THE ADHESION-GPCR CONSORTIUM

Dr. GPCR .com

11TH ADHESION GPCR WORKSHOP

PROGRAM 2024

MEXICO CITY

OCT 23-25

Organized by: **Dr. Antony Boucard** and **Dr. Yamina Berchiche**

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Cinvestav

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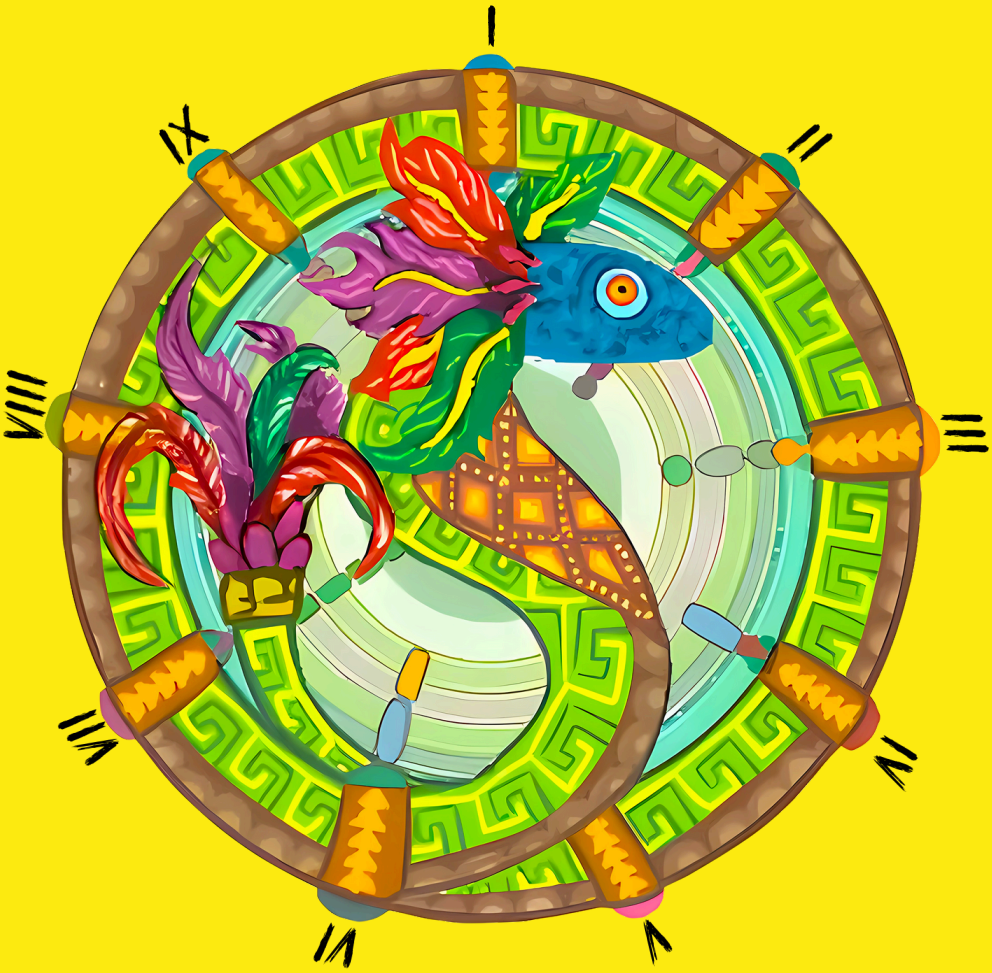
aGEM Award (aGPCR Research Excellence and Mentoring Award)

Rewards graduate students and/or postdoctoral fellows who have shown enthusiasm for research in the aGPCR field through collaborative work highlighted in their oral or poster presentations.

Winner of the Logo Contest



Participants:
María Fernanda Coutiño Rico
María Cruz Enrriquez Arreola
María Fernanda Gómez Chávez
Gilberto Guadalupe Olan Domínguez
Martha Paola Villatoro Gómez

Institution: Universidad Autónoma de Chiapas, Escuela de ciencias Químicas, Ocozocoautla

The logo is inspired by Mexico's rich biodiversity and mythology, combining an alebrije axolotl with elements of the feathered serpent Quetzalcoatl. The feathered serpent is coiled into a circular shape, symbolizing a cell, while within the circle, the axolotl adopts a dynamic posture. G-protein-coupled receptors (GPCRs) are represented by patterns that run through the circular figure, symbolizing the seven transmembrane helices. These patterns integrate with the snake's scales and the axolotl's textures. Small colorful semicircles in the extracellular areas represent ligand binding sites, and different geometric figures connected to the transmembrane helices in the intracellular part symbolize the G proteins. Inspired by Aztec fretwork, geometric patterns decorate the body of the snake and the axolotl, creating a fusion between pre-Hispanic art and modern biology. A vibrant color palette, such as green, brown, bright red, gold, and purple, highlights the different parts of the logo, creating a visually appealing design. This logo is a visual representation that tells a story rich in science and culture, fusing molecular biology with Mexico's cultural heritage.

Welcome to the **Adhesion GPCR Workshop 2024**, hosted in the vibrant heart of Mexico City at the prestigious venue, **CINVESTAV** - Centro de Investigación y de Estudios Avanzados del IPN (in English: Center for Research and Advanced Studies of the National Polytechnic Institute).

About the venue

Cinvestav: Centro de investigación y de Estudios Avanzados del IPN
Av. Instituto Politécnico Nacional no 2508 Mexico City, C.P. 07360

Room: Auditorium Arturo Rosenblueth
*Have an ID card ready (Passport, Driver's license, etc), as this will be required at the venue entrance

AGPCR24 - Full Agenda

OCT 23

Oct 23 - 9:00 AM

Registration & Welcoming Remarks

10:00 AM

Student Flash Presentations

Health and Disease, Metabolism, Nervous System, Proteomics and Transcriptomics, Receptor Structure, Signaling and Activation Mechanism

Abhishek Kumar Singh · Alex Torrelli-Diljohn · Emmanouil Kyrloglou · Vasiliki Karagiannakou · Lara-Sophie Brodmerkel · Rashed Rezwan Parag · Hailey Steichen · Tyler Bernadyn · Jesse Stillwell

12:00 PM

Coffee Break with light snacks

12:30 PM

State of the Art Talk

Adhesion GPCR in Mechanobiology

Tobias Langenhan

1:00 PM

Plenary Lecture

Identification and Functional Characterization of Adhesion GPCRs As Steroid Hormone Receptors and Hearing and Balance Receptors

Jinpeng Sun

2:00 PM

Complimentary Lunch

3:00 PM

Session I

Tethered agonist-dependent/independent activation mechanism in AGPCRs

Signe Mathiasen · Demet Araç · Andrew Dates · Frank Kwarcinski · Peng Xiao

5:00 PM

Leaving for City Center

5:30 PM

Mexico City Nocturnal Tour, Food and drinks

Student Flash Presentations

October 23rd · 10:00 AM

AGPCR 2024

Health and Disease, Metabolism, Nervous System, Proteomics and Transcriptomics, Receptor Structure, Signaling and Activation Mechanism

Adgrg6/Gpr126 is Required for Myocardial Notch Activity and N-cadherin Localization to Attain Trabecular Identity

Abhishek Kumar Singh

Indian Institute of Science Education and Research, Mohali

Investigating The Role of ADGRB3 Loss of Expression in Brain Tumor Formation in Li-Fraumeni Syndrome

Alex Torrelli-Diljohn

University of Alabama at Birmingham

GPR124 Mediates Adhesion Of Leukemic Stem Cells To Their Niche And Leads To Myeloid Skewing

Emmanouil Kyrloglou

University Medical Center Groningen (UMCG)

A single cell GPCR map of thermogenic fat

Vasiliki Karagiannakou

Student Flash Presentations

October 23rd · 11:00 AM

AGPCR 2024

Health and Disease, Metabolism, Nervous System, Proteomics and Transcriptomics, Receptor Structure, Signaling and Activation Mechanism

GAIN Domain Dynamics And Its Relevance For Adhesion GPCR Signaling In Vivo

Lara-Sophie Brodmerkel

University of Leipzig

Novel isoforms of adhesion GPCR B1 (ADGRB1/BAI1) generated from an alternative promoter in intron 17

Rashed Rezwan Parag

University of Alabama at Birmingham (UAB)

Identification of Differentially Expressed Gpr116 (Adgrf5) Transcript Variants in Mouse Kidney

Hailey Steichen

UNM

Elucidating The Role Of GPR97/ADGRG3 In Neutrophil Biology

Tyler Bernadyn

University of Michigan

Next Generation MBD2 inhibitors for Brain Cancer Therapy

Jesse Stillwell

University of Alabama at Birmingham

Student Flash Presentations

October 23rd · 10:00 AM

AGPCR 2024

Health and Disease, Metabolism, Nervous System, Proteomics and Transcriptomics, Receptor Structure, Signaling and Activation Mechanism

Adgrg6/Gpr126 is Required for Myocardial Notch Activity and N-cadherin Localization to Attain Trabecular Identity

Abhishek Kumar Singh

How adhesion G protein-coupled receptors (aGPCRs) control development remains unclear. The aGPCR Adgrg6/Gpr126 has been associated with heart trabeculation. Defects in this process cause cardiomyopathies and embryonic lethality. Yet, how cardiomyocytes attain trabecular identity is poorly understood. Here, we show that Gpr126 regulates Notch activity and N-cadherin localization that are necessary for attaining trabecular identity in zebrafish. Maternal zygotic gpr126stl47 early truncation mutants exhibit hypotrabeculation whereby N-cadherin distributes randomly at apical/basal membranes of compact layer cardiomyocytes. In contrast, gpr126st49 mutants expressing a N-terminal fragment lacking the GPS motif (NTFΔGPS) exhibit a multilayered ventricular wall consisting of polarized cardiomyocytes with normal N-cadherin expression and increased Notch signaling. Notably, endocardially expressed C-terminal fragment (CTF) reinstates trabeculation in gpr126st49 mutants. Collectively, our data indicate domain-specific roles of Gpr126 during trabeculation whereby the NTF maintains cell-cell adhesion and is required for compact wall integrity, while the CTF is essential to provide trabecular identity.

Authors & Affiliations

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1 Experimental Renal and Cardiovascular Research, Department of Nephropathology, Institute of Pathology, Friedrich-Alexander-Universität Erlangen-Nürnberg, Kussmaulallee 12, 91054 Erlangen, Germany

2 Developmental Genetics, Max-Planck-Institute for Heart and Lung Research, Ludwigstrasse 43, 61231 Bad Nauheim, Germany

3 Department of Developmental Biology, Washington University in St. Louis, 660 S. Euclid Ave, St. Louis, MO 63108, USA. Present address: Department of Neuroscience, Kenyon College, 203 North College Road, Gambier, OH 43022, USA"

About Abhishek Kumar Singh

"I am a doctoral student in the lab of Prof. Felix B. Engel. Since my undergraduate studies, I became fascinated with the class of adhesion GPCRs, owing to their potential, scarcity of knowledge on them, diverse expression profile, and the complexity with which they seem to be working. This made me pursue my higher education in the field of adhesion GPCRs. Accordingly, I worked with Prof. Hsi-Hsien Lin as summer intern twice, and finally joined the lab of Prof. Engel. I hope to develop my skillsets so as to be able to establish my own lab in future to work on adhesion GPCRs employing highly interdisciplinary field."

Student Flash Presentations

October 23rd · 10:00 AM

AGPCR 2024

Health and Disease, Metabolism, Nervous System, Proteomics and Transcriptomics, Receptor Structure, Signaling and Activation Mechanism

Investigating The Role of ADGRB3 Loss of Expression in Brain Tumor Formation in Li-Fraumeni Syndrome

Alex Torrelli-Diljohn

Li-Fraumeni syndrome (LFS) is a rare cancer predisposition syndrome caused by a germline mutation in the TP53 tumor suppressor gene. Glioblastoma (GBM) is the most prevalent central nervous system tumor in LFS, with TP53 mutations detected in 30% of sporadic GBMs. GBM is the most aggressive primary brain neoplasm that affects adults, with a median survival of 12-15 months. Recent studies implicate the dysregulation of adhesion G-Protein coupled receptors (GPCRs) in GBM development. Brain angiogenesis inhibitor 3 (BAI3/ADGRB3), a member of the BAI1-3 subfamily of adhesion GPCRS, has been observed to have low expression in brain tumors according to TCGA data, but the significance of this observation has not been explored. However, while its sister protein BAI1 has demonstrated tumor suppressor functions in the brain, it remains unclear whether BAI3 shares this role. To test this, an LFS mouse model (germline Tp53 deletion) with a second floxed allele under the control of Nestin-Cre was crossed to Bai3-/- mice. Preliminary findings indicate that the simultaneous loss of Bai3 and Tp53 expression in our mouse model increased spontaneous brain tumor formation incidence from 34% to 71%, in contrast to the loss of p53 alone. These observations lead me to hypothesize that ADGRB3 functions as a tumor suppressor in the brain, and its silencing, in the context of p53 mutation, facilitates GBM formation. Isolated GBM stem cells were collected for further genomic analyses and to test whether overexpression of BAI3 will save the tumor phenotype.

Authors & Affiliations

"Vukadin L, Park B, Mohamed M, Li H, Elkholy A, Torrelli-Diljohn A, Kim JH, Jeong K, Murphy JM, Harvey CA, Dunlap S, Gehrs L, Lee H, Kim HG, Sah JP, Lee SN, Stanford D, Barrington RA, Foote JB, Sorace AG, Welner RS, Hildreth BE 3rd, Lim SS, Ahn EE. A mouse model of Zhu-Tokita-Takenouchi-Kim syndrome reveals indispensable SON functions in organ development and hematopoiesis. JCI Insight. 2024 Mar 8;9(5):e175053. doi: 10.1172/jci.insight.175053. PMID: 38290089; PMCID: PMC10972584.University of Alabama at Birmingham"

About Alex Torrelli-Diljohn

"Alex completed his undergraduate & master's degrees in Neurobiology & Cognitive sciences from the University of South Florida, where he researched early-onset Alzheimer's disease in the lab of Dr. Angele Parent. He is interested in working on Li-Fraumeni syndrome and helping patients afflicted with this condition. He is also interested in working on Glioma Brain Organoid models.

Health and Disease, Metabolism, Nervous System, Proteomics and Transcriptomics, Receptor Structure, Signaling and Activation Mechanism

GPR124 Mediates Adhesion Of Leukemic Stem Cells To Their Niche And Leads To Myeloid Skewing

Emmanouil Kyrloglou

GPR124, a member of the Adhesion G Protein-Coupled Receptor family, has an established role in the embryonic development of central nervous system vasculature. However, its function in the context of Acute Myeloid Leukemia (AML) remains unexplored. Our transcriptome and proteome studies revealed upregulation of GPR124 in AML CD34+ blasts compared to healthy counterparts. Lentiviral GFP-tagged overexpression of GPR124 showed plasma membrane localization, and enhanced adhesion of leukemic cells to bone marrow stromal cells. In contrast, a splice variant of GPR124 missing exon18 (vDel18), showed intracellular localization and its overexpression did not impact on adhesion. The expression of the full length and the vDel18 isoform was found to differ significantly between patients, and particularly patients with a high wt:vDel18 ratio displayed poor prognosis. Furthermore, GPR124 was found to have an impact on hematopoietic stem cell differentiation within a cord blood CD34+ MLL-AF9 transduction model. GPR124 overexpression skewed transformation towards the myeloid lineage, in line with GPR124 overexpression which is exclusively seen in pediatric AML and not lymphoid leukemia (ALL) patients, both harboring MLL-AF9 translocation. To explore underlying mechanisms and to identify potential ligands for this orphan receptor, we performed various LC-MS/MS based interactome and BioID studies. Candidate ligands and intracellular interacting proteins have been identified and are currently functionally being evaluated using CRISPR-CAS9 mediated KO models and will be discussed. Our ultimate aim is to unravel the role of GPR124 in AML pathogenesis and integrate this knowledge into a targeted therapy protocol against this highly incurable disease.

About Emmanouil Kyrloglou
"Studied medicine at the University of Groningen. Now PhD-candidate at the Experimental Hematology lab of the University Medical Center Groningen (UMCG)."

