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TITLE: Identification of G-Protein-Coupled Receptors (GPCRs) in Pulmonary Artery Smooth Muscle Cells as Novel Therapeutic Targets

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14. ABSTRACT Pulmonary arterial hypertension (PAH) is associated with increased vascular resistance, sustained contraction, and enhanced proliferation of pulmonary arterial smooth muscle cells (PASMCs). The underlying idea of this project is that the currently limited treatments for PAH represent an unmet medical need for soldiers, veterans and those in the general population. We are testing the hypothesis that the discovery of "novel" (i.e., not previously recognized) G-protein-coupled receptors (GPCRs) and their functional activity in the PASMCs from subjects with PAH can reveal new insights into pathophysiology and new therapeutic targets for PAH. Our approach is to isolate PASMCs from PAH subjects and controls, to define the expression and function of their complement of GPCRs, with the goal of identifying GPCRs that have known physiologic agonists and are uniquely expressed and/or prominently up-regulated in PAH-PASMCs and to define their potential as novel therapeutic targets for PAH.						
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Introduction

This project focuses on pulmonary arterial hypertension (PAH), which is associated with enhanced vasoconstriction and proliferation of pulmonary arterial smooth muscle cells (PASMCs). The limited, effective therapies for soldiers, veterans and those in the general population who have PAH represent an unmet medical need. Our overriding hypothesis is that previously unrecognized (“novel”) G-protein-coupled receptors (GPCRs) expressed by PASMCs contribute to the pathophysiology and may be new therapeutic targets for PAH. To test this hypothesis, we isolated PASMCs from PAH subjects and controls, quantified the GPCR expression profile of the PASMCs and sought to identify (and validate in signaling and functional studies) GPCRs that have known physiologic agonists and whose expression prominently increases in PAH-PASMCs. Our working hypothesis is that GPCRs with known agonists would be novel (druggable) targets for treating PAH.

Keywords

Pulmonary arterial hypertension (PAH), pulmonary artery smooth muscle cells (PASMCs), G-protein-coupled receptors (GPCRs), cyclic AMP, hypoxia.

Accomplishments

What were the major goals of this project?

Specific Aim 1/Task 1: The major task of Aim 1 was to isolate control PASMCs from humans, rats and mice and to define the expression profile in these PASMCs of GPCRs with known physiologic agonists, to confirm expression of a subset of GPCRs by independent mRNA analyses, and to analyze expression and responses (functional activities) of receptor proteins. We also sought to determine if GPCR expression profile in PASMCs differs from that of human coronary artery and aortic smooth muscle cells. The sub-Tasks were as follows:

la. Isolate and prepare primary cultures of PASMCs from lung samples obtained from 4 UCSD patients who do not have pulmonary hypertension (PH) **Completed 12/2015**

lb. Prepare RNA and cDNA from PASMCs and use Taqman GPCR arrays to identify and quantify expression of GPCRs with known physiologic agonists. **Completed 5/2017**

le. Prepare RNA and cDNA from commercially available coronary artery and aortic smooth muscle cells; use Taqman GPCR arrays to identify/quantify expression of GPCRs with known physiologic agonists. **Completed 12/2015**

ld. Isolate and culture PASMCs from 8 (4 male; 4 female) 3 -month old rats and mice, prepare RNA and cDNA and assess expression of GPCRs with known physiologic agonists by using Taqman GPCR arrays **Completed 9/2018, modified.**

le. Confirm mRNA expression by independent RT-PCR analyses of the 3 highest expressed PASMC-GPCRs that couple to Gs, Gi, Gq/G11, G12/13 or that are overall the 3 highest expressed GPCRs with known physiologic agonists from PASMCs, coronary artery and aortic smooth muscle cells **Completed 5/2017**

lf. Assess protein expression (immunoblotting, immunofluorescence and immunoprecipitation) with antibodies directed at 2 highly, preferentially expressed human, mouse and rat PASMC- GPCRs each that couple to Gs, Gi, Gq/11 or G12/13. **Completed 9/2018, for 1 GPCR.**

lg. Characterize the function of 2 highly and preferentially expressed PASMCM-GPCRs each that couple to Gs, Gi, Gq/ql1 and G12/13 by assessing signal transduction and cell physiology (cell cycle proteins [p21/p27], DNA synthesis, caspase 3 activation, cell migration) **Completed 8/2018, modified.**

Specific Aim 2/Task 2: The major task was to define the expression profile of GPCRs with known physiologic agonists in PASMCMs from humans, rats and mice with PAH so as to identify alterations in GPCR expression in PAH and to test if GPCRs with altered expression contribute to PAH pathophysiology. The Sub-tasks were to:

2a. Isolate and prepare primary cultures of PASMCMs from 4 patients each with primary and secondary PAH. **Primary (Group 1 PH) PAH: 9 cultures Completed 5/2017; Secondary (Groups 2-5 PH): 1 culture obtained.**

2b. Use RT-PCR and gene-specific primers with samples from at least 15 patients each with primary or secondary PAH to confirm the expression of GPCRs with known physiologic agonists of receptors with the most prominent differences in expression in PAH-PASMCMs (vs. control PASMCMs) and thus, to define a "signature" of the highest expressed such GPCRs, ones with the greatest differences vs. control-PASMCMs and to determine the inter-subject variability in GPCR expression **RNA-seq completed for 10 samples.**

2c. Assess protein expression and functional activity (as in Aim 1) for 2 GPCRs each that are Gs-, Gi-, Gq/11- and G12/13-coupled and whose expression is most prominently altered in PAH and most consistent in primary PAH-PASMCM and secondary PAH-PASMCM. **Completed by 4/2018, for 1 GPCR.**

2d. Set up rat models of PAH (monocrotaline, chronic hypoxia) and a mouse model of PAH (chronic hypoxia) with 4 animals each in each model, confirm PH by right ventricular hypertrophy and PA remodeling, and isolate PASMCMs for culture, assay of expression of GPCRs with known physiologic agonists and functional analyses. **Mouse model completed 8/2018.**

