Preconditioning-induced protection of photoreceptors requires activation of the signal-transducing receptor gp130 in photoreceptors

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Retinal degenerations are a class of neurodegenerative disorders that ultimately lead to blindness due to the death of retinal photoreceptors. In most cases, death is the result of long-term exposure to environmental, inflammatory, and genetic insults. In age-related macular degeneration, significant vision loss may take up to 70–80 years to develop. The protracted time to develop blindness suggests that retinal neurons have an endogenous mechanism for protection from chronic injury. Previous studies have shown that endogenous protective mechanisms can be induced by preconditioning animals with sublethal bright cyclic light. Such preconditioning can protect photoreceptors from a subsequent damaging insult and is thought to be accomplished through induced expression of protective factors. Some of the factors shown to be associated with protection bind and activate the signal transducing receptor gp130. To determine whether stress-induced endogenous protection of photoreceptors requires gp130, we generated conditional *gp130* knockout (KO) mice with the Cre/*lox* system and used light-preconditioning to induce neuroprotection in these mice. Functional and morphological analyses demonstrated that the retina-specific *gp130* KO impaired preconditioning-induced endogenous protection. Photoreceptor-specific *gp130* KO mice had reduced protection, although the Müller cell KO mice did not, thus gp130-induced protection was restricted to photoreceptors. Using an animal model of retinitis pigmentosa, we found that the photoreceptor-specific *gp130* KO increased sensitivity to genetically induced photoreceptor cell death, demonstrating that gp130 activation in photoreceptors had a general protective role independent of whether stress was caused by light or genetic mutations.

IL6 signal transducing receptor | neuroprotection | conditional gp130 knockout | inherited retinal degeneration | light damage

The signal-transducing receptor gp130 is a common receptor for the IL-6 family of cytokines, such as ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), and cardiotrophin-like cytokine (CLC). Use of these cytokines has been shown to be neuroprotective in both the central and peripheral nervous systems, suggesting that this receptor and its signal transduction pathways may be important for the design of neuroprotective therapeutics (1–4).

Up-regulation of protective cytokines by acute or chronic stress has been observed in several studies. Mild light stress has been shown to induce endogenous protection of photoreceptors from a subsequent light damage, and protection coincided with prolonged up-regulation of neurotrophic factors (5). bFGF mRNA expression was up-regulated in mice homozygous for the *rd1* mutation in the PDE6b gene (a model of retinitis pigmentosa) (6). Elevated levels of bFGF and/or CNTF expression in the retina were also found in light-induced as well as inherited models of photoreceptor degeneration in both mice and rats (7, 8). LIF and CLC were also up-regulated by stress from constant light exposure in the mouse retina (9). CNTF and bFGF expression were induced after a single mechanical lesion to the retina and subretinal space in rats (10) and

mice (11), respectively. Collectively, these studies demonstrate that stress in the retina caused by light, mechanical injury, and genetic mutations can induce endogenous up-regulation of neurotrophic factors. Up-regulation of these factors is likely functional, since a number of studies have shown that injection or viral-mediated expression of these factors can protect photoreceptors (12–19).

Determining the mechanism of preconditioning-induced protection has been hampered because multiple factors are induced, which can activate different receptors located on different cell types. The ligands or receptors that are required for preconditioning-induced protection have yet to be identified. In addition, it is not known which cell type is responsible for protection. Thus, the purpose of this study was to determine the role of gp130 activation in endogenous protection induced by bright light preconditioning and by chronic stress caused by genetic mutations. We also sought to determine whether protection of photoreceptors requires activation of gp130 in photoreceptors or in Müller cells. To accomplish this, we generated mice with conditional *gp130* KO in the retina using the Cre/*lox* system. Our data clearly show that chronic stress such as preconditioning and genetic mutations induced endogenous protection of photoreceptors and that loss of gp130 in either the whole retina or specifically in photoreceptors impairs stress-induced protection, although Müller cells do not. This study demonstrates that gp130 activation in photoreceptors is required to prevent death of neurons under stressful conditions and suggests that gp130 may help prevent or delay cell death in neurodegenerative diseases or chronic injury.

