Australian & New Zealand Microcirculation Society

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Australia New Zealand Microcirculation Society
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PROGRAM ABSTRACTS

SESSION 1: BUILDING THE VASCULATURE

01. Cloche-independent blood vessel formation in zebrafish
Naoki Mochizuki

02. Hypertension-induced retinal edema via endothelial transcytosis
Injune Kim

03. Shaping the vasculature – a balance of endothelial cell biomechanics and haemodynamic force
Li-Kun Phng

04. The assembly and guidance of zebrafish facial lymphatics
Jonathan Astin

05. Cellular and molecular basis of the neurovascular link
Yoshiaki Kubota
Department of Anatomy, Keio University School of Medicine, JAPAN

To meet tissue requirements for oxygen and nutrients, blood vessels are properly distributed with an appropriate amount and patterning customized to the function of each organ. In this developmental process, diverse interactions between endothelial cells and other cell types contribute to the establishment of such tissue-specific vascular patternings. Our research is mainly focusing on vascular development in the central nervous system (CNS), particularly that in retina, a part of the central nervous system located in eye balls, now widely utilized as a good model to study the mechanism of angiogenesis. In this presentation I would like to discuss our latest findings regarding the cellular and molecular basis of the neurovascular link which determines the vascular patterning of CNS.

ECR SESSION: SHORT TALKS SELECTED FROM ABSTRACTS

01. Lymphatic endothelial cell identity is controlled by a GATA2 bound enhancer.
Jan Kazenwadel[1], Parvathy Venugopal[1, 2], Sandra G. Piltz[3], Chris Brown[4], Samir Taoudi[5, 6], Paul Q. Thomas[3, 7], Hamish S. Scott[1, 2, 3] and Natasha L. Harvey[1*]
[1] Centre for Cancer Biology, University of South Australia and SA Pathology, Adelaide, Australia; [2] Department of Genetics and Molecular Pathology, SA Pathology, Adelaide, Australia ; [3] School of Biological Sciences, University of Adelaide, Adelaide, Australia.
[4] University of South Australia, Adelaide, Australia; [5] Molecular Medicine Division, Cancer and Haematology Division, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia; [6] Department of Medical Biology, University of Melbourne, Melbourne, Australia; [7] South Australian Health and Medical Research Institute, Adelaide, Australia.

Transcriptional enhancer elements are responsible for orchestrating the temporal and spatial control over gene expression that is crucial for programming cell identity during development. Here, we describe a novel enhancer element important for regulating Prox1 expression in lymphatic endothelial cells. This evolutionarily conserved enhancer is bound by key lymphatic transcriptional regulators including GATA2, FOXC2, NFATC1 and PROX1. CRISPR-Cas9 genome editing of this enhancer element revealed that deletion of only 5 nucleotides encompassing the GATA2 binding site has a dramatic impact on lymphatic vascular development; mice homozygous for this deletion die soon after birth exhibiting profound lymphatic vascular defects. Lymphatic endothelial cells in enhancer mutant mice exhibit reduced levels of genes characteristic of lymphatic endothelial cell identity and acquire characteristics of hemogenic endothelium, including the capacity to generate hematopoietic cells. These data reveal the first transcriptional enhancer element

important for regulating Prox1 expression and lymphatic endothelial cell identity and suggest that Prox1 is important for repressing hemogenic cell identity in the lymphatic endothelium.

02. Myocardial canonical Wnt signaling pathway regulates coronary vessel formation in zebrafish.

Ayano Chiba, Naoki Mochizuki

Department of Cell Biology, National Cerebral and Cardiovascular Center Research Institute

The canonical Wnt signaling pathway is spatiotemporally activated during cardiac development including heart tube looping and atrioventricular valve formation. However, it remains unclear when and where the signaling is exactly activated in cardiomyocytes (CMs) during cardiac development.

We aimed to visualize beta-catenin (Ctnnb)-dependent transcriptional activation in CMs of developing zebrafish heart. Using GAL4/UAS system, we established a transgenic zebrafish that expressed fluorescent protein reflecting Ctnnb-dependent transcriptional activation in CMs (Ctnnb-reporter). Ctnnb-signaling was exclusively activated in CMs at the atrioventricular canal (AVC) region, especially in atrial CMs.

To explore the upstream of Ctnnb signaling, we examined the expression of canonical Wnt ligands. Wnt2bb and Wnt9b were expressed at the AVC. We noticed that the expression of Wnts and the activity of Ctnnb-reporter were dependent on the heartbeat. Heartbeat-dependent Wnt expression seems to activate Ctnnb signaling in CMs at the AVC. Then, we tried to investigate the role of Ctnnb signaling-activated CMs at the AVC. In zebrafish, coronary vessel endothelial cells are derived from endocardial cells at the AVC region. To examine the role of Ctnnb signaling-activated CMs, we ablated them by the Nitroreductase expression. The ablation of Ctnnb signaling-activated CMs resulted in defects of coronary vessel formation. Furthermore, either decreased heart rate or Wnt signaling inhibitor treatment led to the impairment of coronary vessel formation. These data suggest that heartbeat-dependent Ctnnb signaling activation in CMs at the AVC might promote coronary vessel outgrowth.

We here identified Ctnnb signaling-activated CMs and their role for coronary vessel development in zebrafish heart.

03. Effects of obesity on vascular and circulating immune cells in mice.

Tran Vivian, Diep Henry, Sobey Christopher G, Lim Kyungjoon, Drummond Grant R, Vinh Antony, Jelinic Maria

Obesity is associated with low-grade chronic inflammation and the accumulation of immune cells in the perivascular adipose tissue (PVAT) of blood vessels. In obesity, PVAT is a likely cause of vascular dysfunction but the precise mechanisms by which PVAT contributes to a pro-inflammatory state remain unclear. Therefore, we aimed to characterise the aortic immune cell profile in a mouse model of obesity. Six-week-old male C57BL/6 mice were fed a high-fat diet (HFD) for 10 weeks, after which flow cytometry was used to enumerate immune cell populations in the blood and aorta. Compared to mice on a normal diet (ND), HFD mice exhibited higher bodyweight, blood cholesterol and fasting blood glucose levels at the completion ($P<0.05$, n=9-12). The frequencies of total (CD3+) T cells and CD4+ T cells as a proportion of CD45+ cells were reduced in the aorta of HFD vs. ND mice (14±0.6% vs. 23±2% and 32±1% vs. 43±2%; both $P<0.01$). This correlated with a reduced percentage of total T cells in the blood (16±1% vs. 28±3%; $P=0.001$). Conversely, aortic, but not circulating neutrophils (Ly6G+) were more prevalent in HFD mice (35±5% vs. 15±3%; $P=0.006$). Overall, our data demonstrates a shift in the proportion of aortic immune cells during obesity which is independent of changes to circulating immune cells. Ongoing studies are aimed at better understanding the relative contributions of immune cells to vascular dysfunction during obesity.

04. *Ccm2l* deletion aggravates cerebral cavernous malformation in *Ccm2*-deficient mice by activating MEKK3-KLF signalling pathway.

Jaesung P. Choi[1,2]

[1]Centenary Institute, Sydney NSW 2050, Australia; [2]Faculty of Medicine and Health, Sydney Medical School, University of Sydney, Sydney, NSW 2050, Australia

Ccm2-like (Ccm2l) is a paralog of Ccm2 which is selectively expressed in endothelial cells (ECs). CCM2L competes with CCM2 for binding to CCM1 and shown to have antagonistic function to CCM2. CCM2L brings MEKK3 and other potential unknown factors. Our preliminary study demonstrated increased Ccm2l expression in brain ECs with cerebral cavernous malformation (CCM) pathogenesis. The role of Ccm2l has not been studied in CCM. Hence, my aim is to elucidate the role of Ccm2l in CCM and determine whether increased Ccm2l is a causal or compensatory mechanism.

We generated CCM2L knockout mice (Ccm2l-/-) to test our aims in CCM1 (Ccm1iECKO) and CCM2 (Ccm2iECKO) deficient CCM mouse models. Brains were harvested to measure CCM lesion burden using micro-CT. Brain ECs were isolated for real-time PCR and immunoblot analysis.

Micro-CT analysis revealed that complete CCM2L deletion in Ccm2iECKO mice significantly increased CCM lesion burden. Heterozygous CCM2L deletion did not affect CCM lesion burden. Klf2 and Klf4 mRNA expressions were significantly increased in Ccm2iECKOCcm2l-/- compared to controls, which correlates increased lesion burden. Hemizygote MEKK3 deletion significantly reduced lesion burden in Ccm2iECKOCcm2l-/- mouse, suggesting CCM2L regulates MEKK3-KLF signalling pathway in CCM pathogenesis. However, unlike in Ccm2iECKO, CCM2L deletion did not affect CCM lesion burden in Ccm1iECKO mice.

