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Immunomorphological characteristics of pleomorphic adenoma of salivary glands

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SUMMARY

The immunohistochemical profile of 23 pleomorphic adenomas and 7 normal salivary glands was studied. We used antisera to vimentin (V), desmin (D), epithelial membrane antigen (EMA), prostate specific antigen (PSA), pancytokeratin, carcinoembryonic antigen (CEA), glial fibrillary acidic protein (GFAP) and S-100 protein. In the ducts and myoepithelial cells of normal salivary glands immunopositivity to most of the cytoskeletal proteins, EMA and CEA was observed. GFAP was localized only in cells of striated ducts. Major differences in the expression of various antigens among tubular structures, solid sheets, the myxoid and chondroid in the pleomorphic adenoma were encountered. Appearance of GFAP as a sign of stromal transformation into myxoid and chondroid was detected.
Judging from these comparative immunohistochemical characteristics between normal salivary glands and pleomorphic adenomas, we assume that tumour cells originate from the reserve cells of intercalated and striated ducts.

KEY WORDS:

Salivary glands, pleomorphic adenoma, immunohistochemistry.

RÉSUMÉ

Nous avons étudié les caractéristiques immunohistochimiques de 23 adénomes pléomorphes et de 7 glandes salivaires non tumorales. Nous avons utilisé des anticorps pour la vimentine (V), la desmine (D), l'antigène de membrane épithéliale (EMA), antigène prostatique spécifique (PSA), la pancytokératine, l'antigène carcinoembryonnaire (CEA), la protéine gliale fibrillaire acide (GFAP) et la protéine S-100. Dans les canaux et dans les cellules myoépithéliales des glandes salivaires normales c'est l'immunopositivité pour la plupart des protéines du cytosquelette, EMA et CEA qui est observée. GFAP est localisée uniquement dans les cellules des canaux striés. Des différences majeures dans l'expression des divers antigènes ont été rencontrées dans les structures tubulaires, dans les nappes cellulaires et dans les portions myxoïdes et chondroïdes des adénomes pleomorphes. L'apparition de GFAP a été observée comme signe de transformation myxoïde ou chondroïde du stroma.
En nous basant sur la comparaison des caractéristiques immunohistochimiques entre les glandes salivaires normales et les adénomes pléomorphes, nous supposons que les cellules tumorales trouvent leur origine dans les cellules de réserve des canaux intercalaires et des canaux striés.

MOTS CLÉS:

Glandes salivaires, adénome pléomorphe, immunohistochimie.

INTRODUCTION

The complex and not fully clarified histogenesis and morphogenesis of the pleomorphic adenoma (PA) of salivary glands, as well as its high histological variability has attracted the interest of pathologists. Various immunohistochemical studies have been performed [1, 2, 4, 8, 9, 11, 16, 19, 20] in an attempt to give a detailed immunohistochemical picture of cellular types. The data on the expression of intermediate sized cytoskeletal filament proteins and other tumour markers in its diverse structural components have been so far rather controversal [3, 6, 10, 11, 12, 15, 17, 18, 20]. The aim of the present study was to perform a complex immunohistochemical investigation of different structures in PA compared to those in normal salivary glands.

MATERIALS AND METHODS

Paraffin embedded biopsies from 23 PA of major salivary glands were collected from the files of the Department of General and Clinical Pathology, Medical University Varna.

The diagnostic criteria were based on WHO classification (Seifert G., 1992). Seven normal human salivary glands without obvious atrophy or inflammation were used as controls. Sections were processed according to the avidin-biotin immunoperixidase labelling techniques of Hsu *et al.*, (1981). Briefly after blocking of endogenous peroxidase and incubation with normal sera, sections were incubated with primary antibodies.

The series of antibodies are given on Table I. After washing in 0.01M PBS pH 7.4 sections were incubated with biotinylated secondary antibodies (ZYMED, Histostain SP kit, ZYMED Laboratories, USA), washed and incubated in streptavidin-horseradish peroxidase (ZYMED). Sections were then washed in PBS and incubated with AEC, countestained with hematoxillin and mounted in Aquamount.

Slides were examined with Amplival light microscope. Cell counts were carried out during observation on each section to determine the relative number of positive cells of different structures in normal salivary glands and pleomorphic adenomas at five points under X200 magnification. The intensity of reaction was assessed semiquantitatively as follows: (−) for negative, (±) for uncertain immunoreactivity, (+) for weakly positive, (++) for moderate and (+++) for strong positivity.

