9th Adhesion GPCR Workshop

Sept. 13–15, 2018 | The Nines Hotel | Portland, OR, USA
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Welcome from Kelly Monk, Ph.D.

It is with great enthusiasm that I welcome you to the 9th Adhesion GPCR Workshop. The meeting has assembled leaders in the field together with junior faculty and trainees to discuss the latest developments in this fast-growing field.

The meeting begins Thursday morning (13 September) and concludes Saturday afternoon (15 September) and will be held at the Nines Hotel in downtown Portland.

I am very happy that you are able join us in Portland September, 2018. The meeting will offer a fantastic opportunity to present your work, learn about the latest developments in the adhesion GPCR field, and to forge new connections and collaborations.

The meeting also provides a chance to enjoy all that the great city of Portland has to offer as well as an opportunity to explore the stunning Pacific Northwest before or after the meeting.

Welcome to Portland!

Kelly Monk, Ph.D.
Workshop Organizer
Professor and Co-Director, Vollum Institute
Oregon Health & Science University
Keynote Speakers

JoAnn Trejo, Ph.D., UCSD
Professor and Associate Dean, Faculty Affairs
Department Pharmacology
University of California, San Diego

JoAnn Trejo, Ph.D., is best known for her groundbreaking discoveries on cell signaling by protease-activated G protein-coupled receptors. An innovative researcher pioneering new frontiers in cell signaling, she has made numerous novel discoveries related to the regulation of cell signaling in vascular endothelial cells and breast cancer published in prominent journals. Her work has been continuously funded by the NIH, UC Tobacco-related Disease Research Program, Komen Foundation, and the American Heart Association (AHA) including the prestigious AHA Established Investigator Award. She is also a leader in the scientific community and elected by her peers to serve on Council of the American Society for Cell Biology and American Society for Biochemistry and Molecular Biology, as Chair of two Gordon Research Conferences and multiple NIH and HHMI Study Sections.

Dr. Trejo completed her Bachelor of Science degree in toxicology/biochemistry from the University of California, Davis, and her doctorate at the University of California, San Diego. She was recruited to UC San Diego in 2008, and promoted to full professor with tenure in 2012. In 2014, she was appointed Vice Chair of the Department Pharmacology.

Dr. Trejo is a strong advocate for diversity. She has been active in various national committees aimed at increasing the representation of women and under-represented ethnic/racial groups in science. In 2008, Dr. Trejo became director for the Institutional Research and Academic Career Development Award (IRACDA) Postdoctoral Training Program at UC San Diego. The program has been twice renewed by the NIH and has an impressive track record with 65% of the trainees obtaining faculty positions at academic institutions. She has made great strides in institutionalizing faculty diversity, developing and implementing strategies, programs and initiatives for the recruitment, retention and development of a diverse faculty and an inclusive environment.

Michael Bruchas, Ph.D., University of Washington
Professor
Departments of Anesthesiology and Pharmacology
University of Washington, Seattle

Michael R. Bruchas, Ph.D., is a professor in the departments of Anesthesiology and Pharmacology at the University of Washington. His laboratory focuses on understanding how brain circuits are wired, how they communicate with one another, and in dissecting the neural basis of stress, emotion and reward. Dr. Bruchas' work has been continuously funded by the NIH and has led to over 70 peer-reviewed publications, book chapters, and numerous invited lectures worldwide. His laboratory's discoveries have been published in widely regarded journals that include Science, Cell, and Neuron. In addition to peer-review journals, his work has been covered in popular media including public radio, The Wall Street Journal, and The New Yorker.

In 2012, Bruchas received the prestigious NIH Director's Transformative Research Award and an NIH EUREKA (Exceptional Unconventional Research Enabling Knowledge Acceleration) Award in 2013. In 2014 he received the Young Investigator Award from the International Narcotics Research Conference in 2014. In 2016, his laboratory was awarded a BRAIN Initiative grant from the NIH.

In 2012, Bruchas received the prestigious NIH Director's Transformative Research Award and an NIH EUREKA (Exceptional Unconventional Research Enabling Knowledge Acceleration) Award in 2013. In 2014 he received the Young Investigator Award from the International Narcotics Research Conference in 2014. In 2016, his laboratory was awarded a BRAIN Initiative grant from the NIH.

Dr. Bruchas received his Bachelor of Science degree in biology from Creighton University and his doctorate in pharmacology from the same institution in 2004. He then completed a postdoctoral fellowship in neuroscience under Dr. Charles Chavkin at the University of Washington, Seattle, where examined how the brain encodes emotional behaviors and how stress impacts anxiety, depression, and addiction. He was recruited as an assistant professor to Washington University School of Medicine in late 2010. Dr. Bruchas was promoted to associate professor with tenure in July 2015, and was awarded the Henry E. Mallinckrodt Professorship in 2017. In 2018, Dr. Bruchas and his group moved to the University of Washington in Seattle, where he continues his multidisciplinary approaches of GPCR pharmacology, physiology, and behavioral neuroscience.
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<th>Speaker</th>
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<tr>
<td>9:00-9:10</td>
<td>Opening remarks</td>
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<td>9:10-10:30</td>
<td>Session 1: aGPCRs in Development</td>
<td>Simone Prömel, Caroline Formstone</td>
<td>The enigmatic trans function of the Adhesion GPCR Latrophilin acts non-cell autonomously in fertility</td>
<td>Simone Prömel</td>
<td>Leipzig University, Leipzig, Germany</td>
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<td>BAI1/ADGRB1 sculpts the dendritic arbors of hippocampal pyramidal neurons via RhoA-dependent growth restriction</td>
<td>Joseph Duman</td>
<td>Baylor College of Medicine, Houston, TX</td>
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<td>Dystroglycan binds Celsr3 (Adgrc3) to regulate commissural axon guidance</td>
<td>Kevin Wright</td>
<td>Oregon Health &amp; Science University, Portland, OR</td>
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<td>A role for the Adhesion-GPCR Celsr1 in contact-mediated alignment of cell behaviour</td>
<td>Caroline Formstone</td>
<td>Kings College London, University of Hertfordshire, Hatfield, UK</td>
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<td>10:30-11:00</td>
<td>Coffee Break</td>
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<td>11:00-12:40</td>
<td>Session 2: Signaling &amp; Activation I</td>
<td>Ines Liesbscher, Randy Hall</td>
<td>The physiological role of the mechano-responsive aGPCR GPR133</td>
<td>Ines Liebscher</td>
<td>Leipzig University, Leipzig, Germany</td>
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<td>Signaling and trafficking of GPR64/ADGRG2 are regulated by its N-terminal fragment and tethered peptide</td>
<td>Nariman Balenga</td>
<td>University of Maryland School of Medicine, Baltimore, MD</td>
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<td>Coincidence detection of membrane stretch and extracellular pH by a proton-sensing G protein coupled receptor</td>
<td>Maike Glitsch</td>
<td>University of Oxford, Oxford, UK</td>
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<td>Signaling and Regulation of the BAI Sub-Family of Adhesion GPCRs</td>
<td>Randy Hall</td>
<td>Emory University, Atlanta, GA</td>
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<td>12:20-12:40</td>
<td>Lightning Talks</td>
<td>Ryan Gray</td>
<td>A Tale of Two aGPCRs (in Sensory Neuron Myelination): ADGRG6 and ADGRG1</td>
<td>Amit Mogha</td>
<td>Oregon Health &amp; Science University, Portland, OR</td>
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| 12:25-12:30  | **G protein-coupled receptor 56 (GPR56) as a potential functional regulator of normal and leukemic human stem cells**  
Heather Duncan  
*McGill University, Montreal, QC, Canada* |
| 12:30-12:35  | **ADGRG6 (Gpr126) regulates EMT of endocardial cells during valve formation**  
Gentian Musa  
*Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany* |
| 12:35-12:40  | **Role of GPR110 in Breast Cancer**  
Meghana Trivedi  
*University of Houston College of Pharmacy, Houston, TX* |
| 12:40-14:10  | Lunch (provided)                                                        |
| 14:10-15:30  | **Session 3: aGPCRs in Health & Disease**  
Chairpersons: Hsi-Hsien Lin  
Gabi Aust |
| 14:10-14:30  | **The Adhesion G protein-coupled receptor G6 is essential for homeostasis of the intervertebral disc in mice**  
Ryan Gray  
*University of Texas at Austin Dell Medical School, Austin, TX* |
| 14:30-14:50  | **Roles of the ADGRA family in glandular development, lineage commitment and tumorigenesis**  
Elena Spina  
*New York University School of Medicine, New York, NY* |
| 14:50-15:10  | **Regulation of canonical WNT7 signaling by GPR124/ADGRA2**  
Mario Vallon  
*Stanford University, Stanford, CA* |
| 15:10-15:30  | **Together or apart, but always close: Latrophilin-1 mediates axonal attraction induced by proteolytically released Lasso**  
Yuri Ushkaryov  
*University of Kent, Chatham, UK* |
| 15:30-15:50  | **Mechano-dependent phosphorylation of CD97/ADGRE5 at its PDZ-binding motif modulates cellular detachment**  
Gabi Aust  
*Leipzig University, Leipzig, Germany* |
| 15:50-16:15  | Coffee Break                                                            |
| 16:15-17:15  | **KEYNOTE**  
Ubiquitin and Cell Signaling by Protease-activated Receptors  
JoAnn Trejo  
*University of California, San Diego, CA* |
| 17:15-19:15  | Poster session – snacks and bar                                          |
| 17:15-18:15  | Odd numbered posters present                                             |
| 18:15-19:15  | Even numbered posters present                                            |
| 19:15-20:00  | Meeting for aGPCR board  
Ruby Boardroom – **see map located in Visitor Information**  
Other attendees free |
### Day 2 Agenda
Friday, September 14th

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<tr>
<th>Time</th>
<th>Session 4: Structure &amp; Function</th>
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<tr>
<td>9:00-10:20</td>
<td>Chairpersons: Demet Araç</td>
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<td>Antony Boucard</td>
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<tr>
<td>9:00-9:20</td>
<td>Structural and functional basis of adhesion GPCR activation</td>
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<td>Demet Araç</td>
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<td>University of Chicago, Chicago, IL</td>
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<tr>
<td>9:20-9:40</td>
<td>Annotation and quantification of adhesion GPCR splice variants</td>
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<td>Alexander Knierim</td>
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<td>Leipzig University, Leipzig, Germany</td>
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<tr>
<td>9:40-10:00</td>
<td>CryoEM visualization of G protein-coupled receptors</td>
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<td>Georgios Skiniotis</td>
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<td>Stanford University, Stanford, CA</td>
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<tr>
<th>Time</th>
<th>Session 5: Biological Functions I</th>
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<tr>
<td>10:00-10:20</td>
<td>Adhesion G protein-coupled receptors adgrl/latrophilins physically and functionally interact with the actin cytoskeleton</td>
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<td></td>
<td>Antony Boucard</td>
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<td>Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Mexico City, Mexico</td>
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<table>
<thead>
<tr>
<th>Time</th>
<th>Session 5: Biological Functions I</th>
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<tr>
<td>10:20-11:40</td>
<td>Chairpersons: Felix Engel</td>
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<td>Kim Tolias</td>
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<tr>
<td>10:20-10:40</td>
<td>ADGRG6 (Gpr126) is a mechano-responsive gene</td>
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<td>Felix Engel</td>
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<td>Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany</td>
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<td>10:40-11:00</td>
<td>Systematic affinity proteomics identifies functional modules associated with adhesion GPCRs</td>
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<td>Uwe Wolfrum</td>
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<td>Johannes Gutenberg University of Mainz, Mainz, Germany</td>
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<tr>
<td>11:00-11:20</td>
<td>Coincidence detection of membrane stretch and extracellular pH by a proton-sensing G protein coupled receptor</td>
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<td>Cheng-Chih (Andy) Hsiao</td>
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<td>University of Amsterdam, Amsterdam, The Netherlands</td>
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<td>11:20-11:40</td>
<td>The adhesion-GPCR bai1 promotes excitatory synaptogenesis by coordinating bidirectional trans-synaptic signaling</td>
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<td>Kim Tolias</td>
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<td>Baylor College of Medicine, Houston, TX</td>
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<tr>
<th>Time</th>
<th>Community Session</th>
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<tr>
<td>11:40-12:00</td>
<td>Announce 2020 Meeting Location</td>
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<tr>
<th>Time</th>
<th>Lunch and extended poster viewing</th>
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<tr>
<td>12:00-14:00</td>
<td>Lunch provided</td>
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<tr>
<th>Time</th>
<th>Session 6: Signaling &amp; Activation II</th>
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<tr>
<td>14:00-15:40</td>
<td>Chairpersons: Nicole Scholz</td>
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<td>James Bridges</td>
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<td>14:00-14:20</td>
<td>The adhesion-GPCR latrophilin/dCirl shapes the development of the nmj in drosophila</td>
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<td>Nicole Scholz</td>
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<td>Rudolf-Schönheimer-Institute of Biochemistry, Leipzig, Germany</td>
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Day 2 Agenda continued
Friday, September 14th

14:20-14:40  The Role of GPR110-dependent signaling in neurodevelopment and neuroprotection  
Hee-Yong Kim  
National Institutes of Health, Bethesda, MD

14:40-15:00  GPR126 function is regulated by its extracellular region  
Katherine Leon  
University of Chicago, Chicago, IL

15:00-15:20  ADGRB1 suppresses cerebellar transformation by sequestering mdm2 from p53  
Erwin Van Meir  
Emory University, Atlanta, GA

15:20-15:40  Molecular Analysis of ADGRF5 Signaling Determinants Required for Alveolar Homeostasis  
James Bridges  
Cincinnati Children’s Hospital Medical Center, Cincinnati, OH

15:40-16:00  Coffee break

16:00-17:20  Session 7: Biological Functions III  
Chairpersons: Garret Anderson  
Benoit Vanhollebeke

16:00-16:20  Role of Latrophilin Adhesion GPCRs in Synaptic Assembly  
Garret Anderson  
University of California, Riverside, CA

16:20-16:40  Gpr126 NTF regulates cardiomyocyte depolarization and delamination in zebrafish trabeculation  
Swati Srivastava  
Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany

16:40-17:00  Adhesion GPCR: novel targets to modulate glucose homeostasis  
Doreen Thor  
Leipzig University, Leipzig, Germany

17:00-17:20  Neurovascular development via Gpr124/Reck-dependent Wnt7/β-catenin signaling  
Benoit Vanhollebeke  
Université Libre de Bruxelles, Bruxelles, Belgium

19:00-21:00  Baerlic Brewing Co.– optional  
2311 SE 11th Ave,  
Portland, OR 97214  
Max and walking directions located in Visitor Information.
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<tr>
<td>9:30-10:30</td>
<td>KEYNOTE: Dissecting Neuromodulatory Circuits and Signaling in Affective Behavior</td>
<td>Michael Bruchas</td>
<td>Washington University, St. Louis, MO</td>
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<td>Introduction by Kelly Monk</td>
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<tr>
<td>10:40-12:00</td>
<td>Session 8: Signaling &amp; Activation III</td>
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<td>Chairpersons: Gregory Tall, Xianhua Piao</td>
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<tr>
<td>10:40-11:00</td>
<td>Harnessing the AGPCR tethered-peptide-agonist mechanism for high throughput screening of chemical modulators</td>
<td>Gregory Tall</td>
<td>University of Michigan, Ann Arbor, Michigan</td>
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<td>11:00-11:20</td>
<td>Orphan aGPCR GPR110 – a novel potential player in kidney function</td>
<td>Sandra Huth</td>
<td>Leipzig University, Leipzig, Germany</td>
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<td>11:20-11:40</td>
<td>Renal Expression of Adhesion GPCR Gpr116 (ADGRF5) Plays a Role in Urinary Concentration in Mice</td>
<td>Nathan Zaidman</td>
<td>Johns Hopkins University School of Medicine, Baltimore, MD</td>
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<td>11:40-12:00</td>
<td>The adhesion GPCR VLGRI is a part of focal adhesion complexes, cell migration and mechanotransduction</td>
<td>Deva Krupakar Kusuluri</td>
<td>Johannes Gutenberg University of Mainz, Mainz, Germany</td>
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<tr>
<td>12:00-12:20</td>
<td>Oligodendrocyte GPR56/ADGRG1 integrates signals from microglia and the extracellular matrix to regulate developmental myelination and myelin repair</td>
<td>Xianhua Piao</td>
<td>Children’s Hospital and Harvard Medical School, Boston, MA</td>
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<tr>
<td>12:20-12:30</td>
<td>Closing remarks</td>
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THE ENIGMATIC TRANS FUNCTION OF THE ADHESION GPCR LATROPHILIN ACTS NON-CELL AUTONOMOUSLY IN FERTILITY

Simone Prömel 1
Daniel Matúš 1, Franziska Fiedler 1, Julia Luterán 1, Claudia Binder 1, Alexander B. Knierim 1, Torsten Schöneberg 1

1Rudolf Schönheimer Institute of Biochemistry, Medical Faculty, Leipzig University, Leipzig, Germany

G protein-coupled receptors (GPCRs) classically mediate signals via intracellularly activating G proteins. Recently, it was found that members of the class of Adhesion GPCRs have, additionally to mediating such a signal into a cell, a function which is completely independent of seven transmembrane domain (7TM) and C terminus. This trans (7TM-independent) function, which is highly unusual for GPCRs, has been shown for several aGPCRs. However, its details such as how this mode is realised and whether it involves signalling remains enigmatic.