Health and Disease, Metabolism, Nervous System, Proteomics and Transcriptomics, Receptor Structure, Signaling and Activation Mechanism

A single cell GPCR map of thermogenic fat

Vasiliki Karagiannakou

Brown and beige adipocytes upon adrenergic activation increase mitochondrial uncoupling of electron transfer chain resulting in dissipation of energy to heat. Therefore, they are called thermogenic adipocytes. Activation of thermogenesis naturally occurs by exposure to cold temperature and has multiple metabolic benefits. Beta adrenergic agonists are potent activators of thermogenesis, however, have multiple of target effects which make them not attractive targets for drug development. Therefore, identification of novel GPCRs which can activate thermogenesis is desirable. Due to the low abundance and slow mRNA turnover, RNAseq based unbiased approaches are challenging.
Methods:We exposed 10-12 weeks old male, single housed mice to 8oC for 2 weeks. Evercode from Parse Biosciences was used to barcode single nuclei with the split pool combinatorial protocol. Illumina short reads and Pacbio long reads sequencing.
Results: Unsupervised clustering showed an excessive remodeling of the brown and white adipose tissue upon cold stimulation. We sequences more that 90 GPCRs per tissue. Most GPCRs were expressed in more than one cell types, but exhibited cell type specific changes in response to cold induced tissue remodeling. Class A GPCRs showed the strongest changes in response to cold, localized mainly in adipocytes. Interestingly Adhesion GPCRs were the second class of receptors exhibited strong cell type dependent changes and they were predominantly expressed in non-adipocyte cell types. Next to GPCRs we observed strong changes in expression levels of splicing factors suggesting extensive cell type specific activation of splicing during cold induced tissue remodeling. Current ongoing analysis of long reads RNAsequencing will reveal the cell type GPCRs isoforms associated with thermogenesis.
Conclusions: SPLiT-seq combinatorial single nuclei RNA seq offers high resolution for GPCRs. Leveraging on this technology we have generated a single cell map of GPCRs associated with the thermogenic remodeling response of murine brown and white adipose tissue. Adhesion GPCRs were mapped in non-adipocyte cells and showed cell type specific changed in response to cold induced remodeling.

Authors & Affiliations
"Karagiannakou Vasiliki, El Merahbi Rabih, Herzig Stephan , Georgiadi A , Helmholtz Center Munich, Institute of Diabetes and Cancer"

About Vasiliki Karagiannakou
"MSc in Bioinformatics, PhD student since 2022 in the Institute for Diabetes and Cancer IDC, Helmholtz Centre Munich"

Health and Disease, Metabolism, Nervous System, Proteomics and Transcriptomics, Receptor Structure, Signaling and Activation Mechanism

GAIN Domain Dynamics And Its Relevance For Adhesion GPCR Signaling In Vivo

Lara-Sophie Brodmerkel

Over the last years, Adhesion G Protein-coupled receptors (aGPCR) have been shown to play a crucial role in the perception of mechanical signals. However, the molecular details underlying their activation and how mechanical forces are translated into an intracellular response remains largely unknown. Recent Molecular Dynamics (MD) simulations of several aGPCRs predicted two flexible regions, termed flaps, located within the GPCR autoproteolysis inducing (GAIN) domain. These flaps could theoretically enable partial decryption of the Stachel through lateral movement and affect activation of the receptor independent of NTF-CTF dissociation. However, the physiological relevance of flap flexibility on receptor activation and signaling remains unclear. To investigate whether flexibility of GAIN flaps affects aGPCR function under native conditions, we strategically inserted specific mutations into the GAIN domain of the Latrophilin homologue Cirl in Drosophila melanogaster, with the intention to alter flap dynamics. Our goal is to understand if and how flap dynamics influence Cirl function and consequently the mechanosensory faculty of neurons in vivo. To this end, we combine behavioral, biochemical, immunohistochemical and functional readouts, with the overarching ambition to expand our knowledge on the mechanistic details underlying aGPCR activation in mechanosensation.

Authors & Affiliations
"Brodmerkel Lara-Sophie 1, Bormann Anne 1, Seufert Florian 2, Hildebrand Peter 2,3 ´, Ljaschenko Dmitrij 1´, Scholz Nicole 1´

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*contributed equally"

About Lara-Sophie Brodmerkel
"I am a medical student and I´m currently working on my MD thesis in the lab of Dr. Nicole Scholz. We are investigating the relevance of GAIN domain dynamics for aGPCR signaling in Drosophila melanogaster."

Student Flash Presentations

October 23rd · 11:00 AM

AGPCR 2024

Health and Disease, Metabolism, Nervous System, Proteomics and Transcriptomics, Receptor Structure, Signaling and Activation Mechanism

Novel isoforms of adhesion GPCR B1 (ADGRB1/BAI1) generated from an alternative promoter in intron 17

Rashed Rezwan Parag

Brain-specific angiogenesis inhibitor 1 (BAI1) belongs to the adhesion G-protein-coupled receptors, which exhibit large multi-domain extracellular N-termini that mediate cell-cell and cell-matrix interactions. To explore the existence of BAI1 isoforms, we queried genomic datasets for markers of active chromatin and new transcript variants in the ADGRB1 (adhesion G protein-coupled receptor B1) gene. Two major types of mRNAs were identified in human/mouse brain, those with a start codon in exon 2 encoding a full-length protein of a predicted size of 173.5/173.3 kDa and shorter transcripts starting from alternative exons at the intron 17/exon 18 boundary with new or exon 19 start codons, predicting shorter isoforms of 76.9/76.4 and 70.8/70.5 kDa, respectively. Immunoblots on wild-type and Adgrb1 exon 2-deleted mice, reverse transcription PCR and promoter-luciferase reporters confirmed that the shorter isoforms originate from an alternative promoter in intron 17. The shorter BAI1 isoforms lack most of the N-terminus and are very close in structure to the truncated BAI1 isoform generated through GPS processing from the full-length receptor. The cleaved BAI1 isoform has a 19 amino acid extracellular stalk that can serve as a receptor agonist, while the alternative transcripts generate BAI1 isoforms with extracellular N-termini of 5 or 60 amino acids. Further studies are warranted to compare the functions of these isoforms and examine the distinct roles they play in different tissues and cell types.

About Rashed Rezwan Parag

"Rashed is from Bangladesh. He has received his BSc and MS degree from the Department of Biochemistry and Molecular Biology, University of Chittagong, Bangladesh. Before joining UAB as a graduate student, he worked in the EuGEF Research Group to identify novel prognostic biomarkers and therapeutic options for Metastatic Breast Cancer (BC) and Head and Neck Squamous Cell Carcinoma (HNSCC). Currently, he is working to elucidate the role of ADGRB1 and ADGRB3 in medulloblastoma (pediatric brain tumor)."

Health and Disease, Metabolism, Nervous System, Proteomics and Transcriptomics, Receptor Structure, Signaling and Activation Mechanism

Identification of Differentially Expressed Gpr116 (Adgrf5) Transcript Variants in Mouse Kidney

Hailey Steichen

Adhesion G protein-coupled receptors (aGPCRs) are important and understudied modulators of physiological processes. Previous work suggests that aGPCRs, and Adgrf5 in particular, undergo significant tissue-specific mRNA processing that results in holoreceptors with unique and variable N-terminal structures (Knierim et al. 2019). Recently, it was shown that transcripts of the postsynaptic aGPCR Latrophilin-3 (Lphn3/Adgrl3) undergo physiologically relevant alternative splicing, which determined heterotrimeric signaling through Gαs- or Gα12/13- mediated pathways (Südhof et al. 2024). These results demonstrate that identifying precise, tissue-specific transcript variants is critical to understanding the physiological relevance of aGPCRs. Moreover, these studies highlight the possibility that tissue expression of single aGPCRs is likely comprised of multiple transcript variants. We previously demonstrated that kidney-specific Adgrf5/Gpr116 knockout causes luminal membrane accumulation of V-ATPase in acid-secreting A-type intercalated cells (AICs) in the collecting ducts and a significant reduction in urine pH (Zaidman et al. 2020). Renal Adgrf5 is restricted to two distinct populations of cells: AICs and endothelial cells (ECs). We hypothesized that cell-specific Adgrf5 transcript variants are expressed in renal AICs and ECs, and therefore are activated by distinct mechanisms unique to the cellular microenvironment. We detected and aligned three Adgrf5 exons that undergo differential expression in the kidney: exons 2, 12, and 22. Adgrf5 transcripts in FACS-sorted GFP+ ICs do not contain the exon 2 variable region, or the alternative exons 12 and 22, while ECs contain all three. However, EC markers were detected in GFP+ ICs, demonstrating some EC contamination in the sorted ICs. Detection of transcripts that do, and do not, contain multiple variable regions suggests expression of multiple mRNAs in specific cells. These data demonstrate that Adgrf5 transcript variants are cell-specific in the kidney. Moreover, the complete repertoire of aGPCRs expressed in the kidney is undefined. We performed single-nucleus RNA sequencing on male and female kidneys. snRNAseq revealed abundant, cell-specific expression of six aGPCRs (Adgrl4, Adgre5, Adgrf1, Adgrf5, Adgrg1, and Adgrg3). Detection of these, as well as 18 other aGPCRs, was confirmed by PCR screening for GAIN/GPS domains on cDNA from whole-kidney lysates. These results reveal the complete set of aGPCRs expressed in the murine kidney. Future studies will focus on determining the physiological roles and tissue-specific variants of these receptors.

Authors & Affiliations
"Department of Biochemistry & Molecular Biology, University of New Mexico Health Sciences Center
Xue, Jianxiang; Yan, Teagan; Eaton, Krystin, and Zaidman, Nathan"

About Hailey Steichen
"I currently work in Dr. Nathan Zaidman's lab at the University of New Mexico Health Sciences Center. I am researching the physiological relevance of Adgrf5 (Gpr116) transcript variants in specific cell types in the kidney. I have also worked in the laboratory of Dr. James Bridges at National Jewish Health in Denver, CO researching molecular mechanisms of lung injury and repair mediated by Adgrf5. I received my MS in Applied Toxicology from the University of Washington, and my BA in Biology from Vassar College."

Student Flash Presentations

October 23rd · 11:00 AM

AGPCR 2024

Health and Disease, Metabolism, Nervous System, Proteomics and Transcriptomics, Receptor Structure, Signaling and Activation Mechanism

Elucidating The Role Of GPR97/ADGRG3 In Neutrophil Biology

Tyler Bernadyn

Adhesion G protein coupled receptors (AGPCRs) are activated by tethered-peptide agonists (TA) in response to extracellular forces. Cells presenting AGPCRs, or adjacent cells that present AGPCR ligands move to generate the force that dissociates the N-terminal fragment (NTF) and C-terminal fragment (CTF) to expose the AGPCR TA. The decrypted TA binds rapidly to its orthosteric site within the CTF to stabilize the active state of the receptor. Here, we demonstrate that GPR97/ADGRG3 is activated in vitro by urea-mediated dissociation of its NTF/CTF in a TA-dependent manner. TA peptidomimetics stimulated GPR97/ADGRG3 in cell-based luciferase gene reporter assays and in receptor/G protein reconstitution assays. The profile of GPR97 G protein coupling specificity was assigned. GPR97 was shown here by immunoblotting and previously to be present on human polymorphonuclear neutrophils (hPMNs) (1). Neutrophils escape the vascular lumen via extravasation into sites of tissue inflammation. Prior to extravasation, neutrophils are activated, adhere to the endothelium, and undergo actin polymerization to induce cell shape and migratory changes. Multiple membrane-bound proteins facilitate the responses for neutrophil migration; however, we hypothesize that GPR97/ADGRG3 is a missing player in the process. We found that GPR97 activation via TA peptidomimetics stimulated actin polymerization in hPMNs and mouse bone-marrow neutrophils (mBMNs) and induced cell polarization comparably to the established neutrophil ligand, fMLP. GPR97 TA peptidomimetics also stimulated hPMN and mBMN migration in trans-well Boyden chamber assays. Altogether our emerging results demonstrate that ADGRG3/GPR97 activates G proteins via tethered agonism and likely participates in the process of neutrophil chemotaxis and extravasation.

Authors & Affiliations

"Gandhi, Riya; Chandan, Nancy; Kwarcinski, Frank; Smrcka, Alan; and Tall, Gregory G."

About Tyler Bernadyn

"4th year Pharmacology Ph.D. Student in Greg Tall's Lab."

Student Flash Presentations

October 23rd · 11:00 AM

AGPCR 2024

Health and Disease, Metabolism, Nervous System, Proteomics and Transcriptomics, Receptor Structure, Signaling and Activation Mechanism

Next Generation MBD2 inhibitors for Brain Cancer Therapy

Jesse Stillwell

Medulloblastoma (MB) is one of the most lethal pediatric brain tumors. Standard of care for MB includes tumor resection, chemotherapy, and cranio-spinal radiation. This regimen has long lasting side-effects, including neuroendocrine and cognitive problems, and ~ 30% of patients still do not survive 5 years past diagnosis. Clearly, a new, less toxic therapeutic is needed. Our lab has previously shown that expression of adhesion GPCR BAI1 (ADGRB1) is lost by epigenetic silencing in MB. Restoration of ADGRB1 expression slowed tumor growth and improved survival in mice bearing MB xenografts. The ADGRB1 promoter is methylated in MB, and this allows for Methyl CpG Binding Domain protein 2 (MBD2) to silence the gene through recruitment of the NuRD silencing complex. KCC-07 is an inhibitor that prevents MBD2 from binding to DNA, allowing re-expression of BAI1. To further optimize the chemical scaffold, we synthesized KCC07 analogs that we're testing for their ability to reactivate BAI1 expression. The current methods for testing KCC-07's ability to reactivate ADGRB1 expression involve western blotting and RT-qPCR, both of which are semi-quantitative methods that require large numbers of cells and high volumes of analogs, creating a bottleneck in screening. These methods are time consuming, and their inherent variability makes precise quantification difficult. This research focuses on the design of a new endogenous ADGRB1 activation reporter assay to test analogs faster and with more reproducibility.

Authors & Affiliations

"Erwin Van Meir, University of Alabama at Birmingham/Sadanandan Velu, University of Alabama at Birmingham/Takahiro Yamamoto, Kumamoto University"

About Jesse Stillwell

"Jesse Stillwell is a 3rd year graduate student with a research focus in drug development. His project is drug discovery focused, with particular interest in use of a novel epigenetic therapy to reactivate ADGRB1 expression."

State of the Art Talk
October 23rd · 12:30 PM

AGPCR 2024

Tobias Langenhan
Leipzig University

Adhesion GPCR in Mechanobiology

The aGPCR field has been impeded by the lack of direct support for the identity of proteolytic fragments ascribed to GAIN domain proteolysis through protein sequencing or spectrometric analysis of fragment masses, thereby excluding other possibilities for their provenance such as their generation by proteases.
We collected data casting doubt on the autoproteolytic capacity of the ADGRC homolog Flamingo/Starry night (Fmi) in Drosophila melanogaster, with roles in planar cell polarity (PCP) and tissue organogenesis. Previous biochemical analyses suggested that Fmi is self-fragmented at the GPCR proteolysis site (GPS). Genetic removal of fmi results in penetrant embryonic lethality of mutant animals due to defects in axonal fasciculation and dendritogenesis, while milder perturbation of fmi levels or functions are expressed by PCP defects in epithelial appendages and corrupted asymmetric cell division.
fmi alleles with GAIN domain autoproteolysis-inhibiting mutations show that the en-coded Fmi proteins are still cleaved in vivo. Neither embryonic lethality nor defects in PCP in the eye and wing bristles are observed in fmiΔGPS mutants. We generated a genetically encoded NTF release sensor (NRS) and show that the release patterns between Fmi-NRS reporters with intact and mutated GPS are indistinguishable.
Collectively, these results suggest that Fmi is not autoproteolytically processed by the GAIN domain and that modifications to the protein at GPS-homologous positions are inert to the receptor function. Future studies will concentrate on the mechanism behind non-GAIN domain Fmi cleavage and analyse its effects on cell biological and physiological consequences.

About Tobias Langenhan

1997-2004: Medical school and Dr. med. Neuroanatomy (Würzburg, Germany);
2004-2005: M.Sc. Neuroscience (Oxford, UK);
2005-2009: D.Phil. Neuroscience (Oxford, UK);
2009-2016: Group leader, Institute of Neurophysiology (Würzburg, Germany);
2016: Heisenberg professorship (Würzburg, Germany);
2016-to date: Professor and Chair in Biochemistry (Leipzig, Germany)

Plenary Lecture
October 23rd · 1:00 PM

AGPCR 2024

Jin-Peng Sun
Shandong University

Identification and Functional Characterization of Adhesion GPCRs As Steroid Hormone Receptors and Hearing and Balance Receptors

Steroid hormones, with more than 50 known types, are one of the major classes of hormones and play essential regulatory roles in a wide variety of physiological processes. Steroid hormones exert physiological and pharmacological effects mainly through activating their respective nuclear receptors, thereby mediating long-term genomic effects. Intriguingly, research over the past 70 years have revealed that steroid hormones also elicit rapid physiological effects within seconds to minutes, suggesting the existence of membrane receptors for steroid hormones that mediate non-genomic effects. However, the putative membrane receptors of steroid hormones remain a mystery and the underlying regulating mechanisms are largely unknown. Over the last decade, by establishing highly-sensitive and high-resolution GPCR screening and signaling profiling platforms, our group have identified that multiple adhesion GPCR (aGPCR) members serve as the membrane receptors for different steroid hormones and regulate important physiological functions. Specifically, we identified GPR97 as an endogenous membrane receptor for glucocorticoids, mediating adrenocorticotropic hormone-induced release of corticosterone from the adrenal cortex. Moreover, we uncovered that whereas dehydroepiandrosterone (DHEA) is able to activate GPR64 and regulates male fertility through CFTR coupling, progesterone and 17-hydroxyprogesterone are the endogenous ligand of GPR126 and promote the progression of breast cancer. Till now, we have paired more than 20 steroids with their aGPCR receptors. The discovery of aGPCR subfamily as steroid hormone membrane receptors has reshaped our understanding of the regulating mechanism of steroid hormones and provided insight into the potential development of pharmaceutical strategies by targeting these receptors.

