Société Française d'Angiogenèse

10th Congress of the French Society of Angiogenesis

Bordeaux – October 11th-13th 2023

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10th Congress SFA

Bordeaux – 11-13 October 2023

Day 1 – Wednesday, October 11th

12h-13h15
Welcome – Registration, attachment posters
13h15-13h30
Introductory remarks and welcome by the local Organizing committee

Session 1: Vessel tissue interaction in cardio-neuro-vascular diseases

13h30-15h00 - Moderators: Marie-Ange Renault & Geraldine Siegfried

13h30-14h00
Invited speaker: Elizabeth Jones (Leuven, Belgium)
Pericytes in cardiac microvascular dysfunction

14h00 -14h30
Invited speaker: Raquel Del Toro (Sevilla, Spain)
Role of mesenchymal progenitor cells in heart post-ischemic angiogenesis

14h30 -14h45
Short communication: Florian Alonso (Bordeaux, France)
Extracellular matrix protein Fibrillin-1, a new player in the formation of blood vessels

14h45 -15h00
Short communication: Apeksha Shapeti (Leuven, Belgium)
Unraveling the mechanisms of enhanced angiogenesis and endothelial mosaicism in cerebral cavernous malformations: the role of cellular forces and ECM degradation

Poster Session 1: 15h00-16h30:

16h00-16h30: Coffee Break

Session 2: Metabolism regulation of vascular cells

16h30-18h00 - Moderators: Eva Faurobert & Candice Chapouly

16h30-17h00
Invited speaker: Mariona Graupera (Barcelona, Spain)
PIK3CA mutations in vascular malformations

17h00-17h30
Invited speaker: Anais Briot (Toulouse, France)
Adipose depots microvascular endothelial cells in metabolic flexibility

17h30-17h45
Short communication: Stephanie Gayral (Toulouse, France)

A PI3KCIIalpha-dependent autophagy program protects from endothelial dysfunction and atherosclerosis in response to low shear stress

17h45-18h00
Short communication: Yasmin Rida (Grenoble, France)
ALK1 is critical for maintenance of pulmonary and liver endothelium integrity

Session 3: Open public: the role of the blood vessel in pathologies

18h00 – 19h00 Thomas Mathivet (Bordeaux, France); Eva Faurobert (Grenoble, France) k; Elliot Lopez (Paris, France); Romain Boulestrau (Bordeaux, France); Chloe Dujardin (Paris, France); Bérénice Dugué (Bordeaux, France);

19h00 -21h00: Pessac-Leognan Wine tasting

Day 2 – Thursday, October 12th

Session 4: Hematovascular Interaction in Development and Disease

8h30-10h00 - Moderators: Olivier Mansier & Thomas Mathivet

08h30-09h00
Invited speaker: Karen Hirschi (Charlottesville, USA)
"Regulation of Endothelial Cell Specialization"

09h00-09h30
Invited speaker: Chloe James (Bordeaux, France)
Unraveling the role of blood and endothelial cells in JAK2V617F myeloproliferative neoplasm-associated thrombosis

09h30-09h45
Short communications: Alison Domingues (Milan, Italy)
Endothelial cell-driven CXCR4 signalling limits vascular expansion during embryonic brain development

09h45-10h00
Short communications: Théo Morel (Paris, France)
Roles of proteases signaling in late stage development of the murine cardiovascular system

10h00-10h30: Coffee Break / Poster Session

Session 5: Vessel tissue interactions in the tumoral progression

10h30-12h00 - Moderators: Julie Gavard & Andreas Bikfalvi

10h30-11h00
Invited speaker: Holger Gerhardt (Berlin, Germany)

11h00-11h30
Invited speaker: Jean-Philippe Girard (Toulouse, France)
High endothelial venules (HEVs): specialized blood vessels for lymphocyte entry in tumors during cancer immunity and immunotherapy

11h30-11h45
Short communications Lucas Treps (Nantes, France)
Explore endothelial cell interaction & immune functions in a model of multicellular tumor spheroid

11h45-12h00
Short communication: Maeva Totobesola (Nice, France)
LVRF, a new isoform of VEGF, and a relevant therapeutic target in clear cell renal cell carcinoma (ccRCC)

Poster Session 2: 12h00-14h00:

13h00-14h00 : Lunch buffet

Session 6: Advances in vascular imaging

14h00-15h30 - Moderators: Anne Clémence Vion & Cécile Duplàa

14h00-14h30
Invited speaker: Claudio Franco (Lisbon, Portugal)
"Stochastic vascular morphogenesis"

14h30-15h00
Invited speaker: Alexandre Dubrac (Montreal, Canada)
« Spatiotemporal regulation of neurovascular patterning »

15h00-15h15
Short communication: Guy Malkinson (Paris, France)
"*Probing the diversity of brain perivascular macrophages by advanced imaging methods"*

15h15-15h30
Short communication: Laurent Jacob (Paris, France)
Unveiling the 3D Anatomy and Development of Meningeal Lymphatic Vessels: Implications for Neuroimmune Communication and Therapeutic Potential

15h30-15h45
Short communication: *Juliette Vaurs (Bordeaux, France)*

15h45-16h15: Coffee Break

Session 7: Vessel tissue interaction in Diabetes

16h15-17h45 Moderators: Florence Tatin & Kamel Mohammedi

16h15-16h45
Invited speaker: Louis Potier (Paris, France)
Cardioprotective effects of antidiabetic therapies

16h45 -17h15
Invited speaker: Marthe Moldes (Paris, France)
Effects of glucocorticoids on adipose tissue plasticity

17h15 -17h30
Short communication: Ninon Foussard (Bordeaux, France)
ICAM1 expression by the microvasculature impairs capillary perfusion which compromises hind limb ischemia recovery in diabetic mice

17h30 -17h45
Short communication: Anna Rita Cantelmo (Paris, France)
Integrated single-cell transcriptomics reveals mitochondrial calcium modification as a hallmark of endothelial-to-mesenchymal transition

Integrated single-cell transcriptomics reveals mitochondrial calcium modification as a hallmark of endothelial-to-mesenchymal transition

17h45- 18h45: SFA general assembly

20h00: Gala Dinner

Day 3 – Friday, October 13th

Session 8: Lymphatic vasculature

09h00-10h30 Moderators: Barbara Garmy-Susimi & Ebba Brakenhielm

09h00-09h30
Invited speaker: Steven Proulx (Bern, Switzerland)
The role of lymphatic vessels in the drainage of cerebrospinal fluid

09h30-10H00
Invited speaker: Ebba Brakenhielm, (Rouen, France)
The IN and OUT: Immune cell interactions in the heart with lymphatics vs blood vessels

10h00-10h15
Short communication: Kathryn Jacobs (Leuven, Belgium)
Exploring the Role of Autophagy in the Lymph Node Vasculature

10h15-10h30
Short communication: Florence Tatin (Toulouse, France)
3D-imaging reveals spatially restricted architecture of human lymphatic vasculature in close relationship to αSMA positives blood vessels in subcutaneous adipose tissue

Lecture Magistralis:

10h30-11h15: Moderators: Thierry Couffinhal, Bordeaux
Invited speaker: Kenneth Walsh (Charlottesville, USA)
Clonal hematopoiesis and its contributions to age-related and sex-biased diseases"

11h15-11h30 Coffee Break

Session 9: Signaling in vascular development and pathology

11h30-13h00 Moderators: Alexandre Dubrac & Thomas Daubon

11h30-12h00
Invited speaker: Kevin Boyé (Paris, France)
Netrin1 binding to Unc5B regulates blood-CNS barrier integrity

12h00-12h15
Short communication: Gael Genet (Charlottesville, USA)
Induced cell cycle arrest prevents arterio-venous malformations in Hereditary Hemorrhagic Telengiectasia

12h15-12h30
Short communication: Claire Peghaire (Bordeaux, France)
The ubiquitin ligase Tripartite Motif 47 maintains brain homeostasis by promoting the NRF2 antioxidant protective system

12h30-13h00: Oral and Poster prize ceremony and closing remarks

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SHORT COMMUNICATION

Extracellular matrix protein Fibrillin-1, a new player in the formation of blood vessels

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Résumé

Fibrillin-1 is an extracellular matrix protein which assembles into microfibrils ensuring tissue integrity and elasticity. Mutations in the fibrillin-1 gene cause Marfan syndrome, an autosomal dominant disorder associated with severe cardiovascular, skeletal and ocular defects. While it is established that fibrillin-1 mutations cause weakening of the arterial wall, leading to aortic aneurysms and dissections, the consequences of these mutations on the microvasculature have not been explored. Our study reveals that fibrillin-1 is critical for angiogenesis which is compromised by a typical Marfan mutation. During postnatal mouse development, in the brain or retina, fibrillin-1 is present in the extracellular matrix and colocalizes with microfibril-associated glycoprotein-1, MAGP1 at the angiogenic front. At this site, endothelial cells differentiate into motile and invasive tip cells. In a mouse model of Marfan syndrome carrying a fibrillin-1 mutation, retinal angiogenesis is compromised, endothelial sprouting is decreased and tip cell identity is impaired. *In vitro,* fibrillin-1 deficiency alters VEGF/Notch and Smad signalling which regulate the acquisition of endothelial tip cell identity, and we showed that modulation of MAGP1 expression impacts these pathways. Intraocular injection of a wild-type C-terminal fibrillin-1 fragment prevents the angiogenesis defect observed in the retina of the fibrillin-1 mutant mouse. Proteomic analyses reveal that, in endothelial cells, the C-terminal fibrillin-1 fragment upregulates the expression of ADAMTS1, a tip cell metalloprotease involved in matrix remodelling and cell invasion. Collectively, these new findings identify a transient matrix, enriched in fibrillin-1, MAGP1 and ADAMTS1, at the angiogenic front. This matrix plays a critical role during retinal neovascularization, regulating Notch signaling and matrix remodelling for robust endothelial sprouting.

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Unraveling the mechanisms of enhanced angiogenesis and endothelial mosaicism in cerebral cavernous malformations: the role of cellular forces and ECM degradation

Apeksha Shapeti[1], Jorge Barrasa-Fano[1], Janne De Jong[1], Abdel Rahman Abdel Fattah[2], Said Assou[3], Alexei Grichine[4], Adrian Ranga[1], José Antonio Sanz-Herrera[5], Eva Faurobert[4], and Hans Van Oosterwyck*†[1]

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Résumé

Cerebral Cavernous Malformations (CCMs) are tumor like mosaic lesions composed of mutant and wild-type (WT) endothelial cells (ECs), and are associated with increased angiogenesis. ROCK1 and ROCK2 have been previously shown to be important effectors of mutant EC behaviour upon CCM2 silencing. In order to investigate mechanisms of WT EC recruitment and its modulation by ROCKs, we adapted our 3D in vitro angiogenic invasion assay towards culture of mosaics of WT and CCM2-silenced (siCCM2) ECs (using siRNA) and combined it with 3D Traction Force Microscopy (TFM), imaging of matrix degradation, immunostaining and single cell RNA sequencing (scRNAseq). siCCM2 ECs showed increased invasion, matrix degradation, and 3D tractions during sprouting in both mosaic and non-mosaic cultures. Interestingly, WT ECs invaded further and faster in siCCM2 mosaics as well, but were restricted to follower positions in sprouts led by hyper-angiogenic siCCM2 ECs. These observations were dependent on the siCCM2 ECs' ability to exert contractile forces and to degrade the ECM. Both ROCK1 and ROCK2 were required for force exertion while only ROCK1 was necessary for ECM degradation. We could demonstrate that WT ECs experienced cell-cell pulling forces from leading siCCM2 ECs and displayed higher β1-integrin activation in siCCM2 mosaics compared to non-mosaics. This was accompanied by an upregulation of genes in WT ECs involved in matrix deposition and DNA replication. In summary, our study identifies a role for contractile forces by mutant ECs in the recruitment of WT ECs to CCM lesions. During this process, WT follower ECs undergo a (possibly force-dependent) reprogramming leading to increased DNA replication and eventually cell proliferation.

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A PI3KCIIalpha-dependent autophagy program protects from endothelial dysfunction and atherosclerosis in response to low shear stress

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Résumé

Background: The ability to respond to mechanical forces is a basic requirement for maintaining endothelial cell (ECs) homeostasis which are continuously subjected to Low and High shear stresses (LSS and HSS). In arteries, LSS and HSS have a differential impact on EC autophagy processes. However, it is still unclear whether LSS and HSS differently tune a unique autophagic machinery or triggers specific autophagic responses in ECs.
Methods: Using fluid flow system to generate forces on EC and multiscale imaging analyses on ApoE-/- mice whole arteries, we studied the cellular and molecular mechanism involved in autophagic response to LSS or HSS on endothelium.

Results: We found that LSS and HSS trigger autophagy activation by mobilizing specific

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autophagic signalling modules. Indeed, LSS-induced autophagy in endothelium was independent of the class III PI3K VPS34 but controlled by the a isoform of class II PI3K (PI3KCIIalpha). Accordingly, reduced PI3KCIIalpha expression in ApoE-/- mice (ApoE-/-PI3KCIIalpha+/-) led to EC dysfunctions associated to increased plaque deposition in the LSS regions. Mechanistacally, we revealed that PI3KCIIa controls mTORC1 activation since phosphorylation of p70-S6Kinase was increased in absence of PI3KCIIalpha and that treatment by rapamycin in ApoE-/-PI3KCIIalpha+/- mice specifically rescue autophagy in arterial LSS regions. Finally, we demonstrated that absence of PI3KCIIalpha led to decreased endothelial primary cilium biogenesis in response to LSS and that ablation of primary cilium mimics PI3KCIIalpha-decreased expression in EC dysfunction, supporting the idea that this organelle could be the mechanosensor linking PI3KCIIalpha and EC homeostasis.
Conclusions: Our data reveal that mechanical forces variability within arterial system determine EC autophagic response and support a central role of PI3KCIIalpha/mTORC1 axis to prevent EC dysfunction in LSS regions.

ALK1 IS CRITICAL FOR MAINTENANCE OF PULMONARY AND LIVER ENDOTHELIUM INTEGRITY

Yasmin Rida*†[1], Dzenis Koca[1], Agnès Desroches-Castan[1], Aude Salomon[1], Emmanuelle Tillet[1], Laurent Guyon[1], Sabine Bailly[1], and Nicolas Ricard*[1]

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Résumé

Hereditary hemorrhagic telangiectasia (HHT) is a genetic vascular disease characterized by severe nosebleed, gastrointestinal bleeding, cutaneous and mucous telangiectasia, and arteriovenous malformations mainly found in the lungs and liver. HHT is caused by mutations in receptors of the Bone Morphogenetic Protein (BMP) either Activin Receptor-like Kinase 1 (ACVRL1, encoding for ALK1) or Endoglin (ENG). HHT symptoms worsen with age with a total penetrance by 60-year old. This clinical observation suggests that ALK1 signaling could be involved in the maintenance of the quiescent endothelium integrity. Here we aimed to characterize the biological functions of ALK1 in the adult endothelium, focusing on the pulmonary and hepatic endothelia. We used inducible *Acvrl1* endothelial knockout (*Acvrl1*iECKO) mice to invalidate ALK1 in adult (2-months old) mice. We found that endothelial *Acvrl1* deletion in adult mice is lethal within 8 days. Mice present severe pulmonary and gastrointestinal hemorrhages. This phenotype could be related to defects in endothelial adhesion on the basal lamina and in endothelial junctions. In the liver, loss of sinusoidal endothelial cell identity occurs, as seen notably by loss of endothelial fenestration, concomitant activation of stellate cells and disorganized hepatocytes. These observations show that loss of endothelial Acvrl1 leads to phenotypes beyond endothelial cells. 8 days after *Acvrl1* deletion, these mice exhibit either a severe vasodilation or systemic arteriovenous shunts as fluorescent beads injected in the systemic circulation could reach the lungs. Single cell RNAseq analysis supports that following *Acvrl1* deletion in endothelial cells, immune regulation is greatly affected in lungs and liver. We also found that key metabolic enzymes are regulated by the loss of *Acvrl1*. Interestingly, while some affected functions are found in both pulmonary and hepatic endothelia, other functions were tissue-specific.