To investigate this unusual mode of action only mediated via the extracellular domain we studied the Adhesion GPCR Latrophilin in the model organism Caenorhabditis elegans. We found that the trans function of the C. elegans Latrophilin homolog LAT-1 is involved in fertility by independently controlling oocyte maturation and sperm guidance. Thus, it is spatially and temporally distinct from the classical cis signal mediated by the receptor via G proteins in embryonic cell polarity, suggesting that both modes act separately of each other. Interestingly though, for both functions the same domain architecture of the extracellular N terminus is required. For the peculiar trans function, the N terminus of LAT-1 requires membrane-tethering. By structure-function analyses and selective expression of different receptor N terminus versions in distinct reproduction organs we were able to determine the requirements for this aGPCR role in fertility. We have obtained evidence for a model in which this mode acts in a non-cell autonomous manner involving a cross-talk with the Notch-signalling pathway.

The ability to mediate two distinct signals in different biological contexts, one “classical” and one in trans constitutes a novel mechanism for Adhesion GPCRs to signal. Our findings contribute towards shedding light on this dual signalling principle.

Financial support: DFG FOR2149
BAI1/ADGRB1 SCULPTS THE DENDRITIC ARBORS OF HIPPOCAMPAL PYRAMIDAL NEURONS VIA RHOA-DEPENDENT GROWTH_RESTRICTION

Joseph G. Duman1, Yen-Kuei Tu2, Shalaka Mulherkar1, Christopher P. Tzeng1,a, Vasilis C. Mavratsas3,b, Tammy Szu-Yu Ho4 & Kimberley F. Tolias1,2,5

1Department of Neuroscience, Baylor College of Medicine, One Baylor Plaza, Houston, TX, 77030, USA
2Graduate Program in Integrative Molecular and Biomedical Sciences, Baylor College of Medicine, One Baylor Plaza, Houston, TX, 77030, USA
3Rice University, Houston, TX 77005, USA
4Program in Developmental Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX, 77030, USA
5Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX 77030, USA

aCurrent address: Program in Neurobiology, Harvard Medical School, Boston, MA 02115
bCurrent address: Department of Neuroscience, Baylor College of Medicine, Houston, TX 77030

Cognition arises from complex, dynamic neuronal circuitry that relies on dendritic arbor morphologies to stipulate circuit availability and computational functions. An intricate interplay between genes, activity, and extrinsic signals determines the morphological and functional properties of individual neurons. As dendrites grow and branch during development, they navigate an information-laden neuropil that shapes their forms and connectivities. To establish functional synapses, dendrites must form productive interactions with the appropriate axons. On the other hand, dendrites have repulsive interactions with dendrites emanating from the same neuron or neurons of the same type. The A-GPCR brain-specific angiogenesis inhibitor 1 (BAI1)/ADGRB1 plays an important role in excitatory synaptogenesis and synaptic function in pyramidal neurons. We recently showed that BAI1 signals trans-synaptically and promotes the organization of presynaptic active zones. Taken together, these data suggest that BAI1 plays a role in promoting axonal-dendritic functional contacts. Moreover, BAI1 knock-down (Kd) causes dramatic overgrowth of dendritic arbors in CA1 pyramidal neurons in vivo and in culture. Dendritic arbors of BAI1-Kd neurons grow normally until 17 days in vitro (DIV), the stage at which wild-type neurons transition from arbor growth to stability, but BAI1-Kd arbors continue to grow. Conversely, neurons overexpressing (OX) BAI1 exhibit normal growth until 17 DIV, then retract. Previously reported pathways downstream of BAI1 cannot account for this, but BAI1-Kd blunts and delays a previously unreported peak in RhoA activation that occurs as control neurons transition to stability. We find that BAI1 induces RhoA activation in dendrites, which in turn controls growth arrest. Pharmacological manipulation of RhoA signaling corrects both BAI1 Kd and OX defects. Finally, BAI1-Kd causes increased dendritic self-crossing and increased crossing of dendrites with those of nearby neurons, suggesting a tiling defect. Thus, BAI1 shapes neuronal circuits by mediating both attractive axono-dendritic contacts and repulsive dendrito-dendritic interactions.

Supported by R01-MH109511 (to KFT).
DYSTROGLYCAN BINDS CELSR3 (ADGRC3) TO REGULATE COMMISSURAL AXON GUIDANCE

L. Bailey Lindenmeier¹, Nicolas Parmentier², Caying Guo³, Fadel Tissir², and Kevin M Wright¹,⁴

¹Vollum Institute, Oregon Health & Science University, Portland, OR 97239
²Institute of Neuroscience, Université Catholique de Louvain, Brussels, Belgium
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⁴Presenting author

During neural circuit development, instructive extracellular cues signal through cell surface receptors to direct the precise targeting of axons. We have identified the transmembrane glycoprotein dystroglycan as a regulator of axon tract formation in the retina, brain, and spinal cord. Using genetic approaches, we show that dystroglycan functions non-cell autonomously as an extracellular scaffold by binding multiple Laminin G (LG) domain containing proteins through its extensive glycan chains. This allows dystroglycan to regulate axon tract development in multiple ways. First, dystroglycan maintains basement membranes as permissive growth substrates for extending axons. Second, dystroglycan binds the secreted axon guidance cue Slit to regulate its extracellular distribution in vivo. Finally, we have recently identified an interaction between dystroglycan and the adhesion GPCR Celsr3 (Adgrc3).

Celsr3 is required for axon guidance in the forebrain, spinal commissural axons, and peripheral motor projections. In spinal commissural axons, Celsr3 functions within growth cones to direct anterior turning towards the brain after crossing the ventral midline. Commissural axons in dystroglycan or Celsr3 mutants exhibit a randomization of post-crossing trajectory, with axons extending in both anterior and posterior directions. Using in vitro binding assays, we show that dystroglycan directly binds the LG1 domain present in the extracellular portion of Celsr3. To test the importance of this interaction during axon tract development, we generated a Celsr3 knock-in mutant (Celsr3⁸R₁⁵₄₈Q) that disrupts its binding to dystroglycan. Celsr3⁸R₁⁵₄₈Q mutants recapitulate the post-crossing randomization of commissural axons seen in Dystroglycan⁻/⁻ and Celsr3⁻/⁻ mutants, demonstrating that this interaction is required in vivo. These results provide a mechanistic link between dystroglycan and the adhesion GPCR Celsr3, thereby identifying a novel mechanism by which dystroglycan regulates neural circuit development.
A ROLE FOR THE ADHESION-GPCR CELSR1 IN CONTACT-MEDIATED ALIGNMENT OF CELL BEHAVIOUR

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Planar cell polarity (PCP) proteins facilitate multiple aspects of tissue and organ development. PCP is a key to cellular processes in embryonic development because its primary role is to align cell structures, cell shapes and cell rearrangements along particular body axes. Indeed, disruption of PCP protein function in mammals leads to severe birth defects. The adhesion-GPCR Celsr1 (ADGRC1) is a core component of PCP signalling. Celsr1 is employed as a local communicator of cell polarity information: its extracellular cadherin repeats generate molecular bridges between one cell and its neighbours. Our recent studies have used the mammalian embryonic skin (epidermis) as a model to understand how Celsr1 orchestrates tissue morphogenesis. Our investigations suggest that Celsr1, along with its signalling partner Frizzled-6 (Fz6), communicate the long axis orientation of interphase basal epidermal cells to neighbouring basal mitoses so that they align their horizontal division plane along the same body axis. Our hypothesis has parallels with contact-mediated division orientation in early C. elegans embryos suggesting functional conservation with Latrophilin-1 (ADGRL1). These findings will be discussed and compared with new evidence, which suggests an unexpected role for Celsr1 and Fz6 in the axial alignment of cell arrangements in adjacent epidermal layers. At this time Celsr1 and Fz6 exhibit both asymmetric expression across the basal epidermal layer and strong expression in the centre of basal cell rosettes which align with suprabasal cell rosettes in the overlying tissue layer. Reconstruction of these cell arrangements in 3-dimensions (3D) have led to the hypothesis that basal layer cells extend apical cell protrusions expressing Celsr1 and Fz6 to contact suprabasal cells and in doing so co-ordinate the alignment of rosette-based cell rearrangements in both tissue layers. This novel finding is consistent with our model which posits that PCP proteins play a role in 3D tissue and organ morphogenesis.

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THE PHYSIOLOGICAL ROLE OF THE MECHANO-RESPONSIVE AGPCR GPR133

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Several adhesion G protein-coupled receptors (aGPCRs) have been shown to be activated by mechanical stimuli such as vibration, shaking [1, 2], or touch [3, 4] through their tethered agonist sequence. GPR133/ADGRD1 is yet another mechano-responsive aGPCR, which is expressed in tissues that are known to be exposed to mechanical stress or to exert mechanical force like bone, adipose tissue, and muscle. GPR133 has been associated with changes in heart frequency [5], human body height [6], and body weight in mice [7]. To study the physiological role of GPR133 we generated receptor-deficient zebrafish and mouse lines. Gpr133 knock-out (KO) mutants in both species were significantly smaller compared to wild-type (wt) litter mates. Additionally, gpr133 deficient zebrafish displayed a severe spine deformation. High expression of Gpr133 in the notochord of fish and mouse suggest specific functions of GPR133 in vertebral and cartilaginous disc development. RNA-Seq analysis of Gpr133 KO animals revealed considerable changes in lipid metabolism, collagen content, Wnt signaling cascades, and contractile elements, which we followed up through biochemical assays. Together with the finding of altered smooth muscle and cardiac function in Gpr133 KO mice (see abstract Ullmann et al.) and additional in vitro studies, our studies point towards a mechano-receptor function of GPR133.


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SIGNALING AND TRAFFICKING OF GPR64/ADGRG2 ARE REGULATED BY ITS N-TERMINAL FRAGMENT AND TETHERED PEPTIDE

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We have previously shown that GPR64/ADGRG2 regulates secretion of parathyroid hormone via its crosstalk with calcium-sensing receptor and elevation of cAMP levels. To investigate the mechanisms of activation, signaling and trafficking of GPR64 in HEK293 cells, we generated receptor mutants that either lack the N-terminal fragment (NTF) (GPR64ΔNTF) or both NTF and P-15 tethered peptide (GPR64-7TM-P622). A 15-amino acid long peptide (P-15) after the GPCR-proteolysis site (GPS), acts as an agonist of GPR64 and GPR64ΔNTF constitutively activates the Gas-cAMP-Epac/PKA-CREB pathway. However, GPR64-7TM-P622 does not show constitutive activation of Gas protein. Both GPR64ΔNTF and GPR64-7TM-P622 respond to synthetic P-15 via Ga13-RhoA-SRE cascade but neither mutant shows constitutive activation of this pathway. Unlike full length GPR64, GPR64ΔNTF and GPR64-7TM-P622 interact with b-arrestin 1/2 in basal conditions, suggesting an inhibitory role for NTF in both G-protein-dependent and –independent signaling. Knock down of b-arrestins and GPCR kinases (GRKs) increased the total receptor levels and reduced the internalized receptors at basal condition. Surprisingly, despite the increased level of receptors, we observed reduced CREB and SRE activation in cells expressing GPR64ΔNTF and GPR64-7TM-P622, respectively. These data suggest that 1) b-arrestins and GRKs may play a role in internalization/degradation of constitutively active GPR64 mutants, 2) constitutive activity of GPR64 mutants may originate from endosomal compartments and 3) b-arrestins have differential impact on distinct signaling pathways of GPR64, which might be dependent on the presence of an intact endogenous P-15.

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Mechanical forces influence cell shape, proliferation, differentiation and survival, thereby affecting tissue and organ formation and function. Exactly how the different mechanical forces are sensed and transduced remains largely elusive.

Here, we report that Ovarian cancer G protein coupled receptor 1 (OGR1, aka GPR68) acts as coincidence detector of membrane stretch and its physiological ligand, H+. Using fluorescence imaging, substrates of different stiffness, micro-contact printing methods and cell stretching techniques, we show that OGR1 only responds to extracellular acidification under conditions of membrane stretch, and vice versa. The level of OGR1 activity mirrors the extent of membrane stretch and degree of extracellular acidification. Furthermore, actin polymerisation in response to membrane stretch it critical for OGR1 activity and provides a “memory” for past stretch. Cells experience changes in membrane stretch and extracellular pH throughout their lifetime. Since OGR1 is a widely expressed receptor, it represents a unique and widespread mechanism that enables cells to respond dynamically to mechanical and pH changes in their microenvironment.
SIGNALING AND REGULATION OF THE BAI SUB-FAMILY OF ADHESION GPCRS

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The brain-specific angiogenesis inhibitors 1-3 (BAI1-3; ADGRB1-3) comprise a subfamily of adhesion G-protein-coupled receptors (GPCRs). These receptors have important roles at synapses in the central nervous system and also play key roles outside the CNS. Our studies on G protein-mediated signaling by the members of this family have revealed that BAI1 couples predominantly to G-alpha-12/13 to regulate Rho, whereas BAI2 exhibits preferential coupling to G-alpha-z. Removal of the N-terminal regions of BAI1 and BAI2 (up the point of GAIN domain cleavage) strongly enhances receptor signaling, similar to other adhesion GPCRs. However, removal of the membrane-proximal stalk (stachel) region has little or no effect on BAI1 or BAI2 signaling, which is distinct from the stalk-dependent signaling observed with certain other adhesion GPCRs. A disease-associated mutation in BAI2 (R1465W) enhances receptor surface expression and signaling. This mutation does not affect receptor interactions with beta-arrestins, but sharply reduces receptor binding to endophilins. Ongoing studies are focused on achieving a more comprehensive understanding of BAI1-3 with regard to their downstream signaling pathways, physiological actions, and potential as novel drug targets in the treatment of psychiatric and neurological diseases.
A TALE OF TWO AGPCRS (IN SENSORY NEURON MYELINATION): ADGRG6 AND ADGRG1

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In the peripheral nervous system (PNS), Schwann Cells (SCs) form the myelin sheath, which is essential for fast action potential propagation as well as neuronal health and survival. Myelin not only insulates axons, allowing the rapid propagation of action potentials, but SCs also protect and provide vital trophic support to neurons. Disruptions of myelin can lead to permanent neuron loss, significant pain and morbidity, and ultimately paralysis. Our relative lack of understanding of the mechanisms that govern the genetic and molecular control of myelination hinders the ability to design rational treatments for relevant injuries and diseases. While these mechanisms are not fully understood, we have defined members of the adhesion G protein-coupled receptor (aGPCR) class as major regulators of glial cell development and myelination. We established the function of two aGPCRs, Gpr126/Adgrg6 and Gpr56/Adgrg1 in SC myelination (Mogha \textit{et al.}, 2013; Ackerman \textit{et al.}, 2018) as well as the function of Gpr126/Adgrg6 in remyelination after injury (Mogha \textit{et al.}, 2016). Interestingly, our published work (Mogha \textit{et al.}, 2013 and Ackerman \textit{et al.}, 2018) and preliminary data has demonstrated that these two genes are robustly expressed in DRG neurons. However, function of these receptors in sensory neurons has not been elucidated. Preliminary data from sensory neuronal specific Gpr126/Adgrg6 conditional knockout mice show that myelination is, surprisingly, impaired. We have begun to analyze the roles of Gpr126/Adgrg6 and Gpr56/Adgrg1 in the sensory neurons. Our ongoing efforts to analyze the function of these aGPCRs in sensory neuron development and regeneration using conditional mouse models will be discussed.
G PROTEIN-COUPLED RECEPTOR 56 (GPR56) AS A POTENTIAL FUNCTIONAL REGULATOR OF NORMAL AND LEUKEMIC HUMAN STEM CELLS

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Leukemic stem cells (LSCs) sustain acute myeloid leukemia (AML) and must be eliminated to cure a patient. Improved understanding of LSCs is required to improve therapy. We identified G protein-coupled receptor 56 (GPR56) among a gene expression signature common to LSCs and normal hematopoietic stem cells (HSCs). GPR56 is a novel marker of high LSC frequency in AML patients. GPR56 has also been implicated in the development and regulation of murine HSCs, although this is controversial, and was shown to accelerate leukemogenesis in mice. This study aims to establish the functional role of GPR56 in human LSCs and HSCs, providing insight into the commonalities and differences in their molecular regulation.

