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Effect of spatial arrangement of the basement membrane on cultured pleomorphic adenoma cells. Study by immunocytochemistry and electron and confocal microscopy

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Abstract In a cell line from human pleomorphic adenoma (AP2 cells) we studied the response of these cells to basement membrane proteins. The culture was characterized as myoepithelial-like by transmission electron microscopy and immunocytochemistry. AP2 cells were grown in contact with a reconstituted basement membrane (Matrigel). Cells grown on Matrigel showed conspicuous phenotypic alterations, depending on how the substrate was applied. Cells grown on the top of Matrigel developed a dendritic phenotype, exhibiting thin, long and intercommunicating cytoplasmic extensions resembling normal myoepithelial cells. Cells grown inside Matrigel formed multi-layered clusters. Light, confocal and transmission electron microscopy showed that these clusters were formed by double-layered epithelioid cells delimiting luminal spaces. The cells facing the lumen were cuboidal, showing microvilli at the apical plasmalemmal and junctional complexes. The spatial arrangement of basement membrane is a key modulator of morphogenetic changes and cytodifferentiation of tumour myoepithelial cell lineage in culture.

Key words Salivary gland neoplasms · Myoepithelial cell · Extracellular matrix · Matrigel

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Introduction

Pleomorphic adenoma is a salivary gland tumour with a complex histological picture, characterized by epithelial, myoepithelial and modified myoepithelial elements intermingled with tissue of mucoid, myxoid or chondroid appearance [53, 54]. There is general agreement in the literature that myoepithelial cells play a decisive role in the histological diversity displayed by pleomorphic adenoma [1, 3, 5, 13–15, 31, 38]. However, the regulatory factors involved in the different microscopic patterns exhibited by this neoplasm have not been clarified.

Extracellular matrix (ECM) is a good candidate as a regulatory factor of the broad histological spectrum of pleomorphic adenoma. In normal glandular tissues, ECM plays a fundamental role in organogenesis and morphogenesis, and also in the maintenance of glandular differentiated state [32, 37]. Although there is little evidence of the influence of ECM in salivary gland tumours, experiments carried out in different clones of cultured mammary cells have suggested that ECM is important in the differentiation of stem cells into ductal or alveolar elements [39, 44, 47–50].

Among ECM components, basement membrane proteins have been reported to be an important regulatory factor of the phenotype of pleomorphic adenoma. However, most of the relevant studies were based on descriptive findings reflecting the presence of basement membrane proteins in different human salivary gland tumours [6, 10, 35, 51, 55]. There is a lack of in vivo and in vitro studies aimed at elucidating the role of basement membrane proteins in pleomorphic adenoma cells.

We established a cell line derived from human pleomorphic adenoma (AP2 cells), in order to study the response of these cultured cells to basement membrane proteins.We have demonstrated that the spatial arrangement of basement membrane is a key modulator of morphogenetic changes and cytodifferentiation of tumour myoepithelial cell lineage in vitro.

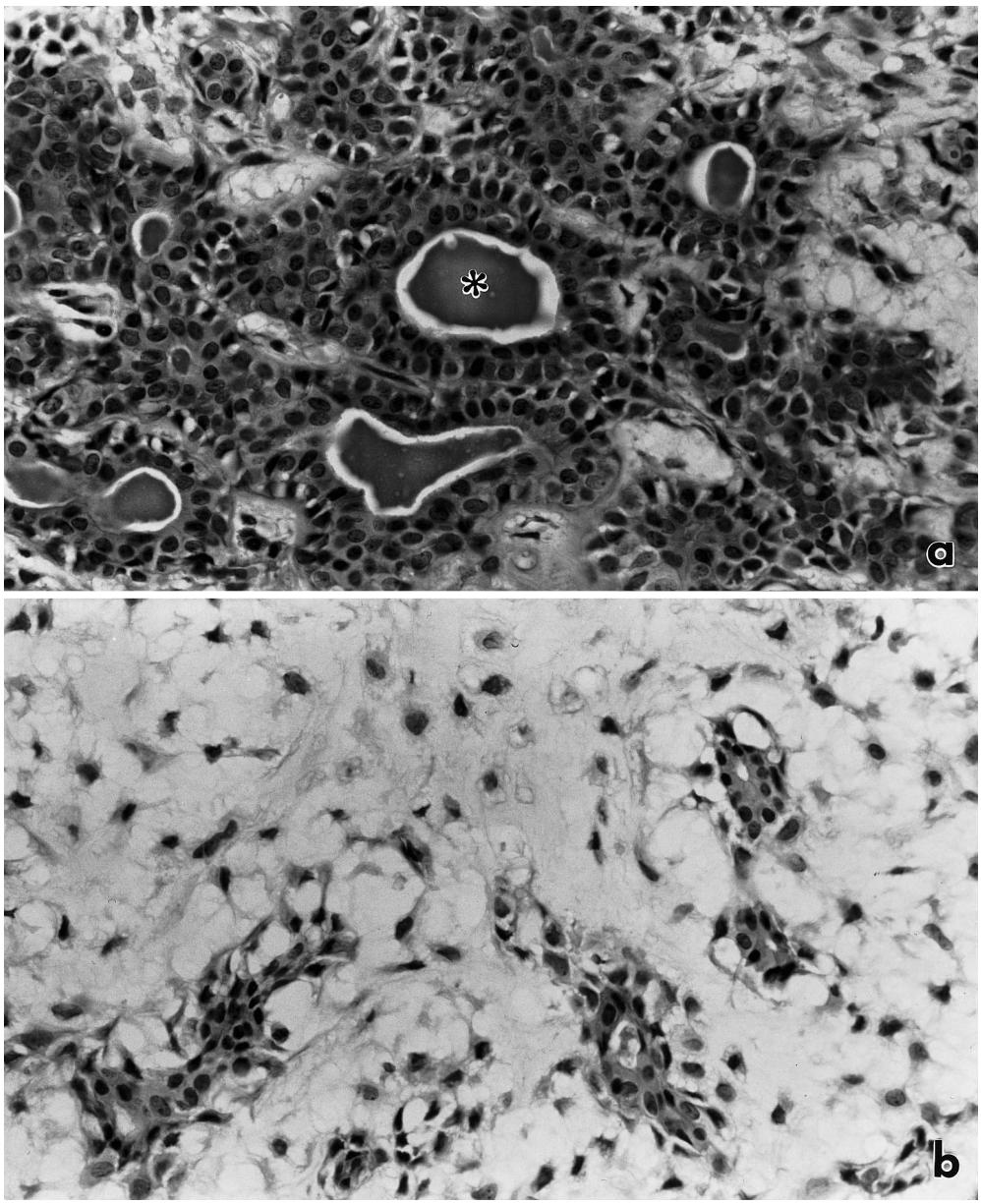


Fig. 1a, b The original tumour, showing typical features of pleomorphic adenoma. Double-layered ductiform structures are present, composed by inner epithelial and outer myoepithelial cells (a). Some ductiform structures are filled with eosinophilic material (a,*). Spindle-shaped and dendritic cells, enmeshed in a myxoid tissue, are also observed (b). H & E, x630

