Virchows Arch (1999) 434:137–143

ORIGINAL ARTICLE

Yuichi Fujita · Toshimichi Yoshida · Yasuo Sakakura
Teruyo Sakakura

Reconstruction of pleomorphic adenoma of the salivary glands in three-dimensional collagen gel matrix culture

Received: 26 March 1998 / Accepted: 1 September 1998

Abstract The morphogenesis of salivary gland pleomorphic adenoma was examined in vitro using three-dimensional (3-D) collagen gel culture. Pleomorphic adenoma cells were isolated from three parotid gland tumours and cultured as monolayers, after which they were subcultured in floating-collagen gel sandwiches. Cells cultured in both conditions were immunohistochemically characterized and compared using antibodies against various proteins representative of each histological component of salivary glands. Monolayers had myoepithelial characteristics, being positive for vimentin and α-smooth muscle actin. In collagen gels, however, the cells assembled in epithelial nests, showing an architecture similar to that of pleomorphic adenoma. The nests were composed of duct-lining epithelial cells that were positive for epithelial markers, surrounded by myoepithelial cells. Collagen gel culture induces multidirectional differentiation of adenoma cells, suggesting that pleomorphic adenomas originate from stem or reserve cells.

Key words Salivary gland · Pleomorphic adenoma · Collagen · Cell culture · Immunohistochemistry

Introduction

The pleomorphic adenoma is the most common neoplasm of human salivary glands. It is characterized by mixtures of epithelial, myoepithelial, and stromal components, with considerable morphological variability within single neoplasms. The epithelial components show various growth patterns, forming solid, cystic, trabecular, or papillary structures consisting of duct-lining epithelial as well as myoepithelial cells.

Immunohistochemical and electron microscopic studies have demonstrated that inner layers of cell nests consist of epithelial elements resembling those of acini or intercalated ducts [6]. These cells are positive for epithelial markers, such as cytokeratin (CK) and epithelial membrane antigen (EMA). Cells of the outer layers, in contrast, stain for α-smooth muscle actin (α-SMA) and vimentin (VN), but are negative for epithelial markers, reflecting their myoepithelial differentiation [3, 5, 6, 9, 11, 17, 20, 23, 24]. The stromal components of the tumour also exhibit variety, with myxoid, chondroid, myxochondroid, fibrous and vascular characteristics, and constituent cells may also have a myoepithelial nature [6]. These observations suggest that the tumour cells of pleomorphic adenomas originate from stem or reserve cells and are multi-potential for differentiation.

The characteristics of cell lines or strains derived from salivary gland pleomorphic adenoma cells have been examined by many authors [9, 20, 25]. In monolayer culture they show strong myoepithelial properties, with only weak staining for epithelial markers including EMA [8, 25]. These characteristics have led to the suggestion that tumour cells are of myoepithelial cell origin [8, 9], but this discrepancy between in vivo and in vitro findings may be due to the influence of different environments on growth and differentiation.

Several studies have shown that culture in three-dimensional (3-D) collagen gels of normal epithelial cells obtained from thyroid [18, 22], mammary [26], tracheal [12], and nasal mucosal glands [10] is associated with organization into tubes and glandular structures. We used this culture system to examine the morphogenesis of pleomorphic adenomas in vitro. For this purpose, tumour cells were isolated from three parotid gland pleomorphic adenomas, cultured in a 3-D collagen gel matrix, and characterized immunohistochemically. They were compared with the same cells grown as monolayers.

T. Yoshida (✉) · T. Sakakura
Department of Pathology, Mie University, School of Medicine,
2-174 Edobashi, Tsu, Mie 514, Japan
e-mail:t-yosida@doc.medic.mie-u.ac.jp
Tel.: +81 59 231 5009, Fax: +81 59 231 5210

Y. Fujita · Y. Sakakura
Department of Otorhinolaryngology, Mie University,
School of Medicine, Tsu, Mie 514, Japan

Materials and methods

Tumour cells were obtained from three male patients, all of whom had undergone partial superficial parotidectomy for resection of their neoplasms. Histopathologically all were pleomorphic adenomas. The cell strains derived from these tumours were designated as PA1, PA2, and PA3.

