10TH ANNUAL

# Young Investigator's Meeting

## on Smooth Muscle in Airways & Vascular Disease

CONFERENCE VENUE

**Thomas Jefferson University**
**Philadelphia, PA**

**May 17-19, 2017**

ORGANIZERS

Dr. Raymond Penn, Ph.D.
Dr. Reynold Panettieri, M.D.
Dr. Deepak Deshpande, Ph.D.
Dr. Steven An, Ph.D.

http://www.jefferson.edu/university/skmc/departments/medicine/our-research/center-translational-medicine/Meeting.html

# Wednesday May 17, 2017

Herbut Auditorium, College Building (Basement level)
Thomas Jefferson University, 1025 Walnut Street, Philadelphia

| | |
|---|---|
| 4:30 – 5:00 PM | Registration |
| 5:00 – 5:10 PM | Welcome and Meeting Overview |
| 5:10 – 6:50 PM | **Plenary Session I** |
| 5:10 – 6:00 PM | "Precision Medicine in Asthma: Endotyping and Biologics"<br>**Reynold Panettieri Jr.**<br>*Rutgers Institute for Translational Medicine and Science, Rutgers University, NJ, USA* |
| 6:00 – 6:50 PM | "Compartmentalized Signaling: The Next Frontier"<br>**Ren Ostrom**<br>*Chapman University, CA, USA* |
| 7:00 PM | **Dinner at CTM**<br>Center for Translational Medicine, 543 Jefferson Alumni Hall, 1020 Locust Street, Philadelphia. |

# Thursday May 18, 2017

Herbut Auditorium, College Building (Basement level)
Thomas Jefferson University, 1025 Walnut Street, Philadelphia

| | |
|---|---|
| 8:00 – 9:00 AM | Breakfast |
| 9:00 – 10:30 AM | **Plenary Session II** (Precision Medicine/Genetics/Epigenetics)<br>*Chairs: Charlotte Billington and Deepak Deshpande* |
| 9:00 – 9:10 AM | Introduction- Deepak Deshpande |
| 9:10 – 9:50 AM | "Substrate Specific Kinase Inhibitors"<br>**Paul Shapiro**<br>*University of Maryland, Baltimore, MD, USA* |
| 9:50 – 10:10 AM | GSTCD knockout mice show a decreased contraction of small airways following LPS treatment<br>**Bo Liu**, *University of Nottingham, UK* |
| 10:10 – 10.30 AM | Sp1 transcription factor promotes c-Abl gene expression and contributes to smooth muscle proliferation<br>**Jiaoyue Long**, *Albany Medical College, USA* |
| 10:30 – 10:40 AM | **Break** |

| | |
|---|---|
| 10:40 – 11:00 AM | Oligonucleotide therapy reduces lung inflammation in HDM-sensitized mice<br>**Sabrina Ramelli**, *University of South Alabama, USA* |
| 11:00 – 11:20 AM | Gene expression signatures of emphysema and terminal bronchiole number in a cross-sectional cohort of COPD<br>**Steven Booth**, *University of British Columbia, Canada* |
| 11:20 – 11:40 AM | microRNA-25 as a potential novel therapeutic target in asthma<br>**Mariam Ba**, *University of Nevada, USA* |
| 11:40 – 11:50 AM | **New Technologies Presentation (ProteinSimple)** |
| 12:00 – 2:00 PM | **Philly Style Lunch**<br>**Poster session**<br>**Exhibitors**<br>First Floor Jefferson Alumni Hall,<br>1020 Locust Street, Philadephia. |
| 2:00 – 5:00 PM | **Plenary Session III** (Regulation of contraction and signaling)<br>*Chairs: Dale Tang, Steven An* |
| 2:00 – 2:10 PM | Introduction - Steven An |
| 2:10 – 2:50 PM | "State of the Art Imaging Approaches for Airway Biology"<br>**Tom Rich**<br>*University of South Alabama, AL, USA* |
| 2:50 – 3:10 PM | $G\alpha_{12}$ mediates human airway smooth muscle cell contraction via a PI3K/ROCK axis-dependent mechanism<br>**Edwin Yoo**, *Rutgers Institute of Translational Medicine, NJ* |
| 3:10 – 3:30 PM | Free fatty acid 4/GPR120 receptor agonist TUG891- a novel bronchodilator of small airways<br>**Chantal Donovan**, *Monash University, Australia* |
| 3:30 – 3:50 PM | Break |
| 3:50 – 4:10 PM | Effects of plant-derived monoterpenes on human airway smooth muscle cell relaxation and calcium signaling<br>**Jessie Huang**, *Johns Hopkins University, USA* |
| 4:10 – 4:30 PM | Selective targeting of Epacs facilitates airway smooth muscle relaxation<br>**Martina Schmidt**, *University of Groningen, The Netherlands* |
| 4:30 – 4:50 PM | TNF$\alpha$-Nitric Oxide axis leads to $\beta$-adrenergic receptor dysfunction in asthma from human airway smooth muscle cells<br>**Manveen Gupta**, *Cleveland Clinic Foundation, USA* |
| 6:00 PM | **Group Dinner (multiple options)**<br>Little Nonna's (Italian) 13$^{th}$ and Locust Street<br>Emei (Chinese) 915 Arch Street<br>Tequila's (Mexican) 1602 Locust Street |

# Friday May 19, 2017

Herbut Auditorium, College Building (Basement level)
Thomas Jefferson University, 1025 Walnut Street, Philadelphia

| | |
|---|---|
| 8:00 – 9:00 AM | Breakfast |
| 9:00 AM – 12 PM | **Plenary Session IV** (Airway Remodeling and Smooth Muscle Growth)<br>*Chairs: Alaina Ammit and Thomas Murphy* |
| 9:00 – 9:10 AM | Introduction- Alaina Ammit |
| 9:10 – 9:50 AM | "Targeting Airway Remodeling: Challenges and Opportunities"<br>**Raymond Penn and Deepak Deshpande**<br>*Thomas Jefferson University, USA* |
| 9:50 – 10:10 AM | Altered ASM mass and contractile responses in an ovalbumin murine model of asthma during the inflammation resolution phase as assessed by PCLS and IHC<br>**Christopher Philp**, *University of Nottingham, UK* |
| 10:10 – 10:30 AM | Caveolar scaffolding domain peptide abrogates hyperoxia-induced remodeling in neonatal mice<br>**Elizabeth Vogel**, *Mayo Clinic, USA* |
| 10:30 – 10:50 AM | **Break** |
| 10:50 – 11:10 AM | EP2 and EP4 receptor agonists inhibit the airway sensory nerves that cause cough, a potential control that is lost in human smokers and a pre-clinical COPD model<br>**Michael Wortley**, *Imperial College, London, UK* |
| 11:10 – 11:30 AM | Signaling and functional consequences of EP receptor subtypes agonism on airway smooth muscle<br>**James Michael**, Thomas Jefferson University, USA |
| 11:30 – 11:50 AM | Airway and lung structure in adult rats previously subject to hypoxia-induced intrauterine growth restriction<br>**Kimberly Wang**, *Telethon Kids Institute, Australia* |
| 11:50 AM – 12 PM | Concluding Remarks, Presentation of Awards<br>Raymond Penn |
| 12:00 PM | Boxed Lunch<br>most heading to ATS; stragglers welcome to hang out at CTM in 543 Jefferson Alumni Hall |

# Posters

**P.1. Targeting GPCR (OGR1) with desmethyl benzodiazepine drugs in an allergen-induced murine asthma model**
Ajay P Nayak[1], Deepak A Deshpande[1], Roslyn Yi[1], Nadan Wang[1], Raymond B Penn[1]

**P2. Genetic variants in Glutathione S-transferase C-terminal domain (*GSTCD*) in COPD**
Bo Liu, S. Azimi, A. Henry, S. Miller, C. Billington, K. Probert, M. Kotlikoff, I. Sayers IP Hall

**P.3. Phosphorylation of GMFγ at Tyrosine 104 by c-Abl Regulates the Localization of Arp2/3 and N-WASP to Promote Airway Smooth Muscle Migration**
Brennan D Gerlach, Rachel A Cleary, Olivia J Gannon, Sixin Jiang, Ruping Wang, Dale D Tang

**P.4. TGF-β1 Induces HASM Cell Shortening and Airway Hyperresponsiveness Through a Smad3-dependent Signaling Pathway**
Christie A. Ojiaku[1,2], Gaoyuan Cao[2], Wanqu Zhu[3], Steven S. An[3], Reynold A. Panettieri[1,2]

**P.5. Epithelial-fibroblast crosstalk via Interleukin-1: the missing link between inflammation and remodeling in the asthmatic EMTU?**
Emmanuel T. Osei[1,2,3], Leila. Mostaco-Guidolin[1], Stephanie. Warner[1], Teal. Hallstrand[5], Wim Timens[2,3], Dirkje S. Postma[3,4], Irene H. Heijink[2,3,4], Corry-Anke Brandsma[2,3] and Tillie L. Hackett[1].

**P.6. miR-363 Modulates DAG Signaling in Asthmatic ASM**
Jon Evasovic, Saul Favela, Cherie A. Singer

**P.7. Cigarette smoke changes cAMP microdomains in lung slices**
Haoxiao Zuo[1, 2, 4], Bing Han[1, 2], Wilfred J. Poppinga[1, 2], Lennard Ringnalda[1], Viacheslav O. Nikolaev[3], Martina Schmidt[1, 2]

**P.8. A new twist to the mechanical cell fate identifies $\alpha_5\beta_1$ and $\alpha_v\beta_3$ as the primitive mechanosensing clusters forcing age-associated stiffening of vascular smooth muscle**
Wanqu Zhu[1,*], Byoung Choul Kim[2,5,6,*], Mingyi Wang[3,*], Abraham Isak[1], Robert Monticone[3], Jessie Huang[1], Jennifer L. Pluznick[4], Taekjip Ha[2,5,6], Edward G. Lakatta[3,†], and Steven S. An[1,7,†]

**P.9. Airway and lung structure in adult rats previously subject to hypoxia-induced intrauterine growth restriction**
Kimberley C Wang[1], Jude S Morton[2], Sandra T Davidge[2], Alexander N Larcombe[1], Alan L James[3], Graham M Donovan[4], Peter B Noble[5]

**P.10. The role of histone arginine methylation in gene expression of airway smooth muscle cells in asthma**
Klaudia Kaczmarek[1], Rachel L. Clifford[1], Jamie Patel[1], Dominick Shaw[1], James Dowden[2], Alan J. Knox[1]

**P.11. $EP_2$ and $EP_4$ receptor agonists inhibit the airway sensory nerves that cause cough, a potential protective control that is lost in human smokers and a pre-clinical COPD model**
Michael A Wortley, Sara J Bonvini, Sarah A Maher, Mark A Birrell, Maria G Belvisi

**P.12. Intrapulmonary Artery and Airway Reactivity is Altered in Precision Cut Lung Slices from a Rat Model of Pulmonary Arterial Hypertension**
Maggie Lam[1], Meaghan FitzPatrick[2], Rebecca Harper[3], Paul N. Reynolds[3], Jane E. Bourke[1]

**P.13. A unique floxed PKI mouse reveals PKA dependence of bronchorelaxing agents**
James V. Michael, Ajay P. Nayak, Roslyn Yi, Tung O. Chan, Raymond B. Penn

**P.14. The mitochondrial pathway in bitter taste receptor agonist-induced airway smooth muscle cell death**
Shi Pan and Deepak Deshpande

**P.15. Potential role for an imbalance between airway smooth muscle laminin α4 and α5 expression in asthma.**
P. Prabhala[1,5,6], D.B. Wright[1,5,6], C. Bitter[1,5,6], P. Robbe[1,5,6], T. Pera[7], B. Hinz[8], G. Kim[8], N.H.T. ten Hacken[2,5], M. van den Berge[2,5], W. Timens[3,5], H. Meurs[1,5,6], B.G.J. Dekkers[1,4,5,6]

**P.16. Maternal eCigarette vaping increases the risk of Th2 driven allergic asthma in the offspring.**
Kielan McAlinden[1,3], Yik Chan[1-3], Anudeep Kota[1,3], Varsha Komalla[1,3], Hui Chen[1-3], Brian Oliver[1-3], **Pawan Sharma**[1-3]

**P.17. Investigating Genome wide DNA methylation in Bronchial and Lung Fibroblasts from healthy individuals and individuals with COPD**
Rachel L. Clifford[1,3], Nick Fishbane[2], Poojitha Rajasekar[1], Julie L. MacIsaac[3], Lisa M. McEwen[3], Andrew J. Fisher[4], Michael S. Kobor[2], Alan J. Knox[1], Tillie-Louise Hackett[2].

**P.18. Functional genetics of lung function associated gene *GPR126***
Robert Hall, Ian Hall & Ian Sayers

**P.19. Identification of Endogenous, Disease Relevant Activators of TRPV4 in the Airways**
Sara J. Bonvini, Michael A. Wortley, Mark A. Birrell, Maria G. Belvisi

**P.20. The DP2/CRTh2 receptor is expressed by ASM cells in asthma and inhibition of its activation suppresses ASM migration**
Ruth Saunders, Michael Biddle, Latifa Chachi, Amanda Sutcliffe, Rachid Berair, Christopher E Brightling.

**P.21. Predictors of Long-term Physical Function Recovery in Survivors of Acute Respiratory Failure**
Sheetal Gandotra, MD[1]; James Lovato, MS[2]; Douglas Case, PhD[2]; Rita Bakhru MD, MS[1]; Kevin Gibbs, MD[1]; Michael Berry PhD[3], D. Clark Files, MD[1]; and Peter E. Morris[4]

**P.22. The role of diacylglycerol kinase in asthma**
Brenal K. Singh[1], Wen Lu[1], Amanda M. Paustian[1], Moyar Ge[2], Reynold A Panettieri[3], Angela Haczku, and Taku Kambayashi[1]

**P.23. GLUCOCORTICOIDS, A DOUBLE-EDGED SWORD IN ANTI-AIRWAY HYPERRESPONSIVENESS THERAPY?**
Benjamin Sheng Gui Tan[1], Hong Lam[2], Steven S. An[2] and Thai Tran[1]

**P.24. The fatty acid amide hydrolase inhibitor URB597 activates TRPA1 ion channels in primary human airway smooth muscle cells**
Xiaochen Liu, Bo Liu, Ian P Hall

**P.25. The role and regulation of c-Abl in airway smooth muscle contractility**
Yinna Wang, Alyssa C. Rezey, Olivia G. Gannon, Ruping Wang, and Dale D. Tang

**P.26. Blocking YKL-40 (chitinase-like protein chitinase 3) attenuates IL-13-induced airway hyper-responsiveness in a human precision cut lung slice model**
C.J. Koziol-White, V. Nezgovorova, L. Cohn, G. Chupp, and R.A. Panettieri Jr.

