Expression of E-Cadherin by Human Retinal Pigment Epithelium: Delayed Expression In Vitro

Janice M. Burke, Feng Cao, Pamela E. Irving, and Christine M. B. Skumatz

Purpose. To determine whether retinal pigment epithelial (RPE) cells, which reportedly express N-cadherin as their major cadherin cell adhesion protein, also express the more common epithelial cadherin, E-cadherin.

Methods. Cadherins expressed by human RPE cells in situ were examined by western blot analysis of extracts prepared from the RPE of human adult eyes. Cadherins expressed in vitro were examined by analysis of confluent and postconfluent human RPE cultures, using the methods of reverse transcription–polymerase chain reaction (RT–PCR) and western blot analysis. Protein distribution was examined by conventional fluorescence microscopy, confocal imaging, or both. Proteins whose expression, distribution, or both correlated with E-cadherin expression in other epithelial cells were examined by similar methods in cultured RPE cells.

Results. In addition to N-cadherin, E-cadherin (and P-cadherin) was found in adult human RPE in situ. In cultured human RPE cells, N-cadherin was ubiquitous, but E-cadherin was limited to patches of cells and was not expressed until several weeks after confluence, a time when several phenotypic variants become prominent. E-cadherin was absent from RPE cells of fusiform shape but was found in only a subset of epithelioid RPE cells. Unlike epithelial cell lines expressing E-cadherin, cultured RPE cells with E-cadherin did not show diminished coexpression of N-cadherin, increased expression of desmosomal proteins, or a preferential expression of the αE- (rather than α-N) isoform of the cadherin linker protein α-catenin. Na/K ATPase distributed to both apical and basolateral membranes in RPE cells with junctional E-cadherin and not preferentially to the basolateral domain as in most epithelial cells with E-cadherin.

Conclusions. RPE cells express E-cadherin, a cadherin found in most other epithelial cells, but which was believed to be absent from RPE. In RPE in vitro, E-cadherin expression is a late developmental event, occurring in late confluence in cells that already express N-cadherin. E-cadherin is an established epithelial morphoregulatory protein, but it does not induce the same properties in RPE cells as in other epithelial cells, suggesting tissue-specific differences in the potential of E-cadherin to determine an epithelial phenotype. (*Invest Ophthalmol Vis Sci.* 1999;40: 2963–2970)

In the process of mediating cell–cell attachment, the adhesion molecule E-cadherin also appears to confer phenotype on cultured epithelial cells. E-cadherin localizes to junctional sites shortly after confluence where it triggers the time-dependent development of polarized plasma membrane domains[1–5] and the acquisition of a grossly epithelial cell shape. In addition to E-cadherin, epithelial cells also often express P-cadherin, although little is known about the morphoregulatory properties of this member of the cadherin family.

In contrast to most monolayer epithelial cells, cells of the retinal pigment epithelium (RPE) have been reported to lack E-cadherin[6–8] and to express N-cadherin,[7,9–12] which is typically found in nonepithelial cells. In embryonic chick development, RPE cells have also been shown to express B-cadherin,[13] which is likely the avian homologue of mammalian P-cadherin.[14] Aside from expressing N-cadherin, RPE cells have several other unusual properties that distinguish them from most epithelia. The RPE monolayer is located between two tissues rather than facing a lumen, the sodium pump of RPE cells is reportedly polarized to the apical rather than basolateral membrane domain,[15–21] and the RPE of many species, including humans, lacks desmosomes.[22] Because E-cadherin plays a role in directing Na/K ATPase polarity[8,23] and desmosome assembly,[8,24] the unusual molecular properties of the RPE compared with other epithelial cells might be attributed at least in part to the absence of E-cadherin in the RPE.

When propagated in vitro, human RPE cells display another feature that differs from epithelial cell lines. Rather than producing cultures consisting of cells with a fairly uniform

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epithelioid phenotype, confluent monolayers of the RPE are morphologically heterogeneous, varying in cell shape from epithelioid to fusiform, and phenotype development occurs over a long postconfluent interval of several weeks.[25,26] However, it is not a common practice to maintain cultured cells for protracted postconfluent periods before analysis of protein expression, so it has not been determined whether cadherin expression by RPE cells may change in late confluence. Considering the morphoregulatory function that has been ascribed to the cadherins, a change in cadherins over time could contribute to late RPE cell shape development.

Here we reevaluated cadherin expression in RPE cells by examining cadherin proteins in postconfluent cultures of human RPE. On observing E-cadherin in some cells in late-stage cultures, we also reexamined cadherins in situ by preparing RPE extracts from adult human eyes. Similarly reexamined in cultured cells were other proteins whose expression, distribution, or both were shown to be related to E-cadherin expression in other cells. These proteins include desmosomal proteins desmoglein (dg) and desmoplakin (dp), isoforms of a cadherin linker protein, αE- and αN-catenin, and Na/K ATPase. We show that, in contrast to previous reports, RPE cells express E-cadherin both within the tissue and in culture, although expression in culture is unusual in that it is restricted to patches of cells and markedly delayed, occurring after weeks at confluence. We also show that RPE cells expressing E-cadherin lack several properties that have been attributed to E-cadherin in other cells, suggesting that the inductive properties of E-cadherin in epithelial cells are not universal but may be modulated by tissue-specific factors.

