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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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# REPORT DOCUMENTATION PAGE

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<b>13. SUPPLEMENTARY NOTES</b>					
<b>14. ABSTRACT</b> 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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## 1. 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 isolate PASMCs from PAH subjects and controls, quantify the GPCR expression profile of the PASMCs and seek to identify (and then validate in signaling and functional studies) GPCRs that have known physiologic agonists and whose expression prominently increases in PAH-PASMCs. We speculate that such GPCRs may be novel therapeutic targets for PAH.

## 2. KEYWORDS

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

## 3. ACCOMPLISHMENTS

### **What were the major goals of the project?**

The 3 Aims/goals of the project are to: 1) Define the expression of GPCRs that have known physiologic agonists in human, rat and mouse PASMCs. 2) Determine if expression of PASMC-GPCRs changes in PAH and if GPCRs with altered expression contribute to the pathophysiology of PAH. 3) Ascertain the therapeutic potential of such PAH-PASMC-expressed GPCRs.

### **What was accomplished under these goals in this reporting period**

#### **1) Major Activities:**

The major task of Aim 1 is to isolate control PASMCs from humans, rats and mice, use the PASMCs to identify and quantify GPCRs that have known physiologic agonists, confirm GPCR mRNA expression (using independent qPCR) and assess protein expression and responses for a subset of GPCRs. Aim 1 will also determine if the GPCR profile of PASMCs differs from that of coronary artery and aortic smooth muscle cells (SMCs). We have made progress on several subtasks during this reporting period, including: **a)** Isolation and preparation of primary cultures of PASMCs from the lungs of subjects who do not have PAH; **b)** Preparation of RNA and cDNA from PASMCs and use of Taqman GPCR arrays and RNA-seq to identify and quantify GPCR expression; **c)** Preparation of RNA/cDNA from commercially obtained coronary artery and aortic SMCs and identification/quantification of GPCR expression; **d)** Isolation and culture of PASMCs from Sprague Dawley rats and C57/BL6 mice prior to preparing RNA/cDNA and assessment of GPCR expression in the PASMCs; and **e)** Confirmation of mRNA expression by independent qPCR analyses of highly expressed GPCRs from PASMCs.

Aim 2's major task is to isolate PASMCs from subjects with PAH and determine if expression of GPCRs that have known agonists differs from that of control PASMCs and if such GPCRs may contribute to the pathophysiology of PAH. The primary subtask in this reporting period has been to isolate and culture PASMCs from PAH subjects and to use a chronic hypoxia model of PAH in rats prior to isolation and culture of PASMCs, RNA/cDNA isolation and GPCR analysis.

The major task of Aim 3 is to determine the therapeutic potential of preferentially expressed PAH-PASMC GPCRs that have physiologic agonists. Criteria we use to choose GPCRs are: GPCRs with the greatest increase in expression in PAH-PASMCs and predicted to impact on PAH pathophysiology, similarity of GPCR changes with PAH in humans and experimental animals, and GPCRs for which drugs (in particular, FDA-approved drugs) are available. As described below, results from efforts in this reporting period suggest that we have identified at least one potential candidate GPCR.

## **2) Specific objectives:**

- a) Collect lung samples from PAH patients and controls
- b) Establish PASMC cultures from patients and controls
- c) Isolate RNA and prepare cDNA from PASMCs; identify and quantify GPCR expression
- d) Confirm RNA expression of GPCRs using independent qPCR.
- e) Obtain and culture commercially available human coronary artery and aortic SMCs, isolate RNA and prepare cDNA from those cells and quantify GPCR expression.
- f) Isolate and grow PASMCs from rats and mice, prepare RNA/cDNA from those PASMCs and quantify GPCR expression
- g) Establish rat (chronic hypoxia, monocrotaline) and mouse (chronic hypoxia and Sugden) models of PAH, isolate/culture PASMCs from the animals, prepare RNA/cDNA from those PASMCs and quantify GPCR expression.
- h) Isolate PASMCs from humans with PAH and determine if those PASMCs have altered GPCR expression, including of GPCRs that may contribute to the pathophysiology of PAH.
- i) Undertake functional studies of GPCRs with increased expression in PAH-PASMCs

## **3) Significant results:**

During this reporting period, we have made progress on each of the Specific objectives above:

- a) and b) The plan established in Year 1, by which we obtain lung samples from UCSD patients who undergo surgery for PAH or other conditions, has yielded samples and PASMC cultures from 4 PAH patients, 5 non-PAH subjects, and 1 patient with pulmonary vascular occlusive disease (PVOD).

