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Pharmacological Properties and Biological Functions of the GPR17 Receptor, a Potential Target for Neuro-Regenerative Medicine

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Abstract

In 2006, cells heterologously expressing the "orphan" receptor GPR17 were shown to acquire responses to both uracil nucleotides and cysteinyl-leukotrienes, two families of signaling molecules accumulating in brain or heart as a result of hypoxic/traumatic injuries. In subsequent years, evidence of GPR17 key role in oligodendrogenesis and myelination has highlighted it as a "model receptor" for new therapies in demyelinating and neurodegenerative diseases. The apparently contrasting evidence in the literature about the role of GPR17 in promoting or inhibiting myelination can be due to its transient expression in the intermediate stages of differentiation, exerting a pro-differentiating function in early oligodendrocyte precursor cells (OPCs), and an inhibitory role in late stage maturing cells. Meanwhile, several papers extended the initial data on GPR17 pharmacology, highlighting a "promiscuous" behavior of this receptor; indeed, GPR17 is able to respond to other emergency signals like oxysterols or the pro-inflammatory cytokine SDF-1, underlying GPR17 ability to adapt its responses to changes of the surrounding extracellular milieu, including damage conditions. Here, we analyze the available literature on GPR17, in an attempt to summarize its emerging biological roles and pharmacological properties.

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Keywords
Differentiation • GPCR • Multiple sclerosis • Myelination • Oligodendrocyte precursor cells

Abbreviations

CNS	central nervous system
cysLT	cysteinyl-leukotrienes
EAE	experimental autoimmune encephalomyelitis
ERK1/2	extracellular signal–regulated kinases 1 and 2
FACS-MS	frontal affinity chromatography-mass spectrometry
GPCRs	G-protein coupled receptors
HM	homology modeling
Lys	lysolecithin
MCAo	middle cerebral artery occlusion
MS	multiple sclerosis
NC-IUPHAR	Nomenclature Committee of the International Union of Pharmacology
OLs	oligodendrocytes
OPCs	oligodendrocyte precursor cells
MBP	myelin basic protein

1 Introduction: The History of GPR17

In 2006, a paper was published where it was demonstrated that cells heterologously expressing the "orphan" receptor GPR17 (i.e., a molecularly identified, 339 amino acid-long G_i-protein-coupled receptor that still lacked a defined ligand) acquired responses to both uracil nucleotides (such as UDP, UDP-glucose, UDP-galactose) and cysteinyl-leukotrienes (cysLTs, like LTC_4 and LTD_4) (Ciana et al. 2006), two chemically unrelated families of signaling molecules that are known to massively accumulate in organs like the brain or the heart as a result of hypoxic/traumatic injuries. Uracil nucleotides and cysLTs were already known to exert multiple biological effects via the activation of separate G-protein-coupled receptors (GPCRs): the eight recognized P2Y receptor subtypes (the $P2Y_{1,2,4,6,11,12,13,14}$ receptors, (Abbracchio et al. 2006) and the two CysLT1 and CysLT2 receptors. Interestingly, the GPR17 sequence had been originally described as the result of a cloning strategy based on the use of RT-PCR degenerate oligonucleotide primers designed on the sequences of the $P2Y_1$ and $P2Y_2$ receptors, with the final aim of identifying new members of this receptor family (Blasius et al. 1998). GPR17 was later found to be at an intermediate structural and phylogenetic position between already known P2Y and CysLT receptors, and GPR99, recently proposed as the third CysLT receptor (also known as 2-oxoglutarate receptor 1, OXGR1) (Kanaoka et al. 2013) (Fig. 1), in the so called "purine receptor cluster" of class A GPCRs (Fredriksson et al. 2003). GPR17 also emerged as represented the closest receptor to a common ancestor that, during evolution, could have generated both P2Y and CysLT receptors (Ciana et al. 2006; Parravicini et al. 2008; Parravicini et al. 2010). To further highlight GPR17 structural similarity to the other members of the P2Y family, a partial sequence of the rat receptor was initially identified from rat striatum by employing oligonucleotide primers specifically designed on the sequence of human $P2Y_{11}$ (Lecca and Abbracchio 2008). Of note, a human GPR17 long splice variant encoding a receptor with a 28-amino acid longer NH2 terminal (for a total of 367 amino acids instead of 339) had been also identified in very early studies aimed at discovering new members of the chemokine receptor family (in this respect, see also Sect. 2.1) (Blasius et al. 1998). Genomic analysis revealed a three-exon structure of the hGPR17 gene, with two putative open reading frames. While the "short isoform" derives from splicing of the