Results

Cre-Mediated Deletion of *gp130* Abolished STAT3 Activation in Response to a gp130 Ligand in a Cell-Specific Manner. To study the cell-specific roles of gp130 in endogenous photoreceptor protection, we generated murine models of conditional *gp130* KO in the retina using the Cre/*lox* system. We mated mice carrying the floxed gp130 allele (*gp130*[f/f]) (20) with *Chx10-cre* (21), *rod-cre* (22), or *VMD2-cre* (23) transgenic lines. The resultant conditional *gp130* KO mice had *gp130* deletion in almost all retinal cells, rod photoreceptors, or Müller glial cells, respectively. All mice were viable and had no apparent phenotypic abnormalities. To test for toxicity induced by Cre expression or by floxed alleles, we used electroretinography (ERG) and histology to examine the retinas of 6-week-old mice for functional and morphological alterations. We did not observe any unexpected abnormalities in
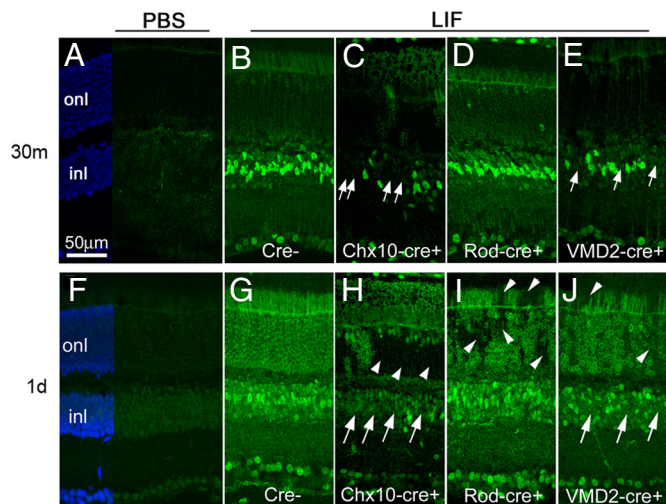
Fig. 1. Retinal cell-specific deletion of gp130 resulted in LIF-induced loss of STAT3 activation. Immunohistochemical detection of STAT3 activation (pSTAT3; green) was used to localize cells that did not responded to LIF 30 min or 1 day after intravitreal injection of LIF. Injection of PBS did not induce detectable STAT3 activation at 30 min *A* or 1 day (*F*). pSTAT3 was detected in Müller cells and all retinal cells 30 min *B* and 1 day (*G*) post-LIF injection in *gp130*^f/f; *cre*^− retinas, respectively. In *gp130*^f/f; *Chx10-cre*^+ retina (*C* and *H*), 80% of the cells lost STAT3 activation including photoreceptors (arrowheads) and Müller cells (arrows). In *gp130*^f/f; *rod-cre*^+ retina, 30% of photoreceptors lost STAT3 activation (arrowheads in *I*), while other cell types were unaffected (*D* and *I*). In *gp130*^f/f; *VMD2-cre*^+ retina, 50% of Müller cells (arrows in *E* and *J*) and <5% of photoreceptors lost STAT3 activation (arrowheads in *J*). Nuclei were counterstained with DAPI (blue). onl, outer nuclear layer; inl, inner nuclear layer.

these mice. Data also show that gp130 deletion did not cause any detectable effect on retinal development, function, and integrity in unstressed animals.

To assess the extent of cell-specific *gp130* deletion in the retina, we initially tested multiple commercially available antibodies against gp130 by using Western blot analysis; however, high non-specific binding produced multiple bands, preventing the use of immunohistochemistry to demonstrate the loss of gp130. To assess the extent of the *gp130* deletion, we measured STAT3 phosphorylation, a known downstream event of gp130 (24). We intravitreally injected 6- to 7-week-old *gp130* KO mice with recombinant human LIF. At 30 min and 1 day, we performed immunohistochemistry for phosphorylated STAT3 on the retinal sections (Fig. 1). Similar to the retinas from WT mice (19), the *gp130*^f/f; *cre*^− retinas had a LIF-induced, robust STAT3 activation in Müller cells 30 min postinjection (Fig. 1*B*) and in all retinal cells 1 day postinjection (Fig. 1*G*). Although injection of PBS did not induce detectable STAT3 activation in any of our *gp130* conditional KO retinas (Fig. 1 *A* and *F*), 80% of the retinal cells, including Müller cells (Fig. 1 *C* and *H*, arrows) and photoreceptors (Fig. 1*H*, arrowheads) in the *gp130*^f/f; *Chx10-cre*^+ mice lost STAT3 activation in response to LIF due to *gp130* deletion. The *gp130* deletion in *gp130*^f/f; *rod- cre*^+ mice abolished STAT3 activation in 30% of rod photoreceptors without affecting STAT3 activation in the other cell types (Fig. 1 *D* and *I*). In *gp130*^f/f; *VMD2-cre*^+ mice, STAT3 activation was lost in 50% of Müller cells (Fig. 1 *E* and *J*, arrows) and in <5% of the photoreceptors (Fig. 1*J*, arrowheads) (23).

