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A GUIDE TO...

A guide to adhesion GPCR research

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Adhesion G protein-coupled receptors (aGPCRs) are a class of structurally and functionally highly intriguing cell surface receptors with essential functions in health and disease. Thus, they display a vastly unexploited pharmacological potential. Our current understanding of the physiological functions and signaling mechanisms of aGPCRs form the basis for elucidating further molecular aspects. Combining these with novel tools and methodologies from different fields tailored for studying these unusual receptors yields a powerful potential for pushing aGPCR research from singular approaches toward building up an in-depth knowledge that will facilitate its translation to applied science. In this review, we summarize the state-of-the-art knowledge on aGPCRs in respect to structure–function relations, physiology, and clinical aspects, as well as the latest advances in the field. We highlight the upcoming most pressing topics in aGPCR research and identify strategies to tackle them. Furthermore, we discuss approaches how to promote, stimulate, and translate research on aGPCRs 'from bench to bedside' in the future.

Abbreviations
7TM, seven transmembrane; ADHD, attention deficit hyperactivity disorder; Adher´n Rise, adhesion GPCR Network: Research and Implementation Set the path for future Exploration; AGC, Adhesion GPCR Consortium; aGPCR, Adhesion G protein-coupled receptor; BAI, Brain-specific angiogenesis inhibitor; CIRL, Calcium-independent receptor for α-latrotoxin; COST, European Cooperation in Science and Technology; CTF, C-terminal fragment; ECM, extracellular matrix; EMR, EGF-like module-containing mucin-like hormone receptor-like; GAIN, GPCR autoproteolysis-inducing; GPCR, G protein-coupled receptor; GPS, GPCR proteolysis site; HSC, hematopoietic stem cell; LPHN, Latrophilin; MoU, Memorandum of Understanding; NK, natural killer; NTF, N-terminal fragment; VLGR, Very large G protein-coupled receptor.

Introduction

G protein-coupled receptors (GPCRs) represent the largest family of membrane-bound receptors, mediating cellular responses to an immense number of diverse signals such as neurotransmitters, photons, and hormones. GPCRs are involved in nearly all biological processes and represent *the* number one therapeutic target for many pathologies, including cardiovascular diseases, hypertension, diabetes, and substance abuse [1]. Indeed, close to 40% of all prescribed drugs target GPCRs [2], positioning these receptors in the focus of academic scientists, clinicians, and pharmaceutical companies.

Adhesion GPCRs (aGPCRs) are the second largest GPCR class (although comprising much fewer members than the largest class, the Rhodopsin-like GPCRs), with unique structural as well as intriguing functional features. aGPCRs are highly conserved and evolutionarily ancient receptors, present in most animals and even in some unicellular eukaryotes [3]. In humans, 33 representative aGPCRs have been identified [4], with numerous splice variants contributing to an even larger structural variety with potential implications for signal transduction in most human tissues [5]. Yet, surprisingly little is known about the characteristics and functionality of aGPCRs in human physiology and disease, making them one of the least understood GPCR class.

To date, three of 33 aGPCRs have been shown to cause human diseases. Mutants of the Very Large G protein-coupled Receptor VLGR1/ADGRV1 were identified in patients suffering from Usher syndrome type II [6]. Several mutations in GPR56/ADGRG1 were found in patients with a severe brain malformation called bilateral frontoparietal polymicrogyria [7]. Recently, a variant of the EGF-like module-containing mucin-like hormone receptor-like 2, EMR2/ADGRE2 was identified in patients with autosomal dominant vibratory urticaria [8]. Even though only these three aGPCRs have been causatively linked to monogenic human diseases so far, it is assumed that this receptor class contributes to polygenic diseases and to undiagnosed causes of embryonic lethality. This assumption is supported by multiple genome-wide association studies, linking various aGPCRs to metabolic or psychiatric diseases, and by knock-out of selected aGPCRs in animal models that cause embryonic death [9,10] or perinatal lethality [11,12]. Some aGPCR knock-out animals that manage to survive to adulthood present with severe organ impairment such as accumulation of alveolar surfactant phospholipids [13], or myocardial hypertrophy [14]. Neuronal cells were shown to require aGPCR function to migrate and assume correct orientation [15,16], to recognize mechanosensory stimuli [17,18], control spatial learning and memory [19], as well as maintain myelination and axon repair [20,21]. Reduction in male fertility has also been observed due to aGPCR downregulation [22,23], while several studies have implicated these receptors in immune defense, metabolism and cancer [24].

In contrast to the obvious essential functions and the untapped pharmacological potential of aGPCRs, our understanding of their various physiological and patho-physiological roles contains huge gaps. These gaps are underscored by the complex architecture of these receptors – most of them extremely large proteins, which can reach up to 6500 amino acids. Their structural features suggest novel modes of signaling and there are indications that in addition to classic G protein-coupled signaling, they also engage actively in cellular adhesion and can be activated by mechanical stimuli [17,18].

Thus, research of the past two decades has revealed that aGPCRs are a class of GPCRs with a diverse spectrum of physiological functions in health and disease, presenting not only tremendous pharmacological potential but also complex modes of action [25]. This complexity extends on many levels – from numerous isoforms per receptor, which can have various functions, to multiple signaling pathways mediated through the modular architecture of aGPCRs – marking them not only a highly interesting class of GPCRs but also a very challenging one to study. Research on many aspects of these receptors, such as elucidating the details of their molecular functions, their network of interactions, or a structural basis for their pharmacological manipulation is still lagging far behind other GPCR classes.

This review provides an overview on the current knowledge on aGPCR structure–function relations, physiological roles, and clinical implications, thereby identifying knowledge gaps and the most pressing challenges in the field. We contemplate how these gaps can most efficiently be filled and the challenges tackled – through connecting academia, industry partners, and other stakeholders on an international level to successfully promote and foster future research on aGPCRs. One approach to achieve this is the currently running COST Action CA18240 *Adher'nRise* (Adhesion GPCR Network: Research and Implementation Set the path for future Exploration).