In summary, our study suggests increased Ccm2l expression in CCM lesions is a compensatory response. Ccm2l regulates CCM pathogenesis only in CCM2-deficient mouse through MEKK3-KLF signalling pathway. Hence, our study demonstrates a complex

05. Novel Role for the Mitochondrial Calcium Exporter NCLX in Controlling Endothelial Cell Signaling and Phenotype

Jacqueline Ku, Melanie Lim, Calum McArthur, Lei Dang, Thuan Thai, Fei Zhong, Shane R. Thomas.

School of Medical Sciences, University of New South Wales, Australia.

Mitochondria are increasingly recognized to control endothelial cell (EC) function and phenotype through the regulation of intracellular calcium signalling and production of reactive oxygen species (ROS). The sodium-calcium-lithium-exchanger (NCLX) is a recently discovered mitochondrial calcium exporter capable of controlling mitochondrial calcium and ROS levels. Here we investigated the role of NCLX in controlling (i) EC calcium and nitric oxide (NO) signaling and (ii) EC phenotypic modulation into mesenchymal-like cells via endothelial-to-mesenchymal transition (EndMT), a process recently identified as a form of endothelial dysfunction driving atherosclerosis. Stimulation of arterial ECs with vascular endothelial growth factor (VEGF) induced the rapid and transient increase in both mitochondrial and cytosolic calcium levels and the calcium-dependent phosphorylation of eNOS at Ser1177 and enzyme activation. Inhibition of NCLX with its pharmacological inhibitor CGP37157 or NCLX-targeted siRNA significantly prolonged the VEGF-induced elevation in mitochondrial calcium and attenuated VEGF-induced cytosolic calcium transients and subsequent Ser1177 phosphorylation and activation of eNOS. Interestingly, NCLX gene-silencing in ECs yielded a change in cellular morphology from the traditional cobble-stone appearance of ECs to a more spindle-shaped morphology of mesenchymal cells. This change in cell morphology was paralleled by the increased production of mitochondrial ROS, enhanced cell migratory capacity in a scratch-wound assay and up-regulated expression of key mesenchymal genes including fibroblast activation protein (FAP), versican and matrix metalloproteinases. This study identifies mitochondrial NCLX as a novel regulatory element controlling (i) intracellular calcium signaling leading to eNOS activation in ECs stimulated with VEGF and (ii) EndMT.

06. Cell-specific functions of TRAIL critical for angiogenesis and vessel stabilization

Manisha Patil [1,2], Siân Cartland[1,2], Ruth Ganss[3], Mary Kavurma[1,2]

1. Vascular Complications, Heart Research Institute, Sydney, NSW, Australia; 2. Sydney Medical School, The University of Sydney, Sydney, NSW, Australia; 3. Vascular Biology and Stromal Targeting, Harry Perkins Institute of Medical Research, Perth, WA, Australia

Angiogenesis is critical for generation of microvascular capillary networks; the cornerstones of nutrient diffusion necessary for tissue development and wound repair. We previously identified TNF-related apoptosis-inducing ligand (TRAIL) as an exciting new molecule that stimulates angiogenesis *in vitro*, and *in vivo* in mice with peripheral artery disease, restoring blood flow, and preserving tissue survival and function. How TRAIL does this is unclear. Our aim was to assess the contribution of endothelial cell (EC)- and pericyte-specific TRAIL expression to angiogenesis *in vivo* and identify the effect of TRAIL on pericyte processes *in vitro*. We generated mice lacking TRAIL specifically from ECs ($Trail^{EC-/-}$) and pericytes ($Trail^{PC-/-}$). The Matrigel plug assay was used to examine angiogenic processes in these mice. EC and mural cell content in plugs from $Trail^{EC-/-}$ was ~50-60% less than in plugs from $Trail^{EC+/+}$ mice, whereas capillary density in plugs of $Trail^{PC-/-}$ mice remained unchanged. Pericyte and angiogenic markers, including RGS5, PDGFRβ, NG2 and VEGF were also reduced ~50% in $Trail^{EC-/-}$ plugs, suggesting that EC-, but not pericyte-expressing TRAIL is critical for angiogenesis. *In vitro*, isolated primary pericytes from $Trail^{-/-}$ or $Trail^{+/+}$ mouse brain had no effect on pericyte proliferation or migration. In contrast, ECs cultured with $Trail^{-/-}$ pericytes displayed significantly less tubule formation than those cultured with TRAIL-expressing cells suggesting that TRAIL regulates pericyte wrapping of capillaries. We have identified novel cell-specific functions of TRAIL critical for angiogenesis and vessel stabilisation. Understanding how TRAIL signals regulate angiogenesis may identify new targets for therapeutic intervention in patients with cardiovascular disease.

PLENARY 1

Endothelium and Aging - Jenny Gamble (Australia)

PLENARY 2

Immunology of microcirculation - Paul Kubes (Canada)

SESSION 2: LYMPHANGIOGENESIS

01. Molecular mode of action of the dominant-negative SOX18 transcription factor: aetiology of a rare lymphatic vascular disorder

Mathias Francois

Australia

02. TGF-β family signals in the formation and maintenance of vascular systems

Tetsuro Watabe

Department of Biochemistry, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University (TMDU), Tokyo, Japan

Formation and maintenance of blood and lymphatic vessels are regulated by various cytokines including transforming growth factor (TGF)-β. Endothelial-mesenchymal transition (EndMT) plays important roles in various physiological and pathological processes, and has been shown to be induced by TGF-β. While we have previously reported that TGF-β signals play important roles in the proliferation and maintenance of blood vascular endothelial cells, the effects of TGF-β signals on lymphatic endothelial cells (LECs) have not yet been fully elucidated. Here, we examined the effects of TGF-β signals on the proliferation and EndMT of LECs. Addition of TGF-β2 to human dermal lymphatic endothelial cells (HDLECs) induced decreased proliferation and mesenchymal transition characterized by increased expression of various mesenchymal markers such as α-smooth muscle actin (α-SMA) and decreased expression of lymphatic endothelial cell markers including LYVE-1. Prox1 transcription factor plays important roles in the differentiation and maintenance of LECs. We found that TGF-β signals decreased Prox1 expression in HDLECs, which led to the loss of LEC identity. Furthermore, we found that LEC specific deletion of TGF-β type II receptor gene caused increased diameter

of lymphatic vessels. These findings suggest that TGF-β signals play important roles in the maintenance of lymphatic vessels.

03. Understanding the effects of cancer therapies on the lymphatic vasculature

Tara Karnezis
Australia

04. Blood-lymph partitioning in mouse embryonic skin

Masanori Hirashima
Division of Pharmacology, Graduate School of Medical and Dental Sciences, Niigata University

Lymphatic endothelial cells (LECs) are derived from venous endothelial cells during embryogenesis, but lymphatic vessels are subsequently established as a vasculature separate from blood vessels in peripheral tissues. Previous studies proposed a critical role of a platelet plug at the lymphovenous valve in preventing blood from entering the lymphatic circulation. In this study, we found peripheral blood-lymph misconnection sites in Phospholipase Cγ2 knockout ($Plcg2^{-/-}$) mice which lack platelet activation. Abnormal blood-lymph connections were randomly detected by fluorescent angiography in back skin of $Plcg2^{-/-}$ embryos. Blood-filled lymphatics were also detected in $Plcg2^{-/-}$ embryos lacking physiological lymphovenous connections at the venous angle, indicating that platelets keep lymphatic vessels separate from blood vessels during lymphatic sprouting. In vitro analysis showed that platelets or activated platelet-conditioned medium inhibited LEC migration and proliferation in a Syk- or Plcg-dependent manner. Time-lapse analysis showed LEC retraction as an immediate response to platelets or activated platelet-conditioned medium. I will discuss a novel role for platelets in blood-lymph partitioning in mouse peripheral tissues.

05. Mesenteric lymphatic dysfunction in obesity promotes insulin resistance

Natalie Trevaskis
Australia

SESSION 3: ANGIOGENESIS

01. Vascular sprouting and integrity: Novel regulators of VEGF receptor signaling

Young-Guen Kwon, Ph.D.
Department of Biochemistry, College of Life Science and Biotechnology, Yonsei University, Seoul, Korea

Patterning of new vascular structure is a coordinated multi-step process that involves sprouting and morphogenesis of endothelial cells (ECs) and requires precisely controlled expression and activation of vascular endothelial growth factor receptors. In order to identify novel genes, which are potentially involved in regulating angiogenesis and vascular patterning, we have set up in vitro EC differentiation model and analyzed gene expression profile during the differentiation by employing DNA microarray and mRNA sequencing methods. We isolated a number of genes, which have unique expression patterns in ECs. Among these genes, we have identified Clec14a, Salm4, and DIXDC1 as novel regulators of VEGFR signaling in ECs. CLEC14A acts in vascular homeostasis by fine-tuning VEGFR-2 and VEGFR-3 levels in ECs and SALM4 specifically regulates VEGFR-2 phosphorylation at Tyr1175. Moreover, DIXDC1 is involved in controlling protein stability of VEGFR-2. These findings suggest usefulness of our system in revealing spatial and temporal involvement of various genes during vascular patterning and offer novel control mechanisms of VEGF signaling in the vasculature.

