Induction of Elongation in Cultured Rat Lens Epithelial Cells By FGF and Inhibition By Selenite

Mitsuyoshi Azuma* and Thomas R. Shearer†

The purposes of this experiment were to: (1) test if fibroblast growth factor (FGF) induced elongation of cultured rat lens epithelial cells (LEC) and (2) determine if selenite affected elongation of LEC. FGF (125–500 ng/ml) reduced the number of colonies of LEC, but it did not induce elongation when cells were cultured on plastic dishes. One hundred micromolar and, to a lesser extent, 10 µmol/l selenite also reduced the number of colonies of LEC. Coculture of FGF and selenite on plastic caused a synergistic reduction in the number of colonies. FGF (125–1000 ng/ml) induced a dramatic morphologic change in LEC. Elongated processes radiated from stellate-like cell aggregates when cells were cultured on reconstituted basement membrane matrix (Matrigel). Again, 100 µmol/l selenite and, to a lesser extent, 10 µmol/l selenite reduced the number of cell aggregates with processes on Matrigel. These results indicated that an inhibitory effect of selenite on the elongation of LEC may be a factor in the development of selenite cortical cataract. Invest Ophthalmol Vis Sci 33:2528–2531, 1992

The lens of the eye consists of two cell types.1 Epithelial cells cover the anterior surface of the lens under the capsule. The other cell type is the fiber cell. Fiber cells are produced by elongation of epithelial cells and they synthesize new crystallins. An interruption of orderly process of mitosis and differentiation, including elongation or aberrant elongation, could affect the transparency of the lens. Thus, the lens is a useful system for studying the relationship among mitosis, differentiation, and transparency.

Fibroblast growth factor (FGF) induces differentiation of epithelial cells into fiber cells in rat lens epithelial explants.2 FGF also has mitogenic and differentiative effects on several other cell types.3 Selenite is a powerful promoter of cataract formation.4 Selenite causes nuclear cataract within 5 d and subsequent cortical cataract within 30 d after injection. We previously hypothesized that the initial site of attack of selenite in nuclear and cortical cataract was the lens epithelium.5 Not known is whether selenite can directly inhibit proliferation and differentiation of LEC. Thus, the purposes of the present study were to determine: (1) whether FGF can induce elongation of LEC, and (2) whether selenite affects FGF-induced elongation.

Materials and Methods

Eyes from 7-day-old Sprague Dawley rats were removed immediately after killing, sterilized by brief immersion in 70% ethanol, and washed thoroughly with culture medium. Culture medium was Eagle’s minimum essential medium (MEM; Gibco Laboratories, Grand Island, NY) with 16% fetal calf serum (FCS; Gibco). Lenses were dissected by a posterior approach under the dissecting microscope, and capsules with attached epithelial cells were peeled off the anterior pole toward the periphery of the lens. These preparations contained negligible contamination from lens fibers.

Isolated capsules with underlying epithelia were incubated for 30 min at 37°C in Hank’s balanced salt solution (calcium- and magnesium-free) with 0.25% trypsin (Sigma, St. Louis, MO). As much trypsin solution as possible was removed, and trypsinization was stopped by dilution with culture medium. Epithelial cells then were dissociated from the capsules by trituration. The suspension was centrifuged twice for 4 min at 1000 rpm, and collected cells were inoculated into 24 well plastic plates (Falcon 3047; Becton Dickinson, Lincoln Park, NJ) at 3 × 10⁶ cells/well with 1 ml culture medium. Collected cells also were inoculated
into 24 well plates at $6 \times 10^4$ cells/well with 1 ml culture medium on reconstituted basement membrane matrix. The initial number of cells plated on plastic or Matrigel (Collaborative Research, Bedford, MA) may have influenced the toxicity of selenite, but it was necessary to inoculate twice the number of cells on Matrigel. A low growth rate probably occurred on Matrigel, and we observed floating unattached cells that could have been a result of poorer plating efficiency. Inoculated cells were observed as single cells without initial clumping.

Reconstituted basement membrane matrix (Matrigel) was produced by the murine Engelbreth-Holm-Swarm (EHS) tumor. The major components of the Matrigel are laminin, type IV collagen, heparan sulfate proteoglycan, entactin, and nidogen. It also contains transforming growth factor-β (TGF-β), basic FGF, tissue plasminogen activator, and other growth factors that occur naturally in the EHS tumor. The plastic culture surface was heated for 2 hr at 37°C before inoculation of wells with 0.2 ml Matrigel.

One day post inoculation medium was replaced in 4 separate groups as follows: (1) MEM (addition of 5% inactive FCS, heated at 56°C for 30 min when cultured without Matrigel); (2) MEM with 125–1000 ng/ml FGF (from bovine brain, Cat. No. 855740, Boehringer Manheim, Indianapolis, IN); (3) MEM with 10–100 μmol/l sodium selenite; and (4) MEM with FGF and sodium selenite. Selenite treatment was performed 1 hr before stimulation by FGF, and agents were present continuously until the end of culture. Phase-contrast micrographs were taken after stimulation to document the effects on morphology. Each colony (>20 cells in a clump were considered a colony) and cell aggregates with processes in each well were counted 3 times under the microscope, and the means were calculated. Standard deviation of the measurement was ±8%.