Materials and Methods

Cell Cultures

For culture, RPE cells were isolated from adult human donor eyes by incubation in trypsin, transferred to culture flasks, and propagated in minimal essential medium supplemented with 10% fetal bovine serum using published methods.[25] A few RPE cultures were examined in early explant, but most were grown to confluence and passaged one or more times by standard trypsin treatment protocols. Some propagated cultures were subjected to a published procedure that partially separates two phenotypically distinct RPE variants, one epithelioid and one fusiform.[26] For analysis, cells were plated in multiwell plates or chamber slides and maintained at confluence without passage for intervals to several weeks. Cultures were regularly examined by phase contrast microscopy to determine when culture confluence was attained and to observe gross cell phenotype.

The human epithelial cell line A431 (American Type Culture Collection) and human corneal stromal fibroblasts were also used for some experiments. The fibroblasts were isolated by enzymatic digestion of the stromas of human corneas as previously described.[27] A431 and stromal cells were grown using the same culture conditions described above for RPE cells.

Analysis of E-Cadherin Expression by Reverse Transcription–Polymerase Chain Reaction and Sequencing

Total RNA was extracted from cultures of RPE cells, A431 cells, or corneal stroma fibroblasts (CSF) using TRI REAGENT (Molecular Research Center). Reverse transcription (RT) reactions were carried out using the GeneAmp RNA PCR Kit (Perkin–Elmer) with the following reaction mixture: 5 mM $MgCl_2$, 1× polymerase chain reaction (PCR) buffer, 1 mM dNTPs, 20 U RNase inhibitor, 50 U MuLV reverse transcriptase, 2.5 μM random hexamer, and 1 μg RNA. The mixtures were incubated at 42°C for 30 minutes for reverse transcription, followed by incubation at 99°C for 5 minutes to inactivate the enzyme.

Primers for the human E-cadherin gene (from Operon Technologies) were as follows: upstream, 5′-GTGACTGATGCTGATGCCCCCAATACC-3′; downstream, 5′-GACGCAGAATCAGAATTAGGAAAGCAAG-3′.[28] PCRs were performed in a DNA Thermal Cycler 480 (Perkin–Elmer) and contained 1.5 mM $MgCl_2$, 1× PCR buffer, 200 μM dNTPs, 0.4 μM primers, 2.5 U DNA polymerase (Ampli*Taq* Gold, Perkin–Elmer), and 1 μl cDNA from the RT reaction. Control RT–PCRs were conducted in the absence of reverse transcriptase or by substituting water for the RNA extract. The cycling conditions were as follows: 1 cycle at 95°C for 10 minutes, 57°C for 2 minutes, and 72°C for 2 minutes; 40 cycles at 94°C for 1 minute, 57°C for 2 minutes, and 72°C for 2 minutes; 1 cycle at 94°C for 1 minute, 57°C for 2 minutes, and 72°C for 10 minutes; followed by a hold at 4°C. Amplified PCR products were electrophoresed in 1% agarose gels containing ethidium bromide. PCR products were sequenced using Ampli*Taq* DNA Polymerase FS and ABI-PRISM fluorescent dye terminator cycle sequencing (BigDye kit; Perkin-Elmer).

Western Blot Analysis

Tissue extracts of RPE cells in situ were prepared for protein blotting from human donors of various ages. To prepare the extracts, the RPE layer was exposed by opening the eye and removing the retina, then RPE cells were dislodged by gentle scraping with a scalpel blade. Samples were transferred to tubes containing Laemmli[29] electrophoresis buffer and boiled for 10 minutes. The extracts were clarified by centrifugation to remove insoluble cytoplasmic granules (melanin and lipofuscin) before electrophoresis and blotting. Extracts were prepared from cultured RPE cells by direct lysis with electrophoresis buffer.

For the RPE extracts prepared from the tissue in situ and from the cultures, aliquots were taken for protein measurements using the method of Lowry et al.,[30] then β-mercaptoethanol (5 mM final concentration) was added to the remainder of the samples. After boiling for 10 minutes, samples were submitted to sodium dodecyl sulfate–polyacrylamide gel electrophoresis, using 6% to 10% separating gels. By methods that have been described previously,[31] proteins were transferred to membranes, blotted with the primary antibodies listed in the following section, and visualized with the ECL detection system (Amersham). Preliminary experiments were performed with each antibody to identify antibody dilution, time of incubation (1 hour to overnight), and protein loading to achieve a specific blotting signal. The protein loadings are reported in the Results section.

Immunofluorescence Microscopy

Wholemounts of human RPE cells in situ were prepared by dissecting the eye to expose the monolayer as described above. Eyecups lined with the RPE layer were fixed by immersion in methanol for 5 minutes followed by rinsing in cold (4°C) phosphate-buffered saline. After fixation, the RPE and underly-

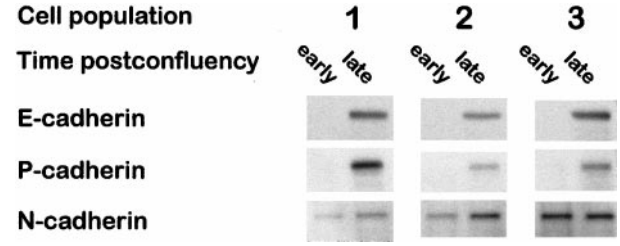

FIGURE 1. Human RPE cell populations cultured from three donors and analyzed in early (2–3 days) and late (8 weeks) confluence. Cultures were passaged 3 to 7 times, then extracts were prepared and blotted with antibodies to E-cadherin or P-cadherin (20 μg protein/lane) or N-cadherin (40 μg protein/lane). E- and P-cadherin antibodies each recognize a single protein migrating at 120 kDa; the N-cadherin band is at 135 kDa.

ing choroid was dissected from the sclera, cut into pieces approximately 5-mm square, processed for immunofluorescence microscopy as for RPE cultures, and immunostained with antibodies to E-cadherin as indicated below.