Materials and methods

A 34-year-old black man had a painless nodule in the right parotid gland. A clinical diagnosis of pleomorphic adenoma was made and the patient submitted to a total parotidectomy with preservation of the facial nerve. A final diagnosis of pleomorphic adenoma was made on the basis of clinical and histopathological examination.

We obtained a cell line by subculturing a primary culture derived from a human pleomorphic adenoma. This cell line is currently at the 20th passage. We used a standard protocol for isolation of epithelial cells, as described elsewhere [20, 29]. Briefly, fragments from this tumour were chemically digested with trypsin and then gently dissociated with Pasteur pipettes. Cells were primary cultured in high glucose Dulbecco's Modified Eagle's Medium (DMEM, Irvine Scientific, Santa Ana, Calif.) supplemented with 10% of fetal bovine serum (Gibco-BRL, Grand Island, N.Y.), 1% glutamine (Irvine Scientific), 1% antibiotic-antimycotic solution (Biofluids, Rockville, Md.) and 0.1% transferrin (Sigma Chemical, St. Louis, Mo.), 0.1% triiodothyronine (T3-Sigma), 0.25% insulin solution (Biofluids), 0.05% hydrocortisone (Sigma) and 0.1% epidermal growth factor (EGF; Biofluids). The cells were maintained in 25-cm^2 flasks in a humidified atmosphere of 5% CO_2 in air at 37°C.

After five passages, the cells were detached from the flasks using a 0.25% trypsin solution (Gibco) and plated either on glass coverslips (15 mm #1 round, Ted Pella, Redding, Calif.) or on polycarbonate filters of 4.7 cm^2 diameter and 0.4 μm pore size (Transwell cell culture inserts, Costar, Cambridge, Mass.).

Cells grown on coverslips were fixed in methanol at –20°C for 10 min and submitted to a standard immunofluorescence protocol [11] to detect cytokeratin (polypetides 14 and 19) and vimentin. Cytokeratin 14 was stained by a mouse monoclonal antibody from BioGenex (BioGenex Laboratories, San Ramon, Calif.), diluted 1/50 in phosphate-buffered saline (PBS). Cytokeratin 19 was labelled by a mouse monoclonal antibody from Sigma, diluted 1/500 in PBS. Vimentin was detected by a mouse monoclonal antibody from Amersham (Amersham, Arlington Heights, Ill.), diluted 1/100 in PBS. A sheep anti-mouse fluorescein conjugate (Amersham) was used as secondary antibody. All incubations were done for 45 min at room temperature. The mounting medium was 0.1%

of paraphenylenediamine (Sigma) and 10% of PBS in glycerol. Epithelial (Madin Darby Canine Kidney Cells, MDCK, American Type Culture Collection, ATCC # CCL 34) and mesenchymal (NIH 3T3, kindly provided by Dr. Silvio Gutkind, NIDR, NIH) cell lines were used as positive controls for cytokeratin and vimentin. Replacement of the primary antibody by PBS was used as negative control.

The observations and photographic recording were carried out in a Zeiss Axiophot fluorescence microscope, using the objectives 63X Plan Neofluor 1.4 NA, and 100X Plan Apochromatic, 1.4 NA.

For transmission electron microscopy, cells grown on polycarbonate filters were fixed by immersion in 3.0% glutaraldehyde, 2.0% paraformaldehyde, in 0.1 M sodium cacodylate buffer solution at pH 7.4 for 2 h, and post-fixed in 1% osmium tetroxide in the same buffer for 45 min. Then, filters were carefully removed from their supports, washed in distilled water, en bloc-stained with 0.5% uranyl acetate for 3 h, rinsed, and dehydrated in graded methanol. After immersion in propylene oxide, samples were embedded in resin (Spurr, Polysciences, Warrington, Pa.) and polymerized for 24 h at 60°C. Semithin sections (1 μm) were cut and stained with a mixture of 1% azure II, 2% methylene blue and 2% borax in distilled water. Ultrathin sections were stained with lead citrate and uranyl acetate and examined in a Jeol 100CXII transmission electron microscope.

Cells grown as described before were plated either on #1 round glass coverslips or on polycarbonate filters coated with a reconstituted basement membrane (Matrigel Basement Matrix, Collaborative Research, Bedford, Mass.). To study cell–Matrigel interactions, two coating procedures were used [29], as follows:

1. Thin gel method, for plating cells on the top of the gel. This preparation creates a two-dimensional environment for growing AP2 cells simulating a disrupted basement membrane: Matrigel was thawed at 4°C overnight. Then, using cooled pipettes, Matrigel was homogenized and diluted in cold serum-free DMEM, to reach a final concentration of 3 mg/ml (stock solution 13 mg/ml). This diluted Matrigel was added (50 μl/cm^2) to either cold coverslips or cold polycarbonate filters. Finally, these coated substrates were placed at 37°C for 30 min. AP2 cells were then harvested from the culture flasks and plated on the top of this gel coating.
2. Thick gel method, for growing cells within a three-dimensional matrix simulating an intact basement membrane: Matrigel was thawed, homogenized as described for the thin gel method, but diluted in cold serum-free DMEM to reach a final concentration of 6 mg/ml (stock solution 13 mg/ml). AP2 cells were then harvested from the culture flasks and resuspended inside the Matrigel preparation. After that, this Matrigel containing AP2 cells was added (100 μl/cm^2) either on cold coverslips or on cold polycarbonate filters, which were placed at 37°C.

In both two-dimensional and three-dimensional preparations, the cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air, for at least 5 days.

For morphological studies, AP2 cells were grown on the two different preparations of Matrigel for 1 week, and then analysed by confocal and transmission electron microscopy (TEM). Transmission electron microscopy studies were carried out in cells grown on polycarbonate filters (controls) and in cells grown on polycarbonate filters coated with Matrigel. Cells were prepared as described before and examined in a Jeol 100 CXII transmission electron microscope.

