Virchows Arch (1997) 430:227–232

ORIGINAL ARTICLE

Isabel Fonseca · Ana Félix · Jorge Soares

Cell proliferation in salivary gland adenocarcinomas with myoepithelial participation

A study of 78 cases

Received: 7 June 1996 / Accepted: 3 October 1996

Abstract We used three markers of cell proliferation – mitotic counts, mitotic index and expression of proliferating cell nuclear antigen – to assess the proliferative activity of a series of 78 low-grade salivary adenocarcinomas with myoepithelial participation classified according to: their histological type, the predominant architectural type, and the predominant cytological type. The series included adenoid cystic carcinomas (40), epithelial-myoepithelial carcinomas (19), polymorphous low-grade adenocarcinomas (12) and basal cell adenocarcinomas (7). The proliferation indicators were found to be similar in the first three groups, being significantly lower than in the last. Tumours formed by basal cells had statistically significant higher mitotic indexes than those predominantly composed of clear cells of myoepithelial type and ductal cells. Tubular tumours, irrespective of the histological classification of the neoplasm, had proliferation indexes similar to those found in cribriform neoplasms. Solid tumours, whether formed by ductal or clear myoepithelial-type cells, had higher indexes than the neoplasms with differentiated (cribriform and tubular) patterns. The highest mean values for every proliferation indicator used were found in tumours with solid organization that were predominantly formed by basal cells. These results agree with the hypothesis that cell proliferation is inversely related to neoplastic differentiation. The identification of the prevalent cell phenotype and architecture may extend our knowledge from adenoid cystic carcinoma, whose solid variant carries a worse prognosis, and supports that the usual classification of this group of salivary adenocarcinomas would benefit to be complemented with information on tumour architecture and cellular composition.

Key words Proliferation · PCNA · Salivary gland neoplasms · Adenoid cystic carcinoma · Mitosis

I. Fonseca · A. Félix · J. Soares[1] (✉)
Departamento de Patologia Morfológica & Centro de Investigação de Patobiologia Molecular (CIPM),
Instituto Português de Oncologia de Francisco Gentil,
Lisboa, Portugal

Mailing-address:
[1] Departamento de Patologia Morfológica,
Instituto Português de Oncologia, Rua Prof. Lima Basto,
P-1093 Lisboa CODEX, Portugal
Tel./Fax: (351) 1 726 6957

Introduction

Information on the proliferation characteristics of normal and neoplastic human salivary gland tissues is scarce, especially in salivary gland carcinomas that express dual differentiation [3, 5, 14, 16, 19, 20, 22]. This group of tumours is formed by cells that exhibit both epithelial and myoepithelial phenotypes and includes four entities: adenoid cystic carcinoma, epithelial-myoepithelial carcinoma, polymorphous low-grade adenocarcinoma and basal cell adenocarcinoma [13, 18]. Similarities in their differentiation characteristics and clinical behaviour and some overlapping of their histological patterns argue in favour of a common morphogenetic pathway for these neoplasms [1, 2]. Neoplastic cell features, as evaluated in haematoxylin and eosin stained histological sections and according to immunohistochemical characteristics, allow the identification of variable proportions of ductal and myoepithelial elements, together with a third cellular type, represented by basal cells, of uncommitted differentiation [3–5, 13, 14, 17].

We investigated the proliferative features of a series of salivary gland adenocarcinomas with myoepithelial participation using three methods: mitotic counts, mitotic index and "proliferating cell nuclear antigen" (PCNA) immunohistochemistry, attempting to improve characterization of this morphologically heterogeneous group of neoplasms. In addition, we aimed to determine whether these proliferation markers correlated with the architectural and cytological tumour patterns potentially related to tumour prognosis and to the process of neoplastic differentiation and tumour histogenesis.

Materials and methods

Seventy-eight cases of salivary gland adenocarcinomas with myoepithelial participation were studied. All the cases were reclassified according to the WHO classification [18] into the following groups: adenoid cystic carcinoma (n = 40), epithelial-myoepithelial carcinoma (n = 19), polymorphous low-grade adenocarcinoma (n = 12) and basal cell adenocarcinoma (n = 7).

The age of the patients ranged between 18 and 83 years (median 56.5 years); 45 were women and 33 were men. The tumours were located in the major glands in 36 and the minor glands in 42 cases.

