# THE THIRD CONGRESS ON THE EPH/EPHRIN SYSTEM

Montreal

## June 1 and 2, 2022

**RESEARCH CENTRE**
**CENTRE HOSPITALIER DE L'UNIVERSITÉ DE MONTRÉAL**
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THE THIRD CONGRESS ON THE EPH/EPHRIN SYSTEM
Montreal June 1 and 2, 2022

# Conference Program

## JUNE 1, 2022 – MORNING SESSIONS

**7:45-8:30 Breakfast – Poster set up**

**8:30-8:35 Welcome – Jiangping Wu,** University of Montreal

**8:35-10:15 SESSION I – SIGNALING-I | Co-Chairs: Max Tognolini and Giovanna Tosato**
University of Parma and NIH

| | Title | Podium Presenter | Institution | Abst. no. |
|---|---|---|---|---|
| 8:35-9:00 | Versatility of EphA2 receptor signaling mechanisms | Elena B. Pasquale | Sanford Burnham Prebys Medical Discovery Institute, La Jolla USA | 01 |
| 9:00-9:25 | Molecular basis of functional oligomerization of EphA2 | Xiaojun Shi | Case Western Reserve University, Cleveland USA | 02 |
| 9:25-9:50 | Eph receptor structures and signaling: of head, core, and tail | Dimitar Nikolov | Sloan-Kettering Institute, New York USA | 03 |
| 9:50:10:15 | Decoding the specific interaction code of EPH Receptors SAM domains provides tools to switch their downstream signaling pathways | Liu Wei (Zoom) | Shenzhen Peking University-The Hong Kong University of Science and Technology, Shenzhen China | 04 |

**10:15-10:45 – BREAK**

**10:45-11:35 SESSION II – NEUROSCIENCES | Co-Chairs: Elena Pasquale and Bingchen Wang**
Sanford Burnham Prebys Medical Discovery Institute and Case Westerm University

| | Title | Podium Presenter | Institution | Abst. no. |
|---|---|---|---|---|
| 10:45-11:10 | WERDS complex regulates apical constriction via crosstalk with Wnt components | Jaeho Yoon | National Cancer Institute, NIH Frederick USA | 05 |
| 11:10-11:35 | Eph/Ephrin signalling regulates gene expression at rhombomere boundaries through the activation of Yap/Taz transcription factors | Jordi Cayuso | The Francis Crick Institute, London UK | 06 |

**11:35-12:00 – GROUP PHOTO**

**12:00-13:30 – LUNCH AND POSTER SESSION**

| Title | Poster Presenter | Institution | Abst. no. |
|---|---|---|---|
| Investigating homotypic interactions of Eph receptor family using PIE-FCCS | Soyeon Kim | Case Western Reserve University, Cleveland USA | 07 |
| Roles of EphA2-ephrin signaling in prostate development and cancer progression | Lingerak Ryan | Case Western Reserve University, Cleveland USA | 08 |

| | | | |
|---|---|---|---|
| Ephrin-A ligands regulate cutaneous tumor etiology and metastasis through cell autonomous and non-autonomous mechanisms | Ji Zheng | Case Western Reserve University, Cleveland USA | 9 |
| Characterization of EPHB1 mutations from a pan-cancer analysis of the TCGA dataset | Snehangshu Kundu | Uppsala University, Uppsala Sweden | 10 |
| Discovery of small molecules selectively inhibiting EPHAs-EPHRIN-As interaction | Massimilliano Tognolini | University of Parma, Parma Italy | 11 |
| EphB2 is a novel regulator of dermal fibrosis during systemic sclerosis | Patirice Mimche | University of Utah, Salt Lake City USA | 12 |
| EPH receptor tyrosine kinases act on the scaffold protein PAR-3 to modulate signaling networks | François J. Chartier | Université Laval, Québec City Canada | 13 |
| Identification of EphR stimulation-dependent and independent proximity partners for EphrinB ligands using TurboID proteomics | Ana Osornio | Université Laval, Québec City Canada | 14 |
| A study of human mutations in the gene encoding the tyrosine kinase receptor EPHB2 | Sung Soon Park | McGill University, Montreal Canada | 15 |
| Role of EPH/EFN in the regulation of neuroblast fate and topographical mapping | Daria Yeroshenko | University of Connecticut, Mansfield USA | 16 |
| Study in Caco-2 3D cellular model reveal a role for EPH tyrosine kinase receptors in epithelial morphogenesis | Noemie Lavoie | Université Laval, Québec City Canada | 17 |

## JUNE 1, 2022 – AFTERNOON SESSIONS

**13:30-15:10 SESSION III – SIGNALING II | Co-Chairs: Andrew Frewald and Nicolas Bisson**
The University of Saskatchewan and Université Laval

| | Title | Podium Pressenter | Institution | Abst. no. |
|---|---|---|---|---|
| 13:30-13:55 | Direct quantification of ligand-induced lipid and protein microdomains with distinctive signaling properties | Kalina Hristova | Johns Hopkins University, Baltimore USA | 18 |
| 13:55-14:20 | Decoding EphA2 interactions in live cells with PIE-FCCS | Adam W. Smith | The University of Akron, Akron USA | 19 |
| 14:20-14:45 | Structural and functional studies of the effects of phosphorylation on ephrin receptor tyrosine kinase, EPHA2, and the relationship with its sam domain as an autoinhibitor | Matthias Buck | Case Western Reserve University, Cleveland USA | 20 |
| 14:45-15:10 | Proteomic analyses of EPH receptors reveal new regulation mechanisms and biological functions | Nicolas Bisson | Université Laval, Québec City Canada; | 21 |

**15:10-15:40 – BREAK**

**15:40-16:55 SESSION IV TUMOR I | Co-Chairs: Massimiliano Tognolini and Elena Pasquale**
The University of Parma and Sanford Burnham Prebys Medical Discovery Institute

| | Title | Podium Presenter | Institution | Abst. no. |
|---|---|---|---|---|
| 15:40-16:05 | Clinical significance of Ephrin receptor (EPH)-B1, -B2, -B4 AND -B6 expression in thymic epithelial tumours | Stamatios Theocharis (Zoom) | National and Kapodistrian University of Athens, Athens Greece | 22 |
| 16:05-16:30 | Cell competition in adult pancreas tissues in vivo requires functional EphA2 | Catherine Hogan (Zoom) | Cardiff University, Cardiff UK | 23 |
| 16:30-16:55 | EphA2 and EphA4 targeting agents for the development of innovative therapeutics in oncology and neurodegeneration | Maurizio Pellecchia (Zoom) | The University of California Riverside, River Side USA | 24 |

**17:30-21:00 – COCKTAIL & DINNER**

**Restaurant Vieux Sénateur in Montreal old port**
**254 Saint-Paul; about 10-minute walk from the meeting venue**

## JUNE 2, 2022 – MORNING SESSIONS

**7:45-8:30 – Breakfast**

**8:30-9:45 SESSION V – TUMOR II | Co-Chairs:** **Elena Pasquale and Andrew Frewald**
The university of Parma and Sanford Burnham Prebys Medical Discovery Institute

| | Title | Podium Presenter | Institution | Abst. no. |
|---|---|---|---|---|
| 8:30-8:55 | EphA3 and ephrin A5 discrete expression gradients function to promote glioblastoma heterogeneity phenotypes | Bryan Day (Zoom) | The University of Queensland, Brisbane Australia | 25 |
| 8:55-9:20 | Inhibition or targeting of EphA3 expression in cancer associated fibroblast subtypes inhibits tumour growth | Peter Janes (Zoom) | Olivia Newton-John Cancer Institute, Melburne Australia | 26 |
| 9:20-9:45 | Identification of Eph receptor Signaling as a Therapeutic Target in Colorectal Carcinoma | Michael DiPrima | National Cancer Institute, Bethesda, USA | 27 |

**9:45-10:15 BREAK**

**10:15-11:30 SESSION VI – NEWLY FOUND FUNCTIONS | Co-Chairs:** **Giovanna Tosato and Binchen Wang**
NIH and Case Western University

| | Title | Podium Presenter | Institution | Abst. no. |
|---|---|---|---|---|
| 10:15-10:40 | Altered Eph-ephrin signaling disrupts brain circuit formation and produces developmental neurological disorders in mouse that mimic human behaviors observed in Autism, OCD, and ADHD | Lawrence Kromer (Zoom) | Georgetown University Medical Center, Washington DC | 28 |
| 10:40-11:05 | EPHB4 regulates pacing cell development and heart rate | Jiangping Wu | l'Université de Montréal, Montreal Canada | 29 |
| 11:05-11:30 | The roles of EphA receptors and ephrinA in memory formation | Raphael Lamprecht | The University of Haifa, Haifa Israel | 30 |

**11:30-12:45 – LUNCH**

## JUNE 2, 2022 – AFTERNOON SESSIONS

**12:45-14:25 SESSION VII CARDIOVASCULAR | Co-Chairs: Jiangping Wu and Phil King**
Université de Montréal and Univerity of Michigan

| | Title | Podium Presenter | Institution | Abst. no. |
|---|---|---|---|---|
| 12:45-13:10 | An EPHB4-RASA1 signaling axis that regulates blood and lymphatic vascular development and function | Phil King | The University of Michigan, Ann Arbor USA | 31 |
| 13:10-13:35 | Function of endothelial EphB4 and ephrin-B2 in angiogenesis, arterio-venous differentiation and heart homeostasis | Mara Pitulescu (Zoom) | Max Planck Institute for Molecular Biomedicine, Münster Germany | 32 |
| 13:35-14:00 | EphA2 serves as a gateway for a fungal pathogen into the central nervous system. | Angie Gelli | The University of California at Davis, Davis CA | 33 |
| 14:00-14:25 | EphA2 contributes to disruption of the blood-brain barrier in cerebral malaria | Tracey Lamb (Zoom) | The University of Utah, Salt Lake City USA | 34 |

**14:25-14:50 – BREAK**

**14:50-15:50 SESSION VIII – TRAINEE PRESENTATION |Co-Chairs: Bingchen Wang and Jiangping Wu**
Case Western University and Université de Montréal

| | Title | Podium Presenter | Institution | Abst. no. |
|---|---|---|---|---|
| 14:50-15:05 | Rhynchophylline, an inhibitor of the EphA4 receptor, modifies sleep architecture in mice | Maria Roig Ballester | Université de Montréal, Montreal Quebec | 35 |
| 15:05-15:20 | Optogenetic Control of EphB Kinase Activity and the EphB-ephrinB Interaction in Filopodial Movement | Matthew Dalva | Thomas Jefferson University, Philadelphia USA | 36 |
| 15:20-15:35 | The ubiquitin ligase and scaffold MYCBP2 is required for EphB2 signaling | Chao Chang | McGill University, Montréal Canada | 37 |
| 15:35-15:50 | Pan-cancer analysis of EPHB1 receptor mutations | Luis Nunes | Uppsala University, Uppsala Sweden | 38 |

**15:50-16:00 – CONCLUDING REMARKS AND ACKNOWLEDGEMENT | Jiangping Wu, Université de Montréal**

**END OF THE CONGRESS**

**Abstracts not presented in the poster session due to Zoom attendance**

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|---|---|---|---|---|
| | Ephrin receptor (EPH)-A1, -A2, -A4 and -A6 expression in thymic epithelial tumours: an immunohistochemical study | Stmatios Theocharis | National and Kapodistrian University of Athens, Athens Greece | 39 |
| | EphB2 a potential therapeutictTarget for paediatric medulloblastoma | Bryan Day | Queensland University of Technology, Brisbane Australia | 40 |

| | | | |
|---|---|---|---|
| Ephrin receptor (EPH)-A2, AND -A4 expression in uveal melanoma: an immunohistochemical study | Stamatios Theocharis | National and Kapodistrian University of Athens, Athens Greece | 41 |
| Mechanisms of EphrinA1-Fc-mediated reduction of acute myocardial ischemia/reperfusion injury | Jitka Virag | East Carolina University, Greenville USA | 42 |
| Effects of EPHB4 receptor tyrosine kinase mutations on MAP Kinase signalling in lymphatic endothelial cells | Kazim Ogmen | St. George's University of London, London UK | 43 |
| A morphogenetic EphrinB/EphB code controls extrahepatopancreatic duct formation | Elke Ober | University of Copenhagen, Copenhagen Denmark | 44 |
| EPH: Ephrin signaling in apical progenitors of the developping neocortex | Alice Davy | Université de Toulouse, Toulouse France | 45 |

# ABSTRACTS

*Abstract 01*
*Session I – Signaling-I*
*June 1, 8:35-9:00 AM*

## VERSATILITY OF EPHA2 RECEPTOR SIGNALING MECHANISMS

Maricel Gomez-Soler[1], Bernhard C. Lechtenberg[2], Marina P. Gehring[1], Mike W. Matsumoto[1], Elmer Zapata-Mercado[3], Taylor P. Light[3], Kalina Hristova[3] and Elena B. Pasquale[1]

*[1] Sanford Burnham Prebys Medical Discovery Institute, La Jolla, California 92037, USA; [2] The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria 3052 and The University of Melbourne, Parkville, Victoria 3010, Australia; [3] Johns Hopkins University, Baltimore, Maryland 21218, USA*

The EphA2 receptor tyrosine kinase has important roles in normal physiological processes as well as in many diseases such as cancer, pathological forms of angiogenesis and inflammation, and parasitic infections. These many diverse functions are made possible by the versatility of EphA2 receptor signaling. EphA2 canonical signaling is induced by the binding of ephrin-A ligands, and a variety of peptide ligands have been engineered that can also activate EphA2. EphA2 canonical signaling relies on kinase activity to achieve receptor autophosphorylation and promote tyrosine phosphorylation of cytoplasmic effectors. Interestingly, we found that peptide ligands that induce completely different dimeric configurations of the EphA2 ligand-binding domains can all efficiently activate EphA2, suggesting that the orientation of the ligand-binding domains is not critical for activation. On the other hand, the juxtamembrane segment linking the transmembrane helix to the kinase domain is required for ligand-induced cross-phosphorylation of EphA2 molecules. Thus, the flexibility of the juxtamembrane segment likely enables reorientation of the kinase domains to enable phosphorylation of tyrosines in different positions, regardless of the arrangement of the EphA2 ligand-binding domains. We also found that different ligands can differentially tune distinct EphA2 signaling responses, suggesting that EphA2 is capable of biased signaling. The EphA2 receptor can also mediate completely different effects through a non-canonical form of signaling that is not well understood, despite its importance, particularly in cancer. Non-canonical signaling involves phosphorylation of a cluster of residues in the EphA2 kinase-SAM linker by multiple serine/threonine kinases. We found that accumulation of linker negative charges, mimicking phosphorylation, induces cooperative changes in the EphA2 intracellular region from more closed to more extended conformations. EphA2 is a promising therapeutic target, and understanding the complexities of its signaling mechanisms can help effective therapeutic exploitation.

