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COMMENTARY

The expanding functional roles and signaling mechanisms of adhesion G protein–coupled receptors

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The adhesion class of G protein–coupled receptors (GPCRs) is the second largest family of GPCRs (33 members in humans). Adhesion GPCRs (aGPCRs) are defined by a large extracellular N-terminal region that is linked to a C-terminal seven transmembrane (7TM) domain via a GPCR-autoproteolysis inducing (GAIN) domain containing a GPCR proteolytic site (GPS). Most aGPCRs undergo autoproteolysis at the GPS motif, but the cleaved fragments stay closely associated, with the N-terminal fragment (NTF) bound to the 7TM of the C-terminal fragment (CTF). The NTFs of most aGPCRs contain domains known to be involved in cell–cell adhesion, while the CTFs are involved in classical G protein signaling, as well as other intracellular signaling. In this workshop report, we review the most recent findings on the biology, signaling mechanisms, and physiological functions of aGPCRs.

Keywords: adhesion G protein–coupled receptor; structural biology; signal transduction; mechanosensation; development; neurobiology; immunology; cancer

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Introduction

The adhesion G protein–coupled receptor (aGPCR) workshop meetings began in 2002 as an effort to encourage informal discussion of findings concerning aGPCR research. Early research in the field focused on the roles of aGPCRs in immunology, neurobiology, and development; however, over the next decade, the biennial workshops gradually revealed increasingly diverse and complex roles for aGPCRs.[1] To this end, an international, open network of academic and nonacademic researchers collectively started the Adhesion GPCR Consortium (AGC) (http://www.adhesiongpcr.org) in 2012 to further foster collaborations in the field. One of the first tasks of the AGC was the introduction of a new nomenclature system in 2015, which has helped to harmonize the aGPCR class across diverse research fields and species.[2]

Two major areas of interest in the field include (1) the discovery and characterization of biological functions of aGPCRs; and (2) the elucidation of signaling mechanisms of aGPCRs. Adhesion GPCRs are expressed in varied cell types and tissues, and the cell- and isoform-specific roles of aGPCRs are still not completely understood. Moreover, aGPCRs exhibit multiple signaling modes, mediated through either classical G protein signaling via their C-terminal fragment (CTF) (*cis* signaling) or through adhesion properties of their N-terminal fragment (NTF) (*trans* signaling). Novel modes of receptor signaling, however, have also recently emerged for some members of the aGPCR class. These critical areas of understanding have gained much traction since the first aGPCR workshop held over 15 years ago (see Ref. 3).

The 9th International Adhesion GPCR Workshop was hosted by the Vollum Institute at Oregon Health & Science University and held at The Nines Hotel in Portland, Oregon, September 13–15, 2018. The workshop included 88 scientists from 11 countries and featured 40 oral presentations and 28 posters focused on aGPCR research in the areas of development, biological functions, signaling and activation, structure, and health and disease.

aGPCRs in development

Simone Prömel (Leipzig University). Prömel presented on the enigmatic *trans* function of latrophilin in *Caenorhabditis elegans* (*C. elegans*) fertility. GPCRs classically mediate signals via intracellularly activating G proteins. Recently, it was found that aGPCRs have, in addition to this classical, seven-transmembrane (7TM)–dependent (*cis*) function, a role completely independent of the C-terminal 7TM. 7TM-independent (*trans*) functions have now been shown for several aGPCRs (reviewed in Ref. 2). However, how this new *trans* function is realized and whether it involves signaling remain enigmatic. To investigate this unusual mode of action that is only mediated via the extracellular domain (ECD), Prömel and colleagues studied the aGPCR latrophilin in the model organism *C. elegans*. Latrophilins (ADGRL/LPHN/CL/CIRL) represent one of the evolutionarily oldest subfamilies of aGPCRs and are present in vertebrates and invertebrates. They were first described as interaction partners for α-latrotoxin, a component of black widow spider toxin.[4]

Recently, Prömel and colleagues found that the latrophilin homolog LAT-1 in *C. elegans* has a *cis* and a *trans* function in two distinct biological settings.[5] The *cis* mode controls spindle directionality and oriented cell division.[6] The signal underlying this function constitutes a classical G protein cascade increasing intracellular levels of the second messenger cAMP upon receptor activation by a tethered agonist.[7] In contrast, the LAT-1 *trans* function is involved in fertility, controlling the number of offspring.[5] By using *in vivo* and *in vitro* approaches, Prömel and colleagues assessed the physiological and mechanistic details of the *trans* mode in this context. A *lat-1* null mutant strain helped to clarify the distinct processes LAT-1 regulates during fertility, and structure-function analyses using a transgenic complementation assay revealed that the same domain architecture of the extracellular LAT-1 N-terminus is required for both the *cis* and the *trans* function. Prömel's data point toward a scenario in which LAT-1 acts noncell autonomously to fulfill its role in fertility. Epistasis assays with different candidate genes potentially interacting with *lat-1* highlighted possible pathways involved in the LAT-1 *trans* function.

The work by Prömel sheds lights on the diverse roles of latrophilin mediated through different modes of action and further adds to the understanding of the previously poorly understood *cis* and *trans* functions of aGPCRs.

Kevin Wright (Vollum Institute, Oregon Health & Science University). Wright presented on the role of ADGRC3 (CELSR3) in regulating commissural axon guidance through the binding of dystroglycan (DAG1). During neural circuit development, instructive extracellular cues signal through cell surface receptors to direct the precise targeting of axons. Wright and colleagues have identified the transmembrane glycoprotein dystroglycan as a regulator of axon tract formation in the retina, brain, and spinal cord. Using genetic approaches, they show that dystroglycan functions noncell autonomously as an extracellular scaffold by binding multiple laminin G (LG) domain–containing proteins through its extensive glycan chains. This allows dystroglycan to regulate axon tract development in multiple ways. First, dystroglycan maintains basement membranes as permissive growth substrates for extending axons.[8] Second, dystroglycan binds the secreted axon guidance cue Slit to regulate its extracellular distribution *in vivo*.[9] Finally, Wright and colleagues have recently identified an interaction between dystroglycan and the aGPCR ADGRC3.[10]

ADGRC3 is required for axon guidance in the forebrain, spinal commissural axons, and peripheral motor projections. In spinal commissural axons, ADGRC3 functions within growth cones to direct anterior turning toward the brain after crossing the ventral midline. Commissural axons in *Dag1* or *Celsr3* mutants exhibit a randomization of postcrossing trajectory, with axons extending in both anterior and posterior directions. Using *in vitro* binding assays, the authors showed that dystroglycan directly binds the LG1 domain present in the extracellular portion of ADGRC3. To test the importance of this interaction during axon tract development, Wright and colleagues generated an ADGRC3 knock-in mutant ($Celsr3^{R1548Q}$) that disrupts its binding to dystroglycan. $Celsr3^{R1548Q}$ mutants recapitulate the postcrossing randomization of commissural axons seen in $Dag1^{-/-}$ and $Celsr3^{-/-}$ mutants, demonstrating that this interaction is required *in vivo*. Wright concluded that these results provide a mechanistic link between dystroglycan and the aGPCR ADGRC3, thereby identifying a novel mechanism by which dystroglycan regulates neural circuit development.

Caroline Formstone (Kings College London, University of Hertfordshire). Formstone presented on the role of the planar cell polarity protein ADGRC1 (CELSR1) in contact-mediated alignment of cell behavior. Planar cell polarity (PCP) proteins facilitate multiple aspects of tissue and organ development. PCP is key to cellular processes in embryonic development because its primary role is to align cell structures, cell shapes, and cell rearrangements along particular body axes. Indeed, the disruption of PCP protein function in mammals leads to severe birth defects.

PCP was originally discovered in *Drosophila* and this model system has elegantly identified a number of core molecular components. ADGRC1 is an essential player (flamingo is the *Drosophila* homolog) employed as a local communicator of global cell polarity information: its extracellular cadherin repeats generate molecular bridges between one cell and its neighbors. ADGRC1 forms a molecular partnership at the cell surface with another GPCR, Frizzled, as well as with the 4-pass transmembrane protein Vangl (Strabismus). Frizzled and Vangl differentially enrich to opposing cell interfaces along the axis of planar polarity to generate molecular asymmetries that act as an internal compass, distinguishing, for example, toward the head or toward the tail in the developing mouse embryo. These asymmetric protein complexes connect downstream to cytoskeletal dynamics.

