Loss of Protein Tyrosine Phosphatase 1B Increases IGF-I Receptor Tyrosine Phosphorylation but Does Not Rescue Retinal Defects in IRS2-Deficient Mice

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PURPOSE. Mice with deletion of insulin receptor substrate (IRS) 2 develop type 2 diabetes and photoreceptor degeneration. Loss of protein tyrosine phosphatase 1B (PTP1B) in diabetic IRS2−/− mice restores insulin sensitivity and normalizes glucose homeostasis. Since insulin-like growth factor (IGF)-IR promotes survival of photoreceptors and is a substrate of PTP1B, we investigated IGF-IR-mediated survival signaling and visual function in PTP1B−/− and double mutant IRS2−/−/PTP1B−/− mice.

METHODS. IGF-IR-mediated Akt signaling was evaluated in IGF1-stimulated retinal explants. Histologic and electroretinogram analysis was performed in wild-type (WT), IRS2−/−, PTP1B−/−, and the double mutant IRS2−/−/PTP1B−/− mice.

RESULTS. IGF1-stimulated the tyrosine phosphorylation of its receptor and Akt activation in retinal explants of WT mice. In PTP1B−/− retinal explants, these responses were enhanced. Conversely, in retinas from IRS2−/− mice, expression of PTP1B was increased, coincident with decreased IGF1-mediated Akt serine 473 phosphorylation. PTP1B deletion in IRS2−/− mice also enhanced IGF1-IR tyrosine phosphorylation but, unexpectedly, did not rescue Akt activation in response to IGF1. One potential explanation is that PTEN was increased in retinas of IRS2−/− and IRS2−/−/PTP1B−/− mice. Histologic evaluation revealed alterations in various structures of the retina in IRS2−/− and IRS2−/−/PTP1B−/− mice, specifically in the outer nuclear layer (ONL) and retinal outer segments (ROS). Electroretinogram (ERG) analysis confirmed that PTP1B deficiency did not restore visual function in IRS2−/− mice.

CONCLUSIONS. Although loss of PTP1B enhances tyrosine phosphorylation of the IGF-IR in retinal explants of IRS2−/− mice, Akt activation remains defective owing to elevated PTEN levels and, thus, structural and functional visual defects persist in this model.

Keywords: IGF-I, IRS2, PTP1B, retinal explants
Increased IGF-IR Phosphorylation in PTP1B<sup>−/−</sup> Retina

Recently, we and others have shown that the hepatic insulin resistance and beta cell failure in diabetic IRS2-deficient mice can be reversed by the inhibition of the protein tyrosine phosphatase 1B (PTP1B).<sup>15</sup>,<sup>16</sup> PTP1B interacts directly with IR and IGF-IR, and catalyzes the dephosphorylation of tyrosine residues in both receptors.<sup>17</sup>,<sup>18</sup> The importance of PTP1B as a modulator of insulin action has been demonstrated in vivo and in cellular models.<sup>19</sup>,<sup>20</sup> In mice, deletion of the Ptpn1 gene increased sensitivity to insulin in liver and skeletal muscle. It also induced resistance to weight gain on a high-fat diet, and increased basal metabolic rate.<sup>21</sup>,<sup>22</sup> Although PTP1B is expressed in rod photoreceptor cells,<sup>23</sup> its role in IGF-IR–mediated signaling in sensorial systems, particularly in the retina, remains unexplored. Therefore, we have analyzed the role of PTP1B in IGF-IR–mediated signaling in the retina, and the impact of its deficiency in the visual function of the IRS2<sup>−/−</sup> mice.

**Materials and Methods**

**Reagents and Antibodies**

IGF-I (human recombinant) was purchased from Calbiochem-Novabiochem, Int., (La Jolla, CA). Antiphospho-IGF-IR (Tyr1165/1166, sc-101704), anti-IGF-IR (sc-713), anti-GFAP (sc-135,921), and anti-PTEN (sc-7974) antibodies were from Santa Cruz Biotechnology (Palo Alto, CA). Anti-IRS1 (06-248) and anti-PTP1B (07-088) antibodies were purchased from Merck-Millipore (Darmstadt, Germany). Anti-Akt antibody (9272) was purchased from Cell Signaling Technology, Inc. (Danvers, MA). Anti-β-actin antibody (A-5441) was purchased from Sigma-Aldrich (Barcelona, Spain).