Specific Aim 3/Task 3: The major task was to determine the therapeutic potential of "novel" GPCRs that we identified as preferentially expressed in PAH-PASMCMs. We sought to use several criteria to choose GPCRs to target: ones with the largest expression changes (vs. control-PASMCMs), similar changes in humans, rats and mice, with available pharmacological agents (in particular, for which FDA-approved drugs existed) and that we predicted would have a pathophysiological impact. The Sub-tasks were:

3a. Choose 2 Gi-coupled, Gq/11-coupled, 2 Gs-coupled and 1 G 12/13-coupled receptors to target using the criteria above and identify potential therapeutic agents **Completed 8/2018**

3b. Use PAH-PASMCMs and normal-PASMCMs as controls and test antagonists of 2 Gi-coupled and 2 Gq/11-coupled GPCRs (chosen in Sub-task 1), with bosentan as a control, in concentration-response studies that assess effects on "basal" signal transduction/functional responses and on the responses to known receptor agonists. **Completed by 8/2018.**

3c. Use PAH-PASMCMs and normal-PASMCMs as controls and test agonists of 2 Gs-coupled GPCRs (chosen in Sub-task 1), with a prostacyclin agonist, as a control in concentration-response studies that assess effects on "basal" signal transduction/functional responses and on the responses to known receptor agonists. **Completed, modified, 8/2018**

3d. Use PAH-PASMCMs and normal-PASMCMs as controls and test a G12/13-coupled GPCR antagonist (chosen in Sub-task 1) in concentration-response studies that assess effects on "basal" signal transduction/functional responses and on the responses to a known receptor agonist. **Completed, modified, 8/2018**

3e. Pending results of studies in Sub-tasks 2-4, test if drugs directed at the "new" GPCRs produce additive (or perhaps synergistic) activity when used in combination with agents approved for the treatment of PAH (bosentan, prostacyclin agonist, PDE 5 inhibitor). **Completed, modified, 8/2018**

3f. Test if commercially available anti-GPCR antibodies are able to antagonize Gi-, Gq/11- and G12/13-coupled GPCRs (chosen in sub-Task I) and evaluated as in Sub-Tasks 2-4. **Not completed, none available.**

What was accomplished under these goals?

1. Major activities

In this final reporting period (in a no-cost extension), we fulfilled several goals of this project and have used samples obtained from animals for further work. In addition to activities described below, we tested multiple drugs on cell proliferation of human PASMCs, discovered a novel GPCR with altered expression in PAH-PASMCs, and found (using samples from animals from which we isolated PASMCs), that the right ventricle (RV) has differences from the left ventricle (LV) that will be investigated in future projects.

Using human PASMCs, we performed RNA-seq and identified multiple GPCRs with altered expression in PAH- compared to control-PASMCs. These results facilitated our generation of a list of potential GPCR targets, whose expression we confirmed by qPCR. For several putative GPCR targets, we utilized cAMP and Ca²⁺ assays to assess their signaling in response to agonists, and also measured proliferation. In addition, we performed RNA-seq. on PASMCs isolated from mice with PAH.

2. Specific objectives

1b. Prepare RNA and cDNA from PASMCs and used Taqman GPCR arrays to identify and quantify expression of GPCRs with known physiologic agonists.

1d. Isolate and culture PASMCs from 8 (4 male; 4 female) 3 -month old rats and mice, prepare RNA and cDNA and assess expression of GPCRs with known physiologic agonists by using Taqman GPCR arrays.

1e. Confirm mRNA expression by independent RT-PCR analyses of the 3 highest expressed PASMC-GPCRs that couple to Gs, Gi, Gq/G11, G12/13 or that were overall the 3 highest expressed GPCRs with known physiologic agonists from PASMCs, coronary artery and aortic smooth muscle cells.

2c. Assess protein expression and functional activity (as in Aim 1) for 2 GPCRs each that couple to Gs-, Gi-, Gq/11- and G12/13 and whose expression was most prominently altered in PAH and most consistent in their expression in primary PAH-PASMCs and secondary PAH-PASMCs.

2d. Set up rat models of PAH (monocrotaline, chronic hypoxia) and a mouse model of PAH (chronic hypoxia) with 4 animals each in each model, confirm PH by RV hypertrophy and PA remodeling, and isolate PASMCs for culture and assays of expression and functional activity of GPCRs with known physiologic agonists.

3a. Choose 2 Gi-coupled, Gq/11-coupled, 2 Gs-coupled and 1 G 12/13-coupled receptor to target using the criteria above and identify potential therapeutic agents.

3c. Use PAH-PASMCs and normal-PASMCs as controls and test agonists of 2 Gs-coupled GPCRs (chosen in Sub-task I), with a prostacyclin agonist as a control in concentration- response studies that assess effects on "basal" signal transduction/functional responses and on the responses to known receptor agonists.

3. Significant results

We conducted multiple experiments that addressed the above objectives. Our results include the following:

A. We isolated RNA from control- and IPAH-PASMCs and performed RNA-seq. These included PASMCs from PAH patients from a collaborator (Jason Yuan [University of Arizona]) and included 3 idiopathic PAH and 2 heritable PAH samples. From this analysis, we identified potential targets that include 9 GPCRs with higher expression in PAH-PASMCs. We confirmed these targets using qPCR as shown in **Figure 1**.