The predominant architectural pattern and the predominant cell composition, where predominant means constituting more than 50% of the tumour area in each case, were evaluated on H&E-stained sections obtained from 10% formalin-fixed, paraffin-embedded tumour tissue.

Three architectural types were considered: cribriform (Fig. 1) and tubular (Fig. 2), both corresponding to a "differentiated" glandular arrangement, and solid (Fig. 3), with less than 50% of the tumour volume composed of discernible glandular structures, and considered "non-differentiated", following Perzin et al.'s proposal [15] for adenoid cystic carcinoma.

The cell types were classified as ductal cells: elements with central nucleus, eosinophilic cytoplasm and displaying unequivocal epithelial differentiation (Fig. 2), clear cells: elements with irregular eccentric nucleus and clear cytoplasm and expressing an immunocytochemical myoepithelial-cell phenotype (Figs. 2, 4), and basal cells: elements with hyperchromatic large round to oval nucleus and scant cytoplasm, and expressing uncommitted features, either epithelial or myoepithelial, varying in proportion and staining intensity (Fig. 4).

The categorization of the tumours according to these three cell types was further achieved by using a panel of antibodies that included: anti-cytokeratins 5, 6, 8, 17 and 18, S-100 protein, alpha smooth muscle actin and vimentin [7] (I. Fonseca, J. Soares, unpublished data). Ductal cells always expressed positivity for low-molecular-weight keratins, clear cells were found to be positive for S–100 protein and smooth muscle actin, and basal cells expressed both epithelial and myoepithelial markers irregularly distributed and with variable frequency.

Mitotic counts were obtained by evaluating the number of mitoses in 10 high power fields (HPF), using a light optic microscope with a field area of 0.16 mm^2. The mitotic index was calculated as the number of mitoses present in a total of 1000 tumour cells and was expressed as a percentage.

A representative section of each tumour was used for staining with PC10/PCNA (Dako, ref. M879) antibody. Briefly, 5-μm sections were collected onto poly-L-lysine (Sigma)-coated slides that were allowed to dry overnight at room temperature (20°C). They were then deparaffinized in xylene, re-hydrated in ethanol of decreasing concentrations and the endogenous peroxidase was blocked using 0.5% hydrogen peroxide in methanol for 10 min. The slides were incubated with the PC10 antibody at a dilution of 1:150 for 30 min at room temperature, followed by the secondary antibody (Dako, ref. Z259, 1:50) and the streptABC complex/HRP (Dako, K377, 1:25) for 30 min each. The reaction was developed using DAB (Sigma). The sections were counterstained with Harris' haematoxylin, dehydrated and mounted with Entellan.

The positive results were represented by brown (light to dark) nuclear staining. In each case approximately 1000 randomly selected nuclei were assessed. An ocular mesh was used to prevent recounting, and the percentage of PCNA-positive cells was calculated.

The results obtained were compared using the Chi-square test for tabulated data and the Kruskal-Wallis test for comparison of means.

Results

Mitotic counts ranged between 0 and 11, with a mean value of 2.06 ± 2.16 mitoses per 1.6 mm^2. Lower counts were observed in the groups of epithelial-myoepithelial carcinoma and adenoid cystic carcinoma, each with a median value of 1 mitosis per 10 HPF (Table 1). The highest count was verified in the group of basal cell adenocarcinoma (median: 4 mitoses per 10 HPF). There was a statistically significant difference between counts of adenoid cystic carcinoma, epithelial-myoepithelial carcinoma and polymorphous low-grade adenocarcinoma vs basal cell adenocarcinoma (P = 0.01; Table 1).

Cribriform and tubular variants of adenoid cystic carcinoma had median mitotic counts of 1 and 2, respectively, lower than that of the solid variant of the tumour (median = 3 mitoses per 10 HPF) or of the basal cell adenocarcinomas (median = 4 mitoses per 10 HPF).

In the groups of adenoid cystic carcinoma and polymorphous low-grade adenocarcinoma, tumours with solid architecture had mitotic counts over their respective median value in 83.0% and 75.0% of the cases.

Tumours predominantly composed of basal cells showed mitotic counts higher than those of tumours predominantly formed of either ductal or clear cells. The difference between median values did not, however, reach statistical significance. The group of adenoid cystic carcinomas predominantly formed by basal cells had lower mean mitotic counts than that of basal cell adenocarcinoma group (2.28 ± 1.97 vs 5.28 ± 3.81 per 10 HPF). Among the clear-cell-predominant tumours, irrespective of their histological type, there were no significant differences in the median values of their mitotic counts.