For primary culture, pieces of the resected tumours were placed in ice-cold Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum (FCS), streptomycin (0.5 mg/ml) and penicillin (500 U/ml) and transferred to the laboratory. The materials were minced into fine pieces with scissors and washed several times with Hank's solution containing antibiotics. Next, the tissue fragments were transferred into DMEM containing 0.1% collagenase (CLS2, Worthington, Freehold, NJ) and agitated gently for 8–12 h at room temperature. The resulting cell suspension was stood for 15 min and the supernatant was collected. After being combined with 2 vol. of fresh DMEM supplemented with 10% FCS, the isolated cells were recovered by centrifugation at 1000 rpm for 5 min. Finally, cell pellets were resuspended in culture medium (DMEM/10% FCS, 0.1 mg/ml streptomycin, 100 U/ml penicillin), plated in T-75 tissue culture flasks and incubated in 5% CO_2/95% air at 37°C. The medium was changed every 3 days. On reaching 80% confluency, the cells were harvested by trypsinization with 0.05% trypsin and 0.02% ethylenediaminetetraacetic acid (EDTA) solution and subcultured onto glass coverslips or transferred to collagen gel cultures.

Preparation of type I collagen from rat tail tendons and collagen gel culture was performed by a modified procedure described by Dunn et al. [4]. In brief, 5 volumes of collagen solution (2.2 mg/ml), 5 vol. of 2× concentrated Iscove's Modified Dulbecco's Medium (IMDM) and 1 vol. of FCS were mixed and poured into the wells of 12-well plastic plates (1 ml/well). The plates were warmed to 37°C for 30 min to let the gels form. Next, 1 ml of cell suspension diluted with culture medium containing $3–5\times10^5$ cells was poured on each formed gel. The cells were allowed to attach to the surface of the gels for 12 h, and the medium and nonadherent cells were then removed, after which 1 ml of a second collagen gel layer was overlaid to form collagen gel sandwiches. After gelatinization, the gels were further covered with 2 ml of the medium, and 1 day after seeding they were released from the walls of the wells and floated in the culture medium. These collagen gel cultures were then maintained for up to 4 weeks with the culture medium changed every 3 days. On days 5, 10, 15 and 30, collagen gel cultures were fixed with 10% formalin in 0.1 M phosphate buffer. Some cultures were treated with 5 mM monensin for 6 h before fixation, to accumulate secretory proteins. The gels were embedded in paraffin and sectioned at 4 μm for histological assessment.

Collagen gel and monolayer cultures of pleomorphic adenoma cells were characterized by immunohistochemistry with antibodies against antigens representative for various histological components of salivary glands. Paraffin sections of the original tumours along with normal portions of salivary glands were also immunostained with the same antibodies as controls. The antigens evaluated in this study were α-amylase (AL), lysozyme (LZ), EMA, CK, VN, α–SMA and S-100 protein. The primary antibodies used are summarized in Table 1. Staining was performed by the labelled streptavidin biotin (LSAB) peroxidase technique. After deparaffinization and rehydration, the sections were exposed to methanol containing 0.3% H_2O_2 to inhibit endogenous peroxidase activity. After incubation with the primary antibodies and treatment with a SensiTek HRP kit (Scytek Laboratories, Logan, Utah), the labelling was visualized with 0.02% diaminobenzidine and 0.01% H_2O_2. Finally, the sections were lightly counterstained with haematoxylin. To provide negative controls, the blocking solution included in the SensiTek HRP kit was substituted for the antibodies.

Monolayer cultures on glass coverslips were stained by the indirect immunofluorescence technique using fluorescein isothiocyanate-conjugated goat anti-mouse IgG or anti-rabbit IgG (MBL, Nagoya, Japan) as the secondary antibodies. In brief, the cells were fixed with 10% formalin for 10 min, and subsequently treated with the same fixative supplemented with 0.02% Triton X-100 for 10 min. After being washed with PBS and treated with 10% normal goat serum for 30 min, the cells were incubated with appropriately diluted primary antibodies for 12 h at 4°C. Labelling was analysed under a fluorescence microscope.

Table 1 Technical data for the antibodies used (*EMA* epithelial membrane antigen, *α-SMA* α-smooth muscle actin)

Antigen	Source	Clonality	Working dilution
α-Amylase	Nordic immunology, Capstrano Beach, Calif.	Poly[a]	1:1000
Lysozyme	DAKO Japan, Kyoto, Japan	Poly[a]	1:1000
EMA	DAKO Japan	E29[b]	1:100
Cytokeratin	Sigma, St Louis, Mo.	C11[b]	1:400
Vimentin	DAKO Japan	V9[b]	1:50
α-SMA	DAKO Japan	1A4[b]	1:50
S-100	DAKO Japan	Poly[a]	1:200

