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Development of Cataractous Macrophthalmia in Mice Expressing an Active MEK1 in the Lens

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Purpose. To characterize the extracellular signal-regulated kinase (ERK) pathway in the lens and to try to understand how this pathway contributes to lens function and cataractogenesis.

Methods. The members of the ERK pathway in the lens were examined by Western blotting, immunohistochemical staining, and kinase assay. A gain-of-function approach was used to perturb the ERK pathway in the lenses of transgenic mice via expression of a constitutively active mutant of the mitogen-activated protein kinase kinase 1 (MEK1(E)), the direct upstream kinase of the ERK1 and ERK2 kinases, under the αA-crystallin promoter.

Results. The presence of an active ERK pathway was found in lens epithelial cells and in differentiating fibers. Transgenic mice that expressed MEK1(E) developed postnatal cataracts as well as macrophthalmia. Distinct morphologic alterations, such as lens enlargement, swelling fiber cells, enlarged extracellular space, and vacuole formation, were observed in the lenses of these transgenic mice. A significant increase in the glucose transporter 1 (GLUT1) level, as well as in the glucose level, was detected in the lens.

Conclusions. The MAP kinase pathway is involved in the regulation of glucose metabolism and balance in the mouse lens. Moreover, the alteration of MAP kinase activity in the lens is sufficient to cause cataract formation with enlarged extracellular space and vacuoles in the differentiating fibers. This transgenic mouse may provide a useful model for understanding the mechanism(s) for some aspects of human cataracts. (*Invest Ophthalmol Vis Sci.* 2001;42:539–548)

The lens is a highly organized, transparent, refractive, and avascular organ. The specific function of the lens is to form an image by refracting and focusing light onto the retina. The mammalian lens consists of a bundle of lens fibers covered by a single layer of epithelial cells on its anterior hemisphere surface.[1] The continuing growth of the lens is dependent on the generation of secondary fiber cells from the epithelial cells. The newly differentiating secondary fibers lie on top of the previous generation of fibers in a concentric manner in the bow region of the lens. Eventually, these differentiating fibers eliminate their intracellular organelles to become mature fibers.[2] The lens epithelial cells and the outer fibers comprising the lens cortex possess the major metabolic machinery of the whole lens. The lenticular body must be organized at the molecular and structural levels, with control mechanisms maintaining short-range order of macromolecules and lens transparency.[3]

Cataracts are the opacification of the lens that causes impaired vision, and they are still the major cause of blindness in humans. Although cataracts appear mostly in elderly populations as an effect of aging or environmental and nutritional stresses, lens opacities may develop rapidly as a consequence of metabolic diseases, such as diabetes.[4] Many studies have attempted to understand diabetic cataracts through the use of a variety of animal or cell models, generated by special dietary administration of different sugars or other chemical reagents with various methods.[5–7] The limitation of these approaches is that they cannot determine whether the initiation event for the alteration of glucose metabolism in the lens is due solely to an elevated intracellular glucose level or whether it is the result of some other event occurring simultaneously, such as an activation of intracellular signaling pathways.[8,9] In particular, it is known that an elevated glucose level in the blood precedes the appearance of diabetic cataracts in human patients.[6]

There is evidence that the mitogen-activated protein (MAP) kinase plays an essential role in controlling cell proliferation, differentiation, and many other cellular responses in a variety of biological systems.[10] The ERK pathway is normally referred to as the classic MAP kinase pathway,[10,11] and it has been reported to be activated by UV radiation, oxidants, high glucose levels, shock, and cytokines.[12,13] There is evidence suggesting that some of these environmental stresses are the risk factors for causing human senile or diabetic cataracts.[5–7] Thus, we have hypothesized that MAP kinase pathways play a role in normal lens development and in pathologic responses to environmental stresses. Furthermore, the MAP kinases are likely to be the downstream signaling members of the growth factors.

Perturbation of the FGF signaling pathway via overexpression of FGFs[14–16] or dominant-negative receptors[17,18] resulted in microphthalmia with alterations in lens epithelial cells and fiber cells in mice. However, the intracellular signaling pathways that mediate these growth factors in the lens are not well understood.[19] To explore the role of the ERK pathway in the lens and to try to mimic a chronic activation of it under environmental stresses during a lifetime, we have selected a gain-of-function approach through an expression in transgenic mice of a constitutively active mutant MEK1(E) kinase, driven to the lens by the αA-crystallin promoter. The MEK1(E) kinase is the direct upstream kinase of the ERK1 and ERK2 kinases.

The present report demonstrates that the expression of MEK1(E) in transgenic mice is associated with severe impairment of the glucose level and/or glucose transport in the lens. This metabolic stress is associated with macrophthalmia and lens opacities that are similar to some aspects of human cataracts.

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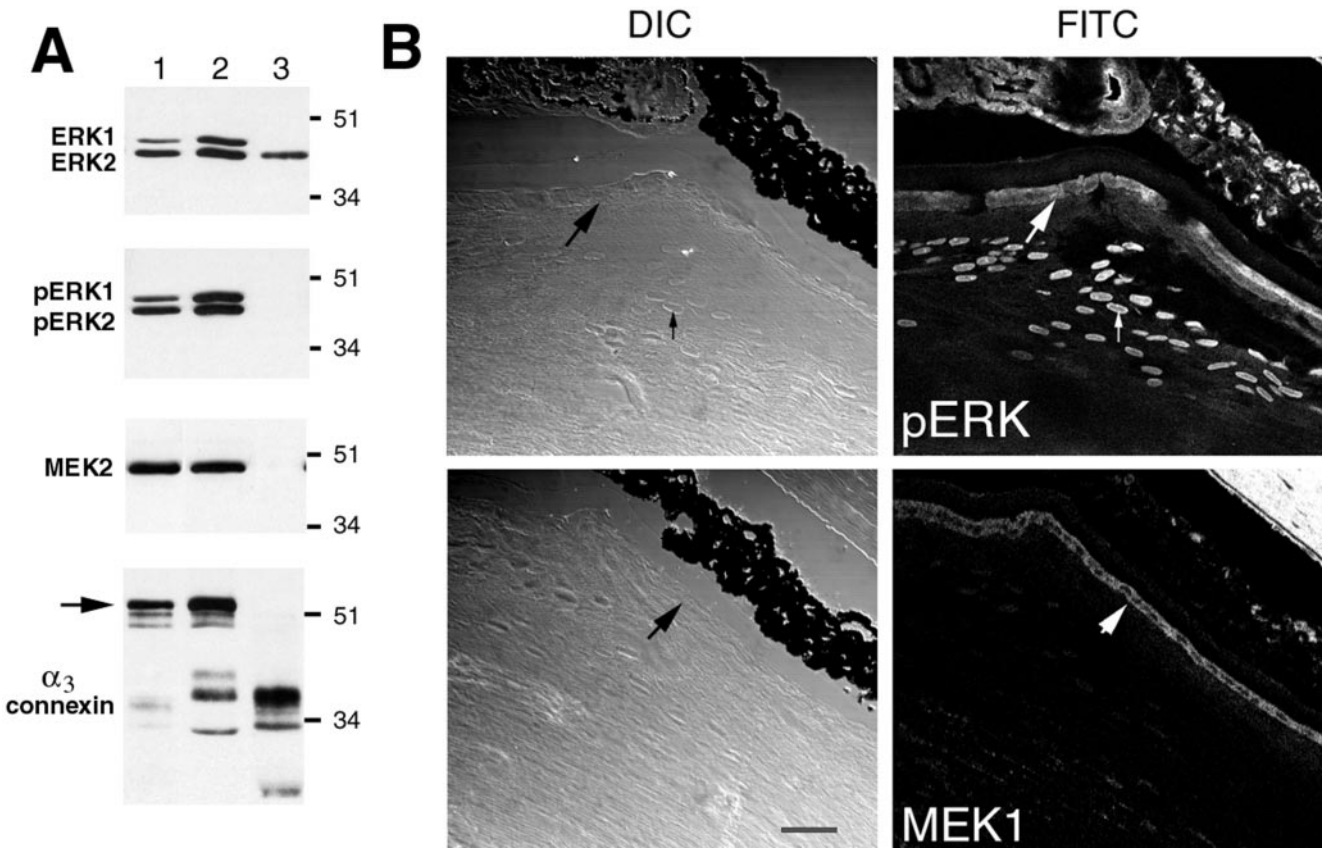

