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4th Joint Dutch-German Vascular Biology Meeting

Groningen, 20-22 September 2023

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Plenary Program

Wednesday September 20th 2023

12.00 – 12.45 Welcome & Registration

12.45 – 13.00 Conference opening,
Guido Krenning (UMC Groningen, NL), Ferdinand Le Noble (Karlsruhe Institute of Technology, D), Liz Jones (KU Leuven, B)

Session 1. Systems Biology

Chair: Jaap van Buul (University of Amsterdam, NL)

13.00 – 13.30 **Catherine Robin (Hubrecht Institute, Utrecht, NL).** Multiple levels of regulation of hematopoietic stem cell production during embryonic development.

13.30 – 13.50 **Daniele Panáková (UKSH Kiel, D).** Cellular drivers of regeneration.

13.50 – 14.00 **Stijn Groten (Sanquin, Amsterdam, NL).** The Proteomic Landscape of in vitro Cultured Endothelial Cells across Vascular Beds.

14.00 – 14.10 **Sarah Steffens (Free University Brussels, Brussels, B).** Genetic phenotyping at single cell resolution in 3D sprouting assay to study the role of microtubules during sprouting angiogenesis.

14.10 – 14.45 *Coffee break*

Session 2. Clinical-Translational Science

Chair: Ed Eringa (Maastricht University / Amsterdam UMC, NL)

14.45 – 15.15 **Bart Loeys (University of Antwerp, B).** New insights from hereditary aortopathies.

15.15 – 15.35 **Hester den Ruijter (UMC Utrecht, NL).** Sex differences in atherosclerosis.

15.35 – 15.45 **Coenraad Withaar (UMC Groningen, NL).**The cardioprotective effects of semaglutide exceed those of dietary weight loss in mice with HFpEF.

15.45 – 15.55 **Mostafa Samak (German Primate Center, Göttingen, D).** Micro-RNA 92a as a therapeutic target for cardiac microvascular dysfunction in diabetes.

15.55 – 16.40 *Coffee break*

Aletta Jacobs Award Session

Chair: Liz Jones (KU Leuven, B)

16.40 – 16.50 **Marina Horvat (Ghent University, B**). Studying cardiovascular effects of fibrillin impairment in zebrafish.

16.50 – 17.00 **Fabienne Podieh (Amsterdam UMC, NL).** Proteomics screen to identify new regulators of endothelial barrier function.

17.00 – 17.10 **Kayleigh van Dijk (Leiden UMC, NL).** Exploring PD-1+ and Tissue Resident Memory T Cells in Atherosclerotic Diseases.

Keynote

Chair: Jaap van Buul (University of Amsterdam, NL)

17.15 – 18.00 **Dirk Duncker, ErasmusMC Rotterdam (NL).** Coronary microvascular dysfunction in cardiovascular disease: a *porcinal* translational view.

Thursday September 21st 2023

Session 3. Mechanotransduction

Chair: Cor de Wit (University of Lübeck, D)

9.00 – 9.30 **Paul Evans (University of Sheffield, UK).** Endothelial responses to shear stress in development and disease.

9.30 – 9.50 **Liz Jones (KU Leuven, B).** Integrating Mechanical Microenvironments the Interplay of Shear Stress Tissue Stiffness and Gene Expression.

9.50 – 10.00 **Ibrahim Hamid (Free University Brussels, B)**. Mechanical role of microtubules during angiogenesis: focus on specific MT subpopulations.

10.00 – 10.10 **Werner Jack van der Meer (Amsterdam UMC, NL).** α-Catenin C-Terminal Extension undergoes tension-induced conformational changes during diapedesis of neutrophils.

10.10 – 10.40 *Coffee break*

Session 4. Metabolism

Chair: Jeffrey Kroon (Amsterdam UMC, NL)

10.40 – 11.10 **Massimo Santoro (University of Padua, I).** Metabolic-dependent mRNA translation regulation in angiogenesis.

11.10 – 11.30 **Iris Bibli (University of Frankfurt, D).** Metabolic surveillance of vascular growth.

11.30 – 11.40 **Oana Sorop (Erasmus MC, Rotterdam, NL).** Reactive Oxygen Species Mitigate Perturbations in Coronary Microvascular Tone in Exercising Swine with Multiple Risk Factors.

11.40 – 11.50 **Anouk Groenen (UMC Groningen, NL).** High advanced glycation end products associate with blood monocytes in diabetes: a cross-sectional study in the population-based LifeLines cohort.

Poster session

11.50 – 14.30 *Lunch & Poster presentations*

'New Talents in Vascular Biology'

Chair: Stephan Huveneers (Amsterdam UMC, NL)

14.30 – 15.00 **Claire Peghaire (University of Bordeaux, F).** The ubiquitin ligase Tripartite Motif 47 (TRIM47): a novel endothelial contributor to cerebral small vessel disease

15.00 – 15.30 **Patrick Sips (Ghent University, B).** Unraveling the Role of TGF-beta signaling in Thoracic Aortic Aneurysm and Dissection Using Fibrillin-1 Mutant Mouse Models

Session 5. Macrovascular Biology

Chair: Rabea Hinkel (German Primate Center, Göttingen, D)

15.30 – 16.00 **Marit Westerterp (UMC Groningen, NL).** Cholesterol efflux pathways control T cell ageing and atherosclerosis.

16.00 – 16.20 **Vivian de Waard (Amsterdam UMC, NL).** Marfan syndrome, a structural or metabolic problem?

16.20 – 16.30 **Marie-José Goumans (Leiden UMC, NL).** Absence of GDF15 aggravates adverse cardiac remodelling in mice hallmarked by perivascular fibrosis and signs of Endothelial-to-Mesenchymal transition

16.30 – 16.40 **Jamie Kane (Amsterdam UMC, NL).** Peritoneal dialysis aggravates and accelerates atherosclerosis in uraemic ApoE$^{-/-}$ mice

16.40 – 17.15 *Coffee break*

Dutch Heart Foundation Keynote

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Chair: Guido Krenning (UMC Groningen, NL)

17.15 – 18.00 **Triantafyllos Chavakis, Uniklinikum Dresden (D).** Trained innate immunity and inflammatory memory.

19.30 – 0.00 *Conference Diner & Party*

Friday September 22nd 2023

Session 6. Neurovascular Biology

Chair: Ferdinand Le Noble (Karlsruhe Institute of Technology, D)

9.00 – 9.30 **Martin Dichgans** *(online)* **(LMU Munich, D).** Stroke Genetics: Discovery, Biology, and Clinical Implications.

9.30 – 9.50 **Isabelle Brunet (INSERM Paris, F).** Development and physio-pathological role of the intra-neural vascularization.

9.50 – 10.00 **Noëlle Bakker (Amsterdam UMC, NL).** Shared pathophysiology in the neurovascular unit of the human retina and Alzheimer's brain in type 2 diabetes

10.00 – 10.10 **Hannelore Kemps (KU Leuven, B).** Unraveling the role of transcription factor Prdm16 in endothelial cells during the progression of ischemic stroke.

FujiFilm Visualsonics Session

Chair: Liz Jones (KU Leuven, B)

10.10 – 10.25 Peter Kesa **(FujiFilm VisualSonics, Amsterdam, NL)**. Vevo F2 LAZR-X for Preclinical Cardiovascular Research.

10.25 – 11.00 *Coffee break*

Session 7. Inflammation & Ageing

Chair: Boy Houben (Maastricht UMC+, NL)

11.00 – 11.30 **Guido de Meyer (University of Antwerp, B).** Prevention of atherosclerotic plaque destabilization: focus on necroptosis, pyroptosis and ferroptosis.

11.30 – 11.50 **Amanda Foks (Leiden University, NL).** Single cell profiling of age-associated immunity in atherosclerosis.

11.50 – 12.00 **Max Grönloh (Amsterdam UMC, NL).** The endothelium functions as an immune modulator between the innate and adaptive immune systems by triggering neutrophil-induced cxcl12 production to promote cd8+ t-cell extravasation.

12.00 – 12.10 **Zhendong Wang (UMC Groningen, NL).** Endothelial NF-κB, MAPK, and STAT3 pathways are heterogeneously activated in mouse kidney microvascular beds in early sepsis.

Awards Ceremony & Closure

12.15 – 12.45 Awards ceremony & Conference closure
Guido Krenning (UMC Groningen, NL), Ferdinand Le Noble (Karlsruhe Institute of Technology, D), Liz Jones (KU Leuven, B)

12.45 – 13.00 *Lunch break & departure*

Keynote Speakers

Keynote Lecture Dirk Duncker (Erasmus MC, Rotterdam, NL)

Coronary microvascular dysfunction in cardiovascular disease: a *porcinal* translational view.

Dirk Duncker is a prominent researcher and medical professional at the Erasmus MC. With a deep passion for cardiovascular physiology, he has dedicated his career to understanding the intricacies of the human heart and its diseases. Graduating with honors in medicine and earning a Ph.D. in Physiology, Duncker has made groundbreaking contributions to the field through his innovative research on myocardial metabolism, coronary circulation, and exercise physiology. His work has not only advanced scientific knowledge but has also translated into practical applications, improving patient care and treatment strategies.

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Dutch Heart Foundation Keynote Lecture Triantafyllos Chavakis, Uniklinikum Dresden (D).

Trained innate immunity and inflammatory memory.

Trian Chavakis is a distinguished academic and accomplished researcher at the Uniklinikum Dresden. With a profound focus on advancing the knowledge and therapeutic strategies in the realms of vascular diseases and immunology, Dr. Chavakis has made seminal contributions to the field of medical science. His scholarly pursuits primarily revolve around the intricate interplay between inflammation, metabolism, and cardiovascular pathophysiology. Renowned for his expertise and innovative methodologies, Dr. Chavakis has authored numerous influential publications that elucidate the intricate molecular mechanisms underlying inflammatory responses and cardiovascular events.

Plenary Session Abstracts

Session 1. Systems Biology

Invited Lecture Catherine Robin (Hubrecht Institute, Utrecht, NL)

Multiple levels of regulation of hematopoietic stem cell production during embryonic development.

Catherine Robin is a prominent researcher based at the Hubrecht Institute. With a focus on developmental biology and stem cell research, Robin has made significant contributions to understanding the mechanisms that shape embryonic development and tissue regeneration. Her expertise lies in the field of cell fate determination and the role of signaling pathways in tissue patterning.

Invited Lecture Daniele Panáková (UKSH Kiel, Kiel, D)

Cellular drivers of regeneration.

Daniele Panáková is an accomplished researcher affiliated with the Max Delbrück Center for Molecular Medicine (MDC) in Berlin, Germany. With a specialization in cardiovascular biology, Panáková has made remarkable contributions to understanding heart development and regeneration. Her research focuses on the mechanisms of cardiac tissue repair, with a particular emphasis on the role of stem cells and genetic regulation.

Selected abstracts:

1. **The Proteomic Landscape of in vitro Cultured Endothelial Cells across Vascular Beds.**

Stijn A Groten[1], Eva R Smit[1], Maartje van den Biggelaar[1*], Arie J Hoogendijk[1*]

[1]Department of Molecular Hematology, Sanquin Research, Amsterdam 1066CX, The Netherlands

[*]Equal contribution

Abstract

Blood vessel endothelial cells (EC) display a large heterogeneity across vascular beds. This functional diversity is at the bases of organ specify and anticipated to drive site-specific vascular pathology and is widely assessed in vivo using transcriptomics, and in vitro using functional assays. How proteomes compare between and across human in vitro cultured ECs originating from different organs remains incompletely characterized. We generated an in- depth human EC proteomic landscape (>8000 proteins) across 6 vascular beds and 2 well- established in vitro models both in steady-state and upon IFNγ-induced inflammation. EC proteomes displayed an overall high similarity and organ specific proteins were limited for the majority of organ-derived ECs. Variation between ECs and donors was mainly based in biological processes underlying proliferation and differentiation. Notably, BOECS and HUVECs represented the extremes of proteomic phenotypes across all assessed ECs. IFNγ responses were highly conserved across cellular sources. Harnessing dynamics in protein abundances we delineated interactor networks of fundamental EC proteins VWF and VE- Cadherin. This EC landscape provides an extensive proteomic addition in studying basic EC biology and heterogeneity of vascular ECs from an in vitro perspective.

2. Genetic phenotyping at single cell resolution in 3D sprouting assay to study the role of microtubules during sprouting angiogenesis.

Sarah Steffens[1], Benoit Vanhollebeke[1] & Maud Martin[1]

[1]Laboratory of Neurovascular Signaling, Departement of Molecular Biology, Université Libre de Bruxelles, Gosselies, Belgium

Abstract

As humans require their skeletal structures to move, cells use their cytoskeleton network to migrate. During sprouting angiogenesis, endothelial cells, and more specifically the leader tip cell, actively migrate through extension of guided protrusions to form new blood vessels. Surprisingly, the role of microtubules (MTs), a key component of the cellular cytoskeleton, has been underexplored in this context, in contrast to actin involvement. Our laboratory has showed that the formation of polarized protrusions in 3D sprouting endothelial cells is regulated by a specific population of MTs (Martin et al., 2018). These observations highlight an important role for MTs during sprouting angiogenesis, but their precise mode of action still needs to be characterized.

Our goal is to address in detail the previously underappreciated role of MTs during angiogenesis. Given their many functions, MTs are regulated by the existence of specialized proteins that can interact with their extremities or their lattice. These MT-associated proteins can be divided into several categories according to their mode of action. To understand which MT regulators and therefore which MT properties, are the most important for tip cell sprouting and stalk cell trailing, we have developed an innovative genetic phenotyping method with single-cell resolution in 3D sprouting assay that will allow medium throughput screening. The sprouting assay consists of 3D cultures where ECs are coated on beads, embedded in collagen gels and, by adding growth factors, form polarized protrusions mimicking the behavior of tip and stalk cells.

About forty protein candidates were selected based on the MT function they regulate (mechanical properties, transport or signaling roles) but also on their endothelial expression and silenced using lentivirus-based microRNA delivery. By associating each microRNA with a barcode sequence composed of 3 tags out of a library of 7 tags, each cell can be identified by a series of sequential immunostainings (multiplex approach) after a 3D sprouting assay. By analyzing the immunostaining signals of the barcode and of the cell membrane using segmentation and automatic profiling analyses, we will be able to associate each phenotype to a genotype. We present here the optimization of the experimental pipeline, a proof of concept of the efficiency of the multiplex immunostaining in 3D and a functional validation of the approach using two candidate genes.

