±1.5 (% viability) CF vs PANC-1, n=3). A lower IC_{50} value for sunitinib was required to exert the same effects on CF (IC_{50}5.2 μM) vs PANC-1 (IC_{50}13.5 μM) cell viability.

These results suggest sunitinib can cause lethal effects in cardiac cells at lower doses than those required to induce pancreatic cancer cell death. Future work will aim to identify cellular mechanisms responsible for these toxic effects. Parallel studies in cardiac and cancer cells will be beneficial in distinguishing how focused anti-cancer drug delivery could be improved to avoid CTX.

7 NOX4 NADPH OXIDASE IS A KEY REGULATOR OF ENDOTHELIAL CELL FUNCTION IN EXPERIMENTAL DIABETES

[1,2]Eleanor K Gill, [1]Kevin S Edgar, [1]Adam J Wilson, [1]Ellen Patterson, [1,2]David J Grieve. *[1]Centre for Experimental Medicine, Queen's University Belfast; [2]British Society for Cardiovascular Research, Belfast, UK*

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Introduction The characteristic hyperglycaemia of diabetes drives reactive oxygen species (ROS) production in endothelial cells (ECs), leading to microvascular dysfunction and cardiovascular complications. NADPH oxidases are enzymes whose primary function is to generate ROS, and which contribute to development and progression of cardiovascular disease. This study aimed to investigate the role of the NOX4 isoform, which is highly expressed in ECs, under hyperglycaemic conditions, in EC signalling and paracrine communication with fibroblasts, as a major determinant of diabetic cardiovascular remodelling.

Methods Human aortic endothelial cells (HAoECs) were treated with normal (NG, 5.5 mM) or high (HG, 25 mM) glucose for up to 5 days with or without NOX4 siRNA knockdown (KD) prior to assessment of mRNA expression (real-time RT-PCR; relative to β-actin) and superoxide generation (DHE fluorescence). NIH 3 T3 fibroblasts were treated with conditioned media from NOX4-modified HAoECs for 24 hour to interrogate effects on paracrine signalling.

Results HG treatment of HAoECs for 5 days increased mRNA expression of NOX4 (NG 1.01±0.06, HG 1.27±0.06; n=9, p<0.05) and associated antioxidant and proinflammatory genes (e.g. NRF2: 0.91±0.04 vs 1.22±0.04; IL-6: 1.03±0.13 vs 2.59±0.58; n=6, p<0.05). This was associated with increased superoxide production (262±12 vs 338±11 arbitrary units; n=4, p<0.05) at 2 but not 5 days. Interestingly, NOX4 KD under HG conditions increased mRNA expression of endogenous antioxidant enzymes after 2 days (e.g. NRF2: 1.90±0.06 vs 2.21±0.06; n=3, p<0.05) whilst normalising increased superoxide production. Furthermore, increased TGFβ-induced differentiation of NIH 3 T3 fibroblasts observed in the presence of conditioned media from HG-treated HAoECs was ablated by NOX4 KD (e.g. α-SMA: scrambled control 1.31 ±0.04, NOX4 KD 0.94±0.07; n=3, p<0.05).

Conclusions HG-induced NOX4 signalling regulates ROS production and endogenous antioxidant expression in ECs, driving paracrine stimulation of fibroblast differentiation. It therefore seems likely that EC NOX4 NADPH oxidase signalling contributes significantly to adverse diabetic cardiovascular remodelling.

8 HEDGEHOG RESPONSIVE STEM CELL ANTIGEN-1/S100β RESIDENT VASCULAR STEM CELLS CONTRIBUTE TO NEOINTIMAL FORMATION

[1]Emma Fitzpatrick, [2]Weimin Liu, [1]Maryam Alshamrani, [2]Jay-Christian Helt, [1]Roya Hakimjavadi, [1]Susan Harman, [1]Abidemi Olayinka, [1]Denise Burtenshaw, [1]Eoin Corcoran, [1]Mariana Di Luca, [3]Catriona Lally, [2]Eileen M Redmond, [1]Paul A Cahill. *[1]Vascular Biology and Therapeutics Laboratory, School of Biotechnology Faculty of Science and Health, Dublin City University, Dublin 9, Ireland; [2]Trinity College Dublin, Trinity Centre For Bioengineering, Trinity Biomedical Sciences Institute, Dublin Ireland; [3]Department of Surgery, University of Rochester Medical Centre, Rochester, NY, USA*

