The multiple signaling modalities of adhesion G protein-coupled receptor GPR126 in development

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The G protein-coupled receptor (GPCR) superfamily is the largest known receptor family in the human genome. Although the family of adhesion GPCRs comprises the second largest sub-family, their function is poorly understood. Here, we review the current knowledge about the adhesion GPCR family member GPR126. GPR126 possesses a signal peptide, a 7TM domain homologous to secretin-like GPCRs, a GPS motif and an extended N-terminus containing a CUB (Complement, Uegf, Bmp1) domain, a PTX (Pentraxin) domain, a hormone binding domain and 27 putative N-glycosylation sites. Knockdown and knockout experiments in zebrafish and mice have demonstrated that Gpr126 plays an essential role in neural, cardiac and ear development. In addition, genome-wide association studies have implicated variations at the GPR126 locus in obstructive pulmonary dysfunction, in scoliosis and as a determinant of trunk length and body height. Gpr126 appears to exert its function depending on the organ system via G protein- and/or N-terminus-dependent signaling. Here, we review the current knowledge about Gpr126, which, due to the variety of its functions and its multiple signaling modalities, provides a model adhesion GPCR to understand general functional concepts utilized by adhesion GPCRs.

Keywords: GPR126; heart; myelination; ear; GPS; GAIN; NTF; CTF; adhesion GPCR

Introduction

The GPCR superfamily is the largest known receptor family in humans comprising around 4% of the entire protein-coding human genome1,2, and GPCRs can be grouped into five classes: Glutamate, Rhodopsin, Adhesion, Frizzled/Taste 2, and Secretin3,4. These receptors are major targets of modern therapeutics, as they play important roles in organ development and disease5. The Adhesion class contains 33 receptors in humans that can be classified in nine distinct families6. They are expressed in most organs playing important roles in development and disease. They contain extended N-termini containing various adhesion domains, which are separated from the 7TM domain by a GPCR autoproteolysis-inducing (GAIN) domain. Cleavage at the GPCR proteolysis site (GPS) in the GAIN domain results in an N-terminal fragment (NTF) and a C-terminal fragment (CTF) that may or may not remain together at the cell surface. This property permits a variety of
signaling modalities including activation of CTF-dependent or -independent signals via agonist interactions at the NTF or the CTF. In addition, NTF can bind to partners at the surface of a neighboring cell or the extracellular matrix or NTF can be shed of to induce non-cell-autonomous signals in neighboring cells or in distant cells (reviewed in [7]). However, how these receptors mediate their physiological and pathological functions is poorly understood.

**Discovery and Structure of GPR126**

GPR126 was first identified by Fredriksson and co-workers by performing blast searches utilizing human sequences of known adhesion GPCRs; they identified GPR123, GPR124, GPR125, GPR126, GPR127 and GPR128 [8]. Phylogenetic analysis placed GPR126 in a cluster with the previously identified GPR64 (HE6), GPR56 (TM7XN1), GPR97 (Pb99), GPR112 and GPR114 (now designated Group VIII) [7,8]. The first experimental evidence for a biological function was provided by Moruguchi and co-workers who cloned the full-length human and mouse GPR126 cDNAs (“DREG” for developmentally regulated G protein-coupled receptor) utilizing human brain and human keratinocyte cDNA libraries [9]. While the groups of Moruguchi and Fredriksson identified GPR126 based on sequence analyses [8,9], Stehlik and co-workers isolated endogenously expressed GPR126 (vascular inducible GPCR, VIGR) from human umbilical vein endothelial cell (HUVEC) cultures by searching for differentially expressed secretory and membrane proteins in order to understand the role of the vascular endothelium in the process of acute inflammation [10].

Domain analyses revealed that GPR126 possesses a signal peptide, a 7TM domain homologous to secretin-like GPCRs, a GPS motif and an extended N-terminus containing a CUB (Complement, Uegf, Bmp1) domain, a PTX (Pentraxin) domain, a hormone binding domain and 27 putative N-glycosylation sites [8-10] (Figure 1). Overexpression experiments of wild-type and mutated forms of human and mouse GPR126 combined with antibody-based assays indicated that GPR126 is cleaved at the GPS motif into a CTF that locates to the plasma membrane and a NTF. Subsequently, the NTF is cleaved by furin at an additional cleavage site (S2 site) between the GPS motif and the PTX domain [9], releasing a putative fragment from the rest of the NTF, which remains non-covalently associated with the CTF (Figure 1). Experiments utilizing tunicamycin, a potent inhibitor of N-glycosylation, confirmed that GPR126 is indeed highly glycosylated and indicated that glycosylation is not essential for the cellular localization of GPR126 [10]. Finally, the cytoplasmic domain contains a potential palmitylation site as well as several potential phosphorylation sites for cAMP dependent kinases/protein kinase G, protein kinase C, and casein kinase II, as well as a potential myristoylation signal and a microbodies C-terminal targeting motif [10].