Session 2. Clinical-Translational Science

Invited Lecture Bart Loeys (University of Antwerp, B)

New insights from hereditary aortopathies.

Bart Loeys is a distinguished researcher associated with the University of Antwerp in Belgium. His expertise lies in the field of medical genetics, particularly in the study of rare connective tissue disorders. Loeys has made significant contributions to unraveling the genetic and molecular basis of conditions such as Marfan syndrome and related disorders. His work has greatly enhanced the understanding and diagnosis of these conditions, leading to improved patient care and management.

Invited Lecture Hester den Ruijter (UMC Utrecht, Utrecht, NL)

Sex differences in atherosclerosis.

Hester den Ruijter is a prominent researcher based at the UMC Utrecht. With a focus on cardiovascular biology, den Ruijter has made significant contributions to the understanding of atherosclerosis and its underlying mechanisms. Her research primarily revolves around sex differences in cardiovascular disease, with a particular emphasis on identifying novel biomarkers and therapeutic targets.

1. The cardioprotective effects of semaglutide exceed those of dietary weight loss in mice with HFpEF

Coenraad Withaar[1], Laura M.G. Meems[1], Edgar E. Nollet[2,3], Jolanda van der Velden[2,3], Carolyn S.P. Lam[2,4], Herman H.W. Silljé[1], Rudolf A. de Boer[1,5]

[1]University of Groningen, University Medical Center Groningen, Department of Cardiology, The Netherlands, Hanzeplein 1, 9713 GZ, Groningen, the Netherlands; [2]Amsterdam UMC location Vrije Universiteit Amsterdam, Physiology, De Boelelaan 1117, Amsterdam, the Netherlands; [3]Amsterdam Cardiovascular Sciences, Heart failure & arrhythmias, Amsterdam, The Netherlands; [4]National Heart Centre, Singapore and Duke-National University of Singapore; [5]Erasmus MC, department of Cardiology, Dr. Molewaterplein 40, 3015GD, Rotterdam, the Netherlands

Abstract

People with heart failure with preserved ejection fraction (HFpEF) often have an unfavorable cardiometabolic profile, and obesity-related HFpEF has become a well-recognized HFpEF sub-phenotype. Targeting this unfavorable cardiometabolic profile may represent a rational treatment strategy, especially for obesity-related HFpEF. The glucagon-like peptide-1 receptor agonist (GLP-1RA) semaglutide has shown to induce significant weight loss in people with overweight/obesity and/or type 2 diabetes mellitus (T2DM) and to improve cardiovascular outcomes in T2DM.

In this study, we systematically investigated the cardiometabolic effects of semaglutide in a representative mouse model of HFpEF and compared it to the effects of weight loss.

Female aged mice (18–22 months) were fed a high fat diet (HFD) and infused with angiotensin-II (ANGII) to induce a cardiometabolic HFpEF phenotype. Mice where treated with semaglutide, vehicle or, weight loss was induced with a pair-feeding (PF) protocol.

Treatment with semaglutide and PF both resulted in significant weight loss with a similar reduction in adipose tissue. Treatment with semaglutide, but not PF, reduced left ventricle hypertrophy and fibrosis, improved diastolic dysfunction, reduced lung congestion and improved exercise capacity. Transcriptomics of LV tissue demonstrated that semaglutide activated pathways in endothelial cells and cardiomyocytes that facilitate cardiac cytoskeleton relaxation and contraction, while proteomics of plasma proteins and transcriptomics of visceral adipose tissue demonstrated that semaglutide restored protective immune responses.

In mice with HFpEF, treatment with semaglutide induces a wide array of favorable cardiometabolic effects beyond the effect of weight loss by PF. GLP-1RAs may therefore represent an important novel therapeutic option for treatment of HFpEF, especially obesity-related HFpEF.

2. Micro-RNA 92a as a therapeutic target for cardiac microvascular dysfunction in diabetes

Mostafa Samak[1,2], Giulia Germena[1,2], and Rabea Hinkel[1,2,3]

[1]Laboratory Animal Science Unit, German Primate Center GmbH (DPZ), Göttingen; [2]German Centre for Cardiovascular Research (DZHK), Partner Site Göttingen; [3]Stiftung Tierärztliche Hochschule Hannover, University of Veterinary Medicine, Hannover, Germany

Abstract

Microvascular dysfunction is a pathological hallmark of the diabetic myocardium, and is central to the etiology of diabetes-associated cardiac events. Herein, previous studies highlighted the role of the vasoactive micro-RNA 92a (miR-92a) in small, as well as large animal models. In this study, the effects of miR-92a in primary human cardiac microvascular endothelial cells (HCMEC) and their mouse equivalents (MCMEC) were explored. We characterized endothelial dysfunction and inflammation in HCMEC from diabetic patients and reported their upregulation of miR-92a. Importantly, inhibition of miR- 92a in diabetic HCMEC rescued angiogenesis and ameliorated endothelial bed inflammation. The in silico analysis identified four conserved targets downstream of miR- 92a with direct relevance to the observed phenotypes. Of novelty, we reported the miR- 92a-dependent downregulation of the coronary essential metalloproteinase, ADAM10, in diabetic HCMEC. This was also shown in diabetic porcine ventricular tissue. Accordingly, downregulation of ADAM10 impaired angiogenesis, sprouting and wound healing in HCMEC and MCMEC. Further, a dysregulation of the anti-inflammatory Krüppel-like factors (KLF) 2 and 4 in diabetic HCMEC and diabetic porcine left ventricles was observed. Indeed, ablation of KLF2 in non-diabetic HCMEC elicited the same inflammatory phenotype as their diabetic counterparts. Upstream of KLFs, dysregulation of myocyte enhancer factor 2D (MEF2D) in diabetic HCMEC and porcine ventricular tissue was demonstrated. By virtue of dual luciferase reporter assays, we confirmed direct interaction between miR-92a and all four targets. Importantly, inhibition of miR-92a was also shown to restore their levels in diabetic HCMEC. Altogether, our results highlight novel molecular mechanisms in the pathogenesis of cardiac microvascular dysfunction in diabetes and strongly qualify miR-92a as a therapeutic target.

Aletta Jacobs Award Session

1. **Studying cardiovascular effects of fibrillin impairment in zebrafish.**

Marina Horvat[1], Karo De Rycke[1], Lisa Caboor[1], Petra Vermassen[1], Julie De Backer[2], Patrick Sips[1]

[1]Department of Biomolecular Medicine, Ghent University, Ghent, Belgium; [2]Department of Cardiology and Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium

Abstract

Marfan syndrome (MFS) is the most common type of fibrillinopathy with potentially severe cardiovascular manifestations. Alongside ocular and skeletal abnormalities, MFS patients are particularly susceptible to progressive aortic dilation leading to potential dissection and wall rupture. While the development of several mouse models of MFS has contributed greatly to our current knowledge, as of yet, no causal treatment is available. Therefore, there is a pressing need for novel animal models with a higher level of flexibility and the ability of in vivo disease modeling.

We aimed to generate a relevant zebrafish model of MFS to gain a better understanding of the molecular mechanisms relating fibrillin defects of the cardiovascular system, that would ultimately lead to finding disease-specific treatment options and improved risk estimation.

The CRISPR/Cas9 system was used to systematically target the three zebrafish fibrillin genes (fbn1, fbn2a and fbn2b) in Tg(kdrl:GFP) reporter lines. For the detailed investigation of their cardiovascular phenotype, we used time-lapse fluorescent microscopy in embryonic stages, and echocardiography and histology in adult zebrafish.

We found that zebrafish lacking fbn1 and/or fbn2a do not show any cardiovascular pathology during early-stage development. However, approximately 50% of homozygous fbn2b mutant (fbn2b-/-) zebrafish embryos show a severe phenotype characterized by atrial endocardial detachment, leading to vascular embolism, pericardial edema, loss of blood flow, and premature mortality at 7-9 dpf. Interestingly, the remaining fbn2b-/- zebrafish survive until adulthood, but during larval stages already develop a dilation of the bulbus arteriosus, a structure anatomically related to the aortic root in humans, the primary location of aortic dilation in MFS patients. In addition, caudal vein of all fbn2b-/- embryos develops abnormally as a cavernous structure lacking vessel integrity. This phenotype is resolved in embryos retaining normal blood flow and aggravated upon its pharmacological inhibition during development. Furthermore, all the adult fbn2b-/- mutant zebrafish have abnormalities in the bulboventricular valve.

These studies indicate that our new fbn2b-/- zebrafish model recapitulates some of the cardiovascular pathology observed in patients with MFS. Thus, it can be considered as a relevant model to study the consequences of fibrillin impairment on the cardiovascular system. Our preliminary data suggest that there is an interplay between fibrillin deficiency and biomechanical signaling, leading to abnormal development of the specific parts of the cardiovascular system.

2. **Proteomics screen to identify new regulators of endothelial barrier function**

Fabienne Podieh[1], Max Overboom[1], Jaco Knol[2], Sander Piersma[2], Richard Goeij-de Haas[2], Connie R Jimenez[2], Peter L. Hordijk[1]

[1]Department Physiology, Amsterdam UMC, Amsterdam, The Netherlands. Amsterdam Cardiovascular Sciences, Amsterdam The Netherlands;[2]Department of Medical Oncology, Oncoproteomics Laboratory, VUmc-Cancer Center Amsterdam, Amsterdam UMC, Amsterdam, The Netherlands

Abstract

Endothelial cells (ECs) form a semi-permeable, dynamic barrier controlling the passage of plasma and leukocytes from the circulation into the tissue. By controlling EC cytoskeletal dynamics and stability of junctions, Rho GTPases are key modulators of endothelial integrity. Signaling by Rho GTPases is regulated in several ways, including ubiquitination-mediated degradation, which requires the sequential activity of E1, E2 and E3 ligases. Previously, it was shown that ubiquitination and degradation of the Rho GTPase RhoB is crucial to preserve quiescent endothelial barrier function. Our recent study shows that ubiquitination has a more generic role in regulating endothelial barrier function and that there are other barrier regulators, next to RhoB, which have a short half-life and are controlled by continuous ubiquitination and degradation.

To identify such proteins and their role in endothelial integrity, we performed short-term inhibition of E1 ligase and Cullin-mediated E3 ligases in primary human ECs and identified up- and downregulated proteins in a proteomics approach. After data analysis and literature search, knockdown of the six most promising hits was performed and the effect on endothelial integrity assessed. Silencing of angio-associated migratory cell protein (AAMP) results in marked improvement of endothelial barrier function. Concomitantly, immunofluorescent staining shows an increase in junctional, VE-cadherin-positive area and in total cell area, indicating increased cell spreading in ECs following loss of AAMP expression. We are currently investigating the mechanism by which AAMP acts on endothelial barrier function, will identify downstream targets of AAMP and analyze if AAMP itself is regulated by ubiquitin-mediated degradation in ECs. Thus, we identified AAMP as novel negative regulator of endothelial integrity. This is important, as knowledge on the regulation of endothelial integrity will contribute to our options to target dysregulation of vascular permeability.

3. Exploring PD-1+ and Tissue Resident Memory T Cells in Atherosclerotic Diseases

K. van Dijk[1], A. de Jong[1], P.H.A. Quax[1], M.R. de Vries[1,2]

[1]Einthoven laboratory for Experimental Vascular Medicine, department of Surgery, LUMC, The Netherlands; [2]Department of Surgery and the Heart and Vascular Center, Brigham & Women's Hospital and Harvard Medical School, Boston, MA, 02115 USA

Abstract

Checkpoint inhibitor therapy (ICI), a potent oncologic treatment, has been associated with an enhanced risk for atherosclerotic cardiovascular events and increased plaque progression. A key player affected by ICI is the tissue resident memory T cell (Trm), a T cell subset residing in tissues and characterized by CD44, CD69, PD-1, and CD103. Trms have also been linked to chronic inflammatory conditions, yet their involvement in atherosclerosis and accelerated atherosclerosis, as observed in postinterventional diseases such as vein graft disease, remains elusive. Here, we present an investigation into the role of PD-1 positive cells and Trms in cardiovascular disease.

Human carotid atherosclerotic plaques were stained with a MOVAT stain and scored to determine plaque stability. 25 Stable and 25 unstable plaques were stained for CD3+ T cells and PD-1. Hypercholesterolemic ApoE3*Leiden mice underwent vein bypass surgery and were sacrificed at t7, t14, t28 (n=6 per time point). The vein grafts were processed for flow cytometry to quantify the Trms population, using markers including CD4+, CD8+, CD44+, CD25-, CD69+, CD103+ and PD-1+. Hypercholesterolemic ApoE3*Leiden mice underwent vein bypass surgery. Biweekly, mice received ICI (anti-PD-L1) or control antibody (n=7). After 28 days vein graft morphology was assed using MOVAT staining and vein graft inflammation by staining MAC3 and CXCL10.

Within the human atherosclerotic plaques, T cells expressing PD-1 were present, mostly in the shoulder regions of the necrotic core. Unstable lesions had significantly higher levels of PD-1 expressing CD3+ T cells compared to stable lesion (p=0.001). Moreover, the percentage of PD-1 expression positively correlated to immune cell infiltration scores (p=0.019). Additionally, we identified Trms in murine vein grafts via flow cytometry. A trend could be observed that the number of CD4 and CD8 Trms increased at t14 compared to t7 Trms (CD8+, CD44+, CD69+/CD103+). At t28 the number of CD4 and CD8 Trms decreased compared to t14. The PD1+ expressing Trms followed a similar trend, with peak count and percentage at t14. Vein grafts of the mice receiving anti-PD-L1 mAb showed no differences in morphology compared to the control group. Nonetheless, we observed significant difference in inflammation in the vein grafts. The anti-PD-L1 treated group had higher number of macrophages (p=0.02) and increased expression of CXCL10 (p=0.008).

Here we demonstrate that PD-1+ T cells are associated with unstable lesions. Trms are increased in numbers in the inflammatory phase and decrease in the stabilizing phase of accelerated atherosclerosis. Trms could be a potential detrimental target of ICI therapy, which is characterized by increased inflammation.

Session 3. Mechanotransduction

Invited Lecture Paul Evans (University of Sheffield, UK)

Endothelial responses to shear stress in development and disease.