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Intimal medial thickening (IMT) and vascular remodelling are hallmarks of arteriosclerotic disease. The origin of neointimal cells and the signalling molecules that direct their fate and function is controversial. Here, we demonstrate that Hedgehog (Hh) responsive $S100\beta^+/Sca1^+$ perivascular stem cells substantially contribute to IMT within carotid arteries of transgenic mice following ligation-induced injury *in vivo*. Genetic lineage tracing analysis using S100β-eGFP/Cre/ERT2 transgenic mice to mark resident vascular stem cells before injury demonstrated that Hh responsive $S100\beta^+/Sca1^+$ cells substantially contribute to IMT, an effect significantly attenuated following treatment with the Hh smoothened inhibitor, cylopamine. *In vitro*, recombinant SHh (rSHh) treatment of multipotent $S100\beta^+/Sca1^+$ resident stem cells increased target gene Gli expression, decreased telomerase activity, and promoted myogenic differentiation and cell growth; effects significantly attenuated following Hh inhibition. These findings suggest that perivascular $S100\beta^+/Sca1^+$ stem cells are a major source of neointimal cells contributing to IMT and suggest that this cohort may be a relevant therapeutic target to prevent arteriosclerosis.

9 ENDOTHELIAL NOX4 NADPH OXIDASE PROTECTS AGAINST ADVERSE CARDIAC REMODELLING ASSOCIATED WITH EXPERIMENTAL DIABETES

Kevin S Edgar, Eleanor K Gill, Ellen Patterson, David J Grieve. *Centre for Experimental Medicine, Queen's University Belfast, Belfast, UK*

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Background Chronic heart failure (CHF) is a major cause of mortality in diabetes due to characteristic alterations in cardiac structure and function. The diabetic heart is typified by increased fibrosis, inflammation and microvascular remodelling together with hyperglycaemia-induced endothelial dysfunction and reactive oxygen species (ROS) generation, which may predispose to cardiovascular stress. The aim of this study was to investigate the specific contribution of endothelial Nox4 NADPH oxidase, a major source of cardiovascular ROS, to cardiac remodelling in experimental diabetes.

Methods Diabetes was induced in endothelial-specific Nox4 transgenic (Tg) mice and WT littermate controls (10–12 weeks of age; n=8–12/group) by streptozotocin (STZ) injection. After 6 months, echocardiography was performed and blood and cardiac tissue collected for metabolic and gene expression analyses, respectively.

Results Endothelial Nox4 overexpression did not affect blood glucose or HbA1c levels in control or diabetic animals.

Significant diastolic dysfunction was observed in WT STZ mice (E/A ratio: control 1.6±0.09 versus STZ 1.3±0.04). However, whilst Tg control mice demonstrated impaired diastolic function (E/A ratio: 1.6±0.09 vs 1.4±0.08), no further dysfunction was seen with experimental diabetes. Consistent with basal diastolic dysfunction, CTGF and MMP2 expression were increased in Tg control animals, without being further altered by STZ, whereas CTGF was increased in WT STZ animals versus controls. Interestingly, increased expression (compared to WT control animals) of SOD1 (WT 31% ±6.3%, Tg 78%±24%) and catalase (WT 24%±14%, Tg 85%±28%) seen in STZ diabetes was greater in Tg than in WT mice, which is likely to at least partly explain protection against further diastolic dysfunction.

Conclusion These data indicate that endothelial Nox4 NADPH oxidase may protect against adverse cardiac remodelling and dysfunction in experimental diabetes, thereby highlighting this major ROS source as a potential therapeutic target for CHF in diabetes.

10 AM2/IMD SECRETION FROM HUMAN PULMONARY SMOOTH MUSCLE CELLS AND PULMONARY FIBROBLASTS IS AUGMENTED IN RESPONSE TO MECHANICAL STRETCH

Gavin Thomas, Mark T Harbinson, Gary McVeigh, Christopher Watson, Malcolm Campbell, Alice Cheung, David Bell. *Centre for Experimental Medicine, The Queen's University of Belfast*

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Introduction Pulmonary hypertension (PHT) is a severe life-limiting condition resulting in progressive shortness of breath, exercise intolerance and heart failure. PHT is defined by increased mean pulmonary arterial pressure (PAP) ≥25 mmHg at rest, and has been attributed to an imbalance between vasodilator and vasoconstrictor influences in the pulmonary microcirculation. Assessment of the vasodilator AM2/IMD, a member of the CGRP/AM peptide family, may have potential application as novel disease biomarker.

