow), ×200

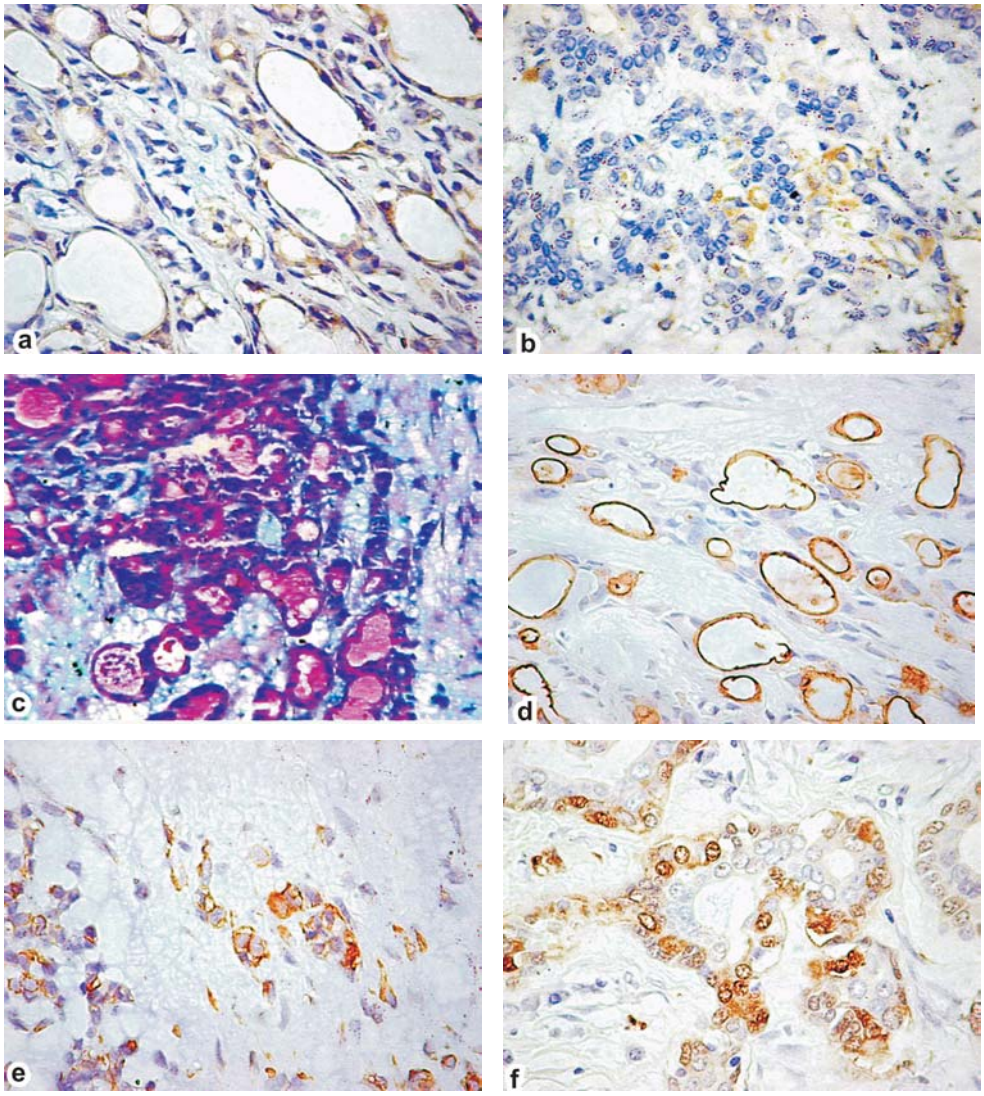

Figure 3 – a) CK8 positive reaction of the luminal cells in the neoplastic areas with tubular pattern predominance (arrow), ×200; b) CK8 positive reaction of same myoepithelial-like cells (arrow) from solid neoplastic proliferations, ×200; c) Pleomorphic adenoma with epithelial predominance – tubular pattern, PAS-Blue Alcian, ×200; d) EMA positive reaction on luminal slope (arrow) of the tubular neoplastic proliferations, ×200; e) Vimentin positive reaction of some cells from the periphery of neoplastic epithelial proliferation and some cells from the chondroid stroma (arrow), ×200; f) S-100 positive reaction in few luminal cells of the tubular neoplastic proliferations (arrows), ×400

