Human Lens Epithelium in Tissue Culture: Biochemical and Morphological Aspects

P. Ringlens*, G. Munoyert†, P. Jap†, P. Ramaekers*, H. Hoenders* and H. Bloemendal*

*Department of Biochemistry and †Department of Cytology and Histology,
Geert Grooteplein N21, 6500 HB Nijmegen, The Netherlands

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Human lens epithelium is cultured and the epithelial origin of the cells is established ultrastructurally. The epithelial cells have a limited growth capacity in vitro; only in rare cases a confluent monolayer is obtained. When primary confluent cultures are split 1:1:8 the cells again form a monolayer; they do not form a monolayer when split at 1:2. The light microscopical and ultrastructural features of early (1 week) and late (older than 1 month) stage cultures are described. Moreover, the ultrastructure of human lens epithelium in situ is presented. Incubation of early and late stage cultures in a 3S-methionine containing medium revealed that the crystallin synthesis in younger cultures is reduced, whereas it has completely ceased in the older ones. These late stage cultures only synthesize cytoskeletal components (43 and 57 K daltons). These observations are confirmed by the indirect immunofluorescence study.

Key words: human lens epithelium; tissue culture; crystallin synthesis; light microscopy; electron microscopy; indirect immunofluorescence; cytoskeleton.

1. Introduction

In cultured calf lens epithelium synthesis of the specific lenticular proteins, the crystallins, rapidly decreases during cell elongation. The presence of one protein of the β-crystallin class has been demonstrated in long-term cultures; however, unlike the situation in vivo (Papaconstantinou, 1967), neither α- nor β-crystallin could be detected upon cell elongation in vitro (Van Venrooy, Groeneveld, Bloemendal and Benedetti, 1974). On the other hand an important role has been ascribed to filamentous structures in lens cell elongation in vitro (Ramaekers, Hukkelhoven, Groeneveld and Bloemendal, 1979).

Only a few reports are available concerning human lens epithelium in tissue culture. Hamada and Okada (1978) examining 25-day-old monolayers, described the occurrence of piles of elongated cells (so-called 'lentoid bodies'), in which they demonstrated the presence of γ-crystallin. These 'lentoid bodies' have also been observed by Tassin, Malaise and Courtois (1979) and by Eguchi and Kodama (1979). An ultrastructural comparison of human lens epithelium in situ and in vitro has been made by Perry, Tassin and Courtois (1979); unfortunately these authors stored their lenses before fixation for 6–12 hr in a balanced salt solution.

The present communication deals with the light microscopical and ultrastructural characteristics of human fetal lens epithelium cells in early (1 week) and late (older than 1 month) stage cultures and the confirmation of their epithelial origin. Furthermore, the proteins synthesized after incubation in a 3S-methionine containing medium are analyzed and the presence of various filamentous components in the in vitro differentiating lens cell is demonstrated with indirect immunofluorescence methods.

Requests for reprints should be addressed to H. Hoenders.
2. Materials and Methods

Tissue culture

Thirty-five human fetal lenses between 8 and 14 weeks of gestation were freshly collected in culture medium. In order to avoid contamination with fibroblasts, the capillary network surrounding the lens at this stage of development was removed by incubation in 0.25% trypsin in Ca²⁺-, and Mg²⁺-free Tyrode’s solution for 5 min; the lenses were collected in tissue culture medium. The basic medium consisted of 80% medium 199 (containing 0.5% lactalbumin hydrolysate) and 20% fetal bovine serum. For cultivation 4 parts of this medium were mixed with 1 part of the same medium conditioned by human embryonic fibroblasts.

To prepare conditioned medium lens cultures contaminated with fibroblasts were used. The latter cells overgrew the lens epithelium and formed closed monolayers about 2 weeks after explantation. The medium of these cultures were centrifuged at 3000 r/min for 20 min and the supernatant frozen and thawed repeatedly to avoid possible contamination of the epithelial cultures by fibroblasts.

The lenses were minced into very small pieces and the material of one pair of lenses was incubated on a coverslip (9 x 50 mm) in a Leighton tube. Occasionally plastic culture dishes were used. For electron microscopy carbon coated coverslips were taken. In rare cases of monolayer formation the cells were subcultured by 0.25% trypsin in Ca²⁺-, and Mg²⁺-free Tyrode’s solution.