Formstone's recent studies have utilized the mouse embryonic skin (epidermis) as a model to understand how ADGRC1 orchestrates organ morphogenesis. PCP in this model can be visualized by the directional down-growth of developing hair follicles in back skin, which are oriented along the head-to-tail axis. Their investigations[11,12] have revealed multiple novel facets of ADGRC1 function that are believed to be critical for understanding how complex organs in mammals are established and maintained during embryogenesis:

(1) ADGRC1 functions in three-dimensional tissue morphogenesis in mammals (e.g., determines radial-superficial to basal-tissue architecture as well as planarity).
(2) The molecular partnership between ADGRC1 and Frizzled, which is an ancient

one through evolution, appears to be less stringent in mammals than in flies, suggesting that ADGRC1 plays important roles in mammalian organ formation via cellular processes that are independent of other core PCP proteins.

(3) ADGRC1 exists as distinct molecular weight protein isoforms with different functions in local (one cell to its immediate neighbors) versus global (pervasive across a large field of cells) communication of PCP.

(4) Our current model for the role of ADGRC1 in the local communication of planar cell division orientation in mouse skin highlights potential functional conservation with that of another aGPCR, ADGRL1 (LPHN1), in early *C. elegans* embryos.

Further study is now necessary to fully understand the role of ADGRC1 in severe birth defects and to aid the design of successful strategies for the repair and regeneration of tissues and organs once diseased or damaged.

Biological functions of aGPCRs

Felix Engel (Friederich-Alexander-Universität Erlangen-Nürnberg). Engel presented on the role of ADGRG6 (GPR126) as a mechanoresponsive gene. ADGRG6 is required for proper heart,[13] ear,[14] skeletal,[15] and myelin[16] development. However, the complete roles of ADGRG6 as well as up- and downstream signaling pathways are poorly understood.[17] A detailed expression pattern on a cellular level might provide novel insight into possible functions of ADGRG6.

As most available expression data are based on RT-PCR of tissue samples,[2] Engel and colleagues generated a "knock out first" allele mouse line utilizing the EUCOMM targeting construct, *Adgrg6*$^{tm1a(EUCOMM)Hmgu}$, which expresses the gene *LacZ* under the control of the *Adgrg6* promoter. The insertion of the cassette causes truncation after exon 6 of *Adgrg6*, resulting in viable heterozygous offspring. LacZ activity could readily be detected, confirming, for example, *Adgrg6* expression in the heart and sciatic nerve. A detailed analysis could not confirm all RT-PCR–based expression patterns but did reveal new cell-types that express *Adgrg6*. Collectively, their data suggested that ADGRG6 is mainly expressed in mechanosensitive cell-types.

Uwe Wolfrum and Deva Krupakar Kusuluri (Johannes Gutenberg University of Mainz). Two talks were presented from the Wolfrum lab that covered: (1) affinity proteomics to identify aGPCR functional modules; and (2) the role of ADGRV1 (VLGR1) in focal adhesion complexes. In the first talk, Uwe Wolfrum presented on affinity proteomics approaches. For this, they applied tandem affinity purifications (SF-TAP) in HEK293 and RPE1 cells[18] expressing systematically tagged sets of different aGPCRs, including ADGRL2 (LPHN2), ADGRE5 (CD97), ADGRA1 (GPR123), ADGRA2 (GPR124), ADGRA3 (GPR125), ADGRB1-3 (BAI1-3), and ADGRV1 (VLGR1). Subsequent mass spectrometry identified the protein and peptide compositions of the recovered protein complexes related to the aGPCRs. For the analysis of the acquired proteomic data, the hits were functionally grouped based on their Gene Ontology terms and related to functional cell modules. Selective complementary *in vitro* and *in situ* experimental analyses support the following annotations:

(1) The analyses confirmed previously described functions of some aGPCRs at synaptic contacts, but also provided remarkable evidence related to functional roles of aGPCR in intracellular membrane networks.

(2) The presented data suggested a direct role of aGPCRs in transcriptional regulation and novel noncanonical signaling modules for aGPCRs.

(3) The data also revealed the association of aGPCRs with gene products related to neuronal diseases.

In a second talk, Kusuluri, a PhD student in the Wolfrum lab, presented on ADGRV1 as a part of focal adhesion complexes and its role in cell migration and mechanotransduction. ADGRV1 is by far the largest aGPCR. It is almost ubiquitously expressed in the body.[19] Mutations in *ADGRV1* cause Usher syndrome (USH), the most common form of hereditary deaf-blindness and can be related to epilepsy. ADGRV1 has been mapped in affected neurons, sensory hair cells, and photoreceptor cells to adhesion complexes and synapses associated with membrane adhesions.[20,21] To decipher components of the functional and cellular modules related to ADGRV1, they performed

affinity proteomics followed by bioinformatics to reveal numerous putative interacting molecules associated with focal adhesion. The presented study provided several lines of evidence that ADGRV1 is a vital component of focal adhesions in diverse cell types sensing mechanical stress. These findings further support the notion that defects in ADGRV1 cause dysregulation of adhesion complexes contributing to the pathophysiology of USH and epilepsy.

Cheng-Chih Hsiao (University of Amsterdam). Hsiao, a postdoctoral fellow working with Jörg Hamann and collaborating with Hsi-Hsien Lin (Chang Gung University), discussed the expression, structure, function, and signaling characteristics of ADGRG3 (GPR97) in granulocytes. ADGRE1-4 (EMR1-4) and ADGRE5 (CD97), members of the subfamily E of aGPCRs, are known for their expression in hematopoietic cells.[22,23] More recently, a cluster within subfamily G comprising ADGRG1 (GPR56), ADGRG3 (GPR97), and ADGRG5 (GPR114) was found to be also expressed in immune cells, and the specific presence of ADGRG1 was demonstrated in human cytotoxic lymphocytes, where it inhibits immediate effector functions.[24,25]

Granulocytes execute highly effective responses against microorganisms. RNA sequencing and mass spectrometry revealed abundant transcription and translation of ADGRG3 in granulocyte precursor cells and terminally differentiated neutrophilic, eosinophilic, and basophilic granulocytes. Using a newly generated monoclonal antibody, Hsiao and colleagues showed that ADGRG3 is a proteolytically processed, *N*-glycosylated bipartite receptor. Immunohistochemistry and microarray analysis confirmed ADGRG3 expression in tissue-infiltrating granulocytes and showed its induction during systemic inflammation in pneumonia or endotoxemia. Antibody ligation of ADGRG3 increased reactive oxygen species production and proteolytic enzyme activity in granulocytes via NF-κB and ERK signaling. By analyzing ADGRG3 signaling, a possible switch from basal $G\alpha_s$/cAMP–mediated signal transduction to a $G\alpha_i$-induced reduction in cAMP levels upon mutation-induced activation of the receptor was detected, in combination with an increase in downstream effectors of $G_{\beta\gamma}$, such as SRE and NF-κB. Hsiao and coworkers concluded that the specific expression of ADGRG3 regulates antimicrobial function in human granulocytes.

Kimberley Tolias (Baylor College of Medicine). Tolias presented on the role of ADGRB1 (BAI1; brain-specific angiogenesis inhibitor 1) in promoting excitatory synapse development. Excitatory synapses mediate information flow and storage in the brain. Most excitatory synapses are located on dendritic spines, which rapidly remodel during development and activity-dependent synaptic plasticity associated with learning and memory. Spine and synapse abnormalities are a common feature of brain disorders including intellectual disabilities, autism spectrum disorders (ASDs), schizophrenia, and Alzheimer's disease, suggesting that their proper regulation is critical for normal cognitive function.

ADGRB1 is a postsynaptic aGPCR that Tolias and colleagues previously identified as a critical regulator of spine and synapse development.[26] Like most aGPCRs, ADGRB1 possesses an extended NTF containing multiple adhesion domains, including five thrombospondin type 1 repeats (TSRs) and a GPCR-autoproteolysis inducing (GAIN) domain located N-terminal to its 7TM. ADGRB1 promotes spinogenesis and synaptogenesis in part by recruiting the Rac1 guanine nucleotide exchange factor (GEF) Tiam1 and the polarity protein Par3 to spines, resulting in localized Rac1 GTPase activation and actin cytoskeleton remodeling that drives spine and synapse growth.[26] ADGRB1 also stabilizes the synaptic scaffolding protein PSD-95 by binding to the E3 ubiquitin ligase MDM2 and preventing it from targeting PSD-95 for degradation.[27] Moreover, genetic ablation of *Adgrb1* results in mice with hippocampus-dependent spatial learning and memory deficits, enhanced long-term potentiation, and impaired long-term depression.[27]

Despite these recent advances, many unanswered questions remain about the function of ADGRB1 at synapses. Understanding the function of ADGRB1 in the nervous system is important because of its implications for neural circuit development and neurological disease. Human *ADGRB1* is located in a hot spot for *de novo* germline mutations in patients with ASD, and *Adgrb1* expression is altered in mouse models of Rett and MeCP2 duplication syndromes and in glioblastoma.[28–30]

Since GPCRs are often successful therapeutic targets for disease intervention, further insight into ADGRB1 regulation and function could facilitate the development of new treatments for these disorders.