c) We isolated RNA and generated cDNA from the patient samples noted above. In prior experiments (in year 1), we used Taqman GPCR arrays (Life Technologies) to identify and quantify expression of ~355 non-chemosensory (other than odorant, taste, visual) GPCRs and additional mRNAs, including ones (e.g., 18S rRNA) used to normalize results for the GPCRs. We cluster the GPCRs based on their coupling to the heterotrimeric G proteins  $G_s$ ,  $G_{i/o}$ ,  $G_{q/11}$ ,  $G_{12/13}$  and use a “weighting” method to define each GPCR’s contribution. As shown in **Table 1**, PASMCS from 4 control subjects expressed 112-144 (mean = 123) GPCRs; 73 GPCRs are shared among the 4 control PASMCS. **Table 2** shows the levels of expression ( $\Delta Ct$  [cycle-threshold] values) of the 20 highest expressed GPCRs in control PASMCS, their G-protein linkage and ligand (if known). **Table 3** shows those linkages for the 50 highest expressed GPCRs; certain GPCRs have multiple such linkages so the total number of GPCRs is > 50. The largest number of GPCRs link to  $G_q$  or have unknown linkages. **Table 4** shows the known physiologic agonist and G-protein linkages of the highest expressed GPCRs for PASMCS for which that information is known. Preliminary RNA-seq data (not shown) confirm the results for GPCRs shown in **Tables 1 and 2**.

**Table 1:** Number of GPCRs expressed in each human smooth muscle cell culture

Sample	# of GPCRs
Control 1 (commercial) PASC	112
Control 2 (commercial) PASC	120
Control 3 (commercial) PASC	116
Control 4 (patient 1) PASC	144
Coronary artery 1 (commercial) SMC	114
Coronary artery 1 (commercial) SMC	114
Aortic (commercial) SMC	129
IPAH, pt1 PASC	126
IPAH, pt2 PASC	103
IPAH, pt3 PASC	126
IPAH, pt4 PASC	Not Yet Run
IPVOD PASC	132

**Table 2:** Top 20 highest expressed receptors in control human PASMCs

Gene Symbol	Gene Name	Ligand	Primary Linkage	$\Delta Ct$ (18s)
EDG2	lysophosphatidic acid receptor 1	LPA	Gi/Go, Gq/G11, G12/G13	14.12
FZD6	frizzled class receptor 6	Wnt-3a	Gi/Go, Gq/G11	14.29
LPHN2	adhesion G protein-coupled receptor L2	Orphan	Unknown	14.57
GPR176	G protein-coupled receptor 176	Orphan	Unknown	14.95
ELTD1	adhesion G protein-coupled receptor L4	Orphan	Unknown	15.01
FZD4	frizzled class receptor 4	norrin	G12/G13	15.62
F2R	coagulation factor II (thrombin) receptor	thrombin	Gi/Go, Gq/G11, G12/G13	15.70
CD97	adhesion G protein-coupled receptor E5	Orphan	G12/G13	15.71
GPR124	adhesion G protein-coupled receptor A2	Orphan	Unknown	15.77
BDKRB1	bradykinin receptor B1	bradykinin	Gi/Go, Gq/G11	15.81
C11ORF4	not found	Orphan	Unknown	16.03
GPRC5B	G protein-coupled receptor, class C, group 5, member B	Orphan	Unknown	16.10
GPRC5A	G protein-coupled receptor, class C, group 5, member A	Orphan	Unknown	16.29
BDKRB2	bradykinin receptor B2	bradykinin	Gs, Gi/Go, Gq/G11	16.30
GPR125	adhesion G protein-coupled receptor A3	Orphan	Unknown	17.21
ADORA2B	adenosine A2b receptor	adenosine	Gs	17.50
GABBR1	gamma-aminobutyric acid (GABA) B receptor, 1	GABA	Unknown	17.73
PTGFR	prostaglandin F receptor (FP)	PGD <sub>2</sub>	Gq/G11	17.99
GPR161	G protein-coupled receptor 161	Orphan	Unknown	18.10