Controls: Specificity of immunolabelling was studied by omission of primary antisera, incubation of irrelevant antibodies, as well as use of normal goat serum (NGS) and normal rabbit serum (NRS) instead of primary antibodies. Positive controls on etalons as well as on built in controls were also performed.

RESULTS

Normal salivary glands. The results of the immunohistochemical staining of tissues are summarized in Tables II and III. Some details are described below.

In all acinar glandular cells (AGC) we observed expression of EMA on cell membranes and CEA in the apical cytoplasm as well as within the lumen of the acinus (Fig. 1a). Only in 25% of cells immunoreactivity to pancytokeratin was established (Fig. 1b). No reaction to D, V, GFAP, S-100 and PSA was detected. Myoepithelial cells were immunoreactive to D, V, and S-100, rarely to pancytokeratin and negative to GFAP, EMA, CEA and PSA. Epithelial cells of intercalated ducts (ID) and striated ducts (SD) were the richest in antigenic structure. Multiple expression of almost all detected antigens was observed (Fig. 1c, 1d). However, PSA-immunoreactivity was absent. Moderate expression of GFAP was observed only in epithelial cells of SD (Fig. 1d).

Pleomorphic adenoma. Assessment of the immunohistochemical profile of PA was hard to perform due to the complexity of histological structures and great variability in the proportion of structural components.

Comparative investigation of the five most common morphological components: tubular structures, solid sheets, regions of squamous metaplasia, the myxoid and chondroid transformation show considerable variability in the expression and distribution of different antigens (Table III). Inner tubular cells were intensely immunostained with EMA and CEA (Fig. 2a, 2b). A considerable number of these cells were positive to pancytokeratin (25-75%), V (25-100%) and D (50-75%) (Fig. 2c). S-100 was encountered only in single cells (<10%). Solid sheet cells are a heterogenous group in terms of immunocytochemical reaction. Comparative survey of serial sections showed that two major cellular types are

TABLE I: Antibody used.
TABLEAU I: Anticorps utilisés.

Antibody	Source	Type	Optimal dilution
Pancytokeratin	Zymed	polyclonal	prediluted
Vimentin	Boehringer	monoclonal	10 µg/ml
	Zymed	monoclonal	prediluted
Desmin	Zymed	monoclonal	prediluted
GFAP	Zymed	polyclonal	prediluted
EMA	Merck	monoclonal	prediluted
S-100	Merck	polyclonal	prediluted
CEA	Zymed	polyclonal	prediluted
PSA	Zymed	monoclonal	prediluted

TABLE II: Immunomorphologic profile of normal salivary gland.
TABLEAU II: Profil immunomorphologique de la glande salivaire normale.

Histological structures Antibodies	Acinar glandular cells	Myo-epithelial cells	Cells of intercalated ducts	Cells of striated ducts
Pancyto-keratin	25 % (+++)	100 % (+++)	100 % (+++)	100 % (+++)
Vimentin	100 % (−)	100 % (+++)	100 % (+++)	100 % (+++)
Desmin	100 % (−)	75-100 % (+)/(+++)	75-100 % (+)/(+++)	100 % (+++)
GFAP	100 % (−)	100 % (−)	100 % (−)	100 % (+++)
EMA	100 % (+++)	100 % (−)	100 % (+++)	100 % (+++)
S-100 protein	100 % (−)	75-100 % (+)/(+++)	100 % (+++)	100 % (+)
CEA	100 % (+++)	100 % (−)	100 % (+++)	100 % (+++)
PSA	100 % (−)	100 % (−)	100 % (−)	100 % (−)

TABLE III: Immunomorphologic profile of pleomorphic adenoma
TABLEAU III: Profil immunomorphologique de l'adénome pléomorphe.