For laser scanning confocal microscopy, cells grown either on the top of Matrigel or inside this substrate were fixed in 1% paraformaldehyde in PBS for 10 min at room temperature, and permeabilized with 0.5% Triton X-100 in the same buffer for 10 min at room temperature [28]. Samples were then labelled to actin with rhodamine phalloidin (Molecular Probes, Eugene, Ore.) in PBS for 45 min at room temperature. The mounting medium was 0.1% of paraphenylenediamine and 10% of PBS in glycerol.

Actin-labelled samples were three-dimensionally reconstructed from the bottom to the top by the laser scanning confocal microscope LSM 10 (Carl Zeiss, Oberkochen, Germany) equipped with an argon laser light source. Rhodamine was excited by a 514-nm wavelength. Optical sections 1 μm apart were taken from cells grown on the top of Matrigel, while cells grown inside this substrate were reconstructed with sections 4 μm apart.

For further investigation of the confocal findings in cells grown inside Matrigel, these samples were studied by light microscopy. Cells were fixed in paraformaldehyde 4% in PBS for 2 h. Filters were then carefully removed from their supports, embedded in paraffin, and stained with haematoxylin-eosin.

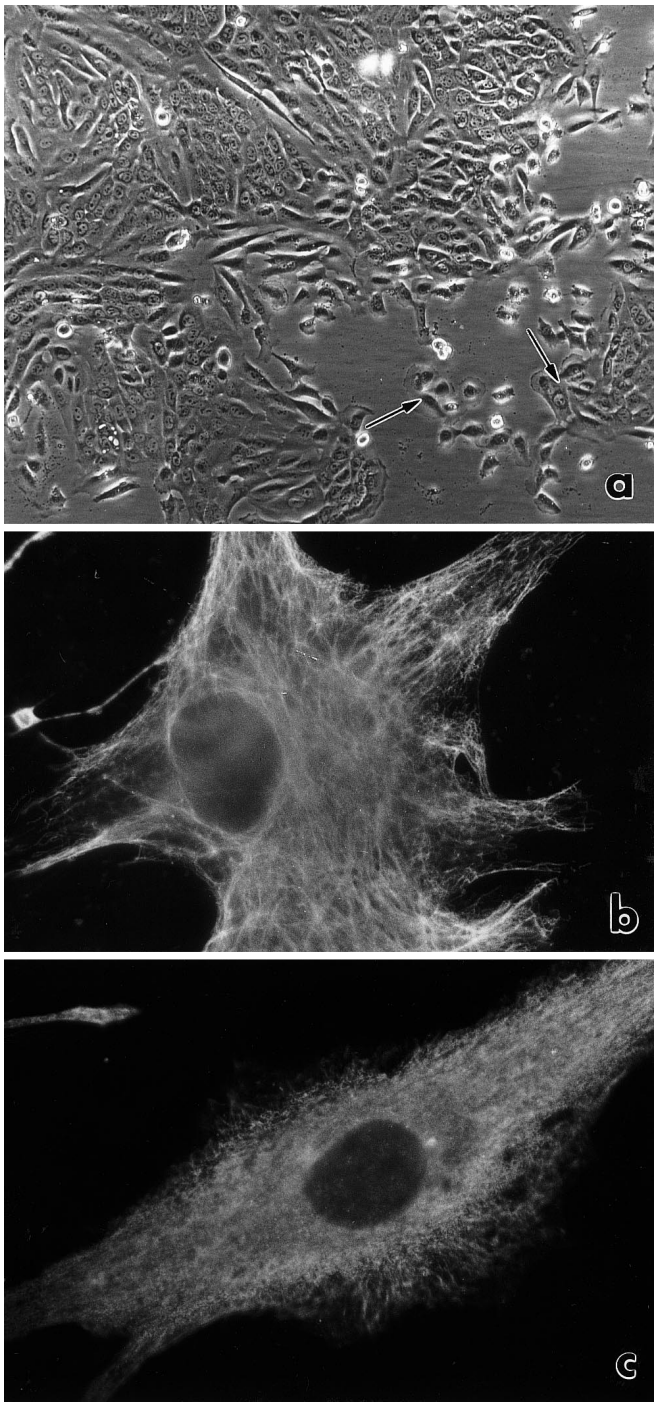
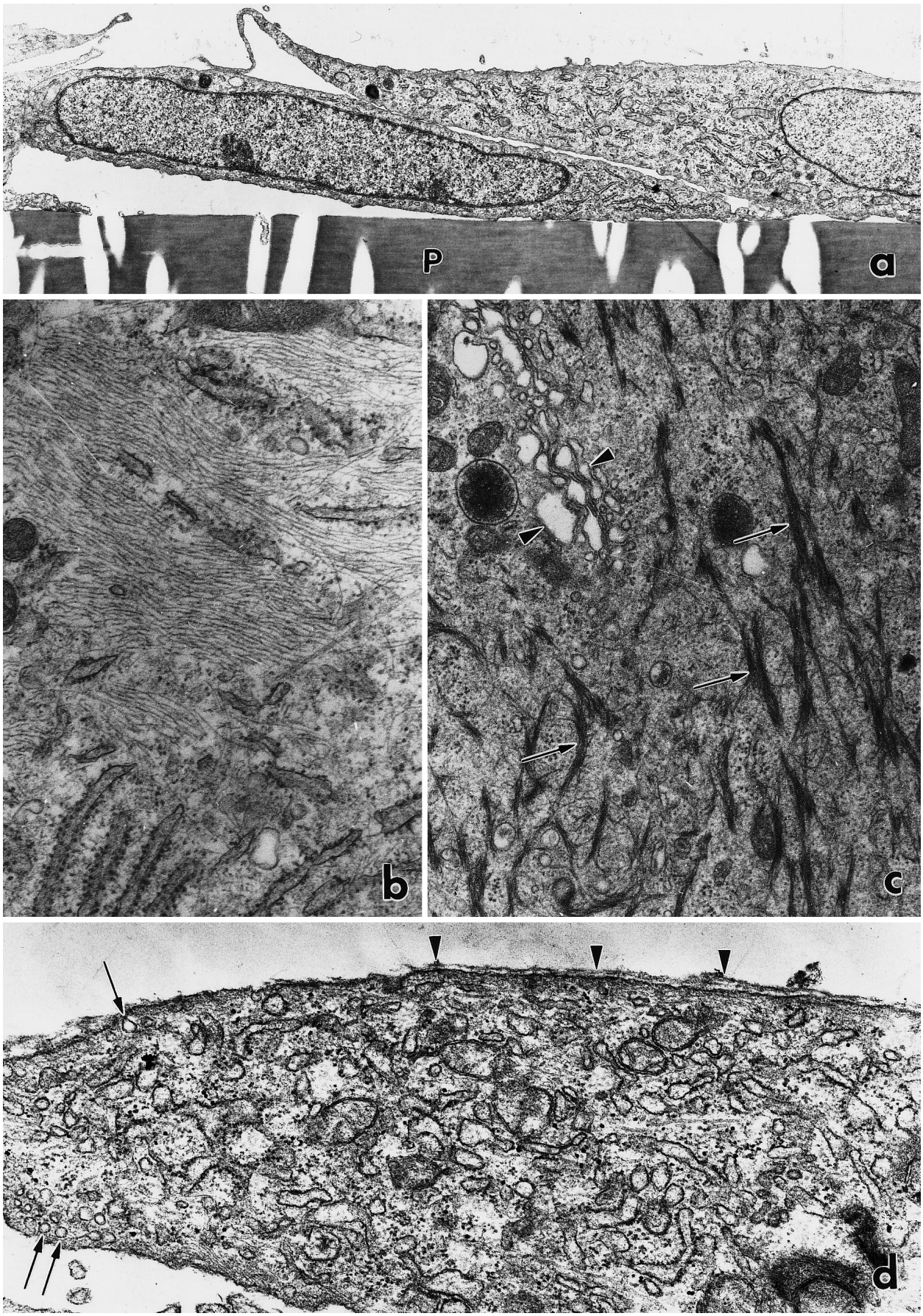