[a] Rabbit polyclonal antibody
[b] Monoclonal antibody

Results

Within 24 h of plating, most cells in primary culture had attached to the surfaces of the flasks and begun to spread and proliferate. Within 2 weeks, confluent monolayers were obtained (Fig. 1A). Cells were harvested from these and plated on collagen gel layers, where they attached and began to form globular or polygonal-shaped clusters within 12 h (Fig. 1B). Other cells, which were free from the clusters, were spindle shaped or spherical. After the second collagen gel layers were overlaid and the gels were released from the plastic substratum of the wells, the cell clusters grew in size and some of them fused. In addition, the gels began to contract and become thick and opaque. This phenomenon went on for a few weeks, and it became impossible to observe the details by phase-contrast microscopy within a few days. Haematoxylin-eosin staining of paraffin sections (Fig. 1C-F) of fixed collagen gel cultures revealed that most cells had formed ductal structures complete with a lumen by day 5 (Fig. 1C). Surrounding these were myoepithelial cells, as in the original tumours. On day 10 some of the myoepithelial cells appeared to have separated from the duct-like assemblies and migrated into the gel matrix (Fig. 1D). On day 30 the epithelial nests had grown, but the architecture described above was preserved (Fig. 1E, F).

The histological characteristics of the three original tumours were assessed using paraffin sections stained with haematoxylin-eosin. The original specimens of PA1 and PA3 consisted of myxoid areas containing nests of duct-acinar structures, which occupied approximately 30% of each section. Half area of the original PA2 tumour showed a myxoid stroma containing a few duct-acinar structures, while the other half consisted of proliferating myoepithelial cells. No chondroid component was seen in any of the three cases.