**Animals**

Wild-type (WT), IRS2<sup>−/−</sup>, PTP1B<sup>−/−</sup>, maintained on a similar mixed genetic background (C57BL/6 × 129 sv), were described previously.<sup>15</sup>,<sup>16</sup> Male IRS2<sup>−/−</sup> and female PTP1B<sup>−/−</sup> mice were intercrossed to yield IRS2<sup>−/−</sup>/PTP1B<sup>−/−</sup> mice. The resulting double-heterozygous mice then were intercrossed to obtain all possible genotypes, including double-null IRS2<sup>−/−</sup>/PTP1B<sup>−/−</sup> mice (dKO). Genotyping was performed by PCR as described.<sup>16</sup> The various genotypes were born with the expected mendelian frequencies. Animals were fed a standard diet ad libitum and had free access to drinking water. All procedures were approved by the Ethical Committee from CSIC, and performed in accordance with the regulations of the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research.

**Culture of Retinal Explants**

Ex vivo assays were performed with retinas from mice of 9 weeks. Briefly, animals were killed by cervical dislocation and their eyes were enucleated. The lens, anterior segment, vitreous body, retinal pigment epithelium, and sclera were removed, and the retina was cultured in R16 medium (kindly provided by Dr. Velasco [Neuroscience Institute of Salamanca, Salamanca, Spain]).

**Western Blotting**

Whole retinas were homogenized in lysis buffer containing 50 mM Tris-HCl, 1% Triton X-100, 2 mM EGTA, 10 mM EDTA acid, 100 mM NaF, 1 mM Na<sub>3</sub>P<sub>2</sub>O<sub>7</sub>, 2 mM Na<sub>2</sub>VO<sub>4</sub>, 100 μg/mL phenylmethylsulphonyl fluoride, 1 μg/mL aprotinin, and 1 μg/mL leupeptin. All debris was removed by centrifugation (1,000g) at 4°C and protein concentration was quantified using the Bio-Rad protein assay (Bio-Rad, Helmel Hempstead, UK) using bovine serum albumin as a standard. Equivalent amounts of protein were resolved using denaturing SDS-PAGE, followed by transfer to polyvinylidene fluoride (PVDF) membranes (Merck-Millipore). Membranes were blocked using 5% non-fat dried milk or 3% BSA in 10 mM Tris-HCl, 150 mM NaCl pH 7.5 (TBS), and incubated overnight with several antibodies (1:2000 unless otherwise stated) in 0.05% Tween-20-TBS. Immunoreactive bands were visualized using the enhanced chemiluminescence reagent (ECL; Merck-Millipore). Densitometry on Western blots was performed using the ImageJ program (available in the public domain at http://rsb.info.nih.gov/ij/download.html). Values in all graphs represented the mean ± SEM. Statistical tests were performed using SPSS for Windows (SPSS, Inc., Chicago, IL). Data were analyzed by one-way ANOVA followed by the Bonferroni t-test. Differences were considered significant at P < 0.05.

**Quantification of Akt Phosphorylation by ELISA**

Alternatively, Akt (Ser473) phosphorylation was analyzed by a sandwich ELISA kit (ab126543; Abcam, Cambridge, MA), in vitro ELISA, accordingly to the manufacturer’s instructions. Absorbance was measured at 450 nM.

**Histologic Analysis and Immunofluorescence**

Eyes were fixed in 4% paraformaldehyde for 4 hours and processed for paraffin sections or cryosections. Paraffin sections were stained with hematoxylin and eosin (H&E) and examined by light microscopy. For quantitation, H&E-stained paraffin retinal sections were viewed using a Leica DM5000B microscope (Leica Microsystems GmbH, Wetzlar, Germany). The thickness of different layers of the retina was measured using the OpenLab software (Improvision, Lexington, MA). In each individual mouse studied, we measured a total of six retinal cross-sections made through the optic nerve head. These data then were averaged, and results obtained from 3 animals of each genotype were analyzed statistically using Student’s t-test.

To assess immunofluorescence analysis, retinal cryosections were washed in TBS containing 0.1% (wt/vol) BSA and 0.1% (vol/vol) Triton X-100 (this buffer was used for all subsequent washes), and then blocked and permeated for 2 hours in TBS containing 3% (wt/vol) BSA and 1% (vol/vol) Triton X-100. The sections were incubated overnight in a humid chamber at 4°C with rabbit anti-GFAP antibody (1:1000) or mouse antitubulin synthetase (1:10000) in blocking solution. Sections then were washed and incubated for 90 minutes with antimouse or antirabbit immunoglobulin antibody conjugated to Alexa 647 (1:2000; Molecular Probes, Eugene, OR). After washing, sections were mounted with medium (Fluoromount G) containing 4′-6-diamidino-2-phenylindole (DAPI). Staining was observed with an inverted laser confocal microscope LSM710 (Carl Zeiss Microscopy GmbH, Göttingen, Germany).

