potential mechanistic link to elite athlete infection susceptibility.

Please refer to page A190 for declarations of interest related to this abstract.

S95

TRANSCRIPTIONAL SIGNATURES OF BLOOD OUTGROWTH ENDOTHELIAL CELLS FROM PATIENTS WITH PULMONARY ARTERIOVENOUS MALFORMATIONS AND HEREDITARY HAEMORRHAGIC TELANGIECTASIA

¹ME Bernabeu Herrero, ¹A Bielowska, ¹D Patel, ²S Srikanan, ¹P Chaves Guerrero, ¹M Nosedá, ³MA Aldred, ¹CL Shovlin. ¹Imperial College London, London, UK; ²London North West University Healthcare NHS Trust, London, UK; ³Indiana University School of Medicine, Indianapolis, USA

10.1136/thorax-2021-BTSabstracts.101

Introduction and Objectives Pulmonary arteriovenous malformations (PAVMs) are most commonly caused by hereditary haemorrhagic telangiectasia (HHT). This multisystemic condition, inherited as an autosomal dominant trait, results from a heterozygous loss-of-function variant in *ACVRL1*, *ENG* or *SMAD4*. Heterozygous endothelial cell phenotypes have proved elusive, hindering preclinical testing of potential therapeutic agents. Here, our objective was to define the transcriptional changes occurring in patient-derived blood outgrowth endothelial cells (BOECs).

Methods With ethical approvals (16/ES/0095), BOECs were established from HHT/PAVM patients heterozygous for a pathogenic variant in *ACVRL1*, *ENG* or *SMAD4*, and from healthy volunteers. HHT gene protein production by patient and control BOECs was evaluated by ³⁵S-methionine pulse chase experiments. Single cell qRT-PCR aimed to verify expression and heterogeneity of 48 transcripts in 40 viable (DRAQ7 negative) BOECs per genotype. Long and short RNA libraries were generated from BOECs prior to Illumina HiSeq sequencing of paired-end reads, and alignment to GRCh38. Differential alignments were used to rank transcripts for discovery gene ontology process identifications.

Results 24 BOEC lines were established from patients heterozygous for one of 10 different nonsense (stop gain) pathogenic variants in *ENG*, *ACVRL1* and *SMAD4*, with a median of two donors per genotype. Pulse chase experiments distinguished the genotypes. Blinded analyses of normalised RNA-Seq alignments also identified the source heterozygous HHT genotypes: *ENG* alignments were lowest in heterozygous *ENG*^{+/−} BOECs (Dunn's *p*=0.0089); *ACVRL1* alignments lowest in heterozygous *ACVRL1*^{+/−} BOECs (*p*=0.0040) and *SMAD4* alignments lowest in heterozygous *SMAD4*^{+/−} BOECs (*p*=0.007). By single cell qRT-PCR, 7/48 (15%) genes were expressed in all BOECs, 7/48 (15%) in no BOECs with 34 genes expressed in a proportion of BOECs. Seven genes displayed differential expression patterns between HHT and control BOECs, confirmed by distribution plots of all 16,807 RNASeq Ensembl transcript alignments in BOECs from different donors. Ranking transcripts by differential alignments in *ENG/ACVRL1/SMAD4* compared to control BOECs, identified consistent gene ontology processes enriched compared to equivalent numbers of randomly-selected transcripts.

Conclusions There are reproducible, transcriptional signatures in pulmonary AVM and HHT patient-derived BOECs distinguishable from healthy volunteer BOEC signatures. Common patterns for *ACVRL1*, *ENG* and *SMAD4* BOECs suggest a shared HHT transcriptome phenotype.

S96

PULMONARY ARTERIOVENOUS MALFORMATIONS – GENETIC VERSUS CLINICAL EVIDENCE OF UNDERLYING HEREDITARY HAEMORRHAGIC TELANGIECTASIA

¹L Sharma, ²A Alsafi, ²T Ferguson, ²J Redhead, ²WL Genomic Medicine Centre, ¹CL Shovlin. ¹Imperial College London, London, UK; ²Imperial College Healthcare NHS Trust, London, UK

10.1136/thorax-2021-BTSabstracts.102

Introduction and Objectives Pulmonary arteriovenous malformations (PAVMs) result in early onset but preventable strokes and other complications. Patients know that PAVMs can be a familial condition, most commonly due to hereditary haemorrhagic telangiectasia (HHT). Since the April 2020 NHS National Genomic Test Directory launch, patients with PAVMs are eligible for gene testing only if they already meet a definite clinical diagnosis of HHT, requiring two further Curaçao Criteria from nosebleeds, mucocutaneous telangiectasia, or first-degree relative with HHT. Our goal was to test the validity of this requirement.

