Islet-Sparing Effects of Protein Tyrosine Phosphatase-1b Deficiency Delays Onset of Diabetes in IRS2 Knockout Mice

Jake A. Kushner,[1,2] Fawaz G. Haj,[3] Lori D. Klaman,[3] Matthew A. Dow,[1] Barbara B. Kahn,[4] Benjamin G. Neel,[3] and Morris F. White[1]

Protein tyrosine phosphatase-1b (Ptp1b) inhibits insulin and leptin signaling by dephosphorylating specific tyrosine residues in their activated receptor complexes. Insulin signals are mediated by tyrosine phosphorylation of the insulin receptor and its downstream targets, such as Irs1 and Irs2. Irs2 plays an especially important role in glucose homeostasis because it mediates some peripheral actions of insulin and promotes pancreatic β-cell function. To determine whether the deletion of *Ptp1b* compensates for the absence of Irs2, we analyzed mice deficient in both *Ptp1b* and *Irs2*. Pancreatic β-cell area decreased in *Ptp1b*$^{-/-}$ mice, consistent with decreased insulin requirements owing to increased peripheral insulin sensitivity. By contrast, peripheral insulin sensitivity and β-cell area increased in *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice, which improved glucose tolerance in *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice and delayed diabetes until 3 months of age. However, β-cell function eventually failed to compensate for absence of Irs2. Our studies demonstrate a novel role for Ptp1b in regulating β-cell homeostasis and indicate that Ptp1b deficiency can partially compensate for lack of Irs2. *Diabetes* 53: 61–66, 2004

Insulin resistance, when combined with sluggish and relatively insufficient insulin secretion, causes type 2 diabetes (1). Although insulin action and secretion had been thought to be unrelated processes, recent work with genetically altered mice suggests that the Irs2 branch of the insulin/Igf-signaling pathway might be a common molecular link (2). The receptors for insulin and IGFs phosphorylate a family of insulin receptor substrates (Irs proteins), including Irs1, -2, -3, and -4. After tyrosine phosphorylation, these scaffold proteins recruit common signal relay molecules, which activate the phosphatidylinositol (PI) 3-kinase→Akt pathway and other downstream signaling cascades (3). During insulin stimulation, the PI 3-kinase cascade stimulates glucose utilization by muscle and lipid storage in adipose tissue, while inhibiting hepatic gluconeogenesis (2). In pancreatic islets, the Irs2→PI 3-kinase cascade promotes islet growth and β-cell survival and function (2).

Irs1 and Irs2 are expressed in all cells and tissues, where they help regulate growth and metabolism. Disruption of *Irs1* reduces body size to ~50% normal, suggesting that it plays a central role in the effects of IGF1 on somatic growth (4–6). Moreover, β-cell mass expands in *Irs1*$^{-/-}$ mice to provide sufficient insulin to compensate for peripheral insulin resistance (4–6). Although both Irs1 and Irs2 contribute to peripheral insulin action, *Irs2*$^{-/-}$ mice develop diabetes at 6–8 weeks of age and die between 14 and 17 weeks due to β-cell failure and uncompensated insulin resistance (5). Irs1 is not required for β-cell expansion in wild-type mice, but it is insufficient in *Irs2*$^{-/-}$ mice, as *Irs1*$^{+/-}$::*Irs2*$^{-/-}$ mice are born with few β-cells and die of diabetes at 30 days of age (7).

Insulin signaling is negatively regulated by several mechanisms, including downregulation of the insulin receptor, serine phosphorylation or degradation of Irs proteins, and dephosphorylation by specific protein tyrosine phosphatases (8). Biochemical and structural studies show that the protein tyrosine phosphatase-1b (Ptp1b) plays a key role in dephosphorylating the insulin receptor (9–13). Ptp1b is localized on the endoplasmic reticulum, where it encounters the insulin receptor during endocytosis (14,15). Ptp1b also inhibits leptin signaling by dephosphorylating Jak2, which decreases hypothalamic leptin sensitivity (16,17). Consequently, *Ptp1b*$^{-/-}$ mice are hypersensitive to insulin and resistant to dietary fat–induced obesity (18,19). Similarly, antisense disruption of *Ptp1b* confirms that Ptp1b inhibition in adult rodents promotes insulin sensitivity and glucose tolerance (20–22). To determine whether increased insulin receptor signaling owing to the disruption of *Ptp1b* can compensate for the absence of Irs2, we evaluated *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice for alterations in glucose homeostasis and β-cell morphology.

From the [1]Howard Hughes Medical Institute, Joslin Diabetes Center, Harvard Medical School, Boston, Massachusetts; the [2]Division of Endocrinology, Children's Hospital, Harvard Medical School, Boston, Massachusetts; the [3]Cancer Biology Program, Division of Hematology-Oncology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts; and the [4]Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts.

L.D.K. is currently affiliated with Wyeth Research, Cambridge, Massachusetts. J.A.K. is currently affiliated with the Division of Endocrinology, Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania.

Address correspondence and reprint requests to Morris F. White, Ph.D., Howard Hughes Medical Institute, Joslin Diabetes Center, 1 Joslin Place, Boston, MA 02215. E-mail: whitemor@joslab.harvard.edu.

Received for publication 26 June 2003 and accepted in revised form 23 September 2003.

AUC, area under the curve; IGF1R, type 1 IGF receptor; IRS, insulin receptor substrate; PI, phosphatidylinositol; Ptp1b, protein tyrosine phosphatase-1b; PTP, protein tyrosine phosphatase.