**Electroretinogram (ERG) Recording**

All the ERG methodologic procedures have been described previously.<sup>24</sup> Briefly, dark adapted mice were anesthetized under dim red light by intraperitoneal (IP) injection of a solution of ketamine (95 mg/kg) and xylazine (5 mg/kg), and maintained on a heated pad at 37°C. The pupils were dilated by...
topic application of 1% tropicamide (Colircus Tropicamida; Alcon Cusi, SA, Barcelona, Spain); a topical drop of 2% Methocel (Ciba Vision AG, Hetlingen, Switzerland) was instilled in each eye immediately before location of the corneal electrode. All recordings were performed with the animal placed on a Faraday cage. Flash-induced ERG responses were recorded from the right eye in response to light stimuli produced with a Ganzfeld stimulator. Light intensity was measured with a photometer (Mavo Monitor USB, Nuremberg, Germany) at the level of the eye. Totals of 4 to 64 consecutive light stimuli were presented. The interval between light flashes applied in scotopic conditions was 10 seconds for dim flashes and up to 60 seconds for the highest intensity; under photopic conditions, the interval between light flashes was fixed at 1 second.

**Figure 1.** PTP1B is upregulated in retinas from IRS2−/− mice before the onset of hyperglycemia in parallel with altered visual function. (A) Retinas from 9-week-old male WT and IRS2−/− mice were dissected. Whole retinal lysates (50 μg) were separated by SDS-PAGE, and Western blot analysis was performed with the anti-PTP1B and anti-IRS1 antibodies. β-actin was used as a loading control. The autoradiograms were quantified by scanning densitometry. Results are expressed as percentage of the WT and are the mean ± SEM (n = 6 mice from each genotype, *P < 0.05). (B) ERG responses from WT and IRS2−/− mice at 9 weeks of age. Rod responses to light flashes of −2 log cd·s·m−2 and mixed responses (rod and cone) to light flashes of −1.5 log cd·s·m−2 were recorded under dark adaptation. (C) Cryostat sections of retinas from WT and IRS2−/− mice at 9 weeks were obtained. Immunostaining for GFAP and glutamine synthetase (GS) was carried out (red), and the structure of the retina was visualized by nuclear DAPI staining (blue). The retinal layers are labeled as ONL, INL, and ganglion cell layer (GCL). Three mice from each genotype were analyzed.
second. The ERG signals were amplified, and band filtered between 0.3 and 10,000 Hz with a Grass amplifier (CP511 AC amplifier; Grass Instruments, Quincy, MA). Electrical signals were digitized at 10 kHz with a Power-Lab data acquisition board (ADInstruments, Chalgrove, UK). Bipolar recordings were obtained using corneal electrodes (Burian-Allen electrode; Hansen Ophthalmic Development Laboratory, Coralville, IA) and a reference electrode located in the mouth; ground electrode was located in the tail.

Rod-mediated responses were recorded under dark adaptation; light flashes ranging from $-4$ to $-1.5 \log \text{cd}\cdot\text{s}\cdot\text{m}^{-2}$ were used. Light flashes ranging from $-1.5$ to $1.5 \log \text{cd}\cdot\text{s}\cdot\text{m}^{-2}$ were
used for recording of the mixed rod- and cone-mediated responses. Cone function was tested in response to light flashes ranging from 0.5 to 2 log cd s m⁻² on a rod saturating background of 30 cd m⁻². Measurements of wave amplitudes were estimated by an observer blind to the experimental condition of the animal, and the statistical analysis was performed using the Student’s t-test.

