PROGRAMME ET ABSTRACTS

Journée Scientifique à l'Occasion de l'Honoris Causa de Michel L. Tremblay
11 Mai 2022
Amphitheatre IECB

9:00-9:10 Introduction de la journée : **Andreas Bikfalvi** (BRIC, INSERM U1312, Bordeaux)

Session Médecine régénérative – angiogenèse

9:10-9:35 **Nicolas L'Heureux** (BIOTIS - Laboratoire de Bioingénierie Tissulaire INSERM, Bordeaux)
Cell-assembled Extracellular Matrix (CAM) as a Biomaterial for Building Vascular Grafts and More.

9:35-10:00 **Pierre Nassoy** (LP2N, CNRS-Institut Optique, Bordeaux)
Self-assembly and mechano-sensing in blood vascular, nervous, or tumor organoids.

10:00-10:25 **Marie Ange Renault** (Biologie des maladies Cardiovasculaires, INSERM, Bordeaux)
Importance of microvascular integrity in the pathophysiology of critical limb ischemia.

10:25-11:00 Pause Café

11:00-11:30 **Barbara Garmy-Susini** (i2MC, INSERM Toulouse)
Lymphangiogenesis in health and disease.

11:30-12:30 Guest Lecture :
Michel L. Tremblay (Institut du Cancer Goodman, Université McGill, Montréal, Québec, Canada)
Protein Tyrosine Phosphatase family: An untap source of targets for disease understanding and novel therapeutics.

12:30-13:45 Buffet

Session Cancer

13:45 -14:15 **Eric Chevet** (Oncogenesis, stress, Signaling" Inserm U1242, Rennes)
Targeting Endoplasmic Reticulum proteostasis in cancer.

14:15-14:40 **Delphine Fessart** (BRIC-INSERM U1312, Bordeaux)
Characterization of the epigenetic and genetic mechanisms governing the fate of adult stem cells (aSCs) in epithelial morphogenesis.

14:40 -15:05 **Lucie Brisson** (BRIC-INSERM U1312, Bordeaux)
Autophagy-Lipophagy & mitophagy and Cancer.

15:05-15:30 **Frédéric Saltel** (BRIC-INSERM U1312, Bordeaux)
Tumor microenvironment remodeling by cancer cells-released tracks on type I collagen.

15:30-15:55 Pause Café

15:55-16:20 **Christine Varon** (BRIC-INSERM U1312, Bordeaux)
Cancer stem cells in gastric cancer.

16:20-16:45 **Majid Khatib** (BRIC-INSERM U1312, Bordeaux)
Proteolytic protein repression mediates tumor T cells infiltration and anti-tumor immune response: A drug-repurposing approach.

16:45-17:30 Table ronde et échange avec les étudiants.

Cell-assembled Extracellular Matrix (CAM) as a Biomaterial for Building Vascular Grafts and More

Nicolas l'Heureux, Biotis, INSERM U1026

Synthetic biomaterials have many convenient qualities (mechanical strength, low cost, sterilizable, etc.), but they are also recognized as foreign materials and can trigger inflammatory responses such as scarring, encapsulation, and calcification. Extracellular matrix assembled by cultured human cells have the potential to avoid immune and inflammatory responses, and to provide a mechanically robust and stable scaffold for tissue engineering. Rolled sheets of Cell-Assembled extracellular Matrix (CAM) have been used to create living, autologous, small diameter tissue-engineered blood vessels (TEBV) for hemodialysis access and have shown reduced complication rates in patients with advanced end-stage renal disease. Non-living, allogeneic, sheet-based grafts have also been implanted in patients and without signs of degradative or immune responses. A new generation of CAM-based grafts can be produced from CAM yarn, using a textile assembly approach, to create TEBV 3 times faster and with highly tunable mechanical properties. Human CAM threads can be woven, braided, or knitted like synthetic threads. However, when implanted *in vivo* (nude rats) these biological threads integrate in the host tissue without encapsulation or chronic inflammation resulting in a remarkable stability (up to 10 months). Human woven vascular grafts produced using this approach have a high burst pressure, substantial suture retention strength, and low transmural permeability. Finally, particles of CAM can provide an injectable form of the material for use as a tissue filler for aesthetic, reconstructive applications or dental pulp regeneration. The CAM, under its various forms, is a true bio-material with tremendous clinical potential.