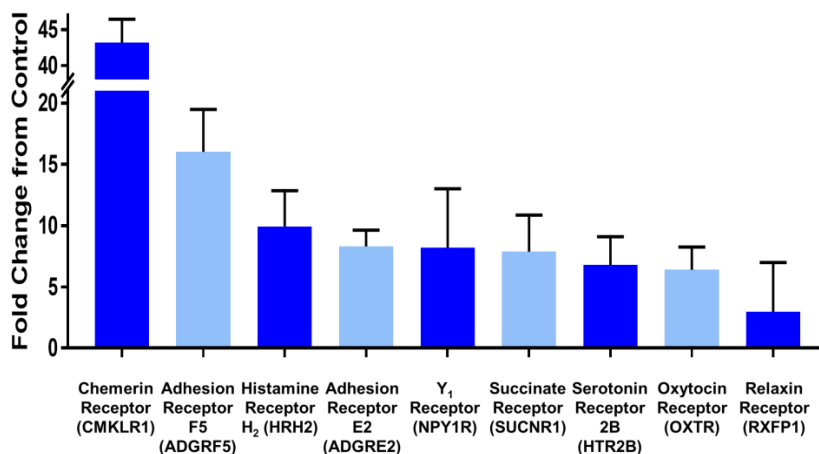


Figure 1: qPCR of target GPCRs expressed higher in PAH-PASMCs compared to control. N=7 IPAH and 4 control PASMCs, all are significantly changed via t-test ($P < 0.05$).

B. Using agonists of the target GPCRs, we assessed second messenger activity, i.e., cAMP assays (DiscoverX) and Ca^{2+} assays (Molecular Devices), of the PASMCs. The cells had increased cAMP accumulation in response to adenosine (as a positive control) and moderate Gi activity (but greater in PAH-PASMCs) in response to succinate (the SUCNR1/GPR91 ligand) or ML290 (a RXFP1 ligand) (**Figure 2**). The PASMCs had an increase in intracellular Ca^{2+} , indicative of signaling via Gq, in response to oxytocin (OXTR agonist) or histamine (H1 histamine receptor agonist) (**Figure 3**), but not to succinate, chemerin, or bradykinin (data not shown).

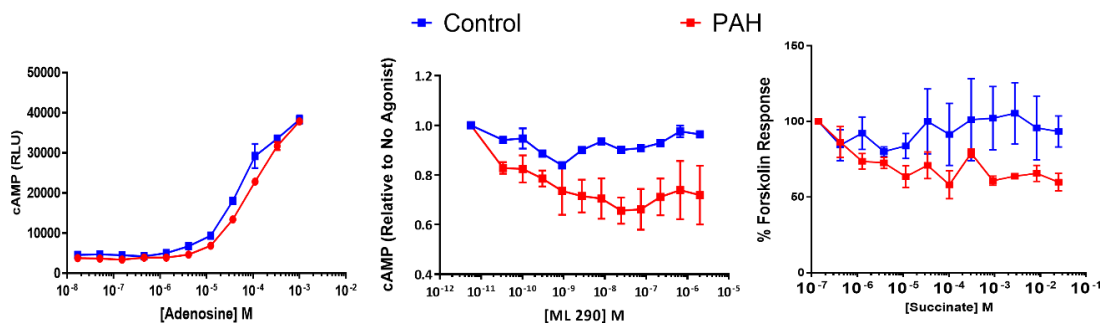


Figure 2: cAMP accumulation in response to agonists for target GPCRs. Data shown are mean \pm SEM for control PASMCs ($n=1$) and PAH-PASMCs ($n=2$).

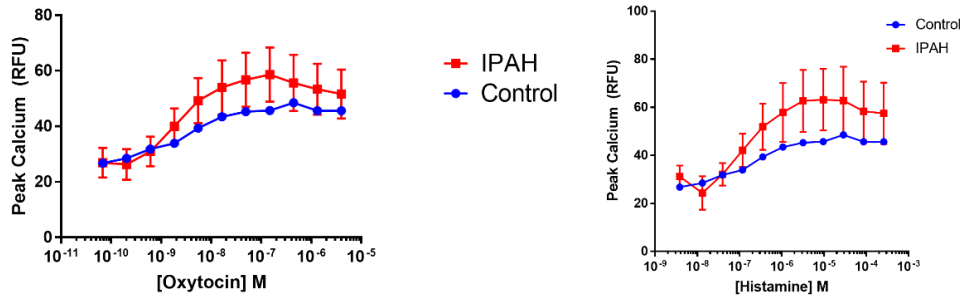


Figure 3: Intracellular calcium in response to agonists for target GPCRs. Data shown are mean \pm SEM for PAH-PASMCs ($n=4$) or control PASMCs ($n=1$).

C. We further analyzed the RNA-seq data for differential gene expression using edgeR (*Bioinformatics* 26: 139-40 [2010]); stringent parameters were used to reduce the false discovery rate (FDR) of GPCRs as PAH targets. Several samples had distinct clustering of gene expression compared to others (**Figure 4**, IPA1 – 3). Based on that result and other data for agonists that lacked signaling activity, we excluded those GPCR and generated a revised list of target GPCRs consistently overexpressed in PAH- vs. control-PASMCs. The list includes PTGER2, LPAR3, and GPR126, which had higher expression in the PAH-PASMCs ($FDR < 0.1$) and were low-expressed in aortic and coronary SMCs (**Figure 5**). Overexpression of GPR126 (also called ADGRG6) significantly reduced PASMC proliferation, and increased cellular cAMP, and knockdown of GPR126 in IPA1-PASMCs caused a distinct, though not significant, increase in cell proliferation (**Figure 6**).

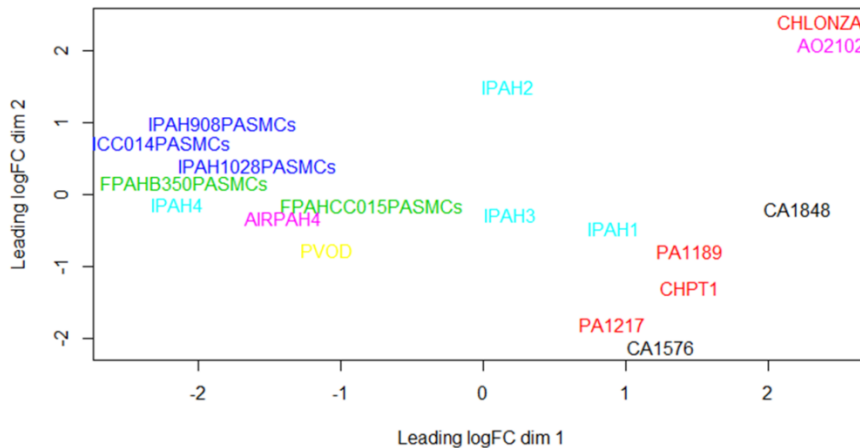


Figure 4: Clustering of gene expression data of smooth muscle cells (SMCs) derived from human tissues.