RESULTS

PTP1B Is Upregulated in Retinas From IRS2⁻/⁻ Mice Before the Onset of Hyperglycemia and Is Associated With Defective Visual Function and Reactive Gliosis

It has been shown that IRS2⁻/⁻ mice display photoreceptor loss at early stages of postnatal development, owing to impaired Akt phosphorylation that results in activation of caspase-3. On the other hand, we recently have reported that mRNA, protein levels, and enzymatic activity of PTP1B were upregulated in the liver of hyperglycemic IRS2⁻/⁻ mice, coincident with decreased IR/IRS1-mediated insulin signaling. Based on these observations, our first goal was to evaluate PTP1B protein levels in whole retinal extracts in 9-week-old WT and IRS2⁻/⁻ mice. PTP1B levels were increased in whole retinas of IRS2⁻/⁻ mice compared to the WT controls, whereas no differences were found in IRS1 levels (Fig. 1A). At this age, visual function is altered in IRS2⁻/⁻ mice as manifest by reductions in the amplitude of the a-wave and b-wave in the scotopic ERG (Fig. 1B). Additionally, we detected early reactive gliosis in mice lacking IRS2, due to elevated levels of glial fibrillar acidic protein (GFAP) and glutamine synthetase (GS; Fig. 1C). Of note, these data were obtained in mice with fasted blood glucose < 120 mg/dL, before the onset of severe type 2 diabetes.

IGF-I Induces Tyrosine Phosphorylation of the IGF-IR and Activation of Akt in Organotypic Cultures From Mouse Retina

To analyze IGF-IR signaling in whole retina, we set up organotypic cultures that were stimulated with various doses of IGF-I for 30 minutes. IGF-I induced tyrosine autophosphorylation of the IGF-IR in retinal explants of WT mice in a dose-dependent manner (Fig. 2A), with maximal effects being elicited at 60 nM concentration. Under these experimental conditions, phosphorylation of Akt at serine 473 was augmented maximally at 30 nM. To confirm the dose-response effect of IGF-I further in retinal explants, we measured Akt phosphorylation by ELISA analysis, a more quantitative procedure. As shown in Figure 2B, maximal Akt (Ser473) phosphorylation also was observed at 30 nM IGF-I concentration. These results indicated that organotypic cultures are a suitable model to study IGF-IR-mediated signaling in mouse retina.

![Figure 3. Increased IGF-I-mediated signaling in retinas from PTP1B-deficient mice.](image-url)
FIGURE 4. PTP1B deficiency does not recover IGF-I–mediated Akt phosphorylation in IRS2−/− mice. (A) Retinas were dissected from 9-week-old male WT, IRS2−/−, and dKO mice, and cultured as described in Materials and Methods. Then, retinal explants were stimulated with IGF-I (30 nM) for 30 minutes. Whole retinal lysates (50 μg) were separated by 8% SDS-PAGE gels and analyzed by Western blot with the antiphospho IGF-IR (Tyr1165/1166), total IGF-IR, antiphospho Akt (Ser473), and antitotal Akt antibodies. Representative autoradiograms are shown. The autoradiograms were quantified by scanning densitometry. Results are expressed as fold increase of IGF-I stimulation and are the mean ± SEM (n = 6 from each genotype/condition, *P < 0.05). (B) Analysis of Akt (Ser473) phosphorylation in retinal explants by ELISA. Retinal explants were stimulated as described in (A). Results are expressed as arbitrary units (normalized by data obtained with the analysis of total Akt) and are the mean ± SEM (n = 4 from each genotype/condition).
PTP1B Deficiency Increased IGF-IR/Akt Activation in Mouse Retina

Since the IGF-IR is a substrate of PTP1B, we compared IGF-I stimulation in retinas from WT and PTP1B-deficient (PTP1B−/−) mice. IGF-I-mediated phosphorylation of the IGF-IR and Akt (Ser473) were significantly higher in retinal cultures from PTP1B−/− mice compared to WT controls (Fig. 3A). Total levels of IGF-IR and Akt were comparable in the two genotypes of mice. The results on Akt (Ser473) phosphorylation also were confirmed by ELISA analysis (Fig. 3B). Next, ERG was used to evaluate photoreceptor function in WT and PTP1B−/− mice. As depicted in Figure 3C, no differences were found in the amplitude of the a-wave and b-wave between the two groups.

These results on visual function are in agreement with the analysis of Rajala et al.23

FIGURE 5. Morphologic alterations in retinas from IRS2−/− mice were not corrected by deletion of PTP1B. Eyes were collected from 9-week-old male WT, IRS2−/−, PTP1B−/−, and dKO mice. Cryostat sections were prepared for light microscopic observation. The thickness of different layers of retina was measured using the OpenLab software (Improvision). Whole retina thickness (A), ONL (B), ROS (C), and INL (D) were analyzed. In each individual mouse studied, we measured a total of six retinal cross-sections made through the optic nerve head. These data then were averaged, and results obtained from three animals of each genotype were analyzed statistically using Student’s t-test (*P < 0.05).