Here, Tolias and colleagues confirmed that ADGRB1 promotes hippocampal spine development in the mouse brain, and they identify three distinct mechanisms by which ADGRB1 mediates its synaptogenic functions.[31] ADGRB1 appears to function as a receptor at synapses, as its extracellular NTF is required for both its spinogenic and synaptogenic functions, and activation of ADGRB1 with a *Stachel*-derived peptide, which mimics a tethered agonist motif found in aGPCRs, drives synaptic Rac1 activation and subsequent spine and synapse development.[31] Their work also reveals a trans-synaptic function for ADGRB1, demonstrating that ADGRB1 induces the clustering of presynaptic vesicular glutamate transporter 1 (vGluT1) in contacting axons, indicative of presynaptic differentiation.[31] Finally, they show that ADGRB1 forms a receptor complex with the synaptogenic cell-adhesion molecule neuroligin-1 (NRLN1) and mediates NRLN1-dependent spine growth and synapse development.[31] Together, their findings establish ADGRB1 as an essential postsynaptic aGPCR that regulates excitatory synaptogenesis by coordinating bidirectional trans-synaptic signaling in cooperation with NRLN1.

Garret Anderson (University of California, Riverside). Anderson presented on the role of latrophilin aGPCRs (ADGRL1-3/LPHN1-3) in synaptic assembly. Synapse assembly likely requires postsynaptic target recognition by incoming presynaptic afferents. Using newly generated conditional knockin and knockout mice, Anderson and colleagues showed that the cell aGPCR ADGRL2 controls the formation of a specific subset of synapses in CA1-region hippocampal neurons, suggesting that ADGRL2 acts as a synaptic target-recognition molecule. In CA1-region pyramidal neurons *in vivo*, ADGRL2 was specifically targeted to postsynaptic sites at dendritic spines in the stratum lacunosum-moleculare hippocampal subregion. There it was found that ADGRL2 functions to regulate synaptic assembly by matching with presynaptic entorhinal cortex afferents. Postsynaptic deletion of *Adgrl2* from CA1 pyramidal neurons selectively decreased spine numbers and impaired synaptic inputs from entorhinal but not from Schaffer-collateral afferents. Behaviorally, loss of ADGRL2 from the CA1-region increased spatial memory retention, but decreased learning of sequential spatial memory tasks. Thus, it was concluded that ADGRL2 appears to control synapse formation in the entorhinal cortex/CA1-region circuit by acting as a domain-specific postsynaptic target-recognition molecule.

Swati Srivastava (Friederich-Alexander-Universität Erlangen-Nürnberg). Srivastava presented work on the role of the ECD of ADGRG6 (GPR126) in regulating cardiac development. Trabeculation is a complex morphogenetic process in heart development that leads to the formation of muscular protrusions in the ventricular lumen.[32] Recently, Srivastava and colleagues have suggested that the ECD of Adgrg6 (Adgrg6/Gpr126-NTF) is required for this process in zebrafish and mouse.[13] However, this conclusion was mainly drawn from zebrafish experiments utilizing splice morpholinos, a technique that has been questioned in recent years regarding its specificity. Therefore, their work focused on analyzing the genetically modified zebrafish lines *adgrg6/gpr126*^{stl47} (full length-deleted mutant) and *adgrg6/gpr126*^{st49} (CTF-deleted mutant expressing NTF).[33,34] Their data regarding trabeculation at 5 days postfertilization verified that the NTF is required for proper trabeculation of the zebrafish heart. In addition, mRNA injection experiments indicate that Gpr126-NTF might be sufficient to partially induce trabeculation, a process including cardiomyocyte selection, depolarization, delamination, and proliferation.[35] Furthermore, they performed a comparison of gross morphological phenotypes with other mutants which exhibit trabeculation defects. Their preliminary analysis revealed that *adgrg6* mutants exhibit characteristics typical for Erbb2 misregulation. Thus, it will be important in the future to determine whether the observed trabeculation phenotypes in *adgrg6* zebrafish mutants are due to altered Erbb2 signaling, and if so, how Adgrg6 contributes to the known Erbb2 signaling pathway.

Doreen Thor (Leipzig University). Thor presented her work on the role of aGPCRs in modulating glucose homeostasis. aGPCRs have many well-appreciated roles within the immune and

central nervous systems and in cell adhesion and development. However, an impact of aGPCR in metabolic processes remains largely unstudied, even though for several metabolically relevant tissues, the regulating properties of GPCRs are well established and high expression of aGPCRs has been shown.

In pancreatic islets, G_s and G_q protein signaling has been linked to hormone exocytosis, while G_i protein signaling leads to a reduced hormone secretion.[36] RNA-seq analyses revealed the expression of 13 aGPCRs in murine pancreatic islets, suggesting the physiological relevance of aGPCRs in glucose homeostasis.[37] Until now, only ADGRG1 (GPR56) has a known function in endocrine pancreas, which is regulating insulin secretion.[38,39] However, Thor and colleagues have also demonstrated high expression of other aGPCRs, such as members of the ADGRL (latrophilin/LPHN) family and ADGRF5 (GPR116), which is partly restricted to specific pancreatic cell types.

They used islet-derived cell lines to analyze expression patterns of the aGPCR group under low- and high-glucose conditions.[40] Furthermore, they took the advantage of *Stachel*-derived peptides to activate the receptors and evaluate hormone secretion in cell lines and primary islets. Comparing islet composition and hormone content of wild-type and knock-out islets will shed light on the influence of aGPCRs in islet development. Metabolic phenotyping of wild-type and knock-out animals will help to understand the physiological function of these receptors in modulating glucose homeostasis. With several aGPCRs expressed in pancreatic islets and other metabolically relevant and endocrine tissues, this might uncover novel targets to intervene with in metabolic dysfunctions (Fig. 1).

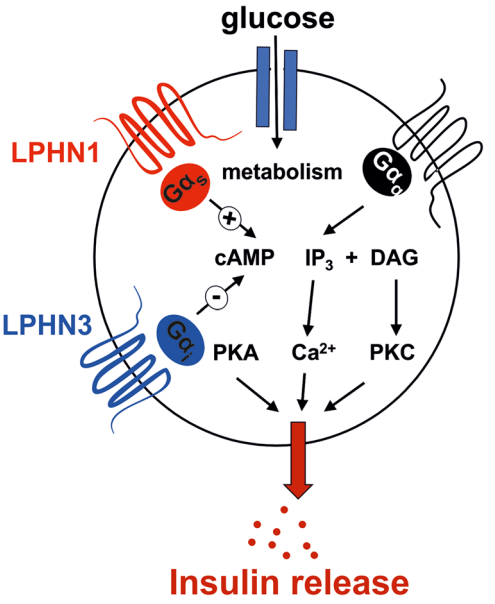

Figure 1. Latrophilin function in pancreatic beta cells. ADGRL3 (LPHN3) coupling to $G_{\alpha i}$ proteins and reducing intracellular cAMP levels reduces glucose-induced insulin secretion in pancreatic beta cells upon activation with *Stachel*-derived peptides.

Benoit Vanhollebeke (Université Libre de Bruxelles) and Mario Vallon (Stanford University). Vanhollebeke and Vallon both presented their findings on the regulation of Wnt7-specific signaling cascades mediated through ADGRA2 (GPR124) and RECK. Cerebrovascular development in vertebrates functionally integrates angiogenic and differentiation programs ensuring that only blood–brain barrier forming vessels penetrate the brain parenchyma. Endothelial Wnt/β-catenin signaling has emerged as a key signaling event in this coupling mechanism. In mammals, retinal endothelial Wnt/β-catenin signaling is regulated by Müller cell–derived Norrin ligand,[41] whereas forebrain and ventral neural tube vascularization is orchestrated by Wnt7 ligands.[42] In order to respond to Wnt7, endothelial cells were shown to require a membrane receptor complex made of ADGRA2 and RECK, a GPI-anchored glycoprotein.[43–46] Time-lapse confocal imaging of genetic mosaics revealed that these proteins control brain vascular invasion by selectively modulating tip cell function, consistent with a nonuniform requirement of Wnt signaling in assembling the cerebral vasculature.[43]

The ADGRA2/RECK complex has been suggested to form higher order receptor complexes with Frizzled receptors and Lrp5/6 coreceptors, in a Wnt7-dependent manner.[44] Genetic analyses in cultured cells confirmed the requirement of Frizzled and Lrp5/6 to transduce Wnt7 signals across the membrane bilayer.[45,46] Understanding of how ADGRA2 and RECK mediate discrimination of Wnt7 ligands from other Wnt isoforms, despite the promiscuous Wnt/Frizzled interaction mode, has been elusive.