FIGURE 1. Activation of the ERK pathway in the lens epithelium and differentiating fiber cells. (**A**) The homogenates of the total lens (1), lens cortex (2), and lens nucleus (3) were examined for ERK1 and ERK2 kinases, the phosphorylated forms of ERK1 and ERK2 (pERK1 and pERK2), MEK2, and α_3 connexin (b) by Western blotting. The connexin α_3 (Cx46) antigen was used as a control for the separation of the lens cortex and lens nucleus. The intact α_3 connexin was detected only in the lens cortical homogenate, whereas the cleaved forms of α_3 connexin were found in the lens nuclear homogenate. The samples were prepared from 6-week-old mice. (**B**) Laser-confocal microscopic images of immunohistochemical stainings of the phosphorylated forms of ERKs (pERK) and MEK1 in the frozen lens sections from 6-week-old mice through the use of their specific antibodies. *Left*: differential interference contrast (DIC) pictures of these sections; *right*: FITC-stained results of the anti-pERK antibody (*top*) and the anti-MEK1 antibody (*bottom*). *Large arrows,* lens epithelium; *small arrows,* cellular nucleus of the differentiating fibers in the bow region. Scale bar, 10 μm.

METHODS

All scientific methods described in this article adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Generation of Transgenic Mice

The constructs that express a constitutively active form of kinases under the control of the αA-crystallin gene promoter[20,21] were generated and injected into fertilized embryos of the inbred C57BL/6J strain to produce the transgenic founder mice (F0).

Rat MEK1(E) cDNAs that encode constitutively active kinases were provided by Jiahuai Han. MEK1(E) cDNA encodes a constitutively active form of MEK1 by replacement of both serine residues (Ser-218 and Ser-222) with glutamic acid residues to mimic phosphorylation in the activated form.[22] The MEK1(E) cDNA (1.2 kb) was subcloned into the CPV2 expression vector provided by Paul Overbeek at Baylor College of Medicine.[23] A 2.5-kb fragment containing an αA-promoter-MEK1(E)-SV40pA region was used to generate the transgenic mice.

A PCR method was used to detect the transgene from mouse tail DNA. The 5′ primer is an oligo Ap (CCCAGAGGCTCCTGTCTGAC 1917–1936 bp of GenBank accession no. S79462). The 3′ primer for MEK1(E) is an oligo K1p (TGTGCTCCATGCAGATGCTG 436–417 of GenBank accession no. D14591). A 550-bp PCR fragment was detected from the tail DNA of CPV2-MEK1 transgenic mice by using this pair of primers, as indicated in Figure 1A.

Western Blot Analysis

A standard Western blot procedure provided by the manufacturer was used to carry out the analyses. The representative data shown in the figures were taken from more than three independent experiments. We prepared homogenates from the total lens, the lens cortex (including epithelial cells), and the lens nucleus of mice. The lens was arbitrarily separated into the lens cortex (including epithelial cells) and the lens nucleus, as we have done in previous articles.[24,25] These lens samples were directly homogenized in 1× sample buffer (60 mM Tris, pH 6.8, 2% SDS, 10 mM DTT, and 10% glycerol) according to the ratio of 10 mg of lens wet weight per 400 μl of buffer. Equal amounts of these homogenates were loaded onto SDS-PAGE for Western blot analysis.

Antibodies against MEK1, MEK2, ERK1, ERK2, GLUT1, GLUT3, and CIC-3 were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). The antibodies against the phosphorylated forms of ERK1/2, p38, and JNK1/2 were purchased from New England Biolabs (Beverly, MA). The antibodies against phospho-tyrosine were purchased from Signal Transduction. Antibodies against α_3 connexin (Cx46), α_8 connexin (Cx50), MP26, and MP20 were rabbit polyclonal antibodies.

Kinase Assay

Lens homogenized samples were prepared from the decapsulated lenses and examined for their kinase activities. Nonradioactive kinase

activity assay kits for ERK1 and ERK2 kinases were purchased from New England Biolabs, Inc. The experimental procedures were performed according to the protocol provided by the manufacturer. Active ERKs phosphorylated an Elk-1 fusion protein in the presence of ATP and kinase buffer. Phosphorylation of Elk at Ser383 was measured through Western blot analysis, using a phospho-Elk-1 (Ser383) antibody provided in the kit.

Histologic Analysis and Immunohistochemical Staining

The hematoxylin and eosin–stained sections were prepared from these mouse eyes by standard methods.[24] Similarly, standard methods were used for the antibody staining of the frozen lens sections.[24] A laser-confocal microscope (model 1024; BioRad, Hercules, CA) was used to collect the staining results.

Transmission Electron Microscopic Analysis

Mouse lenses were fixed in 2.5% gluteraldehyde with 0.075 M of cacodylate buffer, pH 7.2, overnight at 4°C. The fixative was changed daily for a total fixation time of 5 days at 4°C. After extensive washes in 0.1 M cacodylate buffer, lenses were post-fixed in 1% OsO_4 for 1 hour at room temperature, stained with 0.5% tannic acid/0.05 M cacodylate buffer for 1 hour, and neutralized with 1% Na_2SO_4 in 0.1 M cacodylate buffer for 15 minutes. Lenses were then stained en bloc with 1% uranyl acetate/10% ethanol for 15 minutes and further dehydrated in a standard ethanol series with two changes of 95% and 100%. Ethanol was exchanged with HPMA and lenses were infiltrated overnight in a 1:1 ratio of HPMA/LX112 (Ladd Scientific, Burlington, VT) mixture while rotating. Lenses were embedded in LX112 after one change of 100% LX112 for 4 hours and polymerized for 24 to 36 hours at 60°C. Sample blocks were sectioned at 70 to 80 nm. Each grid was contrasted with uranyl acetate and lead citrate before transmission electron microscopic (TEM) inspection.