RESEARCH DESIGN AND METHODS

Mice. *Irs2*$^{+/-}$ mice on a mixed genetic background (C57BL/6 × 129Sv) were intercrossed with *Ptp1b*$^{-/-}$ mice on a similar genetic mixed background (also C57BL/6 × 129Sv) to yield *Irs2*$^{+/-}$::*Ptp1b*$^{+/-}$ mice, which were then intercrossed to yield a subsequent generation of doubly heterozygotic mice. Double heterozygotes were then intercrossed to yield *Irs2*$^{+/-}$ mice and *Irs2*$^{+/-}$::*Ptp1b*$^{-/-}$ mice, which were then intercrossed to yield the genotypes of this study: wild-type mice and *Ptp1b*$^{-/-}$, *Irs2*$^{-/-}$, and *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice. *Irs1*$^{+/-}$ mice were intercrossed with *Ptp1b*$^{-/-}$ mice in a similar fashion. Mice were maintained at a nonbarrier animal facility in the Joslin Diabetes Center and fed Mouse Diet 5020 9F (9% fat calculated by weight, 21.6% fat calculated by kilocalories) (PMI Nutrition International, Richmond, IN). Genotyping of animals was done by PCR, as described previously (5,19). Male mice were used to characterize glucose homeostasis phenotypes and pancreatic pathology. Random-fed glucose and insulin measurements were performed as previously described. Intraperitoneal glucose tolerance tests were performed on mice fasted for 15–16 h with 2 g D-glucose per kilogram body weight as described previously (23). Intraperitoneal insulin tolerance tests were performed on 6-h fasted mice with 0.75 units (6-week-old mice) or 1.5 units/kg (15-week-old mice), similar to previously described methods (23). Area under the curve (AUC) analysis was calculated with trapezoid rule algorithms for individual glucose tolerance tests and reported as grams per deciliter per minute.

Statistics. All results are reported as mean ± SE for equivalent groups. To minimize the effect of statistical variance, statistical comparisons were only performed between *Irs2*$^{-/-}$ mice and *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice (and not other possible combinations) and were compared with independent *t* tests (unpaired and two-tailed) reported as *P* values.

Immunohistochemistry and islet morphometry. Immunohistochemical localization of antigens and double-label immunohistochemistry were performed similarly to previously described methods (23). Pancreas samples were dissected from fed mice and fixed with Bouin's solution overnight. Five-micrometer longitudinal sections of paraffin blocks were rehydrated with xylene followed by decreasing concentrations of ethanol, microwaved in 0.01 mol/l sodium citrate (pH 6.0) for 20 min and permeabilized with 1% Triton X-100 in PBS before primary antisera incubation. Guinea pig anti-insulin, rabbit anti-glucagon antibodies (Zymed Laboratories, South San Francisco, CA) and goat anti-Pdx1 (Santa Cruz Biotech, Santa Cruz, CA) were used as primary antibodies. Secondary antibodies were labeled with fluoroscein isothiocyanate or rhodamine (Jackson ImmunoResearch Laboratories, West Grove, PA). Adult β-cell area was measured by acquiring images at ×10 from 14–20 adjacent nonoverlapping images of insulin sections from 3-month-old male mice, at least six animals per genotype with a Zeiss Axiovert microscope (Carl Zeiss MicroImaging, Thornwood, NY). Images were analyzed for area with Improvision Open Lab software density slice software (Improvision Scientific Imaging, Lexington, MA). Results of β-cell quantification are expressed as the percentage of the total surveyed area containing cells positive for insulin. Islet density and size were calculated from captured insulin-stained images. Results of *Irs2*$^{-/-}$ mice and *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice were compared with independent *t* tests (unpaired two tailed) and reported as *P* values.

RESULTS

We intercrossed *Irs2*$^{+/-}$ mice with *Ptp1b1*$^{-/-}$ mice to generate compound heterozygotes (*Irs2*$^{+/-}$::*Ptp1b*$^{+/-}$), which were backcrossed to produce the nine expected genotypes at the expected Mendelian frequencies (data not shown). Male wild-type, *Irs2*$^{-/-}$, *Ptp1b*$^{-/-}$, and *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice were selected for this investigation to focus on the effect of *Ptp1b* disruption upon glucose tolerance and diabetes in *Irs2*$^{-/-}$ mice (4–6,18,19). We fed all of the mice a regular diet containing 9% fat (calculated by weight) to reduce dietary fat–induced differences in weight that occur between wild-type mice and *Ptp1b*$^{-/-}$ mice (18,19). Consistent with our previous findings, a weight difference was barely perceptible between wild-type and *Ptp1b*$^{-/-}$ mice at 6 weeks of age, whereas *Ptp1b*$^{-/-}$ mice weighed 20% less than wild type at 15 weeks (Fig. 1). By contrast, *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice weighed 20% less than *Irs2*$^{-/-}$ mice at 6 weeks, whereas the difference was almost imperceptible by 15 weeks of age, when *Irs2*$^{-/-}$ mice develop severe diabetes (Fig. 1).

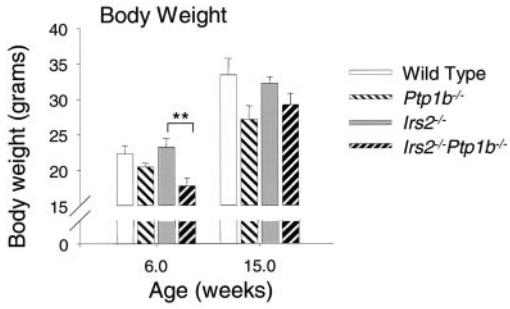

FIG. 1. Growth measurements of *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ intercross mice. Body weights of male wild-type mice, *Irs2*$^{-/-}$ mice, *Ptp1b*$^{-/-}$ mice, and *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice at 6 and 15 weeks of age. **$P < 0.01$, *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ vs. *Irs2*$^{-/-}$. Results represent the mean ± SE of 6–10 mice per genotype.