**Expression pattern of GPR126**

Initially, it had been predicted based on human and mouse ESTs (expressed sequence tags) that GPR126 is expressed in adult lung, liver, testis and skeletal muscle [6]. In recent years a large amount of expression data has been collected based on RT-PCR, in situ hybridization, and genetic labeling. GPR126 is strongly expressed during mouse and zebrafish development predominantly in the somites, the frontonasal process, pharyngeal arches, the heart, the ear and the nervous system. In adult mouse tissues, GPR126 has been detected at a high level in the lung and at low levels in a few other tissues (for details see Table 1). The broad and tightly regulated spatio-temporal expression pattern suggests that GPR126 might play important roles in development and disease.

**Gpr126 in peripheral nerve development**

In 2009, the Talbot lab described the first known function of Gpr126 [11]. Starting with a forward genetic screen for mutations that affect the development of myelinated axons [12], two allelic mutations of gpr126 were uncovered. Myelin is the multilayered membrane generated by specialized glial cells that insulates and protects axons in the vertebrate nervous system; in the PNS, Schwann cells generate the myelin sheath by iteratively wrapping their membrane around a selected neuronal axon. In gpr126 zebrafish mutants, peripheral myelination is specifically impaired: Schwann cells associate with axons but never spiral their membrane to generate the myelin sheath. Analysis of Gpr126 constitutive knockout mice showed that the function of Gpr126 is conserved in mammalian myelination [13]. Like zebrafish mutants, Schwann cells in Gpr126-/ mice ensheath but do not myelinate axons. Unlike zebrafish mutants, all stages of Schwann cell development leading to axon ensheathment were significantly impaired in Gpr126-/ mice. Additional phenotypes observed in mouse knockouts but not zebrafish mutants included significant embryonic lethality, ataxia, limb abnormalities and pronounced axon degeneration [13]. These observations raised the question of which cell(s) require Gpr126 function for proper Schwann cell myelination.

To address the question of cellular autonomy in Schwann cell myelination, Mogha and co-workers recently described a conditional Gpr126 mutant [14]. In this study, the conditional mutant was used to delete...
GPR126 in Schwann cell precursors at E12.5, and Schwann cell-specific Gpr126 mutants recapitulated several defects observed in constitutive Gpr126 knockouts. Specifically, Schwann cells completely failed to myelinate axons, and all stages of Schwann cell development leading to axon ensheathment were severely delayed. This data supports a model in which Gpr126 is required autonomously in Schwann cells for timely axon selection and myelination, consistent with genetic chimera analyses in zebrafish [11]. Interestingly, Schwann cell-specific Gpr126 conditional mutants were viable and did not present with other defects observed in constitutive Gpr126-/- mutants, including ataxia, limb defects and axon degeneration. These data suggest that Gpr126 has essential functions in other cell types and underscore the need for further tissue-specific investigations of this adhesion GPCR.

GPR126 in heart development

Studies on Gpr126 knockout mice have demonstrated that Gpr126 function is also required for cardiac development [15]. This was initially surprising, as previous reports analyzing the zebrafish mutant line gpr126<sup>st49</sup> did not report a heart phenotype [11]. Recently, the analysis of three different mouse lines has demonstrated that disruption of Gpr126 causes a heart phenotype and is embryonic lethal (E11 to E12.5). At E10.5 in Gpr126 knockout mice, the ventricular cardiac wall is trabeculated and appears normal. In contrast, at E11.25, Gpr126 knockout mice have a thinner ventricular myocardial wall, contain fewer trabeculae [15, 16] and exhibit bradycardia along with cardiac arrhythmia [16]. These data suggested that Gpr126 might be required for proper cell–cell contacts and electric coupling. However, immunohistological and electron microscopic analyses indicated that cell–cell contacts were maintained as the localization of cadherins and adherent junction proteins was not affected. In contrast, mitochondria in cardiomyocytes as well as in endocardial cells exhibited unusual characteristics such as a more complex shape, less well developed cristae and electron-dense precipitates, indicating mitochondrial dysfunction. This was further supported by the accumulation of lipid droplets and a decrease in glycogen deposits [16]. The accumulation of lipids might be the cause of the observed cardiac bradycardia as it has previously been demonstrated that mitochondrial respiratory dysfunction or the deficiency of lipid transporter in the mitochondrial membrane leads to the accumulation of lipids causing cardiac arrhythmia [17, 18].
Table 1. GPR126 expression data.