RPE cultures were fixed in 3% paraformaldehyde, permeabilized by detergent treatment, and immunostained as previously described.[25] The following primary antibodies were used: monoclonal antibodies, E-cadherin (Zymed [HECD-1]), P-cadherin, and desmoglein (Transduction Laboratories); desmoplakins I and II (American Research Products); N-cadherin ([clone GC4]; Sigma) and pancadherin (Sigma) and polyclonal antibodies, αE-catenin and αN-catenin (Santa Cruz Biotechnology); Na/K ATPase (Cortex Biochem [$\alpha 1\beta 1$]); and pancadherin (Sigma). Appropriate fluorescein isothiocyanate– or TRITC-conjugated secondary antibodies were from Jackson ImmunoResearch Laboratories.

Preparations were examined and photographed with a Leitz epifluorescence microscope or a Bio-Rad MRC-600 confocal microscope. For the latter, serial 0.2-μm sections were collected through the thickness of the RPE monolayer to generate cross-sectional Z-scans and to produce scans from which composite en face images could be reconstructed.

RESULTS

Cadherins in Cultured Human RPE Cells

As shown by western blot analysis, cultures of human RPE cells do not express E-cadherin in early confluence (Fig. 1). However, when cultures are maintained undisturbed at confluence for several weeks, both E-cadherin and P-cadherin are expressed. N-cadherin is constitutively expressed by RPE cells regardless of time at confluence (Fig. 1).

The expression of E-cadherin genes in late-confluence RPE cultures was confirmed by RT–PCR using E-cadherin–specific primers to amplify a region that encodes part of the extracellular repeat domain of the protein. The PCR product amplified from an RNA extract of RPE cells was of the predicted size and comigrated with the PCR product resulting from parallel amplification of RNA from the human epithelial cell line A431 (Fig. 2). No PCR product was obtained from fibroblasts grown from the human cornea. Analysis of a 524-bp readable portion of the sequence showed 99% identity at the nucleotide and amino acid level of the RPE product with the published sequence for human E-cadherin.

Immunostaining showed that the E-cadherin in late-confluence RPE cultures is localized at the borders of individual cells or clusters of cells rather than uniformly distributed at the borders of all cells (Fig. 3). Costaining of the same fields with a pancadherin antibody, which reacts primarily with N-cadherin,[32] showed pancadherin staining at the borders of essentially all RPE cells. The relative staining intensity for E-cadherin and pancadherin varied among cells. In some E-cadherin–positive cell clusters, pancadherin staining was also prominent (Fig. 3B), whereas in other clusters pancadherin staining was somewhat diminished relative to surrounding cells (Fig. 3D).[33] Similar to E-cadherin, P-cadherin staining in late-confluence RPE cultures was also limited to scattered clusters of cells, although P-cadherin was difficult to detect even in those regions (not shown).

The amount of E- and P-cadherin that was expressed in late confluence varied among cultures from different donors (Fig. 4). In some RPE cell populations, E- and P-cadherin levels were nearly undetectable, even after extended postconfluent periods. In late confluence, RPE cultures vary in phenotype due to the presence of several shape variants that coexist in varying proportions.[25] Higher levels of E-cadherin (and perhaps P-cadherin) appeared to correlate with a more grossly epithelioid culture appearance (Fig. 4).

Occasionally, cultures from a single donor consist largely of two RPE subpopulations with highly divergent phenotypes, one epithelioid and one fusiform,[26] that can be partially separated to produce separate paired cultures (Figs. 5A, 5B). Analysis of extracts of cultures enriched for the epithelioid sub-

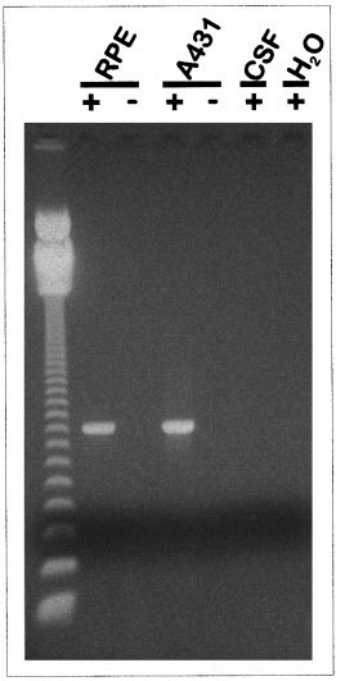

FIGURE 2. Agarose gel of products resulting from RT–PCR of RNA extracts of postconfluent cultures of human RPE and confluent cultures of A431 cells or human corneal stromal fibroblasts (CSF). Reactions were performed in the presence (+ *lanes*) or absence (− *lanes*) of reverse transcriptase, or with water substituted for RNA (H_2O lane). E-cadherin–specific primers were used that predicted a 991-bp product, as found in + *lanes* for RPE and A431. The DNA standard is shown in the *first lane*.