# Abstracts

**Signaling and functional consequences of E-prostanoid (EP) receptor subtype agonism on human airway smooth muscles**

A. Gavrila, T. Pera, A.P. Nayak, A. Ulichney, E. Farrell, T.O. Chan, R. B. Penn
Center for Translational Medicine, Thomas Jefferson University, Philadelphia, PA

**Introduction:** Various studies have noted beneficial and detrimental effects of prostaglandin E2 ($PGE_2$) in the airways. Since $PGE_2$'s effects are due to activation of four E-prostanoid (EP) receptors, we took advantage of recently-developed subtype-selective ligands to investigate the effect of singular or combined receptor activation in regulating signaling pathways and functional outcomes in human airway smooth muscle (HASM) cells.

**Methods:** HASM cells were stimulated with the EP2/3/4 agonists, isoprotenerol (ISO) (1μM) and $PGE_2$ (100nM) in a dose- (1-100nM) and time- (10 min-18 h) dependent manner. Immunoblotting was performed to determine protein kinase A (PKA) activation by assessing VASP and p-HSP20 signals. Cyquant was used to assess ligand effects on PDGF-stimulated cell proliferation.

**Results:** EP2 and EP4 agonists (100nM, 10min) induced nearly complete VASP phosphorylation (90% and 77%, respectively), similar to that induced by ISO (93%) and $PGE_2$ (94%), making it difficult to assess a potential synergistic/additive effect of EP2+EP4. At 1h, the effect of EP2+EP4 (10nM) was modestly superior (73%) to EP2 (50%) and EP4 (66%). At 3h, EP2+EP4 agonism (36%) was superior to EP2 (10%), EP4 (25%) and ISO (29%). This was also seen at 18h (20% by EP2+EP4), whereas PKA activation by single ligands was weak. EP3 agonism had no effect at any of the times assessed. p-HSP20 was induced by EP2 and EP4, but not EP3, agonism. Growth assays revealed that EP2 and EP4 agonists had strong anti-proliferative properties. At 10nM EP4 agonism was superior to EP2 and ISO in inhibiting PDGF-induced growth (47% compared to 16% and 36%). There was a modest cooperativity of the EP2+EP4 (69%) in inhibiting HASM proliferation, although this was constrained by the high efficacy of EP4 agonism (47%).

**Conclusion:** These data suggest that combined activation of EP2+EP4 receptors could account for the superior efficacy of $PGE_2$ over ISO with respect to amount and duration of PKA substrate phosphorylation and anti-mitogenic properties in HASM cells. Given EP3 agonism has been implicated in promoting cough, future studies should investigate whether EP2+EP4 agonism, or $PGE_2$ + EP3 antagonism could have any therapeutic benefits. Supported by grants HL58506 and AI11007

**Targeting GPCR (OGR1) with desmethyl benzodiazepine drugs in an allergen-induced murine asthma model**

Ajay P Nayak[1], Deepak A Deshpande[1], Roslyn Yi[1], Nadan Wang[1], Raymond B Penn[1]
[1] Center for Translational Medicine, Jeff Alumni Hall, Rm 543, 1020 Locust Street, Philadelphia, PA 19107

Asthma is an inflammatory disease driving significant respiratory morbidity and mortality. The disease is accompanied with a dysregulated immune response, excessive mucus secretion, airway hyperresponsiveness (AHR) and airway remodeling. Current clinical management of asthma is suboptimal, thus underscoring the need to develop novel therapeutics for effecting bronchodilation. More recently, desmethyl benzodiazepine drugs that selectively target γ-aminobutyric acid-A ($GABA_A$) receptors in the brain were identified as small molecule regulators of the 'proton-sensing' ovarian-cancer G-protein coupled receptor 1 (OGR1). Using a pre-clinical model for allergen-induced asthma, we evaluated the bronchoprotective competitiveness of two benzodiazepine class of drugs; viz, sulazepam and lorazepam. Wild-type ( WT C57BL/6) and OGR1 knock-out (OGR1KO) mice were treated with vehicle (10% DMSO), sulazepam (10 mM) or lorazepam (10 mM) and challenged with house dust mite (HDM) allergen for 5 days a week for a total of 3 weeks. Twenty-four hours post final challenge; mice were subjected to FlexiVent for evaluation for AHR, followed by collection of bronchoalveolar lavage (BALF) for assessing cellular infiltration and cytokine regulation in the airways, and finally the lung tissue was harvested for evaluating histopathological changes. In WT and OGR1KO mice, treatment with either drug yielded minimal to no attenuation of the HDM-induced inflammatory cellular and cytokine profiles in the airways. Furthermore, broad histopathological observations remained consistent in all experimental groups. However, sulazepam (Gs biased), but not lorazepam (Gq and Gs) treatment achieved protection from methacholine-induced airway hyperresponsiveness. This protection was dependent on OGR1, since sulazepam failed to provide protection from airway hyperreactivity in OGR1KO mice. Preliminary findings in our laboratory highlight the bronchoprotective effects of sulazepam in an OGR1-dependent manner. Current studies in the laboratory are focused on elucidating the mechanism by which the drug contributes to bronchorelaxation.

**Bronchoconstriction *per se* can promote airway smooth muscle contraction and proliferation through the effects of mechanical compression on airway epithelium**

Bo Lan[1#], Jennifer A. Mitchel[1#], Rebecca Hirsch[1], James P. Butler[1], and Jin-Ah Park[1]
[1]Department of Environmental Health, T.H Chan School of Public Health, Harvard University; [#]These two authors contributed equally to this work

**Rationale:** Airway hyperresponsiveness and increased airway smooth muscle (ASM) mass are defining characteristics of asthma, but the underlying etiologies of these processes remain unclear. Classical thought holds that the inflammatory environment plays a critical role in the development of both airway hyperresponsiveness and ASM hyperplasia. Here we propose an alternative: during asthmatic bronchoconstriction, mechanical compression imposed on the airway epithelium provokes epithelial cells to release pathologic mediators that promote airway smooth muscle contraction and proliferation.

**Methods:** Primary human bronchial epithelial (HBE) cells were cultured in air-liquid interface. Well-differentiated HBE cells were subjected to a 30cm $H_20$ pressure mimicking the effect of mechanical compression during bronchoconstriction. At 24hours post-compression, conditioned media were collected from both uncompressed (control) and compressed HBE cells. Minimal medium, not exposed to HBE cells, was used as a vehicle control. Primary human ASM cells were treated with minimal medium and conditioned media (CM) from control and compressed HBE cells for one hour, to determine the effect of CM on ASM contraction. Both baseline and histamine-induced contractions of ASM cells were measured by traction force microscopy. We used a pharmacological inhibitor of ET-1 receptor to test the role of epithelial cell-derived ET-1 on ASM contraction. Further, the ASM cells were incubated with CM for an hour per day for one week, to determine the effect of CM on ASM proliferation. Proliferation of these ASM cells was measured by EdU-staining.

**Results:** Compared to the effect of minimal medium, control CM had no effect on ASM contraction (5±2%, N.S.); by contrast, CM from compressed cells significantly increased their contraction (↑34±6%, $p<0.05$). When challenged with 10uM histamine, ASM cells incubated with control CM showed increased contraction (↑26±11%), whereas ASM cells incubated with compressed CM showed a significant increase over the control level (↑60±10% ($p<0.05$). Pretreatment of ASM cells with PD145065, an ET-1 receptor antagonist, completely blocked the increased baseline traction force caused by compressed CM. Moreover, compared to the effect of control CM, compressed CM treatment lead to increased proliferation of human ASM cells.

**Conclusions:** Our data suggest that even in the absence of inflammatory environment in the airway, mechanical compression induced by bronchoconstriction can lead to increased contraction and proliferation of ASM cells. Thus, through the compression effect on airway epithelium, bronchoconstriction itself can further promote bronchoconstriction and airway remolding. Taken together, these findings uncovered a novel vicious positive feedback cycle that perpetuates asthma pathogenesis.

**GSTCD Knockout Mice Show a Decreased Contraction of Small Airways Following LPS Treatment**

Bo Liu, S. Azimi, A. Henry, S. Miller, C. Billington, K. Probert, M. Kotlikoff*, I. Sayers IP Hall
Division of Respiratory Medicine, University of Nottingham D Floor, South Block, Queen's Medical Centre

**Introduction**: Genome-Wide Association Study (GWAS) meta-analyses have identified a significant association between genetic variants spanning glutathione S-transferase C-terminal domain containing (*GSTCD*) and lung function, suggesting GSTCD may play an important role in development of Chronic Obstructive Pulmonary Disease (COPD). The function of GSTCD in the airways is unclear, therefore we aimed to define the potential role of GSTCD in airway inflammation and contraction using precision cut lung slices from wild (WT) and GSTCD-/- knockout mice (MUT).

**Methods** Murine agarose-filled lungs were sliced (200 μM) from age and gender matched WT and MUT mice using a microtome (OTS-5000). Around 20 slices with cilia beating airways from the left lobe were prepared from each mouse. Contraction was studied after applying either a single dose of Mch (0.1 μM, 3-4 slices each animal) or different doses of Mch (0.001 to 100 μM, 2-3 slices per animal). After washing in HBSS, each slice was treated with 10 μg/ml Lipopolysaccharide (LPS) or vehicle (PBS) control for 24 hours. Microscopic study of contraction of the same airway was repeated before and after LPS treatment. The supernatant was collected and levels of TNFα production was measured (ELISA, R&D). All experiments were conducted blinded to treatment.

**Results** There were no differences in contraction of airways between the WT and MUT mice in response to Mch (EC50 of WT vs MUT animals: 100.01±20.67 vs 107.74±24.46 nM, p=0.855, n=6 animals/group). However, after LPS treatment, there was a 31.6% reduction in contraction in the MUT groups (% contraction of airways before and after LPS treatment: 61.36±2.98 % vs 42.16±6.57 %, p=0.023, n=6 animals). There was no difference between PBS and LPS treatment groups in WT animals. We also observed a significant increase (68.08% increase, $p$=0.0061) in TNFα

production induced by LPS in MUT lung slices compared to the WT LPS treated slices. This difference was not observed under baseline conditions ($p$= 0.2347).

**Conclusion:** *GSTCD* knockout mice showed an increased responsiveness to LPS (as determined by TNFα production) that was accompanied by a reduced contraction of small airways in precision cut lung slices. These data potentially highlight an unrecognised function of GSTCD in mediating inflammatory signals that effect airway calibre.

**Funding:** Medical Research Council, UK (G1000861)

**Phosphorylation of GMFγ at Tyrosine 104 by c-Abl Regulates the Localization of Arp2/3 and N-WASP to Promote Airway Smooth Muscle Migration**

Brennan D Gerlach, Rachel A Cleary, Olivia J Gannon, Sixin Jiang, Ruping Wang, Dale D Tang
Department of Molecular Cellular Physiology, Albany Medical College, New York

Airway smooth muscle migration plays a critical role in the development of the respiratory system, and has been implicated in the pathogenesis of airway remodeling, a cardinal feature of asthma. However, the mechanisms that dictate airway smooth muscle migration have not been completely elucidated. The Arp2/3 complex and its activator N-WASP are critical components for the formation of the lamellipodia and promote directed cell migration. Nevertheless, little is known about how each are regulated during this dynamic process. Glia Maturation factor-γ (GMFγ) is a member of the ADF/cofilin superfamily that is involved in the reorganization of the actin cytoskeleton. Previous studies have found that GMFγ can interact with Arp2 and that its phosphorylation at tyrosine 104 prevents this interaction. In this study, knockdown of GMFγ attenuated net distance, total distance, velocity, and directionality of human airway smooth muscle (HASM) cells as evaluated by time-lapse microscopy. Furthermore, immunofluorescence microscopy revealed that knockdown of GMFγ compromised the spatial distribution of Arp2/3 and F-actin creating a fragmented leading edge. Re-expression of wild-type GMFγ restored cell migration. Moreover, expression of non-phosphorylatable mutant (Y104F) in the knockdown cells inhibited migratory ability. However, expression of phospho-mimic GMFγ constructs (Y104D) restored migration of the knockdown cells. TIRF-live cell imaging revealed that GMFγ Y104F expressing cells exhibited decreased protrusion dynamics and a decreased actin stress fiber network as compared to cells expressing wild type GMFγ. This was accompanied by a shift in localization of GMFγ and phospho-N-WASP (Y256, an indication of N-WASP activation) to vinculin-positive focal adhesions. Expression of the GMFγ Y104D construct was able to shift the localization of phospho-N-WASP back to the leading edge and restored the actin stress fiber network. In conclusion, this study reveals a novel two-tiered regulatory mechanism where the phosphorylation states of GMFγ dictates localization of Arp2/3 and N-WASP within lamellipodia /focal adhesions to promote actin dynamics and smooth muscle migration.

**TGF-β1 Induces HASM Cell Shortening and Airway Hyperresponsiveness Through a Smad3-dependent Signaling Pathway**

Christie A. Ojiaku[1,2], Gaoyuan Cao[2], Wanqu Zhu[3], Steven S. An[3], Reynold A. Panettieri[1,2]
1University of Pennsylvania, Department of Systems Pharmacology and Translational Therapeutics, Philadelphia, Pennsylvania, United States. 2Rutgers The State University of New Jersey, Rutgers Institute for Translational Medicine and Science, New Brunswick, New Jersey. 3 Johns Hopkins University, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

**Introduction:** The mechanisms governing airway hyperresponsiveness (AHR) in asthma are incompletely understood. Transforming growth factor beta 1 (TGF-β1) plays an essential role in airway inflammation and remodeling in asthma. However, the role of TGF-β1 in AHR and human airway smooth muscle (HASM) cell shortening remains unclear. We hypothesize that TGF-β1 induces AHR in HASM cells through a Smad3-dependent signaling pathway.

**Methods:** Human precision-cut lung slices (hPCLS) were treated with TGF-β1 (100 ng/ml) overnight. Bronchoconstriction to carbachol was detected by analyzing changes in airway lumen area using a live-feed microscope and an Image Pro-Plus software macro. Smad3, Smad2, Smad4, or non-targeting siRNA was transfected into HASM cells prior to treatment and collection. Serum-starved HASM cells were treated with 10 ng/mL TGF-β1 for 24 h and/or carbachol (10 uM, 10 min). HASM cell protein was isolated and expression of phosphorylated myosin light chain (pMLC) and phosphorylated myosin phosphatase target subunit 1 (pMYPT1) was determined by immunoblot. Additionally, HASM cell contraction was measured by assessing alterations in cytoskeletal stiffness using magnetic twisting cytometry (MTC).

