Mutations in the human phosphatase and tensin homolog (PTEN) gene cause PTEN hamartoma tumor syndrome (PHTS), which includes cataract development among its diverse clinical pathologies. Currently, it is not known whether cataract formation in PHTS patients is secondary to other systemic problems, or the result of the loss of a critical function of PTEN within the lens. We generated a mouse line with a lens-specific deletion of Pten (PTEN KO) and identified a regulatory function for PTEN in lens ion transport. Specific loss of PTEN in the lens resulted in cataract. PTEN KO lenses exhibited a progressive age-related increase in intracellular hydrostatic pressure, along with, increased intracellular sodium concentrations, and reduced Na⁺/K⁺-ATPase activity. Collectively, these defects lead to lens swelling, opacities and ultimately organ rupture. Activation of AKT was highly elevated in PTEN KO lenses compared to WT mice. Additionally, pharmacological inhibition of AKT restored normal Na⁺/K⁺-ATPase activity in primary cultured lens cells and reduced lens pressure in intact lenses from PTEN KO animals. These findings identify a direct role for PTEN in the regulation of lens ion transport through an AKT-dependent modulation of Na⁺/K⁺-ATPase activity, and provide a new animal model to investigate cataract development in PHTS patients.
AKT activation promotes PTEN hamartoma tumor syndrome–associated cataract development

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Mutations in the human phosphatase and tensin homolog (PTEN) gene cause PTEN hamartoma tumor syndrome (PHTS), which includes cataract development among its diverse clinical pathologies. Currently, it is not known whether cataract formation in PHTS patients is secondary to other systemic problems, or the result of the loss of a critical function of PTEN within the lens. We generated a mouse line with a lens-specific deletion of Pten (PTEN KO) and identified a regulatory function for PTEN in lens ion transport. Specific loss of PTEN in the lens resulted in cataract. PTEN KO lenses exhibited a progressive age-related increase in intracellular hydrostatic pressure, along with, increased intracellular sodium concentrations, and reduced Na⁺/K⁺-ATPase activity. Collectively, these defects lead to lens swelling, opacities and ultimately organ rupture. Activation of AKT was highly elevated in PTEN KO lenses compared to WT mice. Additionally, pharmacological inhibition of AKT restored normal Na⁺/K⁺-ATPase activity in primary cultured lens cells and reduced lens pressure in intact lenses from PTEN KO animals. These findings identify a direct role for PTEN in the regulation of lens ion transport through an AKT-dependent modulation of Na⁺/K⁺-ATPase activity, and provide a new animal model to investigate cataract development in PHTS patients.

Introduction

Germline mutations in the human phosphatase and tensin homolog (PTEN) gene cause a collection of rare syndromes collectively known as PTEN hamartoma tumor syndrome (PHTS; ref. 1). The best-described syndrome within PHTS is Cowden’s disease (2), where PTEN mutations have been identified in more than 80% of patients (1, 3). Clinical presentation of Cowden’s disease is complex, like other PHTS disorders, and covers a broad spectrum of health problems that often involve cancer and include abnormalities of the eye in approximately 13% of cases (4). Lens cataract is a common pathology among Cowden’s disease patients with ocular involvement, and germline PTEN mutations have been confirmed in PHTS cases in which cataract is present (3–7). Although global Pten deletion in mice is lethal (8), many tissue-specific KO models have been generated to examine the role of Pten mutation and/or deletion in different types of cancers linked to PHTS (1). In contrast, no animal models of lens cataract caused by Pten mutation have been described.

PTEN is a ubiquitously expressed dual-specificity enzyme that can act as either a lipid phosphatase that antagonizes class I PI3K signaling or as a protein phosphatase that can dephosphorylate serine and threonine residues (9, 10). Class I PI3Ks are lipid kinases acting downstream of cell surface receptors to phosphorylate the 3′-hydroxyl group of phosphatidylinositol-(4,5)P₂. The generated phosphatidylinositol-(3,4,5)P₃ (PIP₃) activates additional signaling pathways to regulate cell growth, proliferation, motility, and survival (11). One of the most well-characterized functions of PIP₃ is activation of the protein kinase AKT. PTEN dephosphorylates PIP₃ generated by PI3K and negatively regulates AKT activity (12, 13). The interplay among PTEN, PI3K, and AKT is central to the regulation of many cellular processes in a wide variety of tissues, including the ocular lens (14).

