6ème Congrès de la Société Française d’Angiogenèse
Paris
Collège de France - September 24-25, 2015

Invited speakers:
Ralf Adams, Munster - Kari Alitalo, Helsinki - Hellmut Augustin, Heidelberg - Christophe Borg, Besançon
Peter Carmeliet, Leuven - Lena Claesson-welsh, Uppsala - Elisabetta Dejana, Milan
Anne Eichmann, Yale - Holger Gerhardt, London - Gervaise Loirand, Nantes
Paolo Madeddu, Bristol - Stefan Offermanns, Bad Nauheim
Shahin Rafii, New York - Dietmar Vestweber, Munster

Organizers
Catherine Boisson-Vidal, Julie Gavard, Stéphane Germain, Jean-Sébastien Silvestre

With institutional support from
Roche
Bayer HealthCare
Institut National du Cancer
Université Paris Descartes
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ACKNOWLEDGMENTS

The Scientific and Organizing Committees express their gratitude to the following Companies and Institutes for their contribution to the success of the congress.

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Institut National du Cancer

La Ligue contre le Cancer

SBCF

Université Paris Descartes

Île-de-France
Dear Friends and Colleagues,

Research in angiogenesis has been the focus of intense and exciting research activity over the last decade. Recent results in the clinic have shown that anti-angiogenic agents can delay tumor progression or tackle the negative outcome of retinopathies. However, novel mechanisms responsible for disease progression and resistance have emerged and new challenges remain, such as understanding the crosstalk between angiogenesis and the tumor micro-environment. Nevertheless, various cell-based and cell-free therapeutic strategies for stimulating angiogenesis have shown encouraging results, and are often advanced rapidly to clinical testing. However, the benefits of such treatments, if any, remain controversial and are still far from widespread clinical application.

These unexpected challenges and controversies might be attributable, at least in part, to our incomplete understanding of how the vascular compartment develops and regenerates and which intrinsic factors are accountable for the differences in angiogenic capacity in physiological and pathological circumstances. The key to future successful pro- or anti-angiogenic strategies lies in our continuing efforts to unravel and explore the role played by haemodynamic forces and the fate of vascular cells.

The 6th International Conference of the French Society of Angiogenesis will gather leading scientists working on these aspects of angiogenesis to present and discuss recent advances and future research opportunities. The conference will take place 24-25th September 2015, in the prestigious Collège de France in Paris (France), where Claude Bernard performed his pioneering research that identified i) red blood cells carry oxygen and ii) constrictor and dilatory elements of the vasomotor system that determine blood vessel caliber and blood flow.

All participants are invited to present a poster. A number of short communications will be selected from the submitted abstracts.

We look forward to an exciting meeting and welcoming you to Paris.

Catherine BOISSON-VIDAL
Julie GAVARD
Jean-Sébastien SILVESTRE
Stéphane GERMAIN

ORGANIZING COMMITTEE
Organizers
Catherine Boisson-Vidal (Paris) - Julie Gavard (Paris)
Jean-Sébastien Silvestre (Paris) - Stéphane Germain (Paris)

ORGANIZING SECRETARIAT
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angiogenese2015@publiccreations.com
Thursday, September 24

MORNING

09h00-09h15 Welcome and Introduction
The Organizing Committee

09.15-10.45 Session 1
Vascular Network and Morphogenesis
With the support of ARC Foundation

Chairpersons: Jean-Jacques FEIGE - Stéphane GERMAIN

09.15-09.45 Invited speaker: K. ALITALO (Institute of Biomedicine, Helsinki, Finland)
Biological functions and therapeutic potential of vascular endothelial growth factors

09.45-10.15 Invited speaker: R. ADAMS (Max Planck Institute, Münster, Germany)
Cellular and molecular principles of organ-specific blood vessel growth

10.15-10.30 L. SAUTEUR (Biozentrum, University of Basel, Switzerland)
VE-cadherin promotes dynamic cell shape changes during angiogenic sprouting
by cortical actin anchorage and actin polymerization

10.30-10.45 V. GEBALA (Cancer Research UK London, United Kingdom)
Blood flow drives lumen formation by inverse membrane blebbing during sprouting angiogenesis

10.45-11.15 Coffee Break

11.15-12.45 Session 2
Therapeutics: anti-angiogenesis in ophthalmology
Sponsored by BAYER HEALTHCARE

Chairpersons: Olivier CUVILLIER - Fabrice SONCIN

11.15-11.45 S. BAILLIF (Hôpital Saint Roch, Nice, France)
Angiogenesis in ophthalmology

11.45-12.15 C. DOT (HIA Desgenettes, Lyon, France)
Mechanisms and specificities of anti-VEGFs in the eye

12.15-12.45 S. TICK (CHNO des XVX, Paris, France)
Clinical applications
Thursday, September 24

**AFTERNOON**

**12.45-14.15**  
**Lunch & Poster Session**

**14.15-15.45**  
**Session 3**

**Therapeutics: anti-angiogenesis in cancer**

Sponsored by ROCHE

Chairpersons: Julie GAVARD - Gilles PAGÉS

**14.15-14.45**  
**Invited speaker: H. AUGUSTIN** (German Cancer Research center, Heidelberg, Germany)

Targeting Angiopoietin/Tie signaling during tumor angiogenesis: Where do we stand?

**14.45-15.15**  
**Invited speaker: C. BORG** (Institut Régional de Cancérologie de Franche Comté, CHU Besançon)

Anti-angiogenesis in oncology

**15.15-15.30**

T. MATHIVET (Vascular Patterning Laboratory, Leuven, Belgium)

Unraveling mechanisms in vascular patterning during tumor angiogenesis using Multi-Photon in Vivo imaging in mouse glioma

**15.30-15.45**

H. CASTEL (Inserm U982, Mont-Saint-Aignan, France)

The vasoactive peptide urotensin II : a new chemokine exhibiting migration/adhesion mesenchymal and angiogenic properties during glioma development

**15.45-16.00**  
**SPONSORED Session by NANOSTRING**

R. VAN EIJSDEN, PhD. Nanostring Technologies (Netherlands) - Multiplexed Cancer Progression Analysis: Using the nCounter® PanCancer Progression Panel

**16.00-17.15**  
**Coffee break & Poster Session**

**17.15-18.45**  
**Session 4**

**Vascular Barrier & Signaling**

With the support of French Society of Cell Biology

Chairpersons: Julie GAVARD - Danièle MATHIEU

**17.15-17.45**  
**Invited speaker: E. DEJANA** (University of Milan, Italy)

Transcriptional regulation of vascular development in health and disease

**17.45-18.15**  
**Invited speaker: D. VESTWEBER** (Max Planck Institute, Munster, Germany)

Mechanisms that regulate endothelial junctions

**18.15-18.30**

G. BOULDAY (INSERM, UMR-1161, Paris, France)

Integrating transcriptome and interactome data pinpoints a vWF pathway deregulation in Cerebral Cavernous Malformations

**18.30-18.45**

L. TREPS (INSERM U1016, Institut Cochin, Paris, France)

Extracellular vesicle-transported Semaphorin3A promotes vascular permeability in glioblastoma

**20.00**  
**Gala Dinner at the Procope Restaurant**
**Friday, September 25**

**MORNING**

**09.00-10.30 Session 5**

**Regeneration and Vascular Functions**

*Chairpersons: Jean-Sébastien SILVESTRE - Ebba BRAKENHIELM*

- **09.00-09.30**
  - **Invited speaker:** S. RAFII (Cornell University, New-York, USA)
  - Executive functions of tissue-specific vascular niche in stem cell self-renewal and organ regeneration

- **09.30-10.00**
  - **Invited speaker:** S. OFFERMANNS (Bad Nauheim, Germany)
  - Endothelial GPCR signaling in angiogenesis and vascular homeostasis

- **10.00-10.15**
  - O. HENRI (Inserm U1096, Rouen, France)
  - Therapeutic lymphangiogenesis in chronic heart failure

- **10.15-10.30**
  - K.-Y. HOWANGYIN (INSERM UMRS 970, Cardiovascular Research Center, Paris, France)
  - Efferocytosis controls myeloid cell-derived vascular endothelial growth factor release and cardiac remodeling after myocardial infarction

- **10.30-11.00**
  - *Coffee break*

- **11.00-12.30 Session 6**

**Signaling and trafficking in angiogenesis**

*Chairpersons: Catherine MONNOT - Cécile DUPLAA*

- **11.00-11.30**
  - **Invited speaker:** L. CLAESSON-WELSH (Uppsala University, Sweden)
  - VEGF signaling regulating the endothelial barrier

- **11.30-12.00**
  - **Invited speaker:** G. LOIRAND (Institut du Thorax, Nantes, France)
  - Functions and regulations of Rho proteins in endothelial cells

- **12.00-12.15**
  - A. ROSSI (Max Planck Institute, Bad Nauheim, Germany)
  - VEGFR2-independent VEGFA signaling in early zebrafish embryos

- **12.15-12.30**
  - C. PAQUES (GIGA-Research center, Molecular Angiogenesis Laboratory, Liège, Belgium)
  - Growth factors-induced angiogenesis requires the uPAR on the endothelial cells
# SCIENTIFIC PROGRAMME

## Friday, September 25

### AFTERNOON

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<td>12.30-14.00</td>
<td>Lunch</td>
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<td>13.30-14.00</td>
<td>SFA General Assembly</td>
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<td>14.00-15.45</td>
<td>Session 7&lt;br&gt;&lt;b&gt;Molecular Mechanisms of Angiogenesis&lt;/b&gt;&lt;br&gt;Chairpersons: Thierry COUFFINHAL - Virginie MATTOT&lt;br&gt;&lt;br&gt;&lt;b&gt;14.00-14.30&lt;/b&gt; Invited speaker: H. GERHARDT (London Research Institute, UK) Mechanisms in vascular patterning – a tale of fate and forces&lt;br&gt;&lt;b&gt;14.30-15.00&lt;/b&gt; Invited speaker: A. EICHMANN (Yale University, USA) Molecular parallels between neural and vascular development&lt;br&gt;&lt;b&gt;15.00-15.15&lt;/b&gt; F. TATIN (I2MC-INSERM U1048, Toulouse, France) Fat4/Dachsous1 signalling orchestrates collective cell polarization and migration during lymphatic valve morphogenesis&lt;br&gt;&lt;b&gt;15.15-15.30&lt;/b&gt; C. UMANA-DIAZ (INSERM U1050, Collège de France, Paris, France) LOXL2 regulates vascular morphogenesis through collagen type IV assembly in the extracellular matrix&lt;br&gt;&lt;b&gt;15.30-15.45&lt;/b&gt; S. GAUVRIT (Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany) The transcription factor Hhex is necessary for venous sprouting and lymphatic development in zebrafish&lt;br&gt;&lt;b&gt;15.45-16.00&lt;/b&gt; Coffee break</td>
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<td>16.00-17.30</td>
<td>Session 8&lt;br&gt;&lt;b&gt;Metabolism in the Vascular System&lt;/b&gt;&lt;br&gt;Chairpersons: Barbara GARMY-SUSINI - Andreas BIKFALVI&lt;br&gt;&lt;b&gt;16.00-16.30&lt;/b&gt; Invited speaker: P. CARMELIET (Vesalius Research Center, Leuven, Belgium) Angiogenesis revisited: role and (therapeutic) implications of endothelial metabolism&lt;br&gt;&lt;b&gt;16.30-17.00&lt;/b&gt; Invited speaker: P. MADEDDU (University of Bristol, UK) The impact of diabetes on angiogenesis and vasculogenesis&lt;br&gt;&lt;b&gt;17.00-17.15&lt;/b&gt; S. HERKENNE (Department of Biology, University of Padova, Italy) The mitochondrial shaping protein Optic Atrophy 1 (OPA1) controls angiogenesis&lt;br&gt;&lt;b&gt;17.15-17.30&lt;/b&gt; C. ERPICUM (I2MC-INSERM U1048, Toulouse, France) Lymphatic system drains peripheral adipose tissue to promote cachexia&lt;br&gt;&lt;b&gt;17.30-17.45&lt;/b&gt; Closing Ceremony&lt;br&gt;Best poster and best oral presentation Awards</td>
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INVITED LECTURES

Société Française d'Angiogenèse
BIOLOGICAL FUNCTIONS AND THERAPEUTIC POTENTIAL OF VASCULAR ENDOTHELIAL GROWTH FACTORS

Kari ALITALO and collaborators

Translational Cancer Biology Program and Wihuri Research Institute, Biomedicum Helsinki, 00014 University of Helsinki, Finland

Because of the importance of the growth of new blood vessels, or angiogenesis, in tumor progression, the first anti-angiogenic agents have been approved for clinical use. Although these treatments have been successful in the treatment of many types of solid tumors, most patients are either refractory or eventually acquire resistance to anti-angiogenic therapeutics. A combination of angiogenesis inhibitors based on solid knowledge of the major interacting angiogenesis signaling pathways could be used to significantly advance the efficacy of tumor therapy. - The idea of proangiogenic therapy is to grow new functional blood vessels and thus restore blood flow to ischemic tissue. In addition to angiogenesis of blood capillaries, growth of larger arterioles/arteries (arteriogenesis, or collateral formation) is especially beneficial for this goal. Several attempts have been made to stimulate angiogenesis and arteriogenesis in tissue ischemia, with limited success. One of the obstacles has been the property of angiogenic growth factors to promote vascular leakage, leading to tissue edema and fibrin deposition. Despite intensive efforts, growth factors suitable for angiogenic therapy have not yet provided significant help in the treatment of cardiovascular disease. – A better understanding of the biology of the vascular growth factors may facilitate therapeutics development for cardiovascular diseases. - The growth of lymphatic vessels, lymphangiogenesis, is actively involved in a number of pathological processes including tissue inflammation and tumor dissemination but is insufficient in patients suffering from lymphedema, a debilitating condition characterized by chronic tissue edema and impaired immunity. Lymphangiogenic growth factors provide possibilities to treat these diseases.

TARGETING ANGIOPOIETIN/TIE SIGNALING DURING TUMOR ANGIogenesis: WHERE DO WE STAND?

Hellmut G. AUGUSTIN and co-workers

1Vascular Oncology and Metastasis, German Cancer Research Center, Heidelberg (DKFZ-ZMBH Alliance); 2Vascular Biology and Tumor Angiogenesis, Medical Faculty Mannheim (CBTM), Heidelberg University; 3German Cancer Consortium (DKTK) Heidelberg, Germany

It’s more than 20 years since the discovery of the Tie receptors Tie1 and Tie2 and almost 20 years since the discovery of the Tie2 ligands, the angiopoietins (Ang). Much has been learnt about the mechanisms of Ang/Tie signaling during vessel assembly, maturation, remodeling and quiescence in health and disease. Yet, the contextual mechanistic analysis of Ang/Tie signaling continues to be poorly understood with fundamental questions being unanswered. For example, the agonistic Tie2 ligand Ang-1 appears to serve as a constitutive signaling axis controlling the maintenance of the mature endothelium. Yet, conditionally induced Ang-1 deficiency in adult mice can be compensated by hitherto unexplored mechanisms. Likewise, the Tie2 receptor is not constitutively expressed by endothelial cells, but negatively regulated during angiogenesis. The primary antagonistic ligand Ang-2 is now widely recognized as partial Tie2 agonist, but it is not known how agonistic vs. antagonistic functions are determined by the default state of Ang1/Tie2 signaling. Lastly, Tie1 continues to be an orphan receptor whose contribution to Tie2 signaling is mechanistically poorly understood. All of these enigmatic questions persist at a time when the Angiopoietin-targeting therapies are in advanced clinical development. In fact, the first phase III clinical trial in ovarian cancer targeting the angiopoietins has recently been reported to not have met its primary endpoint creating a volatile situation about the future of translational Ang/Tie research. This presentation will summarize the state-of-the-art of current Ang/Tie research with a focus on novel therapeutic windows of opportunity and strategies for the mechanism-guided translation of recent discoveries into clinical application.
INVITED LECTURES

Thursday, September 24 - 14.45 to 15.15

NÉOANGIOGENÈSE ET CONTRÔLE DU MICROENVIRONNEMENT TUMORAL

Christophe BORG
Institut Régional de cancérologie de Franche Comté, CHRU Besançon, UMR1098 INSERM

Résumé:

ANTI-ANGIOGENESIS IN ONCOLOGY

Abstract:
Cancer-associated angiogenesis was previously described as an important mechanism sustaining cancer progression. Beyond the angiogenic switch described by Judah Folkman, the mechanisms controlling cancer-related angiogenesis are also involved in the regulation of the cancer microenvironment. Moreover, the regulation of cancer neoangiogenesis is a multistep process where the number of angiogenic growth factors increase during cancer progression. Recent advances evidenced that angiogenic-related growth factors are also implicated in cancer microenvironment organization, as well as in cancer immune subversion. While hypoxia and VEGF prevent the infiltration of effector lymphocytes in the tumor, angiogenic growth factors directly promote the recruitment and activity of immuno-suppressive regulatory T cells and myeloid derived suppressive cells. A better understanding of these interactions is required to support the development of novel therapeutic strategies combining molecular and immune-related therapies.

Thursday, September 24 - 17.15 to 17.45

VEGF SIGNALING REGULATING THE ENDOTHELIAL BARRIER

Lena CLAESSON-WELSH and co-workers
Uppsala University, Dept. Immunology, Genetics and Pathology, Dag Hammarskjöldsv. 20, 751 85 Uppsala, Sweden.

VEGF was identified as a permeability factor. Although excess vascular permeability is thought to exacerbate diseases such as cancer and retinopathy, it has remained unclear how VEGF-induced permeability, separated from other VEGF-regulated vascular functions, contributes to disease.

Moreover, the impact of VEGF-induced vascular permeability on vascular development and physiology is unclear.

To address these different questions, we wished to selectively suppress VEGF-driven vascular permeability without affecting other aspects of VEGF-regulated vascular function. For this purpose, a tyrosine to phenylalanine exchange mutant at position Y949 in VEGFR2 was created by homologous recombination. This mutation blocks a pathway regulating activation of c-Src at endothelial junctions. While transient exposure of wild type mice to VEGF results in opening of endothelial junctions and molecular extravasation, the junctional barrier of the mutant mouse vasculature remains intact in the presence of VEGF. The mutant mouse is phenotypically normal with regard to vascular development and adult morphology suggesting that vascular permeability is dispensable for development and physiology. Challenge of the vegr2 Y949F/Y949F mouse with different cancer models shows that while suppressing VEGF-driven vascular permeability does not affect inflammation and necrosis in the tumor microenvironment, metastatic spread is reduced and administration of therapeutics is facilitated. Therefore, suppressing VEGF-driven vascular permeability is an attractive strategy in cancer treatment.

Vegetically poorly understood. All of these enigmatic questions persist at a time when the Angiopoietin-targeting therapies are in advanced clinical development.

In fact, the first phase III clinical trial in ovarian cancer targeting the angiopoietins has recently been reported to not have met its primary endpoint creating a volatile situation about the future of translational Ang/Tie research. This presentation will summarize the state-of-the-art of current Ang/Tie research with a focus on novel therapeutic windows of opportunity and strategies for the mechanism-guided translation of recent discoveries into clinical application.

Thursday, September 24 - 17.45 to 18.15

FUNCTIONS AND REGULATIONS OF RHO PROTEINS IN ENDOTHELIAL CELLS

Gervaise LOIRAND
Inserm UMR 1087, l’institut du thorax, 8 Quai Moncousu, Nantes, France

Rho proteins are ubiquitous and complex integrators of cytoskeletal structures and tension generation that can exert multiple functions depending on the cellular context. They act as binary molecular switches regulated by a large variety of upstream signals such as soluble mediators but also mechanical forces. Each of the 20 Rho protein is regulated by guanine nucleotide exchange factors (GEFs, ~80 members) that catalyze activation by exchanging bound GDP for GTP, and GTPase activating proteins (GAPs, ~65 members) that hydrolyze GTP thereby stopping activation. This overrepresentation of regulatory proteins compared to the number of Rho proteins themselves enabling them to fine-tune the actions of Rho proteins in the cell. They define where and when a Rho protein is activated and are thus likely to convey high signaling specificity. This talk will describe how Rho protein activities are regulated and coordinate endothelial cell cytoskeleton remodeling to control processes such as such as adhesion, migration, polarity or proliferation that enable endothelial cells to adequately respond, according to physiological or pathological conditions.
ENDOTHELIAL GPCR SIGNALING IN ANGIOGENESIS IN VASCULAR HOMEOSTASIS

Stefan OFFERMANNS
Max Planck Institute for Heart and Lung Research, Dept. of Pharmacology, Ludwigstr. 43, 61231 Bad Nauheim and Medical Faculty, J. W. Goethe University, Theodor-Stern-Kai 7, 60590 Frankfurt am Main, Germany

Angiogenesis, microvascular permeability and mechanosensitivity of endothelial cells involve a variety of mediators, many of which act through G-protein-coupled receptors (GPCRs). We have systematically characterized the expression of GPCRs in various endothelial cell types and have analyzed the role of G-protein-mediated signal transduction pathways in regulating angiogenesis, vascular tone regulation and vascular permeability by conditional mutagenesis of the genes encoding G-protein subunits. Data will be presented on the expression of GPCRs in endothelial cells as well as on the role of endothelial G-protein-mediated signaling pathways in the control of angiogenesis, vascular tone and vascular permeability under normal and pathological conditions. The relevance of these signaling pathways in the context of cardiovascular regulation through endothelial and systemic mediators will be discussed.
MECHANISMS THAT REGULATE ENDOTHELIAL JUNCTIONS

Dietmar VESTWEBER

Max Planck Institute of Molecular Biomedicine, Roentgenstr. 20, 48149 Muenster, Germany

VE-cadherin is a central adhesion molecule that provides stability of endothelial junctions and that controls junction integrity. In addition to these central functions in the adult organism, VE-cadherin is an essential player in the formation of blood vessels during angiogenesis. In the past our group has studied molecular mechanisms that control the formation and integrity of endothelial junctions. We have focused on VE-cadherin and an endothelial specific receptor tyrosine phosphatase, called vascular endothelial protein tyrosine phosphatase (VE-PTP) that associates with VE-cadherin and supports its adhesive activity and thereby the integrity of endothelial junctions.

The tyrosine kinase receptor Tie-2 had originally been identified as the first substrate of VE-PTP, although no biological function had been demonstrated. More recently, we found that VE-PTP associates with Tie-2 in vivo and antibodies against the extracellular domain of VE-PTP dissociate VE-PTP from Tie-2 and trigger endocytosis and degradation of the phosphatase. This effect stimulated tyrosine phosphorylation of Tie-2 and thereby enhanced proliferation of endothelial cells leading to vessel malformations in explant cultures of embryonic tissues and to vessel enlargement when administered to juvenile mice.

In addition to the important functions of Tie-2 in angiogenesis, it is well established that stimulation of Tie-2 with the agonistic ligand Angiopoietin 1 (Ang1) can counteract inflammation-induced plasma protein leaks in the vasculature. This prompted us to test whether our antibodies against VE-PTP (monoclonal and polyclonal) would be able to block the induction of vascular permeability. We found VEGF and histamine induced vascular leaks in the skin were inhibited by these antibodies. The group of Peter Campochiaro (Johns Hopkins University, Baltimore) could show, in cooperation with us, that neovascularization in the eye in an oxygen-induced ischemic retinopathy model was inhibited with these antibodies and a highly specific inhibitor of VE-PTP.