02. PDGF-C and PDGF-D in angiogenesis

Xuri Li
China

03. New mechanisms and regulators of angiogenesis in diabetes

Khalia Primer[1,2], Emma Solly[1,2], Peter Psaltis[1,2], Joanne Tan[1,2] and Christina Bursill[1,2]

1 Vascular Research Centre, Heart Health Theme, South Australian Health and Medical Research Institute, Adelaide, Australia; 2 Faculty of Health and Medical Science, Adelaide Medical School, University of Adelaide, Adelaide, Australia

Diabetic vascular complications are characterised by impaired angiogenic responses to ischaemia. Our laboratory has identified that high-density lipoproteins (HDL) rescue diabetes-impaired angiogenesis, however full elucidation of the mechanisms remains under investigation. Our laboratory has recently focussed on two new pathways. In the first of these we are determining whether reconstituted HDL (rHDL) increases endothelial cell (EC) tolerance to hypoxia in high glucose via improved metabolic reprogramming responses. We find rHDL increases the metabolic reprogramming protein pyruvate dehydrogenase 4 (PDK4) that prevents glucose-derived pyruvate from entering the tricarboxylic acid cycle. Consistent with this, rHDL attenuated high glucose-induced mitochondrial respiration in hypoxia. Whilst in parallel, rHDL rescued high-glucose impaired EC tubulogenesis and migration responses to hypoxia, key angiogenesis functions. In vivo using a diabetic murine model of wound healing, daily topical application of rHDL increased wound PDK4 expression, wound angiogenesis and the rate of wound closure.

Using a miRNA array on rHDL treated ECs we have identified a new anti-angiogenic miRNA, miR-181c, that is strikingly upregulated in high glucose and downregulated in hypoxia. Inhibition of miR-181c rescues high-glucose impaired in vitro tubulogenesis and increases the mRNA levels of pro-angiogenic VEGFA and anti-apoptotic BCL-2 genes. In vivo, local hindlimb inhibition of miR-181c completely prevented diabetes-impaired blood-flow reperfusion in ischaemic hindlimbs and increased neovessel formation.

In conclusion, rHDL corrects high glucose-impaired metabolic reprogramming responses to hypoxia which may underlie its pro-angiogenic effects. We have also revealed a new anti-angiogenic role for miR-181c, highlighting it as a therapeutic target for the prevention of diabetic vascular complications.

04. Vascular and Immune Cell Network in the Pathogenesis of Atherosclerosis

Goo Taeg Oh

Korea

05. Roles of tumor endothelial cells in tumor progression

Kyoko Hida

Vascular Biology and Molecular Pathology Graduate School of Dental Medicine, Hokkaido University

Tumor growth and metastasis are dependent on angiogenesis. Tumor blood vessels, especially the endothelial cells lining tumor blood vessels (tumor endothelial cells [TECs]), are important targets in cancer therapy. It has been known that tumor blood vessels have a distinctively abnormal phenotype, including morphological alterations. Also, it has been revealed that TECs constitute a heterogeneous population, exhibiting characteristics that are induced by tumor microenvironmental factors. Furthermore, TECs contribute to cancer progression through metastasis. For example, TECs in highly metastatic tumors aberrantly express biglycan, chemoattracting factor which stimulates cancer cell intravasation, in turn they instigate tumor cells to metastasize. Also, TEC intracellular adhesion molecule, VE-cadherin expression was downregulated by tumor-extracellular vesicles (EVs), causing in tumor metastasis. Besides, we have found that TECs express ABCB1, a drug transporter molecule and TEC ABCB1 is upregulated by chemotherapeautic drugs. TEC may also affect to tumor immunity negatively by enhancing PDL-1 expression level in cancer cells. TEC abnormalities related to cancer progression will be to provide insight into new anticancer therapies

SESSION 4: INFLAMMATION AND THE VASCULATURE

01. Impaired neutrophil function with age contributes to the onset of stroke-associated infection

Shu Wen Wen[1] and Connie H. Y. Wong[1]

1 Centre for Inflammatory Diseases, Department of Medicine, School of Clinical Sciences, Monash University, Clayton, Victoria 3168, Australia.

Bacterial pneumonia is a leading cause of death among patients with stroke. We recently reported that the frequency and severity of pneumonia is greater with advanced age, raising the possibility that antibacterial immune responses by neutrophils that normally facilitate bacterial clearance are compromised with advanced age after stroke. Using an experimental mouse model of stroke, we evaluated the effects of ageing on neutrophil function in young (7-10 weeks) and older (12-15 months) C57Bl/6 male mice. Older post-stroke mice showed a slight increase in lung neutrophil numbers despite presenting with a 100-fold increase in culturable lung bacteria when compared to young cohorts, suggesting a possible age-associated impairment of neutrophil recruitment and function. Indeed, while neutrophils isolated from post-stroke aged mice had comparable phagocytic ability to those from young mice, they showed reduced in vitro migration towards potent chemoattractants, fMLP and IL-18. In vivo cremaster intravital imaging experiments to test neutrophil recruitment towards a gradient of IL-18 further showed neutrophils from older mice have reduced transmigration and chemotaxis in the extravascular tissue. For a comprehensive overview on phenotypic differences between neutrophils, we further isolated pulmonary neutrophils from young and older mice after stroke, and characterised them using RNA sequencing. While neutrophil transcripts from young stroke mice were closely associated with positive regulation of immune responses, neutrophils from post-stroke aged mice were enriched in transcripts aligned with stress responses and cell death pathways. Taken together, our results suggests the age-dependent onset of stroke-associated infection may be attributed to impaired neutrophil response and function.

02. Role of Resident Arterial LYVE-1+ Macrophages in Vascular Homeostasis and Disease

Veronique Angeli

Singapore

03. Therapeutic Vaccines for Cardiovascular Diseases

Hironori Nakagami, MD. Ph,D,

Department of Health Development and Medicine, Osaka University Graduate School of Medicine

Vaccines are commonly used worldwide as a preventive medicine for infectious diseases and have recently been applied to cancer. We and others have developed therapeutic vaccines designed for cardiovascular diseases that are notably different from previous vaccines. In the case of cancer vaccines, a specific protein in cancer cells is a target antigen, and the activation of cytotoxic T cells (CTLs) is required to kill and remove the antigen-presenting cancer cells. Our therapeutic vaccine mainly induces the antibody, but not CTLs, which could be used as therapies against common diseases, such as Alzheimer's disease or hypertension. In our system, an immunogenic molecule (i.e., KLH) with adjuvants provides an antigen that supports the activation of helper T cells in the combination of adjuvants. We have reported the Angiotensin II vaccine for hypertension and related diseases (Hypertension 2015, Stroke 2017), DPP-4 vaccine for Diabetes (PNAS 2014) and PCSK9 vaccine for Dyslipidemia (PLoS One 2018) in each animal model. In terms of Angiotensin II vaccine project, the phase I clinical trial has been designed and first-in-patient was enrolled in 2018.

The therapeutic target of our therapeutic vaccine is similar to that of antibody therapy. Recently, multiple antibody-based drugs have been developed for cancer, immune-related diseases and dyslipidemia, which are efficient but expensive. If the effect of a therapeutic vaccine is nearly equivalent to antibody therapy as an alternative approach, the lower medical cost and improvement of drug adherence can be advantages of therapeutic vaccines.