| Organ/Tissue                           | Age       | Detection | Species  | References | KO/KD Phenotype |
|----------------------------------------|-----------|-----------|----------|------------|-----------------|
| embryo                                 | E11/E15/E17| RT-PCR    | mouse    | [9]        | Yes [15, 16]    |
| bone, spine, vertebrae, jaw            | E16.5     | ISH       | mouse    | [25]       | TBD             |
| head, pectoral fin                     | 48 hpf    | ISH       | zebrafish| [11]       | TBD             |
| cartilage                              | adult     | RT-PCR    | human    | [25]       | TBD             |
| ceratobronchiol (gill arches)          | 7.2 hpf   | ISH       | zebrafish| [19]       | TBD             |
| ear, otic vesicle                      | E9.5/E11.5| ISH       | mouse    | [16]       | No [13]         |
| otic vesicle                           | 24 hpf    | ISH       | zebrafish| [19]       | Yes [19]        |
| canal projections                      | 48/72 hpf | ISH       | zebrafish| [19]       | TBD             |
| heart                                  | E9.5/E11.5| ISH       | mouse    | [16]       | Yes [15, 16]    |
| endocardium                            | E9.5/E11.5| multiplex ISH | mouse  | [16]       | Yes [16]        |
| brain                                  | E10/E11   | ISH       | mouse    | [9]        |                 |
| intermediate mesoderm                  | 1/4/23 somite stage | ISH   | zebrafish| [19]       | Yes [16]        |
| kidney                                 | adult     | RT-PCR    | mouse    | [9]        | TBD             |
| collecting duct                        | adult     | RT-PCR    | rat      | [16]       |                 |
| liver                                  | adult     | RT-PCR    | mouse    | [9]        | TBD             |
| lung                                   | P14       | ISH       | mouse    | [14]       | TBD             |
| macula                                 | 48 hpf    | ISH       | zebrafish| [19]       | TBD             |
| nervous system, forebrain              | E15       | RT-PCR    | mouse    | [42]       | TBD             |
| Bergmann glial cells                   | P6        | RT-PCR    | mouse    | [42]       | TBD             |
| DRG neurons                            | P4, P21   | ISH, RT-PCR| mouse  | [11, 14]  | Yes [13]        |
| sciatic nerve                          | P4        | RT-PCR    | mouse    | [11]       |                 |
| brain                                  | adult     | RT-PCR    | mouse    | [9]        | TBD             |
| olfactory epithelium                   | 24 hpf    | ISH       | zebrafish| [19]       | No [13]         |
| olfactory bulb                         | 32 hpf    | ISH       | zebrafish| [11]       | TBD             |
| frontonasal process                    | E9/E10    | ISH       | mouse    | [9]        | TBD             |
| nose                                   | 48/72 hpf | ISH       | zebrafish| [19]       | TBD             |
| brain                                  | adult     | RT-PCR    | zebrafish| [11]       | TBD             |
| Schwann cells of PLLn                  | 3 dpf, adult | ISH, RT-PCR | zebrafish | [11]       | TBD             |
| Schwann cells of ALLn                  | 30 hpf to 4 dpf | ISH   | zebrafish| [11]       | Yes [11, 15, 16]|
| neural crest                           | 48 hpf, 54 hpf | ISH   | zebrafish| [11]       | TBD             |
| pancreas                               | adult     | RT-PCR    | mouse    | [9]        | TBD             |
| pericardium                            | 48 hpf    | ISH       | zebrafish| [16]       | TBD             |
| pharyngeal arches                      | E9/E10    | ISH       | mouse    | [9]        | TBD             |
| placenta                               | adult     | RT-PCR    | human    | [31]       | No [15]         |
| placenta                               | adult     | northern blot | mouse  | [10]       | TBD             |
| trophoblast giant cells                | E10/E11   | LacZ reporter | mouse  | [15]       | No [15]         |
| presomite mesoderm                     | E9        | ISH       | mouse    | [9]        | TBD             |
| post-otic neural crest                 | 24 hpf    | ISH       | zebrafish| [19]       | TBD             |
| skeletal muscle                        | adult     | RT-PCR    | mouse    | [9]        | TBD             |
| somites                                | E9.5/E11.5| ISH       | mouse    | [16]       | No [15]         |
| E10/E11                                | ISH       | mouse    | [9]        |            |                 |
| spleen                                 | adult     | RT-PCR    | mouse    | [9]        | TBD             |
| tail fin tip                           | 48/72 hpf | ISH       | zebrafish| [19]       | TBD             |
| testis                                 | adult     | RT-PCR    | mouse    | [9]        | TBD             |
| uterus                                 | adult     | RT-PCR    | human    | [37]       | TBD             |

