

The peroxisome proliferator–activated receptor β/δ agonist GW0742 has direct protective effects on right heart hypertrophy

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Abstract: Pulmonary hypertension is a debilitating disease with no cure. We have previously shown that peroxisome proliferator–activated receptor (PPAR) β/δ agonists protect the right heart in hypoxia-driven pulmonary hypertension without affecting vascular remodeling. PPAR β/δ is an important receptor in lipid metabolism, athletic performance, and the sensing of prostacyclin. Treatment of right heart hypertrophy and failure in pulmonary hypertension is an emerging target for future therapy. Here we have investigated the potential of GW0742, a PPAR β agonist, to act directly on the right heart in vivo and what transcriptomic signatures are associated with its actions. Right heart hypertrophy and failure was induced in mice using a pulmonary artery banding (PAB) model. GW0742 was administered throughout the study. Cardiovascular parameters were measured using echocardiography and pressure monitoring. Fibrosis and cellular changes were measured using immunohistochemistry. Transcriptomics were measured using the Illumina MouseRef-8v3 BeadChip array and analyzed using GeneSpring GX (ver. 11.0). PAB resulted in right heart hypertrophy and failure and in increased fibrosis. GW0742 reduced or prevented the effects of PAB on all parameters measured. GW0742 altered a number of genes in the transcriptome, with *Angptl4* emerging as the top gene altered (increased) in animals with PAB. In conclusion, the PPAR β/δ agonist GW0742 has direct protective effects on the right heart in vivo. These observations identify PPAR β/δ as a viable therapeutic target to treat pulmonary hypertension that may complement current and future vasodilator drugs.

Keywords: pulmonary hypertension, heart failure, peroxisome proliferator–activated receptor (PPAR) β , right ventricular function, angiotensin-like 4.

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INTRODUCTION

Pulmonary hypertension is a rare but devastating disease in which the normally low pulmonary artery pressure is elevated due to vasoconstriction and remodeling of pulmonary vessels. Increased pulmonary artery pressure results in increased workload on the right side of the heart, causing right heart hypertrophy, fibrosis, and ultimately heart failure. Drugs used to manage pulmonary hypertension are traditionally designed to target the vasculature and enhance vasodilator and antiproliferation pathways, including nitric oxide/cyclic guanosine monophosphate (with phosphodiesterase type 5 inhibitors) and prostacyclin (with prostacyclin and related mimetics), and/or inhibit vasoconstrictor pathways, such as endothelin 1 (with receptor antagonists).^{1,2} However, it is increasingly recognized that,

in addition to the pulmonary vasculature, the right heart is a viable therapeutic target in the treatment of pulmonary hypertension.^{3,4}

We have recently shown, using a hypoxia model of pulmonary hypertension in rats, that GW0742, a ligand for peroxisome proliferator–activated receptor (PPAR) β/δ ,⁵ reduces right heart hypertrophy without influencing pulmonary vascular remodeling.⁶ This was a somewhat unexpected finding, since we and others found GW0742 to relax pulmonary vessels⁶ or vascular cells⁷ and to inhibit lung cell proliferation in vitro.⁸ Despite this, however, the in vivo data suggested that GW0742 might have a direct protective action on the right heart in this model.⁶ PPAR β/δ is expressed in the heart, where it regulates metabolism⁹

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and protects against ischemia reperfusion injury.^{10,11} In addition, a recent study by Liu et al.¹² used a transverse aorta constriction model and showed that left ventricular dilation, fibrosis, and mitochondrial abnormalities were reduced in transgenic mice in which constitutively active PPAR β/δ was expressed. This suggests that PPAR β/δ is an attractive protective pathway in the overloaded heart independent of any effects on blood vessels. Drugs that activate PPAR β/δ also enhance physical endurance when given with exercise by a mechanism involving enhanced metabolism in muscle via the adenosine monophosphate (AMP) kinase/PPAR γ coactivator 1 α pathway.¹³ Importantly, orally active drugs that activate PPAR β/δ have been developed and tested in humans.

In the current study, we have extended our earlier observations to investigate directly the effect of GW0742 on right heart remodeling and the transcriptome in a model that mimics the increased workload to the right side of the heart seen in pulmonary hypertension.¹⁴

METHODS

Experiments were performed according to institutional guidelines that comply with national and international regulations. The local authorities for animal research approved the study protocol.

Animals

Male C57BL/6 mice (Charles River, Sulzfeld, Germany) were used. Baseline cardiovascular characteristics were not different between groups (Table 1).