GPR56 expression was inversely correlated with survival across three microarray AML cohorts (p<0.01). GPR56 expression was higher in samples with worse outcome (p<0.0001) and lower in those with better outcome (p<0.01) predicted by cytogenetics. We confirmed increased GPR56 expression in human LSC- and HSC-enriched fractions versus mature populations by qRT-PCR. Colony forming cell assays were conducted with flow-sorted cells to determine effects on progenitor proliferation and differentiation. Overexpression of GPR56 in AML cell line MOLM-13 (p<0.05) and cultured AML sample 8227 (GPR56dN, p<0.0001) increased colony formation. Conversely, GPR56 overexpression did not significantly alter human hematopoietic progenitor activity. Long-term xenograft assays were performed via intrafemoral injection in immunodeficient mice to determine effects on stem cell function. GPR56 overexpression conferred a significant engraftment advantage (p<0.0001) maintained in secondary 12-week transplants (p<0.05). The lineage distribution and percentage of stem and progenitor cells were unaltered, indicating self-renewal of stem cells.

These data suggest that GPR56 enhances HSC function in vivo, and may regulate human leukemic progenitors, but not hematopoietic progenitors in vitro. Further functional studies will be performed to determine the role GPR56 in LSC function in vivo, and its role in chemotherapy resistance.

*Presenting and corresponding author.
Oral Presentations

ADGRG6 (GPR126) REGULATES EMT OF ENDOCARDIAL CELLS DURING VALVE FORMATION

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The mammalian heart evolves from a simple tubular structure into a highly complex organ. A process critical for proper heart formation is the epithelial-to-mesenchymal transition (EMT) of endocardial cells, which populate the so-called endocardial cushions to initiate valve formation. Our previous studies have demonstrated that Gpr126 is expressed in the ventricular endocardium during development and deletion resulted in embryonic lethality associated with hypotrabeculation. Utilizing the “knockout first” allele Gpr126tm1a(EUCOMM)Hmgu LacZ reporter mouse and anti-GPR126 antibodies, we show here that Gpr126 is expressed also in the mesenchymal cells populating the endocardial cushions at E11.5. Deletion of Gpr126 resulted in fewer invading mesenchymal cells at E11.5. Moreover, fewer migrating endocardial cells were observed in endocardial cushion explant cultures from E9.5 Gpr126 knockout embryos compared to wildtype littermates. In order to verify that Gpr126 expression in the endocardium is required for the survival and proper heart development, we crossed Gpr126tm1b(EUCOMM)Hmgu conditional knockout mouse with Tg(Tek-cre)1Ywa/J resulting in truncation of Gpr126 after exon 6 in endocardial/endothelial cells. Surprisingly, the offspring survived close to Mendelian ratio. In contrast, endocardial/endothelial-specific truncation of Gpr126 after exon 2 in Taconic Gpr126 conditional knockout mice resulted in embryonic lethality. The contrasting results therefore suggest that the remaining fragment of Gpr126 in the Gpr126tm1b(EUCOMM)Hmgu model, which covers part of the extracellular domain, rescued the endothelial related phenotype. This is in concordance with previously published data where the extracellular domain of Gpr126 alone rescued the trabecular defect in zebrafish. Interestingly the germline deletion of Gpr126 in Gpr126tm1b(EUCOMM)Hmgu by means of Tg(EIIa-cre)CS379LmgdJ resulted in embryonic lethality. Taken together these data suggest that the extracellular domain of Gpr126 is sufficient for proper heart development and that Gpr126 regulates EMT of the endocardial cells during heart valve development.

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ROLE OF GPR110 IN BREAST CANCER

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Our long-term goal is to discover adhesion GPCR targets in breast cancer. Our previous studies have found GPR110 to be overexpressed in tumorigenic cell population as well as in anti-HER2 drug-resistant derivatives of HER2+ breast cancer cells. In subsequent studies, we found that GPR110 knockdown inhibited anchorage-independent cell growth, mammosphere formation, and invasion/migration of HER2+ breast cancer cells. Conversely, overexpression of GPR110 by lentiviral delivery of cDNA enhanced anchorage-independent cell growth, mammosphere formation, and invasion/migration in HER2+ breast cancer cells. In addition, GPR110 overexpression led to increase in the % of Aldefluor-positive tumorigenic cell population, further emphasizing the role of GPR110 as a mediator of tumorigenesis in addition to the metastatic processes in HER2+ breast cancer. Among various subtypes of breast cancer, GPR110 expression was higher in HER2+ and basal subtypes, most of which are triple-negative (negative for ER, PR, and HER2), compared to luminal A and B subtypes. GPR110 was either gene amplified or upregulated in 4% of all breast cancers based on the publicly available TCGA dataset. GPR110 overexpression predicted poorer recurrence-free survival in triple-negative breast cancer. Furthermore, GPR110 was overexpressed in brain metastatic lesions compared to mammary tumors in patient-derived xenograft models of triple-negative breast cancer (WHIM2 and WHIM30). Knocking down GPR110 reduced anchorage-dependent and -independent cell growth, mammosphere formation, and invasion/migration of triple-negative breast cancer cells. Overall, our results suggest that GPR110 may be a potential drug target in HER2+ and triple-negative breast cancer. Drug discovery efforts to identify GPR110 antagonists will provide useful pharmacological tools for validating GPR110 as a drug target in breast cancer. Since GPR110 is also overexpressed in various other types of cancer, understanding the mechanism of GPR110 upregulation and signaling in cancer is an important future direction.

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THE ADHESION G PROTEIN-COUPLED RECEPTOR G6 IS ESSENTIAL FOR HOMEOSTASIS OF THE INTERVERTEBRAL DISC IN MICE

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Degenerative changes of the intervertebral disc (IVD) are a leading cause of back pain and disability worldwide. Yet, surprisingly little is known about the homeostatic regulation of the IVD during maturation and aging of the spine. Increased cyclic adenosine monophosphate (cAMP) signaling can promote chondrogenesis during development, as well as attenuate cartilage degradation in injury and disease. However, the pathways that regulate this signaling in vivo are undetermined. Here, we use conditional genetics in mouse and chondrogenic cell culture to demonstrate the necessity of the adhesion G protein-coupled receptor G6 (ADGRG6) for sustained chondrogenic pathways and homeostasis of cartilaginous tissues of the IVD. Using a variety of histological assays, contrast-enhanced microCT imaging, and gene and protein expression analyses we show that ADGRG6 function is dispensable for early IVD development. However, in young adult mice (1.5 months) we observed markers of degeneration and IL6/STAT3 pro-inflammatory signaling commonly observed in osteoarthritis, prior to overt histopathological changes. In older adult mutant mice (6-8 months) we observed accelerated IVD degeneration with endplate-oriented, disc herniation, similar to Schmorl's nodes in humans. Importantly, attenuation of STAT3 phosphorylation (with a small molecule inhibitor, Stattic) resulted in some improvements in the histopathology of IVD tissues in Adgrg6 conditional mutant mice. This study demonstrates a novel role for ADGRG6 function in the homeostasis of cartilaginous tissues in mouse, suggesting a direct regulation of ADGRG6 on IL6/STAT3 signaling for this process. Our findings further suggest that ADGRG6 may provide a promising therapeutic target for cartilage degeneration.

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ROLES OF THE ADGRA FAMILY IN GLANDULAR DEVELOPMENT, LINEAGE COMMITMENT AND TUMORIGENESIS.

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Abstract: We have generated reporter and knock out mouse models to investigate the expression of members of the ADGRA family in ectodermal appendages. Our knock-out mice show defects in ductal elongation and secretory differentiation of lacrimal and mammary glands. Genetic domain analyses demonstrate that the cytoplasmic and transmembrane domains are essential. Expression patterns demarcate early stem/progenitors in hair follicles and in glandular structures at ductal tips that sense directional and growth cues from macrophages. ADGRA-positive cell populations show stem and early progenitor profiles and are amplified in Wnt1 tumors suggesting they lie towards the top of the ductal lineage hierarchy. Tumors with high expression are associated with lineage skewing in favour of stem/progenitors at the expense of differentiated cell populations and with significantly earlier onset in mice. In humans, expression is associated with aggressive tumor subtypes. Within these, high expression correlates with particularly poor survival outcome. Our data indicate that ADGRA are essential for normal ductal development and have potential use as biomarkers of poor prognosis in cancer.
REGULATION OF CANONICAL WNT7 SIGNALING BY GPR124/ADGRA2

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WNT7A and WNT7B are lipid-modified proteins that activate the canonical Wnt/β-catenin signaling pathway in central nervous system (CNS) endothelium, mediating blood-brain barrier formation and maintenance. Canonical Wnt signaling is activated by Wnt-induced heterodimerization of the co-receptors Frizzled (FZD) and LRP5/6. The adhesion G protein-coupled receptor GPR124/ADGRA2 critically regulates WNT7-specific activation of canonical Wnt signaling in CNS endothelial cells by unknown mechanisms. We found that the GPR124 ectodomain, but not its transmembrane and intracellular domains, regulates WNT7 signaling, indicating that GPR124 does not intrinsically transduce canonical Wnt signals. Moreover, we and others found that GPR124 forms a complex with the GPI-anchored protein RECK on the surface of CNS endothelial cells. WNT7A and WNT7B directly bound to purified recombinant RECK, which required the RECK cystine knot motif domain as well as the RECK cysteine-rich domain (CRD) having homology to the Wnt-binding CRD of FZD receptors. Furthermore, we found that active WNT7A is a highly unstable protein that rapidly forms inactive, hydrophilic aggregates after secretion. RECK binding to WNT7A potently stabilized newly secreted WNT7A in its active, hydrophobic, monomeric form. Stabilization of hydrophobic WNT7A by RECK drastically enhanced FZD8:WNT7A complex formation by relay of WNT7A from the lower affinity receptor RECK to the higher affinity receptor FZD8. Overall, the GPR124:RECK complex stabilizes active WNT7 on the cell surface, thereby promoting subsequent FZD/WNT7/LRP5/6 complex formation and canonical Wnt/β-catenin signaling pathway activation.

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TOGETHER OR APART, BUT ALWAYS CLOSE: LATROPHILIN-1 MEDIATES AXONAL ATTRACTION INDUCED BY PROTEOLYTICALLY RELEASED LASSO


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Latrophilin-1, a presynaptic adhesion G-protein-coupled receptor, and its postsynaptic ligand, Lasso/teneurin-2, are cell-surface receptors that mediate cell adhesion and have been implicated in synapse formation. Paradoxically, the extracellular domain of up to 20% of cell-surface Lasso is proteolytically cleaved and released into the medium, making it unable to function in cell adhesion. However, we found that the released fragment of Lasso binds to cell-surface latrophilin-1 on distant cells and axonal growth cones. Using microfluidic devices, we show that a spatio-temporal gradient of the soluble Lasso fragment induces axonal attraction. This effect requires latrophilin-1 (as shown by latrophilin-1 knockout in mice) and involves Lasso-mediated aggregation of latrophilin-1 on the cell surface, increased cytosolic calcium and enhanced exocytosis, processes that are known to induce growth cone turning. This suggests a novel mechanism of axonal pathfinding, with the latrophilin-1-Lasso pair both inducing axonal attraction and supporting synaptogenesis.
MECHANO-DEPENDENT PHOSPHORYLATION OF CD97/ADGRE5 AT ITS PDZ-BINDING MOTIF MODULATES CELLULAR DETACHMENT

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Adhesion GPCRs are potential mechanoreceptors. Here, we examined signaling of CD97/ADGRE5, highly present in force-stressed cells, in the context of mechanotransduction. Mechanical stimuli rapidly induce phosphorylation of CD97 (pCD97) in its C-terminal PDZ-binding motif (PBM). Biochemically, pCD97 disrupts binding to PDZ domains of the scaffold protein DLG1, a here identified CD97 interaction partner. In shear-stressed cells pCD97 appears not only in junctions, retracting fibers and the detachment area, but also in lost membrane patches, indicating cellular detachment at the cytosolic interface, the CD97 PBM. Indeed, this motif is critical for the CD97-dependent mechanoresponse: cells expressing CD97 lacking the PBM show an increased deformability during optical stretching. Under shear-stress they lose cell contacts faster and have an altered actin structure compared with cells expressing full-length CD97. Consistently, CD97 is organized at the membrane in an F-actin dependent manner. In summary, CD97 shapes the cellular mechanoresponse through modulating signaling via its PBM.

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KEYNOTE:

UBIQUITIN AND CELL SIGNALING BY PROTEASE-ACTIVATED RECEPTORS

JoAnn Trejo

Our research goals are to delineate the regulatory mechanisms that control signaling by the G protein-coupled protease-activated receptor-1 (PAR1) and closely-related family members in normal physiology and disease. Our recent efforts have led to the discovery that ubiquitination of a subset of GPCRs including PAR1 promotes p38 mitogen-activated protein kinase (MAPK) inflammatory signaling and not lysosomal degradation of the receptor. We also discovered a novel ubiquitin-independent lysosomal pathway for GPCRs. Contrary to conventional view, we found that ubiquitination of certain GPCRs and canonical ubiquitin-binding ESCRTs are not required for receptor lysosomal degradation. We identified ALG-interacting protein (ALIX) and the alpha-arrestin-related domain containing protein-3 (ARRDC3) as key mediators of this GPCR lysosomal sorting pathway. The ALIX/ARRDC3-dependent pathway bypasses the requirement for GPCR ubiquitination and is distinct from the canonical ubiquitin-dependent endosomal sorting complexes required for transport (ESCRT) lysosomal pathway. Moreover, the ALIX/ARRDC3 pathway appears to be dysregulated in cancer. Our central premise is that ubiquitination offers novel and diverse mechanisms for regulation of GPCR biology. A thorough understanding of the mechanism by which key regulators and mediators of ubiquitination regulate GPCR signaling and trafficking is essential for understanding dysregulated mechanisms in disease and for identifying new drug targets. However, the mechanisms by which GPCRs activate E3 ubiquitin ligases to promote ubiquitin-mediated signaling or trafficking are not known. Here, we present our recent finding that PAR1 stimulates c-Src-mediated tyrosine phosphorylation and activation of the HECT-domain NEDD4-2 E3 ubiquitin ligase to promote p38 signaling and inflammatory responses. Using mass spectrometry, we identified a unique phosphorylated tyrosine (Y)–485 within the 2,3-linker peptide between WW domain 2 and 3 of NEDD4-2 in agonist stimulated cells. Mutation of NEDD4-2 Y485 impaired E3 ligase activity and failed to rescue PAR1-stimulated p38 activation and inflammatory responses. The purinergic P2Y₁ receptor also required c-Src and NEDD4-2 tyrosine phosphorylation for p38 activation. These studies reveal an unexpected role for c-Src in GPCR-induced NEDD4-2 E3 ligase activation, which is critical for driving ubiquitin-mediated p38 inflammatory signaling.
STRUCTURAL AND FUNCTIONAL BASIS OF ADHESION GPCR ACTIVATION

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Adhesion GPCRs have large extracellular regions (ECRs) decorated by numerous adhesion domains and a conserved GPCR Autoproteolysis Inducing (GAIN) domain that mediates self-cleavage of the receptor. The talk will focus on our recent studies on two avenues:

1) Our studies showed that adhesion GPCRs are activated via Stachel-independent mechanisms in addition to Stachel-dependent mechanisms (Salzman et al., PNAS, 2017; Nazarko et al., iScience, 2018). Stachel-independent mechanisms depend on the large extracellular regions of adhesion GPCRs and form the basis for the complex regulation of adhesion GPCR function.

2) We determined the high-resolution structure of teneurin, a large-ligand of latrophilin/ADGRL1 and revealed a unique structure that is similar to bacterial Tc-toxins (Li et al., Cell, 2018). We further showed that an alternatively spliced region within teneurin acts as a switch to regulate trans-cellular adhesion of teneurin to latrophilin. One splice variant activates trans-cellular signaling in a latrophilin-dependent manner, whereas the other induces inhibitory postsynaptic differentiation. These results highlight the unusual structural organization of teneurins giving rise to their multifarious functions.