RECK and ADGRA2 were found to traffic independently to the plasma membrane, where they

interact to synergistically potentiate Wnt7-specific signaling.[43–47] The interaction involves the cystine knot motifs of RECK and the LRR/GAIN (and to a lesser extent the HRM) domains of the ADGRA2 ectodomain.[44,46] Within this complex, RECK confers ligand specificity by binding directly and selectively to Wnt7 with a 1:1 stoichiometry.[45,46] Wnt ligand discrimination involves the RECK cystine knot motifs that bind with single-digit micromolar affinity to peptides derived from the intrinsically disordered linker domain of Wnt7.[45] The Frizzled-like cysteine-rich domain (CRD) of RECK was further shown to be required for the interaction with the full-length Wnt7 protein.[46] The RECK-Wnt7 receptor–ligand interaction has biophysical and signaling implications as it maintains the ligand in an active, monomeric, hydrophobic state better suited to activate Frizzled receptors, as free Wnt7 rapidly forms inactive aggregates that do not bind to RECK or Frizzled.[46] Recombinant soluble RECK protein, in the absence of ADGRA2, promotes the formation of soluble Frizzled CRD:Wnt7 complexes in conditioned medium.[46] However, on the cell surface, ADGRA2 is absolutely required for RECK-bound Wnt7 to become available for Frizzled signaling,[43,45,46] revealing that ADGRA2 is essential to increase the bioavailability of Wnt7 for Frizzled receptors.

Surprisingly, in this function, ADGRA2 does not rely on its GPCR structure. Experimental variants lacking the seven-span transmembrane domain were indeed competent to mediate Wnt7 signaling *in vitro* and promote brain angiogenesis *in vivo*.[45,46]

In zebrafish, the intracellular domain (ICD) of Adgra2, like the Adgra3 (Gpr125) ICD, binds Dishevelled (Dvl)[45,48] and is essential to promote brain angiogenesis.[45] CRISPR/Cas9–mediated gene disruption together with nanobody-based functional complementation assays indeed revealed that the Adgra2–Dvl interaction was necessary to mediate brain vascularization in this model. The function of Dvl was linked to its capacity to polymerize and assemble higher order Adgra2/Reck/Frizzled/Lrp receptor complexes by binding simultaneously to Frizzled and Adgra2 ICDs.[45]

In cultured cells, however, some ectopically expressed ADGRA2 variants lacking the ICD were still able to trigger Wnt7 signaling.[45,46] By extension, the ICD might, context dependently, not be required to initiate signaling *in vivo*. While genetic analyses in the zebrafish revealed an essential function for the ICD, it will be important to address this question additionally in the mouse model. The activity of the ICD-lacking ADGRA2 variants *in vitro* could reflect the fact that the ICD-mediated scaffolding function becomes dispensable when the concentrations of ADGRA2/RECK exceed the threshold values required for stochastic encounters with Frizzled within the two-dimensions of a cell membrane. Accordingly, full-length ADGRA2 triggers higher signaling activities than the ICD-lacking variants, in particular when expressed at low levels.[45] Interestingly, exposing RECK/Wnt7-expressing cells to recombinant soluble ADGRA2 ECD was also sufficient to initiate signaling *in vitro*.[46] However, ADGRA2-mediated RECK/Wnt7 signaling did not involve the regulation of RECK:Wnt7 complex formation on the cell surface.[46] This raises the possibility that the ADGRA2 ECD and ICD render the RECK:Wnt7 complex available for Frizzled signaling by independent mechanisms: The ECD possibly through conformational remodeling of RECK/Wnt7, and the ICD through Dvl-mediated recruitment of Frizzled.

Further investigations are warranted to probe the structural basis of this Wnt7 recognition/signaling module, the first described to confer Wnt ligand discrimination potential to vertebrate cells. The advent of single particle analysis through cryo-EM will likely be pivotal in this endeavor. It will be important as well to investigate if the function of the ADGRA2/RECK module is restricted to ligand discrimination at the level of the plasma membrane, or if the complex additionally affects the downstream signal transduction events. In this context, it will be interesting to test if ADGRA2/RECK could also potentiate noncanonical Wnt signaling cascades downstream of Wnt7. Alternatively, RECK/Wnt7 might activate noncanonical Wnt signaling by default in cells that do not express ADGRA2, whereas in ADGRA2-expressing cells, RECK/Wnt7 signaling is potentially rerouted to the canonical Wnt pathway.

The presenters concluded that ADGRA2 might function through alternative mechanisms in other physiological settings. In these settings, ADGRA2 could operate through signaling mechanisms more generic to aGPCRs, including through G-protein coupling, or downstream of tethered or small molecule agonists. However, unlike many other

aGPCRs, endogenous ADGRA2 does not undergo autoproteolytic cleavage at the GPS,[46] which is not conserved at a critical residue. Given the prominent role of brain endothelial Wnt/β-catenin signaling in the progression of several brain neurovascular disorders,[49] the mechanistic insights recently gained on the ADGRA2/RECK module broaden the therapeutic opportunities for treatment of human disorders through aGPCR targeting strategies.

Signaling and activation

Ines Liebscher (University of Leipzig). Liebscher discussed the physiological role of the mechanoresponsive aGPCR ADGRD1 (GPR133). aGPCRs have been shown to be activated by mechanical stimuli such as vibration and shaking[34] through their tethered agonist sequence. ADGRD1 is another aGPCR that is expressed in tissues that are known to be exposed to mechanical stress or to exert mechanical force, like bone, adipose tissue, and muscle. ADGRD1 has been associated with changes in heart rate frequency,[50] human body height,[51] and body weight in mice.[52] Signaling studies have shown that ADGRD1 couples to Gs and Gi proteins through a tethered agonist or its derived synthetic peptide.[53] There are currently no known ligands for this receptor and the mechanical properties for activation remain to be determined. To study the physiological role of ADGRD1, Liebscher and colleagues generated receptor-deficient zebrafish and mouse lines. They phenotyped these mutant animal models with a focus on organs that normally express ADGRD1 and that are subject to mechanical force. Based on changes in transcription levels in *Adgrd1* knockout (compared with wild-type) animals, potential interaction partners will be identified. Their binding and receptor-activating capacities will be analyzed using standard biochemical methods with or without the addition of mechanical force. RNA sequencing should further indicate changes in signaling pathways that are significantly changed in *Adgrd1*-knockout animals. Liebscher will study the direct contribution of ADGRD1 activation on classical G protein–dependent signaling cascades as well as nonclassical pathways such as Wnt, Notch, or Sonic hedgehog signaling using established agonist peptides and mechanical force.

Nariman Balenga (University of Maryland School of Medicine). Balenga showed that ADGRG2 (GPR64) is highly enriched in human parathyroid glands and is significantly upregulated in parathyroid adenomas from patients with primary hyperparathyroidism compared with normal glands from cadaveric donors.[54] ADGRG2 increases the secretion of parathyroid hormone via its crosstalk with calcium-sensing receptor and elevation of cAMP levels in parathyroid adenoma cells. To investigate the mechanisms of activation, signaling, and trafficking of ADGRG2 in HEK293 cells, Nariman generated a series of receptor mutants that lack either the NTF (ADGRG2ΔNTF) or various residues from the *Stachel* sequence. Using second messenger and reporter assays, Nariman showed that a 15 amino acid–long peptide after the GPS acts as an agonist of ADGRG2. He also showed that ADGRG2ΔNTF constitutively activates the $G\alpha_s$-cAMP-PKA-CREB pathway[54] and is constitutively internalized. The mechanisms of basal signaling and trafficking of ADGRG2 and their regulators were also discussed.

Maike Glitsch (University of Oxford). Glitsch discussed the detection of membrane stretch and extracellular pH by a proton-sensing GPCR. Mechanical forces influence cell shape, proliferation, differentiation, and survival, thereby affecting tissue and organ formation and function. Exactly how the different mechanical forces are sensed and transduced remains largely elusive.

