Transdifferentiation of Retinal Pigment Epithelial Cells From Epithelial to Mesenchymal Phenotype

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Purpose. To describe and evaluate retinal pigment epithelial (RPE) cell transdifferentiation in vitro and to determine its importance to the development of proliferative vitreoretinal disorders.

Methods. Porcine RPE cells from single animals were examined at different passages in culture. The authors examined cellular morphology, contraction of a collagenous matrix, and adhesion to fibronectin and type I collagen-coated substrata. These activities were correlated with loss of epithelial characteristics, redistribution of the actin cytoskeleton, and expression of α-smooth muscle actin (α-SMA), a marker of myoid differentiation.

Results. During routine culture on tissue culture plastic, porcine RPE cells lose epithelial characteristics and acquire a mesenchymal cell-like phenotype. The ability of cultured porcine RPE cells to adhere to and exert tractional forces on an extracellular matrix increases with continued passage in vitro and transdifferentiation. This correlates with the loss of the differentiated epithelial morphology, decreased expression of the epithelial marker cytokeratin 18, redistribution of the actin cytoskeleton, and de novo expression of α-SMA.

Conclusion. Results indicate that RPE transdifferentiate in culture and that this transition is accompanied by a shift in biologic activities. Therefore, morphologic and behavioral transdifferentiation of these cells in culture are influencing factors in experimental pathology. The potential relevance of these extensive changes to the biology of proliferative vitreoretinal disorders is discussed. Invest Ophthalmol Vis Sci. 1995; 36:391–405.

Proliferative vitreoretinal disorders (PVD) are characterized by an intravitreal and periretinal dispersion and/or migration of cells that form a scar-like contractile connective tissue, known as intravitreal or periretinal membranes. Membranes can develop in association with a variety of conditions, including longstanding retinal detachment, retinal reattachment surgery, diabetic retinopathy, eye injury, and inflammatory eye disease. The complex series of cellular and biochemical interactions resemble the proliferative fibroplastic phase of physiological wound healing. Tractional forces developing within the resulting membranes can ultimately lead to traction retinal detachment, causing visual impairment or blindness.

The proliferative tissues have been shown to be potentially composed of cells originating from the retinal pigment epithelium (RPE) and the retinal glia (Müller cells, astrocytes), inflammatory cells (macrophages, lymphocytes), and unidentified mesenchymal-like cells. However, since the first systematic studies of PVD, retinal pigment epithelial cells have received considerable attention and a number of investigators have attributed to these the pivotal role in membrane formation and contraction.

The retinal pigment epithelium (RPE) is a highly specialized monolayer, but its characteristics are dependent on appropriate external signals. There is considerable evidence to indicate that certain environmental changes—such as culture of RPE cells or, under some pathologic conditions, retinal detachment—can induce cell transdifferentiation and give rise to mesenchymal-like cells. However, these observations were primarily based on morphologic studies.

To gain a better understanding of RPE cell metaplasia and to investigate potential changes in cell phe-
FIGURE 1. Morphologies of retinal pigment epithelial (RPE) cells cultured under routine conditions. Primary culture (A) (original magnification, ×100), passage 2 (B) (original magnification, ×100), passage 6 (C) (original magnification, ×40), and passage 10 (D and E) (original magnification, ×40 and original magnification, ×200, respectively) RPE cells from the same animal.

notype, we compared the previously reported morphologic shift that occurs in culture20,21 with changes in biologic activities. We examined the ability of porcine RPE cells from different generations to adhere to certain extracellular matrix macromolecules and exert tractional forces on collagen matrices. This study focused on the relationship between morphologic and behavioral transformation and correlated these changes with the progressive reorganization of the actin cytoskeleton and the de novo expression of α-SMA. This marker for myoid transdifferentiation is thought to be a necessary cytoskeletal component in fibrocontractive diseases22 and may be relevant to the pathogenesis of proliferative vitreoretinal disorders.