About Jin-Peng

"Since starting my laboratory in 2011, I has focused on G protein coupled receptors, in particular, the ligand identification, physiological functions and molecular mechanism of biased signaling of GPCRs. Our first main research aspect is the identification of endogenous ligand of GPCRs. We have identified the receptor subfamily to sense the steroid hormones. For instance, membrane receptor GPR97 is able to sense glucocorticoid to mediate its rapid actions, the progesterone and 17-hydroxyprogesterone membrane receptor are GPR126. We also identified DHEA, DHEAS and DOC are endogenous ligands of GPR64 etc (Nature, 2021a, Nat Chem Biol 2022, PNAS 2022b). Our second main research aspect is dissecting the molecular mechanism underlying sensation of force, ordor, itch and taste by GPCRs. We have elucidated the mechanism of receptors' perception of itch, olfactory and force (Nature 2021b, 2022a, 2022b, 2023a, 2024). Our third main research aspect is working mechanism of GPCR. For arrestin mediated biased signaling, we have proposed the "flute model" and "poly proline region docking theory" etc. to explain the arrestin mediated GPCR functions (Nature communications, 2015, 2021, 2022; PNAS 2021, Molecular Pharmacology, 2017; Recommended by Faculty 1000, Nature Chemical Biology 2018). We identified that arrestin can mediated AT1R/TRPC3 or M3R/TRPC3 coupling by forming a complex of AT1R/β-arrestin-1/PLCγ/TRPC3 or M3R//β-arrestin-1/TRPC3 (Nature communications, 2017, Nature communications, 2018). We also identified that orphan receptor GPR64 forms complex with β-arrestin-1 and CFTR at apical membrane of efferent ductulus to regulate the salt/water metabolism (eLife 2018, Faculty 1000 recommendation). Our fourth main research aspect is ligand coding mechanisms and structural aided drug discovery of GPCR. We have decoded the mechanisms underlying recognition of fish oil (unsaturated fatty acids) and other lipids by GPCRs (Science 2023, Science Advance 2021, PNAS 2023, Nature Metabolism 2023), recognition of amine containing hormones by GPCRs (Cell 2021, 2023, Nature 2023b), bile acids or its derivatives by GPCRs (Nature 2020)."

Session I
October 23rd · 3:00 PM

AGPCR 2024

Tethered agonist-dependent/independent activation mechanism in AGPCRs

Signaling Properties of ADGRL3

Signe Mathiasen

University of Copenhagen

An ECR-Mediated and TA-independent Mechanism of aGPCR Activation: Direct Communication of Extracellular Region with Transmembrane Domain in a Holo-Adhesion GPCR

Demet Araç

University of Chicago

Heterogeneity of Tethered Agonist Signaling in Adhesion G Protein-Coupled Receptors

Andrew Dates

Harvard Medical School

Discriminating between the extracellular scaffolding and G protein signaling roles of GPR56/ADGRG1 via the characterization of a non-cleavable point mutant knock-in mouse, H381S

Frank Kwarcinski

University of Michigan

Tethered Peptide Activation Mechanism of Adhesion GPCRs

Peng Xiao

Tethered agonist-dependent/independent activation mechanism in AGPCRs

Signaling Properties of ADGRL3

Signe Mathiasen

The adhesion G protein-coupled receptor ADGRL3 is known for its role in synaptic connectivity. It serves to bind transsynaptic ligands through a large extracellular architecture comprising an array of adhesive protein domains. The receptor is anchored in the plasma membrane via a transmembrane GPCR domain, that has been shown to impact intracellular G protein signaling activity, which is enhanced by exposure of the internal tethered agonist (TA) of ADGRL3. Upon tethered agonist exposure, ADGRL3 principally activates the intracellular G12/13 signaling pathway. Altogether ADGRL3 encompasses a high degree of functional complexity, and to date it remains largely unknown, whether extracellular adhesive interactions are functionally linked to TA exposure, and the TA dependent enhancement of intracellular signaling.
In the group we aim to establish assays that read out on ADGLR3 signaling activation and to characterize how ADGRL3 signaling activity may be modulated. We use a multidisciplinary approach combining a suite of live cell GPCR signaling assays, with state-of-the-art fluorescence microscopy approaches. We have established assays to investigate ADGRL3 signaling activity downstream of the G12/13 pathway and show that ADGRL3 TA activation leads to enhanced RhoA levels at the plasma membrane. We have further developed live cell assays to monitor adhesive ligand binding, and the goal is to combine these efforts to study the potential impact of adhesive interactions on ADGRL3 intracellular signaling functions.

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About Signe Mathiasen

"2022-present
Assistant Professor (Tenure Track) and Group Leader
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Department of Biomedical Sciences, University of Copenhagen.

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Postdoc / Assistant Professor
Department of Psychiatry, Columbia University, New York, USA.
New York State Psychiatric Institute, Research Foundation for Mental Hygiene, New York, USA.
Postdoc Supervisor Professor Jonathan Javitch

2013:
PhD in Nanoscience/Biophysics. Department of Chemistry, University of Copenhagen, Copenhagen Denmark.
PhD Supervisor Professor Dimitrios Stamou."

Tethered agonist-dependent/independent activation mechanism in AGPCRs

An ECR-Mediated and TA-independent Mechanism of aGPCR Activation: Direct Communication of Extracellular Region with Transmembrane Domain in a Holo-Adhesion GPCR

Demet Araç

According to the Tethered Agonist (TA)-mediated model of aGPCR activation, the ECR acts as a protective cap for the TA peptide to hide it within the GAIN domain. However, several recent observations suggest that other mechanisms of aGPCR activation are possible. For example, some aGPCRs do not undergo autoproteolysis, which is required for TA release. Even the aGPCRs that are cleaved do not always require cleavage for mediating some aspects of wild type functions. It has been suggested that the TA can regulate receptor signaling without coming out of the GAIN domain or by being partially exposed, however the recent TA-bound 7TM structures of multiple aGPCRs showed that the critical phenylalanine residue and other important TA residues have to reach deep into the 7TM orthosteric pocket for receptor activation, suggesting that non-release or partial release of the TA is unlikely to activate the receptor. In this talk, I summarize accumulating data from our lab and the aGPCR field that suggests an additional model in which the conformation of the Extracellular Region (ECR) has a direct role in modulating the 7TM signaling, independently of TA-mediated activation.
Our results provide evidence for the ECR-mediated activation of aGPCR as a complementary mechanism for the TA-mediated activation of aGPCRs. Many biological forces are smaller than 200 pN, the force that is needed to separate the TA from the GAIN domain. To sense these smaller forces, and to regulate aGPCR function on and off, a mechanism that does not depend on ECR dissociation and TA exposure might be at work. At low force or no force conditions, aGPCR may be reversibly regulated by binding and dissociation of a ligand to the ECR without ECR shedding and TA exposure. In this ECR-mediated mechanism of activation, the ECR-7TM communication is altered by transient interactions between ECR and 7TM. The TA peptide remains at its original position and is not involved in signaling. Because the TA-mediated mechanism is a “one and done” mechanism that is irreversible and prevents the receptor from going back to its inactive resting state, the ECR-mediated mechanism may operate in situations where a reversible regulation is needed. The ECR-mediated mechanism may also enable responding to compressing forces on the receptor, that directly “push” on the protein. In cases where a large “pulling” force is executed on the ECR, the ECR may be removed from the 7TM releasing the tethered agonist and activating the aGPCR irreversibly but acutely. ECR-mediated mechanism opens new possibilities for drugging aGPCRs. Future work that dissects different activation mechanisms of aGPCRs in different physiological contexts will shed light on this fascinating family of receptors.

Authors & Affiliations
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1. Department of Biochemistry and Molecular Biology, The University of Chicago, Chicago; 2. Neuroscience Institute, Institute for Biophysical Dynamics, and Center for Mechanical Excitability, The University of Chicago, 3. Department of Molecular Biosciences, Northwestern University; 4. Vanderbilt University"

About Demet Araç
"Demet was an undergraduate at Bilkent University in Turkey, where she majored in Molecular Biology and Genetics. She moved to the University of Texas Southwestern Medical Center at Dallas in 2000 to work with Dr. Jose Rizo-Rey as a graduate student to elucidate the mechanisms of neurotransmitter release. After finishing her graduate training, she joined Dr. Axel Brunger’s lab at Stanford University to study the structure and function of cell-adhesion proteins at the synapse. In 2013, Demet began her independent research career at the University of Chicago within the Department of Biochemistry and Molecular Biology."

Tethered agonist-dependent/independent activation mechanism in AGPCRs

Heterogeneity of Tethered Agonist Signaling in Adhesion G Protein-Coupled Receptors

Andrew Dates

Adhesion G Protein-Coupled Receptor (aGPCR) signaling influences development and homeostasis in a wide range of tissues. In the current model for aGPCR signaling, ligand binding liberates a conserved sequence that acts as an intramolecular, tethered agonist (TA), yet this model has not been evaluated systematically for all aGPCRs side-by-side. Here, we assessed the TA-dependent activities of all 33 aGPCRs in a suite of transcriptional reporter, G protein activation, and β-arrestin recruitment assays using a new fusion protein platform. Strikingly, only ~50% of aGPCRs exhibited robust TA-dependent activation, and unlike other GPCR families, aGPCRs showed a notable preference for G12/13 signaling. AlphaFold2 predictions assessing TA engagement in the predicted intramolecular binding pocket aligned with the TA-dependence of the cellular responses. TA-independent signaling was also observed for a variety of aGPCRs, and ongoing work is focused on understanding how these signals may be regulated at the molecular level. This dataset uncovered tethered agonism for three additional aGPCRs, identified secondary signaling responses for several others, and provides a comprehensive resource to inform the investigation of all human aGPCRs and for targeting aGPCRs therapeutically.

Authors & Affiliations
"Daniel T.D. Jones (Harvard Medical School); Jeffrey S. Smith (Harvard Medical School, Brigham and Women's Hospital); Meredith A. Skiba (Harvard Medical School); Maria F. Rich (University of Cincinnati School of Medicine); Maggie M. Burruss (Harvard Medical School); Andrew Kruse (Harvard Medical School); Stephen C. Blacklow (Harvard Medical School)"

About Andrew Dates
"Drew Dates received his B.S. in Biological Chemistry from Carnegie Mellon University in 2018. As an undergraduate, he studied opioid receptor trafficking and G protein conformational dynamics in the laboratories of Manojkumar Puthenveedu and Roger Sunahara, respectively. As part of his doctoral work in the Blacklow laboratory at Harvard Medical School, Drew studied structure-function relationships in the Adhesion Family of GPCRs."

Tethered agonist-dependent/independent activation mechanism in AGPCRs

Discriminating between the extracellular scaffolding and G protein signaling roles of GPR56/ADGRG1 via the characterization of a non-cleavable point mutant knock-in mouse, H381S

Frank Kwarcinski

GPR56/ADGRG1 is an adhesion G protein-coupled receptor (AGPCR) that binds to extracellular collagen to mediate cell-matrix interactions. Recessively inherited mutations of GPR56 result in a severe brain malformation known as bilateral frontoparietal polymicrogyria (BFPP), a condition characterized by abnormally numerous and small gyri in the frontal lobe of the cerebral cortex. Although GPR56 is essential for proper structural development of the brain, it also has a broad tissue expression profile and critically regulates several other physiological functions including hemostasis, immune responses, and male fertility. Like other AGPCR family members, GPR56 has a GPCR autoproteolysis inducing (GAIN) domain within a large extracellular region that cleaves the receptor into non-covalently associated, N-terminal (NTF) and C-terminal fragments (CTF). Shear force removal of the GPR56 NTF exposes a cryptic tethered peptide agonist (TA) that binds to the orthosteric site of the receptor to initiate G protein-dependent signaling. Nearly all AGPCRs with cleavage-capable GAIN domains signal in this manner, yet it is unknown how other potential activation mechanisms influence the phenotypes observed within this receptor subgroup. To discriminate GPR56 cellular responses attributed to TA-activation from TA-independent functions, we created and characterized a full body, TA-cleavage-deficient, Gpr56 H381S+/+, knock-in mouse model. Through histological analyses, we determined that our newly derived Gpr56-/- mice and Gpr56 H381S+/+ mice demonstrated altered cerebellum lobulation patterns and partial fusions of adjacent lobules consistent with a human BFPP phenotype, along with altered neuronal migration patterns within the anterior cerebellum (neuronal heterotopia). Interestingly, Gpr56 H381S+/+ mice did not have reduced myelin basic protein levels within developing mice embryos (E14), a statistically disparate result from the deficits observed previously, and in our Gpr56-/- mice cohort. These results indicate that cleavage and TA-activation of GPR56 is required for proper neuron migration, but may not be required for the maintenance of the oligodendrocyte precursor cell (OPC) pool to support proper oligodendrocyte-mediated myelination of central nervous system (CNS) nerve axons. An additional breeding study of Gpr56 H381S+/+ male mice demonstrated an infertility phenotype comparable to Gpr56-/- male counterparts, indicating that TA agonism is also required in Gpr56-expressed tissues outside of the CNS. Conversely, preliminary Gpr56 H381S+/+ ex vivo and in vivo mouse platelet assays indicate that presence of the NTF of full length GPR56 is enough for normal platelet activation and adhesion mechanisms to occur. Our characterization of the TA-cleavage-deficient Gpr56 H381S+/+ mouse furthers understanding of the heterogeneity of AGPCR activation mechanisms and provides a foundation to identify future modulators of AGPCRs.

Authors & Affiliations
"Tyler F. Bernadyn, Mariane Nascimento, Xinyi Lu, Pauline L. Pan, Michael Holinstat and Gregory G. Tall
Department of Pharmacology, University of Michigan"

About Frank Kwarcinski
"I am research faculty within the department of Pharmacology at the University of Michigan. I work under the supervision of Dr. Gregory Tall and our research primarily focuses on the structural and biochemical characterization of adhesion GPCRs (AGPCR) for mechanism of action and pathogenesis studies. We utilize several genetically modified mouse models to investigate requirements for receptor activator and continuously work to identify novel chemical modulators of AGPCRs through assay development and high-throughput screening efforts. I have previous work experience at two separate contract research organizations centered on assay development, and I am formally trained as a chemical biologist."

Tethered agonist-dependent/independent activation mechanism in AGPCRs

Tethered Peptide Activation Mechanism of Adhesion GPCRs

Peng Xiao

Adhesion G protein-coupled receptors (aGPCRs) constitute an evolutionarily ancient family of receptors that often undergo autoproteolysis to produce α and β subunits. A tethered agonism mediated by the 'Stachel sequence' of the β subunit has been proposed to have central roles in aGPCR activation. Here, we report the cryogenic electron microscopy (cryo-EM) structures of four aGPCRs β subunit in complex with Gs: ADGRG2, ADGRG4, GPR133 and GPR114. The Stachel sequences assume a U shape and insert deeply into the seven-transmembrane bundles. Constituting the FXφφφXφ motif (in which φ represents a hydrophobic residue), five residues of the receptor extend like fingers to mediate binding to the seven-transmembrane domain and activation of the receptor. We also resolved the structure of the full-length ADGRG2 in complex with the exogenous ADGRG2 Stachel-sequence-derived peptide agonist IP15 (ADGRG2(FL)–IP15–Gs). The structure of the ADGRG2(FL)–IP15–Gs complex reveals the structural basis for the improved binding affinity of IP15 compared with VPM–p15 and indicates that rational design of peptide agonists could be achieved by exploiting aGPCR-β structures. By converting the 'finger residues' to acidic residues, we develop a method to generate peptide antagonists towards several aGPCRs. Collectively, our study provides structural and biochemical insights into the tethered activation mechanism of aGPCRs.

About Peng Xiao

"I joined Prof. Jin-Peng Sun's Lab since I graduated from Shandong University in 2012, and worked under the guidance of Prof. Sun as a postdoc/research associate/assistant professor. Since then, I have been working on dissecting the three-dimensional architecture and underlying molecular signaling mechanism of GPCR using cryo-electron microscopy (cryo-EM). So far, I have published 20 peer-reviewed papers as correspondence (or co- correspondence) or first (or co-first) authors, among which, four papers were published in Nature (2022a, 2022b, 2021, 2020); one paper was published in Cell (2021); on paper was published in Science (2023); two papers were published in Nat Chem Biol. (2022, 2018)."