Moreover, we found that endothelial specific, tamoxifen-inducible gene inactivation of VE-PTP in VE-PTPlox/lox mice had similar effects in the Miles assay as the inhibitor and the antibodies against VE-PTP (Frye et al., submitted). Neutrophil recruitment into the lung of these mice after LPS challenge was also inhibited. Thus, conditional gene ablation of VE-PTP, as well as interference with antibodies or administering a specific VE-PTP inhibitor each stabilized endothelial junctions thereby preventing enhanced permeability and leukocyte extravasation induced by inflammatory mediators. Considering the supportive effect of VE-PTP on the adhesive function of VE-cadherin, these results were unexpected. They suggested that activation of Tie-2 via inhibition of VE-PTP protects endothelial junctions against inflammation-induced destabilization and overrides the negative effect of VE-PTP-inhibition on the adhesive function of VE-cadherin. Mechanisms underlying these effects will be discussed.
MECHANISMS IN VASCULAR PATTERNING – A TALE OF FATE AND FORCES
Holger GERHARDT1,2
1 Max-Delbrueck-Center for Molecular Medicine in the Helmholtz Foundation, MDC, Robert-Rössle-Strasse 10, 13125 Berlin, Germany,
2 Vesalius Research Center, VIB, KU Leuven, Belgium

Formation, expansion and functional adaptation of vascular networks are critical for development and physiology in vertebrates. Endothelial cells are the prime building blocks of these vascular networks, and their collective behavior drives vascular morphogenesis. Using genetic mosaics in 3D in vitro cultures, and in mouse and zebrafish development in vivo, we discovered a surprising degree of motility and plasticity of endothelial cells, not only during the formation of new vascular tubes, but also during remodeling and adaptation after blood flow is already established. The challenges of making new lumened tubes, establishing new connections, remodeling these to adapt the density and branching patterns to serve and meet organ functions, are formidable. How individual endothelial cells meet these challenges, and how they communicate with each other and the surrounding tissue to orchestrate patterning is still poorly understood. Are endothelial cells fated to form vessels of a certain shape and identity or do they dynamically adapt to local requirements? I will illustrate and discuss functions and regulation of endothelial cell dynamics and how they communicate with each other and the surrounding tissue to orchestrate remodeling of vessel connections and on the role and regulation of actin dynamics during formation and stabilization of nascent vascular tubes.

MOLECULAR PARALLELS BETWEEN NEURAL AND VASCULAR DEVELOPMENT
Anne EICHMANN
Cardiovascular Research Center, Yale University School of Medicine, 300 George Street, New Haven, CT 06510-3221, USA

Anatomical parallels between the nervous and the vascular system are readily apparent in peripheral body tissues, where blood vessels and nerves ramify throughout nearly all domains of the body and are usually aligned. To orchestrate the formation of their highly branched, exquisitely wired networks, nerves and blood vessels have developed shared cellular and molecular principles. At the cellular level, axons of developing neurons and capillaries use specialized motile structures to ensure their directional guidance. In axons, a growth cone is situated at the axon extremity and ensures axon guidance towards its distant target. In blood vessels, specialized motile endothelial cells (EC) situated at the capillary tips ensure capillary guidance during sprouting angiogenesis. Molecularily, common signaling molecules guide vascular and axonal outgrowth. Axonal growth cones and tip cells express receptors for axon guidance molecules, including Neuraplin receptors (Nrps), Eph family receptor tyrosine kinases, PlexinD1, Robo4 and UNC5B. Loss-of-function of the genes encoding these receptors leads to defects in vessel formation and in most cases to embryonic death, indicating a critical function of axon guidance receptors in vascular development. Their guidance properties and vascular expression makes them attractive targets for approaches directed at inhibiting tumor angiogenesis, or conversely for guiding new vessels towards ischemic tissue areas.

ANGIOGENESIS REVISITED: ROLE AND (THERAPEUTIC) IMPLICATIONS OF ENDOTHELIAL METABOLISM
Peter CARMELIET, MD, PhD
Laboratory of Angiogenesis and Neurovascular Link, Vesalius Research Center, Leuven Belgium, Department of Oncology, KU Leuven, Leuven, Belgium

Angiogenesis, the growth of new blood vessels, plays a crucial role in numerous diseases, including cancer. Anti-angiogenesis therapies have been developed to starve cancer cells from nutrients. Clinically approved anti-angiogenic drugs prolong the survival of cancer patients, but their success is limited by intrinsic refractoriness and acquired resistance. New strategies are thus needed to block tumor angiogenesis via alternative mechanisms. We recently reported that PFKFB3-driven glycolysis regulates the endothelial tip cell function during vessel sprouting, even capable of overcoming the potent pro-stalk activity of Notch, and that its loss in endothelial cells causes vascular hypobranching defects. Moreover, partial and transient reduction of glycolysis by blocking PFKFB3 reduced pathological angiogenesis in several disease models. Ongoing studies explore the role of lipid and amino acid metabolism in vessel sprouting, and assess the therapeutic potential of targeting these metabolic pathways for anti-angiogenic therapy.

DIABETES, THE BONE MARROW NICHE, AND IMPAIRED VASCULAR REGENERATION
Paolo MADEDDU
Bristol Heart Institute, University of Bristol, Level 7, Bristol Royal Infirmary, Upper Maudlin Street, Bristol BS2 8HW, UK

Diabetes mellitus is a global health problem that results in multiorgan complications leading to high morbidity and mortality. Accumulating evidence indicates that an altered control of angiogenesis and vasculogenesis contributes in worsening cardiovascular complications and their clinical outcomes in diabetic patients. We were the first to document that diabetic microangiopathy can be prevented in preclinical models using gene therapy and cell therapy. Significant examples are represented by treatment of peripheral microangiopathy with human tissue kallikrein and diabetic cardiomyopathy with Pim1 gene therapy. Until recently, the effects of diabetes and hyperglycemia on the bone marrow microenvironment -a site where multiple organ systems con verge and communicate- have been underappreciated. However, several new studies in mice, rats, and humans reveal that diabetes leads to multiple bone marrow microenvironmental defects, such as small vessel disease (microangiopathy), nerve terminal pauperization (neuropathy), and impaired stem cell mobilization (mobilopathy). The discovery that diabetes involves bone marrow-derived progenitors implicated in maintaining cardiovascular homeostasis has been proposed as a bridging mechanism between micro- and macroangiopathy in distant organs. Herein, we report the physiological and molecular bone marrow abnormalities associated with diabetes and discuss how bone marrow dysfunction represents a potential root for the development of the multiorgan failure characteristic of advanced diabetes. Cellular and molecular phenotyping of bone marrow pathology, including quantities and migratory properties of stem cells, and assessment of microRNA expression reportedly help predict combined cardiovascular risk in cohorts of diabetic patients with critical limb ischemia. We also report new data indicating that abrogation of pain perception in diabetes (sensory neuropathy) disrupts a previously neglected nociceptive mechanism that contributes to recruit pro-angiogenic cells to ischemic limb muscles and heart. The notion of diabetes as a bone marrow and stem cell disease opens new avenues for therapeutic interventions ultimately aimed at improving the outcome of diabetic patients.
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ORAL PRESENTATIONS

Société Française d'Angiogenèse
Vascular networks form by sprouting angiogenesis, anastomosis and vessel regression. Only properly patterned vascular trees ensure efficient blood transport and support embryonic growth. All these processes are governed by dynamic cell behaviors, such as proliferation, polarization, rearrangements and cell shape changes, all of which require endothelial cell-cell interactions. We study these cell-cell interactions by in vivo high-resolution time-lapse analysis in transgenic zebrafish embryos. Our previous analyses have shown that vascular tube formation during anastomosis is driven by a stereotypic sequence of events, including endothelial cell contact formation (anastomosis), apical-basal polarization and cell rearrangements that transform initial unicellular to multicellular tubes with patent lumen.

Our recent analysis of cell behaviors during angiogenic sprouting shows that sprout elongation is mainly driven by two distinct mechanisms: cell migration and cell elongation. Whereas cell migration is mainly attributed to angiogenic tip cells, endothelial stalk cells undergo extensive elongation, which lead to sprout extension. We find that loss of VE-cadherin (Cdh5) function does not affect tip cell migration but severely impairs stalk cell elongation. Analysis of 3D cell-cell interfaces showed that cdh5 mutant stalk cells are not able to transform the intercellular surface area from a round to elliptical shapes. Pharmacological inhibition of actin polymerization in wild-type embryos phenocopies these defects in cell shape deformation. These results suggest that Cdh5 and the actin cytoskeleton cooperate to to apply deforming forces onto cell-cell junctions. In agreement with this view, the cortical actin network in cdh5 mutant stalk cells is disturbed. Taken together, our finding support a cellular model for sprout elongation, in which Cdh5 is specifically required in stalk cells to promote cell shape changes by transmitting cytoskeletal forces, which eventually lead to stalk cell elongation and sprout extension.
ORAL PRESENTATIONS

I Thursday, September 24 - 10.30 to 10.45

**C02 - BLOOD FLOW DRIVES LUMEN FORMATION BY INVERSE MEMBRANE BLEBBING DURING SPROUTING ANGIGENESIS**

Véronique GEBAŁA1,2; Li-Kun PHNG3,4; Holger GERHARDT1,3

1Cancer Research UK London Research Institute, London, United Kingdom; 2Max Delbrueck Center for Molecular Medicine, Berlin, Germany; 3Vesalius Research
Laboratory of Experimental Neurosurgery and Neuroanatomy, KU Leuven, Leuven, Belgium; 4National Cerebral and Cardiovascular Center Research Institute, Department of Cell Biology, Osaka, Japan

Studies on in vitro and in vivo models of vascular lumen formation established the concept by which endothelial cells actively generate intra- and intercellular luminal spaces through apical-basal polarisation, apical membrane repulsion and vacuole fusion, similarly to other tubular epithelial structures. Recent studies in zebrafish embryos however suggested that blood flow is required for lumenezation during sprouting angiogenesis, thus raising the question whether and how active endothelial processes and external haemodynamic forces may contribute to lumen formation during angiogenesis in vivo.

Using high resolution imaging in mouse retinas and zebrafish embryos, we found that blood flow drives lumen expansion during sprouting angiogenesis, through a process that we termed inverse membrane blebbing. We show that blood flow induces spherical deformations of the apical membrane of endothelial cells leading to the expansion of the luminal compartment. Furthermore, we find that endothelial cells react to these membrane intrusions by local and transient recruitment and contraction of actomyosin. Loss-of-function experiments show that this contractile behaviour of the apical membrane is required to control blood pressure-driven membrane dynamics, and thus guarantees single, unidirectional lumen expansion in angiogenic sprouts. With this work, we show that blood flow, already known to control the remodeling of vascular networks, plays a key role during blood vessel morphogenesis by driving lumen expansion in angiogenic sprouts, and does so through a previously undescribed mechanism of inverse membrane blebbing.

I Thursday, September 24 - 15.15 to 15.30

**C03 - UNRAVELING MECHANISMS IN VASCULAR PATTERNING DURING TUMOR ANGIGENESIS USING MULTI-PHOTON IN VIVO IMAGING IN MOUSE GLIOMA**

Thomas MATHIVET1; Claire BOULETI 1; Tina VERSCHUERE 1; Fabio STANCHI 1; Matthias VAN WOENSEL 2; Balcer MARLY 1; Massimiliano MAZZONE 1; Steven DE VLEESCHOUWER 1; Holger GERHARDT1,3

1Vascular Patterning Laboratory, Vesalius Research Center, VIB, Department of Oncology, KU Leuven, Leuven, Belgium; 2Department of Neurosciences, Laboratory of Experimental Neurosurgery and Neuroanatomy, KU Leuven, Leuven, Belgium; 3Integrative Vascular Biology Laboratory, Max-Delbrück-Center for Molecular Medicine (MDC), Berlin, Germany

Tumor-angiogenesis field has been fuelled by hopes to identify tumor-specific endothelial markers allowing vascular targeting to improve drug delivery or altering vessel formation. However, the current limitations in non-invasive in vivo imaging and the chaotic nature of tumor vessels provide challenges to our ability to analyze vascular patterning in tumor-angiogenesis.

In order to overcome these limitations, we developed a cranial-window model in mice to allow the examination of tissues that are otherwise inaccessible to light microscopy in vivo. These settings allow us to visualize intracranial structures by multi-photon microscopy. Technically, spheroids of syngenic C57Bl6-mouse glioma are injected 200μm bellow the brain surface after craniotomy and a glass window is cemented above the disclosed brain, allowing repeated visualization of the same animal without need for further surgery. Implantation in mTmG-PDGF-iCRE or mTmG-CSF1R-Mer-CRE mice allows to highlight specifically endothelial-cells or myeloid-cells respectively with GFP-expression. Our preliminary results identify a progressive abnormalization of blood vessels patterning (figure1A) concomitant with a massive recruitment of host macrophages (figure1B): early-stage sprouting thin and perfused blood vessels are associated with M1 cytotoxic macrophages recruiting in tumor microenvironment, macrophages that switch to M2 phenotype in late-stage tumors, relocate close to blood vessels and produce VEGF (figure1C) inducing vessel dilation and leakiness.

Blocking the differentiation of monocytes-precursors, and so macrophages production, with anti-CSF1 antibody treatment resulted in vessels normalization in late-stage tumor. This blood vessel normalization is accompanied with restored perfusion and absence of leakage induced by M2 macrophages. Based on these results, we hypothesize that a switch in macrophage polarization from M1 anti-tumoral state to M2 pro-tumoral and pro-angiogenic state is responsible for deleterious blood vessel function along glioma progression.

*Figure1 : A.Two-photon live imaging on 2-5weeks glioma implanted in mTmG-pdgfCre mouse. Blood vessels switch from sprouting angiogenesis (2weeks) to vessel expansion (5weeks) in BFP expressing tumor B.MRC1 immunocytochemistry on 2-5weeks growth glioma. M2 macrophages are present at low level at 2weeks and accumulate until 5weeks. C.sFlt1 binding-assay on 5weeks growth glioma. M2 macrophages surrounding blood vessels present high amount of VEGF (binding sFlt1) to the neighboring endothelium leading blood vessel from sprouting to expansion.*
MESENCHYMAL AND ANGIOGENIC PROPERTIES DURING GLIOMA DEVELOPMENT

Hélène CASTEL; Vladim LE JONCOUR; Pierre-Olivier GUICHET; Céline LECOINTRE; Nicolas PERZO; Laurence DESRUES; Florent MARGUET; Annie LAQUERRIÈRE; Fabrice MORIN; Pierrick GANDOLFO

In mice, UT agonists stimulated matrigel sponge invasion by macrophages, endothelial and smooth muscle cells, stressing the chemokine and pro-angiogenic system. In the present study, more than 80 patients with oligodendrogliomas (grade II and III) or astrocytomas (grade I to IV) were retrospectively and prospectively included. The immunohistochemical analysis showed a higher expression of UII/UT in astrocytomas compared with oligodendrogliomas and there is a positive correlation with the grade. In GBM, UII and UT co-labeled with the pericyte marker -smooth muscle cell actin or with the hypoxia marker carbonic anhydrase 9, indicating specific localizations in vascular and hypoxic perivascular and pseudopalisadic areas. We thus investigated which signaling pathways activated by the urotensinergic system can be associated with hypoxia and angiogenesis. The U251 GBM cell line was exposed to normoxic or hypoxic conditions, in the absence or the presence of UII, and cell lysates were analyzed via hybridization on protein chips. UII induced significant variation in the expression of 58 proteins (among 247), and the global pathway analysis (Ingenuity Pathway Analysis software) predicted activation of the Lxr-Rfr, Gloma Invasiveness, PI3K/AKT and MIF Regulation of Innate Immunity pathways suggesting a major role in glioma chemotactic mechanisms and cell homing.

In vitro, gradient concentrations of UII induced chemotactic migrating effects and endothelial tube formation. Glioma migration was blocked by UT antagonists and mainly involved the Gq/Rho/ROCK pathway while partially requiring Gq/PI3K components. In contrast, we observed that homogeneous concentrations of UII blocked cell motility and stimulated cell-matrix adhesions through a UT/Gq signaling cascade, partially involving PI3K. Finally, homogeneous concentration of UII allowed translocation of Gq to the UT receptor at the plasma membrane and increased actin stress fibers, lamellipodia formation and vinculin-stained focal adhesions. UII also induced relocalization of UT pre-coupled to Gi in filipodia and initiated integrin-stained focal points.

In mice, UT agonists stimulated matrigel sponge invasion by macrophages, endothelial and smooth muscle cells, stressing the chemokine and pro-angiogenic properties of UII in vivo. In heterotopic GBM xenografted in Nude mice, intratumoral injection of UII accelerated tumor growth, hypoxia and necrosis, and stimulated a tortuous angiogenesis through metalloprotease activation. UT antagonists/biased ligands inhibited tumor growth, angiogenesis and prolonged mice survival. Thus, the peptide chemokine UII promotes the recruitment of pro-angiogenic cells, induces cell adhesion and chemotaxis, and stimulates necrosis associated with angiogenesis, thus accompanying GBM development. The specific blockade of UT signalings by means of antagonists or biased ligands would constitute a new route for the treatment of GBM.

Supported by the University of Rouen, Inserm, Haute-Normandie Région, Géfluc


C05 - VEGFRE-INDEPENDENT VEGFA SIGNALING IN EARLY ZEBRAFISH EMBRYOS

Andrea ROSSI; Sébastien GAVRIT; Michele MARASS; Didier STAINIER

Max Planck Institute, Bad Nauheim, Germany

Despite the importance of Vascular Endothelial Growth Factor (VEGF) signaling in health and disease, information about the role of the various ligand-receptor complexes is in large part based on in vitro binding studies. Using TAL effector nucleases (TALENs), we generated a zebrafish vegfaa mutant line that can be rescued to viability by RNA injections in early embryos. We used this unique reagent as an in vivo test tube to investigate the structural determinants of VEGF-A binding to VEGF receptor 2 (VEGFR-2) and Neuropilin-1 (NRP1). VEGFA-VEGFR-2 interaction was prevented by point mutations in one of two residues F17 and K84. Surprisingly, these mutations completely abolished vegfaa mutant rescue by Vegfa121 but not by Vegfaaa. Interestingly, Vegfaaa rescue of vegfaa mutants was completely lost upon mutation of the R164 residue that has been shown to interact with NRP1 in vitro. Furthermore, Vegfb (Vegfb), Placental growth factor (Pgf) and Fibroblast growth factor-2 (FGF-2) which bind NRP-1 but not VEGFR-2 were also able to rescue vegfaa mutants. VEGFR-2 independent rescue was also confirmed by using chemical inhibitors of VEGFR-2 signaling. Finally, we engineered the first known Vegfaa dominant negative isoforms with antiangiogenic therapeutic potential. Altogether our data indicate that:
1) F17 and K84 are key amino acids for Vegfaa interaction with VEGFR-2 in vivo;
2) Vegfaaa appears to be more effective at signaling in vivo via NRP1 than via VEGFR-2 in vivo;
3) VEGFR-2 and NRP1 signaling can independently lead to the formation of a functional vascular system;
4) the Vegfaa dominant negative isoforms are currently being explored as a tool to inhibit pathological angiogenesis.

Angiogenesis plays a key role in tumor growth and metastasis dissemination. Tumor cells secrete several growth factors including VEGF, which plays a major role in regulation of angiogenesis. Recently, several studies showed that VEGF signaling is not simply mediated by VEGFR2 alone. Indeed, in the signaling research field, accumulating data describes that receptors including VEGFR2 are included in large complexes located at the cell surface.

In our laboratory, we showed that VEGFR2 interacts with uPAR following VEGF stimulation. Moreover, uPAR knockdown impairs VEGF effects in several models of angiogenesis such as retinal vascularization, endothelial cell (EC) migration, proliferation and permeability. Antiangiogenic therapy by blocking VEGF pathway is currently known to induce tumor resistance, notably by upregulating the expression of other growth factors, like bFGF and EGF. We thus speculated that uPAR acting as a co-receptor is not specific to VEGFR2 but by its association with other tyrosine kinase receptors is also required for other growth factors. Then uPAR represents an attractive and ideal target to fight cancer and metastasis dissemination.

First we showed by using Proximity ligation and co-immunoprecipitation assays that uPAR interacts with FGFR1 and EGFR upon bFGF and EGF stimulation. Moreover, uPAR ablation impairs bFGF and EGF effects in vitro. As GPI-anchored protein uPAR, is mainly located in the lipid rafts. Interestingly, ultracentrifugation experiments, we showed that uPAR, FGFR1 and VEGFR2 are also localized in lipid rafts. Then we showed that lipid rafts are required for uPAR/VEGFR2 and uPAR/FGFR1 complexes formation. Moreover disruption of lipid rafts by drugs affects ERK and AKT phosphorylation induced by VEGF, bFGF and EGF.

In summary, these data suggest that uPAR, by interacting with FGFR1 and VEGFR2 in the lipid raft, is required for these two angiogenic agents. Our future work will attempt to identify where and how uPAR is required.

C06 - GROWTH FACTORS-INDUCED ANGIOGENESIS REQUIRES THE UPAR ON THE ENDOTHELIAL CELLS

Cécile PAQUES1, Thomas POLLENUM2, Stéphanie HERKENNE3, Michelle LION1; Ingrid STRUMAN1

1GIGA-Research center, Molecular Angiogenesis Laboratory, University of Liège, Liège, Belgium; 2Dulbecco-Telethon Institute, Venetian Institute of Molecular Medicine, Padua, Italy; 3Department of Biology, University of Padova, Padua, Italy

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C07 - THERAPEUTIC LYMPHANGIOGENESIS IN CHRONIC HEART FAILURE

HENRI ORIANNE1,2; POUEHE Chris1,2; HOUSSARI Mahmoud4; GALAS Ludovic2,3; EDWARDS-LÉVY Florence4; NICOL Lionel1,2,5; HENRY Jean-Paul1,2; DUMESNIL Anais1,2; SCHAPMAN Damien2,3; THUILLEZ Christian1,2; RICHARD Vincent1,2; MULDER Paul1,2; BRAKENHIELM Ebba1

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The pathophysiological role of cardiac lymphangiogenesis has received little attention. Here, we investigated the impact of myocardial infarction (MI) and therapeutic lymphangiogenesis on cardiac lymphatic structure and function during the development of chronic heart failure (CHF).

We hypothesized that

1) insufficient cardiac lymph drainage may contribute to the deleterious cardiac remodeling post-MI; and
2) therapeutic stimulation of lymphatic growth, or lymphangiogenesis, may reduce chronic myocardial edema and inflammation in this setting.

MI was modeled in Wistar rats by coronary occlusion followed by reperfusion. Lymphangiogenesis (VEGFR-3)-selective VEGF-CC152S was delivered intramyocardially using slow-release albumin-alginate microparticles. Animals were divided into: sham, control MI, and treated MI, receiving low or high dose of VEGF-C upon reperfusion. Cardiac function, lymphatics, blood vessel density, cardiac perfusion, edema, levels of infiltrating immune cells, and cardiac fibrosis were evaluated at 3 and 8 weeks post-MI.