Paul Evans is a distinguished researcher based at the University of Sheffield in the United Kingdom, specializing in the field of mechanotransduction. With a profound focus on understanding how cells sense and respond to mechanical forces, Evans has made significant contributions to the field of biomechanics and tissue engineering. His research primarily revolves around investigating the molecular mechanisms that underlie mechanosensitive pathways and their impact on cellular behavior and tissue development.

Invited Lecture Liz Jones (Catholic University Leuven, B)

Integrating Mechanical Microenvironments the Interplay of Shear Stress Tissue Stiffness and Gene Expression.

Elizabeth Jones is a renowned researcher at KU Leuven in Belgium, specializing in the interaction between blood flow dynamics change endothelial cell behavior. Her pioneering work delves into the intricate mechanisms by which endothelial cells perceive and respond to mechanical forces in their environments. Jones' research sheds light on how these processes influence disease development with emphasis on arterial-venous malformations and diastolic heart failure.

1. Mechanical role of microtubules during angiogenesis: focus on specific MT subpopulations

Ibrahim Hamid, Danahe Mohammed, Benoît Vanhollebeke, Maud Martin

Laboratory of Neurovascular Signaling, Department of Molecular Biology, Université Libre de Bruxelles (ULB), Gosselies, Belgium

Abstract

Microtubules (MTs) are increasingly recognized as key regulators of cell migration in different contexts. During sprouting angiogenesis, our laboratory has shown that a specific population of MTs controls endothelial cells (ECs) polarity during formation of cellular protrusions, but the precise underlying mechanism still needs to be characterized. One hypothesis is that MTs could exert a mechanical role by creating protruding or resisting forces needed to initiate or stabilize endothelial protrusions. The MT network is composed of subpopulations bearing different properties and one interesting characteristic is tubulin post-translational modifications (PTMs). Among these PTMs, acetylation and detyrosination, are crucial factors affecting MT functions, including regulation of mechanical resistance and of motor behavior. Here, the goal is to provide a mechanistic understanding of the role of microtubules during angiogenesis, with an emphasis on the mechanical aspect. For that we want to decipher the role of distinct MT populations and PTMs on angiogenic properties.

We first wanted to understand if and how MT acetylation and detyrosination were regulated in response to different angiogenic stimuli. We found that MT acetylation levels were increased during scratch-wound induced cell migration in 2D but surprisingly, they were decreased after VEGF signaling alone. In a more physiological 3D collagen matrix environment, we observed that MTs were more acetylated in the major protrusion compared to the other less stable ones. In order to study the dynamics of these PTMs in different situations, we intend to use recently developed live sensors which track specific tubulin subpopulations both in cultured ECs and in zebrafish. We then aim to ascertain if those MT PTMs are necessary for ECs behavior and characteristics by regulating their levels positively or negatively using mainly siRNAs. We will study their sprouting ability in a 3D matrix and measure their protrusive forces when migrating on 1D microstrips. We believe that these results will provide some insight into MT mechanical functions during angiogenesis. Moreover they could uncover the mechanisms behind their specialization.

2. α-Catenin C-Terminal Extension undergoes tension-induced conformational changes during diapedesis of neutrophils

W.J. van der Meer[1,2], M.L.B. Grönloh[1,2], J.D. van Buul[1,2]

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Abstract

The endothelial monolayer forms a dynamic yet tight barrier between the bloodstream and underlying tissues. Paradoxically, the endothelial junctions must ensure the integrity of this barrier, while also facilitating leukocyte transendothelial migration (TEM) during inflammatory conditions. Vascular Endothelial (VE) cadherin is a major determinant of junctional integrity and forms homotypic dimers with VE-cadherin from neighbouring endothelial cells. Intracellularly, VE-cadherin is linked via beta- and α-catenin to the actin cytoskeleton. α-catenin is a tension sensor and transducer of mechanical forces, with multiple domains that can undergo conformational changes upon myosin-generated tension. Using a tension-sensitive antibody for α-catenin , we found that neutrophil-endothelial cell interactions induced local tension at the cadherin-catenin interphase. This local increase in subcellular tension by transmigrating neutrophils was supported by a local increase in Myosin Light Chain Kinase (MLCK) activity, measured in real-time with a FRET-based MLCK biosensor. These data show that not adhesion but neutrophil diapedesis across the endothelial monolayer induces local tension at the level of the endothelial cell junctions, potentially to transiently destabilize VE-cadherin-catenin complexes to allow neutrophils to paracellularly cross the endothelium.

Session 4. Metabolism

Invited Lecture Massimo Santoro (University of Padua, I)

Metabolic-dependent mRNA translation regulation in angiogenesis.

Massimo Santoro is an esteemed researcher affiliated with the University of Padua in Italy, specializing in the field of vascular biology. With a profound focus on understanding the development and function of blood vessels, Santoro has made notable contributions to the field of cardiovascular research. His research primarily revolves around investigating the molecular mechanisms involved in vascular development, angiogenesis, and vascular pathologies. Through his innovative approaches and findings, Santoro aims to unravel the complexities of vascular biology, paving the way for advancements in therapeutic interventions and treatments for vascular-related disorders.

Invited Lecture Iris Bibli (University of Frankfurt, D)

Metabolic surveillance of vascular growth.

Iris Bibli is an accomplished researcher affiliated with the University of Frankfurt in Germany, specializing in the field of metabolism. With a profound focus on understanding the intricate metabolic processes in the human body, Bibli has made notable contributions to the field of metabolic research. Her research primarily revolves around investigating the molecular mechanisms involved in energy metabolism, nutrient sensing, and metabolic disorders such as obesity and diabetes. Through her innovative approaches and findings, Bibli aims to unravel the complexities of metabolic regulation, paving the way for advancements in personalized medicine and therapeutic interventions for metabolic diseases.

1. Reactive Oxygen Species Mitigate Perturbations in Coronary Microvascular Tone in Exercising Swine with Multiple Risk Factors

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Abstract

Multiple cardiovascular risk factors, such as diabetes mellitus (DM), chronic kidney disease (CKD) and dyslipidemia are known to induce inflammation and microvascular dysfunction contributing to impaired myocardial perfusion. We previously observed that a combination of DM, high fat diet (HFD) and CKD produced an increase in coronary microvascular tone in awake swine, resulting in perturbations in myocardial O2 balance. The increased microvascular tone was mediated by an impaired NO bioavailability and was accompanied by increased myocardial levels of reactive oxygen species (ROS) and increased circulating levels of endothelin (ET). Hypothesis: In the present study in swine, we tested the hypothesis that ROS scavenging and ET receptor blockade reduce coronary microvascular tone, improving myocardial O2 delivery and O2 balance in swine with DM+HFD+CKD.

DM (streptozotocin), HFD and CKD (renal artery embolization) were induced in 13 female swine (DM+HFD+CKD), while 11 healthy female swine on normal pig chow served as controls (Normal). After 6 months, the effects of ROS scavenging and ET receptor blockade on coronary microvascular tone were studied at rest and during graded treadmill exercise.

6 months of sustained hyperglycemia (18.1±1.0 in DM+HFD+CKD vs 8.5±0.6 mmol/l in Normal), hypercholesterolemia (12.3±2.0 vs 1.7±0.1 mmol/l) and renal dysfunction (plasma creatinine: 165±7 vs 119±3 µmol/l) were accompanied by systemic inflammation (TNF 52±5 vs 25±6 pg/ml), elevated ET plasma levels, (36±2 vs 29±2 pg/ml), and oxidative stress (myocardial PGF2 12.9±0.8 vs 10.2±0.5 pg/mg protein, all $P<0.05$ by t-test). Surprisingly, in vivo ROS scavenging (TEMPOL+MPG) reduced myocardial O2 delivery (forcing an increased myocardial O2 extraction) in DM+HFD+CKD swine, indicative of a coronary microvascular constrictor response to ROS scavenging, implying a ROS-mediated vasodilator influence. In vitro experiments, using catalase in coronary small arteries, suggested a vasodilator role for hydrogen peroxide (H2O2) in DM+HFD+CKD but not in Normal swine. A switch from NO towards to H2O2 in the regulation of coronary microvascular tone was further supported by an increase in ceramide production (138±34 vs 45±4 nmol/ml, $P<0.05$) and increased activity of catalase in the myocardium (44±3 vs 31±4 nmol/min/mg, $P<0.05$) of DM+HFD+CKD vs Normal swine. Despite elevated ET plasma levels in DM+HFD+CKD swine, combined ETA/ETB receptor blockade with tezosentan did not affect myocardial O2 balance in either Normal or DM+HFD+CKD swine.

In swine, 6 months exposure to multiple risk factors resulted in increased oxidative stress that paradoxically acted to mitigate perturbations in coronary microvascular tone via an H2O2-mediated coronary vasodilator influence. Despite an increase in circulating ET levels, we found no evidence for an increased ET-1 mediated coronary vasoconstrictor influence.

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2. High advanced glycation end products associate with blood monocytes in diabetes: a cross-sectional study in the population-based LifeLines cohort

Anouk G. Groenen[1], Isabelle A. van Zeventer[2], Benedek Halmos[1], Jonas B. Salzbrunn[2], Marianne L. Mayer[1], Nikita D. La Rose[2], Jan Jacob Schuringa[2], Gerwin Huls[2], and Marit Westerterp[1]

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Abstract

In diabetes, high levels of advanced glycation end products (AGEs) that form due to hyperglycemia, are associated with increased cardiovascular (CV) mortality, but the underlying mechanisms are unknown. In diabetic mice, agonism of the receptor for AGEs increases blood monocyte and neutrophil levels, as well as atherosclerosis. Elevated blood monocyte and neutrophil levels increase cardiovascular risk in humans. We asked whether accumulation of AGEs in humans was associated with blood monocyte and neutrophil levels, and whether this association was strengthened in patients with diabetes.

To examine these associations, we used data on skin autofluorescence (SAF), a non-invasive measurement for AGEs that highly correlates with AGEs in plasma, in participants of the LifeLines (LL) cohort, a prospective population-based cohort from the North of the Netherlands. From 167.729 subjects at baseline, we included 58.923 LL participants that had valid data on SAF, blood monocytes, neutrophils, and covariates, in multivariate regression analyses. We found that SAF positively associated with blood monocytes and neutrophils (B = 0.136, p = 9.37*10-3; B = 0.164, p = 1.43*10-46; respectively), after adjustment for parameters known to affect blood myeloid cells, including age, sex, smoking, pack years, coffee consumption, estimated glomerular filtration rate, plasma high-density lipoprotein-cholesterol, plasma triglycerides, body mass index, diastolic and systolic blood pressure. The strength of the correlation was augmented in 1.726 LL participants with diabetes (B = 0.182, p = 0.001, monocytes; B = 0.288, p = 1.81*10-8, neutrophils). Moreover, we found that the positive association between SAF and blood monocytes was dependent on diabetes (Binteraction = 0.134, p = 0.003). We also found that specific AGEs, similar to those measured via SAF, induced differentiation of CD34+ hematopoietic stem and progenitor cells into granulocyte monocyte progenitors, precursors for monocytes and neutrophils, in Colony Forming Unit assays.

In conclusion, our findings show an association between AGEs (as assessed by SAF) and blood monocytes or neutrophils in the LL participants with diabetes. These data may provide an explanation for the increased cardiovascular risk in patients with diabetes.

New Talents in Vascular Biology

1. **The ubiquitin ligase Tripartite Motif 47 (TRIM47): a novel endothelial contributor to cerebral small vessel disease**

Claire Peghaire[1], Juliette Vaurs[1], Cloé Combrouze[1], Valentin Delobel[1], Sébastien Rubin[1], Romain Boulestreau[1], Béatrice Jaspard-Vinassa[1], Carole Proust[1], Cécile Duplàa[1], Thierry Couffinhal[1]

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Abstract

Cerebral small vessel disease (cSVD) is a leading cause of strokes and a major contributor to cognitive decline and dementia. Growing evidence indicates that the blood brain barrier (BBB) dysfunction may play a significant role in cSVD pathogenesis. However, our understanding of the mechanisms underlying the cause of cSVD is limited. We recently reported a whole-exome association study of a MRI-defined SVD phenotype in population based cohorts which identified a missense variant on TRIM47 locus and showed an inverse correlation between TRIM47 expression and SVD severity. The ubiquitin ligase TRIM47 is highly expressed in brain endothelial cells (EC), indicative of its putative role at the BBB level.

Our data indicate that TRIM47 controls functions of human brain microvascular EC (HBMEC) and display antioxidant properties in vitro. Bulk RNA-sequencing performed on HBMEC treated with TRIM47 siRNA revealed a downregulation of genes driven by Nuclear factor-erythroid factor 2-related factor 2 (Nrf2), a critical transcription factor that regulates antioxidant defense gene expression. Mechanistically, we have established that TRIM47 is upstream NRF2 and cooperate with NRF2 to induce the expression of NRF2-dependent genes. In vivo approach using Trim47 full knockout mouse revealed that Trim47 protects from an oxygen-induced retinopathy model characterized by oxidative stress. Importantly, adult Trim47 KO mice showed lower spatial memory performances at behaviour tests (water/Y maze), associated with brain defects (astrocytes activation, increased BBB permeability) and decreased activation of the protective Nrf2 pathway in brain EC. A diet with an Nrf2 pathway activator (tBHQ) was sufficient to prevent brain lesions, cognitive impairments of Trim47 KO mice and to rescue the Nrf2 pathway.

Together, our results highlight the key role of TRIM47 as a novel contributor of brain physiology and BBB integrity and provide a proof of concept of the relevance of targeting the protective TRIM47/NRF2 axis in patients with cSVD.

2. Unraveling the Role of TGF-beta signaling in Thoracic Aortic Aneurysm and Dissection Using Fibrillin-1 Mutant Mouse Models

Violette Deleeuw[1], Eric Carlson[2], Marjolijn Renard[1,3], Keith D. Zientek[4], Phillip A. Wilmarth[4], Ashok P. Reddy[4], Elise C. Manalo[3], Sara F. Tufa[3], Douglas R. Keene[3], Margie Olbinado[5], Marco Stampanoni[5], Sachiko Kanki[6], Hiromi Yanagisawa[7], Laura Muiño Mosquera[8], Julie De Backer[9], Lynn Y. Sakai[2], and Patrick Sips[1]

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Abstract

Aortic dissection and rupture is the main cause of early cardiovascular mortality in patients with Marfan syndrome (MFS). MFS is caused by a defect in the gene coding for fibrillin-1, a building block for microfibrils which binds transforming growth factor beta (TGF-beta) via interaction with latent TGF-beta binding proteins (LTBPs). Multiple mouse models have been used to investigate the pathophysiology of thoracic aortic aneurysms and dissections in MFS. However, the role of TGF-beta has been controversial, with earlier studies suggesting that excess release of TGF-beta due to decreased interaction with dysfunctional fibrillin-1 leads to aortic dilation and vascular damage, while other studies have shown an important protective effect for TGF-beta. Studies of dedicated mouse models will help to address these discrepancies.