Pulmonary artery banding (PAB)

Briefly, animals were anesthetized with isoflurane (3%), intubated, and mechanically ventilated. Pulmonary arteries were constricted with a small titanium ligating clip

(Hemoclip; Edward Weck, Research Triangle Park, NC) with a width of 0.35 mm, after which the chest was closed and the animal was allowed to recover from anaesthesia. Control mice were subjected to sham operations with either drug or placebo (vehicle) treatments.

Experimental protocol

Seven days after surgery, mice were randomized (10 mice per group) to the following groups: group 1, sham-operated mice plus placebo; group 2, sham + GW0742 at 30 mg/kg/d; group 3, PAB plus placebo; group 4, PAB plus GW0742 at 10 mg/kg/d; group 5, PAB plus GW0742 at 30 mg/kg/d. Treatments (oral gavage) were performed for 14 days until the day of the terminal hemodynamic measurements on day 21.

Echocardiography

Echocardiography was performed before and 14 days after treatment using the Vevo770 high-resolution imaging system equipped by 30-MHz transducer (VisualSonics, Toronto). Anesthesia was induced with 3% and maintained with 1.0%–1.5% isoflurane in room air supplemented with 100% O₂. Cardiac output (CO), tricuspid annular plane systolic excursion (TAPSE), and myocardial performance index (MPI) were measured as described elsewhere.¹⁵ All measurements were performed by an experienced sonographer who was blinded to the results of invasive and morphometric studies.

Hemodynamic and right ventricle (RV) hypertrophy measurements

RV systolic pressure (RVSP) was measured by a catheter inserted into the RV via the right jugular vein; for systemic arterial pressure (SAP), left carotid artery catheterization was performed as described elsewhere.¹⁶ The heart was dissected to separate RV from left ventricle plus septum

Table 1. Baseline echocardiographic characteristics of the studied groups

	Sham + placebo	Sham + PPAR 30 mg/kg	PAB + placebo	PAB + PPAR 10 mg/kg	PAB + PPAR 30 mg/kg
BW, g	24.3 ± 0.4	23.9 ± 0.2	23.0 ± 0.1	23.3 ± 0.2	23.9 ± 0.1
CI, mL/min/BW	0.78 ± 0.01	0.80 ± 0.02	0.61 ± 0.09*	0.60 ± 0.03*	0.59 ± 0.02*
TAPSE, mm	1.59 ± 0.02	1.60 ± 0.03	0.98 ± 0.01*	1.01 ± 0.02*	0.99 ± 0.04*
RV MPI (Tei)	0.72 ± 0.05	0.73 ± 0.04	1.38 ± 0.07*	1.39 ± 0.04*	1.41 ± 0.07*

Note: Data are mean ± SEM. PPAR: peroxisome proliferator-activated receptor; PAB: pulmonary artery banding; BW: body weight; CI: cardiac index; TAPSE: tricuspid annular plane systolic excursion; RV: right ventricle; MPI: myocardial performance index.

* $P < 0.05$ vs. sham-operated mice.

(LV + S), and the ratio RV/(LV + S) was calculated as a measurement for RV hypertrophy.

RV cardiomyocyte size, fibrosis, and vascularization assays

RV cardiomyocyte size, fibrosis, and vascularization were performed as described elsewhere.^{15,17} Briefly, myocardium was fixed in 4% formalin and stained using picosirius red (Sirius Red F3B; Niepoetter, Bürstadt, Germany) for collagen fractions. For cardiomyocyte size determination, transverse sections of RV were stained with fluorescein isothiocyanate (FITC)-conjugated wheat germ agglutinin (WGA-FITC; Sigma-Aldrich, St. Louis, MO) mounted with dako mounting medium. Sections without WGA-FITC were used as a negative control. To quantify the capillaries, transverse cryosections of the RV were stained with rat anti-platelet endothelial cell adhesion molecule (Santa Cruz Biotechnology, Heidelberg, Germany) and rabbit anti-dystrophin (Santa Cruz Biotechnology). Alexa Fluor 555-conjugated anti-rat (Cell Signaling Technology, Beverly, MA) and FITC-conjugated anti-rabbit (Invitrogen, Carlsbad, CA) secondary antibodies were used for fluorescent detection. Nuclei were stained with 4',6-diamidino-2-phenylindole (Invitrogen) and mounted with Dako (Glostrup, Denmark) mounting medium.

Microarray analysis

RNA was extracted from whole right hearts of mice and converted, and samples were hybridized onto probe sets on an Illumina MouseRef-8v3 BeadChip array, as described elsewhere.¹⁸ Data were analyzed using the syntax package R (ver. 2.3.1) with Bioconductor and limma.¹⁴ Data were calculated using a moderated *t* test followed by Benjamini-Hochberg false discovery rate correction and Ingenuity Pathway Analysis software, and selected associations are shown. Transcriptomic changes in the heart associated with PAB have been reported elsewhere.¹⁴ As shown previously,¹⁴ PAB in this study induced significant changes in numerous genes in the heart; transcriptomic analysis of genes in this model have been reported in detail in other studies and are not the direct subject of this report. However, the data obtained have been included for the readers' information in Tables S5–S8; Tables S1–S8 are available online.