MATERIALS AND METHODS

Cells and Culture Conditions

Primary cultures of porcine RPE were established from eyes made available from the laboratory of Dr. William Holman, Department of Cardiovascular/Thoracic Surgery, University of Alabama at Birmingham. Methods used for securing the animal tissue were humane and complied with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The freshly enucleated porcine eyes were transported to the laboratory in ice-cold normal saline and processed using a modification of the method of Flood,23 as previously described.24 Briefly, cells were released from posterior eyecups by treatment with trypsin 0.25% and ethylenediaminetetraacetic acid (EDTA) 0.02% (Gibco, Grand Island, NY). To avoid contamination with other cells, we performed a density gradient centrifugation through a cushion composed of Percoll 40% (Pharmacia Biotech, Piscataway, NJ) with 0.01 mol/L Na2PO4 and 0.15 mol/L NaCl, pH 7.4. After centrifugation at room temperature, the pigmented cells were recovered in the pellet, whereas other cells remained near the top of the cushion. The cell populations isolated from two eyes were routinely estab-
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FIGURE 2. Kinetics of collagen gel contraction by RPE cells at different passages. Varying numbers of RPE cells (•) and human dermal fibroblasts (50,000 cells/gel, ○) attached to collagen gels were incubated in medium containing serum. At the times indicated, gel thickness was measured and the percent contraction was determined. These data represent the averages ± standard deviations from triplicate cultures. Passage 2 (A), passage 6 (B), and passage 10 (C) RPE cells from the same animal. Other details are described in methods. (D) Specific contractility of RPE cells at different passages (2, 6, and 10) after 96 hours of incubation. Linear regression analysis of the data resulted in the following functions. Passage 2: \( y = 0.07x + 0.39; r = 0.96 \). Passage 6: \( y = 1.21x + 1.01; r = 0.99 \). Passage 10: \( y = 3.70x - 1.44; r = 0.99 \).

Established in two 25-cm\(^2\) tissue culture flasks (Corning Glass Works, Corning, NY). Routine subcultivation involved passage of cells from one culture flask into two culture flasks of the same size (1:2) after confluency was achieved. All cells were harvested for subculture or experimentation using trypsin 0.05% and EDTA 0.02%. For experimentation, we routinely used cultures at 70% to 80% of confluency.

Human dermal fibroblasts were used as internal controls. The tenets of the Declaration of Helsinki were followed for the collection and use of human material. The human material used was normally discarded surgical specimens. Primary cultures were established from foreskins obtained at circumcisions, as previously described. \(^{25}\) Cells in passage numbers 5 through 8 were used for these experiments.

Cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM, Gibco) supplemented with 20 mM HEPES (Sigma, St. Louis, MO) and 10% fetal bovine serum (Gibco). Cultures were incubated at 37°C in a humidified atmosphere of 5% CO\(_2\)/95% air with medium changes three times per week. Morphologic changes occurring during culture were documented using an inverted phase-contrast microscope (Nikon TMS, Garden City, NY).

Assay of Cell Adhesion

Fibronectin substrates were prepared by coating tissue culture six-well plates (Corning) with 1 ml/well phosphate-buffered saline (PBS: 0.15 mol/l NaCl and 0.01 mol/l Na\(_2\)HP\(_4\), pH 7.4) containing 0.01 mg of human plasma fibronectin (New York Blood Center, New York, NY). Monomeric collagen substrates were prepared with a type I collagen (Vitrogen 100, Celtrix, Palo Alto, CA) solution at 0.1 mg/ml in 0.012 N HCl. The plates were incubated for 60 minutes at room temperature, after which the excess solution was removed. Nonspecific
FIGURE 3. Morphologies of RPE cells at different passages after 6 hours of incubation under the conditions described in the legend to Figure 2. Passage 2 (A), passage 6 (B), and passage 10 (C) RPE cells from the same animal (original magnification, ×100).

binding sites were blocked for 60 minutes at room temperature with 1% (wt/vol) heat denatured (at 80°C for 30 minutes) bovine serum albumin diluted in PBS. After the plates were washed with PBS, cell suspensions in DMEM were plated for the appropriate time at culture conditions (37°C, 5% CO₂/95% air). At selected time intervals, nonadherent cells were removed by gently rocking the plates, and the cell suspension was harvested for cell counting on a Coulter counter. All assays were performed in triplicate, and each experiment was repeated at least three times. The mean of the number of adherent cells (percentage of originally plated cells) for each incubation time was determined, and the standard deviation was calculated.