**Results:** Overnight TGF-β1 treatment induced a 36% reduction in hPCLS airway lumen diameter over control. Following a carbachol dose response, TGF-β1 pretreated hPCLS exhibited significantly increased area under the curve, sensitivity to carbachol, and total bronchoconstriction. TGF-β1 treatment also significantly increased basal and agonist-induced HASM cell cytoskeletal stiffness, pMLC expression, and pMYPT1 expression. Interestingly, siRNA targeted

against Smad3 – but not Smad2 - significantly attenuated pMLC and pMYPT1 by TGF-β1. Smad4 siRNA also decreased pMLC and pMYPT1 by TGF-β1 to a lesser extent.

**Conclusion:** Our data suggest that TGF-β1 contributes to HASM cell shortening and AHR in asthma through a Smad3-dependent mechanism. While Smad2 and Smad3 are closely related, Smad2 knockdown showed little effect on TGF-β1-induced HASM cell shortening. Together, these data provide a novel role for Smad3 activation in TGF-β1-induced AHR in asthma.

**Free Fatty Acid 4/GPR120 Receptor Agonist TUG891 - a Novel Potent Bronchodilator of Small Airways**

Chantal Donovan[1,2], Jun Chen[3], Mirjam Simoons[2], Jacqueline B Micallef[2], Maggie Lam[2], Bharat Shimpukade[4], Trond Ulven[4], Michael Sanderson[3], Jane E Bourke[2]
[1]Priority Research Centre for Healthy Lungs, School of Biomedical Science and Pharmacy, Hunter Medical Research Institute, University of Newcastle, NSW, Australia; [2]Biomedicine Discovery Inst, Dept of Pharmacol, Monash University, Clayton, VIC, Australia; [3]Department of Microbiology and Physiological Systems, University of Massachusetts Medical School, Worcester, MA, USA; [4]Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Odense, Denmark

**RATIONALE:** Small airways are a major site of airway obstruction in asthmatic and COPD patients. Novel bronchodilator agents targeting these airways are crucial to provide therapeutic alternatives when albuterol (ALB) is ineffective. The free fatty acid receptor 4(FFA4)/GPR120 has recently been identified in human and mouse lungs. TUG891 is able to activate GPR120, however its function in airway smooth muscle is unknown.

AIMS: To assess the effect of TUG891 on mouse small airways in precision cut lung slices (PCLS) and isolated trachea.

**METHODS:** Mouse PCLS were prepared for visualising changes in area of intrapulmonary airways (~200μm) by phase contrast microscopy and calcium oscillations within airway smooth muscle cells using two-photon microscopy. Potential bronchodilator responses to TUG891 following methacholine (MCh) pre-contraction were compared with ALB. The effects of TUG891 on the increase in calcium oscillations induced by MCh were also tested. Mouse tracheal rings (2mm) were mounted in a myograph (37°C) for measurements of changes in MCh-induced tone to TUG891 and ALB.

**RESULTS:** TUG891 elicited concentration-dependent relaxation in PCLS. At 21°C, this occurred at μM concentrations (% relaxation: 100μM TUG891: 90.2±2.2, n=5). However at 37°C, TUG891 was over 100x more potent, eliciting 36.1±8.7% relaxation at 1nM and maximal relaxation at 1μM, and was 10x more potent than ALB at the same temperature. Calcium oscillations induced by 300nM MCh were inhibited by TUG891 over the same concentration range. In mouse trachea, TUG891 elicited complete relaxation, but only at 100μM (n=4).

**CONCLUSIONS:** TUG891 is a potent bronchodilator, eliciting airway relaxation at nM concentrations at 37°C in PCLS, with higher potency than ALB. TUG891 appears to have a novel property of selectivity for small airways, since TUG891-mediated relaxation was only evident at μM concentrations in trachea. These data highlights that FFA4/GPR120 might represent a new target for treatment of obstructive airway diseases.

**$G\alpha_{12}$ Mediates Human Airway Smooth Muscle Cell Contraction via a PI3K/ROCK axis-dependent Mechanism**

Edwin Yoo, Gao Yuan Cao, Hong Lam, Steven An, Reynold A. Panettieri, Jr.
Rutgers Institute for Translational Medicine and Science, New Jersey, USA

**RATIONALE:** Asthma manifests as airway hyperresponsiveness and inflammation. Importantly, G-protein-coupled receptors (GPCRs) regulate contraction of the human airway smooth muscle cell (HASMC), the pivotal cell regulating bronchomotor tone. Although PI3K-dependent activation of Rho Kinase is necessary for HASMC contraction and PI3K inhibition promotes bronchodilation of human small airways, the upstream mechanisms driving GPCR-mediated PI3K/ROCK axis activation remain unclear. Since G12 family proteins regulate smooth muscle tone by activating Rho Kinase, we hypothesize that $G\alpha_{12}$ may regulate HASMC contraction by activation of the PI3K/ROCK axis.

**METHODS:** Primary HASMCs were transfected with siRNA targeting GNA12 or non-targeting control siRNA. HASMC were treated with agonist 4 days post-transfection, and subjected to magnetic twisting cytometry or collected for immunoblotting. Lysates were probed for myosin light chain (MLC), AKT, and myosin-phosphatase target subunit 1 (MYPT1) phosphorylation. Coupling of $G\alpha_{12}$to the M3 muscarinic receptor (M3R) was evaluated using co-immunoprecipitation.

**RESULTS:** siRNA targeting GNA12 resulted in a reduction of $G\alpha_{12}$ protein expression compared to non-targeting siRNA (74% reduction, $p<0.001$, n=3). GNA12 knockdown resulted in basal stiffness reduction (28% reduction, $p<0.001$,

n=689) and attenuated carbachol-induced phosphorylation of AKT (59% reduction, p=0.03, n=3), MYPT1 (65% reduction, p=0.04, n=3), and MLC (58% reduction, p=0.04, n=3). Co-immunoprecipitation demonstrated an association between $G\alpha_{12}$ protein and the M3R (n=5).

**CONCLUSIONS:** GNA12 knockdown resulted in decreased basal HASMC tone and blunted carbachol-induced phosphorylation of AKT, MYPT1, and MLC. Importantly, this is the first demonstration that $G\alpha_{12}$ protein mediates M3R effects in HASMC. Taken together, these data show that $G\alpha_{12}$ signaling regulates HASMC contraction by modulation of the PI3K/ROCK axis, and may serve as a potential therapeutic target in the treatment of asthma.

**Epithelial-fibroblast crosstalk via Interleukin-1: the missing link between inflammation and remodeling in the asthmatic EMTU?**

Emmanuel T. Osei[1,2,3], Leila. Mostaco-Guidolin[1], Stephanie. Warner[1], Teal. Hallstrand[5], Wim Timens[2,3], Dirkje S. Postma[3,4], Irene H. Heijink[2,3,4] , Corry-Anke Brandsma[2,3] and Tillie L. Hackett[1].
[4]University of Groningen, University Medical Center Groningen, Department of Pulmonology; Groningen, the Netherlands, [5]University of Washington Medical Center, Washington, USA

**Introduction**: Asthma is a chronic inflammatory disease associated with airway remodeling that involves all tissues in the lung epithelial-mesenchymal trophic unit (EMTU). We previously showed that airway epithelial cells, through the production of IL-1α, regulate fibroblast phenotype within the lung EMTU. The objective of this study was to assess the effects of epithelial-derived IL-1 on fibroblast-induced inflammation, ECM production and remodeling within the asthmatic EMTU.

**Methods**: Primary airway epithelial cells (PAECs) and primary airway fibroblasts (PAFs) were obtained from both asthmatic and non-asthmatic donor lungs deemed not suitable for transplantation. PAECs were cultured in an air-liquid interface (ALI) and the expression and release of IL-1 family members were determined by RNA sequencing and ELISA respectively. PAFs were cultured alone and in collagen I gels and were stimulated with 1ng/mL IL-1α, IL-1β and IL-33. The release of pro-inflammatory mediators was measured using ELISA and gel contraction over time was quantified using ImageJ software respectively. Collagen I fiber formation was also assessed using second harmonic generation-non linear optical microscopy (SHG-NLOM).

**Results**: We found higher mRNA and protein expression of IL-1 and IL-33 in asthma-derived PAECs compared to controls when cells were in monolayer compared to differentiated ALI cultures. Exogenous stimulation of fibroblasts with IL-1 but not IL-33 significantly increased the release of pro-inflammatory cytokines IL-6, IL-8 and TSLP as well as the growth factor GM-CSF. When comparing the ability of PAFs to contract collagen I, we found that fibroblasts stimulated with IL-1 were less able to contract collagen I gels than those stimulated with IL-33 and unstimulated fibroblasts. SHG-NLOM analysis revealed that IL-1 but not IL-33 inhibited the ability of PAFs to remodel gelatin into fibrillar collagen I. mRNA expression of GLI1, a known regulator of collagen synthesis, and lysyl oxidase, a collagen cross-linking enzyme, were found to be down-regulated with IL-1 treatment in fibroblasts.

**Conclusion**: IL-1 expression is increased in asthma-derived PAECs, and epithelial-derived IL-1 influence the remodeling and pro-inflammatory phenotype of PAFs. This has important implications for IL-1 release during epithelial damage in asthma, and may help in understanding defective collagen deposition and remodeling in asthma, creating potential opportunities for therapeutic intervention.

**miR-363 Modulates DAG Signaling in Asthmatic ASM**

Jon Evasovic, Saul Favela, Cherie A. Singer
Department of Pharmacology, University of Nevada, Reno, USA

**Rationale:** Asthma is a chronic inflammatory disease of the lower airway characterized by hyperresponsiveness mediated by remodeling of airway smooth muscle (ASM); however, underlying mechanisms intrinsic to hypercontractility remain ambiguous. Diacylglycerol-dependent pathways are key regulators of ASM contractility and modulate development of airway obstruction associated with asthma. Parasympathetic $M_3$-stimulated ASM contraction is regulated by phospholipase Cβ and mediated by downstream effectors protein kinase Cε, C-kinase-activated protein phosphatase-1 inhibitor and myosin light chain phosphatase. Previous miRNA bioarray experiments identified downregulation of miR-363-3p (1.36-fold ± 0.03) in human ASM cells stimulated with 10ng/mL IL-1β, TNF-α and IFN-γ. Subsequent bioinformatics analyses predict protein kinase Cε (*PRKCE*) and the regulatory subunit of myosin light chain phosphatase, *PPP1R12A*, as targets of miR-363-3p. **It is hypothesized that dysregulation of miR-363-3p modulates effectors of diacylglycerol such that ASM contractile output is increased.**

**Methods:** Total RNA was extracted from proliferating and 7-day differentiated asthmatic and non-asthmatic ASM cells. Quantitative PCR was performed to assess *PPP1R12A* expression relative to 18S rRNA. Luciferase reporter assays will be used to verify binding of miR-363-3p to the 3' untranslated region of *PPP1R12A* and other bioinformatically predicted targets.

**Results:** Reported miR-363-3p downregulation in cytokine-stimulated ASM cells was correlated with changes in *PPP1R12A* expression in asthmatic ASM cells. *PPP1R12A* transcript levels were significantly decreased (56.48% ±1.82) in asthmatic differentiated ASM cells compared to nonasthmatic counterparts.

**Conclusions:** Decreased *PPP1R12A* expression in differentiated asthmatic ASM would logically impede relaxation through a reduced ability to dephosphorylate myosin light chain. Future experiments seek to further characterize miR-363-3p expression in cytokine-stimulated ASM with regards to *PPP1R12A* and other relevant target gene and protein expression. These results support the continued investigation of miR-363-3p as a mediator of diacylglycerol-dependent pathways underlying airway hypercontractility in asthma.

**Funding Source:** This work is supported by R01 HL127192-01A1 from the NHLBI to CAS.

**TNF-alpha–Nitric Oxide (NO) Axis Leads to beta-Adrenergic Receptor Dysfunction in Asthma from Human Airway Smooth Muscle Cells**

M.K. Gupta1, G. Cao2, Terri Harford, Peidemonte, R.A. Panettieri2, S.V. Naga Prasad1
1Lerner Research Institute, Cleveland Clinic Foundation - Cleveland, OH/US, 2University of Pennsylvania - Philadelphia, PA/US

**Rationale:** beta-agonist activation of βARs leads to desensitization (inactivation) by phosphorylation through G- protein coupled receptor kinases (GRKs) while resensitization occurs by dephosphorylation of endosomal βARs. β2-adrenergic receptor (β2AR) agonist (β-agonist) is the most commonly used bronchodilator for acute relief in asthma but chronic use of β-agonist paradoxically exacerbates airway hyper-responsiveness. Based on our previous published studies on receptor dysfunction in asthmatic HASMCs, we hypothesized if TNF- and NO, which are elevated in asthmatic patients could independently cause receptor dysfunction.

**Methods**: Primary HASMCs isolated from lungs of subjects with non-asthma were treated with TNF- and NO donor NC-18. receptor function was determines in the plasma membranes. Adenylyl cyclase (AC) assays, cAMP, state of receptor phosphorylation and expression of GRKs and β-arrestin were used a measures of desensitization while, resensitization was assessed by protein phosphatase 2A (PP2A) and phosphoinositide-3 kinase (PI3K).

**Results**: HASMCs treated with TNF- and NO donor show -AR phosphorylation with diminished AC activity and cAMP responses. Desensitization components like GRKs and β-arrestins were analyzed. There was a significant

increase in PI3Kγ and reduction in PP2A activity HASMCs treated with TNF- . TNF-a also induces dampening of the relaxation after b-AR stimulation in the tracheal rings.
**Conclusions:** Our study shows that β2-ARs can be desensitized by TNF- /NO donor axis independently of the β2- AR agonist challenge contributing to β2AR dysfunction which may be an additional underlying cause for asthma pathophysiology and loss in asthma control.

**Cigarette smoke changes cAMP microdomains in lung slices**

Haoxiao Zuo[1, 2, 4], Bing Han[1, 2], Wilfred J. Poppinga[1, 2], Lennard Ringnalda[1], Viacheslav O. Nikolaev[3], Martina Schmidt[1, 2]
[1]University of Groningen, The Netherlands, [3]Institute of Experimental Cardiovascular Research, University Medical Centre Hamburg-Eppendorf, 20246 Hamburg, Germany.

**RATIONALE:** Cyclic AMP regulates numerous physiological functions in subcellular microdomains. Such signaling diversity can be in part explained by phosphodiesterases (PDEs) hydrolyzing cAMP, thereby controlling local cAMP levels and creating subcellular microdomains. In chronic obstructive pulmonary disease (COPD), an airway disease primarily provoked by cigarette smoke (CS), cAMP is considered as a therapeutic target for COPD treatment. Due to a still limited usage of real-time imaging techniques, the effect of CS on spatio-temporal cAMP dynamics in the airways is barely understood.