ANNOTATION AND QUANTIFICATION OF ADHESION GPCR SPLICE VARIANTS

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The enormous sizes of adhesion GPCR (aGPCR) are based on complex genomic exon-intron architectures raise to multiple mRNA variants encoding receptor proteins with potential functional differences. We established and validated a bioinformatic pipeline including a new visualization tool to extract and quantify mRNA variants of aGPCR from deep-sequenced transcriptomes. Data analysis shows that all aGPCR investigated (n=15) have multiple transcription start sites suggesting complex expression regulation. Further, we found $20.3 \pm 14.2$ splice variants per aGPCR gene and frequent tissue-specific splicing. Transcripts with an abundance above 1% of all specific aGPCR transcripts are generated from $40.0 \pm 17.8$ exons per aGPCR gene. Considering all exons identified in this study less than half ($42.0 \pm 11.7\%$) of these exons were already annotated. A single aGPCR transcript is built from $16.5 \pm 3.8$ exons showing that not all exons of a given aGPCR gene are always used. About 67% of all investigated aGPCR possess splice variants changing the structure and domain composition of the N terminus. For several aGPCR there we provide strong evidence that the large ectodomain exists without or with an incomplete seven-helix-transmembrane anchor. Experimental analyses of selected aGPCR splice variants revealed functional differences. Summarizing these data, one can estimate over 600 aGPCR variants which are significantly expressed and many of them may also differ in specific functions. We also demonstrate that our analysis provides new support on the evolutionary history of aGPCR and has an impact on a rational design of aGPCR gene-deficient mouse lines.
CRYOEM VISUALIZATION OF G PROTEIN-COUPLED RECEPTORS

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Recent technological breakthroughs have enabled single-particle electron cryo-microscopy (cryoEM) to achieve close to atomic resolution structures of macromolecular complexes. The methodology is now displaying its hidden potential, and, has already become a principal choice of method for characterizing the structure of relatively large macromolecular assemblies including membrane receptors. GPCRs and their complexes have been challenging targets for cryo-EM analysis, both because of their dynamic nature and also due to their relatively small size that can limit accurate alignment for 3D reconstructions. Nevertheless, near atomic resolution cryoEM maps of GPCRs are now within reach, opening up unprecedented opportunities to uncover molecular mechanisms and facilitate drug discovery. Here, I will give an overview our more recent cryo-EM work to obtain the structures of several pharmacologically important GPCR complexes.
ADHESION G PROTEIN-COUPLED RECEPTORS ADGRL/LATROPHILINS PHYSICALLY AND FUNCTIONALLY INTERACT WITH THE ACTIN CYTOSKELETON

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ADGRL/Latrophilin (Lphn) represents a subgroup of the adhesion G-protein-coupled receptors family that comprises three isoforms (Lphn1,2,3) and that are mainly expressed in neurons. Consistent with their neuronal expression, Lphn have been described as synapse stabilizers. Their role in synapse stabilization relies on their adhesion function, which is mediated by heterophilic interactions with adhesion molecules such as Teneurins. While all three isoforms of Lphn share a high sequence homology within the adhesion motifs composing their extracellular N-terminal region, low sequence homology is detected in their cytoplasmic domains. A conserved PDZ binding motif known to recruit scaffolding proteins linked to the actin cytoskeleton decorates the extremity of their otherwise divergent cytoplasmic regions. While there is a better understanding of how Lphn establish intercellular adhesion, very little is known on their ability to link extracellular adhesion to intracellular signaling pathways. Here, we sought to investigate the role of Lphn in relation to the actin cytoskeleton in an intent to probe their signaling mechanism. Individual expression of all three Lphn isoforms in HEK293T cells modulated the formation of actin structures reminiscent of membrane specializations sustaining neuronal migration and function. Cell and nuclear dimensions of Lphn-expressing cells displayed significant changes that resulted in increased volume. In addition, Lphn isoforms were co-purified in a complex with actin that paralleled an activation of the actin-depolymerizing factor coflin1, suggesting the presence of a constitutive physical association with functional implications. Intercellular adhesion involving Lphn ligand Teneurin4, disrupted the co-localization with F-actin of all three Lphn isoforms and restored specific actin structures. We find that the remodeling of the actin cytoskeleton constitutes an important component of Lphn signaling pathway that might contribute to synaptic architecture and function.

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ADGRG6 (GPR126) IS A MECHANO-RESPONSIVE GENE

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Previously it has been shown that Gpr126 is required for proper heart, ear and skeletal development as well as myelination. Yet, the role of Gpr126 as well as up and downstream signaling pathways are poorly understood. A detailed expression pattern on a cellular level might provide novel insight in possible functions of Gpr126. As most available expression data are based on RT-PCR of tissue samples, we have generated a “knock out first” allele mouse line utilizing the EUCOMM targeting construct, Gpr126<sup>tm1a(EUCOMM)Hmgu</sup>, which expresses the LacZ gene under the control of the Gpr126 promoter. The insertion of the cassette results in the truncation of Gpr126 with embryonic lethality of the homozygous offspring, but not the heterozygous. This outcome is in concordance with previously published results. The analysis of this mouse line indicated that Gpr126 is expressed during early development in the endocardium at E9.5, in addition in the somites at E11.5 and joints between E12.5 and E13.5. In the juvenile but not adult mice, LacZ activity was still detected in the endocardium. In addition, it could be detected in adult mice in cells lining tissues such as endothelium of vessels, especially capillary endothelium of the lung, and epithelium of the urinary bladder and urethra. Furthermore, chondrocytes were positive. Because of the focalized endothelial/epithelial expression of Gpr126, and previous reports of GPCR acting as transducers of shear force, we tested weather Gpr126 expression is regulated by shear flow. Our data demonstrate that the expression of Gpr126 in HUVEC cells increases when cells are subjected to laminar flow. In addition we observed a decrease in Gpr126 expression when ablating blood flow in zebrafish embryos by the means of tnt2 morpholino. In summary, our data provide an extensive and detailed Gpr126 expression pattern and indicate that Gpr126 is regulated by shear force.

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SYSTEMATIC AFFINITY PROTEOMICS IDENTIFIES FUNCTIONAL MODULES ASSOCIATED WITH ADHESION GPCRS

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Despite receiving attention of adhesion G protein-coupled receptors (aGPCRs) from a wide spectrum of biomedical fields, little is known about their cellular environment and specific cellular function. The knowledge about aGPCR protein networks is still scarce. For most receptors only few interaction partners are known so far. Here, we aimed to identify novel aGPCR-interacting partners which should shed light on the composition of cellular protein networks and cell modules that rely on aGPCR function. For this, we performed affinity proteomics utilizing tandem affinity purifications of systematically tagged sets of diverse aGPCRs, namely LPHN2, CD97, GPR123, GPR124, GPR125, BAI1, BAI2, BAI3, and VLGR1. We subsequently examined the eluted affinity purified protein complexes with mass spectrometry. For the analysis of the acquired proteomic data we functionally grouped the hits based on their Gene Ontology terms and related them to functional cell modules. Selective complementary in vitro and in situ experimental analyses support the annotations.

Our analyses confirmed previously described functions of some aGPCRs at synaptic contacts, but also provided remarkable evidence related to functional roles of aGPCR in intracellular membrane networks at the ER and the Golgi as well as at mitochondria-associated ER membranes (MAM). Furthermore, our data suggest a direct role of aGPCRs in transcriptional regulation and novel non-canonical signaling modules for aGPCRs. Moreover, our data reveal the association of aGPCRs with gene products related to neuronal diseases.

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THE ADHESION G PROTEIN-COUPLED RECEPTOR GPR97/ADGRG3 IS EXPRESSED IN HUMAN GRANULOCYTES AND TRIGGERS ANTIMICROBIAL EFFECTOR FUNCTIONS

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The adhesion family of G protein-coupled receptors (aGPCRs) comprises 33 members in human, several of which are distinctly expressed and functionally involved in polymorphonuclear cells (PMNs). As former work indicated the possible presence of the aGPCR GPR97 in granulocytes, we studied its cellular distribution, molecular structure, signal transduction, and biological function in PMNs. RNA sequencing and mass-spectrometry revealed abundant RNA and protein expression of ADGRG3/GPR97 in granulocyte precursors and terminally differentiated neutrophilic, eosinophilic, and basophilic granulocytes. Using a newly generated GPR97-specific monoclonal antibody, we confirmed that endogenous GPR97 is a proteolytically processed, dichotomous, N-glycosylated receptor. GPR97 was detected in tissue-infiltrating PMNs and upregulated during systemic inflammation. Antibody ligation of GPR97 increased neutrophil reactive oxygen species production and proteolytic enzyme activity, which is accompanied by an increase in ERK and IKKα phosphorylation. In-depth analysis of the GPR97 signaling cascade revealed a possible switch from basal Gαs/cAMP-mediated signal transduction to a Gαi-induced reduction in cAMP levels upon mutation-induced activation of the receptor, in combination with an increase in downstream effectors of Gβγ, such as SRE and NF-κB. We conclude that the specific presence of GPR97 regulates antimicrobial activity in human granulocytes.

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THE ADHESION-GPCR BAI1 PROMOTES EXCITATORY SYNAPTOGENESIS BY COORDINATING BIDIRECTIONAL TRANS-SYNAPTIC SIGNALING

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Excitatory synapses are specialized cell-cell contacts located on actin-rich dendritic spines that mediate information flow and storage in the brain. The post-synaptic adhesion-G protein-coupled receptor (A-GPCR) BAI1 is a critical regulator of excitatory synaptogenesis, which functions in part by recruiting the Par3-Tiam1 polarity complex to spines, inducing local Rac1 GTPase activation and actin cytoskeletal remodeling. However, a detailed mechanistic understanding of how BAI1 controls synapse and spine development remains elusive. Here, we confirm that BAI1 is required in vivo for hippocampal spine development and identify three distinct signaling mechanisms mediating BAI1’s pro-synaptogenic functions. Using in utero electroporation to sparsely knockdown BAI1 expression in hippocampal pyramidal neurons, we show that BAI1 cell-autonomously promotes spinogenesis in the developing mouse brain. BAI1 appears to function as a receptor at synapses, as its extracellular N-terminal segment is required for both its pro-spinogenic and pro-synaptogenic functions. Moreover, BAI1 activation with a Stachel-derived peptide, which mimics a tethered agonist motif found in A-GPCRs, drives synaptic Rac1 activation and subsequent spine and synapse development. We also reveal for the first time a trans-synaptic function for BAI1, demonstrating in a mixed-culture assay that BAI1 induces the clustering of presynaptic vesicular glutamate transporter 1 (vGluT1) in contacting axons, indicative of pre-synaptic differentiation. Finally, we show that BAI1 forms a receptor complex with the synaptogenic cell-adhesion molecule Neuroligin-1 (NRLN1) and mediates NRLN1-dependent spine growth and synapse development. Together, these findings establish BAI1 as an essential post-synaptic A-GPCR that regulates excitatory synaptogenesis by coordinating bidirectional trans-synaptic signaling in cooperation with NRLN1.
THE ADHESION-GPCR LATROPHILIN/dCIRL SHAPES THE DEVELOPMENT OF THE NMJ IN DROSOPHILA

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Adhesion G protein-coupled receptors (aGPCRs) form one of the largest yet least understood family within the GPCR realm. Several studies indicate that aGPCRs bear receptivity towards mechanical cues. Previously we reported that Latrophilin/dCirl adjusts membrane excitability of sensory neurons in Drosophila mechanodependently.

Here, we report dCIRL expression in most if not all motoneurons of Drosophila larvae, where it appears to be involved in synaptic vesicle (SV) clustering. Interestingly, the decrease in SV density inversely correlates with the size of the extracellular moiety of dCIRL, the portion that shapes the mecanoceptive profile of sensory neurons. Also, motoneurons of dCirl mutants (dCirlKO) are signified by cytoskeletal changes, which may intersect with the SV clustering deficit. In addition, dCirlKO show morphological abnormalities of the postysynaptic subsynaptic reticulum (SSR). Thus, dCIRL may perceive and integrate mechanical information required for the development and maintenance of the NMJ likely through cell-autonomous as well as cell non-autonomous signaling modes.

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THE ROLE OF GPR110-DEPENDENT SIGNALING IN NEURODEVELOPMENT AND NEUROPROTECTION

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GPR110 (ADGRF1) is an adhesion G-protein coupled receptor recently deorphanized to be a target receptor for N-docosahexaenylethanoamine (Synaptamide). Synaptamide is an endogenous metabolite derived from docosahexaenoic acid (DHA, 22:6n-3), an omega-3 fatty acid highly enriched in the brain. At low nanomolar concentrations this DHA-metabolite promotes neurogenesis, neurite outgrowth and synaptogenesis in developing neurons. Synaptamide also attenuates the lipopolysaccharide-induced neuroinflammatory response and ameliorates the deleterious effects of ethanol on neurogenic differentiation of neural stem cells (NSCs). Specific binding of synaptamide to GPR110 causes conformational changes of GPR110, activates Gαs, and induces cAMP production and phosphorylation of protein kinase A (PKA) and the cAMP response element binding protein (CREB). This signaling pathway leads to the expression of neurogenic and synaptogenic genes and suppresses the expression of proinflammatory genes. GPR110 is highly expressed in NSCs and the developmental brain, and its expression diminishes after birth. Nevertheless, the role of synaptamide/GPR110 signaling in the nervous system beyond the developmental stage is evident as GPR110 knockout mice show significant deficits not only in synapse number and memory function but also in recovery outcome after brain injury in adult animals. The GPR110-dependent cellular effects of synaptamide recapitulated in animal models suggests that synaptamide-derived mechanisms may have translational implications. The structural and cellular basis for in vivo implications of synaptamide/GPR110 signaling in the nervous system will be presented.

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GPR126 FUNCTION IS REGULATED BY ITS EXTRACELLULAR REGION

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The adhesion G-protein-coupled receptor GPR126/ADGRG6 is expressed in Schwann cells and is essential for myelination in the peripheral nervous system. GPR126 has, in addition to the canonical signaling transmembrane domain, a large extracellular region that has been shown to mediate cell communication. However, the molecular details of GPR126 and its mechanism of signaling has not been well-studied. Here, we study the function of GPR126 using a combination of biochemical and biophysical methods. Our results suggest that the extracellular region of GPR126 plays an important role in regulating receptor function.

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ADGRB1 SUPPRESSES CEREBELLAR TRANSFORMATION BY SEQUESTERING MDM2 FROM P53

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Adhesion G-protein coupled receptors (ADGRs) are transmembrane proteins involved in cell-cell/matrix interactions. We show the ADGRB1 gene, which encodes Brain-specific angiogenesis inhibitor 1 (BAI1), is epigenetically silenced in human medulloblastomas through a methyl-CpG binding protein 2 (MBD2)-dependent mechanism. Knockout of Adgrb1 in mice augments proliferation of cerebellar granule neuron precursors (GNPs), and increases medulloblastoma penetrance and accelerated death in Ptch1+/− mice. BAI1 prevents Mdm2-mediated p53 polyubiquitination, and its loss substantially reduces p53 levels. Reactivation of BAI1/p53 signaling axis by targeting MBD2 pathway suppresses human medulloblastoma growth in orthotopic xenograft models. Our findings highlight the importance of BAI1 loss in medulloblastoma and demonstrate that epigenetic restoration of its expression with a new brain-permeable MBD2 inhibitor has therapeutic potential. Revealing BAI1 as a physiological tumor suppressor in medulloblastoma unveils a direct crosstalk between ADGRs and p53 signaling, and provides a causal relationship between ADGRs and cancer. The discovery of a novel upstream regulator of the p53 tumor suppressor is highly significant because of this pathway’s involvement in many cancers. Disruption of the BAI1/mdm2/p53 signaling axis through BAI1 silencing reveals a vulnerability in cancer, and offers an opportunity for therapeutic exploitation through epigenetic reactivation. We provide proof-of-principle that this can be achieved with a chemical scaffold targeting MBD2, and this lead molecule is actionable for translation into a first-in-class therapeutic intervention against medulloblastoma, and possibly other cancers (Zhu D et al, Cancer Cell, June 2018 issue; supported by NIH NS096236).
MOLecular Analysis of ADGrF5 Signaling Determinants Required for ALVeolar Homeostasis

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Background: We have previously demonstrated that epithelial expression of the adhesion G protein-coupled receptor Adgrf5 regulates pulmonary surfactant levels and alveolar homeostasis in mice. Mechanistically, activation of ADGRF5 with synthetic peptides that mimic the extracellular ectodomain of the receptor elicit Gnaq/11-coupled responses and actin cytoskeletal rearrangements in primary mouse and human alveolar type II (AT2) cells. The ability to pharmacologically manipulate the Adgrf5 pathway, both positively and negatively, would be a major therapeutic advance for patients with lung diseases associated with pulmonary surfactant disorders. The goal of this study was to define the molecular determinants of ADGRF5 that are essential for activation in vitro and in transgenic mouse models, with the long-term goal of designing small molecule modulators of Adgrf5 to treat pulmonary disease.

Methods: G protein-coupled assays (calcium transients and inositol phosphate (IP) conversion assays) were performed in primary AT2 cells and in HEK293 cells transiently expressing wild-type ADGRF5 or chimeric cDNAs of ADGRF5 that harbored alanine substitutions at sites predicted to be essential for receptor function. A synthetic peptide corresponding to the first 10 amino acids in the ectodomain of the C-terminal Fragment of Adgrf5 (termed GAP10) and a scrambled control peptide (SCR) were used in G protein-coupled activity assays in vitro and administered to WT and Adgrf5H991A mice to determine the impact of ADGRF5 activation on surfactant pool sizes in vivo.