We used Western blots to quantify the loss of STAT3 activation in the whole retina due to *gp130* deletion in each conditional KO line ([Fig. S1](#)). Retinas from *gp130*^f/f; *Chx10-cre*^+ mice had 80% reduction in LIF-induced signaling at all time points when compared to retinas from *gp130*^f/f; *Chx10-cre*^− mice. The retinas from *gp130*^f/f; *VMD2-cre*^+ mice had significantly less signaling activation than the retinas from *gp130*^f/f; *VMD2-cre*^− mice when LIF-induced signaling activation was only detected in Müller cells (30 min) (*P* <
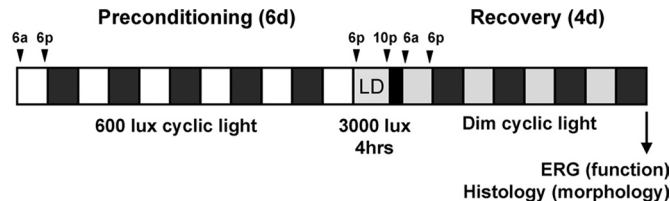

Fig. 2. Bright light preconditioning model. Mice were exposed to moderately bright cyclic light (600 lux; 12 h on/12 h off) for 6 days to induce endogenous protection in the retina. The mice were exposed to 3,000 lux light to induce LD and then to 50 lux cyclic light (12 h on/12 h off) for 4 days to recover. Photoreceptor function and morphology were subsequently assessed by electroretinography and histology.

0.05). The retinas from the *gp130*^f/f; *rod-cre*^+ mice had less STAT3 activation than those from the *gp130*^f/f; *rod-cre*^− mice when photoreceptors were most active (1 day), but no effect on early STAT3 activation when only Müller cells were active (30 min).

Loss of gp130 in the Retina Significantly Impaired Endogenous Protection of Photoreceptor Function and Accelerated Light-Induced Photoreceptor Cell Death. It was previously shown that preconditioning with bright cyclic light can induce endogenous mechanisms of photoreceptor protection against subsequent exposure to damaging light (light damage; LD) (5, 25–27). Ligands of gp130, such as CNTF, are up-regulated in response to light exposure, suggesting that gp130 might play a protective role (8, 9). We found previously that a 6-day exposure to sublethal bright cyclic light (600 lux) was sufficient to induce endogenous protection against subsequent LD in WT albino mice (28). We used this preconditioning model (Fig. 2) to analyze the effect of gp130 loss on endogenous protection of photoreceptors in *gp130*^f/f; *Chx10-cre* mice (Figs. 3 and 4).

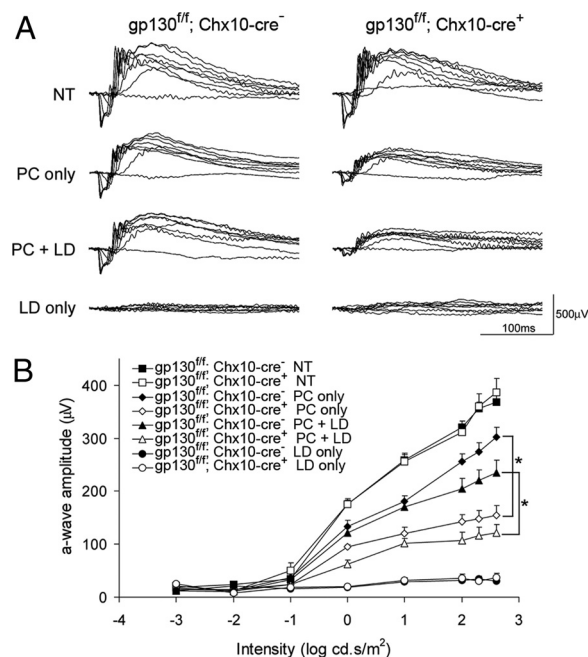To evaluate the effect of gp130 loss on protection of photore-

