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PTEN affects cell size, cell proliferation and apoptosis during *Drosophila* eye development

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SUMMARY

Mutations in the tumor suppressor gene *PTEN* (*MMAC1/TEP1*) are associated with a large number of human cancers and several autosomal-dominant disorders. Mice mutant for *PTEN* die at early embryonic stages and the mutant embryonic fibroblasts display decreased sensitivity to cell death. Overexpression of *PTEN* in different mammalian tissue culture cells affects various processes including cell proliferation, cell death and cell migration. We have characterized the *Drosophila PTEN* gene and present evidence that both inactivation and overexpression of *PTEN* affect cell size, while overexpression of *PTEN* also inhibits cell cycle progression at early mitosis and promotes cell death during eye development in a context-dependent manner. Furthermore, we have shown that *PTEN* acts in the insulin signaling pathway and all signals from the insulin receptor can be antagonized by either *Drosophila* or human *PTEN*, suggesting a potential means for alleviating symptoms associated with altered insulin signaling.

Key words: *Drosophila* development, PTEN tumor suppressor, Cell size, Proliferation, Apoptosis, Insulin-signaling

INTRODUCTION

The tumor suppressor gene *PTEN* is one of the most frequently mutated genes involved in the development of human cancer (Cantley and Neel, 1999; Li et al., 1997a; Steck et al., 1997). *PTEN* mutations are found in a wide variety of tumors such as glioblastomas, endometrial carcinomas, advanced prostate cancers and melanoma cells (Cairns et al., 1997; Guldberg et al., 1997; Li et al., 1997a; Liu et al., 1997; Rasheed et al., 1997; Risinger et al., 1997; Tashiro et al., 1997; Wang et al., 1997). Germ-line mutations in *PTEN* are linked to three rare autosomal dominant syndromes: Cowden Disease, Bannayan-Zonana syndrome and Lhermitte-Duclose disease (Liaw et al., 1997; Marsh et al., 1997). A common feature of these syndromes is a predisposition for the development of hamartomas, benign tumors that have differentiated but disorganized cells.

The PTEN protein contains the phosphatase signature motif HCXXGXXRS/T that is found in all protein tyrosine phosphatases (Denu et al., 1996; Li and Sun, 1997; Li et al., 1997a; Steck et al., 1997). This phosphatase domain is the most common site of germline or sporadic *PTEN* mutations (Myers and Tonks, 1997). In vitro studies have demonstrated that PTEN can dephosphorylate phosphotyrosine, phosphoserine and phosphothreonine residues on artificial substrates (Li and Sun, 1997; Myers et al., 1997). Recently, PTEN has been shown to have strong in vitro and in vivo activity for the 3′ position of phosphatidyl inositol 3,4,5 trisphosphate (PIP3) (Maehama and Dixon, 1998). PIP3 is produced by the catalytic activity of phosphatidyl inositol 3 kinase (PI3K) and can act as a membrane-embedded second messenger for the activation of a variety of signaling molecules.

A number of *Pten* mutant mice have been described (Di Cristofano et al., 1998; Podsypanina et al., 1999; Suzuki et al., 1998). Although the spectrum of disorders affecting the *Pten* mutant mice varies between the different strains, all homozygous *Pten* mutants exhibit early embryonic lethality (E7.5-E9.5) and the heterozygotes display a predisposition to tumor development (Di Cristofano et al., 1998; Podsypanina et al., 1999; Suzuki et al., 1998). Consistent with the role for PTEN as a PIP3 phosphatase, *Pten* mutant cells exhibit increased PIP3 signaling (Stambolic et al., 1998; Sun et al., 1999).

The *C. elegans* gene *daf-18* encodes a distant *PTEN* homolog (Gil et al., 1999; Mihaylova et al., 1999; Ogg and Ruvkun, 1998; Rouault et al., 1999). In addition to a conserved phosphatase domain, *daf-18* encodes a large non-homologous C-terminal region (Ogg and Ruvkun, 1998). *Daf-18* mutants can suppress the mutant phenotypes of *daf-2*, the *C. elegans* insulin-like receptor, and *age-1*, the *C. elegans* PI3K (Gil et al., 1999; Mihaylova et al., 1999; Ogg and Ruvkun, 1998; Rouault et al., 1999).