Histological structures Antibodies	Inner tubular cells	Solid sheets	Squamous cell metaplasia	Myxoid	Chondroid
Pancyto-keratin	25-75 % (++)/(+++)	25-50 % (++)/(+++)	50 % (++)/(+++)	>95 % (−)	>95 % (−)
Vimentin	25-100 % (+++)	25-100 % (+++)	100 % (−)	75-100 % (+++)	75-100 % (+++)
Desmin	50-75 % (+++)	50 % (++)	100 % (−)	75-100 % (+++)	75-100 % (+++)
GFAP	100 % (−)	50 % (++)/(+++)	100 % (−)	50-75 % (++)/(+++)	>95 % (+++)
EMA	100 % (++)/(+++)	50-75 % (++)	100 % (+++)	1-25 % (±)/(++)	100 % (−)
S-100 protein	<10 % (+++)	25 % (++)	100 % (−)	25-50 % (++)/(+++)	100 % (+++)
CEA	100 % (+++)	5 % (+++)	100 % (++)	10 % (+)	25-75 % (++)/(+++)
PSA	100 % (−)	100 % (−)	100 % (−)	100 % (−)	100 % (−)

Fig. 1:
Normal salivary gland. A. CEA - strong expression in the apical region of glandular acinar cells. No counterstaining with haematoxillin. × 160. B. Pancytokeratin - strong expression in striated duct cells and weak expression in glandular acinar cells. Haematoxillin countestain. × 160. C. Vimentin - strong expression in striated ducts and weak expression in glandular cells. No counterstaining with haematoxillin. × 160. D. GFAP - strong expression only in striated ducts. No haematoxillin countestain. × 160.