Measurement of Lens Glucose Concentration

Two lenses from one mouse were deproteinized by homogenization with 150 μl of perchloric acid solution (6% $HClO_4$), followed by centrifugation at 13,000 rpm for 10 minutes at 4°C. The supernatants were neutralized with 35 μl of 2 M K_2CO_3 followed by centrifugation at 13,000 rpm for 10 minutes at 4°C. One hundred microliters of the neutralized supernatant per sample was used for assaying the glucose amount according to the procedure provided by the manufacture (diagnostic glucose [HK] 10 kit; Sigma, St. Louis, MO). The lens glucose concentration was calculated from the glucose amount (per lens) and its volume. Because the mouse lens is almost like a sphere, we calculated the lens volume, $1/6\ \pi D^3$, as if it were a sphere, using the equatorial diameter (D).

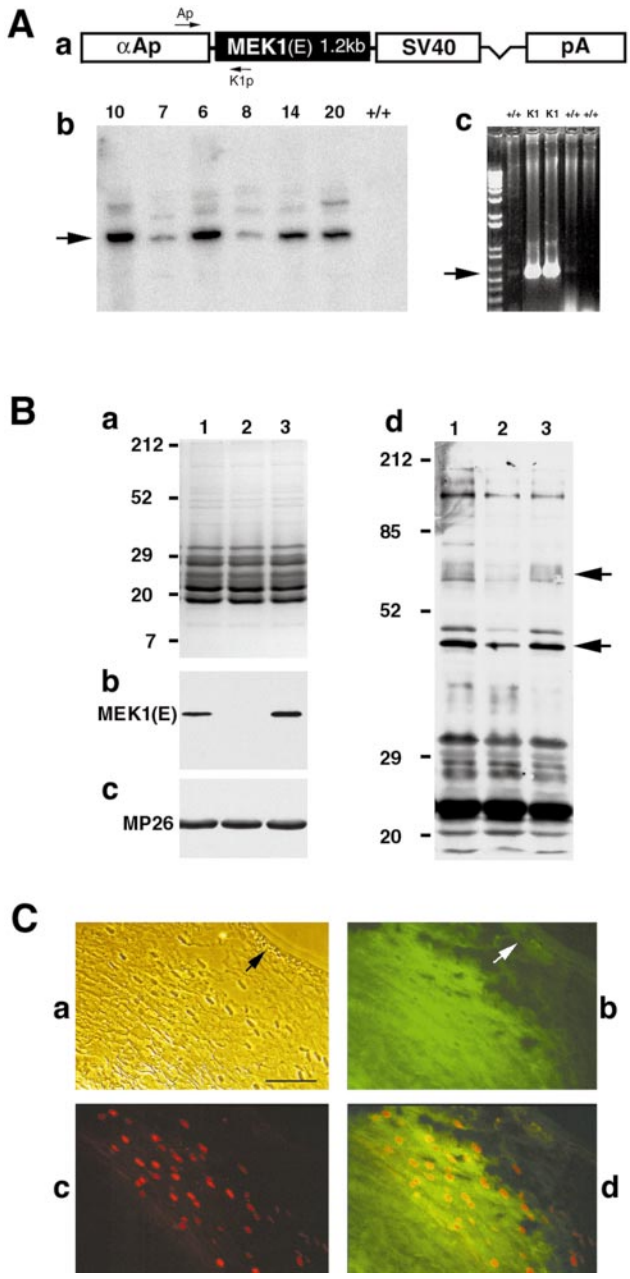

FIGURE 2. Generation and characterization of the MEK1(E) transgenic mice. (**A**) The generation of the transgenic mice. (**a**) The physical map of the MEK1(E) construct used for generating the MEK1(E) transgenic mice. MEK1(E) cDNA was inserted between the αA-crystallin promoter (αAp) and the DNA fragment of an intron and polyadenylation sequence of the small t antigen from the SV40 virus (SV40pA). The primers Ap and K1p (*arrows*) were used for mouse genotyping by PCR. (**b**) The tail DNA samples of six transgenic founder mice and one wild-type mouse were digested with *Bam*HI and examined by Southern blot analysis, using a MEK1(E) cDNA probe. Ten micrograms of DNA was loaded in each lane. An expected 2.8-kb band was detected in all founder mice. (**c**) A 550-bp was detected from the tail DNA of MEK1(E) transgenic mice by PCR, using Ap and K1p primers. One-kilobase maker from Gibco-BRL was used as a standard marker. (**B**) Biochemical characterization of the transgenic mice. (**a**) Total lens homogenates from two 3-week-old F2 transgenic mice (*1* and *3*) and one wild-type littermate were examined by a 10% Coomassie-stained gel. Twenty micrograms of protein was loaded in each lane. (**b**) The same samples in (**a**) were examined for MEK1(E) protein by an anti-MEK1 antibody. The MEK1(E) protein band was detected only in the lens homogenates of the MEK1(E) transgenic mice and not in that of the wild-type mouse. (**c**) The same samples in (**a**) were examined for MP26, the lens major membrane protein, by an anti-MP26 specific antibody. (**d**) An increase in several phospho-tyrosine proteins (*arrows*) was detected in the transgenic samples when compared with that of the wild-type control by an anti–phospho-tyrosine antibody. The molecular weight markers (kDa) are indicated on the *left* side of the figures. (**C**) The immunohistochemical staining of the MEK1(E) and c-maf transcription factor in the frozen lens sections of 3-week-old MEK1(E) transgenic mice by their specific antibodies. (**a**) A phase photograph of the section with the lens epithelial cells indicated by an *arrow.* (**b**) The FITC-stained result of the anti-MEK1 antibody. (**c**) The RITC-stained result of anti-c-maf antibody. (**d**) The merged picture of (**b**) and (**c**). Scale bar, 20 μm. The anti-MEK1 antibody is the same antibody used for the staining of the wild-type lens section in Figure 1B. The transcription factor c-maf was used as the marker for the cellular nucleus of the differentiating fibers in the bow region (**C**,**c**).