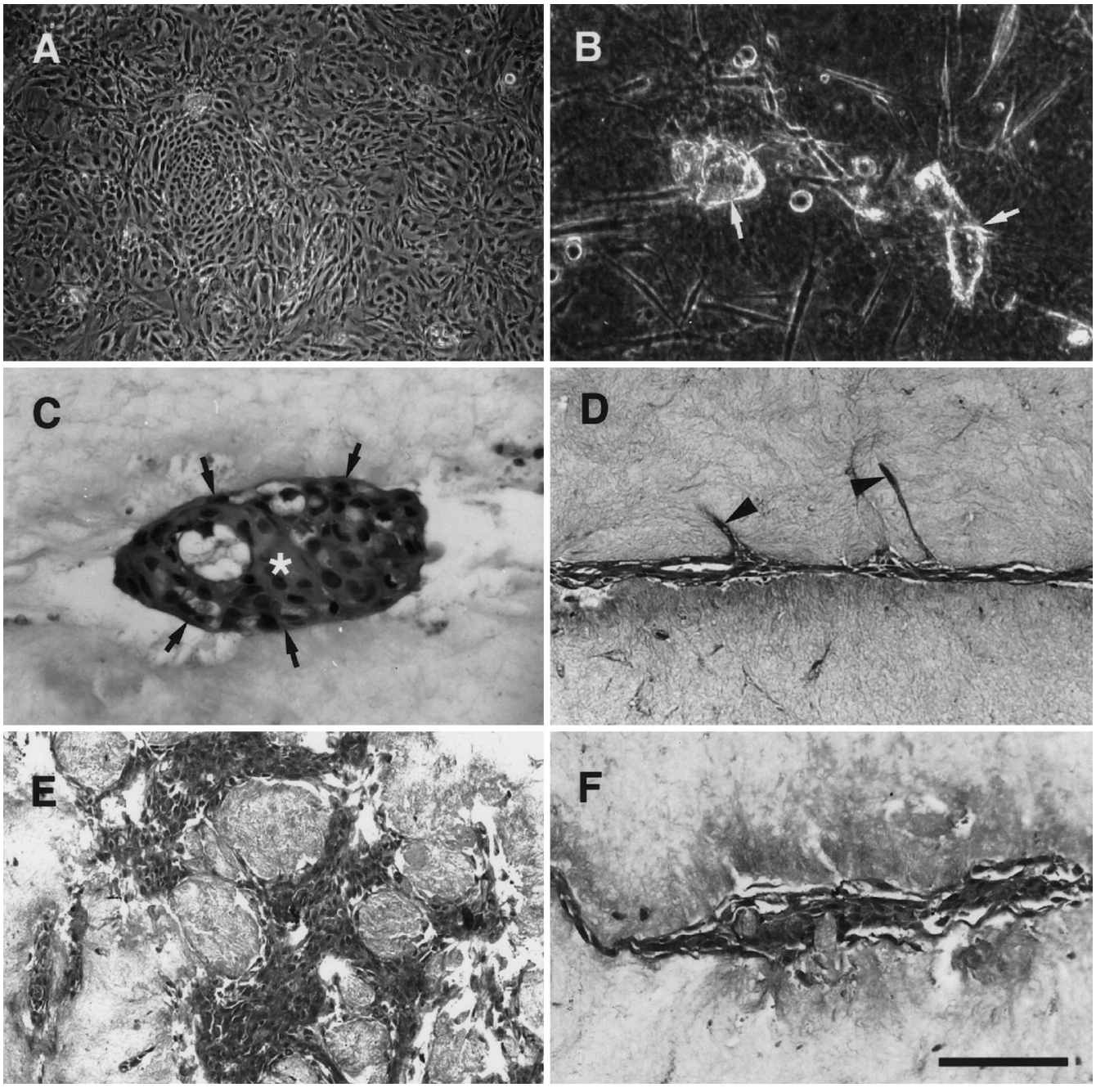

Fig. 1A–F Monolayer and collagen gel culture of pleomorphic adenoma cells. **A** Pleomorphic adenoma cells are polygonal or spindle-shaped when cultivated as conventional monolayers. **B** When transferred to collagen gel culture, the cells form spherical assemblies (*arrows*) within 12 h. Paraffin sections of collagen gel cultures were prepared on days 5, 10 and 30 and stained with haematoxylin-eosin. **C** On day 5, a epithelial cell nest with a lumen (*), surrounded by flattened myoepithelial-like cells (*arrows*), is shown. **D** Myoepithelial cells are apparently migrating away from an epithelial nest (*arrowheads*) on day 10. **E** A tangential section and **F** a cross section of collagen gel cultures on day 30. The cell nests have grown in size, but architectural characteristics of epithelial cells and surrounding myoepithelial cells are preserved. *Bars*: **A, E** 40 μm, **B, C** 25 μm, **D** 100 μm, **F** 50 μm

Immunohistochemical characterization of the cells was performed on the original tumour tissue together with normal portions of salivary glands and monolayer and collagen gel cultures. In normal salivary glands AL was only positive in acinar cells, whereas LZ was present in acinar cells and epithelial cells of intercalated ducts. EMA was positive at the luminal surfaces throughout the duct system, including the intercalated, striated, and excretory ducts. CK was positive in all epithelial cells of the acini and ducts, but negative in myoepithelium. VN and α-SMA were positive only in myoepithelial cells. S-100 protein was positive in acinar cells, and partially in myoepithelium around acini (data not shown). In the tumour tissues, AL was negative and LZ was positive in

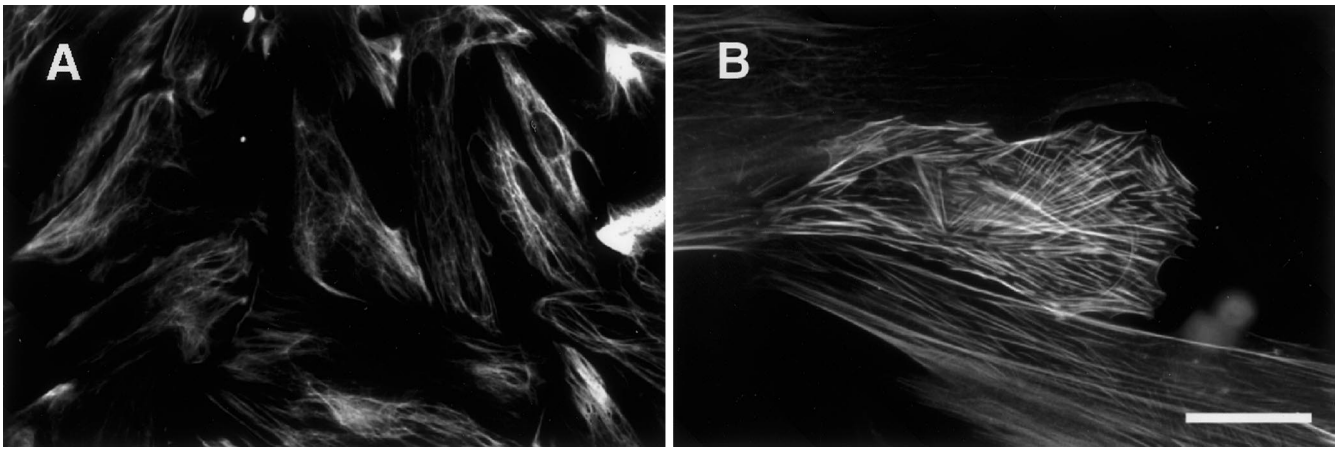

Fig. 2A, B Immunofluorescence of pleomorphic adenoma cells cultured as monolayers. The cells are labelled for **A** vimentin and **B** α-smooth muscle actin. *Bars:* **A** 100 μm, **B** 50 μm