Glitsch and colleagues reported that ovarian cancer G protein–coupled receptor 1 (OGR1, also known as GPR68) acts as coincidence detector of membrane stretch and its physiological ligand, H^+.[55] Using fluorescence imaging, substrates of different stiffness, microcontact printing methods, and cell stretching techniques, they showed that OGR1 only responds to extracellular acidification under conditions of membrane stretch, and vice versa. The level of OGR1 activity mirrors the extent of membrane stretch and degree of extracellular acidification. Furthermore, actin polymerization in response to membrane stretch is critical for OGR1 activity and provides a memory for past stretching. Cells experience changes in membrane stretch and extracellular pH throughout their lifetime. Since OGR1 is a widely expressed receptor, it represents a unique and widespread mechanism that enables cells to respond dynamically to mechanical and pH changes in their microenvironment.

Randy A. Hall (Emory University). The brain-specific angiogenesis inhibitors 1–3 (BAI1-3; ADGRB1-3) constitute a subfamily of aGPCRs with important roles at synapses in the CNS, as well as key roles outside the CNS.[56,57] Prior studies by Hall and colleagues on G protein–mediated signaling by the members of this family have revealed that ADGRB1 couples predominantly to $G\alpha_{12/13}$ to regulate Rho,[58,59] whereas ADGRB2 exhibits preferential coupling to $G\alpha_z$.[60] Removal of the N-terminal regions of ADGRB1 and ADGRB2 (up to the point of GPS cleavage) was found to strongly enhance receptor signaling, similar to other aGPCRs.[58,59] However, removal of the membrane-proximal stalk (*Stachel*) region had little or no effect on ADGRB1 or ADGRB2 signaling, which is distinct from the *Stachel*-dependent signaling observed with certain other aGPCRs.[59] A disease-associated mutation in *ADGRB2* (R1465W) enhanced receptor surface expression and signaling. This mutation did not affect receptor interactions with β-arrestins, but sharply reduced receptor binding to endophilins.[60] Ongoing studies are focused on achieving a more comprehensive understanding of ADGRB1-3 with regard to their downstream signaling pathways, physiological actions, and potential as novel drug targets in the treatment of psychiatric and neurological diseases.

Nicole Scholz (University of Leipzig). Scholz researches aGPCR function utilizing the ADGRL/latrophilin/CIRL (calcium-independent receptor of latrotoxin) homolog expressed in *Drosophila* (dCIRL). Previously, Scholz and colleagues demonstrated the capacity of dCIRL to shape the mechanoceptive profile of larval chordotonal sensory neurons, which leaves $dCirl^{KO}$ larvae less sensitive to gentle touch and sound as well as proprioceptive stimuli.[61,62] aGPCRs have long been known to be subject to alternative splicing of both coding and noncoding receptor moieties,[63–65] yet another feature of aGPCRs that is rather uncommon for canonical GPCRs.

The *Drosophila* genome contains only a single *Cirl* gene. Alternative splicing of the *dCirl* pre-mRNA produces eight transcripts, some of which encode identical receptor proteins, while the rest encode receptor molecules characterized by varying ECD and TM architecture (see flybase.org). Interestingly, isoform-specific alteration of ECD size and complexity has been noticed for other aGPCRs in the past, including ADGRE1/EMR1 and ADGRE5/CD97.[63] Therefore, Scholz hypothesized that this is a more general feature of aGPCRs enabling a certain degree of flexibility with respect to the ligand and activity profile. Moreover, aGPCRs typically localize to the surface of the expressing cell engaging in interactions with adjacent transmembrane receptors or matricellular components.[66] Therefore, alternative splicing may constitute a mechanism to vary the architecture of aGPCR ECDs to match the geometry of the expressing and surrounding tissues.

Furthermore, Scholz reported that alternative splicing results in transcripts encoding dCIRL receptor variants that contain only a single TM domain. As G protein coupling is unlikely to occur for these receptors, the question arises whether they solely serve adhesive functions or if they employ noncanonical signaling pathways to shape cellular biology. Preliminary data suggest isoform-specific expression patterns of different dCIRL isoforms in heterologous expression systems, which warrants the interrogation of the expression pattern of dCIRL isoforms as well as their putative contribution in shaping the physiology of mechanosensory neurons *in vivo*. In sum, it is intriguing to speculate that alternative splicing constitutes a mechanism that increases the functional diversity of aGPCRs. Thus, deciphering putative isoform-specific functions of aGPCRs will be the focus of future studies in the Scholz lab.

Hee-Yong Kim (NIH/NIAAA). Kim presented the role of ADGRF1 (GPR110)-dependent signaling in neurodevelopment and neuroprotection. ADGRF1 is an aGPCR recently deorphanized to be a target receptor for *N*-docosahexaenoylethanoamine (synaptamide).[67] Synaptamide is an endogenous metabolite derived from docosahexaenoic acid (DHA, 22:6n-3), a very long-chain omega-3 fatty acid highly enriched in the brain. At low nanomolar concentrations, this DHA-metabolite promotes neurogenesis,[68] neurite outgrowth, and synaptogenesis in developing neurons.[69] Synaptamide also attenuates the lipopolysaccharide-induced neuroinflammatory response[70] and ameliorates the deleterious effects of ethanol on neurogenic differentiation of neural stem cells (NSCs).[71] Specific binding of

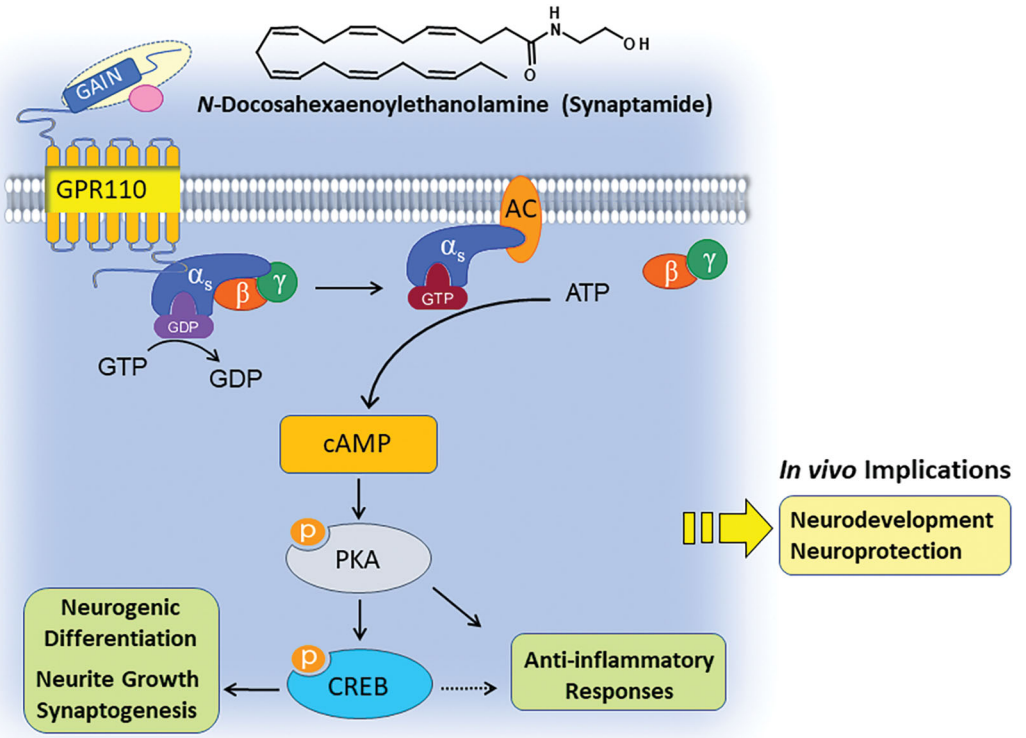

Figure 2. Ligand-activated ADGRF1 (GPR110) signaling with neurodevelopmental and neuroprotective implications.

synaptamide to ADGRF1 causes conformational changes of ADGRF1, activates $G\alpha_s$, and induces cAMP production and phosphorylation of PKA and CREB. This signaling pathway leads to the expression of neurogenic and synaptogenic genes and suppresses the expression of proinflammatory genes. ADGRF1 is heavily glycosylated and contains a GPS in the GAIN domain. The GPS cleavage, which releases the NTF and exposes the *Stachel* sequence of the 7TM domain, is neither induced by synaptamide nor required for ADGRF1 activation by synaptamide. In fact, synaptamide binds to the N-terminal side of GPS and without it, synaptamide does not activate ADGRF1, suggesting that the ligand-induced activation mechanism may be distinctively different from the GPS cleavage–dependent mechanism commonly observed with aGPCRs. ADGRF1 is highly expressed in NSCs and fetal brain, but its expression in the brain diminishes after birth. Nevertheless, the expression of ADGRF1 is sustained in the hippocampal dentate gyrus, where neurogenic capacity is retained throughout life, suggesting a role of ADGRF1 in promoting neurogenesis even after embryonic development. The impact of synaptamide/ADGRF1 signaling on the nervous system beyond developmental stages is further evident, as *Adgrf1* knockout produces significant deficits in memory function in adult mice. The ADGRF1-dependent cellular effects of synaptamide recapitulated in *in vivo* models suggest that synaptamide-derived mechanisms may have translational implications, particularly in neurodevelopment and neuroprotection (Fig. 2).