The crystalline lens is an avascular organ that is uniquely dependent on the activity of numerous membrane channels and transporters to maintain its transparency and prevent cataract (15). The human lens facilitates visual accommodation and contains a single layer of epithelial cells spanning the anterior half of its surface, differentiating fiber cells that constitute the cortex from mature fiber cells in the core that make up the most of the lens mass (14). The lens depends on ion transport to create an internal circulating current, with Na⁺ being the primary current carrier (16). Na⁺ enters the lens at the anterior and posterior poles and flows inward along the extracellular spaces. Within the lens, Na⁺ is driven by its electrochemical gradient to move into the fiber cells, where the direction of flow is reversed and the current flows back to lens surface through gap junction channels (15, 17). Gap junction coupling is concentrated at the equator in peripheral fiber cells, directing the Na⁺ current to the equatorial epithelium, where Na⁺/K⁺-ATPase activity pumps Na⁺ out of the lens to complete the circulatory loop (18). Na⁺ transport is coupled to fluid circulation, creating a microcirculatory system that carries nutrients to the fiber cells and allows removal of metabolic waste. The intracellular passage of fluid through gap junction channels is driven by a standing hydrostatic pressure gradient within the lens (19–21). The ensemble activity of the various membrane channels and transporters that drive this microcirculatory system overcomes the lack of a lens vasculature and supports clarity, consistent with the finding that mutations in many of these channel genes have been linked to congenital cataract (22).

PHTS encompasses a complex set of syndromic disorders that are united by their linkage to germline mutations within PTEN. As somatic mutations of PTEN are involved in many cancers, it is not
Results

Lens-specific Pten deletion causes cataract and lens rupture. Germline mutations in PTEN cause PHTS, which can include cataract (5, 6) as a clinical consequence. Whole-animal KO of Pten resulted in embryonic lethality (8), necessitating the generation of a lens-specific conditional KO to investigate the mechanism whereby cataracts are caused by Pten mutation. To achieve this, mice with a floxed Pten allele (24) were interbred with MLR10-Cre transgenic mice (25) to obtain lens-specific conditional KO mice (referred to herein as PTEN KO). MLR10-Cre mice utilize a modified αA-crystallin promoter containing a Pax6 consensus binding site to specifically express Cre recombinase in both the lens epithelium and fiber cells. Compared with littermate WT controls (i.e., floxed Pten animals without the MLR10-Cre transgene), cataracts were obvious in the intact eyes of adult PTEN KO mice at 24 weeks (Figure 1A, A and B). Lenses isolated from WT mice remained transparent at all ages examined (Figure 1, C–F). In contrast, lenses dissected between 1 and 24 weeks of age from PTEN KO animals showed a progressive pathology. At 1 week, PTEN KO lenses exhibited a ring of densely packed cortical vacuoles (Figure 1G) that by 5 weeks had condensed into smaller opacities dispersed throughout the outer lens (Figure 1H). At 12 weeks, PTEN KO lenses were larger than WT lenses and had variable degrees of opacity (Figure 1I). Between 12 and 24 weeks, lenses became more opaque and began to rupture, ultimately leaving behind cortical fragments and a dense nuclear cataract (Figure 1J). These observations confirmed a specific role for PTEN in the maintenance of lens clarity and validated the PTEN KO model as a tool with which to discern the molecular mechanism of cataract in PHTS patients.