Sequence and structure–function relations across the aGPCR class

Adhesion GPCRs are characterized by a strikingly complex architecture coupled with an extremely large size, which is mainly attributed to the N terminus.

They display the classic hallmarks of GPCRs, including an extracellular N terminus, seven transmembrane helices, and an intracellular C terminus. However, the N-terminal ectodomain of aGPCRs differs from the rest of the superfamily. All aGPCRs, except GPR123/ADGRA1, contain a conserved membrane-proximal GPCR autoproteolysis-inducing (GAIN) domain [26] (Fig. 1). Upstream of the GAIN domain, most aGPCRs contain additional domains that are typically involved in cell–cell and cell–matrix interactions, leading to a dual role in cell adhesion and cellular signal transduction [27]. Structural data for these extracellular domains, which also show how they arrange onto the seven transmembrane domain (7TM), are now beginning to emerge.

Almost all aGPCRs are alternatively spliced into several different gene products [5,25,28], an uncommon feature among other GPCR classes. Splice variants of EMR2/ADGRE2 were found to be differentially expressed in colorectal tumor cell lines [29], while CD97/ADGRE5 displays an altered subset of splice variants in rheumatoid synovial tissue [30]. While it is not known whether all of these variants lead to functional proteins, splice variants were shown to affect ligand binding in the latrophilin LPHN1/ADGRL1 [31,32] and in GPR56/ADGRG1 [33]. In fact, one of the best characterized aGPCRs with respect to splice variants is CD97/ADGRE5, where alternative splicing leads to diverging N termini and thereby to varying interactions with ligands [34-36]. Further, splicing was demonstrated to affect signaling in LPHN3/ADGRL3

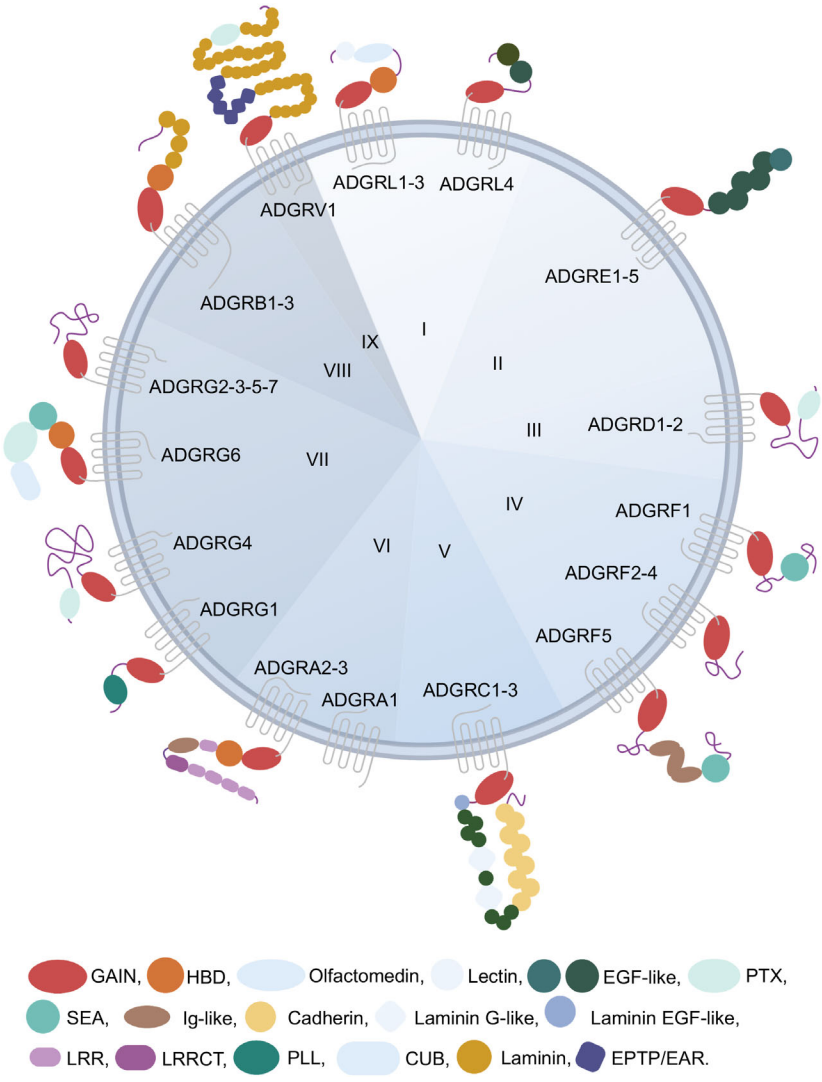

Fig. 1. Structural features of representative aGPCRs from each human group (I–IX). Extracellular regions are shown according to the identified domains, detailed in the caption below the figure. The 33 aGPCRs comprise the following representatives: ADGRL1-3 (LPHN1-3), ADGRL4 (ELTD1); ADGRE1-5 (EMR1-4, CD97); ADGRD1-2 (GPR133, GPR144); ADGRF1 (GPR110), ADGRF2-4 (GPR111, GPR113, GPR114), ADGRF5 (GPR116); ADGRC1-3 (CELSR1-3); ADGRA1 (GPR123), ADGRA2-3 (GPR124, GPR125); ADGRG1 (GPR56), ADGRG2-3-5-7 (GPR64, GPR97, GPR114, GPR128), ADGR4 (GPR112), ADGRG6 (GPR126); ADGRB1-3 (BAI1-3); and ADGRV1 (VLGR1). The image was created with biorender.com.

[37], and to change basal activity in GPR114/ADGRG5 [38]. These findings suggest that alternative splicing increases the functional complexity of aGPCRs, which raises potential implications for activation and signal transduction.