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Endothelial cell-driven CXCR4 signalling limits vascular expansion during embryonic brain development

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Résumé

During embryonic development new vessel sprouts are formed by proliferating and migrating endothelial cells (ECs) in a process termed sprouting angiogenesis. Subsequently, vessel sprouts have to fuse with each other in a process called vascular anastomosis that is promoted by tissue-resident macrophages. The chemokine CXCL12, also known as stromal derived factor 1 (SDF1), primarily acts through the receptor CXCR4 and during development CXCL12-CXCR4 signalling regulates arterial morphogenesis in zebrafish embryos and in mouse heart, mesentery and limb skin. CXCR4 inhibition decreases neovascularization as well as the number of hematopoietic cells recruited in mouse models of eye pathology. Indeed, CXCL12-CXCR4 signalling has been proposed as a potential mediator of EC-macrophage interactions. Even though *in vitro* studies suggest that CXCL12 signalling induces angiogenic responses in ECs, **the precise mechanism by which the CXCL12-CXCR4 axis functions in blood vessel formation remains elusive**. We found that absence of CXCL12 or CXCR4 resulted in a decrease in the number of tissue macrophages in the embryonic organs analysed, suggesting that CXCL12-CXCR4 signalling consistently promotes macrophage colonisation across embryonic organs. Unexpectedly, lack of CXCL12 or CXCR4 caused vascular overgrowth/hyperbranching in the embryonic hindbrain, but not other organs. By combining single cell transcriptomic studies with a mouse genetic reporter line, we established that brain ECs were enriched not only in *Cxcr4* but also in *Cxcl12* transcripts compared to other organ ECs. Indeed, conditional deletion of *Cxcl12* or *Cxcr4* only in ECs showed vascular overgrowth in the embryonic hindbrain, like the complete lack of CXCL12 or CXCR4. **Our results therefore indicate that endothelium-derived CXCL12 can prevent vascular overgrowth during physiological angiogenesis. This work also sheds new insights on the role of CXCL12 in macrophage-EC interactions in vascular morphogenesis regulation.**

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Roles of proteases signaling in late stage development of the murine cardiovascular system.

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Résumé

Protease-activated receptors (PARs) are G protein-coupled receptors that are activated by a unique mechanism involving receptor cleavage at the N-terminus by serine proteases, resulting in release of a tethered ligand and receptor activation. The four receptors that constitute the PAR family (PAR1-4) play important roles in hemostasis, inflammation, and vascular homeostasis. An important role for thrombin-mediated PAR1 signaling in the endothelium was revealed by failed vasculogenesis and partly penetrant mid-gestational lethality in murine embryos deficient in PAR1. Novel protease agonists and a role for protease signaling in epithelial fusion were later revealed by neural tube closure defects associated with compound deficiency of PAR1 and PAR2. Unpublished observations from our laboratory show highly penetrant late gestation lethality in PAR1:PAR2 double knockouts (dKO), suggesting additional roles for protease signaling. Affected embryos display pallor, edema and venous congestion between E13.5 and birth. However, the cause of death remains elusive. To uncover the role of protease signaling in late gestation, we first employed ß-galactosidase reporter mice to map the expression of PAR1 and PAR2 during late gestation. In the embryo, PAR1 expression was observed in the endocardium, angiogenic endothelium, megakaryocytes and vascular smooth muscle cells (VSMCs), while PAR2 was expressed primarily in epithelial cells, skeletal muscle and arterial endothelial cells. In the placenta, PAR1 expression was observed in VSMC and endothelial cells, while PAR2 was expressed in syncytiotrophoblast. Despite little observable PAR2 expression in VSMC, expression of a PAR2 transgene under the control of a VSMC promoter significantly improved survival of dKO embryos. Taken together, these observations suggest a critical but complex role for protease signaling in late gestation development that appears to involve both placental function and the development of vascular resistance.

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Explore endothelial cell interaction & immune functions in a model of multicellular tumor spheroid

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Résumé

Introduction: Beyond their physiological functions, endothelial cells (ECs) serve as a key component of the tumor microenvironment (TME) promoting tumor progression and metastasis. Anti-angiogenic therapies have been developed to target tumor ECs (TECs) and their pro-angiogenic function. However, recent studies using patient-derived tumor biopsies showed that TECs are much more heterogenous than anticipated with new immunomodulatory phenotypes discovered. In line with the new era of immunotherapies in cancer, it is tempting to speculate that the understudied immunomodulatory TECs may affect these therapies.
Method: We developed a model of 3D multicellular tumor spheroid (MCTS) composed of lung tumor cell mixed with several components of the TME: primary human lung fibroblasts, peripheral blood-derived monocytes/macrophages and primary human ECs (HUVECs). To explore MCTS cell heterogeneity we then performed single cell RNA-sequencing (scRNA-seq) and predicted the cell-cell interactome of 48.000 high quality cells.

Results: Besides the angiogenic and proliferative ECs, we also identified a new phenotype expressing pro-inflammatory, mTORC1 signaling and ER stress response gene signatures. We coined these cells immunomodulatory TECs (iTECs), which resemble TECs found in scRNA-seq from lung cancer patients. Interestingly, iTECs were absent in scRNA-seq datasets from TEC or HUVEC 2D monocultures, highlighting the relevance of MCTS to study such cells.

Conclusion: We described a 3D model system whereby (i) the immunomodulatory function of TECs can be studied, and (ii) the effect of clinically approved drugs could be monitored to asses/predict treatment resistance in patients.
Keywords : Tumor microenvironment, Endothelial cells, Cell-cell interaction, Heterogeneity

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LVRF, a new isoform of VEGF, and a relevant therapeutic target in clear cell renal cell carcinoma (ccRCC)

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Résumé

Clear cell renal cell carcinoma (ccRCC) is a cancer known for its hypervascularization, mainly caused by the inactivation of the Von Hippel Lindau (VHL) gene. Its inactivation leads to an accumulation of hypoxic factors: Hypoxia Inducible Factors (HIF), which contribute to the overexpression of VEGF, known as the essential growth factor for blood vessels. Its dysregulation is widely described in various types of cancers, including renal cancer. Thus, anti-angiogenic agents represent a major advancement in the therapeutic management of ccRCC patients. However, the effectiveness of current treatments for kidney cancer is highly debated, varying from one patient to another. To date, no valid answer explains the reasons behind this variability. A recent discovery by our team (Pagès et al., 2023) of a new pro-angiogenic isoform of VEGF-A could provide an answer to this question. Preliminary results from our team describe this new isoform as promoting the proliferation of tumor cells in ccRCC. Interestingly, analysis of patient plasma also revealed a correlation between its presence and a poor response to one of the reference anti-angiogenic treatments for ccRCC: sunitinib.
Our working hypothesis is that its expression is correlated with a poor response to current anti-angiogenic treatments, as well as a more significant tumor development. We started by studying what could induce its expression, particularly under conditions of physical and metabolic stress (RT-qPCR). We also used reference anti-angiogenic agents such as sunitinib and axitinib for the study. Subsequently, we investigated its autocrine role in proliferation, migration, and invasion. Finally, monoclonal antibodies directed against this new isoform demonstrated in vitro and in vivo efficacy, making VEGF NF an interesting therapeutic target in the management of ccRCC patients.

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Probing the diversity of brain perivascular macrophages by advanced imaging methods

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Résumé

Brain perivascular macrophages (brPVM), found in the perivascular spaces, are emerging as important elements with key regulatory roles in waste clearance and in brain homeostasis1,2,3. They express lymphatic vessel endothelial hyaluronan receptor 1 (lyve-1) and take part in the regulation of vascular tone through interactions of lyve-1 with collagen2. We recently demonstrated their unique and diverse spatio-temporal developmental profiles across different brain regions during early postnatal stages in the mouse brain and described their differential association with blood vessels of different calibers and functions, i.e. artery vs vein1. What underlies this diversity, and what properties enable brPVM to perform their physiological role, is largely unknown. Here, we describe advanced imaging and image analysis approaches that we are developing to advance our understanding of brPVM properties at two histological scales. At the vascular scale, we are employing Second Harmonic Generation (SHG) microscopy that enables to achieve "label-free" detection of single collagen fibers without prior immuno-labeling. We are performing SHG with simultaneous detection of brPVM to understand the reciprocal relations between them and collagen around arteries and veins. In a different set of analyses, we are assessing at a larger scale whether brPVM distribution pattern is conserved between animals. To carry this out we are implementing advanced multi-dimensional image analysis to compare the localization of single brPVM-positive vessels in different regions of cleared mouse brain samples. Overall, we expect our efforts to provide a novel perspective on how brPVM interact with their environment, how they are organized across the brain and how they contribute to brain physiology.
References:

1. Karam et al., 2022, doi: 10.1177/0271678X221101643

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Unveiling the 3D Anatomy and Development of Meningeal Lymphatic Vessels: Implications for Neuroimmune Communication and Therapeutic Potential

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Résumé

Meningeal lymphatic vessels (MLVs) play a key role in the clearance of the cerebrospinal fluid (CSF), metabolites and immune surveillance within the brain parenchyma. Nevertheless, the presence of MLVs in the ventral regions of both murine and human skulls, as well as their connections with the glymphatic system and the extracranial lymphatic vasculature, remained uncertain.
In this project, we generated a 3D map of MLV drainage by light-sheet fluorescence microscopy (LSFM) imaging of adult mouse whole-head preparations following fluorescent OVA-555 tracer injections into the CSF. In humans, we performed real-time magnetic resonance vessel wall imaging. Altogether, our results revealed a conserved 3D-anatomy of MLVs in both mice and humans and unveiled an expanded anterior network around the dural cavernous sinuses.

The MLVs development is known to occur during postnatal period, specifically along the dural venous sinuses. However, the precise timing of the maturation of MLVs in various CNS regions, coinciding with functional CSF drainage, remains poorly understood.

To better characterize the postnatal acquisition of the CSF drainage capacity, we are using 3D LSFM imaging associated to CSF tracer injection in pups at different developmental timepoints. With these tools, we are creating an integrated imaging of CSF outflow routes, lymphatic vasculature, venous circuits, and immune cells around brain tissue. We are characterizing the kinetics of the MLVs network progression, the spatial maturation of MLVs-associated dural immune cells and the establishment of the neuroimmune communication (NC) during development.
Furthermore, we are investigating the molecular mechanisms underlying the NC establishment in MLVs development. We targeting S1P-S1PR or VEGFC-VEGFR3 signaling to

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impact MVLs outgrowth and drainage. This strategy of NC modulation may be used as a therapeutic tool to improve clinical outcomes in neuroinflammation disorders as brain tumors or CNS lesions.

Imaging of Perivascular Fibroblast Dynamics in Mouse Cerebral Vasculature

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Résumé

It is known that mural cells, as pericytes and vascular SMCs, regulate vascular integrity in the brain. However, the contribution of other cell types to vascular stabilization has been largely unexplored. Recent studies in mice have identified the presence of perivascular fibroblasts (PVFs) surrounding blood vessels in meninges and in central nervous system. Using fibroblast reporter mice (Col1a1-eGFP), we were able to monitor PVF recruitement along cortical vessels during postnatal development. Using 3D imaging after tissue clearing (CUBIC method), we analyze the setting and migration of PVFs along penetrating arteries of the cortex. We show preferential PVFs migration along all SMA-positive arteries' main trunk between P7 and P14 correlated with cortical vascular plexus expansion and then along SMA positive secondary branches. Additionally, the primary trunk of only larger ascending venules is also covered by PVFs. We have discriminated two populations of PVFs: a population with migrating characteristics and a population wrapped around branching vessels, extending processes that coat arterioles at the bifurcation. At the mechanistic level, we report that PVF recruitement depends of astrocyte secreted Wnt factors.

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ICAM1 expression by the microvasculature impairs capillary perfusion which compromises hind limb ischemia recovery in diabetic mice

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Résumé

Introduction: Chronic limb-threatening ischemia (CLTI), one of the diabetic complications, is associated with a poor limb prognosis. Its pathophysiology is poorly understood, and therapeutic targets are missing.
Objective: Our objective is to explore the contribution of endothelial dysfunction in the development of CLTI in diabetic mice.

Method: Hind limb ischemia (HLI) was induced by ligation and resection of the femoral artery in C57BL6/J male mice, in which insulin resistance was induced by a high fat diet, and hyperglycemia by low dose streptozotocin administrations one month later.

Results: According to our previous investigations, ischemic foot re-perfusion, assessed via laser doppler perfusion imaging, was significantly reduced in diabetic animal 28 days after HLI surgery was performed (ratio blood flow in the ischemic leg vs non ischemic leg=0.27±0.09 vs 0.44±0.21 in control mice; P=0.03), even though angiogenesis was identical in both groups. On the contrary, we found that impaired ischemic foot re-perfusion was associated with an increased endothelial cell activation attested by an increased ICAM1 expression (P< 0.0001). We then hypothesized that ICAM1, by interacting with white blood cells (WBC), may compromise the perfusion of capillaries with a diameter smaller than a WBC. Accordingly, we found that WBC circulation velocity in the microvasculature was diminished in diabetic mice (P=0.004) and associated with a decreased percentage of capillaries perfusion by BS-1 lectin (P=0.03).With the aim to test whether ICAM1 overexpression may be responsible for impaired ischemic foot re-perfusion, diabetic mice were administered with anti-ICAM1 antibodies or isotype control for 14 days, starting 14 days after HLI surgery was performed. We found that anti-ICAM1 therapy increased WBC circulation velocity within the microvasculature (P< 0.0001) and the percentage of perfused capillary (P=0.009).
Conclusion: Altogether, our results demonstrate that ICAM1 overexpression may compromise HLI recovery in diabetic mice by decreasing WBC circulation velocity and impairing capillary perfusion.