**METHODS:** To study the impact of CS on cAMP microdomains, *in vitro*, *in vivo* and *ex vivo* CS exposure models were set up. In the *ex vivo* model, Lung slices of transgenic mice expressing the FRET-based cAMP sensor Epac1-camps were exposed to CS extract for 24 hours. In the *in vivo* model, Epac1-camps mice were exposed to CS for 4 days. Moreover, human airway smooth muscle cells infected with Epac1-camps adenovirus were used as an in vitro model. The $\beta_2$-agonist fenoterol was applied to elevate intracellular cAMP. To achieve PDE subtype selective inhibition, we used the PDE4 inhibitor rolipram and the PDE3 inhibitor cilostamide. The nonselective PDE inhibitor IBMX served as control.

**RESULTS and CONCLUSIONS:** Fenoterol-induced cAMP FRET responses were significantly decreased ($p<0.01$) in mice exposed to CS for 4 days. A significant increase induced by CS could be observed in the PDE3-dependent FRET response of both *in vivo* ($p<0.01$) and *in vitro* ($p<0.05$) models. Under basal conditions, CS treatment increased in all three models inhibition by both PDE4 ($p<0.05$) and PDE3 ($p<0.01$). The increase of PDE4 activity correlated with an upregulation of PDE4D protein, indicating PDE4D might be the potential target for CS. Our findings suggest that CS induced alterations in such cAMP microdomains may contribute to the development of COPD.

**A new twist to the mechanical cell fate identifies $\alpha_5\beta_1$ and $\alpha_v\beta_3$ as the primitive mechanosensing clusters forcing age-associated stiffening of vascular smooth muscle**

Wanqu Zhu[1,*], Byoung Choul Kim[2,5,6,*], Mingyi Wang[3,*], Abraham Isak[1], Robert Monticone[3], Jessie Huang[1], Jennifer L. Pluznick[4], Taekjip Ha[2,5,6], Edward G. Lakatta[3,†], and Steven S. An[1,7,†]
[1]Department of Environmental Health and Engineering, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, USA.

With every beat of the heart, and each thrust of the stroke volume, the central arteries must comply with imminent mechanical stretch and their *instantaneous* elastic properties fashion, in turn, long-lived human physiology. With aging, however, there is a general breakdown of vascular distensibility/elasticity which is marked by inflammation, fibrosis and stiffening in the arterial wall. Using a constellation of single-cell and single-molecule biophysical approaches, here we report compartmentalized stiffening of the structural cell-types (smooth muscle and fibroblast) of the vascular wall derived from thoracic aorta of adult (8 months) *vs.* aged (30 months) F344XBN rats. Specifically, the increased cytoskeletal stiffness and basal tone were localized to isolated vascular smooth muscle (VSM) cells derived from the aged animals and, in the absence of immune inflammatory responses, maintained in culture. This discrete mechanical cell fate was persistent across a wide range of matrix rigidities and, on multiple length and time scales, coupled to a finite number of interactions between the VSM and the extracellular matrix (ECM) through the cell surface integrin receptors $\alpha_5\beta_1$ and $\alpha_v\beta_3$. Strikingly, the pro-fibrotic transforming growth factor $\beta$1 (TGF$\beta$1) emerged as a specific modifier of these slowly evolving receptor clusters forcing the mechanical landscape of arterial aging in VSM. We demonstrate a simple pharmacological perturbation of TGF$\beta$1 signaling is sufficient to rejuvenate *in vitro* the material properties of isolated VSM cells. These studies serve as a proof-of-concept that the broad-based inhibition of TGF$\beta$1 signaling in the aortic VSM may be a useful therapeutic approach to mitigate arterial aging and central arterial wall stiffening.

**Effects of plant-derived monoterpenes on human airway smooth muscle cell relaxation and calcium signaling**

Jessie Huang[1], Wanqu Zhu[1], Prem Rajkumar[2], Jennifer Pluznick[2], and Steven An[1]
[1]Johns Hopkins Bloomberg School of Public Health, [2]Johns Hopkins School of Medicine, Baltimore, MD

Plant-derived essential oils have been a source of alternative medicine use for individuals with asthma, although the research behind the mechanisms and effects of many essential oils on asthmatics is severely lacking. Monoterpenes are a class of volatile terpenes derived from essential oils that show evidence of anti-inflammatory, antispasmodic, and antimicrobial effects in rodents. However, the effects and modes of action of monoterpenes in human airways are unclear. Thus, we hypothesized that monoterpenes can modulate human airway smooth muscle (HASM) function in asthma, and aimed to explore the mechanistic determinants of this phenomenon. Cultured primary HASM cell lines were used to study intracellular Ca2+ mobilization and cell contractility. Intracellular Ca2+ was quantified using ratiometric Ca2+ indicator Fura-2, in which Fura-2-loaded cells were treated with a range of concentrations of three selected monoterpenes – nerol, eugenol, and linalool – in the presence and absence of extracellular Ca2+ and thapsigargin. Cell stiffness, an index for cell contractility, was determined using optical magnetic twisting cytometry. We found that all three monoterpenes induced increases in intracellular Ca2+ in HASM cells. The removal of extracellular Ca2+ ablated the monoterpene-induced increase in intracellular Ca2+, while thapsigargin-mediated depletion of intracellular Ca2+ had no effect. All three monoterpenes induced ASM relaxation in a dose-dependent manner in both non-asthmatic and asthmatic HASM. Using antagonists to target G protein-coupled receptor, transient receptor potential (TRP) channel, and ion channel signaling pathways, we found that 2-APB (TRP and IP3 receptor inhibitor) ablated ASM relaxation induced by nerol, linalool, and eugenol, suggesting that either TRP channels or G protein-coupled receptors are involved. While further investigation is underway to determine the precise signaling mechanisms involved in monoterpene-induced ASM relaxation, our findings suggest that monoterpenes may potentially be used as novel bronchodilators in the treatment of asthma.

**Sp1 transcription factor promotes c-Abl gene expression and contributes to smooth muscle cell proliferation**

Jiaoyue Long , Guoning Liao, Dale D. Tang
Department of Molecular and Cellular Physiology, Albany medical college, USA

The nonreceptor tyrosine kinase c-Abl (Abelson tyrosine kinase, Abl) plays a critical role in regulating smooth muscle cell proliferation, which promote the development of airway remodeling in asthma. However, little is known concerning transcriptional mechanisms regulating c-Abl gene expression. Sequence analysis revealed that the c-Abl promoter region contains Sp1-binding sites. Sp1(Specific protein-1) is a transcription factor that has been implicated in gene expression in nonmuscle cells. In this study, we found that c-Abl expression increased in human airway smooth muscle cells upon stimulation with platelet –derived growth factor (PDGF) and interleukin-33 (IL-33), Sp1 expression was also enhanced in these cells. To further investigate the role of Sp1 in the regulation of the c-Abl expression, RNA interference was used to knock down Sp1 expression. Inhibition of Sp1 protein expression by siRNA resulted in a significant decrease in the expression of c-Abl protein. In addition, Treatment of smooth muscle cells with PDGF or IL-33 increased significantly the phosphorylation level of extracellular signal-regulated kinase (ERK). Pretreatment the cells with the ERK kinase1/2 (MEK1/2) inhibitor U0126 obviously inhibited the proliferation of airway smooth muscle cells evaluated by counting cell number and Brdu incorporation. Furthermore, treatment with U0126 also reduced the expression of c-Abl and Sp1 in smooth muscle cells. These results suggest that Sp1 regulates c-Abl expression in smooth muscle cells during stimulation with PDGF and IL-33, which may controls c-Abl regulated cell proliferation. ERK1/2 is an upregulator of Sp1 in smooth muscle cells.

**Airway and lung structure in adult rats previously subject to hypoxia-induced intrauterine growth restriction**

**Kimberley C Wang**[1], Jude S Morton[2], Sandra T Davidge[2], Alexander N Larcombe[1], Alan L James[3], Graham M Donovan[4], Peter B Noble[5]
[1]Telethon Kids Institute, WA 6008, Australia

**Aim:** Intrauterine growth restriction (IUGR) is associated with asthma in childhood and adulthood. Abnormalities in airway and lung structure accompanying IUGR may predispose children to the development of asthma. The aim of this study was to determine the structural consequences in the airways and lungs of adult offspring born from IUGR pregnancies using a maternal hypoxia-induced IUGR rat model.

**Methods:** Pregnant Sprague Dawley rats were housed under hypoxic conditions (11.5% $O_2$) from gestational days E13-E20 (gestation period = 22 days) and then returned to normoxia (21% $O_2$). A control group of pregnant rats was housed under normoxic conditions throughout pregnancy. Weights of male offspring at birth and 7 weeks of age were recorded at which point bronchoalveolar lavage fluid (BALf) was collected for total cell counts and lungs fixed for morphometry/stereology.

**Results:** Offspring born to dams exposed to hypoxic conditions were lighter at birth compared with Control ($P$<0.01), but not at 7 weeks ($P$=0.49). Total cell number (predominantly macrophages) was increased in BALf of the IUGR offspring ($P$<0.01). There were no differences in mean thickness of the epithelium, airway smooth muscle layer, inner wall, outer wall or total wall, or in the surface area of alveoli, lung volume or parenchymal volume between groups at 7 weeks. However, there was greater anatomical variation in airway lumen area in the IUGR group ($P$<0.05). Based on a more heterogeneous distribution of airway lumen area in the IUGR group, a mathematical simulation predicted increased resistance and elastance during bronchoconstriction.

**Conclusion:** *In utero* growth restriction resulted in a more heterogeneous distribution of airway lumen calibre with potential implications for ventilation and bronchoconstriction. The increased number of lung macrophages in adulthood indicates a phenotypic change that has a fetal origin and warrants further investigation.

**The role of histone arginine methylation in gene expression of airway smooth muscle cells in asthma**

Klaudia Kaczmarek[1], Rachel L. Clifford[1], Jamie Patel[1], Dominick Shaw[1], James Dowden[2], Alan J. Knox[1]
[1]Division of Respiratory Medicine, School of Medicine and [2]School of Chemistry, University of Nottingham

**Introduction:** Asthma is estimated to affect at least 300 million people globally. About 25% of the patients do not respond to therapy; therefore we need to develop novel treatments. ASM cells have a crucial role in asthma, contributing to airway remodelling, inflammation and airflow obstruction. We have previously shown that epigenetic histone modifications, particularly histone lysine acetylation and methylation regulate the secretion of inflammatory mediators from ASM cells. Here we tested the hypothesis that histone arginine changes are also involved. Protein arginine N-methyltransferases (PRMTs) are the enzymes which catalyse histone arginine methylation (HRme, the addition of a methyl group to arginine residues on the N-terminal tails of histones), and inhibiting them represents a strategy to reduce the secretion of inflammatory mediators from ASM cells.

**Methods:** Studies were performed in cultured human ASM cells from asthmatic and non-asthmatic donors at passage 6. PRMT expression in human ASM cells was investigated by qPCR. Protein levels of four PRMTs in human ASM cells were investigated by western blotting. The effect of inhibiting PRMTs on the secretion of eotaxin, IL-6, CXCL8 and IP-10 from healthy ASM cells, under basal conditions and following stimulation with TNF-α (1ng/ml), was investigated by ELISA.

**Results:** We found that ASM cells express the PRMT1, PRMT2, PRMT3, CARM1, PRMT5, PRMT6, PRMT7 and FBX011 mRNA and PRMT1, CARM1, PRMT5, and PRMT6 protein. Two PRMT inhibitors, namely TCE5003 – a PRMT1 inhibitor, and 217531 - a CARM1 inhibitor, significantly reduced the secretion of inflammatory mediators from ASM cells.

**Conclusions:** ASM cells express a number of PRMTs at mRNA and protein levels. The inhibition of PRMTs results in the reduced secretion of inflammatory mediators from ASM cells. PRMTs may have an important role in regulating chemokine production from ASM cells in asthma, and are a promising target for future investigations in asthma.

**$EP_2$ and $EP_4$ receptor agonists inhibit the airway sensory nerves that cause cough, a potential protective control that is lost in human smokers and a pre-clinical COPD model**

Michael A Wortley, Sara J Bonvini, Sarah A Maher, Mark A Birrell, Maria G Belvisi
National Heart & Lung Institute, Imperial College, London, United Kingdom

$PGE_2$ acts on multiple receptor subtypes, and we have shown that $EP_3$ is the receptor responsible for activation of sensory nerves and cough[1]. $EP_2$ and $EP_4$ have protective anti-inflammatory and bronchodilator effects in other lung tissues[2-4], and we therefore hypothesized that $EP_2$ or $EP_4$ receptor agonism could modulate airway sensory nerve activity and aimed to characterise their effects in health and disease.
We assessed the effect of selective $EP_2$ and $EP_4$ agonists±antagonists on induced depolarisation of isolated vagus (sensory) nerve from male Dunkin-Hartley guinea pigs, Brown-Norway rats, or human subjects (tissues unsuitable for transplant) as well as in a COPD model using air-/CS-exposed guinea pigs[5]. We additionally profiled the effect of $EP_2$ and $EP_4$ agonists on capsaicin-induced calcium-flux in isolated primary, airway-terminating, sensory neurons from guinea pig jugular ganglia[5].

Our data shows, for the first time, that $EP_2$ and $EP_4$ receptor activation inhibits sensory nerve depolarization or calcium flux in response to capsaicin activation of guinea pig, rat and human vagus nerves. Furthermore, both $EP_2$ and $EP_4$ mediated inhibition of sensory nerves was lost in CS-exposed guinea pig tissue, and in nerves from human smoker subjects. This tallies with our recent work demonstrating that COPD patients have reduced responsiveness to $PGE_2$[5]. In the current study we additionally show that loss of EP receptor activity was restored by treatment with diclofenac (a COX-2 inhibitor) concomitantly with CS-exposure in guinea pigs, suggesting EP receptor desensitization in response to increased $PGE_2$. This demonstrates that activation of different EP sub-types can mediate either tussive or anti-tussive effects. This modulation of EP receptors functional activity (and perhaps expression) in disease states suggests a role for prostanoids in certain neurophenotypes of chronic cough.
[1]-Maher-2009-AJRCCM-923;8, [2]-Buckley-2011-Thorax-1029;35, [3]-Birrell-2015-Thorax-740;7, [4]-Jones-2016-BJP-992;1004 and [5]-Belvisi-2016-AJRCCM-1364;72.

