

Culture on basement membrane does not reverse the phenotype of lens derived mesenchyme-like cells

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ABSTRACT Definitive epithelia suspended within type I collagen gel give rise to individual, freely migrating cells that express the mesenchymal phenotype. They become elongate in shape, invade collagenous matrices and develop abundant RER. We investigated whether mesenchyme-like cells that derive from lens epithelia retain the mesenchymal phenotype or revert to epithelial phenotype when cultured on basement membrane (BM). Mesenchyme-like cells placed on top of BM gel or lens capsule BM retain the elongate, bipolar morphology of mesenchymal cells. They migrate individually along and into the BM matrix. Mesenchyme-like cells on or in BM ultrastructurally resemble true mesenchymal cells. They extend pseudopodia and filopodia, exhibit a circumferential actin cortex, and contain well developed RER. Mesenchymal products, such as type I collagen, continue to be expressed. We conclude that the phenotype of mesenchyme-like cells derived from definitive epithelia is stable even in or on matrix known to promote the epithelial genetic program. Their behavior, thus, is similar to that of true (secondary) mesenchymal cells in the embryo.

KEY WORDS: *epithelia, mesenchymal cells, extracellular matrix (ECM), basement membrane (BM)*

We have previously reported that definitive epithelia retain the ability to acquire the mesenchymal phenotype (Greenburg and Hay, 1982). After anterior lens epithelia (Greenburg and Hay, 1986) or thyroid follicles (Greenburg and Hay, 1988) are suspended 4-5 days within hydrated type I collagen gels, mesenchyme-like cells detach from the epithelium and migrate freely out into the gel. Mesenchyme-like cells are bipolar, elongate in shape and lack epithelial contacts (tight junctions, desmosomes). As judged by immunohistochemistry, they acquire vimentin cytoskeleton while losing keratin, and they synthesize type I collagen and turn off type IV collagen (Greenburg and Hay, 1986, 1988). Elongated profiles of RER fill the cytoplasm. Thyroid-derived mesenchyme-like cells lose thyroglobulin, and lens-derived cells lose crystallins (Greenburg and Hay, 1986, 1988). The mesenchyme-like cells interact via the actin cortex with extracellular matrix (ECM) on all sides and exhibit mesenchymal polarity (Zuk *et al.*, 1989) of cell surface and cytoplasm.

Epithelial cells, on the other hand, are characteristically cuboidal in shape, contiguous, and show epithelial polarity (Simons and Fuller, 1985). The apical cell surface contains microvilli and membrane proteins not found on the baso-

lateral plasmalemma, and the basal surface contains the actin cortex and receptors for ECM (Sugrue and Hay, 1986). Epithelia other than lens (Ramaekers *et al.*, 1980) typically contain keratin intermediate filaments (Schmid *et al.*, 1979) associated with numerous intercellular junctions. They characteristically produce type IV collagen rather than interstitial collagens like type I (Greenburg and Hay, 1986).

In this paper, we seek additional behavioral and biochemical evidence that the lens derived mesenchyme-like cells are indeed mesenchymal. We isolated the mesenchyme-like cells and grew them on basement membrane (BM) to see if they retain mesenchymal phenotype under conditions that promote the epithelial phenotype. We used reconstituted BM gel (Kleinman *et al.*, 1986), which consists of heparan sulfate proteoglycan, type IV collagen, laminin, and other structural glycoproteins, and frozen-killed lens capsule (Meier and Hay, 1974) as substrata. We report that the elongated lens-derived cells cultured on top of BM retain mesenchymal shape and ultrastructure, in-

Abbreviations used in this paper: ECM, extracellular matrix; BM, basement membrane; rer, rugged endoplasmic reticulum.

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vade the BM, and continue to produce type I collagen. Thus, we can add to the previously described mesenchymal features of cells derived from epithelia in collagen gels the fact that the phenotype is as stable as that of true mesenchymal cells, such as the corneal fibroblasts used as controls in our experiments.