A characteristic feature of aGPCRs is a highly conserved extracellular GAIN domain. Crystal structures of LPHN3/ADGRL3 and the brain-specific angiogenesis inhibitor 3, BAI3/ADGRB3 GAIN domains showed subdomains A and B, which are composed of six α-helices and a twisted β-sandwich, respectively [26]. The previously described GPCR proteolysis site (GPS) is an integral part of subdomain B of the GAIN domain. Autoproteolytic cleavage at this site yields two fragments: the extracellular N-terminal fragment (NTF) and the C-terminal fragment (CTF) [39], which includes the integral tethered agonist sequence (coined *Stachel*), the 7TM domain, and intracellular regions (Fig. 1) [18,40,41,42,43]. Mutations introduced to suppress proteolysis were shown in some aGPCRs to affect receptor trafficking or function [44-46], while others were not impaired by similar mutations [17,47,48]. Recent evidence in several aGPCRs points to a receptor activation mechanism, in which rearrangement within the GAIN domain or release of the NTF lead to the *Stachel* substructure acting as an internal agonist for the 7TM domain, resulting in turn in G protein-dependent signaling [17,18,40,41,42,49]. *Stachel*-mediated activation has been detected *in vitro* in nine aGPCRs [37,38,40,41,42,49,50,51,52,53,54,55,56,57,58], as well as in two *in vivo* [40,49]. Release of the NTF is a model favored by many researchers to explain mechano-sensing of aGPCRs. Hints to this connection include the mechano-sensing of cleavable GPR126/ADGRG6 conveyed through Laminin-211 [18], shear stress downregulation of the cleavable CD97/ADGRE5 [59] or observations of a potentially GPS-destabilizing missense mutation in EMR2/ADGRE2 in patients with vibratory urticaria [8]. However, none of these studies reported actual NTF shedding upon mechano-activation. Furthermore, *Drosophila melanogaster* models showed that sensing of vibration through the latrophilin homolog CIRL/ADGRL (Calcium-independent receptor for α-latrotoxin) was independent of cleavage [17,60,61], and it was found in cell-based studies that the mechano-sensitive aGPCR GPR114/ADGRG5 was uncleavable [38]. These observations argue against a general mechanism for mechano-sensing mediated by NTF shedding, while supporting a possible prebound *Stachel* substructure, which, similar to retinal in Rhodopsin, isomerizes upon external stimuli [38]. Further, there is some indication that the *Stachel* is accessible also in an uncleaved state through GAIN domain flexibility [62]. A third activation scenario describes a *Stachel*-independent or extracellular region-mediated receptor activation. In this model, the extracellular region of aGPCRs contributes to receptor activation via interactions with the 7TM domain in a ligand-dependent and *Stachel*-independent manner [43,63,64].

There is a handful of ligands known to interact with aGPCRs. Among them are proteins of the extracellular matrix (such as glycosaminoglycans [65-67], collagens [68,69] or laminin [18]), other membrane proteins (such as FLRT [31,70], neurexins [71]), phosphatidylserine [72], ethanolamine derivatives such as synaptamide [73], glucocorticoids [74,75], surface cluster of differentiation antigens [76,77] or integrins [34], and soluble proteins (like Wnt [78] or surfactant protein D [79]). Several aGPCRs are also substrates of enzymes (such as furin [80] or matrix metalloproteinase 14 [81]), which catalyze the cleavage of N-terminal receptor portions of different sizes.

Besides the role of the N terminus for the activation level of the CTF, this extracellular part of the receptor has additional aspects in mediating adhesion to the extracellular matrix or neighboring cells [27]. Interestingly, besides this adhesion, the N terminus can also induce a function on its own, a process highly unusual for GPCRs, which is known as the *trans* function [47,82,83,84]. This function, for which 7TM domain and C terminus are not required, evokes a response in neighboring cells. This has, for instance, been shown for BAI1/ADGRB1, which mediates a *trans* function across synapses [84], for the latrophilin homolog LAT-1, which evokes a *trans* function on germ cells [47], or for CD97/ADGRE5 that acts from tumor cells onto platelets [82]. Whether this mode of receptor function is mediated by classical signals, in which the N terminus acts as a ligand for receptors on the opposing cell or whether other mechanisms are involved, remains elusive for most of the described cases of *trans* function.

New and exciting insights into aGPCR structure and function were revealed in several very recent structural studies. These include the structure of the GAIN domains of LPHN1/ADGRL1 and BAI3/ADGRB3 along with the HormR domains [26], the structure of the GAIN domain with a new functional domain of GPR56/ADGRG1 [33], the structure of five domains of the zebrafish GPR126/ADGRG6 extracellular region [85], and the structure of the active conformation of GPR97/ADGRG3 that includes the 7TM domain [75]. As most structures so far only contain the extracellular regions, it will be exciting to retrieve full-length structures in the future. Large latrophilin 'super-complexes' were found that include both FLRT

and the interaction partner uncoordinated-5 [86,87]. This complex opened new questions such as what causes the tetramerization of the latrophilin NTF, and why adhesive (FLRT-latrophilin) complexes are intermixed with repulsive ones (FLRT-uncoordinated-5). Another identified complex contains FLRT-latrophilin-teneurin [88] that functions in synapse development, presumably with an adhesive role, and at earlier developmental stages, where it mediates cell–cell interactions during cell migration [88] and axonal path finding [89]. This work on latrophilins suggests that aGPCR function extends beyond simple 1 : 1 interactions between proteins and a simple on/off internal activation mechanism. Instead, aGPCRs often engage in large signaling hubs that use the large extracellular domains as interaction scaffolds.