Results: Alanine mutation analysis identified four key amino acids within the ectodomain and two in the second extracellular loop of ADGRF5 that are required for full activation. We also identified a conserved amino acid in the GAIN domain of ADGRF5 (H991) that is essential for proper cleavage of the receptor into N- and C-terminal fragments (NTF and CTF, respectively). The H991A chimeric mutant routed to the cell surface and elicited GAP10-induced IP responses and calcium transients comparable to WT ADGRF5, demonstrating that cleavage of the receptor is not essential for peptide-based activation in vitro. To test the hypothesis that cleavage of ADGRF5 is required for activation in vivo, we generated mice that express the H991A mutant from the endogenous ADGRF5 locus (Adgrf5H991A) via CRISPR/Cas9 gene editing. Analysis of 4-week old Adgrf5H991A/H991A mice revealed increased pulmonary surfactant and airspace enlargement, similar to levels observed in Adgrf5−/− mice. These data indicate that cleavage of Adgrf5 into NTF and CTF fragments is essential for receptor function in vivo. Activation of ADGRF5 via GAP10 administration to WT mice suppressed alveolar surfactant secretion and elicited calcium transients in primary AT2 cells from Adgrf5H991A knock-in mice.

Conclusion: Through the use of structure/function studies in vitro and in vivo, we have identified critical residues in ADGRF5 that are essential for activation and function of the receptor. While the endogenous ligand of ADGRF5 is unknown, our data support a model whereby binding of a ligand to the NTF results in separation of the NTF from the CTF, revealing a cryptic tethered peptide that binds the second extracellular loop of ADGRF5, resulting in activation and suppression of surfactant secretion from AT2 cells. Ongoing studies are focused on identification of the endogenous ligand and intracellular signaling events mediating ADGRF5 regulated exocytosis in AT2 cells.

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ROLE OF LATROPHILIN ADHESION GPCRS IN SYNAPTIC ASSEMBLY

Garret Anderson

Synapse assembly likely requires postsynaptic target recognition by incoming presynaptic afferents. Using newly generated conditional knockin and knockout mice, we here show that the cell-adhesion GPCR latrophilin-2 controls the formation of a specific subset of synapses in CA1-region hippocampal neurons, suggesting that latrophilin-2 acts as a synaptic target-recognition molecule. In CA1-region pyramidal neurons in vivo, latrophilin-2 was specifically targeted to post-synaptic sites at dendritic spines in the S. lacunosum-moleculare hippocampal sub-region. There latrophilin-2 functions to regulate synaptic assembly by matching with presynaptic entorhinal cortex afferents. Postsynaptic deletion of latrophilin-2 from CA1 pyramidal neurons selectively decreased spine numbers and impaired synaptic inputs from entorhinal but not from Schaffer-collateral afferents. Behaviorally, loss of latrophilin-2 from the CA1-region increased spatial memory retention, but decreased learning of sequential spatial memory tasks. Thus, latrophilin-2 appears to control synapse formation in the entorhinal cortex/CA1-region circuit by acting as a domain-specific postsynaptic target-recognition molecule.
GPR126 NTF REGULATES CARDIOMYOCYTE DEPOLARIZATION AND DELAMINATION IN ZEBRAFISH TRABECULATION

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Trabeculation is a crucial process during heart development in which cardiomyocytes protrude into the lumen of the ventricular chamber. Recently, we have suggested that the extracellular domain of Gpr126 (NTF) is required for this process in zebrafish. Yet, the underlying mechanism of how Gpr126 regulates trabeculation is unknown. Trabeculation involves an inter-play of different processes like cardiomyocyte selection, depolarization, delamination, and proliferation. Here, we aimed at verifying our initial results and to determine which cellular processes are regulated by Gpr126. For this purpose, we analyzed zebrafish mutants and performed gain of function experiments. The analysis of full length-depleted mutant gpr126stl47 and C-terminal fragment (CTF)-depleted mutant gpr126st49 (expressing NTF), revealed that gpr126stl47 maternal zygotic mutants exhibit hypotrabeculation, whereas the gpr126st49 mutants showed normal trabeculation. This data confirmed our previous hypothesis that NTF is sufficient for trabeculation during heart development. Ectopic expression of NTF by injecting mRNA encoding NTF in single cell-staged zebrafish embryos resulted in a multilayered ventricle at 72 hours post-fertilization (hpf), which was dependent on cardiac contraction and/or blood flow. Furthermore, it promoted depolarization and eventually delamination of cardiomyocytes. Recently, it has been shown that these two processes are regulated in zebrafish by Nrg2a. Therefore, we determined whether gpr126stl47 maternal zygotic mutants phenocopy nrg2a mutants. As both Nrg2a as well as NTF overexpression resulted in similar heart phenotypes but also both the gpr126stl47 maternal zygotic and nrg2a mutants exhibited defects in trabeculation, median fin fold morphogenesis and swim bladder inflation, we hypothesize that both proteins act in the same pathway to control trabeculation. Collectively, our study proves that the NTF of Gpr126 is sufficient for proper heart development and provides evidence that Gpr126 regulates depolarization and eventually delamination of cardiomyocytes possibly in a Nrg2a-dependent manner.

* Presenting author

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ADHESION GPCR: NOVEL TARGETS TO MODULATE GLUCOSE HOMEOSTASIS

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Adhesion GPCR (aGPCR) have many appreciated roles within the immune and central nervous system and in cell adhesion and development. However, an impact of aGPCR in metabolic processes remains largely unstudied, even though for several metabolic relevant tissues high expression of aGPCR has been shown. Own RNAseq analyses revealed expression of 13 aGPCR in murine pancreatic islets indicating physiological relevance of aGPCR in glucose homeostasis. Until now, only GPR56 has an assigned function in endocrine pancreas regulating insulin secretion¹. However, we also demonstrate high expression of other aGPCR, such as members of the Latrophilin family and GPR116, which partly is restricted to specific pancreatic cell types. Here, we demonstrate a specific function for GPR116 in pancreatic delta cells regulating somatostatin release. In primary pancreatic islets Stachel-peptide stimulation induces $G_{q/11}$-mediated $Ca^{2+}$ increase and somatostatin secretion under high-glucose conditions. This effect is lost in GPR116 knock-out mice verifying receptor specificity. Thus, GPR116 activation modulates secretion of other pancreatic hormones due to the paracrine function of somatostatin. Interestingly, while we observe no change in islet somatostatin content in knock-out mice, insulin content and beta-cell mass are significantly reduced leading to enhanced fasting glucose levels in knock-out mice. This highlights an important function of GPR116 in the tightly regulated glucose homeostasis. Since several aGPCR are expressed in pancreatic islets and other metabolic relevant and endocrine tissues, novel physiological functions of aGPCR in metabolic pathways need to be unraveled.


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NEUROVASCULAR DEVELOPMENT VIA GPR124/RECK-DEPENDENT WNT7/B-CATENIN SIGNALING

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Neurovascular biology is a relatively young field and an integrated model of how, when and to what extent neural and vascular development are coordinated is currently lacking. Building this model will greatly benefit from the combined capacity to (i) scrutinize the cellular modalities of the highly dynamic processes of neurovascular development in real time and to (ii) identify experimental settings where the signaling and circulatory functions of the blood vessels can, at least partially, be uncoupled. We previously validated the transparent zebrafish brain as being uniquely endowed with these distinctive attributes, through the generation of mutant larvae displaying fully avascular and yet normoxic brains throughout organogenesis. Taking advantage of the combination of high spatio-temporal resolution, best suited to comprehend the intrinsically dynamic processes of neurovascular development and function, and the absence of confounding hypoxic signaling cascades our laboratory recently revealed that neural-derived Wnt7 signals, important regulators of blood-brain barrier formation, control brain angiogenesis at the single cell level through specific modulation of endothelial tip cell function in perineural vessels at pre-angiogenic stages. In order to recognize and respond in an appropriate manner to the Wnt7 ligands, EC tip cells were shown to assemble a unique receptor complex composed of Gpr124 and its co-receptor Reck. The Gpr124/Reck complex enables brain endothelial cells to selectively respond to Wnt7. This selective Wnt recognition or "decoding" capacity is intriguing because Wnt/Frizzled interactions are largely incompatible with monospecific recognition. Reck was found to bind with low micromolar affinity to the intrinsically disordered linker region of Wnt7. Availability of Reck-bound Wnt7 for Frizzled signaling relies on the interaction between Gpr124 and Dishevelled. Surprisingly, this Gpr124 function does not rely on its GPCR structure. Through polymerization, Dishevelled recruits Gpr124 and the associated Reck-bound Wnt7 into dynamic Wnt/Frizzled/Lrp5/6 signalosomes, resulting in increased local concentrations of Wnt7 available for Frizzled signaling. Gpr124/Reck-dependent Wnt signaling provides insights into the Wnt decoding capacities of vertebrate cells, unravels structural determinants of the functional diversification of Wnt family members and constitutes a paradigm for organotypic angiogenic programs.

References:


KEYNOTE:

DISSECTING NEUROMODULATORY CIRCUITS AND SIGNALING IN AFFECTIVE BEHAVIOR

Michael Bruchas

Stress and affective behaviors are largely controlled by specific neurotransmitters and their receptors in the central nervous system. Many of these signals are conveyed through activation of both neuropeptide (i.e. CRF and Opioid) and monoamine (norepinephrine, dopamine, serotonin) receptor systems. These receptors are seven transmembrane spanning G-protein coupled receptors (GPCR) and they can stimulate a variety of signaling cascades following neurotransmitter/neuropeptide release. The Bruchas laboratory uses a multimodal effort to uncover GPCR-mediated neuromodulation from the receptor, signaling, circuits, and systems level analysis. Here I will describe two recent developments in the laboratory. Neurobiological studies of neuropeptides in motivation and technology development for dissecting neuromodulation in vivo. I will also briefly discuss recent advances in optogenetic technology including development of opto-GPCRs and implementation of wireless devices for in vivo behavioral measures. I focus on presenting unpublished data of a novel brain region subnuclei containing a relatively novel neuropeptide and its cognate GPCR in the peri-ventral tegmental area (dopamine system) that act to gate motivated behavior. We find that chemogenetic and optical control of this neuropeptide-GPCR system results in altered motivation, reward and aversion behavior. We also identify a critical corresponding VTA opioid GPCR system that mediates this neuropeptide’s effects on motivation. In sum, I will highlight some recent biological advances from our laboratory that dissect the role of GPCR-mediate neuromodulation in motivated behavior as well as feature some new technology development associated with these long term efforts.
HARNESSING THE AGPCR TETHERED-PEPTIDE-AGONIST MECHANISM FOR HIGH THROUGHPUT SCREENING OF CHEMICAL MODULATORS

Gregory Tall

The Tall lab investigates mechanisms of adhesion GPCR (AGPCR) activation. Our lab has helped advance the hypothesis that AGPCR orthosteric agonism is manifested by receptor fragment dissociation, which serves to liberate a concealed extracellular peptide emanating from transmembrane domain 1 (TM1). This peptide, termed a tethered-peptide-agonist, presumably binds in intramolecular fashion to a typical GPCR orthosteric agonist binding site to stabilize the active receptor conformation and facilitate G protein signaling. We currently have three research directions that will be discussed:

1. Biochemical and structural studies are ongoing to identify the AGPCR orthosteric binding site through the use of synthetic mimetics of the tethered-peptide-agonist.

2. Through receptor engineering of the AGPCR tethered-peptide-agonists, we developed sets of receptors with a spectrum of activity states from high to low. This permitted the development of high throughput screening (HTS) assays that led to the discovery of a small molecule GPR56/GPR114 partial agonist and antagonist (tool compounds).

3. We are investigating the physiological processes that mediate AGPCR fragment dissociation that lead to tethered-peptide-agonist engagement of the orthosteric sites.

Our current progress in each of these areas will be discussed including a new project in which we have used our pharmacological tool compounds to identify AGPCR activities in a previously unsuspected human tissue system.
Adhesion G protein-coupled receptors (aGPCRs) have essential functions in various biological processes. However, detailed knowledge on the function and biological roles of many aGPCRs such as the GPR110 is scarce. The high evolutionary conservation of \textit{Gpr110} between vertebrates suggests an essential physiological role for this aGPCR rendering the need for a better understanding of its function. Several studies report associations of \textit{Gpr110} variants with prostate, breast and liver cancer, blood pressure, body fat and lipid levels, thus also highlighting the pharmacological potential of this receptor.

To clarify the biological function of GPR110 we made use of a mouse strain knockout for \textit{Gpr110}. Expression analysis revealed a very distinct \textit{Gpr110} expression in collecting ducts of the renal papilla, which are involved in reabsorption of water and small solutes. In this tissue we showed that GPR110, which can couple to Gs and Gq proteins, is activated by a tethered peptide agonist derived from the \textit{Stachel}-sequence. Expression of \textit{Gpr110} in collecting ducts occurs in an early postnatal kidney stage along with the ability of the kidneys to concentrate urine. Consistently with this expression in the papilla, \textit{Gpr110}-deficient mice display a higher urine osmolality compared to the wild-type littermates. Moreover, induced hyperosmolarity leads to \textit{Gpr110} upregulation in cultured primary cells of the renal papilla. RNAseq data revealed several differently expressed genes between knockout and wildtype mice. Our results so far point towards an essential role of GPR110 in renal function, in particular in the urinary concentrating process, water balance or electrolyte homeostasis.

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RENAL EXPRESSION OF ADHESION GPCR GPR116 (ADGRF5) PLAYS A ROLE IN URINARY CONCENTRATION IN MICE

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G-protein coupled receptors (GPCR) are a large and diverse family of integral membrane proteins that recognize a tremendous assortment of extracellular molecules including neurotransmitters, hormones, light and odors. They are a common target of pharmaceutical drug development, and uncovering the function of novel GPCRs in the kidney represents a wealth of untapped therapeutic potential. We previously performed an mRNA screen for novel GPCRs in the kidney to identify promising yet overlooked renal GPCRs. This screen revealed that Gpr116, an adhesion-class GPCR, is highly expressed in the kidney. To understand the role of Gpr116 in renal physiology, we have taken a multidisciplinary approach. Using transfected HEK293 cells expressing cloned Gpr116, we have validated a monoclonal antibody for Gpr116. In murine kidney, this antibody indicates localization of Gpr116 to intercalated cells in the collecting duct of mouse kidneys. This staining is absent in kidneys from targeted knockout (KO) of Gpr116 in renal tubules (Gpr116floxflox, ksp-Cre). Additionally, kidney-specific KO animals have significantly reduced urine osmolality (1552±71 mOsm/kg, N=11) compared to wild-type (WT) (2097±76, N=10, p<0.0001) and heterozygous (2423±86, N=6, p<0.0001) littermates. Lastly, preliminary data of urine osmolality following 12-hour water restriction (dark-cycle) suggests KO animals are capable of concentrating urine, though only to about 75% that of WT littermates (3492±150 vs. 4361±525; N=3, N=4, respectively). Overall, these results suggest a role for Gpr116 in enhancing the ability to concentrate urine. This study establishes a physiologic role of the previously understudied Gpr116 in the murine kidney and demonstrates the scientific potential of future investigations into novel GPCRs.

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THE ADHESION GPCR VLGR1 IS A PART OF FOCAL ADHESION COMPLEXES, CELL MIGRATION AND MECHANOSENSATION

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Adhesion G protein-coupled receptors (aGPCRs) increasingly receive attention from a wide spectrum of biomedical fields, little is known about their specific cellular function. VLGR1 (very large G protein-coupled receptor-1) is by far the largest aGPCR. Mutations in VLGR1 cause the Usher syndrome (USH), the most common form of hereditary deaf-blindness and are also related to epilepsy. In affected neurons, sensory hair cells and photoreceptor cells VLGR1 has been mapped at adhesion complexes and synapses. Nevertheless, VLGR1 is almost ubiquitously expressed in the body. Here, we aimed to elucidate the role of VLGR1 in cellular adhesion, cell spreading, cell migration and mechanotransduction.

To decipher components of the cellular environment of VLGR1 we performed affinity proteomics. Bioinformatics on tandem affinity purifications data revealed 96 molecules associated with focal adhesion. Immunocytochemistry showed consistent localization of VLGR1 in nascent and mature focal adhesions of diverse cell types. siRNA knock-downs of VLGR1 revealed a decrease in the number and length of focal adhesions. In addition, depletion of VLGR1 caused the reduction of the cell surface area and lead to the declaration in cell spreading. In migrating cells, we observed the localization of VLGR1 in focal adhesions as well as at the dynamic membrane of the leading edges. Wound healing assays showed that VLGR1-deficient cells displayed a delay in closure of the cell layer indicating reduced cell motility. Furthermore, we provide evidence that these cell processes are regulated by VLGR1 via the modulation of the focal adhesion kinase (FAK) activity. Finally, shear stress paradigms indicate that VLGR1 senses physical mechanical signals.