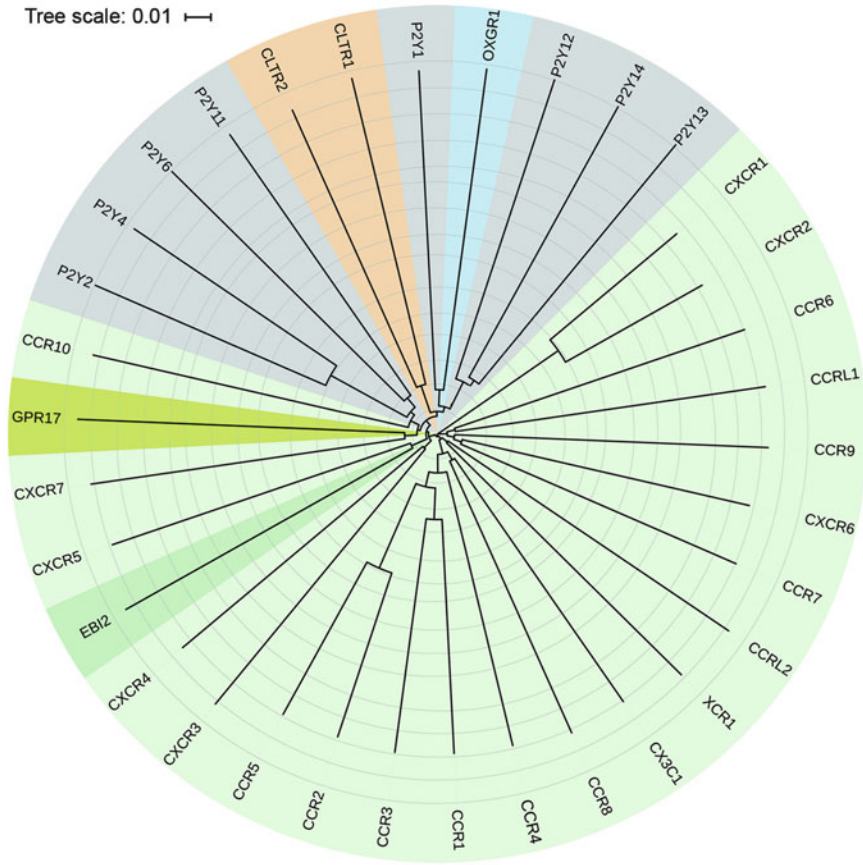

Fig. 1 Phylogenetic tree illustrating the relationship of GPR17 to selected structurally related class-A GPCRs. The evolutionary relationship analysis is based on a multiple sequence alignment performed on homologous GPCR sequences using TM-Coffee, a module of the T-Coffee package optimized for transmembrane proteins (Chang et al. 2012). Receptors belonging to the same family are clustered according to the following color code: *grey* for purinergic receptors (P2Y), *orange* for cysteinyl-leukotriene receptors (CysLT), *light green* for chemokine receptors (CXCRn, CCRn, XCRn), *emerald green* for Epstein-Barr virus-induced G-protein coupled receptor 2 (EBI2), *sky blue* for 2-Oxoglutarate receptor 1 (OXGR1/GPR99), *apple green* for GPR17

second exon, the "long one" contains all three exons of the hGPR17 gene, leading to a transcript which is 1104 bp in length (Blasius et al. 1998; Pugliese et al. 2009). Interestingly, quantitative gene expression studies revealed that GPR17 short isoform is expressed more abundantly in the brain than the long one (a tenfold increase), whereas the opposite was observed in heart and kidney. Pharmacological profile of the long isoform also showed that some differences exist between the two GPR17 receptor isoforms (Pugliese et al. 2009; Benned-Jensen and Rosenkilde 2010).

In 2006, there were already papers reporting functional interactions between "classical" P2Y and CysLT receptors. For example, under some conditions, the CysLT1 receptor antagonist montelukast effectively antagonized the responses evoked by purinergic P2Y receptors (Capra et al. 2005; Mamedova et al. 2005). Conversely, the $P2Y_{12}$ receptor had been reported to be also activated by LTE_4 (Paruchuri et al. 2009), suggesting the existence of some kind of ligand/receptor promiscuity between the P2Y and CysLT receptor families. On this basis, the identification of GPR17 as the first dual member of the "purine receptor cluster" able to respond to

both purinergic and cysLT ligands (Ciana et al. 2006) represented the demonstration of a further level of interaction between these two chemically unrelated, but functionally interconnected, systems. Later studies extended the response profile of this receptor to other classes of endogenous "emergency" molecules connected to oxidative stress, neuroinflammation and neurodegeneration, i.e., oxysterols and chemokine stromal derived factor-1 (SDF-1) (Parravicini et al. 2016), further highlighting the promiscuous behaviour of GPR17. Of note, a phylogenetic analysis among structurally related class-A GPCRs (Parravicini et al. 2008, 2010, 2016; Sensi et al. 2014), suggest that, besides P2Y and CysLT receptors, GPR17 holds a tight evolutionary relationship also with chemokine receptors and Epstein-Barr virus-induced G-protein coupled receptor 2 (EBI2) (Fig. 1). The possibility that GPR17 can be activated by diverse family of ligands underlines the relevance of a new transversal signaling mechanism that synchronizes all these emergency molecules and their receptors under specific neurodegenerative conditions. Such a high promiscuity in receptor behaviour is often found in receptors involved in immunological responses and may, at least in part, depend on GPR17 ability to form dimers with other related receptors, thus widening the array of pharmacological responses (see also Sect. 2.2.1).

In subsequent years, new data have revealed a key role for GPR17 in oligodendrogenesis and myelination (Lecca et al. 2008; Chen et al. 2009). However, while some authors have provided evidence for a stimulatory role of GPR17 in the specification and maturation of oligodendrocyte precursor cells (OPCs), some others have proposed an inhibitory role. Here, we aim at analyzing all the available literature on GPR17 in an attempt to provide an overview of the different biological and pharmacological data emerged from all these papers.