Self-assembly and mechanosensing in bloody, nervous, or tumoral organoid

Pierre Nassoy, Laboratoire de Photonique, Numérique et Nanosciences CNRS/Université de Bordeaux/Institut d'Optique Graduate School

We will first introduce the microfluidic technique that we developed to encapsulate cells and generate multicellular spheroids or organoids. We will then focus on selected examples that highlight specific aspects of self-assembly and mechanotransduction in 3 dimensions. Technological developments related to the difficulty in imaging spheroids in depth or in a high throughput format will be reported. Finally, biomedical applications in oncology and regenerative medicine will also be addressed through some preliminary results.

Importance of microvascular integrity in the pathophysiology of critical limb ischemia
Marie Ange Renault (Biologie des Maladies Cardiovasculaires, INSERM, Bordeaux)

Peripheral arterial disease (PAD) is an atherosclerotic disease increasingly present especially because of the pandemic evolution of diabetes. In addition to causing life style limiting claudication symptoms, uncontrolled disease can progress into chronic limb threatening ischemia (CLTI) of which the diagnosis presages a high rate of limb loss along with patient mortality. Therapeutic angiogenesis for CLTI was unsuccessfully proposed as a supplement or alternative to surgical revascularization and lets about 30% of patients with no therapeutic options. Our objective is to identify new mechanisms responsible for PAD exacerbation into CLI in order to identify new therapeutic options for CLTI patients. Based on the mixed results obtained with pro-angiogenic therapies and our recent data reporting that capillary density is not diminished in the ischemic muscle of CLTI patients, we have hypothesized that muscle ischemia in these patients is rather due to impaired integrity/function of the micro circulation rather than a decreased muscle vascular density.

Accordingly, we have demonstrated that impaired limb perfusion is not associated with altered angiogenesis in in the setting of diabetes, one of the main factors promoting PAD exacerbation into CLTI. Besides, we have demonstrated that improving endothelial cells dysfunction by targeting either Hedgehog signaling or soluble Guanylate cyclase does significantly improve ischemic limb reperfusion. Mechanistically, we found that increased endothelial cell expression of adhesive molecules notably ICAM-1 deceases white blood cell circulation velocity in small capillaries impairing their perfusion. Altogether our results support that while PAD is primarily a large vessel disease caused by atherosclerosis, exacerbation of PAD to CLTI may be due to endothelial dysfunction, especially increased endothelial adhesiveness in small capillaries.

15-Lipoxygenase drives inflammation resolution and Treg trafficking in lymphedema
*Zamora A.1, Coulibaly AM.1, Benuzzi E.1, Morfoisse F.1, David F.1, Pujol F.1, Tatin F.1, Lacazette E.1, Galitzki J.1, Bouloumié A.1, Dubourdeau M.2, Chaput B.3, Malloizel-Delauney J.5, Bura-Rivière A.5, Prats AC.1, **Garmy-Susini B1**§. I2MC INSERM Toulouse*