To further elucidate the role of TGF-beta, we studied the in vivo effects of disrupted sequestration of TGF-beta to fibrillin-1 in mutant mouse models.

We examined the cardiovascular phenotype of mice lacking the first hybrid domain of fibrillin-1, which includes the binding site for LTBPs (Fbn1H1Δ/+), mice with a truncated fibrillin-1 (Fbn1GT-8/+), and mice with a combination of both alleles (Fbn1GT-8/H1Δ). In vivo ultrasound measurements of aortic diameter and ex vivo phase-contrast synchrotron X-ray imaging and histological analysis of the thoracic aorta were performed. Proteins present in the ascending thoracic aorta were quantitated using Tandem Mass Tag (TMT) isobaric-labeling and mass spectrometry, and interesting targets were validated using immunofluorescence staining.

Aortic dilatation and aortic wall damage increased progressively with age in Fbn1GT-8/+ mice, and was even more severe in the compound heterozygous Fbn1GT-8/H1Δ mice, which suffered from aortic rupture starting from the age of 4 months. Surprisingly, while Fbn1H1Δ/+ mice did not show aortic enlargement, areas of 'microdissections' –very localized aortic wall lesions– were observed in the ascending thoracic aorta. Microdissections in Fbn1H1Δ/+ mice were associated with a signature of reduced TGFβ signaling. Both proteomic and immunohistological data indicated an increased

abundance of mast cell proteases in the ascending thoracic aortic wall of Fbn1H1Δ/+ mice, closely associated with sites of microdissection.

Our data indicate that the regulatory role of fibrillin-1 might be compromised when deleting the first hybrid domain. We hypothesize that, in Fbn1H1Δ/+ ascending thoracic aorta, local environments in which TGF-beta signaling is reduced might be permissive for the expansion of mast cells and release of mast cell proteases, which in turn results in the local degradation of elastic lamellae. Our novel fibrillin-1 mutant mouse models of microdissection (Fbn1H1Δ/+) and of aortic aneurysm-with-rupture (Fbn1GT-8/H1Δ) may help to obtain a more comprehensive understanding on how TGF-beta signaling contributes to the molecular pathways leading to aortic dissection and rupture.

Session 5. Macrovascular Pathology

Invited Lecture Marit Westerterp (UMC Groningen, NL)

Cholesterol efflux pathways control T cell ageing and atherosclerosis.

Marit Westerterp is a distinguished researcher at the University Medical Center Groningen (UMCG). With expertise in immunology and atherosclerosis, Westerterp has made significant contributions to our understanding of how the immune system impacts cardiovascular health. Her research focuses on immune cell interactions in atherosclerotic disease, aiming to uncover novel therapeutic strategies.

Invited Lecture Vivian de Waard (Amsterdam UMC, NL)

Marfan syndrome, a structural or metabolic problem?

Vivian de Waard is a distinguished researcher affiliated with Amsterdam UMC in the Netherlands. With expertise in the fields of pharmacology, clinical pharmacy, and Marfan syndrome, de Waard has made significant contributions to personalized medicine and the understanding of this connective tissue disorder. Her research primarily focuses on the genetic and molecular basis of Marfan syndrome, pharmacogenetics, and optimizing medication use in individuals with Marfan syndrome. Through her work, de Waard aims to improve patient outcomes, enhance medication safety, and advance personalized treatment strategies for individuals affected by Marfan syndrome.

1. Absence of GDF15 aggravates adverse cardiac remodelling in mice hallmarked by perivascular fibrosis and signs of Endothelial-to-Mesenchymal transition

M. Wesseling[1,2], G. Sanchez-Duffhues[3], J.J. de Haan[1], J. Tromp[4,5,6], L. Bosch[1], C. van Munsteren[7], M.A.D. Brans[1], J.P.G. Sluijter[1,8,10], G. Pasterkamp[2,10], A.A. Voors[11], M.J. Goumans[3*], S.C.A. de Jager[1,12*]

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Abstract

Growth differentiation factor 15 (GDF15) levels associate with increased mortality and rehospitalisation in heart failure (HF) patients. Whether GDF15 is causally involved in the pathobiology of HF remains largely unknown. Using the transverse aortic constriction (TAC) mouse model, we explored a potential causal role of GDF15 underlying the development of HF, to better understand disease pathology.

To induce HF, mice were subjected to TAC, and we could confirm the increase in circulating GDF15 levels. One-week post-TAC, adverse cardiac remodelling, defined by increased cardiac volumes and myocardial global deformation (decreased contractility), was more pronounced in genetically deficient Gdf15-/- mice compared to wild type (WT) littermates. This further aggravated into severe HF in Gdf15-/- mice over 42 days follow-up. Cardiac remodelling in Gdf15-/- was accompanied by enhanced perivascular fibrosis. In vitro assays using GDF15 knock-down endothelial cells revealed impaired barrier function, while Endothelial-to-Mesenchymal transition (EndMT) was promoted in these cells when exposed to an inflammatory stimulus (Activin A). In line, we observed increased co-localisation of fibroblast and endothelial specific markers in cardiac endothelium of Gdf15-/- mice, suggestive of enhanced EndMT.

The absence of GDF15 in a mouse model of chronic pressure overload results in severe HF induction, hallmarked by perivascular fibrosis and signs of endothelial dysfunction reflected by Endothelial-to-Mesenchymal transition and. Furthermore, our data suggest that GDF15 by nature is a protective factor that aids the maintenance of endothelial integrity.

2. Peritoneal dialysis aggravates and accelerates atherosclerosis in uraemic ApoE$^{-/-}$ mice

Jamie Kane[1,2,3], Winnie G Vos[3], Laura A Bosmans[3], Bram W van Os[3], Myrthe den Toom[3], Sanne Hoeksema-Hackmann[4], Denise Moen-de Wit[4], Marion J Gijbels[3,5], Linda Beckers[3], Aldo Grefhorst[6], Johannes H M Levels[6], Lily Jakulj[1,7], Marc G Vervloet[1], Esther Lutgens[8 *], Etto C Eringa[2,9 *]

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Abstract

Atherosclerosis is highly prevalent in people with chronic kidney disease (CKD) including those receiving peritoneal dialysis (PD). While being life-saving, PD induces systemic inflammation which may promote atherosclerosis. We hypothesise that PD aggravates atherosclerosis via immune cell activation.

Three groups of ApoE$^{-/-}$ mice were fed a high-cholesterol diet (HCD), two groups also underwent a 5/6 nephrectomy to induce CKD, and one additional group received daily peritoneal infusions of 3.86% Physioneal® for 67 days (CKD+PD). Mice were sacrificed twelve weeks after the nephrectomy and assessments of atherosclerotic plaques, and immune responses were performed.

CKD+PD mice displayed more severe atherosclerotic disease than control mice. Plaque area increased, and plaques were more advanced, with a vulnerable plaque phenotype typified by decreased collagen content and fibrous cap thickness. Additionally, iNOS^{+} macrophages and CD3^{+} T-cells infiltrated plaques and perivascular adipose tissue (PVAT) of CKD+PD mice. CKD mice exhibited the vulnerable plaque phenotype and PVAT infiltration of CD3^{+} T-cells.

Only CKD+PD mice showed more CD4^{+} central memory and terminally differentiated Th1 cells, Th17, and vascular homing CX3CR1^{+} CD4^{+} T-cells with less regulatory and effector T-cells. CX3CR1 upregulation was replicable in vitro on CD4^{+} T-cells exposed to PD-fluid and uraemia.

PD-fluid exposure in uraemic mice potentiates inflammation and aggravates atherosclerosis. The CD4^{+} T-cell remodelling toward an inflammatory Th1/Th17 phenotype with more CX3CR1^{+} T-cells is present both in vivo and in vitro. This immune phenotype likely arises due to modifiable patient factors, and may be a future target of treatment.

Session 6. Neurovascular Biology

Invited Lecture Martin Dichgans (LMU Munich, D)

Stroke Genetics: Discovery, Biology, and Clinical Implications.

Martin Dichgans is an esteemed researcher affiliated with the Ludwig Maximilian University of Munich (LMU) in Germany, specializing in the field of neurovascular research. With a profound focus on the intersection of neurology and vascular biology, Dichgans has made remarkable contributions to understanding the complex interplay between the nervous system and blood vessels. His research primarily revolves around investigating the pathogenesis and mechanisms underlying neurovascular diseases, such as stroke and cerebral small vessel disease. Through his innovative approaches and findings, Dichgans aims to advance our knowledge of neurovascular disorders, leading to improved diagnosis, prevention, and therapeutic interventions in the field of neurology.

Invited Lecture Isabelle Brunet (INSERM, F)

Development and physio-pathological role of the intra-neural vascularization.

Isabelle Brunet is a distinguished researcher affiliated with INSERM in Paris, France, specializing in the field of neurovascular research. With a profound focus on understanding the intricate relationship between the nervous system and blood vessels, Brunet has made notable contributions to the field of neurovascular biology. Her research primarily revolves around investigating the molecular mechanisms underlying neurovascular interactions, cerebral blood flow regulation, and the impact of vascular dysfunction on neurological disorders. Through her innovative approaches and findings, Brunet aims to advance our understanding of neurovascular processes, leading to improved diagnosis and treatment strategies for neurovascular-related conditions.

1. **Shared pathophysiology in the neurovascular unit of the human retina and Alzheimer's brain in type 2 diabetes**

Noëlle Bakker, Cornelis J.F. van Noorden, Reinier O. Schlingemann and Ingeborg Klaassen

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Abstract

Type 2 diabetes (T2D) accounts for more than 90% of all diabetes cases worldwide. An association between T2D and dementia has been reported in the literature. Diabetes is known to affect the microvasculature in the brain and the retina. But the pathophysiology of the complex cell-cell interactions in the blood-brain barrier and blood-retinal barrier is not well understood, especially with respect to the vascular aspect of the neurovascular unit (NVU).

Human post-mortem eyes from T2D or diabetic retinopathy (DR) patients and brains from T2D and dementia patients were sectioned. Expression of selective NVU markers for vascular cells, perivascular cells, glial cells, tight junctions and vascular leakage were investigated using immunofluorescence staining followed by confocal microscopy.

In diabetes and dementia, we found that the NVU pathology of the brain is similar to that in the retina based on immunofluorescence analysis. We observed an increase in extravascular fibrinogen, indicating vascular leakage in the diabetic retina and demented brain compared with their non-diabetic and non-demented controls. By studying the tight junctions of the NVU, we detected a sharply localized occludin staining in control retina at the cell border of adjacent endothelial cells, whereas in the DR retina a reduced and more intracellular expression was observed. A similar expression pattern was found in the diabetic and demented brain. Furthermore, astrocyte loss was indicated by reduced glial fibrillary acidic protein (GFAP) expression around capillaries in the DR retina, whereas increased GFAP expression in retinal Müller cell axons suggested gliosis. For the diabetic and demented brain, the number of $GFAP^+$ cells was increased. Since astrocytes are crucial in the water and ion balance of neuronal tissue, we also studied astrocytic water and potassium channels, both of which were affected in pathology in the brain and retina.

The immunofluorescence staining of markers of the NVU provide further evidence for similar dysfunction in the vascular facet of the NVU in the retina and brain for T2D and dementia. Our findings will contribute to the identification of molecular links between the retina and brain in terms of NVU impairment that occurs in T2D and dementia.

2. Unraveling the role of transcription factor Prdm16 in endothelial cells during the progression of ischemic stroke.

Hannelore Kemps[1], Pieter Vrancaert[1], Jore Van Wauwe[1], Sébastien Foulquier[2,3], Aernout Luttun[1]

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Abstract

Ischemic stroke is defined as focal neurological damage to the brain due to vascular insufficiency. During an ischemic event, several cerebrovascular adaptations occur, including alterations in blood-brain barrier (BBB) permeability as well as recruitment of collateral arteri(ol)es, which are both determinant for stroke outcome. Understanding the molecular mechanisms that govern the maintenance and function of collateral arteri(ol)es and BBB capillaries upon cerebral ischemia is crucial to establish new effective therapies. Recently, our lab found that transcription factor Prdm16 in arterial endothelial cells (ECs) supports arterial flow recovery during hindlimb ischemia by maintaining their function. Here, we hypothesize that Prdm16 has a similar protective role during ischemic stroke by preserving cerebrovascular function. While many peripheral tissues feature an arterial-restricted Prdm16 expression pattern, we demonstrate that Prdm16 expression in the brain vasculature is not limited to arteri(ol)es, but is also present in BBB capillaries. In support of a maintenance role for Prdm16, we show that Prdm16 is upregulated within the brain vasculature in the perilesional region. Furthermore, EC-specific Prdm16 loss results in increased infarct sizes in mice subjected to stroke, suggesting a compensatory protective role for Prdm16 following cerebral ischemia. Additionally, EC-specific Prdm16 deficiency tends to decrease cerebral (collateral) perfusion, reduces BBB junctional protein levels, and increases immune cell infiltration within the lesion, indicating a potential role for Prdm16 in preserving both collateral arterial and BBB capillary EC function following stroke. Although cerebral ischemia induced a compensatory increase in Prdm16 in the cerebral vasculature, we show that Prdm16 levels are critically reduced in the aged brain vasculature. Since stroke is a disease of aging, it could be speculated that reduced Prdm16 expression in aged animals primes the endothelium for dysfunction during ischemic stroke. Altogether, targeting Prdm16 may be of major interest to ameliorate cerebrovascular function following stroke, especially within the aged brain.

Session 7. Inflammation & Ageing

Invited Lecture Guido de Meyer (University of Antwerp, B)

Prevention of atherosclerotic plaque destabilization: focus on necroptosis, pyroptosis and ferroptosis.