FIGURE 4. Kinetics of cell adhesion by RPE cells at different passages. RPE cells (30,000 cells/well) were incubated in wells precoated with human plasma fibronectin (○) or type I collagen (●) and incubated in serum-free DMEM. At the times indicated, nonadherent cells were removed and counted, and the percentage of attached cells was calculated. Wells precoated with heat-denatured bovine serum albumin were used as negative controls in each experiment (□). These data represent the averages ± standard deviations from triplicate cultures. Passage 2 (A), passage 6 (B), and passage 10 (C) RPE cells from the same animal. Other details are described in Methods.
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FIGURE 5. Morphologies of RPE cells at different passages adherent to monomeric type I collagen and fibronectin. Passage 2 (A, B), passage 6 (C, D), and passage 10 (E, F) RPE cells from the same animal after 2 hours of incubation on collagen (A, C, E) and fibronectin (B, D, F) precoated wells under the conditions described in the legend to Figure 4 (original magnification, X200).

Assay of Collagen Gel Contraction

Native type I collagen gels were prepared as described previously. Native type I collagen gels were prepared as described previously. Vitrogen 100 was adjusted to physiologic ionic strength, pH, and a concentration of 1.5 mg/ml with 10% of 10 X PBS and 0.1 M NaOH while it was maintained at 4°C. Aliquots (200 µl) of this solution were added to the center of a 12-mm circular score on the bottom of a 24-well tissue culture plate (Corning) and polymerized at 37°C for 90 minutes. The results were hemispherical gels approximately 2 mm thick attached only to the bottom surface of the well.

Cells released with trypsin–EDTA treatment were washed once with growth medium (containing serum) and again with serum-free medium before they were placed in the appropriate number onto the top of the polymerized collagen gels in 50 µl serum-free medium. These were incubated for an additional 30 minutes at 37°C to allow cell adhesion. After cell attachment, each well was flooded with 1 ml of DMEM containing 1 mg/ml fetal bovine serum.
FIGURE 6. Immunolocalization of cytokeratin 18 in primary and transdifferentiated RPE cells.

Phase-contrast (A, C) and immunofluorescence photomicrographs (B, D) of primary culture (A, B; original magnification, ×200) and 9th-passage (C, D; original magnification, ×50) RPE cells probed with a monoclonal anticytokeratin peptide 18 antibody.

Gel contraction was observed as a function of reduced gel thickness. The gel height was measured using an inverted phase-contrast microscope (Nikon) equipped with a Z-axis digitizer (LaSico, Los Angeles, CA) by adjusting the plane of focus from a reference point at the bottom to the cells seeded on the top of the gel and recording the distance of stage movement. For kinetic studies, gel thicknesses were measured after well flooding and were remeasured after appropriate incubation times at 37°C. Percent contraction was determined by dividing the remaining height by the original height and then subtracting this percentage from 100. All assays were performed in triplicate, and each experiment included human dermal fibroblasts (50,000 cells/ml per well) as an internal control. The mean extent of contraction was determined for each incubation time, and the standard deviation was calculated.

Indirect Immunofluorescence

Cells were plated at different densities on sterile glass coverslips (Fisher Scientific, Atlanta, GA) in 35-mm tissue culture dishes (Corning) and incubated for different lengths of time in growth medium. For the examination of cells at “wound” edges and “wound closure,” we created linear wounds (1.5 mm wide) in confluent monolayers of primary RPE cultures with a glass pipette tip. Loose cells were removed by gentle flushing with medium. Morphologic and immunologic examination were performed after appropriate incubation times at 37°C.

Before fixation, specimens were washed in PBS. Normally, cells were fixed and permeabilized with methanol for 10 minutes at -24°C. To reveal F-actin with rhodamine-phalloidin (1 μg/ml; Sigma), cells were fixed with 2% formaldehyde in fixation buffer (0.2 mol/l Na2HPO4, pH 7.0) for 1 hour at room temperature. This was followed by a 10-minute permeabilization step with 0.1% Triton X-100 PBS.