Fig. 2a–c Characterization of AP2 cells. Spindle-shaped cells are shown in phase contrast (a). In some areas epithelioid cells are observed (a, arrows). Immunofluorescence detected vimentin (b) and cytokeratin 19 (c). Magnifications: a) 250x; b, c) 630x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Results

The original tumour exhibited the typical histopathological features of pleomorphic adenoma, being characterized by proliferation of epithelial and myoepithelial cells in different patterns forming nests, sheets and double-layered ductiform structures (Fig. 1a). The luminal structures, which are very often filled with eosinophilic material, were composed of inner epithelial and outer myoepithelial cells. The stromal pattern of the pleomorphic adenoma was also present, displaying a mixture of spindle-shaped and dendritic cells, dispersed in either myxoid or hyaline tissue (Fig. 1b).

The cell line derived from pleomorphic adenoma was named AP2. These cells, grown on substrates without Matrigel, were characterized by means of immunohistochemistry and transmission electron microscopy.The cells were positive for cytokeratin and vimentin (Fig. 2b, c), and subcellular analysis showed spindle-shaped cells with a prominent endoplasmic reticulum, mitochondria, and Golgi apparatus (Fig. 3a). Networks of linear elements were present, characterized by intermediate filaments, tonofilaments and microfilaments (Fig. 3b, c). Tracts of basal lamina were observed apposed to the outer face of the plasma membrane (Fig. 3d, arrowheads). An additional feature was the presence of numerous caveoli (pinocytotic vesicles) close to the inner face of the plasma membrane (Fig. 3d, arrows).

Different preparations of Matrigel created different phenotypes of AP2 cells, with remarkable alterations. Two-dimensional Matrigel preparation exhibited a monolayer of cells displaying a dendritic phenotype, with several thin, long and intercommunicating processes branching out from the central body (Fig. 4). TEM observation displayed spindle-shaped cells with prominent cytoplasmic extensions filled with microfilaments. Organelles were scarce and basically represented by endoplasmic reticulum and Golgi apparatus, located around the nucleus (not illustrated).

AP2 cells grown within three-dimensional Matrigel preparations formed cellular clusters. The examination by conventional fluorescence microscopy showed a great overlap of structures, preventing a detailed analysis of cells composing these clusters. To circumvent this problem, we took advantage of optical sectioning properties of the confocal microscope. Samples were labelled with the specific fluorescent probe to actin, rhodamine phalloidin. The rationale for the use of this probe was to detect the actin cytoskeleton that lies immediately beneath the plasma membrane, which indirectly outlines the profile of cells present inside the clusters. The clusters were then reconstructed optically from the bottom up, with optical sections 4 μm apart. These clusters appeared to be very high when compared with cultures grown in two-dimensional environments. Confocal microscopy showed that while the thickness of a monolayer grown on Matrigel was around 7 μm (Fig. 5a–c), cells grown inside Matrigel formed a three-dimensional organization reaching 30 μm height (Fig. 5d–f). We observed cuboidal cells delimiting a central space resembling a lumen (Fig. 5d). This finding was confirmed by light microscopy and TEM. Light microscopy showed that the clusters were