A variety of cellular functions have been reported for *PTEN* using different mammalian systems. Overexpression of *PTEN* in *PTEN*$^{-}$ glioblastoma cells inhibits cell cycle progression (Cheney et al., 1998; Furnari et al., 1997). Furthermore, homozygous *Pten* mutant mice exhibit regions of increased cell proliferation (Suzuki et al., 1998). These results suggest a role for *PTEN* in regulating cell division. On the other hand, overexpression of *PTEN* in *PTEN*$^{-}$ breast and prostate cancer cells causes apoptosis (Li et al., 1998; Myers et al., 1998), and immortalized *Pten* mutant fibroblasts exhibit an elevated resistance to apoptosis (Stambolic et al., 1998). Thus, *PTEN* could have a distinct role in regulating apoptosis. Furthermore, *PTEN* has been reported to inhibit cell migration by directly dephosphorylating focal adhesion kinase (FAK) and Shc (Gu et al., 1999; Tamura et al., 1998). In *C. elegans, daf-18* has been shown to be involved in dauer formation (Gil et al., 1999; Mihaylova et al., 1999; Ogg and Ruvkun, 1998; Rouault et al., 1999).

To explore the various roles of *PTEN* during development, we have isolated and characterized the *Drosophila PTEN* homolog. We show that fly *PTEN* functions in the insulin signaling pathway, and both human and fly *PTEN* elicit similar effects during *Drosophila* eye development. Using this *Drosophila* system, we demonstrate that *PTEN* affects cell proliferation and apoptosis in a developmental context-dependent manner. Furthermore, we show that *PTEN* plays an essential in the regulation of cell size.

MATERIALS AND METHODS

Molecular biology

The low stringency condition used in cDNA library screening was as described (Tao et al., 1999). Three *dPTEN* cDNA were isolated and sequenced. A 1.8 kb *dPTEN* cDNA, a wild-type human *PTEN* cDNA and the human *PTENC124S* mutant cDNA were cloned into *pUAST* for P-transformation (Brand and Perrimon, 1993). The *dPTEN* genomic region was isolated from P1 clones Ds01978, Ds02806 and Ds05299 (Berkeley *Drosophila* Genome Project). A 6.2 kb *Spe*I genomic fragment was cloned into *pW8* for rescue experiments.

Fly genetics

dPTEN^{c494} is an EMS-induced mutation isolated from a screen for lines that failed to complement the *Df(2L) J1 b*^{1} *Tft*^{1} deficiency. Clones of *dPTEN* mutant cells were generated by X-ray irradiation of *w; P[w*$^{+}$*; ry*$^{+}$*] 30C P[ry*$^{+}$*; hs-neo; FRT] 40A/ dPTEN*^{c494} larvae (Xu and Harrison, 1994). Multiple lines for each P-element construct were established. At least three independent lines carrying *UAS-PTEN* and *UAS-dPTEN* were tested for each *GAL4* cross and they all gave similar phenotype. As controls, five lines carrying *UAS-PTENC124S* or *GAL4* lines alone were treated in parallel. *Dp110*^{DN} was originally called *Dp110*^{D954A} (Leevers et al., 1996). *UAS-p21*, *GMR-GAL4* and *EYE-GAL4* were gifts from I. Hariharan, M. Freeman and W. Gehring.

Flow cytometry

Eye discs from wandering third instar larvae were dissected in PBS and dissociated in PBS with 5 mg/ml porcine trypsin (Intergen) and 1 mM EDTA at room temperature for 2 hours. Dissociated cells were washed 3 times, resuspended in 200 μl of cold PBS containing 6 mM EDTA, and fixed by six stepwise additions of 100 μl each of cold 95% ethanol. After staining with propidium iodide, the cell cycle profiles were determined using a Becton Dickinson FACS Vantage, and the data was analyzed using the Modify 5.2 model (Verity Software House). Approximately 200-250 discs for each genotype were dissociated, and at least 15,000 cells were analyzed for each sample. We found no difference between human and fly *PTEN* transgenes tested.

Histology and immunofluorescence

Eye disc fixing and staining, scanning electron microscopy (SEM) and adult eye sections were performed as described (Xu and Harrison, 1994). Acridine orange staining was performed according to Spreij (1971). For labeling of cells in S phase, eye discs were dissected in PBS solution, incubated in M3 solution (Sigma) containing 500 μg/ml BrdU (Sigma) for 60 minutes at room temperature, and fixed in 4% paraformaldehyde in PBST (PBS with 0.3% Triton X-100) for 30 minutes at room temperature. Signals were detected with anti-BrdU antibody (1:50, Becton Dickinson). Samples were collected using confocal microscopy (BioRad, MRC-1024).