We grew embryonic avian anterior lens epithelia within hydrated type I collagen gels as described by Greenburg and Hay (1982, 1986). Mesenchyme-like cells leave the former free surface of the epithelia, which is now in contact with collagen, and after 2-4 wks fill the ECM around the explant. The epithelial explant was removed and mesenchyme-like cells were isolated enzymatically from the collagen gels. These cells were cultured on top of BM gels (Fig. 1A) or frozen-killed lens capsule (Fig. 1B) for 1-6 wks, during which time they continued to behave as do true mesenchymal cells. Both mesenchyme-like cells (Fig. 1A, B) and corneal fibroblasts (Fig. 1C) on BM retain the elongate, bipolar morphology typically expressed by fibroblasts in type I collagen gels (Greenburg and Hay, 1982, 1986; Tomasek and Hay, 1984). They migrate individually across (open arrows) and into (closed arrows) the BM matrix, appearing at all levels in the gel (Fig. 1A,C) or on the plastic substratum (closed arrows, Fig. 1B) after having migrated through the lens capsule. Both lens mesenchyme-like cells and corneal fibroblasts may occur as networks of elongate cells on BM (Fig. 1A, C), and invade type I collagen gel (data not illustrated), as reported for other mesenchymal cells (Bernanke and Markwald, 1982; Emonard *et al.*, 1987a, b; Bilozur and Hay, 1988). On the contrary, cells isolated directly from differentiated lens epithelium express epithelial phenotype (Greenburg and Hay, 1986) when grown on BM. They sometimes form cysts (Fig. 1D) or tubules.

The ultrastructure of lens-derived mesenchyme-like cells growing on/in BM gel resembles that of true mesenchymal cells, such as corneal fibroblasts. Pseudopodia extend from opposite poles of the cell, organizing the cytoplasm into leading and trailing ends. The first step in invasion of the gel is the extension into the gel of a pseudopodium (p, Fig. 1F). The cytoplasm contains numerous ribosomes and elongated profiles of rough endoplasmic reticulum (rer, Fig. 1F, G). It is not fibrillogranular, since it has lost the lens crystallins that fill the cytoplasm of the lens epithelia from which the mesenchyme-like cells originated (Greenburg and Hay, 1986). Mitochondria (m, Fig. 1G) are scattered throughout the cytoplasm, and the Golgi apparatus (ga, Fig. 1G) is located on one side of the cell.

Intermediate filaments (if) course lengthwise through the cytoplasm, and a well developed actin cortex (ac, Fig. 1G) circumferentially extends around the cell perimeter.