As previously shown, *Irs2*$^{-/-}$ mice displayed fasting and random-fed hyperglycemia between 6 and 15 weeks of age (Table 1). Compared with wild-type mice, *Ptp1b*$^{-/-}$ mice displayed normal fasting blood glucose at 6 weeks and slightly lower values at 15 weeks, whereas no differences were detected during random feeding (Table 1). *Ptp1b* disruption in *Irs2*$^{-/-}$ mice reduced fasting and random-fed glucose at 6 and 15 weeks (Table 1). However at 15 weeks *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice displayed fasting hyperglycemia (Table 1). Still, some *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice survived for as long as 9 months, whereas *Irs2*$^{-/-}$ mice were severely diabetic and invariably died near 15 weeks (data not shown). Thus, disruption of *Ptp1b* improved glucose tolerance, delayed the onset of diabetes, and prolonged survival in the *Irs2*$^{-/-}$ mice.

Next, we assessed glucose tolerance during intraperitoneal glucose injections at 6 and 15 weeks of age. Compared with wild-type mice at 6 weeks, *Ptp1b*$^{-/-}$ mice displayed nearly identical glucose tolerance, with indistinguishable AUCs (Fig. 2*A* and *C*). *Ptp1b*$^{-/-}$ mice displayed greatly improved glucose tolerance by 15 weeks, consistent with previous findings (18,19). *Irs2*$^{-/-}$ mice were severely glucose intolerant at 6 and 15 weeks (Fig. 2). Although *Ptp1b* disruption consistently improved glucose tolerance of the *Irs2*$^{-/-}$ mice, *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice still displayed significant glucose intolerance at 6 and 15 weeks (Fig. 2).

To estimate peripheral insulin sensitivity, fasted mice were tested by intraperitoneal injection of insulin (0.75 units/kg body wt insulin at 6 weeks and 1.5 units/kg at 15 weeks), and glucose levels were measured over the next 60 min. Unlike previous insulin tolerance test results of *Ptp1b*$^{-/-}$ mice on a similar diet at 10–14 weeks, our *Ptp1b*$^{-/-}$ mice displayed a normal response to injected insulin at both ages compared with wild-type controls (Fig. 3) (18,19). The divergence in results may reflect differences in genetic background or testing conditions (in this study we used 1.5 units/kg insulin instead of 0.75 units/kg). Compared with wild-type mice, insulin weakly reduced blood glucose in *Irs2*$^{-/-}$ mice at 6 weeks of age and had no effect at 15 weeks (Fig. 3). Ptp1b deficiency normalized insulin sensitivity in young *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice (Fig. 3*A*); however, by 15 weeks, peripheral insulin sensitivity was diminished in *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice (Fig. 3*B*). Thus, in the absence of Irs2, deletion of *Ptp1b* increased peripheral insulin sensitivity in young mice but had minimal effects as the mice aged.

TABLE 1
Metabolic measurements of *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ intercross mice

Genotype	Fasting blood glucose (mg/dl)		Fed blood glucose (mg/dl)		Fasting serum insulin (ng/ml)		Fed serum insulin (ng/ml)	
	6 weeks	15 weeks	6 weeks	15 weeks	6 weeks	15 weeks	6 weeks	15 weeks
Wild type	74 ± 4	79 ± 4	155 ± 5	140 ± 11	70 ± 48	475 ± 160	1,671 ± 225	2,440 ± 277
Ptp1b$^{-/-}$	73 ± 4	62 ± 5	139 ± 6	140 ± 6	81 ± 57	280 ± 130	1,460 ± 308	1,956 ± 400
Irs2$^{-/-}$	169 ± 29	369 ± 68	434 ± 43	440 ± 72	631 ± 222	476 ± 101	1,373 ± 301	1,709 ± 644
Irs2$^{-/-}$::*Ptp1b*$^{-/-}$	101 ± 8	163 ± 61	185 ± 17	294 ± 82	529 ± 213	651 ± 320	2,340 ± 748	2,826 ± 1,167

Results represent the mean ± SE of 6–10 mice per genotype.

Irs2 mediates peripheral insulin action and plays an essential role in pancreatic β-cell function and survival. Without Irs2, β-cell mass decreases until the mice die between 14 and 17 weeks (5). Genetic manipulation to rescue β-cell function in *Irs2*$^{-/-}$ mice, by upregulation of *Irs2* or *Pdx1* in β-cells or by suppression of *Foxo1*, promotes islet growth, normalizes glucose tolerance, and prevents diabetes (23–25). Because *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice survive beyond 16 weeks, we investigated their insulin levels and β-cell mass. Compared with wild-type mice, fasting and fed serum insulin levels in *Ptp1b*$^{-/-}$ mice were indistinguishable at 6 and 15 weeks, consistent with similar peripheral insulin sensitivity indicated by the glucose and insulin tolerance tests, but different from previous results where *Ptp1b*$^{-/-}$ mice had decreased fed serum insulin values (18,19). This discrepancy could be due to the larger experimental study groups in our previous report or to differences in genetic background. Fasting insulin was elevated 10-fold in both *Irs2*$^{-/-}$ and *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice, indicating compensation for peripheral insulin resistance (Table 1). These results further demonstrate that loss of Ptp1b failed to normalize completely peripheral insulin sensitivity in the *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice (Table 1). Random-fed serum insulin levels were