Changes in animal, eye, and lens size were quantified over time by dissecting and weighing eyes from age-matched PTEN KO and littermate WT mice. Lenses were dissected and photographed at 1, 2, 3, 5, 12, and 24 weeks of age, and their diameters were measured and subsequently used to calculate volumes, assuming spherical geometry. There was no significant difference in overall body size between WT and PTEN KO animals between 1 and 24 weeks (Figure 2A). However, changes in ocular mass were noted in adult mice, with PTEN KO eyes weighing 13% more than WT controls at 12 weeks, increasing to 19% greater PTEN KO eye mass at 24 weeks (Figure 2B). The effect of the PTEN KO phenotype on lens size also developed slowly, with a modest 2%–7% increase in lens volume between 1 and 5 weeks that grew to a 16% increase by 12 weeks and a 29% increase at 24 weeks, in the reduced number of intact lenses found at that age (Figure 2C). We also recorded the frequency of lens rupture between 1 and 24 weeks for WT and PTEN KO littermate animals. WT lenses never exhibited lens rupture in the time period examined, while 9.3% of PTEN lenses had ruptured at 12 weeks, increasing to an 81% incidence of rupture by 24 weeks (Figure 2D). These data suggested that PTEN regulates lens homeostasis, possibly by altering the activities of channels and transporters that control solute concentrations within the lens, ultimately causing lens and eye swelling and lens rupture.

PTEN KO produces a progressive increase in lens intracellular hydrostatic pressure. The lens depends on the coordinated activity of different channels and transporters to create a circulating current, with Na⁺ being the primary current carrier (16, 17). The transport of Na⁺ is coupled to circulation of fluid, and the intracellular passage of fluid through gap junction channels is driven by a hydrostatic pressure gradient (19–21). If the normal lens circulation was altered in PTEN KO animals, a buildup of osmotically active solutes within the lens could increase intracellular pressure at the lens surface, leading to the observed rupture phenotype. We previously developed a technique for directly measuring hydrostatic pressure in the lens (19, 26) and used this method here to determine whether progression of the PTEN KO phenotype correlated

Figure 1
Lens-specific deletion of Pten causes cataract. (A and B) At 24 weeks of age, WT mice had clear eyes (A), while PTEN KO animals displayed obvious cataracts (B). (C–F) Dissected WT lenses remained transparent from 1 to 24 weeks, (G–J) PTEN KO lenses. At 1 week, PTEN KO animals had numerous cortical vacuoles (G, inset) that became punctate opacities by 5 weeks (H). At 12 weeks, PTEN KO lenses were noticeably larger than WT and showed varying degrees of opacity (I). By 24 weeks, the majority of PTEN KO lenses comprised a dense nuclear cataract and cortical lens fragments (J). Scale bars: 1 mm (C–J); 40 μm (G, inset).
with changes in lens intracellular pressure (Figure 3). To derive quantitative values for the central and surface pressures, pooled data were graphed as a function of normalized distance from the lens center, then fit with an equation based on lens structure and experimentally derived transport parameters of the lens (19). At 6 weeks of age, there was extensive overlap between the pressure data derived from WT and PTEN KO lenses (Figure 3A). However, pressure values from PTEN KO lenses were consistently higher than those of WT littermate controls by 8 weeks (Figure 3B), and this difference increased further at the 10-week time point (Figure 3C). Best fit values for hydrostatic pressures at the WT lens surface and center were 0 ± 0 and 336 ± 16 mm Hg, respectively (mean ± SD), with minimal changes between 6 and 10 weeks of age. These data were in excellent agreement with our previously published studies of mouse lens intracellular pressure (19, 26) and confirmed the existence of a standing hydrostatic pressure gradient in the normal lens. In contrast, intracellular hydrostatic pressure in surface cells of the lens increased from 0 to 52 mm Hg between 6 and 10 weeks in PTEN KO mice (Figure 3D). This observation provides a molecular explanation of the cataractous rupture phenotype in PTEN KO mice. In previously studied lenses from various ages and different species, the hydrostatic pressure in lens surface cells was always equal to the pressure in the surrounding bathing solution (26).