Guido De Meyer is a distinguished researcher associated with the University of Antwerp, focusing on the intertwined topics of inflammation and aging. With expertise in cardiovascular biology and immunology, De Meyer has made significant contributions to understanding the molecular mechanisms connecting these processes. His research primarily centers on investigating how chronic inflammation contributes to the aging process and age-related diseases, particularly in the context of cardiovascular disorders. By unraveling these complex interactions, De Meyer aims to identify novel therapeutic targets and strategies for promoting healthy aging and mitigating inflammation-related disorders in older adults.

Invited Lecture Amanda Foks (Leiden University, NL)

Single cell profiling of age-associated immunity in atherosclerosis.

Amanda Foks is a prominent researcher affiliated with Leiden University in the Netherlands. With expertise in the field of immunology and cardiovascular disease, Foks has made notable contributions to understanding the role of the immune system in atherosclerosis and vascular inflammation. Her research primarily focuses on immune cell interactions, immune-mediated vascular damage, and the development of novel therapeutic strategies to combat cardiovascular diseases.

1. **The endothelium functions as an immune modulator between the innate and adaptive immune systems by triggering neutrophil-induced cxcl12 production to promote cd8+ t-cell extravasation**

Max L.B. Grönloh[1,2,3], Sebastián Palacios Martínez[2], Tatum F.J. van Maanen[2], Marianthi Kotsi[1], Roland Immler[4], Markus Sperandio[4], Abraham C.I. van Steen[2], Lanette Kempers[2], Judy Geissler[5], Taco W. Kuijpers[5], Jaap D. van Buul[1,2,3,*]

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Abstract

During inflammation, the innate and adaptive immune system collaborate to fight local infections. To reach those areas, all types of leukocytes need to leave the circulation and cross the endothelial wall in a process called trans endothelial migration (TEM).

For our in vitro experiments, we performed 2D TEM under physiological flow experiments with freshly isolated primary human neutrophils, monocytes and CD4+/CD8+ T cells to study the TEM cascade in high spatiotemporal resolution. These experiments were replicated in our 3D vessel-on-a-chip model. Both HUVECs and mouse cremasters were imaged to confirm the presence and localization of CXCL12.

We found that neutrophils, representing innate immunity, show most efficient TEM during the first hours of endothelial inflammation, whereas CD8+ T-cells, representing adaptive immunity, require the endothelium to be inflamed for a prolonged time for efficient TEM. Strikingly, we show that paracellular CD8+ TEM, but not CD4+ T-cell or monocyte TEM is massively increased after neutrophils have already crossed the endothelium. Mechanistically, a chemokine screen and confocal imaging revealed that neutrophil adhesion triggers the local translation of CXCL12 mRNA in the endothelium to rapidly produce the chemokine CXCL12. This triggers CD8+ T-cells to transmigrate, which can be blocked by endothelial CXCL12 depletion or blockage of CXCR4 on CD8+ T-cells. We found that CXCL12 triggers the activation of b1 integrins on CD8+ T cells, and not on CD4+ T cells, explaining why only CD8+ T cell TEM is enhanced.

In conclusion, these data suggest that neutrophil adhesion triggers local production of endothelial CXCL12 to promote CD8+ T-cells to cross the endothelium. Our data show that the endothelium functions as an immune modulator between the innate and adaptive immune systems.

2. Endothelial NF-κB, MAPK, and STAT3 pathways are heterogeneously activated in mouse kidney microvascular beds in early sepsis.

Zhendong Wang[1], Peter J. Zwiers[1], J. A.A.M. Kamps[1], and Grietje Molema[1]

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Abstract

Sepsis is an uncontrolled systemic inflammatory response to infection, leading to multiple organ dysfunction syndrome which includes the kidneys. Endothelial cells (EC) are one of the first cells that respond to bacterial products such as lipopolysaccharide (LPS) and to pro-inflammatory cytokines produced during sepsis, including tumor necrosis factor-α (TNF-α). These stimuli activate intracellular signal transduction pathways in EC, activating transcription factors to enter the nucleus, which leads to the secretion of pro-inflammatory cytokines and the expression of adhesion molecules. Although studies in vitro have shown that LPS and TNF-α activate endothelial NF-κB p65, MAPK c-Jun, MAPK Junb, and JAK/STAT3 pathways, their activation status is poorly known in renal microvascular beds of septic mice. Understanding the activation status of intracellular signal transduction pathways in EC in the kidney of septic mice will create opportunities to rationally choose inhibitors to interfere with acute kidney injury.

Here, we used mice that underwent cecal ligation and puncture (CLP) as this model has an immune response that is similar to human sepsis. We then investigated the kinetics of activation of NF-κB, MAPK, and JAK/STAT3 pathways in renal microvascular beds in time by immunohistochemical staining, and we confirmed whether their activation occurred in EC by immunofluorescent double staining with the endothelial nuclear marker ERG.

Our studies showed that at early, 4 and 7 hours, time points after initiation of CLP-sepsis, activation of NF-κB p65, MAPK c-Jun, and JAK/STAT3 pathways occurred in all renal microvascular beds, while MAPK Junb activation mainly occurred in glomeruli and peritubular capillaries. Subsequently, activation of these pathways decreased at 24 and 72 hours after CLP-sepsis initiation. In arterioles, nuclear localization of p65 and c-Jun was present in EC, while nuclear pSTAT3 (Y705) was located in smooth muscle cells. In glomeruli and peritubular capillaries, nuclear localization of p65, c-Jun, Junb, and pSTAT3 (Y705) was present in EC and non-EC. In venules, all activated transcription factors except Junb were located in endothelial nuclei. Future studies aim to investigate the effects of pharmacologically inhibiting the activated pathways and study their effects on renal inflammatory activation using the ex vivo precision-cut kidney slices technique.

Poster Abstracts

Poster Abstracts Systems Biology

1. Sex and organ specific differences in fluid extravasation in mice

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Abstract

Female sex might be related to more favorable outcomes following traumatic hemorrhagic shock (THS). Endothelial hyperpermeability contributes to THS-induced organ damage. The endothelial angiopoietin/Tie2 system is an important regulator of endothelial permeability, and activating Tie2 reduces THS-induced vascular leakage and edema in male rodents. However, sex differences have not been taken into account. Therefore, we investigated sex-related differences in vascular leakage and edema formation.

Adult male and female heterozygous Tie2 knockout mice (Tie2+/−) with partial deletion of Tie2 and wild-type controls (Tie2+/+) with normal Tie2 expression were included (n=10 per group). Vascular leakage and edema formation were determined by extravasation of fluoresceinisothiocyanate (FITC)-labeled dextrans (70 kDa) and wet-to-dry weight ratio (WDR), respectively, in the lungs, kidneys, liver, heart and brain.

Tie2+/+ mice show comparable WDR for all organs. Interestingly, females show 27% and 54% higher dextran extravasation in lungs ($p<0.05$) and liver ($p<0.01$), respectively, whereas dextran extravasation was 22% lower in the brain ($p<0.05$) compared to males.

Tie2+/- mice with reduced Tie2 expression did not show differences in dextran extravasation and WDR in all organs compared to Tie2+/+ mice.

Furthermore, comparable WDR were found for all organs between male and female Tie2+/- mice, although the heart and brain show a trend towards lower WDR in females versus males (both $p=0.05$). Tie2+/- females showed 67% and 66% higher dextran extravasation in lungs ($p<0.01$) and liver ($p<0.001$) compared to Tie2+/- males, respectively. No differences in mean arterial pressure and heart rate were found between all groups.

Fluid extravasation differs between sex and vital organs in mice, independent of hemodynamics. Further research is necessary to investigate whether these differences contribute to differences in outcome following THS.

2. Tissue Optical Clearing – set-up of a cardiovascular biogeography

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Abstract

The tissue composition of the heart and in particular its electrophysiology represents a challenging matter not only in the case of cardiac disease with complex tissue changes. Thus, it is not surprising that numerous studies are concerned with the molecular and/or tissue- specific causes of cardiac disease as well as trying to optimize therapeutic approaches for such. In addition to the “classical” histological methods, which have the risk of changing the structure by mechanical sectioning and fixation when needed and only show a small section of the whole heart, optical methods are increasingly being used. Over the recent years significant innovations have occurred in this field, starting with the visualization techniques of excitation conduction in the myocardium, the so-called optical mapping, over the structural three-dimensional elucidation of tissue specifications by means of optical clearing methods.

In this study we utilized the process of optical tissue clearing to set up a cardiac-atlas of the commonly used species in cardiovascular research. This biogeographic atlas elucidating to the differences in different species, allows for a better selection of models in the development of novel (pro-angiogenic) therapeutic approaches. For tissue we used a solvent-based clearing (BABB clearing) set up. For analysis and visualization, the refractive index is adjusted over the tissue band thereby three-dimensional analysis of complete heart (from mice to pig and non-human primates) are performed. For a tissue specific analysis, such as dissection between arterial, venous and lymphatic vessels, staining of these structures is performed by either injection into the vessels or a staining of the whole heart, allowing for in-situ analysis of the heart.

The obtained pictures allow for complex analysis such as e.g. vessel branching, interaction of different vessel types, distribution in healthy and diseased. Thereof we are building up an available database which will allow for selection of the most suitable animal model for specific questions. Moreover this work supports the 3R in animal research, since based on this data 3D models and prediction/simulations can be set up, and thereby might reduce the number of needed animal experiments.

3. Heterogeneous Renal Microvascular Responses in Sepsis-Associated Coagulation and Inflammation

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Abstract

Sepsis is the dysregulated response of the host to infection, which can lead to acute kidney injury (sepsis-AKI), for which no therapies are available despite a high associated mortality. Microvascular endothelial cells play important roles in sepsis pathophysiology by their engagement in coagulation and inflammation, making them interesting target for therapeutics. The contribution of different microvascular beds to these processes is believed to be highly heterogeneous, yet this heterogeneity is at present poorly characterized. Therefore, in this study, we investigated the nature and kinetics of responses of different microvascular beds related to coagulation and inflammation in kidneys of septic mice and humans.

Laser microdissected arterioles, glomeruli, peritubular capillaries, and post-capillary venules from the kidneys of mice subjected to cecal ligation and puncture were analyzed using RNA sequencing. Differential expression and pathway enrichment analyses were used to identify genes involved in coagulation and inflammation. Next, we investigated by RT-qPCR whether these findings were also found in microvascular compartments of renal biopsies from patients with sepsis-AKI. The involvement of the identified genes in lipopolysaccharide-induced endothelial coagulation and inflammatory activation were determined in vitro using siRNA-based gene silencing in HUVEC.

RNA sequencing of renal microvascular compartments revealed approximately 400 genes with altered expression in CLP-sepsis. Their microvascular expression patterns were highly heterogeneous at both mRNA and protein levels for coagulation- and inflammation-related targets in septic mice, including increased microvascular expression of THBD, STAT3 and IFITM3. In patients with sepsis-AKI, we found altered expression of PROCR, SERPINE1, and STAT3 in post-capillary venules, and increased expression of IFITM3 in arterioles and glomeruli. Loss of STAT3 and IFITM3 in vitro resulted in attenuated endothelial coagulation and inflammatory activation.

Renal microvascular compartments in mice and humans exhibited heterogeneous expression of coagulation- and inflammation-related genes in response to sepsis, including STAT3 and IFITM3, which in vitro were shown to mediate endothelial coagulation and inflammatory activation. Additional research aimed at understanding microvascular compartment-specific functional consequences of these and other identified genes is necessary to investigate their potential as pharmacological targets in sepsis.

4. Bridging maternal and fetal circulations: morphogenesis of vitelline and umbilical vessels in the mouse embryo.

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Abstract

Extra embryonic (ExE) tissue s and vasculature support mammalian embryo growth . There are molecular and hemodynamic links between the development of the fetal cardiovascular system and the ExE circulation . For instance, anomalies of the allantois, an ExE tissue that is the precursor for the mouse embryo umbilical cord, can result in cardiovascular abnormalities. Endothelial progenitors first appear in the blood islands within the yolk sac , then in the allantois and the embryo proper at later timepoints.

We will investigate the formation of the vascular network of the yolk sac, umbilical cord, and fetal part of the placenta. Ex vivo culture and live imaging of mouse embryos bearing fluorescent reporters will be used to document the di fferent steps in the morphogenesis of the vitelline and umbilical vessels. S tatic imaging of ex vivo allantois explants cultured either on mesometrial halves of the decidua (c ontaining the chorion and ectoplacental cone)), or on coated surfaces will help provide a better comprehension of chorio allantoic fusion, a process required for placenta labyrinth development. Furthermore, currently existing transcriptomic data will be expanded though the microdissection of ExE tissues followed by single cell RNA sequencing at various timepoints during the development of the ExE vasculature and the cardiovascular system , in order to identify the molecular processes required for the establishment of the ExE vasculature. Application of pha rmacological agents and/or antibodies to disturb these processes will be performed to clarify their functions. Finally, umbilical cord and placenta samples will be obtained from both healthy and pathological human pregnancies to translate our findings form the mouse model to human pathologies.

5. Endothelial struts, a mechanism to generate large lumenized blood vessels de novo

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Abstract

De novo blood vessel formation occurs through coalescence of endothelial cells into a cord-like structure, followed by lumenization either through cell- or cord-hollowing. Vessels generated in this manner are restricted in diameter to 1 or 2 ECs, and these models fail to explain how vasculogenesis can form large diameter vessels. Here, we describe a model for large vessel formation that does not require a cord-like structure or a hollowing step. In this model, ECs coalesce into a network of struts in the future lumen of the vessel, a process dependent upon bmp signaling. The vessel wall forms around this network and consists initially of only a few patches of ECs. Struts gradually prune and ECs from struts migrate into and become part of the vessel wall. Experimental severing of struts resulted in vessel collapse, disturbed blood flow, and remodeling defects, demonstrating that struts enable the patency of large vessels during their formation.

Poster Abstracts Clinical Translation

6. Human pulmonary microvascular endothelial cell barrier response to plasma of patients with hypo- and hyperinflammatory acute respiratory distress syndrome subphenotypes.

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Abstract

Alveolar-capillary hyperpermeability is one of the hallmarks of Acute Respiratory Distress Syndrome (ARDS). Two ARDS subphenotypes have been identified, differing in biological characteristics such as inflammation and endothelial dysfunction. To study the relationship between endothelial permeability and ARDS heterogeneity, this study aimed to examine the effect of ARDS patient plasma with different subphenotypes on pulmonary microvascular endothelial cell (PMVEC) permeability.