Objective To quantify secretion of AM2/IMD from human pulmonary vascular cells cultured under basal, simulated normotensive and hypertensive conditions.

Methods Pulmonary fibroblasts (PF), pulmonary smooth muscle (PSM), human pulmonary artery endothelial cells (HPAEC) and human pulmonary microvascular endothelial cells (HPMEC) were cultured on silicone elastomer-bottomed Flexcell plates pre-coated with Matrigel at rest (un-flexed) or subjected to cyclic mechanical stretch (Flexcell Strain Unit) to simulate pulmonary normotensive (15 mmHg, 2.0 kPA) and hypertensive (40 mmHg, 5.3 kPA) conditions at a frequency of 1 Hz (60 cycles per minute) for 48 hour. AM2/IMD was extracted from the medium of cultured cells and quantified by ELISA (Phoenix Pharmaceuticals Inc. Karlsruhe, Germany).

Results Concentrations of AM2/IMD in culture medium from cells incubated under various conditions were as follows: ng. $ml^{-1,}$ mean ±SE, n=2–12; *difference relative to un-flexed, $p<0.05$; +difference between normotensive and hypertensive condition.

Conclusion Cyclic stretch enhanced secretion of AM2/IMD from PF and PSM, indicating that these cells may be an important source of this vasodilator peptide in the pulmonary microcirculation under physiological conditions. Secretion was not augmented in hypertension relative to normotensive conditions. AM2/IMD is unlikely therefore to be a suitable diagnostic or prognostic biomarker in PHT.

Abstract 10 Table 1

	PF	PSM	HPAEC	HPMEC
un-flexed	6.98±2.01	0.40±0.06	1.28±0.24	12.63±1.38
normotensive	51.49 ±11.25*	106.81 ±59.22	0.48±0.06*	13.53±1.67
hypertensive	41.58±8.57*	83.65 ±20.53*	0.82±0.07+	18.96 ±2.43*

11 THE ROLE OF N-GLYCOSYLATION OF THE NOTCH1 RECEPTOR IN JAGGED1-STIMULATED MYOGENIC DIFFERENTIATION *IN VITRO*

Abidemi Olayinka, Eoin Corcoran, Brendan O'Connor, Paul A Cahill. *Dublin City University, Vascular Biology and Therapeutics Group, School of Biotechnology*

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Resident stem cell fate decisions within the vasculature are crucial to the pathogenesis of vascular diseases, including, arteriosclerosis, atherosclerosis and in-stent restenosis after angioplasty. The Notch signalling pathway regulates stem cell fate and is highly regulated by a number of mechanisms including glycosylation, a post-translational modification.

Our main objective was to define a putative role for N-glycosylation of Notch1 receptor in controlling resident vascular stem cell fate *in vitro*. Utilising ligand-induced Notch signalling assay with Jagged1, qRT-PCR, immunocytochemistry, ectopic expression of Notch1 receptor, siRNA knockdown, pharmacological inhibition and enzyme linked lectin assay (ELLA), alterations in N-glycan decoration of the Notch1 receptor were assessed before evaluation of their effects on Notch signalling and Notch ligand promotion of myogenic differentiation.

N-glycosylation of the Notch1 receptor was assessed using a combination of the HPLC and ELLA assays and confirmed the presence of N-glycans on the receptor, an effect that was abrogated following inhibition of glycosyltransferase activity with tunicamycin and lunatic fringe (Lfng) knockdown. Jagged1- induced Notch activation increased Notch target gene expression and promoted myogenic differentiation of bone-marrow derived mesenchymal stem cells and resident vascular stem cells. Selective knockdown of the Notch1 receptor in stem cells resulted in a significant decrease in Jagged1 stimulated Hey1 expression, a Notch1 target gene, concomitant with a reduction in myogenic differentiation due to decreased smooth muscle differentiation marker expression (CNN1 and MYH11 mRNA and protein levels). Inhibition of N-glycosylation with tunicamycin lead to a down regulation of smooth muscle differentiation markers, CNN1 and MYH11 independent of a reduction in Notch target gene expression. Lfng knockdown lead to a similar significant reduction in Jagged1 induced myogenic differentiation (reduced CNN1 expression). Collectively, these results suggest that N-glycosylation of Notch1 receptor is involved in Notch signalling leading to altered resident vascular stem cell fate.