**Table 3:** Linkage of the 50 highest expressed GPCRs in control subjects and PAH patients

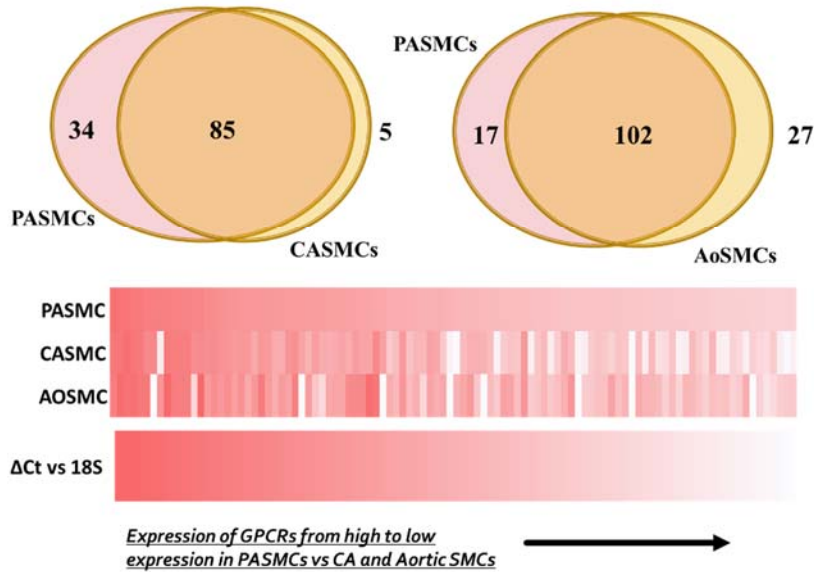
GPCR Classification	# of GPCRs detected in Control Patients	# of GPCRs detected in PAH Patients
GPCRs <i>without</i> known <i>G<math>\alpha</math></i> linkage	18	20
<b>Gi coupled</b>	13	14
<b>G12/13 coupled</b>	6	6
<b>Gq coupled</b>	18	17
<b>Gs coupled</b>	8	6

**Table 4:** Highest expressed GPCRs with known ligands and G protein linkages in control-PASMCs

Gene ID	Gene Name	Ligand	Linkage	Mean $\Delta Ct$ (18s)
EDG2	lysophosphatidic acid receptor 1	LPA	Gi/Go, Gq/G11, G12/G13	14.12
FZD6	frizzled class receptor 6	Wnt-3a	Gi/Go, Gq/G11	14.29
FZD4	frizzled class receptor 4	norrin	G12/G13	15.62
F2R	coagulation factor II (thrombin) receptor	thrombin	Gi/Go, Gq/G11, G12/G13	15.70
BDKRB2	bradykinin receptor B2	bradykinin	Gs, Gi/Go, Gq/G11	16.30
ADORA2B	adenosine A2b receptor	adenosine	Gs	17.50
FZD7	frizzled class receptor 7	Wnt	Gs, Gi/Go	18.62

**d)** We obtained human coronary arterial (n=2) and aortic SMCs (CASMCs, AoSMCs) from a commercial source, cultured these cells, prepared RNA, generated cDNA and used GPCR arrays to identify and quantify GPCRs expressed by the cells. The CASMCs and AoSMCs express a similar number of GPCRs as do the PASMCs. Many of the most highly expressed GPCRs with known agonists are similar in PA, coronary arterial and aortic SMCs (**Table 1; Figure 1**).

**PASMCs uniquely/differentially express a number of GPCRs compared to aortic and coronary arterial SMCs**



**Figure 1:** Similar expression of GPCRs in PASMCs, CASMCs and AoSMCs.

e) We obtained lungs from 3 month-old Sprague-Dawley rats and C57/BL6 mice, isolated and cultured PASMCs, prepared RNA/cDNA and assayed GPCR expression using Taqman GPCR arrays.

f) Rats were subjected to chronic hypoxia (2 weeks, 10% O<sub>2</sub>) to induce PAH. Following chronic hypoxia, PASMCs were isolated, cultured, RNA was isolated, and cDNA was generated. We are currently assessing gene expression via RNA-Seq. **Figure 2** shows preliminary data using Taqman arrays. Many of the highest expressed GPCRs are similar in human, rat and mouse PASMCs. Both male and female rats treated in chronic hypoxia had a higher Fulton index value, a measure of right ventricular hypertrophy (**Figure 3**).