*Abstract 02*
*Session I – Signaling-I*
*June 1, 9:00-9:25 AM*

## MOLECULAR BASIS OF FUNCTIONAL OLIGOMERIZATION OF EPHA2

[1,2]Xiaojun Shi, [1,2]Carmelle Cuizon, [3]Cameron J. Herting, [2,4]Ryan Lingerak, [5]Paul Toth, [6]Juha Himanen, [6]Dimitar Nikolov, [3]Dolores Hambardzumyan [4,5,*]Adam W. Smith and [1,2,4,7,*]Bingcheng Wang

*[1] Department of Medicine at MetroHealth, Case Western Reserve University School of Medicine; [2] Rammelkamp Center for Research, Department of Medicine, MetroHealth Medical Center; [3] Department of Pediatrics, Aflac Cancer and Blood Disorders Center, Children's Healthcare of Atlanta, Emory University School of Medicine; [4] Department of Physiology and Biophysics, Case Western Reserve University; [5] Department of Chemistry, University of Akron; [6] Sloan-Kettering Institute for Cancer Research; [7] Department of Pharmacology, Case Western Reserve University*

Eph receptors constitute the largest family of the receptor tyrosine kinases (RTKs). EphA2 has been reported as the most affected Eph kinases in human cancers. It functions as tumor suppressor through the ligand-dependent activation of its tyrosine kinase, while a ligand-independent signaling mechanism describing the phosphorylation of serine 897 is also reported to promote oncogenic behavior. We set out to investigate the underlying molecular basis of the dual functions of EphA2 with a combination of structural biological, biophysical, biochemical and biological studies. First, we investigated the spatiotemporal organization of EphA2 in live cell membranes by using pulsed interleaved excitation - fluorescence cross-correlation spectroscopy (PIE-FCCS), a time-resolved fluorescence spectroscopy. PIE-FCCS measurements in multiple cell lines showed that unliganded Apo EphA2 receptors underwent multimerization, which is new to the canonical monomer-dimer paradigm of RTKs. Further study located three interfaces that contribute to the formation of Apo EphA2 multimers and ligand-induced EphA2-ephrinA1 polymers. The Sushi-Sushi and LBD-LBD interfaces contribute to head-to-head symmetric contact of EphA2 ecto-domain while the LBD-FN2 interface contributes to head-to-tail asymmetric contact. The function of these two contacts were investigated. Disruption of asymmetric contact affected the spontaneous endocytosis of Apo EphA2 while disruption of symmetric contact abolished both spontaneous and ligand-induced endocytosis. FRET and immunoblotting data confirmed the ligand-induced change in molecular proximity of intracellular domain was mediated through symmetric contact to facilitated transphosphorylation of tyrosine kinases, while the asymmetric contact kept the intracellular domain separated in favor of the phosphorylation of serine 897. With disruption of symmetric contact, the remaining asymmetric contact prompted the resistance to ligand-induced cell rounding behavior and promoted migratory behavior of cells. Finally, in a syngeneic mouse glioma model, mice injected with cells expressing asymmetric contact intact EphA2 showed reduced survival rate in relative to those injected with cells with wild-type EphA2, while disruption of asymmetric contact rescued the survival of mice. Overall, this work advanced the basic understanding of the complex, paradoxical roles of EphA2 in cancers.

*Abstract 03*
*Session I – Signaling-I*
*June 1, 9:25-9:50 AM*

## EPH RECEPTOR STRUCTURES AND SIGNALING: OF HEAD, CORE, AND TAIL

Himanen J, Nikolov D

*Sloan-Kettering Institute, New York*

Eph receptors are activated by ephrin-A and ephrin-B ligands and are divided into two subgroups, A and B, based on ligand binding specificities and sequence conservation. Through their ligand-induced and ligand-independent action, Eph receptors play central roles in diverse biological processes, such as embryonic development, regulation of neuronal signaling and immune responses, vasculogenesis, tumor initiation, progression, and metastasis. The Eph receptor ectodomain (ECD) is an assembly of multiple subdomains, including the ligand-binding domain (LBD), cysteine-rich domain (CRD), and two fibronectin-type-3 (FN3) domains. The intracellular part of the Ephs contains a juxtamembrane region, which has a regulatory function, a tyrosine kinase domain, and sterile alpha (SAM) and PDZ-binding motifs. Earlier structural studies of the ligand-receptor interacting domains revealed details of the Eph/ephrin recognition. Structures of the EphA ECDs have revealed, how receptor clustering, which is required for full biological activity, relies on two separate receptor/receptor interfaces: one in the LBD, and the second one within the CRD. Recent investigations on EphA class ECDs have also shown the existence of a third interacting interface, between the LBD and FN3 domains of adjacent, unliganded Eph molecules. This interaction was suggested to be involved in the fine-tuning of Eph signaling. Overall, organization of Eph receptors in various types of clusters or oligomers on the cell membrane is responsible for the activation of their catalytic function and for defining signaling outputs. While structural analyses of the EphA ECDs have been reported, ECD structures of EphB receptors have not been resolved. Our latest X-ray crystallographic studies on the B-class Eph receptor ectodomains reveal that their overall ECD architecture and the LBD-FN3 interactions are similar to those observed for the A class receptors, suggesting these unusual interactions are of a general importance for Eph function. However, the structures also uncover some differences that help understand the variations in the signaling mechanisms between the A- and the B-class receptors. These findings provide new information on the interactions that the Eph ECDs participate in, which can assist in identifying molecular regions that can be clinically targeted with small molecules, peptides or antibodies.

*Abstract 04*
*Session I – Signaling-I*
*June 1, 9:50-10:15 AM*

## DECODING THE SPECIFIC INTERACTION CODE OF EPH RECEPTORS SAM DOMAINS PROVIDES TOOLS TO SWITCH THEIR DOWNSTREAM SIGNALING PATHWAYS

Wei Liu

*Shenzhen Key Laboratory for Neuronal Structural Biology, Biomedical Research Institute, Shenzhen Peking University-The Hong Kong University of Science and Technology Medical Center, Shenzhen 518036, China*

EPH receptor tyrosine kinases family is the largest subfamily of tyrosine receptor kinases, playing critical roles not only in embryonic and adult development, but also in cell migration, spatial boundaries formation, angiogenesis, neurogenesis and synaptic plasticity. The intracellular part of Eph receptors consists of a juxtamembrane region, a tyrosine kinase domain, a SAM domain and a PDZ binding motif. It has been reported that the EphA2 SAM domain can interact with SHIP2 SAM to affect the endocytosis of EPHA2 receptor. Through biochemical analysis and X-ray crystalography studies, we measured the binding affinity between EphA2 SAM and SHIP2 SAM and determined their complex structure. We found that there was a unique cation-π interaction between EPHA2 and SHIP2, which contributed to the specific interaction between EPHA2 and SHIP2. Based on the crystal structures and further bioinformation analysis, we further discovered the SAMD5 SAM domain as another binding partner of EPH SAM domains and resolved their complex strucutre. Therefore, we artificially divided EPH receptor SAM domains into different categories according to their crystal structures and binding specificities. In addition, by introducing a single point mutation, we successfully manipulated the binding specificity between EPH receptors and its downstream partners both *in vitro* and *in vivo*. In summary, our studies not only elucidated the exquisitely specific target recognition code of EPH SAM, but also provided a tool to switch the downstream signaling pathways between different EPH receptor family members.

*Abstract 05*
*Oral Session II – Neurosciences*
*June 1, 10:45-11:10 AM*

**WERDS complex regulates apical constriction via crosstalk with Wnt components**

Jaeho Yoon[1], Jian Sun[1], Moonsup Lee[1], Yoo-Seok Hwang[1] & Ira O. Daar[1]

[1] *Cancer & Developmental Biology Laboratory, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Frederick, MD 21702, USA*

Apical constriction is a cell shape change along the apical-basal axis that supports tissue morphogenesis, such as neural tube formation. Actin-myosin networks are key components in the generation of the contracting force required for tissue remodeling, but the signaling cue that instructs apical constriction remains unknown. Previously, we showed that ephrinB2 is necessary for neural tube closure, however, the precise mechanism remained elusive. Using a blend of biochemistry, live and fixed cell imaging, gain-of-function and loss-of-function along with rescue experiments using wild-type and mutant constructs *in vivo*, we provide mechanistic insight into how ephrinB2 plays an instructive role in neural tube closure. These experiments led us to the identification of a signaling complex consisting of Wnt4, EphrinB2, Ror2, Dsh2, and Shroom3 (termed WERDS) that is responsible for this ephrinB2-driven process. Moreover, as part of this mechanism, we made the exciting revelation that ephrinB2 antagonizes Wnt/□-catenin signaling through a conformational change in the main Wnt signaling scaffold, dishevelled. This interaction switches dishevelled from canonical to non-canonical Wnt signaling that is required for apical constriction in the neural tube. We believe that these findings provide the profound understanding of how cross-talk occurs between two seemingly separate major signal transduction pathways, Eph/ephrin and Wnt, to coordinate a major morphogenetic event, neural tube formation.

This research was supported by the Intramural Research Program of the National Institutes of Health, National Cancer Institute (Project Number: 1ZIABC010006-26).

*Abstract 06*
*Session II – Neurosciences*
*June 1, 11:10-11:35*

## EPH/EPHRIN SIGNALING REGULATES GENE EXPRESSION AT RHOMBOMERE BOUNDARIES THROUGH THE ACTIVATION OF YAP/TAZ TRANSCRIPTION FACTORS

J. Cayuso[1], Xu Q.[2], D. G. Wilkinson[2]

*1. Research Institute in Healthcare Science, University of Wolverhampton, Wolverhampton, UK.*
*2. Neural Development Laboratory, The Francis Crick Institute, London, UK.*

The establishment of sharp borders between tissue interfaces is critical for the correct organisation of organs and, in some tissues, underlies the formation of signalling centres that regulate growth or patterning of adjacent cell populations. The segmentation of the vertebrate hindbrain is a paradigmatic example in that it is regionalised in rhombomeres with different identities and, at their interfaces, the boundary cells act as signalling centres that pattern neurogenesis. The Ephs and Ephrins, transmembrane proteins mediating bidirectional cell-to-cell communication, are known to initiate heterotypic signalling at segment borders, essential for border sharpening and formation of boundary cells. Cell repulsion and mechanical tension are required for border sharpening, however the mechanism regulating boundary cell formation remains elusive.

Using CRISPR/Cas9, we have built an allelic series of EphA4 that has been instrumental to demonstrate that cell-to-cell interactions at rhombomere interfaces activate Eph-kinase activity, which increases mechanical tension and activates Yap/Taz mechanotransduction, which in turn regulates the expression of boundary cell-specific genes at segment boundaries.

*Abstract 07*
*Poster Session*
*June 1, 12:00-13:30*

## INVESTIGATING HOMOTYPIC INTERACTIONS OF EPH RECEPTOR FAMILY USING PIE-FCCS

Soyeon Kim[1], Xiaojun Shi[2], Bingcheng Wang[2], Adam W. Smith[1]

*[1]Department of Chemistry, The University of Akron, Akron, OH, USA; [2]School of Medicine, Case Western Reserve University, Cleveland, OH, USA*

Eph/Ephrin signaling is crucial for cell adhesion and movement, and abnormal signaling can lead to serious diseases including cancer. Recent work has shown that Eph receptor function is directly connected to its spatial organization in the plasma membrane. Ligand-induced assembly into competent signaling clusters has been demonstrated for several Eph receptors. but the degree of oligomerization before and after ligand binding has not been resolved for the majority of the 14 human Eph receptors. Here we report on an expanded investigation of spatial organization in the EphA subfamily. Our approach is to quantify receptor oligomerization in live cells at physiological expression levels using pulsed interleaved excitation fluorescence cross correlation spectroscopy (PIE-FCCS). Conceptually, PIE-FCCS is a single molecule co-localization assay. The main difference compared to single particle tracking approaches is that PIE-FCCS data is collected in the time domain, which allows us to measure coordinated diffusion and assembly at higher densities. PIE-FCCS data can be used to calculate local densities, diffusion coefficients, proximity, and the degree of oligomerization. With these data we are able to quantify Eph receptor interactions in situ. This approach will help resolve structure function relationships in Eph/ephrin signaling and identify new opportunities for drug development.

*Abstract 08*
*Poster Session*
*June 1, 12:00-13:30*

# ROLES OF EPHA2-EPHRIN SIGNALING IN PROSTATE DEVELOPMENT AND CANCER PROGRESSION

Ryan Lingerak[1,2,3], Aaron Petty[2], Xioajun Shi[1,2], Ji Zheng[1,2], Vera M. Hapiak[2], Bingcheng Wang[1,2,3]

*[1]Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, Ohio, USA, [2]Rammelkamp Center for Research, MetroHealth Medical Center, Cleveland, Ohio, USA, [3]Case Comprehensive Cancer Center, Cleveland, Ohio, USA.*

Prostate cancer (PCa) is the most common cancer in the US men. While usually indolent or benign, a small fraction (~5%) rapidly progress to malignant diseases. PCa is initially responsive to androgen deprivation therapy (ADT) or castration. However, aggressive forms of the disease inevitably become resistant to the therapy, leading progressively to metastatic castration resistant PCa (mCRPC), a fraction of which further progress to neuroendocrine prostate cancer (NEPC) and double negative PCa (DNPC). A major goal of PCa research is to broadly identify molecular and cellular mechanisms aiding nearly inevitable progression to identify vulnerabilities that could be targeted. Multiple receptor tyrosine kinases (RTKs) have been implicated in PCa. A significant body of literature points to an important role of EphA2, a member of the Eph subfamily of RTKs, in PCa. Notably as first reported by Chinnaiyan lab, EphA2 RTK is overexpressed in metastatic CRPC, but not early localized PCa tumors. In tumors where EphA2 is overexpressed, there is loss of the cognate ligand EphrinA1. In fact, Colm Morrissey was the first to discover that EphrinA1 is one of the top three genes whose expression is lost in metastatic PCa, particularly in bone metastases. The Wang lab, a leading group in studying Eph/Ephrin system in cancer biology, discovered that EphA2 has dual opposed roles during tumor development and progression, i.e., a ligand dependent tumor suppressor in the early stage of tumorigenesis and a ligand-independent oncogenic protein in the late stage tumor progression in several cancer types. Our preliminary data indicate that in PCa, EphA2-EphrinA signaling also has tumor suppressor role in early-stage PCa, and pro-oncogenic functions in metastatic CRPC. Excitingly, ongoing studies in our lab show that EphA2 is a potential regulator of tumor immune microenvironment (TIME) in multiple cancer models, including prostate cancer. Understanding the contribution of EphA2 to the status of the prostate TIME could provide a new avenue to target the TIME in hope of alleviating difficulties with treatment using current immunotherapies. Our current hypothesis is that EphA2-ephrinA interaction plays a multifaceted regulatory role in prostate cancer (PCa) development and malignant progression toward late stage PCa. The outstanding questions are addressed by modeling PCa initiation and progression in the context of EphA2/EphrinA signaling using a murine prostate organoid system, a viral, *in vivo* spontaneous initiation of PCa termed RapidCap, and common PCa cell lines that have been previously characterized in the literature.