PTEN KO increases lens intracellular sodium concentration. Sodium transport drives lens circulation, and the intracellular hydrostatic pressure gradient is dependent on Na⁺ concentration (16, 19). The elevation of central and surface hydrostatic pressure in PTEN KO lenses suggested that sodium transport may be compromised, and that Na⁺ may be accumulating within cells. To test this, we used the Na⁺ indicator dye SBFI to measure intracellular sodium concentrations in lenses of 10-week-old WT and PTEN KO mice (Figure 4). A SBFI-containing microelectrode was inserted into the lens near the surface, where the first SBFI injection was made. After diffusion of the dye into a fiber cell, fluorescence emission was recorded using 360- and 380-nm excitation wavelengths. The microelectrode was then advanced further into the lens, and additional injections and fluorescence measurements were made (Figure 4A). Data from several WT and PTEN KO lenses were pooled, and the 360/380 fluorescence emission ratios were calculated and plotted as a function of normalized distance from the lens center (Figure 4B). Depth-dependent calibration curves (27, 28) were then used to convert emission ratios to estimates of the internal Na⁺ concentration at different positions within WT and PTEN KO lenses (Figure 4C). The dashed lines in Figure 4C show fits of the Na⁺ concentration data to a previously described intracellular diffusion model (28). The best fit value for Na⁺ concentration in WT lenses showed a gradient that varied from 14.6 mM at the lens center to 4.4 mM at the lens surface (Figure 4C), consistent with prior measurements in normal mouse lenses (27). In PTEN KO lenses, the gradient had noticeably shifted to higher lens Na⁺ concentrations, with fitted values of 20.4 mM at the center and 7.9 mM at the surface (Figure 4C). Thus, the PTEN KO phenotype caused elevated intracellular...
sodium, which may have increased the osmolarity of surface cells and induced elevated hydrostatic pressure to drive the circulating fluid out of the epithelium.

**Na+/K+-ATPase activity is regulated by AKT and reduced in PTEN KO lens epithelial cells.** The sodium current normally exits the lens through the equatorial epithelium, where Na+/K+-ATPase activity is concentrated (18). The increased Na+ concentration measured within PTEN KO lenses may have resulted from a decrease in Na+/K+-ATPase activity, which in turn could be mediated by elevated PI3K signaling following Pten deletion. To investigate this possibility, we isolated lens epithelial cells from WT and PTEN KO mice and directly measured the Na+/K+-ATPase current in the presence or absence of an AKT inhibitor (Figure 5). The Na+/K+-ATPase current was measured by perfusion of individual lens epithelial cells under voltage clamp with the reversible inhibitor strophanthidin (Figure 5A). After determination of cell capacitance, Na+/K+-ATPase current densities were plotted for WT and PTEN KO epithelial cells (Figure 5B). Na+/K+-ATPase current density in WT epithelial cells was 0.69 ± 0.08 pA/pF (mean ± SD; n = 10). In PTEN KO epithelial cells, current density was significantly reduced to 0.33 ± 0.03 pA/pF (P < 0.05; n = 18). Incubation of PTEN KO epithelial cells with an AKT inhibitor for 30 minutes prior to Na+/K+-ATPase current measurement restored the mean current density to 0.66 ± 0.10 pA/pF (P > 0.05; n = 9), indistinguishable from that of WT cells. To determine whether Na+/K+-ATPase protein levels were altered and confirm that PI3K signaling was elevated in PTEN KO lenses, epithelial cell extracts were probed by Western blot (Figure 5C). WT epithelial cells expressed PTEN, which was completely absent from PTEN KO mice. When blots were probed with an antibody against the α subunit of the Na+/K+-ATPase, both WT and PTEN KO epithelial cells had bands of equal intensity. To ensure equivalent loading, blots were probed with a β-actin antibody, which was also present at equivalent levels in epithelial cells from WT and PTEN KO animals (Figure 5C). Thus, the reduction of Na+/K+-ATPase activity following Pten deletion was not due to reduced levels of Na+/K+-ATPase protein. Consistent with PTEN's antagonistic role toward PI3K signaling, levels of phospho-AKT were highly elevated in PTEN KO versus WT epithelial cells (Figure 5C). This elevation was completely reversed in PTEN KO epithelial cells by preincubation with an AKT inhibitor that has been previously shown to inhibit both AKT activity and phospho-AKT (29). Total AKT levels were not affected by PTEN KO in the presence or absence of the AKT inhibitor. Quantitation of band densities showed no significant differences in levels of β-actin or the Na+/K+-ATPase α subunit between WT and PTEN KO epithelial cells with and without AKT inhibition (P > 0.05; Figure 5D). In contrast, the phospho-AKT/total AKT ratio was elevated more than 10-fold in PTEN KO epithelial cells (P < 0.05) and significantly reduced by AKT inhibitor treatment (Figure 5D). Taken together, these data suggest that the loss of PTEN antagonism of PI3K activity resulted in elevated levels of phospho-AKT, which in turn produced an approximately 50% reduction in Na+/K+-ATPase activity in lens epithelial cells.