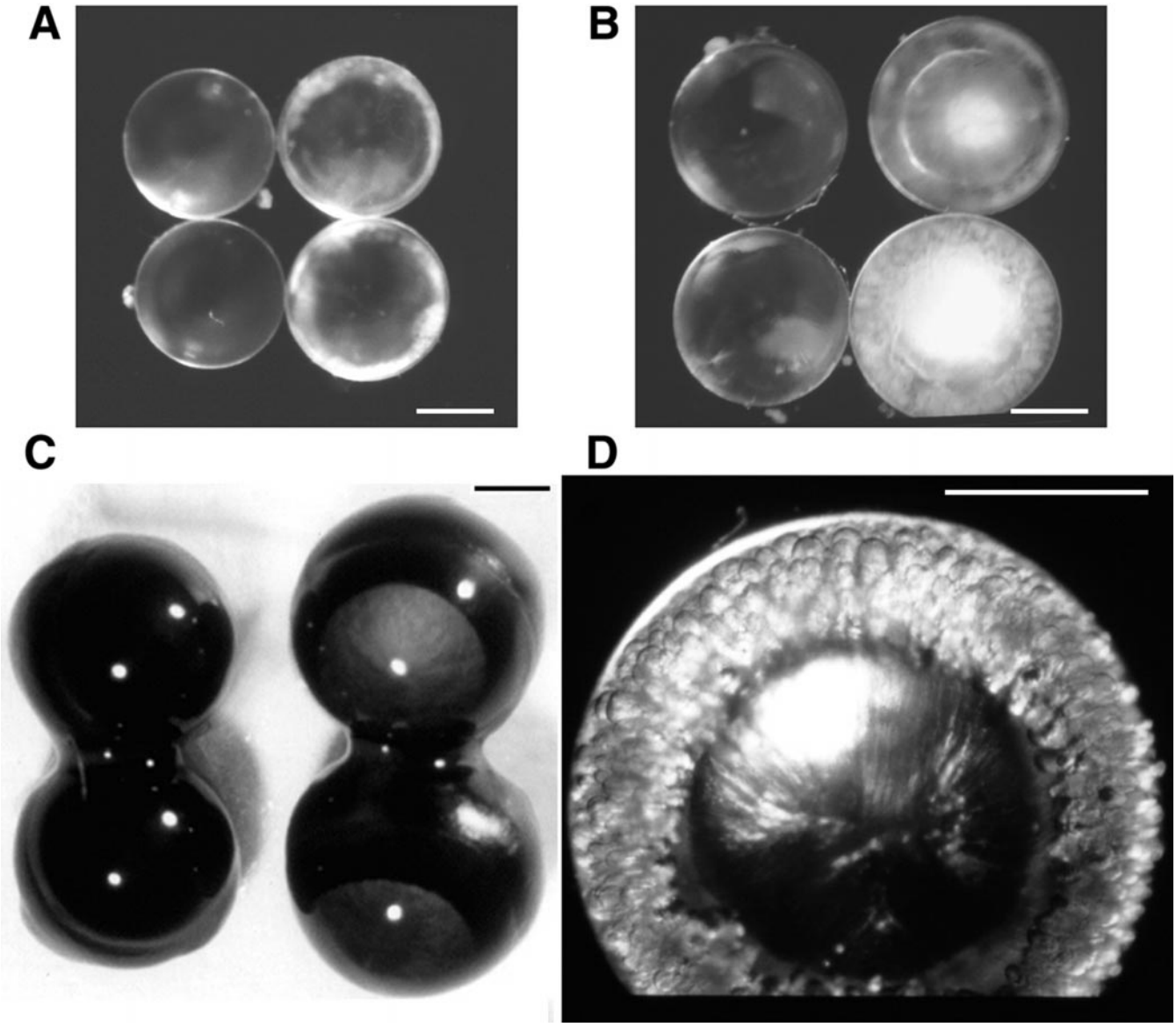

FIGURE 3. Macrophthalmia and cataracts in the MEK1(E) transgenic mice. (**A**) This is an anterior view of two pairs of lenses from a transgenic mouse (the *right* pair) and a wild-type littermate (the *left* pair) at the age of 3 weeks. Cataracts were located in the cortical region of the MEK1(E) lenses. (**B**) Anterior view of two pairs of lenses from a transgenic mouse (the *right* pair) and a wild-type littermate (the *left* pair) at the age of 5 weeks. Both cortical and nuclear cataracts were observed in the MEK1(E) lenses. (**C**) Two pairs of eyes from a transgenic mouse (the *right* pair) and a wild-type littermate (the *left* pair) at the age of 5 weeks. The size of the MEK1(E) eye was significantly larger than that of the wild-type eye. (**D**) This is a magnified anterior view of cataracts in a 5-week-old MEK1(E) lens. Scale bar, 1 mm.

RESULTS

Presence of the Activated ERK Pathway in Lens Epithelial Cells and in Differentiating Fibers

The transcripts of *ERK1*, *ERK2*, *MEK1*, and *MEK2* genes were detected by RT-PCR analysis (data not shown).

MEK2, ERK1, and ERK2 proteins, as well as the phosphorylated (active) forms of ERK1 and ERK2, were detected in the homogenates of the total lens and lens cortex by Western blotting (Fig. 1A, lanes 1 and 2). The ERK2 protein was found in the lens nuclear homogenate, but no phosphorylated forms of ERK2, ERK1 and MEK2 were detected (Fig. 1A, lane 3). The endogenous MEK1 was undetectable in these homogenates through Western blotting, using an anti-MEK1 antibody that recognized both MEK1 and active mutant MEK1(E) proteins (see Fig. 6, the MEK1(E) panel).

Phosphorylated (active) forms of ERK1 and ERK2 were detected with fluorescence by immunohistochemical staining in both the cellular nucleus of differentiating fiber cells and in the lens epithelial cells, using an antibody against the phosphorylated forms of pERK (Fig. 1B, top right). The endogenous MEK1 protein was detected only in lens epithelial cells by the same anti-MEK1 antibody used in the Western blotting (indicated by an arrow in the bottom right of Fig. 1B). The MEK2 antigen was detected in both the epithelial and fiber cells by the anti-MEK2 antibody (data not shown).

Generation and Characterization of the MEK1(E) Transgenic Mice

Six MEK1(E) transgenic founder mice were generated (Fig. 2A). These founder mice were mated with wild-type C57BL/6J mice to generate F1 transgenic mice. F2 mice were generated