*Abstract 09*
*Poster Session*
*June 1, 12:00-13:30*

# EPHRIN-A LIGANDS REGULATE CUTANEOUS TUMOR ETIOLOGY AND METASTASIS THROUGH CELL AUTONOMOUS AND NON-AUTONOMOUS MECHANISMS

Ji Zheng[1,*], Bethany Perez White[3,*], Zhe Shao[1], Nihal Kaplan[3], Aaron Petty[1], Kord Honda[2], Mitchell Denning[5], Miroslav Blumenberg[6], Nickolas Gale[7], Spiro Getsios[3,4,#], and Bingcheng Wang[1,#]

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**These authors contributed equally to the work. #Corresponding Authors.*

Glycophosphatidylinositol (GPI)-anchored ephrin-A ligands target EphA receptor tyrosine kinases (RTKs) to promote keratinocyte differentiation. Accordingly, genetic ablation of the major epidermal EphA subtype, *Epha*2, increases susceptibility to DMBA/TPA-induced cutaneous chemical carcinogenesis. Defining the corresponding role of ligands for EphA2 in skin cancer has been more cumbersome as the three ephrin-A genes (*Efna*1, *Efna*3, *Efna*4) are prominently expressed in skin. We met this challenge by engineering a triple *Efna*1/3/4 knockout (TKO) mice that reflects the pattern of reduced ephrin gene expression found in mouse and human cutaneous squamous cell carcinomas (SCCs). Skin tumors developed earlier and grew faster in mice lacking EphA2 or ephrin-A1, 3, and 4 ligands. Interestingly TKO mice displayed accelerated malignant progression toward invasive SCC that metastasized to the lymph nodes and lungs 25 weeks following DMBA-TPA treatment. Using keratinocyte culture models, we found that ephrin-A ligands act within the epidermis to limit keratinocyte migration in a manner dependent on EphA2 targeting. We also illustrated a key role for ephrin-A ligands in the surrounding tumor microenvironment by re-introducing isogenic SCC cell lines into the skin of wild-type or TKO mice on isogenic FVB background; tumor growth and metastasis was facilitated in mice lacking ephrin-A ligands. Importantly, the invasive phenotype of TKO mouse tumor cell lines was normalized by genetic reintroduction of either *Efna*1 or *Efna*3. Integrating our findings from human tumors, mouse models, primary cell cultures, and allograft models provides strong support that ephrin-A ligands operate within tumor cells and also in the microenvironment to suppress skin tumor initiation and metastasis.

*Abstract 10*
*Poster Session*
*June 1, 12:00-13:30*

## CHARACTERIZATION OF EPHB1 MUTATIONS FROM A PAN-CANCER ANALYSIS OF THE TCGA DATASET

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Approximately 20-25% of CRC patients present with metastatic disease at diagnosis, and another 20-25% will develop metastases later in the disease. Lack of efficient treatment for metastatic disease leads to a high overall mortality rate of 40-45%. Despite the recent progress in cancer genome sequencing, it has proven challenging to associate specific gene mutations with metastasis. It is currently unclear if somatic mutations in specific genes contribute directly to the metastatic process. Resolving this question is of clinical importance to identify patients that require close monitoring to detect recurrence as well as to stratify patients that would benefit most from adjuvant chemotherapy treatment. To this end, we recently demonstrated a link between *EPHB1* inactivating mutations and metastasis of primary CRC through deep sequencing of 672 genes in 107 CRCs (Mathot *et al.*, *Cancer Res* 77(7): 1730-1740, 2017). We could demonstrate that several *EPHB1* mutations observed in CRC reduced the response to Ephrin B ligand in a compartmentalization (cell clustering) assay (Cortina *et al.*, *Nat Med* 39 (11), 2007) as compared to wild-type *EPHB1* (Mathot *et al.*, 2017), which established a potential role in CRC metastasis. Thus, further studies of the contributions of EPH receptor mutations to cancer development and metastasis are warranted. Hence, 10,000 samples from 33 types of tumors from TCGA were analysed bioinformatically to find 2D and 3D hotspot mutations (Gao et al. *Genome Medicine,* 2017). We identified 15 recurring *EPHB1* mutations, which were engineered in lentiviral vectors and transduced into DLD-1 CRC cells for phenotypic studies. Among these *EPHB1* mutation hotspots, the kinase domain mutation D762N and the Ephrin binding domain mutation C61Y showed strongly compromised compartmentalization. To our knowledge, this is the first study of characterizing pan cancer *EPHB1* mutations in context of tumor metastasis.

*Abstract 11*
*Poster Session*
*June 1, 12:00-13:30*

# DISCOVERY OF SMALL MOLECULES SELECTIVELY INHIBITING EPHAs-EPHRIN-As INTERACTION

Carmine Giorgio, Francesca Ferrari, Alfonso Zappia, Lorenzo Guidetti, Riccardo Castelli, Alessio Lodola, Massimiliano Tognolini

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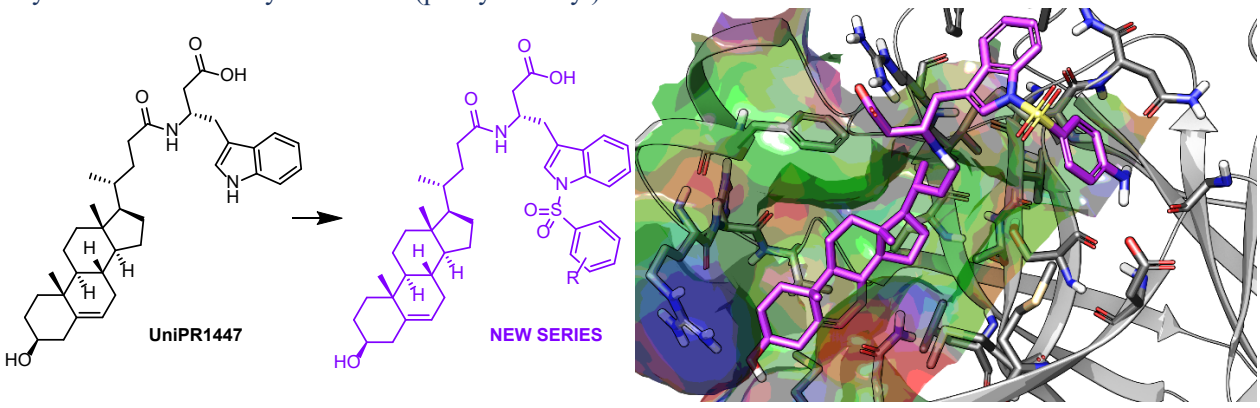
Since human tissues express different members of Eph/ephrin system and their interactions are highly redundant within A and B family, the availability of tools able to discriminate between A and B class would be a valid aid to further clarify the role of this system in different pathophysiology conditions.

The current available tools are highly selective towards a single member of the Eph receptors (i.e. antibodies and peptides) or they completely lack selectivity (kinase and protein-protein interaction inhibitors). The first are well represented by antibodies and peptides where DS-8895 selectively binds to EphA2 Ifobotuzumab to EphA3, YSA and SWL to EphA2 and SNEW to EphB2. The latter are represented by kinase inhibitors (dasatinib, GLPG1790, ALW-II-49-7) and small molecules PPI-inhibitors, including lithocholic and cholenic acid derivatives, whose interactions are extended to almost all the receptors of the family.

Docking simulations of the pan Eph-receptor antagonist UPR1447 (*N*-(3β-hydroxy-$\Delta^5$-cholen-24-oyl)-L-β-homotryptophan) suggest that fun ctionalization of its indole nitrogen can be exploited to obtain selectivity towards EphA-receptor subfamily over the EphB receptor one. This computational hypothesis prompted the synthesis of differently substituted (phenylsulfonyl) indole derivatives.




Binding studies confirmed the ability of newly synthetized compounds to displace ephrin-A1 from all the EphA receptors in the low micromolar range (1-3microM), whereas they resulted completely inactive towards ephrin-B1- EphB1/2 binding. A low potency (15-20microM) was detected towards ephrinB1/2-EphB3/4 interaction.

Our SAR investigation suggests the presence of an accessory pocket in the ligand binding domain of the EphA receptor family, available for binding.

The new compound could represent an interesting pharmacological tool to dissect EphAs and EphBs role in different pathophysiological environment.

*Abstract 12*
*Poster Session*
*June 1, 12:00-13:30*

# EPHB2 IS A NOVEL REGULATOR OF DERMAL FIBROSIS DURING SYSTEMIC SCLEROSIS

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Background: Systemic sclerosis (SSc, scleroderma) is an autoimmune disease characterized by fibrosis of the skin and internal organs. We previously reported that EphB2 is involved in liver fibrosis. However little is known about the implication of EphB2 in skin fibrosis during SSc. The goal of this study is to test the hypothesis that EphB2 activation could promote skin fibrosis in SSc.

Methods: We analyzed transcriptomic data set (GSE45485) comparing genes expression profile of *EPH/EPHRIN* molecules in skin biopsies from individuals with SSc to that of heathy controls. Immunofluorescence confocal microscopy was used to detect EphB2, phospho-EphB2, EphrinB2 and αSMA in skin biopsy specimens of healthy volunteers and patients with SSc. The regulation of EphB2/EphrinB expression on primary human dermal fibroblasts following TGFβ-1 stimulation in the presence or absence of TGFβ/SMAD inhibitors (SB525334 and SIS) and MAPK inhibitor U0126 was evaluated by western blot, quantitative PCR, and immunofluorescence. Mission–esiRNA was used to knockout EphB2 in human dermal fibroblasts and expression of pro-fibrotic genes were evaluated by qPCR and western blot following TGFβ-1 exposure. ELISA was used to detect serum EphrinB2, a ligand for EphB receptors in healthy (n=40) and SSc patients (n=132). Finally, histology, hydroxyproline levels, qPCR and western blots were used to assess the development of skin fibrosis in *EphB2-/-* and *EphB2+/+* mice following subcutaneous injection of bleomycin to induce skin fibrosis.

Results: From the transcriptomic data set, we identified *EPHB2* as the most upregulated *Eph/Ephrin* in skin biopsies of SSc patients compared to controls. Immunofluorescence microscopy showed that EphB2 and phosphoEphB2 are highly expressed in skin biopsies of patients with SSc compared to healthy controls and partially co-localized with the fibroblast marker αSMA. Normal human dermal fibroblasts upregulate EphB2 upon TGF-β exposure and this is mediated via the canonical TGF-β/SMAD signaling. Silencing of EphB2 abrogated dermal fibroblast-to-myofibroblsat conversion upon TGF-β1 exposure. Although, EphrinB2 a ligand for EphB2 was drastically reduced in skin biopsies of SSc patients relative to healthy controls, plasma level of EphrinB2 was instead elevated in patients with SSc. EphB2 expression is elevated in the bleomycin and tight skin (Tsk1/+) mouse models of scleroderma. Finally, we showed that EphB2 is a critical promoter of skin fibrosis in mice because *EphB2-/-* mice exhibit a drastic reduction of bleomycin-induced dermal fibrosis compared to their wild-type littermate controls.

Conclusions: Our findings unveil novel information regarding the potential implication of EphB2 during skin fibrosis in SSc.

*Abstract 13*
*Poster Session*
*June 1, 12:00-13:30*

## EPH RECEPTOR TYROSINE KINASES ACT ON THE SCAFFOLD PROTEIN PAR-3 TO MODULATE SIGNALING NETWORKS

Sara L. Banerjee[1,2,3];François J. Chartier[1,2,3]; Frédéric Lessard[1,2,3]; Kévin Jacquet[1,2]; Valentine Teyssier[1,2,3], Karim Ghani[1,2]; Manuel Caruso[1,2,4]; Jean-Philippe Lambert[1,2,5,6]; Nicolas Bisson[1,2,3,4,*]

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The EPH family of receptor tyrosine kinases (RTKs) is the largest in humans. Ephrins the cognate ligands of EPH receptors (EPHRs) are tethered to the cell surface. EPHR-ephrin signaling mainly involves short-range cell-cell communication events regulating cell adhesion, migration and tissue boundary. EPHRs functions have been broadly studied, however the molecular mechanisms by which they control these processes are still poorly understood. We sought to identify new effector proteins acting downstream of EPHRs and determine their role in EPHR-regulated functions. We applied the mass spectrometry and proximity labelling based approach BioID to the EPHA4, -B2, -B3 and -B4 EPHRs. The resulting interaction network comprises 395 proteins, most of which were not previously linked to EPH signaling. A gene ontology analysis highlighted novel functions associated with EPHR activity. The contribution of a number of selected candidates to EPHR-dependent cell segregation was analyzed. We found that depletion of the signaling scaffold PAR-3 blocked cell sorting. We then delineated a signaling complex involving the C-terminal SRC kinase (CSK), whose recruitment to PAR-3 complexes is dependent on EPHR signals.