Katherine Leon (University of Chicago). Leon, from Demet Arac's group, discussed advances in ADGRL/latrophilin structure and signaling. Due to the recent discovery of aGPCRs and the lack of structural information about their 7TM domains, the activation and regulatory mechanisms of aGPCRs remain relatively uncharted compared with more well-studied GPCR families. However, recent studies on the signaling mechanisms of aGPCRs have largely been aided by the development of signaling assays which can probe the functions of specific receptors. Previous works on ADGRL1

(latrophilin-1) using newly established signaling assays have allowed a better understanding of how aGPCRs function and to probe the signaling effects of cancer mutations.[72] Leon and colleagues found that ADGRL1 is activated by its *Stachel* peptide, similar to other aGPCRs, and studied the residues important for *Stachel*-mediated activation. Furthermore, mutagenesis of residues that are homologous to key conserved residues in other GPCR families was shown to change basal signaling and/or *Stachel* peptide response. In addition, a cancer mutation exhibited high basal activity in the signaling assays and also led to a loss in receptor function *in vivo*.

Complementary to the new discoveries from the signaling study, they also explored the role of cellular communication and adhesion in ADGRL function in a recent study in which they solved the crystal structure of the ADGRL3/FLRT-3 complex that mediates synapse development.[73] Arac's group also showed that ADGRL3, FLRT-3, and UNC-5, another cell-surface molecule important for neural development, form a trimeric complex, which provided further insight into the role of cell adhesion in synapse function.

Erwin Van Meir (Emory University). Van Meir's laboratory is studying the role of ADGRB1-3 (BAI1-3) as tumor suppressors in malignant brain cancers.[74] He showed that the *ADGRB1* gene, which encodes brain-specific angiogenesis inhibitor 1 (BAI1), is epigenetically silenced in both human glioblastoma[75–77] and medulloblastoma[78] through a methyl-CpG binding protein 2 (MBD2)–dependent mechanism. His team previously discovered *trans* functions for BAI1 by demonstrating that its NTF can be cleaved to form fragments called vasculostatins that have antiangiogenic and antitumorigenic properties.[76] He now presented recent work about how ADGRB1 (BAI1) suppresses medulloblastoma formation in the cerebellum in *cis* by sequestering MDM2 from p53 through the protein's 7TM intracellular loop 1.[78]

Knockout of *Adgrb1* in mice augments the proliferation of cerebellar granule neuron precursors, and dramatically increases medulloblastoma penetrance and accelerated death when crossed to *Ptch1*$^{+/-}$ mice. ADGRB1 prevents MDM2-mediated p53 polyubiquitination, and loss of its expression through epigenetic silencing substantially reduces p53 levels. ADGRB1 protects p53 from MDM2-mediated degradation by binding directly to MDM2 through the first intracellular loop of its 7TM and thereby excludes MDM2 from the nucleus. Reactivation of the ADGRB1/p53 signaling axis by targeting the MBD2 pathway with a novel small molecule (KCC07) suppresses human medulloblastoma growth in orthotopic xenograft models. These findings highlight the importance of ADGRB1 silencing in medulloblastoma formation and demonstrate that epigenetic restoration of its expression with brain-permeable KCC07 has therapeutic potential.

These findings establish ADGRB1 as a physiological tumor suppressor in medulloblastoma and reveal a direct connection between aGPCRs and p53 signaling, thus demonstrating a causal relationship between aGPCRs and cancer. The discovery of a new upstream regulator of the p53 tumor suppressor is important due to this pathway's involvement in multiple cancers. Disruption of the ADGRB1/Mdm2/p53 signaling axis through *ADGRB1* silencing unveils a new vulnerability in cancer, which can be therapeutically targeted through epigenetic reactivation. The authors show that this is possible with a new chemical scaffold that prevents MBD2 binding to methylated DNA, and this lead molecule can be further translated into a first-in-class therapeutic for medulloblastoma, and possibly other cancers (Fig. 3).[78]

James Bridges (Cincinnati Children's Hospital Medical Center). Bridges discussed the molecular determinants of the aGPCR ADGRF5 (GPR116) required for pulmonary alveolar homeostasis. It has been previously demonstrated that epithelial expression of ADGRF5 regulates pulmonary surfactant levels and pulmonary alveolar homeostasis in mice.[79–81] Mechanistically, activation of ADGRF5 with synthetic peptides that mimic the extracellular ectodomain of the receptor elicits $G\alpha_{q/11}$-coupled responses and actin cytoskeletal rearrangements in primary mouse and human alveolar type II (AT2) cells.[82,83] The ability to pharmacologically manipulate the ADGRF5 pathway, both positively and negatively, would be a major therapeutic advance for patients with lung diseases associated with pulmonary surfactant disorders. The goal of this study was to define the molecular determinants of ADGRF5 that are essential for activation *in vitro* and in transgenic mouse models, with the

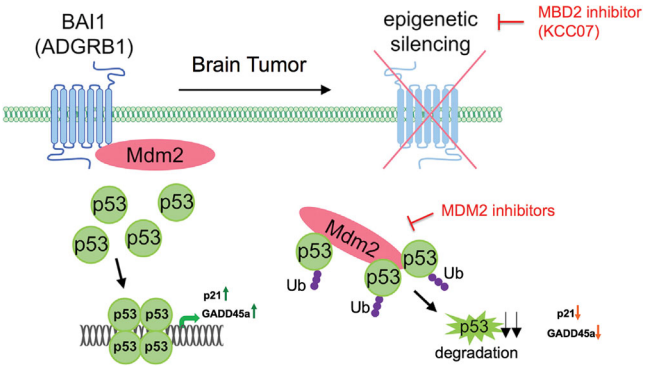

Figure 3. **Working model for ADGRB1 (BAI1) protective effect on p53. When BAI1 is expressed (left), it binds to MDM2 and prevents MDM2-mediated polyubiquitination of p53. P53 target proteins that restrict cell proliferation (p21, GADD45) are induced. When BAI1 expression is lost due to ADGRB1 gene silencing (right), p53 is degraded by the proteasome and cells are more prone to transformation. This mechanism suggests that MDM2 inhibitors or epigenetic reactivation of ADGRB1 with a new MBD2 inhibitor has therapeutic potential. Modified from Ref. 78.**

long-term goal of designing small molecule modulators of ADGRF5 to treat pulmonary disease.

Toward this goal, Bridges' group utilized G protein–coupled assays (calcium transients and inositol phosphate (IP) conversion assays) in primary AT2 cells and in HEK293 cells transiently expressing wild-type ADGRF5 or chimeric cDNAs expressing ADGRF5 that harbored alanine substitutions at sites predicted to be essential for receptor function. A synthetic peptide corresponding to the first 10 amino acids in the ectodomain of the CTF of ADGRF5 (termed GAP10) and a scrambled control peptide were used in G protein–coupled activity assays with chimeric *ADGRF5* mutants *in vitro* and administered to wild-type mice to determine the impact of ADGRF5 activation on surfactant pool sizes *in vivo*. Alanine mutation analysis of ADGRF5 identified four key amino acids within the ectodomain and four in the second extracellular loop of ADGRF5 that were required for full activation. The group also identified a conserved amino acid in the GAIN domain of ADGRF5 that is essential for proper cleavage of the receptor into the NTF and CTF. The ADGRF5 cleavage mutant routed to the cell surface, and elicited GAP10-induced IP responses and calcium transients in HEK293 cells comparable to wild-type ADGRF5, demonstrating that cleavage of the receptor is not essential for peptide-based activation *in vitro*. To test the hypothesis that cleavage of ADGRF5 is required for activation *in vivo*, the authors introduced the cleavage mutation into the endogenous ADGRF5 locus via CRISPR/Cas9–mediated gene editing. Analysis of 4-week old *Adgrf5* cleavage mutant mice revealed increased pulmonary surfactant and airspace enlargement, similar to levels observed in $Adgrf5^{-/-}$ mice. These data indicate that cleavage of ADGRF5 into the NTF and CTF is essential for receptor function *in vivo*.

While the endogenous ligand of ADGRF5 is unknown, these data support a model in which binding of a ligand to the NTF results in separation of the NTF from the CTF, revealing a cryptic tethered peptide that binds to the extracellular loops of ADGRF5, resulting in the activation and suppression of surfactant secretion from AT2 cells. Ongoing studies are focused on identification of the endogenous ligand and intracellular signaling events mediating ADGRF5-regulated exocytosis in AT2 cells.