## GPCR expression is similar in human and rodent PSMCs

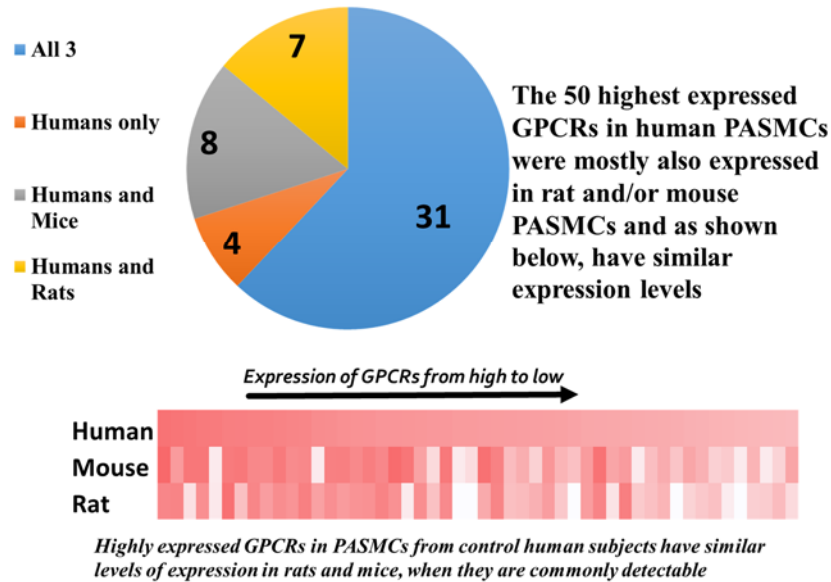


Figure 2: GPCR expression compared in humans, rats and mice

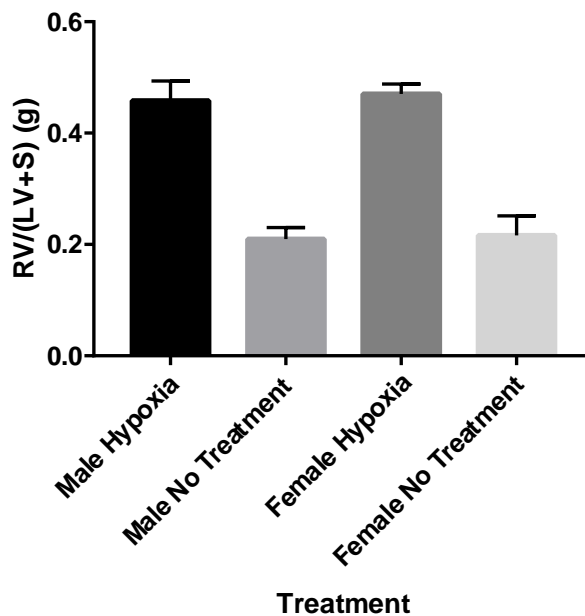


Figure 3: Fulton index (right ventricular [RV] weight divided by left ventricular [LV] weight and septum [LV + S]) in untreated male and female control rats and rats exposed to hypoxia.

h) As noted in c) above, we obtained lung samples and have grown PSMCs from patients with PAH and non-PAH subjects and a patient with PVOD. The primary cultures of PSMCs have been used at low passage for studies of expression of GPCRs and other mRNAs. As shown in **Table 1**, the overall number

of GPCRs was similar in the non-PAH-, PVOD (pulmonary veno-occlusive disease)- and PAH-PASMCs. **Table 6** lists the 20 highest expressed GPCRs in the PAH-PASMCs and their ligands and linkages (if known). The most highly expressed GPCRs are generally similar to those in control PASMCs (**Table 2**) and the G-protein linkage patterns, especially of those with known ligands (**Table 7**), of the GPCRs is similar to that of control-PASMCs (**Table 3**).

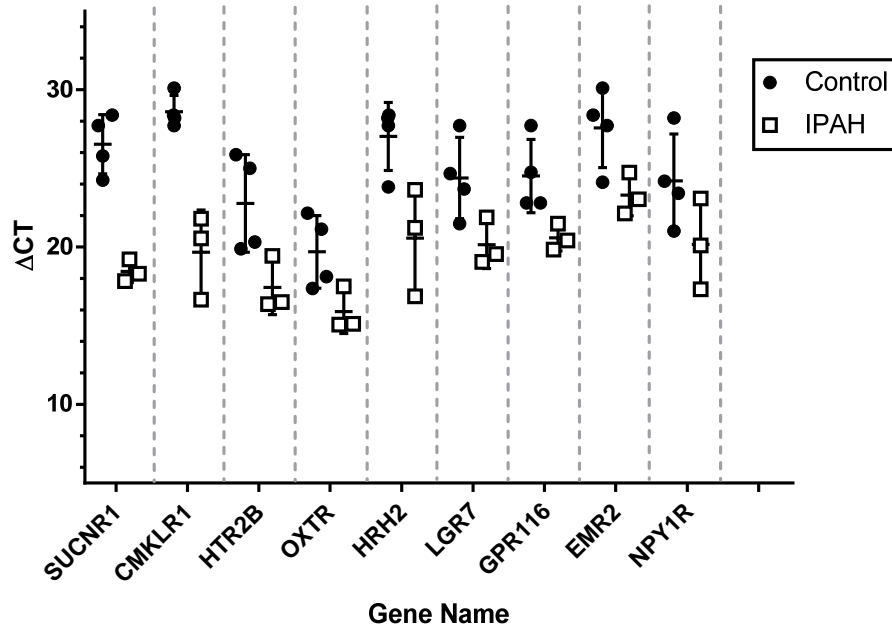
**Table 6:** The 20 highest expressed GPCRs in PAH-PASMCs