Lymphedema is characterized by the accumulation of protein-rich interstitial fluid, lipids and a significant inflammatory cell infiltrate in the limb. It causes a significant morbidity and is a common disabling disease affecting more than 250 million people worldwide, however there is no curative treatment for lymphedema.
Here, we found that dermolipectomies from patient with lymphedema exhibit inflamed gene expression profile compared to normal arm on same patient. After lipidomic analysis, we identified severe decrease in arachidonic acid-derived lipid mediators generated by the 15-lipoxygenase (15-LOX) in lymphedematous arms. Using a mouse model of lymphedema, we reproduced the ethiology of the human pathology including the loss of specialized pro-resolving lipid mediators that play essential roles in resolution of inflammation. This was associated with a lack of regulatory T cells (Treg) recruitment in the injured limb adipose tissue. Importantly, we identified the lymphatic endothelial 15-LOX was responsible for the chemoattraction and transendothelial migration of Tregs. These results were confirmed by an aggravation of lymphedema and deterioration of the lymphatic network in an original transgenic mouse model in which ALOX15 gene is selectively deleted in the lymphatic system (Prox1CreERT2;ALOX15fl/fl). Importantly, this was reversed by the injection of ALOX15 expressing lentivectors. These results provide evidence that lymphatic lipoxygenase may represent a novel therapeutic target for lymphedema by serving as a mediator of Treg invasion into lymphedematous adipose tissue.

Protein Tyrosine Phosphatase family: An untap source of targets for disease understanding and novel therapeutics.

Michel Tremblay, Goodman Cancer Institute and Dept. of Biochemistry, McGill University, Montreal, Canada

The protein tyrosine phosphatase (PTP) gene family encodes for 107 genes in the human genome. This remarkable diversity is reflected in the function of these enzymes in important regulatory axes ranging from growth factor and cytokine signaling, to cell-cell interaction, cytoskeletal regulation, and cell specification. Recent development in the knowledge of both functions and mechanisms of action revived the interest of the biopharmaceutical sector.

As an example of application, nearly 80 of the family members are expressed in the immune system. We have examined the function of two closely related family members to identify the mechanism by which PTP1B (PTPN1) and TC-PTP (PTPN2) overlap and differ in their action towards influencing immune cells properties. We employed both murine models and human primary cells to examine their substrate and signaling modulation to advance preclinical applications of their specific small molecule inhibitors in the immunotherapy field.

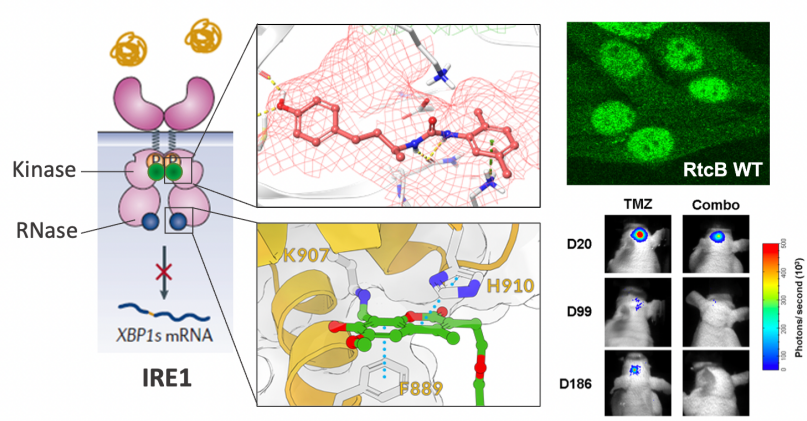
In a second part of the presentation, we will address various function of a sub-family of the PTP4As tyrosine phosphatases (PRL-1,2,3) that are overexpressed in almost all human cancers. Two of which, PRL-1 and PRl-2, have been associated to a remarkable intracellular magnesium sensing to sustain the bioenergetic demands of normal and cancer cells. We will discuss their function and targeting as well as present additional data that several pathogens have either copy these enzymes in their genomes or are targeting this core sensing mechanism to promote their infectious potential.

Therefore, the presentation will discuss the diversity of the PTP family and their function in normal and in disease contexts. Moreover, we will present current approaches to target some of these enzymes and their future application to the clinic.

Targeting Endoplasmic Reticulum proteostasis in cancer

Eric Chevet, INSERM U1242, Université de Rennes, Rennes, France. Centre de Lutte Contre le Cancer Eugène Marquis, Rennes, France.