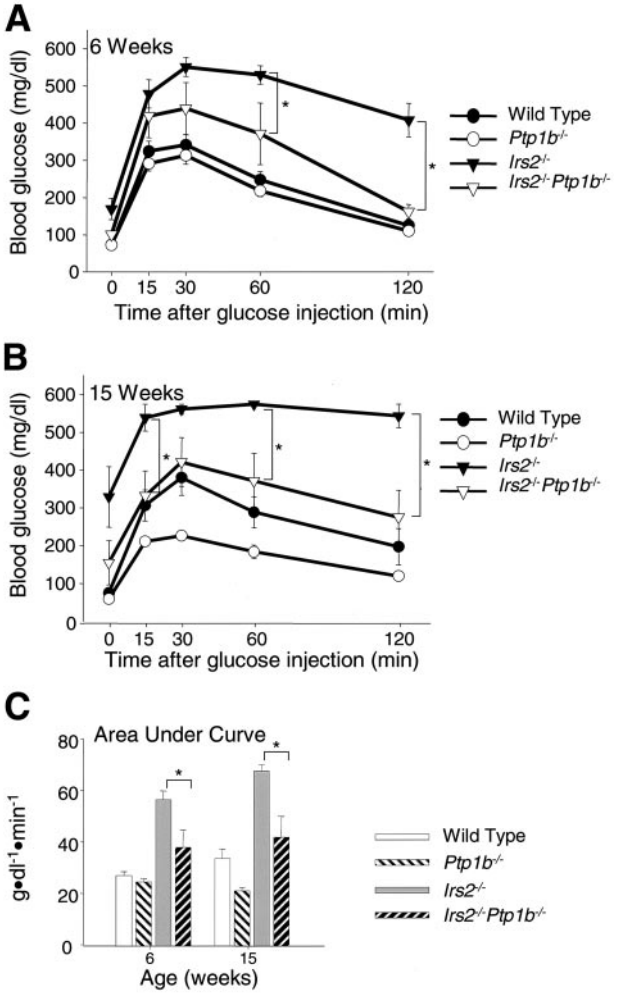

FIG. 2. Glucose tolerance tests of male *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ intercross mice. *A*: Serum blood glucose measurements of 6-week-old mice after intraperitoneal injection of 2 g D-glucose/kg. **P* < 0.05, *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ vs. *Irs2*$^{-/-}$; ***P* < 0.01, *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ vs. *Irs2*$^{-/-}$. *B*: Serum blood glucose measurements of 15-week-old mice after intraperitoneal injection of 2 g D-glucose/kg. **P* < 0.05, *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ vs. *Irs2*$^{-/-}$. *C*: AUC analysis of glucose tolerance tests performed at 6 and 15 weeks of age. **P* < 0.05, *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ vs. *Irs2*$^{-/-}$. Results are expressed as mean ± SE of g · dl^{-1} · min^{-1} for 6–10 animals per genotype.

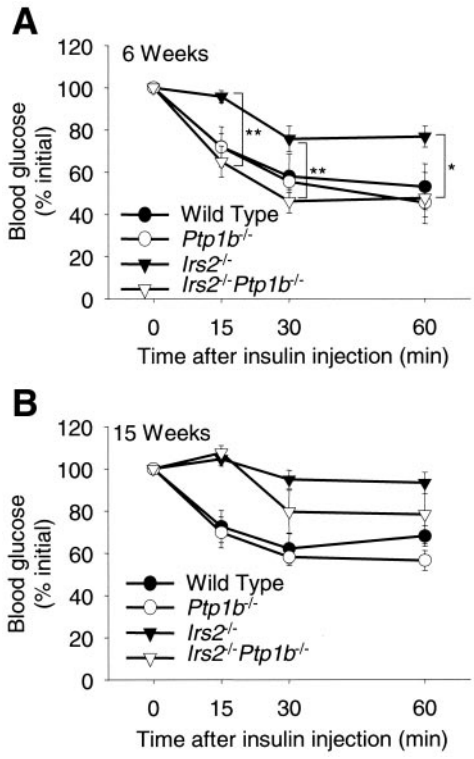

FIG. 3. Insulin tolerance tests of male *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ intercross mice. *A*: Serum blood glucose measurements of 6-week-old mice after intraperitoneal injection of 0.75 units/kg human regular insulin. **P* < 0.05, *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ vs. *Irs2*$^{-/-}$; ***P* < 0.01, *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ vs. *Irs2*$^{-/-}$. *B*: Serum blood glucose measurements of 15-week-old mice after intraperitoneal injection of 1.5 units/kg human regular insulin. Results are expressed as mean ± SE of percentage of initial blood glucose value for 6–10 animals per genotype.