2 GPR17 Characterization

2.1 Receptor Structure, Amino Acid Homology with Phylogenetically Related GPCRs and Binding Sites

GPR17 displays the typical 7-transmembrane (TM) domain topology of GPCRs, with an amino acid identity with the known P2Y and CysLT receptors between 21 and 48% (Abbracchio et al. 2006; Lecca and Abbracchio 2008). All these receptors show partial or complete conservation of a H-X-X-R/K amino acid motif in TM6 (and also of a K-E-X-X-L motif in TM7, in the case of $P2Y_{12}$, $P2Y_{13}$, $P2Y_{14}$) that are important for ligand recognition and have been proposed to represent specific molecular signatures for these receptors (Lecca and Abbracchio 2008) (see also below). Homology Modelling (HM) studies combined with other *in silico* tools have been performed to raise hypothesis on the molecular interaction between GPR17 and its putative endogenous ligands (Parravicini et al. 2008, 2010; Calleri et al. 2010), as well as to identify new potential ligands (Eberini et al. 2011) (see also Sect. 2.2.2). In these studies, *in silico* receptor modeling was performed using different templates, according to the progressive availability of new high-resolution GPCR structures. Starting from bovine rhodopsin, that, since 2000, has represented for many years the only atomistic scaffold for the structural investigation of GPCRs, the recent explosion in the resolution of GPCR crystal structures has given access to detailed structural information previously unavailable, allowing the construction of more and more accurate GPR17 models (Fig. 2).

For example, in 2010, crystallization of human CXCR4 (Wu et al. 2010) provided a significant improvement in the accuracy of GPR17 modelling because this structure enabled to reliably describe the extracellular regions of the receptor, especially extracellular loop 2 (ECL2) and the disulphide bridge linking the N-terminal to ECL3, known to be crucial in ligand molecular recognition (Wheatley et al. 2012), but for which none of the earlier templates was suitable. More

Fig. 2 Three-dimensional homology model of the human GPR17. Topological domains are represented as ribbon and coloured according to their secondary structure: *magenta* for alpha-helices; *yellow* for beta-sheets; white for loops and grey for turns

recently, modelling of GPR17 has been further improved thanks to the atomic resolution of the structures of two members of the P2Y receptor family: the human $P2Y_{12}$ (Zhang et al. 2014) and $P2Y_1$ (Zhang et al. 2015).

Globally, all the *in silico* results on the short isoform of GPR17 suggest that its nucleotide binding pocket is similar to that described for the other P2Y receptors (including the TM6 HXXR/K motif designated to accommodate the phosphate moieties of nucleotide ligands (Parravicini et al. 2008; Jiang et al. 1997), and that this site is also shared by other small molecules identified as GPR17 ligands, including oxysterols and new synthetic compounds (Sensi et al. 2014; Eberini et al. 2011). According to these studies, also the nucleotide-derivative antagonist cangrelor binds to the same binding pocket, behaving as a competitive antagonist for orthosteric ligands.

In both P2Y and CysLT1 and CysLT2 receptors, ligand binding is critically dependent on the basic Arginine residue belonging to the conserved TM6 motif (Parravicini et al. 2010; Temporini et al. 2009). Computational studies suggested that this also holds true for Arg255 of GPR17 (Parravicini et al. 2008). To assess the actual role of this residue in receptor binding, this basic amino acid was mutated to isoleucine, and an *in silico* mutant GPR17 receptor (R255I) was generated. Using steered molecular dynamics simulations (SMD), forced unbinding of the endogenous ligand UDP from both wild type (WT) and R255I receptor models of GPR17 was modeled *in silico*. The energy required to unbind UDP from the nucleotide binding pocket of GPR17 was higher for the WT than for the mutated R255I receptor, and the exit of the ligand from its intracellular cavities occurred earlier in the R255I model compared to the WT receptor. Generation and expression of the mutated receptor in 1321N1 cells confirmed also *in vitro* that the mutation was not silent (Calleri et al. 2010).

Besides the orthosteric binding site, *in silico* studies suggested that GPR17 also possesses an "accessory" binding site in a region formed by extracellular loops ECL2, ECL3 and the N- terminal, which also faces the extracellular space. This external accessory binding site could guide small agonist ligands to the deeper principal binding site in a multistep mechanism of activation. Thanks to further *in silico* investigations, that showed the possibility of GPR17 to be stimulated also by a large peptide ligand such as SDF-1, the extracellular recognition site has been extensively characterized and GPR17 recognition mechanism has been compared to those of some peptide receptors (Parravicini et al. 2016), in which a two-step model of receptor activation, passing through both an extracellular and a TM binding site, has been proposed (Rajagopalan and Rajarathnam 2004).

Due to the intrinsic inaccuracy of the standard template-based HM techniques in predicting conformations of highly flexible and unaligned loop sequences in absence of adequate templates, no modeling studies are yet available for the long isoform of GPR17. Nevertheless, we can speculate that the N-terminal may influence the binding affinity of nucleotide agonists via a different

conformation of the external accessory binding site, resulting in a slightly different pharmacological profile of the long isoform with respect to the short one (Pugliese et al. 2009).