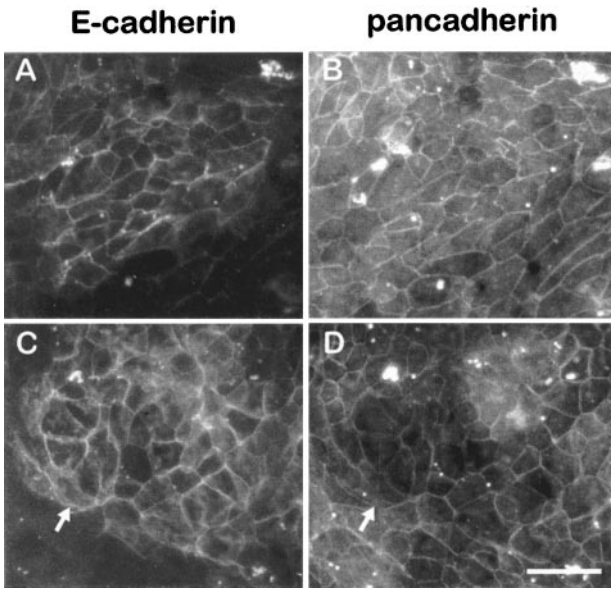

FIGURE 3. Immunofluorescence micrographs of postconfluent RPE cultures showing clusters of E-cadherin–positive cells (**A**, **C**) costained with polyclonal pancadherin antibodies (**B**, **D**) that preferentially recognize N-cadherin[32] (see Fig. 5C). Cells that are E-cadherin–positive are also positive for pancadherin/N-cadherin. Paired micrographs (**A**) and (**B**) show an E-cadherin–positive cell cluster with undiminished levels of pancadherin/N-cadherin costaining. Paired micrographs (**C**) and (**D**) show part of a cluster of E-cadherin–positive cells with slightly diminished pancadherin/N-cadherin (*arrows*). The nonjunctional granular material is autofluorescent lipofuscin or lipofuscin-like material that accumulates in long-term postconfluent cultures.[33] Scale bar, 10 μm.

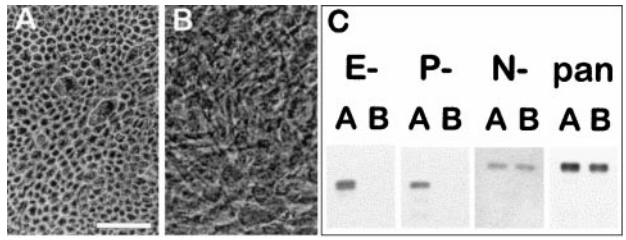

FIGURE 5. Separated RPE cultures enriched for cells of epithelioid (**A**) or fusiform (**B**) phenotype were extracted and blotted with the indicated cadherin antibodies (**C**). E- and P-cadherin were found only in epithelioid cells (*lane A* in all blots), whereas N-cadherin was found in cells of both epithelioid (*A lanes*) and fusiform (*B lanes*) phenotype. N-cadherin is detected by monospecific antibodies or by pancadherin antibodies that preferentially react with a single protein that comigrates with N-cadherin. Similar results were obtained with monoclonal and polyclonal pancadherin antibodies; the result for monoclonal antibodies is shown. Protein loading for gels was as follows: E- and P-cadherin, 20 μg/lane; N-cadherin, 40 μg/lane; and pancadherin, 4 μg/lane. Scale bar, 100 μm.

population of RPE showed E- and P-cadherin, but these cadherins were not detected in cultures of fusiform cells (Fig. 5C). N-cadherin was found at similar levels in RPE cultures of both phenotypes (Fig. 5C). Blotting with monoclonal and polyclonal pancadherin antibodies did not reveal other cadherins, and blotting further illustrated that the pancadherin antibodies recognize a single peptide that coelectrophoreses with N-cadherin (Fig. 5C).

Cadherins in RPE In Situ

As in human RPE cultures in late confluence, human RPE cells in situ also express the epithelial cadherins E-cadherin and P-cadherin (Fig. 6A). A third cadherin recognized by pancadherin antibodies was also found (Fig. 6A). This is likely to be N-cadherin because of the electrophoretic mobility of the reactive band and the high affinity of the pancadherin antibodies for N-cadherin (see Fig. 5C). Also like postconfluent cultures, extracts from different donors varied in amounts of cadherin. Variations did not correlate with donor age, and E-cadherin and pancadherin/N-cadherin tended to be high in the same samples. An exception was an RPE extract from a 2-day-old donor that had markedly high levels of pancadherin/N-cadherin relative to E-cadherin (Fig. 6A).

To determine whether E-cadherin localizes to human RPE junctions in situ, RPE cells in tissue wholemounts and in early explant cultures were examined. E-cadherin localized to the borders of RPE cells in situ (Fig. 6B), although the prominence of the junctional staining varied (not shown), consistent with the donor variability in amount of E-cadherin observed by protein blotting (Fig. 6A). In early explant culture, E-cadherin localized to sites of RPE cell-cell contact, where it often codistributed with pancadherin immunoreactive protein (Fig. 7). Immunostaining was difficult to see in the center of the explant where it was often obscured by autofluorescent lipofuscin granules but was more readily