Forty patients with ARDS were selected based on a hyper- or hypoinflammatory subphenotype (n=20 per classification) from a previously performed prospective observational cohort study. Confluent PMVECs were exposed to 10% citrated plasma for 6 hours and in vitro endothelial barrier function was assessed using electric cell-substrate impedance sensing. The effect of plasma administration on endothelial cell (EC) barrier function was analyzed and clinical and plasma biomarker data was stratified by response.

Patients of the hyperinflammatory subphenotype were characterized by more severe organ failure and higher ventilator requirements. There was no difference in median resistance between hypo- versus hyperinflammatory plasma (0.64[0.6-0.76]Ω vs. 0.67[0.59-1.1]Ω, p=0.59). Dichotomization to above vs. below 0.7 normalized resistance resulted in a group with preserved barrier function (n=24) and a group with increased permeability (n=56) with median normalized resistance of 1.22[1.08-1.33]Ω vs. 0.61[0.58-0.65]Ω (p<0.001). The latter had a lower median SOFA score (9[7-11] vs. 13[11-15], p<0.001) and more favorable measures of organ failure, such as lower lactate levels (3.3[1.7-6.2]mmol/L vs. 8.5[3.8-11.1]mmol/L, p=0.002).

ARDS patient plasma induced a heterogeneous response on in vitro endothelial barrier function, with no difference in endothelial permeability between hypo- and hyperinflammatory ARDS subphenotypes. Unexpectedly, plasma of patients with less severe organ failure caused more severe endothelial barrier disruption, the reason for which is presently unknown.

7. Endothelial activation in COVID-19 patients on ECMO support: a pilot study.

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Abstract

Extracorporeal membrane oxygenation (ECMO) is used as last-resort therapy for critically ill patients with respiratory failure such as severe COVID-19.Nonetheless, extracorporeal circulation also induces systemic inflammation, resulting in endothelial activation and injury. However, knowledge of ECMO on the endothelium is scarce. Therefore, we have explored longitudinal changes in circulating markers of endothelial dysfunction in patients supported by ECMO. Furthermore, we have studied the effect of patient plasma on pulmonary endothelial permeability.

Plasma was obtained from COVID-19 patients on ECMO support before initiation of ECMO, within 48 hours, on day 4, week 1 and week 2 of ECMO support. Circulating levels of TNF-α, ICAM-1, E-selectin, P-selectin, soluble Tie2, angiopoietin-2, angiopoietin-1, syndecan-1, von Willebrand Factor and soluble trombomodulin were measured by Luminex. Human pulmonary endothelial cells were exposed to patient plasma and in vitro endothelial barrier function was assessed using electric cell-substrate impedance sensing.

From April 2020 to January 2022, blood was collected from 14 patients on ECMO support (71.4% male; age 54 [45-62] years). ICAM-1 and E-selectin levels increased over time (ICAM-1: 0.61 [0.43-0.70] µg/ml to 1.06 [0.72-1.13] µg/ml, P time=0.046; E-selectin: 30.99 [21.94-37.93] ng/ml to 43.90 [25.01-60.00] ng/ml, P time=0.001). Circulating angiopoietin-1 levels steadily decreased from 5.88 [3.25-8.88] ng/ml to 1.59 [1.32-2.43] ng/ml (P time<0.001), whereas levels of other circulating markers remained stable. Interestingly, only soluble Tie2 decreased within 48 hours of ECMO initiation (25.84 [13.30-40.46] ng/ml to 18.03 [8.56- 25.13] ng/ml) and increased back to pre-ECMO levels within two weeks (29.01 [17.72-34.47] ng/ml) (P time<0.001). No differences were found between survivors (n=6) and non-survivors (n=8). Furthermore, patient plasma obtained two weeks after ECMO initiation reduced pulmonary endothelial resistance compared to plasma obtained before ECMO initiation (9%, P<0.05).

These pilot data suggest that ECMO support further activates the endothelium, but not induces endothelial damage, in severely ill COVID-19 patients. A larger prospective cohort study is necessary to elucidate whether these effects are specifically due to ECMO or the patients' disease progression.

8. Delivery of Tie2 mRNA to the endothelium as a novel strategy for the treatment of sepsis-induced multiple organ failure.

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Abstract

Sepsis involves a dysregulated host response to infection that can lead to life-threatening multiple organ failure. Due to the unacceptably high mortality and the lack of effective pharmacological intervention, there is an unmet clinical need to develop novel therapeutic approaches to treat sepsis. Endothelial dysfunction is considered a hallmark of sepsis to which the loss of Tie2 signaling, a main regulator of vascular integrity, contributes significantly, making it an attractive therapeutic target. To restore Tie2 receptor signaling, the field has mainly focused on manipulating its circulating ligands – the angiopoietins (Angpt). Yet, reduced Tie2 expression, partially due to Tie2 ectodomain shedding, also contributes to the observed pathological permeability during inflammation. The global success of the SARS-CoV19 vaccines has prompted the use of messenger RNA (mRNA) as a therapeutic modality for transient upregulation of protein expression and modulation of cellular pathways. The sequence of mRNA can be engineered to improve its potency for desired therapeutic applications. In this study, we aim to explore the therapeutic potential of delivering Tie2 mRNA to endothelial cells for the treatment of sepsis-associated multiple organ failure.

Mature 5-Methoxyuridine-modified mRNA encoding the human Tie2 gene (TEK) or a Tie2-eGFP fusion were designed and synthesised. HUVECs were transfected for 24 hrs with Tie2 mRNA to assess mRNA delivery, (phospho)protein expression, cell surface expression and Angpt1-induced receptor translocation by RT-qPCR, Western Blot, flow cytometry, and fluorescence microscopy, respectively.

The evident increase in Tie2 mRNA levels measured in transfected HUVECs was translated into a dose-dependent increase in Tie2 protein expression of up to 7-fold compared to endogenous Tie2 protein levels. Furthermore, Tie2 localisation onto the cell surface, kinase activity and translocation to cellular junctions in response to Angpt1 were confirmed. Early-onset and transient protein kinetic profile was observed favorable for therapeutic use in acute conditions.

Successful delivery of 5moU-modified Tie2 mRNA to endothelial cells resulted in the expression of functionally active Tie2 receptor. Ongoing work is focused on investigating the biological consequence of Tie2 mRNA delivery on endothelial barrier function under inflammatory conditions and developing means for effective mRNA delivery to endothelial cells in vivo.

9. Carcinoma-derived extracellular vesicles promote and stabilize sprouting. The role of miRNA and protein cargo.

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Abstract

Perfusion is essential for cell and tissue survival. Extracellular vesicles (EVs) produced by cancer cells alter the tumor microenvironment promoting tumor growth, angiogenesis, and metastasis. Hypoxic conditions can stimulate the secretory pathway and increase EV secretion. We hypothesized that hypoxia-induced EVs from tumor cell lines augment angiogenesis. EVs from hypoxic and normoxic cervical carcinoma cell lines HeLa and SiHa were isolated, and characterized, and their angiogenic stimulation was assessed in vitro (HUVECs) in complete medium and basal medium. Hypoxia treatment stimulates the production of vesicles ten to a hundredfold, while these have a two to fourfold higher protein content than normoxic EVs.The RNA concentration in EVs was not affected by hypoxia. The expression profile of EV-derived miRNAs from cervical carcinoma cells indicated they share more than 94% of the identified miRNAs. After enrichment analysis more than 60% of all the seq. reads were represented in three miRNA clusters/families: Let-7, miR-23/27/24, miR-17-92 and its paralogues miR-106a-363 and miR-106b-25. The hypoxia effects were more evident in proteomic analysis, where a big part of the cargo alterations was related to angio-metabolic adaptations. Carcinoma-derived EVs increased long-term stabilization of vascular networks in cells cultured in complete medium and restored mitochondrial activity, cell migration, and activated sprouting in cells cultured in basal medium. Hypoxia itself did not increase the already pro-angiogenic effect of the EVs. Tumor-derived EVs transport molecules that redundantly help to recreate a proangiogenic microenvironment, activating cell migration and sprouting. Hypoxia stimulates EV secretion and modestly increases the proangiogenic potential on those vesicles.

10. ALK2/3 signaling inhibition reduces pro-inflammatory cytokine expression in macrophages and prevents murine vein graft failure.

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Abstract

Vein graft (VG) surgery serves as a well-established method for peripheral and coronary revascularization. However, VG disease can lead to compromised clinical outcomes. Transforming growth factor-β (TGF-β) family members modulate inflammatory and fibrotic processes by engaging activin receptor-like kinases (ALK) receptors. This study investigates the impact of ALK2/3 signaling on VG remodeling using the kinase inhibitor LDN193189 (LDN).

Objective: Examining the contribution of the ALK2/3 pathway on murine VG remodeling via vascular smooth muscle cells (SMCs) and macrophages.

C57BL/6J mice underwent VG surgery and were administered either vehicle (n=8) or LDN (n=10) thrice weekly via intraperitoneal injection (3mg/kg). VG remodeling was evaluated at 28 days through morphological and quantitative analyses, including hematoxylin-eosin staining, sirius-red staining (collagen assessment), and immunofluorescent staining for α-smooth muscle actin (α-SMA, SMC marker), CD107b (macrophage marker), and Ki67 (proliferation marker). Additionally, murine bone marrow-derived macrophages were exposed to lipopolysaccharide (LPS, 10ng/mL) for 4 hours followed by vehicle or LDN (125nM-500nM) for 24 hours. Quantitative PCR (qPCR) was conducted to assess downstream ALK2/3 (ID1/3) and pro-inflammatory (TNF-α, IL-6) gene expression. Repeated measures variance was assessed using ANOVA, while two-group comparisons were performed using T-tests (significance threshold: $p<0.05$).

LDN reduced VG wall thickness by 21.3% ($p=0.0186$) in C57BL/6J VGs compared to vehicle at 28 days. No differences were observed in lumen size or relative collagen area. A noticeable trend toward a reduction of 34% in α-SMA area ($p=0.0545$) was identified after LDN treatment, although no differences in proliferating cells and CD107b+ macrophages were observed. Furthermore, LDN exposure led to a concentration-dependent decline in the expression of TGF-β-regulated genes following LPS stimulation in murine macrophages. In addition, LDN treatment was associated with reduced IL-6 and TNF-α expression.

LDN-mediated inhibition of ALK2/3 signaling reduced VG wall thickening and α-SMA+ area in VGs of C57BL/6J mice. A reduced inflammatory response was identified in macrophages upon LDN treatment.

11. Therapeutic inhibition of YAP-TAZ signaling to prevent Vein Graft Atherosclerosis

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Abstract

Venous bypass grafts may have limited patency due to excessive intimal hyperplasia and accelerated atherosclerosis. YAP-TAZ signaling regulates cellular responses to biomechanical cues, such as disturbed flow or vessel distention as seen in vein grafts. YAP activation leads to inflammation and angiogenesis, which contribute to vein graft atherosclerosis. Inhibition of YAP-TAZ attenuates naive atherogenesis, but its potential to inhibit intraplaque angiogenesis and reduce vein graft atherosclerosis has not been explored.

Saphenous vein progenitor cells (SVPs) were subjected to mechanical strain in vitro to assess YAP activation. IHC to assess YAP-expression was performed on vein grafts from hypercholesterolemic ApoE3*Leiden harvested at early, mid- and late-stage disease timepoints to assess optimal treatment window. In a separate experiment, mice underwent bypass surgery and were treated with the FDA-approved YAP-TAZ inhibitor Verteporfin 3 times/week (50 mg/kg) from day 10 until sacrifice (day 28) or vehicle. Vein grafts were harvested and processed for morphometric and compositional analysis.

Mechanical strain induced activation of YAP and its downstream targets (Ctgf, Cyr61) in SVPs, which was inhibited by Verteporfin (confirmed by WB, IP and qPCR). Moreover, YAP expression increased progressively over time after vein graft surgery and reached its peak around day 14, coinciding with the initiation of intraplaque angiogenesis. YAP was predominantly expressed in luminal and adventitial endothelial cells. Therapeutic treatment was started at day 10, which was well-tolerated and did not induce notable side-effects. Intima / media ratio was 36.7% decreased by verteporfin treatment (p=0.001), whilst intraplaque angiogenesis was also reduced (34%, p=0.002). Collagen was not altered between both groups, whereas the vessel wall ACTA2 content was reduced by 42% (p=0.0225).

Verteporfin inhibited YAP-TAZ activation induced by mechanical strain in SVPs in vitro. Therapeutic inhibition of YAP-TAZ using Verteporfin in vivo resulted in a significant decrease of vein graft atherosclerosis and intraplaque angiogenesis. Therefore, targeting YAP-TAZ might be a new therapeutic candidate to improve vein graft patency.

12. Abl inhibition improves endothelial repair by enhancing VEGFR2 Y1175/ERK signalling.

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Abstract

Accumulating evidence points out an important role of rarefaction of the pulmonary microvasculature in emphysema. Vascular maintenance and stability critically depend on the vascular Endothelial Growth Factor Receptor 2 (VEGFR2). In a recent case report, the tyrosine kinase inhibitor imatinib resulted in fast clinical improvement of a patient with emphysema through an unknown mechanism. Here we explored the possible effect of imatinib on endothelial maintenance related to VEGFR2 signalling.

We evaluated the effect of imatinib on VEGFR2 signalling in human pulmonary microvascular endothelial cells (MVECs) and human umbilical vein endothelial cells (HUVECs).

Compared to 0.1% DMSO treated cells, imatinib (0.5, 1, 2, 10µM) increased the phosphorylation of VEGFR2 at tyrosine Y1175 in a dose dependent manner. Consistently, imatinib activated Erk1/2, the downstream of VEGFR2 Y1175, in a dose dependent manner. Further experiments with 10µM imatinib revealed that VEGFR2 Y1175-Erk1/2 signalling was activated by imatinib up to 48hr, with a peak effect between 2 to 8hr, while Y951-Akt signalling was unaltered. Interestingly, repeated imatinib treatment can reactivate Y1175-Erk1/2 signalling in ECs after 46hr. Upon imatinib treatment, the expression of VEGFR2 was reduced, and it was normalized or increased after 24hr. Moreover, imatinib increased the proliferation and wound healing in HUVECs and MVECs. The effect of imatinib on VEGFR2 Y1175 signalling was also observed with Abl1/2 knockdown, in which combined Abl1 and Abl2 knockdown had an additive effect over knockdown of Abl1 or Abl2 alone.