**Cultured Cells**

| Vascular cells (aortic ECs, HUVEC) | RT-PCR | human | [10] | No [15] |
|------------------------------------|--------|-------|------|---------|
| IFTR1 cell line, primary DRG neurons, Schwann cells | antibody | rat | [43] | NA |

KO: knockout; KD: knockdown; ISH: in situ hybridization; EC: endothelial cell; PLLn: posterior lateral line nerve; ALLg: anterior lateral line ganglia; TBD: to be determined; NA: not applicable.
The mitochondrial phenotype, but not the accumulation of lipid deposition, could be recapitulated in zebrafish utilizing morpholinos that deplete Gpr126 \[16\].

The major common phenotype between Gpr126 knockout mice and Gpr126-depleted zebrafish is cardiac ventricular hypotrabeculation \[15, 16\]. With the help of domain-specific morpholinos, rescue experiments in Gpr126-depleted morphants and an in situ protein binding assay using mGpr126-NTF on mouse cardiac sections, Patra and co-workers demonstrated that the NTF fragment NTF$^{\Delta GPR}$ (amino acid 1–783) of Gpr126 is important for cardiac trabeculation \[16\]. Moreover, these data suggested that mGpr126-NTF acts in a paracrine fashion. However, the molecular mechanism utilized by Gpr126 to regulate heart development remains elusive.

**GPR126 in ear development**

In 2013, Geng and co-workers reported that Gpr126 plays a role not only in heart and PNS development, but also in inner ear development \[19\]. The three semicircular canals of the vertebrate inner ear detect angular acceleration (rotational movements). These canals are formed via the movement and fusion of sheets of epithelium (reviewed in \[20\]). In zebrafish, finger-like projections of epithelium grow into the center of the otic vesicle, where they fuse \[21\]. In gpr126 mutants, these projections overgrow and fail to fuse correctly, implicating Gpr126 in the control of projection outgrowth, contact recognition and fusion in the developing ear. A closer analysis revealed that the major difference of gpr126 mutants compared to wild-type zebrafish were changes in the extracellular matrix \[19\]. These data suggest that Gpr126 plays an important role in cell adhesion, signaling and cell-cell or cell-matrix interactions (Figure 1). In the future it will be important to determine in more detail how Gpr126 regulates the cell behavior in zebrafish inner ear development and if this function is conserved between fish and mammals.

**Emerging roles of GPR126 in disease**

To date, there is no human disease known to be caused by a mutation in GPR126. However, genome-wide association studies have implicated variations at the GPR126 locus as a determinant of trunk length and body height \[22-24\], adolescent idiopathic scoliosis \[25\] as well as pulmonary function \[26\]. Body height is determined by trunk and leg length. In contrast to trunk length, leg length is positively associated with nutritional intake in childhood. This suggests that final upper and lower body size is controlled by different pathways \[22, 27\]. To search for loci influencing adult height, several groups have independently associated GPR126 with body height. A genome-wide analysis identified associations with trunk length at SNP rs6570507 in GPR126 \(P\)-value $= 4 \times 10^{-5}$ based on 299,216 single nucleotide polymorphisms (SNPs) and a group of 12,611 adults of Caucasian origin \[22\]. Notably, this SNP was associated in a separate study with adolescent idiopathic scoliosis utilizing 1,819 cases and 25,939 controls \(P\)-value $= 2.25 \times 10^{-7}$ as well as Han Chinese and European-ancestry populations (combined \(P\)-value $= 1.27 \times 10^{-14}$) \[23\]. The association of GPR126 with height is further supported by a study including 11,536 individuals composed of Australian twins, family members, and unrelated individuals ($\sim$550,000 genotyped SNPs) \[23\] and in a study based on a European American pediatric cohort (8,184 children) \[24\]. Finally, GPR126 has also been associated with obstructive pulmonary dysfunction based on the analysis of 20,890 participants of European ancestry identifying 69 SNPs with the top SNP (rs3817928) having a \(P\)-value of $2.60 \times 10^{-10}$ \[26\].