2.2 Pharmacology and Signaling Pathways

2.2.1 Putative Endogenous Ligands and Transduction Systems

In the initial studies, only GPR17 short isoform has been characterized, and both the human, rat (Ciana et al. 2006) and the previously unidentified mouse GPR17 receptors (Lecca and Ceruti 2008) were shown to respond to UDP, UDP-glucose, UDP-galactose and LTC_4 and LTD_4, with comparable profiles that were highly conserved across species (the chemical structures of UDP-glucose and LTD_4 are reported in Fig. 3). Interestingly, the concentration ranges at which uracil nucleotides and cysLTs activated GPR17 (i.e., μM and nM ranges, respectively) were fully consistent with those necessary for these endogenous ligands to activate their already known cognate P2Y and CysLT receptors (Abbracchio et al. 2006; Brink et al. 2003). Very similar agonist responses were detected in a number of different cell lines (1321N1, CHO, COS-7, HEK-293 cells). The 1321N1 cells was the most appropriate cells to test GPR17 responses, since they are one of the few cell lines that do not endogenously express any functional purinergic or CysLT receptors. Responses were highly specific, since no response was ever found in cells transfected with the empty vector. The antagonist response profile of GPR17 was also rather peculiar. Activation by uracil nucleotides was reversed by some typical purinergic antagonists like the $P2Y_1$ antagonist MRS2179 or the $P2Y_{12}$ antagonist cangrelor (Fig. 3). Conversely, responses to cysLTs were inhibited by typical CysLT receptor antagonists like the already marketed drug montelukast (Fig. 3) and pranlukast (Ciana et al. 2006).

GPR17 responses were demonstrated by using [^{35}S]GTPγS binding, a typical functional assay for agonists acting at G_i coupled receptors (Kotani et al. 2001; Marteau et al. 2003; Fumagalli et al. 2004). Under some circumstances, activation of GPR17 could also increase intracellular calcium levels via a phospholipase C mediated pathway; however, this effect occurred only in about 30% of 1321N1 transfected cells, suggesting preferential coupling to the adenylyl cyclase pathway (Ciana et al. 2006). In subsequent studies, GPR17 peculiar profile was confirmed in many other distinct assays independently performed in different laboratories. Concentration-dependent inhibition of forskolin-stimulated adenylyl cyclase was also shown in oligodendendrocyte precursor cells (OPCs), the cell type natively expressing GPR17 at highest levels (Fumagalli et al. 2011a) (Table 1). Inhibition of cAMP was fully counteracted by the same antagonists utilized in the [^{35}S]GTPγS binding. In 2009, another paper appeared where, in GPR17 expressing 1321N1 cells, enhancement of an outward rectifying K^+ current was shown upon addition of either uracil nucleotides or cysLTs (Pugliese et al. 2009). These effects were blocked by MRS2179, cangrelor and montelukast. A few years later, these same authors showed that similar delayed rectifier K^+ currents were stimulated in a concentration dependent manner by GPR17 ligands in a subpopulation of OPCs and pre-oligodendrocytes, but not in terminally mature cells, fully in line with the transient expression of GPR17 during OPC specification (in this respect, see also Sect. 3.1.1) (Coppi et al. 2013). This effect was blocked by MRS2179 and cangrelor and sensitive to the K^+ channel blocker tetraethyl-ammonium. Importantly, the latter also inhibited oligodendrocyte maturation, to support previous literature data on the importance of these currents in OPC differentiation.

Fewer studies are available on hGPR17 long isoform. In the electrophysiological study already mentioned above, no significant differences between the short and long isoforms were detected (Pugliese et al. 2009). In 2010, Benned-Jensen and Rosenkilde independently confirmed the ability of heterologously expressed GPR17 to respond to uracil