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Integrated single-cell transcriptomics reveals mitochondrial calcium modification as a hallmark of endothelial-to-mesenchymal transition

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Résumé

Endothelial cells (ECs) are highly plastic cells able to give raise to other cell types and undergo fate change. The transdifferentiation towards a mesenchymal-like phenotype, a process referred as to endothelial-to-mesenchymal transition (EndMT), is critical for embryonic development, and in adults it is one of the major contributors to the onset of diseases, including cancer, organ fibrosis and a number of cardiovascular disorders. Therefore, EndMT targeting holds therapeutic promise for treating some of the most prevalent diseases. However, the field lacks a precise molecular and functional definition of EndMT, and more importantly, targeting the EndMT master-regulatory transcription factors remains challenging.
Here, we performed single-cell RNA-sequencing on three in vitro models of induced EndMT differentiation to identify novel conserved EndMT regulators. Our analysis revealed that mitochondrial calcium signalling controls EndMT. We functionally validate that mitochondrial calcium uptake influences endothelial transdifferentiation,whereas pharmacological blockade of the mitochondrial calcium uniporter (MCU) prevents EndMT. Conditional deletion of *mcu* in ECs confirms loss of mesenchymal activation in a hind limb ischemia mouse model. Consistent with these findings, human tissues from patients with critical limb ischemia showing features of EndMT express significant higher levels of endothelial MCU.
Together, these data identify mitochondrial calcium signaling as a novel regulatory mechanism of endothelial transdifferentation, thus potentially allowing for the development of new therapeutic interventions for EndMT-based diseases.

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Exploring the Role of Autophagy in the Lymph Node Vasculature

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Résumé

Lymph nodes (LNs) sequester antigens to prime immune cells and mount immunological responses. Under homeostasis, lymphatic vessels (LVs) deliver immune cells and antigens from peripheral tissues and are the major route of lymphocyte efflux, meanwhile high endothelial venules (HEVs) extract lymphocytes from the blood stream to be primed by antigens, thus both are essential to lymphocyte priming. Under homeostasis, HEVs express adhesion molecules to attract naïve lymphocytes, however upon inflammation, they upregulate additional adhesion molecules associated with an inflamed endothelium to allow activated lymphocytes through as well. Autophagy is a major degradative pathway implicated in cellular homeostasis and stress responses. Recently, much attention has been placed on targeting it across a variety of diseases, including cancer, however, potential off-target effects on immune responses must be elucidated. Our data reveal an important role of autophagy in the functioning of HEVs within LNs during inflammation and cancer. Using mouse models with endothelial cell deletion of autophagy (ATG12 /ATG7) we observe loss of HEV "plump" morphology, as well as reduced expression of inflamed adhesion markers in the LNs of knock out mice during inflammation. Functionally, this was accompanied by reduced recruitment of lymphocytes to the LN during inflammation. At the transcriptional level, via single cell RNA seq, we see a reduced expression of mature HEV markers. Together these data imply that it may be an essential pathway for maintaining HEV identity.

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3D-imaging reveals spatially restricted architecture of human lymphatic vasculature in close relationship to αSMA positives blood vessels in subcutaneous adipose tissue.

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Résumé

Alterations of the vascular architecture are involved in a broad spectrum of human disorders such as cardiovascular diseases, atherosclerosis, tumor metastasis, obesity and diabetes. Particularly, the dysfunction of the lymphatic vasculature can result in lymphedema, a common disabling disease characterized by the accumulation of adipose tissue and fibrosis in a limb, aggravated by chronic inflammation. However, the functional relationship between lymphatic vasculature and adipose tissue remodeling is still elusive.
Recent advances highlight the importance of an organ-specific lymphatic vasculature that led us to better define the architecture of lymphatic vessels. We used light-sheet microscopy to revisit the spatial organization of lymphatic vasculature in mouse and human sub-cutaneous adipose tissue. In mice, few lymphatic vessels are visualized in the sub-cutaneous adipose tissue while an important network of pre-collecting vessels with numerous lymphatic valves are partly associated to the dermal adipose tissue. Our work reveals that the architecture of the mouse lymphatic vasculature is strongly organized and correlated to the cellular microenvironment from the skin to the dermal adipose layers.
Surprisingly, human lymphatic vasculature showed a spatially restricted organization in subcutaneous adipose tissue. Lymphatic vessels are preferentially located in septa and fascia of adipose tissue, where numerous lymphatic capillaries were visualized. In addition, all human lymphatic vessels expressed LYVE-1 and Podoplanin, markers initially found in mouse to identify lymphatic capillaries and collecting vessels. Moreover, while any αSMA cells positives could be visualized on lymphatic vessels, we remarkably observed a proximity of lymphatic vessels located alongside to αSMA positives blood vessels. Altogether, our work highlight a species-and tissue-specific features of human lymphatic vasculature and bring novel hypotheses to understand the role of lymphatic vessels in adipose tissue.

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The ubiquitin ligase Tripartite Motif 47 is a novel actor essential to brain physiology by promoting the NRF2 antioxidant protective system

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Résumé

Cerebral small vessel disease (cSVD) is a leading cause of strokes and a major contributor to 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 in population cohorts with SVD which identified a missense variant on *TRIM47* locus. The ubiquitin ligase *TRIM47* is highly expressed in brain endothelial cells (EC), indicative of its potential role on BBB integrity.

Our *in vitro* data indicate that TRIM47 displays antioxidant properties in human brain microvascular EC (HBMEC). Bulk RNA-sequencing performed on HBMEC treated with TRIM47 siRNA revealed a downregulation of genes driven by the antioxidant transcription factor Nuclear factor-erythroid factor 2-related factor 2 (NRF2). Mechanistically, we have established that TRIM47 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 performances at behaviour tests (water/Y maze), associated with brain defects (astrocytes activation, increased BBB permeability) and decreased activation of the Nrf2 pathway in brain EC. Interestingly, a one-month 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 actor of brain physiology and provide a proof of concept of the relevance of targeting the protective antioxidant TRIM47/NRF2 axis in patients with cSVD.

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Induced cell cycle arrest prevents arterio-venous malformations in Hereditary Hemorrhagic Telengiectasia

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Résumé

Background: Distinct endothelial cell cycle states (early G1 vs. late G1) provide different "windows of opportunity" to enable the differential expression of genes that regulate venous vs. arterial specification, respectively. Endothelial cell cycle control and arterial-venous identities are disrupted in vascular malformations including arteriovenous (AV) shunts, the hallmark of hereditary hemorrhagic telangiectasia (HHT). To date, the mechanistic link between endothelial cell cycle regulation and the development of AV malformations (AVMs) in HHT is not known.
Methods: We employed BMP9/10 blocking antibodies and endothelial-specific deletion of *Alk1* (*Alk1*ECiKO) to induce HHT in Fucci2 mice (fluorescent cell cycle reporter) to assess endothelial cell cycle states in AVMs. We also assessed the therapeutic potential of inducing endothelial cell cycle G1 arrest in HHT to prevent AVMs by re-purposing the FDA-approved CDK4/6 inhibitor (CDK4/6i), Palbociclib. Finally, we used Single Cell RNA Sequencing analysis of retinal endothelial cells isolated from *Alk1*ECiKO mice treated or not with Palbociclib to decipher the mechanism of action of CDK4/6 inhibition on AVMs.

Results: We found that endothelial cell cycle state and associated gene expression are dysregulated during the pathogenesis of AVMs in HHT. We also showed that Palbociclib treatment prevented AVM development induced by BMP9/10 inhibition and *Alk1* genetic deletion. Mechanistically, endothelial cell late G1 arrest induced by Palbociclib modulates the expression of genes regulating arterio-venous identity, endothelial cell migration, metabolism and, VEGF-A and BMP9 signaling that collectively contribute to the prevention of vascular malformations.
Conclusion: This study provides new insights into molecular mechanisms leading to HHT by defining how endothelial cell cycle is dysregulated in AVMs due to BMP9/10 and Alk1 signaling deficiencies, and how restoration of endothelial cell cycle control may be used to treat AVMs in patients with HHT.

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POSTER SESSION

COverlap: a versatile Fiji toolset for the 3D co-localization of two fluorescent nuclear markers in confocal images. Application to cerebral angiogenesis.

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Résumé

Angiogenesis is a complex process in which endothelial cells migrate and proliferate to form new blood vessels from the preexisting vascular network. It is abundant in tumor neovascularization, tissue repair, and development, and also occurs, to a lesser extent, in healthy adult tissues. However, assessing it in physiological conditions is challenging due to its scarcity. Angiogenesis is triggered by angiocrine factors synthesized by the endothelium in response to shear stress. Since local variations of neuronal activity prompt local changes in blood flow, tasks that generate such activity could potentially induce angiogenesis. To determine whether an increase in neuronal activity following a memory consolidation task could elicit an angiogenic process in a cortical region of interest, we developed a confocal microscopy image analysis method for identifying newly-formed endothelial cells in brain sections. COverlap is a versatile Fiji toolset that allows the automated quantification of endothelial nuclei in 3D and the detection of their co-occurrence with a proliferation marker. Our approach prioritizes the ease-of-use, traceability, and reproducibility of the analysis.

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Modulation of PI 3-kinaseC2 beta and its product PI3P protect against blood-brain barrier leakage in ischemic stroke

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Résumé

Internalization and degradation of VE-cadherin, the major constituent of adherens junctions, lead to vascular permeability, a situation which can be dramatic in pathological situations such as ischemic stroke. This pathological context is characterized by a profound inflammatory response followed by a rupture of the blood-brain barrier with endothelial junction opening, leukocyte infiltration, edema formation and neuronal death. Thus, the possibility to manipulate the levels of VE-cadherin at vascular adherens junctions is of potential therapeutic interest. Class II PI3KC2β is mainly thought to produce phosphatidylinositol 3-phosphate (PI3P), a phosphoinositide known to control intracellular trafficking. In this study, we investigated whether PI3KC2β and its lipid product could influence VE-cadherin trafficking and endothelial permeability in ischemic stroke. Using two mouse models of stroke, the transient middle cerebral artery occlusion and the thromboembolic model of cerebral ischemia, we found that genetic inactivation of PI3KC2β (C2βD1212A/D1212A) conferred a remarkable protection against ischemia-reperfusion injuries in mouse. Indeed, inhibition of PI3KC2β led to a stabilization of the blood-brain barrier resulting in a reduction of infarct volume, edema and inflammation and improved neurological scores. In vitro, the knock-down of PI3KC2β in human cerebral microvascular endothelial cells (hCMEC/D3), enhanced VE-cadherin delivery to the plasma membrane, and in turn protected it from lysosomal degradation leading to a stabilization of endothelial junctions. Interestingly, PI3KC2β inactivation affected the production of a specific pool of PI3P, controlling early endosomal maturation towards lysosomal degradation and endosomal recycling. These data identify the PI3KC2β as a very attractive therapeutic target in ischemic stroke which inhibition would protect against cerebral ischemia-reperfusion injuries.

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CD160 regulates angiogenesis in activated human endothelial cells.

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Résumé

Angiogenesis, the process of forming new blood vessels from pre-existing ones, plays a pivotal role in various physiological and pathological conditions. CD160, a cell surface receptor known for its modulatory function in the immune response, has recently garnered attention as a surface marker for activated endothelial cells. In this study, we investigate the anti-angiogenic effects of a novel anti-CD160 antibody.
Using the tube formation assay, we evaluated the novel anti-CD160 antibody's ability to inhibit neovascularization potential in activated Human umbilical vein endothelial cells (HUVEC) *in vitro*. Our results revealed a significant reduction in total tube length, the number of branches, and closed networks, indicating the antibody's efficacy in impeding neovascularization. In addition, we explored the molecular mechanisms underlying CD160's anti-angiogenic properties, showing its ability to selectively decrease fibroblast growth factor-2-induced angiogenesis.

Furthermore, we assessed the safety profile of our new anti-CD160 antibody by examining its impact on endothelial cell apoptosis. Encouragingly, our findings demonstrated no induction of apoptosis, suggesting the antibody's selective targeting of angiogenic processes without compromising endothelial cell viability, thus supporting CD160's vascular normalization properties.
In conclusion, our research provides compelling evidence for the anti-angiogenic effects of the CD160 antibody, as evidenced by its inhibition of tube formation without inducing apoptosis in activated endothelial cells. These findings hold promising therapeutic implications for

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targeting angiogenesis in various pathological conditions, warranting further exploration for potential clinical applications..

Impact of Wnt/ROR2 signaling on the organization and function of BBB perivascular fibroblasts

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Résumé

The brain has a unique vascular structure: the blood-brain barrier (BBB). Recently, a new cell type has been identified within the perivascular spaces of the BBB: perivascular fibroblasts (PVF), which cover the penetrating arteries. Because of their location, PVF appear to help maintain vascular stability. These cells also produce the extracellular matrix (ECM) essential for vascular homeostasis and stability. Numerous studies have demonstrated the involvement of the Wnt pathway in the establishment and maintenance of the neurovascular unit. Transcriptomic analysis reveals that brain PVFs express all Wnt pathway receptors, including ROR2 receptor. Interestingly, this receptor has also been described as regulating ECM production in tumor cells. We thus hypothesize that perivascular fibroblasts control endothelial cell functions, and that Wnt/ROR2 pathway expressed by FPVs may promote ECM production and thus play a role in BBB stability. Using an in vitro co-culture approach, we demonstrate that PVFs reduce endothelial cells permeability, by significantly increasing the expression of *CLDN5*, encoding Claudin-5, a tight junction protein. We find an activation of the canonical Wnt pathway in PVFs, in vivo and in vitro, associated with a decrease in *ROR2* expression. Using a siRNA-based approach, we show that deletion of *ROR2* expression in PVFs leads to a concomitant decrease in *MMP9* expression. Conversely, overexpression of ROR2 by fibroblasts, after lentiviral transduction, appears to participate in ECM degradation, notably by decreasing collagens expression and increasing the production of MMP9. Finally, endothelial cell permeability appears to be impaired after treatment with conditioned medium of fibroblasts overexpressing ROR2, via a down-regulation of *CLDN5* expression. These results strongly suggest that PVFs play a functional role within the BBB, modulating endothelial permeability. The Wnt/ROR2 pathway appears to be essential in PVFs, notably by controlling both production and degradation of ECM.

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Cardiac pericytes may regulate lipid metabolism in a sex dependent manner.

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Résumé

Free fatty acids (FFA) are the main substrate of energy used by cardiomyocytes. They result in the lipolysis by lipoprotein lipase (LPL) of circulating triglycerides from VLDL or chylomicrons. Importantly, the bioavailability of FFA is critical, as both a lack and an excess of FFA are deleterious for the heart and may cause heart failure. Lipid metabolism is then extremely regulated. Angiopoietin-like 4 (ANGPTL4), a negative regulator of LPL, whose expression is induced by FFA, acts as a feedback mechanism in FFA uptake, thus limiting lipid accumulation in the heart. Our goal is to identify mechanisms that regulate ANGPTL4 expression in the organ.