Data analysis

Data are mean ± SEM. Differences were considered statistically significant at $P < 0.05$, determined using analysis of variance and the Student-Newman-Keuls post hoc test for multiple comparisons between studied groups.

RESULTS

Effect of the PPAR β/δ agonist GW0742 on cardiovascular parameters induced by PAB

To investigate the effect of GW0742 on RV hypertrophy and function, we used a mouse model of physical pulmonary artery constriction that leads to increased resistance followed by RV hypertrophy, fibrosis, and RV failure. This model allows elucidation of the effects of treatment on the right heart independently of the pulmonary vasculature. We tested the potential for the PPAR β/δ agonist GW0742 to improve RV function at two doses, 10 and 30 mg/kg. These doses were recommended by the supplier, Glaxo-SmithKline, and are consistent with other studies using the compound in vivo.^{6,19}

Noninvasive evaluation of RV function demonstrated that 7 days after PAB mice developed RV dysfunction and failure (Table 1), which progressively worsened from day 7 to day 21. Specifically, PAB increased RVSP and RV mass (RV/LV+S), impaired RV MPI, and decreased cardiac index (CI) and TAPSE. PAB had no effect on SAP (Fig. 1B).

Treatment with GW0742 at a dose of 30 mg/kg had no effect on any parameter measured in sham-operated animals. GW0742 (10 or 30 mg/kg) similarly had no effect on increased RVSP after PAB (Fig. 1A). By contrast, GW0742 (30 mg/kg) significantly decreased RV hypertrophy and improved CI, TAPSE, and RV MPI in PAB mice compared with placebo-treated animals (Fig. 1D–1F). GW0742 (10 mg/kg) significantly prevented further worsening of RV function in PAB mice compared with PAB mice treated with placebo (Fig. 1D–1F). The improvement in MPI in response to GW0742 treatment was associated with reduction in the isovolumic relaxation time with a slight increment in ET interval. Additionally, we found that treatment with GW0742 increased Sm peak velocity, indicating improvement in systolic function of the RV (data not shown).

Effect of GW0742 on fibrosis and vascularization in the RV induced by PAB

Right heart hypertrophy and dysfunction induced by PAB is accompanied by fibrosis and associated with increased levels of collagen, increase in cardiomyocyte size, and reduction in vascularization (number of capillaries; Fig. 2). GW0742 (30 mg/kg) had no effect on collagen, cardiomyocyte size, or capillary density in the RV of sham-operated animals (Fig. 2). However, the protective effects of GW0742 seen on ventricular mass and function were recapitulated at the cellular level in the heart. Increased collagen deposition and cardiomyocyte size were dose-dependently reduced by GW0742 at 10 and 30 mg/kg (Fig. 2A–2C). Vasculariza-