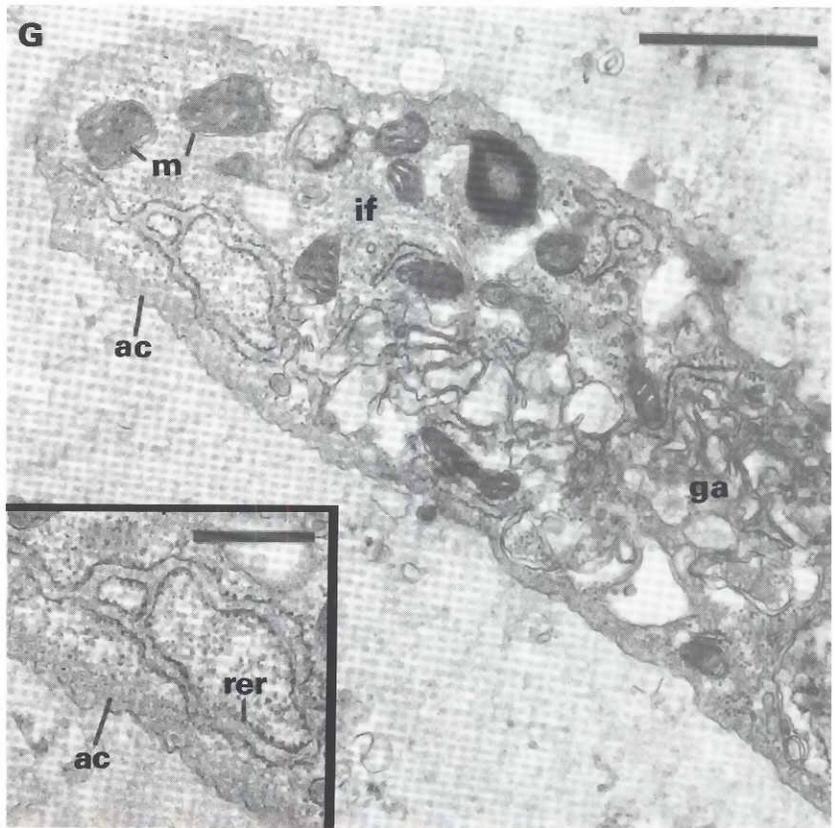
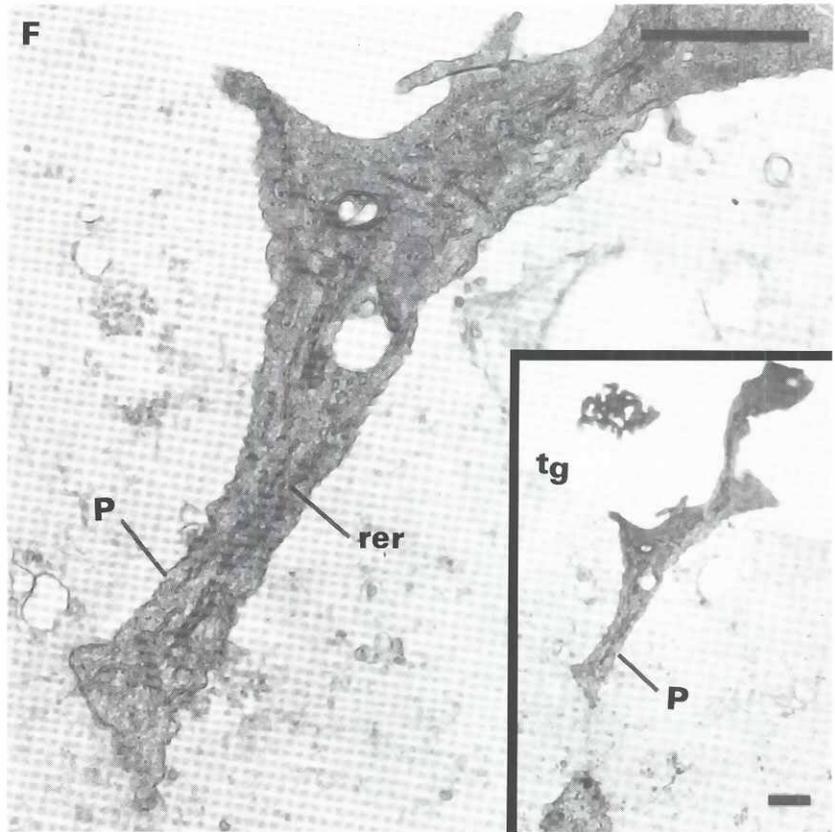
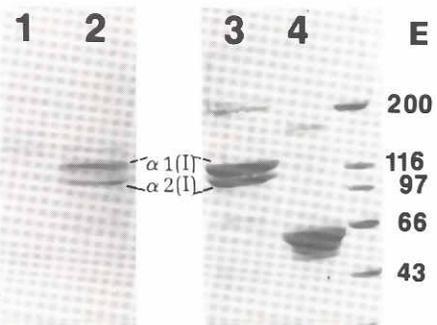
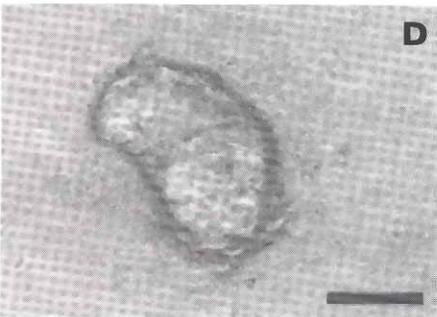
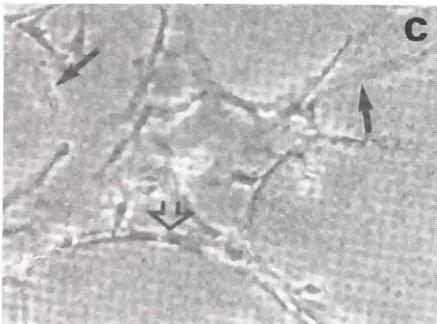
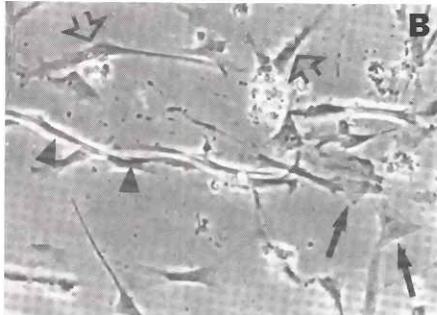
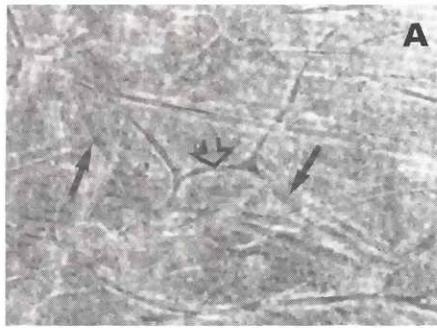
Thus, the lens-derived mesenchyme-like cells pass the behavioral criterion we are using to define fibroblasts: they invade BM, retaining the elongate shape and ultrastructure of fibroblasts. Next, we asked whether or not they continue to synthesize type I collagen, a protein that mesenchymal cells characteristically produce even when grown on BM (Emonard *et al.*, 1987b). The mesenchyme-like cells transforming from lens epithelium in collagen gels are known to activate type I collagen and lose type IV collagen, as judged by immunohistochemistry (Greenburg and Hay, 1986). We metabolically labeled cultures with ^{14}C -proline to assay production of type I collagen. An autoradiogram of SDS-gel shows that mesenchyme-like cells produce type I collagen during the 24 hr pulse (lane 2, Fig. 1E). Type IV collagen, which is produced by anterior lens epithelia *in vitro* (Greenburg and Hay, 1986), was not detected.