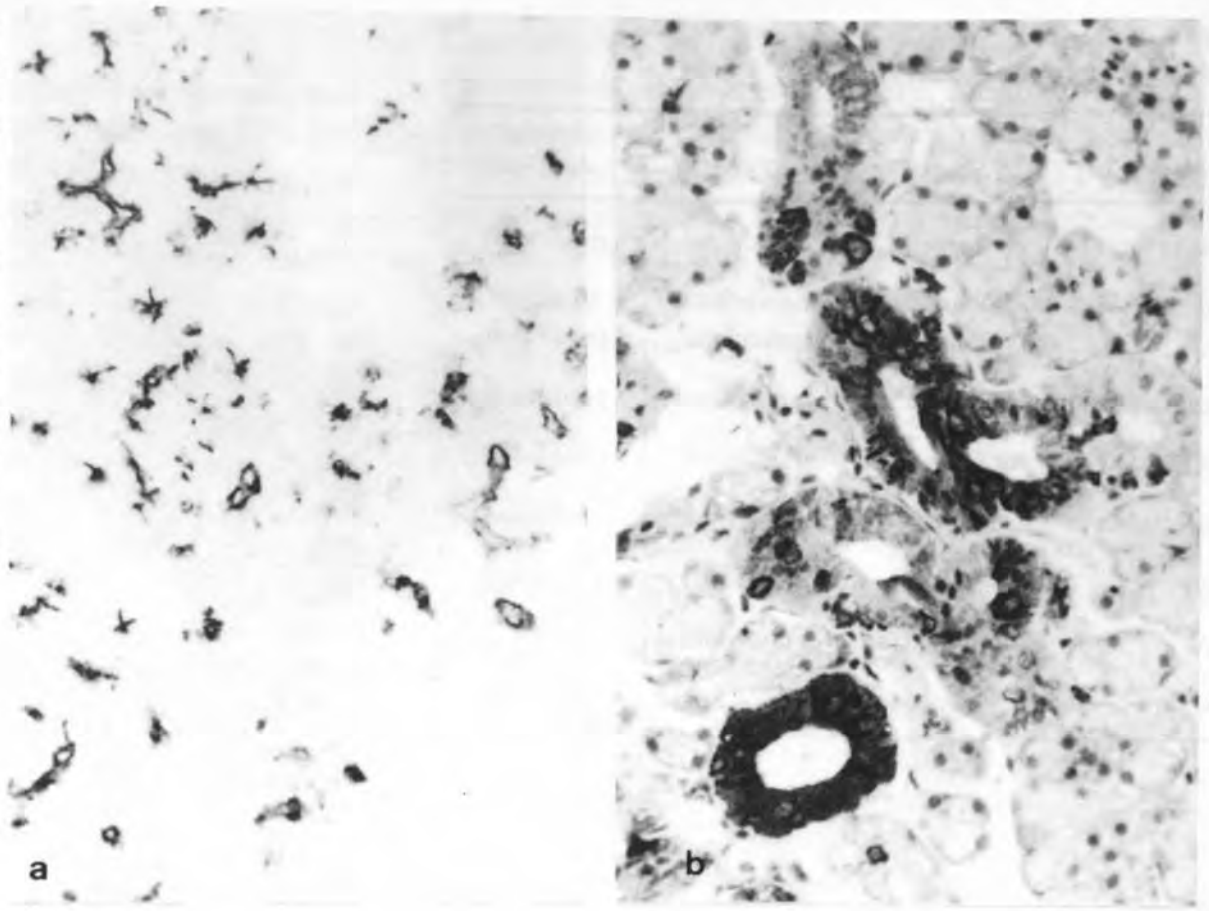

Fig. 1:
Glande salivaire normale. A. Forte expression de CEA dans la région apicale des cellules des acini. Pas de contre-coloration à l'hématoxyline. ×160. B. Pancytokératine. Forte expression dans les cellules des canaux striés de la pancytokératine et faible expression dans les cellules des acini. Contre-coloration à l'hématoxyline. ×160. C. Forte expression de la vimentine dans les canaux striés et faible expression dans les cellules glandulaires. Pas de contre-coloration à l'hématoxyline. ×160. D. Forte expression de GFAP uniquement dans les canaux striés. Pas de contre-coloration à l'hématoxyline. ×160.