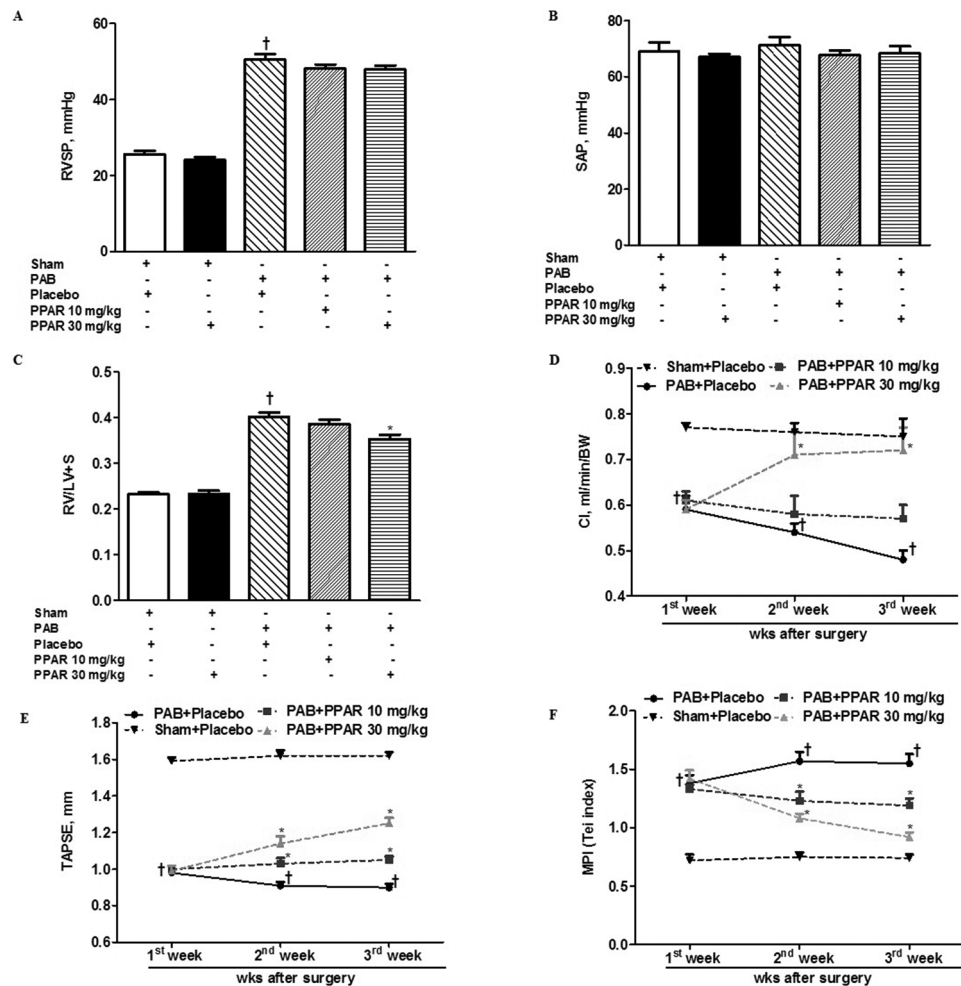


Figure 1. Effects of GW0742 on right heart mass and performance. **A**, Right ventricular systolic pressure (RVSP). **B**, Systemic arterial pressure (SAP). **C**, Ratio of right ventricle (RV) mass to left ventricle and septum (RV/LV+S). **D**, Cardiac index (CI). **E**, Tricuspid annular plane systolic excursion (TAPSE). **F**, RV myocardial performance (or Tei) index. Data are mean \pm SEM. Asterisks indicate $P < 0.05$ versus pulmonary artery banding (PAB) + placebo; daggers indicate $P < 0.05$ for PAB + placebo versus sham + placebo. BW: body weight.

tion was also significantly increased by GW0742 (10 and 30 mg/kg) in a dose-dependent manner (Fig. 2D).

Effect of GW0742 on the transcriptome in sham-operated animals and in animals with right heart failure induced by PAB

PPAR β/δ agonists such as GW0742 are known to cause many or most of their actions through modulating induction of genes.²⁰ In sham-operated animals, treatment with GW0742 induced significant changes (increase or decrease) in 59 genes, with 36 being increased and 23 being decreased. Using a cutoff of genes altered ≥ 1.5 -fold resulted in a list of 20 genes being significantly changed in right heart tissue of sham-operated mice treated with GW0742; 14 genes were increased, and 6 were decreased. Importantly, however, the PPAR β/δ target gene, *Angptl4*, was increased by ≥ 3 -fold in the heart tissue of sham-operated mice treated with GW0742 (Table 2). Pathway analysis of the 59 genes altered by GW0742 in sham-operated mice revealed associations with the following network func-

tions: cell cycle and development/death, amino acid metabolism, and developmental/metabolic disorders (Table 3). These network functions were linked with the following biofunctions: cancer, cell cycle, and connective tissue development and function (Table S1). In line with this, canonical pathways identified in the hearts of sham-operated mice that received GW0742 included the cell cycle, ubiquinone biosynthesis, inositol metabolism, mitochondrial dysfunction, and oxidative phosphorylation (Table S2). In PAB mice, GW0742 treatment resulted in 70 genes being significantly altered, compared with PAB mice treated with placebo. Again taking a cutoff of ≥ 1.5 -fold, GW0742 altered 7 genes in PAB mice (Table 4). Importantly, again, as in sham-operated animals, the target gene *Angptl4* was increased by GW0742 in the right heart tissue of the PAB animals. In fact, *Angptl4* was ranked number one in the list of altered genes with a >3 -fold increase in GW0742- versus vehicle-treated animals. Using the full list of 70 altered genes in PAB mice treated with GW0742, pathway analysis revealed (as was seen in the sham-operated group)