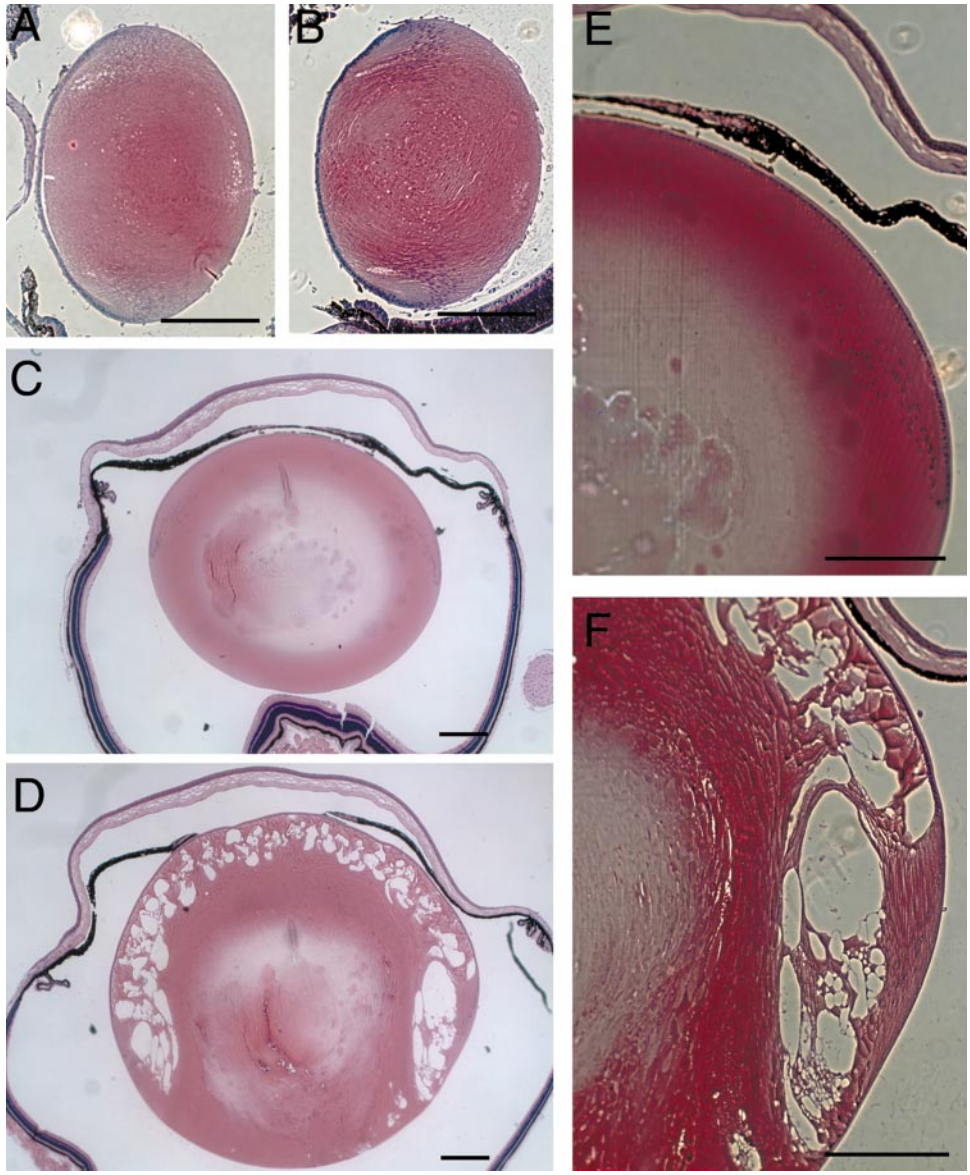

FIGURE 4. Histologic analysis of the MEK1(E) and wild-type lenses. Representative hematoxylin-eosin–stained sections of the lenses of MEK1(E) transgenic and wild-type mice. Scale bar, 0.25 mm. Lens sections along the visual axis from a 1-day-old wild-type mouse (**A**) and a 1-day-old transgenic mouse (**B**). Eye sections along the visual axis from a 5-week-old wild-type mouse (**C**) and a 5-week-old transgenic mouse (**D**). (**E** and **F**) Magnified view at the lens bow regions of (**C**) and (**D**), respectively.

from either an inter-cross between the F1 transgenic mice or an inter-cross between the F1 transgenic mice and F1 wild-type mice. The F1 and F2 mice from three independent founder lines, 7, 20, and 14, were used for various analyses.

Lens Cell Expression of Transgenic MEK1(E) Protein and Changes in Phospho-Tyrosin Proteins in MEK1(E) Transgenic Lenses

No obvious changes in the protein profile of the total lens homogenates between the wild-type and MEK1(E) transgenic mice were observed in a Coomassie-stained gel (Fig. 2B,a). However, the MEK1(E) protein was detected through Western blotting only in samples of MEK1(E) transgenic mice (Fig. 2B,b). There was no detectable change in the level of MP26 (the lens major membrane protein) among the homogenates of MEK1(E) and wild-type lenses (Fig. 2B,c). There was, however, an increase in the signal intensity of several phospho-tyrosine protein bands (indicated by arrows in Fig. 2B,d) in the MEK1(E) lens homogenate when compared with the wild-type control, using an anti-phospho-tyrosine antibody for detection. This result is consistent with the observation of an elevated activity of ERKs in the MEK1(E) transgenic lenses (Fig. 6A, pERK and pELK-1 panels).

Moreover, the MEK1(E) protein was detected in the differentiating fibers by immunohistochemical staining, using an anti-MEK1 antibody (Fig. 2C,b). This is the same anti-MEK1 antibody used for detecting the endogenous MEK1 in the lens epithelial cells in Figure 1C and for the Western blotting. The positive signal in the epithelial cells of the MEK1(E) transgenic mice (indicated by an arrow in the Fig. 2C,b) is relatively lower than that of the wild-type control (shown in 1B), and this is due to the fact that a very intense and diffuse fluorescent signal of the transgene product MEK1(E) was detected in the cytosol of adjacent fiber cells.

These results indicate that the presence of MEK1(E) causes both an elevation in ERK kinase activity and an alteration in the phospho-tyrosine proteins in the mouse lenses.

Postnatal Development of Cataracts and Macrophthalmia in MEK1(E) Transgenic Mice

In comparison to the lenses of their wild-type littermates, all the transgenic mice postnatally developed macrophthalmia

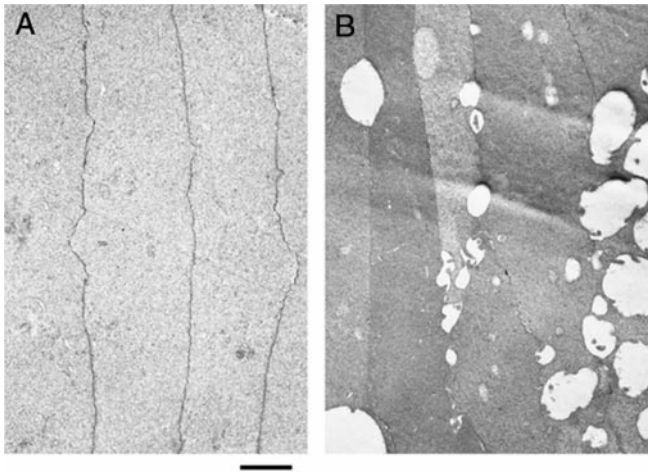

FIGURE 5. Electron micrographs demonstrating the presence of both enlarged extracellular space and vacuoles in MEK1(E) lens fibers. (**A**) Lens fibers from 3-week-old wild-type mice. (**B**) Lens fibers from 3-week-old MEK1(E) mice from line 7. Scale bar, 2 μm.

with cataracts. The offspring of the transgenic mouse lines 7 and 20 were used for the phenotypic analysis.

The increase in the size of the eyeball (Fig. 3C) and the development of enlarged cataractous lenses (Figs. 3A, 3B) can be observed by direct visual examination. Vacuole formation in the lens cortex varied among individual transgenic mice. Disrupted fibers were observed only in the lens cortex of the MEK1(E) transgenic lenses (Fig. 3D). No dramatic lens morphologic changes were detected in 1-day-old transgenic lenses when compared with the wild-type lenses (Figs. 4A, 4B), although a few swollen fibers with no vacuoles were observed in the 1-day-old transgenic lenses (data not shown). However, in a 5-week-old transgenic lens, a number of cytoplasmic vacuoles, or enlarged intercellular spaces, were observed in cortical regions (Fig. 4D). These vacuoles first appeared in the bow regions of the equator and then spread to the anterior side (Fig. 4F). Furthermore, both enlarged extracellular space and vacuoles were detected in MEK1(E) lens fibers by TEM analysis (Fig. 5). No obvious morphologic changes in the lens epithelial cells were observed in the transgenic mice (data not shown). In the 3- to 4-week-old transgenic mice, the other tissues of the eye, including the retina and the cornea, did not show obvious pathologic features aside from an enlargement, which was true of the whole eyeball. No swollen fibers or vacuoles appeared in the lens sections of the wild-type littermates (Figs. 4C, 4E).