OCT 24

Oct 24 - 9:00 AM

Session II

AGPCR signaling pathways and trafficking

Yuling Feng · Monserrat Avila Zozaya · Erwin G. Van Meir · Pal Kasturi

10:30 AM

Coffee Break with light snacks

11:00 AM

Session III

Molecular tools and biosensors directed at AGPCR signaling and function

Stephanie Häfner · Laurent Sabbagh · Ana Lilia Moreno Salinas

12:00 PM

Session IV

AGPCRs signaling in the nervous system

Joseph Duman · Simeon R. Mihaylov · Anne Bormann

1:00 PM

Complimentary Lunch

2:00 PM

Posters

3:00 PM

Session V

Structural mechanisms of AGPCR signaling and function

Fabian Pohl · Sumit Bandekar · Florian Seufert

4:00 PM

Board meeting/General assembly

5:00 PM

Leave for dinner reception

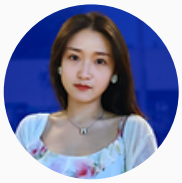
5:30 PM

Complimentary Reception dinner

Session II
October 24th · 9:00 AM

AGPCR 2024

AGPCR signaling pathways and trafficking



Localization of putative ligands for adhesion G protein-coupled receptors in mouse tissues

Yuling Feng

University of Ann Arbor

The ADGRF5/GPR116 receptor is a key regulator of lymphatic endothelial cell identity and function

Monserrat Avila Zozaya

University of North Carolina

Adhesion GPCR BAI1/ADGRB1 can block IGF1R-mediated growth signalling, increase radiosensitivity and augment survival in medulloblastoma

Erwin G. Van Meir

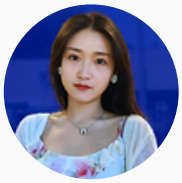
University of Alabama at Birminghan

Site Specific N-Glycosylation Of The N-Terminal Fragment Of ADGRG6 Drives Proteolytic Processing, Trafficking And Signalling

Pal Kasturi

Ashoka University

AGPCR signaling pathways and trafficking



Localization of putative ligands for adhesion G protein-coupled receptors in mouse tissues

Yuling Feng

Adhesion G protein-coupled receptors (aGPCRs), also categorized as Family B2 GPCRs, feature large extracellular regions containing GPCR autoproteolysis-inducing (GAIN) domains. These receptors possess the unique ability to self-cleave and become two protomer receptors with N- and C-terminal fragments (NTF/CTF). Upon protein ligand binding to the NTF and application of cell-movement based extracellular force a tethered agonist of the CTF becomes exposed and binds spontaneously to the orthosteric pocket of the seven-transmembrane domain, thereby initiating intracellular signaling pathways. This distinctive mechanism underscores how aGPCRs exert their biological functions. Despite their significance, many aGPCRs remain orphan receptors. Here, NTF Fc-fusion proteins for GPR56, GPR97, GPR110, GPR114, and GPR116 were produced and used to explore their ligand interactions across various mouse tissues. Notably, the GPR56 NTF probe was found to recognize collagen matrices within cardiovascular tissues, particularly those enmeshing the vascular smooth muscle cell layer within blood vessel walls. The discovery that the GPR56 probe recognized collagen layers in vascular vessel walls highlights our labs previous finding that the platelet-presented GPR56 NTF is a collagen and shear force sensor that participates in G13-mediated platelet shape changes that precede firm platelet adherence at sites of vascular injury (i.e. hemostasis). Intriguingly, we found that the GPR97 probe decorated the luminal endothelium of arterial vessel wall, while showing no affinity for venous endothelium within cardiovascular tissues. The GPR97 probe also decorated the epicardium layer in the heart tissue, a source of progenitor cells contributing to endothelial cell replenishment. Previous studies identified GPR97 expression in neutrophils. The discovery of a putative ligand of GPR97 localized to arterial endothelium highlights the significance of its interaction with arterial endothelium in processes of neutrophil extravasation and leukocyte adhesion. Furthermore, we will present the results of our ongoing investigations aimed at identifying the specific arterial endothelial cells expressing the putative ligand for GPR97, with the potential to elucidate the ligand responsible for mediating neutrophil extravasation.

Authors & Affiliations
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About Yuling Feng
"I am currently a postdoctoral research fellow working with aGPCR pharmacology and physiology in rodents."

AGPCR signaling pathways and trafficking

The ADGRF5/GPR116 receptor is a key regulator of lymphatic endothelial cell identity and function

Monserrat Avila Zozaya

The lymphatic vascular system mediates interstitial fluid homeostasis, immune cell trafficking and intestinal lipid absorption. Disruption of lymphatic vascular permeability or growth can lead to tissue lymphedema and intestinal malabsorption, debilitating conditions for which there are no current G protein-coupled receptor therapies. GPCRs play critical roles in a broad spectrum of physiological and pathological processes. The adhesion G protein-coupled receptor ADGRF5/GPR116 has gained interest due to its involvement in vascular biology, cancer and potential implications in the lymphatic system. However, the precise cellular functions of ADGRF5/GPR116 in lymphatic endothelial cells (LECs) remains largely unexplored. Therefore, we sought to determine the role of ADGRF5/GPR116 in primary human LECs to uncover new therapeutic targets for diseases linked to the lymphatic vascular system. We took advantage of lentiviral transduced shRNAs to knockdown ADGRF5/GPR116 expression in human dermal LECs. Reduction of ADGRF5/GPR116 expression by 70% caused decreased expression of PROX1, LYVE-1 and VE-cadherin, key lymphatic markers essential for LEC identity and establishing intercellular junctions. The disruption of cell-cell contacts was accompanied by disorganized actin stress fibers and decreased activation of Gα13 signaling. Interestingly, ADGRF5/GPR116 knockdown increased the phosphorylation of ERK1/2 and AKT, along with enhanced cellular proliferation, indicating that basal expression of this receptor may generally function to inhibit these signaling pathways. These data support ADGRF5/GPR116 as a multi-functional adhesion receptor regulating molecular aspects of LECs. Future studies will elucidate the potential in vivo implications of these functions in lymphatic vasculature and related conditions.

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About Monserrat Avila Zozaya
"My doctoral research was focused on investigating the cellular effects of missense lung cancer-mutations in the G-protein-coupled receptor Autoproteolysis-Inducing (GAIN) domain of Latrophilin 3 receptor under the mentorship of Dr. Antony Boucard.
I am currently a postdoctoral researcher fellow in Dr. Kathleen Caron's laboratory at UNC. My research focuses on understanding the molecular mechanisms of adhesion GPCRs (aGPCRs) in lymphatic endothelial cells (LECs), a cellular model with unique junction arrangements where aGPCRs are mainly unexplored. "

AGPCR signaling pathways and trafficking

Adhesion GPCR BAI1/ADGRB1 can block IGF1R-mediated growth signalling, increase radiosensitivity and augment survival in medulloblastoma

Erwin G. Van Meir

Medulloblastoma (MB) is one of the most common and aggressive pediatric brain tumors and a better understanding of this disease is warranted to develop new therapeutic approaches. Brain-specific Angiogenesis Inhibitor 1 (BAI1/ADGRB1) is an adhesion GPCR receptor and tumor suppressor epigenetically silenced in MB. We previously reported that BAI1 can bind the MDM2 E3 ubiquitin ligase and relocate it to the cell surface, thereby suppressing its nuclear activity on p53. Whether there are additional MDM2 targets affected by this relocation that may be functionally important in MB development is unknown. We hypothesized that BAI1-mediated relocation of MDM2 might destabilize cell surface oncogenic receptors and found that BAI1 stimulates IGF1R poly-ubiquitination and degradation. Mechanistically, BAI1 fosters MDM2-IGF1R interaction through b-arrestin, an adaptor protein connecting MDM2 to IGF1R. Epigenetic reactivation of BAI1 expression suppressed Stat3 and Akt signaling, and radio-sensitized MB cells, leading to significantly improved survival in orthotopic MB xenograft models in mice regardless of tumor p53 mutation status. These results are important as they demonstrate that BAI1 has dual anti-tumor effects in MB through MDM2 membrane re-localization, stabilizing p53 and blocking IGF1R signaling. They further suggest that epigenetic targeting of BAI1 is a promising therapeutic approach for clinical translation.

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About Erwin G. Van Meir
"Dr. Erwin Van Meir is a professor in the UAB Department of Neurosurgery. He was trained in molecular biology at the Universities of Fribourg and Lausanne, Switzerland where he obtained his Ph.D. in 1989. Dr. Van Meir pursued postdoctoral work at the Ludwig Institute for Cancer Research in San Diego and joined the faculty of Emory University in 1998.

His research interest lies in understanding the molecular basis for human tumor development and how to use this knowledge to devise new therapeutics that will improve patient survival. Van Meir's research examines how genetic alterations and hypoxia induce changes in cell biology that promote tumor formation with particular emphasis on adhesion GPCRs ADGRB1 and ADGRB3. Van Meir has developed novel therapeutic approaches for cancer using oncolytic adenoviruses and anti-angiogenic molecules and is currently developing novel small molecule inhibitors of the hypoxia-inducible factor pathway and the epigenetic reader MBD2 (methyl CpG binding protein 2). His research aims to translate these novel agents to testing in clinical trials with the hope to develop novel medicines for cancer treatment."

AGPCR signaling pathways and trafficking

Site Specific N-Glycosylation Of The N-Terminal Fragment Of ADGRG6 Drives Proteolytic Processing, Trafficking And Signalling

Pal Kasturi

ADGRG6 is a member of the adhesion G-protein-coupled receptor (aGPCR) family, known to play a role in myelination, placentation, blood vessel, and inner ear development. Like many other aGPCRs, ADGRG6 undergoes autoproteolysis at the GPCR-autoproteolysis site (GPS) enclosed within the larger GAIN domain to generate the N-terminal (NTF) and C-terminal fragments (CTF). These cleaved fragments join to form the heteromeric ADGRG6 receptor complex. ADGRG6 NTF has multiple extracellular domains like CUB, PTX, SEA, hormone binding domain, and the GAIN domain, which regulate G-protein signaling by binding to extracellular matrix proteins and mechanotransduction. The short stachel sequence at the extreme N-terminal end of the CTF functions as a tethered agonist to activate cAMP signaling. GPCR signaling and trafficking can be regulated by several different post-translational modifications (PTM). Stehlik et al. have reported that ADGRG6 expressed in lipopolysaccharide stimulated human umbilical vein endothelial cells is N-glycosylated. However, it is unclear which domains of ADGRG6 are N-glycosylated and how this might affect the overall molecular pharmacology of the receptor. Furthermore, are there spatial roles of N-glycosylation in ADGRG6 processing, trafficking, signalling and in-vivo functions? To address these gaps in knowledge, we used biochemical and cell-biological approaches using cell-lines overexpressing wild-type and N-glycosylation mutants of ADGRG6. We observed that N-glycosylation specifically takes place in the NTF and not the CTF of ADGRG6. Our results demonstrate that specific N-glycan residues in different domains of the extracellular NTF of ADGRG6 have distinct roles in ADGRG6 autoproteolysis, furin cleavage, membrane trafficking, and G-protein signalling. In the future, we plan to decipher the roles of N-glycosylation of ADGRG6 in organogenesis and tissue development using zebrafish models.

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Nayonika Chatterjee: Department of Biology, Ashoka University
Prateek Sibal: Department of Biology, Ashoka University"

About Pal Kasturi
"I received my bachelor's degree in Physiology from Presidency College, University of Calcutta and went on to complete my masters from Madurai Kamaraj University. During my PhD training, I worked in the laboratory of Dr. Kathryn Defea at the University of California, Riverside. For my PhD thesis, I worked on non-canonical, scaffold driven signaling by protease activated receptor-2 (PAR2). I joined University of Texas Southwestern Medical Center, for my postdoctoral training. Here, I worked on the regulation of the Sonic Hedgehog pathway by GPCRs which localized to the primary cilia. I then joined the laboratory of Dr. Velia Fowler, at the Scripps Research Institute, as a Judith Graham Poole postdoctoral fellow to work on the role of cytoskeletal proteins in megakaryocyte to platelet differentiation. I joined the Department of Biology at Ashoka University in 2020 as an assistant professor."

Session III
October 24th · 11:00 AM

AGPCR 2024

Molecular tools and biosensors directed at AGPCR signaling and function

The NTF Release Sensor Approach for Drug Discovery for Human Adhesion GPCRs

Stephanie Häfner

Universität Leipzig

bioSens-All: A Multiparametric BRET-Based Platform for Comprehensive Profiling of adhesion GPCR Signaling and Pharmacology-Enabling Drug Discovery

Laurent Sabbagh

Domain Therapeutics

Characterizing hADGRE5/CD97 Activation and Signaling: A Mechanical Stimulation BRET-Based Approach (MS-BRET)

Ana Lilia Moreno Salinas

Université de Sherbrooke

Molecular tools and biosensors directed at AGPCR signaling and function

The NTF Release Sensor Approach for Drug Discovery for Human Adhesion GPCRs

Stephanie Häfner

G Protein-coupled receptors (GPCRs) are common drug targets, yet no approved drugs exist for the Adhesion G Protein-coupled receptors (aGPCRs or ADGRs). This gap is due to their unique autoproteolytic cleavage in the GAIN domain, creating a heterodimer of an N-terminal fragment (NTF) and a C-terminal fragment (CTF), posing challenges for traditional drug discovery.
To address this, we developed the NTF release sensor (NRS), a genetically encoded reporter that facilitates visualization and quantification of aGPCR NTF-CTF separation events both in vitro and in vivo. The NRS fuses the extracellular region of any given aGPCR with a cleavage module from a Notch receptor. Upon NTF dissociation, an intracellular transcription factor (reporter module) is released, generating a specific, measurable biochemical signal.
The NRS system has recently been validated in vivo by targeting the latrophilin-type aGPCR Cirl/ADGRL in Drosophila, revealing NTF release and receptor dissociation within the developing nervous system. It was then adapted for the human aGPCRs CD97/ADGRE5 and Latrophilin/ADGRL3 and tested in HEK293T cells using a luciferase assay to detect NTF release events.
After validating the functionality of the NRS and demonstrating its utility for monitoring aGPCR dissociation across different species, we plan to adapt this technology for high-throughput screening of pharmacological compound libraries to identify potential therapeutic substances for aGPCRs. By leveraging self-cleavage and NTF release, the NRS technology offers a novel approach distinct from conventional GPCR drug discovery methods. This tailored system aims to expedite the identification of drugs targeting the unique aGPCR receptor family and customize the method for disease-relevant human aGPCRs.

Authors & Affiliations
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About Stephanie Häfner
"I am a trained chemist with extensive biochemical experience. After earning my Master of Science in Chemistry, I pursued my PhD in Dr. Michael Schaefer's group at Leipzig University, Germany, focusing on drug screening and utilizing electrophysiological and imaging techniques to study TRP ion channels.
Immediately following my PhD, I joined Dr. Guillaume Sandoz's group in 2019 as a postdoctoral research scientist at Université Côte d'Azur, France. There, I investigated Two-Pore-Potassium channels using electrophysiology, molecular and chemical biology techniques, and fluorescence imaging.
In 2021, I joined Dr. Tobias Langenhan's group, where I currently manage a project to establish a drug screening assay for Adhesion GPCRs using a specialized sensor system and mentor PhD students."

Molecular tools and biosensors directed at AGPCR signaling and function

bioSens-All: A Multiparametric BRET-Based Platform for Comprehensive Profiling of adhesion GPCR Signaling and Pharmacology-Enabling Drug Discovery

Laurent Sabbagh

GThe 3rd generation bioSens-All platform combines BRET-based biosensors that are highly adaptable to the needs of discovery projects for small molecules, peptides, and antibodies. The platform has been successfully used internally to identify biased small molecule negative allosteric modulators for protease-activated receptor 2 (PAR2). The platform revealed different mechanisms-of-action of our lead compound when benchmarked against other antagonists of PAR2. In addition, the platform was used to develop assays for high-throughput screening for challenging adhesion GPCRs. These examples will demonstrate how the bioSens-All platform was used to advance projects from discovery to preclinical candidate nomination and to provide the tools to advance adhesion GPCR biology.

Authors & Affiliations
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About Laurent Sabbagh
"Laurent holds a Ph.D. in immunology from McGill University. Following his doctoral degree Dr. Sabbagh undertook post-doctoral fellowships at the Ontario Cancer Institute and the University of Toronto before being recruited by University of Montreal as an assistant professor working on the role of TNF receptors in immunological memory, inflammation and hematological malignancies. In the fall of 2013, Dr. Sabbagh was recruited by Vertex Pharmaceuticals (Canada) where he worked on biomarker discovery for inflammatory bowel disease and small molecules drug discovery for polycystic kidney disease. Subsequently, Dr. Sabbagh led research projects aimed on drug discovery of small molecules for the treatment of inflammatory disorders and cancer at Paraza Pharma Inc. in Montreal. Laurent is currently leading DTNA discovery group working on GPCRs in immuno-oncology to discover new molecules and antibodies."