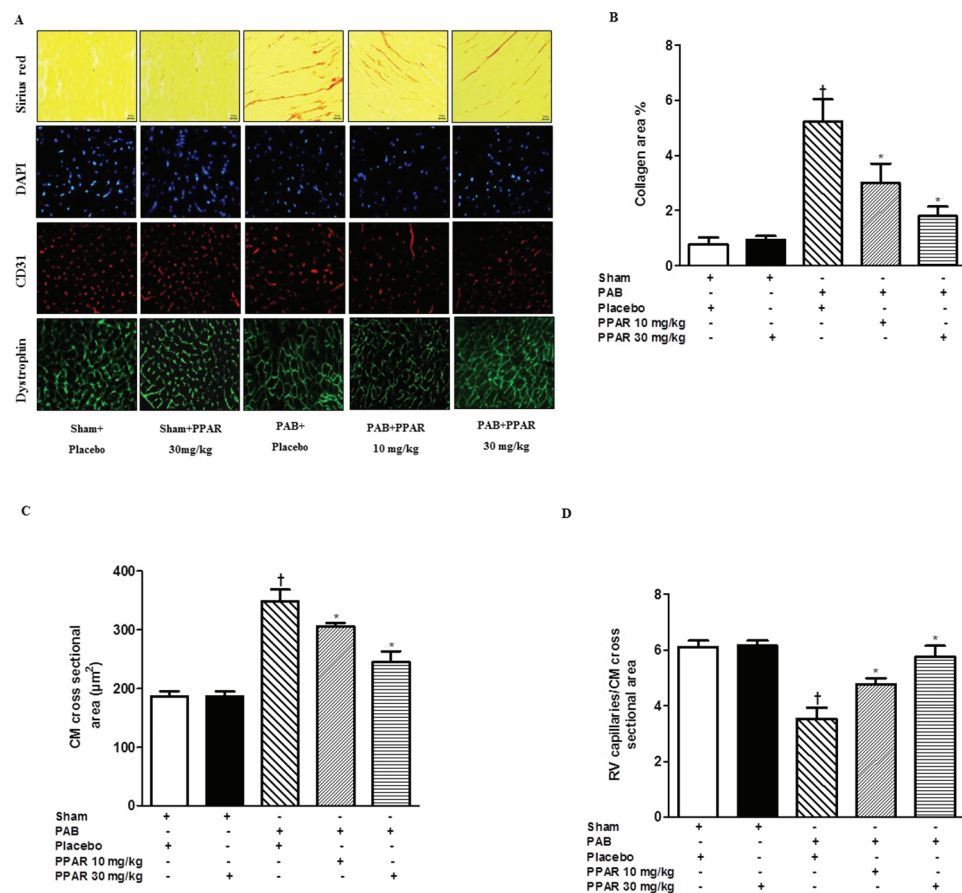


Figure 2. Effects of GW0742 on right ventricle (RV) fibrosis and vascularization. *A*, Representative images of right ventricular staining. *B*, Right ventricular collagen contents (collagen area). *C*, Right ventricular cardiomyocyte (CM) cross-sectional area. *D*, Right ventricular vascularization of the different groups. Data are mean \pm SEM. Asterisks indicate $P < 0.05$ versus pulmonary artery banding (PAB) + placebo; daggers indicate $P < 0.05$ for PAB + placebo versus sham + placebo.

associations with cellular development and cell death network functions (Table 5). Biofunctions associated with GW0742 treatment in this group included cardiovascular disease (Table S3). Four of the canonical pathways identified as being linked to gene alteration by GW0742 treatment in sham-operated animals were also linked in animals treated with GW0742 in the PAB model: inositol metabolism, ubiquinone biosynthesis, mitochondrial dysfunction, and oxidative phosphorylation (Table S4).

DISCUSSION

Pulmonary hypertension is a rare but debilitating disease with no known cure. As the disease progresses, the additional workload on the right heart causes hypertrophy, fibrosis, and failure. As such, the right heart has now become a valid therapeutic target in the treatment of pulmonary hypertension. We have previously shown that the PPAR β/δ agonist GW0742 inhibits right heart hypertrophy in a hypoxia-driven model of pulmonary hypertension and speculated that this may be due to actions directly on the heart.⁶ In the current study, we have addressed this possibility directly and used a PAB model to induce changes in the right heart consistent with those seen in

pulmonary hypertension. Using this model, we have demonstrated that the PPAR β/δ agonist GW0742 reduces hypertrophy and fibrosis. Analysis of the entire mouse transcriptome in the right heart tissue of animals with PAB treated with GW0742 revealed the PPAR β/δ target, *Angptl4*, as the top gene induced. These data support the idea that PPAR β/δ is a therapeutic target in the treatment of pulmonary hypertension and indicate that GW0742 and similar drugs are the first therapies to directly target right heart failure in pulmonary hypertension.