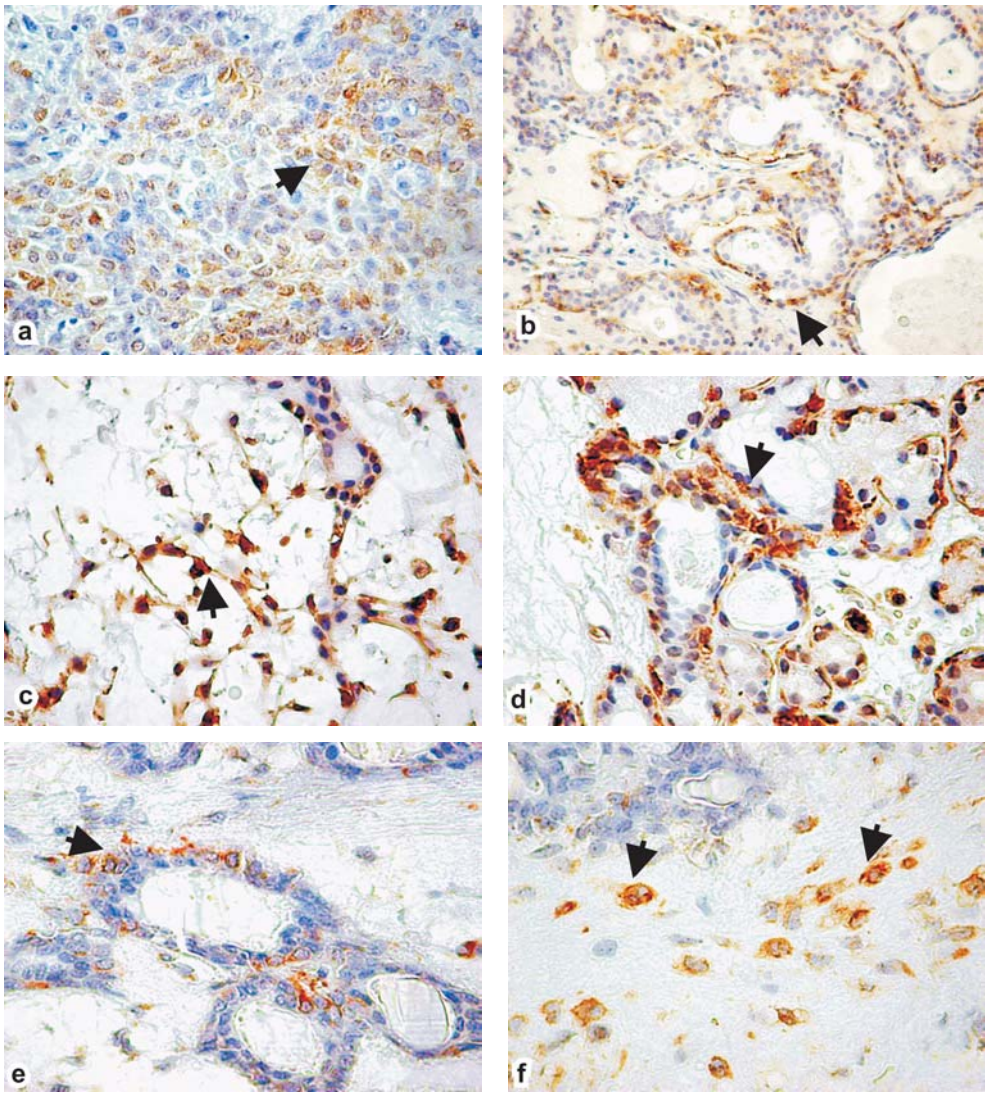

Figure 4 – a) S-100 positive reaction in cytoplasm and nucleus (arrow) of the myoepithelial neoplastic cells from the solid tumoral areas, ×200; b) α-smooth muscle actin positive reaction into the cytoplasm of abluminal neoplastic cells (arrow) from the tubular proliferative zones, ×100; c) α-smooth muscle actin positive reaction in to the cytoplasm of myoepithelial-like neoplastic cells (arrow) from the mxyoid stromal areas, ×200; d) calponin positive reaction in the cytoplasm of abluminal cells (arrow) from the tubular proliferative zones, ×200; e) GFAP positive reaction in the cytoplasm of abluminal cells (arrow) from the tubular proliferative zones, ×200; f) GFAP positive reaction in the cytoplasm of neoplastic stromal cells (arrow) from the chondroid tumoral areas, ×200