RESULTS AND DISCUSSION

Isolation and characterization of the *Drosophila PTEN* gene

We have identified a *Drosophila PTEN* (*dPTEN*) homolog by screening an imaginal disc cDNA library using a human *PTEN* probe at low stringency (Materials and Methods; Fig. 1). The fly and human *PTEN* gene products are similar in length and share sequence similarities in both the N-terminal phosphatase domain and the C-terminal region (overall 44% amino acids (aa) identical; Fig. 1B). We determined the genomic organization for *dPTEN* and mapped the gene to the cytological region 31C-D (Fig. 1A). To isolate mutations in *dPTEN*, we carried out a genetic screen and isolated ten lethal complementation groups using the *Df(2) J1* deficiency, which removes *dPTEN* as well as other genes (Fig. 1A). The lethality of one group consisting of a single allele (*dPTEN*^{c494}) can be fully rescued by P-element transformants carrying *dPTEN* genomic DNA (Fig. 1A). Two lines of evidence indicate that this mutation is a null or strong *dPTEN* allele. First, sequence analysis reveals a G135E change in the coding region (Fig. 1B). This is an invariant residue in the active-site motif of the catalytic domain and is required for phosphatase activity (Denu and Dixon, 1998). Indeed, an identical change in human *PTEN* has been previously found in human melanomas (Guldberg et al., 1997). Moreover, both *dPTEN*^{c494} homozygotes and *dPTEN*^{c494}*/Df(2)J1* trans-heterozygotes die at a similar late embryonic/early first instar larval stage. Thus, *dPTEN* encodes a vital function that is needed for *Drosophila* early development.

Loss of *dPTEN* affects cell size but not patterning during eye development

To gain insight into the functions of *dPTEN* during development, we examined clones of cells that lack *dPTEN* in the *Drosophila* eye. The compound eye provides a useful model for studying cell proliferation and differentiation and yet it is dispensable for viability. *dPTEN*^{c494} mutant ommatidia in the mosaic eyes appear larger in size compared to their neighboring wild-type ommatidia (Fig. 2A). Sections of these mosaic eyes revealed that the mutant ommatidia have normal photoreceptor cell composition and orientation (Fig. 2B,D). However, the sizes of the individual mutant cells are much larger than their neighboring wild-type cells (the average size of a mutant ommatidium is approximately 2.5 times of that of

a wild-type one; Fig. 2B-D). Careful examination of the chimeric ommatidia that contain both mutant and wild-type cells revealed that the *PTEN* mutant phenotype is cell autonomous, as every mutant cell has an enlarged cell body while its immediate wild-type neighbors have normal cell body sizes (Fig. 2C). Unlike the *Drosophila* tumor suppressor genes such as *lats* that we have previously analyzed (Tao et al., 1999; Theodosiou et al., 1998; Xu et al., 1995), we did not observe an obvious overproliferation defect in *PTEN* mutant clones in the eye. This could be because *dPTEN* may not normally

Fig. 1. *Drosophila PTEN* (*dPTEN*) genomic organization and predicted amino acid sequence. (A) The genomic region and *dPTEN* transcript map are shown with the *dPTEN* coding region labeled in black. The deficiency utilized to isolate the *dPTEN* mutant and the genomic region used to generate a *dPTEN* transgene are indicated above the genomic map. Bg, *Bgl*II; B, *Bam*HI; S, *Spe*I. (B) Alignments of the *PTEN* homologs among human, *Drosophila* and *C. elegans*. Only amino acids from *Drosophila* and *C. elegans* that are identical to human PTEN (*hPTEN*) are highlighted. The G→E amino acid substitution found in the *dPTEN*^{c494} mutant is noted above the alignment. The GenBank accession number for *Drosophila PTEN* cDNA is AF144232.