GW0742 is a highly selective PPAR β/δ agonist⁵ shown to be active in a range of cell types, including lung fibroblasts,⁸ platelets,²¹ and—relevant to this study—cardiomyocytes.²² In vivo, GW0742 has been shown to protect against atherosclerosis,²³ colon cancer,²⁴ ischemia reperfusion injury in the heart,^{11,25} acute lung injury,^{26,27} and septic shock.²⁷ In addition, there is mounting evidence that PPAR β/δ activated by other means plays an important role in the heart. Of direct relevance to this study, others have shown that PPAR β/δ activation in adult hearts facilitates mitochondrial function and improves cardiac performance under pressure-overload conditions.¹² In our study, we found that GW0742 reduced right heart hypertrophy and fibrosis. These obser-

Table 2. Transcripts altered ≥ 1.5 -fold in sham-operated C57BL/6 mice that were treated with GW0742

Symbol	Probe ID	Accession no.	Definition	FC	P
<i>Egr2</i>	ILMN_2623983	NM_010118.1	Early growth response 2	4.18	1.52×10^{-5}
<i>Errfi1</i>	ILMN_2714031	NM_133753.1	ERBB receptor feedback inhibitor 1	3.38	5.29×10^{-5}
<i>Angptl4</i>	ILMN_2759365	NM_020581.1	Angiopoietin-like 4	3.22	6.75×10^{-4}
<i>Erg1</i>	ILMN_2662926	NM_007913.5	Early growth response 1	2.71	1.03×10^{-6}
<i>Slc41a3</i>	ILMN_3154553	NM_001037493.1	Solute carrier family 41, member 3 (Slc41a3), transcript variant 2	2.42	2.06×10^{-2}
<i>Fos</i>	ILMN_2750515	NM_010234.2	FBJ osteosarcoma oncogene	2.23	1.49×10^{-3}
<i>Junb</i>	ILMN_1220034	NM_008416.1	Jun-B oncogene (Junb)	2.20	9.81×10^{-5}
<i>Cyr61</i>	ILMN_2794645	NM_010516.1	Cysteine-rich protein 61	2.19	6.99×10^{-4}
<i>Cyr61</i>	ILMN_2710253	NM_010516.1	Cysteine-rich protein 61	2.18	2.30×10^{-4}
<i>Fosb</i>	ILMN_2778279	NM_008036.2	FBJ osteosarcoma oncogene B	1.87	1.03×10^{-6}
<i>Nfkbiz</i>	ILMN_2755008	NM_030612.2	Nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor, zeta	1.87	1.89×10^{-3}
<i>Clock</i>	ILMN_2610973	NM_007715.5	Circadian locomoter output cycles kaput	1.57	1.84×10^{-4}
<i>Slc41a3</i>	ILMN_3075513	NM_001037493.1	Solute carrier family 41, member 3, transcript variant 2	1.56	2.07×10^{-2}
<i>Zfp36</i>	ILMN_3162407	NM_011756.4	Zinc finger protein 36 (Zfp36)	1.54	2.84×10^{-2}
<i>Podn</i>	ILMN_1229370	NM_172874.2	Podocan	0.66	1.89×10^{-3}
<i>Prei4</i>	ILMN_2601884	NM_001042671.1	Mus musculus preimplantation protein 4, transcript variant 3	0.57	8.94×10^{-3}
<i>EG241041</i>	ILMN_2911009	NR_002858.1	Predicted gene, EG241041 on chromosome 1	0.50	1.52×10^{-3}
<i>Prei4</i>	ILMN_1251682	NM_001042672.1	Preimplantation protein 4 (Prei4), transcript variant 4	0.48	1.52×10^{-5}
<i>Coq10b</i>	ILMN_3031099	NM_001039710.1	Coenzyme Q10 homolog B (<i>S. cerevisiae</i>) (Coq10b), transcript variant 1	0.47	1.89×10^{-3}
<i>2210407C18Rik</i>	ILMN_1226767	NM_144544.1	RIKEN cDNA 2210407C18 gene	0.38	5.76×10^{-5}

Note: Animals were sham operated and treated with placebo (vehicle) or 30 mg/kg/d GW0742. After 14 days, the right ventricle was excised, and RNA was extracted from each sample. Gene expression was measured using the Illumina MouseRef-8v3 BeadChip array, and data were analyzed using the syntax package R (ver. 2.3.1) with Bioconductor and limma. Genes that were significantly altered were calculated using a moderated *t* test followed by Benjamini-Hochberg false-discovery rate correction. FC: fold change.