Fig. 3. Loss of gp130 in *gp130*^f/f; *Chx10-cre*^+ mice significantly impaired preconditioning-induced endogenous protection of photoreceptor function. (*A*) Representative ERG traces of *gp130*^f/f; *Chx10-cre*^− (*Left*) and *gp130*^f/f; *Chx10-cre*^+ (*Right*) before preconditioning (NT), after preconditioning (PC only), preconditioning and then LD (PC + LD), and LD (LD only). (*B*) Photoreceptor response (a-wave amplitude) at different intensities of light flashes. *, *P* < 0.05 with *t* test (*n* = at least 6 per group). Error bars represent SEM.
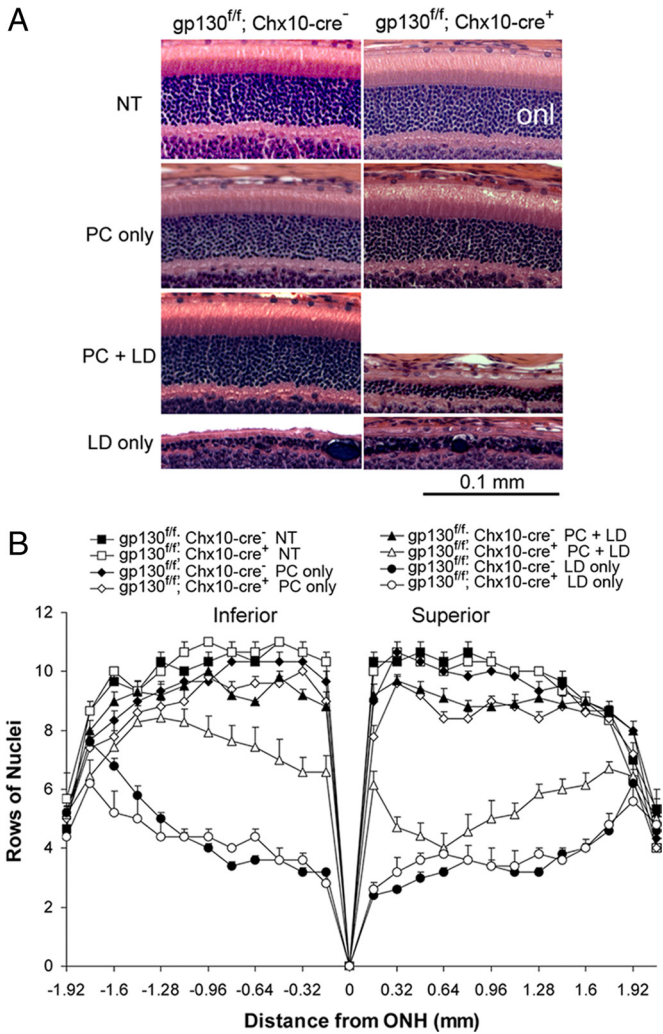
Fig. 4. Loss of gp130 in $gp130^{f/f}$; $Chx10$-cre^+ mice significantly impaired endogenous protection of photoreceptors. The degree of photoreceptor loss was quantified by counting the number of rows of photoreceptor nuclei along the retina from the optic nerve head (ONH). (*A*) Representative histological sections are from the superior retina at 0.48 mm from the ONH. (*B*) The mean number of rows of photoreceptor nuclei in the $gp130^{f/f}$; $Chx10$-cre^+ retinas was significantly fewer than the number in the $gp130^{f/f}$; $Chx10$-cre^- retinas after preconditioning and light damage (PC + LD) (open and closed triangles, respectively). onl, outer nuclear layer. Error bars represent SEM (n = at least 6 per group).

ceptor function, we performed electroretinography (ERG) (Fig. 3). Decreased a-waves in scotopic ERG recordings are an indication of reduced photoreceptor function. Both wild-type ($gp130^{f/f}$; $Chx10$-cre^-) and gp130 KO retinas ($gp130^{f/f}$; $Chx10$-cre^+) had normal responses to light stimulation, indicating that neither the $gp130$ floxed allele (Fig. 3B, closed squares) nor retina-specific loss of gp130 by $Chx10$-cre (Fig. 3B, open squares) affected photoreceptor function under normal conditions. LD without preconditioning resulted in complete loss of photoreceptor function in both $gp130^{f/f}$; $Chx10$-cre^- and $gp130^{f/f}$; $Chx10$-cre^+ retinas (Fig. 3B, circles), showing susceptibility of nonpreconditioned photoreceptors to light-induced oxidative damage. Retinas from $gp130^{f/f}$; $Chx10$-cre^- mice were significantly protected from LD after preconditioning (Fig. 3B, closed triangles). This response was similar to the preconditioning-induced protection of normal mice that we described in ref. 28. In $gp130^{f/f}$; $Chx10$-cre^+ retinas, LD resulted in significant reduction in photoreceptor function (Fig. 3B, triangles). In contrast, preconditioning-induced protection of photoreceptors was not im-

paired in $gp130^{f/+}$; $Chx10$-cre^+ retinas compared to $gp130^{f/+}$; $Chx10$-cre^- retinas (Fig. S2), demonstrating that the expression of Cre was not toxic to photoreceptors nor making photoreceptors more susceptible to light exposure. These data suggest that loss of gp130 in the retina abolishes endogenous protection of photoreceptor function against light-induced oxidative damage.

To determine whether loss of function was the result of photoreceptor cell death, we quantified photoreceptor survival by morphometric analyses (Fig. 4). Representative histological sections of the superior retina, a region most susceptible to light damage, are shown in Fig. 4A. The number of rows of photoreceptors was counted at 0.16-mm intervals from the optic nerve head (Fig. 4B). LD after preconditioning resulted in loss of photoreceptor cells in $gp130^{f/f}$; $Chx10$-cre^+ retinas (PC + LD; Fig. 4B, open triangles), but not in $gp130^{f/f}$; $Chx10$-cre^- retinas (Fig. 4B, closed triangles), suggesting that gp130 expression in the retina is essential for preconditioned-induced protection.