To do so, we first exposed male and female mice to a high-fat diet (HFD) to induce hyperlipidemia. Surprisingly, we found the HFD regimen increases Angptl4 mRNA expression in the heart of females but not in males, suggesting sex hormones may be involved in the regulation of ANGPTL4 in the heart. To test this, we submitted female mice, ovariectomized (OVX) or not, to the HFD regimen. Interestingly, ovariectomy increased Angptl4 mRNA expression in mice fed with a normal diet, whereas in mice fed with a HFD regimen, Angptl4 mRNA expression was lower in OVX females than in non-OVX mice. This result suggests that female hormones may modulate the cardiac response to hyperlipidemia. To identify the cell type producing ANGPTL4 in the heart, we isolated cardiomyocytes, cardiac endothelial cells, and cardiac pericytes and found Angptl4 mRNA expression was higher in pericytes. Finally, we treated cultured cardiac pericytes with palmitate and found that it strongly enhanced Angptl4 mRNA expression, identifying cardiac pericytes as potential actors in the regulation of lipid metabolism in the heart. Whether and how sex hormones may regulate Angptl4 expression in cardiac pericytes remains to be identified.

Our work identifies cardiac pericytes as new potential regulators of lipid metabolism in the heart through ANGPTL4 expression and that the role of cardiac pericytes may be influenced by sex hormones.

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Impact of non-small cell lung cancer cell secretome on endothelial cell plasticity

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Résumé

Introduction : Over the last decades, dogmas in oncology have evolved from a vision restricted to tumor cells to one that considers the tumor microenvironment (TME). Within this TME, the vascular compartment (including endothelial cells, ECs) will help promoting tumor growth and dissemination through angiogenesis and the endothelial-to-mesenchymal transition (EndMT). The EndMT is widely described in different tumor types including non-small cell lung cancer (NSCLC), and has been described as a possible cause of chemoresistance. Hence, the main aim of our study was to determine the impact of the secretome of NSCLC cell lines on ECs.
Method : Five NSCLC cell lines were chosen according to their diverse mutational and aggressiveness traits (A549, H1755, H23, H1437, H1975). The impact of their secretomes (dubbed conditioned media, CM) was assessed on ECs after 48h and 72h by immunofluorescence, proliferation and viability tests, flow cytometry (endothelial (CD31/vWF) & mesenchymal (a-SMA/CD44 proteins)) and functional tubulogenesis assays. Finally, NSCLC secretomes were investigated by proteomic analysis.

Results : ECs treatment with NSCLC CMs resulted in the acquisition of mesenchymal properties such as the appearance of stress fibers, a higher proportion of ECs expressing mesenchymal-like protein (a-SMA), and a loss of their ability to form mature networks as early as 48h. However, ECs retain certain endothelial traits, and their ability to proliferate, suggesting a partial EndMT. Interestingly, mass spectrometry revealed the presence of EndMT-related proteins found in the CM of cancer cells including FSP1 or SPARC.

Conclusion : We report that CM from NSCLC cell lines can induce a partial EndMT on ECs. Subsequently, the validation of tumor cell-secreted EndMT inducers may open new perspectives in the search for new potential targets in the treatment of NSCLC.

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Different cardiovascular and pulmonary phenotypes for single- and double-knock-out mice deficient in BMP9 and BMP10

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Résumé

BMP9 and BMP10 mutations were recently identified in patients with pulmonary arterial hypertension, but their specific roles in the pathogenesis of the disease are still unclear. We aimed to study the roles of BMP9 and BMP10 in cardiovascular homeostasis and pulmonary hypertension using transgenic mouse models deficient in Bmp9 and/or Bmp10.
Single- and double-knockout mice for Bmp9 (constitutive) and/or Bmp10 (tamoxifen inducible) were generated. Single-knock-out (KO) mice developed no obvious age-dependent phenotype when compared with their wild-type littermates. However, combined deficiency in Bmp9 and Bmp10 led to vascular defects resulting in a decrease in peripheral vascular resistance and blood pressure and the progressive development of high-output heart failure and pulmonary hemosiderosis. RNAseq analysis of the lungs of the double-KO mice revealed differential expression of genes involved in inflammation and vascular homeostasis. We next challenged these mice to chronic hypoxia. After 3 weeks of hypoxic exposure, Bmp10-cKO mice showed an enlarged heart. However, although genetic deletion of Bmp9 in the single- and double-KO mice attenuated the muscularization of pulmonary arterioles induced by chronic hypoxia, we observed no differences in Bmp10-cKO mice. Consistent with these results, endothelin-1 levels were significantly reduced in Bmp9 deficient mice but not Bmp10-cKO mice. Furthermore, the effects of BMP9 on vasoconstriction were inhibited by bosentan, an endothelin receptor antagonist, in a chick chorioallantoic membrane assay.
Our data show redundant roles for BMP9 and BMP10 in cardiovascular homeostasis under normoxic conditions (only combined deletion of both Bmp9 and Bmp10 was associated with severe defects) but highlight specific roles under chronic hypoxic conditions. We obtained evidence that BMP9 contributes to chronic hypoxia-induced pulmonary vascular remodelling, whereas BMP10 plays a role in hypoxia-induced cardiac remodelling in mice.

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Development of a method for the detection of the JAK2V617F mutation in human circulating endothelial cells

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Résumé

Background:
Circulating endothelial cells (CECs) are mature endothelial cells (ECs) that detach from the vascular wall. Increased CEC concentration is known to be associated with the presence of vascular injury. Myeloproliferative neoplasms (MPNs) are hematological malignancies, that most often result from the acquisition of the *JAK2*V617F mutation in hematopoietic stem cells (HSC). Some teams have reported the presence of the *JAK2*V617F mutation in ECs in some MPN patients. We hypothesize that CECs can be used as a marker to identify the presence of *JAK2*V617F positive endothelial cells (ECs).

Aim:

Our objective was to develop a fluorescence activated cell sorting (FACS) protocol to look for *JAK2*V617F positive CECs using droplet digital polymerase chain reaction (ddPCR).

Methods:

Mononuclear cells (MNCs) were obtained by Ficoll gradient and labelled with a panel of 3 antibodies (CD45-KO, CD34-APC, CD146-PE) associated with DAPI (cell viability marker). CECs were identified as CD45- CD34+ CD146+ cells and sorted using a FACS Aria cytometer. Immunofluorescence experiments were performed to confirm the endothelial nature of the sorted CECs. DNA from sorted CECs was pre-amplified using the Prelude PreAmp kit (Takara©) and *JAK2*V167F mutation screening was performed using ddPCR.

Results:

We confirmed that sorted CECs were ECs by immunofluorescence analysis as they were CD31+ vWF+ CD45-. CEC sorting was performed in 9 *JAK2*V617F+ patients with a history of thrombosis: portal thrombosis in 4, Budd-Chiari syndrome in 2, typical arterial or venous thrombosis in 3. The WT allele of *JAK2* was efficiently detected by ddPCR but the

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*JAK2*V617F mutation was not detected in any of the patients' CECs.

Conclusion:

We here present a reliable method to sort CECs by FACS and search for *JAK2*V617F mutation by ddPCR. The analysis of 13 *JAK2*V617F+ MPN patients did not reveal the presence of *JAK2*V617F+ CECs. Results with higher numbers of patients will be presented at the meeting.

A Role for the Linear Ubiquitin Chain Assembly Complex in the Brain Endothelial Barrier

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Résumé

LUBAC (linear ubiquitin chain assembly complex) is a multiprotein E3 ligase complex composed by 3 subunits (HOIL-1L, HOIP, and SHARPIN) with the unique ability to add linear ubiquitin chains to target proteins. Over the years, LUBAC has been shown to be important for inflammation, cell death and immune signalling pathways, especially through the NF-kB activation. Recently, few studies have shown that the LUBAC is essential for embryonic development and angiogenesis. Endothelial cells (ECs) play a critical role in maintaining the barrier function of the vasculature lining the lumen of all blood vessels. The formation of adhesive structures between adjacent cells is essential for the maintenance of the tissue integrity. The principal cell-cell adhesion molecule is vascular endothelial cadherin (VE-Cadherin). The cytoplasmic tail associates with p120, b-catenin and actin cytoskeleton, providing stability and strength to the junctions. VE-Cadherin has been implicated in many aspects of blood vessel formation and is dynamically regulated alongside angiogenesis. In this study, we investigated the role of LUBAC in the integrity of brain endothelial barrier by engineering human cerebral microvascular endothelial cells (hCMEC/D3) deficient for HOIP, the catalytic subunit of LUBAC. So far, our results showed that HOIP knockdown reduced VE-Cadherin protein levels, compromising the cell-cell adhesion structures and attenuating endothelial integrity. Moreover, HOIP-silenced ECs did not form properly tubes and showed reduction in collective cell migration. This phenotype was further aggravated in the presence of tumor secretome from brain tumor cells (*ie* patient-derived glioblastoma stem-like cells, GSCs). Together, our results suggested that HOIP might regulate VE-Cadherin levels, which ultimately might govern brain endothelial barrier integrity and angiogenesis. Keywords: HOIP, VE-Cadherin, endothelial integrity, permeability, angiogenesis

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BMP9 is a key player of capillary identity preventing the development of arteriovenous malformations

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Résumé

BMP9 is a high affinity ligand of ALK1 and endoglin receptors that are mutated in the rare genetic vascular disorder Hereditary Hemorrhagic Telangiectasia (HHT). We have previously shown that loss of *Bmp9* in the 129/Ola genetic background leads to spontaneous liver fibrosis via capillarization of liver sinusoidal endothelial cells (LSEC) and kidney lesions. To further address the role of BMP9 in liver homeostasis, we performed a RNAseq analysis on LSEC from adult WT and *Bmp9*-KO mice and identified over 2000 differentially expressed genes. Gene ontology analysis showed that *Bmp9* deletion led to a decrease in BMP and Notch signaling, but also LSEC capillary identity while increasing their cell cycle. The gene ontology term "glomerulus development" was also negatively enriched in *Bmp9*-KO mice versus WT supporting a role for BMP9 in kidney vascularization. Indeed, we found that loss of *Bmp9* led to vascular enlargement of the glomeruli capillaries associated with alteration of podocytes. Interestingly, the loss of *Bmp9* led to spontaneous arteriovenous malformations (AVMs) in the liver, gastro-intestinal tract and uterus. Altogether, these results demonstrate that BMP9 plays an important role in vascular quiescence of many organs by regulating endothelial capillary differentiation markers. It also reveals that loss of *Bmp9* is sufficient to induce spontaneous AVMs, supporting a key role for BMP9 in the pathogenesis of HHT.

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Elucidating the interaction between SarsCov-19 and the vasculature using the vesseloid system

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Résumé

Conflicting results exist about the mechanisms by which SARS-CoV-2 virus acts on the vasculature. The presence of the virus has been reported in endothelial cells in patient's samples. However, the ACE2 receptor seems not be presented in endothelial cells when analysed by RNA analysis. Furthermore, in vitro models that recapitulate the participation of the vasculature during SARS-CoV-2 infection *in vivo* do not exist.Thus, it is important to develop more suitable *in vitro* models that allow to understand how SARS-CoV-2 infection impacts the vascular system. We, therefore, investigated the interaction between SARS-CoV-2 and the vasculature by using our previously developed 3D vesseloid model (Andrique *et al*; 2019) and SARS-CoV-2 virus. We firstly compared gene expression between our vesseloid 3D and a 2D culture model by RNA sequencing. Endothelium-specific genes are significantly more expressed in the 3D model when compared to 2D. We then assessed whether SARS-CoV-2 infects directly endothelial cells in the vesseloid model. In absence of ACE2 in the endothelial cells, no infection could be documented. When ACE2 is overexpressed in endothelial cells, a small uptake of viral particles in the endothelial cells is observed with no efficient viral production. We then looked for an indirect effect by using co-cultures between epithelial cells and vesseloid. After infection of epithelial cells, a significant inflammatory reaction can be detected in endothelial cells. We, furthermore, demonstrated that several cytokines are implicated in the crosstalk between epithelial cells and endothelial cells in our vesseloid system, the major one being CXCL10. The functional importance of this regulation is under investigation. We then investigated the clinical relevance of our finding by using blood samples of SARS-CoV-2 infected patients from Bordeaux (COLCOV collection). We could demonstrate that the patient's cytokine profiles matched our *in vitro* finding, and thus supported the validity of our analysis.

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SARS-Cov-2 impact on vascular endothelium

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Résumé

The vasculature is heavily impacted by Sars-cov-2 infection. Conflicting results exist about the mechanisms by which sars-cov-2 virus acts on the vasculature. The presence of the virus has been reported in endothelial cells in patients' samples. However, the ACE2 receptor is not detected in endothelial cells when analysed by RNA analysis. Furthermore, in vitro models that recapitulate the in vivo role of the vasculature upon SARS-Cov2 infection do not exist. Thus, it is important to develop more suitable in vitro models.
We, therefore, investigated the interaction between Sars-Cov-2 and the vasculature by using our previously developed 3D vesseloid model. We firstly compared gene expression by RNA sequencing between a standard 2D culture model and the 3D vesseloid. Endothelium-specific genes are significantly more expressed in the 3D model when compared to 2D. We then assessed whether Sars-Cov-2 directly infects endothelial cells in the vesseloid model. In the absence of ACE2 in endothelial cells, no infection could be documented. When ACE2 is overexpressed in endothelial cells, a low uptake of viral particles in endothelial cells is observed without efficient viral production. We then investigated the indirect effect of Sars-Cov2 infection by maintaining in culture vesseloids with epithelial cells. After infection of epithelial cells, a significant inflammatory response was detected in endothelial cells. Furthermore, we demonstrated that several cytokines are implicated in the crosstalk between epithelial cells and endothelial cells within the vesseloids, the major cytokine is CXCL10, whose regulatory function is currently investigated in depth. Finally, we investigated the clinical relevance of our findings by using blood samples from Sars-CoV-2 infected patients from Bordeaux (Bordeaux, COLCOV collection). We could demonstrate that the patients' cytokines profiles match the in vitro finding, and thus support the validity of our analysis.

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Diastolic dysfunction is associated with cardiac small vessel disease in ovariectomized females but not in males.

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Résumé

Introduction: Coronary microvascular disease has been proposed to be responsible for heart failure with preserved ejection fraction (HFpEF) about 10 years ago. However, to date the role and phenotype of the coronary microvasculature has still been poorly considered and investigated in animal models of HFpEF.
Objective: To characterize the phenotype of the coronary microvasculature in male and female mice with diastolic dysfunction.

Method: We assessed cardiac function and characterized the coronary microvasculature in two mouse models of HFpEF: mice fed with a high fat diet (HFD) + L-NAME regimen and Leptin receptor deficient (*Leprdb/db)* mice. Notably, our study was done in males, females and ovariectomized (OVX) female mice in order to search for possible sexual dimorphisms.

Results: Upon a HFD + L-NAME regimen, both males and OVX females but not non OVX females develop diastolic dysfunction attested by an increased end diastolic pressure. In Leprdb/db mice, both Male and non OVX female mice develop diastolic dysfunction. Notably female mice have reduced estradiol and progesterone level mice mimicking ovariectomy.

We found that both OVX and non OVX females but not males display increased endothelial activation attested by increased ICAM1 expression, endothelium leakage attested by increased Fibrinogen and IgG extravasation and decreased arteriole diameter suggesting vasoconstriction. The same results were found in *Leprdb/db* mice.
Conclusion: Diastolic dysfunction is not always associated with cardiac small vessel disease since *Leprdb/db* males and C57BL/6J males fed with a high fat diet (HFD) + L-NAME regimen develop diastolic dysfunction in the absence of endothelial dysfunction. Also endothelial dysfunction may not be sufficient to induce diastolic dysfunction since non OVX female mice fed with a high fat diet (HFD) + L-NAME regimen display endothelial dysfunction while they do not develop diastolic dysfunction.