By inhibiting Abl1/2, imatinib can improve endothelial cell integrity by modulating VEGFR2 Y1175-Erk1/2 signalling. This finding suggests a connection between Abl and VEGFR2 signalling in endothelial cells, and Abl inhibition may be a promising target to enhance endothelial integrity and prevent microvascular rarefaction.

13. Association between Coronary Vasomotor Function Measured via Invasive Coronary Function Testing and Peripheral Vasomotor Function.

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Abstract

Coronary vasomotor dysfunction (cVMD), an important underlying cause of non-obstructive coronary artery disease (ANOCA), consists of coronary vasospasm and coronary microvascular dysfunction (i.e. endothelial dysfunction (CED) and/or endothelium-independent dysfunction (CEID)) and is assessed by invasive coronary function testing (ICFT). In this study we evaluated vasomotor function in the skin as a non-invasive correlate of cVMD.

35 ANOCA patients underwent ICFT for the assessment of cVMD, consisting of intracoronary acetylcholine (ACh) and adenosine, with Doppler flow measurements. Current guidelines were used to define cVMD. Cutaneous microvascular function was assessed using Laser Speckle Contrast Analysis in the forearm, combined with endothelium-dependent vasodilator ACh and endothelium-independent vasodilator sodium-nitroprusside (SNP). The reactive hyperemia index (RHI) was assessed by EndoPAT.

Patients (80% women) had a mean age of 59 ± 9 years. cVMD was observed in 28 patients, including 23 with coronary vasospasm, 20 with CED and 4 with CEID, with overlapping endotypes. Patients with and without cVMD had a similar RHI. In contrast, cVMD was associated with lower peripheral perfusion responses to ACh and SNP.

Coronary vasomotor dysfunction is highly prevalent in ANOCA patients. These patients have a lower peripheral response to ACh and SNP, while no difference in RHI is seen. This study provides the first evidence for the potential value of peripheral vasomotor function for non-invasive detection of cVMD.

Poster Abstracts Mechanotransduction

14. A model for physiological transmural flow at the protein level.

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Abstract

In most vessels of the body, blood does not only flow down the vessel but a certain amount of the fluid flows through the endothelial layer. This t r a n s m u r a l fl o w creates a shear stress on the junctions. To understand the effect of transmural flow on endothelial cells, an in vitro flow model is needed that can separate the closely entangled mechanical forces of shear stress on the endothelial apical side and the shear stress in the junctions . Transmural flow is caused by a pressure difference between various regions of the capillary network. Transmural flow can generate shear stress, but it can also indirectly signal by imposing a strain and elastic stress to the extracellular matrix fibres, to which the cells are attached via integrin receptors. Given the small intracellular space , physiologically relevant shear stress levels can still be present regardless of transmural flow being two orders of magnitude slower than blood flow. Current transmural flow models are microfluidic models, excluding the possibility of collecting large amounts of sample. Thus, we created an in vitro transmural flow model to combat the shortcomings of the existing microfluidic image based models , creating the ability to collect protein or RNA, allowing the analysis of signalling pathways and gene regulation. Our system also gives the opportunity to study transmural flow without having to expose cells to shear stress We first assessed the physiological range of transmural flow in vivo using flow data combined with a computational model of fluid flow. We are studying the effect of physiological levels of transmural flow on proteins involved in cell adhesion, inflammation, and flow response as well as on the jagged patterns of cell cell junctions. The studied proteins include VE cadherin and its phosphorylation, ICAM 1 , VCAM 1 , and NF □ B and its phosphorylation.

15. VoC model to study endothelial barrier function and polarity.

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Abstract

Endothelial cells form a single layer of cells that forms the innermost layer of blood vessels. As such, they form a semi–permeable barrier between the blood on the apical side, and surrounding tissue on the basal side [1]. To prevent pathological endothelial dysfunction, the switch between stable and instable barrier has to be tightly regulated. In this regard, two major systems are at play: I) Molecular signaling cascades in endothelial cells (for example via small Rho-GTPases), that facilitate the strength of cell-cell contacts between endothelial cells; and II) multicellular crosstalk with cells that surround endothelial cells basally, namely vascular smooth muscle cells (VSMCs) and pericytes. As a result of their physiological environment, endothelial cells are exposed to distinct signals from the apical blood flow, and basal surrounding tissue (VSMCs and Pericytes) [2, 3].

Our group has recently defined novel regulators of cell-cell and cell-matrix adhesion in healthy and diseased endothelial cells. We aim to show that these regulators are vital to maintain endothelial cell polarity and barrier integrity. We will approach this question in conventional 2D cell models and more complex 3D Vessel on Chip systems (VoC), that include ECs, VSMCs and/or pericytes to study endothelial polarity in a highly translatable environment. To further increase physiological relevance, we will work with iPSC derived cells. This approach allows to generate isogenic multicellular VoCs and opens up the opportunity to include patient derived cell lines that carry mutations known to perturb endothelial polarity. This project aims to identify novel key players that maintain endothelial apico-basal polarity, which is valuable information in a translational context for CVDs that show impaired EC polarity.

References:

1. Kruger-Genge, A., et al., Vascular Endothelial Cell Biology: An Update. Int J Mol Sci, 2019. 20(18).
2. Beckers, C.M., V.W. van Hinsbergh, and G.P. van Nieuw Amerongen, Driving Rho GTPase activity in endothelial cells regulates barrier integrity. Thromb Haemost, 2010. 103(1): p. 40-55.
3. Mendez-Barbero, N., C. Gutierrez-Munoz, and L.M. Blanco-Colio, Cellular Crosstalk between Endothelial and Smooth Muscle Cells in Vascular Wall Remodeling. Int J Mol Sci, 2021. 22(14).

16. Empagliflozin prevents oxidative stress in human coronary artery endothelial cells via the NHE/PKC/NOX axis.

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Abstract

Sodium glucose co-transporter 2 inhibitor Empagliflozin (EMPA) ameliorates reactive oxygen species (ROS) generation in human endothelial cells (ECs) exposed to 10% cyclic stretch (pathological stress). However, the underlying mechanisms are still unclear. Our previous studies have demonstrated that EMPA attenuates ROS generation by inhibiting the activity of sodium hydrogen exchanger 1 (NHE1) in ECs subjected to Tumor Necrosis Factor (TNF) -α or enhanced stretch. It is speculated that pathological stretch activates protein kinase C (PKC), thereby activating nicotinamide adenine dinucleotide phosphate oxidase (NOX) and promoting ROS production in human ECs. We hypothesized that EMPA inhibits stretch-induced NOX activation and ROS generation through preventing NHE1 and PKC activation.

Human coronary artery endothelial cells (HCAECs) were pre-incubated for 2 h with either vehicle, EMPA, or the PKC inhibitor LY-333531, followed by exposure to cyclic stretch (5% (control) or 10% (stress)). PKC activity, NOX activity, and ROS production were detected after 24 h. Furthermore, NHE inhibitor cariporide was applied to explore the involvement of the NHE/PKC/NOX pathway in the ROS inhibitory capacity of EMPA.

Compared to 5% stretch, 10% stretch significantly increased PKC activity (5+Veh: 1.07±0.23 vs 10+Veh: 4.03±0.57, $P<0.05$). Treatment with EMPA and LY- 333531 effectively inhibited the stretch-induced increase in PKC activity (10+EMPA: 0.83±0.40, 10+LY: 0.47±0.17, P both<0.05 vs 10+Veh). EMPA and LY-333531 also reduced NOX activity and ROS production in HCAECs exposed to 10% stretch. The endothelial protective effects were not augmented by combined treatment with both drugs, suggesting that EMPA inhibits NOX activation and ROS generation by suppressing PKC activity in 10%-stretched HCAECs. Furthermore, cariporide prevented the increase in PKC activity induced by 10% stretch (5+Veh: 1.07±0.23, 10+Cari: 1.90±0.30, P both <0.05 vs 10+Veh: 2.72±0.74), and the PKC inhibitory effect of cariporide was not augmented when combined with EMPA.

EMPA reduced NOX activity via attenuation of the NHE/PKC axis, leading to less ROS generation in HCAECs exposed to 10% stretch.

Poster Abstracts Metabolism

17. Deficiency of Abca1- and Abcg1-mediated cholesterol efflux pathways in smooth muscle cells enhances vasoconstriction and bladder smooth muscle cell transdifferentiation but does not affect atherosclerosis.

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Abstract

Smooth muscle cells (SMCs) regulate blood flow distribution via vasoconstriction mediated by α-adrenergic receptors (α-ARs). Plasma membrane cholesterol may affect α1-AR signaling, but consequences for SMC-mediated vasoconstriction are unclear. Cholesterol loading promotes SMC to macrophage transition in vitro, which may enhance atherosclerotic plaque vulnerability. However, the role of ATP Binding Cassette A1 and G1 (ABCA1/G1)-cholesterol efflux pathways in SMC-mediated vasoconstriction and atherogenesis remains poorly understood.

We generated mice with SMC-specific Abca1/g1 deficiency on the low-density lipoprotein receptor deficient ($Ldlr^{-/-}$) background by crossbreeding $Abca1^{fl/fl}Abcg1^{fl/fl}Ldlr^{-/-}$ mice with Myh11-Cre^{ERT2} transgenic mice and feeding them tamoxifen-diet. To induce SMC cholesterol accumulation and atherogenesis, we fed Myh11-$Cre^{ERT2}Abca1^{fl/fl}Abcg1^{fl/fl}Ldlr^{-/-}$ and Myh11-$Cre^{ERT2}Ldlr^{-/-}$ mice Western-type diet (WTD) for 16 weeks.

Combined SMC-Abca1/g1 deficiency increased vasoconstriction in aortic rings induced by the α1-AR agonist phenylephrine, with reversal by methyl-β-cyclodextrin, substantiating its cholesterol-dependency. Unexpectedly, SMC-Abca1/g1 deficiency induced urinary bladder enlargement by >20-fold, resembling bladder outlet obstruction (BOO). This was reversed by the α1-AR antagonist tamsulosin, indicating its dependence on SMC constriction. Moreover, SMC-Abca1/g1 deficiency decreased SMC markers and increased macrophage- and fibroblast-markers in the bladder wall, enhancing collagen deposition, consistent with SMC transdifferentiation. However, after 16 weeks WTD, SMC-Abca1/g1 deficiency did not affect atherosclerotic lesion size, fibrous cap thickness, necrotic core, collagen, or macrophage content, suggesting that SMCs in atherosclerotic plaques were not affected. This may be due to low Abca1/g1 expression in intimal SMCs.

We uncover a new role of SMC cholesterol efflux pathways in suppressing α_1-AR mediated vasoconstriction and bladder SMC transdifferentiation, decreasing BOO. Our data may provide a mechanistic link for the association between BOO and diabetes in humans, particularly because diabetes is associated with decreased cholesterol efflux.

18. INVESTIGATING THE INFLAMMATORY- METABOLIC AXIS IN VALVE INTERSTITIAL CELLS AS TARGET FOR REDUCING THE PROGRESSION OF AORTIC VALVE STENOSIS.

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Abstract

Aortic valve stenosis (AVS) is a disease affecting the aging population characterized by inflammation-induced cellular changes and extensive valve calcification. Recently, lipoprotein (a) [Lp(a)] has been considered to play a crucial role in AVS pathophysiology, while the exact molecular underpinnings remain elusive. Despite open heart surgical valve replacement is the conventional treatment, there is an urgent demand for low-risk, non-invasive pharmaceutical interventions. Valve Interstitial Cells (VICs) play a crucial role in the progression of AVS. Notably, AVS and atherosclerosis exhibit shared characteristics, prompting our hypothesis that Lp(a) can modulate the metabolic-inflammatory axis in VICs, ultimately causing calcification.

Using 18F-FDG PET/CT, we found increased inflammatory activity (TBRmax 1.6 vs. 1.4) in heart valves of high Lp(a) (> 50 mg/dl) vs. low Lp(a) (< 50mg/dl) patients, indicating elevated glucose uptake. In vitro Lp(a)-stimulated VICs a(100 mg/dl) exhibited a 40-fold IL-8, 2-fold IL-6 and 7-fold MCP-1 increase, at the transcriptomic level. This coincided with a simultaneous increased glucose consumption and lactate secretion (1.25-fold). Radioactive tracer experiments using tritium-labeled glucose corroborated these findings of increased glycolysis.

Inhibiting the key-glycolytic enzyme PFKFB3, using KAN0438757 reduced glucose uptake, glycolytic activity, as well significantly reduced the maximal glycolytic capacity, as measured by Seahorse Flux Analysis. This aligned with a 50% reduction in cytokine secretion (IL-6 and MCP-1), emphasizing the intricate link between PFKFB3-driven glycolysis and inflammation in VICs. These findings highlight metabolic reprogramming as a crucial factor in the inflammatory status of VICs during AVS initiation.

In order to investigate the effects of PFKFB3-driven glycolytic inhibition in VICs on immune cell (PBMC) influx into a co-culture model of arterial endothelial cells and VICs, we aimed to mimic the interplay between these cell types. Lp(a) stimulation of VICs (without modulation of the endothelial component), led to a profound increase in PBMC influx in the subendothelial compartment, which could be reduced after glycolytic inhibition. This model shows the importance of VIC metabolic modulation in reducing the inflammatory microenvironment of the heart valve. Collectively, this research provides insights into potential therapeutic strategies for targeting metabolic mechanisms underlying Lp(a)-induced AVS.

Poster Abstracts Macrovascular Pathology

19. Chloride-channels contribute to constrictions-induced by phenylephrine or thromboxane A2 in arteries in vitro as evidenced by reductions after NKCC or TMEM16A blockade.

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Abstract

The membrane potential (MP) is a crucial determinant of vascular tone. It depends on ion membrane conductivity and high potassium conductance generates a negative MP. The chloride equilibrium potential depends on intracellular chloride concentration and thus relies on inward transport provided by the sodium-potassium-chloride cotransporter (NKCC). Due to its activity, the chloride equilibrium potential is more positive than MP and opening of chloride-channels induces depolarization. We hypothesized that chloride-channels contribute to agonist-induced constriction and studied the effect of blockade of NKCC and chloride-channels in arteries in vitro.