In conclusion, VLGR1 is a vital receptor for the regulation of the dynamics of adhesion complexes. Defects in VLGR1 may cause dysregulation of adhesion complexes in sensory cells contributing to the pathophysiology of USH.

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OLIGODENDROCYTE GPR56/ADGRG1 INTEGRATES SIGNALS FROM MICROGLIA AND THE EXTRACELLULAR MATRIX TO REGULATE DEVELOPMENTAL MYELINATION AND MYELIN REPAIR

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In the central nervous system (CNS), myelin formation and repair are precisely regulated by oligodendrocyte (OL) lineage cells sensing signals from their environment, including microglia and extracellular matrix (ECM). However, the signaling pathways that coordinate this complex communications are poorly characterized. The adhesion G protein-coupled receptor, GPR56/ADGRG1, is an evolutionarily conserved regulator of OL development in humans, mice, and zebrafish, although its activating ligand in this cell type is unknown. Here, we report that microglia-derived transglutaminase-2 (TG2) signals to GPR56 on OL precursor cells (OPCs) in the presence of the extracellular matrix protein laminin and that TG2/laminin activation of GPR56 promotes OPC proliferation. Importantly, signaling by TG2/laminin to GPR56 on OPCs is also required for efficient remyelination in vitro and in vivo. These findings document a tripartite module that signals through an adhesion G protein-coupled receptor to promote myelin formation and repair, and suggest new strategies to enhance remyelination.
STACHEL-INDEPENDENT MODULATION OF GPR56/ADGRG1 SIGNALING BY SYNTHETIC LIGANDS DIRECTED TO ITS EXTRACELLULAR REGION

Gabriel S. Salzman, Shu Zhang, Ankit Gupta, Akiko Koided,, Shohei Koide, and Demet Araç, University of Chicago

Adhesion G protein-coupled receptors (aGPCRs) play critical roles in diverse biological processes, including neurodevelopment and cancer progression. aGPCRs are characterized by large and diverse extracellular regions (ECRs) that are autoproteolytically cleaved from their membrane-embedded signaling domains. Although ECRs regulate receptor function, it is not clear whether ECRs play a direct regulatory role in G-protein signaling or simply serve as a protective cap for the activating “Stachel” sequence. Here, we present a mechanistic analysis of ECR-mediated regulation of GPR56/ADGRG1, an aGPCR with two domains [pentraxin and laminin/neurexin/sex hormonebinding globulin-like (PLL) and G protein-coupled receptor autoproteolysis-inducing (GAIN)] in its ECR. We generated a panel of high-affinity monobodies directed to each of these domains, from which we identified activators and inhibitors of GPR56-mediated signaling. Surprisingly, these synthetic ligands modulated signaling of a GPR56 mutant defective in autoproteolysis and hence, in Stachel peptide exposure. These results provide compelling support for a ligand induced and ECR-mediated mechanism that regulates aGPCR signaling in a transient and reversible manner, which occurs in addition to the Stachel-mediated activation.

ORTHOGONAL MANIPULATION OF KETCHUP AND MAYO, TWO NOVEL ADHESION GPCR GENES IN DROSOPHILA

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Only two homologs associated with adhesion G protein-coupled receptor (aGPCR) subfamilies I (CIRL/Latrophilin; ADGRL) and IV (Flamingo/Starry night; ADGRC) have been described in Drosophila to date limiting the use of the fly as a model to investigate other adhesion GPCR layouts. We re-screened the Drosophila genome for the presence of aGPCR genes and uncovered two previously un-annotated bona fide aGPCR candidates, CG15556 and CG11318, which we renamed ketchup and mayo, respectively. Preliminary evolutionary comparisons with all human aGPCR homologs indicate that ketchup/CG15556 and mayo/CG11318 are related to subfamilies VIII and IX, and are highly homologous to each other. To investigate the function of the ketchup/mayo gene pair, we aimed to create novel fly models to study the function of these aGPCR in the fly. While both genes are genetically linked in a 25-kb region on chromosome III, standard Drosophila genomic engineering protocols currently allow for the replacement of only one locus per animal with an attP site for later phiC31-assisted transgenesis and structure-function analyses. Here we present a method to permit the manipulation and independent genomic engineering of linked loci through established phiC31 integrase resources. We show that two syntenic homologous genes can be genomically engineered to harbor orthogonal attP sites of different recognizability and specificity to the integrase. We show that these landing pads can independently receive transgenes through phiC31-assisted integration and facilitate the manipulation and analysis of either gene in the same animal. We have utilized this approach for the generation of ketchup and mayo single as well as double knock-out/knock-in strains including transcriptional reporters. These experiments show non-overlapping expression domains for ketchup in the excretory system and for mayo throughout the gastrointestinal canal and provide a basis for loss-of-function studies of the respective single and double null mutants.

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ADGRG6/GPR126: A NOVEL TARGET FOR PULMONARY ARTERIAL HYPERTENSION?

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Pulmonary arterial hypertension (PAH) is a chronic and fatal lung disease; its 3-year survival rate is 50-60\%, as current therapies have limited effect on mortality. A critical factor in PAH is increased proliferation of pulmonary arterial smooth muscle cells (PASMCs). We cultured patient-derived PASMCs and performed RNA-Seq. on PASMCs from control and PAH lungs. We found that the expression of ADGRG6 (adhesion GPCR G6/GPR126) is \sim 10\text{-}fold higher in PAH patient PASMCs compared to control PASMCs. To test its functional activity, we overexpressed ADRGR6 in control PASMCs and found proliferation of the cells decreased drastically 48 hours after transfection. Using qPCR, we observed that expression of cell cycle/proliferation markers CDK1, CDK2, and MKI67 decreased after 48 hours, while expression of apoptosis markers VIM, ITGAM, and PCDH7 was increased. Additionally, we found that cells overexpressing ADGRG6 have increased cAMP in comparison to mock-transfected cells. Other preliminary data using flow cytometry revealed that cells overexpressing ADGRG6 are stalled at the S/G2 cell cycle checkpoint. Together, these data suggest that ADRGRG6 activation, likely acting via cAMP, reduces proliferation in PASMCs and may be a novel therapeutic target for PAH.
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BAI1 EXPRESSION IN MACROPHAGES?

Cheng-Chih Hsiao, Jörg Hamann

We were intrigued and followed closely work by the Ravichandran, Casanova and Ernst labs that, starting with a Nature publication in 2007, described the adhesion GPCR BAI1 as ultimate phagocytic receptor, able to bind phosphatidylserine on apoptotic corpses and lipopolysaccharide on Gram-negative bacteria, both resulting in ELMO1/Dock180 recruitment and subsequent internalization of the corpses/microbes. Over the years, we noticed that genome-wide expression profiling efforts – from early microarrays to current RNA sequencing and mass spectrometry – consistently fail to identify BAI1 gene expression in monocytes and macrophages. Nevertheless, papers on the role of BAI1 in professional phagocytes continue to appear in top journals like, such as PNAS, Nat Commun and Sci Signal. We have evaluated >20 data sets on gene expression in monocytes, macrophages, and microglia and found no evidence that these cells express biologically meaningful amounts of BAI1. We included almost all entities, in which BAI1 expression has been reported before, yet, failed to confirm that findings. Furthermore, we want to challenge the idea that BAI1 is involved in the functioning of macrophages. Of note, we do not question the role of BAI1 as phagocytic receptor in non-professional phagocytes. We actually believe that the link between BAI1 and macrophage biology, first presented in Nature exactly 10 years ago, needs to be reassessed in light of the actual cellular distribution of BAI1.

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PROBING OF THE CONFORMATIONAL CHANGES OF GPR110 (ADGRF1) IN LIVING CELLS SUPPORTS AN ACTIVATION MECHANISM VIA THE GAIN DOMAIN-LIGAND INTERACTION

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The molecular mechanism for the activation of aGPCRs, particularly for the involvement of the ligand and extracellular domain, is still incompletely understood. We have recently deorphanized the adhesion GPR110 as a functional receptor for N-docosahexaenoylthanolamine (synaptamide), an endogenous metabolite of docosahexaenoic acid that is essential for proper brain development. The binding of synaptamide to GPR110 triggers the cAMP-dependent signal transduction, promoting neurite growth and synaptogenesis in developing neurons. To understand the molecular basis of the GPR110 activation, we probed in this study the ligand-induced conformational changes of GPR110 by in-cell chemical cross-linking and mass spectrometry. This approach allowed us to demonstrate for the first time that the 3-D structure of GPCR can be monitored in living cells. The molecular details revealed by quantitative analysis of cross-linked peptides combined with computer modeling and mutagenesis indicated that the in-cell conformational changes take place in the GAIN domain where the endogenous ligand binds to, and in the intracellular regions presumably involved in Gs protein binding. Our data strongly support a ligand-induced and GAIN-mediated mechanism that regulate aGPCR signal transduction, highlighting the potential utility of novel GAIN domain-targeted drugs for aGPCR-related human disease conditions.
GPR56/ADGRG1 ACTIVATION ENDCUES IL-6 PRODUCTION AND MORPHOLOGICAL CHANGE IN MELANOMA CELL

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GPR56/ADGRG1, a member of the adhesion G protein-coupled receptor family, is involved in tumorigenesis. The role of GPR56 in melanoma development is unresolved. Here, we show that GPR56 receptor ligation by immobilized CG4 mAb in melanoma cell enhanced the production of IL-6, which promoted cell migration and invasion. CG4 also inhibited cell spreading of melanoma cells. The IL-6 level was decreased in CG4-ligated melanoma cells expressing the dominant negative RhoA N19, or treated with exoenzyme C3, and the ROCK inhibitors (Y27632 and H1152). Inhibited cell spreading was recovered while CG4-ligated melanoma cells were treated with ROCK inhibitors. We conclude that GPR56-induced IL-6 production and cell morphology change in melanoma cells is mediated through the RhoA-ROCK signaling pathway. Our results indicate a critical role of GPR56 in melanoma progression.
SYNAPTAMIDE, AN ENDOGENOUS LIGAND OF GPR110, ENHANCES AXON REGENERATION AND VISUAL ACTIVITY AFTER OPTIC NERVE INJURY

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The central nervous system is resistant to regeneration, and therapeutic approaches for repairing damaged axons have been challenging. Previously, we demonstrated that N-docosahexaenoylethanolamine (synaptamide), an endogenous ligand to GPR110 transmitting cAMP/PKA signaling, potently promotes neurite outgrowth and synaptogenesis in developing neurons. Here, we demonstrate that synaptamide/GPR110-mediated cAMP/PKA activation enables robust optic nerve regeneration in adult mice. In vitro, synaptamide significantly increased the neurite extension in axotomized cortical neurons and retinal tissue cultures derived from wild type but not from gpr110 knockout mice. In a mouse model of eye injury caused by optic nerve crush (ONC), intravitreal injection of synaptamide immediately following injury stimulates retinal ganglion cells (RGCs) axon regeneration in a GPR110-dependent manner, leading to improved visual function. Furthermore, optic nerve injury induced gpr110 expression in RGCs of adult retina within 30 min, suggesting that gpr110 is a rapid injury-response gene mediating synaptamide-induced axon repair. Our findings demonstrate a novel strategy for axon regeneration that employs a mechanism for developmental neurite outgrowth, namely synaptamide/GPR110-mediated cAMP/PKA signaling. We propose that synaptamide may have therapeutic potential for central nervous system (CNS) injury, offering a new possibility for clinical translation.
STRUCTURE OF THE CIRCUIT-WIRING PROTEIN TENEURIN UNCOVERS HOMOLOGY TO BACTERIAL TOXINS

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Teneurins (TENs) are cell-surface adhesion proteins with critical roles in tissue development and axon guidance. Here we report the 3.1-Å electron cryo-microscopy structure of the human TEN2 extracellular region (ECR), revealing a striking similarity to bacterial Tc-toxins. The ECR includes a large β-barrel that partially encapsulates a C-terminal domain, which emerges to the solvent through an opening in the mid-barrel region. An immunoglobulin (Ig)-like domain seals the bottom of the barrel while a β-propeller is attached in a perpendicular orientation. We further show that an alternatively spliced region within the β-propeller acts as a switch to regulate trans-cellular adhesion of TEN2 to latrophilin (LPHN), a transmembrane receptor known to mediate critical functions in the central nervous system. One splice variant activates trans-cellular signaling in a LPHN-dependent manner, whereas the other induces inhibitory postsynaptic differentiation. These results highlight the unique structural organization of TENs giving rise to their multifarious functions.

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LIGATION OF EMR2/ADGRE2 INDUCES INFLAMMASOME ACTIVATION IN THP-1 CELLS VIA THE Gα16/MAPK/NF-κB SIGNALING PATHWAYS

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The assembly and activation of inflammasomes represent one of the important innate immune responses to many pathogenic infections. Inflammasome activation in professional innate immune cells usually requires two distinct steps, namely priming and activation, but the mechanisms involved remain incompletely characterized. EMR2/ADGRE2 is an adhesion G protein-coupled receptor (aGPCR) expressed predominantly in human myeloid cells and is known to involve in the migration and activation of neutrophils and macrophages. However, the role of EMR2 receptor in inflammasome activation has not been investigated. In this study, we examine the expression level and activation status of inflammatory cytokines, IL-1β and IL-18, as well as critical inflammasome proteins such as caspase-1, ASC, and NLRP3 following the ligation and activation of EMR2 receptor in THP-1 cells. Our results show that IL-1β and IL-18 are activated and secreted after EMR2 is cross-linked and activated by its specific 2A1 mAb. EMR2 activation induces the assembly and activation of NLRP3 inflammasome. We further show that EMR2 mediate the inflammasome activation specifically via the Gα16/MAPK/NFκB signaling pathways. Taken together, we conclude that EMR2 plays an important role in the inflammasome activation of human myeloid cells.
LATROPHILIN-1/3 IS EXPRESSED AND FUNCTIONAL IN THE NEURONAL SUBPOPULATION OF THE PAIN PATHWAY IN RATS AND DROSOPHILA

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Latrophilin (LPHN) is expressed in mechanosensitive neurons of Drosophila, sharpening the response to innocuous mechanical stimuli. In mammals, transcriptome analysis showed expression of LPHN-1 and -3 in the dorsal root ganglion (DRG). The function in nociceptive neurons in Drosophila, the precise distribution of LPHNs in vertebrates’ peripheral nervous system and its regulation in neuropathy are unknown.

The role of Drosophila LPHN in nociceptors was evaluated by combining behavioural analyses with a chemical neuropathy model and optogenetic manipulation of cyclic AMP levels in vivo. In rats inflicted with a chronic constriction injury (CCI) the expression of LPHN-1 and -3 mRNA was quantified by qPCR followed by in-situ hybridization. Further, the distribution in cellular subpopulations of the DRG identified by immunohistochemistry was evaluated and quantified.

In Drosophila, LPHN functions as a down regulator of nocifensive behaviour by modulating nociceptive neurons under physiological conditions and in a neuropathic model. CCI in rats elicits mechanical and thermal hypersensitivity. LPHN-1 and -3 mRNA are expressed in the DRG and the peripheral nerve. Whole nerve and DRG qPCR revealed upregulation of LPHN-1 and -3 mRNA in the sciatic nerve. In the DRG LPHN-1 and -3 mRNA are expressed in neurofilament-200⁺ thick, myelinated sensory neurons, isolectin B4⁺ non-peptidergic nociceptors and calcitonin gene related peptide⁺ (CGRP⁺) peptidergic nociceptors. LPHN-3 is mainly present in the glutamine synthetase⁺ satellite glial cells. Expression of LPHN-1 is highest in CGRP⁺ cells. LPHN expression in subpopulations is altered 7 days after CCI.

In summary, mechanosensory and nociceptive signal modulation by LPHN differs between neuronal subpopulation in Drosophila. In rats, LPHN-1 and LPHN-3 are expressed in nociceptive neurons and are modulated by neuropathy. The function of LPHN-1 and -3 in pain further needs to be evaluated in mammals and human patients, deciphering the clinical relevance of these receptors in chronic neuropathic pain.

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G PROTEIN SIGNALING MEDIATED BY ADGRL3

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Adhesion GPCRs (aGPCRs) form the second largest, yet most enigmatic class of the GPCR superfamily, with the majority of receptors still orphan with respect to their agonists and signaling properties. A number of aGPCRs (of a total of 33) have been shown to bind and, in some cases, signal through G proteins. In this study we focus on characterizing the interaction partners and signaling pathways of latrophilin 3 (ADGRL3, historically LPHN3). We show that mouse ADGRL3 constitutively enhances cAMP accumulation, and that ADGRL3-mediated constitutive enhancement of cAMP is increased substantially when the entire N-terminal fragment (up to the GPS cleavage site) is removed. Signaling is suppressed in this constitutively active construct by removing the first 4 residues after the GPS, or by mutating conserved key amino acids in the peptide stretch immediately following the GPS.