some of the epithelial cells forming ductal structures and in the spindle cells in the myxoid and chondroid areas. EMA was positive in the epithelial cells comprising the walls of ductal structures and at the luminal surfaces (Fig. 3A). Anti-CK antibodies labelled the epithelial components but not the surrounding myoepithelial cells (Fig. 3B), which were positive for VN and α-SMA (Fig. 3C). VN was also positive in the stromal cells and in the spindle-shaped myoepithelial cells forming solid clusters, whereas α-SMA was negative. S-100 protein showed only weak positivity in the parts of the epithelial cell nests not forming ductal structures (data not shown).

In monolayer cultures, AL was negative in all the cell strains. EMA and LZ were negative in PA1 and PA3, but positive in less than 5% of the PA2 cells. Cells positive for CK were less than 5% of PA1–3, but almost all of cells were labelled with VN (Fig. 2A). α-SMA was positive in degrees varying (approximately 5–80%) among the strains (Fig. 2B). The majority of PA1 cells and a fraction of PA2 and PA3 cells were positive for S-100 protein.

In 3-D collagen gel cultures, AL was found to be positive within the lumina and in a few epithelial-like cells of ductal structures (Fig. 3D). LZ was weakly labelled in all types of cells forming nests in PA3 and in a few scattered epithelial-like cells in PA2. EMA was positive in luminal surfaces of the ductal structures and a few scattered epithelial-like cells (Fig. 3E). CK was strongly positive in the epithelial-like cells, but negative in the myoepithelial-like cells (Fig. 3F). Myoepithelial-like cells adjacent to collagen substratum were labelled by VN (Fig. 3G) in PA1–3 and by α-SMA (Fig. 3H) in PA1 and PA2, whereas the epithelial cells were negative. Cells positive for S-100 protein showed a scattered distribution in the nests. Isolated cells in the gel matrix were strongly positive for VN and S-100 protein (Table 2). Mucinous substances within the lumina in collagen gel cultures stained strongly with periodic acid-Schiff (PAS) and weakly with Alcian blue (AB). The gel matrix around cell nests was also positive for PAS and AB (Fig. 3I, K).

Discussion

The present study clearly demonstrates that the phenotype of salivary gland pleomorphic adenoma cells in culture depends on the environmental conditions. Culture of pleomorphic adenoma cells in a gelatin sponge matrix results in the appearance of an epithelial-like arrangement of cells that are nonepithelial in conventional monolayer culture [21]. In Matrigel, the pleomorphic ad-

Table 2 Expression of histological markers by pleomorphic adenoma cells in monolayer and collagen gel culture. Positivities in each cell strain for PA1/PA2/PA3, respectively. In monolayer culture, –, +, 2+ and 3+ denote that positive cells are absent, or comprise <30%, 30–70%, and 70%+, of the total, respectively. In collagen gel culture, –, +, and 2+ represent negative, scattered or weak positivity, and strong positivity in the majority of cells, respectively. (*Free* isolated cells not belonging to cell assemblies)

Markers	Monolayer culture	3-D collagen gel culture			
		Epithelial cells	Myoepithelial cells	Free	Mucus
α-Amylase	–	+/+/+	–	+/–/–	2+/–/–
Lysozyme	–/+/–	–/+/+	–/–/+	–/–/+	–
EMA	–/+/–	+/+/+	–	–	–
Cytokeratin	+/+/+	2+/2+/2+	–	–	–
Vimentin	3+/3+/3+	–	2+/2+/2+	2+/2+/2+	–
α-SMA	+/3+/2+	–	2+/+/–	–	–
S-100	2+/+/+	+/+/+	+/+/+	+/+/+	–