Unexpectedly, we observed a significant loss of photoreceptor function in $gp130^{f/f}$; $Chx10$-cre^+ retinas after preconditioning (Fig. 3B, open diamonds). Despite the loss of function, the number of photoreceptors did not differ significantly between $gp130^{f/f}$; $Chx10$-cre^- and $gp130^{f/f}$; $Chx10$-cre^+ retinas after preconditioning (Fig. 4B, diamonds). This suggests that the reduction in function after preconditioning was not due to cell loss. The mechanism for loss of function in preconditioned $gp130^{f/f}$; cre^+ mice is unknown, but the results suggest that loss of gp130 alters photoreceptor responses to light stress.

gp130 Expression in Rod Photoreceptors, But Not Müller Cells, Is Essential for Endogenous Protection of Photoreceptors Against Light-Induced Oxidative Stress. While our data from the $gp130^{f/f}$; $Chx10$-cre mice demonstrate that gp130 is essential for stress-induced protection (Figs. 3 and 4), it is not clear whether protection is an effect of gp130 activation in photoreceptors or in Müller cells. In mice with photoreceptor specific deletion of $gp130$ ($gp130^{f/f}$; rod-cre^+), preconditioning-induced protection was dramatically reduced (Fig. 5A, compare triangles). In agreement with ERG functional analysis, histological analysis revealed significantly more photoreceptor loss in the $gp130^{f/f}$; rod-cre^+ retinas after LD compared to $gp130^{f/f}$; rod-cre^- retinas (Fig. 5C, *Left*, and D, triangles). Expression of Cre in rod-cre^+ retinas did not show impairment in preconditioning-induced protection (Fig. S3), further supporting that the loss of protection observed in $gp130^{f/f}$; rod-cre^+ retinas can be attributed to loss of gp130 in photoreceptors rather than potential toxicity associated with Cre expression. Both functional and histological data demonstrate that gp130 in rod photoreceptors was essential for endogenous protection of photoreceptors from light-induced oxidative stress.

Preconditioning-induced protection of photoreceptor function was not impaired in $gp130^{f/f}$; $VMD2$-cre^+ retinas (Fig. 5B, triangles). Histological analyses support these findings and revealed the loss of gp130 in Müller cells did not reduce preconditioning-induced protection of photoreceptors from LD (Fig. 5C, *Right*). These results demonstrate that deletion of gp130 in rod photoreceptors, but not Müller cells, impairs stress-induced endogenous protection of photoreceptors, and suggests gp130 activation in photoreceptors rather than in Müller cells is essential for endogenous protection of photoreceptors.

gp130 in Rod Photoreceptors Plays an Essential Role in Endogenous Protection of Photoreceptors from Genetically Induced Cell Death. Light damage is an experimental model of retinal degeneration that may or may not reflect mechanisms of inherited retinal degeneration. Preconditioning studies suggest retinas under stress induced gp130-mediated endogenous protection. Inherited mutations likely induce chronic stress in which gp130 may also play a role in delaying or reducing photoreceptor cell death. To test this, we mated $gp130^{f/f}$; rod-cre^+ mice with VPP transgenic mice (29), which express a
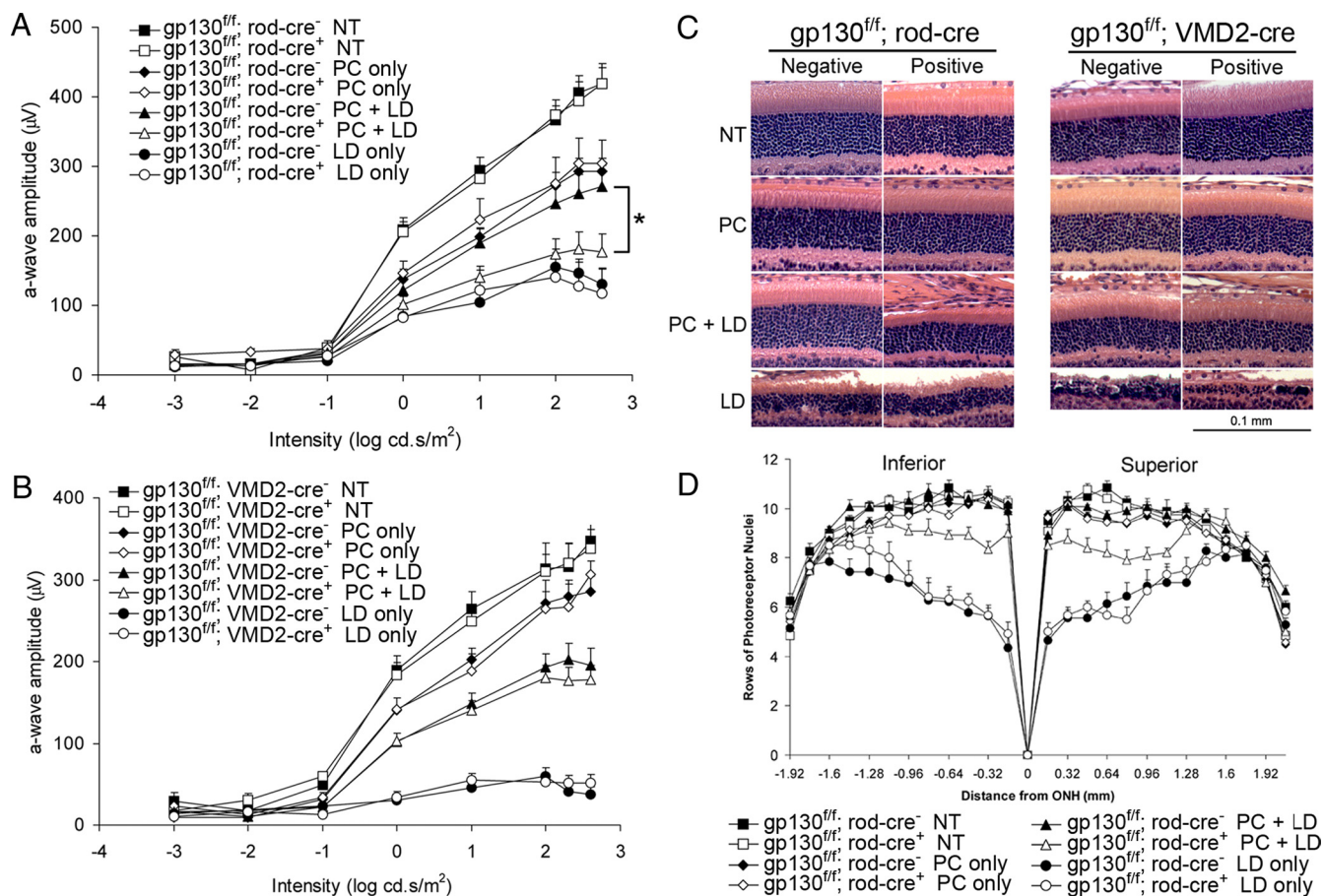
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Fig. 5. Loss of gp130 in rod photoreceptors, but not Müller cells, significantly impaired preconditioning-induced endogenous protection of photoreceptors. (*A*) The mean a-wave amplitude was significantly lower for *gp130*$^{f/f}$; *rod-cre*$^+$ mice than for *gp130*$^{f/f}$; *rod-cre*$^-$ mice after preconditioning followed by light damage (PC + LD) (triangles). (*B*) Loss of gp130 in 50% of Müller cells in the *gp130*$^{f/f}$; *VMD2-cre*$^+$ retina did not affect gp130-mediated endogenous protection of photoreceptor function (open and closed triangles). (*C*) Representative histological sections of the outer nuclear layer at the superior retina 0.48 mm from the ONH. (*D*) Mean number of rows of photoreceptors after PC + LD in *gp130*$^{f/f}$; *rod-cre*$^+$ retina was significantly less than in *gp130*$^{f/f}$; *rod-cre*$^-$ retina after PC + LD (open and closed triangles, respectively). Error bars represent SEM (*n* = at least 8 per group). *, *P* < 0.05 with *t* test.

