A critical role for Egr-1 during vascular remodelling in pulmonary arterial hypertension

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Aims Pulmonary arterial hypertension (PAH) is characterized by the development of unique neointimal lesions in the small pulmonary arteries, leading to increased right ventricular (RV) afterload and failure. Novel therapeutic strategies are needed that target these neointimal lesions. Recently, the transcription factor Egr-1 (early growth response protein 1) was demonstrated to be up-regulated early in experimental neointimal PAH. Its effect on disease development, however, is unknown. We aimed to uncover a novel role for Egr-1 as a molecular inductor for disease development in PAH.

Methods and results In experimental flow-associated PAH in rats, we investigated the effects of Egr-1 down-regulation on pulmonary vascular remodelling, including neointimal development, and disease progression. Intravenous administration of catalytic oligodeoxynucleotides (DNA enzymes, DNAzymes) resulted in down-regulation of pulmonary vascular Egr-1 expression. Compared with vehicle or scrambled DNAzymes, DNAzymes attenuated pulmonary vascular remodelling, including the development of occlusive neointimal lesions. Selective down-regulation of Egr-1 in vivo led to reduced expression of vascular PDGF-B, TGF-β, IL-6, and p53, resulting in a reduction of vascular proliferation and increased apoptosis. DNAzyme treatment further attenuated pulmonary vascular resistance, RV systolic pressure, and RV hypertrophy. In contrast, in non-neointimal PH rodents, DNAzyme treatment had no effect on pulmonary vascular and RV remodelling.

Conclusions These results indicate that Egr-1 governs pulmonary vascular remodelling and the development of characteristic vascular neointimal lesions in flow-associated PAH. Egr-1 is therefore a potential target for future PAH treatment.

Keywords Congenital heart disease • Right ventricular failure • Pulmonary vascular remodeling • Endothelial cell • Proliferation

1. Introduction Pulmonary arterial hypertension (PAH) is a fatal and progressive form of pulmonary hypertension with, so far, unknown origin. It is characterized by the development of unique so-called neointimal lesions that result in vascular proliferation and apoptotic dysregulation. PAH is considered irreversible when these neointimal lesions have formed, and eventually results in death due to right ventricular (RV) failure. Current treatment strategies may beneficially affect disease progression, but certainly do not result in curing of the disease. Therefore, new treatment strategies are needed that target this irreversible form of pulmonary vascular proliferation.

In PAH associated with congenital heart diseases, the role of increased pulmonary blood flow is seen as an important trigger.
PAH models, the addition of increased pulmonary blood flow has extensively been shown to induce neointimal development that reflects human PAH more truly than the monocrotaline or chronic hypoxia PH models. Still, the mechanism by which increased blood flow leads to neointimal development and subsequently RV failure is unknown.

For this study, we hypothesized that the transcription factor Egr-1 (early growth response protein 1) is an important pathogenic inductor for neointimal development in PAH. Recently, we identified Egr-1 to be up-regulated by increased pulmonary blood flow in the pulmonary vessels. Furthermore, we confirmed Egr-1 to be up-regulated in human end-stage PAH.

The transcription factor Egr-1 is known to play a major role in a variety of cardiovascular processes including systemic arterial remodelling. However, it is unknown whether in vivo down-regulation of Egr-1 will beneficially affect PAH progression. Targeting Egr-1 in vivo has previously been described using pharmacological treatment (including peroxisome proliferator activated receptor-γ (PPAR-γ) ligands) or using specific gene silencing methods, including so-called DNA enzymes (DNAzymes). DNAzymes are catalytic oligodeoxynucleotides that are able to bind to specific RNA sequences resulting in RNA degradation. These DNAzymes exhibit greater catalytic efficiency than oligonucleotides such as ribozymes and are more stable in serum in vivo. For this study, we therefore investigated whether in vivo down-regulation of Egr-1, using both DNAzymes and the PPAR-γ ligand pioglitazone, would prevent the development of pulmonary vascular neointimal lesions in rats with flow-associated PAH.

2. Methods

2.1 DNAzyme preparation

DNAzymes (Double RP-HPLC Purification, with a phosphodiester backbone and an inverted T at 3′ terminus for increased stability; Trilink Biotechnology, Limerick, Ireland) were designed to target the Egr-1 3′ untranslated region (5′-ACGUCCGGGAUUGCACCGGGAU-3′). The DNAzyme includes a 5′ phosphodiester backbone for increased stability and resistance to serum nucleases, an inverted T at the 3′ terminus, and a catalytic core (underlined in Figure 1A) to promote RNA cleavage.