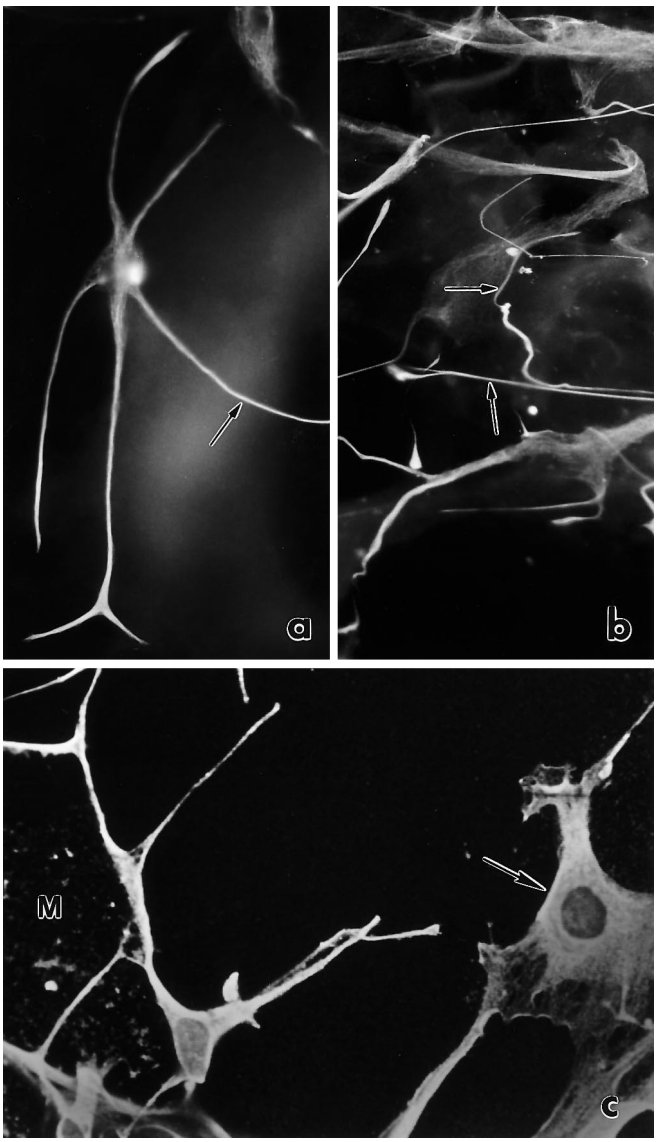


Fig. 4a–c AP2 cells grown on two-dimensional preparations of Matrigel. Dendritic cells are observed (a to c), with thin, long and intercommunicating processes (a, b arrows) branching out from the central body. Fig. 4c shows the interface glass-Matrigel. The cell attached to Matrigel (M) exhibits a dendritic phenotype, while the cell grown without Matrigel presents a bipolar shape (c, arrow). All pictures display cells labelled to vimentin. Magnifications: 630x

◀ **Fig. 3a–d** Subcellular analysis of AP2 cells grown on polycarbonate filters (a, P). Low magnification shows spindle-shaped cells with a prominent endoplasmic reticulum (a). Intermediate filaments (b) and tonofilaments (c, arrows) are present. Fig. 3c also displays cisternae of Golgi apparatus (arrowheads). Tracts of basal lamina are observed, apposed to the outer face of the plasma membrane (d, arrowheads). Caveoli are present, close to the inner face of the plasma membrane (d, arrows). Magnifications: a) 8100x; b, c) 80000x; d) 30000x

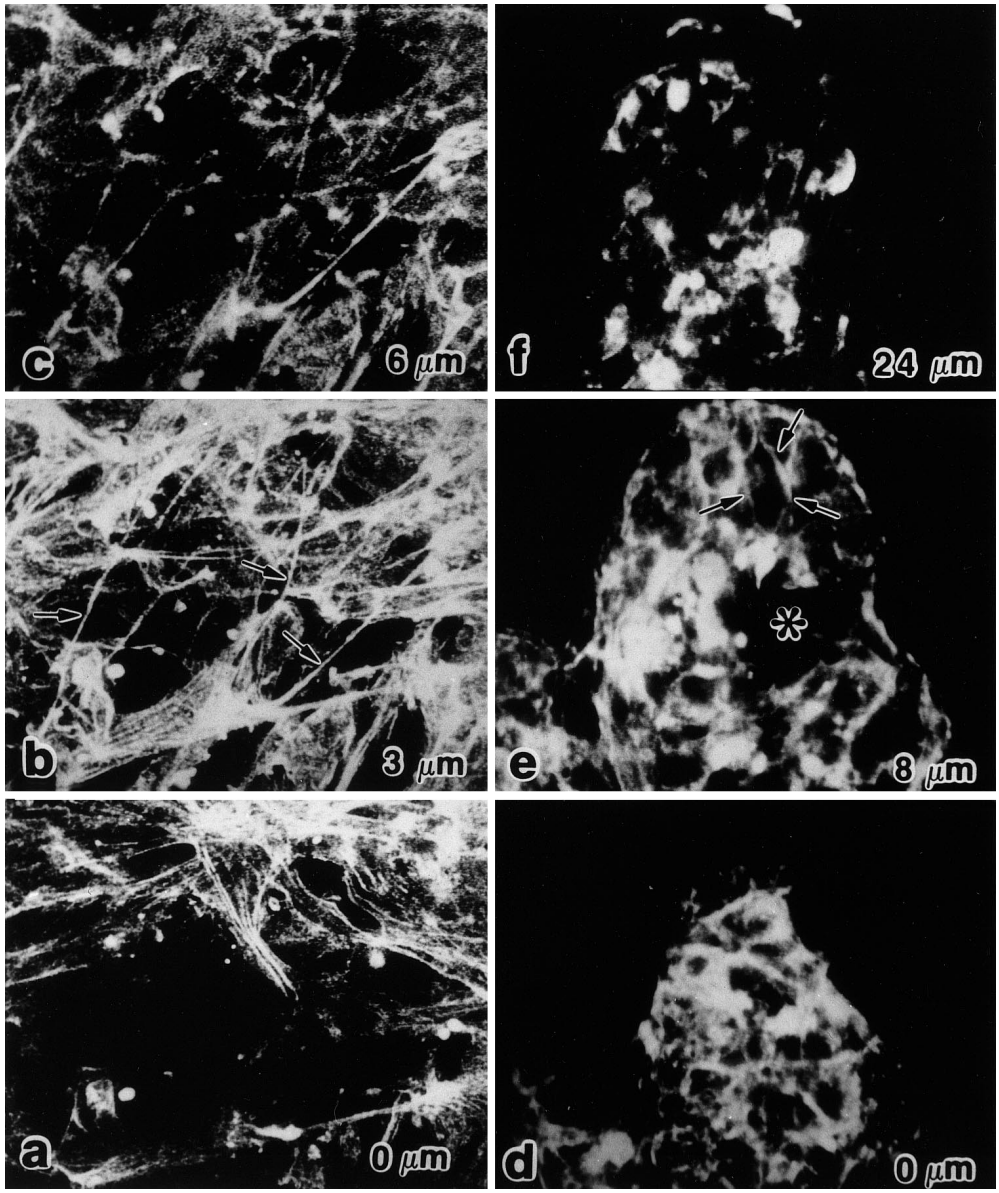
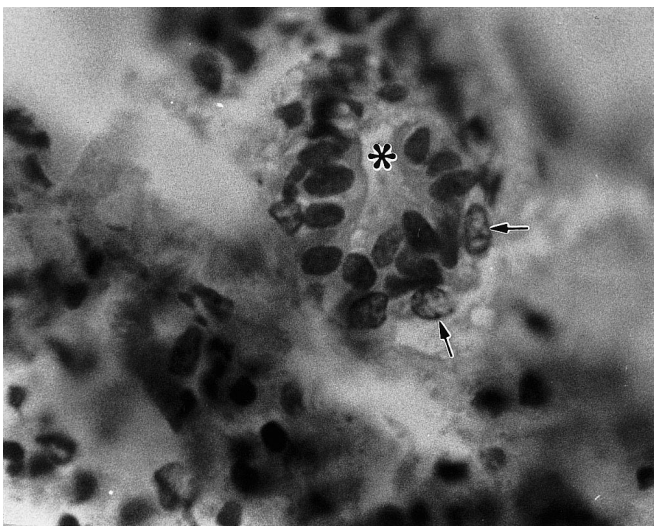