Electron microscopy

For electron microscopy lens material obtained directly was fixed in diluted Karnovsky fixative (mixture of 2% paraformaldehyde and 2% glutaraldehyde); cell cultures were fixed for 1–1.5 hr in 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2; 415 mosm) at 4°C, rinsed in this buffer and postfixed for 1 hr in 1% osmic acid in the same phosphate buffer. Following dehydration in an ascending series of aqueous ethanol the specimens were transferred via a mixture of propylene oxide and epoxy resin (1:1) into pure Epon 812 as embedding medium. In the case of tissue cultures the coverslips were coated with Epon. After polymerization appropriate areas were selected under a phase contrast microscope. The coverslips were removed using dry ice and the selected areas glued to Epon blanks and sectioned parallel to the surface with glass knives on a Reichert OM U3. The sections were picked up on copper grids, double contrasted with uranyl acetate and lead citrate and examined in a Philips Electron Microscope EM 300. A total of 67 cells were investigated.

Labeling of lens cell cultures, preparation of cell lysates and gel electrophoresis

Cultured cells were incubated for 20 hr in a methionine-free culture medium, supplemented with 35S-methionine (5 μCi/ml). After incubation the medium was removed and the cells were rinsed twice with Tyrode solution. Then the cells were harvested with a rubber policeman, washed with Tyrode solution and centrifuged at approximately 2000 g; this procedure was repeated three times. The cells were then centrifuged at 10000 g for 15 min and the pellet dissolved in 50 μl of sodium dodecyl sulphate (SDS) sample solution, frozen and thawed and left at room temperature for 4 hr. SDS gel electrophoresis was performed as described before (Ringens, Boenders and Bloemendal, 1981); staining and destaining were carried out according to Weber and Osborn (1962). For the detection of the labeled proteins the procedure of Bonner and Laskey (1974), in combination with the drying procedure by Berns and Bloemendal (1974), was used.

Immunofluorescence

Cells grown on coverslips were fixed in −10°C methanol for 6 min. After a brief rinse in phosphate buffered saline (PBS) one of four antisera (anti-actin, anti-prekeratin, anti-vimentin and anti-tubulin) was added as the first antibody for 45 min at 37°C. After washing with PBS, the FITC labeled second antibody was added for another 30 min at 37°C. The coverslips were then mounted in Moviol 4-88 and observed in a Zeiss photomicroscope.
Fig. 1: Phase contrast photomicrographs of human epithelial lens cell cultures. (a) 2 days in culture, (b) dissociation of cells, (c) trabecular organization, 6 days in culture, (d) dense aggregate of cells, 7 months in culture. Filamentous structures (+), (b)-(d) x 1250; (e) x 225.
Fig. 2. Phase contrast photographs of epithelial monolayer. (a) primary culture after 1 month. (b) first subculture, 5 weeks. (c) senescent cells in first subculture, 3½ months. (a) and (b) x 200; (c) x 240.
3. Results

Light microscopy [Fig. 1(a)–(d); Fig. 2(a)–(c)]

During the first 24 hr of culture most of the pieces attached to the glass or plastic surface and the first signs of cell growth were seen at the edges of the fragments [Fig. 1(a)]. During subsequent days growth continued and epithelial sheaths surrounding the lens fragments could be observed. The cells first showed bleb-like formations at their periphery [Fig. 1(b)], revealed later a more regular surface and finally either degenerated or elongated. As a result of this continuous process the epithelium had expanded at the end of the first week, showing simultaneously vacuolar degenerative changes, polygonal cells and some already elongated cells. Thus a dissociation of the original monolayer occurred rapidly, resulting in a trabecular organization of the epithelial cells [Fig. 1(c)]. The process of simultaneous growth and dissociation/destruction took place for several weeks. However, after approximately 3 months in vitro the cells were nearly all elongated and signs of cellular aging appeared [Fig. 1(d)]. Especially in the elongated cells the presence of filamentous structures, running parallel with the axis of elongation, was obvious [Fig. 1(d), arrow].