Proteostasis imbalance is emerging as a major hallmark of cancer, driving tumor aggressiveness. Genetic and pharmacological evidence suggest that the endoplasmic reticulum (ER), a major site for protein folding and quality control, plays a critical role in cancer development. This concept has been validated in triple negative breast cancer, prostate cancer as well as in glioblastoma multiform (GB), the most lethal primary brain cancer with an overall survival of 15 months and no effective treatment. We demonstrated that the ER stress sensor IRE1 contributes to GB progression, impacting tissue invasion and tumor vascularization. IRE1 is a dual Kinase/RNase that signals by catalyzing the non-conventional splicing of the mRNA encoding the transcription factor XBP1, and in addition by regulating RNA stability through a process known as Regulated IRE1 Dependent Decay (RIDD). We further investigated the contribution of IRE1 signaling to GB and defined a specific expression signature that when confronted to human GB transcriptomes showed the antagonistic roles of XBP1 mRNA splicing and RIDD on tumor characteristics and outcomes. Moreover, using this signature we have explored the role of IRE1 signaling in tumor cells in reshaping the tumor microenvironment. These data identified IRE1 as an actionable therapeutic target which allowed us to use develop pharmacological approaches to enhance the efficacy of GB standard of care in mouse models.



Recent publications

Plizzari-Raymundo D, Doultsinos D, Pineau R, Sauzay C, Koutsandreas T, Carlesso A, Gkotsi E, Negroni L, Avril T, Chatziioannou A, Guillory X*, Eriksson LA*, Chevet E*. A novel blood brain barrier-permeable IRE1 kinase inhibitor sensitizes glioblastoma to chemotherapy in mice. chemRxiv DOI: 10.26434/chemrxiv-2022-2ld35

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Lhomond S, Avril T, Dejeans N, Voutetakis K, Doultsinos D, McMahon M, Pineau R, Obacz J, Papadodima O, Jouan F, Bourien H, Logotheti M, Jégou G, Pallares-Lupon N, Schmit K, Le Reste PJ, Etcheverry A, Mosser J, Barroso K, Vauléon E, Maurel M, Samali A, Patterson JB, Pluquet O, Hetz C, Quillien V, Chatziioannou A, Chevet E. Dual IRE1 RNase functions dictate glioblastoma development. EMBO Mol Med. 2018 Mar;10(3):e7929.

Characterization of the epigenetic and genetic mechanisms governing the fate of adulte stem cells (aSCs) in epithelial morphogenesis

Delphine Fessart, Team 2 BRIC INSERM U1312

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To better understand mammalian development, as well as to exploit the tremendous therapeutic potential of organoid models, it is necessary to identify and characterize the epigenetic and genetic mechanisms governing the fate of aSCs. Organoids development relies on the self-organizing properties of adult stem cells to create structures which recapitulate the architecture, functionality, and genetic signature observed in original tissues. Little is known about of the exact nature of the intrinsic cell properties at the origin of mammary organoid generation, and of the signaling pathways governing their plasticity and differentiation. Herein, we carried out a microRNA screen to functionally track adult stem cell from human mammary organoids epithelial cell culture. We identified a previously uncharacterized miRNA, miR-106a-3p, and its target genes that play a key role in such process. Transcriptomic profiling of miR-106a-3p transduced cells revealed overlapping genetic programs with other stem and progenitor cells suggesting common features with ESC like cells or some intermediate cellular states. Overall, our results highlight the importance of miR-106a-3p in the maintenance of adult stem-cell derived organoids and provide some clues about the mechanism underlying organogenesis.