We found that, compared to sham, control MI rats displayed lymphatic remodeling with increased lymphatic capillary density but rarefaction of epicardial pre-collector and collector vessels, associated with cardiac lymphatic dysfunction. In accordance with insufficient lymph drainage, chronic myocardial edema and low grade inflammation were found at both 3 and 8 weeks post-MI as determined by gravimetry, Magnetic Resonance T2 Imaging, and immunohistochemistry. VEGF-C treatment led to a dose-dependent increase in lymphatic capillary density and reduced rarefation of pre-collectors and collector lymphatic vessels, without any effect on the endogenous cardiac angiogenic response. As a consequence, myocardial edema (sham, 75.5±0.1; control MI, 77.9±0.2; VEGF-C MI, 77.3±0.1% water content; p<0.05) and macrophage infiltration levels were both reduced already by 3 weeks post-MI. Further, cardiac interstitial fibrosis was completely prevented by 8 weeks, even by low dose VEGF-C (sham, 3.3±0.1; control MI, 6.0±0.6; VEGF-C MI, 3.4±0.1% fibrosis; p<0.001), leading to reduced diastolic dysfunction, as evaluated by cardiac LV pressure-volume loops.

In conclusion, our data reveal that the lymphangiogenic response post-MI is insufficient to resolve the chronic edema and inflammation that contribute to cardiac fibrosis and development of CHF. Further, our targeted VEGF-C therapy improved cardiac lymphatic function leading to expedited resorption of both myocardial edema and infiltrating cardiac macrophages. Our study suggests that therapeutic lymphangiogenesis may represent a new therapeutic target in CHF.
Elisabeth Tournier-Lasserve, Benno Schwikowski
AP-HP, Groupe Hospitalier Saint-Louis Lariboisiere-Fernand-Widal, F-75010, Paris, France; Systems Biology Group, Institut Pasteur, F-75015, Paris, France

Gwenola Boulday, Frederik Gwinner, Claire Vandiedonck, Minh Arnold, Cécile Cardoso, Coralie De-Lucia

IN CEREBRAL CAVERNOUS MALFORMATIONS

C09 - INTEGRATING TRANSCRIPTOME AND INTERACTOME DATA PINPOINTS A VWF PATHWAY Deregulation

Kiave-Yune Howangyin, Min Yin, Clement Cochain, Marie Guenguen, Anta Ngkelo, Ivana Zlatanova, Jose Vilari, Nancy Chaaya

AND CARDIAC REMODELING AFTER MYOCARDIAL INFARCTION

Mathilde Lemtre, Philippe Bonnin, Xavier Loyer, Christian Stockmann, Chantal Boulangier, Ziad Mallat, Jean-Sebastien Silvestre

IN CEREBRAL CAVERNOUS MALFORMATIONS

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Gwenola Boulday, Frederik Gwinner, Claire Vandiedonck, Minh Arnold, Cécile Cardoso, Coralie De-Lucia

1INSERM UMR-S 970 – Paris Cardiovascular Research Center, Université Paris Descartes, Sorbonne Paris Cité, Paris, France; 2Division of Cardiovascular Medicine, University of Cambridge, Addenbrooke’s Hospital, Cambridge, United Kingdom; 3Université Paris-Diderot, Sorbonne Paris Cité, INSERM, Unité 965, Assistance Publique Hôpitaux de Paris, Hôpital Lariboisière, Paris, France

Inflammation and its resolution are crucial components of tissue remodeling after myocardial infarction (MI). Efferocytosis is mainly carried out by the membrane protein tyrosin kinase merk (MerTK) and the milk fat globule epidermal growth factor (Mfge8) and may balance inflammation resolution after MI. We reasoned that efficient MerTK and Mfge8-dependent clearance of apoptotic debris by infiltrated myeloid cells is critical to the angiogenic and regenerative fine-tuning of the ischemic cardiac tissue. We first demonstrated that the left ventricle ejection fraction assessed by echocardiography was decreased in irradiated WT mice transplanted with bone marrow-derived cells isolated from MerTK-/-, Mfge8-/- and double deficient animals when compared to wild-type animals (WT), 14 days after the onset of ischemia (p<0.001, N=8 per group). These effects were associated with an increased infarct size, fibrotic area as well as apoptotic cells number. In cardiac tissue, the amount of CD11b+Ly6G+Ly6Cdim and CD11b+Ly6G-Ly6C++ monocytes as well as that of CD11b+Ly6G-Ly6Cdim/F4/80+ macrophages was unaffected in infected heart of MerTK-/-/Mfge8-/- compared to WT mice. In contrast, deficient efferocytosis was related to a downregulation of the pro-angiogenic growth factor VEGF-A. Notably, in vivo FACS-sorted CD11b+Ly6G monocytes/macrophages from ischemic cardiac tissue of MerTK-/-/Mfge8-/- mice expressed lower levels of VEGF-A when compared to WT cells. In vitro, MerTK-/-/Mfge8-/- bone-marrow-derived macrophages had a clear pro-inflammatory phenotype and secreted significantly less IL-10 and VEGF-A compared to WT-bone-marrow derived macrophages (p<0.01, N=5 per group). In addition, we showed by echocardiography that specific ablation of IL10 or VEGF-A in bone-marrow derived cells decreased ejection fraction 14 days after MI (p<0.05, N=8 per group). In particular, we demonstrated that specific myeloid ablation of VEGF-A significantly increased collagen content and decreased capillary density (p<0.001, N=6 per group) in the heart after MI. Hence, inhibition of efferocytosis leads to an adverse cardiac remodeling after MI through phenotypic alterations of phagocytic cells.

C08 - EFFEROCYTOSIS CONTROLS MYELOID CELL-DERIVED VASCULAR ENDOTHELIAL GROWTH FACTOR RELEASE AND CARDIAC REMODELING AFTER MYOCARDIAL INFARCTION

Method: Affymetrix GeneChip analyses were performed on venous tissues from iCCM1-3 and control mice. A statistical method, termed the “P* method” was developed, that integrates transcriptomic data with publicly available protein interaction networks (STRINGv9.1 database) to define subnetworks of deregulated proteins. The P* method evaluated each gene’s neighborhood in the STRING network, detecting an enrichment of deregulated genes as compared to a background model (Fig1).

Results: We identified 90, 214 and 157 proteins using the P* approach, with neighborhoods significantly enriched for deregulation in iCCM1/2/3 veins compared to controls, respectively. Since features of the disease are similar independently of the CCM gene ablated, we crossed the results obtained from the 3 groups in order to determine shared targets and signaling pathways. Two unexpected genes from the coagulation cascade, factor VIII and von-Willebrand-factor (vWF) were detected in all iCCM1-3 groups. Despite the absence of vWF mRNA deregulation using a classical gene-by-gene analysis, hypergeometric enrichment test for deregulation of known vWF-related genes (literature-based list of 56 genes) revealed statistically significant deregulation of the pathway (p=5x10^-5). Strikingly, we detected abundant vWF strings in cerebral and retinal lesions in iCCM2 animals (Fig2), confirming a dysfunction of the vWF pathway in vivo.

In conclusion, the P* approach was able to predict a novel candidate pathway that was not detected with a classical gene-by-gene approach. Our current goal is to investigate how the vWF pathway could participate in the CCM pathogenesis.

Background: Familial Cerebral Cavernous Malformations (CCM) are vascular malformations caused by loss of function mutations in any of the 3 CCM genes. We established inducible, endothelial-specific CCM1-3 knock-out mouse models (iCCM1-3) which faithfully reproduce human lesions [1]. Consequences of endothelial loss of CCM proteins affect only capillaro-venous vessels and lesion development correlates with intense angiogenesis periods. CCM are scaffold proteins suggesting that the CCM phenotype could be mediated by abnormal protein-protein interactions. Computational methods integrating transcriptomic with protein interaction data have been shown to be powerful in deregulated networks identification (for a review see [2]).

Methods: We identified 90, 214 and 157 proteins using the P* approach, with neighborhoods significantly enriched for deregulation in iCCM1/2/3 veins compared to controls, respectively. Since features of the disease are similar independently of the CCM gene ablated, we crossed the results obtained from the 3 groups in order to determine shared targets and signaling pathways. Two unexpected genes from the coagulation cascade, factor VIII and von-Willebrand-factor (vWF) were detected in all iCCM1-3 groups. Despite the absence of vWF mRNA deregulation using a classical gene-by-gene analysis, hypergeometric enrichment test for deregulation of known vWF-related genes (literature-based list of 56 genes) revealed statistically significant deregulation of the pathway (p=5x10^-5). Strikingly, we detected abundant vWF strings in cerebral and retinal lesions in iCCM2 animals (Fig2), confirming a dysfunction of the vWF pathway in vivo.

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Fig 1: Schematic representation of the p'-method. The p'-approach evaluates each gene’s neighborhood in the STRING network detecting an enrichment of deregulated genes. A protein “g” with p partners in the STRING network will be given a new score representing the enrichment of deregulated genes in its direct network neighborhood.


Fig 2: Abnormal vWF distribution on cerebral (A-B) and retinal (C-D) vascular lesions in the CCM mouse models. vWF (green) dotted staining detected in vessels (PECAM staining in red) from controls (A and C) has been replaced by long vWF strings on CCM lesions in the brain (B) as well as in the retina.

C10 - EXTRACELLULAR VESICLE-TRANSPORTED SEMAPHORIN3A PROMOTES VASCULAR PERMEABILITY IN GLOBLASTOMA

Lucas TREPS1; Sébastien EDMOND1; Elizabeth HARFORD-WRIGHT1; Eva GALAN-MOYA1; Alain SCHMITT1; Sandy AZZI1; Antoine CITERNE1; Nicolas BIDERE1; Damien RICARD3,4; Julie GAVARD2

1Institut Cochin, Inserm U1016, Cnrs UMR8104, Université Paris Descartes, Paris, France; 2Centre de Recherche en Cancérologie, CNRS UMR6299, Inserm U892, Nantes, France; 3Hôpital d’Instruction des Armees du Val-de-Grace, service de neurologie, Paris, France; 4Cnrs UMR8257 COGNAC G, Paris, France

Glioblastoma are malignant highly vascularized brain tumours, which feature large oedema resulting from tumour-promoted vascular leakage. The pro-permeability factor Semaphorin3A (Sema3A) produced within glioblastoma was linked to the loss of endothelial barrier integrity. Here, we report that extracellular vesicles (EVs) released by patient-derived glioblastoma cells disrupt the endothelial barrier (Figures 1a-b). EVs expressed Sema3A at their surface (Figure 2a), which accounted for in vitro elevation of brain endothelial permeability and in vivo vascular permeability, in both skin and brain vasculature. Blocking Sema3A or its receptor Neuropilin1 (NRP1) hampered EVs-mediated permeability (Figure 2b). In vivo models using ectopically and orthotopically xenografted mice revealed that Sema3A-containing EVs were efficiently detected in the blood stream. In keeping with this idea, sera from GBM patients also contain high levels of Sema3A carried in the EV fraction that enhanced vascular permeability, in a Sema3A/NRP1-dependent manner. Our results suggest that EV-delivered Sema3A orchestrates loss of barrier integrity in glioblastoma and may be of interest for prognostic purposes.

Figure 1. Glioblastoma stem-like cells-released extracellular vesicles induce endothelial permeability. (a) Electronic microscopy analysis of purified glioblastoma stem-like cells (GSC)-shed extracellular vesicles (EVs). Smaller (30-100 nm exosomes) and larger vesicles (microvesicles) are displayed. Scale bar: 100 nm. (b) Confocal analysis of VE-cadherin (VE-cad, red) staining in control brain endothelial cells (ECs) (Unt.) or challenged for 1h with PKH-67-labeled purified GSC EVs (green). Nucleic acid staining is shown in blue (DAPI). Scale bar: 20 μm.

Figure 2. EV-transported Sema3A enhances vascular permeability through Neuropilin-1. (a) Confocal analysis of purified GSC EVs stained with Sema3A (green). Nucleic acid staining is shown in blue (DAPI). Scale bar: 500 nm. (b) ECs were pre-treated with blocking antibodies preventing Sema3A- (aNRP1A) or VEGFA- (aNRP1B) binding to NRP1 receptor. Human immunoglobulins (IgG) were used as control. ECs were then exposed to purified GSC-derived EVs. **, p<0.01.

Keywords: brain tumour, endothelial barrier, neuropilin, semaphorin, exosome, microvesicles.
C11 - FAT4/DACHSOU1 SIGNALLING ORCHESTRATES COLLECTIVE CELL POLARIZATION AND MIGRATION DURING LYMPHATIC VALVE MORPHOGENESIS

Francoise PUJOL°†, Tina MARTIN°, Anne-Catherine PRATS°‡, Danelle DEVENPORT°, Taija MÄKINEN°, Philippa FRANCIS-WEST°, Barbara GARMY-SUSINI°‡, Florence TATИ°‡

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Lymphatic valves ensure unidirectional lymph flow to collect tissue fluid and to maintain homeostasis. Defects in lymphatic valves result in accumulation of interstitial fluid and lead to tissue swelling and lymphedema. Recently, mutations of the gene Fat4, a large cadherin involved in cell polarity has been associated to primary lymphedema. Yet, the role of Fat4 in lymphatic vasculature hasn’t been investigated.

Here, we demonstrated that Fat4 and its ligand Dachsous1 play a crucial role in lymphatic valve formation. We showed that Dachsous1 (Dchs) is specifically expressed to cellular junctions of valve endothelial cells (VECs) that is maintained during valve formation. Therefore, Dchs organize the polarity of VECs within lymphatic vessels. Fat4 and Dchs1 deficient mice embryos display a well-developed lymphatic vasculature indicating that lymphatic sprouting and growth is not affected. Interestingly, lymphatic valves are abnormal at birth suggesting a restricted role to valve formation. By measuring the angle orientation of Prox1high cells relative to the vessel axis, we found that Fat4 and Dchs1 cadherins are required for the polarisation of VECs. Interestingly, Celsr1 is well-expressed in Fat4 and Dachsous1 deficient mice suggesting a Fat4/Dachsous1 signalling core-PCP independent.

Taken together, our data highlight an essential role of Fat4/Dachsous1 in collective cell polarization and migration of valve endothelial cells during lymphatic valve morphogenesis.

C12 - LOXL2 REGULATES VASCULAR MORPHOGENESIS THROUGH COLLAGEN TYPE IV ASSEMBLY IN THE EXTRACELLULAR MATRIX

Claudia UMANA-DIAZ°; Cathy PICHOL-THIEVEND°; Marilyn MALBOUYRES°; Laurent BIDAULT°; Alain BARRET°; Catherine MONNOT°; Christophe GUILLUY°; Florence RUGGIERO°; Laurent MULLER°; Stéphane GERMAIN°

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Angiogenesis plays a crucial role in development and cardiovascular ischemic pathologies and is associated with extensive hypoxia-driven extracellular matrix (ECM) remodeling. We showed that lysyl oxidase like 2 (LOXL2), a secreted enzyme involved in collagen and elastin crosslinking, is expressed in endothelial cells both during vascularization of rat retina and in growing intersegmental vessels (ISV) of zebrafish embryos. In vivo, knocking-down LOXL2 prevents proper formation of ISV. In vitro, LOXL2 is required for capillary formation in 3D culture models. We found that LOXL2 is colocalised with collagen IV in the vascular basement membrane (BM) of growing capillaries. Using time-lapse TIRF microscopy, we observed dynamic association of GFP-tagged LOXL2 with the basal ECM of endothelial cells. Furthermore, we showed that LOXL2 depletion affects collagen IV assembly in endothelial ECM leading to decreased ECM stiffness by a three-fold factor. We thus hypothesized that LOXL2 could regulate angiogenesis through organization of the BM which will provide the structural features and stiffness required for capillary morphogenesis.

As LOXL2 consists in four N-terminal SRCR domains and a C-terminal catalytic domain, we assessed the role of these different domains in capillary formation and collagen IV deposition in the ECM. Neither pharmacological inhibition nor mutation of the catalytic site of lysyl oxidase activity affected capillary formation in vivo and in vitro. Likewise, deletion of the whole catalytic domain did not affect capillary formation and collagen IV assembly. Furthermore, restricting LOXL2 expression to the two N-terminal SRCR domains was enough to restore both processes using LOXL2-depleted HUVEC. Unexpectedly, addition of exogenous LOXL2 or its truncated SRCR forms did not restore capillary formation in 3D culture model, nor collagen IV assembly, suggesting that LOXL2 SRCR domains act as important intracellular partners of collagen IV that regulate both ECM assembly and capillary morphogenesis. Indeed, we detected intracellular interaction between LOXL2 and type IV collagen using the Proximity Ligation Assay. Altogether, these data suggest that LOXL2 expressed by endothelial cells regulates capillary morphogenesis through the assembly of collagen IV in the ECM via SRCR-dependent mechanisms.
Opa1-dependent mitochondrial dynamics is a targetable component of angiogenesis. Conditional Opa1 ablation substantiates its role in mouse and zebrafish angiogenesis and in lymphangiogenesis mediated tumor metastatization. Thus, genetic increase. Genetic here we show that Opa1 is a crucial component of the angiogenetic program. Upon endothelial cells angiogenic stimulation, mitochondria elongate and OPA-1 level through which new blood vessels form from pre-existing ones, has not been addressed. Mitochondria not only synthesize most of the cellular ATP, but they are also centrally placed in intermediate metabolism Ca2+ signaling, redox homeostasis and apoptosis. The multifunctional inner mitochondrial membrane mitochondrial fusion protein Optic Atrophy 1 (OPA-1) is placed at the crossroad of fusion, cristae biogenesis, metabolism, apoptosis and regulation of cardiomyocyte differentiation, yet the role of mitochondrial dynamics in angiogenesis, the physiological process through which new blood vessels form from pre-existing ones, has not been addressed. Here we show that Opa1 is a crucial component of the angiogenetic program. Upon endothelial cells angiogenic stimulation, mitochondria elongate and OPA-1 level increase. Genetic Opa1 ablation signals retrogradely from mitochondria to the nucleus to modify angiogenic genes expression and therefore inhibit all features of angiogenesis. Conditional Opa1 ablation substantiates its role in mouse and zebrafish angiogenesis and in lymphangiogenesis mediated tumor metastatization. Thus, Opa1-dependent mitochondrial dynamics is a targetable component of angiogenesis.

C13 - THE TRANSCRIPTION FACTOR HHEX IS NECESSARY FOR VENOUS SPROUTING AND LYMPHATIC DEVELOPMENT IN ZEBRAFISH

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During development, formation of a vascular network is necessary for the efficient transport of gases, liquids, signaling molecules and cells to the different tissues of the growing embryo. To fulfill these needs, the endothelium forms a complex network of tubes consisting of different subtypes: arteries, veins and lymphatics. The concomitant differentiation of the endothelium into arterial, venous and lymphatic cell types is controlled by intrinsic and extrinsic factors. One of these intrinsic factors is the transcription factor Hhex (hematopoietically expressed homebox), which is expressed at early stages by endothelial and blood precursors. In mouse, a null mutation in Hhex results in multiple cardiovascular abnormalities but its precise role in endothelial cells is not well understood. Here, we took advantage of the optical properties of the zebrafish embryo to investigate the role of hhex during sprouting angiogenesis. We generated a hhex mutant using TAL effector nucleases (TALENs) to target the exon encoding the DNA binding domain. In this mutant, the first wave of intersegmental vessels which sprout from the dorsal aorta does not appear to be affected. However, the second wave of intersegmental vessels which sprout from the posterior cardinal vein fails to occur. These venous sprouts give rise to intersegmental veins and lymphatic precursors, and their formation also depends on the Vegfc/Flt4 signalling pathway. We have confirmed by in situ hybridization that vegfc and flt4 expression is affected in hhex mutants. Our results identify Hhex as a novel regulator of the Vegfc/Flt4 signalling pathway during venous sprouting and lymphatic development in zebrafish.

C14 - THE MITOCHONDRIAL SHAPING PROTEIN OPTIC ATROPHY 1 (OPA1) CONTROLS ANGIOGENESIS

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Mitochondria not only synthesize most of the cellular ATP, but they are also centrally placed in intermediate metabolism Ca2+ signaling, redox homeostasis and apoptosis. The multifunctional inner mitochondrial membrane mitochondrial fusion protein Optic Atrophy 1 (OPA-1) is placed at the crossroad of fusion, cristae biogenesis, metabolism, apoptosis and regulation of cardiomyocyte differentiation, yet the role of mitochondrial dynamics in angiogenesis, the physiological process through which new blood vessels form from pre-existing ones, has not been addressed. Here we show that Opa1 is a crucial component of the angiogenetic program. Upon endothelial cells angiogenic stimulation, mitochondria elongate and OPA-1 level increase. Genetic Opa1 ablation signals retrogradely from mitochondria to the nucleus to modify angiogenic genes expression and therefore inhibit all features of angiogenesis. Conditional Opa1 ablation substantiates its role in mouse and zebrafish angiogenesis and in lymphangiogenesis mediated tumor metastatization. Thus, Opa1-dependent mitochondrial dynamics is a targetable component of angiogenesis.

C15 - LYMPHATIC SYSTEM DRAINS PERIPHERAL ADIPOSE TISSUE TO PROMOTE CACHEXIA

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The link between lymphatic system and adipose tissue drainage in physiology has been established for a long time. In the opposite, there is a tremendous lack of knowledge aiming to identify how adipose tissue can promote the activation of lymphatic vessels to induce unbounded emaciation in pathological conditions. Despite large body of evidences, this study will be the first aiming to identify the role of lymphatic system during cachexia, a multifactorial syndrome associated with a pathological loss of body weight.

Here, we postulated that initiation of cachexia is controlled by a dynamic mechanism mediated by the lymphatic system. Indeed, since the lymphatic vascular system is essential for lipid absorption in physiological conditions, we postulated that lymphatic vessels are recruited by lipolytic adipocytes to drain fat and thus participate to adipose tissue reduction. In a mouse model of pancreatic adenocarcinoma (Pdx1Cre; LSLKrasG12DInk4a+/- mice), we showed that lymphatic vessels develop at the interface of pancreatic lesion and adipose tissue. Moreover, we observed the formation of lymphatic vessels in early lesions (PanIN III) before the adenocarcinoma development. Interestingly, the injection of a blocking antibody directed against the main pro-lymphangiogenic receptor, VEGFR-3, delayed the apparition of cachexia. In two models of cancer-associated cachexia, resulting from the orthotopic injection of melanoma or carcinoma cells, we analysed the lymphatic vessels distribution and recruitment in several peri-tumoral or distant adipose tissue (perigonadal, intrascapular and inguinal adipose tissues). The capacity of these vessels to drain lipids was determined by using Nile-Red immunolabelling. Importantly, we demonstrated for the first time that lipid lymphatic drainage is not restricted to the intestine, but occurs in peripheral tissues.