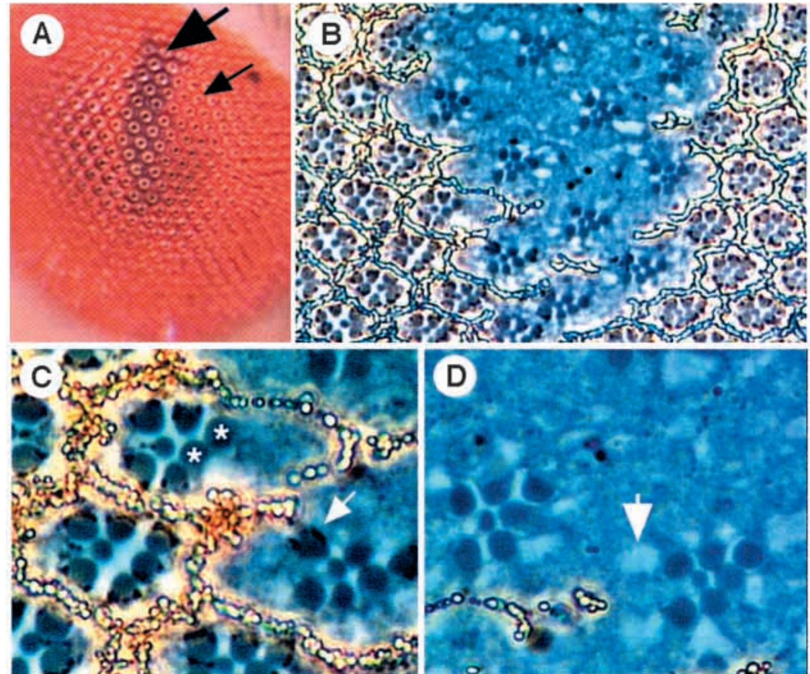

Fig. 2. *PTEN* loss-of-function mutant phenotype in the eye. (A) An adult mosaic eye containing a *dPTEN*^{c494} clone (black shadow area, big arrow) with ommatidia larger than neighboring wild-type ommatidia (small arrow). (B-D) Cross sections of the same eye. Sections revealed enlarged *dPTEN* mutant ommatidia (B) and their photoreceptor cells (C,D). (C) Mutant cells (*) in a chimeric ommatidium have enlarged bodies whereas a wild-type cell in a chimeric ommatidium (arrow) has a normal body size as indicated by the distance between rhobdomere and pigment cells. (D) In non-apical sections, vacuole-like structures are often located near the rhabdomeres of *dPTEN* mutant ommatidia (arrow).

function in the eye in regulating cell proliferation, or alternatively, there may be redundancy for *PTEN*-like molecules in the eye.

Overexpression of *PTEN* inhibits cell proliferation during eye development

To explore other potential roles of *dPTEN* during eye development, we used the *UAS/GAL4* system to overexpress both human and fly *PTEN* in *Drosophila* (Brand and Perrimon, 1993). Interestingly, the phenotypes caused by overexpression of human *PTEN* and *dPTEN* are indistinguishable, suggesting that the functions of the two homologs are conserved. For simplicity, we will use the name *PTEN* in text while specifying between human *PTEN* and *dPTEN* in figures when it is necessary. To verify whether the effects that we observed with expression of *PTEN* are related to its enzymatic activity, we also tested a construct, *UAS-PTENC124S*, in which a critical residue in the phosphatase domain has been mutated. This change also leads to elimination of catalytic activity in vitro (Li and Sun, 1997; Li et al., 1997b). While ectopic expression of wild-type *PTEN* causes specific phenotypes (see below), expression of *PTENC124S* under the same conditions has no effect. Together with the *PTEN*^{c494} mutant, these experiments demonstrate that phosphatase activity is necessary for *PTEN* to exert its functions during development.

Since ubiquitous expression of *PTEN* directed by heat-shock induction of GAL4 causes lethality during embryonic and larval stages, we expressed *PTEN* specifically in the developing eye using the *eyeless GAL4* line (*EYE-GAL4*). The *eyeless* enhancer directs gene expression in the young developing eye disc where cells are actively proliferating