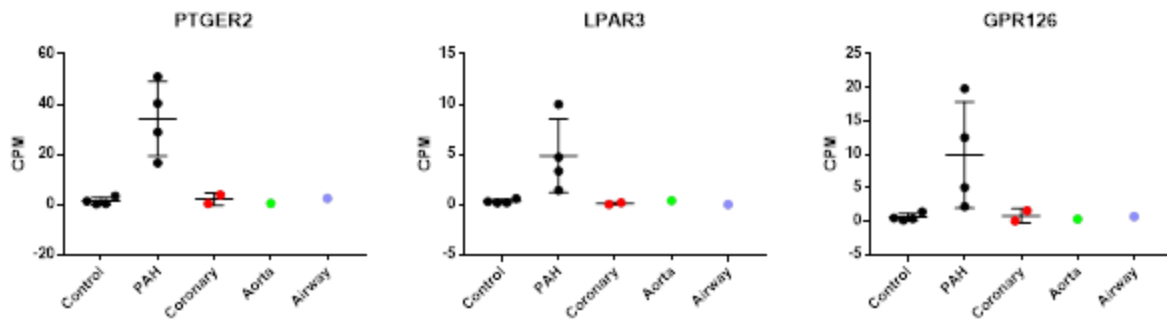


Figure 5: Target GPCRs increased in PAH compared to control PSMCs. PAH samples are significantly changed ($P < 0.05$ via t -test) vs. control.

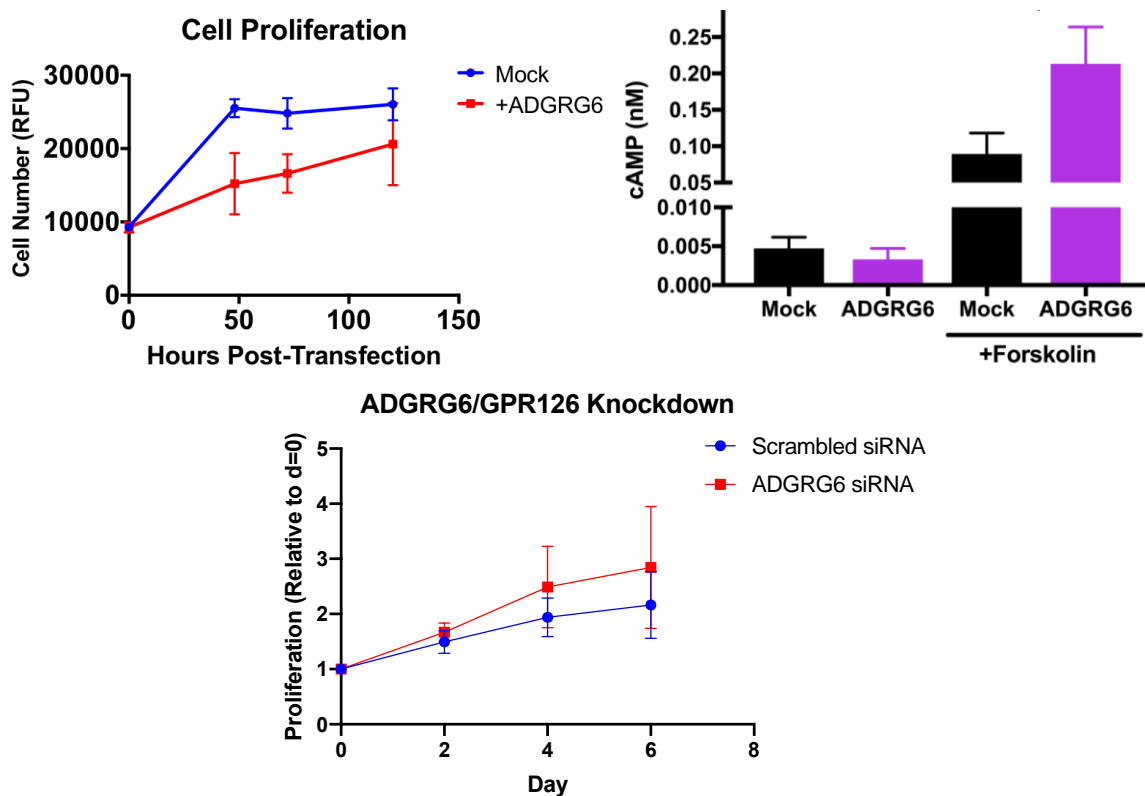


Figure 6: Proliferation (top left) and cAMP production (top right) of control-PASMCs transfected with GPR126/ADGRG6. $N=2-4$ biological replicates.

D. Further analysis of differentially-expressed genes from RNA-seq revealed many genes with significantly altered expression in PAH-PASMCs (**Figure 7**). Using edgeR, we found that 2378 genes were altered with a FDR < 0.1 : 985 were increased at least 2-fold, and 1086 were decreased at least 2-fold in IPAH samples compared to control-PASMCs. These genes were used for a gene ontology analysis (GO, Panther) for biological processes. **Table 1** lists the 10 processes with the highest overrepresentation ($P < 0.05$). Of note, all of the up-regulated processes relate to proliferation, a hallmark of PAH-PASMCs.