**Regulation of GPR126 expression**

In order to understand the function and physiological role of GPR126 and how to modulate it in disease it is important to understand how its expression is regulated. In zebrafish, the expression pattern of gpr126 during development in ear and PNS development suggested that gpr126 might be regulated by the transcription factor Sox10 (Figure 1), which is known to be expressed in the neural crest and ear \[11, 13, 19\] and to be important for ear development \[28\] as well as Schwann cell development \[29, 30\]. Importantly, Geng and co-workers showed that gpr126 expression is partially lost in colorless (sox10) zebrafish mutants. A detailed analysis of these mutants suggested that gpr126 expression is in the ear and the PNS but not in the heart and posterior mesoderm sox10-dependent \[19\]. In the future it will be important to elucidate the relationship between Sox10 and Gpr126.

Very little is also known about signaling pathways that induce GPR126 expression. Stehlik and co-workers presented the first and so far only evidence that GPR126 expression can be induced \[10\]. They demonstrated that stimulation of HUVECs with lipopolysaccharide (LPS) or thrombin resulted in transiently increased GPR126 mRNA levels peaking at 12 h and declining to background levels at 24 h of LPS treatment. However, this effect was cell-type dependent. While no GPR126 expression was observed in primary skin microvascular endothelial cells (ECs), constitutive expression was detectable in aortic ECs. Recently, it has also been shown that Gpr126 is expressed in endocardial cells in the developing mouse heart \[16\]. Induction of GPR126 expression in HUVECs was independent of the major pro-inflammatory transcription factor NF-κB as well as
p38 mitogen-activated protein kinase. However, specific inhibition of JNK and MEK1 completely abolished GPR126 expression \[^{10}\] (Figure 1).

Alternative splicing is one important way gene function can be regulated to increase proteome diversity in higher eukaryotic organisms. GPR126 is a complex protein. It is encoded by 26 exons in mice \[^{16}\], is widely expressed in many tissues and plays distinct roles in several organs. Thus, it appears likely that different splice isoforms of GPR126 exist. Indeed, several alternatively spliced forms have been described in mouse that differ in the presence of exon 6 (including: isoform 1; excluding: isoform 2) and exon 25 changing the usage of termination codons (including: α isoforms; excluding: β isoforms) \[^{9}\] (Figure 1). Subsequently, the existence of different splice isoforms has been confirmed in human, monkey \[^{31}\], and zebrafish \[^{16}\]. However, the importance of these splice-isoforms remains unclear. In the future, it will be interesting to determine how splice-isoforms exist, where they are expressed, and how they differ in their signaling modalities and function.

**GPR126 signaling**

Until recently, it was unclear whether the class of adhesion-GPCRs functioned primarily as adhesion molecules or if members could also function as traditional GPCRs which couple to heterotrimeric G proteins to elicit downstream signaling cascades. In recent years, however, great strides have been made to elucidate signaling capabilities of adhesion GPCRs \[^{32}\] (reviewed in \[^{7, 33}\]), and it is now accepted that many adhesion-GPCRs are indeed capable of G protein-coupling.

The first piece of evidence suggesting that Gpr126 could function as a canonical GPCR came from pharmacological studies in zebrafish. Addition of forskolin, an adenylate cyclase activator which increases cAMP levels, suppressed defects in gpr126 mutants and restored myelination \[^{11}\]. Furthermore, studies using transgenic zebrafish demonstrated that expression of activated PKA in Schwann cells could also rescue myelination in gpr126 mutants \[^{34}\]. Similarly, cAMP elevation and PKA activation could suppress myelin defects in Gpr126-/- mouse mutants \[^{14}\]. These studies supported a model in which Gpr126 elevates cAMP, presumably through interactions with Gαs; recently, more direct biochemical evidence for this model has been reported. Using heterologous cells, Mogha and co-workers showed that GPR126-transfected COS-7 cells showed a concentration-dependent increase in cAMP levels, strongly supporting Gs-protein coupling capabilities for this receptor \[^{14}\]. In the same study, chimeric G-protein assays demonstrated that GPR126 can couple to Gαi-proteins as well as Gαs-proteins \[^{14}\] (Figure 1). Interestingly, Schwann cell-specific Gpr126 mutants also phenocopy Schwann cell-specific Rac1 mutants \[^{14, 35, 37}\] and future work is required to determine the relationship between Gpr126 and Rac1.