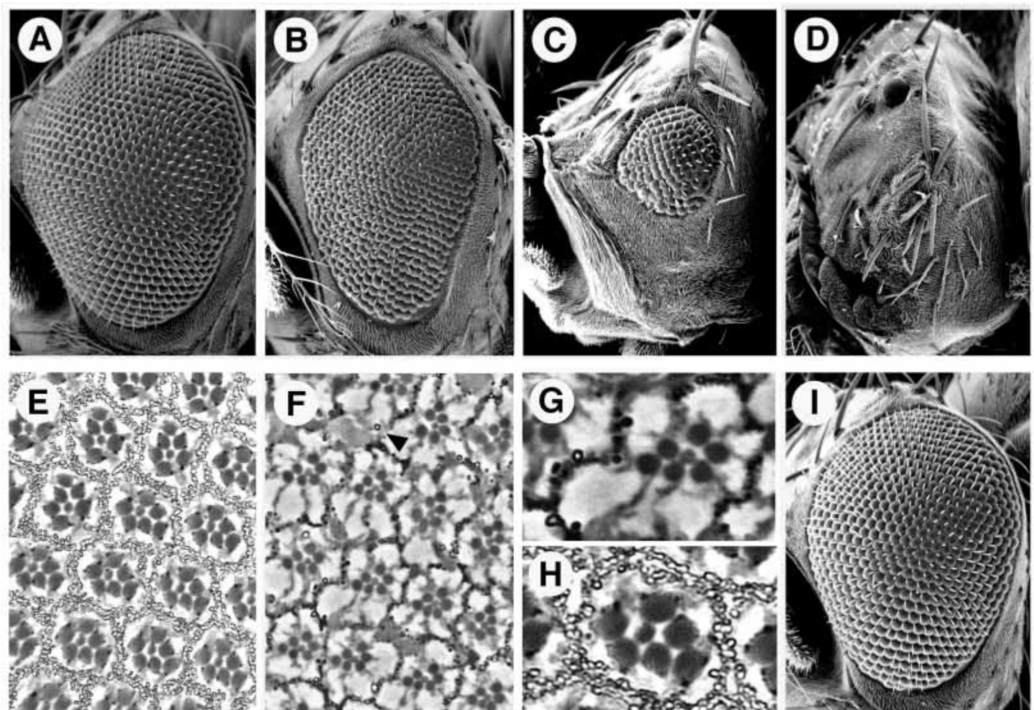

Fig. 3. *PTEN* overexpression eye phenotypes. Scanning electron micrographs (SEMs) (A-D,I) and tangential sections through adult eyes (E-H) are shown. While overexpression of wild-type *PTEN* leads to smaller, flattened adult eyes (B-C) or even no eyes (D), overexpression of a mutant *PTEN* with the inactive phosphatase domain has no effect (I). In the cross section of *GMR-GAL4, UAS-PTEN/UAS-PTEN* (F), photoreceptor cells have enlarged and irregular shaped cell bodies. Some pigment cells are missing (F, arrowhead). (A,E,H) wild-type. (B,F,G) *GMR-GAL4/+; UAS-dPTEN/UAS-dPTEN*. (C,D) *EYE-GAL4/+; UAS-PTEN/UAS-PTEN.* (I) *EYE-GAL4/UAS-PTENC124S; UAS-PTENC124S/+*.

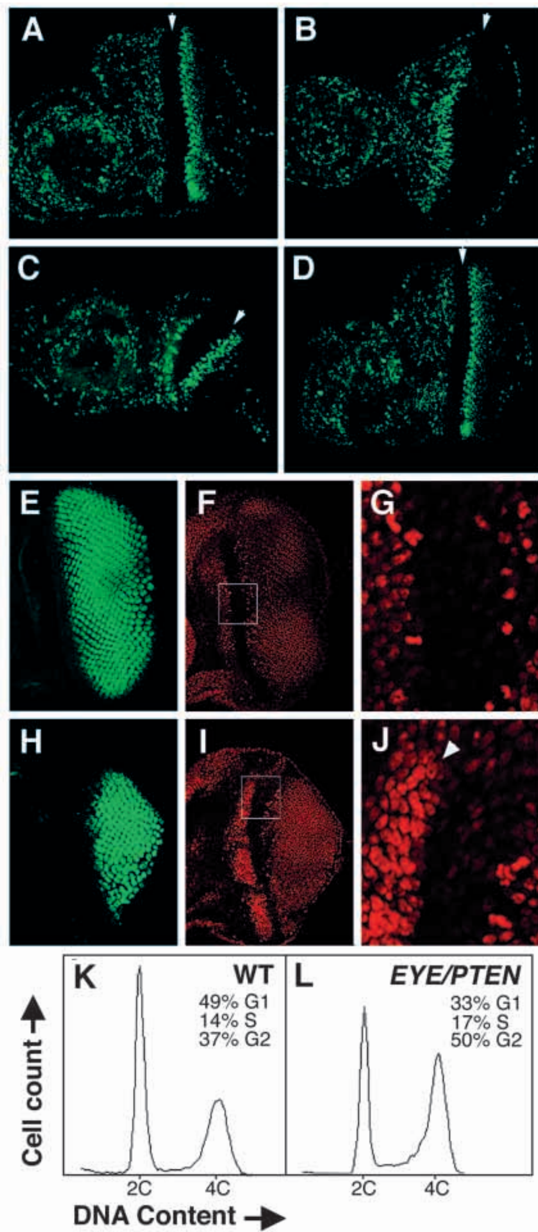

Fig. 4. Overexpression of *PTEN* affects cell-cycle progression in proliferating cells. While the BrdU-staining patterns are indistinguishable between wild-type and *GMR/dPTEN* discs (A,D), the number of staining cells are reduced in the anterior region of the *EYE/dPTEN* third instar eye disc (C). BrdU incorporation is abolished in the *GMR/p21* disc posterior to the MF (B). Arrows indicate the MF, and anterior is to the left. Confocal images of third instar eye discs stained with anti-Elav antibody showing that neural differentiation is relatively normal in *PTEN*-overexpressed small third instar eye disc (H). (F,G) Propidium iodide staining shows that in a wild-type eye disc few post-replication cells with bright nuclear staining can be seen along regions anterior and posterior to the MF (G is an enlarged image of the boxed region in F). (I,J) The anterior region of a *EYE/PTEN*-expressing disc accumulates many bright staining cells with a DNA content of 4C (arrow in J, which is an enlarged region of the boxed image in I)). Histograms of FACS analysis display DNA content and cell numbers (K,L). Similar results were obtained from three repeated experiments. (A, E-G,K) wild type. (C,H-J,L) *EYE-GAL4/+, UAS-dPTEN/UAS-dPTEN*. (B) *GMR-GAL4/UAS-p21*. (D) *GMR-GAL4/+, UAS-dPTEN/UAS-dPTEN*.