Thus, it appears that lens mesenchyme-like cells synthesize mesenchymal matrix and retain the invasive behavior of fibroblasts whether cultured on BM or type I collagen. The anterior lens epithelial cells from which they arose do not exhibit these characteristics, but behave the same as other epithelial cells on BM or collagen, forming cysts or sheets of cells (Meier and Hay, 1974; Overton, 1977; Greenburg and Hay, 1988; Sanders and Prasad, 1989).

Definitive epithelia grown on top of type I collagen gel retain epithelial phenotype (Meier and Hay, 1974), whereas embryonic epithelia programmed to give rise to mesenchyme do so on collagen (Bernanke and Markwald, 1982) or in BM gel (Bilozur and Hay, 1988). It would seem that in the embryo, the epithelia destined to give rise to mesenchyme activate the mesenchymal genetic program before cells leave the epithelium. To trigger this program in definitive epithelia requires complete suspension of the epithelium in type I collagen for a number of days (Greenburg and Hay, 1982). Once activated, this mesenchymal program appears stable, as it is in the embryo. None of the secondary or true mesenchymal cells in the embryo, such as sclerotome, form epithelium again (Hay, 1968).

By suspending lens epithelium in collagen gels, we seem to have triggered the same genetic program that codes for the mesenchymal phenotype in the embryo. The change to mesenchymal behavior, shape, ultrastructure and ECM synthesis appears to be complete. It will be interesting in future studies to evaluate the developmental potential of the lens-derived mesenchymal cells.

Fig. 1. Morphology and behavior of mesenchyme-like cells derived from lens (A, B, E-G), corneal fibroblasts (C) and dissociated anterior lens epithelia (D) on BM gel (A, C-D, F-G) or frozen-killed lens capsule (B). Bipolar mesenchyme-like cells (A, 12 days in culture) and corneal fibroblasts (C, 5 days) are elongate in shape and migrate across the matrix (open arrows) and into the BM gel (closed arrows, A, C). When grown on lens capsule, the mesenchyme-like cells (B, 3 days in culture) migrate through the matrix as elongated cells (open arrows, B) which flatten (closed arrows, B) when they reach the plastic substratum beneath it. The arrowheads in (B) point to folds in the lens capsule. Dissociated anterior lens epithelia sometimes form hollow cysts or spheres (D, 11 days in culture). Fluorography of ^{14}C collagens (E) after SDS-PAGE recovered from the matrix (lane 2) fraction of lens mesenchyme-like cells on BM gel shows production of $\alpha 1(I)$ and $\alpha 2(I)$ chains of type I collagen, not present in the medium (lane 1). The proteins are stained with Coomassie in lanes 3 (matrix) and 4 (medium). The lens mesenchyme-like cells on top of BM gel (tg, inset F) extends a pseudopodium (p) into the BM gel. The cytoplasm of mesenchyme-like cells contains elongated profiles of rer (F, G) and numerous free ribosomes. A well developed circumferential actin cortex (ac, G) is present. m, mitochondria; if, intermediate filaments; ga, Golgi apparatus. Bars: A-D, 100 μm ; F, G, 1 μm ; inset G, 0.5 μm .