**Intrapulmonary Artery and Airway Reactivity is Altered in Precision Cut Lung Slices from a Rat Model of Pulmonary Arterial Hypertension**

Maggie Lam[1], Meaghan FitzPatrick[2], Rebecca Harper[3], Paul N. Reynolds[3], Jane E. Bourke[1]
[1]Monash University, Melbourne, VIC, AUS, [2]University of Melbourne, Melbourne, VIC, AUS, [3]Royal Adelaide Hospital, Adelaide, SA, AUS

**Introduction:** Contraction and relaxation of small intrapulmonary arteries and airways (<500μm diameter) can be visualised *in vitro* using precision cut lung slices (PCLS). PCLS has been applied to provide insights into mechanisms underlying altered airway reactivity in models of lung disease and to identify novel bronchodilators. To date, there have been no studies using PCLS from a model of pulmonary arterial hypertension (PAH), where altered reactivity of intrapulmonary resistance arteries can play a key role.

**Aim:** To establish artery and airway constrictor and dilator responses in rat PCLS, and assess whether reactivity is altered in PCLS from a validated monocrotaline model of PAH.

**Methods:** PCLS were prepared from 6-7 week male Sprague-Dawley rats, 8–10 days after saline (control) or monocrotaline (MCT) treatment (60mg/kg s.c.). Changes in artery and airway lumen area were visualised under phase-contrast microscopy during perfusion with endothelin-1 (ET-1), 5HT, sildenafil and iloprost, as these are relevant to PAH and its treatment.

**Results:** ET-1 elicited contraction in both control arteries and airways with similar potency and maxima. In pre-contracted arteries, sildenafil and iloprost caused relaxation with μM and pM potencies respectively. In the MCT group, maximal arterial contraction to ET-1 was increased (%reduction in area: control 66±8% n=5; MCT 97±4% n=3) with no change in potency. In contrast, ET-1 maxima and potency were relatively decreased in airways (max: control 80±6%, MCT 48±4%; pEC50: control 8.1±0.2, MCT 7.2±0.5).

**Conclusion:** Increased *ex vivo* contraction in intrapulmonary MCT arteries is consistent with the known contribution of ET-1 to *in vivo* PAH. The physical influence of this increased arterial contraction may contribute to the decreased contraction of airways in close proximity. Further studies are required to determine if relaxation to current vasodilator therapies are impaired by MCT to provide validated conditions to assess urgently needed novel treatments for PAH.

**microRNA-25 as a potential novel therapeutic target in asthma**

Mariam Ba, Jennifer Hall, Lauren Schultz, Annemarie Aiyuk, Miguel Hernandez, Jon Evasovic, Karla Hernández and Cherie A. Singer
Department of Pharmacology, University of Nevada, Reno, Nevada 89557, USA

Asthma is a chronic inflammatory disease of the airway characterized by airflow obstruction, airway hyperresponsiveness (AHR) and remodeling. Existing asthma therapies alleviate obstruction and inflammatory symptoms but do not address adequately AHR and remodeling. Therefore, it is crucial to develop novel therapeutic strategies targeting ASM function and structure. MicroRNAs (miRNAs) are small, non-coding RNAs that negatively regulate post-transcriptional gene expression. Previous data from our laboratory demonstrated a decreased miR-25 expression following a pro-inflammatory stimulus in human ASM cells and in mouse model of allergic inflammation indicating of potential role of this miRNA in asthma. We hypothesized that expression of miR-25 attenuates asthma symptoms by altering ASM phenotype. We have generated and characterized a mouse model with smooth muscle-targeted miR-25 expression ($Tg^{SM\text{-}miR25}$). These mice were exposed to acute OVA sensitization and challenge and were used to determine the role of miR-25 in lung function, ASM inflammation, proliferative/contractile protein expression and chemokines/cytokine secretion. $Tg^{SM\text{-}miR25}$ mice treated with OVA displayed a significant reduction in pulmonary resistance and total cell count in BAL fluid compared to wild-type (WT) littermates. This was accompanied by reduced inflammation and ASM mass in the airways, as well as PCNA expression levels indicative of reduced proliferation in $Tg^{SM\text{-}miR25}$ animals compared to WT littermates. Further, miR-25 expression in OVA treated mice led to the decreased secretion of eotaxin, MIG and MIP-2 chemokines, and restored IL-1α, IL-12-p40 and IL-12-p70 cytokines production to levels seen in control animals. These results demonstrate that overexpression of miR-25 improves lung function and alleviates key features of allergic inflammation including airway inflammation, remodeling, cytokine/chemokine production, as well as proliferative and contractile protein expression in response acute OVA treatment. Overall, these findings support development of miR-25 as a potential therapeutic target for allergic inflammation and asthma that affects multiple aspects of ASM phenotype and function.

**Selective targeting of Epac's facilitates airway smooth muscle relaxation**

Martina Schmidt[1],2, Bing Han[1,2], Emanuel Kennedy Feitosa[1], Carolina Elzinga[1], Harm Maarsingh[3], Anouk Oldenburger[1]
[1]University of Groningen, Department of Molecular Pharmacology, Groningen, The Netherlands. [2]University of Groningen, University Medical Center Groningen, Groningen Research Institute for Asthma and COPD, GRIAC, Groningen, The Netherlands. [3]Palm Beach Atlantic University, Lloyd L. Gregory School of Pharmacy, Department of Pharmaceutical Sciences, West Palm Beach, FL, USA

**RATIONALE:** Airway smooth muscle (ASM) tone is dysregulated in obstructive airway diseases. To induce airway relaxation, $\beta_2$-agonists are used due to their ability to elevate cAMP. Next to the main cAMP effector PKA, inhibition of bronchial constriction involves Epac by yet poorly defined mechanisms[1]. Epac -/-mice showed distinct inflammation and remodeling pattern *in vivo*[2], but isoprenaline-induced relaxation remained unchanged in Epac-/- mice. Unexpectedly, pharmacological inhibition of Epac's increased isoprenaline-induced relaxation, effects blunted in Epac-/- mice. Here we studied the mechanisms underlying the Epac driven elevation of airway smooth muscle relaxation.

**METHODS:** ASM tone was performed in control mice, Epac -/- and in human trachea smooth muscle strips. Immortalized human airway smooth muscle cells (hTERT) were as an in vitro model. Phosphorylation of MLC and VASP was analysed by Western blot. CE3F4 (Epac1), ESI-05 (Epac2) and specific PKA inhibitors were used, and specific Epac activator's served as control.

**RESULTS and CONCLUSIONS:** We reported previously that isoprenaline induced relaxation of ASM, partly independent of PKA. Isoprenaline-induced relaxation was not altered in Epac -/- mice. Selective targeting of Epac1 by CE3F4 and Epac2 by ESI-05 increased isoprenaline-induced relaxation, effects blunted in Epac -/- mice. Studies in human trachea strips showed that inhibition of Epac1 - to a lesser extent of Epac2 - reduced methacholine-induced airway smooth tone, a measure for the induction of relaxation. In search for underlying molecular mechanisms, studies

in hTERT cells demonstrated that inhibition of Epac1 - to a lesser extent Epac2 - increased the isoprenaline-induced phosphorylation of VASP but decreased the methacholine-induced phosphorylation of MLC. Selective targeting of Epac's seem to facilitate airway relaxation most likely by skewing the balance between MLC and VASP phosphorylation towards PKA.
Dutch Lung Foundation (3.2.09.34), CNPq (Proc40187/2013-6).[1]Roscioni et al., J Cell Mol Med, 15, 1551-1563 ;[2]Oldenburger et al., FASEB J, 28, 4617-4628.

**A unique floxed PKI mouse reveals PKA dependence of bronchorelaxing agents**

James V. Michael, Ajay P. Nayak, Roslyn Yi, Tung O. Chan, Raymond B. Penn
Department of Medicine, Thomas Jefferson University, Philadelphia, PA, USA

**Rationale** The cyclic adenosine monophosphate (cAMP)- dependent protein kinase (PKA) signaling cascade was the first identified second messenger system and has been extensively studied ever since. However, despite many attempts to study the role of PKA via inhibitory strategies *in vivo*, *ex vivo*, or *in vitro*, many technical limitations exist. In addition to the nonspecific effects of commercial inhibitors, genetic strategies have failed, as knockout of PKA catalytic subunits results in embryonic lethality. To overcome this issue we have developed an inducible system that utilizes a floxed GFP-tagged (PKA inhibitory peptide) PKI mouse resulting in viable and fertile progeny, and demonstrate here its utility in addressing the controversial issue of which cAMP effector mediates bronchodilation.

**Methods** PKI-GFP was inserted into the ROSA locus under the expression of a CAG promoter. Southern blotting was used to screen mice positive for the transgene. Precision cut lung slices (PCLS) were generated from PKI-GFP or littermate controls and transduced with adenovirus to express CRE recombinase. CRE and PKI-GFP expression was assessed, as was the effect of bronchorelaxant reagents on signaling and function in PCLS.

**Results** CRE recombinase-driven expression of PKI was assessed by visualizing $GFP^+$ signal or measuring GFP in lung tissue protein lysate. In contrast to WT controls, PKI expressing PCLS, exhibited an ~4-fold decrease in ability to relax following $PGE_2$ treatment. Moreover, phosphorylation of downstream signaling protein VASP was reduced by 91.9 ± 2.9% in PKI-positive slices. These data indicate a reliance on PKA signaling to mediate agonist-induced relaxation of lung tissue. Finally, we were able to demonstrate the utility of this mouse as a tool to observe effects of PKA *in vivo* in airway smooth muscle and other systems. Crossing loxP-PKI-GFP mice with mesP-CRE mice yielded GFP fluorescence in cardiac progenitor cells in the thoracic region of mouse embryos day E10.5 close to predicted Mendelian frequency (6 of 16 pups 37.5%, predicted 25%).

**Conclusions** Herein, we describe the generation of a genetic model system to inhibit PKA signaling *in vivo*. Addition of CRE recombinase *via* viral transduction or by crossing with CRE-expressing mice yields consistent and measurable PKI expression. We further demonstrate the dependence of PKA signaling on pro-relaxation signaling and bronchorelaxation *ex vivo*. This mouse model system is highly adaptable to applications for assessing PKA-dependent signaling in various tissues, requiring a cross of PKI-GFP mice with other strains of tissue-specific CRE expression.
Funding: HL58506, AI110007

**The mitochondrial pathway in bitter taste receptor agonist-induced airway smooth muscle cell death**

Shi Pan and Deepak Deshpande
Center for Translational Medicine, Thomas Jefferson University, 1020 Locust Street, Philadelphia, PA, 19107

**Rationale:** Our previous studies have demonstrated the expression and functional importance of bitter taste receptors (TAS2R) in airway smooth muscle (ASM) cells via inhibition of ASM contraction and growth factor-induced proliferation. Recent studies suggest that mitochondria play critical role in the maintenance of cellular energetic status, redox balance and calcium homeostasis. In this study we aimed at determining the molecular mechanism and the role of mitochondria in the regulation of ASM cell death by chronic treatment with TAS2R agonists.

**Methods:** Human ASM cells were treated with platelet-derived growth factor (PDGF) with or without pretreatment with TAS2R agonists for 24 hours. Mitochondrial morphology and membrane potential were determined by live cell imaging using confocal microscopy. Cell survival was measured by CyQuant assay and dynamic proteins were assayed by western blotting. In select experiments, cells pre-incubated with mitochondrial fission inhibitor (mdivi-1) or expressing DN-Bnip3 were used.

**Results:** TAS2R agonists induced ASM mitochondrial fragmentation, which was attenuated by Mdivi-1 and DN-Bnip3. The mitochondrial morphology alterations were not associated with changes in the expression of mitochondrial dynamic proteins mitofusin 1, and 2 (Mfn1 and 2), Optic atrophy 1(Opa1) and dynamin-like protein 1 (DLP1). TAS2R treatment significantly modulated mitochondrial membrane potential. Further, treatment of ASM cells with Mdivi-1 and DN-Bnip3 reversed mitochondrial membrane potential alterations induced by TAS2R agonists. TAS2R agonist increased

expression and translocation of Bnip3 to mitochondria in ASM cells. Preventing Bnip3 translocation to mitochondria with DN-Binp3, not down-regulating Bnip3 expression with siRNA, mitigated TAS2R agonist-induced ASM cell death.

**Conclusion:** TAS2R agonists modulate ASM cell survival partly via mitochondrial dynamics. Inhibition of mitochondrial fission attenuates mitochondrial damage and increases ASM cell survival in human ASM cells treated chronically with TAS2R agonists. These studies establish molecular mechanisms involved in the anti-mitogenic effect of TAS2R agonists.

**Potential role for an imbalance between airway smooth muscle laminin α4 and α5 expression in asthma.**

P. Prabhala[1,5,6], D.B. Wright[1,5,6], C. Bitter[1,5,6], P. Robbe[1,5,6], T. Pera[7], B. Hinz[8], G. Kim[8], N.H.T. ten Hacken[2,5], M. van den Berge[2,5], W. Timens[3,5], H. Meurs[1,5,6], B.G.J. Dekkers[1,4,5,6]

*[1]Department of Molecular Pharmacology, Department of Pulmonology University Medical Center Groningen, [3]Department of Pathology and Medical Biology [4]Department of Clinical Pharmacy and Pharmacology, [5]Groningen Research Institute for Asthma and COPD, University Medical Center Groningen, and [6]Groningen Research Institute for Pharmacy, University of Groningen, Groningen, The Netherlands, [7]Center for Translational Medicine, Thomas Jerfferson University, Philadelphia, USA [8]Laboratory of Tissue Repair and Regeneration, Matrix Dynamics Group, University of Toronto, ON*

**Rationale:** Asthma is a chronic inflammatory disease of the airways with hallmarks of reversible airway obstruction, airway hyperresponsiveness (AHR) and remodeling. Airway remodeling is characterized by structural changes, including altered extracellular matrix deposition and increased airway smooth muscle (ASM) mass and contractility, which can contribute to AHR and a decline in lung function. Laminins represent a critical component of the basement membranes underlying the epithelial and endothelial layers and surrounding ASM cells. Using laminin α4 and α5 deficient human ASM cells, we have recently demonstrated that endogenous laminin α4 promotes a contractile and fibrotic ASM phenotype, whereas laminin α5 suppresses the contractile phenotype. In the present study, we investigated the potential clinical relevance of these findings, by measuring ASM laminin α4 and α5 expression in airway wall biopsies of asthmatic patients in relation to lung function and asthma severity.

**Methods:** 20 healthy and 32 asthmatic human biopsy sections were stained with laminin α4 and laminin α5 antibodies. Qualitative and semi-quantitative analysis was then performed on the scans of the biopsy sections. The staining intensity of each section was scored three times by two independent investigators in a blinded manner. These scores were then correlated with asthma severity relating to lung function ($FEV_1$).