The mean value for the mitotic index was of 7.92 ± 6.00% in the whole series (Table 1). The limit values were observed in the adenoid cystic carcinoma group (6.86 ± 4.52%) and the basal cell adenocarcinoma group (16.27 ± 9.47%). The mitotic indexes of adenoid cystic carcinomas, epithelial-myoepithelial carcinomas and polymorphous low-grade adenocarcinomas were statistically significantly lower than that of basal cell adenocarcinomas (P = 0.04; Table 1).

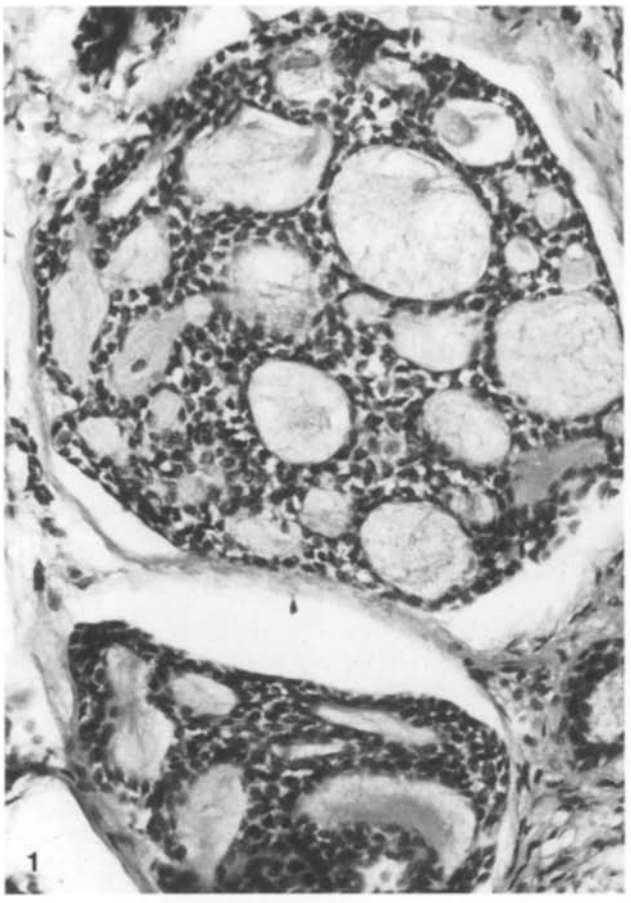
When the tumours were grouped according to the aforementioned architectural types, solid tumours as a whole showed higher mitotic indexes than the two differ-

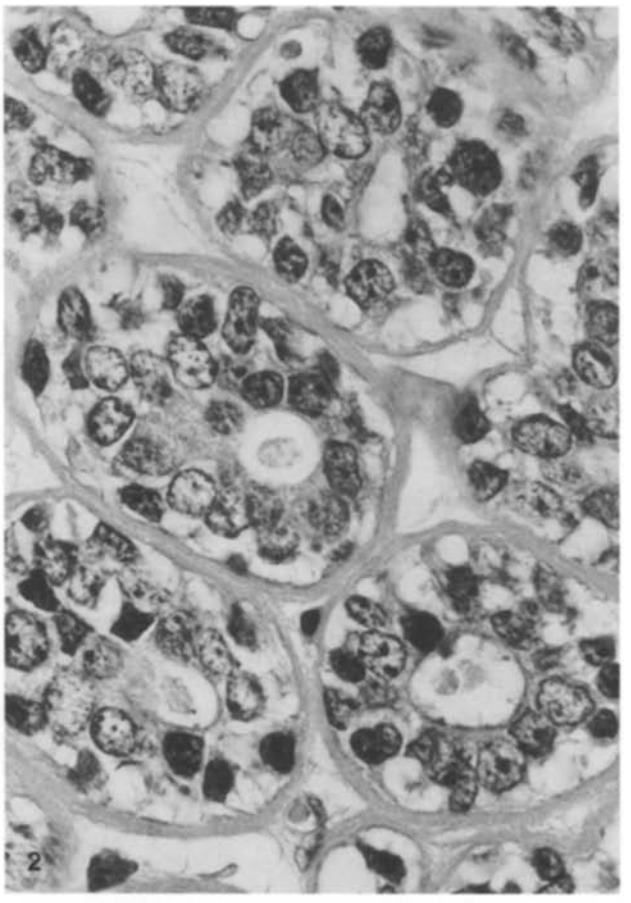
Fig. 1 Low-magnification of a cribriform adenoid-cystic carcinoma. H&E, ×185 ▶