Gene Symbol	Gene Name	Ligand	Primary Linkage	$\Delta Ct$ (18s)
LPHN2	adhesion G protein-coupled receptor L2	Orphan	Unknown	14.01
FZD6	frizzled class receptor 6	Wnt-3a	Gi/Go, Gq/G11	14.80
GPR176	G protein-coupled receptor 176	Orphan	Unknown	14.94
EDG2	lysophosphatidic acid receptor 1	LPA	Gi/Go, Gq/G11, G12/G13	15.19
CD97	adhesion G protein-coupled receptor E5	Orphan	G12/G13	15.24
F2R	coagulation factor II (thrombin) receptor	thrombin	Gi/Go, Gq/G11, G12/G13	15.25
GPRC5A	G protein-coupled receptor, class C, group 5, member A	Orphan	Unknown	15.54
FZD4	frizzled class receptor 4	norrin	G12/G13	15.76
C11ORF4	not found	Orphan	Unknown	15.82
GPR124	adhesion G protein-coupled receptor A2	Orphan	Unknown	16.21
OXTR	oxytocin receptor	arginine vasotocin	Gq/G11	16.38
ELTD1	adhesion G protein-coupled receptor L4	Orphan	Unknown	16.56
GPRC5B	G protein-coupled receptor, class C, group 5, member B	Orphan	Unknown	17.27
GPR153	G protein-coupled receptor 153	Orphan	Unknown	17.43
EDG3	sphingosine-1-phosphate receptor 3	sphingosine 1-phosphate	Gi/Go, Gq/G11, G12/G13	17.61
FZD7	frizzled class receptor 7	Wnt	Gs, Gi/Go	17.98
GPR1	G protein-coupled receptor 1	chemerin	Unknown	18.16
HTR2B	5-hydroxytryptamine (serotonin) receptor 2B, G protein-coupled	5-hydroxytryptamine	Gq/G11	18.18
GPR125	adhesion G protein-coupled receptor A3	Orphan	Unknown	18.20
TM7SF1	G protein-coupled receptor 137	Orphan	Unknown	18.21

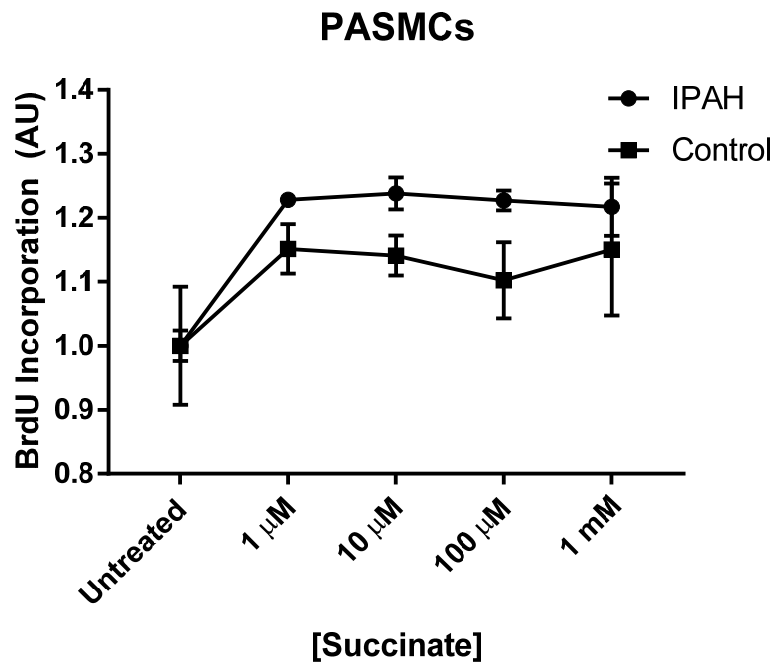
**Table 7:** Highest expressed GPCRs with known ligands and linkages in the PAH-PASMCs