*Abstract 14*
*Poster Session*
*June 1, 12:00-13:30*

## IDENTIFICATION OF EPHR STIMULATION-DEPENDENT AND INDEPENDENT PROXIMITY PARTNERS FOR EPHRINB LIGANDS USING TURBOID PROTEOMICS

Ana Isabel Osornio Hernandez[1,2,3], Sara Banerjee[1,2,3], Melanie Laurin[1,3], Nicolas Bisson[1,2,3,4]

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Reverse signaling downstream of transmembrane Ephrins B (EfnBs) can be classified as pTyr-dependent or PDZ-dependent. The pTyr-dependent reverse signaling initiates when EfnBs bind to Eph receptors (EphRs). This event promotes the phosphorylation of Tyr residues within the EfnBs intracellular domain. The resulting pTyr residues are thought to serve as docking platforms for signaling proteins bearing Src Homology (SH) 2 or Phopho- Tyrosine Binding (PTB) domains, which can then recruit additional signaling proteins in order to control cellular processes such as cell migration, cell adhesion or modulate cell morphology. In addition to pTyr-dependent signaling, EfnBs can initiate downstream signaling pathways through their PDZ binding motif, which mediates protein interactions with PDZ domain proteins. Despite our current knowledge on reverse signaling, the identity of multiple EfnB signaling effectors remains to be elucidated in order to better understand the molecular mechanisms underlying the function of EphR-EfnB signaling in cellular processes. We hypothesized that proximity labeling proteomics of EfnB ligands will allow the identification of new effectors involved in reverse signaling. Hence, we performed experiments with WT and non-phosphorylable EfnB(1-3) fused to miniTurbo in steady-state and following EphR stimulation. This allowed us to identify 170 novel EfnB proximity partners, from which we could distinguish three main groups: (i) EphR stimulation- dependent candidates; (ii) EphR stimulation-independent candidates and (iii) candidates that are negatively modulated by EphR stimulation. Interestingly, we found that most of the EphR stimulation-dependent interactions are lost with the EfnB-Y/F mutants, suggesting that these are pTyr-dependent interactions. Likewise, we found that the majority of EphR stimulation-independent interactions are pTyr-independent. We are currently working to validate these candidates and to investigate their EfnB-dependent functions. Overall, our findings will provide a better picture of signaling networks downstream of EfnBs.

*Abstract 15*
*Poster Session*
*June 1, 12:00-13:30*

## A STUDY OF HUMAN MUTATIONS IN THE GENE ENCODING THE TYROSINE KINASE RECEPTOR EPHB2

Sung Soon Park, Gergely Nagy, Brian Zhang, Chiara La Morgia, Janneke Weiss, MW Elting, Gunnar Houge, Pia Ostergaard, Valerio Carelli, Flavia Palombo, Eloisa Herrera, Christel Depienne, Yvonne Jones, and Artur Kania

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Aberrant neural development results frequently in neurological disorders affecting nervous system function and may even be fatal. In the nervous system, contact-mediated cell to cell signaling between Erythropoietin-producing hepatoma receptor tyrosine kinases (Eph RTKs) and Eph-receptor interacting ligands (Ephrins), so-called the Ephrin-Eph signalling, regulates axon guidance and synaptic function. Dysfunction of EphB2, has been shown to generate neurodevelopmental defects in animal models. However, to date, there is no credible cohort of patients with *EPHB2* mutations and nervous defects. Thus, the link between neuropathology and human *EPHB2* mutations remains tenuous. A worldwide network of clinicians, assembled a cohort of patients with mutations in the *EPHB2* gene, sharing corpus callosum defects. Protein sequence alignment suggests that many of our mutations are located at highly conserved sites across species and Eph receptor paralogues. Based on these human *EPHB2* mutation profiles, we proceeded with functional assays on EphB2. Our data show that one mutation, located at ligand binding domain, decreases surface EphB2 expression and ligand binding. Structure analysis suggests that this mutation causes protein misfolding and reduces binding affinity with B-type ephrins by abolishing disulfide bond formation. Our results suggest that mutations in the gene encoding the human EphB2 receptor could be a risk factor for corpus callosum neurodevelopmental defects in humans.

*Abstract 16*
*Poster Session*
*June 1, 12:00-13:30*

## ROLE OF EPH/EFN IN THE REGULATION OF NEUROBLAST FATE AND TOPOGRAPHICAL MAPPING

Daria Yeroshenko, Mashwiyat Mosharraf, Isabella Livingston, Sarah Bellizzi, Joanne C Conover

*University of Connecicut*

This project represents the initial phase of identifying signaling mechanisms that support cell-cell physical interactions that direct migration and topographical mapping in the postnatal brain. In rodents, the only postnatal long-range pathway is the forebrain rostral migratory stream (RMS). It is known that this migration pathway consists of fasciculated chains of neuroblasts that migrate through a dense meshwork of astrocytes to integrate within the olfactory bulb. However, the molecular cues that coordinate this extensive migration and guide new neuron distribution remain unclear.

Receptor tyrosine kinases Ephs and their ephrin ligands are known for coordinating and directing cell migration through direct cell-cell contact and are abundantly expressed at the ventricular-subventricular zone, RMS, and the olfactory bulb, making them potential candidates for regulating the neuroblast migration. Previously, our group found that EphA4 is a key player in RMS organization, as EphA4-/- mice show disorganization of the astrocyte meshwork, loss of neuroblast fasciculation and uncharacteristic neuroblast migration deviating from the RMS. (Todd et al. J Neurosci 37, 3331–3341, 2017) Immunohistochemistry and single-cell analysis also revealed unique neuroblast and astrocyte subpopulations based on EphA4/ephrin expression patterns.

Here, we present Eph/ephrin distribution patterns within the olfactory bulb, including the specific localization of activated (phosphorylated) receptors and ligands. We hypothesize that the differential co-expression of specific Ephs/ephrins in newly migrated inhibitory interneuron populations plays a role in their guidance. Single-cell expression patterns will be used to infer specific migratory mechanisms of each neuroblast subpopulation across the olfactory bulb development.

*Abstract 17*
*Poster Session*
*June 1, 12:00-13:30*

## STUDY IN CACO-2 3D CELLULAR MODEL REVEAL A ROLE FOR EPH TYROSINE KINASE RECEPTORS IN EPITHELIAL MORPHOGENESIS

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During development and adult tissue homeostasis, various processes are required to maintain tissue architecture. For instance, in epithelial tissues, coordination of apical and basal membrane morphogenesis and cell-cell adhesion are key molecular mechanisms that must be tightly regulated to maintain epithelial structure and function. Dysregulation of any of these processes can lead to defects in epithelial architecture and contribute to the progression of diseases such as cancer. In our laboratory, we are studying EPHRs-ephrin signaling. By using a mass spectrometry (MS)-based approach, namely BioID proximity labeling, we obtained a composite signaling network from EphA4, -B2, -B3 and -B4 receptors. Bioinformatics analysis of this network has revealed that EPHRs partners are involved in processes such as the establishment and maintenance of epithelial cell polarity and cell junctions. We thus hypothesized that EPHRs play a central role in the coordination of epithelial morphogenesis. To test this hypothesis, we used Caco-2 three-dimensional spheroids. We first showed that at least two of the fourteen EPHRs (EPHA1 and -B4) are expressed in polarized Caco-2 epithelial cells. Analysis of the subcellular localization of these two EPHRs in spheroids revealed that EPHA1 and EPHB4 are localized at the basolateral domain. To further study the role of EPHRs in epithelial morphogenesis, we performed loss-of-function experiments. Interestingly, EPHA1 or EPHB4 depletion disrupted spheroid morphogenesis. To better understand the phenotype, we explored two processes that, when deregulated, can lead to defects in tissue organization: maintenance of apical-basal polarity and mitotic spindle orientation. Rather than affecting the integrity of apical-basal polarity, our results suggest that this phenotype stems from defects in mitotic spindle orientation of dividing cells, which is crucial for maintaining a cell monolayer during epithelial tissue morphogenesis. Since this monolayer organization is lost in cancerous tissues, our work could lead to a better understanding of how these receptors contribute to epithelial morphogenesis and homeostasis in the context of cancer progression.

*Abstract 18*
*Session III – Signaling II*
*June 1, 13:30-13:55*

## DIRECT QUANTIFICATION OF LIGAND-INDUCED LIPID AND PROTEIN MICRODOMAINS WITH DISTINCTIVE SIGNALING PROPERTIES

Dr. Daniel Wirth[1], Dr. Michael D. Paul[2], Prof. Elena B. Pasquale[3] and Prof. Kalina Hristova[1,2*]

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Lipid rafts are ordered lipid domains that are enriched in saturated lipids, such as the ganglioside GM1. While lipid rafts are believed to exist in cells and to serve as signaling platforms through their enrichment in signaling components, they have not been directly observed in the plasma membrane without treatments that artificially cluster GM1 into large lattices. Here, we report that microscopic GM1-enriched domains can form, in the plasma membrane of live mammalian cells expressing the EphA2 receptor tyrosine kinase in response to its ligand ephrinA1-Fc. The GM1-enriched microdomains form concomitantly with EphA2-enriched microdomains. To gain insight into how plasma membrane heterogeneity controls signaling, we quantify the degree of EphA2 segregation and study initial EphA2 signaling steps in both EphA2-enriched and EphA2-depleted domains. By measuring dissociation constants, we demonstrate that the propensity of EphA2 to oligomerize is similar in EphA2-enriched and -depleted domains. However, surprisingly, EphA2 interacts preferentially with its downstream effector SRC in EphA2-depleted domains. The ability to induce microscopic GM1-enriched domains in live cells using a ligand for a transmembrane receptor will give us unprecedented opportunities to study the biophysical chemistry of lipid rafts.

*Abstract 19*
*Session III – Signaling II*
*June 1, 13:55-14:20*

## DECODING EPHA2 INTERACTIONS IN LIVE CELLS WITH PIE-FCCS

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A critical aspect of Eph receptor function is assembly into clusters, which regulates kinase activity and downstream signaling. Resolving membrane protein interactions in a live cell environment is challenging because of the chemical diversity and spatial heterogeneity of the PM. My presentation will describe a fluorescence technique called pulsed interleaved excitation fluorescence cross-correlation spectroscopy (PIE-FCCS) that is ideally suited to quantify membrane associations in live cells. PIE-FCCS is a two-color fluorescence fluctuation method that can simultaneously measure the concentration, mobility, proximity, and oligomerization state of membrane proteins in situ. It has several advantages over related approaches like single molecule tracking (SMT) and Forster Resonance Energy Transfer (FRET), including that it measures all the properties listed above in a single measurement. Another advantage is that PIE-FCCS is sensitive at the physiological expression levels for many membrane proteins rather than the very low or high levels typical in other techniques. I will describe recent work from our team that resolves the dimerization interfaces of EphA2 and how each of the interfaces mediate processes like protein activity, endocytosis, cell migration and tumorigenesis.

*Abstract 20*
*Session III – Signaling II*
*June 1, 14:20-14:45*

# STRUCTURAL AND FUNCTIONAL STUDIES OF THE EFFECTS OF PHOSPHORYLATION ON EPHRIN RECEPTOR TYROSINE KINASE, EPHA2, AND THE RELATIONSHIP WITH ITS SAM DOMAIN AS AN AUTOINHIBITOR

ZhenLu Li[1], Xiaojun Shi[2], Fatima Razelle Javier[1], Deanna Bowman[3], Jeannine Mueller-Greven[1], Belinda Willard[4], Bing-Cheng Wang[5], Adam W. Smith[6], Matthias Buck[1]

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Erythropoietin-producing hepatocellular (Eph) receptors are the largest subfamily of the single membrane-crossing receptor tyrosine kinase (RTK) family. Eph receptors have significant roles during embryonic development, cell maturation, and adulthood. Furthermore, a drastic increase in the expression of Eph receptors is detected in tumor cells of numerous cancers, such as melanoma and glioblastoma. Indeed, oncogenic activities have been observed by a non-canonical unliganded Ephrin type-A receptor 2 (EphA2) signaling mechanism. This project aims to probe the effects of phosphorylation on the intracellular domain (ICD) interactions of EphA2 in solution and bound to membranes [1]. Results from this study indicate that deletion of the sterile α motif (SAM) domain leads to an increased binding between kinase domains in solution [2]. Interestingly, upon oligomerization in vitro, a reduced kinase activity is observed, compared to that of the monomeric state of the EphA2 ICD. Thus, intriguingly, while deletion of the SAM domain increases oligomerization in case of the phosphorylated ICD, it appears that such protein-protein interactions are not required for kinase activity. Also docking and dynamics simulations only support a weakly bound kinase dimer in solution and suggest that the mechanism of activation is *in cis*, not requiring receptor cross phosphorylation *in trans* as has been observed in EGFR, for example and many other RTKs. Rather, activation likely involves an allosteric mechanism by disrupting SAM domain-kinase domain and/or SAM domain-membrane interactions. Data from this study will ultimately aid the design of EphA2 intracellular inhibitors or agonists.

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*Abstract 21*
*Session III – Signaling II*
*June 1, 14:45-15:10*

## PROTEOMIC ANALYSES OF EPH RECEPTORS REVEAL NEW REGULATION MECHANISMS AND BIOLOGICAL FUNCTIONS

Ugo Dionne[1,2,3], Sara L. Banerjee[1,3], Noemie Lavoie[1,3], Ana Isabel Osornio[1,3], Francois Chartier[1,3], Kevin Jacquet[1], Frederic Lessard[1,3], Patrick Laprise[1], Christian Landry[2,3], Nicolas Doucet[3,4], Nicolas Bisson[1,3]

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The EPH family of receptor tyrosine kinases (RTKs) is the largest in humans. In contrast to other RTKs, EPH receptors (EPHRs) cognate ligands, ephrins, are tethered to the cell surface. This results in EPHRs-ephrin signaling being mainly involved in short-range cell-cell communication events that regulate cell adhesion, cell migration and tissue boundary formation. However, the molecular mechanisms by which EPHRs mediate these processes are far from being understood. To address this question, we first sought to investigate the mechanisms by which EPHRs initiate and terminate the assembly on the plasma membrane of protein interaction networks that drive downstream signalling. Using mass spectrometry, we showed that activated EPHA4 directly phosphorylates NCK1/2 adaptor proteins, previously described EPHA4 effectors, on an evolutionary-conserved Tyr residue within their Src-Homology (SH) 3 domains. We demonstrate that phosphorylation of this Tyr leads to the disruption of the interaction between SH3 domains with target peptides *in vitro*, and to the collapse of signalling networks *in vivo*. This uncovered a novel, conserved mechanism through which EPHA4 rapidly and reversibly terminate downstream signalling while remaining on the plasma membrane in a catalytically active state.