After the coverslips were rinsed with PBS for 10 minutes, they were incubated with blocking buffer (PBS containing 20% normal goat serum (The Binding Site, San Diego, CA) and 5% bovine serum albumin for 1 hour in a moist chamber at room temperature. Washes with PBS (3 × 10 minutes) was followed by incubation (overnight at 4°C) with the primary antibodies. These were diluted in PBS containing 1% normal goat serum and 3% bovine serum albumin. The primary antibodies consisted of monoclonal mouse anti-α smooth muscle actin (clone 1A4, Sigma) and monoclonal mouse anti-cytokeratin...
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Figure 7. Changes in the actin cytoskeleton associated with transdifferentiation. Primary culture (2 weeks) (A; original magnification, ×100) and 9th-passage (B; original magnification, ×200) RPE cells labeled with rhodamine-phalloidin to visualize the actin cytoskeleton.

tin peptide 18 (clone CY90, Sigma). In control incubations, the primary antibody was omitted. After incubation with the primary antibody, the coverslips were washed with PBS (3 × 10 minutes) and were incubated for an additional 60 minutes with rhodamine-conjugated goat anti-mouse IgG (Sigma). After additional washes of 3 × 10 minutes in PBS, the coverslips were finally mounted onto slides with Fluoromount G (Fisher Scientific). Staining was visualized using a Nikon Optiphot-2 microscope (Nikon) equipped with epifluorescence and phase-contrast optics and appropriate filters.

Materials
Methanol, formaldehyde, and bovine serum albumin and were purchased from Fisher Scientific. X-100 Triton and other chemicals were obtained from Sigma.

RESULTS
Porcine RPE Cells Undergo Morphologic Transdifferentiation During Culture
Primary cultures of freshly isolated cells were routinely plated at relatively high density (see Methods) and, as a result, rapidly achieved confluency. These cells remained a well-differentiated, homogenous, pigmented cell monolayer that resembled differentiated RPE cells in vivo (Fig. 1A). Although all cells were pigmented, spots of dense pigmentation could be recognized, suggesting isolated clumps.

Routine subcultivation (1:2) of these primary cultures preserved the cobblestone morphology. However, the cells appeared less tightly packed at the same stage of confluency and more flat (Fig. 1B). The cellular content of pigment granules decreased during subcultivation and appeared inversely correlated with the progressive transdifferentiation of these cells from an epithelioid to a mesenchymal-like phenotype (Figs. 1C, 1D). As demonstrated in these two micrographs, 6th- and 10th-passage cells display a progressively increased degree of spreading. A characteristic feature of transdifferentiated cells, seen in the high magnification photomicrograph of 10th-passage cells, is the prominent cytoskeleton with well-organized stress fibers (Fig. 1E).

Morphologic Transdifferentiation Coincident With Increased Contraction and Adhesion Potentials
To determine if morphologic transdifferentiation is associated with changes in biologic activities, we examined the contraction and adhesion potentials of cells from the same animal at different passages. Cultures of RPE cells were seeded onto polymerized collagen gels in numbers varying from 0 cells/gel to 200,000 cells/gel. Human dermal fibroblasts at a single density of 50,000 cells/gel were used as internal controls in each experiment.

In one series of separate experiments, we examined cells from the same animal for contractile and adhesive activities at passages 2, 6, and 10. During the course of a 96-hour incubation, 2nd-passage RPE cells produced significant changes in gel thickness (<20%) only at relatively high cell densities (>100,000 cells) (Fig. 2A). These cells, incubated for 6 hours under the described conditions, did not spread, remained round, and were apparently unable to produce a significant tractional force (Fig. 3A). Fifty thousand 6th-passage cells reduced gel thickness by nearly 60% compared to 84% of the same number of human dermal fibroblasts (Fig. 2B). These cells manifested different degrees of spreading. Some cells did not spread, whereas other cells possessed short pseudopodia. Several cells extended long processes and produced visible striations of the underlying collagen matrix (Fig. 3B). Fifty thousand 10th-passage RPE cells were highly contractile, reducing gel thickness by 75%, which was comparable to the reduction generated by human dermal fibroblasts (84% contraction) (Fig. 2C). These cells were well spread and, after 6 hours of incubation,
extended broader lamellipodia causing tensional folds (Fig. 3C). We used the data in Figures 2A to 2C to determine the specific contractility of RPE cells at the examined passages. Linear regression analysis of these data yields functions describing the linear aspects of each activity profile, the slope of which reflects the percent contraction per thousand cells and the remarkable increase in tracional activity associated with passage number and transdifferentiation (Fig. 2D).