Figure 1  ED5 in the pulmonary vasculature. (A) Schematic representation of the DNAzyme sequence targeting Egr-1 (ED5). This molecule, with a phosphodiester backbone and an inverted T at 3′ terminus, consists of a catalytic core (underline) and two recognition arms that attach to the Egr-1 translational start site. (B) Vascular localization of ED5 24 h after injection. ED5 was commercially labelled with 6-carboxyfluorescein, combined with DOTAP, and injected into the jugular vein. Positive fluorescent signalling was mainly seen in the vascular bed. Both pre- and intra-acinar vessels showed positive signalling for ED5. (C) Single ED5 injection resulted in a decrease of vascular Egr-1 expression after 1 day. This effect was lost 3 days after injection (n = 2). (D) Localization of both ED5 signalling and Egr-1 expression in the intra-acinar vessels. Data are presented as mean values ± SEM. *P < 0.05. Scale bar represents 50 μm.
Biotechnologies, Santa Cruz, CA, USA) were sequenced as followed: Egr-1 DNAzyme (ED5; Figure 1): 5′-CCG CTG CCA GGC TAG CTA CAA CGA CCC GGA CGT Ti-3′; scrambled (inactive) DNAzyme (ED5scr): GCC AGC CGC GGC TAG CTA CAA CGA TGG CTC CAC Ti-3′, as described previously. Based on our result from a pilot study, DOTAP was used as the transfection carrier as described in Supplementary material online.

2.2 Animal procedures and groups

2.2.1 PAH animal model

The Institutional Animal Care and Use Committee approved animal care and experiments. One hundred and twenty-three male Wistar rats (270–300 g) were used. Experimental flow-associated PAH was created using a monocrotaline injection (60 mg/kg) followed by an abdominal aortocaval (av) shunt 1 week later, as described previously.9,10,18

2.2.2 Egr-1 down-regulation in PAH animal model

For the main DNAzyme study, 82 rats were used. Blood was drawn perioperatively for baseline blood analyses. A pilot study revealed that rats needed to receive EDS treatment every 48 h (Figure 1C and D, and see Supplementary material online). Rats were randomly divided to receive intravenous treatment of either (i) NaCl 0.9% (PAH_VEH; n = 24), (ii) scrambled DNAzyme–DOTAP solution (PAH_SCR; n = 18), or (iii) DNAzyme EDS–DOTAP solution (PAH_ED5; n = 20). Treatment started directly after shunt creation (induction of increased flow) (Figure 2A). Rats received treatment every 48 h through an externally fixed jugular vein catheter. Randomization was blinded for the investigators. Repeated EDS delivery did not result in a systemic immune response or decreased liver function (see Supplementary material online, Figure S2). For each group, rats were sacrificed 1 week (n = 6–12 per group) or 3 weeks (n = 10–12 per group) after shunt creation. Sham-operated rats served as control and received either NaCl 0.9% (n = 12) or EDS–DOTAP solution.
and haemodynamic measurements.

Our Institutional Animal Care and Use Committee does not allow the conduction of survival studies due to ethical reasons and dictates to sacrifice rats when suffering due to signs of heart failure occurs. Nevertheless, in order to gain an insight in the effect of Egr-1 down-regulation on PAH survival, we set up the experiment as a ‘case–control study’ in seven animals of each group. In this setting, a treated animal was paired with an untreated animal. If one animal met the endpoint for sacrifice (i.e. signs of heart failure), their matched counterparts were sacrificed simultaneously.

2.2.3 Egr-1 down-regulation in a non-neointimal PH animal model

In addition, to investigate the effects of Egr-1 down-regulation in a non-neointimal PH model, additional rats received a single injection of monocrotaline only. One week later, rats were randomly divided to receive either NaCl 0.9% (MCT_VEH; n = 3) or DNAzyme EDS–DOTAP solution (MCT_ED5; n = 3) via externally fixed jugular vein catheters. Rats were sacrificed 3 weeks after monocrotaline injection after echocardiography and haemodynamic measurements.