IGF-I-Mediated IGF-IR Tyrosine Phosphorylation Was Unable to Restore Akt Phosphorylation in Retinas From IRS2−/−/PTP1B−/− Mice

Since PTP1B deficiency enhanced the Akt branch of the IGF-IR signaling cascade in the retina (Fig. 3A), we hypothesized that defects in this pathway observed in IRS2−/− mice could be reversed by deleting PTP1B. Thus, we evaluated IGF-I-mediated Akt signaling in retinal organotypic cultures from the double mutant IRS2−/−/PTP1B−/− mice in comparison with WT or IRS2−/− mice. Consistent with results from another laboratory, phosphorylation of Akt at serine 473 in response
to IGF-I was reduced significantly in IRS2−/− mice compared to the WT controls (Fig. 4A), due to elevated basal levels. Although IGF-I-mediated phosphorylation of the IGF-IR was enhanced in retinas from IRS2−/−/PTP1B−/− mice compared to WT or IRS2−/− mice, as it could be expected due to the loss of PTP1B, basal Akt phosphorylation was increased and the IGF-I response was absent as observed for IRS2−/−/C0 mice. Of note, ELISA analysis of Akt (Ser473) phosphorylation confirmed the elevated basal levels, and the lack of effect of IGF-I in IRS2−/− and double mutant IRS2−/−/PTP1B−/− mice compared to WT controls (Fig. 4B).

**Morphologic Alterations in Retinas From IRS2−/− Mice Were Not Corrected by Deletion of PTP1B**

Next, we analyzed the morphology of the retinas of the four genotypes of mice at 9 weeks of age. Histologic analysis did not detect differences in retinal thickness between WT and PTP1B−/− mice (Fig. 5A). However, in agreement with data from Yi et al., a significant decrease in total retinal thickness was observed in IRS2−/− mice compared to the WT control. Similar reductions of retinal thickness were detected in the double mutant IRS2−/−/PTP1B−/− mice. This effect was the...
Increased IGF-IR Phosphorylation in PTP1B−/− Retina

**Figure 7.** Differential expression of PTEN in retinas from WT, IRS2−/−, and double mutant IRS2−/−/PTP1B−/− mice. The retinas were dissected from 9-week-old male WT, IRS2−/−, and dKO mice. Whole retinal lysates (50 μg) were separated by SDS-PAGE. Western blot analysis was performed with the anti-PTEN antibody. β-actin was used as a loading control. The autoradiograms were quantified by scanning densitometry. Results are expressed as percentage of the WT and are the mean ± SEM (n = 8–9 mice from each genotype, *P < 0.05).

result of decreases in the outer nuclear layer (ONL, Fig. 5B) and retinal outer segments (ROS, Fig. 5C). Of note, no differences were observed in the inner nuclear layer (INL, Fig. 5D).

**Visual Defects in IRS2−/− Mice Were Not Restored by PTP1B Deletion**

To explore further whether enhanced IGF-IR tyrosine phosphorylation was sufficient to delay the loss of visual function in IRS2−/− mice, we analyzed the double mutant IRS2−/−/PTP1B−/− mice by ERG. Visual function was monitored in IRS2−/− and IRS2−/−/PTP1B−/− mice at 5 and 10 weeks of age. However, no differences were observed between the two groups with respect to the ERG wave amplitudes (Fig. 6A). Average values of ERG wave amplitudes and statistical analyses are shown in Figure 6B.

**IRS2-Deficiency Increases the Expression of PTEN Which Is Not Normalized by Loss of PTP1B**

The dissociation between IGF-IR tyrosine phosphorylation and Akt serine 473 phosphorylation in the double mutant IRS2−/−/PTP1B−/− mice prompted us to explore additional negative modulators of this pathway. Among them, the expression of PTEN was highly elevated in retinas from IRS2−/− mice compared to WT controls (Fig. 7). This effect was less pronounced in retinas from IRS2−/−/PTP1B−/− mice, although PTEN levels still were elevated significantly compared to the WT controls.

**DISCUSSION**

IRS2 coordinates IGF-I/IGF-IR signaling in the nervous system and, accordingly, its deletion in mice leads to reduced neural proliferation during development,9 defects in appropriate timing of myelination,25 and loss of retinal photoreceptors by increased apoptosis.14 By contrast, PTP1B deletion confers protection against apoptosis in insulin/IGF-I-sensitive mammalian cells.26,27 Here, we investigated IGF-IR-mediated activation of Akt in organotypic cultures of retina from mice lacking IRS2 and/or PTP1B that constitute two critical nodes of the insulin/IGF-I cascade.