Overall, progress in recent years highlighted some complex structural mechanisms that characterize aGPCRs. However, many aGPCRs are still orphan receptors and activation mechanisms and domain-specific function are still mostly unknown. The details and functional importance of alternatively spliced isoforms also remains mostly unresolved. Therefore, teasing out the conserved or idiosyncratic mechanisms of how individual aGPCRs function on the molecular level and understanding how this translates to domain-specific functions *in vivo* remain a major challenge for the future.

The biology of Adhesion GPCRs

In mammals, aGPCRs can be found in virtually every organ system [3,24] where they mediate a myriad of biological processes [24,90,91,92] (Table 1). For instance, GPR126/ADGRG6 and the LPHN/ADGRL group govern cardiac development [10,83,93,94,95,96,97], while GPR124/ADGRA2 and GPR126/ADGRG6 regulate angiogenesis and brain vascularization, the former supposedly upon proteolytic NTF-CTF cleavage [67,85,98,99,100,101,102,103,104]. Similarly, GPR126/ADGRG6- and GPR116/ADGRF5-mediated mechanosensing may provide the mechanistic basis for their importance in lung physiology [58,105,106,107,108,109], even though mechano-activation has yet to be shown for GPR116/ADGRF5. In the endocrine system, GPR116/ADGRF5 modulates sensitivity to insulin [110,111], while GPR56/ADGRG1 [112,113] and BAI3/ADGRB3 exert opposing effects on insulin secretion [114], and LPHN3/ADGRL3 and LPHN1/ADGRL1 modulate insulin release [37]. Furthermore, aGPCRs such as BAI3/ADGRB3 and GPR64/ADGRG2 control steroidogenesis in Leydig cells and hormone release from parathyroid glands, respectively [53,115].

Adhesion GPCR function further impinges on key aspects of development, architecture, and function of the nervous system. Among the best studied aGPCRs in the nervous system are the members of the ADGRC, ADGRG, ADGRB, and ADGRL subfamilies, all of which shape several neurobiological processes [116-119]. For example, ADGRC proteins are involved in neuronal migration, and axon guidance, as well as dendritogenesis and synaptogenesis [11,12,120,121,122]. GPR56/ADGRG1 regulates migration of cortex neurons and development of oligodendrocytes [7,123,124]. ADGRL proteins regulate synapse formation and function [31,70,71,86,125,126,127] as well as their invertebrate homolog CIRL modulates the physiology of mechanosensory neurons [17,61]. More recently, the function of GPR110/ADGRF1 was linked to neurodevelopmental benefits of omega-3 fatty acids and was suggested to positively affect cognitive function postnatally, for example, spatial orientation and memory formation [73,128]. Moreover, aGPCRs, such as GPR126/ADGRG6 or GPR56/ADGRG1, are involved in the development of myelinating glial cells and are, therefore, relevant for proper neuronal signal propagation [18,116,117,124]. Thus, aGPCRs are key players in a plethora of neurobiological contexts in the developing and adult nervous system.

The hematopoietic system expresses three aGPCR gene clusters, belonging to the ADGRE and ADGRG subfamilies [129,130]. While the ADGRE group members EMR1-4/ADGRE1-4 are well-established as markers of myeloid cell subsets [131-134], CD97/ADGRE5 has a much wider cellular distribution, also outside the immune system [135]. EMR2/ADGRE2 induces inflammatory responses in human neutrophils [136], monocytes [137,138], and macrophages [139]. A role for EMR2/ADGRE2 in mechanosensing by mast cells was revealed in a study describing a missense substitution associated with vibratory urticaria [8]. The ADGRG group comprises three members expressed by hematopoietic cells. GPR56/ADGRG1 contributes to the generation and maintenance of the hematopoietic stem cell (HSC) pool [140], while the balance of GPR56/ADGRG1 and GPR97/ADGRG3 in HSCs is important for their development and differentiation [141]. In acute myeloid leukemia, GPR56/ADGRG1 identifies cells with high repopulating potential [142], while CD97/ADGRE5 regulates leukemic stem cell function [143]. Moreover, GPR56/ADGRG1 is a surrogate marker and regulator of the cytotoxic capacity of human lymphocytes [144], and negatively regulates natural killer (NK)-cell effector functions [145]. Tissue-resident memory T cells, which are controlled by a multitude of inhibitory receptors, express GPR56/

Table 1. Physiological function of aGPCRs. Summary of available published results on the function of aGPCRs, obtained from humans and mouse models. Table 1 was adapted from [24] and updated with current data. Note that human EMR4/ADGRE4 and mouse GPR144/ADGRD2 are pseudogenes [195,196], while EMR2/ADGRE2 and EMR3/ADGRE3 do not exist in mice [197].

ADGRG1 [146,147]. In the brain, GPR56/ADGRG1 is also expressed by healthy, but downregulated by disease-associated microglia [148], and a splice variant of it has been demonstrated to mediate microglia-mediated synaptic pruning [72]. Furthermore, this receptor has been reported as a platelet collagen receptor that senses shear force during hemostasis [149], while GPR97/ADGRG3 is expressed on granulocytes and triggers their antimicrobial functions [150].

These examples illustrate the broad spectrum of aGPCR involvement in diverse tissues and essential physiological functions. Nevertheless, our knowledge is still incomplete with respect to the functional involvement of so far uncharacterized aGPCRs such as GPR123/ADGRA1 or GPR144/ADGRD2 as well as with regard to the functional mechanisms underlying these processes.

Clinical aspects of aGPCR function and potential therapeutics

Overall, GPCRs have well-established pharmacological tractability, but currently no approved therapies target any of the 33 members of the human aGPCR class. However, human genetics and preclinical research have established strong links between aGPCRs and diseases. This, together with a greater understanding of their functional complexity, has led to growing interest in aGPCRs as drug targets. A few of the receptors have been shown to monogenically cause pathologies, such as VLGR1/ADGRV1 (Usher syndrome type II) [6], GPR56/ADGRG1 (bilateral frontoparietal polymicrogyria) [151], and EMR2/ADGRE2 (vibratory urticaria) [8].