2.2.4 Pioglitazon treatment in the PAH animal model

To investigate the effects of the PPAR-γ ligand pioglitazon on pulmonary vascular Egr-1 down-regulation in PAH, we first conducted a dose-finding study: 24 flow-associated PAH rats were randomly divided to receive either (i) vehicle treatment (n = 6), (ii) pioglitazon 20 mg/kg (n = 9), or (iii) pioglitazon 100 mg/kg (n = 9). Treatment was mixed into rat chow and rats were fed ad libitum. Pioglitazone treatment was started the day after surgery. Rats were sacrificed 1 day (n = 3–5 per group) and 1 week (n = 3–4 per group) after start of increased blood flow. Vehicle-treated sham rats from the main study served as control. After pioglitazon 100 mg/kg was found to be most effective, we aimed to investigate the effects of pioglitazon on PAH development. An additional 20 rats were randomly divided to receive either vehicle treatment (n = 10) or pioglitazon 100 mg/kg (n = 10) and sacrificed after 3 weeks after echocardiography and haemodynamic measurements.

2.3 Echocardiography, haemodynamic measurements, pathology, and biomolecular analyses

Echocardiography, haemodynamic measurements, morphometric analyses, immunohistochemistry, and RT-PCR technique were performed as previously described and in more detail in Supplementary material online.

2.4 Statistical analysis

Data are presented as mean ± standard error of the mean. Differences between groups were determined by one-way analysis of variance (ANOVA) with the Bonferroni or Dunnet test where applicable. For data not normally distributed, Mann–Whitney U-test was performed. A P-value of <0.05 was considered to be significant.

### Table I Animal characteristics

<table>
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<tr>
<th></th>
<th>Con</th>
<th>PAH_1wk</th>
<th>VEH</th>
<th>SCR</th>
<th>EDS</th>
<th>PAH_3wks</th>
<th>VEH</th>
<th>SCR</th>
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<td>Body weight at sac (g)</td>
<td>361 ± 7</td>
<td>306 ± 6*</td>
<td>304 ± 11</td>
<td>309 ± 6</td>
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<td>RV weight (mg)</td>
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<td>0.25 ± 0.01</td>
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<td>0.49 ± 0.02*</td>
<td>0.49 ± 0.02</td>
<td>0.39 ± 0.02‡</td>
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<td>LV + IVS weight (mg)</td>
<td>0.68 ± 0.01</td>
<td>0.74 ± 0.17</td>
<td>0.69 ± 0.03</td>
<td>0.71 ± 0.02</td>
<td>0.83 ± 0.02*</td>
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<td>RV/BW (mg/g)</td>
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<td>0.81 ± 0.04</td>
<td>0.60 ± 0.02†</td>
<td>1.49 ± 0.07*</td>
<td>1.53 ± 0.06</td>
<td>1.2 ± 0.06‡</td>
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<td>LV + IVS/BW (mg/g)</td>
<td>1.92 ± 0.02</td>
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<td>2.33 ± 0.14</td>
<td>2.30 ± 0.05</td>
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<td>0.38 ± 0.02†</td>
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<td>0.78 ± 0.02</td>
<td>0.64 ± 0.03‡</td>
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<td>CO (ml/min)</td>
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<td>108 ± 9</td>
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Vascular remodelling

**Pre-acinar**

| Outer diameter (µm) | 101 ± 8 | 96 ± 15 | 104 ± 11 | 106 ± 10 | 124 ± 16 | 115 ± 13 | 110 ± 10 |
| Wall thickness (µm) | 6 ± 1    | 6 ± 1    | 5 ± 1    | 6 ± 1    | 14 ± 1   | 13 ± 1   | 9 ± 1†   |

**Intra-acinar**

| Outer diameter (µm) | 28 ± 1   | 36 ± 1*  | 37 ± 1   | 35 ± 1   | 34 ± 1   | 36 ± 1   | 35 ± 1   |
| Wall thickness (µm) | 0.3 ± 0.0 | 2.3 ± 0.4* | 2.1 ± 0.2 | 1.0 ± 0.1† | 5.6 ± 0.7 | 5.8 ± 0.7 | 3.0 ± 0.4‡ |

Remodelling (% of total vessels)

| Non-muscular (%)     | 79 ± 1   | 22 ± 3  | 24 ± 1   | 31 ± 8†  | 13 ± 3   | 10 ± 2   | 18 ± 2†   |
| Partially muscularized (%) | 26 ± 1    | 35 ± 3  | 35 ± 3   | 43 ± 3†  | 13 ± 2   | 12 ± 2   | 22 ± 5†   |
| Totally muscularized (%) | 6 ± 1    | 35 ± 3  | 33 ± 5   | 20 ± 2.5† | 39 ± 5   | 41 ± 4   | 48 ± 4    |
| Occlusive lesions (%) | 0 ± 0    | 12 ± 2* | 11 ± 1   | 2 ± 1†   | 33 ± 3   | 32 ± 3   | 11 ± 2†   |

Data presented as mean ± SEM.