Autophagy, lipophagy and mitophagy in cancer

Lucie Brisson, INSERM U1312 BRIC Team 1 Tumor and vascular biology laboratory

Tumors are complex tissues composed of cancer and non-cancer cells in a hypoxic and nutrient-deprived microenvironment. In order to survive, tumor cells need to adapt to these harsh conditions. One main property of cancer cells is their capacity to reprogram metabolism and take advantage of substrates available in the surrounding microenvironment. Among the cellular processes used by cancer cells, autophagy may play an important role in the adaptation to the stress conditions found in the tumor microenvironment. This process allows the degradation and recycling of proteins and organelles following the fusion between an autophagosome and a lysosome which provides hydrolytic enzymes and protons. In addition to the non-selective form of autophagy, specific forms of autophagy such as mitophagy and lipophagy may participate in metabolic plasticity by selectively degrading intracellular components linked with metabolism such as mitochondria and lipid droplets. The involvement of autophagy during tumor progression is complex, and it is now well admitted that autophagy can have two roles in cancer: protective at early stage of the tumor but promoting tumor growth at later stages. Therefore, a better understanding of the regulation of autophagy by the tumor microenvironment is needed to identify new targeting strategies.

Autophagy is highly regulated by the presence or absence of metabolic substrates in the microenvironment. Therefore, several signaling pathways controlled by substrate availability in the tumor microenvironment have been associated with autophagy. In particular, we reported that lactate sustains lysosomal function and autophagy in human cancer cells. Lactate dehydrogenase B (LDHB), catalyzing the conversion of lactate to pyruvate, controls lysosomal acidification, vesicle maturation and intracellular proteolysis. In cancer cells preferentially to normal cells, targeting LDHB blocks autophagy thus preventing cell survival and tumor growth in mice.

Autophagy is also influenced by non-cancer cells found in the tumor microenvironment. We have shown that the presence of adipocytes increases autophagy in breast cancer cells, through the acidification of lysosomes, leading to cancer cell survival in nutrient deprived conditions. Mechanistically, the disturbance of membrane phospholipid composition with a decrease in arachidonic acid content is responsible for autophagy activation by adipocyte proximity. Interestingly in androgen-independent prostate cancer cells, adipocyte proximity decreases autophagy but activates the degradation of lipid droplets by lipophagy. In human prostate cancer samples, we observed a significant correlation between autophagy markers and prostate cancer aggressiveness.

Therefore, selective, and non-selective forms of autophagy might be central cellular mechanisms of the interactions between cancer cells and the surrounding microenvironment and thus participate in cancer progression. We will next investigate the role of different forms of autophagy is glioblastoma, in the context of intra-tumor heterogeneity and metabolic adaptation.

Cancer stem cells in gastric cancer

Christine Varon,. Leader of team 4 in INSERM U1312 BRIC unit

Gastric cancer is the fourth leading cause of cancer death in the world. Detected in most cases at an advanced stage, its treatment remains mainly based on surgery for non-metastatic cases and conventional chemotherapy. Chronic infection with *Helicobacter pylori*, a bacterium classified as a level 1 carcinogen by the WHO, is the leading infectious cause of cancer worldwide. It is responsible for more than 90% of distal gastric cancers (GC), which are the long-term consequences of chronic infection of the gastric mucosa. The discovery of cancer stem cells (CSCs) at the origin of the disease has opened new perspectives for a better understanding of the process of carcinogenesis and the development of new therapeutic strategies targeting CSCs. Our group focuses on studying the cellular and molecular mechanisms leading to the emergence of CSC and gastric adenocarcinoma in the context of chronic *H. pylori* infection. Using mouse xenograft models of primary tumors derived from gastric cancer patients these mouse models as well as *in vitro* tumor organoids, we have

characterized certain surface markers allowing the detection and isolation of CSCs, including CD44 and ALDH. In a mouse model of gastric carcinogenesis induced by *H. pylori* infection, we have shown that chronic infection by this bacterium induces an epithelial-mesenchymal transition (EMT) leading to the emergence of CD44 + cells possessing properties of CSC and at the origin of gastric carcinomas. We have identified certain signaling pathways involved in the process of carcinogenesis and in active in CSCs, and proposed different experimental strategies for targeting gastric CSCs to inhibit tumor growth and metastatic spread *in vivo*. Based on these complementary models *in vitro*, *in vivo*, and on the study of tumors derived from patients, our current research aims to characterize the signaling pathways involved in the tumorigenic and invasive properties of CSCs in gastric cancer, in order to propose new biomarkers with prognostic value and new therapeutic targets for this cancer with a poor prognosis.