Fig. 5e–f Comparison between AP2 cells grown on the top of Matrigel (a to c) with cells grown within this substrate (d to f). Cells were labelled to actin with rhodamine phalloidin and optically reconstructed by the confocal microscope. Reconstruction was made through the entire depth of AP2 cells. Cells grown on the top of this substrate (a to c) are dendritic, forming a monolayer 6 μm thick. Cells grown within Matrigel form a cluster 24 μm thick (d to f). This cluster is composed by epithelioid cells delimiting empty spaces (e, *). Actin distribution was different in cells grown on different preparations of Matrigel. Dendritic cells present mostly globular actin, with few stress fibers (b), while epithelioid cells exhibit the actin belt of polarized epithelia (**e** *arrows*). *Labels* indicate *Z*-axis positioning of the optical sections. *Z* = 0 μm represents the bottom of the samples. x400



formed by double-layered ductiform structures. Cells facing the lumen were clearly cuboidal, while the outer layer cells had a spindle-shaped morphology (Fig. 6). In addition to these light microscopy findings, TEM showed polarized cells facing the luminal spaces (Fig. 7). These cells presented microvilli at the apical plasmalemma. Junctional complexes were observed at the basolateral plasmalemma of luminal cells. These complexes were formed by desmosomes and areas of membrane fusion resembling tight junctions (Fig. 8).

The experiments with AP2 cells grown either on plain substrates (glass or polycarbonate filters without Matrigel) or in contact with Matrigel were carried out at least

Fig. 6 Histopathology (H&E) of AP2 cells grown inside Matrigel. Double-layered ductiform structures is present (✻), composed by inner cuboidal and outer spindle-shaped cells (*arrows*). x400

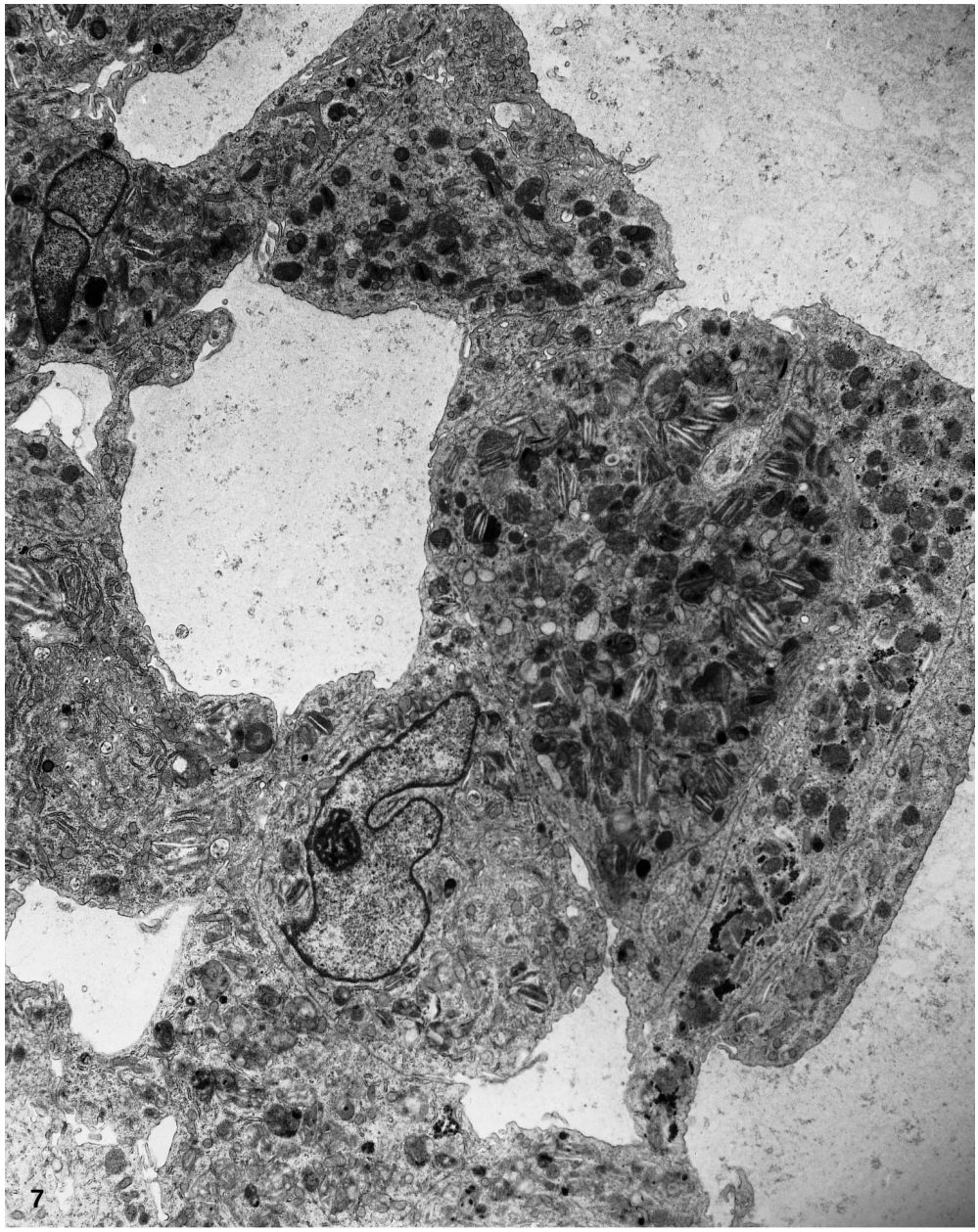


Fig. 7 Transmission electron microscopy of cells grown inside Matrigel. Clusters appear as multi-layered epithelioid cells delimiting luminal spaces. The outer layer present spindle-shaped cells. x5600