**Results:** Significant differences in laminin intensity were found within the ASM layer of sections from control subjects and patients with mild, moderate and severe asthma. ASM laminin α4 intensity was significantly reduced in mild asthmatics when compared to healthy control subjects. Interestingly this reduction was significantly reversed in patients with severe asthma with laminin α4 levels slightly exceeding the control levels. Similar to laminin α4, laminin α5 intensity in the ASM layer was reduced in mild asthmatics, however unlike laminin α4 intensity, the laminin α5 intensity remained attenuated in the moderate and severe patient groups. Consequently we found that the ratio of laminin α4 to laminin α5 gradually increased with the severity of asthma with a significant increase noted between control subjects and patients with severe asthma. In addition we also observed a significant correlation between a reduction in lung function ($FEV_1$) and intensity of laminin α4 present in the ASM of asthmatics.

**Conclusions:** Our findings suggest that an imbalance in laminin α4 and laminin α5 expression in ASM of asthmatic patients contributes to reduced lung function and more severe asthma, which is in line with our previous observations in laminin α4 and α5 deficient ASM cell cultures.

**Maternal eCigarette vaping increases the risk of Th2 driven allergic asthma in the offspring.**

Kielan McAlinden[1,3], Yik Chan[1-3], Anudeep Kota[1,3], Varsha Komalla[1,3], Hui Chen[1-3], Brian Oliver[1-3], Pawan Sharma[1-3]

[1]Molecular Biosciences Team, [2]School of Life Sciences, University of Technology Sydney, NSW, Australia, and [3]Respiratory Research Group, Woolcock Institute of Medical Research, Sydney, NSW, Australia, 2037

**Rationale**: Maternal E-cigarettes (eCig)-vaping may be considered as a safer cigarette smoke (CS)-replacement during pregnancy while their long-term safety and effect on lung pathophysiology are not known. Thus the effect of maternal eCig vaping needs further assessment, particularly the effect this has on offspring and development of allergic asthma later in life. Combining mouse models of maternal vaping and allergic asthma *in vivo* and human airway smooth muscle cells (ASM) *in vitro* we tested whether maternal eCig vaping enhances features of allergic asthma in the offspring.

**Methods**: Female BALB/c mice were vaped with either eCig vapour (± nicotine) or room air (control group). The eCig vaping was started prior to mating and continued during gestation and lactation. The female offspring from these mothers were subjected to an ovalbumin (OVA)-induced allergic asthma model. 24 hours after the last aerosolized OVA

or saline challenge, key parameters of allergic asthma: airway inflammation, airway remodeling and airway hyperresponsiveness were measured. Human ASM cells were treated with varying concentrations of eCig liquid condensate and key parameters of mitochondrial function were measured.

**Results**: Repeated allergen-exposure induced Th2-driven inflammation in OVA-exposed mice, characterized by massive influx of leukocytes predominantly eosinophils. The effect of allergen on airway eosinophilia was significantly enhanced in the offspring from eCig (+Nic)-exposed mothers when compared with eCig (-Nic) or OVA-only animals. eCig (+Nic)-exposure significantly increased total lung collagen and mucus staining (airway remodeling) when compared with eCig (-Nic) or OVA animals. Functionally we found significant increase in airway resistance (flexiVent) in the eCig-OVA (+Nic) group when compared to eCig-OVA (-Nic) or OVA animals. Mechanistically, eCig (± Nic) dose-dependently decreased cell viability (MTT assay, 24h), mitochondrial transmembrane potential (JC10, 6h) and reduced mitochondrial oxygen consumption rate (seahorse bioanalyzer, 24h) which may partly explain enhanced features of allergic asthma in the offspring through mitochondrial dysfunction these effects were independent of nicotine *in vitro.*

**Conclusions**: Our study suggests that maternal eCig vaping enhanced and worsened key features of allergic asthma and this could be attributed to aberrant mitochondrial function.

**Altered ASM Mass and Contractile Responses in an Ovalbumin Murine Model of Asthma during the Inflammation Resolution Phase as assessed by PCLS and IHC**

Christopher J. Philp[1], Charlotte K. Billington[1], Michael R. Hill[2] Andrew Bullock[2], Bo Liu[1], Anthony N. Habgood[1], Suzanne Miller[1], Reuben D. O'Dea[2], Amanda L. Tatler[1], Simon R. Johnson[1], Bindi S. Brook[2]
[1]Division of Respiratory Medicine, University of Nottingham, Nottingham, United Kingdom, [2] School of Mathematical Sciences, University of Nottingham, Nottingham, United Kingdom

Pathophysiological changes occurring during airway inflammation onset and progression in asthma are intensively researched. However, resolution of this state, particularly over a timescale of weeks as opposed to minutes or hours, is barely examined. A mathematical model published by our group suggests that the speed of inflammation resolution post-exacerbation is critical in determining long term airway remodelling (1). We sought to assess how inflammation resolves over four weeks following a regime of Ovalbumin (OVA) sensitisation known to induce airway remodelling (2).

26 balb-c mice underwent OVA-sensitisation or PBS-exposure (2). PCLSs were prepared on the final day of sensitisation (day 34) and 2,3 and 4weeks after. Airways were subjected to increasing concentrations of methacholine (1nM-100uM) and analysed as described (3). Mice with matched treatments were sacrificed 1,2,3 and 4 weeks post-sensitisation and their lungs analysed via immunohistochemistry (IHC). A custom MATLAB program quantified airway smooth muscle (ASM).

On the final sensitisation day, airway remodelling was clearly evident in the lungs of OVA- but not PBS-control mice, with thickened ASM, disorganised epithelium and inflammatory cell infiltration. ASM mass analysis revealed a 2.77 mean fold increase in OVA vs PBS controls (0.051±0.019 vs 0.14±0.12, $p<0.05$, 10-22 airways).
Airways from OVA mice contracted to a greater extent than those exposed to PBS (88±3.4% vs 74±8.3%, five airways per mouse). Airway slices from OVA mice 2,3 and 4 weeks post-sensitisation (i.e. during resolution) exhibited a trend towards decreased contraction as opposed to the increase observed at day 34. Interestingly, there was a consistent trend towards *relaxation* at low doses of methacholine in airways from OVA animals in the resolution phase.

IHC analysis revealed that just one week following the end of the OVA-sensitisation period, ASM mass had reduced to near baseline (0.057±0.05, 10-13 airways).

These data suggest that although ASM mass returns to baseline levels in the weeks following an inflammatory episode, the airways are still affected in perhaps counter-intuitive ways.

**Investigating Genome wide DNA methylation in Bronchial and Lung Fibroblasts from healthy individuals and individuals with COPD**

Rachel L. Clifford[1,3], Nick Fishbane[2], Poojitha Rajasekar[1], Julie L. MacIsaac[3], Lisa M. McEwen[3], Andrew J. Fisher[4], Michael S. Kobor[2], Alan J. Knox[1], Tillie-Louise Hackett[2].
[1] Division of Respiratory Medicine and Nottingham Respiratory Research Unit, University of Nottingham, Clinical Sciences Building, City Hospital, Nottingham, United Kingdom
[2] University of British Columbia, Centre for Heart Lung Innovation, Dept of Anesthesiology, Pharmacology and Therapeutics, Vancouver, BC, Canada.
[3]Centre for Molecular Medicine and Therapeutics, Child and Family Research Institute, Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada.
[4] Department of Respiratory Medicine, Newcastle Upon Tyne Hospitals NHS Foundation Trust, Newcastle Upon Tyne, UK Institute for Ageing, Newcastle University, Newcastle Upon Tyne, UK

**Rationale:** Lung fibroblasts are implicated in respiratory disease pathology including chronic obstructive pulmonary disease (COPD). Phenotypic differences between fibroblasts isolated from the bronchi versus the lung parenchyma have been described but no studies have compared the cell types on a genome wide scale. DNA methylation is a reversible modification of the DNA structure with the ability to affect cell function via the alteration of gene expression. Here we compared genome wide DNA methylation profiles from bronchial and lung fibroblasts and assessed modification to these profiles in cells isolated from individuals with COPD.

**Methods:** Site specific, quantitative genome wide methylation was determined using the Illumina 450K Infinium Methylation BeadChip array. Linear modelling and DMRcate identified differentially methylated sites and regions respectively between BrF and LgF and from cells isolated from healthy individuals versus those with COPD.

**Results:** 3980 CpG sites significantly differed, following Bonferroni correction, between BrF and LgF isolated from healthy individuals. 240 CpG sites displayed a difference in methylation of >50%. 78 of these validated in a second cohort of matched BrF and LgF isolated from the same individuals. DMRcate was used to refine the individual CpG sites to 5 regions of interest associated with 5 genes; HLX, TWIST1, CREB5, SKAP2 and PRDM16. Differences in methylation were less pronounced when comparing cells isolated from healthy individuals to those with COPD. In BrF 47 DMRcate regions were identified with a maximum difference in methylation of at least 20%. In LgF 3 DMRcate regions were identified with a maximum difference in methylation of at least 20%.

**Conclusions:** DNA methylation profiles are significantly different between BrF and LgF but only small modifications are associated with COPD. Future work will focus on validating a methylation based marker of lung versus bronchial fibroblasts to differentiate cell types.

**Oligonucleotide Therapy Reduces Lung Inflammation in HDM-Sensitized Mice**

Sabrina C. Ramelli[1,3], Jared M. McLendon, Ph.D.[4], Andrew Ferretti, Ph.D.[5], Jason Sparks, Ph.D.,[6], Majed Matar[6], Robert Barrington, Ph.D,[2,3], and William T. Gerthoffer, Ph.D.[1,3].
Depts. of [1]Biochemistry & Molecular Biology and [2]Microbiology & Immunology, [3]Center for Lung Biology, University of South Alabama College of Medicine, Mobile, AL, [4]Dept. of Internal Medicine, University of Iowa Carver college of Medicine, Iowa City, IA, [5]Dept. of Medicine Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA., and [6]Celsion Corp., Huntsville, AL

Th2 high asthma is due to imbalance of Th1/Th2 responses leading to high production of Th2 cytokines, and sometimes reduced type 1 interferons. Since type 1 interferons are important in Th1 responses, identifying molecules that upregulate interferon signaling may be therapeutic in asthma. TLR9 signaling is activated by oligonucleotides with unmethylated CpG motifs elevating type 1 interferons. Intranasal and inhaled CpG oligonucleotides have anti-inflammatory effects in mouse models of rhinitis and asthma. We previously found a miR-145 antisense oligonucleotide or a non-targeting oligonucleotide with CpG motifs reduced lung inflammation in house dust mite-sensitized (HDM) mice. We did not identify the mechanism(s) by which these oligonucleotides reduce inflammation. We addressed this by measuring mRNAs differentially expressed after treatment with oligonucleotides of varying chemical structure.

After sensitization with HDM, five cohorts of ten mice were treated with dextrose, antimiR-145, or one of the three non-targeting oligonucleotides. All oligonucleotides were administered IV complexed with a cationic lipid nanoparticle (Therasilence). A sixth cohort of age-matched control mice were not sensitized with HDM but were treated with dextrose. RNA-seq was performed to assess inflammatory gene expression in whole lung extracts. Histological methods were used to assess airway remodeling and inflammation.

Oligonucleotides delivered from the vascular compartment distributed to most cells in the lung parenchyma. Challenge with HDM increased mucus metaplasia, immune cell infiltration, and obstructive airway remodeling. Each oligonucleotide reduced indicators of inflammation. RNAseq followed by gene set enrichment analysis (GSEA) showed all treatments reduced Th2 inflammatory gene expression.

The results show that antagonizing miR-145 function in the lung significantly reduces inflammation in an acute HDM mouse model of asthma. In addition, administering any oligonucleotide with CpG motifs reduces inflammation to varying degrees. We hypothesize that the remarkable anti-inflammatory effects of non-targeting oligonucleotides may be due to immune modulation by unmethylated DNA containing CpG motifs.

**Functional genetics of lung function associated gene *GPR126***

Robert Hall, Ian Hall & Ian Sayers
Division of Respiratory Medicine, Queen's Medical Centre, University of Nottingham, United Kingdom

Chromosome 6q24 has reproducibly been associated with lung function in genome wide association studies (GWAS). The association signal includes a haplotype encompassing a non-synonymous single nucleotide polymorphism (SNP) (rs17280293, Ser123Gly, MAF = 2.9%) in the coding region of the G-protein coupled receptor *(GPR)126*. We aimed to further define i) potential causal variants in the haplotype, ii) the expression of GPR126 in lung and iii) the effects of the amino acid change (Ser123Gly) on GPR126 function.

Bioinformatic analysis, using HaploReg and GTEx, was used to identify any expression quantitative trait loci (eQTL) effects. *In silico* analysis, using SIFT and PolyPhen-2, was carried out to assess the potential pathogenicity of the amino acid change Ser123Gly. Quantitative PCR on a range of cell types and tissues including lung tissue and airway relevant cells was used to profile *GPR126* mRNA expression.

Nine variants were found to be in linkage disequilibrium ($r^2$=0.2) with the sentinel SNP associated with lung function, however only one variant, rs17280293 (Ser123Gly) results in an amino acid substitution. The Ser123Gly variant lies in a domain which is important for binding collagen type IV and is predicted to be damaging to the protein. GPR126 mRNA is expressed in a range of airway relevant cells and whole lung tissue with an expression profile; Human bronchial epithelial (HBEC) cells > Whole lung tissue > Human airway smooth muscle (HASM) cells (n=2-4 donors). GPR126 also shows high levels of staining in smooth muscle cells on Protein Atlas.

*GPR126* is expressed in the lung and in airway relevant cells and harbors a variant associated with lung function which have damaging effects on protein function. Ongoing studies aim to use an overexpression system to measure downstream GPR126 signaling activity in Ser123 and Gly123 receptor variants.

**Identification of Endogenous, Disease Relevant Activators of TRPV4 in the Airways**

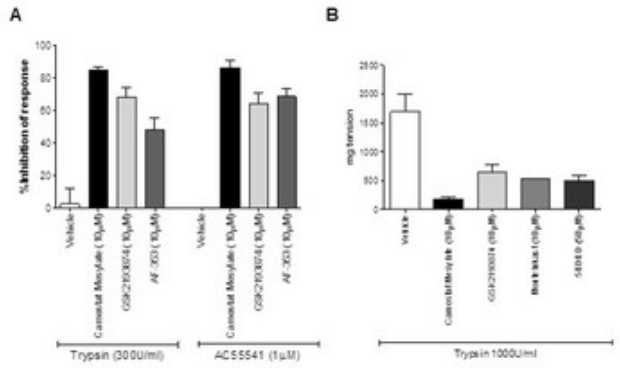
Sara J. Bonvini, Michael A. Wortley, Mark A. Birrell, Maria G. Belvisi
Imperial College, London

**Rationale:** Activation of TRPV4 has been shown to cause firing of airway afferent nerves via release of ATP and activation of P2X3[1]. A role for the TRPV4-ATP axis has also been shown in airway smooth muscle (ASM) with TRPV4 inducing the release of ATP and activating P2X4 on mast cells to cause contraction of human ASM via release of cysteinyl leukotrienes[2]. However, much of the work has used the synthetic ligand GSK1016790a and an endogenous disease relevant stimulus has not been identified. Activation of Protein Activated Receptor 2 (PAR2) has been reported to directly activate TRPV4 r[3], and endogenous ligands of PAR2 such as tryptase are increased in chronic lung diseases such as asthma[4] where enhanced sensory nerve activity and bronchoconstriction are seen.