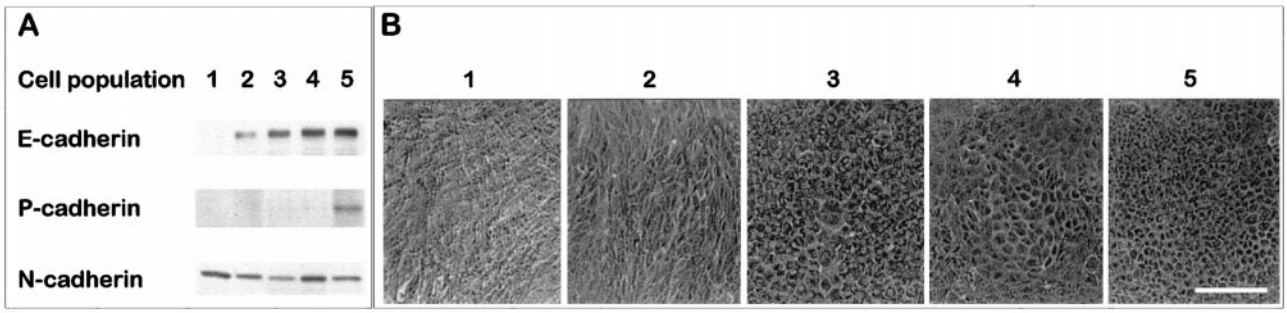

FIGURE 4. Illustration of culture variability in cadherin expression levels indicated by western blot analysis (**A**), with comparison to phase contrast phenotype (**B**) for human RPE cells in postconfluent culture. RPE cell populations from five donors were passaged two or more times, then maintained at confluence for 8 to 12 weeks. Cultures were photographed by phase microscopy, then extracts were prepared and blotted for the indicated cadherins using the antibodies and protein loadings given with Figure 1. Samples were ordered from lowest (population 1) to highest (population 5) levels of E-cadherin. Scale bar, 200 μm.

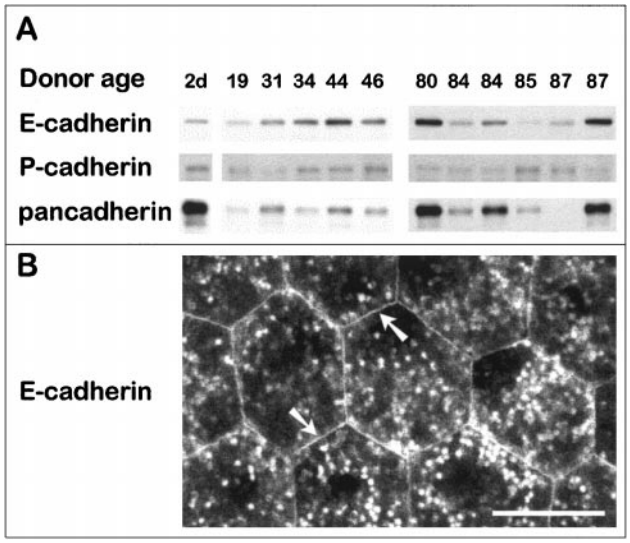

FIGURE 6. Western blot analysis of RPE cell extracts prepared from human donor eyes blotted with monoclonal E-cadherin, P-cadherin, or pancadherin antibodies (**A**). Samples were from a group of younger donors (age range, 2 days–46 years) or older donors (age range, 80–87 years). Protein loading for all samples was 20 μg/lane. E-cadherin localization to the borders of human RPE cells in situ (*arrows*) is illustrated by a confocal image in the plane of the adherens junction (**B**). Scale bar, 5 μm.

seen where cells had begun to spread and granules became displaced from cell borders, revealing cell–cell junctions. At the edges of the explant where cells were beginning to proliferate as indicated by the dilution of lipofuscin granules, E-cadherin staining was lost (Fig. 7A), but pancadherin/N-cadherin staining was retained, albeit in a less complete zonular band (Fig. 7B).

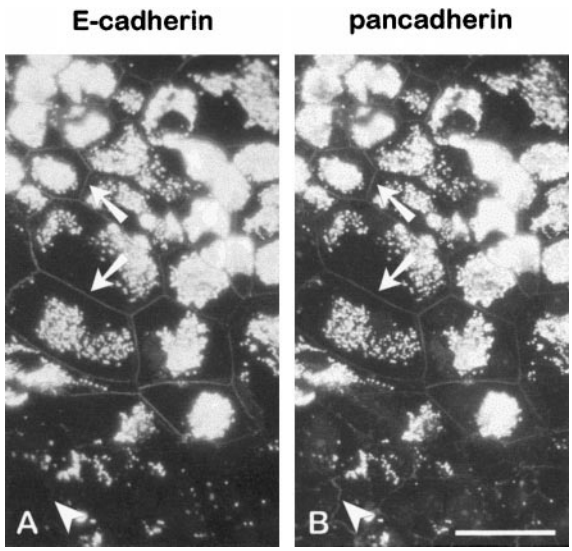

FIGURE 7. Immunofluorescence microscopy of human RPE explants in early culture, dually stained with monoclonal E-cadherin (**A**) and polyclonal pancadherin antibodies (**B**). The prominent granules in the cytoplasm are lipofuscin, which has a broad spectrum autofluorescence. E-cadherin and pancadherin codistribute at cell borders where cells have attached and begun to spread near the center of the explant (**A** and **B**, *arrows*). In the cells in the outgrowth zone at the edge of the explant, E-cadherin staining disappears, but incomplete pancadherin staining remains (**A** and **B**, *arrowheads*). Scale bar, 5 μm.