We further sought to identify new downstream effector proteins for EPHRs and to determine their requirement for EPHR-regulated functions. To unravel EPHR-associated signaling complexes under native conditions, we applied a mass spectrometry-based proximity labeling approach, BioID. We obtained a composite signaling network around 4 EPHRs that is comprised of 395 proteins, most of which not previously linked to EPH signaling. We examined the function of a subset of candidates in EPHR-controlled cell sorting. We showed that depletion of a few candidate proteins, including the scaffold PARD3, blocked cell sorting, suggesting that their function is required to transmit EPHR signals. We further demonstrated the existence of a pTyr-dependent signalling mechanism involving PARD3 and the tyrosine kinase CSK. Our ongoing work will lead to a better understanding of the mechanisms by which EPHRs signal at the membrane and will give insight into how deregulation of these pathways contributes to tissue boundary disruption in disease states.

*Abstract 22*
*Session IV – Tumor I*
*June 1, 15:40-16:05*

# CLINICAL SIGNIFICANCE OF EPHRIN RECEPTOR (EPH)-B1, -B2, -B4 AND -B6 EXPRESSION IN THYMIC EPITHELIAL TUMOURS

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**BACKGROUND** Thymic epithelial tumours (TET) are primary anterior mediastinal neoplasms ranging from locally agressive to frankly malignant. They are further classified according to WHO 2015 subtype and Masaoka stage. Chemotherapy is usually the treatment option for locally advanced and metastatic disease. Ephrins (ephs) and their receptors (EPHs)-the latter members of the receptor tyrosine kinases (RTKs) superfamily-are implicated in tissue development and homeostasis and are aberrantly expressed in tumors. Importantly, several preclinical animal studies have not revealed particular toxicity for a variety of EPH-targeting factors, which renders them attractive anti-cancer agents. In EPH-B-deficient thymi the epithelial network is disrupted, but thymocyte development is generally spared. Hence, inhibition of EPH-Bs may constitute a strategy against TETs, without producing immune deficits. To our knowledge EPH-B expression has not been previously studied in TETs.

**OBJECTIVE** To examine the clinical significance of EPH-B1, -B2, -B4 and -B6 expression in TETs.

**METHODS** Tissue microarrays (TMAs) were constructed out of FFPE tissues from 98 TETs excised between 2009 and 2019, from an equal number of patients (55 females and 43 males; 29 to 85 years old), including 12 type A, 22 type AB, 17 type B1, 18 type B2, 14 type B3, 2 micronodular thymomas and 13 thymic carcinomas, according to the WHO 2015 classification). Three to five cores from each tumour were included so as to capture tumour heterogeneity. Survival data were available for 36 patients with an average follow-up of 42 months (11 to 134 months). The TMAs were immunohistochemically stained for EPH-B1, -B2, -B4 and -B6 and the immunohistochemical protein expression score IRS was evaluated. Pearson's chi-square test was applied for statistical analysis of the relationship between the IRS score and tumour WHO subtype and Masaoka stage. Survival data were analyzed by Cox proportional hazards model.

**RESULTS** EPH-B1 nuclear and cytoplasmic expression pattern was noted in the epitelial compartment of all TET cases, with variations mainly in their lymphocytic component. EPH-B2 was weakly and focally expressed in the cytoplasm of the epithelial cells and mostly weakly in lymphocytic nuclei in almost half of the cases. EPH-B6 nuclear expression pattern was variable, more common in lymphocytes than in the epitelial neoplastic cells, and present in about 2/3 of the tumours. EPH-B4 was not expressed either in epitelial neoplastic or lymphocytes of TETs. Nevertheless endotelial cells only were weakly positive for EPH-B4 in in the vast majority of tumours. The lymphocytic population in thymomas (especially in subtypes AB and B1), which consists of accompanying immature T-cells, presented higher EPH-B6 IRS compared to lymphocytes in thymic carcinomas ($P<.001$), where they probably represent antitumoral immune reaction; conversely, lymphocytic EPH-B1 expression was higher in carcinomas ($P=0.026$). Thymomas of low Masaoka stages (I and II) presented higher lymphocytic ($P=0.043$) and lower epithelial ($P=0.010$) EPH-B6 IRS. Lymphocytic EPH-B1 expression was less strongly correlated with Masaoka stage ($P=0.059$). On the other hand correlation of EPH-Bs IRS with patients' survival was not reached.

**CONCLUSIONS** According to our study EPH-B1, -B2 and -B6 are expressed in both the epitelial and the lymphocytic component of TETs. The expression level of EPH-B1 and -B6 correlates with established prognostic parameters, i.e. tumour subtype and Masaoka stage, suggesting involvement of these RTKs in thymic neoplasia, as well as their potential utility as treatment targets.

*Abstract 23*
*Session IV – Tumor I*
*June 1, 16:05-16:30*

# CELL COMPETITION IN ADULT PANCREAS TISSUES IN VIVO REQUIRES FUNCTIONAL EPHA2

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Cells carrying cancer-causing genetic mutations compete with normal neighbours for space and survival in tissues via a process of cell competition. In general, cell competition removes aberrant or mutant cells from tissues, which if retained would decrease tissue health. The mechanisms underlying mutant-normal competition in adult tissues and the relevance of this process to cancer remain incompletely understood. In simple epithelia, we have previously shown that EphA2-ephrinA signalling drives the segregation and expulsion of RasV12-expressing cells from normal cells. Since oncogenic Kras (KrasG12D) is a key driver mutation in early pancreatic cancer, we investigated whether cell competition is required to maintain pancreas tissue health *in vivo*, and whether functional EphA2 is required in this process. Using mouse models of pancreatic cancer, we find that when present in tissues in low numbers, KrasG12D mutant cells are outcompeted and eliminated from exocrine and endocrine tissue compartments *in vivo*. We identify EphA2 receptor as an essential signal in the removal of KrasG12D cells from all tissue compartments. In the absence of functional EphA2, KrasG12D cells are retained in tissues. Retention of KRasG12D cells leads to an increased burden of premalignant pancreatic intraepithelial neoplasia (PanINs) in tissues. Our data show that adult pancreas tissues remodel to clear KrasG12D cells and maintain tissue health. This study provides evidence to support a conserved functional role of EphA2 in Ras-driven cell competition in epithelial tissues and suggests that EphA2 is a novel tumour suppressor in pancreatic cancer.

*Abstract 24*
*Session IV – Tumor I*
*June 1, 16:30-16:55*

# EPHA2 AND EPHA4 TARGETING AGENTS FOR THE DEVELOPMENT OF INNOVATIVE THERAPEUTICS IN ONCOLOGY AND NEURODEGENERATION

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In most solid tumors, overexpression of EphA2 is invariably associated with poor prognosis and development of aggressive metastatic cancers. Likewise, EphA4 overexpression is associated with poor prognosis in ALS patients. Over the past several years, we focused on the derivation of potent agents that target the ligand binding domains (LBD) of these receptors, mimicking their natural ligands (the ephrins). Guided by our recently solved X-ray structure of the complex between one such agents and EphA2-LBD[1], I will report on our novel EphA2 targeting agents (Baggio *et al*., in preparation) with low-nanomolar affinity in biochemical and biophysical assays. In cell-based assays, our agent, we termed Targefrin, is as effective as the natural dimeric ligands (ephrinA1-Fc) in inducing cellular receptor internalization and degradation. Targefrin is very effective in suppressing cell migration in several cancer cell lines and in preventing metastases in orthotopic mouse models.[1-5] When our agents are conjugated with chemotherapy, these can effectively deliver their cargo to EphA2 expressing tumor cells in various models.

Using similar NMR- and structure-guided design strategies, we also derived first-in-class ligands tailored to the EphA4 subtype targeting its ligand binding domain. [6-7] X-ray structural studies with one such ligand guided further optimizations leading to agents with nanomolar affinity. In cellular assays such agents exert marked protection of motor neurons from ALS patient derived reactive astrocytes (Dennis, Baggio *et al*., submitted). I will reiterate the strategies used to derive these innovative agents and their pharmacological properties in cellular assays and *in vivo*. We are happy to share small amounts of these agents for research purposes and/or for collaborative studies.

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*Abstract 25*
*Session V – Tumor II*
*June 2, 8:30-8:55*

## EPHA3 AND EPHRIN A5 DISCRETE EXPRESSION GRADIENTS FUNCTION TO PROMOTE GLIOBLASTOMA HETEROGENEITY PHENOTYPES

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Glioblastoma (GBM) is an aggressive brain cancer associated with a very poor prognosis; new therapeutic approaches are urgently needed. The EphA3 receptor is frequently elevated in GBM, particularly in the most aggressive de-differentiated mesenchymal subtype. In this study, we sought to investigate the contribution of the EphA3 high affinity ligand, ephrin A5 to GBM tumourigenesis. Immunofluorescence and immunohistochemical expression analysis revealed discrete EphA3 and ephrin A5 tissue gradients present within GBM specimens. EphA3 tumour fractions were shown to be more proliferative and migratory consistent with a more mesenchymal phenotype. In contrast, ephrin A5 fractions displayed low Ki67 staining, appeared less motile and displayed strong staining for the glial marker GFAP, and phosphorylated ERK, indicating a more differentiated slower growing phenotype.

To further explore function, we over-expressed ephrin A5 in primary GBM neurosphere cultures. This led to a decrease in stem cell-like features, loss of neurosphere formation and promoted a differentiated less aggressive phenotype. Ephrin A5 over-expressing cells were engrafted orthotopically into the brains of immunocompromised mice to assess tumour formation. In each model tested, ephrin A5 over-expression significantly delayed tumour progression, compared to control, which was most pronounced in pro-neural subtype GBM models. We next conducted spatial transcriptomics on ephrin-A5 over-expressing xenografted tumours. Results revealed a reduction in Ki67, MCM7 and PCNA marker expression compared to control. In addition, we detected an increase in expression of astrocytic-like GBM cell-state markers concomitant with a reduction in mesenchymal and neural progenitor-like cell-states. Taken together, these findings reveal a functional role of EphA3 and ephrin A5 in modulating GBM heterogeneity cell-states. Our future studies will now focus on dual antibody-based receptor and ligand targeting studies to better capture GBM heterogeneity and extend survival in this aggressive disease setting.

*Abstract 26*
*Session V – Tumor II*
*June 2, 8:55-9:20*

## INHIBITION OR TARGETING OF EPHA3 EXPRESSION IN CANCER ASSOCIATED FIBROBLAST SUBTYPES INHIBITS TUMOUR GROWTH

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Tumour progression relies on interactions between transformed tumour cells and the tumour microenvironment (TME), including the stroma (literally 'mattress'), vasculature and immune cells. Stromal cells include cancer-associated fibroblasts (CAFs), which can promote angiogenesis, tumour growth and immune evasion. Eph receptors and their cell-bound ephrin ligands control cell-cell interactions guiding vascular and neural patterning during normal development, and re-emerge in tumours and the TME. EphA3 is over-expressed in a range of tumour types, and we previously found this expression is not only in tumour cells, but more commonly in supportive stromal and vascular tissues in human tumour samples and in mouse xenografts.

To investigate whether EphA3 expression in the TME supports tumour growth, we generated mice with transgenic inducible shRNA expression to reduce EphA3 expression in the TME. We confirmed specific EphA3 knock-down in mesenchymal stromal progenitor cells (MSCs) from mice, which also displayed reduced angiogenic capacity in vitro. We then analysed syngeneic Lewis Lung Carcinoma tumours in EphA3 knockdown in mice, which showed a reduction of MSC/CAF-like cells, with reduce vessel formation and tumour growth. We found EphA3 was particularly expressed on perivascular and myofibroblast-like CAFs (myCAFs), both in mouse tumours and in human breast and pancreatic cancers, as evident from single cell RNA sequencing analysis. Targeting these cells with a novel anti-EphA3 antibody-drug conjugate inhibited tumour growth in mice, demonstrating potential therapeutic utility. Our results thus indicate that stromal EphA3 expression plays an important and targetable role in supporting tumour growth.

*Abstract 27*
*Session V – Tumor II*
*June 2, 9:20-9:45*

## IDENTIFICATION OF EPH RECEPTOR SIGNALING AS A THERAPEUTIC TARGET IN COLORECTAL CARCINOMA

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Despite advances in prevention, early diagnosis and surgical treatment, colorectal carcinoma is a leading cause of death worldwide. We have identified a phosphotyrosine-dependent Eph receptor signaling as a vulnerability in colorectal carcinoma cells, thus uncovering a pro-survival pathway in colorectal carcinoma cells, which is dependent upon Eph receptor tyrosine kinase signaling. Colorectal cancers express higher levels of the EphrinB2 ligand and Eph receptors than all other cancer types analyzed (no. 9) and increased levels of EphrinB2 expression predict a lower colorectal cancer patients survival probability. In addition, protein content of EphrinB2 and Eph2, EphB3 and EphB3 receptors is significantly higher in colon cancer tissues compared to the normal adjacent colonic tissue. *In vitro*, genetic and biochemical inhibition of Eph tyrosine kinase activity or depletion of the Eph ligand EphrinB2 reproducibly induces colorectal carcinoma cell death. Autophagy was identified as the underlying death pathway. Consistent with this, Spautin and 3-methyladenine, inhibitors of early steps in the autophagic pathway, significantly reduced autophagy-mediated cell death that follows inhibition of phosphotyrosine-dependent Eph signaling in colorectal cancer cells. A specific and potent inhibitor of the Eph kinase, NVP-BHG712 ($IC_{50}$ for EphA2: 15.2nM and $IC_{50}$ for EphB4: 28.4nM in cell-based kinase assays) or its regioisomer NVP-Iso, markedly reduce human colorectal cancer cell growth *in vitro* in 10/10 cell lines, but not the growth of control cells and attenuate tumor growth in mice. These results support Eph signaling inhibition as a potential new strategy for the broad treatment of colorectal carcinoma.

*Abstract 28*
*Session VI – Newly found functions*
*June 2, 10:15-10:40*

## ALTERED EPH-EPHRIN SIGNALING DISRUPTS BRAIN CIRCUIT FORMATION AND PRODUCES DEVELOPMENTAL NEUROLOGICAL DISORDERS IN MOUSE THAT MIMIC HUMAN BEHAVIORS OBSERVED IN AUTISM, OCD, AND ADHD

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Formation of the intricate topographic organization of neural circuits in the telencephalon rely on precisely coordinated and temporally regulated expression levels of cell surface receptors that control neuronal migration and axonal guidance. Over the past 2 decades numerous studies have identified a constellation of receptors and their ligand binding partners in these developmental processes. These studies indicate that the large family of Eph receptors and ephrins are important components in these developmental events. To further clarify how ephrins can produce global alterations in telencephalic circuits that result in associated behavioral changes, we investigate how altered expression of different subsets of A-class ephrins resulting from germline deletions of efn-A2 and efn-A5, either individually or in combination, produce unique and overlapping behavioral changes in mice that mimic a spectrum of behavioral changes observed in individuals with neurodevelopmental spectrum disorders.