Experimental procedures

Lenses were dissected from 11-day old chick embryos. The anterior epithelium resting on an intact BM (lens capsule) was cut into 1 mm² squares, according to methods previously described (Piatigorsky *et al.*, 1973; Greenburg and Hay, 1986). The initial explant was free of mesenchyme (Greenburg and Hay, 1986), because there is no mesenchyme around the lens *in vivo*. The explant was quickly suspended in a gelling solution of rat tail tendon type I collagen (Elsdale and Bard, 1972; Zuk *et al.*, 1989). After the gel polymerized, media was added [Ham's F-12 containing 10% fetal calf serum, 1% fungizone, 1% glutamine, 1% ascorbic-acid and 0.1% gentamycin sulfate (Gibco Laboratories, Grand Island, NY)]. Following 2-4 wks in culture, the epithelial explant was excised and the gel containing mesenchyme-like cells digested in 0.2% collagenase (Worthington Biochemical Corporation, Freehold, NJ) in Hank's Balanced Salt Solution (HBSS, Gibco Laboratories) for 30 min at 37°C. Fibroblasts in control experiments were isolated from 11-day old corneas (Tomasek and Hay, 1984). Anterior lens epithelia were isolated as above and the cells dissociated by 0.2% collagenase in HBSS (30 min) followed by 0.05% trypsin (Gibco Laboratories), 0.02% EDTA in HBSS without Ca²⁺ and Mg²⁺ (15 min, 37°C; Greenburg, 1985).

Eight thousand cells were seeded onto a 50 µl droplet of polymerized BM gel (Kleinman *et al.*, 1986), Matrigel (Collaborative Research, Lexington, MA), or polymerized type I collagen gel (Zuk *et al.*, 1989). For lens capsules (basement membrane), explants of anterior epithelia were frozen (-70°C) and thawed five times (Meier and Hay, 1974) and rinsed with F-12 media prior to use. Cultures were observed with phase microscopy and photographed. For electron microscopy, they were fixed in glutaraldehyde and paraformaldehyde in cacodylate buffer, followed by osmium tetroxide, as previously described (Zuk *et al.*, 1989).

Newly synthesized collagens were assayed by a modification of the procedure of Gibson *et al.* (1984). Mesenchyme-like cells, cultured on BM gels for 2-6 wks, were labeled for 24 hrs with ¹⁴C-proline (12.5 uCi/ml; ICN Radiochemicals, Irvine, CA). The radiolabeled media was removed and the cells plus matrix were washed with 0.1 M Tris-HCl (pH 7.4) containing 0.4 M NaCl and the washings added to the media (media+washings). Proteinase inhibitors were added (2 mM phenylmethylsulfonyl fluoride, 25 mM EDTA, and 50 mM 6-aminohexanoic acid; Sigma Chemical Co., St. Louis, MO). Radiolabeled proteins (media+washings fraction) were precipitated with (NH₄)₂SO₄ and the pellet redissolved in Tris-HCl buffer and proteinase inhibitors. The matrix fraction was digested with pepsin (1 mg/ml) in 0.5 M acetic acid for 12-18 hrs at 4°C. Rat tail tendon type I collagen (200ug/ml) was added as carrier. Samples were centrifuged and radiolabeled collagens in the supernatant precipitated by adding NaCl to 2.5M, then redissolved in 0.5M acetic acid, and lyophilized.

Radiolabeled proteins were resolved after denaturation by SDS-PAGE according to the method of Laemmli (1970) using a stacking gel of 3% polyacrylamide and a linear gradient separating gel of 3-15%. The gels were stained with Coomassie Brilliant Blue, fixed and impregnated with Autofluor (National Diagnostics, Manville, NJ). The gels were dried onto filter paper and exposed to Kodak X-OMAT AR film at -70°C.

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