In conclusion, this study should not only provide new knowledge in the still poorly explored field of lymphangiogenesis induction, but also open new therapeutic perspectives for fat drainage in metabolic syndromes.
Ocular angiogenesis, or ocular neovascularization, is responsible for the leading causes of blindness in the developed countries. It affects many ocular tissues such as the cornea, the iris, the retina, and the choroid.

The cornea is a transparent and avascular tissue, which account for two-thirds of the eye's total optical power. Neovascularization of the cornea causes vision loss as it alters its refractive surface, and obstructs the visual axis. Corneal inflammation, hypoxia and limbal stem cell destruction are the major stimuli for corneal angiogenesis.

Iris neovascularization leads to neovascular glaucoma when the fibrovascular membrane grows over the trabecular meshwork in the anterior chamber angle. Neovascular glaucoma can result in complete vision loss associated with severe ocular pain. Iris neovascularization is almost always secondary to diseases complicated with retina ischemia such as retinal vein occlusion, proliferative diabetic retinopathy or radiation retinopathy.

In retinal neovascularization, new vessels extend from the inner retina into the avascular vitreous gel. With time, these new vessels become fibrotic and traction retinal detachment or vitreous hemorrhages may develop. Retinal neovascularization often originates in the vicinity of areas of ischemic retina. The most frequent diseases leading to retinal neovascularization are diabetic retinopathy and, central vein occlusion but it also occurs in hemoglobinopathies or retinopathy of prematurity.

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The choroid is a vascularized and pigmented tissue that nourishes the outer layers of the retina and plays a role in heat dissipation. Choroidal neovascularization occurs most exclusively in the central macular regions of the retina. New vessels grow from the choriocapillaris, break the Bruch’s membrane end develop into the retinal pigment epithelium and subretinal spaces. Choroidal neovascularization is the trademark of age-related macular degeneration but is also associated with high myopia or in retinal pigment epithelium changes.

Angiogenesis is involved both in anterior and posterior segment of the eye. We can observe it in pathological conditions when ischemia or inflammation occurs, and when VEGF is over-expressed. It leads to new vascular vessels formation that have 2 specificities : the permeability and a high risk of hemorrhage. Three diseases are of particular interest in the eye : the neovascular glaucoma, the age macular degeneration (AMD) and all retinal neovascularization secondary to large retinal ischemia (after retinal vein occlusion, ischemic retinal diseases). Since 2007, approval of anti-VEGFs had dramatically changed the prognosis of them. For the first time we have observed an improvement or a stabilization in visual acuity in AMD. Ocular angiogenesis is characterized by its localized development in an encapsulated organ. Thanks to specific technologies, especially angiography and optical coherence tomography (OCT), we can diagnose the disease, visualize directly the neovascular network and follow it during anti-VEGF treatment. This treatment is directly injected in the eye by an intravitreal procedure under local anesthesia. This allows a very small and efficient dose taking into account the very specific pharmacokinetics of the eye.

The follow-up is performed with OCT and/or angiography that permits to identify the control of the disease, or its recurrence with special ocular criteria like the presence of sub-retinal fluid, reflecting the vascular permeability of the new vessels. Contrary to the retinal neovascularization, new vessels in AMD do not disappear after anti-VEGF therapies but become only quiescent. Other therapies like anti-PDGF will be needed in the future to reduce and destroy the neovascular network and better control this disease. The intravitreal injections must be repeated in AMD according to a monthly or bimonthly regimen, the interval can be then expanded depending on the evolution of the disease. This differs from the anti-VEGF used bevacizumab (off label), ranibizumab or aflibercept.
ANTI-VEGF AND OCULAR PATHOLOGIES. CLINICAL APPLICATIONS

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History of anti-vegf treatment in ocular pathologies started in 2005 with systemic Bevacizumab Therapy for Neovascular Age-Related Macular Degeneration. Soon after, ophthalmologists began injecting anti-vegf directly into the vitreous cavity as an off-label use in the treatment of Neovascular Age-Related Macular Degeneration (NVAMD), which represents the first cause of visual impairment after the age of 50 in western countries. Intravitreal injection of bevacizumab was found to be effective in the treatment of NVAMD, with minimal systemic adverse effects, which led to the first studies to demonstrate an improvement in visual function in patients with NVAMD. For the first time, in 2006, two pivotal studies showed improvement in visual outcomes for all forms of choroidal neovascularization in NVAMD. Based on this evidence, the first intravitreal anti-vegf was approved by the FDA in June 2006, for the treatment of NVAMD (ranibizumab, Lucentis®) and aflibercept, Eylea®, using another anti-vegf strategy has been approved in 2011.

NVAMD was the first ocular pathology treated with anti-vegf. Since, those drugs have been approved for the treatment of choroidal neovascularization in myopia, macular edema in diabetic maculopathy and retinal vein occlusions. They are also used for neovascularization in retinopathy of prematurity or hemoglobinopathies and studied for corneal neovascularization, radiation retinopathy and neovascular glaucoma.

We will expose indications and different treatment strategies and also describe local and systemic side effects of anti-vegf use in the eye.

MULTIPLEXED CANCER PROGRESSION ANALYSIS: USING THE NCOUNTER® PANCANCER PROGRESSION PANEL

R. VAN EIJSDEN

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The NanoString® nCounter® Analysis System utilizes a novel digital color-coded barcode technology that is based on direct multiplexed measurement of gene expression and offers high levels of precision and sensitivity. The technology uses molecular «barcodes» and single molecule imaging to detect up to 800 molecules (DNA or RNA targets) in one single reaction without the need for sample amplification (in the majority of applications).

nCounter® applications can be run on purified RNA and DNA, but also directly on cell lysates from cell lines or blood. The technology is extremely well compatible with FFPE extracted RNA and DNA. Besides custom CodeSets, NanoString also offers a collection of off-the-shelf panels. With a strong focus on cancer, NanoString® has developed three cancer focused panels: the PanCancer Pathways Panel, the PanCancer Immune Profiling Panel, and the PanCancer Progression panel, which can be used to answer the three major questions: (1) How did the tumor develop?, (2) How does the body respond?, and (3) How does the tumor progress?

The PanCancer Progression Panel focuses on the processes of angiogenesis, epithelial-to-mesenchymal transition (EMT), extracellular matrix remodeling (EMT) and metastasis. The soon to be released Multi-Omics application even enables you to combine RNA analysis with protein analysis in one assay!
Société Française d'Angiogenèse
Majority of modern-day microscopy techniques focus on increasing resolution which, is achieved at the expense of cost, compactness, simplicity, and field of view. The substantial decrease in the field of view limits the visibility to a few tens of single cells at best and prevent from tackling quantifications at mesoscopic scales. To catch a global view of a cell population with significant statistics both in terms of cell numbers, space and time, we developed a lensfree video microscope based on digital in-line holography. This system allows capturing the kinetics of thousands of cells simultaneously directly inside the incubators, opening up new horizon to cell culture monitoring and biology of the cell. Here we are assessing the use of the Iprasense Cytonote lensfree video microscope (Fig. 1) in combination with a customized version of the "Angiogenesis Analyzer ImageJ plug-in. This turns into a unique platform to exhaustively and precisely study angiogenesis and cellular network formation over large field of view (29.4 mm²) and extended period of acquisition (days to weeks). As a first case study, we have analyzed and quantified the spontaneous network formation of HUVEC endothelial cells on three-dimensional (3-D) extracellular matrices (Fig. 2). We were able to define three different steps for the network formation: initiation, a stabilization period and then the fusion of meshes (Fig. 3). During the first 4 hours, the network is in formation: the number of meshes increases, and so do the number of segments and junctions (see figure 3d for the definitions). Following this, for 6 hours, the network remains stable. Towards the end, the meshes merge to form larger meshes. After 24 hours, the networks present the following architecture: total meshes area of 9mm², with 60 meshes, with an average size of 0.15 mm². In sum, we demonstrate that the lensfree video microscope in combination with the Angiogenesis Analyzer ImageJ plug-in provides a unique mean to monitor and quantify angiogenesis and to screen ex vivo anti-angiogenic therapies. Since the methods is label-free, high-throughput, ease of use, working directly inside the incubator and relatively low-cost, we expect that it will irreversibly change the quantification of cell culture, and angiogenesis quantification in particular.
P02 - IMAGING OF CAPILLARY FORMATION IN 3D CULTURE HYDROGELS USING LIGHT-SHEET MICROSCOPY

Yoann ATLAS1; Jérome TEILLON1; Philippe GIRARD1; Laurent MULLER1; Stéphane GERMAIN1
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Endothelial cell (EC) activation is a cellular process by which EC – quiescent in mature vessels – are activated under various environmental stimuli such as VEGF, histamine, thrombin or sheer stress. EC activation occurs in vascular homeostasis during healing/reparation or inflammation events, and leads to EC loss of quiescence and Weibel-Palade Bodies (WPBs) exocytosis. WPBs are endothelial-specific storage organelles whose primary constituent is von Willebrand Factor (vWF). WPBs also contain pro-inflammatory molecules (P-selectin, Interleukin-8) and pro-angiogenic factors such as Angiopoietin-2 (Ang2), a ligand for Tie-2 involved in tip cell migration and endothelium destabilization. EC activation is also the first step of angiogenesis, a process allowing new vessels development from pre-existing ones. However, if the role of WPBs is well studied in contexts of vascular homeostasis, little is known about the role of WPBs in angiogenesis. We aimed to characterize the role of WPBs exocytosis during angiogenesis by using mouse retinal models.

In this model, we observed by vWF immunofluorescence staining that retina vasculature of adult mice contained WPBs in all types of vessels (arteries, veins and capillaries). We studied WPBs behavior during the formation of the vessel network of mouse retina. The density of WPBs in the developing vessel network of P6 mice was heterogeneous; vessels close to the vascular front – that are actively angiogenic and exposed to higher levels of VEGF – contained less WPBs than vessels close to the optic nerve. Targeting VEGF signaling pathway with a chimeric antibody directed against VEGF (VEGF-trap) significantly reduced VEGF-induced WPBs exocytosis in EC, and intravitreal injection of VEGF increased the density of WPBs in remodeling vessels of developing retina. These results suggested that WPBs exocytosis participated in the remodeling process of the vascular network.

We next analyzed the localization of Ang2, and observed that Ang2 co-localized with vWF in EC of the retina vascular network, suggesting that Ang2 is contained in WPBs of the retina vessels. Ang2 is known to regulate pericyte recruitment to vasculature and we showed in vitro that Ang2 silencing in EC was sufficient alone to improve pericyte migration. Similarly to WPBs distribution, vessels close to the optic nerve were more covered by pericytes than vessels close to the vascular front, suggesting a functional correlation between WPBs exocytosis and pericyte recruitment during retina angiogenesis. Therefore we studied pericyte coverage after intravitreal injection of VEGF trap. Inhibition of WPBs exocytosis by VEGF-trap increased pericyte density on vessels close to the vascular front but had no effect on vessels close to the optic nerve.

To conclude, we propose a model in which WPBs act as regulators of angiogenesis in the different regions of developing retina. Vessels close to the vascular front are exposed to high levels of VEGF that induce WPBs exocytosis and Ang2 secretion. The presence of Ang2 inhibits pericytes recruitment to vasculature. On the contrary, vessels close to the optic nerve are less exposed to VEGF. WPBs remain stored in EC and the absence of Ang2 release enables pericytes recruitment to newly formed vessels.

P03 - GATA1 IMPAIRS HAEMATOPOIETIC STEM CELL DEVELOPMENT IN THE ABSENCE OF BLOOD FLOW

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In zebratfish, akin to mammals, the first definitive haematopoietic stem cells (HSCs) egress from the ventral region of the dorsal aorta (DA) endothelium. Blood flow is known to have major influence on endothelial cell (EC) behavior during angiogenesis and in EC maturation. Particularly during HSC formation flow plays a key role by regulating the expression of HSC markers such as the transcription factor cmyb, which is an important regulator of haematopoiesis. Nevertheless, the specific role of blood flow during the initial stages of HSC generation remains uncertain without the identification of the gene targets activated in ECs in response to flow forces. In this work we used EC mRNA expression profiling to demonstrate that the endothelial gene expression network is sensitive to early blood circulation despite the role of blood flow during the initial stages of HSC generation remains uncertain without the identification of the gene targets activated in ECs in response to flow forces. Moreover, the absence of flow leads to a disorganized DA ventral wall containing ECs that are rounded, abnormally expressing gata1 and are often misplacated, invading the lumina. Using fluorescence-activated cell sorting and live imaging we quantified the number of cmyb+ kdrl+ ECs in control embryos, in the absence of flow and in the absence of flow combined with gata1 knockdown revealing that knocking down gata1 in the absence of flow rescues cmyb expression and HSC formation. Altogether these data indicate that blood flow regulates the initial steps of HSC generation by limiting gata1 expression, which in turn seems to repress HSC formation in the absence of flow.
**P05 - OPTIMIZATION OF THE USE OF ANTI-ANGIOGENICS IN GlioBLASTOMA USING MATHEMATICAL MODELING**

Karima EL ALAOUI-LASMAILI1; Jean-Baptiste TYLCZ1; El-Hadi DJERMOUNE1; Noémie THOMAS1; Béatrice FAIVRE1,2
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**Introduction.** Glioblastoma is a highly angiogenic type of tumor and glioblastoma patients have a low survival rate in spite of all the therapeutic procedures unleashed (surgical resection, radio- and chemotherapy). According to the literature, anti-angiogenic therapies such as the anti-VEGF bevacizumab (Avastin®), can temporarily induce changes in the tumor vasculature that result in improved tissue oxygenation which is crucial to the success of oxygen-dependent radiation therapy. Combining bevacizumab and radiation therapy seems to be a prospective way to improve patient survival. However, finding the best treatment sequence is not an easy task to achieve and no consensus has yet been established because of the lack of knowledge regarding the time and duration of the increased oxygenation window provided by bevacizumab. Hence it is indispensable to define the morphological and functional effects of bevacizumab on the tumor vascular network that impact on the oxygenation to find the perfect timing for its association with radiation therapy.

To provide clinicians with tools to determine the best time to treat with radiation after bevacizumab, our research team is developing mathematical models of the tumor vascularization using in vivo data of the tumor vascular network obtained with non-invasive imaging.

**Methods & Results.** Glioblastoma tumor fragments were implanted in skinfold chambers borne by nude mice. The evolution of the vascular network in tumors treated or not with bevacizumab at 10 mg/kg/day was observed using intravital microscopy and analyzed with mathematical algorithms created by our research team to quantify daily the effects of bevacizumab on the vascular network. We showed a significant drop in the vascular density and the appearance of a vascular stabilization in the bevacizumab group that was comforted by immunostaining the blood vessels in formalin-fixed tumors. The vascular density data obtained allowed us to create a prototype behavioral-model of the tumor response that shows a perfect simulation of the experimental data both in the control and treated group (Figure 1).

**Conclusion.** Using our biological data, we present here an original approach to characterize and analyze the anti-angiogenic response with a behavioral-model that will in the future help us predict the therapeutic outcome.

![Figure 1](image_url) — Mice bearing skinfold chambers are treated with an anti-angiogenic drug (bevacizumab, Avastin®) and the vascular network is visualized using intravital microscopy. The images are analyzed with mathematical algorithms to extract the data of the vascular density which is used to create a behavioral-model reproducing the tumor response to anti-angiogenics. *From Tylicz, J.-B.; El-Alaoui-Lasmali, K. et al. Biomed. Signal Process. Control, 2015.*
Three main reasons explain why most of the critical events driving normal and pathological scenarios had been less investigated: they occur rarely in space and time, and will provide important and unexpected insights into the mechanisms of tumor invasion, angiogenesis and metastasis remain to be elucidated. Intravital imaging has opened the door to detection mechanisms at play at the cellular scale allowing the developing endothelium to sense flow-mediated forces. This allowed us to study, in its most details, the highly dynamic, they differ when studied in situ in an entire living organism.

More recently, we developed a multimodal correlative approach allowing us to rapidly and accurately combine functional imaging with high-resolution ultrastructural analysis of invasive and angiogenic tumor cells in a relevant pathological context. Metastasis is the primary cause for cancer-related mortality, but its mechanisms remain to be elucidated. Intravital imaging has opened the door to in vivo functional imaging in animal models of cancer, however it is limited in resolution. Ultrastructural analysis of tumor metastasis in vivo has so far been hindered by the limited field of view of the electron microscope, making it difficult to retrieve volumes of interest in complex tissues. This reliable, versatile, and fast workflow offers access to ultrastructural details of metastatic and stromal cells with an unprecedented throughput and will provide important and unexpected insights into the mechanisms of tumor invasion, angiogenesis and metastasis in vivo.

**P06 - TRACKING RARE AND DYNAMIC EVENTS IN VIVO AT HIGH RESOLUTION USING MULTIMODAL CORRELATIVE MICROSCOPY**

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Three mains reasons explain why most of the critical events driving normal and pathological scenarios had been less investigated: they occur rarely in space and time, they are highly dynamic, they differ when studied in situ in an entire living organism.

Using a correlative combination of live-cell imaging at high temporal resolutions and electron microscopy coupled to electron tomography, we dissected the mechano-detection mechanisms at play at the cellular scale allowing the developing endothelium to sense flow-mediated forces. This allowed us to study, in its most details, the behavior, the nature and the architecture of endothelial primary cilia and how it is capable of relaying the biomechanical information carried by blood flow.

More recently, we developed a multimodal correlative approach allowing us to rapidly and accurately combine functional imaging with high-resolution ultrastructural analysis of invasive and angiogenic tumor cells in a relevant pathological context. Metastasis is the primary cause for cancer-related mortality, but its mechanisms remain to be elucidated. Intravital imaging has opened the door to in vivo functional imaging in animal models of cancer, however it is limited in resolution. Ultrastructural analysis of tumor metastasis in vivo has so far been hindered by the limited field of view of the electron microscope, making it difficult to retrieve volumes of interest in complex tissues. This reliable, versatile, and fast workflow offers access to ultrastructural details of metastatic and stromal cells with an unprecedented throughput and will provide important and unexpected insights into the mechanisms of tumor invasion, angiogenesis and metastasis in vivo.

**P07 - HEPARANASE AND SYNDECAN-4 ARE INVOLVED IN LOW MOLECULAR WEIGHT FUCOIDAN-INDUCED ANGIOGENIC PROPERTIES**

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**Background**

Induction of angiogenesis is a potential treatment for chronic ischemia. Low molecular weight fucoidan (LMWF), a sulfated polysaccharide from brown seaweeds, has been shown to promote revascularization in a rat model, eliciting endothelial colony-forming cells to adopt a proangiogenic phenotype in vitro and increasing angiogenesis in vivo.

**Objectives**

In this study, we hypothesized that LMWF can also modify the amount and the distribution of heparan sulfate (HS) chains exposed at the endothelial cell surface and of two major heparan sulfate membrane proteoglycans, namely the syndecan-1 and -4 (SDC-1 and SDC-4), causing modifications of cell properties related to proangiogenic abilities.

**Methods**

Thus, we have investigated the effects of LMWF on key actors of HS biosynthesis and on SDC-1 and -4 cell expression in vitro and in vivo in rat model of intimal hyperplasia. We have also assessed the impact of these key elements on phenotypic tests (cell migration, spreading and vascular network formation) in human vascular endothelial cell (HUVECs) model.

**Results**

Confocal microscopy showed that LMWF induced the formation of lamellipodia and stress fibers which characterize a migration phenotype and reorganized actin cytoskeleton. In addition, our results showed that LMWF increases HUVEC migration and vascular network formation. Besides, we report that LMWF decreases the amount of HS chains exposed at the membrane associated to lower levels of EXT2 (enzyme responsible for GAG chain elongation). In parallel, the expression and activity of the HS-degrading enzyme heparanase (HPSE) was increased in LMWF-treated cells. The phenotypic tests of LMWF-treated and EXT2- or HPSE-siRNA transfected cells indicated that EXT2 or HPSE expression significantly affect the angiogenic potential of LMWF. In addition, LMWF increased SDC-1, but decreased SDC-4 cell expressions and the effect of LMWF depends on SDC-4 expression. Finally, silencing EXT2 or HPSE leads to an increased expression of SDC-4, providing the evidence that EXT2 and HPSE are involved in the expression of SDC-4.

**Conclusion**

Altogether, these data indicate that EXT2, HPSE and SDC-4 are involved in the proangiogenic effects exerted by LMWF. Significance: HS metabolism changes linked to LMWF-induced angiogenesis offer opportunity for new therapeutic strategies.

**P08 - VASCULAR AND ANGIOGENIC ACTIVITIES OF CORM-401, A REDOX SENSITIVE CARBON MONOXIDE-RELEASING MOLECULE**

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Carbon monoxide (CO), a byproduct of heme degradation by heme oxygenase-1 (HO-1) has emerged as an important signaling molecule with a therapeutic potential in cardiovascular and inflammatory diseases. To exploit the beneficial effect of CO our group has synthesized different CO-releasing molecules (CO-RMs) as pharmacological compounds capable of releasing controlled amounts of CO to biological tissues. Here we report on the biochemical and vascular activities of CORM-401, a new manganese-based metal carbonyl that releases 3 CO/mole with a slow kinetic.

Since manganese is a redox-sensitive metal, we assessed the effect of biologically-relevant oxidants on the release of CO by CORM-401. A spectrophotometric myoglobin assay, where the conversion of ferrous myoglobin into carboxymyoglobin is followed over time, showed that oxidants significantly enhanced the CO release by this molecule. CORM-401 also effectively delivered CO intracutaneously as assessed by COP-1, a CO-sensitive fluorescent probe. CORM-401 induced significant vaso dilatation in rat-isolated aortic rings and promoted endothelial cell (EA.hy926) migration in agarose droplets in a concentration-dependent manner. The addition of H2O2 significantly enhanced the vasodilatory effect of CORM-401 without affecting cell migration. In addition, CORM-401 increased the mRNA expression of several angiogenic factors, including VEGF, IL-8 and HO-1, and increased protein expression of HO-1, NrF2 and activated p38 MAP kinase. In contrast, treatment of endothelial cells with tin protoporphyrin (SnPPIX), an inhibitor of heme oxygenase activity, or SB 203580, an inhibitor of p38 MAP kinase, decreased the migration mediated by CORM-401. These effects were not observed when inactive CORM-401, which is unable to liberate CO, used as a negative control in our experiments.

In conclusion, we report that CORM-401 is a redox-sensitive compound which exerts pronounced vasodilatory effects and promotes marked endothelial cell migration in vitro via liberation of CO. Our data also identify important roles for HO-1 and phosphorylated p38 MAP kinase in mediating the angiogenic effect of CO.
**P09 - BENEFICIAL EFFECT OF ESTROGEN ON LYMPHATIC SYSTEM IS INHIBITED BY HORMONE THERAPY TO PROMOTE LYMPHEDEMA**

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More than 10 percent of breast cancer survivors develop secondary lymphedema within weeks and months after surgery and radiotherapy. Lymphedema refers to a condition of lymphatic dysfunction, which results in a massive fluid and fat accumulation. Although it is a common disabling disease, treatment for this chronic pathology remains limited and largely ineffective.