mutant rhodopsin and display photoreceptor degeneration occurring after 2 weeks of age (Fig. 6). Deletion of gp130 (VPP$^+$; *gp130*$^{f/f}$; *rod-cre*$^+$) resulted in accelerated degeneration compared to VPP mice with gp130 (VPP$^+$; *gp130*$^{f/f}$; *rod-cre*$^-$) (Fig. 6 *A* and *B*). The accelerated degeneration was the result of gp130 loss and not the result of Cre toxicity since the rate of degeneration caused by the VPP transgene was the same in Cre$^+$ and Cre$^-$ mice, which had one wild-type allele of *gp130* (Fig. S4). These results clearly demonstrate gp130 in photoreceptors plays a role in preventing or delaying cell death from genetically induced chronic stress.

Discussion

We previously showed that sublethal light stress (preconditioning) induces endogenous neuroprotection, which is highly effective at protecting photoreceptors from a subsequent exposure to LD (28). Therefore, identifying the mechanism for endogenous protection may lead to the development of neuroprotective therapies. We and others have previously shown that application of exogenous gp130 ligands promotes photoreceptor survival in light-induced and inherited models of retinal degeneration in many species (12–19). Thus, gp130 activation could be one of the key events mediating endogenous neuroprotection. Our conditional *gp130* KO data clearly show that gp130 plays an essential role in endogenous neuroprotection and that this protection is mediated by activation of gp130 in photoreceptors. Data further suggest that the indirect effect of gp130 activation in Müller glial

cells is not as critical, since inactivating *gp130* in 30% of rod photoreceptors reduced protection while inactivating *gp130* in 50% of Müller glial cells did not.

The light history of an animal greatly affects the susceptibility of its retinas to subsequent light damage (25, 30, 31). For example, albino rats raised in darkness were more susceptible to light-induced retinal degeneration than albino rats raised in cyclic light (30). Previous studies demonstrated that moderate bright light stress induced endogenous protection of photoreceptors from subsequent LD in both mice and rats (5, 26, 27, 32). In addition, preconditioning has been shown to coincide with prolonged up-regulation of neurotrophic factors (5). Elevated levels of bFGF, CNTF, LIF, and CLC have been found in mouse and rat retinas in light-induced and inherited models of photoreceptor degeneration (7–9).