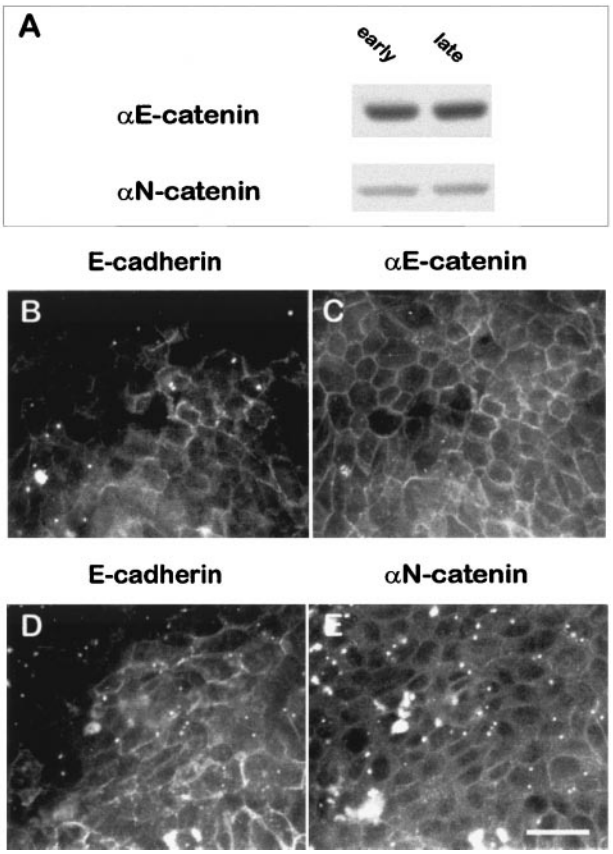

FIGURE 8. Cell extracts from human RPE cultures in early (1 week) or late (16 weeks) confluence, loaded at 15 μg protein/lane and blotted with antibodies to αE- or αN-catenin (**A**). Immunofluorescence micrographs of late-confluence cultures show E-cadherin in clusters of cells (**B** and **D**), and in the same fields αE-catenin (**C**, paired with **B**) or αN-catenin (**E**, paired with **D**) distributes to all cells. For an explanation of the granular autofluorescence, see Figure 3. Scale bar, 10 μm.

α-Catenin Isoforms, Desmosomal Proteins, and Na/K ATPase in RPE Cells

Two isoforms of a cadherin linker protein αE-catenin and αN-catenin appear to be preferentially expressed in cells expressing E-cadherin and N-cadherin, respectively.[34,35] Because RPE cells in vitro express both cadherins but nonuniformly among cells, we examined the α-catenin isoforms to determine whether they correlated with cadherin type. Cultured human RPE cells expressed both αE- and αN-catenin (Fig. 8A), and the expression of αE-catenin was not limited to late confluence cultures as for E-cadherin (Fig. 1). In postconfluent cultures both α-catenin isoforms were localized to the borders of essentially all cells, regardless of whether the cells expressed E-cadherin (Figs. 8B through 8E).

Human RPE cells have been reported to lack desmosomes[22]; however, because E-cadherin may induce desmosomal protein expression[8] and because E-cadherin was found here in human RPE, we reexamined human RPE for desmosomal proteins desmoglein and desmoplakin. Both desmosomal proteins were found in extracts of RPE cultures (Fig.

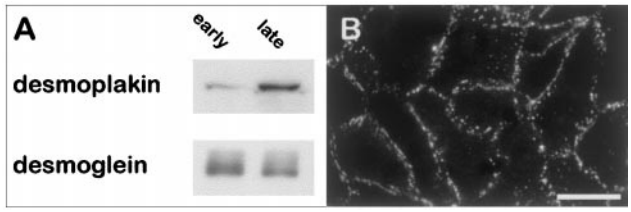

FIGURE 9. Cell extracts from human RPE cultures in early (1 week) or late (16 weeks) confluence, loaded at 40 μg protein/lane and blotted with antibodies to desmoglein or desmoplakin (**A**). Immunofluorescence microscopy of a late-confluence culture stained with desmoplakin antibodies (**B**), which localize to prominent puncta at the borders of some cells. This is an unusually large group of cells with punctate desmoplakin staining. Scale bar, 5 μm.

9A). Desmoplakin expression increased somewhat with time at confluence, but both desmoplakin and desmoglein were also found in early-confluence RPE cultures (Fig. 9A), in contrast to E-cadherin (Fig. 1). In postconfluent cultures desmoplakin localized to prominent puncta at the borders of individual cells or small clusters of cells (Fig. 9B).

Evidence suggests that E-cadherin contributes to polarization of the sodium pump to the (baso)lateral membrane domain of epithelial cell lines.[8,23] The observation of E-cadherin in RPE cells, which are reported to have an apical polarization of Na/K ATPase,[15–21] led us to examine sodium pump distribution in clusters of E-cadherin–positive cells in postconfluent cultures. As shown (Fig. 10), RPE cells with E-cadherin showed Na/K ATPase localized to apical, lateral, and basal cell surfaces. Notably, prominent staining was seen on apical microvilli in cells expressing E-cadherin.