Here, we developed a new model of mice secondary lymphedema consisting in a mastectomy associated to axillary and brachial lymph nodes dissection. We observed lymphedema formation after 2 weeks associated with a massive dermal lymphatic leakage and lipid accumulation.

To study the effect of estrogen on lymphatic endothelium, mice were ovariecotomized and treated with constant delivery of 17βEstradiol (80mg/kg/d). Surprisingly, we found that estradiol protects from edema and restore lymphatic flow. Estradiol insures a functional lymphatic network by promoting hyaluronan synthesis in the skin in vivo and inducing lymphatic endothelial cells migration, tubulogenesis and filopodia formation in vitro. We found that the effect is dependent of the nuclear receptor ERα, but not beta.

To evaluate the effect of hormone therapy on lymphedema, mice were treated with tamoxifen, an estrogen receptor antagonist and the major therapy for breast cancer. We found that the protective effect of estradiol on lymphatic endothelium is abolished in tamoxifen-constant delivery treatment, but is not affected by bolus injections. We observed a massive disruption of the lymphatic network associated with an inhibition of the lipid drainage function. More importantly, we found that this deleterious effect is associated with a modification of the extracellular matrix content and the lymphatic endothelial cell adhesion and filopodia formation.

In conclusion, this study demonstrates for the first time the beneficial effect of estradiol on lymphatic endothelium. We showed that the hormone therapy abolished this effect by disrupting the lymphatic network and modifying the microenvironment. Our work should thus bring new insights for a better understanding on the lymphedema prevalence and constitute a first step to the treatment of secondary lymphedema.

**P10 - BMP9 AND BMP10 VIA ENDMT INDUCTION ARE NECESSARY FOR PROPER CLOSURE OF THE DUCTUS ARTERIOSUS**

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The transition to pulmonary respiration following birth requires rapid alterations in the structure of the mammalian cardiovascular system. One dramatic change is the closure of the duc tus arteriosus (DA), an arterial connection in the fetus that directs blood flow away from the pulmonary circulation. Failure of its closure at birth is a major cause of mortality, particularly in preterm neonates. Two members of the TGFβ family, BMP9 and BMP10, have been recently involved in postnatal angiogenesis both being necessary for remodeling of newly formed microvascular beds. Both BMP9 and BMP10 are present in blood and their circulating levels are particularly elevated in mice around birth suggesting that they could play a role in pre- and postnatal development. The aim of the present work was to study whether BMP9 and BMP10 could be involved in closure of the DA.

For this, we have used Bmp9-KO mice, which are a neutralizing anti-BMP10 antibody. In mice, DA closure involves a two-step process: (1) functional closure occurs at birth and is followed by (2) anatomical closure which involves a profound remodeling which takes several days. We found that Bmp9 knockout in mice led to an imperfect closure of the DA. Further, addition of a neutralizing anti-BMP10 antibody at postnatal day 1 (P1) and P3 in these pups exacerbated the remodeling defect and led to a re-opening of the DA at P4. Transmission electron microscopy images and immunofluorescence stainings demonstrated that this could be due to a defect in intimal cell differentiation from endothelial to mesenchymal cells (endMT) associated with a lack of extracellular matrix deposition within the center of the DA. This result was supported by the demonstration that BMP9 and BMP10 induce the expression of several genes known to be involved in this process (SNAI1, SNAI2, ZEB2, TWIST1 and FOXC2). The involvement of these BMPs was further supported by human genomic data, as we could define a critical region in chromosome 2 encoding eight genes including BMP10 that correlated with the presence of a patent DA.

This work thus identifies the BMP9/10 pathway in the anatomical closure of the DA.
P11 - BMP9 REGULATES EARLY LYMPHATIC COMPETENCE DURING IN VITRO MOUSE EMBRYONIC STEM CELL DIFFERENTIATION THROUGH MOBILIZATION OF THE CALCINEURIN/NFATC1 SIGNALING PATHWAY

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The lymphatic vasculature is essential for immune surveillance and the maintenance of tissue fluid balance. While several key factors regulating lymphangiogenesis have been identified, those involved in the regulation of the initial steps of lymphatic endothelial development remain largely unknown. Bone Morphogenetic proteins (BMPs) have been characterized as essential factors for early embryonic development and stem cell fate. Some recent data documented a role for BMP2 as a negative regulator of lymphatic specification using zebrafish and mouse models. A role for BMP9 in lymphangiogenesis, lymphatic collecting vessel maturation and valve formation has also recently been established as well as the involvement of its receptor ALK1 in the regulation of mouse postnatal lymphatic vessel development.

Here, we have examined the potential involvement of BMP9 during the first steps of lymphatic endothelial differentiation. We have used an in vitro model based on the coculture of vascular precursor generated by mouse ES (embryonic stem) cell differentiation with OP9 stromal cells, which recapitulates the initial features of lymphatic specification. We found that BMP9 increases the formation of LYVE-1+ cells at concentrations between 0.1 and 0.5 ng/ml. BMP9 induced a functional coupling with the phosphorylation of Smad1,5,8 proteins on ES cell-derived vascular precursors, in accordance with the mobilization of the canonical BMP9 downstream signaling pathway. The quantitative RT-PCR analysis of transcription factors known to be involved in the commitment and the early differentiation into the lymphatic endothelial cell lineage revealed that expression of COUP-TFI, Sox18 and Pirx-1 was unchanged 24h after BMP9 stimulation. In contrast, BMP9 was found to significantly induce NFATC1 expression in vascular precursors. The involvement of the calcineurin phosphatase/NFATC1 signaling pathway was confirmed after addition of cyclosporin-A which inhibited BMP9-induced lymphatic differentiation commitment.

Our results provide evidence for a BMP9 role in the lymphatic endothelial cell fate specification being in large part mediated through calcineurin/NFATC1 signaling. We are currently investigating the receptor subtypes and further molecular mechanisms involved in this process.

P12 - SVEGFR1 A SPLICE VARIANT OF VEGFR1: A DUAL FUNCTION IN THE RESPONSE TO ANTI-ANGIOGENIC THERAPIES IN SQUAMOUS CELL LUNG CARCINOMA

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Vascular endothelial growth factors (VEGFs) and their receptors are primary regulators of physiological and pathological angiogenesis. Although targeting angiogenesis has become a key therapeutic strategy for cancer treatment, trials evaluating anti-angiogenic (AA) therapies have failed to identify any benefits in patients with squamous cell lung carcinoma (SCC). Rather, patients are at higher risk of bleeding complications when exposed to Bevacizumab (BVZ), a humanized monoclonal anti-VEGF-A antibody. The soluble VEGF receptor-1, namely sVEGFR1, is a truncated version of the cell membrane-spanning VEGFR1 that only retains the first six N-terminal Ig-like extracellular motifs of VEGFR1 owing to intron 13 retention and polyadenylation. Interestingly, some recent data highlighted sVEGFR1 as a potential biomarker of response to BVZ treatment in some cancer types, while the molecular bases of such clinical observation remain unexplored. In this study, we investigated the effects of AA therapies on the expression of sVEGFR1 splice variant and its downstream signaling in SCC histological subtype. First, using various SCC cell lines, we showed that BVZ increases the intra- and extra-cellular levels of sVEGFR1. Furthermore, in NCTU-induced SCC tumorgrafts models and as detected by phospho-Smad1 in ES cell-derived vascular precursors, in accordance with the mobilization of the canonical BMP9 downstream signaling pathway. Of note, these increases were never observed in the lung adenocarcinoma histological sub-type treated in the same conditions. Moreover, at the molecular level, we unraveled an original signaling network by which sVEGFR1 negatively but also unexpectedly positively controls a VEGFR1/VEGFR2/VEGF165 autocrine loop leading to either cellular apoptosis or proliferation in SCC cells. Preliminary data indicated that sVEGFR1 is connected with NRP1 and beta-1 integrin to differentially regulate VEGF’s kinase activity, such a network allowing to discriminate between AA-sensitive or -resistant SCC cells. To conclude, our results provide the first evidence that AA therapies control sVEGFR1 expression in SCC cells, while sVEGFR1 exerts a dual function on VEGFR-dependent autocrine loop in this model. These results might help to define SCC patients eligible to anti-angiogenic therapies as well as might help to explain why SCC but not lung adenocarcinoma patients treated with Bevacizumab exhibit severe complications.

P13 - BIOMIMETIC CROSS-LINKING OF TYPE I COLLAGEN HYDROGELS AS BIOMATERIAL FOR ENHANCED TISSUE CONSTRUCT VASCULARIZATION

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Tissue engineering is aimed at repairing and regenerating injured tissue using designed biomaterials able to promote in situ repair through both vascularization and cell delivery. Such tissue constructs should ideally display mechanical properties which support mechanist stress and also preserve their ability to promote capillary formation. One of the strategies to achieve this goal consists in in vitro pre-vascularizing the tissue construct before its implantation in vivo in order to enhance perfusion via promoting anastomoses with the host vasculature. To perform this goal, interest for hydrogels has been growing due to their resemblance to native ECM.

The aim of this study was thus to synthesize a cross-linked type I collagen matrix that would combine optimal handling properties and efficient cell invasion. For this purpose, we favored a biomimetic approach based on the use of lysyl oxidase like 2, a copper-dependent secreted enzyme that physiologically cross-links collagens and elastin. We found that human recombinant LOXL2, purified in our lab, cross-links type I collagen hydrogels and increases their stiffness as measured by oscillatory rheology thereby allowing the synthesis of easily handling hydrogels (Elastic modulus >1kPa). LOXL2 also affects collagen fibrillogenesis as observed using multiphoton microscopy and both second harmonic generation and electron microscopy. Finally, LOXL2-mediated cross-linking of collagen hydrogels improved capillary formation by HUVEC co-cultured with fibroblasts. Altogether, these features result in easy-to-handle collagen hydrogels, obtained by LOXL2 cross-linking, that show better vascularization in vitro and in vivo.

In conclusion, we were able to synthesize collagen hydrogels with tunable stiffnesses, biomimetically cross-linked by LOXL2. These hydrogels display increased angiogenic properties in vitro and in vivo. They thus constitute promising biomaterials for fast vascularization in tissue engineering applications.
P15 - NUCLEOLIN ANTAGONIST PEPTIDE N6L IMPAIRS THE PROGRESSION OF PANCREATIC DUCTAL ADENOCARCINOMA AND PROMOTES TUMOR VESSEL NORMALIZATION THROUGH ANG-2 INHIBITION

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Background: Nucleolin is a nuclear protein regulating ribosome and cell cycle progression, and is overexpressed in tumor cells. Shuttling to the cell surface of cancer cells and tumor vessels. Nucleolin is a marker of neoplastic tissues constituting an interesting target for cancer therapy. Recently, we developed a family of nucleolin antagonist pseudopeptides (NucANT). One of these molecules, the N6L peptide, strongly inhibits human tumor growth by inducing apoptosis of tumor cells. Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal human malignancies, and one of the explanation of the failure of the treatments, is the lack of efficacy of tumor vascularization to deliver drugs to tumors. Stemming from these findings, we sought to investigate whether targeting nucleolin, by N6L, on both cancer cells and tumor vasculature could represent a promising new strategy for the treatment of PDAC.

Methodology/Findings: In this study we used mice model of oxygen-induced retinopathy (OIR) to explore the involvement of Fzd7 during initial vaso-obliterration (VO) and subsequent neovascularization (NV) phases. First we observed that Fzd7 was expressed in pathologic neovessels. Second, by transgenic approaches, we observed that specific deletion of fzd7 in the endothelium (fzd7cko) resulted in increased retinal tissue sensitivity to hypoxia during the vaso-obliterative phase of the OIR model. Moreover, fzd7 deletion in EC reduced the ectopic growth of pathologic neovessels into the vitreous during the second phase of the OIR model demonstrating that Fzd7 was involved in pathologic neovascularization formation in mice retina after ischemia. To determine the molecular mechanisms by which Fzd7 may regulate ischemic retinopathy, we explored canonical -catenin and Notch signaling during the two different phases of OIR. Preliminary results showed that transcript expressions of lef1 and axin2 and partners of Notch signaling were strongly decreased in fzd7cko retina mice as compared to control after OIR.

Conclusion: By controlling pathogenesis of ischemic retinopathy, Fzd7 could be an efficient and specific therapeutic target to develop anti-angiogenic drugs in the treatment of ischemic retinopathies.

P16 - ANTI-ANGIOGENESIS THERAPIES INDUCES AN INCREASE OF VEGFC IN RCC

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The first line treatment for renal cell carcinoma (RCC) is currently an anti-angiogenesis targeted therapy, the tyrosine kinase inhibitor sunitinib. Despite an increase in the time to progression, patients ineluctably relapse. We hypothesized that the development of a lymphatic network is at the origin of the progression observed after an average of eleven months. Indeed, the lymphatic vessels induce dissemination of the most aggressive tumor cells allowing the development of new metastatic niches. Sunitinib is supposed to act on endothelial cells but tumor cells expressed VEGF receptors, its major targets. Hence, this aberrant expression resulted in adaptation of tumor cells that developed new ways to survive and to disseminate in the presence of the drug. We observed that sunitinib stimulated the expression of VEGFC, the main growth factor for lymphatic endothelial cells, in RCC cell lines and in primary RCC cells. We showed that sunitinib: i) stimulated the transcription of the VEGFC gene through activation of the transcription factor NFkB and ii) increased VEGFC mRNA half-life. Sunitinib activated p38 MAP Kinase resulting in up-regulation/activity of the AU-rich element binding protein HuR. In parallel, p38 phosphorylated and inactivated the AU-rich binding protein tristetraprolin, the natural competitor of HuR that further participates in VEGFC mRNA stabilization. In experimental tumors in mice, sunitinib stimulated VEGFC expression and the development of lymphatic vessels. Therefore, we showed for the first time that the treatment of reference for RCC was at the origin of the development of an alternative vessel network that is probably responsible of the patients’ relapse. Our results paved the way toward new therapeutic associations targeting both the vascular and the lymphatic systems.
P17 - RESISTANCE TO SUNITINIB IN RENAL CLEAR CELL CARCINOMA RESULTS FROM SEQUESTRATION IN LYSOSONES AND INHIBITION OF THE AUTOPHAGIC FLUX

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Metastatic renal cell carcinomas (mRCC) are highly vascularized tumors that are a paradigm for the treatment with anti-angiogenesis drugs targeting the Vascular Endothelial Growth Factor (VEGF) pathway. The available drugs increase the time to progression but are not curative and the patients eventually relapse. In this study we have focused on the attention on the molecular mechanisms leading to resistance to sunitinib, the first line treatment of mRCC. Because of the anarchic vascularization of tumors the core of mRCC tumors receives only sub-optimal concentrations of the drug. To mimic this in vivo situation, which is encountered in a neo-adjuvant setting, we exposed sunitinib-sensitive mRCC cells to concentrations of sunitinib below the concentration of the drug that gives 50% inhibition of cell proliferation (IC50). At these concentrations, sunitinib accumulated in lysosomes, which down-regulated the activity of the lysosomal protease cathepsin B (CTSB) and led to incomplete autophagic flux. Amino acid deprivation, which initiates autophagy enhanced sunitinib resistance through the amplification of auto-lysosome formation. Sunitinib stimulated the expression of the ATP binding cassette, sub-family B (MDR/TAP), member 1 (ABCB1), which participates in the accumulation of the drug in auto-lysosomes and favor its cellular efflux. Inhibition of this transporter by elacridar or the permeabilization of lysosome membranes with Leu-Leu-OMethyl (LLOM) re-sensitized mRCC cells that were resistant to concentrations of sunitinib superior to the IC50. Proteasome inhibitors also induced the death of resistant cells suggesting that the ubiquitin-proteasome system compensates inhibition of autophagy to maintain a cellular homeostasis. Based on our results we propose a new therapeutic approach combining sunitinib with molecules that prevent lysosomal accumulation or inhibit the proteasome.

P18 - FGF-2 PRIMING OF DENTAL PULP STEM CELLS IMPROVES THEIR ANGIOGENIC POTENTIAL THROUGH INCREASED RELEASE OF HGF AND VEGF

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One of the major hurdles of tissue engineering is rapid perfusion of the tissue constructs for providing nutrients and oxygen to implanted stem cells (SC). Whereas the angiogenic potential of various MSC has been extensively described, strategies to accelerate and increase angiogenesis are still in development. Here, our aim is to characterize and improve the angiogenic potential of Dental Pulp Stem Cells (DPSC), a promising cell type for tissue regeneration.

Using 3D co-culture models of angiogenesis, we demonstrated that DPSC are able to promote capillary network formation in hydrogels. We identified HGF and VEGF as the two major cytokines released by DPSC, using antibody arrays. We found that whereas hypoxia stimulated only VEGF release, FGF-2 increased both cytokines and limited the inhibition of HGF induced in hypoxia. Further analysis in the co-culture model using conditioned media demonstrated that :

- i) HGF directly targets endothelial cells and
- ii) HGF improves VEGF-induced capillary growth but has no effect on its own.

In order to investigate DPSC-induced angiogenesis in vivo, we subcutaneously implanted collagen hydrogels on the back of SCID mice. These implants were cellularized with DPSC that had been preconditioned by either hypoxia or FGF-2. Capillary formation was analyzed in vivo by micro-CT after IV injection of contrast agent at 4 weeks post-implantation. Vascularization was also quantified by immunohistochemistry using vascular markers. The angiogenic potential of DPSC was further increased by both primings, with FGF-2 generating more numerous and larger vessels compared to hypoxia. Altogether, these results showed that priming DPSC with FGF-2 enhances vascularization in engineered tissue through high release of both HGF and VEGF. Furthermore, our in vitro experiments suggest that DPSC could be used for prevascularizing tissue constructs before implantation. Combining both strong angiogenic potential and differentiation capacity, makes DPSC great candidates for SC-based regenerative medicine therapies.
P19 - EMPLOYING MULTIPLE APPROACHES TO IMPAIR Glioblastoma INITIATING CELL SURVIVAL

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Glioblastoma multiforme (GBM) is the most aggressive cancer of the central nervous system, which is associated with rapid vascular proliferation and an extremely poor prognosis. A subpopulation of cells within GBM has been identified that reside in close proximity to the brain vasculature and possess the characteristics of progenitor cells such as self-renewal and multipotency. Glioblastoma initiating cells (GICs) are a relatively quiescent population of cells, the survival of which may be regulated by external cues from endothelial cells. GICs have the potential to evade current treatments, as conventional therapies predominately target rapidly dividing cells. Consequently, it has been suggested that GICs are responsible for therapeutic resistance and tumour recurrence in GBM. As such, identifying different approaches to target resistant GICs is of great importance.

In order to investigate the potential of GICs as a potential therapeutic target in GBM, we have employed two different methods for GICs intervention. Firstly, to directly target the GICs themselves by employing a large-scale chemical screen of 1280 FDA and EMEA approved drugs on patient derived GICs to identify potential therapies. The second approach involves pharmacologically blocking the endothelial-secreted factors that promote GIC survival in an in vivo xenograft model of GBM.

The initial screen of 1280 compounds resulted in 9 hits with >40% inhibition of GIC viability in two patient derived GIC lines. This compound resulted in a significant (p<0.001) reduction in GIC viability, impaired neurosphere formation and increased apoptosis in vitro. Alternatively, targeting the action endothelial secreted factors on GICs in vivo resulted in increased survival in GIC bearing mice.

These preliminary findings indicate that we have identified a compound that has the ability to directly target GIC survival in vivo. Additionally, blocking endothelial-secreted factors with an alternate compound impairs disease progression in vivo. Further studies are required to fully elucidate the molecular mechanisms by which these compounds are acting on GICs. However, these results indicate that multiple approaches to targeting GICs may be beneficial in the treatment of GBM.
P22 - ENDOTHELIAL PROGENITOR CELLS AS A PREDICTIVE MARKER OF THE RESPONSE TO NEOADJUVANT SUNITINIB IN METASTATIC RENAL CELL CARCINOMA (MRCC) PATIENTS (PREINSUT TRIAL)

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Sunitinib is approved in first-line therapy for mRCC, but nephrectomy remains recommended in these metastatic patients. The PREINSUT trial was conducted to evaluate sunitinib as a neoadjuvant in mRCC patients prior to nephrectomy, and determine biological, pathological and imaging markers of response.

Patients were treated with two cycles of sunitinib (50 mg 4/6 weeks) before nephrectomy. Biological parameters measurement and perfusion CT were performed on the first day and at the end of each cycle of treatment. Among the biomarkers studied, circulating endothelial progenitor cells (CD34+/CD45-/CD146+, EPCs) were quantified by flow cytometry. Correlations between EPCs, size reduction of the primary renal tumor under therapy and perfusion imaging parameters were studied using a Spearman correlation coefficient ($r$).

The level of EPCs at baseline were inversely correlated with the size reduction of the tumor ($n=12$, $r=-0.762$, $p=0.004$). Interestingly, EPC level at baseline were positively correlated with microvessel permeability as measured by perfusion imaging ($r=0.8$, $p=0.0096$).

A high level of EPCs could reflect an active tumor angiogenesis, characterized by a higher permeability of tumor vessels. From these results, EPCs could be an early predictive marker of the final 3-months response of the renal tumor to sunitinib.

P23 - THE PREVALENCE OF OVARIAN VARICES IN PATIENTS WITH ENDOMETRIOSIS

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Endometriosis and ovarian varices manifest with similar symptomologies and the hormone estradiol, oxidative stress, and angiogenesis are implicated in both. We decided to investigate the possible association between them.

The sample consisted of 48 female patients between the ages of 18 and 50 years. A total of 25 patients had been diagnosed with endometriosis, including 15 confirmed by surgery and histopathology and 10 confirmed by nuclear magnetic resonance. There were also 23 patients without endometriosis who were placed in a control group.

The prevalence of ovarian varices in the endometriosis patients was 80%, whereas that in the controls was only 26.1%. The elevated percentage of ovarian varices in the endometriosis patients was highly significant, with a difference of 53.9% and a 95% confidence interval of 30% to 78%. The criterion for significance was set at $p<0.05$. Statistical analysis was performed using SAS statistical software, version 6.11 (SAS Institute, Inc., Cary, North Carolina).

Varices in the lower limbs are associated with OS, leucocyte adhesion, endothelial dysfunction, and can cause alterations in the microcirculation of the skin, resulting in chronic venous insufficiency with hyperpigmentation, eczema, lipodermatosclerosis and ulcerations (69). We also found tissue damage provoked by varices in the scrotal sack. Varicocle in men is associated with OS, endothelial dysfunction, DNA fragmentation of the sperm, alterations in hormonal production and an imbalance in the function of the hypothalamic-pituitary-testes axis and even atrophy of the testicle (39,40).

It is possible that ovarian varices as well provoke OS, endothelial dysfunction, and alterations in the formation of the follicles and oocytes, causing an imbalance in the genetic, hormonal, and immunological composition of the ovary. The high prevalence of ovarian varices in patients with endometriosis suggests that more studies be carried out in order to evaluate the role of pelvic varicosce veins in infertility and endometriosis.