Concomitant with changes in their contractile potentials, transdifferentiating cells demonstrated increased adhesion potentials both for fibronectin and monomeric collagen. Parallel cultures of RPE cells were incubated in fibronectin and type I collagen-coated wells for as long as 120 minutes under culture conditions. Wells coated with heat-denatured bovine serum albumin were used as a negative control for each experiment. Second-passage RPE cells adhered to fibronectin and to type I collagen, but the levels of adhesion were significantly different. After 2 hours, 66% of RPE cells were attached to plates adsorbed with fibronectin, but only 15% adhered to collagen.
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FIGURE 9. Cell morphologies and kinetics of collagen gel contraction by RPE cells cultured at different densities. Freshly isolated cells from the same animal were cultured at high (A) and low (B) density (original magnification, ×50). (C) Second-passage (routine high density, total of 2 weeks) and (D) primary culture (low density, 2 weeks) RPE cells from these cultures attached to collagen gels (0 to 200,000 cells/gel) were incubated in medium containing serum (○). Human dermal fibroblasts (50,000 cells/gel) were used as an internal control (■). At the times indicated, gel thickness was measured and the percent contraction was determined. These data represent the averages ± standard deviations from triplicate cultures. Other details are described in Methods.

type I-coated surfaces (Fig. 4A). Both groups of cells adhering to the different substrata did not spread and remained rounded (Figs. 5A, 5B). Sixth-passage cells demonstrated an increased capacity to adhere both to fibronectin (78% adherent cells after 2 hours) and collagen (48% adherent cells after 2 hours) (Fig. 4B). However, there was still a significant difference between the levels of adhesion, which, at this time, was also accompanied by different degrees of cell spreading. Cells adherent to surfaces coated with fibronectin spread to a greater extent (Fig. 5D) than cells attached on type I collagen-coated plates (Fig. 5C). These cells remained relatively round and extended only thin lamellipodia. Tenth-passage RPE displayed the highest level of adhesion for fibronectin (85% adherent cells after 2 hours) and for collagen (77% adherent cells after 2 hours) (Fig. 4C). Although the degree of adhesion of the RPE cells on both substrata was no more significant, cell spreading on fibronectin-coated surfaces was substantially higher (Figs. 5E, 5F).

In a related series of experiments, we examined the acquisition of enhanced contractile activity. We analyzed each passage of cells established from a single animal. As expected, well-differentiated 1st- and 2nd-passage RPE cells displayed low contractile activities. Transdifferentiating 3rd-passage RPE cells began to exhibit enhanced tractional capabilities (results not shown). The acquisition of these increased tensile forces was also coincident with the morphologic transformation of the 3rd-passage cells.

Transdifferentiated RPE Cells Have Decreased Cytokeratin 18 Expression and an Altered Actin Cytoskeleton

To characterize further the phenotypic changes associated with transdifferentiation, we examined the
expression of established markers typical for differentiated RPE cells.\textsuperscript{15-21,25} We examined the expression of cytokeratin 18 and the distribution of filamentous actin in well-differentiated primary cultures and fully transdifferentiated 9th-passage RPE cells. Retinal pigment epithelial cells express the keratin intermediate filament in primary cultures (Figs. 6A, 6B), but lose it after transdifferentiation (Figs. 6C, 6D). Reactivity could be also recognized as a decrease from the center to the periphery of primary colonies.