PAH: pulmonary arterial hypertension; Con: control; VEH: vehicle treatment; SCR: scrambled DNAzyme treatment; EDS: DNAzyme treatment; Sac: sacrifice; RV: right ventricle; LV: left ventricle; IVS: intraventricular septum; BW: body weight; CO: cardiac output; PAAT: pulmonary artery acceleration time; TAPSE: tricuspid annular plane systolic excursion.

*P < 0.05 Con vs. VEH.

†P < 0.05 SCR vs. EDS.
3. Results

3.1 Increased pulmonary blood flow resulted in progressive PAH with neointimal development

Rats that received monocrotaline administration and an av shunt (flow-associated PAH) showed progressive pulmonary vascular remodelling over time (Figure 2A and Table 1). One week after shunt creation (PAH_1wk), intra-acinar vessels exhibited muscularization, and sporadic neointimal lesions were observed. In end-stage disease (PAH_3wks), occlusive neointimal lesions were seen in the majority of intra-acinar vessels (Figure 2 and Table 1). Occlusive neointimal lesions consisted of endothelial cells (ECs) (staining positive for von willebrand factor) surrounded by smooth muscle cells (SMCs) (staining positive for α-smooth muscle actin) (Figure 2C). Vessel occlusion resulted in symptomatic PAH 3 weeks after induction of increased blood flow, including increased peak RV systolic pressure (pRVSP), increased pulmonary vascular resistance, and RV hypertrophy (RVH) (Figure 2B). These parameters correlated with pulmonary vascular remodelling (see Supplementary material online, Figure S4).

Four rats did not reach the endpoint of the study: one died perioperatively (ED5 group) and three rats were sacrificed (2 ED5 group and 1 SCR group) because of severe dyspnoea due to air emboli during intravenous injection. These rats were excluded from further analyses.

3.2 Intravenous ED5 delivery reduced vascular Egr-1 expression in flow-associated PAH

Compared with control, pulmonary vascular Egr-1 protein expression increased 1 week after increased pulmonary blood flow (PAH_1wk) and progressed during disease development (PAH_3wks) (Figure 3). Chronic ED5 delivery (PAH_ED5) reduced pulmonary vascular Egr-1 protein expression with 35% and 43% at 1 and 3 weeks after increased
flow, respectively (Figure 3D). Egr-1 was targeted throughout the vessel wall (see Supplementary material online, Figure S5). Pulmonary vascular Egr-1 expression correlated with pulmonary vascular remodelling (Figure 3G).

3.3 Egr-1 down-regulation attenuated neointimal formation and flow-associated PAH development

Vascular Egr-1 down-regulation due to ED5 delivery (PAH_ED5) reduced vessel occlusion and wall lumen ratio of the intra-acinar vessels at both 1 week and 3 weeks after induction of increased blood flow (Figure 4 and Table 1). Egr-1 down-regulation furthermore resulted in a reduction of the pRVSP, pulmonary vascular resistance, and RVH (Figure 4B). Scrambled DNAzyme treatment (PAH_SCR) had no effect on any of the vascular remodelling or haemodynamic parameters. ED5 treatment had no effect on left ventricular mass (Table 1).

To gain an insight on the effect of Egr-1 down-regulation on PAH survival, we set up the experiment as a ‘case–control study’ in seven animals of each group. Six of seven PAH-VEH rats, one PAH-SCR, and zero of seven PAH-ED5 rats determined the time of sacrifice. This indicates that untreated rats (i.e. without Egr-1 down-regulation) showed poorer survival.

3.4 Egr-1 down-regulation attenuated neointimal development through early attenuation of vascular cell proliferation and end-stage increase of apoptosis

In vehicle- and scrambled DNAzyme-treated rats, both vascular proliferation and apoptosis peaked 1 week after the induction of increased flow (Figure 5A). Egr-1 down-regulation reduced early vascular cell proliferation and increased end-staged vascular cell apoptosis, predominantly in the endothelial layer (Figure 5B and see Supplementary material online, Figure S6).