P24 - A NEW MODEL FOR CXCR3 ACTIVITY AND TRAFFICKING: ROLE OF LRP1 IN INTERNALIZATION AND RECYCLING

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CXCR3 belongs to the G-protein-coupled receptors (GPCRs) and plays important roles in development, angiogenesis, inflammation and cancer. CXCR3 has been reported as a functional receptor for CXCL4 and its variant CXCL4L1 by mediating their angiostatic activity. However, the interaction with CXCR3 is rather complex due to the presence of three distinct spliced isoform: CXCR3-A, CXCR3-B and CXCR3-alt. Depending on the CXCR3 isoform expressed in the cells, ligands have opposing effects. CXCR3-A is reported to regulate cell proliferation, survival and migration while CXCR3-B mediates growth-inhibitory activity. Another receptor for CXCL4 is the low-density lipoprotein receptor-related protein 1 (LRP1), an endocytic receptor reported to be involved in physiological processes such as vascular development, but also in critical pathological situations, including cancer and neurological disorders. Our aim is to determine the mechanisms of action and the impact of CXCL4 and CXCL4L1 and their receptors in the signaling of tumor cells. Using HeK-293 and glioblastoma cells overexpressing CXCR3-A or CXCR3-B, we demonstrate an interaction between CXCR3 and LRP1 and show that LRP1 plays a key role in the localization, trafficking and activity of CXCR3. Both isoforms of CXCR3 are rapidly internalized into EEA1-positive endosome, where LRP1 is enriched, and are recycled in a clathrin-dependent manner and through the trans-golgi pathway. We thereby demonstrate that both CXCR3-A and CXCR3-B signal to the ERK and PI3K pathways. Thus, segregation of CXCR3 isoforms into distinct endocytic compartments regulates CXCR3 activation and receptor turnover and is dependent of LRP1 in glioma cells.
P25 - CROSSTALK BETWEEN VEGF AND BMP9 SIGNALING PATHWAYS

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Hereditary Hemorrhagic Telangiectasia HHT (or Rendu-Osler syndrome) is a genetic disease causing severe nosebleeds, telangiectasias, arteriovenous malformations and ultimately cardiac dysfunction. HHT1 is due to mutations of ACVRL1 encoding Activin receptor-Like Kinase-1 (ALK1) whereas HHT2 is due to mutations of ENG encoding Endoglin. ALK1 is a BMP (Bone Morphogenetic Protein) receptor specifically expressed on endothelial cells, whose physiological ligands, BMP9 and BMP10 have been identified by our team in 2006. Endoglin is a co-receptor for these ligands that potentiates the intracellular signaling triggered by BMP9 and BMP10. Ligand binding stimulates intracellular Smad1/5/8 phosphorylation, association of these phospho-Smads with Smad4 and nuclear translocation of the complex. This Smad complex binds Smad response elements on the promoters of target genes and promotes transcription in association with other transcription factors. Interestingly, patients treated with anti-VEGF antibody (AvastinTM) show an impressive decrease of their symptoms, suggesting that the BMP9/ALK1/Endoglin pathway crosstalks with the VEGF pathway.

In this context, we decided to analyze this crosstalk in vitro on primary endothelial cells. We treated human endothelial cells from umbilical vein overnight with BMP9 (1 ng/ml) or VEGF (40 ng/ml) and then stimulated the cells respectively with VEGF or BMP9 for 15min. Activation of both signaling pathways was then analyzed by Western blots. Our first results show that the VEGF-induced phosphorylations of p38MAPK and p42/p44MAPK are decreased after an overnight treatment by BMP9. The ratio of phospho-VEGR2 (phosphosite 1175)/VEGFR2 was not altered after BMP9 treatment. Further characterization of the mechanism of this cross-regulation is ongoing and will be presented on the poster.

P26 - MYOFERLIN: AN INDISPENSABLE COMPONENT IN VEGF-A SECRETION BY PANCREAS CANCER CELLS

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Ferlin family proteins have been reported to participate in plasma membrane fusion, repair, and endocytosis concretely but not exclusively, it has been reported in skeletal muscle development and repair (myoferlin and dysferlin) and presynaptic transmission in the auditory system (otoferlin). While some reports have implicated a member of ferlin family proteins, myoferlin, in cancer; the extent of its expression in and contributions to cancer are not well established.

Myoferlin, a member of the ferlin protein family was recently identified in our laboratory as a new accessible biomarker for human pancreatic ductal adenocarcinoma. In addition to its potential suitability for targeted therapy, we aim to examine the biological role of this protein in the development of pancreatic cancer. SiRNA-mediated myoferline-silencing significantly reduced the volume of BxPC-3 tumors developed onto the chorioallantoic membrane of fertilized chicken eggs. Intriguingly, aside their reduced volume, myoferlin-silenced tumors appeared whitish and exhibited a significant decrease of blood vessel density as shown by FITC-conjugated Sambucus nigra agglutinin staining. This observation suggested that, in addition to an inhibition of BxPC-3 cell growth after myoferlin silencing, this protein may exhibit a pro-angiogenic activity. Accordingly, we next showed that myoferlin-silencing significantly inhibited VEGF-A secretion without decreasing VEGF-A gene expression. Western blotting and Immunofluorescence reveal that VEGF-A seemed to accumulate in the cytosol in the vicinity of the plasma membrane. Knowing the previously reported role of myoferlin in membrane fusion processes, we proposed that myoferlin could play an important role in VEGF-A secretion by BxPC-3 cells. Currently, exocytosis and exosomes pathways have been explored showing, in BxPC-3 cells, that VEGF-A seems to be secreted by an alternative myoferlin-dependent pathway.

P27 - THE HYPOXIC RESPONSE IN NATURAL KILLER CELLS: LINKING IMMUNE SURVEILLANCE AND TUMOR ANGIOGENESIS

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Hypoxia-inducible transcription factors (HIFs) are central mediators of cellular adaptation to low oxygen and play a pivotal role for the inflammatory response in cells of the innate and adaptive immune system. Natural Killer (NK) cells, blurring the borders between innate and adaptive immunity, are a subset of cytotoxic innate lymphoid cells. In addition to their ability to secrete Interferon (IFN-)γ to kill «aberrant» cancer cells while sparing «normal» cells. Owing to these unique features, NK cells are able to restrict primary tumor growth and limit metastatic spread. By genetic targeting HIFs in NK cells, we define a crucial role of HIF-1α in NK cell function and the immune surveillance of circulating tumor cells. Furthermore, we show that NK cells preferentially infiltrate into hypoxic zones of solid primary tumors and that HIF-1α-deficiency in NK cells rather slowed down the growth of primary tumors. Surprisingly, this was largely due to decreased HIF-dependent expression of various angiostatic factors within the tumor microenvironment, resulting in unproductive tumor angiogenesis, characterized by immature and non-functional vessel, severe hemorrhage and increased tumor hypoxia. This suggests that the hypoxic response in NK cells slows down overall angiogenesis in order to allow for vessel formation in a more coordinated fashion.

In summary, we define HIF-1α as a critical mediator of NK cell effector function and cancer immune surveillance. Secondly, show that HIF-1α in NK cells acts as a negative regulator of tumor angiogenesis that ensures the fine tuning of the angiogenic response. These results indicate that exploiting HIF signaling in NK cells may represent a novel therapeutic avenue.
P28 - LYMPHOCYTE INFILTRATION IS INCREASED UNDER LOW DOSE SUNITINIB WITHOUT TUMOR VESSEL NORMALIZATION
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To develop, tumors need to induce vascularization and to escape immunity. However, tumor angiogenesis results from an imbalance in favour of proangiogenic factors, leading to an abnormal vascularization with structural disorganization and loss of vessel functionality. This finally contributes to the development of hypoxia within tumors. Tumor vascular normalization has been described with low dose of antiangiogenic treatments, like anti-VEGF receptor. These drugs can also decrease tumor immunosuppression. To assess the potential link between vascular normalization and the increase of immune response, we assessed tumor vasculature and lymphocyte infiltration in an in vivo renal cell carcinoma tumor model.

Tumor cells from murine renal cell carcinoma (RENCA) were injected subcutaneously in mice flank. After tumor size had reached 10 mm², mice were daily treated with sunitinib, a tyrosine kinase inhibitor, at 40 mg/kg, 10 mg/kg or with PBS-DMSO, by oral gavage. Tumor volume was measured twice a week. At days 4 and 11, tumors were frozen. Lymphocyte infiltration and vessel characterization, e.g. vascular density and pericyte coverage, were assessed by immunofluorescence staining.

Tumor growth was significantly reduced after 11 days of treatment both in mice treated with 10 and 40 mg/kg of sunitinib (p<0.01 and final volume of 190, 113 and 96 mm³ for PBS-DMSO, sunitinib 10 and 40 mg/kg respectively). Vascular density were significantly decreased at both day 4 and 11 with sunitinib 40 mg/kg (p<0.01). In this group, pericyte coverage seemed increased at day 4 but was significantly higher than other groups only at day 11 (p<0.01). CD8+ and FoxP3+ cell infiltration were significantly higher in mice treated with sunitinib 10 mg/kg, from day 4 to day 11 (with p<0.05 for both).

In RENCA tumors, sunitinib delayed tumor growth, even at low dose. Interestingly, lymphocyte infiltration was increased with low dose of sunitinib, without modification of pericyte coverage.

P29 - HUMAN ANGIOGENIC MONOCYTES RECRUITMENT ACUTE VERSUS TUMOR-RELATED INFLAMMATION
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Human angiogenic monocytes are part of resident monocytes characterized by Tie2 expression, essential for solid tumor growth, angiogenesis and refractoriness to anti-angiogenic therapies. However, the actual subset of resident monocytes exhibiting proangiogenic activity as well as their recruitment mechanisms remain elusive. Herein, we show that non-classical or patrolling monocytes constitute a proangiogenic monocyte subpopulation able to spontaneously secrete high amount of matrix metalloproteinase 9, which activation induces extracellular matrix proteolysis, the release the matrix-bound growth factors including VEGF that activate endothelial cells and lead to angiogenesis through MAP kinase and PI3K-dependent cell sprouting, proliferation and survival. Interestingly, patrolling monocytes that we found to be angiogenic were previously reported to remain on endothelial luminal surface and patrol without transmigration through quiescent or inflamed endothelium. We investigated how these angiogenic monocytes are recruited by developing a time-lapse imaging assay of monocyte recruitment under flow. Therefore we defined two main inflammations namely acute inflammation, induced by inflammatory cytokines such as TNFa or IFNg, and tumor-related inflammation, which is induced by inflammatory cytokines combined to angiogenic factors. We found that in acute inflammatory conditions, activated endothelium allows angiogenic monocyte adhesion, crawling on endothelial luminal surface but not transendothelial migration under flow. Conversely in tumor-related inflammatory conditions, endothelium activation status allows angiogenic monocyte transendothelial migration in addition to adhesion and crawling. We report for the first time, conditions for angiogenic monocyte transmigration and show that they exclusively transmigrate in tumor-related inflammatory conditions, constituting a milestone toward the development of new therapeutic strategies in targeting solid tumor inflammation.

P30 - ROLE OF MACROPHAGE SUBSETS IN THERAPEUTIC ANGIOGENESIS VS. LYMPHANGIOGENESIS
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Macrophages play a decisive role during pathophysiological angiogenesis but also lymphangiogenesis. Here, we investigated whether macrophages may similarly modulate vascular responses to targeted growth factor therapy. Mouse matrigel plug assay and rat myocardial infarction (MI) model were used to assess angiogenic therapy with either VEGF-A or the combination of FGF-2 with HGF (F+H). Growth factors were delivered locally via albumin-alginate microcapsules. Blood vessel and lymphatic vessel densities, and infiltration levels of classical M1-type and alternative M2-like (CD206/MRC-1+) macrophages were assessed. Clodronate was used to prevent macrophage recruitment, and the VEGFR2 blocking antibody, DC101, to prevent VEGF-A signaling.

We found that the combination therapy (F+H) led to increased M2-like macrophage infiltration in matrigel plugs vs. control and VEGF-A plugs, correlating with angiogenic and lymphangiogenic responses. In contrast, VEGF-A preferential recruited M1-type macrophages and showed much weaker angiogenic and lymphangiogenic effects as compared to F+H in this model.

In agreement with a direct role of M2-like macrophages in F+H-induced vessel growth, clodronate radically decreased angiogenesis as well as lymphangiogenesis. Further, DC101 reduced F+H-induced angiogenesis, without altering macrophage infiltration, revealing macrophage-derived VEGF-A as a crucial determinant of tissue responsiveness. In contrast, F+H-induced lymphangiogenesis was largely unaffected by blockage of VEGFR2. In the rat heart, we found increased cardiac M2-like macrophage infiltration following F+H therapy post-MI, with strong correlation between macrophage levels and the arteriogenic response. However, the cardiac lymphangiogenic response was not altered.

In conclusion, M2-like macrophages play a decisive role, linked to VEGF-A production, in regulation of tissue responsiveness to angiogenic therapies including the combination of F+H. Similarly, tissue macrophages are actively involved in the lymphangiogenic response to F+H therapy.
P31 - BOOSTING THE HYPOXIC RESPONSE IN SCAR-ASSOCIATED MYELOID CELLS ACCELERATES THE RESOLUTION OF LIVER FIBROSIS

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We have recently shown that targeting Vascular Endothelial Growth Factor (VEGF) specifically in scar-infiltrating myeloid cells prevented remodeling of the resolution of murine liver fibrosis. Furthermore, we showed that the resolution of liver fibrosis is associated with increased expression of Matrix Metalloproteases (MMP)-2 and -14 as well as decreased expression of Tissue Inhibitor of Metalloproteases (TIMP)-1 and 2 confined to sinusoidal endothelium, thereby unmasking an unanticipated link between angiogenesis and resolution of fibrosis. In a gain of function approach, we wanted to test the impact of VEGF overexpression in myeloid cells on fibrosis. We observe that genetic inactivation of the von Hippel Lindau protein (VHL), a negative regulator of Hypoxia-inducible factors (HIF) in myeloid cells, leads to increased VEGF expression and most importantly, accelerated matrix degradation and fibrosis resolution after CCL4 challenge. This association is enhanced with reduced expression of MMP-2 and -14 in liver endothelial cells and improved sinusoidal infiltration of the fibrotic scar. In addition, we report overall increased expression of MMP-7, -9 and -13 as well as improved liver regeneration upon ablation of VHL in myeloid cells.

Discussion: Boosting the hypoxic response, HIF signaling and VEGF release in scar infiltrating myeloid cells could: represent a promising therapeutic avenue for the treatment of liver fibrosis.

P32 - BIOLOGICAL OUTCOME AND MAPPING OF FACTOR CASCADES IN RESPONSE TO HYPOXIA DURING REGENERATIVE ANGIOGENESIS IN ZEBRAFISH

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Hypoxia-based angiogenesis is emerging as a new therapeutic approach since it constitutes the primary angiogenic stimulus. However, the complex factor cascades induced by hypoxia are currently incompletely mapped and the biological outcome and side effect of hypoxia-based treatments remain unknown. Here we investigate the impact of hypoxia on vascular regeneration and provide tools for strategies aiming to use hypoxia-based angiogenesis, as a therapeutic approach. Using the Tg(fli1:EGFP) zebrafish fin regeneration model, vessels regeneration, and tissue remodeling were analyzed under normoxic and hypoxic conditions. Gene expression arrays combined to gene functional enrichment analysis were used for Mapping of Factor Cascades in Response to Hypoxia, Regeneration or both. Following fin amputation, exposure to hypoxia prevents vessel differentiation by maintaining the vascular plexuses of regenerating vasculature in an immature state. Gene expression arrays combined to gene functional enrichment analysis revealed that regenerative processes control organogenesis and development by regulating exclusively the expression of genes involved in development, cytoskeleton and cell cycle and motion. Hypoxia in turn, regulated expression of genes associated with cell stress response and cellular homeostasis, highlighting the pleiotropic effect for hypoxia. The function clusters that were common to hypoxia and regeneration were linked to DNA replication, proteasome and cytoskeleton. Finally, regeneration under hypoxia involved genes associated with cell differentiation, consistent with the immature vascular plexuses in hypoxic regenerates. Our data improved the biological understanding of how hypoxia impacts on regenerative angiogenesis and provide a framework to develop gene network leading to regenerative processes under hypoxic conditions. These findings may also serve to strategies aiming to induce hypoxia-induced signaling directly through pharmacological means for possible shift into clinical trials.

P33 - AUTOPHAGY IS REQUIRED FOR MAINTENANCE OF ENDOTHELIAL FUNCTION & SLOWS THE DEVELOPMENT OF DIABETIC NEPHROPATHY

LENOIR Olivia1; BEAUJEAN Céline6; Cdh5.Cre-Atg5lox/lox: We generated mice with an endothelial-specific disruption of the autophagy pathway using the Cre-lox system (Cdh5.Cre-Atg5lox/lox). We assessed blood pressure (BP) via tail cuff plethysmography, cardiac structure and function by Doppler ultrasound and end-organ injury. Endothelial function was assessed ex vivo in isolated mesenteric arteries and in vivo using cremaster arterial intravital microscopy and by Doppler ultrasound. We then induced diabetic nephropathy (DN) – a model characterized by endothelial dysfunction and microvascular injury – in Cdh5.Cre x Atg5lox/lox and control mice.

Results: At baseline, adult Cdh5.Cre. Atg5lox/lox mice had an elevated BP associated with increased peripheral vascular resistance, especially renal vascular resistance, compared to littermate controls. Therefore, we investigated endothelial function. Whereas, endothelial-dependent and endothelium-independent vascular function was similar between Cdh5.Cre. Atg5lox/lox and controls in isolated mesenteric arteries ex vivo, flow mediated vasodilatation was impaired in Cdh5.Cre x Atg5lox/lox mice as assessed by ultrasound-Doppler measurement of hypercapnia-induced basilar artery vasodilatation. In addition, we found reduced capillary density within the kidneys and heart of Cdh5.Cre. Atg5lox/lox mice, possibly further contributing to increased vascular resistances. Disturbed endothelial function and increased peripheral vascular resistances were accentuated upon streptozotocin-induced diabetes mellitus. Likewise, genetic deletion of endothelial cell autophagy dramatically sensitized mice towards the development of DN with Cdh5.Cre. Atg5lox/lox mice demonstrating not only glomerular endothelial cells ultrastructural defects but also accelerated glomerulosclerosis with albuminuria, podocyte foot process effacement and thickening of the glomerular basement membrane when control mice showed only minor glomerular lesions. Finally, we found that endothelial autophagy is also required for recovery after myocardial infarction (MI). Indeed, Atg5 selective-endothelial invalidation leads to a decrease of the left ventricular fractional shortening after MI.

Discussion

These results support the important role of endothelial cell autophagy in maintaining endothelial function and regulating the development of DN. Autophagy may represent a potentially novel therapeutic target for DN.
**P34 - INHIBITION OF SEMAPHORIN-3A AS A NOVEL STRATEGY FOR THERAPEUTIC ANGIOGENESIS AND NERVE REGENERATION**

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The class 3 semaphorins are secreted molecules known to control axonal guidance. In addition to its inhibitory effect on nerve regeneration, Semaphorin-3A (Sema-3A) has been shown to hamper angiogenesis in tumor models. The objective of this study was to elucidate the role of Sema-3A in post-ischemic revascularization and axon regeneration.

Control and type 1 diabetic (i.e. injection of streptozotocin) C57Bl6 mice were submitted to surgical ligation of femoral artery (n=14 per group). Sema-3A protein levels peaked at day 4 after the onset of ischemia in both skeletal muscle and sciatic nerve in reference to that of non ischemic leg. In addition, Sema-3A content was higher in diabetic mice when compared to control mice whatever the time point after ischemia. Expression of the Sema-3A receptor, Neuropilin-1, was unaffected by ischemia but was reduced by 40% in diabetic mice (p<0.01). In contrast, protein levels of the other Sema-3A receptor, plexin-A1, were up regulated mainly at day 7 after ischemia in both control (1.41-fold) and diabetic (2.06-fold) animals. Treatment with the Sema-3A inhibitor, SM-345431, increased foot perfusion (1.46-fold), angiographic score (1.52-fold) and capillary density (1.39-fold), when compared to PBS-treated animals (p<0.05 for each parameter). SM-345431 increased small vessels number in the sciatic nerve and improved the regeneration of small nerve fibers in the skin. Behavior tests revealed an increased in the motor score after treatment with Sema-3A inhibitor. Conversely, SM-345431 had no significant effects on large nerve fibers. Interestingly, SM-345431 also improved post-ischemic revascularization in diabetic mice compared to untreated diabetic animals. This effect was not related to alterations in the immune-inflammatory reaction.

These results indicate that inhibition of Sema-3A may be a novel strategy for therapeutic angiogenesis and nerve regeneration in the setting of ischemia.

**Key words:** Sema-3A; Ischemia; Angiogenesis; Nerve regeneration

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**P35 - CHARACTERIZATION OF PROANGIOGENIC POTENTIAL BY HIGHLY SULFATED FUCOIDAN: ROLE OF THE CHEMOKINES AND THE GLYCOSAMINOGLYCANS**

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Fucoidans are natural sulfated polysaccharides used as glycosaminoglycans (GAG) mimetics. Both GAGs and chemokines are important to regulate angiogenesis and wound healing. Fucoidans interacts with pro-angiogenic chemokines, such as RANTES/CCL5 and SDF-1/ CXCL12, leads to revascularization (Luyt et al., 2003) and increases endothelial cell migration (Hlawaty et al., 2011). However this pro-angiogenic activity remains unclear and depends of the type of fucoidan. The lack of treatment regenerating the vasculature after ischemic event let the opportunity to develop innovative vascular therapies based on well characterized GAG mimetic.

Upstream of developing a tissue engineering therapy, we propose to understand the beneficial action of fucoidans on angiogenesis by a structure-function study. We hypothesize that depending on their size and sulfation level, fucoidans could regulate the chemokines affinity and modulate the pro-angiogenic response.

We purified and characterized fractionated-fucoidans according to their sulfuration rate. We measured their affinity to chemokines (Surface Plasmon Resonance) and their functional effects on endothelial cells migration and microvascular network formation. We also analyzed these effects on endothelial cells depleted in endogenous GAG expression. Finally we studied the fucoidan-induced mechanisms such as the signalling pathways involved in angiogenesis, the localization of a small fluorescent fucoidan in endothelial cells in kinetic and its endophytic pathway.

The structural analysis of fractionated-fucoidans (5-27kg/mol) releaved their composition in fucose, sulfate and uronic acid. The most sulfated fraction (5kg/mol with 1.55 sulfate/fucose) presented high affinity to the chemokines SDF-1/CXCL12 and RANTES/CCL5. Furthermore, this fucoidan significantly increased endothelial cell migration and microvascular network formation compared to other fractions on healthy endothelial cells and on the cells depleted in endogenous GAGs. This pro-angiogenic potential used both the MAPK and PI3K/Akt pathways. Fucoidan is located in intracellular vesicles and internalized via clatrin mediated endocytosis. The 5kg/mol fucoidan showed the highest pro-angiogenic effects on endothelial cells. This sulfate-rich fucoidan confirmed our hypothesis than small and highly sulfated fucoidan is an attractive candidate to develop therapies based on revascularization. Our team now focus to develop regenerative cell therapy of ischemic tissue based on the injection of bio-prosthesis homed by progenitors cells coupled with pro-angiogenic fucoidan and chemokines.