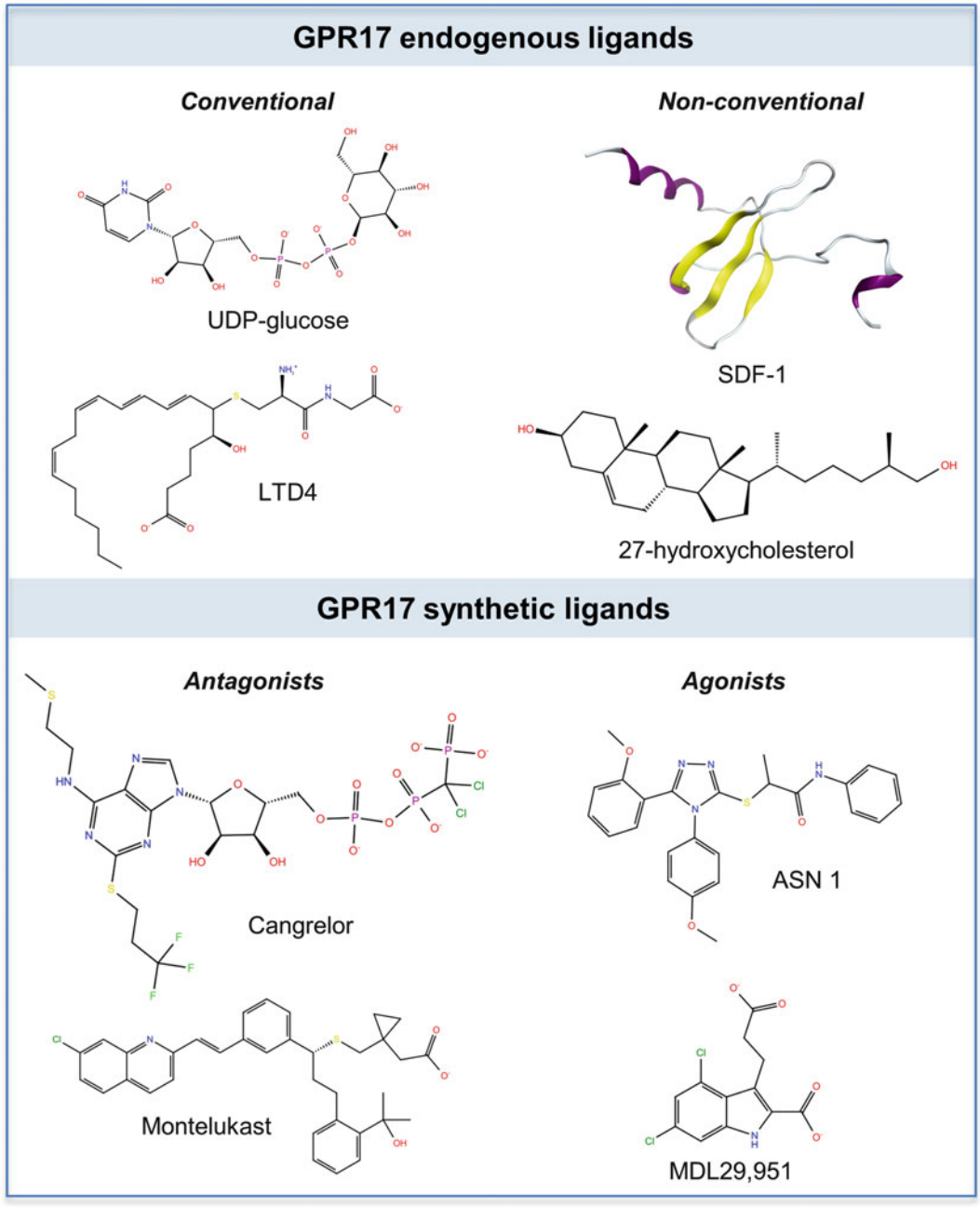

Fig. 3 Chemical structures of endogenous and synthetic compounds reported to bind GPR17. CAS Registry number of synthetic ligands are the following: MDL29,951 #130798–51-5; ASN-1: #483283–39-2. For SDF-1, a representative X-ray structure deposited in the Protein Data Bank is reported (Pdb code: 1QG7) AU1

nucleotides in a cAMP response element binding (CREB) trans-reporter luciferase assay in HEK293 cells (Benned-Jensen and Rosenkilde 2010). Both UDP, UDP-glucose and UDP-galactose activated GPR17 short isoform with EC_{50} values exactly in the same μM range that had been previously reported in both the [^{35}S]GTPγS binding (Ciana et al. 2006; Lecca et al.

t.1 **Table 1** GPR17 signaling in native systems and relevant pharmacology

t.2 Tested ligand	Type of cells	Signaling	EC_{50}/IC_{50} values	Reference
t.3 UDP-glucose	Rat primary OPCs	Inhibition of cAMP production	IC_{50}: 424.7 ± 125 nM	Fumagalli et al. (2011a)
t.4	Rat primary OPCs	Outward K^+ currents	EC_{50}: 4.6 μM	Coppi et al. (2013)
t.5	Rat primary OPCs	Association to GRK5	N.A.	Daniele et al. (2014)
t.6	Rat primary OPCs	β-arrestin dependent ERK1/2 activation	N.A.	Daniele et al. (2014)
t.7	Oli-neu cells	Clathrin-mediated endocytosis	N.A.	Fratangeli et al. (2013)
t.8	PC12 cells	ERK1/2 and p38 phosphorylation	N.A.	Daniele et al. (2010)
t.9 UDP LTD_4	Rat primary OPCs	Inhibition of cAMP production	IC_{50}: 1.29 ± 0.07 μM	(Fumagalli et al. 2011a)
t.10	Rat primary OPCs	Inhibition of cAMP production	IC_{50}: 2.85 ± 0.89 nM	Fumagalli et al. (2011a)
t.11	Rat primary OPCs	Association to GRK2	N.A.	Daniele et al. (2014)
t.12	Rat primary OPCs	CREB activation	N.A.	Daniele et al. (2014)
t.13	PC12 cells	ERK1/2 and p38 phosphorylation	N.A.	Daniele et al. (2010)
t.14	Oli-neu cells	Clathrin-mediated endocytosis	N.A.	Fratangeli et al. (2013)
t.15 LTE_4	Rat primary OPCs	Inhibition of cAMP production	IC_{50}: 51.8 ± 6.6 pM	Fumagalli et al. (2011a)
t.16 MDL29,951	Rat primary OPCs	Inhibition of cAMP production	N.A.	Hennen et al. (2013)
t.17	Rat primary OPCs	$Ca^{2+}{}_i$ increase	N.A.	Hennen et al. (2013)

t.18 N.A.: Not available

2008) and in frontal affinity chromatography-mass spectrometry (FAC-MS) studies (Calleri et al. 2010; Temporini et al. 2009). Much lower potencies to uracil nucleotides were observed for the long receptor isoform, with a 50–170 fold increase in EC_{50} (Benned-Jensen and Rosenkilde 2010). Moreover, no responses to cysLTs were detected either on the short or long isoform, nor were cysLTs able to induce GPR17 removal from the membrane and internalization. This is in contrast with subsequent studies on cells natively expressing the receptor (Fratangeli et al. 2013) (see below). This may depend on differences in the conformation/ability of the recombinant receptor to respond to agonists compared to the native one, as well as on the fact that, in heterologously expressing systems, constitutive activity of transfected receptors may significantly alter ligand behavior (Kenakin 2001; Im 2013) (see also Conclusions). In this respect, Benned-Jensen and Rosenkilde indeed reported a notable constitutive activation of recombinant GPR17 resulting in potent inhibition of forskolin stimulated adenylyl cyclase in the absence of any endogenous ligand (Benned-Jensen and Rosenkilde 2010).