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Identification of Mutated in colorectal cancer (MCC) protein as a novel partner of PDZRN3 during Blood brain barrier development

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Résumé

Wnt signaling is crucial in blood-brain barrier (BBB) development and stability. Any disruption in this pathway can lead to a breakdown of the BBB, which is critical in neurological disorders pathophysiology. Previously, we showed that an excessive activation of the ubiquitin ligase PDZRN3, a key component of Wnt signaling, can destabilize the BBB. However, the mechanism by which PDZRN3 operates in this context remains unresolved.
We employed a differential screening technique (BioID) to identify potential interacting partners of PDZRN3.Mutated in Colorectal Cancer (MCC) was then identified as a potential candidate. Through mass spectrometry and biochemical assays, we have demonstrated that in endothelial cells (EC), MCC undergoes phosphorylation by the kinase CKIϵ, which is a critical regulator of the Wnt pathway. Importantly, PDZRN3 inhibits this phosphorylation. Subsequently, we have linked this phosphorylation state of MCC to the stabilization of the BBB. In mice, MCC undergoes a transition from an unphosphorylated to a hyperphosphorylated state during BBB maturation. Interestingly, increased expression of *Pdzrn3* in brain EC disrupted this MCC phosphorylation transition. This was associated with a decrease of endothelial junctions proteins level.

We further explore MCC's role in EC functions. Knocking down *MCC* impaired the directed migration and polarization of EC under flow conditions *in vitro*. We also characterized MCC as a regulator of centrosome proteins, which are crucial for EC migration. To validate the function of MCC, we used mice with specific knockout of *Mcc* in EC. This deletion was enough to delay retinal vascularization, increase vessel permeability, and alter the organization of the vascular bed in the cortical brain during BBB postnatal maturation.
To conclude, this study positions MCC as a novel central player in the dynamic maturation of blood vessels. Indeed, PDZRN3 prevents MCC CKIe-induced phosphorylation, redirecting MCC to the centrosome, facilitating EC polarization and migration.

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Les hématopoïèses clonales de potentiel indéterminé sont associées avec une augmentation du taux des marqueurs plasmatiques de NET

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Résumé

Les hématopoïèses clonales de potentiel indéterminé (CHIP) résultent de l'acquisition de mutations somatiques dans les cellules souches hématopoïétiques sans signe d'hémopathie maligne. Les CHIP sont principalement associées à des complications cardiovasculaires, notamment l'athérothrombose et l'insuffisance cardiaque. Cependant, la physiopathologie de ces complications reste à déterminer. La plupart des études se sont concentrées sur le rôle des monocytes/macrophages mutés qui présentent des propriétés pro-inflammatoires. Toutefois, le rôle des autres types cellulaires porteurs de la mutation reste inconnu, notamment celui des polynucléaires neutrophiles qui représentent pourtant la majorité des leucocytes mutés. Parmi les phénomènes par lesquels les neutrophiles pourraient être impliqués dans les complications cardiovasculaires associées aux CHIP, les *Neutrophils Extracellular Traps* (NETs) représentent un bon candidat, car ils ont été associés à la thrombo-inflammation ainsi qu'à l'athérosclérose. Dans cette étude, nous avons étudié 81 patients ayant présenté un premier infarctus du myocarde après 75 ans. Nous avons déterminé que les patients porteurs d'une CHIP présentent des niveaux plus élevés de biomarqueurs plasmatiques de NETs que les patients sans CHIP. Cependant, ceci n'était pas associé à une inflammation plus importante, ni à une charge athéromateuse accrue. Si ces données doivent être confirmées dans une cohorte de plus grande taille, notre étude est la première à montrer que les NETs pourraient représenter un nouvel acteur dans la physiopathologie des pathologies cardiovasculaires associées aux CHIP.

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Abstract de vulgarisation pour la Session Open Science - Rôle des cellules endothéliales de la rate dans la thrombose des patients atteints d'une Néoplasie MyéloProliférative (NMP)

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Résumé

Les néoplasies myéloprolifératives (NMP) sont des maladies acquises au cours de la vie caractérisées par une production anormalement élevée de cellules sanguines. La complication la plus fréquente de ces maladies est la formation de caillots dans les vaisseaux sanguins (thrombose) qui est la principale cause de décès.
Actuellement, les mécanismes responsables de la thrombose dans ces maladies ne sont pas encore clairement élucidés. Cependant, nous savons déjà que les cellules constituant la paroi des vaisseaux sanguins (cellules endothéliales) jouent un rôle dans la survenue de ces thromboses. En effet, la mutation JAK2 V617F est fréquemment retrouvée dans les cellules du sang chez les patients porteurs de ces maladies. Or, nous avons démontré au cours des dernières années que chez la souris, les cellules endothéliales porteuses de la mutation JAK2 V617F favorisent les thromboses.

Par ailleurs, les thromboses chez les patients atteints de NMP interviennent dans des territoires particuliers tels que les vaisseaux de la rate, ce qui est très rare dans la population générale. Ainsi nous pensons que les cellules endothéliales de cet organe jouent un rôle particulier dans ces thromboses.

Mon travail de thèse vise donc à étudier le rôle des cellules endothéliales de la rate dans la survenue des thromboses chez les patients atteints de NMP et présentant la mutation JAK2 V617F.
Pour cela, nous allons étudier quels sont les gènes exprimés de façon différente par les cellules endothéliales des échantillons de rate provenant de patients ayant eu une thrombose par rapport à des patients sans thrombose, via une technologie appelée transcriptomique spatiale. Cette technologie permet également de voir où sont situées les cellules dans l'espace, ce qui permettra d'étudier la communication entre les cellules de cet organe, et en particulier d'étudier les interactions existant entre les cellules des vaisseaux sanguins et les autres cellules de la rate.

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Development of a 3D structured hydrogel membrane for cellular therapy of the outer blood-retina barrier

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Résumé

The outer blood-retina barrier (oBRB), composed of a retinal pigment epithelium (RPE) above the vascularized choroid, is disrupted in many retinal dystrophies. The main current strategy to repair the oBRB consists in implanting a RPE monolayer, but without including the choroid, which will alter the graft integration and survival. Here, we aim to develop a structured membrane for cellular therapy, mimicking the entire oBRB and co-cultured with RPE and endothelial cells (EC) derived from human induced pluripotent stem cells (hiPSC).
Membrane design. A 200 μm thick pullulan-dextran hydrogel was synthesized and freeze-dried to tailor its porosity. After optimization, we obtained on one side a porous side connected to the inner porosity for the pre-vascular network development and, on the other side, a smooth non-porous surface intended for the RPE. To enhance cellular adhesion, the membrane was coated with collagen I in the pores and on the surfaces.

Mono-culture seeding. Mono-culture experiments were first conducted to optimize the seeding protocols. hiPSC-derived ECs were seeded on the porous side. After a week, results showed that the EC entered the inner structure, where they adhered to the side of the pores and proliferated, to form a pre-vascular network. hiPSC-derived RPE cells were cultured on the smooth side for a month. The cells adhered on the smooth side without entering the porous network and proliferated to form a monolayer within 10 days, with the expression of typical RPE markers increasing over time.
Co-culture seeding. Once the monoculture protocols optimized, co-culture experiments were conducted. The RPE cells were seeded on the smooth side, and after 10 days, once the RPE formed, the ECs were added on the rough side. The collagen functionalization was still efficient after 10 days allowing ECs to adhere to the pores. For up to a week, each cell type stayed on their attributed side and expressed typical markers. A full characterization of the cellular interactions is on-going to analyse the benefits of the co-culture.

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Role of intra-nervous vascularization in oxaliplatin induced peripheral neuropathy

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Résumé

Chemically induced peripheral neuropathies (CIPN) are common dose-dependant adverse effects of anti-cancer drugs, including oxaliplatin. Patients experience paresthesia and allodynia especially in hands and feet and have more severe symptoms when the cumulative dose becomes important. To date, there is a lack of specific treatment against CIPN mostly because its etiology remains poorly understood. Patients are left with untreated neuropathic pain, which often lead to chemotherapy reduction or cessation. Because oxaliplatin is injected intravenously, we hypothesized that changes in intra-nervous vasculature could be involved in CIPN development. Indeed, changes in the properties of intra-nervous blood vessels could alter nerve homeostasis and participate in the development of neuropathic symptoms. Thus, we injected oxaliplatin intravenously 3 time in mice and performed behavioral tests. Our "acute" model recapitulate CIPN symptoms but did not exhibit nervous nor vascular defect. A transcriptomic analysis on isolated blood vessels from sciatic nerves from sham and oxaliplatin-treated mice shows an upregulation of genes involved in vasoconstriction in oxaliplatin-treated group. This data leads to the intiguiging possibility that CIPN etiology could be initiated by an increased intra-nervous vasoconstriction, limiting nerve proper perfusion with a disturbed nerve homeostasis. We then administered two different vasodilator molecules prior and together with oxaliplatin. Our results show a significant reduction of CIPN symptoms with both vasodilators treatments (n=12), confirming that the blood vessels, through their contractile function, are indeed involved in the onset of CIPN. Along this line, our data suggest that animals with CIPN have a higher level of hypoxia than untreated animals or animals receiving oxaliplatin and at least one vasodilator molecule. These findings represent an important therapeutic hope for patients suffering from neuropathic pain following chemotherapy treatment.

*Intervenant

A pro-tumor role of IL34/CSF1R axis in the immune-vascular crosstalk in Renal Cell Carcinoma

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Résumé

Therapy of metastatic Renal Cell Carcinoma (RCC) mainly targets the angiogenic and immunosuppressive tumor microenvironment (TME), but drug resistance is almost inevitable. Lack of validated biomarkers and scarce knowledge in mechanisms of RCC progression are main reasons of therapy failure. In our previous work, we identified Interleukin-34 (IL34) as potential biomarker of RCC progression. Here, using a syngeneic mouse model of RCC, we show that IL34-enriched TME displays immunosuppression and non-functional vasculature, two features of therapy resistance. This was associated with an accumulation of tumor-associated macrophages (TAM) in IL34-overexpressing primary tumors or lung metastasis through CSF1R signaling. Finally, we show that blockade of CSF1R by using Pexidartinib in combination with Sunitinib, an anti-angiogenic drug used in RCC therapy, was more efficient in reducing metastatic growth compared to monotherapy. Altogether, our data highlight the involvement of the IL34/CSF1R axis in regulating the tumor immune-vascular crosstalk in RCC, and the association Pexidartinib plus Sunitinib as therapeutic alternative for RCC.

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Role of Orai1 in post-ischemic angiogenesis

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Résumé

Background: Post-ischemic neovascularization is required for tissue repair and heart recovery after myocardial infarction (MI). This process is controlled by many factors, especially vascular endothelial growth factor (VEGF), which triggers signaling pathways involving the increase in the intracellular Ca2+ concentration ((Ca2+)i). Recently, we demonstrated that Orai1-dependent store-operated calcium entry (SOCE) plays a key role in angiogenesis. However, the function of SOCE in post-ischemic angiogenesis remains unknown.
Methods: Cytokines levels wereexamined using ELISA in serum obtained from healthy volunteers and patients with ST-segment elevation MI (STEMI) who underwent primary percutaneous coronary intervention (pPCI). Human Umbilical Vein Endothelial Cells (HUVEC) was treated with patients' serum to assess *in vitro* angiogenesis using tube formation and wound healing assays, and Organ-on-a-chip 3D cell culture. Changes in (Ca2+)i were measured using FURA-2 AM in HUVEC.

Results: Our findings showed that STEMI patients have higher serum levels of VEGF-A and IL-17A than healthy controls. The incubation of HUVEC seeded on Matrigel® with STEMI patients' serum boosted vessels-like formation, as assessed by the increase in the number of meshes and junctions. Likewise, wound healing assay demonstrated that ischemic serum promoted faster HUVEC migration. Interestingly, using Organ-on-a-chip 3D culture of HUVEC we demonstrated that ischemic serum stimulated *tip* EC migration that was attenuated by Orai1 silencing. Furthermore, long-term incubation of HUVEC with ischemic serum stimulated an exacerbated SOCE induced by thapsigargin, which correlated with Orai1 overexpression. Finally, we found that knockdown of Orai1 inhibited significantly *Notch1*, *Hes1* and *Hey1* expression induced by STEMI patients' serum.
Conclusion: Our data show for the first time that serum from STEMI patients promotes angiogenesis, involving Orai1 upregulation and the activation of Notch1 signaling pathways.

*Intervenant

Impaired balance between formation of neutrophil extracellular traps and their degradation by DNases in COVID-19 patients

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Résumé

Background

During severe SARS-CoV2 infection, coagulation and inflammation occur concurrently, leading to increased thromboinflammation, microthrombosis, and multi-organ dysfunction. Damaged endothelial cells, activated platelets, neutrophils and monocytes are observed during disease exacerbation, particularly Neutrophil Extracellular Traps (NETs). The mechanisms explaining the increase of NETs markers are unknown. We hypothesized that decreased DNase activity could be associated with increased NETosis and clinical worsening in COVID-19 patients.

Objectives

Our objectives were to compare the balance between NET biomarkers and the amount of functional DNase, based on disease severity, and to study the mechanisms responsible for a possible imbalance.

Methods

Biological samples were collected from the COLCOV19-BX study for inpatients (15 with severe disease and 37 with critical disease at the time of sampling) and the COVERAGE trial (control arm) for 93 outpatients with non-severe disease.

Results

The functional DNase level was lower in the most severe patients, coinciding with an impaired balance between NET markers and DNase functional activity. While DNase1 antigen levels increased in outpatients, it decreased in the most severe ones. DNase1L3 antigen levels

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remains similar in all subgroups and did not increase with NET markers. DNASE1 polymorphisms were associated with decreased DNase1 antigen levels while a quantitative defect of plasmacytoid dendritic cells (pDC), the main cells expressing DNase1L3, was observed in critical patients. Besides, analysis of public single cell RNAseq data revealed that pDC from COVID-19 patients express less DNase1L3.

Conclusion
Severe and critical COVID-19 were associated with an imbalance between NETs and both the functional activity and the amount of DNases. Early identification of patients with NETosis imbalance could allow targeted therapies to prevent clinical worsening, such as DNase administration.