Methods We audited ClinVar-listed variants in the major HHT genes, and with ethical approval, case notes of patients with PAVMs who had undergone NHS genetic testing. Tests were ordered predominantly between 2015–2019 through Mainstreaming Genomics initiatives via gene-test panels, or whole genome sequencing through the 100,000 Genomes Project.

Results ClinVar lists 2,804 variants in *ENG*, *ACVRL1* and *SMAD4*, including 909 likely pathogenic/pathogenic variants that diagnose HHT. Most are loss-of-function frameshift, stop-gain and splice site variants (390/645 [60%] for *ENG/SMAD4*; 126/264 [47%] for *ACVRL1*). At least 50% of people with one of these variants would be expected to have PAVMs. At our institution, 124 patients with PAVMs were the first in their family to have a gene test. Of these, 83 (67%) tested positive for HHT, i.e. were found to be heterozygous for a likely pathogenic or pathogenic variant in *ENG*, *ACVRL1* or *SMAD4*. Focussing on the 83 patients with PAVMs and genetically-diagnosed HHT, only 63/83 (76%) met three or more Curaçao criteria. For the remaining 20 patients with PAVMs and a positive HHT gene test, none met the family history criterion for HHT. While 14 (70%) described nosebleeds as an adult, only 3 (15%) had classical HHT telangiectasia, and the cohort included families where pulmonary AVMs (single or multiple) were the only HHT clinical feature across individuals with *ENG* pathogenic variants in two generations.

Conclusions There is a high burden of deleterious variants in HHT genes. Two-thirds of unselected PAVM patients have genetically-confirmed HHT, but of these, 1 in 4 display few if any clinical features of HHT. Wider gene testing is recommended.

S97

INVESTIGATING THE PRO-FIBROTIC EFFECTS OF GALECTINS IN IPF – A POTENTIAL ROLE FOR GLYCAN-MEDIATED INTERACTIONS WITH INTEGRINS

^{1,2}JF Calver, ²G Harris, ^{1,2}RM Lithgo, ³R Slack, ^{1,2}DJ Scott, ⁴RG Jenkins, ⁴AE John. ¹University of Nottingham, Nottingham, UK; ²Research Complex at Harwell, Oxford, UK; ³Galecto Biotech, Copenhagen, Denmark; ⁴Margaret Turner Warwick Centre for Fibrosing Lung Disease, NHLI, Imperial College London, London, UK

10.1136/thorax-2021-BTSabstracts.103

Introduction Integrins are a family of transmembrane heterodimer proteins differentially expressed on the cell surface of many lung cell types. Integrin-mediated activation of the key

pro-fibrotic mediator TGF- β 1, plays a critical role in the pathogenesis of Idiopathic Pulmonary Fibrosis (IPF). Both galectin-1 and galectin-3 potentiate this TGF- β 1 signaling pathway to promote fibrogenesis, although the exact mechanism is unclear. Integrins are glycoproteins thus their activity can be facilitated by glycan-mediated interactions with N-linked glycosylation the most well studied.¹

Objective To investigate whether galectin-1 and galectin-3 interact directly with α v β 1, α v β 5 and α v β 6 integrins using biophysical methods.

Methods Integrin-galectin interactions were determined by surface plasmon resonance (SPR) in the presence of divalent cations and the effect of extensive integrin deglycosylation or removal of N-linked oligosaccharides alone explored. SPR was used to assess integrin-galectin interactions in the presence of small molecule galectin inhibitors, GB1107 (galectin-3 selective inhibitor) or GB1490 (galectin-1 selective inhibitor). Binding of both galectins to N-Acetyl-D-glucosamine was assessed by isothermal titration calorimetry (ITC).

Results SPR data showed that both galectin-1 and galectin-3 bind to recombinant human α v β 1, α v β 5 and α v β 6 in a glycosylation-dependent manner. Minimal integrin-galectin binding was observed following integrin protein deglycosylation or in the presence of small molecule galectin inhibitors which act via the galectin carbohydrate binding domain (CBD). However, the removal of integrin N-linked oligosaccharides alone resulted in only a partial decrease in integrin-galectin binding. Additionally, ITC demonstrated that both galectin-1 and galectin-3 were unable to bind N-Acetyl-D-glucosamine; the α 5 β 1 terminal sugar required for α 5 β 1-fibronectin binding.