Expression of PDGF-B, TGF-β, IL-6, and p53, all downstream targets of Egr-1 and known to play a role in vascular proliferative remodelling in human PAH, was increased in the pulmonary vessels during neointimal development (PAH_VEH and PAH_SCR) at both 1 and 3 weeks (Figure 6 and see Supplementary material online, Figure S6). Egr-1 inhibition (PAH_ED5) resulted in a significant reduction of PDGF-B, TGF-β, IL-6, and p53 expression, compared with vehicle and scrambled treatment (Figure 6 and see Supplementary material online, Figure S6).

3.5 ED5 delivery had no effect on vascular remodelling in non-neointimal non-flow PH

To further test whether the effects of Egr-1 down-regulation are specific for flow-induced neointimal formation, we investigated the effects

**Figure 4** Effects of pulmonary vascular Egr-1 down-regulation. At 1 and 3 weeks after increased flow, Egr-1 down-regulation using ED5 significantly attenuated both (A) pulmonary vascular remodelling and (B) PAH development as shown by a reduction in pulmonary vascular resistance, pRVSP, and RVH (n = 7–12 per group). Data are presented as mean values ± SEM. *P < 0.05 ED5 vs. VEH and SCR.
of ED5 delivery in a non-neointimal, non-flow-associated model of pulmonary hypertension, namely monocrotaline-only model (MCT_VEH vs. MCT_ED5). MCT injection (MCT_VEH) resulted in a significant increase in vessel wall thickness, increased pRVSP, and RVH compared with control (Figure 7C). Neointimal lesions were not observed (Figure 7B). In MCT-only PH rats, vascular Egr-1 expression was only sporadically seen compared with its expression in flow-associated PAH rats (Figure 7A). Also, ED5 treatment (MCT_ED5) did not alter vascular remodelling, pRVSP, or RVH (Figure 7C) in MCT-only PH rats.

### 3.6 Pharmacological down-regulation of Egr-1 using pioglitazone also attenuated vascular remodelling in flow-associated PAH

Using the PPAR-γ ligand pioglitazone, we investigated whether Egr-1 could be pharmacologically inhibited. First, pioglitazone treatment activated PPAR-γ, as confirmed by increased peroxisome proliferator activated receptor gamma co-activator 1 (coactivator of PPAR-γ) and MCAD (downstream gene of PPAR-γ) gene expression (see Supplementary material online, Figure S7). Pioglitazone reduced vascular Egr-1 expression in a dose-dependent manner after increased pulmonary blood flow (Figure 8A and B). This was accompanied with attenuation of pulmonary vascular remodelling at both 1 and 3 weeks, including reduction of neointimal lesions (Figure 8C). Improvement of vascular remodelling resulted in a reduction of pRVSP RVH and pulmonary vascular resistance, compared with vehicle-treated PAH rats (Figure 8D).

### 4. Discussion

This study in experimental flow-associated PAH demonstrates that in vivo Egr-1 down-regulation, both via intravenous administration of catalytic oligodeoxynucleotides (DNAzymes) and pharmacological intervention using pioglitazone, attenuates pulmonary vascular remodelling, including the development of occlusive neointimal lesions, and reduced PAH progression. These results indicate that Egr-1 plays a
critical role in the induction and development of pulmonary vascular remodelling in flow-associated PAH. Egr-1 is therefore a putative target for future PAH treatment.

Egr-1 is a transcription factor with pleotropic effects. Egr-1 is poorly expressed in the normal arterial wall, but can be activated by various stimuli including changes in shear stress. In systemic vessels, Egr-1, once activated, has been shown to act as an early ‘master switch’ during vessel wall remodelling, including intimal thickening, vascular proliferation, and vessel inflammation. A role of Egr-1 in pulmonary vascular remodelling in PAH had not been described until now.

Here we demonstrate, for the first time, a functional role of transcription factor Egr-1 in pulmonary vascular remodelling in PAH. Our results indicate that Egr-1-inhibition in vivo attenuates apoptotic dysregulation and reduces vascular proliferation. In human PAH development, vascular proliferation and apoptosis-resistant ECs are recognized hallmarks of pulmonary neointimal formation. Egr-1 has been previously shown to induce neointimal formation in systemic arteries, in atherosclerotic disease, and in-stent restenosis, mediated through both anti-apoptotic and pro-proliferative pathways. PDGF-B and TGF-β1 are known to induce proliferation of both SMCs and ECs and are up-regulated in human PAH. Egr-1 is known to bind to the promoter regions of these genes resulting in increased expression. The results of the current study, showing increased PDGF-B and TGF-β1 expression in experimental PAH and its reduction through inhibition of Egr-1, support the working mechanism of Egr-1-induced vascular proliferation through PDGF-B and TGF-β1 (Figure 9). In addition, we show that down-regulation of Egr-1 results in a reduction of IL-6 (vascular inflammation) and p53 (vascular apoptosis), both downstream targets of Egr-1 and known genes in the pathogenesis of human PAH.