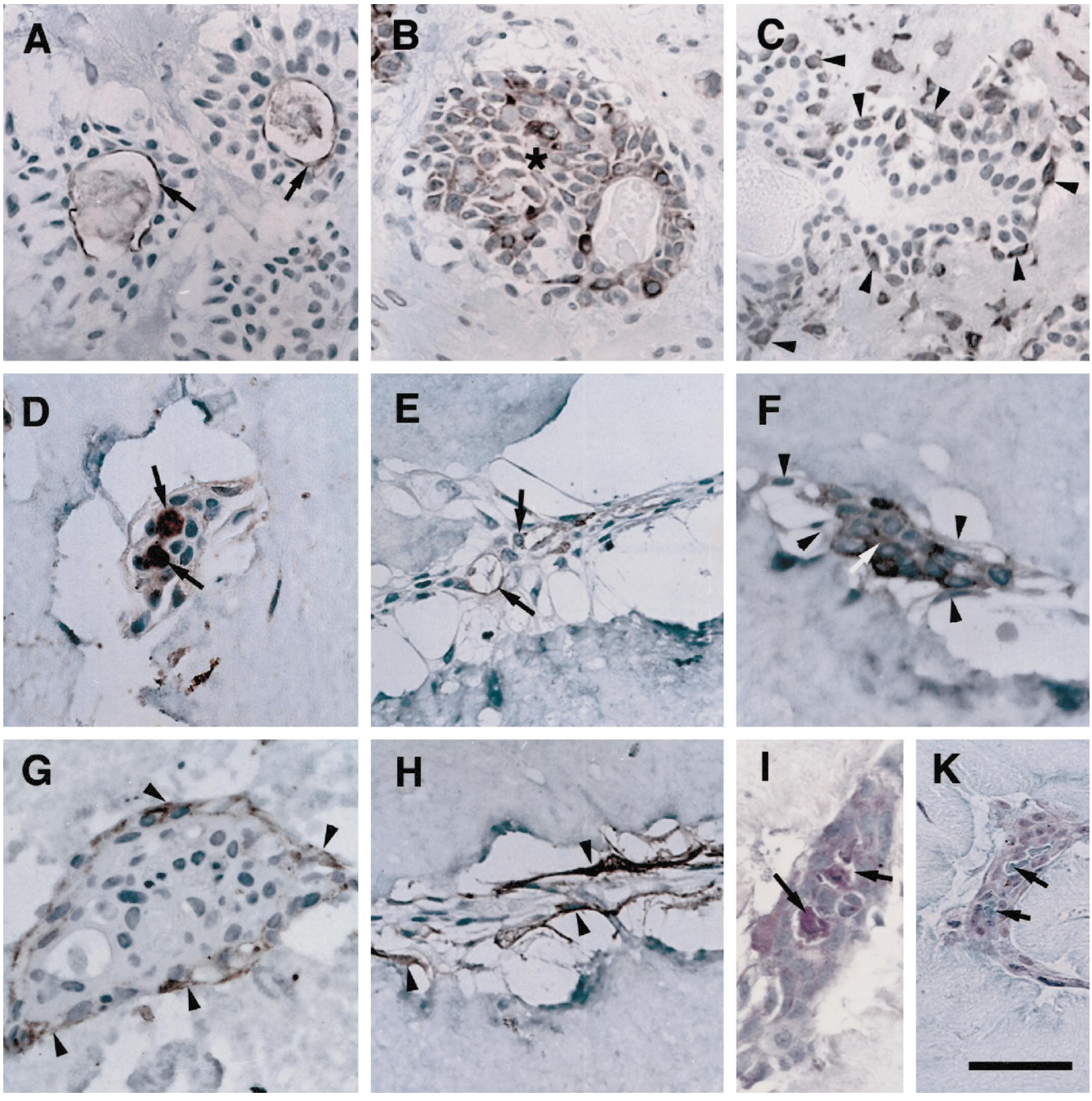

Fig. 3 Immunohistochemistry of **A–C** tumour and **D–K** collagen gel cultures of pleomorphic adenoma. In the tumour inner cells in duct-acinar structure are labelled for **A** epithelial membrane antigen (EMA, *arrows*) and **B** cytokeratin (*). **C** The outer cells are positive for vimentin (*arrowheads*), suggesting a myoepithelial nature. **D** In collagen gel cultures, α-amylase is positive in mucinous material within a ductal lumen (*arrows*). **E** Luminal surfaces of ducts are positive for EMA (*arrows*). **F** Cytokeratin is present in epithelial (*white arrow*), but not myoepithelial, cells (*arrowheads*). **G** Vimentin and **H** α-SMA are labelled only in myoepithelial cells (*arrowheads*). Mucinous material in lumina is positive for **I** periodic acid–Schiff and **K** alcian blue (AB). Gel matrix surrounding the cell nests is also weakly positive for AB. *Bars*: **A–C, D, F, G, I** 25 μm, **E, H, K** 50 μm

enoma cell lines, UNC4 and AP2, grow in organized clumps and ductules [13, 25]. Recently, the floating-collagen gel sandwich culture technique used in this study has attracted increasing interest for the in vitro study of epithelial cells [18, 19]. Not only is the architectural organization preserved, but cellular functions resembling those in vivo can be maintained. We found that pleomorphic adenoma cells cultured in such collagen gels reorganize into epithelial nests similar to the original tumour tissues, with functional differentiation as indicated by the expression of epithelial and myoepithelial markers. The patterns were compatible with those found for these tumours in previous immunohistochemical studies [3, 5, 6, 11, 17, 23, 24].