Gene ID	Gene Name	Ligand	Linkage	Mean $\Delta Ct$ (18s)
FZD6	frizzled class receptor 6	Wnt-3a	Gi/Go, Gq/G11	14.80
EDG2	lysophosphatidic acid receptor 1	LPA	Gi/Go, Gq/G11, G12/G13	15.19
F2R	coagulation factor II (thrombin) receptor	thrombin	Gi/Go, Gq/G11, G12/G13	15.25
FZD4	frizzled class receptor 4	norrin	G12/G13	15.76
FZD7	frizzled class receptor 7	Wnt	Gs, Gi/Go	17.98
BDKRB2	bradykinin receptor B2	bradykinin	Gs, Gi/Go, Gq/G11	18.67
ADORA2B	adenosine A2b receptor	adenosine	Gs	18.70

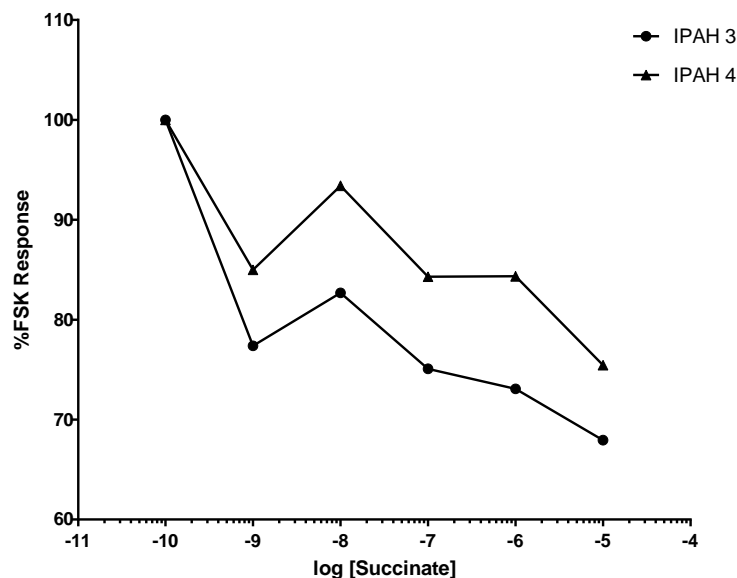
i) We identified several GPCRs with increased expression (lower  $\Delta Ct$ ) in IPAH- PASMCs compared to control PASMCs (**Figure 4**). We initiated functional studies to assess the role of the SUCNR1/GPR91, a GPCR that is activated by the metabolite succinate, in PAH- and control-PASMCs. Our initial studies suggest that activation of SUCNR1 with succinate, its cognate agonist, increases the proliferation of PAH-PASMCs to a greater extent than of control PASMCs (**Figure 5**). Succinate also decreases cAMP levels in PAH-PASMCs, a result consistent with the reported linkage of SUCNR1 to the  $G_i$  protein (**Figure 6**).



**Figure 4:** Increased expression of GPCRs in IPAH-PASMCs compared to control-PASMCs. Each point indicates an individual subject, bars indicate mean and standard deviation. The data are expressed as  $\Delta CT$  (cycle-threshold change), such that lower  $\Delta CT$  values indicate higher expression.



**Figure 5:** Succinate-stimulated BrdU Incorporation in PASMCs from IPAH and Control subjects (n=1 each). Error bars indicate standard deviation of technical replicates.



**Figure 6:** Percent response (cAMP accumulation) to *varying* succinate *concentrations* in the presence of forskolin, an adenylyl cyclase *activator*, in PASMCs from two IPAH patients

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

Mathew Gorr, PhD joined our laboratory in February, 2016 as a new post-doctoral fellow and has devoted most of his efforts to this project. He has received support from an NHLBI-funded Cardiology Training Grant and has participated in training grant activities, including having made two presentations to faculty and trainees about his findings on this project and assisted as an organizer of a one day retreat of participants on that Training Grant. Dr. Krishna Sriram, another post-doc working on this project, presented a poster on our findings at the 2016 American Thoracic Society (ATS) meeting and participated in activities for trainees at that meeting.

### **How were the results disseminated to communities of interest?**

Poster presentations of findings from this project were given at the 2016 Experimental Biology meeting (April, 2016) and ATS meeting (May, 2016). Abstracts for these posters are attached below.