A single point mutation Y685F within the VE-cadherin cytoplasmic domain affects gene expression in lung: evidence for FOXF1 mediated S1PR1 upregulation to stabilize vessels in mice

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Résumé

Abnormal features of tumor blood vessels and irregular interendothelial junctions are not well understood. In highly vascularized human glioblastoma, VE-cadherin is a target for Src-mediated Y685 phosphorylation. We have previously published a tyrosine–> phenylalanine (Y–> F) point mutation (Y685F) within the cytoplasmic domain of VE-cadherin in a mouse. Given that VE-cadherin is exclusively expressed in endothelial cells (ECs), it was reasonable to hypothesize that a single mutation in the protein would affect the anchoring of its binding partners including b-catenin and thus modulate the endothelial gene expression. Here we took advantage of this animal model to demonstrate that the ECs from lung of these mice have angiogenic abnormalities as well as a global profiling of transcripts modified. RNA-seq analyses revealed changes in the expression of 884 genes of which 766 genes were downregulated and 118 genes were upregulated. Focusing on angiogenic genes, we found an upregulation of S1PR1 gene in mutated ECs. Mechanistically, chromatin immunoprecipitation assay (ChIP) demonstrated that in KI ECs, FOXF1 directly binds to the S1PR1 promoter 7-fold stronger compared to WT. Consequently, the increase in S1PR1 mRNA and protein was greater in KI compared to WT as well as the localization of VE-cadherin at the membrane. Of importance, we found that VE-cadherin mutant was still phosphorylated on Y731 and is associated with active Src but to a lesser extent with b-catenin which translocates to the nucleus. The lung morphometric analysis showed less vessels and vascular remodeling with less fibrosis in mutated mice. Thus in vitro and in vivo studies demonstrate that the VE-cadherin mutant activates S1PR1 through FOXF1 to stabilize adult lung vessels. Hence, our findings provide new insights in the role of a single tyrosine of VE-cadherin which would participate in an endothelial-specific transcription program important for tumor vessels and therapeutic targets.

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New molecular underpinnings of blood-brain barrier dysfunction in Multiple Sclerosis

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Résumé

Introduction: Blood-brain barrier (BBB) breakdown is amongst the earliest pathological hallmarks observed in multiple sclerosis (MS). The mechanisms leading to BBB dysfunction are incompletely understood and are generally thought to be a consequence of the autoimmune neuroinflammatory process in MS. **Objective:** We challenge this view and ask if intrinsic alterations in BBB endothelial cells manifested at the genetic or epigenetic, transcriptional, and ultimately phenotypic level cause or contribute to altered BBB function. **Methods:** We made use of human induced pluripotent stem cells (hiPSCs) derived from 3 healthy controls (HC) and 4 MS patients and differentiated them into brain microvascular endothelial cell (BMEC)-like cells as in vitro model of the BBB employing our recently developed extended endothelial cell culture (EECM) method. We compared the transcriptional profile of HC and MS-derived EECM-BMEC-like cells stimulated or not with TNF-α and IFN-γ. **Results:** The RNA sequencing analysis showed differentially expressed genes between EECM-BMEC-like cells from HC and MS patients. These findings underscore that intrinsic alterations of the BBB may directly contribute to MS pathogenesis. Specifically, we found a strong modulation of the Semaphorin-4D (SEMA4D) signalling pathway in MS-derived EECM-BMEC-like cells when compared to their HC counterparts. Protein expression of SEMA4D and its downstream effectors, RHOB and ROCK2 in EECM-BMEC-like cells were confirmed by Western Blot and immunostaining. As in MS serum levels of SEMA4D are enhanced we exposed EECM-BMEC-like cells to soluble SEMA4D which resulted in modulation of SEMA4D expression. **Conclusion:** Our study suggests that SEMA4D and its downstream effectors may contribute to BBB dysfunction in MS.

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Mitochondrial derived vesicles and endosomes work synergically to get a regulated angiogenesis

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Résumé

Because of the mostly glycolytic nature of endothelial cell metabolism, the role of mitochondria and mitochondrial shape in angiogenesis, the new blood vessel formation from existing vasculature, has not been extensively studied. So far we shown that mitochondrial dynamic plays a crucial role in endothelial cells activation. Now, we have discovered that alteration of mitochondrial fragmentation and mitochondrial derived vesicles biogenesis and trafficking induce a dysregulated angiogenesis in the mouse retinal and zebrafish angiogenesis model. This dysregulated angiogenesis is caused by an hyperactivation of VEGFR2 and ERK signalling pathways. Mechanistically, we highlighted a new direct communication between mitochondria and early endosomes. Ablation of this communication curtails the maturation of early endosomes towards late endosomes and therefore induce uncontrolled signalling pathways.

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Putative role of miR-126-3p in protecting from vascular damage induced by ischemia reperfusion injury

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Résumé

In kidney transplantation, donor criteria selection has evolved to face organ shortage and includes the use of marginal donors. However, these organs are more sensitive to ischemia reperfusion injury (IRI) which is associated with early and long-term kidney graft dysfunction. Despite the progress in pre-transplant conditioning, IRI is unavoidable and is associated with vascular damage. Evidence suggests that endothelial cell damage and dysfunction are central in the role of renal IRI and preserving endothelial function may contribute to the reduction of renal damage occurring during IRI. This study aims to develop new strategies to reduce vascular dysfunction induced by IRI.
Accumulating evidence suggests that miRNAs are key regulators of biological processes through repression or degradation of their target. MiR-126-3p is highly enriched in endothelial cells and participates in the regulation of angiogenesis and vascular integrity. In previous studies, down-regulation of miR-126-3p expression was observed at reperfusion in an ex vivo model of IRI in human kidneys and human fibrotic kidneys. MiR-126-3p was down-regulated in human endothelial cells in vitro undergoing endothelial-to-mesenchymal transition and overexpression of miR-126-3p was able to partially prevent this transition. Recently, in an in vitro model of hypoxia-reoxygenation, expression of miR-126 was down-regulated in endothelial cells during reoxygenation alongside increased endothelial cell activation.
We conclude that reduced miR-126-3p expression may act as a marker of endothelial damage. Ongoing studies are investigating the protective effect of miR-126-3p on the endothelium post-IRI.

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LYVE-1+PVMs state modulation in Alzheimer's disease and brain edema.

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Résumé

While the brain lacks a lymphatic vasculature, a macrophage population, exhibiting a major lymphatic marker (LYVE-1) and found in the **perivascular space**, has been proposed to be a regulator of the cerebro-spinal fluid (CSF) transport and drainage. These LYVE-1+Perivascular macrophages (PVM) emerge as the main movers and sweepers at the interface between the neuropil and the vasculature. In our study, we have provided new information on LYVE-1+PVMs identity, brain-wide localization and implication in brain-drainage defect pathologies such as Alzheimer's disease (AD) and brain edema. We were able to show that LYVE-1+PVMs exhibit a spatio-temporal dynamic within five important brain regions and during postnatal development. We categorized PVMs into three sub-populations on the basis of CD206 and LYVE-1 expression. We also identified, for the first time, a LYVE-1+PVMs' arterio-venous zonation with respect to their coverage pattern and LYVE-1 expression. We furthermore highlighted two opposite PVMs state modulations in neurodegenerative disorders (such as AD) and in brain edema disorders (such as Megalencephalic Leukoencephalopathy with cysts – MLC). PVMs seem to be modulating their molecular expression following environmental cues. Therefore, LYVE-1+PVMs downregulated their LYVE-1 receptor in areas burdened with amyloid beta deposits. However, an opposite dynamic was observed in edematous brains exhibiting defects in their astrocytic end-feet coverage and CSF drainage, where a LYVE-1+PVMs accumulation was noted. Understanding changes in PVMs state, molecular composition and functions is the challenge of the forthcoming years, especially for exploring innovative therapies in brain-related diseases.

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Impact of direct interactions of non-small cell lung cancer cell on healthy endothelial cell transcriptome

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Résumé

Introduction: Lung cancers account for over 1,7 million deaths worldwide every year, with non-small cell lung cancer (NSCLC) representing the major subtype. Today, immunotherapy (IT) is the mainstay for NSCLC and is based on the understanding of the tumor microenvironment (TME). Notably, the vascular compartment contributes significantly to the TME promoting tumor growth through angiogenesis, and modulating the immune system access to the tumor site. However, the immunomodulatory ability of endothelial cells (ECs) remains largely understudied.
Method: Three NSCLC cell lines were selected according to their diverse mutational and aggressiveness traits and cocultured directly or indirectly with ECs (HUVEC) during 48h. Thereafter, ECs were isolated and studied by 3'RNA-seq to compare the impact of NSCLC tumor cells coculture. Key targets and signature related to the immune function were further validated, and our data compared to normal/tumor ECs, previously isolated from 13 NSCLC patients.

Results: ECs coculture revealed massive transcriptional remodeling, among which we identified a common signature to all NSCLC cell lines, while a cell line showed its own specificity. Interestingly, gene set enrichment analysis showed altered expression of genes involved in immunoregulation. Key adhesion molecules were up-regulated (e.g. ICAM-1, VCAM-1), thus enabling extravasation of circulating leukocytes into tissues (validated by qPCR and functional assays). Finally, the main candidate presumably driving most of the immune-related functions appears to be related to a major metabolic pathways that is under investigation.

Conclusion: We described a co-culture system in which NSCLC cell lines can impact ECs transcriptome and behavior (either physically or at distance). In the future, this model may represent an interesting surrogate to study the immuno-modulatory function of EC.
Keywords : Lung cancer, Tumor microenvironment, Endothelial cells, Immune cells

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Serglycin at the Glia Limitans, a key player of neuro-inflammation pathophysiology

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Résumé

Introduction: During neuroinflammation, astrocytes undergo morphological and molecular changes called "astrogliosis" enabling them to communicate with endothelial cells at the blood brain barrier (BBB) by producing pro-inflammatory factors. To characterize astrocytes signature during astrogliosis, we performed a RNA sequencing on "basal" (hBA) versus "reactive" human astrocytes (hRA) and identified Serglycin (SRGN), a proteoglycan, as highly expressed by hRA.
Objective and hypothesis: Our goal is to unravel the contribution of astrocytic SRGN to neuro-inflammatory behavior. Our hypothesis is that SRGN promotes glial scar formation and BBB breakdown.

Methods: *In vitro,* we transfected hbA with control or SRGN siRNAs (siSRGN) before inducing astrogliosis with IL-1β treatment. Cell lysates and conditioned media were then harvested to perform transcriptomic and proteomic analysis. In parallel, Human Brain Microvascular Endothelial Cells (hBMECs) were incubated with hRA conditioned medium for 24 hours to perform transcriptomic analysis.

Results: siSRGN treated hRA present a disturbed actin cytoskeleton with stress fibers assembly associated to a slower and disorganized migration and decreased expression of the tight junction Claudin1. Therefore SRGN seems to play an important role in glial scar formation. On the other hand, siSRGN treated hRA secrete less pro-inflammatory factors notably IL-6 and IL-8 which are trapped in the golgi apparatus. Moreover, conditioned medium of hRA treated with siSRGN inhibits endothelial activation markers (ICAM1, VCAM1 and PECAM1) in hBMECs. Therefore SRGN seems to promote BBB activation by promoting and facilitating inflammatory cytokine secretion.
Discussion: Collectively, our results demonstrate that SRGN in hRA drives astrogliosis properties, during neuro-inflammation, involved in glial scar formation and BBB homeostasis.

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Sema3A/Neuropilin1 pathway drives myeloid cell recruitment and vessel dysmorphia in Glioblastoma microenvironment.

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Résumé

Among primary brain tumors, glioblastoma (GB) is the most prevalent high grade glioma. GB displays highly necrotic, hypoxic and mitotic areas, hallmarks of high grade neoplasms. Current therapy consists of debulking, followed by chemoradiotherapy, resulting in a median survival of 14.6 months. Novel clinical treatment regimens have so far had little impact on GB patient survival. The field of immunotherapy offers promising new avenues with the example of recent clinical trials targeting PD-1 in order to regulate the immune checkpoint in GB. Despite numerous clinical trials, the poor prognosis for GBM patients remains largely unchanged. The uniquely immune-priviledged microenvironment of the central nervous system proves to be particularly challenging for the efficacy of immunotherapy in GBM. Therefore, the development of new therapies with improved activity at the tumor site will require a deeper understanding of the dynamic tumor micro environment (TME) in GBM.
Lately, looking for a novel anti-angiogenic therapy to overcome the resistance observed in response to classical anti-VEGF therapy in gliomas, we evaluated the potential of Sema3A and its receptor Neuropilin1.
Surprisingly enough, depletion of Sema3A, either by targeting its expression with shRNA in tumor cells resulted in: i) limiting peripheral macrophages recruitment, ii) blocking the phenotypic switch of macrophages from cytotoxic to tumor supportive, iii) normalizing vasculature, and iv) inhibiting glioma progression.
These results suggest that innate immunity might have been underestimated to the benefit of adaptative immunity in the quest for effective immunotherapeutic agents. In fact, we identified the crucial role of myeloid cells recruitment and polarization in the organization of glioblastoma stroma, and their deleterious effects on tumor vasculature, promoting blood vessel dysmorphia, leakage and perfusion defects, limiting chemotherapeutic delivery and immunity derived cytotoxicity.

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Blood vessels-on-chip for studying the effects of anti-cancer therapies on the vascular barrier

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Résumé

Blood vessels play key roles as a molecular and cellular barrier between blood and tissues. This vascular barrier is mainly made by endothelial cells which line the inner side of the vessels and by perivascular cells. Historically, blood vessels are the targets of anti-angiogenic therapies. A body of evidence reveals that vascular cells are also responsive to immunotherapies, and that the combinations of these therapies, such as those used in clinics may directly affect the vascular barrier.
We use microfabrication, microfluidics, and vessels-on-chip (VoC) multicellular 3D approaches (1) to study the functional effects of anti-angiogenic and anti-PD-L1 therapies on the vascular barrier, notably by using an optimized permeability assay and an activation test.

We show that endothelial cells express PD-L1 and not PD-1, and that the repression of PD-L1 in these cells does not affect the permeability of the barrier, but it induces the molecular activation of the endothelium. Surprisingly, the latter does not translate into a functional change in the ability of the immune cells to interact with the endothelium, suggesting a post-transcriptional repression of this activation.

Regarding the tested therapies, anti-angiogenics disturb the vascular barrier functions and integrity, whereas anti-PD-L1 therapeutic antibodies used alone do not seem to induce these effects.

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Treatments of VoC with combinations of these therapies will be addressed next in order to identify the most interesting and less detrimental ones.

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METFORMIN ATTENUATES MITOCHONDRIAL DYSFUNCTION IN THE LYMPHATIC ENDOTHELIUM INDUCED BY 5-FLUOROURACIL

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Résumé

5-Fluorouracil (5-FU) is a widely used anticancer drug with a structure similar to that of the pyrimidine molecules. Interfering with nucleoside metabolism, 5FU can be incorporated into RNA and DNA, leading to cytotoxicity and mitochondrial dysfunction which affects both malignant and healthy cells. It explains the high incidence of serious adverse events raising from this medication use. Administration of 5FU in mice was reported to cause the severe injury to the lymphatic endothelium. Metformin is a first-line drug used for the treatment of type 2 diabetes with the verified benefits for the variety of diseases. However, the impact of metformin on the lymphatic system remains elusive.
The aim of the current study is to investigate the mitochondrial dysfunction in human dermal lymphatic endothelial cells (HDLEC), induced by 5-FU, and the protective potential of metformin.