In our studies we used a global connectome approach, in conjunction with a panel of behavioral tests, to identify common and unique circuit changes in cortico-striatal, cortico-hippocampal, and prefrontal cortico-cortical projections that produce co-morbid or unique changes in the behavioral readout from the different mutant mouse lines. The following behavior were evaluated using multiple behavioral tests: 1) cognitive and motor learning and memory, 2) anxiety and fear responses, 3) social and exploratory actions, and 4) sensory processing. Pilot data indicate that efn-A2 KO (2KO) mice were not impaired on motor learning (Rotarod) or on spatial learning (Morris Water Maze), but both efn-A5 KO (5KO) and efn-A2/A5 double mutants (2/5DKO) were impaired in both learning paradigms. On the elevated plus maze, 2KO mice spent significantly less time in the closed arm compared to Controls; whereas both the 5KO and the 2/5DKO mice exhibited the opposite behavior and spent more time in the closed arm. These data indicate that altered EphA-efnA signaling during brain development due to efn-A2 versus efn-A5 deletions results in unique differences in learning and exploratory behaviors; with the loss of both efn-A2 and efn-A5 producing behavioral changes similar to behaviors observed in efn-A5 only deletions.
Neurohistological data from these mice also demonstrate that there are differences in the compartment organization in the striatum and connections in the hippocampus in the different mutant mice. For example, mice with efn-A5 deletions (both 5KO alone and 2/5DKO mutations) exhibit the greatest disruption of the matrisome compartment within the striatum that contains neurons expressing EphA7 receptors, which are high affinity binding partners for efn-A5. Likewise, there are greater disruptions in hippocampal connections, (e.g. granule cells mossy fibers) in mice with efn-A5 deletions than is observed when efn-A2 alone is deleted.

Our results support a dominant developmental role for efn-A5 in striatal neuron compartment organization and hippocampal axonal circuits that are involved in regulating important cognitive and motor behaviors affected in multiple neurodevelopmental spectrum disorders. Since the timing and expression levels of ephrins and Eph receptors are tightly regulated in different brain regions at different developmental stages, epigenetic factors that alter the normal regulation of the promoters for these cell-cell signaling molecules could play a significant role in the underlying molecular mechanisms that lead to subtle differences in the behavioral dysfunctions observed in the spectrum of individuals exhibiting developmental neurological abnormalities.

*Abstract 29*
*Session VI – Newly found functions*
*June 2, 10:40-11:05*

## EPHB4 REGULATES PACING CELL DEVELOPMENT AND HEART RATE

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EPHs/EFNs play essential roles in many biological systems and tissues. Recently, we have revealed that these molecules are novel regulators of blood vessel smooth muscle contractility and blood pressure, based on mouse gene knockout and human genetic studies. During the course of our study, we noticed that the female but not male mice with smooth muscle cell (SMC)-specific EPHB4 deletion (Cre expression driven by the smooth muscle myosin heavy chain promoter (smMHC-Cre)) manifested a lower heart rate. Such a finding prompted us to investigate the role of EPHB4 in heart rate regulation and pacing cell development.

We confirmed that there was EPHB4 deletion in cardiomyocytes of the KO mice due to leaky smMHC-Cre expression. Neither the female nor male KO mice had abnormal EKG findings, and their P-R intervals were normal, indicating that there was no AV block. On the contrary, the expression of hyperpolarization-activated cyclic nucleotide-gated channel 4 (HCN4), which is obligatory for pacing activity, was significantly reduced in the sinoatrial node (SAN) of KO female but not male adult mice. This suggests either functional defects of the SAN or compromised SAN development in the female KO mice.

To investigate the role of EPHB4 in SAN development and HCN4 expression, we used suramin to drive the differentiation of WT and KO female embryonic stem (ES) cells to pacing cells. The KO ES cells presented drastically reduced EPHB4 expression, again due to the leaky smMHC-Cre expression. The KO ES cells showed a reduced proliferation rate. After the suramin treatment, they had a delayed appearance and a reduced number of beating centers. In the beating centers derived from the female KO ES cells, the HCN4 mRNA and protein expression was delayed and decreased. Furthermore, the beating rate of the beating centers derived from the KO ES cells was significantly lower than that of their WT counterparts. Interestingly, the reduced beating rate of the female KO beating centers was not affected by exogenous estrogen or testosterone. These results indicate that EPHB4 is essential in pacing cell development and controls HCN4 expression, and sex but not sex hormone is a cofactor for its effect in this regard.

In the HCN4 gene, there are two enhancers, *i.e.*, AP1- and MEF2-binding sites, that regulate HCN4 expression. The treatment of WT ES cells with suramin clearly influenced the activation of multiple signaling molecules, such as p38MAPK, c-JUN, ERK5, ERk1/2, and MEK1/2, that are upstream of AP1 and MEF2. We are actively assessing how EPHB4 modulates the activation of these signaling molecules and hence regulates HCN4 expression.

Another related observation is that an EPHB4 inhibitor, NVP-BHG712, which inhibits EPHB4 and other EPHB kinases, effectively repressed the WT ES cells to develop into pacing cells and reduce their HCN4 expression. Further, this inhibitor rapidly and dangerously reduced heart rates of WT mice from 600 beats/min to 200 beats/min within 60 min. This finding shall give a very chilly warning to EPHB4 inhibitor developers for the use of such inhibitors in tumor therapy.

In summary, EPHB4 has newly found functions in regulating pacing cell development and heart rate.

*Abstract 30*
*Session VI – Newly found functions*
*June 2, 11:05-11:30*

## THE ROLES OF EPHA RECEPTORS AND EPHRINA IN MEMORY FORMATION

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Eph receptors regulate glutamate receptors functions, neuronal morphology and synaptic plasticity, cellular events believed to be involved in memory formation. In the studies described here we aim to explore the roles of EphA receptors and their cognate ephrinA ligands in learning and memory. Toward that end, we examined the roles of EphA4 receptors and ephrinA4 in fear conditioning memory formation. To assess possible roles of ephrinA4 in fear memory formation we designed and used a specific inhibitory ephrinA4 mimetic peptide (pep-ephrinA4) targeted to EphA binding site. We show that this peptide, composed of the ephrinA4 binding domain, interacts with EphA4 and inhibits ephrinA4-induced phosphorylation of EphA4. Microinjection of the pep-ephrinA4 into the lateral amygdala (LA), a brain region that mediates long-term fear memory, 30 min before training impaired long- but not short-term fear conditioning memory. Microinjection of a control peptide derived from a nonbinding E helix site of ephrinA4, that does not interact with EphA, had no effect on fear memory formation. Microinjection of pep-ephrinA4 into areas adjacent to the amygdala had no effect on fear memory. Acute systemic administration of pep-ephrinA4 1 h after training also impaired long-term fear conditioning memory formation. Our research shows that ephrinA4 binding sites may serve as a target for pharmacological treatment of fear and anxiety disorders. To examine the roles of EphA4 in memory formation we explored CaMKII-cre;EphA4(lx/-) mice where EphA4 is removed from pyramidal neurons of the forebrain. CaMKII-cre;EphA4(lx/-) mice are impaired in long-term fear conditioning memory formation. Mutant mice with targeted kinase-dead EphA4 (EphA4(KD)) exhibit intact long-term fear conditioning memory showing that EphA4 kinase-mediated forward signaling is not needed for fear memory formation. The ability to form long-term conditioned taste aversion (CTA) memory is not impaired in CaMKII-cre;EphA4(lx/-) mice. Thus, EphA4 mediates long-term fear conditioning memory formation in a kinase-independent manner. We also examine EphA receptors potential downstream effectors to further obtain key information on the functions of EphA receptors in memory formation. For example, we show that Rac1 GTPase and its effector p21-activated kinase (PAK) are involved in long-term but not short-term fear memory formation in lateral amygdala.

*Abstract 31*
*Session VII – Cardiovascular*
*June 2, 12:45-13:10*

## AN EPHB4-RASA1 SIGNALING AXIS THAT REGULATES BLOOD AND LYMPHATIC VASCULAR DEVELOPMENT AND FUNCTION

Di Chen[1], Elizabeth D. Hughes[2], Thomas L. Saunders[2,3], Jiangping Wu[4], Magda Hernandez Vasquez[5], Taija Makinen[5], Michael J. Davis[6] and Philip D. King[1]

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Multiple lines of evidence from human and mouse genetic studies indicate that Ephrin receptor B4 (EPHB4) and p120 Ras GTPase-activating protein (p120 RasGAP or RASA1) function in the same intracellular signaling pathway to regulate the development and function of the blood and lymphatic vascular systems. In humans, inherited inactivating mutations of *EPHB4* and *RASA1* genes are both causative of the vascular anomalies, capillary malformation-arteriovenous malformation (CM-AVM) and vein of Galen malformation (VGAM) as well as lymphatic disorders including lymphatic-related hydrops fetalis (LRHF) and central conducting lymphatic anomaly (CCLA). In mice, constitutive or induced disruption of *Ephb4* or *Rasa1* genes results in the same phenotypes of impaired developmental, neonatal and pathological angiogenesis, as well as impaired development and maintenance of lymphatic, venous and lymphovenous valves. Further studies of EPHB4- and RASA1-deficient mouse models support the view that EPHB4 and RASA1 function together to guard against excessive activation of the Ras-MAPK pathway in endothelial cells (EC). Consequently, loss of an EPHB4-RASA1 signaling axis in EC results in dysregulated Ras-MAPK signaling leading to intracellular accumulation of the extracellular matrix protein, collagen IV, which accounts, at least in part, for vascular abnormalities. The basis of the functional relationship between EPHB4 and RASA1 in the control of Ras-MAPK activation in the vasculature is currently unknown. Upon recognition of its ephrin B2 ligand, EPHB4 phosphorylates itself upon two tyrosine residues (Y590 and Y596) present in the EPHB4 juxtamembrane domain. Subsequently, RASA1 binds to EPHB4 through SH2 domain recognition of these phosphorylated tyrosines. Therefore, one possibility is that auto-phosphorylated EPHB4 serves to recruit RASA1 to the plasma membrane promoting its juxtaposition to Ras-GTP necessary for Ras inactivation. To address this, we generated a novel 2YP EPHB4 knockin mouse strain in which Y590 and Y596 were mutated to phenylalanine and P593 and P599 were mutated to glycine. The proline mutations were introduced to restore EPHB4 protein tyrosine kinase activity that would otherwise be blocked by the tyrosine mutations alone. Unexpectedly, developmental, neonatal and pathological angiogenesis were all normal in EPHB4 2YP/2YP mice showing that physical interaction between EPHB4 and RASA1 is not required for these events. In contrast, lymphatic valve function was severely compromised in adult EPHB4 2YP/2YP mice revealing a role for EPHB4-RASA1 physical interaction in lymphatic valve maintenance. These studies reveal differences in a requirement for EPHB4-RASA1 physical interaction between vascular compartments and structures and have important implications for the understanding and treatment of vascular disorders in humans with inherited *EPHB4* and *RASA1* mutations.

*Abstract 32*
*Session VII – Cardiovascular*
*June 2, 13:10-13:35*

## FUNCTION OF ENDOTHELIAL EPHB4 AND EPHRIN-B2 IN ANGIOGENESIS, ARTERIO-VENOUS DIFFERENTIATION AND HEART HOMEOSTASIS

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Eph receptors and their ephrin ligands are essential regulators of cell-cell interactions, mediating repulsion, adhesion and migration processes. In vascular endothelium, ephrin-B2 marks arteries, while EphB4 predominantly labels veins. Our work indicates that during development, ephrin-B2-mediated signaling is important for blood vessel and lymphatic vessel growth, processes through which new vessels emerge from preexisting ones. In this context, ephrin-B2 modulates VEGF signaling by promoting VEGF receptor internalization, downstream signal transduction and turnover. Our preliminary experiments using HUVECs and genetically modified mouse models suggest an important role of EphB4/ephrin-B2 signaling in arterio-venous differentiation.

Furthermore, organ homeostasis in the adult organism, such as maintaining heart function, relies on the appropriate provision of nutrients and specialization of the local vasculature. We have used mouse genetics, imaging and cell biology approaches to investigate how homeostasis in the adult heart is controlled by endothelial EphB4/ephrin-B2 signaling. We show that inducible and endothelial cell-specific inactivation of *Ephb4* in adult mice is compatible with survival, but leads to rupturing of cardiac capillaries, cardiomyocyte hypertrophy, and pathological cardiac remodeling. In contrast, EphB4 is not required for integrity and homeostasis of capillaries in skeletal muscle. Our analysis of mutant mice and cultured endothelial cells shows that EphB4 controls the function of caveolae, cell-cell adhesion under mechanical stress and lipid transport. Therefore, we propose that EphB4 maintains critical functional properties of the adult cardiac vasculature and thereby prevents dilated cardiomyopathy-like defects.

*Abstract 33*
*Session VII – Cardiovascular*
*June 2, 13:35-14:00*

## EPHA2 SERVES AS A GATEWAY FOR A FUNGAL PATHOGEN INTO THE CENTRAL NERVOUS SYSTEM

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Systemic fungal disease can be extremely serious and often life-threatening for individuals with a compromised immune system. Every year fungal disease causes between 1.5 to 2 million deaths world-wide. Among the most devasting are fungal brain infections and the primary cause is *Cryptococcus neoformans* (*Cn*). Following inhalation of its spores from the environment, *Cn* enters the lung where it can cause a pneumonia-like illness. Dissemination to the brain occurs primarily in individuals with a weakened immune system particularly those with advanced AIDS. Indeed 15% of AIDS-related deaths are due to cryptococcal meningitis (CM). Although a few anti-fungal agents can penetrate the blood-brain barrier (BBB) such as flucytosine or fluconazole, it is nearly impossible to completely eradicate the fungus once it is discovered in the brain. Even following treatment, individuals often experience severe neurological sequelae.