Mice were sacrificed and femoral arteries (200µm diameter) were examined at isometric conditions using a myograph. Vessels were exposed to the vasoconstrictors KCl (100mM), phenylephrine (PE, 0.01-100µM) and the thromboxane A2 (TxA2) analogue U46619 (0.001-0.3µM). Experiments were repeated during blockade of NKCC (furosemide, FURO, 1mM) and/or of TMEM16A-channels [N-((4-methoxy)-2-naphthyl)-5-nitroanthranilic acid, MONNA, 3µM]. Force was recorded continuously (2Hz) using custom-made software. The maximal force attained during the entire experiment was taken as 100% to calculate relative constrictions.

PE and U46619 caused concentration-dependent constrictions with a maximal constriction for PE comparable to KCl (~75%) and for U46619 of ~90%. FURO decreased PE-constrictions (1µM: from 50±6 to 31±5%) but not KCl. In different experiments, FURO was applied initially followed by washout. Here, PE-constrictions increased after FURO washout (from 33±5 to 58±4%). Similarly, U46619-constrictions (0.1µM) amounted to 44±6% during FURO and increased to 89±2% after washout. MONNA also reduced PE- (from 43±5 to 14±3%) and U46619-constrictions (from 87±2 to 48±4%) considerably. In the presence of MONNA, FURO led to a further decrease of these constrictions (to 6±2 and 25±4%, respectively).

These data demonstrate the importance of TMEM16A chloride-channels in vasoconstrictions induced by PE or TxA2. After abrogation of NKCC-mediated inward chloride transport by FURO the chloride equilibrium potential is presumably shifted to more negative values. We suggest that FURO attenuates vasoconstrictions by an effect on chloride concentration. Because FURO exhibited an additional effect after MONNA other chloride-channels may also contribute in PE and TxA2-constrictions. In addition to its diuretic effect, this mechanism may add to the antihypertensive properties of FURO.

20. **H2S is involved in the regulation of intracellular pH in human umbilical vein endothelial cells.**

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Abstract

Intracellular pH (pHi) is involved in several cellular and physiological processes such as homeostasis maintenance, regulation of membrane flow, regulation of growth and proliferation of cells, among other functions. In human umbilical vein endothelial cells (HUVECs) from healthy pregnancies pHi is regulated by sodium proton exchanger 1 (NHE1), but how this transporter is regulated in HUVECs remains unclear. In rat cardiomyocytes, NHE1 activity is inhibited by hydrogen sulfide (H2S). H2S is synthesized by cystathionine gamma-lyase (CSE), and H2S increases nitric oxide (NO) synthesis in endothelium. We evaluated whether endogenous- or exogenous-H2S regulates the NHEs activity, thereby modulating pHi in HUVECs from healthy pregnancies.

HUVECs were isolated from umbilical cord from anonymous healthy pregnancies (n=6) (conform the Declaration of Helsinki) at the University Medical Center Groningen. HUVECs were cultured in M199 plus 20% FBS and exposed to 0-3000 µmol/L sodium hydrosulfide (NaHS, 30 min, H2S donor), aminooxyacetic acid (AOAA, 24 h, CBS inhibitor) or propargylglycine (PAG, 24 h, CSE inhibitor). The acid pulse protocol (NH4Cl) was used for pHi measurements in HUVECs preloaded with the pH-sensitive probe BCECF-AM (12 µmol/L, 10 min) in the absence or presence of 5 µmol/L 5-N,N-hexamethylene-amiloride (HMA, NHEs inhibitor). Basal pHi and pHi recovery rate (dpHi/dt) were calculated. Protein lysates were prepared and separated by polyacrylamide gel (10%) electrophoresis and transferred onto Immobilon-P polyvinylidene difluoride membranes. The proteins were probed against NHE1, CBS and CSE overnight at 4°C. Total RNA was extracted from cultured HUVECs using TRIzol Reagent. 1 ug RNA was converted to cDNA using SuperScript II reverse transcriptase and random hexamer primers. CSE (assay Hs00542284_m1) and CBS (assay Hs00163925_m1) mRNA expression was measured with a Taqman Gene expression assays.

Basal pHi was reduced with NaHS (pHi 7.4 ± 0.1 vs 7.1 ± 0.1, respectively) but unaltered by PAG (7.4 ± 0.1) or AOAA (7.4 ± 0.1) (mean ± SEM, $P<0.05$, two way-ANOVA). The dpHi/dt was reduced by 56 ± 15 % from 0.1 µmol/L NaHS but unaltered by PAG or AOAA. The dpHi/dt in the presence of HMA (i.e. NHEs-mediated) recovery was inhibited by NaHS (from 0.1 µmol/L). The NHE1 protein abundance was unaltered by inhibition of CSE. However, CBS protein abundance was lower (31 ± 4%) compared to CSE. CSE mRNA relative expression was lower (92 ± 38%) than CBS mRNA expression in HUVECs.

The pHi is regulated by exogenous H2S in an NHE-dependent manner, whereas CSE- or CBS-synthesized endogenous H2S did not affect pHi.

Poster Abstracts Neurovascular Biology

21. The role of adrenomedullin in the regulation of angiogenesis and endothelial barrier function.

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Abstract

Diabetic Retinopathy (DR) is the leading cause of visual loss among adults in the developed world. In DR, the blood-retinal barrier suffers alterations preceding or accompanying the development of angiogenesis which can cause visual loss and blindness. The peptide hormone Adrenomedullin (ADM) has been reported to be increased in patients with DR. ADM acts as a regulator of vascular development, vasodilation and stabilization of the endothelial barrier function (EBF). However, how ADM regulates these processes in the retina is still unknown. Therefore, we aim to study the underlying mechanism by which ADM controls angiogenesis and EBF.

To confirm tip cell-specificity, the mRNA expression of ADM in the developing mouse retina was verified by RNAscope in situ hybridization combined with Immunofluorescence (ISH-IF).The effect of ADM knockdown (ADM-KD) by small interfering RNA or short hairpin RNA (shRNA) was studied in the in vitro CD34+ tip cell model using FACS analysis and the spheroid sprouting assay. The effect of exogenous administration of ADM was investigated in the spheroid sprouting assay, in the presence or absence of vascular endothelial growth factor (VEGF). The effect of ADM on vascular permeability was investigated by measuring the transepithelial electrical resistance (TEER) with the CellZscope system, both in the presence and absence of VEGF. The effect of exogenous ADM on the endothelial adhesion molecule VE- cadherin was analyzed by immunofluorescence (IF) staining.

RNAscope ISH-IF revealed a higher ADM expression in cells residing in the angiogenic front, including tip cells. ADM-KD using siRNA or shRNA resulted in a decrease on the percentage of CD34+ tip cells and a significant reduction in the number and length of sprouts in the spheroid assay. However, exogenous ADM did not promote sprouting of spheroids. ADM administration lead to endothelial barrier strengthening, reflected by increased TEER values, partially rescued the disruption of EBF caused by the treatment with VEGF and did not cause changes in VE-cadherin staining.

These results indicate that ADM is necessary but not sufficient to promote angiogenic sprouting and that ADM may control sprouting angiogenesis through the regulation of CD34+ tip cell formation. In addition, ADM was able to reduce VEGF-induced vascular permeability without interfering with VE-cadherin adherens junctions. Therefore, ADM may play an essential role in pathological angiogenesis and vascular permeability.

22. Identifying PLVAP-associated proteins involved in endothelial transcytosis.

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Abstract

Deregulation of endothelial transport has been associated with various endothelial barrier-related diseases such as diabetic retinopathy, acute ischemic stroke, Alzheimer's, Parkinson's, multiple sclerosis, and brain cancer. Paracellular transport, the extensively studied movement of substances across the endothelium in between adjacent cells, was initially considered the predominant regulator of barrier integrity. Yet, the movement of substances through the cytoplasm known as transcellular transport is now recognized as a key process for the selective transport of specific molecules across barrier endothelium, its regulation remains elusive. In this study, we aim to investigate the regulation of transcytosis, a distinct form of transcellular transport associated with endothelial transport vesicles named caveolae. More specifically, we focus on the modulation of transcytosis through Plasmalemma vesicle-associated protein (PLVAP or PV-1), an endothelium-specific integral membrane protein that is the only known constituent of the stomatal diaphragms located on top of caveolae. To identify proteins interacting with PLVAP that may be involved in PLVAP-mediated transcytosis, we intend to use mass spectrometry. To overcome the problem associated with the low PLVAP levels at caveolae in conventional endothelial cell cultures, the expression of caveolae-associated PLVAP needs to be upregulated. Therefore, we assessed the effect of phorbol 12-myristate 13-acetate (PMA), a known PLVAP stimulator, on the expression and localization of PLVAP. We showed that PMA-stimulated PLVAP is localized to caveolae, suggesting that PMA can be used for caveolae-associated PLVAP upregulation. This approach will enable the isolation of sufficient caveolae-associated PLVAP for the identification of novel PLVAP-associated proteins that might be of key importance to transcytosis. Overall, this project will contribute to the understanding of selective permeability via transcytosis in endothelial cells that form blood-tissue barriers. Ultimately, this may lead to the development of more effective strategies for drug delivery into barrier tissues, like the brain and the eyes.

Poster Abstracts Inflammation & Ageing

23. Neutrophils locally translate CXCL12 mRNA in endothelial cells during transendothelial migration.

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Abstract

As part of the inflammatory response leukocytes migrate towards the site of infection, where they cross the vascular wall through a multistep process called transendothelial migration (TEM). Previously, our lab demonstrated that the chemokine CXCL12 is secreted within 10 minutes by endothelial cells when they interact with neutrophils and is required for subsequent transendothelial migration of CD8+ T cells. However, how it is presented in the endothelial monolayer is still yet to be investigated. Here, we studied how CXCL12 is able to be so quickly and locally released.

CXCL12 protein was measured with ELISA after EC-neutrophil interaction, in the presence of transcription and translation inhibitors. To mimic TEM, HUVEC monolayers were placed under physiological flow with isolated human neutrophils. Puromycin immunofluorescence (IF) stains were performed as a marker for translation, while single molecule fluorescence in situ hybridization (smFISH) and IF stains were combined to look at CXCL12 mRNA and protein at the same time. We performed (smFISH) to stain CXCL12 mRNA and Proximity Ligation Assay (PLA) for puromycin and CXCL12 as a marker for newly synthesized CXCL12 protein.

Our ELISA showed that inhibiting translation, but not transcription, blocked CXCL12 secretion upon neutrophil adhesion. smFISH showed that CXCL12 mRNA is present in the EC endoplasmic reticulum (ER) before neutrophils adhere, suggesting that neutrophil interaction with the endothelial cells induces CXCL12 translation. To support that, HUVEC monolayers show a striking increase in PLA signal between puromycin and CXCL12 protein in ECs were neutrophils adhered.

We conclude that CXCL12 translation is locally induced upon neutrophil interaction with the endothelial monolayer.

24. Vascular heterogeneity determines leukocyte transendothelial migration efficiency.

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Abstract

Upon inflammation, leukocytes of the innate- and adaptive immune system cross the endothelial wall via a process called transendothelial migration (TEM). Leukocytes typically follow a chemokine gradient and have been observed to leave the blood vessel through endothelial cell-cell junctions, called the paracellular route, or through the endothelial cell body, called the transcellular route. However, the number of leukocytes that leaves the vasculature and which route they take differs per organ. It is unclear what the underlying mechanism is for these differences. Using our in vitro TEM under flow assays, we found that neutrophils crossed pulmonary endothelial cells with much higher efficiency than endothelial cells from other vascular beds. Moreover, neutrophils predominantly choose the paracellular route of diapedesis, independent of the endothelial origin. When crossing endothelial cells from umbilical cords, neutrophil TEM was more efficient during short-term inflammation, i.e, 4h, compared to long term inflammation, i.e., 24h. However, on pulmonary endothelium, no difference in TEM efficiency between long- and short-term inflammation was observed. Mechanistically, we identified chemokines RANTES, CXCL10 and CCL14 to be more abundantly secreted by pulmonary microvascular endothelium upon inflammation than by umbilical cord-derived endothelial cells. Future research is focused on exploring the role of these chemokines in leukocyte TEM across different vascular beds. Taken together, our data show that the efficiency of leukocyte TEM is determined by endothelial cells from different vascular beds. Moreover, this vascular bed heterogeneity is also observed for the release of different chemokines upon inflammatory stimuli.

25. **Senolytic treatment ameliorates expression of senescence associated- and inflammatory markers in cultured human atherosclerotic plaque slices.**

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Abstract

Atherosclerotic cardiovascular disease cause one-third of all deaths worldwide. Despite the availability of a variety of drugs that target specific pathways involved in disease development, a significant risk remains, which highlights the urgent need for additional treatment strategies. Recent studies have demonstrated the presence of senescent cells in atherosclerotic plaques: these cells contribute to plaque instability by their production of inflammatory cytokines, chemokines and matrix- degrading peptides. Clearance of senescent cells by senolytic agents may, thus, hold promise for the treatment of atherosclerosis.

Aim: To establish the effects of the senolytic agents dasatinab and quercetin (D+Q) on human atherosclerotic plaque slices cultured ex vivo for three days.

Human atherosclerotic plaque material was obtained from carotid endarterectomy surgery performed at the University Medical Center Groningen (UMCG) (n=5). Plaques were cut in circular segments of 2 mm thick and were cultured on collagen sponges at the medium-air interface to remain oxygenated. Plaque slices were cultured for 3 days with either a low (D+Q1; D: 100nM; Q: 10µM) or a high (D+Q2; D: 200nM; Q: 20µM) concentration of D+Q or DMSO (control). We cultured 3 slices per condition for histology, gene expression and proteomics analysis.

We observed a downregulation of senescence-related (p16 and MMP9; $p<0.05$) and inflammation-related (TNFα, IFNγ, MCP-1; $p<0.01$) genes in D+Q1 and D+Q2 treated plaque cultures compared to controls. In addition, with untargeted proteomics we found 652 (D+Q1) or 1182 (D+Q2) differentially expressed proteins in D+Q-treated plaque cultures compared to controls ($q<0.05$; $FC>1.5$). Most of these proteins were downregulated (D+Q1: 580; D+Q2: 1100) and predominantly involved in immune system-related pathways.

Our findings indicate that D+Q treatment of cultured human plaque slices leads to a reduction in inflammatory- and senescence-related markers. This effect is likely attributed to the removal of senescent cells, highlighting a potential therapeutic approach for stabilizing atherosclerotic plaques.