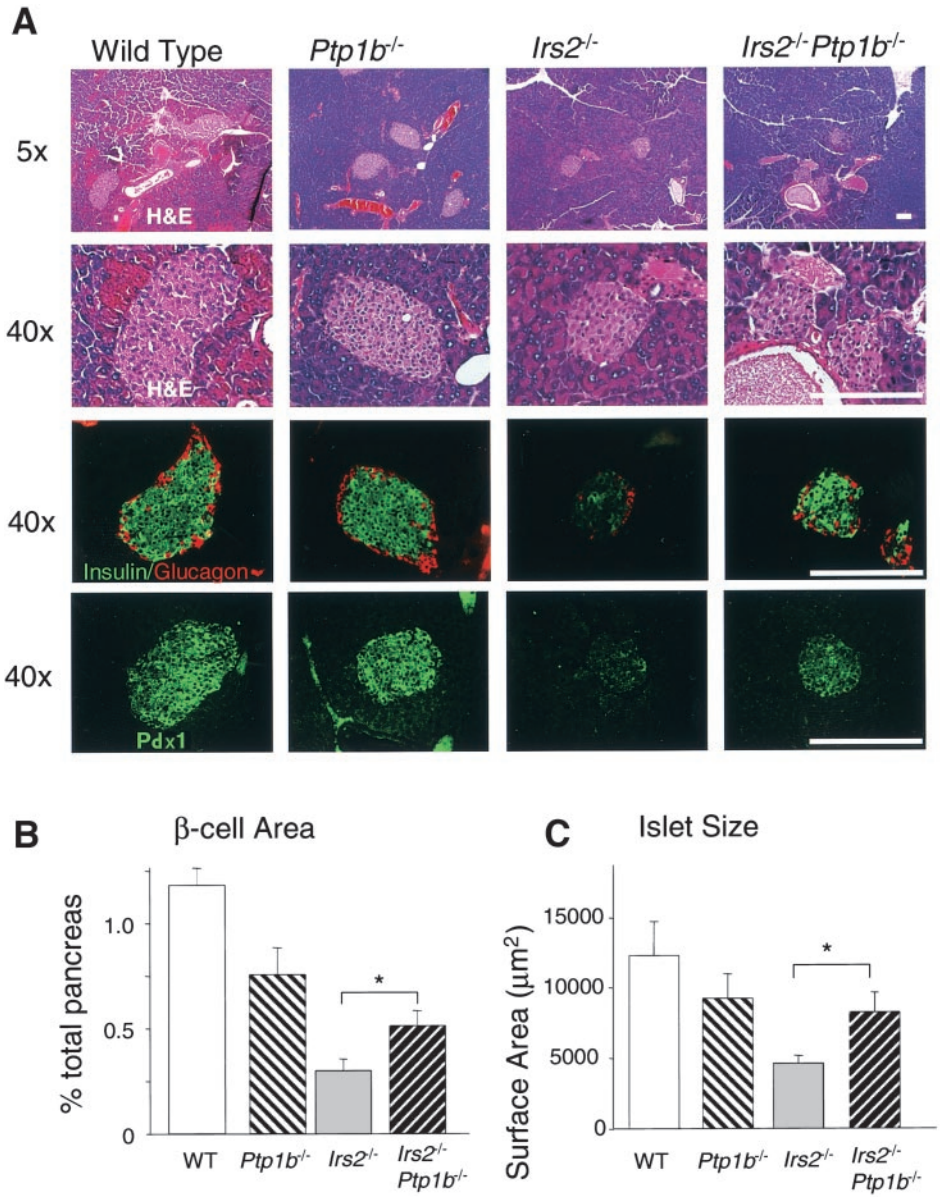

FIG. 4. Islet histology and morphometric analysis of male *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ intercross mice. *A*: Representative islet histology from pancreas sections from 15-week-old male mice. Hematoxylin and eosin (H&E) staining at ×5 (*A*, top panels) and ×40 (*A*, second from top panels). Immunostaining with antibodies against insulin (green) and glucagon (red) at ×40 (*A*, third from top panels). Immunostaining with antibodies against Pdx1 (green) at ×40 (*A*, bottom panels). Scale bars: 200 μm. *B*: Mean cross-sectional β-cell area, reported as percentage of total pancreas area. **P* < 0.05, *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ vs. *Irs2*$^{-/-}$. *C*: Islet size calculated by mean cross-sectional area of multicelled islets reported as microns^2/islet. **P* < 0.05, *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ vs. *Irs2*$^{-/-}$. Results are expressed as mean ± SE of at least six animals per genotype.

quite variable, and while the average serum insulin was higher in *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice than in *Irs2*$^{-/-}$ mice at both 6 weeks and 15 weeks, these differences did not reach statistical significance.

Next we investigated pancreatic histopathology to establish the effect of *Ptp1b* disruption on islet size at 15 weeks. Previous studies show that islet mass is inversely related to peripheral insulin sensitivity (1). We found that pancreatic cross-sectional β-cell area was reduced in *Ptp1b*$^{-/-}$ mice, consistent with their increased peripheral insulin receptor signaling from *Ptp1b* disruption (Fig. 4*B* and *C*). As previously shown, β-cells were barely detected in *Irs2*$^{-/-}$ islets, as β-cell area and islet size in pancreatic sections were decreased (even though *Irs2*$^{-/-}$ mice are severely insulin resistant) (Fig. 4). In contrast to findings in wild-type mice, *Ptp1b* deletion increased insulin and Pdx1 immunostaining as well as β-cell area and islet size in *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice. These findings suggest that in the absence of Irs2, Ptp1b deficiency improved β-cell function, possibly through increased insulin receptor or Irs1 signaling (Fig. 4). Although *Ptp1b* deletion promoted compensatory islet growth and function in *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice, islet function was still insufficient and diabetes developed at 15 weeks.

DISCUSSION

Our results illustrate that superimposed *Ptp1b* disruption in *Irs2*$^{-/-}$ mice improves glucose tolerance by partially increasing peripheral insulin sensitivity in young mice and promoting some compensatory β-cell function. These compensatory effects are insufficient to completely restore islet function, however, and diabetes eventually occurs in *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice.

Ptp1b normally inhibits insulin signaling by dephosphorylating the insulin receptor as it passes by the endoplasmic reticulum during recycling (15). Without *Ptp1b*, the rate of receptor inactivation is reduced, which increases insulin sensitivity. In this study *Ptp1b* disruption in wild-type mice fed a low-fat diet (9%) resulted in mild fasting hypoglycemia, greatly improved glucose tolerance, and slight increases in peripheral insulin sensitivity by 15

weeks of age (Figs. 1–3). In this study, we found that superimposed *Ptp1b* disruption in *Irs2*$^{-/-}$ mice almost completely normalized peripheral insulin sensitivity at 6 weeks of age (Fig. 3*A*). However, 6-week-old *Irs2*$^{-/-}$ and *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice displayed fasting serum insulin values that were equally hyperinsulinemic, revealing persistent insulin resistance that is not entirely corrected by the absence of Ptp1b (Table 1). Supporting this idea, by 15 weeks of age *Ptp1b* disruption had little effect to rescue peripheral insulin resistance of *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice (Fig. 3*B*). Thus, we conclude that *Ptp1b* disruption partially improves peripheral insulin sensitivity in *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice.