**Na+/K+-ATPase activity modulates intracellular hydrostatic pressure in the intact lens.** If downregulation of the Na+/K+-ATPase by AKT underlies the phenotype in PTEN KO lenses, then modulation of Na+/K+-
ATPase activity should alter intracellular hydrostatic pressure in the intact ex vivo lens. To examine this prospect, freshly dissected WT and PTEN KO lenses were impaled with pressure-sensing electrodes near the surface (at a radial position of 0.92 ± 0.02; mean ± SD) and perfused with strophanthidin and/or AKT inhibitor while intracellular pressure was continuously monitored (Figure 6). The initial pressure in WT lenses was 15.0 ± 1.0 mm Hg (n = 5), and this value remained stable for 20 minutes until the perfusion solution was changed to strophanthidin, which caused the pressure to rise to a new steady-state value of approximately 38.2 ± 1.2 mm Hg (P < 0.05; Figure 6A). As expected, PTEN KO lenses initially had an elevated pressure of 42.2 ± 1.6 mm Hg (n = 6), which dropped to a new steady-state value of 17.3 ± 0.8 mm Hg after perfusion with the AKT inhibitor (P < 0.05; Figure 6B). To confirm that these drugs were regulating the same process, we sequentially perfused PTEN KO lenses with the AKT inhibitor followed by strophanthidin (Figure 6C). The initial pressure in these lenses was also elevated, at 42.0 ± 2.0 mm Hg (n = 3), and dropped to 18.7 ± 1.2 mm Hg after AKT inhibitor perfusion. This new level of pressure remained stable for 20 minutes, then strophanthidin was added to the perfusion solution, and the pressure returned to ≥90% of its initial value over the next 80 minutes (38.0 ± 4.0 mm Hg; Figure 6C). These data showed that direct inhibition of Na+/K+-ATPase activity could reduce vacuole formation in neonatal PTEN KO lenses, pregnant females were injected with vehicle or an AKT inhibitor once daily for the last 3 days of gestation (31). Lenses were dissected from pups at birth, fixed, sectioned, and stained with H&E (Supplemental Figure 1; supplemental material available online with this article; doi:10.1172/JCI70437DS1). Newborn PTEN KO lenses from vehicle-injected mothers developed extensive equatorial vacuoles, as expected (n = 8). In contrast, lenses of PTEN KO neonates from AKT inhibitor–treated mothers either displayed a substantial reduction in vacuole formation (56%; n = 9) or developed vacuoles to a similar extent as vehicle-treated controls (44%; n = 7). Combined with the ex vivo lens data in Figure 6, these results suggest that pharmacological inhibition of AKT can ameliorate many of the phenotypic defects underlying cataract formation and lens rupture following Pten deletion in mice.

**Discussion**

In hereditary syndromic disorders like PHTS, it can be difficult to determine whether the development of cataract in some patients is secondary to other widespread systemic abnormalities, or a pri-
mary consequence of lost function within the lens of the mutated causative gene. By generating a tissue-specific KO mouse lacking Pten only within the ocular lens, we identified a novel function for PTEN in the regulation of lens ion transport and homeostasis. Our PTEN KO animals displayed a cataractous rupture phenotype that developed over the first 6 months of life. PTEN KO lenses exhibited a progressive increase in hydrostatic pressure that correlated with increased intracellular sodium concentration and decreased Na+/K+-ATPase activity, leading to lens swelling, opacities, and ultimately organ rupture. AKT was highly activated in PTEN KO lenses, and pharmacological inhibition of AKT restored Na+/K+-ATPase activity to normal levels in primary cultured lens cells from PTEN KO animals, in addition to reducing pressure in intact PTEN KO lenses. These data confirmed a specific role for PTEN in the maintenance of lens clarity and provided a new animal model with which to investigate cataract development in PHTS patients.