04. Monocyte subset contributions to glomerular inflammation

Michael Hickey

Australia

05. TBA

Masayuki Yoshida

Japan

SESSION 5: STEM CELLS AND THE VASCULATURE

01. Identification of adult sinusoidal endothelial stem cells with revascularization potential

Peter AG McCourt[1†], Ana Oteiza [†], Melanie J Domingues[2,3], Christian M Nefzger[3,4,5], Melonie J Storan[2], Brenda Williams[2,3], Chad K Heazlewood[2,3], Karen K Sørensen[1], Songhui Li[2,3], Fernando J Rossello[3], Peter F Choong[6], John F Ouyang[7], Owen JL Rackman[7], Yoshiaki Kubota[8], Benjamin Cao[2,3], Jose M Polo[3,4] and Susan K Nilsson[2,3]

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Hematopoietic stem cells (HSC), the only cells capable of sustained mature blood cell production, reside in a bone marrow (BM) stem cell niche; a specialised microenvironment comprised of many different cell types. BM blood vessels are the interface between circulating blood cells and hemopoiesis. Although the vascular compartment of the BM niche has long been identified as one of the major components involved in HSC regulation and maintenance, little is known about the characteristics or functional capacity of BM sinusoidal endothelial cells. In this study, we identified, prospectively isolated and characterized BM scavenging sinusoidal endothelial cells (BMSEC) utilising their immense endocytic ability as a functional marker. FITC-labelled advanced glycation end-product modified bovine serum albumin (FA), a ligand for endocytic scavenger receptors specifically labels BM sinusoidal vasculature and was used to identify multiple BM sinusoidal endothelial sub-populations. Analysis of these sub-populations revealed a hierarchically organized system with a stem cell population (BM-ESSC; CD45-VE- Cadherin+ CD31+ ESAM+ CD300lg +VEGFR3 +α1+Kdr+Mpl-Stab1+Nrp+kitlo/-Runx1lo/-) capable of serial long-term BM revascularization and reconstitution of the entire BMSEC hierarchy following transplant at the apex. Interestingly, this BM-ESSC population was only found in the endosteal bone marrow region and at a frequency similar to that of HSC. However, importantly HSC and BM-ESSC populations were shown to be mutually exclusive in terms of both cell surface phenotype as well in their molecular signature at the population and importantly at the single cell level. Moreover, we identified human endothelial counterparts suggesting their use in transplantation may lead to improved clinical outcomes as vascular recovery is intrinsically linked to hematopoietic recovery post-transplant.

02. Cell fate conversion and its application

Yoo Wook Kwon

Korea

03. Therapy of Ischemic Diseases using Human Induced Pluripotent Stem Cells

Jaeho Kim

Korea

04. Altering the bone marrow microenvironment to promote haematopoetic stem cell mobilisation

Andrew Murphy

Australia

05. Formation and function of platelets

Dr Samir Taoudi,

The Walter and Eliza Hall Institute, Melbourne.

Platelets are produced by megakaryocytes to maintain vascular integrity and are transfused clinically to prevent bleeding. How platelets are produced by megakaryocytes in vivo remains controversial despite more than a century of investigation. Megakaryocytes readily produce proplatelet structures in vitro, however visualization of platelet release from proplatelets in vivo has remained elusive. Here, by combining three- and four-dimensional quantitative imaging, we show that within the native prenatal and adult environments the low frequency of proplatelet formation is incompatible with the physiological demands of platelet replacement. We resolve this inconsistency by describing a previously uncharacterized platelet-forming mechanism: plasma membrane budding. Megakaryocyte membrane budding occurs at high frequency and results in the sustained release of platelets directly into the peripheral circulation during both fetal and adult life without induction of cell death or proplatelet formation.

PLENARY 3

Title TBA – Nobuyuki Takakura (Japan)

SESSION 6: DIABETES AND THE VASCULATURE

01. Latest updates on the microbiome

Emad M El-Omar

Australia

02. Endothelial function in small arteries from women with gestational diabetes

Tim Murphy

Australia

03. Diabetes, platelets and thrombosis - an unholy trinity

James McFadyen

Australia

04. Microvascular insulin resistance in skeletal muscle

Dino Premilovac, PhD

School of Medicine, College of Health and Medicine, University of Tasmania

Skeletal muscles comprise ~40% of body mass and are important regulators of whole-body metabolism. More than 50% of all post-prandial glucose uptake occurs in skeletal muscles and its transport into muscle cells is dependent on insulin. Within muscle, an important, and at times overlooked action of insulin is to first increase microvascular blood flow via an Akt/nitric oxide-dependent mechanism. By dilating the microvasculature, insulin enhances the delivery of both glucose and insulin to muscle cells to facilitate

increased insulin-stimulated glucose uptake. In insulin resistance, this microvascular action of insulin is lost early and predicts the reduction in insulin-mediated muscle glucose uptake. Importantly, this microvascular insulin resistance precedes any changes in muscle cell insulin sensitivity, suggesting that the microvasculature is acutely affected by alterations in whole body metabolism. While we know that insulin normally acts to increase microvascular blood flow in muscle, identifying exactly where this occurs in the vascular tree and therefore where this vasodilatory effect is lost in insulin resistance has remained elusive. We have new data showing that, (1) pericytes are numerous in muscle capillary networks; (2) pericytes have increased Akt phosphorylation when exposed to insulin in vitro; and (3) pericyte coverage of muscle capillaries changes in type 2 diabetes. This indicates that pericytes are insulin responsive and therefore may contribute to vasodilatory actions of insulin in the muscle microvasculature. This talk will use unpublished data to highlight the potential role capillary-bound pericytes may play in this important physiological process and how this may change in insulin resistance.

05. Relationship between plaque destabilization and glucose / nucleic acid metabolism.

Akifumi Kushiyama, Masahiro Takahashi, Hironori Nakagami

Meiji Pharmaceutical University, Osaka University

Atherosclerosis in diabetic complications tends to be severe, although the mechanism is unknown. Plaque destabilization is important for the onset of cardiovascular events, and foam formation of macrophages plays a major role. Although studies about macrophage foamability have been previously reported mainly using acetylated LDL as modified LDL, we now found that the type of lipid administered to macrophages causes differences in the intracellular metabolic pathways on which foam formation is dependent.

Specifically, acetylated LDL is taken up by macrophages without any relationship to glucose or pyruvate concentration, but intracellular uptake of VLDL dose-dependently requires the presence of glucose and pyruvate. The uptake of VLDL is also completely suppressed by 2-deoxyglucose and a xanthine oxidase inhibitor. That is, glucose and pyruvate metabolite is necessary for VLDL uptake expansion, and suppression of nucleic acid metabolism by XO inhibition can inhibit VLDL uptake. In vivo, XO inhibition in macrophages leads to improved insulin resistance in studies in tissue-specific XOKO mice, and administering a peptide vaccine of XO improved inflammatory cell infiltration of the liver.

In conclusion, whether macrophage foamability is affected by diabetes or hyperglycemia might depend on the type of lipid to be incorporated in cellular model, and inhibition of glucose and/or nucleic acid metabolism completely suppresses foam formation. A new mechanism is inferred to suppress atherosclerosis by regulating metabolism.

06. Fibre prevents high salt diet-induced gut permeability, stem cell mobilisation and atherosclerosis

Man K.S. Lee[1], Olivia D. Cooney[1], Annas Al-sharea[1], Michelle Flynn[1], Gerard Pernes[1], Helene Kammoun[1], Graeme Lancaster[1], Andrew J. Murphy[1]

[1]Baker Heart & Diabetes Institute, Melbourne, Australia

A high salt diet (HSD) is a key risk factor for atherosclerotic-cardiovascular disease (CVD). We show that a HSD (3.6% NaCl) promotes the maturation of pathogenic TH17 cells in the bone marrow (BM) and promotes the breakdown of the BM niche, by reducing leptin receptor mesenchymal cells (LepR+MSCs) and increasing the number of osteoclasts in atherosclerotic-prone Apoe-/- mice. This lead to the egress of haematopoietic stem and progenitor cells (HSPCs) into the spleen, inducing monocytosis via extramedullary myelopoiesis which plays a causal role in atherosclerosis. Administering anti-IL17A antibody and zoledronic acid restored LepR+MSCs and osteoclast levels in the BM back to control (NSD; 1% NaCl) while preventing HSPC egress and atherogenesis. Recently, a HSD has been found to alter the microbiome, promoting intestinal permeability and TH17 maturation. Thus, we supplemented HSD-mice with fibre (400mg/kg), to restore the microbiome and prevent gut permeability. Fibre prevented HSD-induced intestinal permeability and TH17 maturation. Fibre also reduced BM TH17 cells and restored the levels of HSD-induced LepR+MSCs and osteoclasts back to control levels, preventing HSPC egress to the spleen. This reduced the number of inflammatory monocytes entering into the atherosclerotic lesion, inhibiting atherogenesis. These findings demonstrate that fibre

supplementation can restore inflammatory effect in the gut and also prevent the breakdown of the BM niche, thus preventing HSD-induced atherogenesis.

SESSION 7: MECHANOSENSING IN THE BLOOD AND VASCULATURE/DEVICES

01. Mechanisms of mechanotransduction at cadherin cell-cell junctions

Alpha Yap
Australia

02. TBA

Charles Cox,
Australia

03. Mechanotransduction in monocytes and consequences for inflammation

Sara Baratchi,
Australia

04. Vascular intraluminal pressure inhibits angiogenic endothelial cell movement and branch elongation via vascular wall stretch

Koichi Nishiyama (1), Shinya Yuge (2), Yuichiro Arima (1), Yasuyuki Hanada (1), Sanshiro Hanada (1), Ryuji Yokokawa (3), Takashi Miura (4) and Shigetomo Fukuhara (2)
Int. Natl. Res. Ctr. for Med. Sci. (IRCMS), Kumamoto Univ.; Dept. of Mol. Pathophysiol., Inst. of Adv. Med. Sci., Nippon Med. School; Dept. Micro Eng., Kyoto Univ.; Dept. Anatomy and Cell Biol., Kyushu Univ.