We have used this set of receptor constructs in combination with a panel of gene expression signaling assays and CRISPR knockout cell lines that lack the main G protein families Gαs, Gαq/Gα11, Gα12/Gα13 and Gαz. This has allowed us to systematically evaluate the signaling properties of ADGRL3 in a screen of the major G protein signaling pathways by reintroducing one Gα protein species at a time. We find that full length ADGRL3 mediates a receptor concentration dependent baseline cAMP activity whereas the constitutively active truncated receptor construct signals promiscuously with activity in several G protein pathways, including most prominently Gαq and Gα13. We are currently investigating the hypothesis that upon activation the receptor undergoes switching between G protein signaling pathways.

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DEFINING THE FUNCTIONAL ROLES OF ADHESION G PROTEIN-COUPLED RECEPTORS (AGPCRS) IN MYELIN FORMATION AND HOMEOSTASIS USING REVERSE GENETIC AND CHEMICAL SCREENS IN ZEBRAFISH

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Myelin is the lipid-rich, multilayered membrane formed by specialized glial cells in the vertebrate nervous system. In the central nervous system (CNS), myelin is formed by oligodendrocytes (OLs), while in the peripheral nervous system (PNS), myelin is formed by Schwann cells (SCs). During development, myelin functions as an essential insulator for rapid action potential propagation along axons and provides critical support in axonal transport and long-term survival. The loss of myelination (or demyelination) can lead to permanent neuronal damage and is a hallmark of many disorders such as multiple sclerosis (MS) and peripheral neuropathies. The inability to remyelinate damaged axon tracts has prevented the proper treatment of myelin diseases, which is due partly to a poor understanding of the genetic and molecular mechanisms that control myelination.

Zebrafish have emerged as a premiere vertebrate species to study the mechanisms of OL and SC development. A previous forward genetic screen in zebrafish identified the then orphan adhesion G protein-coupled receptor (aGPCR) Gpr126 as essential for SC development and myelination. The function of Gpr126 is conserved in humans, highlighting the utility of the zebrafish model to study myelination. Although Gpr126 is essential for SC development and myelination, it has no known function in OLs. Previously published data sets indicated that Gpr56, also of the aGPCR family, is highly expressed in OL precursor cells (OPCs) in the developing mouse CNS. Complementary studies using both zebrafish and mouse Gpr56 mutants demonstrated that Gpr56 is, in fact, an evolutionary conserved regulator of OL development. Recent studies have also shown that additional aGPCRs are expressed in the vertebrate nervous system. Given the essential roles of Gpr126 and Gpr56 in myelination, we hypothesize that other aGPCRs play important roles in both OL and SC development and myelination.

We are defining the functional roles of aGPCRs in myelin formation and homeostasis through reverse genetic screen in zebrafish using clustered regularly interspaced palindromic repeat (CRISPR)/Cas9 technology by knocking out additional aGPCR family members to provide a rapid assessment of the functional roles of aGPCRs in OL and SC myelin formation and homeostasis. We are also performing large-scale chemical library screens in zebrafish to identify compounds that modulate myelination in aGPCR mutants. These screens will delineate pathways downstream of and in parallel to aGPCRs of interest to provide both insight into aGPCR function and potential leads for therapeutics for myelin disorders.
MECHANISM OF MAST CELL ACTIVATION IN PATIENTS WITH VIBRATORY URTICARIA

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Upon dermal vibration, patients with vibratory urticaria (VU) experience localized hives and increased histamine levels in serum. These responses are caused by hyperreactivity of skin mast cells to a mechanical stimulus. We found that patients with a familial form of VU harbor a missense substitution in an adhesion G-protein coupled receptor (aGPCR), ADGRE2, that renders mast cells more susceptible to vibration-induced degranulation. However, the mechanisms of activation of ADGRE2 and how this mutation enhances the ability of mast cells to respond to a mechanical stimulus are not understood. In the present study, we used wild type ADGRE2 and mutated ADGRE2 (C492Y) constructs to express the receptor in a mast cell line, LAD2 and confirmed that ADGRE2 (C492Y)-expressing cells have increased degranulation compared to WT-ADGRE2 expressing cells when attached onto dermatan sulfate (DS)-coated surfaces and subjected to vibration. We show that under these vibratory conditions, ADGRE2 (C492Y)-expressing cells had stronger calcium responses and activation of the PI3K and ERK signaling pathways. We further define the inter-relation of these signaling pathways and demonstrate that calcium and signals derived from PI3K but not ERK pathways are required for the degranulation of mast cells with this variant. Our studies provide insights into previously unexplored signaling pathways induced by ADGRE2 in response to a physical stimulus and identify possible therapeutic targets for patients with VU.

This work was supported by the Division of Intramural Research of the National Institute of Allergy and Infectious Diseases at the National Institutes of Health.
DEVELOPMENT OF A KO MODEL AND IDENTIFICATION OF SMALL MOLECULE SYNTHETIC INVERSE AGONISTS FOR ADGRG4 (GPR112) SELECTIVELY EXPRESSED IN ENTEROENDOCRINE CELLS

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ADGRG4 is the second largest member of the aGPCR family and like most other aGPCRs, the receptor is orphan. The receptor has a very large potentially O-glycosylated mucin-like ‘stalk’ segment connecting an N-terminal plexin-like domain with its GAIN domain.

Through in situ hybridization combined with immunohistochemistry and qPCR analysis of FACS-purified enteroendocrine cells (EECs) we find ADGRG4 highly expressed and one of the highest enriched GPCR in EECs, i.e. CCK, GIP, PYY, GLP-1, somatostatin and 5-HT cells of the small intestine.

ADGRG4-deficient mice displayed few phenotypic traits and had e.g. normal number of EECs. However, fasting/re-feeding experiments revealed significantly reduced GIP secretion and so was the amount of 5-HT in biopsies of the small intestine. As was chromogranin A and secretin mRNA levels. Importantly, at the systemic level the ADGRG4-deficient mice had a significant lower fat percentage, compared to wildtype littermates.

In transfected cells, ADGRG4 signals through several G-proteins, with constitutive activity through Gaₛ. Upon N-terminal fraction removal, signalling increases 8 to 11-fold, through Gaₛ, Ga₁₂/₁₃ and Ga₁₁ in a stalk-dependent manner, which was corroborated by mutational analysis of the stalk and rescue by use of synthetic stalk peptides.

A suitable in vitro signalling assay was established to screen the chemical libraries of European Lead Factory, which resulted in the identification of two chemical series of highly efficient ADGRG4 small molecule synthetic inverse agonists. SAR analyses indicated that these chemical series could potentially be developed into pharmacological tools and perhaps lead compounds for drug development.

ADGRG4 structure-functionally is a classical aGPCR which is selectively expressed in EECs of the proximal small intestine. Initial characterization indicates its importance in the function of EECs and indirectly for metabolic function of adipose tissue. Importantly, synthetic small molecule ligands have been identified which together with ADGRG4-deficient mice can be used to identify physiological roles and pharmacological potential of ADGRG4.
ADHESION GPCR56 AND 114 PEPTIDE AGONIST INTRAMOLECULAR BINDING SITE IDENTIFICATION BY PHOTO AFFINITY LABELING AND MASS SPECTROMETRY

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The adhesion GPCRs GPR56 and GPR114 belong to a receptor sub-class distinguished by the presence of an extracellular GPCR auto proteolysis inducing (GAIN) domain that is sufficient to self-cleave the receptor into two fragments. AGPCR fragment dissociation reveals a hidden peptide that serves as a tethered-peptide-agonist that possibly binds intracellularly to the GPCR fragment to initiate G protein signaling. To better understand how agonist peptides interact with their cognate receptors, we developed a photo-crosslinking approach to map the interaction between P7 (TYFAVLM) and P18 (TYFAVLMQLSPALVPAEL) agonist peptides to target receptors GPR56 and GPR114. Analogue peptides containing the unnatural amino acids BzP (4-Benzoyl-L-Phenylalanine) or AzP (4-Azido-L-Phenylalanine) were synthesized to promote a UV-light-dependent crosslink to the receptors when in close proximity. Similarly to the natural tethered-peptide agonists, the synthetic analogue probes were able to activate G proteins as determined by $[^{35}S]$ GTPγS binding assay using GPR56 and GPR114 membranes reconstituted with G13 and Gs, respectively. First, to optimize conditions for crosslinking and detection of the peptide-receptor complexes, we used the synthetic agonist peptides conjugated to biotin (P7(AzP or BzP)-biotin and P18(AzP or BzP)-biotin. With these biotinylated probes we demonstrated UV-dependent probe crosslinking to membranes prepared from cells that expressed the receptor. An immunoblot analysis of the samples showed UV-dependent bands when probed with fluorescently-tagged streptavidin conjugates at the molecular mass of the receptor. This indicates the existence of a covalent complex between P7 (AzP or BzP)-biotin and GPR56, and between P18(AzP or BzP)-biotin and GPR114. No evidence was attained using membranes that do not contain receptor. Here, we show that this methodology can be used to probe the binding interface between receptor and synthetics agonist peptide. The application of our findings should prove useful for defining adhesion GPCR orthosteric binding sites and to aid the pharmacological design of small molecule inhibitors and activators.

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THE ROLE OF ADHESION GPCRS IN THE REGULATION OF ENERGY HOMEOSTASIS IN THE MODEL ORGANISM C. ELEGANS

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G protein-coupled receptors (GPCRs) are central molecules in the regulation of energy homeostasis and thus, represent prime targets for therapeutical intervention of conditions related to the consequences of a misbalanced energy homeostasis such as obesity or diabetes. Several recent studies have provided evidence for the involvement of Adhesion GPCRs in different aspects of energy homeostasis. However, their underlying mechanisms are insufficiently understood precluding an indepth evaluation of their impact on metabolic regulation. Compared to the 33 members in mammals, the genome of the nematode Caenorhabditis elegans harbours three aGPCRs, the Latrophilins lat-1 and lat-2 and the Flamingo/CELSR homologue fmi-1 facilitating analyses on the role of these receptors in metabolic processes and rendering C. elegans an ideal model in this context.

This study aims at characterising the role of aGPCRs in metabolism of C. elegans. Our data show that a lat-1 as well as a fmi-1 null mutant display a prolonged lifespan compared to wild-type individuals. One possible reason for this effect could be caloric restriction, as it is described in mammalian systems. For lat-1 we were able to demonstrate this correlation, lat-1-deficient nematodes show a reduced bacteria ingestion, suggesting that the receptor plays a role in food intake. Interestingly, the cause for increased lifespan in fmi-1 mutants is not caloric restriction but alterations in metabolism indicated by smaller body size, elevated fat levels and resistance to thermal stress. Analyses of the mechanisms underlying FMI-1 function have revealed several candidate pathways such as the insuline-like signalling pathway.

Taken together, our data indicate that aGPCRs fulfil different functions in the regulation of energy homeostasis by controlling food intake and metabolic regulation.
NEAR ATOMIC RESOLUTION CRYO-EM STUDIES OF THE HUMAN CANNABINOID RECEPTOR

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Our understanding of GPCR mechanisms of action has been greatly enhanced by the outstanding progress in structure determination of these receptors, with several hundreds of high-resolution GPCR structures determined by X-ray crystallography. Notwithstanding, the difficulty of obtaining crystals remained the limiting factor in determining the structures of many GPCR family members, where capturing snapshots of these receptors along with their downstream signaling partners further increases the challenges to this technique. Advances in single particle electron cryo-microscopy (cryo-EM) now make it possible to capture protein complexes at great details, thus avoiding the bottlenecks of crystallization. Here we report the active 3-Å cryo-EM structure of the human cannabinoid receptor 1 (CB₁R) bound to a highly potent agonist, and a trimeric Gi-protein complex. The human cannabinoid receptors CB₁R and CB₂R mediate the function of the endogenous lipid messengers anandamide and 2-Arachidonoylglycerol (2-AG) as well as of the widely used phytocannabinoid Δ⁹-tetrahydrocannabinol (THC). Modulation of the cannabinoid receptor activity has been associated with various physiological effects ranging from analgesia to control of disorders such as epilepsy, obesity and inflammation. As such cannabinoid receptors have been the target of intensive drug discovery efforts. The structure provides a high-resolution glance into the ligand binding pocket and supplements structural insights into G protein coupling promiscuity and preference of CB₁R. Our results highlight the promise of using cryo-EM as an alternative approach for studying GPCRs and provide a framework for elucidating CB₁R activation mechanisms that could in turn be used for the design of future therapeutics.
Mumps virus (MV) is a re-emerging pathogen that causes painful swelling of parotid salivary glands and associated with meningitis, encephalitis, orchitis and pancreatitis. It is highly neurotropic with evidence of brain infection in half of the cases. Current vaccine programs have not managed to eliminate mumps and infections also occur in vaccinated individuals, indicating a need for better treatments. MV encodes a small hydrophobic (SH) transmembrane protein of unknown function. We identified an interaction of the SH-protein with the human adhesion GPR125, performing yeast-two-hybrid-, co-immunoprecipitation- and label-free whole-cell impedance-based experiments.

Developing antibodies targeting different extracellular domains of GPR125 we detected GPR125 expression in the choroid plexus (blood-cerebrospinal fluid [CSF] barrier) and Sertoli cells (blood-testis barrier). Both barriers could be important for MV entry into the brain and testis, and we hypothesize that the interaction of the SH-protein with GPR125 interferes with the potential barrier function of the receptor, thereby leading to uncontrolled virus or immune cell entry.

We determined that TAMRA-labeled SH-protein binds specifically to mouse choroid plexus and testis and that the SH-protein is expressed on the surface of MV-infected CD14+ monocytes.

In future in vivo studies using genetic zebrafish- and mouse models we will estimate if the potential function of GPR125 in keeping the blood-CSF and blood-testis barrier tight is disturbed by the interaction with the SH-protein encoded by MV.

Our results suggest that MV exploit GPR125 to promote virus entry into organs and that this is a conserved mechanism of RNA viruses to enter organs by interaction with adhesion GPR125.

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BIOCHEMICAL ANALYSIS OF THE SYNAPTIC REGULATOR C1QL3 AND ITS BINDING PARTNERS

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Synapses are specialized neuronal cell-cell junctions, which allow for the formation of neural networks by promoting fast, efficient, and stable communication between neurons. C1QL3 is a secreted protein that is expressed in a small subset of brain regions, mostly in the limbic system in the adult brain. C1QL3 is a known high affinity ligand of ADGRB3, an adhesion-class GPCR that is located post-synaptically when expressed in neurons in the central nervous system. It has been shown that C1QL3 regulates synapse formation and/or maintenance by in vitro experiments, global and conditional C1QL3 knockout mice. This knockout also results in abnormalities in fear memory likely due to the reduction in excitatory synapse density. The mechanism by which C1QL3 influences synapse density is currently unknown but could involve a trans-synaptic adhesion complex between C1QL3, ADGRB3, and an identified, but yet-to-be-validated, presynaptic binding partner. Through a series of biochemical experiments, we hope to map the C1QL3 binding surfaces with its known binding partners and study the functional relevance of these interactions in vivo. In addition, we hope to determine binding constants for each C1QL3 binding partner. This may provide insights about how C1QL3 and its interaction with ADGRB3 influence the formation and/or maintenance of synapses.

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ADGRG6 (GPR126) IS INVOLVED IN DIFFERENTIATION AND FUNCTION OF MOUSE 3T3-L1 ADIPOCYTES

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Genomic variants of Adgrg6 have been correlated with obesity associated traits, namely lipid levels, body mass index, low density lipoprotein particle size, and blood glucose level [1, 2]. Adgrg6 is a member of the aGPCR subfamily VIII with Collagen IV and Laminin 211 as identified binding partners [3, 4]. Here, we investigate the role of Adgrg6 in mouse adipocytes using the pre-adipocyte 3T3-L1 cell line. We observed significant, 4.5-fold decrease in expression of Adgrg6 during early differentiation into adipocytes. Similarly, this decrease of Adgrg6 expression was observed in primary cells by comparing stromal-vascular fraction and matured adipocytes. To examine the importance of Adgrg6, we transiently knocked-down the Adgrg6 expression in 3T3-L1 cells. Depletion of Adgrg6 transcripts leads to lower triacylglycerol accumulation and generally smaller size of lipid droplets in the course of differentiation of 3T3-L1 pre-adipocytes into the adipocytes. Yet, MALDI-TOF analysis did not detect changes in the triacylglycerol composition, indicating that Adgrg6 knock-down has an effect on quantity rather than quality of stored lipids. Further, expression analysis revealed that adipogenic factors such as PPARγ and mSREBP1 are significantly lowered in Adgrg6 transiently knock-down 3T3-L1 cells. Additionally, depletion of Adgrg6 transcripts prevented release of free glycerol during lipolysis in cells treated with Adgrg6 agonist or Isoprenaline. Based on these experimental results, we hypothesize that Adgrg6 plays a significant role in adipocyte differentiation, maturation and function.