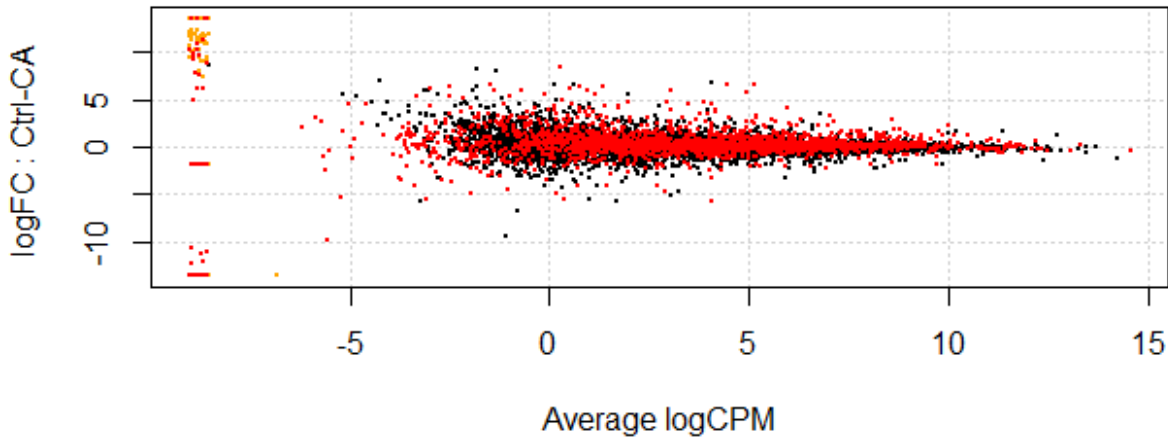


Figure 7: Smear plot of genes with significantly altered expression in PAH. Each dot represents a gene, red dots indicate genes with false discovery rate (FDR) < 0.1.

Table 1: Gene ontology (GO) analysis for the 10 most up-regulated and down-regulated processes in IPAH.

GO Biological Processes Up-regulated in IPAH	GO Biological Processes Down-regulated in IPAH
Positive regulation of DNA-directed DNA polymerase activity	PERK-mediated unfolded protein response
DNA unwinding involved in DNA replication	Positive regulation of transcription from RNA polymerase II promoter in response to endoplasmic reticulum stress
Lagging strand elongation	Intrinsic apoptotic signaling pathway in response to endoplasmic reticulum stress
CENP-A containing nucleosome assembly	Positive regulation of vascular endothelial growth factor production
Kinetochores assembly	Lipopolysaccharide-mediated signaling pathway
Protein localization to kinetochores	Cellular response to glucose starvation
Telomere maintenance via semi-conservative replication	IRE1-mediated unfolded protein response
DNA replication initiation	Cardiac muscle tissue morphogenesis
Regulation of mitotic cell cycle spindle assembly checkpoint	Type I interferon signaling pathway
Mitotic chromosome condensation	Regulation of response to endoplasmic reticulum stress

E. We made additional progress on rat and mouse models of PAH. We isolated PSMCs from mice exposed to hypoxia (10% O₂) plus weekly injections of SU5416 (SUGEN) for 3 weeks. The isolated PSMCs were stained for smoothelin to confirm their SMC identity. Compared to the normoxic controls (which also received vehicle injections), mice injected with SU5416 had increased RV weight (i.e., RV hypertrophy) relative to the LV and septum (S) (RV/[LV + S]):Fulton index) (**Figure 8**). RNA-seq analysis of PSMCs from control and SUGEN mice had few differences in gene expression, which contrasts with data shown above for PSMCs in PAH patients (**Figure 7; Table 1**).

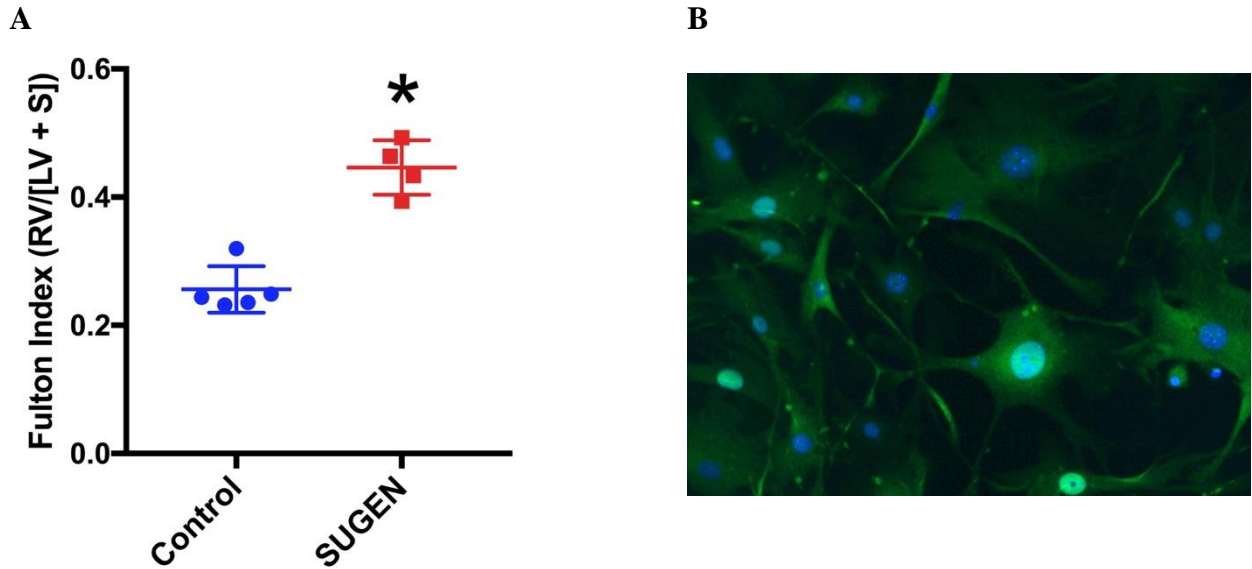
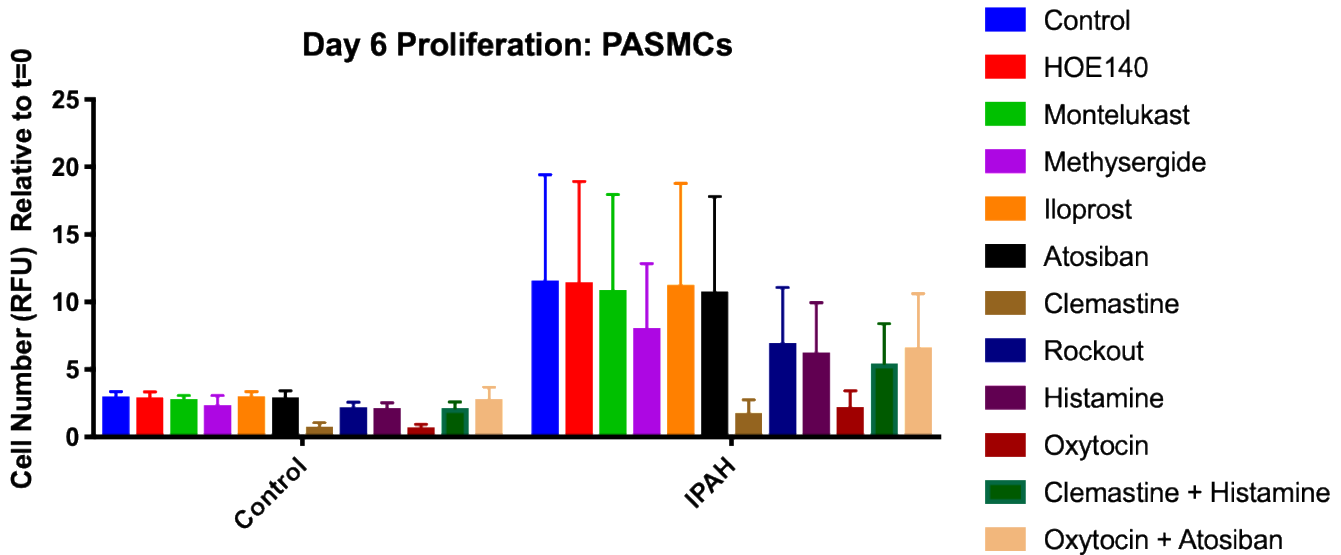