Besides this direct involvement of monogenic variants in human pathologies, numerous aGPCRs have been associated with human conditions or disorders. Given their high expression and roles in the development of the nervous system [9,152], it is not surprising that several neurodevelopmental disorders have been associated with aGPCR dysfunction and variants [118]. Many of these result in severe phenotypes, including neural tube defects [11], spina bifida [153], and brain malformation [7]. When fully expressed, these phenotypes are often embryonically lethal and, therefore, difficult to target therapeutically. However, there are aGPCR variants that lead to milder symptoms that present only after birth, offering the potential for pharmacological treatment. For example, a loss-of-function variant of LHPN3/ADRGL3 confers susceptibility to attention deficit hyperactivity disorder (ADHD) [154-157]. Genetic knock-down of LHPN3/ADRGL3 in *Drosophila* and zebrafish models showed a rearrangement of dopaminergic neurons in the brain [158,159], and an increased expression of the dopamine and serotonin transporter genes in mice [160]. These changes resulted in ADHD-like behavior, which could be rescued through pharmacological intervention with ADHD drugs [158,159]. These studies suggest that targeting LHPN3/ADRGL3 with specific agonists may provide an avenue to treat an ADHD phenotype.

Cellular functions of aGPCRs such as cell adhesion, migration, cell polarity, and guidance are highly relevant for tumor cell biology. Consistently, members from all aGPCR groups are associated with cancer, for example, GPR56/ADGRG1, CD97/ADGRE5, GPR133/ADGRD1, ELTD1/ADGRL4, GPR110/ADGRF1, GPR116/ADGRF5, BAI1/ADGRB1, GPR124/ADGRA2, and GPR125/ADGRA3 [161-167]. For some of these aGPCRs, there is evidence for incorrect expression or changes in receptor activity (e.g., VLGR1/ADGRV [168], GPR133/ADGRD1 [169], and LPHN/ADGRL [170]), whereas for others, detailed knowledge exists about the molecular role they play in tumorigenesis (e.g., CD97/ADGRE5 [34,171,172], ADGRF5/GPR116 [173,174], GPR56/ADGRG1 [51,175,176,177], BAI1/ADRGB1 [178-180], GPR124/ADGRA2 [181], and GPR125/ADGRA3 [182]). Therefore, the receptors are potential therapeutic targets for the treatment of cancer. For instance, BAI1/ADRGB1 is epigenetically silenced in malignant glioma, suggesting that tumorigenesis may select for BAI1/ADRGB1 silencing or inactivation [183]. Exogenous restoration of BAI1/ADRGB1 expression reduces growth and vascularization of tumors derived from gliomas suggesting that BAI1/ADRGB1 is a tumor suppressor. Treatment with 5-aza-2′-deoxicytidine (5-Aza-dC) resulted in re-expression of the receptor along with restoration of its functional activity *in vitro* and *in vivo* [183], suggesting BAI1/ADRGB1 as a potential new drug target for the treatment of gliomas.

Given the complex structure of aGPCRs, it would be theoretically possible to pharmacologically target several topographically distinct regions of the receptors (Fig. 2). The integral agonist mechanism of aGPCRs naturally lends itself to the use of peptide-based ligands for pharmacological modulation. Indeed, synthetic peptides that mimic the *Stachel* sequence of a given aGPCR have been shown to activate their cognate receptor [40,41,52]. Furthermore, the therapeutic potential of *Stachel*-derived peptides was demonstrated in zebrafish using loss-of-function mutations in GPR126/ADGRG6 [40]. Here, a synthetic GPR126/ADGRG6 *Stachel* peptide partially rescued the Schwann cell hypomyelination phenotype [40]. Similarly,