Sprouting angiogenesis is a multicellular morphogenesis, in which vascular endothelial cells (ECs) concertedly elongate the branch, forming lumen structure. We reported sprouting angiogenesis to be driven by dynamic collective EC movement including directed migration mode. However, it is still unknown whether or not and how blood flow-mediated vascular intraluminal pressure can affect the angiogenic EC movement.

To dissect the issue, first we established a reconstitution assay system, in which 3D sprouting angiogenesis was induced in a microfluidic device based on an existing design (Lab Chip 2013), using HUVECs, and further, vascular intraluminal pressure could be reproduced by placing hydrostatic pressure on the reconstructed vascular lumen. Using this assay system, interestingly, we identified that hydrostatic pressure load inhibits branch elongation. Time-lapse imaging at cellular and subcellular levels and whole-mount immunostaining revealed that hydrostatic pressure load extended vascular lumen with circumferential stretch of ECs, which abruptly caused failure of directed migration with anterior-posterior polarity loss in ECs including tip cells. We further found that Arp2/3 complex failed to be formed locally in the leading front of ECs in the very acute phase of the load, followed by suppression of proper F-actin bundling and remodeling and lamellipodia formation, which resulted in impaired directed ECs migration. These results indicate a novel function of vascular intraluminal pressure as an inhibitory regulator of sprouting angiogenesis, and provide a mechanobiological insight into blood flow-dependent angiogenic mechanisms. We are now investigating the mechanosensing mechanism and the mechanistic involvement in physiological and pathological angiogenesis.

05. Analyses of flow-dependent angiogenesis in zebrafish

Hiroyuki Nakajima1 and Naoki Mochizuki1, 2
1. National Cerebral and Cardiovascular Center Research Institute; 2. AMED-CREST

Blood flow-mediated mechanical stimuli are important for vascular development, maintenance, and remodeling. While flow-dependent vascular maintenance and remodeling have been extensively studied, it is poorly understood how flow-mediated mechanical stimuli control angiogenesis. To analyze the relevance of flow to angiogenesis, we simultaneously visualized both blood flow and shape of blood vessels using

genetically modified zebrafish lines expressing fluorescence in blood vessels and by fluorescence-labelled beads. We here report that new vessel formation connecting aortic arch arteries (AAs) depended on blood flow. Inhibition of heart beats by drugs and morpholinos targeting for cardiomyocytes resulted in suppression of both budding from the AAs and elongation of the sprouts. It is of note that sprouting from the AA appeared to be bud-shaped angiogenesis with cell-cell junction, apico-basal polarity, and less filopodia in clear contrast to the tip-stalk type angiogenesis. Apical membrane of the endothelial cells (ECs) in the bud was continuously exposed to the flow. Our high-speed imaging analyses revealed that blood flow was disturbed in the lumen of the bud. In those budding ECs, intracellular Ca2+ oscillations were observed in a flow-dependent manner, suggesting that the budding ECs mechanically responded to blood flow.
Thus, we have uncovered a novel mode of angiogenesis driven by blood flow in the AA that is required for the development of pharyngeal arches.

SESSION 8: ATHEROTHROMBOSIS AND THROMBOINFLAMMATION

01. Mechano-redox control of integrin Mac-1 in thromboinflammation

Freda Passam
Australia

02. Regulation of platelet redox by platelet receptors

Elizabeth Gardiner
Australia

03. Tricky play of dying endothelium with red blood cells and platelets

Mike Wu
Australia

04. Investigation of Recanalization and Cerebral Perfusion with Adjunctive Thrombolytic Therapies in the iCAT Mouse Model of Ischemic Stroke

J. Maclean[1,2], A. Tomkins[1], B. Hofma[1], S. Sturgeon[2], A. Samson[1,2], S. Schoenwaelder[1,2] S. Jackson[1,2].
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Background: Thrombolytic therapy with recombinant tissue plasminogen activator (rtPA) remains the primary pharmacological therapy for ischemic stroke. However, the extent of arterial recanalization achieved with thrombolysis remains suboptimal in many patients. Additionally, recanalization does not always achieve successful cerebral reperfusion. This demonstrates the need for adjunctive therapies to enhance both large vessel recanalization and cerebral microvascular perfusion.
Aim: To investigate the effects of adjunctive therapies on rtPA-mediated large artery recanalization, sustained vessel patency and cerebral perfusion in a mouse model of stroke.
Methods: The in situ carotid artery thrombo(ly)sis (iCAT) model employs electrolytic injury to the carotid artery (CA) to form a platelet and fibrin-rich occlusive thrombus. Ipsilateral occlusion is then coupled with transient stenosis (60mins) of the contralateral CA to achieve controlled hypoperfusion and ipsilateral cerebral infarction.
Results: In the iCAT stroke model, rtPA therapy (10mg/kg/30mins bolus/infusion) induced transient CA recanalization. Combining rtPA with anticoagulant Argatroban (80ug/kg-bolus; 40ug/kg/min-24-hour infusion) enhanced recanalization (41.9% vs 22.2% rtPA alone) and significantly improved cerebral perfusion, leading to a moderate reduction in infarct volumes (TTC). Combination therapy resulted in a marked surgical bleeding (>75% incidence) yet was associated with thrombotic reocclusion in 80% of recanalized vessels. Histological analysis confirmed that reocclusion was due to the formation of platelet-rich thrombi. Consistent with this, rtPA plus anti-platelet agent integrilin achieved CA recanalization in up to 70% of treated mice.

Conclusion: The iCAT model is a useful preclinical model to examine the effects of adjunctive thrombolytic agents on large artery recanalization, microvascular cerebral perfusion and stroke outcomes.

05. TRAIL-expressing monocyte/macrophages are critical for reducing inflammation and atherosclerosis

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Aim: To investigate if monocyte/macrophages are a significant source of TRAIL in the healthy circulation, and if TRAIL-expressing monocyte/macrophages play a role in reducing inflammation and atherosclerosis in mice.

Methods: Plasma cytokines were measured in people +/- CAD; monocytes were isolated and gene expression quantified. Atherosclerosis was assessed in two in vivo models: (i) bone marrow (BM) chimeras where TRAIL was expressed only in BM (BM-TRAIL), or everywhere except BM (parenchymal-TRAIL); (ii) macrophage depletion in Trail-/-Apoe-/- using clodronate liposomes. Ex vivo, macrophages were differentiated from Trail-/-Apoe-/- and Apoe-/- BM. Functional studies were performed, and mRNA expression of inflammatory markers and cholesterol transport genes were measured.

Results: TRAIL mRNA was reduced in CAD monocytes (>60%) concomitant with reduced plasma levels (>70%) and negatively associating with IL-18, but not IL-6 or IL-1β. These suggest that IL-18 may negatively regulate TRAIL expression in diseased monocytes. Indeed, exposure healthy monocytes to IL-18 significantly reduced TRAIL mRNA via NFκB. Importantly, BM-TRAIL mice showed markedly attenuated atherosclerosis, reduced macrophage accumulation, altered cytokine expression and increased iNOS levels. Macrophage depletion also attenuated atherosclerosis, confirming that a significant number of cells in Trail-/-Apoe-/- plaque were monocyte/macrophages. Moreover, Trail-/-Apoe-/-macrophages were more inflammatory, with reduced migration to CCL-19, reduced efferocytosis, and reduced expression of reverse cholesterol transport and nitric oxide-controlling genes.

Conclusions: Here, we identified for the first time that monocyte/macrophages are a significant source of TRAIL, and a modulator of cell phenotype and function in atherosclerosis. Manipulating TRAIL levels in monocyte/macrophages highlights a new therapeutic avenue in the treatment of atherosclerosis.

SESSION 9: CEREBROVASCULAR BIOLOGY AND DISEASE

01. Trans-endothelial migration of leucocytes from patients with Multiple Sclerosis under immunomodulation

Simon Hawke

Australia

02. Circulating immune cells in multiple sclerosis

Felix Marsh

Australia

03. Allogeneic cell therapy for ischaemic stroke - from preclinical discovery to clinical translation

Rebecca Lim

Australia

04. The roles of PDGFRβ in pathogenesis and regeneration of the CNS

Seiji Yamamoto, Noriko Okuno, Takeru Hamashima, Masakiyo Sasahara

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In the central nervous system (CNS), platelet-derived growth factor receptor beta (PDGFRβ) expresses in neural stem/progenitor cells (NSPCs), neurons and vascular pericytes; however, the roles of PDGFRβ in pathogenesis in CNS remain elusive.