Eye Weight, Lens Weight, Lens Total Proteins, and Water-Soluble Crystallins as a Function of Age in MEK1(E) Transgenic Mice

The growth curves of both the eye and the lens were determined by measuring their total wet weight. An increase in the weight of the eye and the lens of the MEK1(E) transgenic mice was found after 2 weeks of age, compared with that of the wild-type mice (Table 1 and Figs. 6A, 6B). The increase in the volume of the lens and the eye was directly correlated with their increase in weight (representative data shown in Table 2). There was a 40% increase in the wet weight of the MEK1(E) lenses of 4-week-old mice compared with that of the wild-type controls (Table 2). However, the lens dry weight of the MEK1(E) lenses was almost the same as that of their wild-type littermates (Fig. 6C). These data were obtained from the transgenic mice of line 7. The phenotypes of the transgenic mice of line 20, macrophthalmia with enlarged cataractous lenses, progressed much faster and more severely than those of line 7. The average increase in the eye weight was 50% when compared with their wild-type littermates at the age of 4 weeks, whereas the average wet weight increase in their lenses was 65% (results taken from 4 transgenic and 4 wild-type mice). The phenotypic variation between line 20 and 7 is possibly due to the fact that the 20 founder mouse had four times more MEK1(E) transgene copies than the seven founder mouse, according to the intensity of the hybridized bands (in Fig. 2A,b), as defined by phosphoimager analysis. It is also possible that, aside from the higher copy number of the transgene, other factors may have contributed to the severe phenotype in line 20.

There were no significant changes in the total lens proteins in the heavier transgenic lenses when compared with the wild-type lenses at 3 and 4 weeks of age (Fig. 6D).

Water-soluble homogenates were prepared from the lenses of both transgenic and wild-type mice at the ages of 2 weeks, 4 weeks, and 2.5 months and were then studied by gel filtration analysis. No obvious changes in the distribution of α-, β-, and γ-crystallins were found in the samples from transgenic lenses of 2- to 4-week-old mice when compared with that of wild-type mice at the same age. A representative result is shown (Fig. 6E). However, a significant loss of γ-crystallin was found in the sample of 2.5-month-old transgenic lenses with nuclear cataracts (Fig. 6F).

Selective Upregulation of GLUT1 and an Elevated Glucose Level in MEK1(E) Transgenic Lenses

We have found that lens-specific expression of the MEK1(E) led to an approximately twofold increase in the phosphorylated ERK1 and ERK2 kinase (pERK), as well as in their kinase activities (measured via pELK-1; Fig. 7A). These results were determined by densitometric measurements from more than three sets of experiments. No detectable changes in the other MAP kinases, such as the MEK2 kinase, the phosphorylated form of p38α kinase, or the phosphorylated form of c-Jun N-terminal kinase 1 (pJNK1), were found in the MEK1(E) lens homogenates by Western blotting (Fig. 7A). Similarly, there were no detectable changes in the levels of several major lens membrane proteins, including MP26, MP20, connexin α_3 and α_8, and the chloride channel

TABLE 1. Comparison of the Eye Weight and the Lens Weight of Wild-Type and MEK1(E) Mice at Different Ages

	Age					
	1 Week	**2 Weeks**	**3 Weeks**	**4 Weeks**	**5 Weeks**	**6 Weeks**
Wildtype lenses (mg)	1.53 ± 0.12 (6)	2.68 ± 0.12 (6)	3.67 ± 0.21 (8)	3.76 ± 0.12 (8)	4.60 ± 0.22 (4)	4.75 ± 0.39 (14)
Wildtype eyes (mg)	9.56 ± 0.23 (5)	13.8 ± 0.26 (6)	14.2 ± 0.35 (8)	15.0 ± 0.64 (13)	16.5 ± 1.0 (6)	
MEK1(E) lenses (mg)	1.43 ± 0.19 (11)	3.28 ± 0.31 (6)	4.26 ± 0.14 (6)	5.13 ± 0.42 (8)	6.10 ± 0.71 (6)	6.38 ± 0.42 (7)
MEK1(E) eyes (mg)	9.01 ± 0.59 (10)	15.4 ± 0.71 (6)	16.5 ± 0.58 (6)	18.3 ± 1.3 (13)	20.9 ± 2.1 (8)	

Values in parentheses are number of mice.