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**P36 - ROLE OF EPHRINE-A4 IN THE DEVELOPMENT AND IN THE PHYSIOLOGY OF ARTERIAL INNERRATION**

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Arteries receive a sympathetic innervation which is crucial to control their contraction level. Arterial innervation settles from post-natal day 2 (P2) to adult in mice. EphrinA4 is an axonal guidance molecule which interacts with EphA4 to induce repulsion, which is suprising at this stage. We wanted to assess the role of EphrinA4/ EphA4 signalisation in the development of arterial innervation in mice.

We investigated arterial innervation and anatomical defects in ears and mesentary from adult EphA4+/mice by immunostaining. We assessed the role of EphrinA4 on primary culture of sympathetic neurons from Superior Cervical Ganglia (SCG). EphrinA4+/ mice showed increased arterial innervation in ear skin and mesentary, but also on heart and kidney during development, and this enhanced innervation remain in adult animals. Added in culture media, EphrinA4 binds to sympathetic neurons and induces collapse of WT sympathetic growth cones, but not EphA4+ growth cones. We are investigating arterial innervation and anatomical defects in ThCre-EphA4flox/flox mice.

EphrinA4 is expressed by arteries and interacts with EphA4 to induce collapse of sympathetic growth cones in vitro. In vivo this can be associated with elimination of inappropriate or outnumbered contacts between sympathetic neurons and arterial smooth muscle cells to assure the optimal amount of arterial innervation. As peripheral vascular resistance is involved in the regulation of blood pressure, understanding development and physiology of arterial innervation could help us to open new therapeutic ways for hypertension.
Endothelial barrier function is assured through a dynamic interplay between endothelial cell-extracellular matrix adhesions, cytoskeleton networks and cell-cell of the endothelium and identify ROCK1 as an important potential therapeutic target. Depletion of ROCK1 in CCM-depleted HUVEC restored a normal contractile and junctional phenotype as well as a correct permeability barrier anchored stress fibers. Upon CCM loss, overactivation of ROCK1 due to ROCK2 delocalization had major consequences on the contractile and junctional phenotypes controlled the balance between ROCK1 and ROCK2 kinase activities, allowing the organization of a cortical junctional actin ring and repressing the formation of ECM-between ROCK1 and ROCK2. We observed that ROCK2 protein is a major target of CCM proteins. By localizing ROCK2 to intercellular junctions, CCM complex of the two isoforms of ROCK, ROCK1 and ROCK2, in these processes have never been addressed. We provide the first evidence for a balance of catalytic activity stimulating the formation of VE-cadherin-dependent junctions and regulating acto-myosin contractility through the RhoA-ROCK pathway. However the specific roles these lesions with deleterious consequences for the surrounding neural tissue. It has been shown that the CCM1/2 complex controls endothelial permeability by controlling the balance between ROCK1 and ROCK2 activities. We investigated the role of MARCH3 in this balance. The effect of MARCH3 on endothelial barrier, we assessed whether the endosome/lysosome-located MARCH family members can contribute to endothelial barrier modification in response to inflammatory permeability-inducing factors. We thus deployed a small-scale siRNA library screen that targets 9 MARCH members and identify MARCH3 as a regulator of endothelial permeability through various signalling pathways; that can involve transcriptional control of VE-cadherin and post-translational modifications, such as phosphorylation of VE-cadherin and its partners, its internalization and membrane destabilization. Ubiquitylation can also take part in trafficking and degradation of adhesion molecules, as envisioned by early reports on cadherin trafficking. To date, the ubiquitin ligases involved are not known. In the search for mediators of the endothelial barrier, we assessed whether the endosome/lysosome-located MARCH family members can contribute to endothelial barrier modification in response to inflammatory permeability-inducing factors. We thus deployed a small-scale siRNA library screen that targets 9 MARCH members and identify MARCH3 as a regulator of endothelial permeability process. We further demonstrated that MARCH3 acts through the FOXO/AKT pathway and tight junctions protein expression.

**P37 - SOLUBLE CD146 PRIMING BOOSTS SURVIVAL AND REGENERATIVE PROPERTIES OF ENDOTHELIAL COLONY-FORMING CELLS**

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Endothelial colony-forming cells (ECFC) constitute an endothelial progenitor fraction with a promising interest for the treatment of ischemic cardiovascular diseases. As soluble CD146 (sCD146) was described as a new factor promoting angiogenesis, we examined whether sCD146 priming could improve the therapeutic potential of ECFC and defined the involved mechanism. The effect of sCD146 priming on ECFC properties was investigated in vivo and in vitro. Soluble CD146 priming improved the survival of ECFC in an animal model of matrigel plug that mimicked a hypoxic environment and boosts their revascularization potential in a mouse model of hind limb ischemia. These results were confirmed in vitro by a DNA fragmentation assay and proliferation/migration experiments. The observed effects were mediated through a signalosome, located in lipid rafts, containing specifically the short isoform of CD146 (shCD146), angiopoietin, and VEGFR1/VEGFR2 and involved the transcription of genes related to angiogenesis (eNOS) and cell viability (FADD, Bcl-xl).

These findings establish that activation of shCD146, in particular with sCD146 priming, constitutes a new pathway to improve ECFC regenerative properties for the treatment of cardiovascular diseases.

**P38 - CCM1/2 COMPLEX CONTROLS ENDOTHELIAL TENSIONAL INTEGRITY BY REGULATING THE BALANCE BETWEEN ROCK1 AND ROCK2 ACTIVITIES**

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Endothelial barrier function is assured through a dynamic interplay between endothelial cell-extracellular matrix adhesions, cytoskeleton networks and cell-cell adhesions. The CCM1/2 complex functions as a signaling node between VE-cadherin- and integrin-dependent adhesions. Loss-of-function mutations in ccm1 or ccm2 genes in humans lead to cerebral vascular malformations consisting in clusters of capillary-like caverns. Defective cell-cell junctions cause the bleeding of these lesions with deleterious consequences for the surrounding neural tissue. It has been shown that the CCM1/2 complex controls endothelial permeability by stimulating the formation of VE-cadherin-dependent junctions and regulating acto-myosin contractility through the RhoA-ROCK pathway. However the specific roles of the two isoforms of ROCK, ROCK1 and ROCK2, in these processes have never been addressed. We provide the first evidence for a balance of catalytic activity between ROCK1 and ROCK2. We observed that ROCK2 protein is a major target of CCM proteins. By localizing ROCK2 to intercellular junctions, CCM complex containing specifically the short isoform of CD146 (shCD146), angiopoietin, and VEGFR1/VEGFR2 and involved the transcription of genes related to angiogenesis (eNOS) and cell viability (FADD, Bcl-xl).

These findings establish that activation of shCD146, in particular with sCD146 priming, constitutes a new pathway to improve ECFC regenerative properties for the treatment of cardiovascular diseases.

**P39 - THE E3 UBIQUITIN LIGASE MARCH3 CONTROLS THE ENDOTHELIAL JUNCTIONS THROUGH THE FOXO PATHWAY**

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The vascular endothelium forms a semi-permeable selective barrier that allows the controlled passage of fluids, molecules and cells from and into the bloodstream. This function relies, in part, on the organization of cell-cell junctions, notably orchestrated through VE-cadherin-based contacts. Angiogenic and inflammatory factors can elevate endothelial permeability through various signalling pathways; that can involve transcriptional control of VE-cadherin and post-translational modifications, such as phosphorylation of VE-cadherin and its partners, its internalization and membrane destabilization. Ubiquitylation can also take part in trafficking and degradation of adhesion molecules, as envisioned by early reports on cadherin trafficking. To date, the ubiquitin ligases involved are not known. In the search for mediators of the endothelial barrier, we assessed whether the endosome/lysosome-located MARCH family members can contribute to endothelial barrier modification in response to inflammatory permeability-inducing factors. We thus deployed a small-scale siRNA library screen that targets 9 MARCH members and identify MARCH3 as a regulator of endothelial permeability process. We further demonstrated that MARCH3 acts through the FOXO/AKT pathway and tight junctions protein expression.
P40 - MOLECULAR MECHANISMS OF ANGPTL4-INDUCED REGULATION OF VASCULAR INTEGRITY

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Cardiovascular ischemic injuries are associated with vascular damage induced by loss of endothelial junction integrity. Deciphering the mechanisms involved in the regulation of vascular integrity is of major interest in order to develop relevant therapeutic cardioprotective approaches to achieve tissue protection. Our team identified angiopoietin-like 4 (ANGPTL4) as a hypoxia-induced target and a key regulator of vascular integrity by reinforcing interendothelial cell junctions. However, ANGPTL4 receptors and downstream signaling pathways which mediate vasoprotective effect remain poorly investigated. Using both in vitro binding (Surface Plasmon Resonance, SPR) and functional in vivo assays, we show that ANGPTL4 binds \( \alpha_v \beta_3 \) integrin and that this interaction is necessary to mediate vasoprotective effects. In addition, ANGPTL4 induces phosphorylation of Tyr773 of \( \beta_1 \) integrin subunit and reorganization of focal adhesions in endothelial cells. Mechanistically, binding of ANGPTL4 to \( \alpha_v \beta_3 \) leads to Src recruitment and its sequestration away from VEGFR2 combined to a diminished Src signaling downstream VEGFR2, thereby inducing stabilization of both VEGFR2/VE-cadherin and VEGFR2/\( \alpha_v \beta_3 \) complexes. Thus, ANGPTL4 strengthens maturation of adherens junctions, characterized by a transition from a zipper to linear organization of VE-cadherin organization.

Altogether, our results identify a novel mechanism by which ANGPTL4 counteracts hypoxia-driven vascular permeability through \( \alpha_v \beta_3 \) binding, modulation of VEGFR2/Src kinase signaling and endothelial junction stabilization.

P41 - TARGETING VASCULAR ENDOTHELIAL GROWTH FACTOR IN MYELOID CELLS ENHANCES NATURAL KILLER CELL RESPONSES TO CHEMOTHERAPY AND AMELIORATES CACHEXIA

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Chemotherapy remains a mainstay of cancer treatment but its use is often limited by the development of adverse reactions. Severe involuntary loss of body weight ( cachexia) is a frequent cause of death in cancer patients and is exacerbated by chemotherapy. We show that genetic inactivation of Vascular Endothelial Growth Factor (VEGF)-A in myeloid cells prevents chemerin-induced cachexia by inhibiting the lipolysis of white adipose tissue. It also improves clearance of senescent tumour cells by natural killer cells and inhibits tumour regrowth after chemotherapy. The effects depend on the adipokine and chemotactrant chemerin, which is released by the tumour endothelium in response to chemotherapy. The findings define chemerin as a critical mediator of the immune response elicited by chemotherapy as well as an important inhibitor of cancer cachexia. Targeting VEGF signaling should impede the lipolysis and weight loss that is frequently associated with chemotherapy, thereby dramatically improving the therapeutic outcome.

P42 - IMPLICATION OF SIRTUIN 1 IN THE SYNOVIAL ANGIOGENESIS OF RHEUMATOID ARTHRITIS

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Background/purpose: Rheumatoid arthritis (RA) is the most frequent chronic inflammatory rheumatic disorder involving primarily the synovium. Neangiogenesis is crucial for the development of the pathologic synovium. Endothelial cells derived from circulating endothelial progenitor cells (EPCs) have been implicated in synovial neangiogenesis. Our aim was to determine the gene expression profiles of endothelial cells derived from circulating EPCs issued from patients with RA compared to healthy controls.

Methods: EPC-derived endothelial cells were isolated from the peripheral blood of 18 RA patients and 11 matched healthy controls. Affymetrix Human Genome HGU133 plus 2.0 Array microarray were used. Validation of genes of interest was performed by quantitative RT-PCR. Protein expression of identified mediators was then assessed in RA and control EPC-derived cells by western blots and immunofluorescence, and in synovial tissue sections by immunohistochemistry.

Results: Hierarchical clustering allowed a correct segregation of the 18 RA patients and the 11 controls. Supervised analysis according to the fold change and the p-value identified 15 top genes differentially expressed between RA patients and controls. Among these 15 genes, the downregulation of the NAD-dependent protein deacetylase sirtuin-1 (SIRT1) in RA EPC-derived cells was of particular interest in the context of RA synovial neangiogenesis, since SIRT1 is implicated in cell survival/proliferation, inflammation and angiogenesis. Reduced SIRT1 mRNA levels in EPC-derived endothelial cells issued from RA patients were further confirmed by quantitative RT-PCR. In addition, we observed a significant decrease of SIRT1 protein expression in RA EPC-derived endothelial cells compared to control cells both by western blot and immunofluorescence. Reduced expression of SIRT1 was also observed in the vessels of the synovial tissue of RA patients. In addition, two additional targets negatively regulated by SIRT1, the tumor suppressor p53, playing a central role in cell proliferation, and Cysteine-rich angiogenic inducer 61 (CYR61), a strong regulator of angiogenesis, were overexpressed both in RA EPC-derived cells and in the vessels of the synovial tissue of RA patients.

Conclusion: Reduced SIRT1 expression, together with upregulation of p53 and CYR61 may contribute to the proliferative, activated and proangiogenic profile of endothelial cells observed in RA synovium. The functional role of SIRT1 in synovial neovascularization is now under investigation.
P43 - INFERENCE OF LOW AND HIGH-GRADE GLIOMA GENE REGULATORY NETWORKS DELINEATES THE ROLE OF RND3 IN ESTABLISHING MULTIPLE HALLMARKS OF CANCER

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Gliomas are a highly heterogeneous group of brain tumours that are refractory to treatment, highly invasive and pro-angiogenic. Glioblastoma patients have an average survival time of less than 15 months. Understanding the molecular basis of different grades of glioma, from well differentiated, low-grade tumours to high-grade tumours, is a key step in defining new therapeutic targets. Here we use a data-driven approach to learn the structure of gene regulatory networks from observational data and use the resulting models to formulate hypothesis on the molecular determinants of glioma stage. Remarkably, integration of available knowledge with functional genomics datasets representing clinical and pre-clinical studies reveals important properties within the regulatory circuits controlling low and high-grade glioma. Our analyses first show that low and high-grade gliomas are characterised by a switch in activity of two subsets of Rho GTPases. The first one is involved in maintaining normal glial cell function, while the second is linked to the establishment of multiple hallmarks of cancer. Next, the development and application of a novel data integration methodology reveals novel functions of RND3 in controlling glioma cell migration, invasion, proliferation, angiogenesis and clinical outcome.

P44 - TIE2-DEPENDENT DELETION OF ALPHA-6 INTEGRIN SUBUNIT IN MICE REDUCES TUMOR GROWTH AND ANGIOGENESIS

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Integrins play key roles in tumour progression and angiogenesis, and neutralizing antibodies against αvβ3, αvβ5 and αvβ1 are currently under development as potential cancer therapies. In this work, we focused on αv integrin subunit. Its role has been demonstrated in the progression of several malignancies such as breast cancer, prostate cancer, glioblastoma and pancreatic cancer. We have previously shown that αv is involved in endothelial cell adhesion, migration, pseudotube formation and post-ischemic vascular repair. Therefore we used the cre-lox system to generate a mouse line, αvfl/fl-Tie2Cre+, with αv gene deletion specifically in Tie2-lineage cells (i.e. endothelial cells, pericytes, subsets of hematopoietic stem cells, and Tie2-expressing monocytes/macrophages (TEMs)) to study tumour growth and tumoral angiogenesis. In a murine B16F10 melanoma model, loss of αv expression in αvfl/fl-Tie2Cre+ mice reduced tumour growth. Immunohistological analysis was performed on tumours, 12 days after implantation or when tumours have identical size in the two groups. Analysis of the tumours showed that Tie2-dependent αv gene deletion was associated with reduced tumour vascularization as assessed by vessel surface area, number of vessels and vessel diameter but did not change significantly pericyte coverage. Finally, we showed that, in tumour, the infiltration of proangiogenic Tie2–expressing macrophages was reduced. Our results thus confirm that αv subunit plays an important role in tumour growth and angiogenesis, by promoting neovessel formation and tumour infiltration by proangiogenic TEMs. Therapeutic targeting of αv might affect the invasive properties of tumour cells, endothelial cells and TEMs, and could thereby reduce tumour growth and invasiveness.

P45 - MITOCOCHONDRIAL DYNAMICS AND ANGIOGENESIS: THE ROLE OF THE PRO-FISSION FACTOR DRP1 IN ENDOThELIAL CELLS

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Angiogenesis requires activation of quiescent endothelial cells, remodeling and new tissue formation and therefore a higher demand in ATP. Mitochondria are essential organelles with pivotal role in processes of life and cell death and they are the main source of ATP. Mitochondria are extremely dynamic structures, their morphology is modulated through processes of fusion and fission in order to fulfill their diverse functions. Mitochondrial morphology is orchestrated by a number of dynamin-like GTPases. Fusion depends on mitofusin 1 (MFN1), mitofusin 2 (MFN2) and Optic Atrophy 1 (OPA1). DRP1 (Dynamin-related protein 1) is the key regulator of mitochondrial fission. However, the role of mitochondria in the endothelium is poorly understood. Our study is approaching the role of mitochondrial shaping protein DRP1 in endothelial cells and during angiogenesis.

Here, we show that VEGF treatment of HUVECs decreases DRP1 protein levels. Parallel to this, DRP1 is upregulated when cells are treated with the inhibitor of angiogenesis 16K prolactin. Furthermore, we evaluated angiogenic parameters in HUVECs with down-regulation of DRP1 (siRNAs) and we observed an increase in cell’s migration, proliferation and permeability.

Our preliminary data are suggesting a key role for mitochondrial dynamics in angiogenesis and a new function for DRP1 in endothelial cells. Our goal is to identify how pro and anti-angiogenic agents affect DRP1 levels and which are the downstream molecular pathways through which the protein is involved in angiogenesis.
P46 - REGULATION OF TUMOR ANGIOGENESIS BY THE STAR (SIGNAL TRANSDUCTION AND ACTIVATION RNA) PROTEIN SAM68: INTERPLAY BETWEEN SIGNAL TRANSDUCTION AND ALTERNATIVE TRANSCRIPT DIVERSITY

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Alternative splicing (AS) represents a major step in gene expression that affects transcripts from greater than 90% of human multi-exon genes and thus contributes significantly to genomic diversity by generating protein isoforms with distinct activities. Deregulation of AS has been shown to play a role in carcinogenesis, by production of oncogenes and tumor suppressors and progression of Epithelial-Mesenchymal Transition in cancer cells. However, the role and regulation of AS in non-malignant cells of the tumor microenvironment, such as angiogenic endothelial cells, is not well established or understood. It is known that AS of genes encoding proteins with “oncofetal domains” (present in tumors and fetal tissues) can confer oncogenic functions to proteins and enhance their pro-tumoral activity. One such functionally relevant AS event in angiogenic endothelial cells is the inclusion of exons encoding oncofetal “Extra Domains” B and A (EDB and EDA) in the mRNA of fibronectin (FN). FN is a large multi-domain ECM molecule with a fundamental role in vascular physio-pathology. FN isoforms harboring the EDB and EDA domains are upregulated in angiogenic blood vessels where they participate in blood vessel remodeling/maturation and recruitment of immune cells and endothelial progenitors. Importantly, as specific markers of angiogenic blood vessels in tumors, these alternatively spliced domains provide clinically relevant targets for antibody-mediated delivery of imaging agents and toxic payloads.

Recently, using a candidate gene approach to determine which splicing factors are involved in the differential expression of FN isoforms in human endothelial cells we identified Sam68. Sam68 belongs to the STAR (Signal Transduction and Activating RNA) family of RNA-binding proteins with a dual function in signal transduction and RNA processing. Through the presence of protein-interacting motifs and post-translational modifications, this protein can link responsiveness to external stimuli to the generation of alternative transcripts.

Here we show that silencing of Sam68 in endothelial cells decreases the levels of alternatively spliced FN mRNAs, impacts fiber assembly, cell motility and adhesive properties, suggesting that Sam68 regulates the angiogenic phenotype of endothelial cells. Investigations of Sam68-regulated AS in endothelial cells and the mechanistic links between external angiogenic cues and intracellular signaling pathways are underway.

P47 - IMPLICATION OF MIRNAS IN THE COMMUNICATION BETWEEN ENDOTHELIAL CELLS AND VASCULAR MUSCLE CELLS

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MicroRNAs are small non-coding RNAs regulating many genes by targeting mRNAs. MicroRNAs influence many processes in the cell, such as angiogenesis. Recent studies showed that microRNAs can be released in nanovesicles called exosomes, which further can target other cells. This transfer of genetic material represents a new mechanism of cell communication.

Interaction between endothelial cells and mural cells is a crucial step for tumor angiogenesis. Understanding how those cells communicate to each other and participate to tumor progression might help to design new therapeutic approaches for cancer.

In this study, we aimed to determine if microRNAs are involved in the communication between endothelial cells and mural cells. As shown by DLS analysis we showed that HUVECs and HUVMCs produce exosomes. Using exosomes stained with a fluorescent dye we saw that HUVECs and HUVMCs can exchange exosomes. To determine if these cells can exchange microRNAs via exosomes, we transfected HUVECs with an exogenous pre-microRNA and we analyzed the transfer of this microRNA to HUVMCs. The reverse experiment was also performed and we obtained similar results.

In order to establish the repertoire of microRNAs exported by HUVECs and HUVMCs, we performed microRNA profiling on co-cultured cells. We observed that some microRNAs are significantly more exported into endothelial exosomes whereas others are more exported into VSMC exosomes in co-cultures experiments. The role of these microRNAs is currently analyzed in different functional assays. Future experiments will attempt to determine the role of those microRNAs in tumor angiogenesis.

With these experiments we will have contributed to unravel the role of microRNAs in communication between endothelial and the smooth muscle cells and their implications in tumor progression.

This work is supported by the FRIA, the FNRS, the Centre Anticancéreux, the Belgian Foundation against Cancer and the University of Liège.

P48 - NADPH OXIDASE-1 REGULATES LYMPHATIC ENDOTHELIAL CELLS BRANCHING CAPACITIES

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Tumor growth depends on its microenvironment; extracellular matrix and various cell types including endothelial cells, pericytes, immune inflammatory cells, cancer-associated fibroblasts, as well as stem cells. Most cancer therapies are directed against tumor cells, however a growing number of cancer therapeutics that targets tumor microenvironment were developed this last decade. Particularly, endothelial cells are targeted via inhibition of the vascular endothelial growth factor (VEGF) signaling pathway, but with modest long-term benefits and eventual development of resistance to therapy. Therefore, new drugs are necessary to complement existing therapies. As lymphatic vessels are used by tumor cells to metastase to distant organs it would be important to block tumor lymphangiogenesis. Reactive oxygen species (ROS) are signaling intermediates that induce cell proliferation, migration and apoptosis. NADPH oxidases (NOX) are ROS producing enzyme, which transport electrons across membranes, thereby reducing oxygen into superoxide. We show recently that in blood endothelial cells, the isofoms NOX1, NOX2 and NOX4 are expressed at different level and depending on the activation status of cells and that NOX1 regulate tumor angiogenesis (Garrido-Urbanı S et al. PLoS One 2011).