Molecular tools and biosensors directed at AGPCR signaling and function

Characterizing hADGRE5/CD97 Activation and Signaling: A Mechanical Stimulation BRET-Based Approach (MS-BRET)

Ana Lilia Moreno Salinas

The human adhesion GPCR (aGPCR) hADGRE5/CD97, primarily expressed on immune cells and upregulated in many cancers, is a potential drug target in oncology/immuno-oncology. Current approaches for studying hADGRE5's signaling often use receptor variants lacking the N-terminal fragment (NTF) exploiting the exposed tethered agonist (TA). While useful, these methods hinder the identification of allosteric modulators acting on the NTF of hADGRE5. Here, we utilized bioluminescence resonance energy transfer (BRET)-based sensors to evaluate hADGRE5's G protein and β-arrestin (β-Arr) engagement. We also developed a novel in cellulo mechanical stimulation (MS) BRET-based assay (MS-BRET) to detect the activity of full-length hADGRE5 through physiological MS. Our findings reveal that full-length hADGRE5 and a truncated N-terminal form (Δ1-437) constitutively activate Gαs, with Δ1-437 also significantly engaging Gα12, Gα13, Gαz, Gi3, Gq, and β-arr. MS of HEK293 cells transfected with full-length hADGRE5 resulted in β-Arr2 recruitment to the plasma membrane, a response abolished by mutating the GPCR proteolysis site (GPS), underscoring the importance of GPS cleavage and TA exposure in aGPCR activation. GPS cleavage-mediated β-Arr2 recruitment was further validated using a synthetically activatable hADGRE5 construct. We further showed that prolonged MS leads to hADGRE5 internalization. To define the mechanistic underpinnings of these observations, we found that a neutralizing antibody to CD55/Decay-accelerating factor (DAF) (a hADGRE5 ligand) significantly dampened MS-induced hADGRE5 β-Arr2 recruitment. This novel methodology offers a valuable tool for deciphering the mechanistic aspects of physiological hADGRE5 (and other aGPCRs) activation and facilitates the identification of ligands that modulate hADGRE5 activity through orthosteric and allosteric receptor sites.

Authors & Affiliations
"Arturo Mancini (2), Samya Aouad (2,3), Herthana Kandasamy (2), Sandra Morrissette (2), Arhamatoulaye Maiga (4), Michel Bouvier (4), Richard Leduc (1), Laurent Sabbagh (2)

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About Ana Lilia Moreno Salinas
"I am currently part of a dynamic research team dedicated to advancing the understanding of G protein-coupled receptors (GPCRs), with a particular focus on the adhesion GPCRs (aGPCRs) family. My expertise lies in exploring the biological properties and signaling pathways activated by aGPCRs, investigating their roles in both normal physiological and pathological conditions. Our research aims to leverage this knowledge to identify novel pharmacological targets and contribute to the development of innovative treatments for a range of diseases, including psychiatric disorders and cancer."

Session IV
October 24th · 12:00 PM

AGPCR 2024

AGPCRs signaling in the nervous system

BAI1/ADGRB1-mediated Regulation of Mitochondrial Morphology in Axons

Joseph Duman

Baylor College of Medicine

Bai1 Is A Novel Neuronal Substrate Of The Psychiatric Risk Kinase TNIK

Simeon R. Mihaylov

The Francis Crick Institute

Intricacies Of Complex Assembly And Ligand Interaction In The Adhesion GPCR Latrophilin/Cirl

Anne Bormann

Leipzig University

AGPCRs signaling in the nervous system

BAI1/ADGRB1-mediated Regulation of Mitochondrial Morphology in Axons

Joseph Duman

Neurons exhibit mitochondrial specialization: axonal mitochondria are smaller and more motile than their dendritic counterparts and can thus provide targeted metabolic support, Ca2+ buffering, and redox signaling. These properties are critical for maintaining active presynapses and affect axonal branching. The signaling that stipulates axonal mitochondrial plasticity is not fully understood. Brain-specific angiogenesis inhibitor 1 (BAI1/ADGRB1) is an adhesion-GPCR largely restricted to the central nervous system that regulates excitatory postsynaptic development, excitatory long-term potentiation, synapse stabilization, presynapse induction, restriction of axonal and dendritic growth, phagocytosis, and angiogenesis. We report that BAI1 regulates the morphology of axonal mitochondria in excitatory hippocampal neurons. Loss of BAI1 from cultured rat hippocampal neurons and in vivo mouse models results in abnormally long axonal mitochondria. BAI1 loss does not affect the baseline motility of axonal mitochondria but increases ATP production. Molecular replacement of BAI1 with mutants affecting specific domains reveals a requirement for BAI1's PDZ-binding motif and GPCR moiety in the determination of mitochondrial morphology in axons. BAI1's cryptic autoagonist (Stachel sequence) also plays a role therein. Pharmacology implicates the small GTPase RhoA downstream of BAI1 in axonal mitochondrial regulation, implying a role for G12/13. Further investigations test the agonists involved in and consequences of BAI1-mediated axonal regulation. These data suggest a cellular signaling mechanism in which some signal(s) relay information through BAI1 to stipulate axonal morphology and identify the first instance of which we are aware in which BAI1 functions though its Stachel-GPCR signaling axis in neurons.

Authors & Affiliations
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About Joseph Duman
"Joseph Duman is an Assistant Professor in the Department of Neuroscience at Baylor College of Medicine, where he studies BAI1's role in the brain and the radiobiology of treatments for brain cancer. He trained at the University of California at Berkeley with John Forte and the University of Washington with Bertil Hille, before joining Kim Tolias' lab at Baylor College of Medicine."

AGPCRs signaling in the nervous system

Bai1 Is A Novel Neuronal Substrate Of The Psychiatric Risk Kinase TNIK

Simeon R. Mihaylov

Schizophrenia (SZ) is a complex and debilitating neuropsychiatric disorder affecting approximately 0.3-0.7% of the global population. It typically manifests in early adulthood and is characterized by a combination of positive symptoms, such as delusions and hallucinations, and negative symptoms, such as social withdrawal and a lack of motivation. The etiology of SZ is still not fully understood, but it is generally believed to arise from a combination of genetic, environmental, and neurodevelopmental factors. TNIK (TRAF2 and NCK-interacting kinase) is a brain-enriched kinase, localized at the post-synaptic density (PSD) of excitatory neurons. Genome-wide association studies have identified three intronic single-nucleotide polymorphisms (SNPs) in TNIK that have been linked to SZ. Additionally, the transcription start site of TNIK was reported to be hypermethylated in SZ patients corresponding to decreased mRNA levels in peripheral blood. However, the precise biological role of TNIK in brain development and disease remains elusive. Using the Shokat chemical genetics approach in conjunction with quantitative TMT labelling mass spectrometry, we identified the adhesion GPCR Bai1 as a novel neuronal substrate of TNIK. Bai1 is enriched in the brain and localized at the PSD. Its role is important for synaptogenesis and synaptic plasticity through regulation of Rac1 and Rho GTPases. Interestingly, Bai1 was also recently functionally associated with schizophrenia. Currently, we are in the process of characterizing the effect of Bai1 phosphorylation by TNIK in the brain, including dendritic spine morphogenesis and binding partner interactions using primary rat neuronal cultures and APEX2 biotin-labeling, respectively.

Authors & Affiliations
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1Kinases and Brain Development Laboratory, The Francis Crick Institute, UK

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About Simeon R. Mihaylov
"I am a postdoctoral researcher in the kinases and brain development laboratory led by Dr Sila Ultanir at the Francis Crick Institute in London, England. I undertook my BSc in Biochemistry and Genetics at the University of Sheffield followed up by obtaining a PhD in molecular neuroscience at the Sheffield Institute for Translational Neuroscience. I then moved to King's College London, where my interest and passion for kinases in brain health and disease developed. I initially worked on mTOR in the pathogenesis of Tuberous Sclerosis Complex and then moved to the Francis Crick Institute working on the psychiatric risk kinase TNIK. I also work on multiple other kinases in our laboratory implicated in various neurodevelopmental and neurodegenerative disorders. My expertise includes biochemical approaches, proteomics and transcriptomics to name a few. I have recently also developed a strong interest in adhesion GPCRs and in particular, Bai1."

AGPCRs signaling in the nervous system

Intricacies Of Complex Assembly And Ligand Interaction In The Adhesion GPCR Latrophilin/Cirl

Anne Bormann

In Drosophila, multiple Latrophilin/ADGRL/Cirl isoforms are generated through intron retention of the Cirl transcript. The resulting six polypeptides are grouped into molecules with seven transmembrane domains (7TM), which is comparable to other GPCR, and 1TM, which lacks the classical GPCR signaling unit. We recently showed that these two Cirl isoform types require each other to tune mechanosensitivity of sensory neurons. This cooperative effect of the isoforms depends on their direct interaction mediated through the N-terminal fragment. The only known ligand of Cirl is the Toll-like receptor 8/Tollo. However, if and how Tollo interacts with Cirl dimers in sensory neurons is not known. Thus, we aim to dissect the geometry and signaling of Cirl complexes and study Tollo's relevance for Cirl-dependent function in mechanosensation.

Authors & Affiliations
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About Anne Bormann
"I am a biochemist by training and studied at Leipzig University from 2015 to 2020. During my Bachelor's in 2018, I sought practical lab experience and found a position as a student assistant in Dr. Nicole Scholz's lab. My main topics were protein biochemistry, Drosophila husbandry, and genetics. I was fortunate that Nicole offered me an opportunity to do my Master's and later on a PhD thesis in her group. Since then, I have broadened my horizons with many more techniques in vivo and in vitro, with a main emphasis on the Adhesion GPCR Latrophilin/Cirl. Currently, I am in the final stages of my PhD, and I am looking forward to new projects and ideas."

Poster Sessions

October 24th · 2:00 PM

AGPCR 2024

Interrogating The Role Of CELSR1 (ADGRC1) In Breast Cancer

Caroline Formstone

University of Hertfordshire

Generation and characterization of collecting duct specific GPR56 knockout mice

Jianxiang Xue

University of New Mexico

Anti-Tumorigenic Role of Brain Angiogenesis Inhibitor 3 (BAI3) in WNT-Activated Medulloblastomas

Virginea de Araujo Farias

University of Alabama at Birmingham (UAB)

Conformational And Functional Coupling Between Extracellular and Transmembrane Regions of a Holo-Adhesion GPCR

Szymon P. Kordon

University of Chicago

Deorphanization Of The Adhesion GPCRs GPR110 and GPR116

Tingzhen Shen

University of Michigan, Ann Arbor

Self-Cleavage of GPR110 SEA Domain and Its Impact on GAIN Domain Autoproteolysis

Bill Huang

NIH

Poster Sessions

October 24th · 2:00 PM

AGPCR 2024

Tethered Agonist Dependent ADGRL3 Signaling Activity In The G12/13 Pathway

Júlia Rosell

University of Copenhagen

The CELSR/ADGRC Homolog Flamingo Is Not Autoproteolytically Processed By The GAIN Domain

Tobias Langenhan

Leipzig University

Structural Insights into the Activation Mechanisms of Adhesion GPCRs

Mao Chunyou

Zhejiang University

GPR110 modulates anxiety-like behaviors and memory function in mice potentially through neuronal and neuroimmune alterations during neurodevelopment

Mariam Melkumyan

NIH

Endocytic Cues Determine the Signaling Profile of Adhesion GPCR ADGRL1 / Latrophilin-1

Sheila Ribalta-Mena

Cinvestav-IPN

Interrogating The Role Of CELSR1 (ADGRC1) In Breast Cancer

Caroline Formstone

Breast cancer is the most common form of cancer amongst women. Ductal carcinomas are increasingly diagnosed but identifying which will progress to invasive disease remains difficult highlighting an urgent need for new biomarkers that distinguish ductal carcinomas on this basis. Planar cell polarity (PCP) proteins contribute to tumour growth and invasion. Recent studies identify CELSR1, a key PCP gene, as a novel biomarker for early-stage breast cancer. CELSR1 is reactivated in luminal-type ductal carcinomas. The impact of CELSR1 on cancer progression, however, is unclear. Our working hypothesis is that distinct CELSR1 protein isoforms differentially regulate tissue adhesiveness by influencing the stability/plasticity of cell-cell and cell-matrix contacts. Notably, our pilot data from luminal-type breast cancer cell lines representative of breast carcinomas with lower versus higher invasive potential reveal differential enrichment of CELSR1 protein isoforms. To test the specific hypothesis that biased expression of CELSR1 isoforms will predict invasive potential of a luminal breast carcinoma we will (a) determine, via loss-of-function assays in vitro and in vivo, whether CELSR1 protein isoforms differentially influence the stability of cell-cell and/or cell-matrix adhesions to dictate breast tumour invasive mechanism (b) quantify CELSR1 isoform expression (mRNA and protein) within patient luminal carcinoma samples exhibiting non-invasive or invasive features, the latter including heterogeneous tumours with mixed pathology. Through study of known protein isoforms of CELSR1, which would be missed in gene expression microarray analyses, we hope to illuminate the prognostic potential of CELSR1 for early-stage breast cancer.

Authors & Affiliations
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About Caroline Formstone
"Cell and developmental biologist with a focus on how planar cell polarity drives complex tissue morphogenesis. I study the cell and tissue level consequences of its failure in foetal development and of its reemployment in cancer"

Generation and characterization of collecting duct specific GPR56 knockout mice

Jianxiang Xue

GPR56 is a multifunctional adhesin G protein-coupled receptor involved in diverse biological processes. The role of GPR56 in the kidneys has been understudied. A recent study demonstrated that GPR56 in the glomerular endothelial cells promoted diabetic kidney disease progression via regulation of eNOS. Using RNAscope in situ hybridization (ISH) for GPR56, aquaporin 2 and NKCC2 (thick ascending limb, TAL marker), we detected GPR56 mRNA highly expressed in the collecting duct and TAL of the loop of Henle with limited expression in the proximal tubule. To determine the physiological role of GPR56 in the collecting duct, we generated a collecting duct-specific GPR56 knockout (GPR56CD-KO) mouse model by crossing GPR56flox (Control) with cadherin 16 Cre mice. The deletion of GPR56 in the collecting duct was confirmed by RNAscope ISH. GPR56CD-KO mice were born at predicted Mendelian frequencies, appeared grossly indistinguishable from Con mice, and developed normally. For baseline phenotypic characterization, blood gas analysis showed no differences in blood pH, blood HCO3-, blood Na+, or blood K+ between GPR56CD-KO and control mice. Metabolic cage experiments demonstrated no differences in fluid intake, urine volume, urinary pH or urine osmolality between genotypes in baseline. 24hr water deprivation experiment showed that GPR56CD-KO mice can concentrate urine as effectively as control mice. In conclusion, we successfully generated collecting duct-specific GPR56 knockout mouse and found no defective urine concentrating ability in GPR56CD-KO mice. This mouse model will be useful to delineate the collecting duct-specific role of GPR56 for renal function, including acid-base regulation.

Authors & Affiliations
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About Jianxiang Xue
"I am a postdoctoral researcher working in the Department of Biochemistry and Molecular Biology, University of New Mexico. I earned my PhD degree in Biomedical Sciences from the University of South Florida. During my graduate studies, using various transgenic mouse models and expertise in intestinal and renal physiology, I systematically characterized the function of sodium/hydrogen exchanger 3 in the intestine and kidneys for fluid and electrolyte homeostasis and acid-base balance. My predoctoral work was supported by an American Heart Association fellowship. Since staring my postdoctoral training, I have continued to develop my expertise to answer fundamental questions on adhesion GPCR in renal physiology and pathology. In my free time, I enjoy reading, workouts, and hiking."

Anti-Tumorigenic Role of Brain Angiogenesis Inhibitor 3 (BAI3) in WNT-Activated Medulloblastomas

Virginea de Araujo Farias

Brain Angiogenesis Inhibitor (BAI) proteins are members of group VII of the adhesion G protein-coupled receptor (aGPCR) family. BAI1-3 are highly expressed in the brain, where they participate in synaptogenesis and synapse maintenance. In cancers, BAI1-3 expression can be lost through epigenetic silencing, copy number loss or truncating mutations. In medulloblastomas (MB), BAI3 (ADGRB3) expression is specifically reduced in the WNT-activated group (WNT-MB), but not in the other three molecular groups. WNT pathway activation in WNT-MB is driven by mutations of the CTNNB1 gene, activating ß-catenin-dependent signaling; however, no interactions between BAI3 and the WNT signaling pathway have been described so far. MAGI3, a PDZ-containing scaffolding protein is known to downregulate WNT signaling by interacting with ß-catenin in gliomas, but it is unknown whether this involves BAI3. To explore a possible connection between BAI3 and ß-catenin signaling through MAGI3 in WNT-MB, we probed for potential protein-protein interactions using co-IP experiments. We found an interaction between BAI3 and MAGI3 in mouse brain lysates. Therefore, we hypothesize that re-expression of BAI3 in WNT-MB cells will restrain ß-catenin activity through the formation of a BAI3/MAGI3/ß-catenin complex, reducing their tumorigenic properties. To test this hypothesis, we created WNT-like MB cell lines stably expressing tet-on wild-type BAI3 or a BAI3 lacking the C-terminal PDZ-binding motif (PBM). We will present the effects of BAI3 re-expression on WNT-MB cells oncogenic properties and signaling.

Authors & Affiliations
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About Virginea de Araujo Farias

“My primary research interest revolves around understanding the mechanisms underlying solid tumor development and developing innovative strategies to overcome this significant challenge. My previous postdoctoral work delved into the radiobiological properties of mesenchymal stromal cells (MSCs) and explored their therapeutic potential for managing radiation-induced side effects in patients. We showed that MSC-released exosomes could significantly enhance the efficacy of radiation therapy for treating melanoma. More recently, using engineered MSCs as vectors for BMP4-based differentiation therapy, I successfully sensitized glioma stem cells to radiation.
In April 2023 I joined Dr. Van Meir's research group as an associate scientist at the University of Alabama at Birmingham, where my focus is on investigating the role of the adhesion G protein-coupled receptor B3 (ADGRB3/BAI3) in WNT-medulloblastoma tumor formation.”