The initial experiments performed in retinal extracts from WT mice demonstrated that IGF-I rapidly induced tyrosine phosphorylation of the IGF-IR as well as Akt phosphorylation at serine 473. Moreover, our results confirm that IGF-IR is a substrate of PTP1B in the retina, since its autophosphorylation in response to IGF-I was significantly augmented in organotypic cultures from PTP1B−/− mice compared to the WT controls. A recent study reported increased IR tyrosine phosphorylation in rats treated with an intravitreal injection of a PTP1B inhibitor upon exposure to light stress.23 However, to our knowledge our study is the first to demonstrate increased IGF-IR activation upon ligand binding in retinas from PTP1B−/− mice. Downstream of the IGF-IR, activation of Akt, which is a key mediator of survival signaling in many mammalian cells, also was augmented in retinas from PTP1B−/− mice. These data are consistent with the protection against caspase-3 cleavage during light stress in PTP1B−/− mice, strongly suggesting that increased Akt-mediated survival signaling upon IGF-IR activation is the molecular mechanism of retinal neuroprotection in these mice. Of note, PTP1B-deficient mice did not present an obvious retina phenotype at the age studied compared to the WT control.

Yi et al. reported the impairment of Akt phosphorylation in response to IGF-I in retinas from IRS2−/− mice.14 Our study demonstrated that IGF-I-mediated tyrosine phosphorylation of the IGF-IR is higher in retinas of IRS2−/− compared to the WT mice, despite elevated PTP1B expression (Figs. 1A, 4A). These data differ from those recently reported by our group in the liver from hyperglycemic IRS2−/− mice, showing that elevated PTP1B expression is associated with a severe impairment of IR tyrosine phosphorylation in response to insulin.28 Importantly, the present study has been performed with IRS2−/− mice with fasting glucose levels < 120 mg/dl to avoid secondary effects derived from severe hyperglycemia and not exclusively to IRS2 deletion. Therefore, in the absence of peripheral hyperglycemia, it is possible that other molecules, such as proinflammatory cytokines, might increase PTP1B expression in the retina from IRS2−/− mice as occurs in the liver of obese mice29 and cochlea in the inner ear.29 Besides elevation of PTP1B in retinas from IRS2−/− mice, it also is possible that the increased IGF-IR tyrosine phosphorylation constitutes a compensatory mechanism to maintain downstream signaling via the alternative substrate IRS1 that also is expressed in mouse retina. Another possible explanation for such effect could be the absence of the negative feedback loops mediated by mTOR/S6K1 on serine phosphorylation of the IGF-IR. In any event, these mechanisms were not able to mediate normal Akt phosphorylation in response to IGF-I, and this might explain the inability of PTP1B deletion to rescue the severe loss of retinal thickness and visual function observed in IRS2−/− mice. These results suggested that, in addition to PTP1B, other factors must intervene to impair IGF-IR-mediated Akt phosphorylation and retinal survival in IRS2-deficient mice. Interestingly, expression of PTEN was enhanced drastically in retinas from IRS2−/− mice compared to the WT controls. This effect was decreased in the double mutant IRS2−/−/PTP1B−/− mice, but still was significantly higher than in retina of WT mice. These data suggest strongly that elevated PTEN expression bypasses the positive effects of PTP1B deficiency on IGF-IR tyrosine phosphorylation, such that IGF-I-mediated Akt phosphorylation is impaired not only in IRS2−/−, but also in the double mutant IRS2−/−/PTP1B−/− mice. It is intriguing that the administration of a PTP1B inhibitor protects against retinal damage in rats,23 but
its deficiency does not rescue photoreceptor loss in IRS2−/− mice. The developmental defects in the retina of IRS2−/− mice14 might account for such differences.

In summary, the results presented in our study demonstrated, for the first time to our knowledge, a direct role of PTP1B in the modulation of IGF-I-mediated Akt signaling in the retina. In the retina of IRS2−/− mice, the upregulation of PTEN impairs IGF-IR-mediated signaling at the level of Akt. Therefore, deletion of PTP1B in IRS2−/− mice is unable to restore Akt phosphorylation or visual function in adult mice. Altogether, our data pinpoint the complexity of the IGF-IR signaling network in the retina, and suggest that therapies aimed to decrease PTEN and improve Akt-mediated signaling might be of benefit against retinal neurodegeneration.

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