Primary cultures of highly differentiated porcine RPE cells display a differentiated circumferential (that is, compact cortical rings of microfilaments) actin cytoskeleton (Fig. 7A). In contrast, transdifferentiated 9th-passage cells exhibit a linearly arranged cytoskeleton composed of numerous stress fibers spanning the cytoplasm (Fig. 7B).

**Transdifferentiating RPE Cells Exhibit a De Novo Expression of \(\alpha\)-SMA**

To determine further the phenotypic modulation RPE cells undergo, we examined the correlation between the contractile phenotype and the expression of the myoid marker \(\alpha\)-SMA. Retinal pigment epithelial cells from the same animal were examined at passages 1, 5, and 14. Highly differentiated cells in primary culture were completely negative for \(\alpha\)-SMA (Figs. 8A, 8B). In contrast, 29% of transdifferentiated 5th-passage RPE cells, some of which still contained pigment granules, expressed \(\alpha\)-SMA (600 counted cells) (Figs. 8C, 8D). The number of \(\alpha\)-SMA positive cells increased to 93% in cultures of highly transdifferentiated 14th-passage cells (600 counted cells) (Figs. 8E, 8F).

**Transdifferentiation Dependent on Spatial Conditions Rather Than Culture Time**

Our results confirmed that in culture, RPE cells acquire an altered phenotype that includes \(\alpha\)-SMA expression and acquisition of enhanced contractile capacities. To explore whether this transdifferentiation is dependent on continuous subcultivation or on culture conditions, we analyzed the effect of cell density on the phenotypic changes.

We compared the contractile activities of freshly isolated RPE cells from the same animal initially cultured at different cell densities after an equal length of time. Cells from one single eye were established as routine 1st-passage culture (25-mm\(^2\) flask) (Fig. 9A) with a routine subcultivation (1:2) to the 2nd passage. After 2 weeks
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Figure 11. Morphology of RPE cells responding to "wounding" in culture. A primary culture (2 weeks) of highly differentiated RPE cells (A) was wounded (1.5 mm wide, linear scratch) and observed at 0 hours (B), 24 hours (C), 48 hours (D), and 72 hours (E) after manipulation (original magnification, ×50). Asterisks indicate fixed reference point.

...in culture, these cells were only minimally contractile, reducing gel thickness by less than 5% (200,000 cells/gel) during a 48-hour incubation period (Fig. 9C). A parallel culture of cells from the fellow eye was established as a "sparse culture," initially introduced into a 75-cm² culture flask rather than a 25-cm² flask. These cells were also examined after 2 weeks in culture and after reaching the appropriate confluency (70% to 80%). The population was composed of multiple cell colonies with a gradient of different cell morphologies. Cells in the central part were differentiated (cobblestone configuration, pigmented, tightly packed), whereas cells at the edge of the colony possessed the features of transdifferentiated cells (Fig. 9B). During the course of a 48-hour incubation period, these cells, seeded at the maximal density of 200,000 cells, reduced the gel thickness by more than 70% (Fig. 9D).

To examine this mechanism further, we used freshly isolated RPE cells to examine α-SMA expression in sparse cultures. Retinal pigment epithelial cells from the same animal were established as parallel low-density cultures on coverslips and were stained after 2 and 5 days, respectively. After 2 days, the populations still consisted of highly differentiated RPE cells and were completely negative (0 out of 500 counted cells) for α-SMA (Figs. 10A, 10B). After 5 days, 3% of the cells expressed a specific reactivity for α-SMA. These cells (Figs. 10C, 10D) were characteristically located at the edge of the growing colony and displayed features of transdifferentiated cells.

These results indicate that the length of time in culture is not the primary factor inducing transdifferentiation. Retinal pigment epithelial cells, given appropriate space, will spread out and/or proliferate and will more rapidly lose their epithelial characteristics.

The Mesenchymal Phenotype in RPE Cultures Derives From Transdifferentiation and Not Contamination

To provide conclusive evidence that phenotypic changes are based on transdifferentiation and not on...
contamination with an unrelated cell type, we examined cells outgrowing from the "wound edge" of a highly differentiated primary RPE cell culture.