![Fig. 1. Phase contrast microscopy of lens epithelial cells (LEC) cultured for 2 d. Original magnification ×100. (A) LEC on plastic substrate without matrigel, showing typical flat shape. (B) Reduction of colony size when LEC were cultured with 250 ng/ml FGF on plastic substrate. (C) LEC on matrigel, showing cluster-like aggregates. (D) Inducement of elongated cells by 1000 ng/ml FGF on matrigel, showing radiation of elongated processes from stellate-like cell aggregate. One division on ruler indicates 10 μm.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933394/)
Results

LEC exhibited a typical flat shape when cultured on plastic without Matrigel (Fig. 1A). FGF (125–500 ng/ml) did not induce elongation when LEC were cultured on plastic without Matrigel (Fig. 1B). FGF reduced the number of colonies (Fig. 2). One hundred micromolar selenite markedly reduced the number of colonies, but 10 μmol/l selenite had less effect. FGF and selenite caused a synergistic reduction in number of colonies.

LEC formed cluster-like aggregates when cultured on Matrigel with or without FGF, and initial morphology of LEC was a rounded shape (Fig. 1C). Within 1 d after stimulation, elongated processes radiated from stellate-like cell aggregates with or without FGF. However, FGF (1000 ng/ml) dramatically enhanced the number of cell aggregates with processes. Length and thickness of elongated processes were increased (Fig. 1D). FGF of 125, 250, and 500 ng/ml was similar to 1000 ng/ml FGF in its ability to enhance elongation. Two days after stimulation, elongated processes were thicker and longer, and the number of cell aggregates with processes increased (Fig. 3). The results in Figure 3 were observed in two separate experiments. One hundred micromolar selenite reduced the number of cell aggregates with processes resulting from FGF, but 10 μmol/l selenite had less effect on day 1 or 2 (Fig. 3). One hundred micromolar selenite also caused elongated processes to become thinner and shorter. Statistical testing between different treatment groups was not possible because collecting enough primary culture LEC to perform multiple well plating was not practical. However, the inhibitory trends in the 3 selenite groups over 0–500 ng/ml FGF were clear with no overlap, except for one point in the 10 μmol/l selenite group on day 1.

Discussion

The results above showed that elongation of LEC could be induced on Matrigel alone and enhanced by FGF. FGF is found in extracellular matrix. To our knowledge, this is the first demonstration that cul-

---

**Fig. 2.** Number of colonies when lens epithelial cells were cultured for 2 d on plastic substrate without matrigel. Each point on the graph represents all colonies counted in one well, and different platings were done for each FGF concentration.

**Fig. 3.** Number of elongated cells when lens epithelial cells were cultured for 1 (A) and 2 (B) d on matrigel. One point is data from one well, and different platings were done for each FGF concentration. This is a representative experiment and was repeated two times.
tured LEC on Matrigel can be induced to elongate by FGF. Extracellular matrix plays an important role in growth and differentiation of several other cell types. FGF strongly interacts with heparin sulfate, which is one of components of Matrigel. This binding converts FGF to the biologically active form. Elongation of LEC due to FGF in rat lens explants also is described by Chamberlain et al. These investigations show the importance of extracellular matrix (lens capsule) for promotion of LEC elongation. In our studies, FGF reduced the number of colonies when cultured on plastic without Matrigel. This may indicate that FGF inhibits proliferation and shunts cells into differentiation.

Selenite causes loss of sulphhydryl groups in membranes of the lens epithelium. This may allow influx of calcium and activation of calpain. Calpains (EC.3.4.22.17) are calcium-activated, nonlysosomal, cysteine proteases, which are very widely distributed. Activation of calpain is associated with selenite nuclear cataractogenesis. However, not known is how selenite causes cortical cataract, which forms after selenite nuclear cataract. Selenite reduces the synthesis of DNA, RNA, and protein in cultured HeLa cells. Injection of selenite also affects DNA damage, repair, and replication in rat lens. Selenium also inhibits DNA synthesis and cell migration in the germinative zone of rat LEC in the S or pre-S phase of the cell cycle, and selenium in vivo depresses the differentiation of lens epithelial cells to fibers. In HeLa cells, micromolar concentrations of selenite inhibited colony formation. Our studies did not determine whether selenite caused cell death, interfered with cell growth, or affected plating efficacy. The present results showed that selenite reduced the number of colonies on plastic substrate and also reduced the number of cell aggregates with processes cultured on Matrigel. The concentration of selenite needed for this effect was comparable (>10 μmol/l) to the amounts of selenium in the whole lens from animals with selenite cataract (approximately 9 μmol/l). Our previous results with radioactive selenium show that most of the selenium is concentrated in the outer portion of the lens, where it may reach higher local concentrations. The results of the present investigation suggest that selenite reduces proliferation and elongation of lens epithelial cells and may cause abnormal fibrogenesis and cortical cataract in vivo.

Key words: FGF, selenite, elongation, lens epithelial cell, rat, extracellular matrix

Acknowledgments

The authors thank Dr. R. S. Anderson for helpful discussions.

References