Figure 8: (A) Fulton index of mice exposed to normoxia or hypoxia with SUGEN (SU5416) injections for three weeks. * indicates $P < 0.05$ via t-test vs. control (B) Mouse PASMCs probed by immunocytochemistry for smoothelin, a SMC marker).

F. We utilized the CyQuant assay (Thermo Fisher Scientific) to measure proliferation in human PASMCs in response to various agonists. **Figure 9** shows the response of IPAH-PASMCs and control PASMCs to HOE140 (bradykinin 2 receptor antagonist), Montelukast (leukotriene receptor antagonist, methyl sergide (serotonin antagonist), iloprost (prostacyclin PGI2 analogue), atosiban (oxytocin receptor antagonist), clemastine (histamine receptor 1 antagonist), Rockout (Rho kinase inhibitor), histamine and/or oxytocin. We additionally exposed cells to hypoxia (1% O₂ for 24 hrs) in combination with clemastine and/or histamine. None of the agents significantly altered PASMC proliferation although proliferation was greater for IPAH-PASMCs.



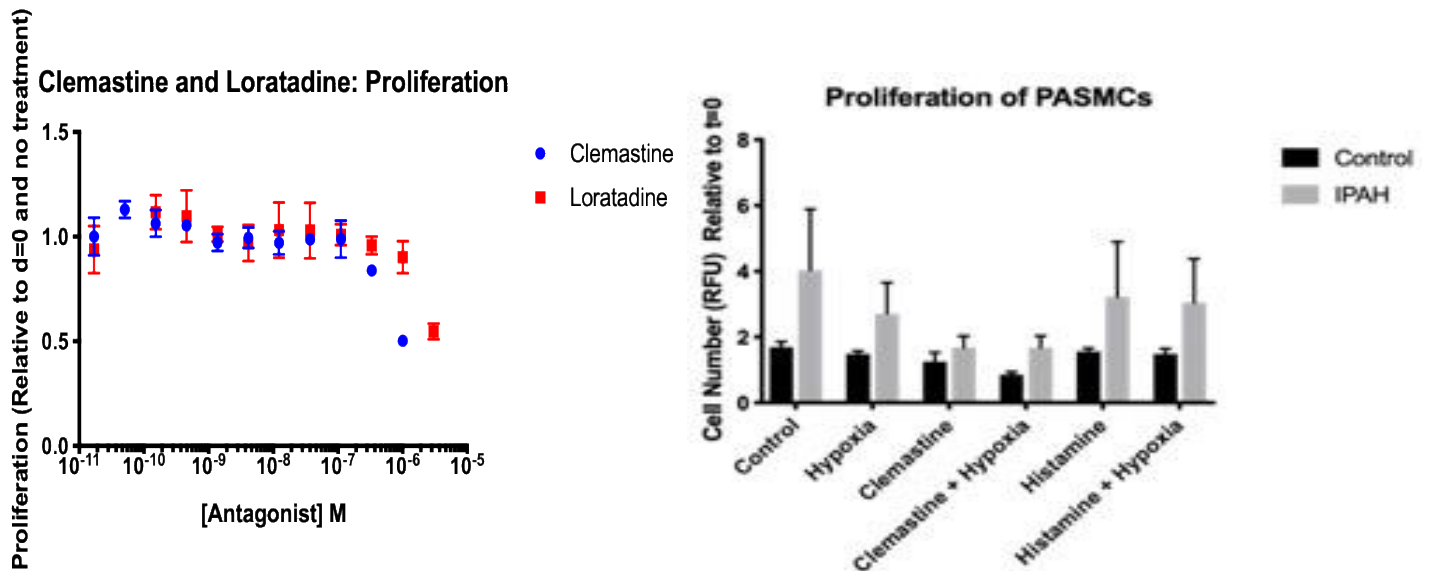


Figure 9: Top: Proliferation of PAMSCs from control and IPAH lungs in response to GPCR agonists and antagonists. PAMSCs from control (n=3) and IPAH (n=2) patients were treated with HOE140 (100 nM, bradykinin 2 receptor antagonist), Montelukast (100 nm, leukotriene receptor antagonist, Methysergide (1 μ m, serotonin antagonist), Iloprost (1 μ m, prostacyclin PGI2 analog), Atosiban (10 μ m, oxytocin receptor antagonist), Clemastine (1 μ m, histamine receptor 1 antagonist), Rockout (50 μ m, Rho kinase inhibitor), histamine (10 μ m) and/or oxytocin (100 nm). Treatments were applied daily for 6 days. IPAH significantly increased proliferation, but