The anti-CK antibody used in this study reacts with human CK 4, 5, 6, 8, 10, 13 and 18. It has been reported that the subtypes of CKs expressed differ between myoepithelial and epithelial cells of normal salivary glands, with acinar and ductal epithelial cells being positive for CK 6, 8 and 18 and myoepithelial cells staining for CK 14, 17 and 19 [1,14]. Using this antibody, we were able to distinguish epithelial components from myoepithelial cells. We also confirmed positive labelling of myoepithelial-like cells in culture using an anti-CK 17 antibody (data not shown).

Many hypotheses have been proposed to account for the histogenesis of salivary gland tumours, including pleomorphic adenomas. The bi-cellular theory is a current histogenic concept [7], which assumes the presence of reserve or stem cells located in the intercalated and excretory ducts as a source for cell renewal and induction of tumours. Many types of salivary gland tumours, including pleomorphic adenomas, adenoid cystic carcinomas, Warthin's tumours, oncocytomas and acinic cell tumours, are considered to originate from such intercalated duct cells. Other types, such as mucoepidermoid carcinomas and squamous cell carcinomas, may originate from excretory duct cells, although a common stem cell might be the origin of all types [16]. While this theory is plausible, direct evidence for the existence of a stem cell or reserve cell is lacking. In addition, all cell types in the normal salivary gland can rapidly enter the cell cycle in steady state and pathophysiological conditions, suggesting that they could all be targets for neoplastic transformation [2, 15]. While studies using cell or tumour lines have suggested that myoepithelial cells or their precursors may give rise to pleomorphic adenoma, this conclusion was reached mainly on the basis of the clearly myoepithelial characteristics observed in monolayer culture, and this is clearly not a situation analogous to the in vivo case [9, 20, 25]. When cultured in floating-collagen gel sandwiches, the tumour cells reconstructed ductal structures and redifferentiated into epithelial and myoepithelial cells, in addition to which the proportion of cells with epithelial characteristics was significantly increased. All cell strains formed similar cell nests in collagen gel culture, despite the various histological patterns of the original tumours. This suggests that pleomorphic adenoma cells can change their phenotype for that of acinar or ductal epithelial cells or that of myoepithelial cells, depending on the environment. This might explain the different characters of tumour cells in monolayer cultures and tissues. Although not solving the problem of the origin of myoepithelial or stem cells, the multi-potentiality of pleomorphic adenoma cells might be responsible for their demonstrably complicated histological features.

The present 3-D culture system appears to be a useful in vitro model for understanding the mechanisms of morphogenesis of salivary gland tumours and of similarly unusual lesions in other organs.

Acknowledgements We thank Ms. Satsuki Ogihara for her technical expertise and Dr. Malcolm A. Moore for editorial assistance.