### **What do you plan to do during the next report period to accomplish the goals?**

We will undertake additional analysis using RNA-seq to assess GPCR expression and relate the expression of GPCRs to that of other RNAs, whose expression may change with PAH. We seek to increase the number of subjects for studies in Aims 1 and 2 and in particular to determine if there is a “PAH-specific GPCR expression profile”. We will expand the studies related to SUCNR1/GPR91, an unexpected, potential contributor to the pathophysiology of PAH and perhaps a therapeutic target. The studies will include protein analysis (using antibodies and other proteomic techniques), and assessment of functional activities, including signaling events, DNA synthesis and effects on cell growth and death. Pending the

outcome of those studies and available time, we will also initiate studies of other GPCRs (**Figure 4**) with enhanced expression in PAH-PASMCs.

#### **4. IMPACT**

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

Our findings strongly suggest that multiple types of vascular SMCs express >70 GPCRs, each of which has the potential to regulate SMC function. GPCRs with known agonists (the focus of this project), may have previously unappreciated effects in normal and diseased PASMCs. Of particular note is our discovery of SUCRN1/GPR91 (the succinate receptor) as a GPCR whose expression differs between PAH-PASMCs and control-PASMCs. SUCRN1 and perhaps other GPCRs with enhanced expression in PAH-PASMCs (**Figure 4**) may contribute to the pathophysiology of PAH and may thus be therapeutic targets for PAH.

##### **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, assessing prognosis and/or serve as 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

#### **5. CHANGES/PROBLEMS**

Nakon Aroonsakool, a staff member, who was vital to our efforts on this project (and was first author on an abstract and presented a poster on findings on this project at the EB2016 meeting), died unexpectedly in April, 2016. Others have “stepped up” to continue the many efforts in which Nakon participated but his loss has been wrenching for everyone working on this project.

#### **6. PRODUCTS**

##### **Publications, conference papers and presentations**

##### **Journal publications:**

K Sriram, N Aroonsakool, AV Michkov, PA. Insel. GPCR expression in pulmonary arterial smooth muscle cells: Novel targets for pulmonary hypertension. A27. ADVANCES IN PEDIATRIC SLEEP. American Thoracic Society (Publisher) (2016).

N Aroonsakool, K Sriram, AV Michkov, PA Insel. G protein-coupled receptor (GPCR) expression in pulmonary artery smooth muscle cells of human and experimental models of pulmonary arterial hypertension. *The FASEB Journal* 30 (1 Supplement), 774.22-774.22 (2016)

K Sriram, N Aroonsakool, A Michkov, PA Insel GPCRs as novel targets in pulmonary artery smooth muscle cells in pulmonary arterial hypertension. *The FASEB Journal* 30 (1 Supplement), 709.8-709.8 (2016)

## **7. 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 and a contract from Bristol-Myers Squibb

Name: Nakon Aroonsakool (deceased April, 2016)

Project Role: Lab Assistant

Researcher Identifier: 93600

Nearest person month worked: 6

Contribution to Project: Obtained lung samples, prepared and cultured PSMCs, assisted with preparation of RNA, cDNA and with GPCR arrays; set up animal models of PAH

Funding Support: this project

Name: Krishna Sriram

Project Role: Post-doctoral fellow

Researcher Identifier: 791836

Nearest person month worked: 3

Contribution to Project: Obtained lung samples, performed GPCR analysis and analyzed all GPCR data

Funding Support: this project and a contract from Bristol-Myers Squibb

Name: Matthew Gorr

Project Role: Post-doctoral fellow

Researcher Identifier: 306535

Nearest person month worked: 8

Contribution to Project: Set up animal models for PAH, obtained lung samples, prepared and cultured PSMCs, isolated RNA and prepared cDNA, performed GPCR data analysis, undertook protein and functional studies of GPR91

Funding Support: this project and a T32 NHLBI Cardiology Training Grant



terms of coupling to G-proteins (e.g. we find that Gq/G11- coupled GPCRs are the highest expressed receptors in all SMCs), so as to help evaluate their therapeutic potential based on signaling mechanisms as well as expression level. Independent qPCR validated the GPCR array data for PASMC-expressed GPCRs. In addition, GPCR profiles in rodent PASMCs correlated with the expression in human PASMCs.

These data thus show that human, rat and mouse PASMCs express ~120 GPCRs, a number of which appear to be potential drug targets for the treatment of PAH. Signaling and functional studies in progress seek to provide further evidence for this idea.