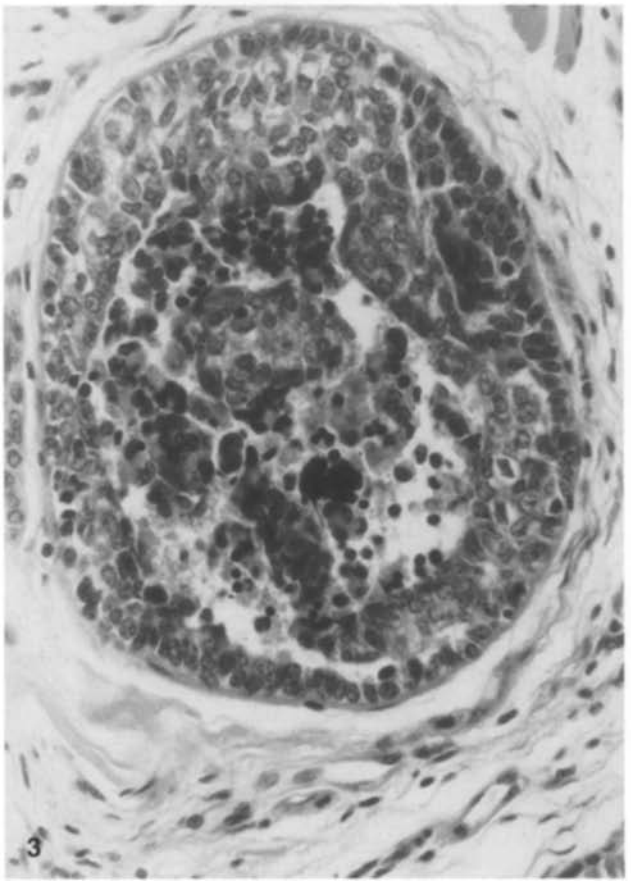
Fig. 2 Epithelial-myoepithelial carcinoma: a tubular arrangement forming small neoplastic ducts. A dual cell population is apparent, with an outer layer of clear cells surrounding the darker, smaller duct cells. H&E, ×928

Fig. 3 Basal cell adenocarcinoma: solid tumour nodule formed by basal cells, with a central, comedo-type necrosis. H&E, ×371

Fig. 4 Basal cell adenocarcinoma: higher magnification of an area in which the two cell types, clear and basal, are sharply apparent. H&E, ×928


1


2


3

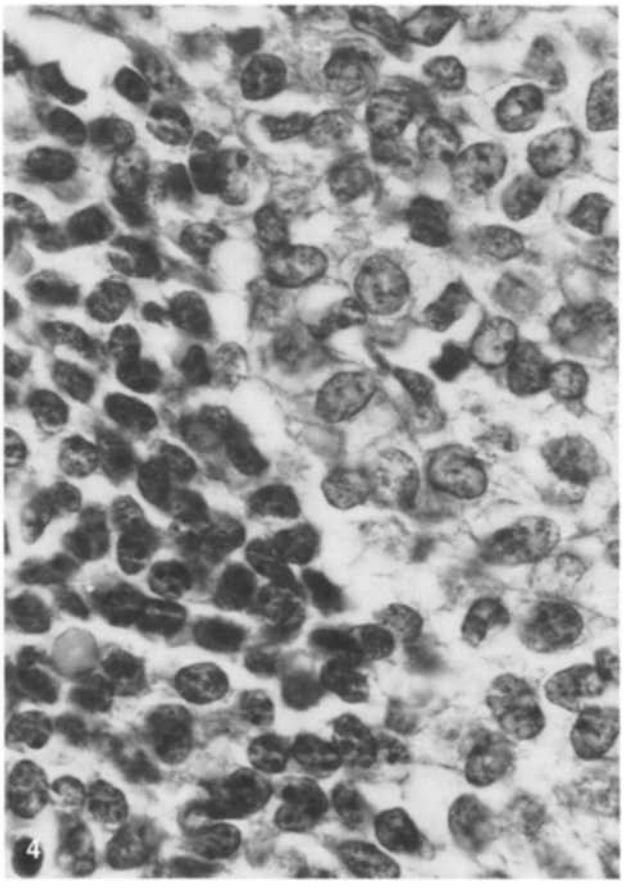

4

Table 1 Median mitotic counts (*MC*), mitotic index (*MI*) and percentage of PCNA-positive nuclei of the whole series