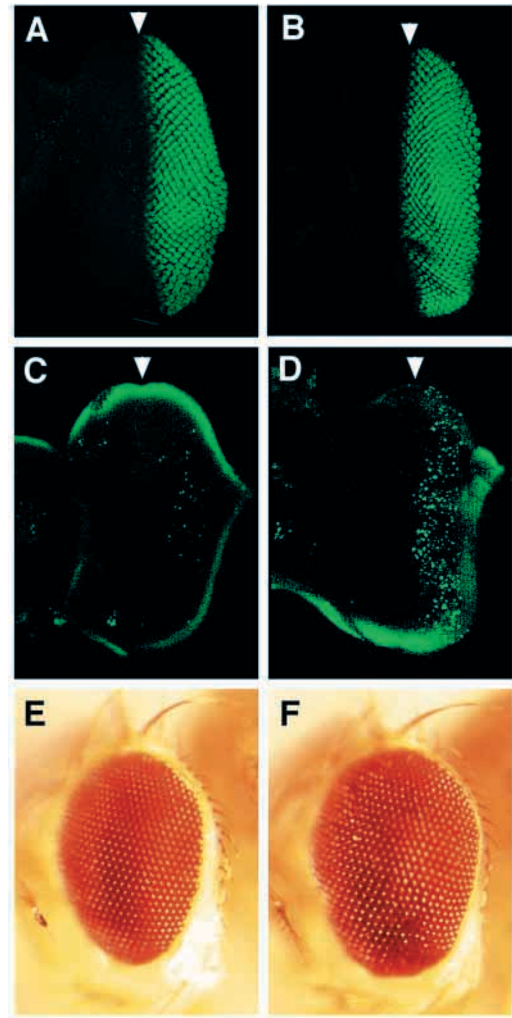

Fig. 5. Overexpression of *PTEN* causes cell death in the differentiating cells of the eye disc. Anti-Elav staining shows that the pattern of photoreceptor differentiation in *dPTEN*-expressing third instar eye disc (B) is comparable to that of a wild-type disc (A). Acridine orange staining shows few apoptotic cells in a wild-type eye disc (C) and massive cell death in the posterior region of the *dPTEN*-expressing disc (D). The *GMR-GAL4/UAS-dPTEN* adult eye phenotype can be largely rescued by coexpression of *p35*. The slight disorganization of the eye in (F) is also seen in flies with *GMR-p35* alone (Hay et al., 1994). Arrowheads indicate the MF, and anterior is to the left. Similar results were obtained with human *PTEN* (data not shown). (A,C) Wild type. (B,D) *GMR-GAL4/+; UAS-dPTEN/UAS-dPTEN*. (E) *GMR-GAL4/+; UAS-dPTEN/+*. (F) *GMR-GAL4/+; UAS-dPTEN/UAS-p35*.

(Halder et al., 1998). Overexpression of *PTEN* in proliferating cells of the eye disc resulted in dramatic reduction of eye sizes in a dosage-dependent manner (Fig. 3C). In fact, multiple copies of *UAS-PTEN* can completely eliminate the eye (Fig. 3D). This small adult eye phenotype could be caused either by the inhibition of cell proliferation by *PTEN*, which would result in eye discs of smaller than normal size, or by the failure of ommatidium differentiation in eye discs of normal size. The sizes of eye discs dissected from third instar larvae carrying *EYE-GAL4/UAS-PTEN* were dramatically reduced (Fig. 4C,H), suggesting a defect in cell proliferation. Consistent with