DISCUSSION

RPE Cadherins In Situ and In Vitro

Cells in monolayer cultures derived from human RPE are not uniformly epithelioid,[25] but the molecular basis for their phenotypic heterogeneity is unknown. Studies of epithelial cell lines implicate an important role for E-cadherin in the development of an epithelial phenotype.[3–5] However, cultured RPE cells have been previously shown to express N-cadherin,[7,11,12] which is more typically found in nonepithelial cells. In a previous study of phenotype development in RPE, we found that N-cadherin developed a zonular band of detergent-resistant protein after confluence in some cells, concomitant with the development of a prominent epithelial cell shape.[12] The process of phenotype development in epithelioid RPE cells resembled the similar process in epithelial cell lines expressing E-cadherin, except in terms of timing. For RPE cells, a postconfluence interval of weeks rather than days is required for both epithelioid cells[12] and other RPE phenotypes to fully manifest their discrete cell shapes.[25]

The slow and variable development of phenotype by RPE cells raised the possibility that the expression of morphoregulatory proteins like the cadherins changes over time after confluence. This possibility was explored here, where it was observed that E-cadherin (and to a lesser extent P-cadherin) is expressed by human RPE cultures, but only in cultures that are maintained undisturbed at confluence for extended periods. E-cadherin in late-confluence cultures varied in amount among cell populations from different donors, as well as among cells within a population. Postconfluent culture populations with high levels of E-cadherin were overall more grossly epithelioid than cultures with low E-cadherin levels, and even adjacent clusters of cells differing in E-cadherin levels could be distinguished by cell appearance. This observation, coupled with the observation that E-cadherin was not detected in cells with a highly fusiform phenotype, suggests a relationship between epithelioid cell shape and E-cadherin expression in RPE cells. However, many RPE cell clusters of epithelioid phenotype lacked detectable levels of junctional E-cadherin. E-cadherin has a morphoregulatory function in epithelial cell lines,[3–5,8] but in RPE cells it is unclear whether E-cadherin induces an epithelial phenotype, or rather is expressed in some epithelioid cells after phenotype is established. Because E-cadherin expression is delayed until several weeks after confluence, epithelial morphogenesis occurs largely in its absence. Rather it is N-cadherin that undergoes postconfluent changes in distribution during phenotype development, and E-cadherin is then expressed in RPE cells that already contain N-cadherin stabilized at junctions.[12]

E-cadherin was not previously detected in cultured RPE cells, presumably because it is rapidly lost with the onset of

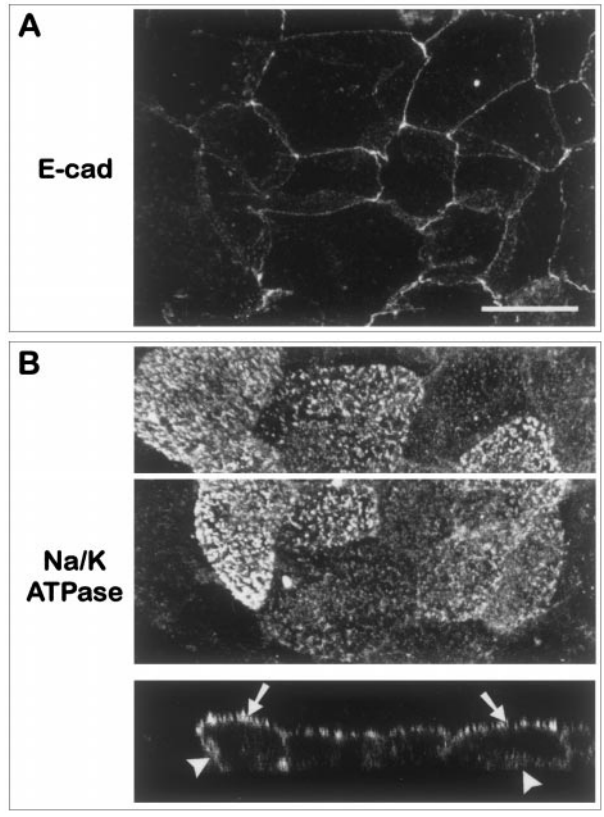

FIGURE 10. Confocal images of a cluster of E-cadherin–positive cells (**A**) in a postconfluent human RPE culture costained with Na/K ATPase antibodies (**B**). Composite images through the thickness of the monolayer are shown for both. The lower part of (**B**) shows a cross-sectional Z-scan for Na/K ATPase at the position indicated by the *horizontal line* in the composite image. Cell height and levels of Na/K ATPase staining vary among cells. Na/K ATPase is distinct on apical microvilli (*arrows*) but is also seen on some basal and lateral surfaces (*arrowheads*). Lateral borders are not "vertical," so lateral staining is indistinct. E-cad, E-cadherin. Scale bar, 20 μm.

growth in culture, and only slowly and variably reexpressed after confluence. Indeed, E-cadherin is undetectable even in primary cultures, where in situ protein expression patterns are more likely to be retained. In one previous study, an RPE cell line (RPE-J cells) was specifically analyzed for E-cadherin, but none was detected at either the mRNA or protein level.[8] There may be species differences in E-cadherin expression by RPE cells; and rat RPE, unlike human cells, may not express it. Another possibility is that E-cadherin would also be expressed by normal rat cells in culture, provided that they are maintained for an extended period of growth quiescence after confluence. Preliminary reports indicate that the expression of E-cadherin by RPE cells is not restricted to human cells but that it is also found in porcine RPE.[36]

E-cadherin was also found here in extracts of RPE cells taken from adult human eyes, indicating that the protein is expressed in situ, albeit variably among eyes from different donors. The donor variability could not be attributed to donor age, time postmortem, topographical location within eyes, or other technical issues, but rather it appears to be a naturally occurring heterogeneity. Others have attempted to detect E-cadherin in RPE cells in situ, using tissues from the rat[6] or embryonic chick.[7] Failure to detect E-cadherin in these specimens could again reflect a species difference. Another difference that may account for the absence of E-cadherin in the chick specimens is that these extracts were prepared from embryonic eyes. If E-cadherin expression is delayed during development until a late stage of morphogenesis, as it appears to be in vitro, then E-cadherin may be absent in the embryonic eye. Developmentally delayed expression of E-cadherin in RPE cells would be consistent with the low levels of E-cadherin, relative to N-cadherin, found in the sample from the one young human donor (the 2-day-old infant) that was analyzed.