Our PTEN KO mice gradually developed cataract between birth and 6 months of age. In Cowden’s disease patients with PTEN mutations, cataract is often diagnosed between childhood and middle age. In one case report, the patient was in good health up to the age of 16 years, when he presented with initial symptoms that included blurred vision, and underwent cataract surgery at age 28 (6). In a second case, a male child presenting with PHTS features was found to have cataract at 6 years of age, which was then corrected surgically (5). We also found highly elevated levels of activated AKT in PTEN KO mice that were correlated with inhibition of lens Na+/K+-ATPase activity and elevated lens pressure. Although the status of lens AKT activity in Cowden’s disease patients with cataract has not been described, it is interesting to note that patients with Proteus syndrome, another highly variable overgrowth syndrome caused by activating mutations in AKT1 (32), also can develop childhood cataract (33). Thus, there is good congruence between our PTEN KO mouse model and forms of human syndromic cataract that involve inactivating mutations in PTEN or activating mutations in AKT1. These observations argue that PTEN, PI3K, and AKT play direct roles in the maintenance of lens clarity and that cataract in PHTS and other overgrowth syndromes is likely a primary pathology, not a secondary consequence of other systemic defects.

Our working hypothesis is that the loss of PTEN antagonism of PI3K signaling in the lens activates AKT, resulting in a reduction of Na+/K+-ATPase activity in equatorial epithelial cells. Sodium and water are then retained within lens cells, leading to increased intracellular hydrostatic pressure and ultimately lens cataract and rupture. Thus, perturbation of the balance among PTEN, PI3K, and AKT disrupted the lens circulation, which depends on the coordi-
Like Na+/K+-ATPase activity, connexin activity must be maximal in order to establish and maintain the lens circulation has been previously highlighted by studies of lens connexin channels. The importance of signal transduction pathways in regulating ion transport to establish and maintain lens clarity. The importance of signal transduction pathways in regulating the activity of diverse channels and transporters in many tissues, and that loss of this regulation may contribute to various disease states.

The hydrostatic pressure in WT lenses increased 2.5-fold after perfusion with strophanthidin (n = 5). (B) Pressure in PTEN KO lenses dropped 2.4-fold after perfusion with the AKT inhibitor (n = 6). (C) Perfusion of PTEN KO lenses with the AKT inhibitor resulted in a 2.2-fold decrease in lens pressure, which nearly recovered to the initial value by sequential perfusion with strophanthidin (n = 3). Data were derived from 10-week-old mice.

One early manifestation of the PTEN KO phenotype was formation of a ring of densely packed cortical vacuoles in neonatal mice (Figure 1G) that preceded the elevation of lens hydrostatic pressure in adult animals by several weeks. Treatment in utero with an AKT inhibitor greatly reduced, or eliminated, vacuole formation in 54% of PTEN KO neonatal lenses (Supplemental Figure 1), which suggests that, like the hydrostatic pressure increase, this was also an AKT-dependent phenomenon. We have not measured the lens surface pressure in lenses of newborn PTEN KO mice and are uncertain how vacuole formation is related to the hydrostatic pressure changes linked to Na+/K+-ATPase activity in adult lenses. In addition, while there was good agreement between our pharmacological manipulation of Na+/K+-ATPase activity in isolated epithelial cells and hydrostatic pressure in intact lenses (Figures 5 and 6), the histological damage phenotype was more pronounced in the cortical fiber cells than in the epithelium itself. One possibility is that signaling regulated by PTEN could affect additional channels and transporters that contribute to the lens circulation — such as the connexins or aquaporins (15, 17), which are highly expressed in the cortical lens fibers — and that these proteins also contribute to changes in lens pressure and fiber cell morphology. Regardless of the precise mechanism of vacuole formation, our data suggest that pharmacological interventions based on AKT inhibition may show promise for alleviating some of the ocular manifestations of PHTS.