Classification	*n*	MC/10 HPF	MI%	PCNA%
Adenoid cystic carcinoma	40	1.0	6.86±4.68	21.56±11.42
Epithelial-myoepithelial carcinoma	19	1.0	7.35±5.90	20.68±14.99
Polymorphous low-grade adenocarcinoma	12	1.5	7.51±4.96	18.91±11.09
Basal cell adenocarcinoma	7	4.0*	16.27±9.47**	43.16±25.38***

* P=0.01; ** P=0.04; *** P=0.02

Table 2 Mitotic counts, mitotic indexes and percentage of "PCNA positive" nuclei correlated with the cytoarchitectural patterns of the tumour collective (mean±SD); median mitotic counts are shown in round brackets)

	Mitotic counts (median)	Mitotic index (%)	PCNA index (%)
Architectural pattern			
Differentiated/glandular	1.71±1.79 (1)	6.81±4.54	20.72±11.62
Cribriform	1.71±2.08 (1)	6.35±4.52	20.66±12.25
Tubular	1.72±1.17 (2)	7.58±4.61	20.83±10.81
Non-differentiated/solid	2.65±2.59 (2)*	9.80±7.60**	26.68±19.29***
Cytological type			
Basal	2.71±2.61 (2)	9.05±7.02	26.61±18.41
Clear	1.42±1.16 (1)	7.05±5.71	20.31±14.45
Ductal	1.76±1.98 (1)	7.19±4.64	20.44±9.43

* P=0.05; ** P=0.03; *** P=0.08 (glandular versus solid)

entiated, cribriform and tubular, glandular types (9.80 ± 7.60 versus 6.81 ± 4.54) (Table 2). The mitotic index of the solid variant of adenoid cystic carcinoma, with 8.67 ± 4.52%, was similar to that found in the solid forms of epithelial-myoepithelial carcinoma (8.00 ± 6.43%), and both these were significantly lower than the miotic index of solid basal cell adenocarcinoma (P = 0.04). Tubular forms of adenoid cystic carcinoma and epithelial-myoepithelial carcinoma also had similar mitotic indexes (6.93 ± 2.89% and 5.93 ± 4.75%, respectively).

The neoplasms in which basal cells predominated presented higher mitotic indexes than tumours mostly formed of either clear or ductal cells (Table 2). Adenoid cystic carcinoma and polymorphous low-grade adenocarcinoma in which basal cells predominated had similar mitotic indexes (7.05 ± 4.70% and 7.02 ± 4.89%), which were lower than that found in basal cell adenocarcinomas.

The mean value of the PCNA index (PCNA% positive) nuclei of the whole series was of 22.94 ± 15.10%. Mean values found in the epithelial-myoepithelial carcinoma group (20.68 ± 14.99%) and the adenoid cystic carcinoma group (21.68 ± 11.42%) were identical, but significantly (P = 0.02) lower than that verified in the basal cell adenocarcinoma group (43.16 ± 25.38%).

Solid architecture was associated with a PCNA index higher than the one verified in cribriform and tubular types (Table 2). Among the glandular differentiated tumours, cribriform neoplasms had lower mean PCNA indexes than tubular tumours in the groups of adenoid cystic carcinoma (cribriform, 21.32 ± 12.20; tubular, 22.36 ± 4.77) and polymorphous low-grade adenocarcinoma (cribriform, 14.46 ± 13.25; tubular, 23.32 ± 11.56).

When tumour cytology was taken into consideration, tumours mostly made up of clear cells had lower mean PCNA indexes than did tumours mostly made up of ductal cells. In the adenoid cystic carcinoma group, tumours predominantly composed of basal cells had higher PCNA indexes than the two other cytological groups (Table 2).

Irrespective of the histological classification, tumours that associated a predominance of basal cells and solid architecture showed the highest PCNA indexes, matching the findings with mitotic counts and indexes.

In this particular group of tumours, immunocytochemical determination of proliferative activity gave parallel results to the traditional indicators of cell division assessed in H&E-stained slides (mitotic counts and mitotic index).