At the same time, another paper suggested GPR17 as a negative regulator of the CysLT1 receptor (Maekawa et al. 2009). This effect was proposed to depend on the formation of a CysLT1-GPR17 heteromer, as suggested by co-immunoprecipitation studies in CHO cells. The interaction between GPR17 and CysLT1 was further confirmed in primary human monocyte cells and in a rodent knock out GPR17 model, thus extending to GPR17 the previously

reported interaction and promiscuity between different members of the "purine receptor cluster". These data indicate that, besides working on its own, GPR17 may also modify the function of other related receptors by the formation of heteromers.

In 2010, the first paper describing the characteristics of GPR17 in a *native* system (rat pheocromocytoma PC12 cells) was published (Daniele et al. 2010) (Table 1). GPR17 was not expressed in undifferentiated PC12 cells but was specifically induced by a 10-day NGF treatment, suggesting a role in the control of neuronal differentiation. Both UDP-glucose and LTD_4 induced a significant pro-survival effect on PC12 cells. By *in vitro* silencing experiments with small interfering RNAs and by using receptor antagonists, these effects were confirmed to be mediated by the selective activation of GPR17. In differentiated PC12 cells, UDP-glucose and LTD_4 caused a significant increase in extracellular signal–regulated kinases 1 and 2 (ERK1/2) phosphorylation. ERK activation induced by the two agonists occurred with different kinetics: LTD_4 induced a transient ERK activation that returned to basal value within 120 min. In contrast, ERK phosphorylation induced by UDP-glucose was maintained over basal values for 120 min and the activation kinetics appeared to be biphasic with two peaks, one at 15 and the other one at 120 min. In addition, incubation of cells with the purinergic antagonist cangrelor completely counteracted UDP-glucose effects at all tested incubation times (Daniele et al. 2010). These data confirmed the responses to uracil nucleotides and cysLTs already seen on the recombinant receptors, and suggested, for the first time, that endogenous GPR17 ligands can couple to distinct G proteins and intracellular pathways, a finding that was later confirmed by other studies (Hennen et al. 2013; Daniele et al. 2014). The signaling pathways of native GPR17 are summarized in Table 1.

In 2011, in another study that independently confirmed the purinergic component of GPR17, Buccioni and coworkers (Buccioni et al. 2011) exploited an innovative and non-radioactive functional cAMP assay to monitor GPR17 activation (and the effects of various ligands) through changes in intracellular cAMP concentrations by using a mutant form of Photinus pyralis luciferase into which a cAMP-binding protein moiety had been inserted (GloSensor cAMP reagent). In HEK293 cells stably transfected with the GloSensor reagent, transient expression of hGPR17 resulted in the appearance of highly specific concentration-dependent responses to both UDP, UDP-glucose and UDP-galactose and to a series of UDP and ATP derivatives that behaved as either agonist or antagonists, with EC_{50} values that were very similar to those obtained in parallel on [^{35}S]GTPγS binding. In this system, cysLTs were not tested, due to the high constitutive expression of traditional CysLT receptors in the HEK293 cells (Ciana et al. 2006; Buccioni et al. 2011).

2.2.2 GPR17 Non-conventional Ligands

In the last years, the increasing number of class-A GPCR solved structures allowed the scientific community to recognize some common features that are crucial for their operability (Levit et al. 2014); however, these studies also revealed an unexpected heterogeneity and complexity in GPCR recognition, challenging the classical pharmacology paradigms of the 'monogamous' interaction between a specific class of natural ligands and a single GPCR (Haupt et al. 2013). In line with the growing promiscuity of GPCRs, ligand dependent transactivation has been demonstrated for GPR17, already known as a "dual" receptor: similarly to EBI2 (Hannedouche et al. 2011; Liu et al. 2011a), and the CXC chemokine receptor 2 (CXCR2) (Raccosta et al. 2013). Specifically, it was shown that, GPR17 could act as a molecular target for oxysterols, oxidized derivative of cholesterol that, in the CNS, are involved in activities not strictly associated with cholesterol metabolism. Of note, these activities are particularly relevant for neurodegenerative disorders, including demyelinating (Raccosta et al. 2013; Garenc

et al. 2010). More in detail, three selected oxysterols (27-Hydroxycholesterol, 7 α-Hydroxycholesterol and 22R–Hydroxycholesterol) were tested in 1321N1 cells stably expressing GPR17, showing that all the tested compounds were able to stimulate GTPγS binding, in a concentration-dependent manner, with EC_{50} values of 4.99 ± 0.78 nM, 0.70 ± 0.09 nM and 0.21 ± 0.03 nM, for 27-Hydroxycholesterol, 7α-Hydroxycholesterol and 22R–Hydroxycholesterol, respectively.