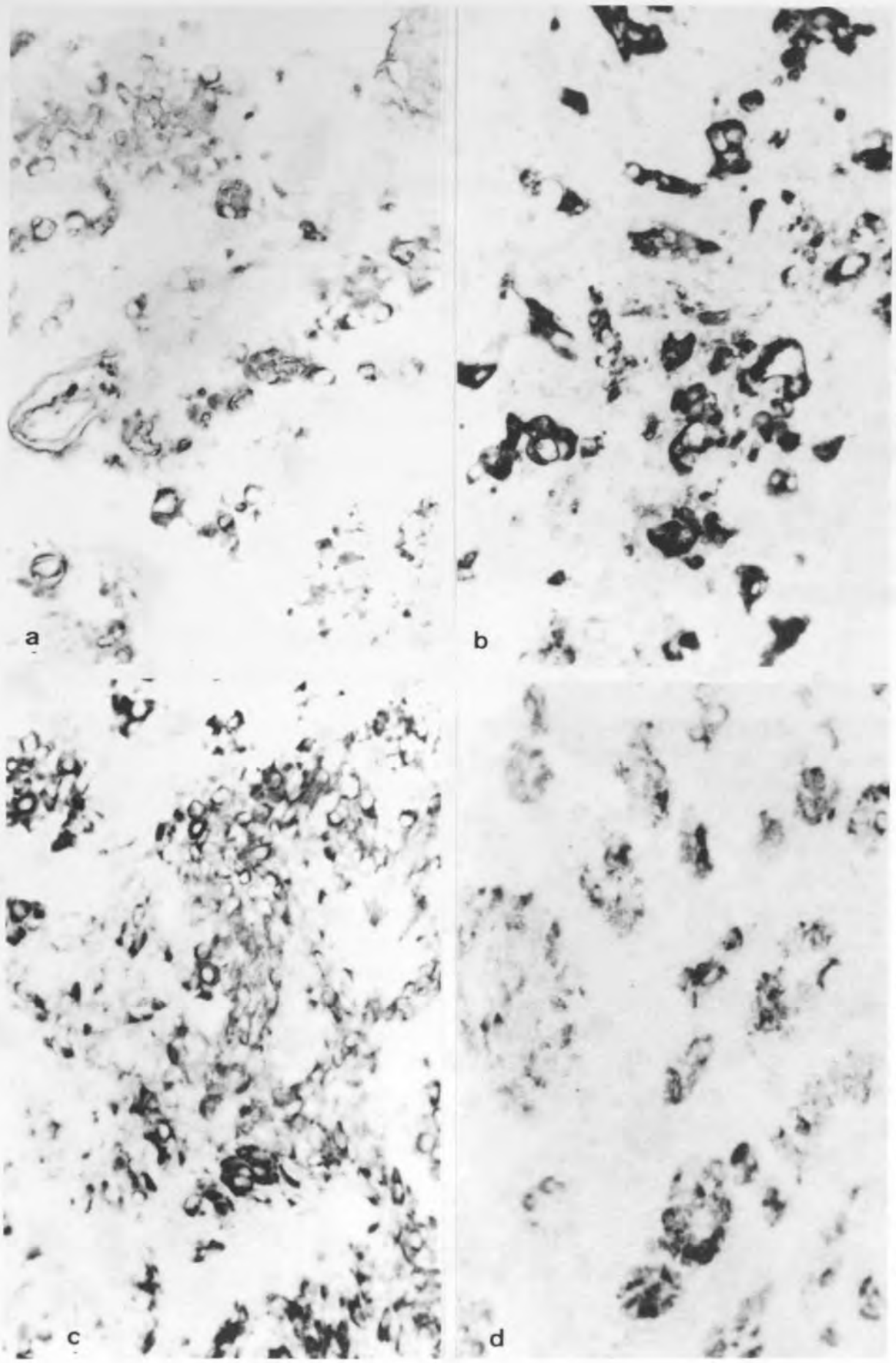

Fig. 2:
Pleomorphic adenoma - tubular structures and solid sheets. A. EMA - strong expression in the apical regions of cellular membranes of tubular structures and solid sheets. No haematoxillin counterstain. × 160. B. CEA - strong expression in cells of tubular structures. No haematoxillin counterstain. × 160. C. Pancytokeratin - strong immunoreactivity in cells of solid sheets. No haematoxillin counterstain. × 160. D. Desmin - moderate expression in cells of tubular structures. No haematoxillin counterstain. × 160.

Fig. 2:
Adénome pléomorphe. Structures tubulaires et nappes cellulaires. A. Forte expression de EMA dans les régions apicales des membranes cellulaires des structures tubulaires et des nappes solides. Pas de contre-coloration par l'hématoxyline. ×160. B. Forte expression de CEA dans les cellules des structures tubulaires. Pas de contre-coloration à l'hématoxyline. ×160. C. Forte immunoréaction pour la pankératine dans les cellules des nappes solides. Pas de contre-coloration à l'hématoxyline. ×160. D. Expression modérée de la desmine dans les cellules des structures tubulaires. Pas de contre-coloration par l'hématoxyline. ×160.

Fig. 3:
Pleomorphic adenoma - solid sheets, myxoid and chondroid. A. GFAP - strong expression in solid sheets and in the myxoid. No haematoxillin staining. × 160. B. Pancytokeratin - no expression in the myxoid, positive immunoreactivity is evident only in tubular structures. No haematoxillin counterstaining. × 160. C. Vimentin - strong expression in cells of the chondroid and weak in solid sheets. Haematoxillin counterstaining. × 160. D. GFAP - strong expression in the cells of the chondroid. No haematoxillin counterstaining. × 160.

Fig. 3:
Adénome pléomorphe. Nappes cellulaires, myxoïdes et chondroïdes. A. Forte expression de GFAP dans les nappes cellulaires denses et myxoïdes. Pas de contre-coloration à l'hématoxyline. ×160. B. Absence d'expression de pancytokératine dans les régions myxoïdes. Une immunoréaction positive est évidente uniquement dans les structures tubulaires. Pas de contre-coloration à l'hématoxyline. ×160. C. Forte expression de la vimentine dans les cellules des régions chondroïdes et expression faible dans les nappes cellulaires denses. Contre-coloration à l'hématoxyline. ×160. D. Forte expression de GFAP dans les cellules des territoires chondroïdes. Pas de contre-coloration par l'hématoxyline. ×160.