The results of our study demonstrate that 1 mM metformin exerts the beneficial influence on the mitochondrial function of the lymphatic endothelium that undergoes severe damage during 5FU-provocated stress. The Seahorse assay reveals an increase in mitochondrial respiratory activity in metformin-treated HDLECs, stressed by 5FU, as compared to untreated cells. Metformin reverses inhibitory effect of 5FU on maximal respiration, ATP production and spare respiratory capacity. In HDLECs, exposed to 5FU, the reduction in mitochondrial mass is observed while metformin rescued this negative effect. In addition, excessive production of superoxide by mitochondria, dramatically stimulated by 5FU, is abolished by metformin suggesting its potential in oxidative stress prevention.
Altogether, our data demonstrate that metformin could efficiently prevent the alterations in the mitochondrial function, caused by 5FU. This investigation represents a promising strategy to counteract the 5FU-induced side effects in the lymphatic system.

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Growth and study of tumor spheroids behavior in a biomimetic vascularized platform.

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Résumé

Cancer remains a global health challenge and asks for more accurate models for drug development. Conventional monolayer cultures fail to recapitulate the complex tumor microenvironment (TME), leading to limited efficacy in drug testing. In particular, they do not incorporate a vascular compartment, which plays a pivotal role in cancer development and in therapies' outcome. Conversely, in vivo models exhibit limited tunability and present escalating financial and ethical implications. Therefore, three-dimensional biomimetic systems emerge as promising candidates for mimicking the pathophysiological complexity of a tumor by integrating biochemical and mechanical cues as well as enabling coculture in highly tunable matrices. Besides, it holds the possibility to add liquid microenvironments to integrate the vascular or lymphatic system. Herein, we have developed a polysaccharide-based platform, composed of two compartments: first, a microwells network of tunable stiffness, porosity and geometry, in which cancer cells are seeded and form spheroids after 3 days. This component has been validated by assessing the response of several cell lines to chemotherapies. A 2 to 5-fold increase in resistance is witnessed for spheroids versus monolayers for most cell types. The enrichment of this TME with cancer-associated fibroblasts within the hydrogel pores allows studying the interplays between cells in culture, monitored by microscopy. The second part of the platform consists in microchannels layered by endothelial cells that are matured to form tubular constructs, and sprouting towards the pores. This vascularized gel will then be combined with the spheroids network and incubated for a few days to allow for tissue interactions. It permits to quantify the vascularization and spreading of the spheroids. Overall the goal is to provide an innovative tri-culture platform combining stromal, cancer, and endothelial cells in a biomimetic substrate in order to investigate interplays between spheroids and a vascularized TME.

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Lymphangiogenesis is modulated by PRL2

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Résumé

Lymphangiogenesis is the process of creating novel lymphatic vessels. These vessels are essential for tissue fluid homeostasis, immune cell trafficking, and dietary fat absorption. Lymphatic and blood vessels share similarities as the lymphatic vasculature takes its origin in veins. In recent collaborative work, we showed that the protein tyrosine phosphatase PRL2 (PTP4A2) modulates vascular development (Poulet *et al.*, 2020). Based on the close relationship between these two types of vasculatures, we hypothesized that PRL2 would likely also play an essential function in forming lymphatic vessels. By functional assay, we demonstrate that silencing PRL2 in lymphatic endothelial cells (LECs) induces a drastic decrease in cell proliferation, migration, and sprouting. Further examination of this phenotype points to some critical signaling cascade modulated by PRL2. Under VEGF-A or VEGF-C stimulation, ERK and Akt signaling pathways are affected by the absence of PRL2, decreasing and increasing phosphorylation respectively. Moreover, Notch1/DLL4 downstream signaling is also reduced in these cells.

We follow-up on the role of PRL2 since it is often upregulated in cancer, particularly within metastatic lymph nodes (Hardy *et al.*, 2018), and we undertook *in vivo* experiments to determine if the absence of PRL2 expression in LECs could inhibit lymphangiogenesis, thus reduce metastasis in tumour-bearing mice. Our preliminary findings suggest that the known increase in PRL2 phosphatase in many cancers may contribute to the lymphatic vessels formation towards the tumor and potentially promote cancer metastasis. Further studies are ongoing to validate this hypothesis using mice with a specific PRL2 gene knock-out in LECs.

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Delta like 4 at the Glia Limitans, a key player of neuro-inflammation pathophysiology

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Résumé

Introduction: During neuro-inflammation, astrocytes undergo morphological and molecular changes named "astrogliosis" and drive the conversion from acute inflammatory injury to chronic neurodegenerative state. To characterize astrocyte signature during astrogliosis, a RNA sequencing on reactive astrocytes was performed and identified delta-like 4 (Dll4) as highly expressed during astrogliosis.
Aims: Our goal is to unravel the contribution of Dll4-Notch1 signaling to astrogliosis and neurovascular unit (NVU) disruption.

Methods: We induced neuro-inflammation in both conditional astrocytic Dll4 KO (Dll4ACKO) mice vs controls and in C57BL/6 mice treated with an anti-DLL4 monoclonal antibody vs IgG. Lesion size, inflammation, demyelination and clinical disability were measured. In parallel, an in vitro analysis has been performed on human reactive astrocytes KO for DLL4 vs control.

Results: In vivo, both Dll4ACKO and anti-DLL4 mAb treated mice exhibited a milder pathology and astrogliosis than controls. It was correlated to the decreased expression of IL-6 and pro-permeability factors. In vitro, we demonstrated that the Dll4-Notch1 juxtacrine signaling in reactive astrocytes directly controls IL-6 transcriptional level and that blocking IL-6 receptor decreases astrogliosis and pro-permeability factor expression.
Discussion: Collectively, these data suggest that the Dll4-Notch1 signaling drives astrogliosis during neuro-inflammation via IL-6 up regulation promoting NVU disruption and pathology severity. Thus, DLL4 might be a new therapeutic target in neuro-inflammatory disorders.

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Hypergravity inducing motion sickness also increases blood-brain barrier permeability in mice.

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Résumé

Spaceflights induce generalized adaptation of mammalian physiology, including vascular, brain, muscle, bone and immunity functions and their earliest effect is an alteration in vestibular function responsible for the motion sickness. As a crucial interface between the vascular system and the brain, the blood-brain barrier (BBB) acts as a filter to protect neurons from toxins, pathogens and inflammation. Spaceflights are characterized by modifications of gravity levels including short hypergravity phases and longer period of microgravity. The easiest ground facility to reproduce variations of gravity level is the centrifugation-induced hypergravity. To evaluate the effects of gravity changes on BBB, we have firstly compared the effects of several protocols of hypergravity induced by centrifugation and whole-body vibrations (WBV) on BBB integrity by immunohistochemistry revealing immunoglobulin G (IgG) extravasation from blood to hippocampal parenchyma of mice. Secondly, we have characterized the BBB leakage by using molecules with different molecular weigth (dextrans and oligonucleotides) injected retro-orbitally into mice blood. Finally, the RT-qPCR analysis, performed on brain extracts, supports the dysregulation in the tight junctions of endothelial cells forming the BBB. Taken together our results suggest that the motion sickness induced by short period of hypergravity is linked to an increase of BBB leakage probably due to the modification of tight junctions.

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Role of Lysyl Oxidase in the pathological interplay between extracellular matrix and lymphatics in secondary lymphedema

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Résumé

It has long been assumed that lymphatic injury is the defining feature of the lymphedema pathophysiology. However, during lymphedema development, pathological changes occur both in the lymphatics and in the surrounding tissue. In particular, lymphedema-associated fibrosis appears to be the main aggravating factor in this pathology. Fibrosis is characterized by a pathological accumulation and remodeling of extracellular matrix (ECM) components. As lymphatic endothelial cells (LECs) directly attached to the ECM, in particular to collagen I, a defect in ECM remodeling will induce changes in lymphatic endothelial function.
Our work aims to provide a comprehensive view of lymphedema-associated fibrosis in order to identify new therapeutic targets for this unmet medical need. We first employed RNA sequencing to identify the molecular actors responsible for lymphedema-induced ECM remodeling. We strikingly observed an upregulation of lysyl oxidase (LOX) a key regulator of collagen crosslinking, accumulation and ECM stiffening. In a murine model of lymphedema, we demonstrated that LOX inhibition decreased collagen accumulation, ECM stiffening and lymphedema development while its overexpression worsened lymphedema. Using a single cell RNA sequencing approach, we provide new insights into the cell heterogeneity of lymphedematous ECM by highlighting a specific fibroblast subpopulation responsible for the synthesis of collagen, ECM proteins and collagen-modifying enzymes such as LOX. Finally, thanks to an original organ-on-chip lymphatic microvessel model, we demonstrate how a LOX-induced ECM remodeling is able to disrupt the lymphatic endothelium integrity creating nonfunctional leaky vessels.
Taken together, our results showed for the first time the implication of LOX in lymphedema-associated fibrosis. This project is thus the first step towards the development of new therapeutic strategies, targeting no more lymphatic vessels alone but normalizing the microenvironment to restore them.

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Characterization of Nestin+ progenitor cells in angiogenesis after myocardial infarction

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Résumé

Heart failure is a complex and progressive disease associated with substantial mortality and morbidity rates. The clinical syndrome of heart failure predominantly results from adverse ventricular remodeling after myocardial infarction (MI), prominently characterized by coronary vessel obstruction. These structural and molecular alterations, mainly due to the ischemia and derived inflammation of the tissue, include excessive collagen production, interstitial fibrosis and new cardiac vessel formation via angiogenesis. Therefore, finding new therapies that promote tissue revascularization and alleviate ischemic-derived damage turns out to be essential. It has been described that a specific population of bone marrow mesenchymal stem cells (MSCs) that express Nestin, differentiate into a variety of lineages like osteoblasts, adipocytes or stromal cells. They also modulate inflammatory cell trafficking to blood in response to acute and chronic inflammation. In chronic inflammatory vascular diseases such as atherosclerosis, these cells proliferate and differentiate into fibroblasts and endothelial cells. Moreover, Nestin+ cells participate in the remodelling of coronary vessels during cardiac development by activating SoxF family of transcription factors (TFs). The presence of Nestin+ cells in the adult myocardium suggests a potential role of these progenitor cells after MI by modulating new vessel formation. Using line-tracing mice models, we observed that derived cardiac Nestin+ cells exhibit endothelial markers which proliferates after the myocardial injury. In addition, we studied the expression of endothelial TFs regulating this switch towards a migrative and proliferative phenotype. In-vitro studies with endothelial cell lines showed that these TFs increases after ischemia stimulus and their targets genes were upregulated. These data confirm cardiac Nestin+ MSCs as source of endothelial cells in post-ischemic angiogenesis.

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NET1 a new mechanosensitive RhoGEF in vascular smooth muscle cells under stretch

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Résumé

Intracranial aneurysms (IA) are abnormal dilations of cerebral artery wall, whose rupture causes subarachnoid hemorrhage. IA develop at arterial bifurcations of the circle of Willis where hemodynamic forces are high. In arteries, pulsatile blood pressure induces cyclic stretch of smooth muscle cells (SMCs) which maintains their contractile phenotype. Changes in blood pressure, and consequent changes in stretch or its perception lead to aberrant SMC behavior and were associated with vascular disorders. Cytoskeleton remodeling is essential in SMC response to these forces, which largely depends on Rho protein activity itself controlled by RhoGEFs. We hypothesized that a deregulation of RhoGEFs signaling in SMC can be involved in the defective adaptation to mechanical forces favoring IA formation. To identify stretch-sensitive genes, we performed 3'-SRP *in vivo* in cerebral arteries of control (WKY), Spontaneously Hypertensive (SHR) and SHR-stroke prone (SHR-SP) rats, and *in vitro* in rat aortic SMC exposed to physiological (10%) or pathological (20%) cyclic stretch (1Hz). Net1 protein expression was studied also in human aortic SMC by immunoblot and subcellular fractionation was used to assess the cellular distribution of Net1.

Using 3'SRP approaches, we observed that Net1 transcript expression was increased in high blood pressure conditions (SHR and SHR-SP) compared to normal condition. Net1 was highly expressed in cerebral arteries compared to mesenteric arteries. *In vitro,* Net1 transcript was also increased in rat SMC subjected to stretch. We confirmed that at protein level in human SMCs. Furthermore, we demonstrated that stretching induced Net1 translocation from the nucleus to the cytosol.
Altogether, we identified Net1 as a mechanosensitive RhoGEF in vascular SMC which expression and localization are regulated by stretch. This suggests that stretch activated Net1, which lead to RhoA activation and cytoskeleton reorganization. Further studies are necessary to assess this hypothesis.

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Role of endothelial- and neural-expressed connexin 43 in post-natal retinal angiogenesis and retinopathies.

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Résumé

In the retina, interactions between endothelial cells (ECs) and the astroglial network are necessary for proper vascular development. Defective cross-talk between astrocytes and ECs leads to retinopathy of prematurity, which is the most vision-impairing disease in childhood. Herein, we investigate the role of EC and/or astroglial cell connexin 43 (Cx43)-comprised gap junctional interactions in the regulation of angiogenesis in the developing and pathological retinal vasculature. ScRNAseq of retinal ECs showed that Cx43 is expressed by all retinal ECs subtypes. We also found that Cx43 is expressed in and between IB4+-ECs and Desmin+-pericytes, and by GFAP+-astroglial cells. Using Tamoxifen inducible mouse models, Cdh5CreiERT2*;Cx43*fl/fl (*Cx43*ECiKO) and GlastCreiERT2;C*x43*fl/fl (*Cx43*GlastiKO), we evaluated the effects of *Cx43* deletion in ECs and astroglial cells, respectively, on retinal vascular development under normal conditions and in a preclinical model of retinopathy, the oxygen-induced retinopathy (OIR) model. In P7 Cx43ECiKO vascular progression was impaired and vascular density was significantly increased. In P7 *Cx43*GlastiKO, vascular progression was not impaired, but the number of arterial branchpoints and vascular density was significantly increased. Moreover, vascular regression assessed by collagen IV/IB4 staining was impaired in *Cx43*GlastiKO retinas, while aSMA coverage was not. Investigation is ongoing to assess whether impaired retinal vascularization observed in the absence of Cx43 in either ECs or astroglial cells is due to impaired EC migration, proliferation and/or specification. Next, we assessed the specific contribution of astroglial-expressed Cx43 in the development of retinopathies by subjecting *Cx43*GlastiKO animals to the OIR model. In P17 *Cx43*GlastiKO retinas post-OIR we observe an increase in avascularization and a decrease in sprouting area from veins. These results show that absence of Cx43 in astroglial cells prevents pathological neo-angiogenesis.

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Unraveling vascular and telocytes remodeling in cutaneous infantile hemangiomas using large-volume 3D imaging

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Résumé

Infantile hemangioma (IH) is the most frequent tumor in newborns, representing up to 1 in 10 births. This benign vascular tumor is characterized by a fast-growing phase followed by a slow involution and propranolol was uncovered highly efficient to accelerate its regression. While mechanisms of actions are still largely unknown, previous work of the team has demonstrated a key role of a new stromal cell: the telocyte (TC). Involved in cellular communication and organization, we hypothesized that TCs could participate in IH involution via vascular remodeling processes, enhanced by propranolol. Our aim is to study the vascular linearization and normalization phenomenon during IH regression, via an architectural study of patient tumor resections. To highlight remodeling deeply and accurately, imaging large volume of tissue is needed. However, working on thick samples require tissue- clearing, as developed for mouse brains. Nonetheless, numerous adaptations are required for skin, a highly pigmented and matrix-dense tissue. Optimizations of iDISCO protocol and compatible antibodies enabled us to highlight a limited number of tortuous vessels in the tumors. Furthermore, TCs are arranged differentially around lesional capillaries. They either form sheets or branching networks that respectively cover, totally or partially vessels, associated with capillaries morphology. These results suggest that natural or propranolol-induced IH involution is accompanied by a dynamic TCs reorganization, resulting in a vascular remodeling. A better understanding of propranolol mechanisms would offer therapeutic prospects for other vascular tumors with unmet therapeutic needs.