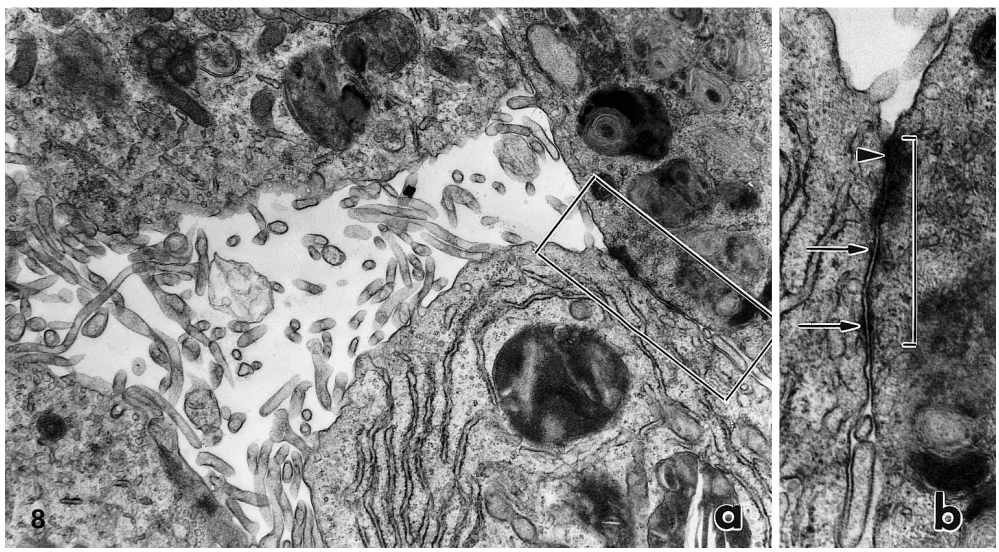


Fig. 8a, b Electron micrograph of cells delimiting a luminal space. **a** Cells facing the lumen are cuboidal with microvilli at the apical plasmalemma x12 800. **b** Higher magnification of the *boxed area* shows a junctional complex (*arrows*). An electron-dense area suggestive of membrane fusion is observed (*arrowhead*). x20 000

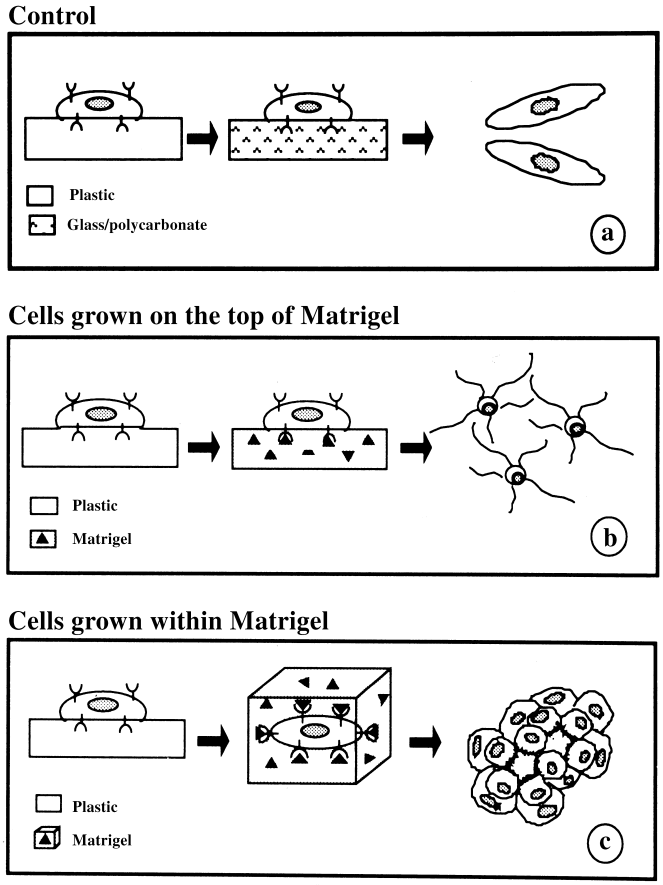


Fig. 9a–c Schematic diagram of the results obtained from different preparations of Matrigel. **a** Cells grown either on glass coverslips or on polycarbonate filters are bipolar and spindle-shaped. **b** Cells grown on the top of Matrigel change their phenotype, from bipolar spindle-shaped to multipolar dendritic shape. **c** Cells grown within Matrigel form multi-layered clusters

five times, and a minimum of 50 cells was examined each time. The results were consistently reproduced.

Discussion

A new cell line named AP2 has been propagated from human pleomorphic adenoma. These cells showed immunocytochemical and subcellular features compatible with those described for neoplastic myoepithelial cell lineages [1–3, 8, 9, 12, 14–16, 30, 31, 35, 38, 40–42, 56]. These cells presented cytokeratin and vimentin. In addition, they showed microfilaments, tonofilaments, basal lamina and peripheral vesicles. By plating AP2 cells in contact with Matrigel, a mixture of basement membrane proteins, we demonstrated that neoplastic myoepithelial cells in culture can differentiate into either ductal or myoepithelial phenotypes. Moreover, these differentiation states are modulated by the spatial arrangement of basement membrane in the cellular environment.

At immunocytochemistry level, the expression of different proteins has been used to characterize neoplastic myoepithelial cells, such as smooth-muscle actin [7, 17, 26, 62], glial fibrillary acidic protein (GFAP) [23, 52], and S-100 protein [24, 52]. However, we have consistent evidence showing that cytokeratin and vimentin are the most reliable markers of neoplastic myoepithelial lineage [1–3, 30]. Thus, we decided to use these two cytoskeletal proteins to characterize AP2 cells. There is some literature describing the acquisition of vimentin as a reaction to the tissue culture conditions [36, 57]. However, this is not the case for AP2 cells. These cells are derived from pleomorphic adenoma, which already expresses vimentin. Furthermore, this intermediate filament is considered the ideal marker for neoplastic myoepithelial lineage [1]. Thus, the presence of vimentin in AP2 cells should not be interpreted as a result of the interaction between these cells and the tissue culture environment.

It has been speculated that modified myoepithelial phenotype in pleomorphic adenoma is developed when neoplastic myoepithelial cells become separated from the duct units by a large amount of basement membrane [14, 15, 35]. In addition, Saku et al. [51] have demonstrated that the histological variety of pleomorphic adenoma stroma is accompanied by differential concentrations of extracellular matrix proteins. These assumptions were based on descriptive findings reporting the presence and distribution of ECM proteins in salivary gland tumours. In order to test these theories we developed an in vitro assay. The main idea was to plate individual neoplastic myoepithelial cells in contact with different amounts of basement membrane proteins.