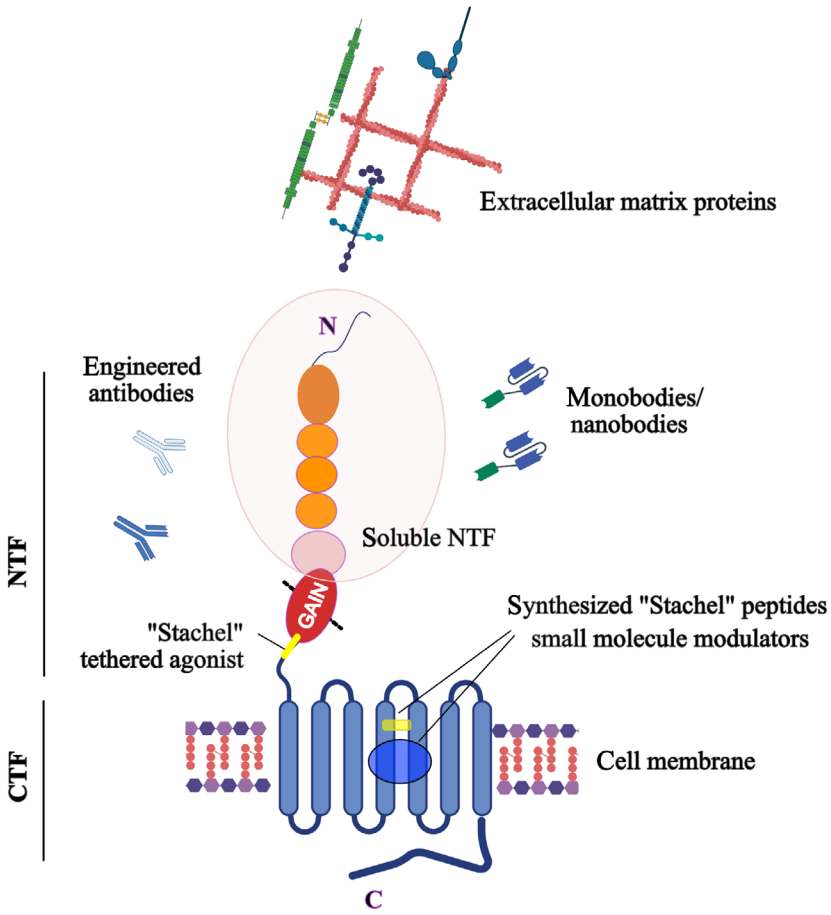

Fig. 2. Potential therapeutics to target aGPCRs. The NTF (N-terminal fragment) and CTF (C-terminal fragment) of Adhesion GPCRs are targeted by different novel therapeutic approaches, including antibodies, monobodies/nanobodies, *Stachel* sequence-derived peptides, extracellular matrix (ECM) molecules, and small molecule modulators. Further, the soluble NTF can act as therapeutic itself. The image was created with biorender.com.

Stachel-derived peptides of GPR116/ADGRF5 could suppress surfactant phospholipid secretion in mice [58]. However, as several *Stachel*-derived peptides display agonist promiscuity and are able to activate aGPCRs from different groups [52], peptides with high cross-reactivity would likely be unsuitable as therapeutic agents due to off-target effects and would require further modification for enhanced specificity.

Receptor-specific small molecule (partial) agonists have been characterized for GPR97/ADGRG3 [74], GPR126/ADGRG6 [184], GPR56/ADGRG1, and GPR114/ADGRG5 [185]. Only one small molecule antagonist has been described so far – the rotenone derivative dihydromunduletone, which can bind both, GPR56/ADGRG1 and GPR114/ADGRG5 [186]. These small molecules target the 7TM domain and were thought to modulate aGPCR function by binding to the orthosteric binding site of the tethered peptide. Nevertheless, future studies will need to identify the exact location and mechanism of these molecules.

Another exciting avenue for targeting aGPCRs is suggested by recent advances in engineering antibodies as GPCR therapeutic agents [187]. Similarly, mono- or nanobodies might be conceivable. The large NTFs and GAIN domains of aGPCRs present a variety of potential epitopes against which extracellular-modulating anti-, mono-, or nanobodies could be generated. Indeed, antibodies have already been characterized for GPR56/ADGRG1 [188], EMR2/ADGRE2 [136], and CD97/ADGRE5 [189]. These antibodies were shown to inhibit their respective receptor function *in vitro*, affecting the adhesion and migration of neutrophils [136], granulocyte migration [189], as well as the migration of neural progenitor cells [188], but their efficacy in disease-relevant *in vivo* models still needs to be elucidated. An anti-EMR1/ADGRE1 antibody, however, induced natural NK cell-mediated removal of eosinophils *in vivo* in monkeys [190], while in a clinical phase-I study recombinant antibodies against LPHN3/ADGRL3 [191] resulted in beneficial effects on the secretion of inflammatory mediators through downregulating of NF_kB signaling pathways [191]. Thus, aGPCR-targeted antibodies also offer an exciting approach to therapeutically target aGPCRs.

Besides targeting the *cis* function of aGPCRs, *trans* signaling can also be therapeutically exploited. As has

been shown experimentally, the soluble NTF of CD97/ADGRE5 can stimulate tumor angiogenesis through the binding of integrins and chondroitin sulfate [34,46,171]. Also, the NTF of BAI1/ADGRB1 has been discovered to have anti-tumorigenic activity, as this 120 kDa fragment inhibited angiogenesis *in vitro* and also suppressed subcutaneous tumor growth [179]. Thus, recombinant expression of truncated receptor segments can be used in therapeutic approaches.

How to advance future research on Adhesion GPCRs

Despite the essential functions and pharmacological potential of aGPCRs, there are still vast gaps in the understanding of their exact roles and, thus, their impact on human conditions. To overcome these limitations, the current knowledge on aGPCRs needs to be structured, and better tools to specifically study aGPCRs need to be developed. Thus, it is vital to direct intense basic and applied research toward the most pressing questions as outlined below and shown in Box 1.

What is the cause and consequence of the huge sequence repertoire and resulting variants of aGPCRs?

Publicly available genome databases and genome-wide association studies in humans offer the unparalleled opportunity to generate an information map on how many different variants of each aGPCR exist in specific physiological contexts and which variants are associated with diseases.

For instance, RNA sequencing data sets show that aGPCRs have many splice variants, which exhibit distinct expression patterns [5,37]. Yet, little is known about the function of these naturally occurring variants and how they differ in biochemical processes. Mapping them across species and profiling their intracellular signaling repertoire are crucial to understand their emergence, roles, and preservation across evolution.

Box 1. Future directions to develop aGPCR research

- Systematically map available data on aGPCRs across species and profile their intracellular signaling repertoire.
- Acquire informative structures on aGPCRs.
- Make use of engineered aGPCR versions employing the toolbox of GPCR-tailored technologies to study receptor trafficking, compartmentalization, and signaling.
- Study the impact of aGPCRs on human conditions.
- Translate fundamental research into clinical application (collect clinical study data, combine this information with basic research towards a more clinically relevant approach).

How is the complex structure of aGPCRs related to their function *in vitro* and *in vivo*?

Understanding the complex structures of aGPCRs presents a significant but intriguing challenge toward a more comprehensive knowledge of their function. Studying engineered aGPCR versions using the toolbox of GPCR-tailored technologies, for instance, in immortalized cell lines, will provide insights into the role of the numerous diverse aGPCR domains. CRISPR/Cas9-mediated genome-editing then enables the expression of these synthetic versions in model organisms to translate the findings into *in vivo* systems. While invertebrate animal models such as *Drosophila melanogaster* and *Caenorhabditis elegans* can be particularly useful to discover key receptor concepts in embryonic development, for many aGPCRs not present in invertebrates, murine and zebrafish models have been shown to be promising systems for structure–function relationship studies. Advances in proteomic techniques further offer the ability to map the interactome of aGPCRs *in vivo* and test the physiological relevance of ligands.