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STUDYING THE ROLE OF GPR126 SIGNALING DURING HEART DEVELOPMENT

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Trabeculae formation and remodelling ensure the configuration of a thick ventricular wall sustaining every heartbeat throughout life. Notch signaling is a key regulator of both processes through a temporal sequence of ligand signaling. Gene profile analysis of Notch mutant hearts identified the adhesion G protein-coupled receptor 126 (Gpr126) as a candidate molecule guiding trabeculation downstream of Notch. Previous studies demonstrated that Gpr126 is required for cardiac trabeculation in mice and zebrafish; however, its downstream signalling mechanism is not known. Here, we generated various standard and conditional knockout mouse models using the CRISPR/Cas9 technology. Targeted in-frame deletion of exons 3 and 4, encoding the adhesive domains of the extracellular N-terminal fragment (NTF), does not cause any obvious cardiovascular defect, but impairs peripheral nervous system (PNS) development. In contrast, standard or conditional deletion of exon 6 produces a premature stop codon that results in the near absence of \textit{Gpr126} mRNA and fully penetrant embryonic lethality. Mutant embryos die between E11.5 and E13.5 and exhibit severe cardiac defects, including thinning of the ventricular wall, septal defects and blood accumulation in the chambers. Expression of various chamber-patterning and metabolic markers appears unaffected in Gpr126 standard mutants, thus we are currently deepening their characterization in terms of cellular proliferation and gene profiling analysis. Ultimately, endocardial-specific conditional ablation of Gpr126 and in vivo rescue experiments will allow us to test whether the phenotype observed is due to the loss of endocardial Gpr126. Thus, we have developed new mouse lines as useful tools to disclose not only the precise role of Gpr126 in heart development, but also its potential interaction with the Notch pathway.

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THE ADHESION G PROTEIN-COUPLED RECEPTOR GPR133/ADGRD1 IS INVOLVED IN SMOOTH MUSCLE FUNCTION OF MICE

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Genome wide association studies (GWAS) indicated a link between single nucleotide polymorphisms at the GPR133/ADGRD1 locus and changes in heart frequency, human body height and body weight in mice [1-3]. Published and own transcript analyses showed expression of GPR133 particularly in smooth muscle, skeletal muscle, adipose tissue, bone, and the reproductive system. We performed broad phenotyping of GPR133 knock-out (KO) mice and found increased heart frequency in KO animals compared with wild type siblings, while there was no effect on blood pressure under basal conditions. Altered heart function was confirmed using echocardiography and a significant reduction of the endurance level was found when KO mice were forced to run on a treadmill. We also observed significantly reduced smooth muscle contractility of the urinary bladder and the gastrointestinal system. Considering the lack of expression of GPR133 in cardiomyocytes, we concluded that the observed changes in heart function is likely be secondary to a reduced contractility of smooth muscle cells in the vascular system. Our data indicate an important role of GPR133 in smooth muscle contractility and may point towards a mechanofunction also of this adhesion GPCR.


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StaR® ENGINEERING: STABILISATION OF ADHESION GPCRs TO AID DEVELOPMENT OF NOVEL THERAPEUTICS

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G-protein-coupled receptors (GPCRs) represent one of the largest and most important classes of membrane proteins for drug discovery. However, due to their inherent flexibility and instability outside of the membrane, they are challenging targets for structural and biophysical studies. Here we demonstrate how these challenges have been overcome for a wide range of GPCRs through protein engineering, by identifying a minimal number of thermostabilising mutations that can lock a receptor in a single conformation and enhance its survival in a detergent environment. This technique is now being successfully applied to a member of the adhesion GPCR family, ADGRD1 (GPR133), a receptor that has been shown to have a key role in glioblastoma. The addition of five mutations has increased the thermostability of ADGRD1 by more than 10 °C. Following the addition of further stability, this receptor will be taken forward for structure determination by X-Ray crystallography and cryo-electron microscopy.
FUNCTIONAL DOMAINS OF THE STACHEL SEQUENCE WITHIN ADHESION GPCRS

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Several studies about the activation of adhesion G-Protein-coupled receptors (aGPCRs) reported that they are activated through a tethered peptide agonist, coined the Stachel sequence [1, 2, 3, 4]. The Stachel sequence is the most C-terminal part of the highly conserved GPCR autoproteolysis-inducing domain within the N-terminus [1]. Analysis of several representatives of aGPCRs regarding their activation mechanism revealed a highly conserved agonistic core sequence within the N-terminal part of the Stachel sequence [1, 3], which is essential for activation and which allows for agonist promiscuity of various Stachel sequence-derived peptides. In-depth mutational analysis of the C-terminal part of the Stachel peptide revealed biased peptide agonists. We suspect that the C-terminal part of the Stachel sequence represents a positioning region being responsible for the proper structural conformation of the agonistic core region to the putative binding pocket, which might allow for different positioning of the peptide agonist with subsequent differences in the downstream signaling pathways. Similarly, functional analysis of the full length GPR114/ADGRG5 supported the importance of the highly conserved core sequence of Stachel for activation, which is independent from cleavage yet sensitive to mechanical force. The role of the C-terminal positioning region is for GPR114 established through the tissue specific expression of two isoforms, which structurally vary only in the existence of one amino acid within this portion of the Stachel, while displaying vast differences in basal receptor activity and response to mechanical force [3].

We believe that our findings will help to elucidate the still enigmatic mechanisms behind the activation of aGPCRs and will be help to establish more specific and biased agonistic peptides.


Source of funding:
RU2149 project 5.
Latrophilin-3 (ADGRL3) is an adhesion G protein coupled receptor (AGPCR) enriched in synaptic membranes. Latrophilins (1-3) are important for development of synapses and maintenance of synaptic integrity. The mechanism(s) by which Latrophilins become activated and the downstream G protein pathways that they may activate are unclear. Latrophilins are typical AGPCRs that are self-cleaved at the conserved GPCR-autoproteolysis-inducing (GAIN) domain site ~20 residues N-terminal to the start of the first transmembrane span (TM1). We hypothesize that one mechanism of Latrophilin activation is via a tethered-peptide-agonist unmasking process that requires dissociation of its N-terminal and C-terminal fragments. To test our hypothesis, we reconstituted Latrophilin-3-enriched membranes with representative members of all four heterotrimeric G protein subfamilies (Gq, G13, Gi, Gs) and measured the kinetics of receptor-stimulated G protein GTPγS binding. Latrophilin-3 is shown to robustly couple to G13, exhibited appreciable Gi and Gq coupling, but did not activate Gs. Dramatic enhancement of G protein activation was observed when the Latrophilin-3 NTF was dissociated, indicating that the release of the tethered-peptide-agonist from the GAIN domain hydrophobic core was critical for receptor activation. We then serially truncated single, N-terminal residues of the Latrophilin-3 CTF tethered-peptide-agonist and tested the engineered receptors for activation of the G13-dependent Serum Response Element (SRE)-luciferase gene reporter. The first three N-terminal Latrophilin-3 CTF residues were critical for signaling. A small peptide library comprising the Latrophilin-3 tethered-peptide-agonist was synthesized and tested for the ability to activate Latrophilin-3. Latrophilin-3 synthetic peptides comprising sequences N'-TFAVLM(Xn), but not GPR56/114 derived peptides comprising sequences N'-TYFAVLM(Xn) activated Latrophilin-3. Our work demonstrates that Latrophilin-3 is activated via a tethered-peptide-agonist mechanism, which may be a general paradigm for AGPCR activation. Ongoing work is examining our new finding that Latrophilin-3 is predominantly coupled to G13. This may provide important new insight into the role Latrophilins have in shaping synaptic architecture.

Funding support: GM120110 (NIH).
IMAGING GPCR SIGNALING IN SPACE AND TIME

Haining Zhong1, Lei Ma1, Bart C. Jongbloets1, Wei-Hong Xiong1, Joshua B. Melander1,3, Maozhen Qin1, Tess J. Lameyer1, Madeleine F. Harrison2, Boris V. Zemelman2, and Tianyi Mao1

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Regulations via GPCRs impose powerful control over the brain function, from neuronal development and plasticity to animal behavior. My lab studies the subcellular signaling events underlying GPCRs, in particular the cAMP/PKA signaling pathway downstream of both Gs and Gi proteins. In order to investigate PKA signaling in real time, we recently set out to establish the capacity of PKA imaging in vivo. By screening for best available PKA sensors, further developing the best sensor, and integrating with the right imaging modality, we can now carry out longitudinal in vivo imaging of PKA activity in behaving mice. I will present our ongoing progress of imaging and analyzing the population properties of neuromodulatory PKA signaling occurring in the mouse cortex and in deep brain structures in response to distinct brain states and motor behavior. Similar approaches may be used to study the intracellular signaling underlying adhesion GPCR function. This work was supported by two NIH BRAIN Initiative awards U01NS094247 (H.Z. and T.M.) and R01NS104944 (H.Z. and T.M.), an NINDS R01 grant R01NS081071 (T.M.) and a NINDS R21 grant R21NS097856 (H.Z.).
The adhesion GPCR GPR56/ADGRG1 and GPR64/ADGRG2 are involved in the regulation of murine adipocyte differentiation and metabolism

Christian Zieschang, Ines Liebscher, Torsten Schöneberg, Doreen Thor

Rudolf Schönheimer Institute of Biochemistry, Molecular Biochemistry, Medical Faculty, University of Leipzig, 04103 Leipzig, Germany.

Expression and genome wide association studies have linked several adhesion GPCR (aGPCR) with adipose tissue function and metabolic dysfunctions. Although RNAseq analyses of adipose tissue revealed high expression of a number of aGPCR, the physiological function in fat tissue still remains elusive. So far, only GPR116/ADGRF5 has been shown to modulate insulin sensitivity in adipocytes.

To analyze aGPCR function in adipose tissue we studied aGPCR expression during adipocyte differentiation using 3T3-L1 cells, a model cell line for adipocytes, primary murine adipocytes, and primary murine preadipocytes. We found significant >10-fold upregulation of GPR56/ADGRG1 and GPR64/ADGRG2 expression, indicating potential functions of these aGPCR during adipocyte differentiation. We show that Stachel-peptide stimulation of GPR64/ADGRG2 activates the Gs protein/adenylyl cyclase pathway which increases intracellular cAMP levels and stimulates lipolysis in both, differentiated 3T3-L1 cells and primary murine adipocytes. Vice versa, knock-down of GPR64/ADGRG2 causes lipid accumulation changes in differentiated 3T3-L1 cells.

Ongoing work focuses on the physiological function of these receptors in adipocytes in respect of glucose uptake and adipokine secretion. Our data highlight the previously unappreciated role of aGPCR in controlling the metabolic and endocrine functions of adipose tissues.


Funding: CRC1052 project B6.
G PROTEIN-COUpled RECEPTOR 56 (GPR56) AS A POTENTIAL FUNCTIONAL REGULATOR OF NORMAL AND LEUKEMIC HUMAN STEM CELLS

Heather Duncan, MSc1*, Karin G. Hermans, PhD2, Sara Chisling, BSc3, Mark D. Minden, MD, PhD4, John E. Dick, PhD4, Kolja Eppert, PhD5

1Division of Experimental Medicine, McGill University, Montreal, QC, Canada; 2Program of Developmental and Stem Cell Biology, Peter Gilgan Centre for Research and Learning, The Hospital for Sick Children, Toronto, ON, Canada; 3Faculty of Medicine, University of Montreal, Montreal, QC, Canada; 4Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada; 5Department of Pediatrics, McGill University, Montreal, QC, Canada;

Leukemic stem cells (LSCs) sustain acute myeloid leukemia (AML) and must be eliminated to cure a patient. Improved understanding of LSCs is required to improve therapy. We identified G protein-coupled receptor 56 (GPR56) among a gene expression signature common to LSCs and normal hematopoietic stem cells (HSCs). GPR56 is a novel marker of high LSC frequency in AML patients. GPR56 has also been implicated in the development and regulation of murine HSCs, although this is controversial, and was shown to accelerate leukemogenesis in mice. This study aims to establish the functional role of GPR56 in human LSCs and HSCs, providing insight into the commonalities and differences in their molecular regulation.

GPR56 expression was inversely correlated with survival across three microarray AML cohorts (p<0.01). GPR56 expression was higher in samples with worse outcome (p<0.0001) and lower in those with better outcome (p<0.01) predicted by cytogenetics. We confirmed increased GPR56 expression in human LSC- and HSC-enriched fractions versus mature populations by qRT-PCR. Colony forming cell assays were conducted with flow-sorted cells to determine effects on progenitor proliferation and differentiation. Overexpression of GPR56 in AML cell line MOLM-13 (p<0.05) and cultured AML sample 8227 (GPR56dN, p<0.0001) increased colony formation. Conversely, GPR56 overexpression did not significantly alter human hematopoietic progenitor activity. Long-term xenograft assays were performed via intrafemoral injection in immunodeficient mice to determine effects on stem cell function. GPR56 overexpression conferred a significant engraftment advantage (p<0.0001) maintained in secondary 12-week transplants (p<0.05). The lineage distribution and percentage of stem and progenitor cells were unaltered, indicating self-renewal of stem cells.

These data suggest that GPR56 enhances HSC function in vivo, and may regulate human leukemic progenitors, but not hematopoietic progenitors in vitro. Further functional studies will be performed to determine the role GPR56 in LSC function in vivo, and its role in chemotherapy resistance.

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Visitor Information

**General Addresses and Contact Information**

<table>
<thead>
<tr>
<th>Name</th>
<th>Address</th>
<th>Phone</th>
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<tbody>
<tr>
<td>Bobbi Chamberlain</td>
<td>General questions and concerns about the event</td>
<td>(503) 575-6743</td>
</tr>
<tr>
<td>The Nines Hotel</td>
<td>525 SW Morrison, Portland, OR 97204</td>
<td>(877) 229-9995</td>
</tr>
<tr>
<td>Oregon Health &amp; Science University</td>
<td>3181 SW Sam Jackson Park Road, Portland, OR 97239</td>
<td>(503) 494-8311</td>
</tr>
<tr>
<td>Baerlic Brewing Co. and Taproom</td>
<td>2235 SE 11th Avenue, Portland, OR 97214</td>
<td>(503) 477-9418</td>
</tr>
<tr>
<td>US Bank Main</td>
<td>321 SW 6th Avenue, Portland, OR 97204</td>
<td>(503) 227-1104</td>
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<tr>
<td>Non-emergency dispatch</td>
<td>1111 SW 2nd Ave, Portland, OR 97204</td>
<td>(503) 823-3333</td>
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**Transportation**

**Car Rentals**

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<td>Enterprise</td>
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<td>Dollar Rent-A-Car</td>
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<td>Hertz</td>
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<td>National Rent-A-Car</td>
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**Public Transportation**

Tri-Met is the main method of transportation around the city of Portland. Portland offers a variety of buses around town as well as a light rail train (called “the max”) and the Portland Streetcar. Maps, schedules, a transit tracker and a trip planner are all available online at: [https://trimet.org](https://trimet.org).

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<td>7-17 or high school</td>
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Visitor Information

To Baerlic Brewing Co. from The Nines:

Via Tri-Met - Max
Estimated travel time: 23 minutes

1. Walk 381 ft. to Pioneer Place/SW 5th Max Station (Stop ID 7646)
2. Board Max Orange Line Milwaukie
3. Ride for 15 minutes
4. Disembark at Clinton St/SE 12th Ave Max Station
5. Walk about 7 min to Baerlic Brewing.

Via Walking:
Estimated travel time: 38 minutes

1. Head east on SW Morrison St. toward SW 5th Ave
2. Turn left onto SW 2nd Ave
3. Turn right onto SE Morrison Bridge
4. Turn right
5. Turn left
6. Keep right to continue toward SE Water Ave
7. Turn right onto SE Water Ave
8. Turn left onto SE Hawthorne Blvd
9. Turn right onto SE 3rd Ave
10. Continue onto SE Division St.
11. Turn left onto SE 7th Ave
12. Turn right onto SE Sherman St.
13. Turn left onto SE 11th Ave, destination will be on the left.
Visitor Information

The Nines

The concierge is available on the main level (8th floor) at the front desk every day from 7am-11pm. Amenities available include: ATM, fax machine, copier and scanner.

Portland Tourist Highlights


Powell’s City of Books 1005 W. Burnside Street, Portland, Oregon
Portland Japanese Garden 611 SW Kingston Ave, Portland, Oregon
Washington Park 4033 Southwest Canyon Road, Portland, Oregon
Forest Park W 29th Avenue and Upshur Street to Newberry Road, Portland, Oregon
Portland Art Museum 1219 SW Park Ave, Portland, Oregon
Multnomah Falls 5000 E Historic Columbia River Hwy, Bridal Veil, Oregon
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Cover image courtesy of Demet Araç, University of Chicago