Conformational And Functional Coupling Between Extracellular and Transmembrane Regions of a Holo-Adhesion GPCR

Szymon P. Kordon

Adhesion G Protein-Coupled Receptors (aGPCRs) are key cell-adhesion molecules involved in numerous physiological functions. aGPCRs have large multi-domain extracellular regions (ECR) that mediate cell adhesion and play roles in transmitting extracellular signals to the inside of the cell. Ligand binding and mechanical force applied on the ECR regulate receptor function. However, how the ECR communicates with the seven-pass transmembrane domain (7TM) remains elusive, because the relative orientation and dynamics of the ECR and 7TM within a holoreceptor is unclear. Here, we describe the cryo-EM reconstruction of an aGPCR, Latrophilin3/ADGRL3, and reveal that the conserved GAIN domain, that directly precedes 7TM, adopts a parallel orientation to the membrane and has constrained movement. Single-molecule FRET experiments unveil three slow-exchanging FRET states of the ECR relative to the 7TM within the holoreceptor. GAIN-targeted antibodies, and cancer-associated mutations at the GAIN-7TM interface, alter holoreceptor conformations, and modulate downstream receptor signaling. Altogether, this data demonstrates conformational and functional coupling between the ECR and 7TM, suggesting an ECR-mediated mechanism for aGPCR activation.

Authors & Affiliations
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1. Department of Biochemistry and Molecular Biology, The University of Chicago, Chicago, IL, USA; 2. Neuroscience Institute, Institute for Biophysical Dynamics, and Center for Mechanical Excitability, The University of Chicago, Chicago, IL, USA; 3. Department of Molecular Biosciences, Northwestern University, Evanston, IL, USA; 4. Current affiliation: Department of Structural Biology, Genentech, South San Francisco, CA, USA"

About Szymon P. Kordon
"I am a postdoctoral scholar in the Araç Lab at The University of Chicago, studying the structure and function of aGPCRs. Utilizing synthetic antibody fragments, I aim to understand better the structural basis of the aGPCRs activation and signaling and to characterize ECR-mediated signal transduction at the molecular level."

Deorphanization Of The Adhesion GPCRs GPR110 and GPR116

Tingzhen Shen

University of Michigan, Ann Arbor

The current activation model proposes that the adhesion GPCRs are activated by mechanical force-induced dissociation of the ECR from the 7TM domain. To fully understand how the receptors become activated in physiological contexts, there is a critical need to understand how force-based receptor fragment dissociation occurs beginning with identification of the interacting partners (protein ligands) of their adhesive modules. Here, we developed a toolbox of N-terminal fragment / Fc fusion protein probes and used them to stain cells for protein ligand identification and discovery. We sought ligands for GPR110 and GPR116, as both receptors are cancer biomarkers. Using flow cytometry and confocal microscopy, we discovered that GPR110 and to a lesser degree, the GPR116 probes labeled one mammalian cell line out of many tested, indicating that this cell line may express a cell surface ligand that binds to the receptors adhesive domain(s). The putative GPR110/GPR116-ligand interactions were glycosylation-independent, but trypsin-sensitive, suggestive of a protein-protein interaction (PPI). We are currently isolating the putative ligand(s) from the cell surface using proximity labeling and using mass spectrometry. Preliminary results demonstrate specific proximity labeling by the GPR110 ECR-Fc fusion probe using the mammalian cell line over the negative probes or with other negative cell lines. We are also trying to label the cells with GPR110/GPR116 fusion probes, then pulldown the putative ligand(s) based on a crosslinking strategy. After the AGPCR-ligand interactions are vetted in biochemistry / cell-based experiments, we will investigate how GPR110 and GPR116 fragment dissociation is manifested in physiological contexts.

Authors & Affiliations
"Frank E. Kwarcinski, Gregory G. Tall (University of Michigan, Ann Arbor)"

About Tingzhen Shen
"A graduate student from Tall Lab, department of Pharmacology, University of Michigan, Ann Arbor."

Self-Cleavage of GPR110 SEA Domain and Its Impact on GAIN Domain Autoproteolysis

Bill Huang

The adhesion G-protein coupled receptor (aGPCR) GPR110 contains a self-cleaving SEA (Sperm protein, Enterokinase, and Agrin) domain preceding the conserved GPCR-autoproteolysis-inducing (GAIN) domain in its extracellular region. While GAIN domain autoproteolysis is important for aGPCR activation, the cleavage of the SEA domain and its biological implications remain elusive. To identify the SEA domain cleavage site, we transfected HEK cells with GPR110 variants including the mutation of the putative cleavage site (S207A). Cleaved products of GPR110 that were pulled down by immunoprecipitation were analyzed by high resolution mass spectrometry (MS). The MS analysis combined with mutagenesis revealed that the GPR110 SEA domain cleavage occurs between G206 and S207. Interestingly, we found that either S207A mutation or SEA domain deletion decreases GAIN autoproteolysis, suggesting that the presence of SEA domain and its self-cleavage are crucial for maintaining proper autoproteolysis activity of the GAIN domain. Given the differential roles of the GAIN self-cleavage in various GRR110-induced G protein signaling, we evaluated the effect of S207A and SEA domain deletion in downstream G-protein signal transduction pathways using bioluminescence resonance energy transfer (BRET)- based assays. As expected, S207A and SEA domain deletion impair the stalk peptide-induced GPR110/Gq signaling which is known to be dependent of GAIN autoproteolysis while having no impact on the Gs signaling triggered by synaptamide-like ligand. Our study reveals for the first time the biological relevance of the GPR110 SEA domain and its self-cleavage.

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About Bill Huang
"Researcher"

Tethered Agonist Dependent ADGRL3 Signaling Activity In The G12/13 Pathway

Júlia Rosell

The adhesion GPCR Latrophilin 3 (ADGRL3) has been associated with an elevated risk of attention deficit hyperactivity disorder (ADHD) and substance use in human genetic studies. Furthermore, knockout in several species reveal a hyperlocomotor phenotype and alterations in dopaminergic signaling. ADGRL3 constitutes a large protein structure with several functional properties. The N-terminal adhesive domains are known to stabilize the synapse by binding transsynaptic ligands, and the transmembrane GPCR domain has been shown to regulate intracellular G protein signaling pathways. Despite standing out as a potential target for treatment of neuropsychiatric disorders involving dopamine dysfunction, the intracellular signaling properties of ADGRL3 remain to be fully established. Here, we use an acute activation strategy in combination with a panel of bioluminescent energy transfer (BRET) assays, to map the intracellular signaling pathway of ADGRL3 downstream G12/13. We employ an ADGRL3 construct containing an enterokinase (EK) cleavage site immediately upstream of the internal tethered agonist (TA). Upon enzymatic cleavage by EK, the exposed TA stabilizes an active form of ADGRL3. We measured the translocation and activation of effectors downstream of G12/13, and found that ADGRL3 activation leads to increased p115-RhoGEF and RhoA levels at the plasma membrane. To control that the signal is G12/G13-dependent, we used a KO cell line lacking the relevant G-alpha subunits. RhoA signaling involves actin cytoskeleton re-organization, suggesting that TA activation of ADGRL3 can lead to changes in cell morphology and potentially migration.

Authors & Affiliations
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(2) Department of Psychiatry and Molecular Pharmacology and Therapeutics, Columbia University Vagelos College of Physicians and Surgeons, New York, USA; Division of Molecular Therapeutics, New York State Psychiatric Institute, New York, USA"

About Júlia Rosell
"I am a first-year PhD student with two years of experience in the adhesion GPCR field. I completed my Master's thesis on ADGRL3, where I conducted research involving mammalian cell cultures and techniques such as BRET assays and gene expression assays. Currently, my research focuses on the intracellular signaling of ADGRL3 from a single-molecule perspective and investigating how the binding of extracellular transsynaptic ligands modulates ADGRL3 activity, aiming to elucidate their interplay."

The CELSR/ADGRC Homolog Flamingo Is Not Autoproteolytically Processed By The GAIN Domain

Tobias Langenhan

The aGPCR field has been impeded by the lack of direct support for the identity of proteolytic fragments ascribed to GAIN domain proteolysis through protein sequenc-ing or spectrometric analysis of fragment masses, thereby excluding other possibili-ties for their provenance such as their generation by proteases.
We collected data casting doubt on the autoproteolytic capacity of the ADGRC homolog Flamingo/Starry night (Fmi) in Drosophila melanogaster, with roles in pla-nar cell polarity (PCP) and tissue organogenesis. Previous biochemical analyses suggested that Fmi is self-fragmented at the GPCR proteolysis site (GPS). Genetic removal of fmi results in penetrant embryonic lethality of mutant animals due to de-fects in axonal fasciculation and dendritogenesis, while milder perturbation of fmi levels or functions are expressed by PCP defects in epithelial appendages and cor-rupted asymmetric cell division.
fmi alleles with GAIN domain autoproteolysis-inhibiting mutations show that the en-coded Fmi proteins are still cleaved in vivo. Neither embryonic lethality nor defects in PCP in the eye and wing bristles are observed in fmiΔGPS mutants. We generated a genetically encoded NTF release sensor (NRS) and show that the release pat-terns between Fmi-NRS reporters with intact and mutated GPS are indistinguisha-ble.
Collectively, these results suggest that Fmi is not autoproteolytically processed by the GAIN domain and that modifications to the protein at GPS-homologous positions are inert to the receptor function. Future studies will concentrate on the mechanism behind non-GAIN domain Fmi cleavage and analyse its effects on cell biological and physiological consequences.

Authors & Affiliations
"Tobias Langenhan, Nicole Scholz, Genevieve M. Auger, Helen Strutt, David Strutt"

About Tobias Langenhan
"1997-2004: Medical school and Dr. med. Neuroanatomy (Würzburg, Germany); 2004-2005: M.Sc. Neuroscience (Oxford, UK); 2005-2009: D.Phil. Neuroscience (Oxford, UK); 2009-2016: Group leader, Institute of Neurophysiology (Würzburg, Germany); 2016: Heisenberg professorship (Würzburg, Germany); 2016-to date: Professor and Chair in Biochemistry (Leipzig, Germany)."

Structural Insights into the Activation Mechanisms of Adhesion GPCRs

Mao Chunyou

Adhesion G protein-coupled receptors (aGPCRs) represent a relatively understudied class of GPCRs, yet they are implicated in various physiological and pathological processes. A comprehensive understanding of their signaling mechanisms is essential for the development of modulators for diseases such as cancer, immune disorders, and neurological conditions. In our previous work, we reported the structures of a prototypical aGPCR CD97 in both inactive and active states, revealing a compact inactive conformation and significant conformational changes upon activation, particularly on the intracellular and extracellular sides. We also identified key motifs involved in aGPCR activation. Recently, we have elucidated the high-resolution structures of GPR97 transitioning from its inactive state to both G protein-coupled and arrestin-coupled states. Our findings highlight pronounced conformational shifts across the receptor, especially involving transmembrane helices TM5 and TM6. Notably, we discovered that activation by small molecule ligands and peptide tethered ligands induces markedly different mechanisms of ligand recognition, activation, and coupling, resulting in differential signaling pathways. These insights contribute to a deeper understanding of the signaling mechanisms of aGPCRs, which could inform future therapeutic strategies.

Authors & Affiliations
"Zhang Yan,Zhejiang University"

About Mao Chunyou
"Dr. Mao Chunyou is a researcher at the Zhejiang University School of Medicine and the affiliated Shao Yifu Hospital, as well as a doctoral supervisor and a recipient of the national-level young talent program. He has focused on researching novel regulatory mechanisms related to receptors associated with major diseases and discovering new intervention methods. As the first author or corresponding author, he has published over 20 papers in internationally renowned journals, including Nature (5 papers), Science (2 papers), Cell, Cell Research (3 papers), Molecular Cell (2 papers), and Science Advances."

GPR110 modulates anxiety-like behaviors and memory function in mice potentially through neuronal and neuroimmune alterations during neurodevelopment

Mariam Melkumyan

GPR110, an adhesion G protein coupled receptor (GPCR), is widely expressed in developing brains but diminishes in adult stage except in the hippocampus, a region involved in learning and memory. Ligand-induced GPR110 signaling stimulates neurogenesis and synaptogenesis during development, and the absence of the ligand-induced signaling causes object recognition and spatial memory deficits in adulthood and increased neuroinflammatory responses. Nevertheless, the role of GPR110 signaling in behavioral consequences has not been fully explored.

This study aimed to understand the effects of GPR110 on mouse behaviors in relation to neurodevelopmental and neuroimmune gene and protein expression. Anxiety and memory function were tested using both male and female mice at 5-6 month of age. GPR110 knockout (KO) mice displayed trends for increased anxiety-like behaviors in the elevated plus maze test and in the open field test. Memory tests, including the novel object test and the radial 8-arm maze showed worsened spatial and reference memory in the GPR110 KO mice compared to wildtype mice. The y-maze showed a significant sex by genotype interactions with GPR110 KO male mice having increased number of correct alterations and errors, while the GPR110 KO females had fewer correct alterations and errors.

RNAseq data indicated significantly impaired developmental gene expression for neuronal differentiation, axonogenesis, and synaptogenesis, as well as altered neuroinflammatory marker expression in GPR110 KO mouse brains. Further studies exploring the protein expression and neural activity of these mouse brain will give insight on the mechanism underlying the behavioral consequences associated with the GPR110 receptor.

Authors & Affiliations
"Joel Toro, Bill Huang, Hee-Yong Kim

Laboratory of Molecular Signaling, National Institute of Alcohol Abuse and Alcoholism, NIH"

About Mariam Melkumyan
"Mariam Melkumyan is a postdoctoral fellow at the Laboratory of Molecular Signaling studying the role of GPR110 in neurotransmission and neuroimmune activity involved in learning and memory, anxiety, and alcohol use. Mariam, originally from Armenia, completed her bachelor's degree in Neuroscience at American University in Washington, DC and her dual-title PhD in Neuroscience and Clinical and Translational Sciences at Penn State College of Medicine in Hershey, PA. Mariam started her postdoctoral training in February 2024 and is hoping to become an academic professor and mentor the next generation of scientists."

Endocytic Cues Determine the Signaling Profile of Adhesion GPCR ADGRL1 / Latrophilin-1

Sheila Ribalta-Mena

Cinvestav-IPN

Endosomes recently emerged as signaling hubs accounting for the sustained signaling of G protein-coupled receptors (GPCRs). Sustained signaling in endosomes is produced by “megaplexes” comprising GPCRs (G Protein-Coupled Receptors), G proteins, and β-arrestins upon prolonged ligand exposure. However, the formation of these complexes diverges from the previously proposed canonical model describing GPCR signaling which posited that β-arrestins’ recruitment leads to the disassembly of receptor-G protein complexes into a process named desensitization and thus resulting in mutually exclusive receptor complexes with either β-arrestins or G proteins. Adhesion GPCRs (aGPCRs) exhibit persistent ligand-receptor exposure due to the intrinsic presence of a N-terminal tethered agonist. However, the contribution of endosomes in aGPCR signaling remains elusive. Here, we describe the localization of the adhesion GPCR latrophilin-1 (Lphn1, also known as ADGRL1) in endosomal compartments as evidenced by its coincident localization with intracellular β-arrestins, in both intrinsic as well as ligand-induced conditions. Furthermore, the ability of Lphn1 to functionally couple to different G proteins was severely impeded when expression levels of β-arrestins were altered using miRNA-mediated targeting approaches. Similarly, the co-expression of a dominant-negative mutant of the endocytic factor dynamin largely abrogated Lphn1-G protein coupling as well as downstream signaling. Cell membrane and endosome-specific BRET-based biosensors unveiled that Lphn1 elicited compartimentalized signaling dependent on the presence of β-arrestins and to a minor extent dynamin activity. Supporting the endosomal signalosome formation, we reveal that Lphn1 activity promotes the assembly of complexes comprising specific sets of G protein/ β-arrestins pairs. Altogether our work suggests that endosomal translocation dictates the signaling profile of aGPCR Lphn1.

About Sheila Ribalta-Mena
Cell Biology PhD student

Session V
October 24th · 3:00 PM

AGPCR 2024

Structural mechanisms of AGPCR signaling and function

Structural Determinants Of GAIN Domain Autoproteolysis And Cleavage Resistance Of Adhesion G Protein-Coupled Receptors

Fabian Pohl

University Leipzig

Structural studies of the CELSR1 extracellular region reveal a compact multidomain module of fourteen domains which regulates signaling

Sumit Bandekar

University of Chicago

Unveiling the GPS Cleavage Mechanism in ADGRL1 with QM/MM

Florian Seufert

Leipzig University

Structural mechanisms of AGPCR signaling and function

Structural Determinants Of GAIN Domain Autoproteolysis And Cleavage Resistance Of Adhesion G Protein-Coupled Receptors

Fabian Pohl

The GPCR autoproteolysis-inducing (GAIN) domain is a hallmark feature of adhe-sion G-protein coupled receptors (ADGRs), as this extracellular domain contains an integral agonistic sequence (Stachel) for activation via binding to the 7-transmembrane (7TM) helical domain of the receptor. Many ADGRs are autoproteo-lytically cleaved at the GPCR proteolysis site (GPS), an HXS/T motif within the GAIN domain. However, several ADGRs can be activated without GPS cleavage. We de-termined the crystal structure of the human ADGRB2/BAI2 hormone receptor (HormR) and GAIN domains and found that this ADGR is resistant to autoproteolysis despite the presence of a canonical HLS sequence at the GPS. By structural com-parison and with the help of molecular dynamics (MD) simulations we identified several unique structural features that are important for autoproteolytic cleavage, beyond the canonical HXS/T motif. Disruption of these features reduced autoproteo-lytic activity in ADGRL1/LPHN1 and restored cleavage competence of AD-GRB3/BAI3. Furthermore, conservation analysis indicates that wild type ADGRB2 and ADGRB3 are GPS cleavage-incompetent receptors.