none of the drugs had a significant affect (2-way ANOVA, $P>0.05$). Bottom left: Clemastine and an additional HRH1 antagonist, loratadine, were tested at lower concentrations and had no significant effect on proliferation of PAMSCs after 3 days of treatment. Bottom right: cells were treated with hypoxia (1% O₂ for 24 hours) or drugs as indicated and proliferation was measured.

G. We performed RNA-seq. on tissue isolated from control rats and rats exposed to hypoxia (10% for 14 days) from the RV and LV. We found significant transcriptomic changes (**Figure 10**; red dots) in the RV compared to the LV in both normoxia and hypoxia; in the RV, hypoxia increased the number of genes with significant changes in expression. Unexpectedly, the transcriptomic analysis indicated that many genes involved in immune function (e.g. CD3, MHC class II antigens) had higher expression in the RV. Therefore, we performed flow cytometry and found that the RV in both normoxia and hypoxia contains a significantly greater percentage of CD45⁺/CD11b⁺/c⁺ cells (**Figure 11**), results implying an increased number of macrophages in the RV than in the LV. This finding will be further evaluated in future studies.

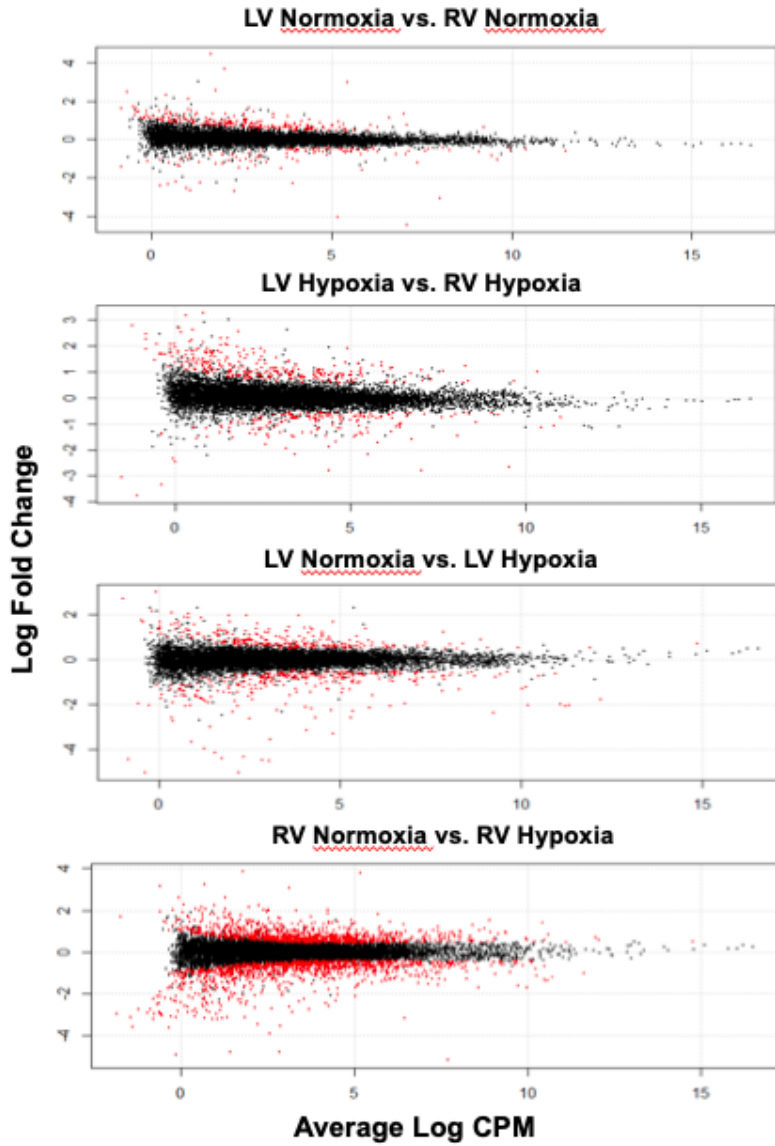


Figure 10: RNA-seq. of RV and LV tissue from rats treated with hypoxia or control (normoxia). Y-axis indicates log fold change, and x-axis indicates average log counts per million (CPM, measure of expression)

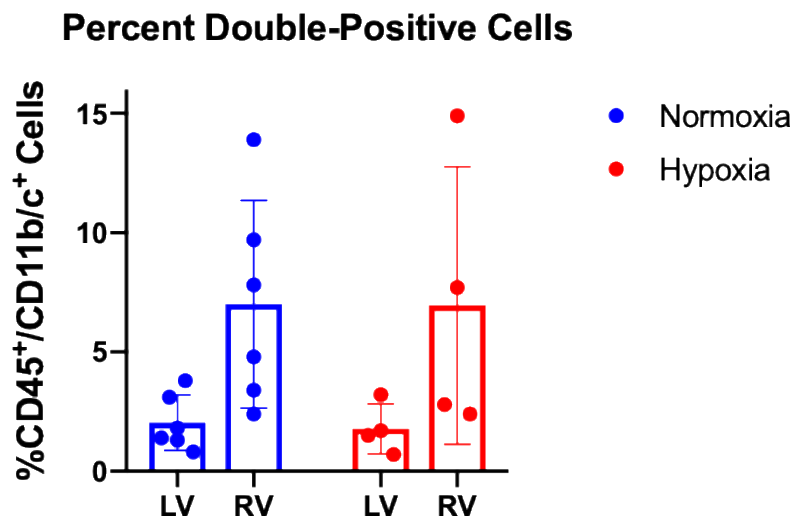


Figure 11: Flow cytometry of LV and RV cells of normoxia- and hypoxia-treated rats. P<0.05 for ventricle difference, two-way repeated measures ANOVA, n=4-6 rats.