We have previously measured preconditioning-induced expression of several factors including FGF2, BDNF, LIF, oncostatin M (OSM), and CLC (28). Several of the induced cytokines including LIF, CNTF, OSM, and CLC signal through the common receptor gp130, suggesting that this cytokine/gp130 system may be important for neuroprotection. However, other factors that do not activate gp130 were also up-regulated, suggesting the possibility that other factors or receptors may also be involved. Since multiple cytokines and growth factors are up-regulated by stress, it has been difficult until now to determine which ligand-receptor systems are essential for stress-induced protection. We previously used antagonists to the
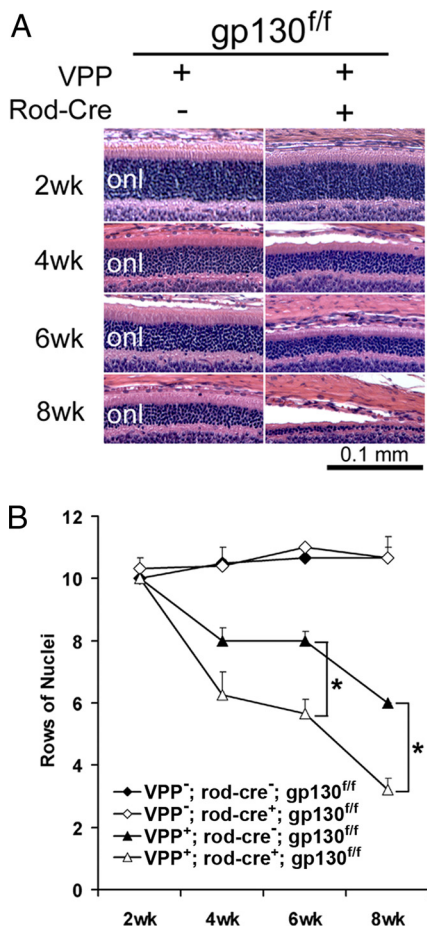
A   gp130^f/f

VPP	+	+
Rod-Cre	−	+

2wk, 4wk, 6wk, 8wk — onl

0.1 mm

B

VPP⁻; rod-cre⁻; gp130^f/f
VPP⁻; rod-cre⁺; gp130^f/f
VPP⁺; rod-cre⁻; gp130^f/f
VPP⁺; rod-cre⁺; gp130^f/f

Rows of Nuclei (y-axis 0–12), x-axis 2wk, 4wk, 6wk, 8wk

Fig. 6. Loss of gp130 in rod photoreceptors accelerated the rate of photoreceptor cell death in an inherited model of retinal degeneration. Rod photoreceptor-specific *gp130* KO mice were crossed with transgenic mice expressing a mutant from of opsin (VPP) to determine whether loss of gp130 accelerated retinal degeneration. (*A*) Representative histological sections are from the superior retina at 0.32 mm from the ONH. Progressive loss of photoreceptors was observed in VPP⁺ retina (*Left* column). (*B*) Mean number of photoreceptors in the region shown in panel *A* is reported. Loss of gp130 in 30% of rod photoreceptors (VPP⁺; *gp130*^f/f; *rod-cre*⁺) (open triangles) significantly accelerated the rate of genetically induced photoreceptor cell death. onl, outer nuclear layer. Error bars represent SEM (*n* = at least 5 per group). *, $P < 0.001$ with *t* test.

LIF receptor (LIFR) and found that ligands, including LIF, CNTF, OSM, and CLC, that used LIFR are the most likely candidates for involvement (28). A recent study using LIF KO mice suggests that LIF is the key cytokine for protection from inherited and light-induced retinal degenerations (33, 34). LIF's role in preconditioning-induced protection is yet to be determined, but based on its induction and the requirement for LIFR, LIF is the most logical candidate (28). In the current study, loss of gp130 in 80% of all retinal cells in the *gp130*^f/f; *Chx10-cre*⁺ mice resulted in near complete loss of preconditioning-induced protection (Figs. 3 and 4). Our data clearly demonstrate that stress-induced neuroprotection requires the signal-transducing receptor gp130.

Activation of the PI3K/Akt pathway can promote cell survival in many tissues, including the retina. Li et al. found that Akt2, one of the three isoforms of Akt kinases, has a role in protecting photoreceptors from acute light-induced cell death, suggesting an essential neuroprotective role of PI3K/Akt pathway during acute insults (35). Acute bright light stress can activate the insulin receptor (IR), which in turn activates the PI3K/Akt pathway in photoreceptors (36). Moreover, rod-specific deletion of the IR increased acute light-induced photoreceptor apoptosis, suggesting a neuroprotec-

tive role of the IR/PI3K/Akt signaling during acute light insults (37). We observed no increase in sensitivity to LD in the absence of preconditioning in any of the conditional *gp130* KO mice (Figs. 3, 4, and 5, LD-only group). This was expected since induction of gp130 ligands requires exposure to chronic stress. Without ligands, loss of the receptor should have no effect. This suggests that there are two nonredundant pathways for protection of photoreceptors; one is the acute response that involves the IR and signaling through Akt2 (35–37) and the other, as shown in this current study, is induced when ligands of gp130 are elevated after prolonged light stress or by genetic mutations (5, 8, 9, 28).