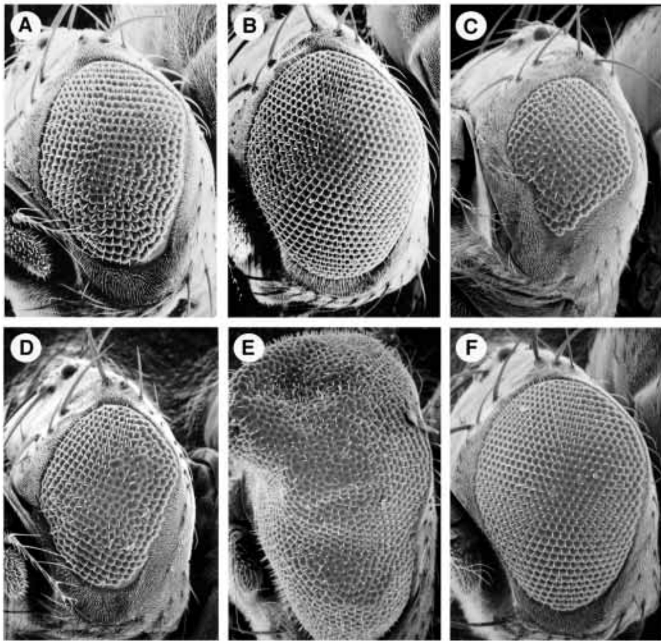

Fig. 6. Genetic interactions of *PTEN* with genes involved in insulin signaling. SEMs (A-F) are shown. (A) Expression of *PTEN* is dose-sensitive; one copy of a *EYE/PTEN* transgene causes a moderately reduced eye phenotype. (B) Coexpression of a wild-type *D-PI3K* (*Dp110*) suppresses the eye phenotype caused by *EYE/PTEN*. (C) Coexpression of a dominant-negative *D-PI3K* enhances the *EYE/PTEN* eye phenotype. (D) Removing one copy of *chico* also enhances the *EYE/PTEN* eye phenotype. (E) At 20-22°C, overexpression of *Inr* with *EYE-GAL4* results in a hyperplastic growth eye phenotype. (F) Coexpression of *EYE/PTEN* suppresses the *Inr* eye phenotype and, in turn, the *EYE/PTEN* eye phenotype is suppressed by overexpression of *Inr*. Similar interactions were observed when the human *PTEN* transgenes were tested (data not shown). (A) *EYE-GAL4, UAS-dPTEN/+*. (B) *EYE-GAL4, UAS-dPTEN/UAS-Dp110^{wt}*. (C) *EYE-GAL4, UAS-dPTEN/+; UAS-Dp110^{DN}/+*. (D) *EYE-GAL4, UAS-dPTEN/chico^{1}*. (E) *EYE-GAL4/UAS-Inr^{wt}*. (F) *EYE-GAL4, UAS-dPTEN/UAS-Inr^{wt}*.

this idea, sections of these small adult eyes showed that all types of photoreceptor cells were present and the pigment lattice was essentially regular (data not shown), indicating that cell differentiation and pattern formation are not disrupted. Moreover, although these eye discs were much smaller, neural differentiation in the posterior region occurs normally as indicated by neuronal-specific anti-Elav staining (Fig. 4H) (Robinow and White, 1991).

Overexpression of *PTEN* does not block G_1/S transition in the developing eye disc

Suppression of cell proliferation in mammalian tissue culture cells by *PTEN* has been reported to act through blocking cell cycle progression in the G_1 phase (Furnari et al., 1998; Li and Sun, 1998). To determine whether *PTEN* blocks G_1/S transition in the developing eye, we examined eye discs with *GMR-GAL4* driven *PTEN* expression. The *GMR* promoter directs gene expression in the region posterior to the morphogenetic furrow (MF) in the eye imaginal disc (Hay et al., 1994). While most cells in this region are differentiating, a stripe of cells posterior to MF undergo a synchronized, last round of cell division. It has been previously shown that *GMR*-directed expression of *p21$^{C1P1/WAF1}$*, a human cyclin-dependent kinase inhibitor, blocks this last round of division by preventing these cells from entering S phase (de Nooij and Hariharan, 1995) (Fig. 4B). Unlike the *GMR*-directed *p21* expression, BrdU labeling showed that these *PTEN*-expressing discs have the characteristic stripe of staining similar to that of the wild-type discs (Fig. 4A,D), suggesting that expression of *PTEN* does not block G_1/S transition. In contrast, propidium iodide staining of the *EYE-GAL4/UAS-PTEN* eye discs revealed that the discs accumulate many cells with brighter nuclear staining consistent with a DNA content of 4C (Fig. 4I,J). FACS analysis of dissociated cells from these discs also showed an increased percentage of cells in G_2 (Fig. 4K,L) (Neufeld et al., 1998). These results suggest that overexpression of *PTEN* during eye development causes cell cycle arrest at the G_2 or G_2/M phase.

Overexpression of *PTEN* in differentiating cells triggers apoptosis

Overexpression of *PTEN* under the direction of *GMR* results in adult eyes that are not only smaller but also rough (Fig. 3B). Sections of these adult eyes revealed a complex phenotype with varied degrees of severity. While the apical lenses of the ommatidia are smaller (Fig. 3B), the sizes of photoreceptor cells in the cross sections are larger than normal (Fig. 3F,G). Furthermore, these *GMR/PTEN* adult eyes have a flattened appearance with a narrowed retina (Fig. 3A,B; data not shown), suggesting the mutant phenotype could result from changes in both cell shape and size. In addition, different from the phenotype caused by inactivation of *PTEN*, the rhobdomeres of the *PTEN*-overexpressed photoreceptors are reduced in size (Fig. 3G). Moreover, these sections showed that some ommatidia had missing pigment or photoreceptor cells (Fig. 3F; data not shown). We therefore stained third instar eye discs with the anti-Elav antibody to monitor retinal neuron differentiation (Robinow and White, 1991). Elav staining revealed a neuronal differentiation pattern comparable to wild type (Fig. 5B), indicating that photoreceptor differentiation is not affected. To pinpoint the cause of the phenotype, we further assessed whether cell death might have contributed to the *GMR-GAL4/UAS-PTEN* eye phenotype by staining these eye discs with acridine orange. Unlike wild-type third instar eye discs which have few dying cells (Wolff and Ready, 1991), the *GMR-GAL4/UAS-PTEN* eye discs have a substantially increased amount of cell death in the posterior region of the eye disc, where *GMR-GAL4* is expressed (Fig. 5D). To further verify the contribution of cell death to the eye phenotype, *GMR-GAL4* was used to coexpress *PTEN* with the *p35* baculovirus gene, which can block apoptotic cell death (Hay et al., 1994; White et al., 1996). The eye phenotype induced by *GMR/PTEN* was largely rescued by coexpression of *p35* (Fig. 5F).