Discussion

Proliferative activity measured by our methods was found to be similar among tumours of the adenoid cystic carcinoma, epithelial-myoepithelial carcinoma and polymorphous low-grade adenocarcinoma groups (Table 1). In contrast, the three proliferation indicators were significantly higher in the basal cell adenocarcinoma group, demonstrating that these neoplasms have a higher capacity for division than the three other histological groups. This statistically significant difference fits well with the fact that basal cell adenocarcinomas, by definition, almost invariably exhibit a solid organization of cells of basaloid morphology, the opposite being true for the majority of tumours of the three other histological types. This conclusion strongly supports the relationship of the cellular composition and tumour organization with proliferative activity in bidifferentiated low-grade adenocarcinomas of the salivary glands.

It is generally accepted that, despite a certain degree of variation, three main architectural patterns of tumour organization and an identical number of cytological subtypes are recognized among salivary gland adenocarcinomas with myoepithelial participation [6, 11]. Solid architecture is frequently associated with non-differentiated neoplastic organization, usually opposed to the differentiated pattern of tumours having glandular arrangements, typified by cribriform and tubular architecture and, less

frequently, by concurrent papillary areas. Papillary arrangements, mostly found in polymorphous low-grade adenocarcinoma, never achieved a predominant pattern in the tumour architectural pattern and were not considered in the study.

To compare the proliferative activity between architectural groups (glandular/differentiated versus solid/non-differentiated) and cytological groups (epithelial-type versus myoepithelial-type versus basal-type), we included in the same category neoplasms from each of the four histological types. For this purpose, adenoid cystic carcinoma, epithelial-myoepithelial carcinoma, polymorphous low-grade adenocarcinoma and basal cell adenocarcinoma were considered as a single "family" of entities.

In structural terms, we found that the group of tumours showing differentiated glandular patterns, either tubular or cribriform, presented mean values of the proliferation markers studied lower than the group of solid tumours, as is shown in Table 2. This difference had statistical significance.

It is commonly accepted that differentiation of normal tissues is inversely related to the proliferating activity of their cells and that the loss of tumour cell proliferative capacity is associated with acquisition of a well-differentiated pattern [10, 12, 21]. In a previous study of a series of epithelial-myoepithelial carcinomas, the salivary tumour type that mostly resembles normal intercalated ducts, we showed that neoplastic proliferation was restricted to modified myoepithelial cells and that in epithelial cells forming typical ductal structures with a terminally differentiated phenotype no evidence of proliferative activity was disclosed by immunohistochemistry [8]. The typical glandular appearance (tubular and/or cribriform) of adenocarcinomas associated with a remarkable biphasic cell composition is associated with a low rate of cell division and should express a well-differentiated neoplastic organization compared with the composition of normal tissue [1, 2].

The three aforementioned indicators of cell proliferation also demonstrated that basal cells have higher proliferation rate than myoepithelial-type cells and the latter a slightly higher rate than ductal cells. These results allow us to conclude that the cytological phenotype of the elements that predominate in the composition of the neoplasms might anticipate their proliferative capacity, while identifying potential clinical behaviour as usually correlated with the histological pattern of the neoplasms.

Previous studies on salivary gland tumour proliferation have mostly been restricted to the group of adenoid cystic carcinomas and have used different methodologies, namely S-phase cytometric determination [16], Ki-67/MIB1 [14, 19] and PCNA immunocytochemistry [22], which biases the comparison of the different results. However, all these studies [14, 16, 19, 22] like ours, demonstrate very consistently that solid adenoid cystic carcinoma has proliferation index values double those of more differentiated, cribriform and/or tubular forms.

Overall, our results in this group of salivary adenocarcinomas with myoepithelial participation fit well with the view that tumour differentiation correlates inversely to proliferation activity and that solid tumours, predominantly formed of basal cells, have increased potential for local growth [9]. The proliferative indicators studied are not informative markers of tumour prognosis and are of no value for the evaluation of the individual case. However, in this particular group of neoplasms, cytodifferentiation and architectural differentiation are morphological indicators of potential clinical utility in addition to the histological classification, extending to all salivary adenocarcinomas with myoepithelial participation prognostic criteria that have been suggested for adenoid cystic carcinoma [15] and for epithelial-myoepithelial carcinoma [7].