Both Irs2 and Ptp1b appear to be critical regulators of body weight. Irs2 mediates antiobesity signals that are observed in healthy female *Irs2*$^{-/-}$ mice, possibly caused by impaired hypothalamic leptin signaling (26). In contrast, Ptp1b appears to antagonize metabolic signals that promote normal body weight: *Ptp1b*$^{-/-}$ mice are resistant to obesity and can partially rescue loss of leptin signaling (16–19). Supporting previous findings, we found that *Ptp1b* disruption modestly reduced body weight in wild-type mice by 15 weeks (19). *Ptp1b* disruption had a profound effect in the *Irs2*$^{-/-}$ background, reducing body weight in 6-week-old *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice 20% below *Irs2*$^{-/-}$ mice (Fig. *1*). However, as *Irs2*$^{-/-}$ mice developed frank diabetes, this difference was lost and *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice and *Irs2*$^{-/-}$ mice had equivalent body weights (Fig. *1*). Future studies will be required to determine the reason for the greater decrease in body weights of young *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice compared with *Ptp1b*$^{-/-}$ mice.

Ptp1b deletion had differential effects on islet formation in wild-type and *Irs2*$^{-/-}$ mice. β-Cell area decreased in *Ptp1b*$^{-/-}$ mice consistent with improved glucose tolerance, fasting hypoglycemia, and slightly improved peripheral insulin sensitivity. This inverse relation between insulin sensitivity and insulin secretion is well established in rodents and humans (1). However, the relation between peripheral insulin action and islet mass is entirely disrupted in *Irs2*$^{-/-}$ mice because β-cell area decreases as peripheral insulin sensitivity decreases. Relative to *Irs2*$^{-/-}$ mice, peripheral insulin action improves in the *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice and β-cell area increased as the islet-trophic signals are partially restored. However, this compensatory β-cell function is eventually insufficient to meet insulin requirements, and diabetes occurs in *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice.

Although *Irs2*$^{-/-}$ mice are severely resistant to insulin, several genetic maniupulations can prevent diabetes in *Irs2*$^{-/-}$ mice by promoting islet expansion and restoring β-cell function. Transgenic expression of Irs2 itself in pancreatic and β-cells prevents diabetes in *Irs2*$^{-/-}$ mice (25). Furthermore, changes that mimic the effects of Irs2 signaling restore β-cell function in *Irs2*$^{-/-}$ mice. For example, genetic downregulation of the forkhead transcription factor Foxo1 restores β-cell function and prevents diabetes in *Irs2*$^{-/-}$ mice (24), as does upregulation of *Pdx1* (23). These results also suggest that β-cell failure, and not peripheral insulin resistance, is the major cause of diabetes in *Irs2*$^{-/-}$ mice. Indeed, we found that islet Pdx1 immunostaining was increased in pancreatic sections from *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice compared with *Irs2*$^{-/-}$ mice. Genetic background also appears to play an important role to modify islet function in *Irs2*$^{-/-}$ mice as *Irs2*$^{-/-}$ mice on different genetic backgrounds with higher basal Pdx1 expression (CBA and C57BL/6) have milder defects in islet function (27,28).

Previous work suggests that the type 1 IGF receptor (IGF1R) plays an important role in β-cell growth and survival; however, the exact location where IGF1R action is required is not clear because β-cell–specific *Igf1r* disruption does not reduce β-cell mass (29). In contrast, β-cell–specific disruption of the insulin receptor results in decreased β-cell mass as the mice age (30). Although Ptp1b has never been demonstrated to be expressed in β-cells, our RT-PCR analysis suggests that *Ptp1b* mRNA is expressed in isolated islets (data not shown). The disruption of *Ptp1b* could therefore increase insulin receptor and/or Igf1 receptor islet signaling, although β-cell expansion via these pathways is still not sufficient to fully restore β-cell mass of *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice. In the absence of Irs2, Irs1 signals presumably would be used to mediate insulin receptor signaling. Previous experiments show that Irs1 promotes partial β-cell function in the *Irs2*$^{-/-}$ mice, but it is not sufficient for long-term β-cell function (7). We cannot exclude the possibility that in the absence of Ptp1b, other Irs proteins in islets may also contribute to β-cell survival or proliferation. Thus, glucose tolerance is improved in the *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice and diabetes is delayed relative to *Irs2*$^{-/-}$ mice, but compensatory β-cell expansion is not sufficient to completely prevent diabetes. Nevertheless, in human patients with type 2 diabetes PTP1b might be expected to promote both peripheral insulin sensitivity and β-cell function, as the disease does not result from a complete lack of IRS2 (31).

Even though *Ptp1b*$^{-/-}$ mice display improved glucose tolerance, they do not develop tumors. In contrast, *PTEN*$^{+/-}$ mice develop tumors from hyperactivity of the PI 3-kinase cascade (32,33). The different outcome might be related to the specificity of Ptp1b to act upon the insulin receptor and not the IGF1R receptor in the whole organism context. Supporting this idea, *Ptp1b*$^{-/-}$ mice do not display an overgrowth phenotype. Notably, however, in fibroblasts Ptp1b may downregulate IGF1R signaling (34). Indeed, some distinct developmental roles of Irs2 that might be mediated by the IGF1R are not restored in *Irs2*$^{-/-}$::*Ptp1b*$^{-/-}$ mice, including ovarian function and female fertility (data not shown). To further test the role of Ptp1b in IGF→Irs protein–related somatic growth signaling, we have also created *Irs1* and *Ptp1b* double knockout mice. Preliminary data suggest that Ptp1b deficiency does not modify the growth phenotype of *Irs1* knockout mice: *Irs1*$^{-/-}$::*Ptp1b*$^{-/-}$ mice were ~60% normal size at various ages, similar in size to *Irs1*$^{-/-}$ mice (data not shown). These data support the hypothesis that Ptp1b has tissue-specific roles to dephosphorylate and downregulate tyrosine kinase–dependent signaling in vivo and that *Ptp1b* disruption does not promote general somatic growth. This finding diminishes concerns that Ptp1b inhibitors might promote tumor growth. However, an alternative explanation could be that enhanced Irs2 signaling cannot completely compensate for Irs1 deficiency. The specificity of PTP1b toward the insulin receptor and the lack of onco-