What opportunities for training and professional development has the project provided?

Mathew Gorr, PhD, a post-doctoral fellow, has devoted most of his efforts to this project. He also received support from an NHLBI-funded Cardiology Training Grant and participated in training grant activities, including making two presentations to faculty and trainees regarding his findings on this project. Dr. Gorr also served as an organizer of a one day retreat for participants on this Training Grant. Additionally, he presented findings from this project in two additional presentations at the 2018 Experimental Biology meeting in addition to those at the 2017 Experimental biology meeting. Additionally, this project provided training for three volunteer-based UC San Diego undergraduate students, Esther Wu, Jason Liao and Abinaya Muthusamy, each of whom subsequently enrolled in independent research elective courses to continue work on this project.

How were the results disseminated to communities of interest?

During the past year, poster and oral presentations from this project were given at the 2018 Experimental Biology meeting (April 2018). Abstracts are listed below. Additionally, three manuscripts are in preparation that have derived from this work.

Impact

What was the impact on the development of the principal discipline(s) of the project?

Our findings indicate that multiple types of vascular smooth muscle cells, including PASMCs, coronary artery SMCs and aortic SMCs, express >70 GPCRs that may regulate cellular signaling and function. Of particular note, work on this project discovered that the adhesion GPCR ADGRG6/GPR126 has increased expression in PASMCs from PAH patients and appears to regulate PASMC proliferation. GPCRs with known agonists (the focus of this project), appear to have previously unappreciated effects in normal and diseased PASMCs. We also discovered that multiple GPCRs have different expression in PAH- and control-PASMCs. GPCRs with increased expression in PAH-PASMCs may contribute to PAH pathophysiology and may be therapeutic targets for PAH. Additionally, this project has revealed that the right ventricle (RV) may be an important but previously overlooked factor in PAH and other diseases.

What was the impact on other disciplines?

The notion that individual cell types express many more types of GPCRs than were previously known is potentially important for the regulation of cells and tissues in health and disease. The results have impact on cell biology, biochemistry, physiology, pharmacology and pathology, as well as clinical medicine, especially if the newly recognized GPCRs can further understanding of pathophysiology and be used to aid in diagnosis, assessment of prognosis and/or be therapeutic targets in disease states.

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

CHANGES/PROBLEMS

None.

PRODUCTS

Publications, conference papers and presentations from this work during this entire work:

Targeting the Right Ventricle as a Treatment Strategy for Pulmonary Arterial Hypertension MW Gorr, W Liang, A Muthusamy, K Sriram, PA Insel FASEB JOURNAL 32 (1) Presented at Experimental Biology 2018, San Diego, CA.

ADGRG6/GPR126 as a Novel Therapeutic Target in Pulmonary Arterial Hypertension A Muthusamy, MW Gorr, AM Chinn, PA Insel FASEB JOURNAL 32 (1) Presented at Experimental Biology 2018, San Diego, CA.

Transcriptomic analysis of the right ventricle reveals novel biology in rats. Gorr, M.W., Sriram, K., Chinn, A.M., Muthusamy, A., Insel, P.A. *In preparation*.

Transcriptomic analysis of pulmonary arterial smooth muscle cells from patients with pulmonary arterial hypertension reveals ADGRG6/GPR126 as a novel therapeutic target. Gorr, M.W., Muthusamy, A., Sriram, K., Insel, P.A. *In preparation*.

Cellular GPCRomics as a tool for drug discovery. Insel, PA, Sriram K, Gorr, MW, Michkov A, Wiley SZ, Chinn AM , *Trends in Pharmacological Sciences*, in preparation.

MW Gorr, K Sriram, PA Insel. GPCRs in Pulmonary Arterial Smooth Muscle Cells as Novel Targets in Pulmonary Arterial Hypertension. Experimental Biology, April 2017, San Diego, CA. Winner of ASPET Postdoc Award. The FASEB Journal 31 (1 Supplement), 664.11-664.11

MW Gorr, K Sriram, PA Insel Transcriptomic Analysis of the Right and Left Ventricle in Normoxia and Hypoxia (a Model of Pulmonary Arterial Hypertension). Experimental Biology, April 2017, San Diego, CA. The FASEB Journal 31 (1 Supplement), 884.8-884.8

Krishna Sriram, Nakon Aroonsakool, Alexander V. Michkov, Paul A. Insel GPCR expression in pulmonary arterial smooth muscle cells: Novel targets for pulmonary hypertension. American Thoracic Society International Conference, May 2016. San Francisco, CA.

Nakon Aroonsakool, Krishna Sriram, Alexander V. Michkov, Paul A. Insel. GPCRs as novel targets in pulmonary artery smooth muscle cells in pulmonary arterial hypertension. Experimental Biology, April 2016. San Diego, CA.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Paul A. Insel

Project Role: Principal Investigator

Researcher Identifier: 402799

Nearest person month worked: 2

Contribution to Project: Directed all phases of the project

Funding Support: this project

Name: Krishna Sriram

Project Role: Post-doctoral fellow

Researcher Identifier: 791836

Nearest person month worked: 3

Contribution to Project: Performed RNA-Seq analysis; analyzed RNA-seq, GPCR data

“Caveolin 3 and cardiac fibrosis”

Major goals: To determine the role of cardiac myocyte-expressed caveolin-3 in regulating fibrotic activity of cardiac fibroblasts with aging in mice.

What other organizations were involved as partners?

None

8. SPECIAL REPORTING REQUIREMENTS

None

9. APPENDICES

None