In lymphatic endothelial cells, the role of NADPH oxidases has not yet been studied. We show here that lymphatic endothelial cell lines (LyEnd.1, LyEnd.5 and LyEnd.7) express NOX4, NOX1, NOX2 and DUOX1/2 genes. Then we have analyzed the branching capacity of lymphatic cell lines in 3D fibrin gel, using large anti-oxidant inhibitors such as DPI or Apocyanin and siRNA specific for NOX1. These reagents inhibit lymphatic cell branching to about 40 %. This result suggests that NOX1 is implicated in lymphatic endothelial cell sprouting. We are now developing an assay to study ex-vivo lymphatic vessel branching using thoracic duct extracted from NOX1 deficient mice.

Our results show that NOX1 is also implicated in lymphangiogenesis meaning that by targeting NOX1 we could affect two arms of tumor microenvironment, blood and lymphatic vessels.
Tumor microenvironment, besides tumor cells, comprises stromal cells like fibroblasts, inflammatory, immune and endothelial cells (ECs) [1, 2]. Tumor-associated ECs have a crucial role in organizing the stroma [3]. Firstly, vicinal ECs are recruited locally. Secondly, the distant recruitment of bone marrow endothelial precursors (EPCs) occurs [4]. These processes are induced by tumor hypoxia - a critical parameter of the tumor microenvironment [5,6] and can be mediated by angiogenic miRNAs – angiomiRs [7]. Therefore, we propose a 3-D cellular model, in which crucial characteristics of the tumor microenvironment are preserved. As model of murine melanoma, B16F10 cell line grew as spheroids. Precursor endothelial cells from mouse embryo (MAgEC 10.5 & 11.5) and mature endothelial cells from mouse lungs (Lung FVB) were added to mimic in vivo angiogenic processes. Matrix composition was optimised for cell migration. In our 3D model we demonstrated recruitment of ECs by B16F10 spheroids, increased for progenitors in hypoxia, which also promoted formation of pseudo-vessels. Lung FVB and MAgEC 11.5 extracted from the model expressed higher levels of miR-21, comparing to single cell cultures. Moreover expression of miR-210-3p, which is usually upregulated in single cell cultures in hypoxia, was increased in cells from our 3D model already in normoxia. We believe that this indicates that we were able to recreate pro-angiogenic microenvironment promoting tumor progression.

REFERENCES
P51 - WHAT IS THE BEST ANIMAL MODEL TO SPECIFICALLY TARGET ENDOTHELIAL CELLS, WITHOUT CONCOMITANT INVOLVEMENT OF THE HEMATOPOIETIC LINEAGE?

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Background: Models commonly used to target a gene in endothelial cells (EC) are constitutive (β-actin and Vascular Endothelial Growth Factor (VEGF)) or inducible by Tamoxifen (VEGFR2 and PDGFβR). In the constitutive models, an expression of Cre in the hematopoietic compartment is reported, but it appears variable depending on the model. With inducible ones no hematopoietic expression has been reported previously. To clarify the tissue specificity of the different models, we used mice with a reporter transgene whose green or red fluorescence depends on the expression of Cre [1]. We then used mice expressing JAK2V617F conditionally to unmask any hematopoietic expression, knowing that JAK2V617F hematopoietic cells acquire a proliferative advantage.

Methods and results: We used β-actinCreERT2, VEGFR2CreERT2, and PDGFβRCreERT2-mT/mG mice. Tissues expressing Cre turn green instead of red. As expected, in the constitutive models Cre is expressed both in EC and most blood progenitors. In contrast, in inducible models Cre is expressed specifically in EC. Interestingly, in VEGFR2CreERT2-mT/mG mice we observed a lower expression of GFP in the mesentery 1 month after injection of tamoxifen, suggesting that Cre is not expressed in all EC. We then used mice expressing JAK2V617F under the control of these promoters to unveil the “non-specificity” of models. As expected, constitutive models develop a “Myeloproliferative disorder” (MPD). One month after tamoxifen induction inducible mice models have a normal phenotype. However, 2 months after, PDGFβRCreERT2-JAK2V617FmT/mG mice develop a MPD with hematopoietic progenitors expressing JAK2V617F, thus reflecting an hematopoietic expression of Cre. In contrast, VEGFR2CreERT2-JAK2V617FmP/mP mice keep normal blood counts after injection of tamoxifen.

Conclusion: Our data show that PDGFβRCreERT2 mice are strictly endothelial specific 1 month after induction, but not 2 months after. This is not observed in the VEGFR2CreERT2 which remain endothelial specific long after induction.


P52 - CONTRIBUTION OF ENDOTHELIAL AND TUMORAL LOXL2 TO TUMOR ANGIOGENESIS

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Hypoxia-driven remodeling of the microenvironment is a regulated process involved in angiogenesis. Hypoxia regulates composition of the extracellular matrix (ECM) by modulating its degradation, deposition, post-translational modifications and cross-linking. Lysyl oxidase-like-2 (LOXL2) belongs to the lysyl oxidase family of enzymes involved in ECM crosslinking. We demonstrated that LOXL2 is a hypoxia-target, which is secreted by endothelial cells, regulates angiogenesis and is required for type IV collagen assembly (Bignon, Blood 2011). Moreover, inhibition of extracellular LOXL2 impedes fibrosis and angiogenesis, i.e. the development of pathologic microenvironment in cancer (Barry-Hamilton, Nat. Med. 2010). Here, we aimed at evaluating the contribution of LOXL2 secreted by endothelial and tumor cells in angiogenesis.

As the relative contribution of the cell types involved in LOXL2 expression is unknown, the expression of loxl2 mRNA was investigated in human renal carcinoma (RCC). We showed a differential expression of loxl2 mRNA in endothelial and tumor cells, depending on primary tumors with or without associated metastasis.

We investigated the role of LOXL2 using an in vitro 3D angiogenesis model with HUVEC spheroids embedded in a Matrigel/fibrin gel. When added into the gel, recombinant LOXL2, thereby mimicking tumor microenvironment, increased endothelial tubulogenesis. When using LOXL2-overexpressing endothelial spheroids, tubulogenesis was increased as compared to controls. The in vivo effects of endothelial LOXL2 were assessed using Matrigel/fibrin plug containing spheroids implanted in mice. We demonstrated an increased vascularisation of plugs, which contain LOXL2-overexpressing HUVEC spheroid as compared to controls.

We analyzed the role of LOXL2 produced by tumor cells (RCC4 cells), using co-culture of LOXL2-overexpressing RCC4 cells in the in vitro angiogenesis model and in vivo by implanting Matrigel/fibrin plugs containing LOXL2-overexpressing RCC4 cells in mice. These studies showed that LOXL2-overexpressing tumor cells stimulated in vitro and in vivo angiogenesis. We demonstrated that secretion medium of LOXL2-overexpressing RCC4 cells increased endothelial cell proliferation and migration. Preliminary data suggest that the angiogenic roles of endothelial and tumor LOXL2 are mediated by the PI3K pathway.

In conclusion, LOXL2 from endothelial and tumor cells participates to angiogenesis. Further studies are ongoing to determine the role of LOXL2 catalytic activity in tumor angiogenesis.

P53 - THE GENE ENCODING VITAMINE K-DEPENDENT ANTICOAGULANT PROTEIN S ENHANCES, PROLIFERATION, MIGRATION AND CAPILLARY TUBULE OF ENDOTHELIAL CELLS DURING ANGIOGENESIS

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Coagulopathy and angiogenesis are among the most consistent host responses associated with cancer. Clinical observations support the contention that these two respective processes are intricately linked within tumors and contribute to the tumour-associated thrombosis. Protein S (PS) is now emerging as a key anticoagulant enzyme involved in angiogenesis. We used adenoviral overexpression of PS and generated stable knock-down endothelial cell lines expressing shRNA targeting PS. We now demonstrate that regulating PS expression using either a replication-incompetent adenovirus or antisense PS, respectively, modifies the transformed phenotypes of endothelial cells, including cell proliferation (i.e., colony formation), migration, and vascular tube-like structures. A direct relationship is observed between PS expression in endothelial cells and increased phosphorylation between of ERK1/2 and p38/MAPK pathways. Additionally, treatment of endothelial cells with TAM receptors shRNA lentiviral particles expressing sense PS or antisense PS, selectively, target the tissue-specific phenotypes of endothelial cells, including cell proliferation and migration.

In conclusion, LOXL2 from endothelial and tumor cells participates to angiogenesis. Further studies are ongoing to determine the role of LOXL2 catalytic activity in tumor angiogenesis.
P54 - INTERLEUKIN-34, A NEW ACTOR IN TUMORAL ANGIogenesis

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IL-34 is a newly-discovered cytokine sharing a common receptor with M-CSF. The implication of M-CSF in oncology was initially suspected by the reduced metastatic dissemination in knock-out mice, due to angiogenesis impairment. Based on this observation, our work studied the involvement of IL-34 in the pathogenesis of osteosarcoma.

The in vivo effects of IL-34 were assessed on tissue vasculature and macrophage infiltration in a murine preclinical model based on a paratibial inoculation of human osteosarcoma cells overexpressing or not IL-34 or M-CSF. In vitro investigations using endothelial cell precursors and mature HUVEC cells were performed to analyse the involvement of IL-34 in angiogenesis and myeloid cell adhesion. The data revealed that IL-34 overexpression was associated with the progression of osteosarcoma (tumour growth, lung metastases) and an increase of neo-angiogenesis. In vitro analyses demonstrated that IL-34 stimulated endothelial cell proliferation and vascular cord formation. Pre-treatment of endothelial cells by chondroitinases/heparinases reduced the formation of vascular tubes and abolished the associated cell signalling.

In addition, IL-34 increased the in vivo recruitment of M2 Tumour Associated Macrophages into the tumour tissue. IL-34 increased monocyte/CD34+ cell adhesion to activated HUVEC monolayers under physiological shear stress conditions. This work demonstrates that IL-34 is expressed by osteosarcoma cells, is regulated by TNF-a, IL-1b and contributes to osteosarcoma growth by increasing the neo-angiogenesis and the recruitment of M2 macrophages.

By promoting new vessel formation and extravasation of immune cells, IL-34 may play a key role in tumour development and inflammatory diseases.

Baud’huin M, Renault R, Charrier C, et al. Interleukin-34 is expressed by giant cell tumours of bone and plays a key role in RANKL-induced osteoclastogenesis. J Pathol 2010;221:77–86


P55 - MOLECULAR IMPACT OF TENASCIN-C ON DRIVING ABERRANT TUMOR ANGIogenesis

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Employing the neuroendocrine Rip1Tag2 insulioma model to generate mice with stochastic tumorigenesis and abundant (TNCwt, TNC-overexpression) and no tenascin-C (TNC-knockout (TNCKO)) we had provided formal proof that tenasin-C promotes several steps in tumor progression. Tenascin-C, organized in matrix tacks (1), promoted survival, proliferation, invasion, angiogenesis - with poorly functional blood vessels - and lung micrometastasis. This correlated with Wnt activation and low expression of the Wnt inhibitor Dickkopf-1 (DKK1). We showed that tenascin-C inhibits transcription of DKK1 through blocking actin stress fiber formation.

High DKK1 inhibited tumor angiogenesis and blocked Wnt signaling in endothelial and tumor cells. Altogether our results suggest that tenascin-C generates a pro-tumorigenic microenvironment involving repression of DKK1 (2).

We had identified a gene expression signature characterising the angiogenic switch that we named «AngioMatrix» where tenasin-C amongst other matrisomal molecules is one of the most highly induced glycoproteins. We had proven an important role of tenasin-C in the angiogenic switch since in the absence and abundance of tenasin-C less and more angiogenic islets, respectively were counted (2, 3). These results suggest that tenasin-C is a driver of the angiogenic switch. High expression of a 110 gene signature of the «AngioMatrix» correlated with angiogenesis and worsened survival in colon cancer and glioblastoma patients suggesting a role of this signature beyond the angiogenic switch for poor prognosis (3).

By using ex vivo and in vitro assays we had observed tenasin-C angiogenesis inhibitory and promoting effects. We showed that the anti-angiogenic function of tenasin-C is linked to disruption of the actin cytoskeleton in endothelial cells resulting in downregulation of pro-angiogenic molecules (mechanism will be presented). In contrast, tenasin-C imposed a paracrine effect on endothelial cells through induction of soluble factors (will be presented) in tumor cells and cancer associated fibroblasts that promoted endothelial cell survival, proliferation and tubulogenesis. These mechanisms provide an explanation for tenasin-C promoting more but poorly functional blood vessels as we had seen in cancer tissue (2, Rupp et al., in preparation).

1.- Spenlé et al., 2015, Cell Adh & Migr 5, 9, 2.- Sapeu et al., 2013, Cell Reports 5, 482, 3.- Langlois et al., Oncotarget 5, 10529
P56 - MODULATION OF THE ANTI-(LYMPH)ANGIOGENIC RESPONSE IN SQUAMOUS CELL CARCINOMA CELLS FOLLOWING PROTON VERSUS PHOTON IRRADIATION

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Introduction: The treatment of head and neck squamous cell carcinoma (SCC) by standard radiotherapy is challenging because of the proximity of organs at risk. Due to its higher precision in tumor targeting, proton therapy could become the treatment of choice for SCC. Additionally, recent studies have shown that proton irradiation suppresses angiogenic genes and impairs tumor cells invasion/growth.

Objective: We investigated in SCC cells the gene expression profile of the cytokines implicated in (lymph)angiogenesis/metastasis following proton versus photon irradiation.

Materials and Methods: CAL33 SCC cells were subjected to either proton or photon irradiation (1,2,4,6,8 Gy). Both freshly irradiated (FC) and surviving cells (SC) at 48h and 2 weeks post-irradiation, respectively, were analyzed. Their proliferation potential was documented by cell counting. The relative biological effectiveness (RBE) following proton vs. photon irradiation was assessed by clonogenicity assays. IL-6, IL-8, CCL2, VEGF-A and VEGF-C gene expression was quantified by real time PCR.

Results: Cell proliferation decreased with the irradiation dose in both FC and SC, being lower following proton irradiation. RBE for the proton-irradiated group was 1.1. The higher differences in the analyzed genes expression were observed after 8 Gy: VEGF-A gene expression was induced in FC only following 8 Gy, with no differences between the irradiation groups. IL-6, IL-8 and CCL2 genes were systematically less expressed after proton irradiation in FC, whereas in SC no differences were observed, depending on the irradiation type/dose; however, in both irradiation groups, these genes were overall less expressed in SC as compared to FC, whereas the opposite was observed for VEGF-C.

Conclusion: Our preliminary data show that proton irradiation led to a less expressed pro-lymphangiogenic gene profile in CAL33 cells. This might suggest the acquisition of a less invasive phenotype by SCC after proton therapy, with a potentially lower relapse rate. However, our hypothesis needs to be confirmed by in vivo experiments in nude mice. Our research is highly relevant for the identification of new predictive markers of proton/photon therapy efficacy, as well as novel therapeutic targets for more efficient combined treatment approaches for SCC.

Acknowledgement: FP7 Marie Curie grant IF-“VELYMPH”-No.626449/2015-2016 (ML-P); University of Nice Sophia-Antipolis/Aix Marseille University-Master II program (AC).

P57 - FUNCTION OF EPIGENETIC ENZYMES (PRMTs) DURING BLOOD VESSEL FORMATION

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The protein arginine methyltransferases (PRMTs) are the family members of enzymes catalyze arginine methylation and are implicated in a myriad of cellular pathways including transcription activation/inhibition, or signal transduction. PRMT1, PRMT% and CARM1 are expressed in zebrafish and human endothelial cells but very little is known about their function in those cells. Our preliminary data in zebrafish indicate that these three proteins could play a role for blood vessel formation. More interestingly, the knock-down of prmt5 leads to angiogenesis defects, such as (i) delayed sprouting from the dorsal aorta, (ii) some sprouts never reach the most dorsal part of the trunk vasculature and (iii) the dorsal longitudinal anastomotic vessel (DLAV) is only partially formed. In order to further investigate the role, expression and subcellular localization of these proteins during blood vessel formation, we are using the CRISPR/Cas9 system to generate zebrafish mutants as well as transgenic lines. Moreover, we will knock-out these genes in HUVEC (Human Umbilical Vein Endothelial Cells) and analyze the proliferation, migration and survival of those cells comparing to control cells. Finally, we will identify the gene regulatory network on downstream of PRMTs, by crossing the data of massive parallel sequencing of the transcripts with the data of CHIP-sequencing from CARM1 and/or PRMT5 in HUVEC. Altogether, those experiments should help us to decipher the role of epigenetic enzyme during angiogenesis.

P58 - SOLUBLE CD146 PROMOTES ANGIOGENIC EFFECTS ON ENDOTHELIAL COLONY-FORMING CELLS THROUGH BINDING ON ANGIOMOTIN

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The aim of this study was to identify the binding protein of soluble CD146 (sCD146) on endothelial colony-forming cells (ECFC).

Using peptide pulldown and mass spectrometry, we identified angiomotin as a sCD146-binding protein in ECFC. Interaction between angiomotin and sCD146 was evidenced by ELISA, Homogeneous Time-Resolved Fluorescence (HTRF) and binding of sCD146 on both immobilized recombinant angiomotin and angiometin-transfected cells detected by flow cytometry. Silencing angiomotin in ECFC inhibited sCD146 effects on migration, proliferation, and capacity to form capillary-like structures in Matrigel. In addition, sCD146 effects were inhibited by the angiometin inhibitor angostatin and competition with recombinant angiomotin. Finally, binding of sCD146 on angiomotin triggered the activation of several transduction pathways that were identified by antibody array.

These results delineate a novel signaling pathway where sCD146 binds to angiomotin to stimulate a proangiogenic response. This result is important to find novel target cells of sCD146 and for the development of therapeutic strategies based on ECFC in the treatment of ischemic diseases.
P60 - HIGH PROTEIN - LOW CARBOHYDRATE DIET INDUCES ALTERATIONS OF ANGIOGENIC PROCESS IN ADULT MICE

Alison DOMINGUES1,2; Blandine DIZIER1; Tatiana BEDARIDA1,2; Stéphanie BARON1,4; Anna LOKAJCZYK1; Pascale GAUSSEM1,2,5; Catherine BOISSON-VIDAL1,2; Valérie NIVET-ANTOINE1,2

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Cardiovascular complications represent one of the first causes of morbi-mortality in elderly patients. Indeed, both arterial and metabolic aging are risk factors for ischemic pathologies. To respond to hypoxia, the capacity of vessels to develop a collateral network will determine their ability to limit ischemic complications. Endothelium plays a central role in the angiogenesis, particularly during hypoxia. Yet, aging endothelial cells become pro-oxidative, pro-inflammatory and pro-thrombotic leading to endothelial dysfunction that can impair the angiogenesis. We have developed a model of vascular accelerated aging consisting in a three-month High protein-Low carbohydrate (HP-LC) diet given to 6 month old mice. We reported that this diet induced a marked glucose intolerance responsible for cardiovascular damages, associated with a decreased aortic distensibility and left ventricle dysfunction, as a probable consequence of arterial dysfunctions due to major oxidative stress and inflammation.

In this work, we hypothesized that HP-LC diet would induce a defect of angiogenic process with ischemic damage in adult mice. In a first approach, we studied the angiogenic process “in vivo” in the model of accelerated vascular aging. After a three-month HP-LC diet, a hindlimb ischemia (the femoral vein and arteries were ligated and then excised down) was performed on adult mice. Immediately after surgery, mice were imaged on a near infrared Laser Doppler Imager measuring the perfusion at days 0, 5, 7, 13 and 20 and showing a decrease of blood flow associated with a major release of LDH at day 20 after 3 months of HP-LC diet. These results were associated with an increased number of gastrocnemius fibers in necrosis and to an increased necrosis score of Shireman measured at days 5,7,13 and 20. An “ex vivo” approach using organotypic culture with the aortic ring assay was then considered. Sprouting angiogenic responses on Matrigel were assessed by the quantification of the number of endothelial microvessel sprouts growing out from the aortic rings and measuring vessel area. HP-LC diet decreased the vessel area and the number of branch points. A lowering of VEGF expression in aorta endothelium and aorta media by HP-LC diet was observed with a decrease of VEGF receptor 2 phosphorylation.

This work shows the first observation of a defect in angiogenic process with ischemic damage induced by HP-LC diet in adult mice.
Inference of Low and High-Grade Glioma Gene Regulatory Networks Delineates the Role of Rnd3 in Establishing Multiple Hallmarks of Cancer.

Targeting Vascular Endothelial Growth Factor in myeloid cells enhances natural killer cell responses to chemotherapy and ameliorates cachexia.

The E3 Ubiquitin ligase march3 controls the endothelial junctions through the foxo pathway.

Soluble CD146 priming boosts survival and regenerative properties of endothelial colony-forming cells.

Role of Ephrine-A4 in the development and in the physiology of arterial innervation.

Characterization of proangiogenic potential by highly sulfated fucoidan: role of the chemokines and the glycosaminoglycans.

Inhibition of Semaphorin-3A as a novel strategy for therapeutic angiogenesis and nerve regeneration.

Autophagy is required for maintenance of endothelial function & slows the development of diabetic nephropathy.

Boosting the hypoxic response in scar-associated myeloid cells accelerates the resolution of liver fibrosis.

Human angiogenic monocytes recruitment in acute versus tumor-related inflammation.

Lymphocyte infiltration is increased under low dose sunitinib without tumor vessel normalization.

The hypoxic response in Natural Killer cells: Linking immune surveillance and tumor angiogenesis.

Soluble CD146 promotes angiogenic effects on endothelial colony-forming cells through binding on angiomotin.

Function of epigenetic enzymes (PRMTs) during blood vessel formation.

Molecular impact of tenascin-C on driving aberrant tumor angiogenesis.

Cell Orientation and Polarity, a Combined Role of Shear Stress and VEGF?

High protein - low carbohydrate diet induces alterations of angiogenic process in adult mice.
GENERAL INFORMATION

ADMISSION
The participant’s name badge is provided at the registration desk, located at the level-1 of Collège de France. All participants are requested to wear the badge throughout the Congress.

COFFEE BREAKS AND LUNCHES
During the Congress breaks, coffee, tea and refreshments are served free of charge to all registered participants wearing Congress badges. A lunch will be served to the participants on Thursday, September 24th and 25th in Collège de France.

LANGUAGE
The official language of the Congress is English. Simultaneous translation is not provided for this congress.

LIABILITY
The Congress organizers shall only be liable for the delegates and their accompanying persons in case of gross negligence or willful misconduct in the deliverance of their obligations under the event agreement. The Congress organizers shall accept no responsibility or liability for events such as accidents, thefts or losses of property etc, as the delegates and accompanying persons are responsible for their own insurance. Registration does not include insurance.

GALA DINNER
The Gala dinner will be held on Thursday, September 24th in Procope Restaurant at 8.00 p.m. 13, rue de l’Ancienne Comédie - 75006 PARIS. Registration to the gala dinner is required.

SECRETARIAT DESK
The Secretariat desk will be open during the following hours:

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<td>Friday, September 25th</td>
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