Authors & Affiliations
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About Fabian Pohl
"Mar 2023 – Today Postdoc, University Leipzig, Group of Prof. Langenhan

Apr 2016 – Nov 2022 PhD candidate, University Leipzig, Group of Prof. Sträter

Oct 2011 – Mar 2016 Master of Science in chemistry, University Leipzig

Oct 2008 – Sep 2011 Bachelor of Science in chemistry, University Leipzig"

Structural mechanisms of AGPCR signaling and function

Structural studies of the CELSR1 extracellular region reveal a compact multidomain module of fourteen domains which regulates signaling

Sumit Bandekar

Cadherin EGF Laminin G seven-pass G-type receptors (CELSRs) are conserved adhesion G protein-coupled receptors; they are essential for embryogenesis and neural development. CELSRs have large and enigmatic extracellular regions (ECRs) with nine cadherin repeats and a variety of adhesion domains which couple cell adhesion to signaling. CELSRs regulate planar cell polarity, including the closure of the neural tube. Despite numerous cell and animal studies, molecular details on CELSR proteins are sparsely available, precluding an integrative understanding of CELSR biology. Here, we report the 3.8 Å cryo-EM reconstruction of the CELSR1 ECR which enables unambiguous assignment of the 14 domains within the structure. These domains form a compact module mediated by robust and evolutionarily conserved interdomain interactions. This compact module provides a plethora of potential ligand binding sites for the various adhesion domains within the structure and hints at a model where the compact module could be pulled apart by robust mechanical force. We present biophysical evidence that the CELSR1 ECR forms an extended dimer in the presence of Ca2+, which we propose represents the cadherin repeats dimerizing in a configuration similar to protocadherins. We employ cellular assays with full-length CELSR1 and truncation constructs to assess the adhesive and signaling functions of this protein. We assign the N-terminal CADH1-8 module as necessary for cell adhesion and we show the C-terminal CAHD9-GAIN module regulates signaling. Our work provides molecular context to the literature on CELSR function and lays the groundwork for further elucidation of structure/function relationships.

Authors & Affiliations
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About Sumit Bandekar
"I am an NIH F32 postdoctoral fellow in the Araç Laboratory at the University of Chicago. I study adhesion GPCRs using structural biology perspective and I am interested in how the large multidomain extracellular region regulates receptor function. In my free time, I enjoy biking around Chicago and trying new breweries and restaurants."

Structural mechanisms of AGPCR signaling and function

Unveiling the GPS Cleavage Mechanism in ADGRL1 with QM/MM

Florian Seufert

Adhesion G-protein coupled receptors (aGPCR) are a family of 32 mammalian proteins with a defining conserved GPCR autoproteolysis inducing (GAIN) domain that catalyzes receptor self-cleavage at a GPCR proteolysis site (GPS). The autoproteolytic mechanism has been previously proposed, but remains to be validated.
A previous computational study has uncovered variable flexible protein regions, whose dynamics mediate solvent-accessibility of the catalytically active GPS triad HL|S/T, however classical molecular dynamics approaches fall short of explaining the chemical reaction.
Using a multiscale QM/MM approach - combining computational quantum mechanics with classical molecular dynamics - to study the GAIN domain cleavage mechanism of ADGRL1 reveals the sequence of events at the electronic level, suggesting relative energies for the individual states during the reaction, and provides insight into the structural determinants for a successful GPS cleavage exceeding the catalytically active GPS triad.
By directly scanning and comparing energetic sequences of reaction steps, the most likely pathway and the individual contribution of surrounding protein residues can be elucidated. A stable π-edge contact with a conserved phenylalanine and a protonated glutamate side-chain catalyze the reactant conformation. MD simulations with the parameterized ester intermediate reveal a protonation-dependent dynamic desolvation of the GPS for subsequent ester hydrolysis by restricting water conformations.
Mutational experiments on residues of interest showed that restoring the Phe-His interaction in the uncleaving ADGRB3 GAIN domain partially re-instates cleavage, while its deletion reduces cleavage in the ADGRL1 GAIN domain. We present a two-step GPS cleavage model and respective determinants of the reaction.

Authors & Affiliations
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3 Institute of Bioanalytical Chemistry, Leipzig University, Germany"

About Florian Seufert
"Florian Seufert has studied Biochemistry in Leipzig, before joining the Hildebrand Lab in Leipzig for his PhD."

OCT 25

Oct 25 - 9:00 AM

Session VI

AGPCRs shaping the nervous system

Yimin Zou · Dimitris Placantonakis · Nicole Perry-Hauser

10:00 AM

Coffee Break with light snacks

10:30 AM

Session VII

Physiological and pathological roles of AGPCRs in the nervous system

Beatriz Blanco Redondo · Willem Berend Post

11:10 AM

Dr. GPCR Communication Presentation

Breaking Barriers: My Journey from Mexico to the Heart of the Dr. GPCR Ecosystem and Beyond

Monserrat Avila Zozaya

11:30 AM

Session VIII

Physiological and pathological roles of AGPCRs in the periphery

Cheng-Chih Hsiao · Anastasia Georgiadi · Douglas Tilley

12:30 PM

Complimentary Lunch

1:30 PM

Session VIII

Physiological and pathological roles of AGPCRs in the periphery

Tobias Langenhan · Hee-Yong Kim · Alain Garcia De Las Bayonas · Gabriela Aust

1:30 PM

Session IX

Technology capsule: Light on aGPCR signaling and function

Novoprotein

3:20 PM
Coffee Break with pastries announcement of the aGEM award

4:00 PM
Closing remarks

5:00 PM
Departure

Session VI
October 25th · 9:00 AM

AGPCR 2024

AGPCRs shaping the nervous system

ADGRCs in glutamatergic synapse formation, maintenance and degeneration

Yimin Zou

University of California, San Diego

Antibody-drug conjugates targeting CD97 in glioblastoma

Dimitris Placantonakis

NYU Grossman School of Medicine

Adhesion G protein-coupled receptor latrophilin-3 (ADGRL3) modulation of dopaminergic neurotransmission

Nicole Perry-Hauser

Columbia University

AGPCRs shaping the nervous system

ADGRCs in glutamatergic synapse formation, maintenance and degeneration

Yimin Zou

ADGRCs (Celsr1-3) are components of the conserved planar cell polarity (PCP) pathway, which establishes and maintains cell and tissue polarity along the tissue plane in all tissues. Work from our lab showed that the PCP components, including ADGRC2 and ADGRC3, are localized in the developing and adult synapses and interact with synaptic scaffold proteins and glutamate receptors and are responsible for the formation and stability of the vast majority of glutamatergic synapses in the mammalian brain. Initial impairment of synaptic functions, which occurs early in Alzheimer's disease, and subsequent massive loss of synapses are closely correlated with the decline of cognitive function. We showed that oligomeric Aβ binds to ADGRC3 on the same domain required for the interaction with Frizzled3, weakens their interaction and assists Vangl2 in disassembling synapses. Conditionally knocking out Ryk, required for Vangl2 function, protected synapses and preserved cognitive function in a mouse model for Alzheimer's. Massive synapse loss in the prefrontal cortex is a hallmark of massive depressive disorder. Injection of low-dose ketamine, an antidepressant, can lead to acute (in several hours) and sustained (up to several weeks) antidepressive effects. Restoration of synaptic connections induced by low-dose ketamine has been found associated with the sustained antidepressive effects. We showed that ADGRC2 and ADGRC3 are required for the restoration of glutamatergic synapses in prefrontal cortical neurons of chronically stressed animals and their behavioral remission induced by low-dose ketamine. I will also present ongoing work on the signaling mechanisms of how ADGRCs regulate synapse formation, maintenance and plasticity.

About Yimin Zou

"I received Ph.D from University of California at Davis and San Diego in 1995 and then postdoctoral training from University of California, San Francisco in 2000. I was an assistant and then associate professor with tenure at the University of Chicago from 2000 to 2006 and moved to University of California San Diego as an Associate Professor in 2006. I became full professor in 2011 and Vice Chair of the Neurobiology Department at UC San Diego in 2012. I served as the Chair of the Neurobiology Department at UC San Diego from 2014 to 2017. My research focus is the mechanisms of neural circuit development, function and disease."

AGPCRs shaping the nervous system

Antibody-drug conjugates targeting CD97 in glioblastoma

Dimitris Placantonakis

Glioblastoma (GBM) is the most common and aggressive primary brain malignancy. Several adhesion G protein-coupled receptors (aGPCRs) have recently been shown to play critical roles in GBM biology. We showed that CD97 (ADGRE5), in particular, drives tumor growth via effects on GBM stem cell self-renewal and metabolism, but also has a therapeutically favorable expression pattern: it is highly expressed in all GBM specimens, but is absent from healthy brain tissue. To exploit this expression profile, we have developed antibody-drug conjugates (ADCs) targeting CD97, by screening a synthetic human antibody library. We initially tested the ADC using in vitro WST-8 viability assays in human GBM cell lines and cell types that lack CD97. We observed significantly lower LD50 values in patient-derived and U87 GBM cell cultures vs. CD97-lacking cells. We also found significantly lower LD50 values when treating human GBM cells with the ADC (0.6788 nM), as compared to control ADC targeting RSV glycoprotein F (19.964 nM). In vivo intratumoral administration of the ADC in patient-derived GBM xenografts in the brain of immunodeficient mice resulted in significant reduction of tumor growth and prolongation of survival of host mice. Collectively, these data suggest that ADCs targeting CD97 impair tumor growth in preclinical GBM models and are promising candidates for future clinical trials.

Authors & Affiliations
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New York University Grossman School of Medicine"

AGPCRs shaping the nervous system

Adhesion G protein-coupled receptor latrophilin-3 (ADGRL3) modulation of dopaminergic neurotransmission

Nicole Perry-Hauser

Columbia University

Adhesion G protein-coupled receptor latrophilin-3 (ADGRL3) is a cell adhesion molecule highly expressed in the nervous system. Polymorphisms in ADGRL3 are associated with increased risk of attention-deficit/hyperactivity disorder and substance use disorder. In addition, disruption of ADGRL3 leads to hyperactivity and altered dopaminergic neurotransmission across animal species. Given that dopamine (DA) is crucial for numerous basic functions, including movement, memory, reward, and motivation, ADGRL3 presents a novel target for modulating dopaminergic neurotransmission, with potential therapeutic implications for neuropsychiatric disorders involving DA dysfunction.

Striatal DA is linked to reinforcement learning and is released in response to reward-predicting cues. Previous findings in ADGRL3 KO mice indicate increased impulsivity and motivation in behavioral tasks, yet the connection to altered DA neurotransmission remains unclear. In this study, we explored the role of the DA system in reward processing in ADGRL3 knockout mice using in vivo fiber photometry with the biosensor dLight1.2. Mice underwent the progressive ratio schedule of reinforcement task, a standard method for assessing motivation, while imaging was conducted in the nucleus accumbens.

Our results indicated that ADGRL3 KO mice exhibit comparable levels of motivation to wild-type mice during the progressive ratio task. However, they consistently showed delayed reward retrieval compared to wild-type counterparts. Additionally, DA levels in ADGRL3 KO mice during these tasks are significantly lower, particularly when aligning photometry signals to lever extension and dipper up events. Future research will investigate whether these lower DA levels indicate reduced DA availability or if baseline DA levels in these animals are inherently higher.

About Nicole Perry-Hauser

I am an associate research scientist endeavoring to build a productive, independent scientific research career in adhesion G protein-coupled receptor (aGPCR) biology. My long-term research interests involve resolving signaling pathways downstream of aGPCRs and establishing how/if these receptors' adhesive properties influence signaling events, and in turn whether signaling impacts synapse formation and neuronal wiring. I initially became interested in GPCR signal transduction during my graduate training in the Department of Pharmacology at Vanderbilt University where I studied under the co-mentorship of Dr. Vsevolod V. Gurevich and Dr. Tina M. Iverson. I then pursued a postdoctoral research position under the mentorship of Dr. Jonathan A. Javitch in the Department of Psychiatry at Columbia University Irving Medical Center.

Session VII

October 25th · 10:30 AM

AGPCR 2024

Physiological and pathological roles of AGPCRs in the nervous system

Uncovering the signaling pathway of the ADGRA homolog Remoulade in Drosophila

Beatriz Blanco Redondo

Uniklinikum Leipzig

The Adhesion GPCR Latrophilin Interacts With The Notch Pathway To Control Germ Cell Proliferation

Willem Berend Post

Institute for Cell Biology, Heinrich Heine University Düsseldorf

Physiological and pathological roles of AGPCRs in the nervous system

Uncovering the signaling pathway of the ADGRA homolog Remoulade in Drosophila

Beatriz Blanco Redondo

The Drosophila genome contains five loci encoding adhesion G-protein coupled receptors (aGPCRs). Phylogenetic analysis revealed that the remoulade (remo) gene is a homologue of the vertebrate aGPCR ADGRA family, sharing the same overall receptor domain structure.

In vivo expression profiling has shown Remo expression in the central (CNS) and peripheral nervous systems (PNS) of third-instar larvae (L3) and adults. In L3 PNS specimen Remo is expressed in a subset of neurons expressing the DEG/ENaC channel pickpocket (PPK), which is involved in transduction of sensory information like nociception. remoKO larvae and animals, in which remo was knocked down in ppk-neurons through RNA interference, show a higher nocifensive response compared to wildtype remorescue controls indicating that remo is required in PPK-neurons for this behaviour.

Furthermore, with the aim to analyse the biochemical properties of Remo, we performed immunoprecipitation analysis. We found that the receptor is cleaved despite the lack of a consensus GPS sequence. Hence, Remo is proteolytically processed, either by the GAIN domain or an alternative protease that cleaved Remo near the GPS.

We also aimed at identifying the signaling pathway that Remo is involved in. The mammalian Remo homolog ADGRA2/Gpr124 cooperates with other GPCRs of the Frizzled family, and the transmembrane proteins RECK and Lrp5/6. Collectively these proteins form a cell surface complex that acts as a recognition platform for Wnt ligands. Knowledge of the structural dynamics of this complex is limited and pharmacological and in vivo systems that would allow its characterization are scarce. Remo may serve a role in this peculiar signaling pathway and require further analysis.

Authors & Affiliations
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2Laboratory of Neurovascular Signaling, Department of Molecular Biology, ULB Neuroscience Institute, Université libre de Bruxelles (ULB), Gosselies B-6041, Belgium"

About Beatriz Blanco Redondo
"I studied Biomedicine at the University of Barcelona. After my bachelors, I moved to Germany where I obtained my Master's of Science and PhD degree in Dr. Buchner's group at the University of Wuerzburg.
Shortly after receiving my PhD, I joined Dr. Neil Shneider's group as a postdoctoral research scientist at Columbia University, New York, where I studied the mechanisms of motor neuron degeneration in Amyotrophic Lateral Sclerosis (ALS).
In 2017, I joined the group of Prof. Langenhan where I am studying and characterizing newly generated adhesion GPCR receptors in Drosophila as a model organism for future pharmacological applications."

Physiological and pathological roles of AGPCRs in the nervous system

The Adhesion GPCR Latrophilin Interacts With The Notch Pathway To Control Germ Cell Proliferation

Willem Berend Post

The processes of cell proliferation and differentiation, along with their regulation, are fundamental to many biological functions. Dysregulation can lead to severe consequences, such as uncontrolled cell division. A crucial pathway involved in these processes is the Notch signaling pathway. Here, we show that the Latrophilin/LPHN/ADGRL homolog LAT-1 in the nematode Caenorhabditis elegans interacts with the Notch ligand and DSL homolog LAG-2 to influence cell proliferation.
A lat-1 null mutant has a reduced number of mitotic cells and overall germ cells in the proliferation zone of the nematode's gonad. This effect is cell non-autonomous as it can be ameliorated to wild-type levels by expressing solely the aGPCR N terminus tethered to the membrane. Using reporters as well as epistasis analyses, we revealed that LAT-1 enhances Notch activity. Similar to other organisms, in C. elegans, the Notch ligand (LAG-2) activates the Notch receptor (GLP-1) present on proliferating germ cells. Upon activation, the intracellular portion of the Notch receptor translocates into the nucleus to function as a transcription factor. Our findings indicate that in lat-1 mutants, this translocation rate is reduced, leading to diminished Notch activity.
Using molecular modelling as well as in vitro BRET and in vivo BiFC analyses, we show that the LAT-1 N terminus directly interacts with the Notch ligand LAG-2, thereby positively modulating Notch activity. We further provide insights into the molecular details of this interaction by delineating the receptor domains essential for this interaction.
Thus, our data identifies LAT-1 as a direct modulator of the Notch pathway.