In one case the early epithelial sheath around the lens fragments did not dissociate, but formed a confluent monolayer [Fig. 2(a)] throughout the culture vessel. After 6 weeks in vitro subcultivation was possible and the cells were seeded at approximate ratios of 1:1-5 and 1:2. At the higher cell density a new monolayer was formed [Fig. 2(b)]; however, at the lower cell density only local confluent sheaths developed. After 3 months cell elongation, the increased amounts of filaments and signs of cellular senescence were also evident in these cultures.

Ultrastructural aspects [Fig. 3(a)–(i)]

Lens 'in situ' [Fig. 3(a)–(c)]

The epithelium is surrounded by a fibrous capsule. The anterior face is covered by the superficial cuboidal epithelial cells. Electron-light and -dense cells can be distinguished [Fig. 3(a)]. A few rough endoplasmatic reticulum (RER) profiles and Golgi areas, as well as a fair amount of juxtanuclear mitochondria are observed. Apart from free ribosomes and polysomes, many glycogen particles (α- and β-form) are striking.

The underlying cortex consists of nuclei-containing lens fibers. Their nuclei show a spotty nucleoplasm with a reticular nucleolus. Close to the surface epithelial layer the parallel fibers demonstrate the same distribution of organelles as described above [Fig. 3(b)]. But deeper towards the interior of the lens the cytoplasmic appearance changes gradually with a loss of organelles. It should be emphasized that, though in decreased amount, α- and β-particles and Golgi complexes are still well recognizable in the most interior cells.

As for the cell membranes, it is shown that the surface epithelial layer is directly adjacent to the multilayered capsular fibrils and under the plasmalemma thin bundles of microfilaments are localized. The majority of the junctions between the cells are fasciae adherentes and closer to the underlying cortex gap junctions are present, too. The first signs of 'ball and socket' formation are demonstrated in the epithelial layer but this process is more prominent in the interior. Accumulations of microfilaments are present, particularly in these formations [Fig. 3(c)].
1 day in vitro [Fig. 3(d) and (e)]

At the edges of the explanted fragments an epithelioid growth is observed, consisting of large cells with lobulated nuclei and an extended cytoplasm. Apart from the regular organelles, the abundance of polysomes and glycogen particles as well as lysosomal structures should be noted. Both microtubules and intermediate-sized filaments can be discerned [Fig. 3(d)]. The junctions are made up by fascia adherens-like structures, to which many microfilaments attach [Fig. 3(e)].

1 month in vitro [Fig. 3(f) and (g)]

Different shapes of growing cells are conspicuous at this stage. Nuclei may have capricious forms with distinct nucleoli. The majority of the cells demonstrates an abundance of organelles. The RER is rather dilated and extensively distributed; in between are long, slender mitochondria and an ample amount of free polysomes. Golgi areas and some lipid droplets are localized centrally, while lysosomes and glycogen particles are found dispersed as well as in patches throughout the cytoplasm. The cytoskeletal feature is as follows: microfilaments as well as intermediate-sized filaments and a few microtubules surrounding the nucleus with an increasing amount of microfilaments towards the periphery. The epithelioid nature is obvious as most cells are in contact with each other by their adjacent cell membranes and by fascia adherens-like junctions [Fig. 3(f)]. Additionally, cells with a different (electron-light) cytoplasm are found too [Fig. 3(g)]. Their organelles are decreased in number and are distributed unevenly, few mitochondria, polysomes, granulated lysosomes and scattered glycogen granules are consistently found but bundles of microfilaments are sometimes observed.

3 months in vitro [Fig. 3(h) and (i)]

At this stage the lens cell elongates. The nucleus is bizarre with nucleoli and electron-dense chromatin spots. Centrally few RER and Golgi areas are seen. Mitochondria and lysosomal structures as well as glycogen particles, both scattered and in clusters, are detected. Also a few lipid droplets are found. Most striking, however, is the increase of all types of filamentous structures, especially that of the microfilaments [Fig. 3(h)], particularly at the periphery. The same junctions as described above are found at this stage, albeit in greater quantity and longer [Fig. 3(i)].