Egr-1 could be a putative therapeutic target for future PAH treatment. We have previously shown that Egr-1 is up-regulated in human end-stage PAH. In these patients, Egr-1 was located at sites that are of interest for treatment targeting, namely in vessels with media hypertrophy and in neointimal lesions. The positive effects of Egr-1 inhibition on vascular remodelling and PAH progression in this study support future outlook of Egr-1 as a treatment target. The fact that these effects were seen in the current PAH model that mimics human pathological trigger, complex neointimal development, and human PAH progression makes the critical role of Egr-1 in PAH development more convincing. The observations in both human and now in experimental PAH justify future investigation on Egr-1 intervention either via gene silencing techniques or pharmacological treatment in human PAH.
The rapid developments in the design of gene silencing agents could facilitate a novel approach for therapeutic intervention for the treatment of PAH. First antisense oligonucleotide-based drugs have reached the clinic and have proved to be effective against for instance cytomegalovirus infections and hypercholesterolaemia. New generations of oligonucleotides such as DNAzymes or small interfering RNAs (siRNAs) have entered the clinical stage. In the current study, we demonstrated that, in PAH, Egr-1 can be interfered in vivo using RNA interfering agents. Several types of clinically suitable carriers for siRNA delivery into pulmonary ECs have been developed and could serve as an alternative for DOTAP-mediated delivery in the future and increase specificity. ED5 could possibly have a direct effect on pressure-induced RVH. However, since we show that Egr-1 down-regulation (i) attenuates pulmonary vascular resistance in flow-associated PAH rats, (ii) has no effect on RVH in MCT-only treated rats, and (iii) has no effect on left ventricular mass, it is most likely that the positive effects of ED5 treatment are to Egr-1 down-regulation in the pulmonary vasculature.

Pharmacological down-regulation of Egr-1 with clinically used drugs could also be an interesting pursuit. One of these pharmacological possibilities is the use of PPAR-γ ligands, such as pioglitazone. PPAR-γ ligands have previously shown to inhibit Egr-1 expression in vivo. These studies describe Egr-1 as a key repressive target gene of PPAR-γ in different pathophysiological mechanisms. Activation of PPAR-γ has been shown to prevent ERK phosphorylation in ECs and SMCs, which may affect Egr-1 activation pathways. In addition, recent studies have suggested that BMPR2 signalling dysfunction in PAH leads to failure of PPAR-γ activation, resulting in SMC proliferation in PAH. PPAR-γ ligands are also shown to have a positive effect on vascular remodelling in other pulmonary hypertension models. However, these studies were conducted in a hypoxic PH model that is known to fail to induce the typical neoointmental

Figure 7  ED5 treatment in non-flow non-neointimal PH. (A) Egr-1 protein expression and localization (B) in the intra-acinar vessels at 3 weeks. In rats where pulmonary blood flow was not increased (MCT-only rats), Egr-1 was only sporadically seen and no neointimal lesions were formed. (C) In these non-flow non-neointimal rats, ED5 administration had no effect on vascular remodelling, RVH, or pRVSP (N = 3–7 per group). Data are presented as mean values ± SEM. *P < 0.05. Scale bar represents 50 μm.
lesions and progressive PAH development, which limits translation to human disease. Our results in a neointimal model of PAH show that pioglitazone prevents pulmonary vascular remodelling, and that this positive effect is associated with a down-regulation of vascular Egr-1 expression. This justifies further investigation of oral thiazolidinediones (PPAR-γ ligands) in both preclinical and clinical settings, also focusing on possible side effects.

This study was designed to investigate the mechanistic role of Egr-1 in the development of neointimal lesions. Therefore, the question whether targeting Egr-1 also results in reversal of established PAH (when neointimal lesions are already present) should now be addressed subsequently. However, since Egr-1 is expressed in remodelled vessels of both experimental and human end-stage PAH, Egr-1 could very well be a target in established pulmonary vascular remodelling.

5. Conclusions
This study indicates that the transcription factor Egr-1 governs flow-induced pulmonary vascular remodelling and the development of the characteristic vascular neointimal lesions in flow-associated PAH. Egr-1 therefore is a potential target for future PAH treatment.

Supplementary material
Supplementary material is available at Cardiovascular Research online.
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References