variations are in line with others showing that activation of PPAR β/δ with the selective agonist L-165041 inhibits hypertrophy in neonatal rat cardiomyocytes²⁸ and proliferation of cardiac fibroblasts through inhibition of nuclear factor $\kappa\beta$ signaling.²⁹ In our study, the effects of GW0742 on fibrosis in the right heart were associated with reduced collagen expression. Of direct relevance to our work, others have shown that activation of PPAR β/δ using GW501516 inhibits collagen synthesis in cardiac fibroblasts.³⁰ Thus, reduced fibrosis may in part help to explain the reduction in right heart remodeling and subsequent improvements in CO and performance observed in our model. However, it should be noted that a reduction of pressure overload-induced RV hypertrophy by pharmacological intervention might be either beneficial or deleterious for heart function. The overall effect may depend on many factors, including fibrosis, vascularization, and intrinsic metabolic demand. Indeed, since the mecha-

nisms of transition from compensatory RV hypertrophy to maladaptive RV remodeling are not completely understood, it is difficult to make firm conclusions regarding the overall clinical benefit of PPAR β/δ agonists in the complex setting of clinical pulmonary hypertension. Nevertheless, in our study we have clearly demonstrated that PPAR β/δ activation reduced RV hypertrophy and improved vascularization and heart function. This observation, together with our previous work showing that GW0742 improved RVSP and reduced RV hypertrophy in a hypoxic model of pulmonary hypertension,⁶ suggests that this pathway warrants further study. In line with this and of direct relevance to patients with pulmonary hypertension, where exercise capacity is reduced, it should also be noted that PPAR β/δ activation in association with exercise or activators of the AMP kinase pathway improves athletic performance at the level of muscle metabolism.¹³ Small-molecule activators of AMP kinase have been

Table 3. Top five networks in sham-operated C57BL/6 mice that were treated with GW0742

Associated network functions	Molecules in network	Focus molecules	Score
Cell cycle, cellular development, cellular growth and proliferation	BMP4, CCNA1, CCNE1, CCNE2, CDC25A, CDK2, CDKN1A, CDX2, CHEK1, CSF1 (includes EG:12977), CUL1, cyclin A, cyclin E, ELAVL1, FOXM1, HDAC3, HDAC4, histone H1, KLF4, LGALS1, MAP2K1, NCOR1, NCOR2, NPM1, OSM, PCNA, POLR2A, PRKCE, PYHIN1, RBL2 (includes EG:100331892), SFN, SKP2 (includes EG:27401), SP3, TOPBP1, TSPYL2	2	3
Amino acid metabolism, small-molecule biochemistry, connective tissue disorders	PPAT, SREBF1	1	3
Amino acid metabolism, auditory and vestibular system development and function, cellular development	DIO2, MARCH6	1	3
Cell death, protein synthesis, cellular development	DGAT2, ERN1	1	3
Developmental disorder, genetic disorder, metabolic disease	CTSD, HEXB, MREG	1	3

Note: Animals underwent a sham operation and after several days were orally dosed with vehicle or GW0742 (30 mg/kg). After 14 days, the right ventricle was excised, and RNA was extracted from each sample. Gene expression was measured using the Illumina MouseRef-8v3 BeadChip array, and data were analyzed using the syntax package R (ver. 2.3.1) with Bioconductor and limma. Genes that were significantly modified, as calculated using a moderated *t* test followed by Benjamini-Hochberg false-discovery rate correction, were imported into Ingenuity Pathway Analysis software. The table shows the top five networks identified from genes differentially expressed in C57BL/6 mice treated with GW0742 compared with sham-operated controls.

developed,³¹ and, while it is beyond the scope of our current study to investigate this pathway, it is tempting to speculate that these drugs may act in synergy with PPAR β/δ agonists in the treatment of pulmonary hypertension. Indeed, the AMP kinase activator AICAR inhibits cardiac fibroblast pro-

liferation^{32,33} and protects against myocardial injury after ischemia reperfusion.³³

PPAR β/δ mediates its effects via genomic³⁴ and non-genomic^{20,35} pathways. In the current study, we analyzed genomic influences by monitoring global gene induction

Table 4. Transcripts altered ≥ 1.5 -fold in C57BL/6 mice undergoing pulmonary artery banding that were treated with placebo or GW0742

Symbol	Probe ID	Accession no.	Definition	FC	<i>P</i>
<i>Angptl4</i>	ILMN_2759365	NM_020581.1	Angiopoietin-like 4	3.50	6.38×10^{-4}
<i>Serpina3g</i>	ILMN_2725927	NM_009251.1	Serine (or cysteine) peptidase inhibitor, clade A, member 3G	2.30	4.79×10^{-2}
<i>Fos</i>	ILMN_2750515	NM_010234.2	FBJ osteosarcoma oncogene	1.77	4.22×10^{-2}
<i>Junb</i>	ILMN_1220034	NM_008416.1	Jun-B oncogene	1.5	4.22×10^{-2}
<i>Mertn</i>	ILMN_2739843	NM_113719	Meteorin, glial cell differentiation regulator	0.65	1.52×10^{-2}
<i>Aqp1</i>	ILMN_2980661	NM_007472.1	Aquaporin 1	0.63	4.08×10^{-3}
<i>Aqp1</i>	ILMN_2980663	NM_007472.1	Aquaporin 1	0.62	4.08×10^{-3}

Note: Animals were subjected to pulmonary artery banding and treated with placebo (vehicle) or 30 mg/kg/d GW0742. After 14 days, the right ventricle was excised, and RNA was extracted from each sample. Gene expression was measured using the Illumina MouseRef-8v3 BeadChip array, and data were analyzed using the syntax package R (ver. 2.3.1) with Bioconductor and limma. Genes that were significantly altered were calculated using a moderated *t* test followed by Benjamini-Hochberg false discovery rate correction. FC: fold change.