Our data showing that this receptor can induce protection suggests that gp130 ligands may be effective at preventing or delaying neurodegeneration in human diseases. However, little is known regarding the mechanism of protection. In addition, several adverse consequences of overstimulation of this receptor have been reported, including gliosis (38), inhibition of neural function (17, 19, 39, 40), and altered developmental states (41–43). Therefore, specific knowledge of the mechanism for gp130-induced protection is essential for the development of neuroprotective therapies to avoid these side effects. Several studies have suggested that photoreceptor protection is mediated by gp130 activation in Müller cells through an indirect mechanism (44, 45). Evidence for this model has been based solely on activation of STAT3 and/or ERK1/2 signaling in Müller cells. This model suggests that Müller cells are activated by gp130 ligands and in response release other factors that would protect photoreceptors. We previously demonstrated that LIF activates signaling in all retinal cells, suggesting the possibility of direct protection by gp130 activation in photoreceptors (19). In our current study, loss of gp130 in only 30% of rod photoreceptors significantly impaired stress-induced photoreceptor protection (Figs. 5 *A*, *C*, and *D*, and 6). On the other hand, loss of gp130 activation in 50% of Müller cells (*gp130*^f/f; *VMD2-cre* mice) did not affect endogenous protection (Fig. 5 *B* and *C*). These two observations suggest that protection through gp130 activation in Müller cells is not as critical as it is in rod photoreceptors. Other recent studies have shown that Müller cells are the source of LIF in stressed retinas (33, 34). Together, these studies suggest that Müller cells respond to an unknown stress signal to produce protective factors including LIF. LIF then directly activates gp130 on photoreceptors to induce their survival.

In summary, we provide direct evidence for the essential role of gp130 in stress-induced endogenous protection of photoreceptors. These data support the findings of recent studies showing that photoreceptors with inherited retinal degeneration die faster in the absence of LIF (33) or in the presence of a LIFR antagonist (28). The current study extends these findings by demonstrating that photoreceptor protection by gp130 activation is mediated by direct mechanism in photoreceptors. Thus, we hypothesize that photoreceptors under stress stimulate Müller cells to express LIF (33, 34), which then activates LIFR (28) and gp130 signaling in photoreceptors to induce protection. The mechanism by which stressed photoreceptors signal to Müller cells has not yet been identified. The stress-induced endogenous protection by gp130 activation may be playing an important role to help prolong neuronal survival in neurodegenerative and neuroinflammatory diseases in which neurons are under chronic stress.

Methods

Intravitreal Injection. Recombinant human LIF (gp130 ligand) was produced in house and injected intravitreally as described in ref. 19. Briefly, 0.5 µg rLIF (1 µL final volume) was injected into one eye of 6- to 7-week-old mice to induce gp130-dependent signaling activation. PBS (vehicle) was injected in the other eye of each mouse as a control.

Immunohistochemistry. Loss of STAT3 activation was used as a marker for loss of gp130. After 30 min and 1 day, the cornea and lens were removed from LIF- and PBS-injected retinas, and the eye cups were fixed with 2% paraformaldehyde/1× PBS for 30 min. Eye cups were then cryoprotected with sucrose solution (10%, 20%, and then 30%), and cross-sections of the retina were obtained by using a cryostat. Retinal cross-sections were immunostained by using the anti-pSTAT3 antibody (Cell Signaling Technology), and imaging was performed by using a FluoView FV500 confocal laser scanning microscope (Olympus). Imaging and microscope settings were identical in all samples.

Bright Light Preconditioning. Unanesthetized mice were exposed to diffuse, cool, white fluorescent light coming from the top of the cage. Food and water were provided ad libitum, but were placed inside the cage to avoid blocking exposure to light. The light preconditioning model (Fig. 2) was used to induce endogenous protection (600 lux; 12 h on/12 h off for 6 days). Mice were then exposed to 3,000 lux light for 4 h to induce LD, which was followed by a 4-day recovery in 50-lux cyclic light (12 h on/12 h off).

Electroretinogram. Photoreceptor function was measured by using a Colordome ERG instrument (Diagnosxys). After dark adaptation, mice were deeply anesthetized with a single i.p. injection of xylazine (7 mg/g) and ketamine (40 mg/g). Pupils were dilated with tropicamide and phenilepherine, and gold wire electrodes were placed centrally on the cornea to record full-field scotopic ERGs for both eyes.

Morphometric Analysis. After the ERG recordings, mice were killed by CO_2 asphyxiation, and the eyes were collected for histology. Conditional *gp130* KO mice carrying the VPP transgene were collected at the postnatal weeks 2, 4, 6, and 8. Paraffin sections of the eye through the optic nerve head were stained with H&E. The number of photoreceptors lying in a single column spanning the outer nuclear layer (at 0.16-mm intervals) was counted under light microscopy.

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