AP2 cells were plated in two different preparations of Matrigel. Cells were plated within a three-dimensional preparation of Matrigel, to simulate in vitro a situation in which neoplastic myoepithelial cells are involved with large amount of basement membrane. These cells showed remarkable phenotype alterations. Surprisingly, instead of developing modified myoepithelial cell phenotypes, AP2 cells were organized as three-dimensional clusters composed of multi-layered epithelioid structures delimiting luminal spaces. The cells facing the lumen were cuboidal, showing microvilli at the apical plasmalemma and junctional complexes with desmosomes. This observation indicates that when completely involved with an intact basement membrane the neoplastic myoepithelial cells undergo cytodifferentiation originating the duct-myoepithelial units rather than modified myoepithelial phenotypes. It confirms that although the neoplastic myoepithelial cells are restrained by the basement membrane they do not originate modified myoepithelial phenotypes present in the tumour stroma.

We plated AP2 cells on the top of Matrigel to simulate a situation in which only some of the cells are in contact with basement membrane. Thus, the cells are in contact with a disrupted basement membrane. Cells grown on the top of the Matrigel were transformed from bipolar spindle-shaped cells to multipolar dendritic phenotype. Morphologically, these dendritic cells resemble some modified myoepithelial cells present in myxoid and chondroid areas of pleomorphic adenoma. Subcellular

analysis of the dendritic cells showed features of myoepithelial cell lineage, such as basement membrane and pinocytotic vesicles (caveoli). However, these dendritic cells showed no subcellular features such as are described in modified myoepithelial cells, e.g. tonofilaments [12, 14, 15, 35, 42]. On the basis of this result, we may infer that these dendritic cells are less well differentiated than modified myoepithelial cells. Thus, we suggest that AP2 cells grown on two-dimensional preparations of Matrigel showed striking morphological alterations, but were unable to differentiate into either ductal or modified myoepithelial cell phenotypes.

Pleomorphic adenoma displays cells with a variety of phenotypes, either dispersed in the tumour stroma or forming double-layered ductiform structures or units [14, 15]. We have obtained some cell phenotypes present in pleomorphic adenoma, such as epithelial-myoepithelial duct units. However, our experiments did not induce the development of modified myoepithelial cells, which would have been represented in the tumour by plasmacytoid, chondroid and dendritic cells. This indicates that the development of these phenotypes could be regulated either by more complex mechanisms rather than cell–basement membrane interactions alone, or by the presence of ECM containing additional interstitial proteins, including collagen type I and chondroitin sulfate.

Manipulation of AP2 cells by different preparations of Matrigel provided interesting insights into the histomorphological features of pleomorphic adenoma. Figure 9 summarizes our findings with different preparations of Matrigel. These data strongly suggest that AP2 culture depends on a common precursor of myoepithelial and ductal cells [18, 45, 58]. We also suggest that pleomorphic adenoma is derived from a single cell type. This assumption is supported by an important feature of the primary culture technique. Primary culture allows the study and experimental manipulation of isolated clones of neoplastic cells [19]. Once a primary culture is subcultured it becomes known as a cell line, partially reflecting the heterogeneous lineage of the neoplasm [19]. As the cell line proliferates and is subcultured, a selection process occurs that narrows the range of cell phenotype within the line [19]. The AP2 cell line has been cultured for at least 20 passages. We have conducted a thorough study of the AP2 phenotype, by immunofluorescence and transmission electron microscopy, in most of the passages. Since the 5th passage, all cells have maintained the same markers and modifications of the phenotype have been observed only in Matrigel experiments. Thus, we should assume that a natural subcloning procedure occurred with AP2 cells, creating a monoclonal population. Depending on the spatial arrangement of the basement membrane in the cellular environment, this monoclonal population of cells would differentiate into either epithelial–myoepithelial duct units or undifferentiated myoepithelial cell phenotype.

Matrigel has been used in many laboratories as means of preserving, enhancing or inducing differentiates phenotypes of a variety of epithelial cells [33], including salivary submandibular glands [32, 46]. Matrigel is similar to basement membranes, which contain supramolecular networks of laminin and collagen IV; glycoproteins such as entactin; and proteoglycans [4, 33, 60, 61]. All of these molecules can interact either in group or independently with epithelial cells through a variety of cell surface receptors, including the signal transducing class of heterodimeric receptors known as integrins [25, 27, 59]. Our experiments have shown that when Matrigel interacts with membrane receptors present only in one surface of AP2 cells a multipolar dendritic phenotype develops. However, when this substrate interacts with a larger number of receptors, which are present in all the surfaces of AP2 cells, these cells undergo morphodifferentiation and cytodifferentiation into authentic ductiform structures. Thus, we may infer that the spatial status of basement membrane in contact with neoplastic myoepithelial cells is crucial for the development of the histological variety present in pleomorphic adenoma.

Phenotype alterations of AP2 cells were also accompanied by reorganization of their actin cortical cytoskeleton. It is well known that when a cell migrates on a substrate, membrane receptors (integrins) diffuse laterally into the newly formed adhesion points [21]. The actin cytoskeleton is linked to integrins through actin-binding proteins, such as actinin, vinculin and tallin, and follows integrin reorganization [21, 27]. Although a detailed study of actin networks is beyond the scope of this paper, we have observed that AP2 cells grown on different preparations of Matrigel show some degree of actin rearrangement. Confocal microscopy has shown that cells plated on the top of Matrigel presented mostly globular actin (G-actin) and stress fibres, while cells grown within Matrigel showed the actin circumferential belt that is characteristic of polarized epithelia [22].

ECM are always in close apposition to cells, and it has been well demonstrated that basement membranes do not only provide a mechanical support, but also influence cellular behaviour [21, 27, 33, 34, 43]. Although there is little evidence of the influence of the ECM in salivary gland tumours, we have demonstrated that basement membrane proteins are key modulators of morphogenetic changes and cytodifferentiation of AP2 cells.

We have directly demonstrated that most of the histological diversity of pleomorphic adenoma depends on different spatial interactions between tumour cells and ECM proteins. There are many speculations regarding the role played by ECM in the histological spectrum of pleomorphic adenoma. However, so far no other experimental evidence of this role has been demonstrated. Establishment of the precise molecular mechanisms that generate differences in the morphology of myoepithelial cells induced by ECM is only an initial step towards understanding the effect of cell–substrate interactions on morphogenesis of salivary gland tumours.

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