genic effects from *Ptp1b* disruption in vivo provide further evidence that pharmacological PTP1b antagonists could be safe and effective diabetes treatments, if residual IRS2-like signals persist in β-cells of human patients with type 2 diabetes.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health Grants to M.F.W. (DK55326, DK43808, and DK38712), to B.G.N. (CA 49152 and DK60838), and to B.B.K. (DK60839) and by Howard Hughes Medical Institute (HHMI) funds to M.F.W. J.A.K. was supported by a Lawson Wilkins Pediatric Endocrine Society Fellowship, a National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) training grant, a Juvenile Diabetes Research Foundation Fellowship, and a Charles H. Hood Foundation Child Health Research Grant.

REFERENCES

1. Kahn SE: The relative contributions of insulin resistance and β-cell dysfunction to the pathophysiology of type 2 diabetes. *Diabetologia* 46:3–19, 2003
2. White MF: IRS proteins and the common path to diabetes. *Am J Physiol Endocrinol Metab* 283:E413–E422, 2002
3. White MF, Yenush L: The IRS-signaling system: a network of docking proteins that mediate insulin and cytokine action. *Curr Top Microbiol Immunol* 228:179–208, 1998
4. Tamemoto H, Kadowaki T, Tobe K, Yagi T, Sakura H, Hayakawa T, Terauchi Y, Ueki K, Kaburagi Y, Satoh S, Sekihara H, Yoshioka S, Horikoshi H, Furuta Y, Ikawa Y, Kasuga M, Yazaki Y, Aizawa S: Insulin resistance and growth retardation in mice lacking insulin receptor substrate-1. *Nature* 372:182–186, 1994
5. Withers DJ, Gutierrez JS, Towery H, Burks DJ, Ren JM, Previs S, Zhang Y, Bernal D, Pons S, Shulman GI, Bonner-Weir S, White MF: Disruption of IRS-2 causes type 2 diabetes in mice. *Nature* 391:900–904, 1998
6. Araki E, Lipes MA, Patti ME, Bruning JC, Haag B III, Johnson RS, Kahn CR: Alternative pathway of insulin signalling in mice with targeted disruption of the IRS-1 gene. *Nature* 372:186–190, 1994
7. Withers DJ, Burks DJ, Towery HH, Altamuro SL, Flint CL, White MF: Irs-2 coordinates Igf-1 receptor-mediated beta-cell development and peripheral insulin signalling. *Nat Genet* 23:32–40, 1999
8. Tonks NK, Neel BG: Combinatorial control of the specificity of protein tyrosine phosphatases. *Curr Opin Cell Biol* 13:182–195, 2001
9. Ahmad F, Li PM, Meyerovitch J, Goldstein BJ: Osmotic loading of neutralizing antibodies demonstrates a role for protein-tyrosine phosphatase 1B in negative regulation of the insulin action pathway. *J Biol Chem* 270:20503–20508, 1995
10. Kenner KA, Anyanwu E, Olefsky JM, Kusari J: Protein-tyrosine phosphatase 1B is a negative regulator of insulin- and insulin-like growth factor-I-stimulated signaling. *J Biol Chem* 271:19810–19816, 1996
11. Dadke S, Kusari J, Chernoff J: Down-regulation of insulin signaling by protein-tyrosine phosphatase 1B is mediated by an N-terminal binding region. *J Biol Chem* 275:23642–23647, 2000
12. Goldstein BJ, Ahmad F, Ding W, Li PM, Zhang WR: Regulation of the insulin signalling pathway by cellular protein-tyrosine phosphatases. *Mol Cell Biochem* 182:91–99, 1998
13. Salmeen A, Andersen JN, Myers MP, Tonks NK, Barford D: Molecular basis for the dephosphorylation of the activation segment of the insulin receptor by protein tyrosine phosphatase 1B. *Mol Cell* 6:1401–1412, 2000
14. Frangioni JV, Beahm PH, Shifrin V, Jost CA, Neel BG: The nontransmembrane tyrosine phosphatase PTP-1B localizes to the enodpasmic reticulum via its 35 amino acid c-terminal sequence. *Cell* 68:545–560, 1992
15. Haj FG, Verveer PJ, Squire A, Neel BG, Bastiaens PI: Imaging sites of receptor dephosphorylation by PTP1B on the surface of the endoplasmic reticulum. *Science* 295:1708–1711, 2002
16. Zabolotny JM, Bence-Hanulec KK, Stricker-Krongrad A, Haj F, Wang Y, Minokoshi Y, Kim YB, Elmquist JK, Tartaglia LA, Kahn BB, Neel BG: PTP1B regulates leptin signal transduction in vivo. *Dev Cell* 2:489–495, 2002
17. Cheng A, Uetani N, Simoncic PD, Chaubey VP, Lee-Loy A, McGlade CJ, Kennedy BP, Tremblay ML: Attenuation of leptin action and regulation of obesity by protein tyrosine phosphatase 1B. *Dev Cell* 2:497–503, 2002
18. Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, Loy AL, Normandin D, Cheng A, Himms-Hagen J, Chan CC, Ramachandran C, Gresser MJ, Tremblay ML, Kennedy BP: Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. *Science* 283:1544–1548, 1999