Stimulus of cell membranes with different oxysterol concentrations after treatment with the purinergic ligand UDP-glucose showed a left-shift of the concentration-response curves or an enhancement of their maximal [^{35}S]GTPγS binding stimulation, suggesting that these ligands may cooperate under neuroinflammatory conditions.

In parallel, the effect of different concentration of the GPR17 receptor antagonist cangrelor on oxysterol-stimulated [^{35}S]GTPγS binding was evaluated, demonstrating that cangrelor can counteract GPR17 activation by oxysterols through a competitive mechanism, with IC_{50} values in a sub-nM range. These results are also in agreement with *in silico* data suggesting a common orthosteric molecular recognition mechanism for oxysterols and other small GPR17 ligands, despite different local arrangements in the TM binding site (Sensi et al. 2014).

Among other non-conventional ligands, further evidence showed that SDF-1, historically known as the endogenous ligand for CXCR4 and CXCR7 receptors, is able to transactivate GPR17 *in vitro*, specifically increasing the [^{35}S]GTPγS binding to membrane of GPR17-expressing cells, with affinity constant values of 0.14 ± 0.03 nM. The effect of SDF-1 in modulating GPR17 responses *in vitro* was further assessed in primary OPC cultures natively expressing GPR17. In this model, treatment with physiological concentrations of SDF-1 significantly increased the number of cells expressing the Myelin Basic Protein MBP compared to control, thus accelerating OPC differentiation towards a mature phenotype. The specific involvement of GPR17 in these effects was unequivocally demonstrated by further experiments showing that, in presence of the GPR17 antagonist cangrelor, SDF-1 induced no increases of either [^{35}S]GTPγS binding to cell membranes, or MBP-expression in OPC cultures. Moreover, the mechanism by which GPR17 and SDF-1 can directly interact to each other has been predicted and extensively characterized *in silico* through molecular modeling (Parravicini et al. 2016).

These results are in line with literature data, that propose a role of SDF-1 in orchestrating OPC differentiation and maturation also via CXCR4/CXCR7-axis (Li et al. 2012; Patel et al. 2010; Carbajal et al. 2011).

Interestingly, not only GPR17, CXCR4 and CXCR7, but also others chemokine receptors, like CXCR2, have demonstrated roles in regulating OPCs. As previously mentioned (see Sect. 2.1), besides sharing the same ligands, GPR17 and chemokine receptors are phylogenetically related to each other, and all participate to CNS reparative responses. This raises the hypothesis that, under neurodegenerative demyelinating conditions, oxysterols and other pro-inflammatory ligands, such as SDF-1, act as non-conventional molecules with a transversal regulatory role, representing a conserved, "unspecific" signaling mechanism, by which emergency molecules synchronize multiple receptors involved in inflammatory/immune responses.

2.2.3 New GPR17 Synthetic Ligands

In 2009 and 2010, two papers reported the development of a new FAC-MS binding method for the analysis of GPCRs (Calleri et al. 2010; Temporini et al. 2009). In this assay, UDP was found to bind to GPR17 with a Kd value of 1612.0 ± 708 nM that was very similar to the Kd value (1140.0 nM) obtained by Ciana et al. and Lecca et al. in the [^{35}S]GTPγS-binding (Ciana et al. 2006; Lecca and Ceruti 2008). This paper also unveiled a number of previously unreported GPR17 ligands, some of which were able to increase [^{35}S]GTPγS binding, with

potency values in the μM and sub-nM range. For example, the ATP analogue 2-Phenylethynyladenosine-5′-monophosphate Compound N. 4 behaved as a very potent agonist with an EC_{50} value of 36 pM. In contrast, other ligands (e.g.: N^6-Benzoyl-2′-deoxyadenosine 3′,5′-Bis phosphate, referred by the authors as Compound N. 12) did not induce any increase in [^{35}S]GTPγS binding, but counteracted stimulation induced by UDP-glucose with an antagonist profile and an affinity constant in the nM range comparable to that reported for its analogue derivative MRS2179. Both the newly identified agonists and antagonists displayed similar behavior in the FAC-MS binding assay (Calleri et al. 2010). A comparison between these data and [^{35}S]GTPγS binding results have been also reported in a recent review article on GPR17 (Marucci et al. 2016).