Tumor microenvironment remodeling by cancer cells-released tracks on type I collagen

Léa Normand[1#], Lucile Rouyer[1#], Sylvaine Di-Tommaso[1,2], Cyril Dourthe[1,2], Anne-Aurélie Raymond[1,2], Jean-William Dupuy[3], Nathalie Allain[1], Nathalie Dugot-Senant[4], Anthony Bouter[5], Alexandre Favereaux[7], Violaine Moreau[1,2], Manon Ros[1] and* ***Frédéric Saltel[1,2*]***

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Metastasis is the leading cause of cancer-related deaths. During this process, tumor cells acquire invasive and migratory capacities in order to invade the surrounding tissues. To achieve this, the tumor microenvironment is modified to facilitate cancer cells proliferation and dissemination. Multiple mechanisms are involved in this evolution, including cell-cell communications through the tumor microenvironment and the extracellular matrix modifications. Indeed, extracellular vesicles such as exosomes or migrasomes are already known to induce protumor features such as migration, promoting tumor development and metastasis formation. Here we describe a new type of extracellular vesicles (referred as tracks) specifically released by cancer cells along type I collagen fibers during cell migration. We characterized these tracks, their structure as well as their composition in terms of proteins and nucleic acids (miRNA), and could show that they are different from classical extracellular vesicles known so far. These tracks are characterized by a discoidin domain receptor 1 (DDR1) staining in the extracellular space. Moreover, these tracks are very stable structures and can be internalized by neighboring cells. After internalization, they modify the differentiation status of cells able to internalize these tracks, promoting epithelial–mesenchymal *transition* and cancer cell proliferation, invasion and also matrix degradation.

These data suggest that these collagen-associated tracks have a role in cell-cell communication and participate in the remodeling of the tumor microenvironment. Even if their function and in vivo relevance needs to be fully elucidated, these tracks seem to be a new player in the tumor invasion process and could provide a better understanding underlying this process.

Proteolytic protein repression mediates tumor T cells infiltration and anti-tumor immune response: A drug-repurposing approach.

Majid Khatib, BRIC INSERM U1312

Immune checkpoints, such as programmed death-1 (PD-1) are involved in the regulation of T cell effector function, are now exploited for the treatment of various solid and hematologic cancer. However, although therapies targeting PD-1 were clinically effective in various preclinical models and cancer patients, several patients with solid tumors are still refractory to
these treatments. Indeed, solid tumors evade anti-cancer immune control by establishing immune privileged niches within the tumor microenvironment that reduce proliferation, viability, and/or activity of cytotoxic T lymphocytes (CTL). Interestingly, a wide range of proteins involved in the expression of PD-1 and CTL function require proteolytic activation by the proprotein convertases (known as PCs). Using general protein-based inhibitors of the PCs we previously reported the implication of the PCs in PD-1 expression and T cell exhaustion. In the current study we identified small molecule convertase inhibitors through virtual ligand screening and drug repurposing approach that inhibit the activity of the convertases. Using organoids culture, we found that some of these molecules were able to repress cancer cells viability, proliferation and invasion. These molecules were also able to mediate potent repression of PD-1 expression on activated T cells. In vivo, subcutaneous inoculation of mice with syngeneic cancer cells revealed their anti-tumoral efficacy that associated increased intratumoral T cell infiltration in the developed tumors. The treated mice showed improved overall survival while compared to controls. These and other findings highlight the potential use of PC inhibitors to increase the anti-tumoral immune response and could act as novel immunotherapeutic approach in cancer used alone or as adjunct therapy.