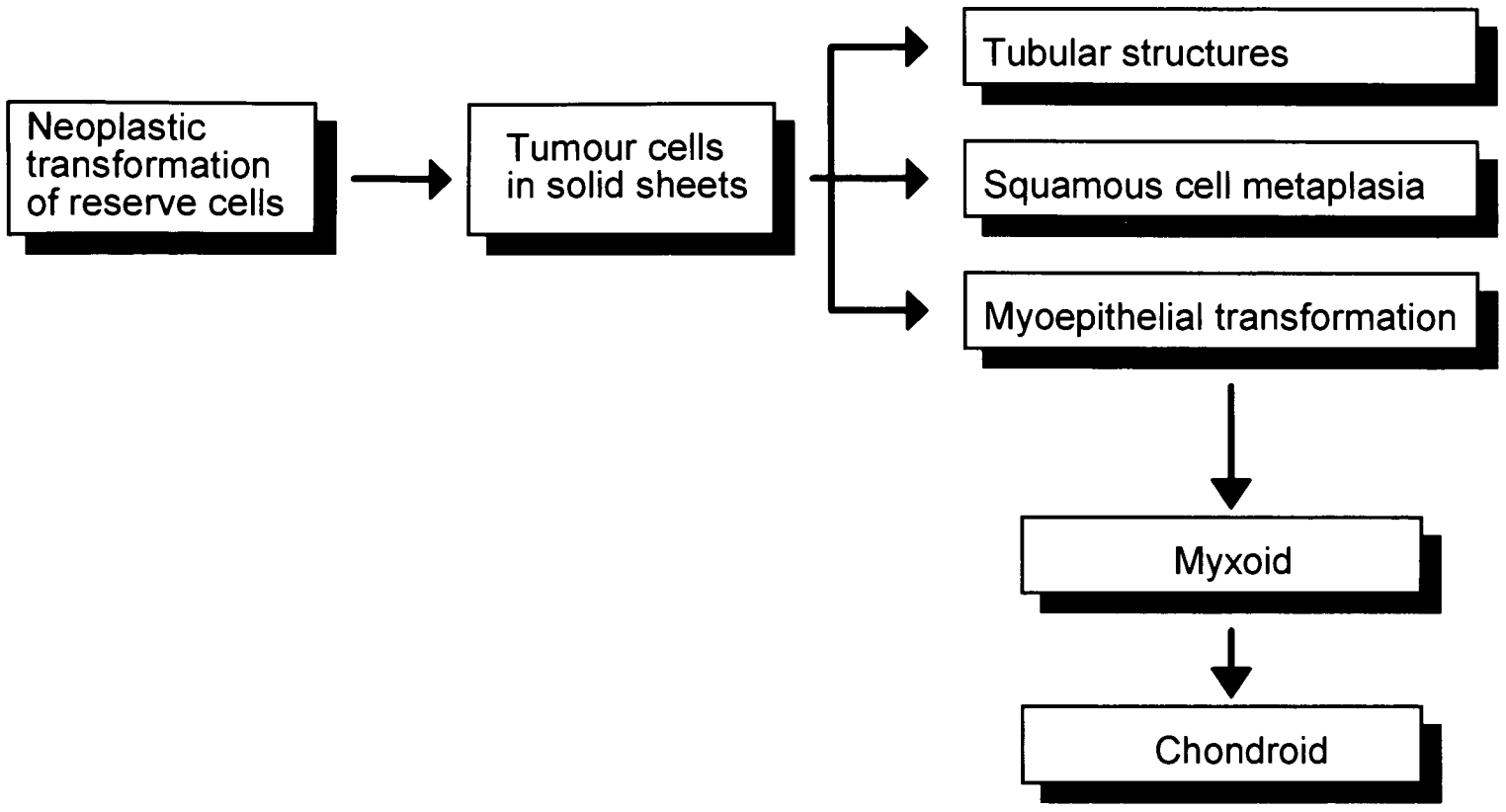

Fig. 4: Histogenesis and morphogenesis of pleomorphic adenoma.

Fig. 4: Histogenèse et morphogenèse de l'adénome pléomorphe.

dominating. In the first cell type coexpression of pancytokeratin (Fig. 2d) and EMA and rarely of V was found. Multiple expression of V, D and GFAP was characteristic for the second cell type (Fig. 3a). In the second group a small part of cells was positive to S-100. CEA was encountered in single cells of both types. In regions of squamous cell metaplasia all cells expressed EMA and CEA; in 50% of cells immunoreactivity to pancytokeratin was detected. Immunoreactivity to V, D, GFAP, S-100 and PSA was negative. Cells of the myxoid were immunopositive to V and D (75-100%). In 50 to 75% of cells expression of D, V and GFAP and in 25-50% with S-100 was detected. Pancytokeratin-immunoreactivity was negative (Fig. 3b), while EMA was observed only in single cells. Reaction to CEA was weak and existed in 10% of cells. Cells of the chondroid showed multiple expression of GFAP, S-100, V, D and CEA (in 75% of cells) (Fig. 3c, 3d); reaction to pancytokeratin and EMA was negative.