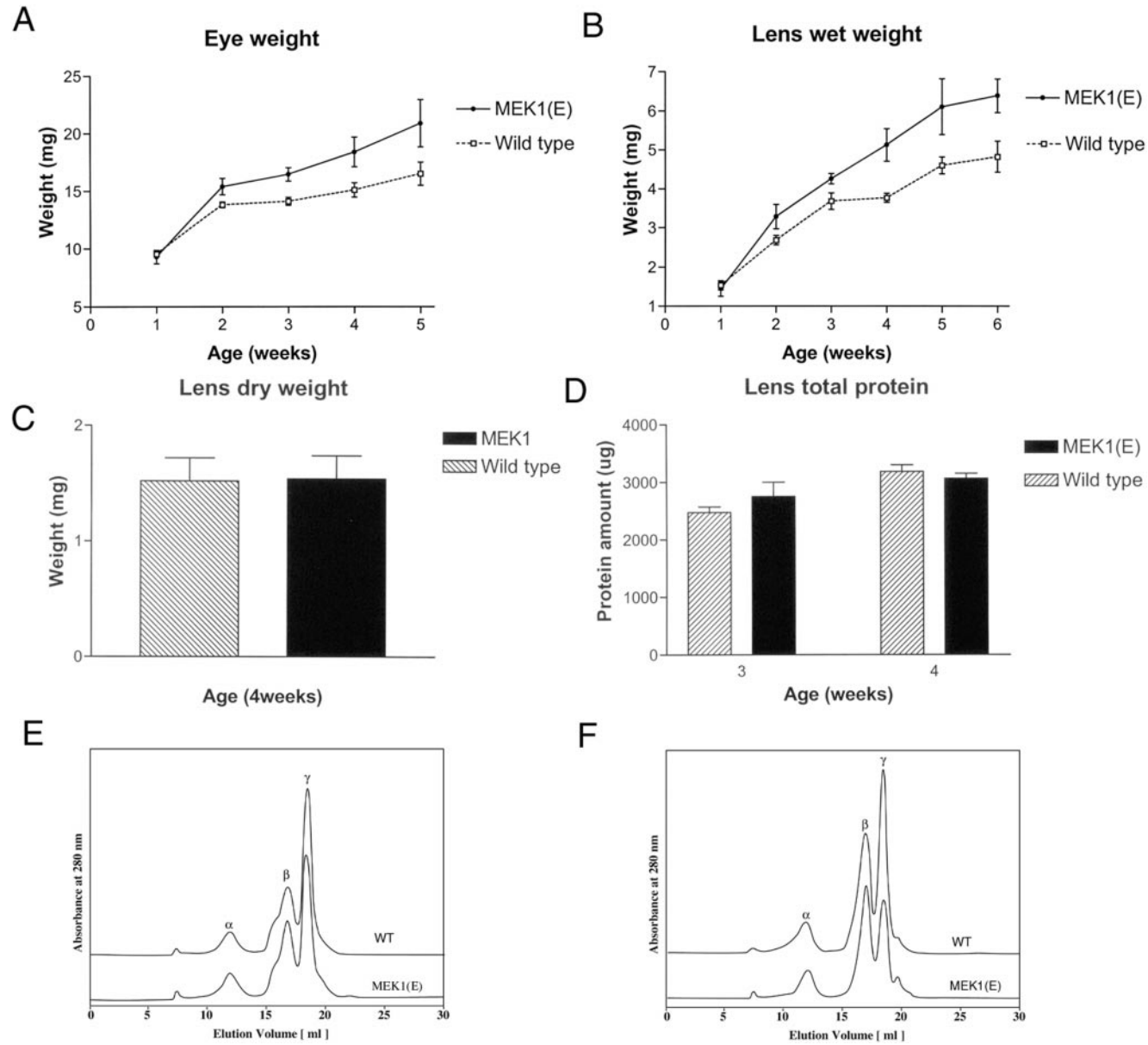

Figure 6. Comparison of the eye weight, the lens weight, lens total proteins, and water-soluble crystallins between MEK1(E) transgenic and wild-type mice. (**A**) Eye wet weight as a function of mouse age. (**B**) Lens wet weight as a function of mouse age. (**C**) Lens dry weight as a function of mouse age. (**D**) Lens total protein amount as a function of the mouse age. (**E**) Elution profiles of the total water soluble proteins from two lenses of MEK1(E) transgenic and wild-type mice at the age of 4 weeks. α, β, and γ peaks correspond to the three major classes of crystallins. Gel filtration analysis was performed as described in the text.[25] At least three pairs of lens samples from each of the transgenic and wild-type mice of the same age were analyzed by the gel filtration method. (**F**) Elution profiles of the total water-soluble proteins from the lenses of the MEK1(E) transgenic and wild-type mice at the age of 2.5 months. α, β, and γ peaks correspond to the three major classes of crystallins.

CIC3 (Fig. 7A). However, we have found that there was at least a fourfold increase in the glucose transporter 1 (GLUT1) protein level in the decapsulated MEK1(E) lens homogenates when compared with that in the wild-type controls. This was determined through a densitometric analysis from Western blot results of six sets of experiments. This increase in the GLUT1 level was also detected in lens samples of 2-week-old MEK1(E) mice (data not shown). No

Table 2. Comparison of the Increase of Weight and Volume of the Eyes and Lenses of Wild-Type and MEK1(E) Mice at Age of 4 Weeks

	Eye Weight (mg)	Eye Volume (mm^3)	Lens Weight (mg)	Lens Volume (mm^3)
Wild type	14.8 ± 0.7 (5)	15.7 ± 1.3 (5)	3.66 ± 0.19 (5)	3.78 ± 0.31 (5)
MEK1(E)	19.6 ± 0.5 (5)	22.7 ± 1.1 (5)	5.15 ± 0.26 (4)	5.82 ± 0.42 (4)
Increase of MEK1(E) (%)	32	44	40	54

Values in parentheses are number of mice.

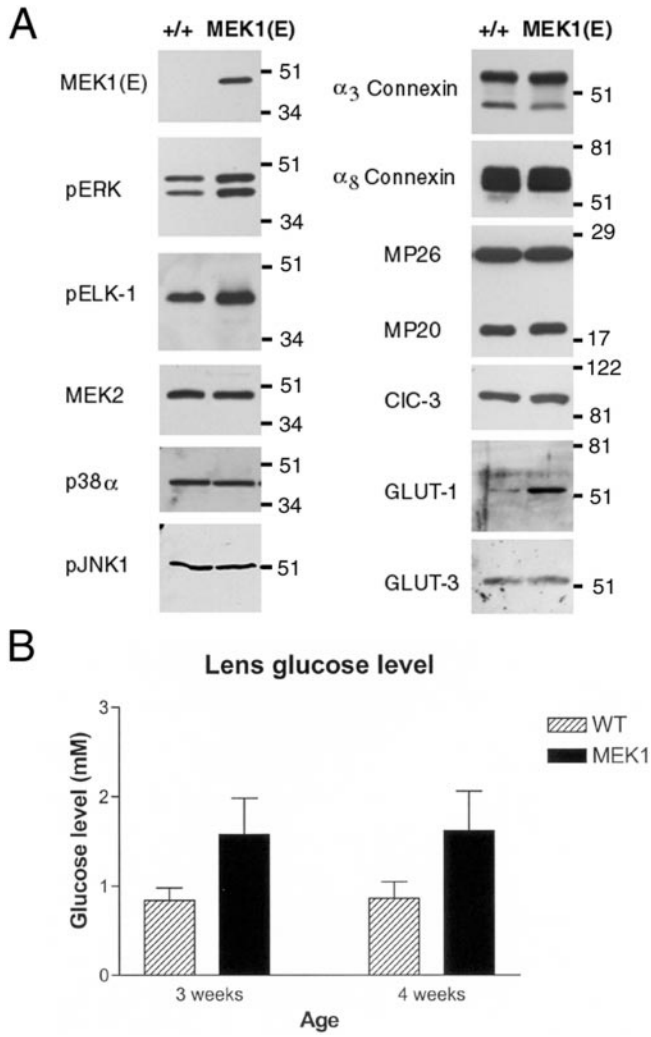

FIGURE 7. Elevated levels of GLUT1 and glucose in the lenses of the MEK1(E) transgenic mice. (**A**) Homogenates prepared from the decapsulated lenses of the 3-week-old F2 transgenic and wild-type mice were used for both the kinase activity assay and Western blot analysis. Representative results are from more than three different sets of experiments. The pELK-1 panel was determined by the kinase assay of the ERK activity by using a recombinant ELK-1 protein as the substrate of ERK1 and ERK2 kinases; all other panels are lens endogenous proteins detected by their specific antibodies. There was an approximately twofold increase in the phosphorylated forms of ERKs (pERK) as well as in their activities (pELK-1) in the MEK1(E) lens sample when compared with that of the wild-type control. There were no detectable changes in MEK2, p38α (a phosphorylated form), pJNK1 (a phosphorylated form), α_3 connexin, α_8 connexin, MP26, MP20, or the chloride channel CIC-3 in the MEK1(E) lens. However, there was a significant increase in the GLUT1 protein level (at least fourfold, as determined by a densitometric analysis) in the decapsulated MEK1(E) lens homogenate when compared with the wild-type control. There were no detectable changes in the GLUT3 level in these MEK1(E) samples. The molecular weight markers (kDa) are indicated on the *right* side of the figures. (**B**) Lens glucose level as a function of age in the wild-type mice ($n = 6$) and transgenic mice ($n = 16$). The lens glucose level was measured from the deproteinized total lens homogenates of the mice at 3 and 4 weeks of age.

detectable change in the glucose transporter 3 (GLUT3) protein level was observed (Fig. 7A). There was a more than 80% increase in the glucose level in the MEK1(E) lenses when compared with that in the wild-type mice (Fig. 7B).