We have examined the role of PDGFRβ in NSPCs after stroke. A transient middle cerebral-arterial occlusion was introduced into the mice that is neural tissue specific conditional deletion of Pdgfrb-gene named N-PRβ-KO (Nestin-Cre;PdgfrbFlox/Flox). The migration of the DCX+ neuroblasts from the subventricular zone toward the ischemic lesion was highly increased in N-PRβ-KO compared with control mice. CXCL12, a potent chemoattractant for CXCR4-expressing NSPCs, was upregulated in the ischemic lesion of N-PRβ-KO mice. In addition, integrin α3 expressed in NSPCs that mediates extracellular matrix-dependent migration was upregulated in N-PRβ-KO. These findings suggested that PDGFRβ is involved in neurogenesis through the regulation of lesion-derived chemoattractant as well as intrinsic signal of NSPCs.

In the retina, N-PRβ-KO showed tractional retinal detachment. Depletion of Nestin-Cre-sensitive NG2+αSMA− pericytes suppressed pericyte-coverage and induced pathological angiogenesis. Nestin-Cre-insensitive NG2+αSMA+ pericytes detached from the vascular wall, and subsequently changed to myofibroblasts in proliferative membrane to cause retinal traction. PDGF-BB were significantly increased in the retina of N-PRβ-KO, suggesting PDGF-BB contributes to the pericyte-fibroblast transition (PFT). The PDGF-BB-PDGFRβ signal may be the relevant therapeutic targets to protect eyes from DR.

05. Pericyte deficiency sequentially induces inflammation, barrier breakdown, and fibrosis in retina

Akiyoshi Uemura[1], Yumi Komine[1], Naomi Inoue[1], Kaori Kurata[1], Wataru Ikeda[2], Hideaki Ogasawara[2], Eri Nishioka[2], Koichi Nishiyama[3], Hiroki Kurihara[4], Toshio Imai[2]

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In the central nervous system (CNS), pericytes are pivotal for maintaining the blood-neural barrier. Although the loss of pericytes has been implicated in the pathophysiology of certain CNS diseases such as diabetic retinopathy (DR), how pericyte deficiency affects the vascular and neural functions remains obscure. Here we show that depletions of pericytes from retinal vessel walls sequentially reproduced characteristic features of early and advanced DR, including inflammation, breakdown of blood-retina barrier (BRB), and fibrosis. Single intraperitoneal injections of an anti-PDGFRβ antibody to neonatal mice inhibited recruitment of pericytes to developing retinal vessels, which directly evoked inflammatory responses in endothelial cells (ECs) and perivascular infiltrations of macrophages. In this setting, ECs and macrophages formed a cycle of vessel damage via VEGF-A, placental growth factor, and angiopoietin-2, thereby sustaining BRB breakdown and retinal edema. Subsequently, remaining pericytes dissociated from ECs and transdifferentiated into myofibroblasts. During the progression of retinal fibrosis, the properties of macrophages and the expression pattern of inflammatory cytokines synchronously changed. The RNA-Seq analyses using pericyte-deficient retinas have identified a number of macrophage-derived signals under acute and chronic inflammation, which may be involved in the distinct disease steps including BRB breakdown and fibrosis. Our study will contribute to the discovery of new drug targets, not only for DR but also for the CNS diseases associated with pericyte deficiency, such as Alzheimer's disease.

SESSION 10: MICROVASULATURE ADAPTATIONS (IN PREGNANCY AND PERINATAL OUTCOMES)

01. Targeting the vascular dysfunction of the pregnancy disorder preeclampsia

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Preeclampsia (PE) affects 1 in 20 pregnancies and remains a leading cause of maternal and fetal morbidity and mortality worldwide. PE is characterised by hypertension after 20 weeks gestation with proteinuria, uteroplacental dysfunction and/or maternal organ dysfunction. Women with PE have widespread vascular dysfunction caused by placental-derived toxins and oxidative stress. The aims of this study were to investigate if the antioxidant sulforaphane (from cruciferous vegetables) could improve endothelial function and reduce enhanced constriction in arteries exposed to placental toxins. Human umbilical vein endothelial cells (HUVECs) were extracted from the umbilical cord of healthy women undergoing caesarean (Monash Health, Clayton, Australia). Oxidative stress was induced in HUVECs (n=6) by incubation with transforming growth factor-α (TNF-α, 1-100ng/mL) with or without sulforaphane (5-20μM). Cells were assessed for expression of endothelial activation markers endothelin-1, VCAM1, ICAM1 and E-selectin. Small mesenteric arteries from C57BL/6J mice (n=8/dose) were incubated for 24h in normal or conditioned media (exposed to placental cells: PCM; containing placental toxins) with or without sulforaphane (5-20μM). Then, vascular responses of the mesenteric arteries to a variety of constrictors were assessed using wire myography. Sulforaphane significantly reduced TNF-α mediated HUVEC secretion of endothelin-1, VCAM1, ICAM1 and E-selectin. Furthermore, sulforaphane significantly reduced PCM-induced enhanced response of the mesenteric arteries to angiotensin II, endothelin-1 and phenylephrine. In conclusion, sulforaphane reduces endothelial oxidative stress and reduces vasoconstriction. We believe sulforaphane may be a new therapeutic approach for the treatment of preeclampsia.

02. Optometrist office retinal photography; a practical assessment of microvascular retinal structure in the modern world

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Introduction: Retinal microvascular photography is an established technique for assessment of microvascular structure from the newborn to the elderly. It has been associated with both antecedent risk factors and later hypertensive and cardio-metabolic disease. Acquiring the photographs has been costly in terms of equipment and trained staff. Many high street optometrists have introduced pan-retinal photography as a standard service. In the context of a pilot study of children born following gestational diabetes we aimed to evaluate the feasibility the optometrist office as retinal photographic assessment site.

Methods: 33 participants aged 8 to 17 years in our pilot study were asked to obtain a retinal photograph from a designated optometrist within a few weeks of their in-house assessments. The optometrist offered a full visual review if the child was eligible and also a retinal photograph of each eye (Canon). Images were transferred via a University-hosted secure file exchange facility for semi-automated retinal vascular assessment, by experienced assessors, using SIVA software (Singapore).

Results: 23 (70%) completed within the allotted time frame. Baseline characteristics of those who completed were not different from non-attenders. Non-attenders were also more likely to not complete other tasks (p=0.031) All photographed children had software assessable vasculature, the vast majority in both eyes. No concerns about the methodology were expressed by parents, children or staff obtaining photographs.

Conclusion: With retinal photography rolling out to the high street, a valuable tool for microvascular structural assessment is potentially widely available. We have demonstrated the technical and patient

acceptability. Our pilot suggests appropriate use of reminders, combined with a geographical network of participating centres could further increase uptake.

03. Girls Rule: The "female advantage" following preterm birth and the role of antenatal glucocorticoids in the programming of early- and long-term (cardio)vascular adaptations

Rebecca Dyson

Australia

04. Pravastatin targeting of uterine radial arteriole endothelium-dependent vasodilator signalling mechanisms in preeclampsia

Shaun L Sandow,[1,2] Leo Leader,[3] Victoria Hinkley,[1] Nathan Luque,[1,2] Marianne Tare,[4] Sandra Lowe,[3] Timothy V Murphy[2]

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Preeclampsia (PE) causes significant maternal and fetal morbidity and mortality. Maternal endothelial dysfunction is the hallmark of PE, including in the uterine myometrial vasculature, resulting in systemic organ dysfunction. No therapies have been identified and treatment remains supportive. Early data support beneficial effects of the cholesterol-lowering drug pravastatin in reducing blood pressure and moderating PE by altering vascular endothelial (dys)function, although the mechanism is unknown.

This work identifies key endothelial signalling pathways in human myometrial radial arterioles, and their alteration by pravastatin in PE, to reflect potential mechanisms for improving uterine vascular endothelial function.

Methods: Myometrial radial arterioles from caesarean-section normotensive (NT), gestational (GH) and PE hypertensive patients were examined as control and in vitro pravastatin (2mM/6h)-incubated segments. Electron microscopy, immunohistochemistry and pressure myography with pharmacological intervention characterized vessel structure and function. Protocols were approved by local Ethics Committees.

Caveolae density and caveolin-1 expression is reduced in arterioles from hypertensives, and further reduced by pravastatin incubation. PE is accompanied by decreased vasodilator NO and endothelium-derived hyperpolarization (EDH) activity; and altered expression of NO and EDH signalling components, including myoendothelial gap junction and channels modulating calcium entry. Endothelium-independent smooth muscle relaxation is unchanged in arterioles from GH/PE compared with NT pregnancies. Pravastatin incubation restored endothelium-dependent relaxation by ~50% in PE samples.