Xianhua Piao (Harvard Medical School). Piao presented her group's work on how oligodendrocyte ADGRG1 (GPR56) integrates signals from microglia and the extracellular matrix to regulate developmental myelination and myelin repair. Myelin, a fatty membrane that wraps around axons to ensure both efficient impulse conduction and the health of nerve fibers, is produced and maintained by special glial cells called oligodendrocytes (OLs) in the CNS. OLs arise from a lineage-restricted, proliferative pool of OL precursor cells (OPCs) during development, and are also abundant in the adult CNS, generating new OLs and new

myelin under conditions of myelin damage, as is seen in demyelinating diseases and in rodent models of demyelination. Local environmental cues, including neighboring cells and extracellular matrix, influence OPC development. In particular, microglia regulate OPC proliferation and differentiation during development and remyelination.[84,85] However, the molecular signaling pathways that mediate communication between microglia and OL lineage cells during development and repair have not been fully delineated. Piao presented the discovery jointly made by her group and Kelly Monk's group, elucidating how OPCs integrate signals from both microglia and matrix during developmental myelin formation and repair.

ADGRG1 is an evolutionarily conserved regulator of OL development in zebrafish, mice, and humans.[86,87] Loss-of-function *ADGRG1* mutations cause the devastating human brain malformation called bilateral frontoparietal polymicrogyria, which comprises a constellation of structural brain defects, including CNS hypomyelination.[88] Conditional deletion of *Adgrg1* in OL lineage cells in mice results in CNS hypomyelination, and this is specifically caused by deficiencies in ADGRG1 signaling in OPCs.[87] Loss of ADGRG1 in mice and zebrafish decreases OPC proliferation, thereby leading to a reduced number of mature myelinating OLs and fewer myelinated axons in the CNS.[86,87] Through a combination of unbiased *in vitro* biotinylation proteomics, biochemistry, *in vitro* OPC culture, and mouse and zebrafish genetics, Piao and colleagues discovered the relevant ADGRG1 ligand during CNS myelination is microglia-derived tissue transglutaminase (TG2, mouse gene symbol *Tgm2*). Interestingly, TG2 signaling to OPC ADGRG1 requires the presence of the extracellular matrix protein laminin, and the TG2/laminin activation of ADGRG1 promotes OPC proliferation. Importantly, signaling by TG2/laminin to ADGRG1 on OPCs is also required for efficient remyelination *in vitro* and *in vivo*.[89] These findings document a tripartite module that signals through an aGPCR to promote myelin formation and repair, and suggest new strategies to enhance remyelination.

Gabriela Aust (University of Leipzig). Aust and colleagues identified a mechanism by which an aGPCR might transduce mechanical stimuli inside the cell. One third of all aGPCRs contain a PDZ-binding motif (PBM) at their intracellular C-terminus.[66] Together with PDZ domain–containing scaffold proteins, aGPCRs can thereby build intracellular signaling complexes near the membrane. Disruption of such networks by mutation of a key player protein may result in pathophysiological signaling.

Aust and colleagues demonstrated that mechanical stimuli induce rapid phosphorylation of the aGPCR ADGRE5 (CD97) at its PBM, and that this biochemical modification has functional consequences.[90] At the biochemical level, phosphorylation of ADGRE5 (pADGRE5) at S740 in the PBM disrupts binding of the receptor to the PDZ domain–containing scaffold protein DLG1. Aust described the identification of protein kinases with a phorbol ester/DAG-responsive C1 domain as kinases able to phosphorylate ADGRE5 S740.

At the cellular level, loss of the PBM results in altered mechanical properties and an enhanced retraction of cells under shear stress, both of which are related to alterations in the structure of the actin cytoskeleton. Indeed, membrane localization of ADGRE5 depends on an intact F-actin cortex. The loss of pADGRE5 S740–positive membrane patches ("footprints") from shear-stressed retracting cells in the dish indicates a cytosolic detachment of the cells that occurs between the ADGRE5 PBM and intracellular proteins. The footprint phenomenon is well known in the rear detachment of a migrating cell, forming characteristic tracks that mark the direction the cell has taken.[91] Unlocking cell contacts inside the cell between the PBM and intracellular proteins, which is not at the GPS between the NTF and CTF or between the NTF of an aGPCR and the dish, releases attachment and is likely to prevent cell injury. Aust postulated that phosphorylation might determine the threshold of forces transmitted inside the cell and terminate the junctional function of this aGPCR. Phosphorylation at the PBM may subsequently permit the binding of ADGRE5 to other intracellular proteins.

Importantly, Aust and colleagues detected pADGRE5 S740 *in situ* in tumor cells, located at the invasion front of colorectal carcinomas, and in infiltrating tissue leukocytes, thus providing the evidence of a pathophysiological relevance of ADGRE5 S740 phosphorylation.

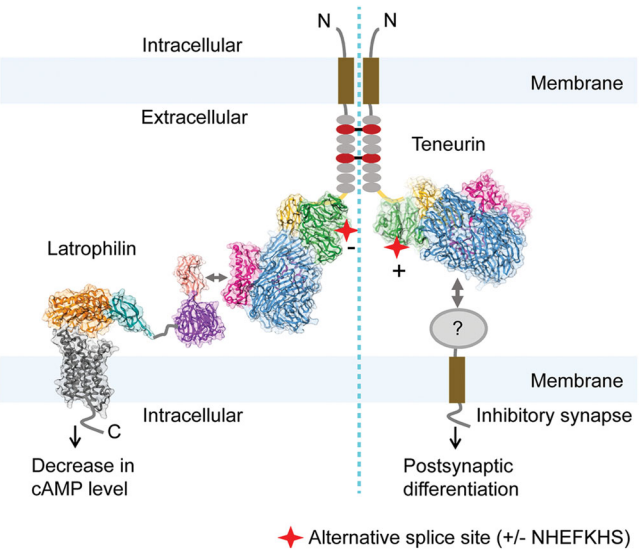

Figure 4. Model for the regulation of ADGRL1 (latrophilin)/teneurin interaction in an alternative splice–dependent manner. The model depicts how alternative splicing acts as a molecular switch to determine which adhesion partner teneurin 2 binds to, and, accordingly, which cellular functions teneurin 2 mediates. Left: the teneurin 2 variant lacking the β-propeller splice insert (–) interacts with ADGRL1 and modulates cAMP levels in the neighboring cell. Right: teneurin 2 variant including the splice insert (+) is unable to interact with ADGRL1, but it induces inhibitory synapses by interacting with unknown ligands. The left and right sides of the teneurin 2 dimer represent various cell–cell junctions and inhibitory synapses, respectively. The teneurin structure (PDB ID: 6CMX), membranes, and distance between synaptic membranes are drawn to scale. The molecules on the postsynaptic side are drawn schematically and are not to scale. The alternative splice site is shown by a red star. From Ref. 93.

Structure and function of aGPCRs

Demet Araç (University of Chicago). Araç presented her group's latest research on the structural and functional basis of aGPCR activation. aGPCRs have large extracellular regions (ECRs) decorated by numerous adhesion domains and a conserved GPCR autoproteolysis inducing (GAIN) domain that mediates self-cleavage of the receptor. Two avenues of research from her group were discussed:

(1) Araç and colleagues showed that aGPCRs are activated via *Stachel*-independent mechanisms in addition to *Stachel*-dependent mechanisms.[72,92] *Stachel*-independent mechanisms depend on the large ECRs of aGPCRs and form the basis for the complex regulation of aGPCR function.
(2) They also determined the high-resolution structure of teneurin, a large ligand of ADGRL1 (latrophilin/LPHN1) and revealed a unique structure that is similar to bacterial Tc toxins.[93] They further showed that an alternatively spliced region within teneurin acts as a switch to regulate transcellular adhesion of teneurin to ADGRL1. One splice variant activates transcellular signaling in an ADGRL1-dependent manner, whereas the other induces inhibitory postsynaptic differentiation. These results highlight the unusual structural organization of teneurins that give rise to their multifarious functions (Fig. 4).