These experiments suggested the possibility that cell death might also contribute to the *EYE/PTEN* small eye phenotype. However, we found that *EYE/PTEN* eye discs do not have increased acridine orange staining and that the *EYE/PTEN* small eye phenotype was not suppressed by coexpression of *p35* (data not shown). Thus, cell death appears not to be a major factor in PTEN-mediated inhibition of cell proliferation. Instead, these results suggest that cell death is a consequence

of cells at different developmental stages in response to the *PTEN* product.

PTEN negatively regulates insulin signaling in *Drosophila*

We have further examined the relationship between *PTEN* and the *Drosophila PI3 kinase* (*D-PI3K*) gene, *Dp110* (Leevers et al., 1996). At 25°C, flies do not survive to adult when wild-type *D-PI3K* is expressed with *EYE-GAL4*. Interestingly, this lethality can be rescued by coexpression of *PTEN*. Furthermore, the small eye phenotype of *PTEN* overexpression is suppressed by overexpression of wild-type *D-PI3K* and enhanced by overexpression of dominant negative *D-PI3K* (Fig. 6A,B). These results clearly indicate that *PTEN* and *PI3K* function antagonistically in *Drosophila*.

The recent characterization of *chico*, a *Drosophila* IRS1-4 homolog, showed that *chico*, *D-PI3K* and *insulin receptor* (*Inr*) act as positive elements in a *Drosophila* insulin signaling pathway to regulate cell proliferation and cell size (Bohni et al., 1999). Consistent with the role of *PTEN* as a negative regulator in this insulin pathway, removal of one copy of the *chico* gene genetically enhances the *EYE/PTEN* eye phenotype (Fig. 6D). Overexpression of *Inr* (*EYE/Inr*) causes lethality at 25°C. At room temperature, few animals survive with overproliferated eyes (Fig. 6E). Strikingly, cooverexpression of *PTEN* completely rescues lethality and the overproliferation phenotype (Fig. 6F). This suggests that all signals from the insulin receptor can be antagonized by *PTEN* function. Together with the previous findings that mammalian and *C. elegans* PTEN molecules interact with components of the insulin pathway (Furnari et al., 1998; Maehama and Dixon, 1998; Myers et al., 1998; Ogg and Ruvkun, 1998; Stambolic et al., 1998; Sun et al., 1999), our genetic data argues that *PTEN* functions as a major conserved negative regulator in the insulin signaling pathway.

The role of *PTEN* in tumor suppression is not fully understood. Results from experiments involving different mammalian cell systems favor different theories. In this study, we have taken a genetic approach to analyze *PTEN*'s function in *Drosophila*. Sequence analysis has shown that *Drosophila PTEN* is a close homolog of human *PTEN*, sharing high sequence similarity in both the N-terminal phosphatase domain and in the C-terminal region. Furthermore, expressing human and fly *PTEN* in *Drosophila* elicits similar effects during development. These results indicate that *Drosophila* is a useful model for studying *PTEN* functions. Phenotypes caused by overexpression of *PTEN* depend on the developmental status of the cells: overexpression of *PTEN* arrests cell-cycle progression in proliferating cells while promoting apoptosis in differentiating cells during eye development. These results suggest that *PTEN* may suppress tumorigenesis by preventing damaged cells from dividing and/or promoting a response to apoptotic signals in a cell context-dependent manner. Although it has been reported that *PTEN* affects cell migration in mammalian cell cultures (Tamura et al., 1998), we did not observe effects of *PTEN* on cell migration in *Drosophila* (H. H. and T. X., unpublished data). In addition to previously reported effects, we have shown for the first time that *PTEN* function is essential in the regulation of cell size. The finding that *PTEN* acts in the *Drosophila* insulin pathway, together with previous reports that *Inr*, *chico* and *PI3K* affect cell size (Bohni et al., 1999; Leevers et al., 1996), supports a pivotal role for the insulin signaling pathway in cell-size regulation. The conservation of this pathway raises the possibility that it may be involved in the regulation of cell size in different organisms. Finally, the observation that all signals from the insulin receptor can be antagonized by either *Drosophila* or human *PTEN* indicates that modulation of PTEN activity could be an effective means for alleviating symptoms associated with altered insulin signaling.

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