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**G protein-coupled receptor (GPCR) expression in pulmonary artery smooth muscle cells of human and experimental models of pulmonary arterial hypertension.** N Aroonsakool, K Sriram, AV Michkov, PA Insel. *The FASEB Journal* 30 (1 Supplement), 774.22-774.22 (2016)

Pulmonary Arterial Hypertension (PAH) occurs as a primary (idiopathic) disease (IPAH) and secondary to various conditions. Both types are characterized by increased pulmonary vascular resistance and remodeling of the pulmonary artery, which derives in part from the proliferation of pulmonary artery smooth muscle cells (PASMCs), and can lead to right ventricular (RV) heart failure. Current treatments for PAH are inadequate, especially in terms of impact on mortality; thus, new therapies are needed. Animal models that mimic features of human PAH have been used to assess pathophysiology and evaluate treatments for PAH and include the exposure of rodents to chronic hypoxia (in the absence or presence of the VEGFR inhibitor SU5416) or treatment of rats with monocrotaline. Our studies are testing the hypothesis that previously unrecognized G protein-coupled receptors (GPCRs) may contribute to PAH pathophysiology and may be new therapeutic targets. To begin to test this hypothesis, we used TaqMan® GPCR arrays as an unbiased approach to identify and quantify GPCRs in PASMCs of humans, rats and mice, including from patients with PAH and experimental PAH animal models. Development of PAH and RV hypertrophy in the animal models was confirmed by immunostaining/histology and quantified using the Fulton index (the [right ventricle]/[left ventricle + septum] weight). We found that PASMCs express >100 GPCRs and that the GPCR expression profiles of mouse and rat PASMCs are similar to that of human PASMCs. Moreover, we find numerous changes (of at least 2-fold) in GPCR expression of PASMCs from PAH patients compared to control PASMCs. GPCRs with altered expression include receptors that link to G proteins whose signaling effectors (e.g., cAMP, Ca<sup>++</sup>, etc.) are predicted to regulate PASMC proliferation and tone. These results show that PASMCs express many more GPCRs than previously known and that the GPCR expression profile differs in PAH-PASMCs. GPCRs with altered expression may contribute to altered function in PAH and thus may be novel therapeutic targets for this disease.

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**GPCRs as novel targets in pulmonary artery smooth muscle cells in pulmonary arterial hypertension.** K Sriram, N Aroonsakool, AV Michkov, PA Insel. *The FASEB Journal* 30 (1 Supplement), 709.8-709.8 (2016)

There is an unmet need for new therapeutics to treat pulmonary arterial hypertension (PAH): currently approved therapy has limited benefit in terms of overall mortality and many such treatments not only reduce vascular resistance in the pulmonary arterial tree but also have side-effects, including systemic hypotension. We sought to determine if certain G-protein-coupled receptors (GPCRs) are more highly expressed in pulmonary artery smooth muscle cells (PASMCs) from patients with PAH compared to controls and thus might be novel therapeutic target for the treatment of PAH.

**METHODS:** We performed GPCR expression profiling using qPCR-based Taqman GPCR arrays that identify and quantify mRNA expression of non-chemosensory endo-GPCRs. Profiling was done on PASMCs isolated from PAH and non-PAH patients. We also assessed GPCR expression in PASMCs from mice and rats and in SMCs from the human aorta and coronary artery.

**RESULTS:** PASMCs expressed ~120 GPCRs, including ~45 orphan (without known physiologic agonist) GPCRs. Numerous adhesion and frizzled receptors were detected: LPHN2, CD97, ELTD1 and GPR124 were among the highest expressed GPCRs in all human SMCs profiled. Expression and identities of the highest expressed GPCRs were consistent among biological replicates of non-diseased human PASMCs. GPCR expression profiles were similar, between non-IPAH PASMCs and SMCs from the aorta and coronary artery but PAH-PASMCs showed changes in GPCR expression. We identified ~20 receptors with at least 2-fold increases in expression in PASMCs from PAH patients compared to controls. These include numerous orphan receptors as well as GPCRs previously not known to play a role in PAH. In addition, a similar number of GPCRs had reduced expression in PAH-PASMC relative to control-PASMCs, indicating a subset of GPCRs that might be considered for gene-based therapies of PAH. GPCR expression profiling in mouse and rat PASMCs revealed a high degree of similarity with PASMCs from human subjects, highlighting the utility of animal models and cells for further investigation of the physiological/functional role for the GPCRs we identified.

**CONCLUSION:** Numerous orphan and previously under-studied GPCRs are expressed by PASMCs with a number of GPCRs having increases/decreases in PAH compared to control PASMCs. GPCR expression in PASMCs from rats and mice are similar to that of human PASMCs, highlighting the potential for use of animal models to study these GPCRs as potential therapeutic targets for PAH

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