Properties of Epithelial Cells Related to E-Cadherin Expression

Some protein expression patterns and structural features characteristic of epithelial cells that express E-cadherin may be induced by the cadherin. For example, desmosomes are found in most epithelial cells and their formation may depend on E-cadherin to induce desmosomal protein synthesis[8] and to trigger the assembly of the adhesion plaque.[24] RPE cells from some species have desmosomes, but human RPE cells in situ reportedly lack them.[22] As shown here, however, human RPE cells in culture express desmosomal proteins, and some cells also appear to assemble desmosomes as indicated by puncta of desmoplakin staining at cell borders. Such structures were infrequent, however, and if desmosomes are equally rare in situ, they would be difficult to locate in the ultrastructural analyses usually used to identify them. There was no apparent relationship between E-cadherin expression and desmosomes in RPE cells. E-cadherin is expressed in human tissue in situ, where desmosomes appear to be rare or absent,[22] and desmosomal proteins are synthesized in vitro in early confluence, before the expression of E-cadherin.

Epithelial cells that express E-cadherin do not usually coexpress N-cadherin, but here both cadherins were found to be coexpressed and codistributed at cell junctions in some RPE cell clusters in postconfluent cultures. In carcinoma cell lines, an inverse relationship between levels of expression of E- and N-cadherin has been shown,[37] but no such relationship was apparent in normal RPE cells in which both cadherins may be coexpressed at apparently high levels in the same cells.

Cells that express E-cadherin reportedly preferentially express the αE-isoform of α-catenin rather than αN-catenin.[34] In RPE cells, however, there appears to be no preferential coexpression between E-cadherin and αE-catenin. Cultured RPE cells constitutively expressed both αE- and αN-catenin, and both catenin isoforms distributed to the borders of essentially all cells in postconfluent cultures, even though E-cadherin expression was limited to scattered cells or cell clusters.

One of the prominent correlations with E-cadherin expression in epithelial cells is the polarization of Na/K ATPase to the lateral membrane, basal to the adherens junction. The process of polarizing the sodium pump has been studied in some detail in epithelial cell lines, and E-cadherin appears to play a role in segregating the pump to the basolateral domain.[8,23] RPE and choroid plexus epithelial cells are unusual in that the sodium pump is reportedly apical rather than basolateral.[15–21,38] How the sodium pump becomes asymmetrically enriched on the apical surface of RPE cells is unknown, and the failure of most cultured cells to develop an apical polarity has made it difficult to identify the mechanism. Although the mechanism of apical polarization is unknown, a lack of E-cadherin might be considered a prerequisite due to its basolateral inductive property. After identifying E-cadherin in some RPE cells in postconfluent cultures, we examined Na/K ATPase distribution to determine whether the pump was preferentially basolateral in those cells. We found that even in the presence of E-cadherin, the sodium pump was well represented on the apical surface of RPE cells. This distribution is different from that observed by Marrs and coworkers,[8] who transfected RPE cells to express E-cadherin and found that a basolateral polarity of the sodium pump was induced. The difference in outcome might be explained by differences in the cells that were used and in the timing of the expression of E-cadherin. In the previous study, E-cadherin was expressed early in culture in an immortalized rat RPE cell line, whereas here RPE cells were grown from adult human eyes and E-cadherin upregulation was a naturally occurring event that was delayed until late confluence. It appears that E-cadherin does not induce a basolateral Na/K ATPase polarity under all conditions in all epithelial cells, and other factors such as tissue of origin or maturational state of the cells may modulate the molecule's inductive properties.

The observation of a delayed expression of E-cadherin by RPE cells, which were previously believed to lack this epithelial morphoregulatory protein, provides a stimulus for investigating late stages of RPE morphogenesis to determine whether and how molecular phenotype is affected by E-cadherin expression. The detection of E-cadherin in RPE cells may also stimulate a reevaluation of the functions of the cadherin in epithelial cells. Most information regarding E-cadherin comes from studies of epithelial cell lines, and functions attributed to the protein are generalized to all epithelial cells, including those from normal tissues. However, RPE cells with E-cadherin lack several properties attributed to E-cadherin induction in other epithelial cells, suggesting that cell type modulates cadherin function. With regards to E-cadherin expression, RPE cells differ from most epithelial cells in that E-cadherin is expressed in cells that simultaneously express the nonepithelial cadherin N-cadherin and that E-cadherin is expressed late in morphogenesis when an adherens junction comprised of N-cadherin is already formed. The effect on cells of the simultaneous expression of different cadherins has received at-

tention,[37,39,40] but the issue of timing of cadherin expression relative to the morphogenetic state of the cells has yet to be considered.

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