Acknowledgements This work was partially supported by a grant from the Núcleo Regional do Sul da Liga Portuguesa Contra o Cancro. The authors acknowledge skilful technical support from Cristina A. Teixeira and Teresa Pereira.

References

1. Batsakis JG (1980) Salivary gland neoplasia: an outcome of modified morphogenesis and cytodifferentiation. Oral Surg Oral Med Oral Pathol 49:229–232
2. Batsakis JG, Regezi JA, Luna MA, El-Naggar A (1989) Histogenesis of salivary gland neoplasms: a postulate with prognostic implications. J Laryngol Otol 103:939–944
3. Dardick I (1991) Histogenesis and morphogenesis of salivary gland neoplasms. In: Ellis GL, Auclair PL, Gnepp DR (eds) Surgical pathology of the salivary glands. Saunders, Philadelphia, pp 108–128
4. Dardick I, Nostrand AWP van (1987) Morphogenesis of salivary gland tumors. A prerequisite to improving classification. Pathol Ann 22:1–53
5. Dardick I, Byard RW, Carnegie JA (1990) A review of the proliferative capacity of major salivary glands and the relationship to current concepts of neoplasia in salivary glands. Oral Surg Oral Med Oral Pathol 69:53–67
6. Evans HL, Batsakis JG (1984) Polymorphous low-grade adenocarcinoma of minor salivary gland origin. Report of 14 cases of a distinctive neoplasms. Cancer 53:935–942
7. Fonseca I, Soares J (1993) Epithelial-myoepithelial carcinoma of the salivary glands. A study of 22 cases. Virchows Arch [A] 422:389–396
8. Fonseca I, Soares J (1993) Proliferating cell nuclear antigen (PCNA/cyclin) immunohistochemistry in epithelial-myoepithelial carcinoma of the salivary glands. Arch Pathol Lab Med 117:993–995
9. Fonseca I, Soares J (1996) Basal cell adenocarcinoma of minor and sero-mucous glands of the head and neck region. Semin Diagn Pathol 13:128–137
10. Hall PA (1992) Differentiation, stem cells and tumour histogenesis. Recent Adv Histopathol 15:1–15
11. Luna MA, Batsakis JG, Ordonez NG, Mackay B, Tortoledo ME (1987) Salivary gland adenocarcinomas: a clinicopathologic analysis of three distinctive types. Semin Diagn Pathol 4:117–135
12. Mackillop WJ, Ciampi A, Till JE, Buick RN (1989) A stem cell model of human tumor growth: implications for tumor clonogenic assays. J Natl Cancer Inst 70:9–16
13. Morinaga S, Shimosato Y (1989) Myoepithelial cells in normal and neoplastic salivary gland tissue. Dig Dis Pathol 11:127–145

14. Murakami M, Ohtani I, Hojo H, Wakasa H (1992) Immunohistochemical evaluation with Ki-67: an application to salivary gland tumors. J Laryngol Otol 106:35–38
15. Perzin KH, Gullane P, Clairmont AC (1978) Adenoid cystic carcinoma arising in salivary glands. A correlation of histologic features and clinical course. Cancer 42:265–282
16. Saka T, Yamamoto Y, Takahashi H (1991) Comparative cytofluorometric DNA analysis of pleomorphic adenoma and adenoid cystic carcinoma of the salivary glands. Virchows Arch [B] 61:255–261
17. Seifert G (1996) Classification and differential diagnosis of clear and basal cell tumors of the salivary glands. Semin Diagn Pathol 13:95–103
18. Seifert G, Sobin LH (1991) Histological classification of salivary gland tumors. In: World Health Organization. International histological classification of tumors. Springer, Berlin Heidelberg New York
19. Simpson RHW, Skálová A (1995) MIB1 in polymorphous low grade adenocarcinoma and adenoid cystic carcinoma of the salivary glands (abstract). Pathol Res Pract 191:779–780
20. Skálová A, Leivo I (1996) Cell proliferation in salivary gland tumors. Gen Diagn Pathol 142:7–16
21. Snell S, Pierce GB (1994) Maturation arrest of stem cell differentiation is a common pathway for the cellular origin of teratocarcinomas and epithelial cancers. Lab Invest 70:6–22
22. Yang L, Hashimura K, Qin C, Shrestha P, Sumitomo S, Mori M (1993) Immunoreactivity of proliferating cell nuclear antigen in salivary gland tumours: an assessment of growth potential. Virchows Arch [A] 422:481–486