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Neuropilin 2, vascular development and flow-migration coupling

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Résumé

Neuropilin 2 (Nrp2) is a single transmembrane protein with no known catalytic function. Nrp2 was originally identified as a receptor for secreted class III semaphorins mediating repulsive signals during axonal growth. Nrp2 is also expressed in veins and lymphatic vessels where its role remains poorly understood. We generated a novel inducible VEGFR3Cre line, and showed that Nrp2 is essential for lymphatic development. Nrp2;VEGFR3Cre embryos exhibit edema at E14.5 and abnormal patterning of the lymphatic vasculature postnatally. Furthermore, we show that Nrp2 mutant neonates display decreased meningeal LV network without changing their drainage function. Mechanistically, NRP2 regulates FAK phosphorylation and activation in lymphatic endothelial cells resulting in decreased cell migrations. This mechanism is conserved in blood endothelial cells as endothelial specific NRP2 knockout showed decreased FAK activation resulting in decreased flow-migration coupling and decreased tip cell migration toward arteries in the developing retina.

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Warning regarding hematological toxicity of tamoxifen activated CreERT2 in young Rosa26CreERT2 mice

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Résumé

The Cre-lox system is a versatile and powerful tool used in mouse genetics. It allows spatial and/or temporal control of the deletion of a target gene. The Rosa26-CreERT2 (R26CreERT2) mouse model allows ubiquitous expression of CreERT2. Once activated by tamoxifen, CreERT2 will enter into the nuclei and delete floxed DNA sequences. We observed that intraperitoneal injection of tamoxifen in young R26CreERT2 mice leads to morbidity and mortality within 10 days after the first injection, in the absence of a floxed allele. Activation of CreERT2 by tamoxifen led to severe hematological defects, with anemia and a strong disorganization of the bone marrow vascular bed. Cell proliferation was significantly reduced in the bone marrow and the spleen resulting in the depletion of several hematopoietic cells. However, not all cell types or organs were affected to the same extent. We realized that many research groups are not aware of the potential toxicity of Cre recombinases, resulting in misinterpretation of the observed phenotype and in a waste of time and resources. It can be necessary to include tamoxifen injected CreERT2 controls lacking a floxed allele in experimental designs. It is also necessary to improve the communication about the limitations of Cre-lox mouse models among the scientific community.

*Intervenant

Thrombosis in the coronary microvasculature may be responsible for impaired cardiac relaxation and diastolic dysfunction

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Résumé

• Introduction :
Heart Failure with Preserved Ejection Fraction (HFpEF) is proposed to be caused by endothelial dysfunction in cardiac small vessels. We previously identified Hhipl1,as a gene upregulated in the coronary vasculature of Leptin receptor deficient mice (Leprdb/db) a well-established mouse model of HFpEF. Importantly, Hhipl1 encodes for a decoy receptor of Desert Hedgehog (DHH) which is k
nown to be critical for endothelial integrity.

• Objective :
Our objective is to investigate the functional consequences of impaired Hedgehog (HH) signaling in the adult heart in order to identify novel mechanisms underlying the development of diastolic dysfunction.

• Method :
To do so, Cdh5-Cre/ERT2, DhhFlox/Flox (DhhECKO) mice and their control littermates were administered with tamoxifen at 8 weeks of age to induce Dhh KO. Their cardiac function, exercise tolerance, and the phenotype of their coronary vasculature were assessed one month later.

• Results :
DhhECKO mice presented significantly reduced exercise tolerance, increased end diastolic pressure (EDP) and Tau, with no change in their ejection fraction consistent with diastolic dysfunction. At molecular and cellular level, impaired cardiac relaxation in DhhECKO mice was associated with a significantly decreased phospholamban phosphorylation on Thr17 and an alteration of sarcomeric shortening in ex-vivo. Besides, as expected, DhhECKO mice exhibited phenotypic changes in their coronary vasculature including a prominent prothrombotic phenotype (63±6,2 vs 25±5,2 thrombi/mm^2; p< 0,001) leading to an impaired

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capillary perfusion and local hypoxia. Notably, antiaggregant therapies (aspirin and clopidogrel) prevented the occurrence of both diastolic dysfunction and exercise intolerance in DhhECKO mice demonstrating for the first time that thrombosis may promote diastolic dysfunction. Importantly, we confirmed the critical role of thrombosis in Leprdb/db mice which also displayed increased cardiac small vessel thrombosis in comparison to control mice. Alike DhhECKO mice, we found that antiaggregants decreased EDP (6,3±0,4 mmHg in aspirin-treated vs 11,3 ± 0,79 in control mice; p=0,001) and improved exercise tolerance in Leprdb/db mice (34 ± 2,52 min in aspirin-treated vs 24 ± 3,55 in control mice; p=0,004).
• Conclusion :
Altogether, these results demonstrate that small vessel thrombosis may participate in the pathophysiology of heart failure with preserved ejection fraction.

Targeting Unc5b induces blood-brain barrier permeability and improves chemotherapy delivery and efficacy in glioblastoma.

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Résumé

The Blood-Brain Barrier (BBB) protects the CNS from toxins and pathogens and maintain homeostasis and proper function of the CNS. However, the unchallenged impermeability of the CNS impedes drug and treatment delivery to the brain during various CNS pathologies including glioblastoma tumors. Thus, the development of tailored therapeutic strategies capable of increasing CNS barrier permeability on demand is a holy grail of CNS therapy.

GBM represents the most common form of brain tumors and are associated with high resistance to conventional radiotherapy and chemotherapy. With a median survival between 12-15 months after diagnosis and less than 5% survival at 5 years, GBM is also the most severe form of brain tumors. While BBB integrity within the tumor core is impaired, the degree of breakdown is not homogeneous throughout the tumor and the highly invasive behavior of tumor cells into the BBB-competent brain parenchyma strongly limits efficacy of chemotherapeutic molecules.
We previously showed that Unc5B is a major regulator of BBB integrity via regulation of the Wnt/β-catenin signaling and systemic administration of anti-Unc5B blocking antibody induced a transient (1-8h) and size-selective (up to 70KDa) BBB opening. In this project, we investigated the spatial distribution and molecular mechanisms underlying the BBB's regulation by anti-Unc5B delivery. Using iDISCO/LSFM imaging, we showed prominent region-specific leakage in the medial dorsal cortex, hippocampus, thalamus, and cerebellum. Moreover, we showed that anti-Unc5b treatment induced increased doxorubicin delivery into GBM-bearing mice and led to reduced tumor size and increased tumor cells apoptosis. Therefore, anti-Unc5b mAb treatment enhanced the delivery and effectiveness of chemotherapy in GBM mouse model and offers a promising avenue to optimize therapeutic approaches of brain tumors.

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CTGF/CCN2 plays a key role in the mechanism of resistance to anti-angiogenic drugs in clear cell renal cell carcinoma

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Résumé

Clear cell renal cell carcinoma (RCC) is one of the most vascularized tumors. Sunitinib and axitinib are two anti-angiogenic tyrosine kinase inhibitors used in metastatic renal cell carcinoma (mRCC). Unfortunately, patients often relapse after one year of treatment. The anti-angiogenic role of these treatments on endothelial cells is well described, but their role on tumor cells is poorly understood. Proteomic analysis of sunitinib and axitinib treated as well as resistant cells to these treatments, showed an increase in pro-inflammatory proteins and secreted factors. Among them, we identified Connective Tissue Growth Factor (CTGF). CTGF is a signaling factor that promotes cancer development, progression and metastasis. We hypothesize that CTGF is involved in resistance to anti-angiogenic treatments. We demonstrated an increase in CTGF mRNA levels and the secreted form of CTGF in mRCC cells treated or resistant to sunitinib and axitinib. We also demonstrated that invalidation of CTGF reduced proliferation, migration, and invasion of untreated cells, treated with sunitinib and axitinib and resistant cells. In addition, recombinant CTGF protein increases the migration of mRCC cells. Invalidation of CTGF in mRCC cells decreases metastasis formation in the zebrafish model. Finally, a high level of CTGF in the plasma of mRCC patients is associated with shorter progression-free survival. Our results suggest that CTGF may play a key role in the sunitinib and axitinib resistance of mRCC, as well as in tumor cell aggressiveness, and need to be further explored.

*Intervenant

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Que sais-je ? L'angiogenèse, part de la biologie de nos vaisseaux

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Résumé

Prêt de cent mille kilomètres de vaisseaux sanguins irriguent notre corps. La majeure partie d'entre eux naissent du processus d'angiogenèse (ou création d'un néo-vaisseau à partir d'un vaisseau préexistant). Ce mécanisme complexe et étroitement contrôlé permet d'édifier un réseau fonctionnel acheminant oxygène et nutriments à tous nos organes. Par ailleurs, en plus de son rôle dans l'édification du réseau vasculaire au cours du développement, l'angiogenèse sera réactivée à l'âge adulte, entre autres au cours des processus pathologiques afin de réparer les tissus lésés.
Nous vous proposons un bref voyage le long de l'arbre vasculaire.

*Intervenant

Influence des vaisseaux sanguins sur le développement d'une tumeur

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Résumé

A l'intérieur du corps humain, les tissus sont vascularisés, c'est-à-dire qu'ils sont reliés à un réseau dense de vaisseaux sanguins qui assurent le transport de l'oxygène et des nutriments jusqu'aux cellules. C'est en particulier important dans le cas d'une tumeur, où la densité de cellules est très importante, et où les cellules se sont " déréglées " et consomment davantage de nutriments. La rapidité de croissance de la tumeur, ainsi que sa capacité à se disséminer dans le corps humain dépendent ainsi de cet approvisionnement. Nous essayons de construire un modèle de vaisseau sanguin artificiel pour observer la croissance de tumeurs dans cet environnement afin de mieux comprendre ces phénomènes.

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L'angiogenèse tumorale : comment utiliser les vaisseaux contre le cancer

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Résumé

La tumeur, comme tout tissu en croissance, nécessite un apport en énergie sous la forme d'oxygène et nutriments. A cette fin, elle va appeler la croissance de néo-vaisseaux via le processus d'angiogenèse. Il est à noter que la présence de vaisseaux dans la tumeur, bien que véhiculant ces sources d'énergie soutenant la progression de la pathologie, permet aussi l'acheminement des traitements anti-cancéreux. Néanmoins, les vaisseaux issus de cette croissance tumorale sont généralement chaotiques, le programme strict de cette organisation n'étant pas respecté.
Nous vous proposons de découvrir les stratégies thérapeutiques étudiées ciblant la biologie vasculaire dans le traitement des cancers.

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Quand des mécaniciens volent au secours des biologistes pour comprendre la cavernomatose cérébrale

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Résumé

Mes travaux de recherche portent sur une pathologie vasculaire cérébrale qui atteint une personne sur 200 nommée Cavernomatose ou Angiome caverneux. Cette maladie peut être d'origine génétique, transmise des parents aux enfants. La moitié des personnes atteintes présente des troubles comme des migraines, de l'épilepsie ou des paralysies. Ces symptômes proviennent de lésions focalisées dans le cerveau pouvant mesurer jusqu'à quelques centimètres de diamètre suite à un empilement de vaisseaux malformés, dilatés et hémorragiques. Il n'existe pas de traitement contre cette maladie. Une recherche internationale active dans les domaines de la médecine et de la génétique cherche à comprendre l'origine de ces malformations vasculaires. J'ai choisi d'emprunter une approche originale qui prend en compte les propriétés mécaniques de l'environnement des cellules dans ces lésions, notamment la force du flux sanguin qui passe à leur surface ou bien la dureté du tissu qui les entoure. En collaboration avec des " mécaniciens " de la cellule, nous avons pu montrer que les cellules mutées ont perdu la capacité de s'adapter à ces stimuli mécaniques et que ceci a des conséquences majeures sur leur biologie.

*Intervenant

Hypertension Artérielle : une recherche dynamique au service d'une pathologie vasculaire largement répandue

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Résumé

Vous avez certainement déjà entendu parler d'"Hypertension Artérielle". Et pour cause, cette pathologie vasculaire est la plus fréquente du monde! C'est aussi la cause la plus fréquente d'années de vie en bonne santé perdues, et pourtant sa prise en charge n'est pas encore optimale en France et dans le monde. Nous verrons ensemble lors de la session comment une simple élévation de la pression dans les artères peut conduire à l'insuffisance cardiaque, à l'insuffisance rénale ou encore l'accident vasculaire cérébral et la démence. Nous évoquerons les outils dont disposent les équipes de recherche pour mieux comprendre la survenue de l'hypertension artérielle, de ses complications et mettre au point de nouveaux moyens pour améliorer la prise en charge des patients.

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Développer un implant pour combattre les maladies dégénératives de la rétine

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Résumé

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Etude du rôle des vaisseaux sanguins de la rate dans la formation de caillots chez les patients atteints de néoplasie myéloprolifératives

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Résumé

Les néoplasies myéloprolifératives sont des maladies rares mais potentiellement mortelles, caractérisées par une production excessive de cellules sanguines qui présentent fréquemment une mutation au niveau du gène JAK2. La principale complication de ces maladies est la formation de caillots dans les vaisseaux qui bloquent la circulation sanguine et peuvent provoquer la mort des patients.
Actuellement, les mécanismes responsables de la formation de caillots sanguins chez ces patients ne sont pas encore clairement élucidés mais notre équipe a montré chez la souris que les cellules constituant la paroi des vaisseaux sanguins semblent jouer un rôle important lorsqu'elles portent la mutation du gène JAK2.

Par ailleurs, chez les patients atteints de néoplasies myéloprolifératives, les caillots apparaissent souvent au niveau des vaisseaux de la rate, ce qui est extrêmement rare dans la population générale. Pour cette raison, nous pensons que les vaisseaux de cet organe jouent un rôle spécifique dans la formation des caillots sanguins. Or, il est important de comprendre le mécanisme en jeu pour pouvoir efficacement cibler les vaisseaux à risque et prévenir le blocage de la circulation sanguine.

Mon travail de thèse vise donc à étudier le rôle des vaisseaux de la rate dans la formation de caillots sanguins chez les patients atteints de néoplasies myéloprolifératives et présentant la mutation du gène JAK2.
Pour cela, j'utilise une technologie relativement récente, appelée transcriptomique spatiale, qui permet d'étudier l'activité d'un organe en analysant les gènes exprimés par ses différents types cellulaires. Ainsi, nous pourrons mieux comprendre l'activité des vaisseaux de la rate chez les patients et comment ces vaisseaux interagissent avec les cellules environnantes dans le cadre de la maladie.

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