No expression of PSA in all PA was detected.

DISCUSSION

Our results show that as in the normal salivary glands, PA express a very rich immunohistochemical profile. In certain cellular types and histological structures all intermediate cytoskeletal filament proteins detected were present as well as were EMA, S-100 and CEA. Others have failed to show expression of V, D, GFAP and S-100 in normal glands [11, 13]. However in our studies V, D, S-100 was observed in myoepithelial cells as well as in cells of ID and SD. Immunolocalization of S-100 in myoepithelial cells was reported by Nakazato *et al.* (1985) and Kahu *et al.* (1985). Regezi *et al.* (1991) have localized S-100 in myoepithelial cells and ID. Vimentin has been also present in myoepithelial cells (Nakazato *et al.* 1991, Shamsudinov 1991). The expression of GFAP in cells of SD is of particular interest since this protein has been characteristic primarily for glial cells. Similar results have been reported by Regezi *et al.* (1991), in myoepithelial cells.

Comparative immunohistochemical profile analysis of different cells in normal glands and PA tempts us to speculate on the histo- and morphogenesis of PA. Salivary glands originate from pluripotential epithelial cells derived from the ectoderm (parotid) and the endoderm (sumbandibular). Three major cellular types are then differentiated – (i) secretory acinar cells, (ii) myoepithelial cells and (iii) ductal cells. It has been widely accepted that part of the basal cells remain undifferentiated and function as

reserve cells [2, 16, 17]. Regarding the histogenesis of PA two hypotheses are dominating. According to the first, tumor cells originate principally from myoepithelial cells [1, 2, 12] while according to the other – they are derivatives of reserve cells [1, 2, 9, 14]. However dual origin of neoplastic cells from a ductal-acinar unit composed of intercalated ductal cells and myoepithelial cells can not be excluded [18]. A high proliferative activity of reserve cells in ID in comparison to others has been detected by Fujita *et al.* (1992), which is in support of the second hypothesis. Reserve cells have been reported to be pluripotential. They have the ability to differentiate either towards epithelial or myoepithelial direction [11]). In normal salivary glands immunopositivity to almost all antigens detected in this study was observed in the cells of SD and ID. The weakest immunoreactivity has been shown in highly differentiated acinar cells.

Tumour cells in PA most often show great variability and more frequent multiple expression of antigens compared to normal glands. However cells in structures with diverse differentiation show greater immunohistochemical heterogenity. This phenomenon is regarded as a sign of the expression of an intrinsic genetic «instability» of tumour cells accompanied by an altered ballance in the expression of cytoskeletal intermediate filament proteins (Su *et al.*, 1993).

The cells of solid sheets expressed the most diverse pattern of immunohistochemical staining. They have been regarded as the progenitor cells of different structural components of PA [9]. Compared to normal glands these cells react similarly to cells of SD and ID. It can be therefore accepted that tumour cells in solid sheets originate from reserve cells (Fig. 4). It has to be noted also that the cellular population of the solid sheets is immunohistochemically heteregenious; in part of cells multiple expression of pancytokeratin with EMA, in others – with D, V, GFAP and S-100 was observed. Most probably the first group of cells shows signs of initial epithelial differentiation and is a progenitor of tubular epithelial structures. The other group expressing similar immunoreactivity to the myxoid and chondroid may be regarded to differentiate to myoepithelial direction. No immunoreactivity to pancytokeratin was detected in the myxoid, while the relative number of cells positive to EMA and CEA diminished greatly. The myxoid/chondroid transformation leads to a disappearance of epithelial markers. In contrast the number of cells expressing GFAP increases. Cross-reactivity has been excluded [1]. GFAP has been reported to be characteristic for poorly-differentiated myoepithelial cells (Anderson *et al.* 1990). Since GFAP in PA and glial cells have similar molecular weights [12] its expression in PA is most probably a result of phenotypic changes and closely related to oncogenesis [1, 11, 13].

The present study did not provide evidence of PSA expression as earlier reported by van Krieken (1993).

In conclusion, expression of various cytoskeletal intermediate filament proteins and a great variety of tumour markers in different cells of PA is most probably determined by their embryological origin from pluripotential cells as well as by their neoplastic transformation and diverse differentiation leading to changes in phenotype. Further studies are needed to clarify this problem.

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