These data suggest that caveolae and non-caveolae sites change their form and microdomain- and endothelium-dependent signalling functions a key contributor to myometrial radial arteriole activity in PE. Further, pravastatin acts at these sites to correct the endothelial-dysfunction associated with PE.

05. Development of a Thermal Challenge to Investigate Cardiovascular Dysfunction in Preterm-born Guinea Pigs.

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2 Centre for Translational Physiology, University of Otago, Wellington

Background: Cardiovascular stress tests are critical for understanding dynamic system function in at-risk populations. Otherwise healthy ex-preterm individuals have impaired heart function during exercise stress tests, and poor recovery following. Animal models used to understand such results must pair physiology with mechanistic insights. However, exercise stress tests can be difficult to implement. A passive thermal stress test offers a clinically-relevant cardiovascular stress test that can be applied in our guinea pig model.

Aim: To design a thermal stress test capable of (1) manipulating skin and core body temperature and (2) eliciting cardiovascular homeostatic responses in term- and preterm-born guinea pigs.

Method/Results: The final thermal stress test comprises two arms (heating and cooling) conducted on separate days, each containing three phases: induction and titration of anaesthesia, thermal challenge, and recovery. Animals are anaesthetised under 0.8-1.0% isoflurane + 70% N2O throughout testing. Cardiovascular parameters measured include ECG, non-invasive blood pressure, microvascular perfusion, respiration rate, oxygen saturation, skin and rectal temperature. Thermal stress is applied by manipulating temperature of a custom-made water-perfused wrap secured tightly around the animals.

Through an iterative process, the thermal challenge has been developed as separate heating and cooling challenges conducted on separate days. The challenges are sufficient to induce significant deviations in skin and core body temperature, as well as responses in cardiovascular parameters.
Conclusion: Preterm birth is associated with significant changes in central and peripheral cardiovascular function. We have established a method for eliciting and measuring cardiovascular strain in term- and preterm-born guinea pigs across the lifespan.

SESSION 11: VASCULAR REMODELLINGAND MECHANISMS (SMOOTH MUSCLE/ENDOTHELIUM/NERVES)

01. Clonality and plasticity of plaque cells in atherosclerosis

Ashish Misra
Australia

02. Endothelial dysfunction and reduced compliance in Schlager hypertensive (BPH/2J) mice

Maria Jelinic
Australia

03. Small-molecule formyl peptide receptor ligand, Compound 17b is a vasodilator in the mouse aorta

Chen Huei Leo[1,2], Sarah A Marshall[3], Maria Jelinic[4], Kelly O'Sullivan[2], Minh Deo[5], Jess Walsh[5], Mandy Li[5], Laura J Parry[2], Rebecca H. Ritchie[5,6,7], Chengxue Xue Qin[5,6]; *[1]Science and Maths Cluster, Singapore University of Technology & Design, Singapore; [2]School of BioSciences, The University of Melbourne, Victoria, Australia; [3]The Ritchie Centre, Department of Obstetrics and Gynaecology, School of Clinical Sciences, Monash University, Clayton, Victoria, Australia; [4]Department of Physiology, Anatomy & Microbiology, La Trobe University, Bundoora, Victoria, Australia; [5]Department of Heart Failure Pharmacology, Baker Heart & Diabetes Institute, Victoria, Australia; [6]Department of Pharmacology, University of Melbourne, Victoria, Australia; [7]Department of Medicine, Monash University, Victoria, Australia.*

The formyl peptide receptor (FPR) family are a group of G-protein coupled receptors that plays an important role in the regulation of inflammatory processes. It is well-established that activation of FPRs has cardioprotective properties. Recently, more stable and smaller molecules of FPR agonists were developed, Compound 17b (Cmpd17b) and Compound 43 (Cmpd43). Both activate a range of biased-signaling pathways in human-engineered FPRs cells, including ERK1/2, Akt, cAMP and Ca^{2+} mobilisation. However, it is unknown whether these FPR agonists have any vascular effects. Therefore, we compared the vasodilator effects of Cmpd17b and Cmpd43 in mouse aorta. Abdominal aortae were isolated from male C57BL/6 mice and vascular reactivity was analysed using wire-myography. The presence and localisation of FPR receptors were shown by qPCR and immunohistochemistry. Specifically, FPR1 and FPR2 were expressed in the aorta and predominately localised to the vascular smooth muscle cells. Cmpd17b (pEC_{50}:5.09±0.03, n=5) but not Cmpd43 evoked a concentration-dependent relaxation (R_{max}:62±5%, n=5) of the mouse aorta. Removal of endothelium or blockade of endothelium-derived relaxing factors using pharmacological inhibitors had no effect on Cmpd17b-evoked relaxation. Further investigations into the mechanisms of Cmpd17b-induced

relaxation with 50mM K^+ concentration, ODQ or glibenclamide inhibition also revealed no significant effect on the responses to Cmpd17b in the mouse aorta. In aorta primed with elevated K^+ concentration, increasing concentrations of $CaCl_2$ evoked concentration-dependent contraction, which is abolished by Cmpd17b (10 µM). Our data indicates that only Cmpd17b is a direct endothelium-independent vasodilator, which may involve the inhibition of Ca^{2+} mobilisation via voltage-gated calcium channels.

04. Dexmedetomidine reduces noradrenaline requirements and preserves renal macro- and micro-circulatory perfusion, oxygenation and function in ovine septic acute kidney injury

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Introduction: Noradrenaline is the primary vasopressor used to restore mean arterial pressure (MAP) in patients with septic acute kidney injury (AKI). However, restoring blood pressure with noradrenaline has the potential to worsen renal medullary hypoxia during septic AKI.

Aims: To examine whether dexmedetomidine, an α_2-adrenoreceptor receptor agonist, can decrease noradrenaline requirements and attenuate renal medullary hypoxia in a pre-clinical ovine model of septic AKI.

Methods: Sheep were instrumented with renal artery flow probes, and laser Doppler and oxygen-sensing probes in the renal cortex and medulla. Conscious sheep received an infusion of live *Escherichia coli* for 30 h. Sheep were randomized to receive either noradrenaline or noradrenaline with dexmedetomidine from 24-30 h of sepsis (both N=8).

Results: Sepsis reduced MAP (84±3 to 67±4 mmHg), renal medullary perfusion (1250±256 to 730±176 perfusion units), medullary oxygenation (40±6 to 21±6 mmHg) and creatinine clearance (2.50±1.10 to 0.78±0.40 mL/Kg/min) ($P<0.01$). Noradrenaline restored MAP (to 83±6 mmHg) but worsened medullary hypoperfusion (to 330±150 units) and medullary hypoxia (to 9±5 mmHg). Dexmedetomidine (0.5 µg/kg/h) co-administration reduced the noradrenaline dose (0.8 to 0.4 µg/kg/min; $P<0.001$) required to restore MAP, attenuated medullary hypoperfusion (to 606±300 units; $P=0.001$), decreased medullary tissue hypoxia (to 29±7 mmHg; $P=0.001$), and improved creatinine clearance (to 1.8±0.4 mL/Kg/min; $P<0.001$).

Conclusion: In ovine septic AKI, dexmedetomidine reduces noradrenaline requirements, attenuates its adverse effects on the renal medulla, and improves renal function. Therefore, dexmedetomidine as an adjunct therapy may be a useful catecholamine-sparing strategy to offer a degree of reno-protection from hypoxic injury in septic AKI.

05. Homeostasis in NFAT1-Down syndrome critical region-1 signaling is critical for regulation of proper vessel formation and vascular integrity

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1 Kumamoto University, Japan. 2 U. Penn, USA. 3 Nagoya City University, Japan.

Epidemiological studies suggest that although individuals with Down syndrome have increased risks of leukemia and neuronal diseases, they have a considerably reduced incidence of most solid tumors and advanced atherosclerosis. We previously reported the Down syndrome critical region (DSCR)-1 gene lies on chromosome 21 encoding a feedback modulator of VEGF-calcineurin-NFAT signaling in endothelial cell (EC)s. NFAT/DSCR-1 signaling axis critically regulates septic mortality, angiogenic balance, and susceptibility of preferential tumor metastasize to lung.