Gel electrophoresis [Fig. 4]

Figure 4 shows the SDS gel electrophoretic patterns and the corresponding autoradiographs of cultured lens epithelium after 1 week [Fig. 4(a)], and 6 weeks [Fig. 4(b)].
Fig. 4. (a) (1) Autoradiograph of human epithelial lens cell proteins after SDS gel electrophoresis; 1
week in culture; (2) stained pattern of the sample shown in lane 1; (3) markers: bovine serum albumin
(68 K daltons); ovalbumin (43 K); bovine α-crystallin (20 and 22 K) and cytochrome c (14.5 K). (b) (1)
Markers; (2) stained pattern of human epithelial lens cell proteins after SDS gel electrophoresis; 6 weeks
in culture; (3) autoradiograph of the pattern shown in lane 2.

In young cultures [Fig. 4(a)] a 20 K dalton chain is present and a fair amount of
radioactivity is incorporated in this polypeptide. Moreover, a weak chain is observed
at approximately 35 K daltons with no distinct corresponding radioactive band. A
relatively high amount of radioactivity is seen in the 40–50 K dalton region and in the
higher molecular weight region.

The older cultures [Fig. 4(b)] reveal a 20 K dalton polypeptide with no corresponding
band on the autoradiograph. However, two bands with a clearcut incorporated
radioactivity (at 43 and 57 K daltons, respectively) are observed.

Immunofluorescence (Fig. 5)

The results of the indirect immunofluorescence study are presented in Fig. 5. The
identity of actin, vimentin and tubulin could be confirmed with highly specific
antibodies but no prekeratin was detected.

In young polygonal cells a rather random distribution of the filaments is observed.
In elongated cells strong arrays of parallel stress fibers are seen in the actin pattern
[Fig. 5(b)], often running across the whole length of the cell in the direction of the
elongation. For vimentin and tubulin this organization can not be observed with cell
Fig. 5. Immunofluorescence patterns of human epithelial lens cells after 1 week (a), (c) and (e), and 8 weeks (b) top and bottom, (d) and (f) in vitro. Incubations were performed with antibodies against actin (a) and (b), top and bottom, vimentin (c) and (d) and tubulin (e) and (f).

elongation, although for the former some adaptation to the changed cell shape can be seen.

4. Discussion

In contrast to calf and chick lens human lens epithelium has a limited growth capacity, while elongation generally occurs rapidly. According to some recent reports the so-called 'lentoid bodies' have been described by several investigators (Hamada
and Okada, 1978; Eguchi and Kodama, 1979; Tassin, Malaise and Courtois, 1979); these piles of elongated cells are believed to be in vitro differentiated lens cells as they are not only elongated but also are reported to contain γ-crystallin. However, it cannot be excluded that the suspected presence of γ-crystallin might be due to cross-reactivity of the anti-serum.

In the experiments described here the epithelial origin of the cultured cells is established; however, 'lentoid bodies' are not seen. This may be due to different culture conditions (Vornhagen and Rink, 1979). It is therefore of interest that Reddan and McGee (1979) also found limited growth capacity of human lens epithelium in vitro and moreover, their results also sustain our finding that only some cultures reach confluency and are amenable to subculture.

Van Venrooy et al. (1974) have not been able to show α- or γ-crystallin in established calf lens epithelium in vitro and found one β-crystallin polypeptide; Rink and Vornhagen (1979) found γ-crystallin in cultured rat lens epithelium in stage B, but not in later stages of subculturing. In our hands young cultures of human lens epithelium show a reduced synthetic activity compared to the in situ situation, whereas in the older cultures no radioactivity at all is incorporated in the crystallin region.

On the other hand, the high amount of radioactivity in the 43 and 57 K dalton bands in the 6-week-old cultures is striking. These bands comigrate with purified actin and vimentin, and additionally, specific antisera directed against these two cytoskeletal proteins react positively upon indirect immunofluorescence. These results are confirmed by our ultrastructural observations. Ramaekers et al. (1979) also described the presence of actin bundles (stress fibers) in elongated calf lens cells in vitro, whereas intermediate-sized filaments of the vimentin-type are known to occur in cultured epithelial cells from diverse vertebrates (Franke, Schmid, Winter, Osborn and Weber, 1979).

The results of our biochemical, immunofluorescence and morphological investigations show both an increase and a reorganization of filamentous structures of the actin type which points to a role for these structures in the maintenance of shape and elongation in vitro, similar to what has been described for calf lens cells (Ramaekers et al., 1979).

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