19. Klaman LD, Boss O, Peroni OD, Kim JK, Martino JL, Zabolotny JM, Moghal N, Lubkin M, Kim YB, Sharpe AH, Stricker-Krongrad A, Shulman GI, Neel BG, Kahn BB: Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. *Mol Cell Biol* 20:5479–5489, 2000
20. Zinker BA, Rondinone CM, Trevillyan JM, Gum RJ, Clampit JE, Waring JF, Xie N, Wilcox D, Jacobson P, Frost L, Kroeger PE, Reilly RM, Koterski S, Opgenorth TJ, Ulrich RG, Crosby S, Butler M, Murray SF, McKay RA, Bhanot S, Monia BP, Jirousek MR: PTP1B antisense oligonucleotide lowers PTP1B protein, normalizes blood glucose, and improves insulin sensitivity in diabetic mice. *Proc Natl Acad Sci U S A* 99:11357–11362, 2002
21. Rondinone CM, Trevillyan JM, Clampit J, Gum RJ, Berg C, Kroeger P, Frost L, Zinker BA, Reilly R, Ulrich R, Butler M, Monia BP, Jirousek MR, Waring JF: Protein tyrosine phosphatase 1B reduction regulates adiposity and expression of genes involved in lipogenesis. *Diabetes* 51:2405–2411, 2002
22. Gum RJ, Gaede LL, Koterski SL, Heindel M, Clampit JE, Zinker BA, Trevillyan JM, Ulrich RG, Jirousek MR, Rondinone CM: Reduction of protein tyrosine phosphatase 1B increases insulin-dependent signaling in *ob/ob* mice. *Diabetes* 52:21–28, 2003
23. Kushner JA, Ye J, Schubert M, Burks DJ, Dow MA, Flint CL, Dutta S, Wright CV, Montminy MR, White MF: Pdx1 restores beta cell function in Irs2 knockout mice. *J Clin Invest* 109:1193–1201, 2002
24. Kitamura T, Nakae J, Kitamura Y, Kido Y, Biggs WH, III, Wright CV, White MF, Arden KC, Accili D: The forkhead transcription factor Foxo1 links insulin signaling to Pdx1 regulation of pancreatic beta cell growth. *J Clin Invest* 110:1839–1847, 2002
25. Hennige AM, Burks DJ, Ozcan U, Kulkarni RN, Ye J, Park S, Schubert M, Fisher TL, Dow MA, Leshan R, White MF: Up regulation of Irs2 in pancreatic β-cells prevents diabetes. *J Clin Invest* 112:1521–1532, 2003
26. Burks DJ, de Mora JF, Schubert M, Withers DJ, Myers MG, Towery HH, Altamuro SL, Flint CL, White MF: IRS-2 pathways integrate female reproduction and energy homeostasis. *Nature* 407:377–382, 2000
27. Kubota N, Tobe K, Terauchi Y, Eto K, Yamauchi T, Suzuki R, Tsubamoto Y, Komeda K, Nakano R, Miki H, Satoh S, Sekihara H, Sciacchitano S, Lesniak M, Aizawa S, Nagai R, Kimura S, Akanuma Y, Taylor SI, Kadowaki T: Disruption of insulin receptor substrate 2 causes type 2 diabetes because of liver insulin resistance and lack of compensatory β-cell hyperplasia. *Diabetes* 49:1880–1889, 2000
28. Terauchi Y, Matsui J, Suzuki R, Kubota N, Komeda K, Aizawa S, Eto K, Kimura S, Nagai R, Tobe K, Lienhard GE, Kadowaki T: Impact of genetic background and ablation of insulin receptor substrate (IRS)-3 on IRS-2 knock-out mice. *J Biol Chem* 278:14284–14290, 2003
29. Xuan S, Kitamura T, Nakae J, Politi K, Kido Y, Fisher PE, Morroni M, Cinti S, White MF, Herrera PL, Accili D, Efstratiadis A: Defective insulin secretion in pancreatic beta cells lacking type 1 IGF receptor. *J Clin Invest* 110:1011–1019, 2002
30. Kulkarni RN, Bruning JC, Winnay JN, Postic C, Magnuson MA, Kahn CR: Tissue-specific knockout of the insulin receptor in pancreatic β cells creates an insulin secretory defect similar to that in type 2 diabetes. *Cell* 96:329–339, 1999
31. Kalidas K, Wasson J, Glaser B, Meyer JM, Duprat LJ, White MF, Permutt MA: Mapping of the human insulin receptor substrate-2 gene, identification of a linked polymorphic marker and linkage analysis in families with type 2 diabetes: no evidence for a major susceptibility role. *Diabetologia* 41:1389–1391, 1998
32. Di Cristofano A, Pesce B, Cordon-Cardo C, Pandolfi PP: Pten is essential for embryonic development and tumour suppression. *Nat Genet* 19:348–355, 1998
33. Podsypanina K, Ellenson LH, Nemes A, Gu J, Tamura M, Yamada KM, Cordon-Cardo C, Catoretti G, Fisher PE, Parsons R: Mutation of Pten/Mmac1 in mice causes neoplasia in multiple organ systems. *Proc Natl Acad Sci U S A* 96:1563–1568, 1999
34. Buckley DA, Cheng A, Kiely PA, Tremblay ML, O'Connor R: Regulation of insulin-like growth factor type I (IGF-I) receptor kinase activity by protein tyrosine phosphatase 1B (PTP-1B) and enhanced IGF-I-mediated suppression of apoptosis and motility in PTP-1B-deficient fibroblasts. *Mol Cell Biol* 22:1998–2010, 2002