**Objective:** To investigate the role of PAR2 in TRPV4 mediated functional responses in the airways.

**Methods**. Using an *in vitro* guinea pig (GP) and rat vagus nerve depolarisation system, two selective PAR2 ligands; trypsin (30-1000U/ml) and AC55541 (0.01-10μM) were assessed and the effect of a serine protease inhibitor camostat mesylate (10μM), the TRPV4 antagonist GSK2193874 (10μM) and the P2X3 antagonist AF-353 (10μM) on the subsequent depolarisation investigated. The contractile effects of both trypsin (1000U/ml) and AC55541 (1μM) were evaluated on human ASM, and the role of TRPV4, Cystlt1 and P2X4 investigated using GSK2193874 (10μM), montelukast (10μM) and 5BDBD (50μM), respectively.

**Results**: Both trypsin and AC55541 caused depolarisation of GP and rat vagus. Depolarisation induced by both ligands was inhibited by camostat, GSK2193874 and AF-353 (Fig 1A), implicating the TRPV4-ATP axis. In human tracheal strips, both trypsin and AC55541 caused a large contraction (>2g) which was inhibited by camostat, GSK2193874, montelukast and 5BDBD (Fig1B) indicating that PAR2 induced contraction also involved the TRPV4-ATP axis.




**Conclusions**: These data suggest that PAR2 is a disease relevant endogenous activator of the TRPV4-ATP axis. As there are increased levels of PAR2 activators in the diseased airways, this suggests that this pathway may contribute to the increased symptoms observed in chronic lung disease and highlights TRPV4 as a global target.

1:BonviniS.J.*etal*.(2016).JACI;*138*:249-261, 2:BonviniS.J.*etal.*(2015)ERJ:OA3253, 3:Poole P.*etal.*(2013)JBC:288:5790-802, 4:Aubier M.*e al*.(2016)JACI:138:729-739.

**The DP2/CRTh2 receptor is expressed by ASM cells in asthma and inhibition of its activation suppresses ASM migration**

Ruth Saunders, Michael Biddle, Latifa Chachi, Amanda Sutcliffe, Rachid Berair, Christopher E Brightling.
University of Leicester, Leicester, UK

Activation of the DP2/CRTh2 receptor by prostaglandin D2 (PGD2) in the airway mediates pro-inflammatory responses from immune cells and epithelial remodelling. The airway smooth muscle (ASM) is dysfunctional in asthma with an increase in mass and infiltration by mast cells which release PGD2 following allergen challenge. The role of the PGD2/DP2 axis in ASM in asthma has not been studied extensively.

Expression and function of DP2 in primary ASM cells was assessed by flow cytometry, migration in a wound healing assay, metabolic activity using the MTS assay, and $\alpha$-SMA expression and cell size/granularity by flow cytometry. Gene expression was assessed by gene array.

ASM cells expressed cell surface DP2 (DP2/isotype control geometric mean fluorescence intensity (gMFI): 1.3±0.1, $p<0.001$, n=15) and mRNA for genes involved in $PGD_2$ synthesis (COX1 and L-PGDS: 5.2±2.5% and 8.0±2.4% of $\beta$-actin mRNA, respectively, n=6). ASM cells secreted $PGD_2$ with an increase following wounding (129±19 and 181±25 $pg/ml/10^5$ cells respectively, p=0.0195, n=10). Inhibition of DP2 activation by endogenous $PGD_2$ using the DP2 specific antagonist CAY10471 resulted in inhibition of ASM migration after 24h, although other measures of ASM phenotype/function were unaffected (see Table 1).

ASM migration is reduced in the presence of the DP2 antagonist CAY10471, suggesting a role for the PGD2/DP2 axis in recruitment of ASM cells to the ASM bundle in asthma, which could contribute to the increased ASM mass observed in asthma. This recruitment could be driven by release of $PGD_2$ by ASM cells into the extracellular milieu following injury, we show here that ASM cells release more $PGD_2$ following wounding, or from mast cells present in the ASM bundle in asthma. DP2 could represent a novel therapeutic target for patients with airway remodelling, who are unresponsive to other therapies.

| Table 1: | 10nM CAY10471 | 50nM CAY10471 | 100nM CAY10471 |
|---|---|---|---|
| ASM cell wound healing (D cells moved into wound after 24h % vehicle control) | -9.9 [10.8]* | -7.3 [19.4]* | -11.0 [17.5]* |
| ASM cell $\alpha$-SMA expression ($\Delta$ gMFI $\alpha$-SMA/isotype control antibody, % vehicle control) | -0.05 [19.4] | -3.1 [15.2] | -0.3 [15.1] |
| ASM cell size ($\Delta$ geometric mean forward scatter, % vehicle control) | -0.36 [1.1] | -0.7 [1.9] | -0.6 [2.4] |
| ASM cell granularity ($\Delta$ geometric mean side scatter, % vehicle control) | -0.8 [1.4] | -0.2 [4.0] | 0.4 [5.5] |
| ASM cell metabolic activity after 24h ($\Delta$ formazan absorbance at 490 nm, % vehicle control) | 3.9 [26.7] | 4.7 [33.5] | -0.1 [20.4] |

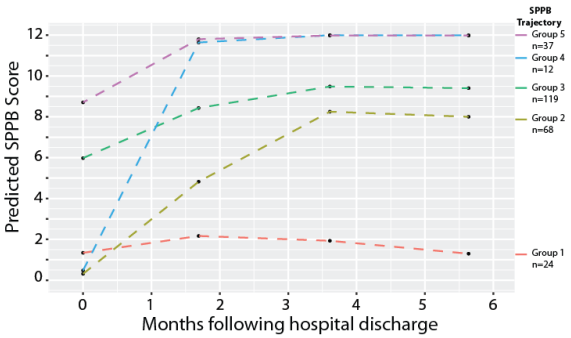
**Predictors of Long-term Physical Function Recovery in Survivors of Acute Respiratory Failure**

Sheetal Gandotra, MD[1]; James Lovato, MS[2]; Douglas Case, PhD[2]; Rita Bakhru MD, MS[1]; Kevin Gibbs, MD[1]; Michael Berry PhD[3], D. Clark Files, MD[1]; and Peter E. Morris[4]
1-Section on Pulmonary, Critical Care, Allergy, and Immunologic Disease, Wake Forest University, Winston Salem, North Carolina; 2-Department of Biostatistical Sciences, Wake Forest University, Winston Salem, North Carolina; 3-Department of Health and Exercise Science, Wake Forest University, Winston Salem, North Carolina; 4-Division of Pulmonary, Critical Care, and Sleep Medicine, University of Kentucky, Lexington

**Rationale:** Factors mediating functional recovery following critical illness are poorly understood. Subject heterogeneity may influence response to interventions, such as a structured early rehabilitation program. Here we examine functional trajectories of critically ill patients enrolled in a randomized controlled trial that included long-term functional outcomes.

**Methods:** We use a latent class analysis in the form of trajectory modelling to identify distinct subgroups of subjects with similar recovery patterns, in a trial evaluating standardized rehabilitation therapy (SRT) in patients with acute respiratory failure at an academic tertiary care medical center. The Short Physical Performance Battery (SPPB), an objective physical function measurement designed for low-functioning individuals, is used for this trajectory analysis. Trajectories span from hospital discharge through six months post-discharge. Chi-squared tests and one-way ANOVA were used to determine variables associated with trajectory membership. Subsequently, a multinominal nominal regression analysis was performed to help identify variables associated with group membership. Patients who died or failed to return for follow-up were excluded from the analysis.

**Results:** Analysis of the SPPB identified five trajectory groups (Fig 1). SPPB trajectory groups differed by gender (p=0.01), age (p<0.0001), hospital length of stay (LOS) (p=0.0017), and proportion of continuous sedation days (p=0.0020). Subject trajectories demonstrating functional recovery (Groups 2 and 4) consisted of younger females with a shorter LOS. The subject trajectory that failed to functionally recover (Group 1) consisted of older males with the longest LOS. By multinominal nominal regression, age (p>0.0001), gender (p=0.0118) and LOS (0.0061) were the most significant variables contributing to trajectory group membership.




**Conclusions:** We identified distinct functional trajectories of SPPB recovery following critical illness. Age, gender and hospital length of stay significantly impact the trajectory of functional recovery after critical illness. Further examination of these groups may assist in clinical trial design to tailor interventions to specific subgroups.

**Gene expression signatures of emphysema and terminal bronchiole number in a cross-sectional cohort of COPD – Uniting microCT imaging with transcriptomics**

Steven Booth[1,2], Hyun-Kyoung Koo[1,2], Jackson Steinkamp[4], Jake Kantrowitz[4], Guohai Zhou[1], Dragoş M. Vasilescu[1,3], Marc Lenburg[4], James C. Hogg[1,3], Avrum Spria[4], Tillie-Louise Hackett[1,2]
[1]Centre for Heart Lung Innovation, University of British Columbia (UBC), [2] Department of Anesthesiology, Pharmacology and Therapeutics, [3] Department of Pathology, UBC, Vancouver, B.C. Canada[4] Department of Medicine, University of Boston, Boston, USA

**Background**: We have previously demonstrated that gene-expression changes associated with microCT morphological measurements can provide insights into the pathogenesis of end-stage emphysema (Campbell et al 2013). The objective of this study was to determine the genes associated with emphysema and small airways disease in mild and moderate Chronic Obstructive Pulmonary Disease (COPD) patients in order to uncover molecular determinants involved in the early disease stages.

**Methods:** Lung samples were obtained from 5 smokers, 5 mild, 5 moderate, and 6 severe COPD patients undergoing surgical resection for lung cancer treatment or whole lung transplant. Lungs were inflated, frozen, sliced and 8 randomly sampled lung tissue cores per case were scanned by microCT to determine, the mean linear intercept (Lm) and number of terminal bronchioles. 30mg of tissue from each sample was used for RNA extraction and microarray analysis. Linear mixed effect models and WGCNA were used to correlate morphometry with gene expression, and gene set enrichment analysis was used to identify key pathways.

**Results:** We report that 887 genes were significantly correlated with Lm in all subjects (P<0.05), and these genes were enriched for the previously reported 127 gene signature for end-stage emphysema (FDR<0.001). Of the 887 genes, 418 were upregulated and enriched for genes involved in inflammatory/immune response, oxidative phosphorylation, and p53 pathways. Furthermore WGCNA identified networks of co-expressed immune response and Tcell genes which were significantly correlated with Lm (P<0.0001). The remaining 469 down regulated genes were enriched in TGFβ and Hedgehog signaling pathways. The total number of terminal bronchioles per/ml of lung tissue were significantly decreased by 57% in mild (P<0.05), 50% in moderate (P<0.05), and decreased by 71% in severe COPD patients (P<0.001) compared to smokers with normal lung function, and we identified 68 genes significantly correlated with total terminal bronchiole number (FDR<0.10). Genes which were differentially expressed when correlated with terminal bronchiole number were enriched for pathways involved in oxidative phosphorylation, protein secretion, IFN-a response, epithelial to mesenchymal transition and the Kras signaling pathway.

**Conclusions:** We demonstrate that combining tissue pathology imaged by microCT with whole tissue transcriptomics can uncover causal molecular determinants of emphysema and small airways disease across the spectrum of COPD.

**The role of diacylglycerol kinase in asthma**

Brenal K. Singh[1], Wen Lu[1], Amanda M. Paustian[1], Moyar Ge[2], Reynold A Panettieri[3], Angela Haczku, and Taku Kambayashi[1]

1Department of Pathology and Laboratory Medicine, Perelman School of Medicine at the University of Pennsylvania, USA, [2]University of California, Davis, USA, [3]Rutgers, the State University of New Jersey, Rutgers Institute for Translational Medicine and Science, USA

Asthma is a respiratory disease characterized by Th2 airway inflammation with concomitant increases in mucus production and smooth muscle contraction, leading to airway obstruction. Inhibition of both inflammatory and structural components of the asthma response is needed for optimal control of asthma symptoms. In this study, we identify diacylglycerol kinase (DGK), an intracellular enzyme that regulates the diacylglycerol signaling pathway, as a novel target in asthma. Airway hyperresponsiveness and lung Th2 inflammation in a mouse model of allergic asthma was abolished in DGK-deficient mice compared to wildtype mice. The reduction in airway hyperresponsiveness and lung inflammation was mediated by DGK deficiency in different cell types. While the reduction in inflammation was conferred by DGK deficiency in hematopoietic cells, the reduction in airway hyperresponsiveness was mediated by structural non-hematopoietic cells. Importantly, we also observed a reduction in airway hyperresponsiveness and Th2 inflammation by treatment with a DGK inhibitor. Thus, our data suggest that the inhibition of DGK blocks the asthmatic airway response by affecting function of both hematopoietic and structural cells in the airway and may represent a novel target for asthma therapy.

**GLUCOCORTICOIDS, A DOUBLE-EDGED SWORD IN ANTI-AIRWAY HYPERRESPONSIVENESS THERAPY?**

Benjamin Sheng Gui Tan[1], Hong Lam[2], Steven S. An[2] and Thai Tran[1]

[1]Department of Physiology, National University of Singapore, Singapore 117597, [2]Department of Environmental Health and Engineering, Johns Hopkins Bloomberg School of Public Health, Baltimore, USA 21205

**Background:** Glucocorticoids (GCs) are used as first-line therapy in the treatment of inflammation-associated asthma but has little impact on reducing airway hyperresponsiveness (AHR). We previously implicate, the laminin receptor, CD151, as a determinant of AHR in vitro and in vivo, but its regulation by GCs was not explored. Therefore, we aim to determine whether GCs regulate CD151 expression to impact airway smooth muscle (ASM) cell stiffness and thus AHR.

**Results:** Histamine concentration-dependently increased CD151 protein levels and this was reduced by the histamine H1 receptor antagonist, mepyramine. Surprisingly, GCs, dexamethasone, alone concentration-dependently increased CD151 protein abundance which was inhibited by GCs antagonist, RU486, when measured by western blotting. Interestingly, co-treatment of dexamethasone and histamine on ASM cells caused a synergistic increase in CD151 protein levels, but not when Dexamethasone was added 1h before histamine stimulation. However, quantitative real-time PCR showed neither histamine nor dexamethasone induced CD151 transcript level at 2,4,6,8h stimulation. In concordant with increased CD151 protein level, dexamethasone increased the stiffness of non-asthmatic ASM cells in a dose- and time-dependent manner, both at baseline and when stimulated with histamine as measured by magnetic twisting cytometry. Interestingly, asthmatic ASM cells had similar stiffness to dexamethasone induced non-asthmatic ASM cells.