Table 5. Top five networks in C57BL/6 mice undergoing pulmonary artery banding (PAB) that were treated with 30 mg/kg GW0742

Associated network functions	Molecules in network	Focus molecules	Score
Cellular development, embryonic development, cell death	ACADS, ADAM12, ALDOA, ANKRD40, APP, ARHGEF2, CFL2, CKAP2, CRT1, CSRP3, DYRK1A, ENSA, EPN2, HGF, HSPH1, IGF1R, IL5, KIF5C, LBR (includes EG:368360), LIF, LMO7, MAP4, MAPK1, NDUFV3, NUMBL, PACSIN3, PLOD1, PLXDC1, PSMB5, RAE1, RGS12, SH3BP4, TLR3, TP53 (includes EG:22059), YWHAG	13	27
Cell death, cellular movement, cell morphology	ADAM10, APOA1, AQP1, AQP7, CACNA2D1, calcineurin protein(s), calpain, DNAI1, DNAJB6, ERK, FCER1G, FHOD1, FOS, GATA6, HBB, HOXA10, HTT, JUP, KLF1, LCN2, LPAR1, MAP7D1, MDH2 (includes EG:17448), PAK1, PCYT1A, PDGFB, PECAM1, PRKCB, REM1, RGS5, SIRPA, SUZ12, TGFA, TGFB1 (includes EG:21803), VASP	12	25

Note: Animals' pulmonary arteries were occluded, and after several days animals were orally dosed with vehicle or 30 mg/kg GW0742. After 14 days, the right ventricle was excised, and RNA was extracted from each sample. Gene expression was measured using the Illumina MouseRef-8v3 BeadChip array, and data were analyzed using the syntax package R (ver. 2.3.1) with Bioconductor and limma. Genes that were significantly modified, as calculated using a moderated *t* test followed by Benjamini-Hochberg false discovery rate correction, were imported into Ingenuity Pathway Analysis software. The table shows the top five networks identified from genes significantly expressed in PAB C57BL/6 mice treated with GW0742 compared with vehicle controls.

in the right hearts of animals treated with GW0742. PAB itself has profound effects on gene induction in both the right and the left heart, which has been described in a previous study.¹⁴ In other systems, the PPAR target gene, *Angptl4*, is consistently induced by PPAR β/δ agonists,³⁴ including GW0742.³⁶ In line with this, we found that *Angptl4* was the top gene induced from the entire transcriptome in right heart tissue from PAB mice treated with GW0742. We also found that *Angptl4* was among the top three genes induced by GW0742 in the right heart of sham-operated animals. As we saw previously,⁶ GW0742 had no effect on cardiovascular or cardiac parameters in sham-operated animals. These observations suggest that the changes in the transcriptome are protective and, therefore, are only apparent functionally when there is a pathological change. *Angptl4* is a member of the angiopoietin-like family and regulates angiogenesis and lipid metabolism.³⁷ While no data currently exist relating *Angptl4* to idiopathic pulmonary hypertension, it has recently been associated with high-altitude adaptation in Tibet.³⁸ *Angptl4* has also been associated with left heart failure; a recent study found *Angptl4* to protect against myocardial infarction and no reflow through preservation of vascular integrity.³⁹ It was demonstrated that vascular permeability, hemorrhage, edema, inflammation, and infarct severity were increased in *Angptl4*-deficient mice, whereas injection of recombinant human *Angptl4* reduced myocardial infarct size and the extent of no reflow in mice and rabbits. These observations support the idea that *Angptl4* is a viable mechanism by which PPAR β/δ activation leads to cardioprotection.

PPAR β/δ agonists induce *Angptl4* gene expression, protect the right heart at the level of reduced fibrosis, and improve CO and failure in a PAB model. These findings identify the right heart as a viable therapeutic target in the treatment of pulmonary hypertension and add further weight to the hypothesis that PPAR β/δ agonists may be of benefit to patients with the disease. It should, however, be noted that drugs like GW0742 may interact with other therapies used to treat pulmonary hypertension, and more preclinical studies should be carried out⁶ using combinations of drugs before clinical trials begin.

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