Alexander Knierim (University of Leipzig). Knierim presented evidence for new and previously undescribed splice variants of several aGPCRs. Even though the enormous sizes of aGPCRs and the complex genomic exon–intron architecture strongly suggest a large variety of different transcript variants, an up-to-date study for the whole aGPCR class is missing, and only splice events for single receptors were reported in the past.[94–96] Knierim and colleagues established a bioinformatics pipeline to assemble splice variants for aGPCRs out of large RNA-seq datasets. The pipeline includes a quality check with strict

inclusion criteria and a new visualization tool suited to the comparative analysis of transcripts with many exons. With the new pipeline, the number of exons encoding aGPCR transcripts doubled. Knierim and colleagues found an average of 18 significantly expressed variants for each receptor, with splice events occurring in the ectodomains, the 7TM region, and the intracellular part. Experimental evidence was provided for significant changes in the surface expression and signaling of some splice variants, indicating the functional relevance of alternative splicing for these receptors. The unexpectedly large number of transcript variants in the aGPCR class may have an impact on the rational design of aGPCR gene–deficient mouse lines, primers, and antibodies in the future.

Antony Boucard (Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Mexico City). Boucard has been studying the role of adhesion molecules in the formation of neuronal synapses for many years. His recent studies focus on a subfamily of previously orphan aGPCRs, the ADGRLs/latrophilins, which he contributed to deorphanizing by identifying and characterizing various endogenous ligands that support bidirectional signaling.[97,98] The mammalian ADGRL subfamily comprises three isoforms (ADGRL1, 2, and 3) that are mainly expressed in the brain, and, consistent with their expression profile, these aGPCRs are involved in determining interneuronal adhesion.[97,99] ADGRLs stabilize cell–cell contacts through their N-terminal region, which mediates interactions with endogenous ligands spanning opposite cell membranes such as teneurins, neurexins, or FLRT proteins.[97,98] ADGRL isoforms possess a high degree of protein sequence homology, with the N-terminal region displaying the most conserved sequences, whereas the cytoplasmic domains that are coupled to the intracellular machinery are more divergent.[100] Thus, ADGRL-dependent adhesion events rely on the presence of extracellular adhesion motifs involved in multiple protein–protein interactions, the stabilization of which can lead to the formation or maintenance of interneuronal contacts at the neuronal synapse, for example. Consequently, ADGRLs have the potential to initiate intracellular cascades in a way to convert extracellular adhesion signals into the formation of structures that support or maintain the adhesive properties of the cell.

Common but divergent molecular characteristics imply that all ADGRL isoforms can form similar adhesion complexes with shared ligands, but that the elicited intracellular signals might lead to different activation patterns. This conundrum prompted Boucard and collaborators to conduct a comparative study aiming at deciphering how the different isoforms affect cell morphology. Although ADGRLs have been described as important stabilizers of neuronal synapses, evidences suggest that their physiological role might not be restricted to synaptogenic events. Indeed, their presence in tissues, such as kidney, immune cells, lung, and heart, hints at a ubiquitous role in cell adhesion.[98,101] To identify a unifying function for these receptors, Boucard explored the convergent but distinct functions in adhesion events of ADGRL isoforms expressed by neuronal and non-neuronal cells alike. Boucard presented his strategy to monitor the cellular function of ADGRLs involving the role of these receptors in trans-adhesion and adhesion-independent events. Using imaging through confocal microscopy and biochemical assays to reveal cell signaling pathways, Boucard characterized the cell morphological structures that are modulated by ADGRLs signaling. The molecular determinants that support their involvement in the genesis of cell adhesion structures were also discussed. Boucard described the constitutive function of ADGRLs in determining the genesis of cell structures as well as their ability to reorganize intracellular complexes upon trans-adhesion with endogenous ligands.

aGPCRs in health and disease

Ryan Gray (University of Texas at Austin). Gray presented his group's research focused on the essential function of the aGPCR ADGRG6 (GPR126) in homeostasis of the intervertebral disk (IVD) in mice. Degenerative changes of the IVD are a leading cause of back pain and disability worldwide. Yet, surprisingly little is known about the homeostatic regulation of the IVD during maturation and aging of the spine. Using conditional genetics in mouse and chondrogenic cell culture, the authors demonstrated the necessity of ADGRG6 for sustained chondrogenic pathways and homeostasis of cartilaginous tissues of the IVD. Interestingly, ADGRG6 function is

dispensable for early development of cartilaginous tissue of the spine. However, by 1.5 months and prior to obvious histopathology, ADGRG6-deficient IVDs displayed biomarkers associated with degeneration and commonly observed in osteoarthritis. In older adult mutant mice (6–8 months old) IVDs, the authors reported obvious histopathology coupled with increased degenerative marker expression. This study demonstrates a novel role for ADGRG6 function in the homeostasis of cartilaginous tissues in mouse spine, suggesting a direct effect of ADGRG6 on the regulation of chondroprotective and catabolic gene expression. These findings further suggest that ADGRG6 may provide a promising therapeutic target for cartilage degeneration.

Yuri Ushkaryov (University of Kent). Ushkaryov described that ADGRL1 (latrophilin-1) mediates axonal attraction induced by proteolytically released Lasso. ADGRL1 is a presynaptic aGPCR. When stimulated by its exogenous agonist α-latrotoxin (from black widow spider venom), ADGRL1 activates the $G\alpha_q$/phospholipase C/inositol-1,4,5-trisphosphate cascade and release of intracellular Ca^{2+}, leading to massive exocytosis of neurotransmitters.

In 2004, the authors hypothesized that the extracellular NTF of ADGRL1 must bind an endogenous protein which, based on its predicted characteristics, was called latrophilin-1-associated synaptic surface organizer (Lasso).[102] Using affinity chromatography on the NTF of ADGRL1, the authors isolated its hypothetical ligand from rat brain and identified it as teneurin-2.[103] Lasso/teneurin-2 is the strongest endogenous ligand of ADGRL1 and is also the only protein isolated by ADGRL1 affinity chromatography.

Lasso/teneurin-2 is a type 2 membrane receptor of ∼300 kDa, whose N-terminus is localized inside the cell, while the large C-terminal domain containing 8 EGF repeats is extracellular. It is a dimer of two subunits linked by two disulfide bridges. Lasso/teneurin-2 is constitutively cleaved by furin within its ECD, but the large extracellular fragment remains tightly tethered to the cell surface due to its noncovalent interaction with the transmembrane domain. Lasso/teneurin-2 is widely expressed in the brain. While it is mostly present on dendrites and dendritic spines, ADGRL1 is largely presynaptic, and the two proteins form a strong trans-synaptic receptor pair that mediates cell adhesion[103] and has been implicated in synapse formation.[98]

Paradoxically, the ECD of up to 20% of cell-surface Lasso is shed into the medium as a result of regulated proteolytic cleavage at another position, which releases the whole ECD containing the constitutive cleavage site.[104] This makes the released ECD unable to function in cell adhesion. However, the authors found that the released fragment of Lasso binds to cell-surface ADGRL1 on distant cells and axonal growth cones and causes intracellular signaling.[104,105] This indicated that the interaction of the shed ECD of Lasso/teneurin-2 with ADGRL1 could have a function in growth cone behavior. Using microfluidic devices, the authors further showed that a spatiotemporal gradient of the soluble Lasso/teneurin-2 ECD induces axonal attraction, without increasing the length of axons. This effect requires ADGRL1 (as shown by *Adrgl1* knockout in mice) and involves Lasso-mediated aggregation of ADGRL1 on the cell surface, increased cytosolic Ca^{2+}, and enhanced exocytosis, processes that are known to induce growth cone turning.[105] This suggests a novel mechanism of axonal pathfinding, where the ADGRL1/Lasso pair mediates axonal attraction and supports synaptogenesis.

Conclusions

The 9th International Adhesion GPCR Workshop concluded with significant progress in the field on multiple fronts. Through the years, many research groups have serendipitously discovered functions of individual aGPCRs based on basic cellular expression patterns, but as a class, aGPCRs are now beginning to emerge as important regulators in many different systems of various organisms. New investigators have joined the ever-expanding aGPCR community since the 8th International Adhesion GPCR Workshop in Leipzig, Germany. The collaborative effort of the consortium has enabled numerous advances in elucidating new mechanisms and functions of aGPCRs in development, neuroprotection, myelination, mechanosensation, cancer, the immune system, and other systems. There have been significant efforts in understanding how these receptors activate and transduce signals, and the field has also made considerable progress in understanding aGPCR structure, cellular and isoform

differences, and functions in diverse tissues. Newly developed methods to modulate these receptors will be very valuable tools for generating therapeutic strategies in the future. With a more elaborate understanding of the aGPCR class, we will certainly see a better advancement in their characterization and application in health and disease in the near future.

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Supporting information

Additional supporting information may be found in the online version of this article.

Figure S1. Participants of the 9th International Adhesion GPCR Workshop at the Nines Hotel in Portland, Oregon.

Appendix S1. 9th International Adhesion GPCR Workshop meeting program.

Competing interests

The authors declare no competing interests.

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