How do aGPCRs contribute to physiology in health and disease, and how can they be exploited to design therapeutic strategies?

Adhesion GPCRs have a large impact on the maintenance of health and disease progression. Yet, exploring the significance of individual aGPCRs in (patho-) physiological processes remains an advancing field of investigation. Using the approaches discussed above combined with siRNA/shRNA reagents and receptor-specific anti-/nanobodies represent an established approach to dissect their contribution to physiological processes and to identify disease state-specific diagnostic markers and targets for drug development.

One example for this approach is GPR123/ADGRA1. Very limited information is currently available on its physiological roles, partially owing to its short extracellular N terminus, hampering the development

of receptor-specific antibodies to map its expression. While RNA-based approaches detected wide-spread distribution of GPR123/ADGRA1 in the central nervous system [192,193], immunohistochemical staining with a C-terminally binding antibody recently demonstrated that it is an independent biomarker of bladder cancer [194]. Proteomics analyses of patient samples or disease animal models can speed up the detection of GPR123/ADGRA1 functions in other processes and boost the quest for receptor-selective drugs through high-throughput screenings or *in silico* techniques.

Establishing the approaches mentioned above can only be accelerated through joint efforts of international and interdisciplinary scientists and research centers. Collaborations between technology platforms focused on CRISPR/Cas9-mediated gene knock-out or high-throughput screening can speed up the discovery of aGPCR ligands. Likewise, open-access collection of the functional impacts of disease-related aGPCR mutations will foster further investigations by basic scientists, industrial researchers, and clinicians.

Expanding and advancing the aGPCR field through networking

The aGPCR field is relatively young and our understanding of these enigmatic receptors has only begun to take shape. A key to addressing the most pressing questions in the field, to filling the gaps and to moving research 'from bench to bedside', is to form a critical mass regarding expertise, access to technologies, tools, and specimens, as well as know-how. This can only be achieved by engaging and bringing together a network of appropriate groups of professionals from different fields of expertise, as well as other stakeholders (Fig. 3).

Obviously, basic researchers from an academic environment are the key players in generating new knowledge on aGPCRs. Increasing interdisciplinary approaches in the aGPCR field will be achieved by bringing together researchers from various backgrounds such as biological sciences (biochemistry, signal transduction, structural biology, and biophysics), bioinformatics (databases, data mining, data curation, and computational modeling), and basic medicine (pharmacology, pharmacogenomics, drug discovery and design, and drug therapy). The field would particularly benefit from inclusion of scientists experienced with GPCRs and non-GPCR adhesion receptors. Moreover, the presence of highly specialized researchers from unrelated fields could enrich the aGPCR research with new sophisticated technologies.

Within this group of scientists, non-tenured principal investigators (PIs), early career investigators, and students are of particular importance as they represent a large proportion of the current scientific community and its future. Training of early career investigators to become the next generation of leaders in the aGPCR field can lead to its expansion and to the development of novel ideas along with securing long-term progress of this research area. Therefore, experienced PIs are essential to provide professional and scientific advice as mentors for these junior colleagues.

Given the relevance of aGPCRs for human health, clinicians play a crucial role in translating research 'from bench to bedside' and introducing aGPCR research into a clinical setting. Furthermore, aGPCR researchers particularly benefit from medical institutions with access to human tissues and patient data.

Another important group of stakeholders in aGPCR research are employees of the pharmaceutical industry and biotechnology companies. Their interest in the field stems from the untapped pharmacological potential and druggability of aGPCRs. Networking between pharma representatives and basic researchers helps bridging the gap between fundamental research and therapeutic innovation. For instance, while basic researchers can provide assays for drug screens, companies would perform such screenings on a large scale. These interactions are currently being established, but are still in their infancy.

Finally, the main beneficiaries of aGPCR research include pharmaceutical/biotechnology companies, patients, educators, and the general public. While companies could utilize the research data to develop new treatments and create revenue that would help their sustenance, patients would benefit from these therapeutics. Educators could use the research results to advance their knowledge and transmit it to students as well as to other interested individuals from the general public that represent the key components in popularizing and increasing recognition of the field.

Strategies to promote and fuel successful aGPCR research

Bringing together a network of diverse scientists and stakeholders to tackle the scientific challenges of aGPCR research and to orchestrate efforts to fill the existing knowledge gaps is one of the major challenges to date. Especially the proposed vision for the future development on basic and translational research on aGPCRs is not attainable without combined efforts.

Huge progress has already been achieved through financing of single projects by national funding bodies in many countries and interactions within the framework of the Adhesion GPCR Consortium (AGC,