Several studies have shown that *Cn* can move freely in the bloodstream or co-opt monocytes, lodge within the lumen of the capillaries and cross the BBB directly. We initially examined the transcriptome of brain microvascular endothelial cells exposed to *Cn* in an *in vitro* model of the human BBB. Upon mapping the transcriptome to known canonical signaling, we identified the EPH-EphrinA1 (EphA2) TKR-signaling pathway and demonstrated that the EphA2 receptor mediated the migration of *Cn* across the BBB in a CD44-dependent manner. Silencing the EphA2 transcript or inhibiting EphA2 activity with an antibody or an inhibitor (dasatinib) prevented *Cn* from crossing the BBB, whereas activation of EphA2 with the ephrinA1 ligand or an agonist (doxasozin) enhanced crossing of *Cn*. The EphA2 receptor was phosphorylated during *Cn* infection, but phosphorylation was prevented by dasatinib, consistent with less cryptococci crossing the BBB when treated with dasatinib. Localization studies of *Cn* and EphA2 in human brain endothelial cells, live-cell recording of HEK293T cells expressing EphA2, and protection assays demonstrated a clear association between *Cn* and EphA2, consistent with a role for EphA2 in internalizing *Cn*. Animal studies involving EphA2-/- knockout mice have demonstrated that the lack of EphA2 is protective consistent with our in vitro data.

Our working model proposes that *Cn* associates with CD44 on the luminal side of the BBB and induces EphA2 phosphorylation via a CD44-mediated transactivation of EphA2. Once activated, EphA2 may promote signaling that reorganizes the actin cytoskeleton and internalizes *Cn* via macropinocytosis. We are currently investigating the molecular basis of EphA2 in upregulating vesicular traffic of *Cn* across the BBB and using BBB spheroids to resolve the interactome of EphA2. We anticipate that EphA2 or its signaling partners might serve as targets for small molecules or drugs that could be used to prevent fungal brain infections. In addition, our studies may assist with better informed design strategies for technologies geared toward crossing the BBB and delivering cargo to the brain.

*Abstract 34*
*Session VII – Cardiovascular*
*June 2, 14:00-14:25*

## EPHA2 CONTRIBUTES TO DISRUPTION OF THE BLOOD-BRAIN BARRIER IN CEREBRAL MALARIA

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Disruption of blood-brain barrier (BBB) function is a key feature of cerebral malaria. Increased barrier permeability occurs due to disassembly of tight and adherens junctions between endothelial cells, yet the mechanisms governing junction disassembly and vascular permeability during cerebral malaria remain poorly characterized. We found that EphA2 is a principal receptor tyrosine kinase mediating BBB breakdown during *Plasmodium* infection. Upregulated on brain microvascular endothelial cells in response to inflammatory cytokines, EphA2 is required for the loss of junction proteins on mouse and human brain microvascular endothelial cells. Furthermore, EphA2 is necessary for CD8+ T cell brain infiltration and subsequent BBB breakdown in a mouse model of cerebral malaria. Blocking EphA2 protects against BBB breakdown highlighting EphA2 as a potential therapeutic target for cerebral malaria.

*Abstract 35*
*Session VIII – Trainee Presentation*
*June 2, 14:50-15:05*

## ***RHYNCHOPHYLLINE*, AN INHIBITOR OF THE EPHA4 RECEPTOR, MODIFIES SLEEP ARCHITECTURE IN MICE**

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EphA4 is a cell adhesion molecule involved in neurotransmission by modulating spine retraction, receptors at the synapse and neuron-glia communication. It is expressed in the cerebral cortex, hippocampus and the suprachiasmatic nucleus. Interestingly, *EphA4* knockout mice (KO) had shown altered sleep encephalogram (EEG) including decreased REM sleep in the light phase and shorter duration of non-rapid eye movement (NREM) sleep slow waves. However, it remains to be defined whether these changes originate from neurodevelopmental effects or from a role of EphA4 in neuronal function. We hypothesize that repressing EphA4 in adult mice will replicate effects observed in *EphA4* KO mice.

Accordingly, the EEG was recorded in C57BL/6J mice receiving the EphA4 inhibitor *Rhynchophylline* (RHY). RHY (50 or 100 mg/kg) or saline was injected intraperitoneally at light onset (ZT0) and 1h before light offset (ZT11). Effects on sleep architecture and slow wave properties were investigated together with sex differences. After the injection day, brains were sampled to examine protein and gene expression in the cortex, thalamus, hippocampus and striatum.

Results show that RHY decreases the time spent in rapid eye movement (REM) sleep during the light phase and importantly decreases the time spent in wakefulness during the dark phase. In addition, RHY produced a pronounced fragmentation of wakefulness and NREM sleep during the light phase (more individual bouts of shorter duration). Sex-dependent variations and the impact of RHY on EphA4 downstream effectors (e.g., Cdk5, GLUR1, GLT1) are now being examined.

This research reveals that the EphA4 inhibitor RHY modifies sleep in a way that resembles the phenotypes found in *EphA4* KO mice. This supports a considerable role of EphA4 in the regulation of sleep stages. Nevertheless, effects of RHY differing from previous KO experiments (like the increased fragmentation) may suggest EphA4-independent effects of RHY. Our findings increase the understanding of molecular pathways contributing to sleep regulation.

FUNDING: NSERC discovery grant, VANIER fellowship, and Canada Research Chair in Sleep Molecular Physiology

*Abstract 36*
*Session VIII – Trainee Presentation*
*June 2, 15:05-15:20*

## OPTOGENETIC CONTROL OF EPHB KINASE ACTIVITY AND THE EPHB-EPHRINB INTERACTION IN FILOPODIAL MOVEMENT

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The proper function of the brain relies on specific connections between synapses. Abnormal synapse formation is related to devastating diseases such as autism, intellectual disability, schizophrenia, Alzheimer's, addiction and epilepsy. During brain development, neurons extend protrusions from dendrites and axons to initiate synapse formation. It remains unknown how the initial stage of synapse formation is regulated. In previous research, we found that the postsynaptic receptor tyrosine kinase EphB2 acts as a decision-maker in the tips of dendritic filopodia to determine synapse initiation. EphB kinase activation regulates filopodial retraction with a fast signal (<1min rise time) and drives filopodial stabilization/synapse initiation with a slow signal (>4min). However, it is unclear how EphB kinase activity is modulated to drive these distinctive cellular behaviors. One important but a little-studied feature of Eph kinase signaling is that Ephs and their ligand ephrins can bind in *cis*. Here using biochemistry, immunocytochemistry, and proximity ligation assay, we show that EphB2 *cis*-interacts with ephrinB3 via the EphB fibronectin type III (FN3) domain. We also find that both EphB2 and ephrinB3 localize in the tip of dendritic filopodia. Based on these results and evidence from studies indicating that EphrinAs can *cis*-attenuate EphA phosphorylation, we propose that the EphB2-ephrinB3 *cis* interaction alters EphB kinase activity and filopodial movement. Using genetically encoded indicator and live-cell imaging, we find EphB2-ephrinB3 *cis* interaction downregulates EphB kinase activity. To regulate EphB2-ephrinB3 *cis* interaction, we invented optogenetic tools to spatiotemporally induce EphB2-ephrinB3 *cis* interaction. In previous research, we found that focal activation of EphB2 drives fast EphB activation and filopodial retraction. Here we demonstrate focal induction of the EphB2-ephrinB3 *cis* interaction alters the fate of filopodial movement to drive filopodial stabilization. These data are consistent with the hypothesis that EphB activity controls the filopodial movement. Results from these experiments will expand our understanding of the basic mechanisms underlying synapse development and provide key information for neurodevelopmental diseases.

*Abstract 37*
*Session VIII – Trainee Presentation*
*June 2, 15:20-15:35*

## THE UBIQUITIN LIGASE AND SCAFFOLD MYCBP2 IS REQUIRED FOR EPHB2 SIGNALING

Chao Chang[1,2], Sung-Soon Park[1,2], Muriel Desbois[3], Sara Banerjee[1,6], Brian Zhang[4], Brock Grill[3,5] and Artur Kania[1,2,6]

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Eph receptor tyrosine kinases play important roles in versatile developmental and adult processes. Dysregulation of Eph receptor signaling is implicated in neurodevelopmental defects, neurodegeneration and cancer, among others. Despite years of studies, the mechanisms governing the magnitude and duration of Eph receptor intracellular signaling evoked by its ligand ephrin, are still mostly elusive.

To gain insights into Eph receptor mediated signaling, we performed affinity purification coupled with mass spectrometry to characterize the EphB2 interactome that fluctuates in response to ephrin-B2 ligand. Among potential EphB2-interacting proteins we identified MYCBP2, a member of the PHR protein family. MYCBP2 is composed of Guanine Exchange Factor (GEF), F-box binding and E3 ligase domains. It forms a ubiquitin ligase complex with FBXO45 and regulates target protein stability. Remarkably, MYCBP2 is implicated in axon guidance and synaptic growth, processes also regulated by EphB2, but the upstream effectors of MYCBP2 remain unknown.

Using co-immunoprecipitation, we demonstrated that EphB2-MYCBP2 interaction is facilitated by FBXO45, and inhibition of FBXO45-MYCBP2 association by the FBD1 peptide disrupts the EphB2-MYCBP2 interaction. Moreover, *MYCBP2* knockout cells exhibited a reduced ephrin-B2-evoked cell retraction and ephrin-B2 stripe avoidance compared to control cells. Similar effects were also observed in cultured cells and neurons expressing the FBD1 peptide. MYCBP2 loss-of-function resulted in decreased EphB2 expression levels as well as enhanced ligand-induced EphB2 degradation, suggesting that MYCBP2 stabilizes EphB2. Intriguingly, although loss of MYCBP2 reduced EphB2 levels, EphB2 activation-mediated pERK1/2 signaling was increased, suggesting that MYCBP2 may play roles in multiple aspects of EphB2 signaling. We are now studying the mechanism of EphB2-MYCBP2 association and its specific function in developing neurons.

Our work positions MYCBP2 as a key effector of EphB2 receptor tyrosine kinase signalling and describes a conceptually novel mechanism by which MYCBP2 regulates EphB2 level and activity. Given that MYCBP2 and EphB2 loss-of-function exhibit similar neurodevelopmental phenotypes, MYCBP2 may be an important effector of EphB2 function in axon guidance and synaptic plasticity, and their links may broaden our understanding of neurological disorders.

*Abstract 38*
*Session VIII – Trainee Presentation*
*June 2, 15:35-15:50*

## PAN-CANCER ANALYSIS OF EPHB1 RECEPTOR MUTATIONS

Luis Nunes[*,1], Snehangshu Kundu[1], Jeremy Adler[1], Ivaylo Stoimenov[1], Lucy Mathot[1] and Tobias Sjöblom[1]

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Background: Ephrin receptors (EPH) have been associated with metastatic disease, for example, reduced *EPHB1* expression in colon cancer has been linked to poor differentiation and increased invasive capacity. Previously, we have shown that somatic *EPHB1* mutations observed in colorectal cancer (CRC) led to a reduction in cell compartmentalization. The aim of this project is to find recurrent *EPHB1* mutations though integration of pan-cancer and pan-EPH mutational data for further functional evaluation.

Materials and Methods: The Cancer Genomes Atlas (TCGA) database was used as the source of somatic mutations for 33 different cancer types. The likely functional impact was assessed for all exonic missense mutations annotated through ANNOVAR dbNSFP version 3.3a. Recurrent 1D and 3D hotspot mutations were identified by projecting the mutations on an EPH consensus protein sequence and by HotSpot3D-1.3, respectively. DLD-1 CRC cells were then transduced with wild-type and prioritized mutant *EPHB1*-GFP variants by lentiviral transduction and assessed for compartmentalization.

Results: In total, 8.231 EPH exonic variants were collected from TCGA, of which 2.850 were missense mutations. Of the 201/984 amino acids mutated in *EPHB1*, 61 were predicted to be associated with another mutated residue in the 3D hotspot screening. Of these, four mutations were in the ligand binding domain and four mutations in the kinase domain were selected based on high average functional impact. *EPHB1* 1D hotspot mutations were then selected from the other 140 amino acids according to highest mutation prevalence: one in the fibronectin type-III 1 domain, four in the kinase domain, and two located outside of known domains. Cell compartmentalization was strongly compromised for the *EPHB1* p.C61Y and p.D762N mutants.

Conclusions: Through the application of 1D and 3D approaches we identified 15 recurring *EPHB1* mutations. Two of these mutants showed strongly compromised compartmentalization phenotypes indicating that they could contribute to invasion and metastasis in different cancer types.

**Posters not displayed due to Zoom presentation**

*Abstract 39*

## EPHRIN RECEPTOR (EPH)-A1, -A2, -A4 AND -A6 EXPRESSION IN THYMIC EPITHELIAL TUMOURS: AN IMMUNOHISTOCHEMICAL STUDY

Christos Masaoutis[1,2], Panagiotis Sarantis[1], Mattheos Bobos[3], Nikolaos Tsoukalas[1,4], Paraskevi Alexandrou[1], Dimitra Rontogianni[2], Stamatios Theocharis[1]

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BACKGROUND Thymic epithelial tumours (TET) are primary anterior mediastinal neoplasms ranging from locally agressive to frankly malignant. They are further classified according to WHO 2015 subtype and Masaoka stage. Chemotherapy is usually the treatment of locally advanced and metastatic disease. Ephrins (ephs) and their receptors (EPHs)-the latter members of the receptor tyrosine kinases (RTKs) superfamily-are implicated in tissue development and homeostasis and are aberrantly expressed in tumors. Importantly, several preclinical animal studies have not revealed particular toxicity problems for a variety of EPH-targeting factors, which renders them attractive anti-cancer agents [1]. Lack of EPH-A4 seems to alter thymic epithelial cytoarchitecture and block T-cell precursor differentiation [2]. Hence, EPH-A inhibition may constitute a strategy against TETs. To our knowledge EPH-A expression has not been previously studied in TETs.

OBJECTIVE To examine the clinical significance of EPH-A1, -A2, -A4 and -A6 in TETs.

METHODS Tissue microarrays (TMAs) were constructed out of FFPE tissues from 98 TETs excised between 2009 and 2019, from an equal number of patients (55 females and 43 males; 29 to 85 years old), including 12 type A, 22 type AB, 17 type B1, 18 type B2, 14 type B3, 2 micronodular thymomas and 13 thymic carcinomas, according to the WHO 2015 classification. Three to five cores from each tumour were included so as to capture tumour heterogeneity. Survival data were available for 36 patients with an average follow-up of 42 months (11 to 134 months). The TMAs were immunohistochemically stained for EPH-A1, -A2, -A4 and -A6 and the immunohistochemical protein expression score IRS was evaluated. Pearson's chi-square test was applied for statistical analysis of the relationship between the IRS score and tumour subtype and Masaoka stage. Survival data were analyzed by Cox proportional hazards model.