After a well-differentiated monolayer of highly pigmented cells (Fig. 11A) was "wounded" (1.5 mm wide, linear scratch) (Fig. 11B), the RPE cells along the "wound" edge began to proliferate and migrate toward the denuded surface. During the period of active proliferation and migration, the cells at the wound margin showed various degrees of spreading (Figs. 11C to 11E). Cells closer to the wound edge spread to a greater degree (Fig. 11E) than those further back (Fig. 11E). This gradual loss of the typical morphology and transdifferentiation was concomitant with a rearrangement of the actin cytoskeleton. Differentiated cells distant from the wound edge exhibited a differentiated circumferential actin ring. In contrast, transdifferentiated cells at the outgrowing wound edge manifested a linear distribution of the microfilaments that seems to occur parallel to the movement direction (data not shown).

In a parallel experiment, we examined α-SMA expression by the outgrowing transdifferentiating cells within the wound. After 2 days of repopulation to cover the denuded area, cells at the edge of the wound had the morphologic features of transdifferentiated mesenchymal-like cells with prominent stress fibers (Fig. 12C) and α-SMA expression (Figs. 12B and 12D). The identity of these cells was confirmed not only by their origin within the monolayer, but also by the fact that they still contained some pigment granules (Fig. 12C). Acquisition of transdifferentiated morphology, stress fibers, and α-SMA expression strongly suggests these cells would have had similar contractile activities as the subcultured cells with the same phenotype.

DISCUSSION

Retinal pigment epithelial cells in situ possess strong intercellular contacts with neighboring cells to form a continuous monolayer. Freshly isolated cells appear to preserve these contacts when seeded at high densities, which permits confluency to be achieved in 5 to 7 days. Low-density culture conditions enable continuous proliferation for more than 2 weeks, greater degrees of cell spreading, and more rapid transdifferentiation. It appears that contact inhibi-
tion of either growth or spreading is inversely related to these changes. Diminished support by cell–cell contacts (for example, low-density culture or outgrowing cells at the wound edge) apparently promotes a shift from cell–cell to cell–substrate contacts (for example, focal adhesion) and seems to be responsible for the observed redistribution of the actin cytoskeleton. Opas proposed that these changes are not an artifact of growth in vitro. Retinal pigment epithelial cells grown on their native basement membrane exhibit the same morphologic and cytoskeletal changes. His conclusion was that elastic forces operating within the substratum determine directly the allowable cell traction and indirectly the architecture of the cytoskeleton. Tightly interconnected cells, however, seem to be less dependent on the mechanical properties of the substrata.

Investigators have provided evidence that RPE cells play a pivotal role in the development of proliferative vitreoretinal disorders and vitreoretinal traction. The goal of this study was to draw conclusions about the significance of RPE cell metaplasia in these pathologic conditions from the transdifferentiation occurring in vitro (Figs. 1A to 1E). Our results indicate that RPE cells in culture undergo not only the previously described morphologic changes but also an impressive change in biologic activities.

Freshly isolated, highly differentiated RPE cells in vitro have been shown to contain a contractile cytoskeleton. Nevertheless, consistent with a previous study, substantial contraction of collagen matrices could be only reached with high densities of RPE cells (200,000 cells reduced the gel thickness by an average of 20% after 4 days). We suggest that the contractile activity of these cells results from isometric contraction between continuously interconnected neighboring cells forming a tensile sheet not unlike their function in situ. In contrast, fully transdifferentiated RPE cells (10th passage) approximate the phenotype under pathologic conditions and may acquire a-SMA expression.
forces. We observed that this ability correlates with an increase of cell adhesion and α-SMA expression. It remains to be determined if one or both components reflect the condition when non of tractonal force development. We propose that morphologic and behavioral changes are concomitant with biochemical differences between differentiated RPE cells and the transdifferentiated mesenchymal phenotype. This should be considered and defined in future studies that evaluate the effects of pharmacologic therapies for proliferative vitreoretinal disorders.

**Key Words**

retinal pigment epithelium, transdifferentiation, adhesion, contraction, vitreoretinopathy

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