Here, we investigate the effects of constitutive Dscr-1 expression and following to NFAT inhibition in vivo, we generated EC-specific conditional DSCR-1 transgenic (TgEC) mice. Highly DSCR-1 expression resulted in embryonic lethal due to lacking the EC proliferations. Thus, conditionally reduced DSCR-1 expression rescued to the birth, whereas similar to the Down syndrome model mice, the embryo sizes were much smaller than the wild-type littermate controls. DSCR-1 stable expression resulting to the NFAT1 inactivation in ECs revealed the malformations of Dll4-Notch-mediated tip/stalk cell balances and the proper branch formations, therefore total blood vessel densities were markedly reduced. NFAT/DSCR-1 signaling in ECs was predominantly activated in developmental and pathological angiogenesis steps. Thus, DSCR-1 TgEC demonstrated growth normally after the birth, except behavioural disorder due to the loss of neuronal cells volume. Taken together, our studies provide new insights into mechanisms underlying angiogenesis with NFAT/DSCR-1 signaling, which would be helpful for both Down syndrome and cancer patients in future.

SESSION 12: THE TUMOUR VASCULATURE

01. Vascular remodelling and anti-cancer therapy

Ruth Ganss
Australia

02. Tumour vasculature, a story of convenience

Lih Tan[1], Emma Thompson[1], Michaelia Cockshell[1], Mark de Nichilo, Kay Khine Myo Min[1], Jeff Holst[2], My Mahoney[3], Mark Shackleton[4], Lisa Ebert[1] and Claudine Bonder[1]
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Formation of new blood vessels in a solid tumour are critical for cancer growth and metastasis. Until recently, tumour vasculature was thought to occur exclusively via angiogenesis using endothelial cells (ECs). However, there is increasing evidence that many solid tumours are capable of creating an independent blood supply via their own cancer cells, a process known as vasculogenic mimicry (VM). Like angiogenesis, VM content in solid tumours correlates with poor prognosis for patients.

We have identified a number of regulatory mechanisms that mediate VM in breast cancer, melanoma and pancreatic cancer. For example, the adhesion molecule desmoglein-2 (DSG2) is an important cadherin of the vasculature that promotes both angiogenesis and VM. Moreover, increased DSG2 expression correlates with poor outcome for patients with melanoma. Herein we reveal that the VM capability of melanoma cells positively correlates with the adhesion/recruitment of peripheral blood mononuclear cells. Using the parallel plate flow chamber and transwell migration assays we observed that monocytes are selectively recruited across a melanoma cell monolayer via select adhesion molecules and chemokines. We observed that growth of the mouse melanoma cell line B16-F10-GFP-P2A-luc is significantly reduced in mice with loss-of-function Dsg2 (Dsg2lo/lo) when compared to control mice (WT) and mice wherein Dsg2 has been restored (Dsg2R/R). Histologically, tumours from Dsg2lo/lo mice reveal restructured tumour vasculature with increased cancer killing CD8+ T lymphocytes and reduced FoxP3+ Treg infiltrate.

Taken together our results suggest that DSG2 plays an underappreciated role in regulating tumour vasculature and the infiltration of leukocytes. Ongoing investigations are underway to understand how modulating expression of DSG2 can reshape the tumour vasculature for increased tumour infiltrating leukocytes and whether DSG2 is a potential target to treat melanoma.

03. Characterization of extracellular vesicles in cancer: from lab bench to clinic

Elham Beheshti
Australia

PLENARY 4

Extracellular vesicles and tumour microenvironment - Dolores Di Vizio (USA)

PLENARY 5

Novel insights from functional siRNA/CRISPR screens of endothelium - Steve Stacker (Australia)

POSTERS

#	Surname	First Name	Abstract Title
1	Bello	Idris	Pharmacological Inhibition of Myeloperoxidase Protects against Aortic Aneurysm in Angiotensin II-Infused Apolipoprotein E Gene Knockout Mice
2	Besnier	Marie	Newly identified miR-6770-3p dramatically induces angiogenesis in human endothelial cells while decreasing proliferation
3	Cannizzo	Carla	miRNA-200b: A therapeutic target for diabetes impaired angiogenesis
4	Chiu	Joyce	Regulation of GPIIb/IIIa conformation by an allosteric disulphide bond inthe α subunit
5	Crawshaw	Jessica	A computational model of vascular regression
6	Faqihi	Fahimeh	Autophagy function in endothelial surface translocation of mitochondrial PDCE2 in response to radiation: a novel vascular target?
7	Han	Jin Han	Tetrahydrobiopterin stimulates mitochondrial biogenesis through increased PGC-1alpha expression in mice hearts
8	Hayashi	Hiroki	Inflammation-associated NO regulates chemokine signaling via CX3CR1
9	Hoang	Thu	A novel cervical lymph cannulation method in rats to evaluate clearance from the brain into the lymphatics
10	Hwee Ying	Lim	Cellular cholesterol content mediates macrophage VEGF-C to promote efficient lymphatic vessel transport
11	Iba	Tomohiro	Identification and characterization of endothelial cell heterogeneity at single cell resolution.
12	Kim	Young-Myeong	NF-κB-responsive miR-155 induces functional impairment of vascular smooth muscle cells by downregulating soluble guanylyl cyclase
13	Krishna	Smriti	Novel insights from experimental models of Abdominal Aortic Aneurysm
14	Lam	Alina	Intra-articular injection of macromolecular immunotherapy drugs is a novel method to target the lymphatics that drain inflamed joints
15	Lee	Wookjin	A Numerical Approach for Predicting the Aortic Valve Calcification using Computational Fluid Dynamics
16	Lee	Eunhyeong	Sox7 promotes tumor growth by increasing VEGFR2-mediated vascular abnormality in high-grade glioma

17	Maeng	Yong-Sun	Immune regulation and lymphatic vessel development in decidua during pregnancy
18	McRobb	Lucinda	Vascular targeting of an atypical surface-translocated αB-crystallin for the treatment of irradiated brain arteriovenous malformations
19	Naim	Hussein	Effect of dietary nicotinamide mononucleotide (NMN) on vascular endothelial function in aged mice.
20	Nakaoka	Yoshikazu	gp130-mediated signaling in CD4-positive cells has a critical role for the pathogenesis of pulmonary arterial hypertension
21	Pennings	Gabrielle	Rapid release of Interleukin-1β from human platelets is independent of NLRP3 and caspase
22	Phie	James	Quercetin does not improve exercise performance in old Apolipoprotein E deficient mice with sustained hind limb ischemia
23	Rayner	Benjamin	Pharmacological inhibition of lysyl oxidases attenuates cardiac fibrosis and preserves heart function following acute ischemia reperfusion injury.
24	Rayner	Benjamin	Selenomethionine supplementation reduces lesion burden, improves vessel function and modulates the inflammatory response within the setting of atherosclerosis.
25	Reddel	Caroline	Enhanced fibrinolysis and altered extracellular vesicles after remote ischaemic preconditioning in non-diabetic coronary artery disease patients
26	Sashindranath	Maithili	Characterisation of Ischemia and Haemorrhage Mouse Stroke Models for Efficient Drug Development
27	Ta	Hang	Novel nanomaterials based on metal and metal oxide for advanced diagnosis and treatment of cardiovascular disease
28	Tan	ZheHao Paul	The glomerular Endothelial Surface Layer (ESL) impedes monocyte recruitment but promotes neutrophil recruitment to the glomerulus
29	Thomas	Shane	Vascular, but not circulating, levels of myeloperoxidase significantly increase with time post-symptom onset in non-ST elevation acute coronary syndrome patients
30	Wang	Jiaqiu Wang	Carotid Bifurcation with Tandem Stenosis – A Patient-specific Case Study Combined Imaging, Histology and Computational Simulation
31	Wang	Luping Wang	Coupled modeling of vascularization and bone regeneration within a biodegradable polymer scaffold loaded with VEGF
32	Wise	Lyn	A novel suppressive role for Langerhans cells during cutaneous wound healing

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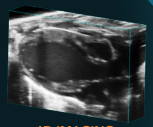
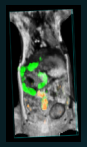
CARDIOLOGY



- Myocardial Perfusion
- Myocardial Ischemia
- Myocardial Strain Imaging
- Myocardial Drug Delivery
- Systolic & Diastolic Functions
- Cardio Toxicity

4D IMAGING

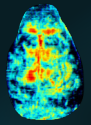
ONCOLOGY



- Angiogenesis
- Hypoxia & Therapy Monitoring
- Biodistribution & Perfusion
- Tumor Implantation & Drug Delivery
- Earliest Tumor Detection
- 2D/3D Volume Quantification

WHOLE BODY IMAGING

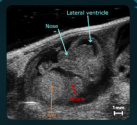
NEUROLOGY



- Cerebral Ischemia
- Cerebral Thrombosis
- Biodistribution
- Implantation & Biopsy
- Hemodynamics
- Cerebral Perfusion

BRAIN IMAGING

EMBRYOLOGY



- Placental Hypoxia
- Placental Vascularity
- Congenital Heart Disease
- Embryo Manipulation
- Embryonic Development
- Embryo Morphology

EMBRYO IMAGING

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