DISCUSSION

The lens may be viewed as an asymmetrical syncytial organ characterized by a functional and vectorial organization. The maintenance of the electrochemical gradient across the lens relies on the presence in the epithelial monolayer and in the outer cortical differentiating fibers of sources for active metabolism and energy production for active transport.[26] Cell–cell communication between epithelial and fiber cells throughout the lenticular mass depends on the presence of junctional domains of low electrical resistance and membrane sites of increased permeability for small ions and water.[27–30]

It is noteworthy that active forms of ERK1 and ERK2 were found in both the lens epithelial cells and in the differentiating fiber cells, suggesting that mitogen-activated protein kinases operate in a lens domain where the maintenance of the electrochemical gradient across the lens is actively regulated. In this study, we have selected a constitutively active mutant of MEK1(E) instead of an active mutant of MEK2 to activate the ERK pathway in the lens. This is for three reasons: (1) MEK1 and MEK2 are equivalent in terms of the activation of ERKs; (2) we can distinguish the transgene MEK1(E) from the endogenous MEK2 in the lens fibers by Western blotting; and (3) the endogenous MEK1 in the lens fibers is undetectable by Western blotting. It is striking that, in transgenic mice expressing MEK1(E) that was driven to the lens by the αA-crystallin promoter, cataracts and macrophthalmia developed. It is therefore likely that the lens-specific expression of MEK1(E) and/or an elevated ERK pathway activity selectively alters properties of downstream targets, including glucose metabolism and, in turn, the cataract formation of the lens. Given the fact that the ERK pathway plays an essential role in controlling cell proliferation and differentiation, it is surprising that a disruption of secondary fiber cells is the predominant defect in the MEK1(E) transgenic lenses. The fact that there were no obvious changes in the lens epithelial cells might be due to the absence of the MEK1(E) transgene product, because the αA-crystallin promoter has been reported to be inactive in the epithelial cells.[15] Currently, we do not know why we did not observe any obvious defects in the primary fibers, where transgenes should be expressed. Because we were unable to distinguish MEK1(E) from endogenous MEK1 in these cells with anti-MEK1 antibodies, further studies will be required for any interpretations about the primary fibers of MEK1(E) lenses.

The question remains, however, whether the actual mechanism generating intra-cytoplasmic vacuoles and enlarged intercellular spaces is restricted to the cortical lens region. We assumed that water is retained in the lens cortical region in parallel with an increase in the pressure via an elevated level of ions and other osmolytes. The presence of extensive swelling of cytoplasmic membrane compartments and intercellular spaces would reflect dramatic changes in the function of specific membrane transporters in the cortical fibers of the transgenic lenses. It is likely that this transporter is one of the downstream targets of the ERK pathway, which regulates the balance in the cortical fibers of the lens. Several published articles have shown that similar cataracts were observed in lenses after they were treated with chloride channel blocker 5-nitro-2-(3-phenylpropylamin) benzoate (NPPB) and tamoxifen.[31,32] Although there were no changes in the protein level of chloride channel CIC3 or in the water channel protein MP26[33] in the MEK1(E) lens fibers, we still do not know whether their functions are normal. It has been reported that the MEK1 kinase may have an ERK-independent downstream pathway,[34] although we have no evidence as of yet to show this novel pathway in the lens.

Glucose Transporter and Cataractogenesis

Diabetic cataract formation is associated with swelling fibers and vacuole formation in the lens cortex, which subsequently cause nuclear cataracts.[6] Cataract formation in the MEK1(E) transgenic mice, to a certain degree, resembled some of the lens pathologic processes in diabetic cataractogenesis.

Our hypothesis is that the selective upregulation of GLUT1 partly contributes to the lens phenotype of the MEK1(E) transgenic mice. An elevated GLUT1 level could cause an increase in the influx of glucose into the lens (the glucose level is ~1 mM)[35] from the aqueous or vitreous humor (~15–30 mM).[36] Subsequently, an altered glucose metabolism in the lens cells can lead to an increase in the sorbitol level via the reaction catalyzed by aldose reductase,[37,38] nonenzymatic modification of lens proteins,[6] and oxidative stress.[7] All these changes eventually induce cataract formation.

It was recently reported that GLUT1 is expressed and located in the epithelial cells and in the newly differentiating fiber cells.[39] It has also been reported that the rate-limiting factor for the influx of glucose into the cells is dependent on the level of the glucose transporter in the plasma membrane in vivo and in vitro.[40] An overexpression of GLUT1 in the muscle cells of transgenic mice caused an increase in the influx of glucose into these cells.[41] Certain specific *cis*-elements in the GLUT1 gene promoter were responsible for the stimulation of the ERK pathway.[42] Activation of the ERK pathway can stimulate expression of the GLUT1 gene in cardiac myocytes[43] and adipose tissue.[44] Therefore, it is likely that glucose transport and metabolism are regulated by the ERK pathway in the lens.

We may then hypothesize that the ERK pathway is involved in cataract formation. The high glucose levels not only cause an increase in the influx of glucose into the lens cells but also activate the ERK pathway. An elevated intracellular glucose level via activated ERK pathways is one of the factors for subsequent cataract formation. Certainly, the other downstream targets of the ERK pathways may also contribute to cataract formation in MEK1(E) transgenic mice. We are in the process of identifying these other downstream targets of the ERK pathway in the lens.

Regulation of Eye Size

The phenotype of the MEK1(E) transgenic mice, macrophthalmia with cataractous lenses, is unique. Dozens of different transgenic mice have been reported to develop various eye and/or lens phenotypes by using the same αA-crystallin promoter to express many different genes, including FGF, FGF receptor,[45] and myo-inositol transporter.[46] However, although some did develop microphthalmia, neither these transgenic mice nor gene knockout mice[47,48] have been reported to develop macrophthalmia.

The factors that control the growth of the eyeball are largely unknown.[49] There are human genetic diseases linked to macrophthalmia.[50] A recently published article suggests that an enlarged eye could be the result of an increase in the embryonic eye field.[51] However, the phenotype of the MEK1(E) transgenic mice suggest that the size of the eye may be postnatally regulated by the size of the lens. Therefore, there are at least two distinct mechanisms that control the growth of the eyeball.

In summary, we believe that the MEK1(E) transgenic mice may be useful for studying not only the molecular mechanism that controls cataract formation but also the mechanisms that control the size of the entire eye.

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E R R A T A

Erratum in: "Macular Pigment in Donor Eyes with and without AMD: A Case-Control Study" by Bone et al. (*Invest Ophthalmol Vis Sci.* 2001;42:235–240).

In the Discussion on p. 238, the two citations to Figure 5 were incorrectly stated. The first reference should have read: Figure 5 (filled circles) shows the odds ratios plotted against the median values for these quartiles.

The second citation should read: Odds ratios, shown in Figure 5 (open circles), could then be calculated from an analysis of these numbers and the "control" numbers in the first column.

Erratum in: "Identification of Ocular Cicatricial Pemphigoid Antibody Binding Site(s) in Human $\beta 4$ Integrin" by Kumari et al. (*Invest Ophthalmol Vis Sci.* 2001;42:379–385).

On p. 379 and in the Table of Contents, two authors' names were inadvertently misspelled. The names should have read: Mohammed S. Razzaque and A. Razzaque Ahmed.

Accordingly, the corresponding author's email address should also read: razzaque_ahmed@hms.harvard.edu.

The online version of this article was corrected on January 31, 2001.