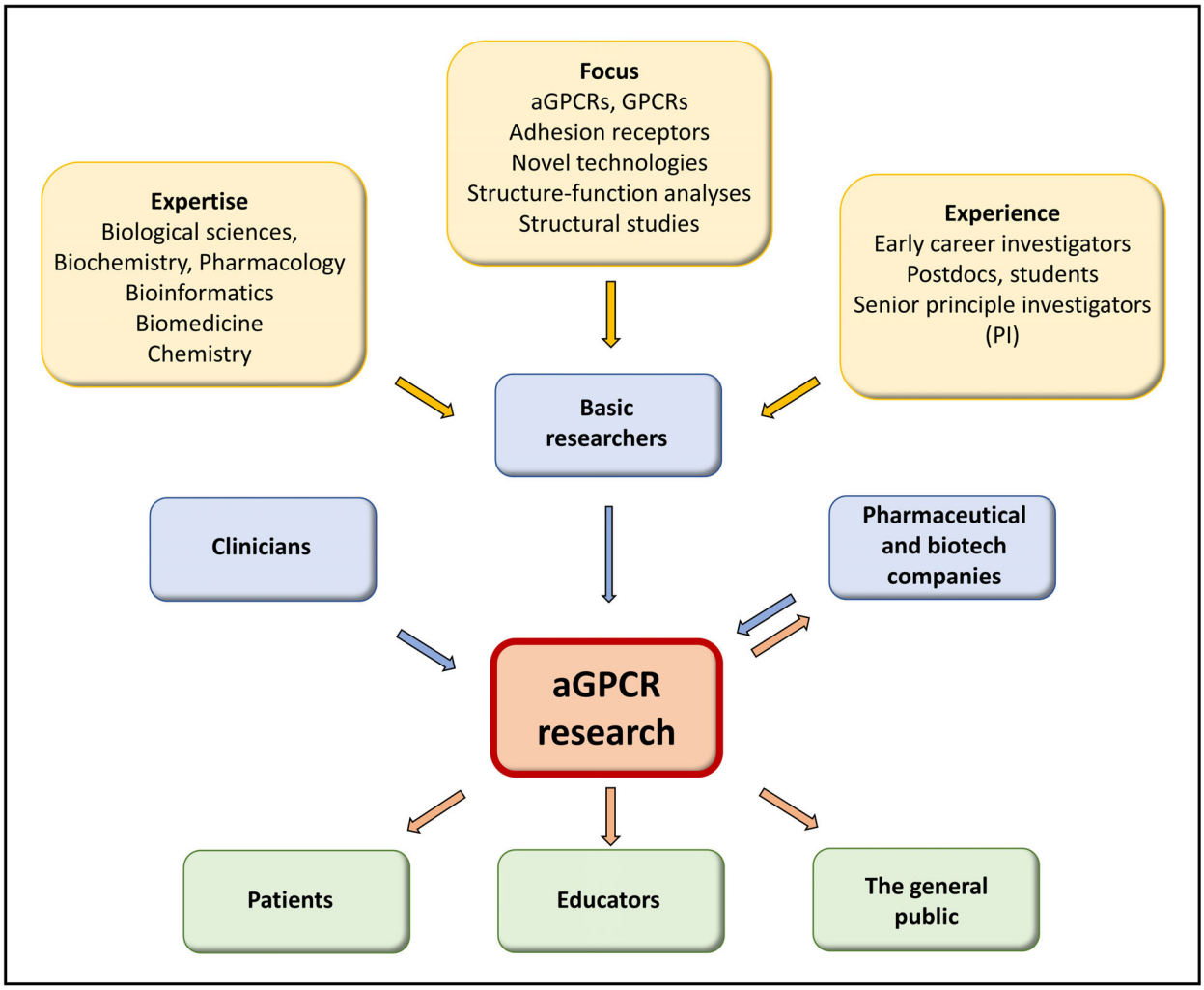

Fig. 3. Network of stakeholders in the aGPCR field. Many groups are interliaising to promote and progress aGPCR research. Major contributors include various basic researchers and clinicians, whereas beneficiaries are patients, educators, and the general public. Pharmaceutical/biotechnology companies act as both, contributors and benefactors, of aGPCR research.

https://www.adhesiongpcr.org/). Both have supported individual research projects and helped foster first ties between researchers. While the Adhesion GPCR Consortium (AGC) offers a conceptual framework for scientists to gather and exchange information, it does not provide any economic support, while national grants are usually limited to a certain country and are often hard to obtain, especially for early career investigators.

A bottom-up network to fill these gaps has recently been established. This network is funded by the European Cooperation in Science and Technology (COST), which is a funding organization for research and innovation networks. The COST Action CA18240 *Adher´nRise* is a multi-national and multi-stakeholders interdisciplinary research network guided by a MoU (Memorandum of Understanding) aiming to promote, stimulate, and translate research on aGPCRs 'from bench to bedside' in Europe. With more than 112 members from 27 countries it has started to shape the aGPCR landscape. Committed participants create a shared understanding and make relevant, transparent, and effective decisions. Engagement of fellow scientists and stakeholders in general is pivotal for the success of the Action, especially as it aims to build a structured and cohesive community of researchers working on aGPCRs. Committed participants will create a shared understanding and make relevant, transparent, and effective decisions.

The flagship cooperation tool of *Adher´nRise* is an Action webpage (https://www.adhernrise.eu) providing a hub for all stakeholders to join or interrogate the action and exchange information about the members,

meetings and workshops, job offers, and links to other webpages containing useful information on aGPCR research. Moreover, it will soon host a comprehensive technology database featuring a collection of technologies and expertise, including access to protocols, animal models, cell lines, reagents/tools, technology platforms, and mutation data collections.

In order to promote and broaden acquaintance and interactions among the multiple partners, strengthening the ties for an environment of continuous collaboration is essential. Further, frequent exchange of scientific knowledge, joint symposia, and collaborative meetings are often held by the network and exchange between laboratories is encouraged and funded. Especially young scientists are in the focus of these network efforts.

Furthermore, given the vast pharmacological potential of aGPCRs, this Action is committed to bridge the gap between fundamental research and therapeutic innovation. Therefore, attracting more representatives from the pharmaceutical industry, biotechnology companies, and clinicians is paramount. The action already includes collaborators from large international industrial partners and continues to seek additional prospective partners to better promote technology transfer. Finally, to increase the visibility of aGPCRs and to raise awareness of their importance for health and disease, the general public is frequently addressed by the Action´s efforts.

Overall, research on aGPCRs has been steadily increasing as these enigmatic receptors have become the focus of several different scientific areas. To support these advances, a network of stakeholders has emerged that is supported and structured within the framework of the COST network CA18240 *Adher'n-Rise* guided by the ambitious aim to widely establish aGPCRs as disease-relevant molecules and accepted therapeutic targets.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

All authors wrote the manuscript.

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