PTEN/PI3K regulation of ionic activity may be a general homeostatic mechanism that is perturbed in other disease states in addition to cataract. For example, it was recently shown that downregulation of PI3K signaling prolonged the QT interval of the cardiac action potential by affecting multiple ion channels, providing a possible mechanism to explain why some tyrosine kinase inhibitors in clinical use as anticancer therapies are associated with increased risk of arrhythmias like long QT syndrome (41, 42). In the central nervous system, PTEN regulation of synaptic transmission may help to explain abnormal social and cognitive behaviors observed in humans with PHTS and other neurological syndromes, such as autism spectrum disorders (43, 44). In the present study, we have shown that syndromic cataract linked to Na+/K+-ATPase activity in adult lenses. In addition, while the increase of static pressure in intact lenses (Figures 5 and 6), the histological damage phenotype was more pronounced in the cortical fiber cells than in the epithelium itself.

**Methods**

*Generation of PTEN KO mice.* Pten homozygous floxed mice (24) were interbred with MLR10-Cre mice (25) to obtain PTEN KO mice, with lens-specific conditional KO of *Pten*. Genotypes were verified by PCR of genomic tail DNA using sense (5′-CTCCCTCCTACTCCATTCCC-3′) and antisense (5′-ACTCCACCACAATGACACAA-3′) primers to detect the floxed *Pten* allele. The MLR10-Cre transgene was amplified as previously described (25, 45).
Microelectrode along a track between the surface and center 45° between the posterior pole and equator. The ratios of emission at 360/380 nm excitation were compared with sodium calibration curves that were determined as previously described for intracellular calcium (28).

Measurement of lens epithelial cell Na+/K+-ATPase activity. Lens epithelial cells were dissociated by incubation of dissected capsules with 0.25% trypsin for 5–10 minutes at 37°C. Na+/K+-ATPase currents were measured as previously described (48), with the exception that 5 mM strophantidin (Sigma-Aldrich) was used as an inhibitor instead of dihydro-ouabain. 5 mM strophantidin was chosen as a saturating concentration of inhibitor for mouse Na+/K+-ATPase based on species variation in sensitivity (46, 47). Briefly, isolated lens epithelial cells were placed in a chamber in which the perfusion solution could be exchanged. Individual cells were subjected to whole-cell patch clamp using an Axopatch 1B amplifier (Axon Instruments), and membrane capacitance was determined in current clamp mode. To measure the Na+/K+-ATPase current, cells were perfused with 5 mM strophantidin while voltage was clamped at 0 mV. The change in membrane current due to superfusion of strophantidin was recorded to provide a direct measure of Na+/K+-ATPase activity, then divided by the cell capacitance to determine the current density. In some experiments, epithelial cells were incubated in the presence of 5 μM AKT inhibitor VIII (EMD Millipore) for 30 minutes prior to measurement of the Na+/K+-ATPase current.

Western blotting. Lenses were dissected from eyes and transferred to calcium- and magnesium-free PBS. The lens capsule was then peeled away from the fiber cell mass using fine forceps. For Western blotting, capsules were transferred to 2× sample buffer, separated on SDS-PAGE gels, and transferred to nitrocellulose membranes. Blots were probed with rabbit monoclonal antibodies against PTEN and mouse monoclonal antibodies against serine 473 phospho-AKT (Cell Signaling Technology), or with rabbit polyclonal antibodies against the α subunit of the Na+/K+-ATPase and total AKT1/2/3 (Santa Cruz Biotechnology). Peroxidase-conjugated goat anti-rabbit (Jackson ImmunoResearch) or sheep anti-mouse (GE Healthcare) secondary antibodies were used prior to ECL detection. In some experiments, dissected lenses were incubated in the presence of 5 μM AKT inhibitor VIII for 30 minutes prior to lens capsule dissection. Band densities from 4 independent blots were quantified using ImageJ (49).