**Conclusions**: We show for the first time that histamine induces CD151 expression which could have implications for its role in modulating ASM contraction via regulation of CD151 expression at the protein but not mRNA level. Interestingly, Dexamethasone alone also induces CD151 protein levels, supporting the notion that lack of beneficial effects of GCs on reducing AHR observed in some asthma patients, could be explained by its effects on increasing CD151 expression and thereby increasing ASM cell stiffness. Furthermore, the order of treatment with GCs may impact its effectiveness in reducing AHR.

**Caveolar Scaffolding Domain Peptide Abrogates Hyperoxia-induced Remodeling in Neonatal Mice**

Elizabeth R. Vogel, MD, PhD[1], Logan J. Manlove, BS[1], Ine Kuipers, PhD[1], Michael A. Thompson, BS[1], Yun-Hua Fang, BS[2], Michelle R. Freeman, BS[1], Rodney D. Britt, PhD[2], Arij Faksh, DO[3], Y. S. Prakash, MD, PhD [1, 2], Christina M. Pabelick, MD[1, 2]

Departments of [1]Anesthesiology, [2]Physiology and Biomedical Engineering, and [3]Obstetrics and Gynecology, Mayo Clinic, Rochester, MN

**Background:** Reactive airway diseases remain significant sources of pulmonary morbidity in neonatal and pediatric patients. Supplemental oxygen is a common perinatal exposure that may contribute to airway remodeling, characterized by increased airway smooth muscle (ASM) mass and extracellular matrix (ECM) deposition. Decreased caveolin-1 (CAV1) expression has been implicated in airway remodeling and hyperreactivity. We hypothesized that moderate hyperoxia exposure would decrease ASM CAV1 expression leading to airway remodeling and hyperreactivity while treatment with a CAV1 analog, caveolar scaffolding domain peptide (CSD), would ameliorate these effects.

**Methods:** Litters of B61295F2J mice (Jackson Laboratories) were randomized to room air (RA) or 50% hyperoxia (O2) exposure with or without CSD treatment (10μL of 0.25mM CSD daily via intraperitoneal injection). Following 7 days of exposure, all pups were returned to RA. At 21 days, airway resistance, compliance, and response to methacholine challenge were assessed using a SCIREQ FlexiVent system. Lung tissue was flash frozen for laser capture microdissection (LCM) or inflated with paraformaldehyde for histologic assessment.

**Results:** Compared to RA/PBS control mice, O2/PBS mice demonstrated increased airway reactivity and decreased compliance with methacholine challenge. CSD treatment prevented these changes. Histology and immunofluorescent staining demonstrated significant airway wall thickening and increased ASM mass in the O2/PBS group compared to control. These changes were improved in CSD treated mice. mRNA from ASM isolated via LCM demonstrated significant decrease in CAV1 and cavin-1 in the O2/PBS animals, while cavin-3 and collagen I were significantly increased ($p<0.05$).

**Conclusions:** These data show that moderate hyperoxia exposure is detrimental to the developing airway and may predispose to airway reactivity and remodeling. Loss of CAV1 is one mechanism through which hyperoxia may produce these deleterious effects. Supplementation of CAV1 using CSD or another form of CAV1 analog may represent a new therapeutic avenue for prevention of hyperoxia-induced pulmonary damage in neonates.

**The fatty acid amide hydrolase inhibitor URB597 activates TRPA1 ion channels in primary human airway smooth muscle cells**

Xiaochen Liu, Bo Liu, Ian P Hall

Division of Respiratory Medicine, University of Nottingham D Floor, South Block, Queen's Medical Centre

**Introduction:** Transient receptor potential ankyrin subfamily, member 1 (TRPA1) are excitatory cation ion channels activated by noxious environmental compounds and are involved in respiratory pathophysiology. Numerous TRPA1 agonists have been identified; however, they lack selectivity to TRPA1 and activate other channels in the TRP family. Recent study has identified URB597, a potent inhibitor of fatty acid amide hydrolase (FAAH) shown to activate transiently expressed TRPA1 channels (Niforatos et al. 2007). This study aims to 1) investigate whether URB597 can activate endogenously expressed TRPA1 in primary human airway smooth muscle (HASM) cells and 2) determine if URB597 activation of TRPA1 can affect small airway contraction in lung tissue.

**Methods:** Intracellular $Ca^{2+}$ changes were assessed using FLIPR5 calcium assay in HASM cells. Different concentrations A967079 and URB597 were injected into the cell culture media to obtain dose-dependent responses; ratiometric fluorescence changes after compound injections were detected using the FlexStation3 reader. Time-lapse high contrast microscopy (VL21, Phase Focus Ltd) was used to observe HASM cell contraction. Rat agarose-filled lung slices (200 μm) were precision cut using a microtome (OTS-5000). Contractile studies were done in airways of similar sizes perfused with URB597 and Methacholine.

**Results:** URB597 produced a significant increase in intracellular $Ca^{2+}$ ($EC_{50}$ = 199.7±32.8 μM, n=5). URB597-associated $Ca^{2+}$ influx was inhibited by the TRPA1 specific antagonist, A967079 ($IC_{50}$ = 33.03±5.8 nM, n=5). Significant HASM cell contraction was observed when 100 μM of URB597 was added, while A967079 abolished the contractions (n= 10 cells). We observed oscillating non-sustained small airways contractions from precision-cut rat lung slices perfused with 100 μM of URB597 (n=5).

**Conclusion:** We have demonstrated that URB597 induces $Ca^{2+}$ influx via endogenous TRPA1 channel activation in HASM cells. These data suggest TRPA1 may play a role in $Ca^{2+}$ homeostasis in airway smooth muscle.

References: Niforatos, W et al. Mol Pharmacol 71(5): 1209-1216.

**The role and regulation of c-Abl in airway smooth muscle contractility**

Yinna Wang, Alyssa C. Rezey, Olivia G. Gannon, Ruping Wang, and Dale D. Tang
Department of Molecular and Cellular Physiology, Albany Medical College, New York, USA

c-Abl (c-Abelson tyrosine kinase) is a nonreceptor protein tyrosine kinase that has a role in regulating actin dynamics, as well as smooth muscle contraction, and plays an essential role in the progression of airway chronic disease, especially asthma. The role and regulation of c-Abl in smooth muscle are not fully understood. In this study, acetylcholine (ACh) stimulation increased the tyrosine phosphorylation of p130CAS (an adapter protein implicated in smooth muscle contraction) in mouse tracheal smooth muscle. Conditional knockout of c-Abl in smooth muscle reduced the ACh-induced p130CAS phosphorylation. Moreover, c-Abl knockout also reduced the ACh-induced the interaction of p130CAS and profilin-1 (Pfn-1), and inhibited an increase in F/G actin ratios and tracheal contraction without affecting myosin light chain phosphorylation. Furthermore, Traf2- and Nck-interacting kinase (TNIK) and Src are tyrosine kinases implicated in the actin cytoskeleton. To understand whether these two kinases are involved in c-Abl phosphorylation (an indication of c-Abl activation), we determined the effects of the TNIK inhibitor KY-05009 and the Src inhibitor PP2 on c-Abl phosphorylation and tracheal contraction. Treatment of tracheas with KY-05009 and PP2 diminished the ACh-induced c-Abl tyrosine phosphorylation. These results suggest that c-Abl may regulate airway smooth muscle contraction by controlling p130CAS phosphorylation and interaction with Pfn-1. TNIK and Src are able to mediate c-Abl activation in smooth muscle during contractile stimulation.

**Blocking YKL-40 (chitinase-like protein chitinase 3) attenuates IL-13-induced airway hyper-responsiveness in a human precision cut lung slice model**

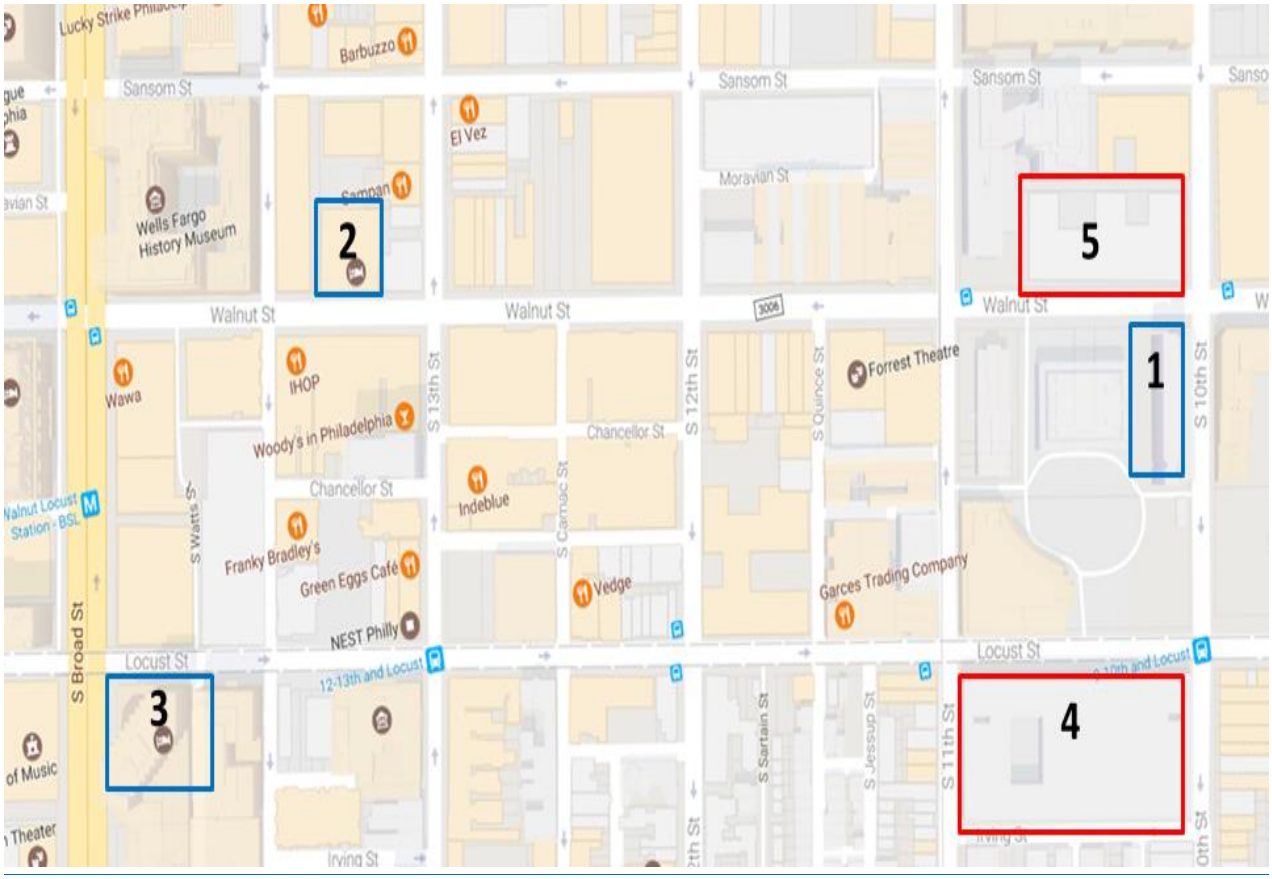
C.J. Koziol-White, V. Nezgovorova, L. Cohn, G. Chupp, and R.A. Panettieri Jr.
Rutgers University, New Brunswick, NJ; Yale University, New Haven, CT, USA

**RATIONALE:** Asthma manifests as a heterogeneous syndrome characterized by airflow obstruction, inflammation, and hyperreactivity (AHR). Increased release of both IL-13 and a chitinase-like protein YKL-40 have been found in the lungs of allergic asthmatics following segmental allergen challenge. Others demonstrate that IL-13 induces expression of YKL-40, binds to the IL-13 receptor a2 subunit and induces signaling. Further, IL-13-induced inflammatory responses are blunted by genetic ablation of YKL-40. We posit a central hypothesis that states blocking YKL-40 will reverse IL-13-induced airway hyper-responsiveness of human airways.

**METHODS:** Human precision cut lung slices (PCLS), each containing a small airway, were preincubated with a YKL-40 blocking antibody prior to overnight stimulation with IL-13 (100 ng/ml). The airways were then constricted to a dose response of carbachol and bronchoconstriction measured. Human airway smooth muscle (HASM) cells derived from donors with and without asthma were assessed for expression of IL-13 receptor α2, as well as phosphorylation of ERK1/2, Akt, and STAT6 following stimulation with YKL-40.

**RESULTS:** Airway responsiveness to carbachol-induced bronchoconstriction was augmented by overnight IL-13 treatment (LogEC50 control vs IL-13, -0.19 vs -0.97 μM). Preincubation with an YKL-40 blocking antibody attenuated IL-13-induced airway hyper-responsiveness (LogEC50 IL-13 vs YKL-40 antibody + IL-13, -0.97 vs -0.39). RNA-seq results indicate that both asthma and non-asthma HASM express IL-13 receptor α2. YKL-40 induces acute phosphorylation of ERK1/2, but not Akt or STAT6, in non-asthmatic HASM but does not induce this signaling in asthmatic HASM.

**CONCLUSIONS:** These data suggest that YKL-40 partially modulate airway hyper-responsiveness induced by IL-13 exposure, and that HASM may be a target for its effects. This may serve as unique therapeutic target to modulate both the increased airway reactivity and inflammation associated with allergic asthma.




## Hotel Information

1. **Martin Hall**
   201 S. 11th Street
   Philadelphia, PA 19107

2. **Holiday Inn**
   Holiday Inn Express Midtown
   1305 Walnut Street
   Philadelphia, Pa 19107
   Tel: 215-732-1045
   Fax: 215-732-2593

3. **Double Tree**
   237 S. Broad Street,
   Philadelphia, PA 19107
   Tel: 215-893-1600
   Fax: 215-893-1663

## Conference Venues

4. **Jefferson Alumni Hall**
   1020 Locust Street
   Philadelphia, PA 19107

5. **College Building**
   1025 Walnut Street
   Philadelphia, PA 19107

## Tourist Attractions in Philadelphia

- Liberty Bell Center
- Independence Hall
- Reading Terminal Market
- Valley Forge National Historical Park
- The Philadelphia Zoo
- Franklin Square
- The Franklin Institute
- Philadelphia Museum of Art
- National Constitution Center
- Penn Museum

## Getting Around

- Walking
- Bus
- Trolley
- Subway (Market-Frankford line)
- Regional Railway

Visit www.septa.org

Our sincere gratitude to all the sponsors