References

1. Araujo VC, Sousa SO (1996) Expression of different keratins in salivary gland tumours. Eur J Cancer [B] 32:14–18
2. Bassett RE, Dardick I, Burford-Mason AP, MacKay A, Burgess K, Gullane PJ (1995) Postirradiated submandibular gland: a potential model to study salivary gland radioprotection and tumorigenesis. J Otolaryngol 24:184–190
3. Caselitz J, Lunau U, Hamper K, Walther B, Schmiegelow P (1985) The pleomorphic adenoma of salivary glands transplanted on athymic mice. A light microscopical and immunohistochemical investigation. Virchows Arch [A] 408:191–209
4. Dunn JC, Tompkins RG, Yarmush ML (1992) Hepatocytes in collagen sandwich: evidence for transcriptional and translational regulation. J Cell Biol 116:1043–1053
5. Egan M, Crocker J, Nar P (1988) Localization of salivary amylase and epithelial membrane antigen in salivary gland tumours by means of immunoperoxidase and immunogold-silver techniques. J Laryngol Otol 102:242–247
6. Erlandson RA, Cardon-Cardo C, Higgins PJ (1984) Histogenesis of benign pleomorphic adenoma (mixed tumour) of the major salivary glands. An ultrastructural and immunohistochemical study. Am J Surg Pathol 8:803–820
7. Eversole LR (1971) Histogenetic classification of salivary tumours. Arch Pathol 92:103–112
8. Gallo O, Bani D (1994) Increasing evidence that the myoepithelial cell is the actual ancestor of pleomorphic adenoma of the salivary gland. Arch Otolaryngol Head Neck Surg 120:675
9. Gallo O, Bani D, Toccafondi G, Almerigogna F, Storche OF (1992) Characterization of a novel cell line from pleomorphic adenoma of the parotid gland with myoepithelial phenotype and producing interleukin-6 as an autocrine growth factor. Cancer 70:559–568
10. Guo Y, Majima Y, Fujita Y, Shimizu T, Yoshida T, Sakakura T, Sakakura Y (1998) Cultured human nasal gland cells in a three-dimensional collagen gels. In Vitro Cell Dev Biol Anim 34:16–18
11. Gusterson BA, Lucas RB, Ormerod MG (1982) Distribution of epithelial membrane antigen in benign and malignant lesions of the salivary glands. Virchows Arch [A] 397:227–233
12. Jacquot J, Spilmont C, Burlet H, Fuchey C, Buisson AC, Tournier JM, Gaillard D, Puchelle E (1994) Glandular-like morphogenesis and secretory activity of human tracheal gland cells in a three-dimensional collagen gel matrix. J Cell Physiol 161:407–408
13. Jaeger MMM, Araujo VC, Jaeger RG (1997) Effect of spatial arrangement of the basement membrane on cultured pleomorphic adenoma cells. Study by immunocytochemistry and electron and confocal microscopy. Virchows Arch 430:467–477
14. Li C, Okamoto Y, Ohmura H, Ogawa K, Shrestha P, Mori M (1996) Expression of cytokeratins in Warthin's tumour (adenolymphoma) of parotid glands: specific detection of individual cytokeratin types by monoclonal antibodies. Eur J Cancer [B] 32:352–358
15. Norberg L, Dardick I, Burford-Mason AP (1996) Differentiating myoepithelial and acinar cells in rat neonatal parotid gland and histogenetic concepts for salivary gland tumours. J Oral Pathol Med 25:474–480
16. Roger JL, Margaret KB (1996) Neuroectodermal antigens persist in benign and malignant salivary gland tumour cultures. Arch Otolaryngol Head Neck Surg 122:551–558
17. Saito I, Teratani K, Inoue M, Saito A, Funatsu K, Moro I (1984) Immunohistochemical characterization of functional markers in human minor salivary gland tumours. J Oral Pathol 13:525–534
18. Sasaki M, Sawada N, Minase T, Satoh M, Mori M (1991) Collagen-gel-embedded three-dimensional culture of human thyroid epithelial cells: comparison between the floating sandwich method and the dispersed embedding method. Cell Struct Funct 16:209–215
19. Sasaki M, Sawada N, Chiba H, Miyao N, Tsukamoto T, Satoh M, Mori M (1994) Cellular polarity correlates with vimentin

distribution, but not to keratin, in human renal cell carcinoma cells in vitro. Cell Struct Funct 19:115–121
20. Shirasuna K, Sato M, Miyazaki T (1980) A myoepithelial cell line established from a human pleomorphic adenoma arising in minor salivary gland. Cancer 15:297–305
21. Takeuchi J, Sobue M, Yoshida M, Esaki T, Kato Y (1975) Pleomorphic adenoma of the salivary gland. With special reference to histochemical and electron microscopic studies and biochemical analysis of glycosaminoglycans in vivo and in vitro. Cancer 36:1771–1789
22. Toda S, Sugihara H (1990) Reconstruction of thyroid follicules from isolated porcine follicle cells in three-dimensional collagen gel culture. Endocrinology 126:2027–2034
23. Toto PD, Hsu DJ (1985) Product definition of pleomorphic adenoma of minor salivary glands. J Oral Pathol 14:818–32
24. Warner TF, Seo IS, Azen EA, Hafez GR, Zarling TA (1985) Immunocytochemistry of acinic cell carcinomas and mixed tumours of salivary glands. Cancer 56:2221–2227
25. Witsell DL, Weissler MC, Livanos E, Bova-Hill C, Gilmer TM (1993) Unique chromosomal rearrangement and mucin production in a novel salivary myoepithelial cell strain. Arch Otolaryngol Head Neck Surg 119:1151–1157
26. Yang J, Elias JJ, Petrakis NL, Wellings SR, Nandi S (1981) Effects of hormones and growth factors on human mammary epithelial cells in collagen gel culture. Cancer Res 41:1021–1027