A role for cAMP in Schwann cell development and myelination has long been hypothesized, as increased levels of this second messenger promote Schwann cell differentiation in vitro (e.g. \[^{58, 39}\]); thus, the ability of Gpr126 to couple to Gαs and to elevate cAMP as well as the results of the phenotypic analysis of mutants in zebrafish and mouse strongly supports the hypothesis that Gpr126 is the major, if not the receptor, that modulates cAMP in Schwann cells to promote myelination. cAMP elevation can also rescue the ear defects observed in gpr126 zebrafish mutants, supporting a model in which Gαi-protein interactions are critical for canal projection development in this tissue \[^{19}\]. Given that Gpr126 is expressed in a wide range of tissues, it will be interesting in the future to determine the function of Gαi-protein and Gs-protein coupling in other organ systems.

While evidence is accumulating that Gpr126 signals through G-proteins, the consequence of this is poorly understood. The analysis of zebrafish mutants has demonstrated that gpr126 expression is required for oct6 expression in Schwann cells, which is required for the expression of krox20 and mbp myelin basic protein \[^{11, 34}\] (Figure 1). The analysis of Gpr126 knockout mice showed that this function of regulating Oct6 (also known as Pou3f1) is conserved in mammals \[^{13}\]. During ear development loss of Gpr126 function results in the misregulation of genes that encode extracellular matrix (ECM) proteins (hapln1a, hapln3, vcana, vcanb) or ECM-modifying enzymes (chsy1, ugdh, has3). In addition, gpr126 mutants were characterized by substantial changes in the expression of other semicircular canal marker genes, including aldha1a3, bmp7b and sox9b \[^{19}\] (Figure 1). It will be important to identify the direct targets of Gpr126 signaling resulting in the observed phenotypical changes such as ECM modulation.

The function of the NTF of adhesion GPCRs has been implicated in adhesion, cellular migration and dimerization of adhesion GPCRs \[^{40}\]. However, although most different protein domain types found in adhesion GPCRs have the ability to mediate contact with cellular or matrix-associated molecules, there is little information available about the functional role of NTFs (reviewed in \[^{7}\]). The importance of the NTF has been indicated through the detailed analysis of why the zebrafish mutant gpr126 \[^{40}\] exhibits no heart phenotype \[^{16}\]. The gpr126 knockout zebrafish mutants have a range of defects including altered craniofacial morphology as well as significantly increased cell death in the dorsolateral retinal ganglion cell layer. Additionally, gpr126 mutants have reduced sensitivity to water, suggesting a role in chemosensory function. These findings support the hypothesis that GPR126 signaling in development in this tissue is highly specialized.
mutant allele carries a single point mutation that introduces a stop codon immediately before the GPS motif \[^{[11]}\]. This suggested that the NTF of Gpr126 might function during heart development independently of its CTF, which plays an important role in Schwann cell myelination. Rescue experiments in Gpr126-depleted morphants demonstrated that the NTF fragment NTF\(^{AGPS}\) (amino acid 1-783) rescued the cardiac phenotype but not the myelin nor ear phenotype \[^{[16]}\], which both depend on G-protein signaling \[^{[11, 14, 19]}\].

How the NTF functions during heart development is poorly understood. Based on multiplex \textit{in situ} hybridization experiments on mouse heart sections, \textit{Gpr126} is expressed in the endocardium. This appears to secrete the NTF that binds to the myocardium, as incubation of tissue sections with recombinant Gpr126-NTF\(^{AGPS}\) resulted in cardiomyocyte-specific binding in the heart \[^{[16]}\]. As endocardium and myocardium exhibit defects in \textit{Gpr126} knockout mice, it is possible that the NTF is required for proper cardiomyocyte function while the CTF might be required for endocardial function (Figure 1). However, it is also possible that the endocardial phenotype is due to the disruption of endocardial-cardiomyocyte interactions. In the future it will be important to delete \textit{Gpr126} in the heart with cell-type-specificity and to perform rescue experiments utilizing the CTF and NTF.

\textbf{Conclusions}

\textit{Gpr126} is expressed in a variety of organs and tissues. It regulates neural, cardiac and ear development via G-protein- and/or N-terminus-dependent signaling. In addition, genome-wide association studies have implicated variations at the \textit{GPR126} locus in obstructive pulmonary dysfunction, scoliosis and as a determinant of trunk length and body height. The variety of functions of Gpr126 and its multiple signaling modalities identify Gpr126 as model adhesion GPCR to better understand general functional concepts utilized by adhesionGPCRs.

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\textbf{Conflicting interests}

The authors declare that they have no conflict of interest.

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