RESULTS There was universal nuclear and cytoplasmic high EPH-A4 expression with some variation in intensity in TETs. EPH-A2 and EPH-A6 were variably expressed in the cytoplasm of the epitelial cells in the vast majority of tumours. The accompanying immature lymphocytes showed variable nuclear positivity mainly for EPH-A2 and less frequently for EPH-A6. EPH-A1 was not expressed in TET cases examined. The more epithelial-rich TET subtypes (B2, B3 and carcinoma) presented higher cytoplasmic EPH-A6 IRS ($P<0.001$); nuclear localization of EPH-A6 was rare and less strongly correlated with WHO classification subtypes ($P=0.097$). B3 thymomas and carcinomas were also more likely to intensely express EPH-A4 ($P=0.011$). High lymphocytic EPH-A6 IRS was infrequent, but more probable to be seen in the lympocyte-rich B1 subtype ($P=0.015$). No correlation was found between EPH-A expression and Masaoka stage or survival rates.

CONCLUSIONS According to our study Type A EPHs (-A2, -A4 and -A6) are expressed in TETs and their expression levels statistically significantly correlate with tumour subtype, suggesting participation of these RTKs in carcinogenesis in the thymus.

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*Abstract 40*

## EPHB2 A POTENTIAL THERAPEUTIC TARGET FOR PAEDIATRIC MEDULLOBLASTOMA

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Medulloblastoma (MB) is the most frequent malignant brain tumour to occur in children and remains the leading cause of cancer-related mortality in childhood.

Eph family receptors predominantly function during embryonic development and are typically either not expressed or expressed at low levels in normal healthy tissues. It is now established that many Eph receptors are re-expressed and functional in human cancers making them attractive, relatively tumour-specific targets. In this study, we have generated compelling preliminary data showing that EphB2 is functional and overexpressed in paediatric MB and could therefore be a valid tumour-specific therapeutic target for the treatment of MB.

EphB2 mRNA and protein expression analysis show that EphB2 is elevated in both primary cell lines and tumour tissue specimens. Furthermore, we show that EphB2 is expressed in a subgroup specific pattern, which is in concordance with expression analysed via the Medulloblastoma Advanced Genomics International Consortium (MAGIC) dataset. The high affinity ligands ephrinB1-Fc and ephrin B2-Fc are able to actively initiate EphB2 receptor activation and downstream signalling leading to reduced tumour cell proliferation and invasion. An SNEW EphB2 blocking peptide inhibited EphB2 and subsequently FAK phosphorylation and rescued MB cell growth upon ephrin B ligand stimulation. Lastly, EphB2 shRNA mediated knockdown significantly delayed tumour cell proliferation *in vitro* and significantly delayed tumour formation in orthotopic MB animal models.

This study identifies EphB2 has a functional elevated receptor in MB which could prove effective as a novel tumour-specific therapeutic target for future validation studies.

*Abstract 41*

## EPHRIN RECEPTOR (EPH)-A2, AND -A4 EXPRESSION IN UVEAL MELANOMA: AN IMMUNOHISTOCHEMICAL STUDY

Eougken Dana[1], Georgia Levidou[1], Pawel Gajdzis[2], Christos Masaoutis[1], Piotr Donizy[2], Penelope Korkolopoulou[1], Jerzy Klijanienko[3], Stamatios Theocharis[1,3]

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Background-Aim: Uveal melanoma (UM) represents the most common primary intraocular malignancy in adults, exerting high metastatic potential and poor prognosis. Ephrins (ephs) and their receptors (EPHs)-the latter members of the receptor tyrosine kinases (RTKs) superfamily-are implicated in tissue development and homeostasis and are aberrantly expressed in tumours. Aim of this study was to assess EPH-A2 and -A4 expression in UM and to evaluate its possible clinical significance.

Patients and Methods: EPH-A2 and -A4 expression was examined immunohistochemically in 49 UM tissue specimens. EPH-A2 and -A4 expression (percentage and intensity of staining as categorical variables, and their product IRS, classified into 4 levels: negative, mild, moderate and strong) was correlated with tumours' clinicopathological characteristics, BAP1 positivity, presence of tumour infiltrating lymphocytes (TILS) and with patients' overall (OS) and disease free survival (DFS).

Results: Cytoplasmic pattern of EPH-A2 expression was noted in UM cases with mild/moderate IRS in 35/49 (71%) of them. Nuclear and cytoplasmic pattern of EPH-A4 expression was noted in UM cases with mild/moderate IRS in 32/49 (65%) and 33/49 (67%) of them, respectively. Negative EPH-A2 IRS (3/4, 75%) was more oftener noted in UM located in the iris when compared to those that did not involve it (11/45, 24.44 %) (p= 0.032). Negative or mild EPH-A2 IRS (8/9, 88.89%) was noted in UM cases with non intrascleral location when compared to those that involved it (20/40, 50%) (p= 0.033). The majority of positive for BAP1 UM cases (12/19, 63.16%) presented either negative or mild EPH-A2 IRS compared with negative for BAP1 cases which more often exerted moderate EPH-A2 IRS (5/6, 83.33%) (p=0.047). Nuclear EPH-A4 expression was not correlated with any of the examined parameters. UM Patients with moderate cytoplasmic EPH-A4 IRS were younger than those with negative/mild one (p= 0.0388), All UM cases with epithelioid morphology presented negative or mild cytoplasmic EPH-A4 IRS (12/12, 100%), whereas moderate cytoplasmic EPH-A4 IRS was noted in cases with mixed (4/25, 16%) and pure spindle cell (5/12, 41.67%) morphology (p=0.028). UM cases with mild/moderate cytoplasmic EPH-A4 IRS presented higher mitotic activity when compared to negative ones (p=0.0150). A positive correlation between nuclear and cytoplasmic EPH-A4 IRS was also noted. Cytoplasmic EPH-A2 IRS was marginally correlated with UM patients' OS, as cases with mild/moderate IRS presented a marginally better, although non statistically significant, prognosis compared to negative ones (log rank test, p=0,0712).

Conclusion: Our study verified the expression of EPH-A2 and -A4 in UM and revealed correlations with various clinicopathological parameters.

*Abstract 42*

## MECHANISMS OF EPHRINA1-FC-MEDIATED REDUCTION OF ACUTE MYOCARDIAL ISCHEMIA/REPERFUSION INJURY

Victoria Chan[1], Caitlin Friello[1], Eleftherios Vlahos[1], Uma Sharma[1], Abigail Neininger[2], Amy Stark[2], Dylan Burnette[2], and Jitka Virag[1]

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EphrinA1 is a cell membrane-anchored protein expressed in mouse and human cardiomyocytes (87% homology). Administration of ephrinA1-Fc at the time of onset of ischemia in mice significantly attenuates acute myocardial ischemia/reperfusion injury (IRI). We have recently reported that this is due to preservation of the cardiomyocyte cytoskeletal ultrastructure which in turn maintains the interconnected function of mitochondria, enabling them to sustain adequate ATP production and structural integrity for contraction. The mechanism by which this occurs is not understood and the contribution of other cell types to the observed protection is unknown.

In order to understand the sequence of signaling events and the intercellular dynamics that occur to elicit ephrinA1-Fc-induced cardioprotection, we are using both in vivo and in vitro approaches in mice as well as human cardiomyocytes derived from iPCS. Specifically, heart tissue collected from intact mice that have undergone 30min I/24hr R was used for immunostaining and protein was extracted from whole left ventricular homogenates. Preliminary data indicate that p-tubulin/tubulin was increased and cleaved caspase-3/caspase-3 level is reduced, corroborating previously published findings that cardiomyocyte architecture is preserved and apoptosis is attenuated. Further analyses to identify key signaling mediators and their tissue distribution profile are in progress. In vitro, viability of hypoxic human cardiomyocytes differentiated from iPSCs is preserved by ephrinA1-Fc and studies to compare the signaling mediator expression profile to mice are underway. Evaluation of the profibrotic response in cultured primary murine cardiac fibroblasts in response to TGF-□ □shows that MMP-9, MMP- 2, and TIMP-1 expression levels in EA1-treated +/TGF-β primary cardiac fibroblasts are modulated in a dose-dependent manner and low dose of EphrinA1-Fc alone increased DDR2 expression as well as p-SMAD2/3/SMAD2/3 expression in primary cardiac fibroblasts whereas the high dose reduced expression of these compared to TGF- β alone or together with either the low or high dose of EA1. Similar studies using isolated peritoneal macrophages in response to TNF-α stimulation in the presence and absence of a hi and low dose of ephrinA1-Fc are in progress to determine the effects of ephrinA1-Fc on the pro- inflammatory vs resolution phase macrophage phenotype.

Taken together, these findings indicate that the role of ephrinA1-Fc in mediating protection from I/R injury is a complex phenomenon not restricted to cardiomyocytes alone but rather, is a constellation of effects produced by activation and/or inhibition of the combination of EphA receptors expressed by each cell type that coordinately behave to mitigate the injury response. The intricate nature of the Eph/ephrin signaling system requires sophisticated molecular and cellular techniques in vitro as well as in vivo models to tease apart their participation in the wound healing response. These highly conserved proteins are a new and rapidly growing area of research that influence a range of cellular behaviors and biological processes, thus possessing enormous translational potential for the treatment of human diseases.

*Abstract 43*

## EFFECTS OF EPHB4 RECEPTOR TYROSINE KINASE MUTATIONS ON MAP KINASE SIGNALLING IN LYMPHATIC ENDOTHELIAL CELLS

Ogmen K[1], Martin-Almedina S[1], Sackey E[1], Grigoriadis D[1], Karapouliou C[1], Josephs K[3], Mortimer PS[1,2], Jeffery S[1], Gordon K[1,2], Mansour S[1,3] and Ostergaard P[1]

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*EPHB4* gene mutations have been associated with lymphatic-related fetal hydrops (LRFH) as well as other vascular anomalies (Martin-Almedina et al 2016; Martin Almedina et al 2021). However, our understanding of how EPHB4 tyrosine kinase signalling controls lymphatic endothelial cell behaviour is not well understood.

Preliminary data generated in our lab shows that under normal conditions EPHB4 tyrosine kinase activity plays a key role in negatively regulating MAP kinase (MAPK) signalling by reducing ERK1/2 phosphorylation in lymphatic endothelial cells. However, two different EPHB4 mutations that result in loss of the receptor kinase activity cause upregulation of ERK1/2 phosphorylation.

We therefore hypothesise that loss of EPHB4 kinase activity, and in turn deregulation of MAP kinase pathway, might be associated with lymphatic-related disorders, for instance primary lymphedema. We speculate that pharmacological targeting of deregulated MAP kinase signalling with well-characterised MEK inhibitors (such as Trametinib) could provide benefits for individuals with EPHB4-related lymphatic diseases.

Objective and Methods

The main aim is to investigate whether the pharmacological treatment with MEK inhibitors can reduce ERK1/2 phosphorylation to basal levels in human primary lymphatic endothelial cells (HDLECs) expressing EPHB4 mutations. In addition to this, physiological function of EPHB4 in MAPK signalling in HEDLECs will be determined. Missense variants of EPHB4 will then be used to determine the pathological impact on MAPK signalling before determining the effect of Trametinib on EPHB4 driven MAPK signalling in HDLECs.

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Martin Almedina S. et al (2021) Genetics in Medicine; 23, 1315-1324.

*Abstract 44*

## A MORPHOGENETIC EPHRINB/EPHB CODE CONTROLS EXTRAHEPATOPANCREATIC DUCT FORMATION

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The extrahepatopancreatic ducts (HPD) connect the intrahepatic and intrapancreatic ducts to the intestine and ensure the afferent transport of the bile and pancreatic enzymes, respectively. Obstruction or paucity of the developing extrahepatic ducts causes severe liver damage in infants, nevertheless the development of the extrahepatic ducts is largely unknown.

To unravel the cell biological processes controlling duct differentiation, we have analysed a combination of membrane and polarity markers during zebrafish HPD development. This revealed that duct formation starts with the formation of multiple microlumina, which subsequently remodel into a single duct. We find this process is highly dynamic, including ductal progenitor cell rearrangement, lumen merging and cell intercalation supported by actomyosin contractility. Searching for molecular regulators of this dynamic morphogenetic process, we found that two ligands and two corresponding receptors of the EphrinB/EphB families are expressed in a regionalized pattern in the endoderm and surrounding mesoderm along the entire HPDs. Newly generated zebrafish mutants exhibit dysmorphic HPDs that fail to resolve into mature ducts with a single lumen. These defects are region-specific, e.g. *ephrinb1* mutants show dysmorphic extrahepatic and common bile ducts while *ephb3b* mutants in addition exhibit a short and dysmorphic extrapancreatic duct. These findings indicate that EphrinB/EphB signaling controls dynamic cell rearrangements driving domain-specific HPD remodeling during development, making them good candidates for congenital cases of bile duct abnormalities.

In summary, our studies show that the differentiation of the EHPDs into mature, patent ducts encompasses a multi-step cord hollowing process. The underlying cell behaviours are regulated by a ‘morphogenetic EphB/EphrinB code’ of multiples ligands and receptors directing duct differentiation and gall bladder formation in a region-specific fashion.

*Abstract 45*

## EPH:EPHRIN SIGNALING IN APICAL PROGENITORS OF THE DEVELOPPING NEOCORTEX

Alice Davy

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Apical neural progenitors (aNP) are self-renewing dividing cells that give rise to projection neurons in the developing neocortex. Apical NP, which are derived from neuroepithelial cells, have an atypical polarized and elongated morphology and remain organized as a pseudo-epithelium throughout corticogenesis. It is now recognized that aNP play a dual role in the developing neocortex: first, they collectively act as a scaffold, providing rigidity to the tissue and offering migration routes to newborn neurons; second they act as stem (or progenitor) cells, sequentially giving birth to intermediate progenitors, projection neurons and glial cells. In the last few years, work in my team has revealed that Eph:ephrin signaling controls both of these aNP functions. By studying *Efnb1* and *Efnb2* loss-of-function mutant embryos, we discovered that ephrinB1, partly via reverse signaling, plays an important structural role in the developing neocortex, controlling aNP attachment and tissue organization. In contrast, ephrinB2 is required to balance self-renewal vs neuronal differentiation at early stages of corticogenesis and this involves forward signaling and an interaction with a metabolic pathway. I will discuss these and more recent findings highlighting current challenges in the study of Eph:ephrin signaling in aNP in vivo.

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**Smith Adam**
**Wu Jiangping**
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## Virtual Presenters

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**Lamb Tracey**
**Liu Wei**
**Pellecchia Maurizio**
**Pitulescu Mara Elena**
**Theocharis Stamatios**

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**Kim Soyeon**
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