Conclusion Galectins are able to bind to integrins via their post-translational glycosylation sites. Collectively, these data suggest that the presence of both N-linked and O-linked glycan residues are essential for integrin-galectin binding, and that this binding may occur at the galectin galactoside-binding pocket. Understanding the precise role of galectins in integrin-mediated TGF- β 1 activation and IPF pathogenesis may be critical for the continued development of more effective and selective treatments for IPF patients.

REFERENCE

1. Janik ME, et al. Cell migration-the role of integrin glycosylation. *Biochim Biophys Acta*. 2010;1800(6):545–55.

Please refer to page A190 for declarations of interest related to this abstract.

S98 DISSECTING HUMAN PLEURA AT SINGLE-CELL RESOLUTION

¹J Obacz, ²TS Adams, ²JC Schupp, ²N Kaminski, ³G Aresu, ³AS Coonar, ³A Peryt, ⁴DM Rassl, ⁵RC Rintoul, ^{1,7}SJ Marciniak. ¹Cambridge Institute for Medical Research (CIMR), University of Cambridge, Cambridge, UK; ²Section of Pulmonary, Critical Care, and Sleep Medicine, Yale School of Medicine, New Haven, USA; ³Thoracic Surgery, Royal Papworth Hospital NHS Foundation Trust, Cambridge, UK; ⁴Department of Histopathology, Royal Papworth Hospital NHS Foundation Trust, Cambridge, UK; ⁵Department of Thoracic Oncology, Royal Papworth Hospital NHS Foundation Trust, Cambridge, UK; ⁶Department of Oncology, University of Cambridge, Cambridge, UK; ⁷Department of Medicine, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK

10.1136/thorax-2021-BTSAbstracts.104

The mesothelium is a serous membrane lining of the coelomic cavities, which comprise the pleura, pericardium, peritoneum, tunica vaginalis testis and tunica serosa uteri. It is a dynamic

Abstract S98 Figure 1 Human parietal pleura scRNAseq of freshly prepared cells. T-distributed stochastic neighbour embedding (tSNE) of jointly analysed single-cell transcriptomes from 12,162 cells from 2 pneumothorax patients. Vasc_Endo, vascular endothelial cells; SMC_Per1, smooth muscle cells; T_ILC, T cells, innate lymphoid cells; B_pDCs, B cells, plasmacytoid dendritic cells; Lymph_Endo, lymphatic endothelial cells

structure important for tissue homeostasis by regulating inflammation and wound healing. Defects of the pleura are involved in the pathogenesis of pleural fibrosis and adhesions, and in malignant mesothelioma, an aggressive cancer associated with previous exposure to asbestos.

Currently, there is an inadequate understanding of pleural biology in health, which impedes the development of treatments for these pleural pathologies. To address this, we aimed to establish a reproducible protocol for the isolation and culture of mesothelial cells from human pleural tissue. Moreover, using single-cell RNA profiling, we explored the cellular heterogeneity of human pleura in 8 patients treated for pneumothorax (figure 1). This resulted in the generation of a comprehensive atlas composed of mesothelial, stromal and immune cells, providing a valuable resource for further pleural research.

S99 FLUORESCENCE-LIFETIME IMAGING: A NOVEL DIAGNOSTIC TOOL FOR SUSPECTED LUNG CANCER

¹SE Fernandes, ¹GOS Williams, ¹E Williams, ^{1,2}N Finlayson, ¹Q Wang, ^{1,3}DA Dorward, ⁴C Dhaliwal, ³WA Wallace, ¹AR Akram, ¹K Dhaliwal. ¹Translational Healthcare Technologies Group, Centre for Inflammation Research, Queen's Medical Research Institute, The University of Edinburgh, Edinburgh, UK; ²Institute for Integrated Micro and Nano Systems, The University of Edinburgh, Edinburgh, UK; ³Department of Pathology, Royal Infirmary of Edinburgh, Edinburgh, UK; ⁴Department of Pathology, Western General Hospital, Edinburgh, UK

10.1136/thorax-2021-BTSAbstracts.105

Introduction and Objectives Lung cancer is the commonest cause of cancer-related deaths. Early detection improves outcomes, however, the diagnostic yield of existing sampling techniques is suboptimal. Fluorescence-lifetime imaging microscopy (FLIM), an autofluorescence-based technique which measures endogenous fluorophore decay rates, may aid identification of optimal biopsy sites in suspected lung cancer. We describe the