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

3 Department of Molecular and Cellular Physiology, Stanford University, Stanford CA, USA

4 Department of Anaesthesiology and Intensive Care, Medical Faculty, Leipzig University, Leipzig, Germany

5 Institute for Drug Discovery, Faculty of Medicine, Leipzig University

6 School of Medicine, University of Global Health Equity, Kigali, Rwanda"

About Willem Berend Post
"Willem Berend Post is a PhD student in Cell Biology at Heinrich Heine University in Düsseldorf, Germany. His research focuses on the relevance of aGPCRs in physiology and signaling using both in vitro and in vivo approaches."

Dr. GPCR Community Presentation

October 25th · 11:10 AM

AGPCR 2024

Monserrat Avila-Zozaya
Dr. GPCR Volunteer

The Dr.GPCR Ecosystem platform aspires to provide opportunities to connect, grow, and thrive as a great GPCR Community.

Breaking Barriers: My Journey from Mexico to the Heart of the Dr. GPCR Ecosystem and Beyond

About Monserrat Avila-Zozaya

“My doctoral research was focused on investigating the cellular effects of missense lung cancer-mutations in the G-protein-coupled receptor Autoproteolysis-Inducing (GAIN) domain of Latrophilin 3 receptor under the mentorship of Dr. Antony Boucard.

I am currently a postdoctoral researcher fellow in Dr. Kathleen Caron's laboratory at UNC. My research focuses on understanding the molecular mechanisms of adhesion GPCRs (aGPCRs) in lymphatic endothelial cells (LECs), a cellular model with unique junction arrangements where aGPCRs are mainly unexplored.”

Session VIII
October 25th · 11:30 AM

AGPCR 2024

Physiological and pathological roles of AGPCRs in the periphery

ADGRG1/GPR56 regulates survival of terminally differentiated CD8+ T cells

Cheng-Chih Hsiao

Amsterdam University Medical Center

Adhesion GPCR GPR116/Adgrf5 controls a lineage of anti-thermogenic adipocytes with implications for adaptive thermogenesis during prolonged cold exposure

Anastasia Georgiadi

Helmholtz Centre Munich

ADGRF5-mediated regulation of cardiac health and disease

Douglas Tilley

Temple University

The CELSR/ADGRC Homolog Flamingo Is Not Autoproteolytically Processed By The GAIN Domain

Tobias Langenhan

Leipzig University

Characterization of Phenotypes Associated with GPR110 Deletion

Hee-Yong Kim

NIH

The Adhesion GPCR Cupidon Regulates Mating In The Closest Relatives Of Animals

Alain Garcia De Las Bayonas

Howard Hugues Medical Institute , UC Berkeley

Critical role for CD97/ADGRE5 in the induction of allergic airway inflammation

Gabriela Aust

University Leipzig

Physiological and pathological roles of AGPCRs in the periphery

ADGRG1/GPR56 regulates survival of terminally differentiated CD8+ T cells

Cheng-Chih Hsiao

Amsterdam University Medical Center

Introduction |
A loss-of-function mutation in ADGRG1, encoding the adhesion GPCR GPR56, is known for its causative role in the severe human brain malformation bilateral frontoparietal polymicrogyria (BFPP). Besides its presence in developing neurons, GPR56 expression is found on terminally differentiated NK and T cells. Functional studies suggest that GPR56 regulates their cell migration and effector functions, acting as an inhibitory immune checkpoint. Gene expression in NK and T cells in BFPP patients has not been studied.

Experimental design |
Of n=4 BFPP patients and n=7 age-matched controls, we investigated NK- and T-cell phenotypes with flow cytometry and studied bulk transcriptomes of sorted NK and T cells.

Results |
Compared to controls, CD8+ T cells from BFPP patients were skewed towards increased proportions of CD27+CD45RA+ naïve and reduced proportions of CD27-CD45RA+ terminally differentiated cells. Terminally differentiated CD8+ T cells from BFPP patients expressed lower levels of ribosomal protein L and S families and anti-apoptotic genes (BAG1, BCL2L1-AS1, FASTKD1). Genes up-regulated in terminally differentiated CD8+ T cells from BFPP patients were associated with cytotoxicity (MPEG1/perforin-2), mediation of signal transduction (CARD11, TSPAN6/tetraspanin 6, TMEM67, USP42), and immune activation (FCER1G, HDAC9, LAT2, LGR6).

Conclusion |
Loss of GPR56 function in BFFP is associated with reduced abundance of terminally differentiated CD8+ T cells, coinciding with differences in cell activation and survival. These findings indicate the role of GPR56 in regulating cellular cytotoxicity.

About Cheng-Chih Hsiao
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Physiological and pathological roles of AGPCRs in the periphery

Adhesion GPCR GPR116/Adgrf5 controls a lineage of anti-thermogenic adipocytes with implications for adaptive thermogenesis during prolonged cold exposure

Anastasia Georgiadi

Aim: Most abundant GPCRs in brown adipose tissue belong to class A and adhesion GPCRs. Previous studies have focused on class A GPCRs, however, the role of Adhesion GPCRs in thermogenesis has not been explored. Here, we followed an unbiased single nuclei approach to identify temperature regulated adhesion GPCRs in BAT. We found that amongst the 15 cold altered adhesion GPCRs, GPR116/Adgfr5 to be the most abundant. The aim of this study is to understand the role of GPR116/Adgrf5 in BAT cold induced remodeling.
Methods: Single nuclei: Split Pool Ligation-based Transcriptome sequencing combinatorial barcoding. Cold exposure: WT and transgenic mouse lines of exon2 deletion of GPR116 (GPR116ex2del) were exposed for 2 weeks at 8oC. Metabolic phenotyping was performed with indirect calorimetry.
Results: We found that whole GPR116ex2del mice did not show any abnormal phenotype at room temperature, but manifested impaired thermogenesis upon prolonged cold exposure. Single nuclei analysis in BAT of GPR116ex2del revealed a big population of anti-thermogenic adipocytes (Cyp2e1+, Aldh1a1+) and adipocytes populations which were Ucp1+, but Adrb3low and showed a glycolytic and lipogenic, rather a fat oxidative gene signature. RNA velocity analysis and in vitro experiments supported a subpopulation of preadipocytes and endothelial cells, as possible lineages for the GPR116 dependent emergence of anti-thermogenic adipocytes.
Conclusion: GPR116 controls a lineage of anti-thermogenic adipocytes. Ongoing experiments investigate the mechanism via which the extracellular part of GPR116 control a preadipocyte and endothelial cell subpopulations lineage of anti-thermogenic adipocytes in BAT.

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Physiological and pathological roles of AGPCRs in the periphery

ADGRF5-mediated regulation of cardiac health and disease

Douglas Tilley

The impact of adhesion GPCRs (AGPCRs) on cardiac function normally or during disease has been largely understudied. We evaluated AGPCR expression from left ventricular (LV) samples of adult mice, of which Adgrf5 was one of the most highly expressed. Notably, decreased ADGRF5 expression is observed in both mouse and human failing LV tissue, and specifically in cardiomyocytes. To investigate the impact of CM-specific ADGRF5 on the heart, we crossed floxed ADGRF5 (ADGRF5f/f) and αMHC-Cre mice to generate constitutive, CM-specific ADGRF5 knockout mice (F5cmKO). These mice display normal cardiac structure and function at 12 weeks of age as assessed via echocardiography, gravimetrics and immunohistochemistry. However, F5cmKO mice develop cardiac dysfunction, maladaptive remodeling, and increased mortality over time, even in the absence of pathologic insult. RNAseq analysis of LV tissue from 12-week-old F5cmKO mice led to the identification and subsequent validation of Scn1b as significantly upregulated in F5cmKO CM. Scn1b encodes for the β subunits of voltage-gated sodium channels (Scnβ1/β1B), alterations of which could lead to arrhythmias. Indeed, conscious electrocardiogram (ECG) readings via telemetry indicated F5cmKO mice had a high burden of premature ventricular contractions (PVC), including ventricular tachycardia associated with cardiac enlargement. Using a combination of biomolecular reporters and gene expression analyses in neonatal rat ventricular myocytes (NRVM), we confirmed that ADGRF5 controls Scn1b expression in a Gαq/11 protein-dependent manner. Thus, loss of CM-specific ADGRF5 leads to increased Scn1b expression with enhanced susceptibility to stress, arrythmias and sudden death, thus may provide a novel target to regulate cardiac rhythm.

About Douglas Tilley

"Research in the Tilley laboratory focuses primarily upon aspects of GPCR regulation of cardiac function, inflammation and remodeling during HF or following acute cardiac injury. Much of this work centered on elucidating novel mechanisms by which β-adrenergic receptors impact cardiac structure and function, and has evolved to encompass their roles in regulating immune cell response to acute cardiac injury or chronic stress. Additionally, the lab has begun to investigate potential roles for previously unrecognized cardiac-expressed GPCRs in the regulation of physiologic/pathologic function in the heart in an effort to uncover novel therapeutic directions for HF, including adhesion GPCRs (AGPCRs). In all, research in the Tilley lab spans molecular pharmacology to pathophysiology studies focused primarily in the cardiovascular realm."

Physiological and pathological roles of AGPCRs in the periphery

The CELSR/ADGRC Homolog Flamingo Is Not Autoproteolytically Processed By The GAIN Domain

Tobias Langenhan

The aGPCR field has been impeded by the lack of direct support for the identity of proteolytic fragments ascribed to GAIN domain proteolysis through protein sequencing or spectrometric analysis of fragment masses, thereby excluding other possibilities for their provenance such as their generation by proteases.
We collected data casting doubt on the autoproteolytic capacity of the ADGRC homolog Flamingo/Starry night (Fmi) in Drosophila melanogaster, with roles in planar cell polarity (PCP) and tissue organogenesis. Previous biochemical analyses suggested that Fmi is self-fragmented at the GPCR proteolysis site (GPS). Genetic removal of fmi results in penetrant embryonic lethality of mutant animals due to defects in axonal fasciculation and dendritogenesis, while milder perturbation of fmi levels or functions are expressed by PCP defects in epithelial appendages and corrupted asymmetric cell division.
fmi alleles with GAIN domain autoproteolysis-inhibiting mutations show that the en-coded Fmi proteins are still cleaved in vivo. Neither embryonic lethality nor defects in PCP in the eye and wing bristles are observed in fmiΔGPS mutants. We generated a genetically encoded NTF release sensor (NRS) and show that the release pat-terns between Fmi-NRS reporters with intact and mutated GPS are indistinguisha-ble.
Collectively, these results suggest that Fmi is not autoproteolytically processed by the GAIN domain and that modifications to the protein at GPS-homologous positions are inert to the receptor function. Future studies will concentrate on the mechanism behind non-GAIN domain Fmi cleavage and analyse its effects on cell biological and physiological consequences.

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About Tobias Langenhan
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Physiological and pathological roles of AGPCRs in the periphery

Characterization of Phenotypes Associated with GPR110 Deletion

Hee-Yong Kim

G-protein coupled receptor 110 (ADGRF1, GPR110), an adhesion GPCR recently deorphanized, plays an important role in in the development of neurons and cognitive function. Synaptamide, an endogenous ligand for GPR110, binds to the N-terminal G-protein autoproteolysis-inducing (GAIN) domain of GPR110, and activates GPR110/cAMP signaling. This activation promotes neurogenic differentiation of neural stem cells, neurite growth, and synaptogenesis of developing neurons. In addition, a significant role of GPR110 in blood brain barrier (BBB) function has been discovered. GPR110 is highly expressed in mouse and human NPCs and neurons, while its expression was absent in astrocytes. GPR110 is also highly expressed in the kidney, however, little is known about the function of this receptor in renal physiology. To extend our understanding of the role of GPR110 signaling in kidney, we evaluated the urine albumin level in mice devoid of GPR110 gene (GPR110 KO) compared to the wild type (WT). To provide the molecular basis for the renal phenotype, we analyzed in parallel differential expression of kidney proteins in GPR110 KO and WT mice by label-free LC-MS/MS and pathway analysis. We found that the albumin to creatinine ratio was significantly elevated in urine samples obtained from GPR110 KO mice, indicating glomerular filtration dysfunction. The change in protein expression of key proteins including VEGFA is associated with the abnormal renal phenotype of albumin urea in GPR110 KO mice. In addition to the central nervous system phenotype such as learning and memory deficit and BBB dysfunction, our study revealed a new renal phenotype associated with lack of GPR110 signaling.

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Physiological and pathological roles of AGPCRs in the periphery

The Adhesion GPCR Cupidon Regulates Mating In The Closest Relatives Of Animals

Alain Garcia De Las Bayonas

All animals develop through the recognition, adhesion, and fusion of a differentiated sperm and egg. Although fundamental, the evolution of gametogenesis and fertilization in animals is poorly understood. Recently, evidence for sex has been described in choanoflagellates, the closest living relatives of animals. Under nutrient depletion, the model choanoflagellate Salpingoeca rosetta forms distinct cell types that aggregate, fuse, and undergo meiotic recombination. Additionally, the bacterium Vibrio fischeri also induces mating in S. rosetta cultures, suggesting that multiple environmental cues can trigger sex. Importantly, the signaling pathways underlying sexual reproduction in these different contexts have not been investigated.

In this study, we report the discovery of an adhesion GPCR, named Cupidon, that regulates the switch from vegetative growth to sexual reproduction in S. rosetta. We found that the knock-out of cupidon induces a gain in cell adhesion and cell fusion, resembling the mating behavior of wild-type cells under nutrient depletion. Cupidon mutants, similar to starved wild-type cells, upregulate various extracellular matrix-related genes, including teneurins and metalloproteases. Finally, we showed that nutrient availability controls the dissociation of the N-terminal fragment in Cupidon.

Together, our results suggest that Cupidon prevents sexual reproduction in S. rosetta under high nutrient availability, by inhibiting genes involved in gamete recognition.

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About Alain Garcia De Las Bayonas
"Hi everyone! I am currently finishing my postoc in the laboratory of Pr Nicole King at UC Berkeley where I am studying the evolution of GPCR families in choanoflagellates, the sister group of animals. I have a particular interest in understanding the premetazoan function of adhesion GPCRs."

Physiological and pathological roles of AGPCRs in the periphery

Critical role for CD97/ADGRE5 in the induction of allergic airway inflammation

Gabriela Aust

Allergic asthma, a common chronic disease affecting more than 340 million people worldwide, is caused by an inappropriate T helper 2 (TH2) cell–mediated immune response to environmental allergens such as house dust mite (HDM). The adhesion GPCR CD97/ADGRE5 is highest expressed on immune cells but also present on epithelial cells, especially those of the lung, in human and mouse. Notably, CD97 in lung (patho)physiology has not been characterized yet.
Here, we investigated the role of CD97 in the development of allergic asthma. Loss of CD97, induced either genetically in Cd97-/- mice or by targeting CD97 with a CD97 antibody (Ab), reduces allergic asthma in ovalbumin- and HDM-induced acute and chronic mouse asthma models. The absence of CD97 increased eosinophilic inflammation in the broncho-alveolar lavage and in lung tissue and enhanced allergen-specific IgE and TH2 cytokine levels when compared with the appropriate controls. The CD97-dependent effect is mainly caused by loss of CD97 from immune, not lung epithelial cells as conditional Cd97-/- mice show. The fact that only a single CD97 Ab application just before HDM sensitization induced an increase in allergic airway inflammation indicates a role of CD97 in the early immune response. Indeed, transfer of allergen-primed bone marrow-derived dendritic cells of Cd97-/- mice induced airway inflammation in untreated BALB/c mice. Furthermore, immediately after HDM sensitization Cd97-/- mice showed activated lung dendritic and CD4+ T cells not seen in the appropriate controls. In summary, CD97 plays a critical role in allergic asthma by attenuating the induction of the TH2 cell–mediated immune response.

Technology capsule
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AGPCR 2024

Gavin Zhang

Novoprotein North America

Light on aGPCR signaling and function: NovoiSMART - A new platform for GPCR antibody drug discovery

Developing monoclonal antibody drugs against GPCRs and other multi-pass transmembrane targets, such as ion channels, remains a significant challenge. Novoprotein developed a NovoiSMART technology, utilizing mRNA-based immunization, which can overcome these obstacles by producing high-quality antibodies that more accurately mimic natural protein structures. This approach contrasts with other antigen forms like peptides or DNA, which face limitations in structural integrity and immunogenicity. mRNA technology, demonstrated in the success of COVID-19 vaccines, is emerging as a promising method for antibody discovery. Several case studies of GPCR and other multi-pass transmembrane targets are presented, including GPRC5D, Claudin 6 and Napi2b. These studies show that mRNA immunization yields higher antibody titers and greater epitope diversity compared to other methods. These examples underscore the potential of NovoiSMART technology in developing highly specific antibodies for complex targets, with implications for overcoming challenges like drug resistance and tumor escape.

About Gavin Zhang

Gavin is a currently a director of business and operations at Novoprotein Scientific. His research experience includes phylogenetics and cancer epigenetics.

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