In the same year, an advanced *in silico* HM procedure combined with high-efficiency virtual screening of more than 120,000 compounds from the Asinex Platinum Collection (http://www.asinex.com/), a lead-like structural library, on the modeled receptor led to the selection of 5 chemically diverse molecules (the ASINEX compounds, see Fig. 3 for the chemical structure of one representative compound, 2-[[5-(2-Methoxyphenyl)-4-(4-methoxyphenyl)-4H-1,2,4-triazol-3-yl]thio]-N-phenylpropanamide, also referred as ASN 1), that were completely unrelated to already known ligands. These compounds were tested *in vitro* in the [^{35}S]GTPγS binding assay, revealing a sub-nM potency for GPR17 (Eberini et al. 2011) (see also below). None of these compounds could have been expected 'a priori' to act on GPR17, and all of them behaved as much more potent ligands than GPR17 endogenous activators (Eberini et al. 2011). Finally, in 2013, MDL29,951 was reported as an additional small molecule agonist at GPR17 (Hennen et al. 2013) (Fig. 3). In a variety of different heterologous expression systems, MDL29,951-stimulated GPR17 engaged the entire set of intracellular adaptor proteins for GPCRs: G proteins of the Gαi, Gαs, and Gαq subfamily, as well as β-arrestins. This was visualized as alterations in the concentrations of cyclic adenosine monophosphate and inositol phosphate, increased Ca^{2+} flux, phosphorylation of ERK1/2, as well as multifeatured cell activation recorded with label-free dynamic mass redistribution and impedance biosensors. pEC_{50} values for MDL29,951 at GPR17 ranged between 5 and 8.80, depending upon the transfected cell type and the used read out. MDL29,951-stimulated GPR17 effects were counteracted in a concentration-dependent manner by pranlukast and, to a lesser extent, by montelukast. This is fully in line with the activities of these antagonists on recombinant GPR17 in previous studies, in which pranlukast was significantly more potent than montelukast in antagonizing LTD4-stimulation of GPR17 (Ciana et al. 2006). In OPCs, MDL29,951 rapidly mobilized intracellular Ca^{2+} in a concentration-dependent manner and engaged both $G_{\alpha i}$ and $G_{\alpha q}$, but not $G_{\alpha s}$ signaling pathways, further suggesting differences in GPR17 responses between transfected and native systems (see also Conclusions). This is at variance from previous studies reporting $G_{\alpha i}$ coupling and decreases of intracellular cAMP as a primary transduction pathway of GPR17 in OPCs (Daniele et al. 2014; Fumagalli et al. 2011b). However, it has to be emphasized that, despite being selective for GPR17 inside the "purine receptor cluster" (Hennen et al. 2013), MDL29,951 also significantly interacts with the glycinergic site of the glutamate NMDA receptor (Salituro et al. 1992). This may be at the basis of the ability of MDL29,951 to activate multiple signaling pathways in both transfected cells and in OPCs, and of the data reported for this compound on myelination (see also Sect. 3.1.1).

2.2.4 Agonist-Induced Desensitization and Internalization

In 2011, the first complete agonist-induced GPR17 desensitization/resensitization study was published (Daniele et al. 2011). By using [^{35}S]GTPγS binding and cAMP measurements in 1321N1 cells expressing hGPR17, both UDP-glucose and LTD_4 were shown to induce a time- and concentration-dependent loss of

GPR17 response (homologous desensitization). GPR17 homologous desensitization was accompanied by internalization of receptors inside cells, as assessed by biotin labeling of cell surface receptors. Desensitization occurred in a time-dependent manner, with similar kinetics for both agonists. Upon agonist removal, receptor resensitization occurred with the typical kinetics of GPCRs. Finally, activation of GPR17 by UDP-glucose induced a partial heterologous desensitization of LTD_4-mediated responses (but not *vice versa*), suggesting that nucleotides have a hierarchy in producing desensitizing signals.

The pattern of GPR17 desensitization and internalization was fully confirmed and further expanded in differentiated oligodendroglial Oli-neu cells that natively express GPR17 (Fratangeli et al. 2013) (Table 1). Agonist-induced internalization, intracellular trafficking and membrane recycling of GPR17 were analyzed by biochemical and immunofluorescence assays using an *ad hoc*-developed new antibody against the extracellular N-terminal of GPR17. Both UDP-glucose and LTD_4 increased GPR17 internalization, although with different efficiency. At early time points, internalized GPR17 co-localized with transferrin receptor, whereas at later times it partially co-localized with the lysosomal marker Lamp1, suggesting that a portion of GPR17 is targeted to lysosomes upon ligand binding. Internalization of GPR17 occurred via clathrin-dependent endocytosis (Fratangeli et al. 2013). Analysis of receptor recycling and degradation demonstrated that a significant fraction of GPR17 is recycled to the cell surface. These results provided the first data on the agonist-induced trafficking of native GPR17 in oligodendroglial cells and may have implications in fine-tuning cell responses to demyelinating and inflammatory conditions when these ligands accumulate at lesion sites (see also Sect. 3.1.2). More recently, GPR17 downregulation by uracil nucleotides and cysLTs was confirmed in primary cultured OPCs, and the role of the GRK/β-arrestin machinery in receptor desensitization and intracellular signaling was also extensively investigated (Daniele et al. 2014). It was shown that, following OPCs treatment with the two classes of purinergic and cysLT ligands, different GRK isoforms were recruited. Specifically, cysLT-mediated GPR17 desensitization mainly involved GRK2 via a G protein-dependent mechanism (Daniele et al. 2014). This kinase promoted transient binding of the receptor to β-arrestins, rapid ERK phosphorylation and sustained nuclear CREB activation. Furthermore, GRK2, whose expression paralleled that of the receptor during the differentiation process, was required for cysLT-mediated OPCs maturation (see also Sect. 3.2.). On the other hand, purinergic ligands exclusively recruited GRK5 via a G protein-independent/β-arrestin-dependent mechanism. This kinase induced a stable association between the receptor and β-arrestin, followed by slower and sustained ERK stimulation and marginal CREB activation (Daniele et al. 2014). These results show that, through activation of GPR17 and recruitment of specific GRK isoforms, purinergic and cysLT ligands engage distinct intracellular pathways.

Recently GPR17 desensitization (and its relationship to terminal OPC maturation) has been linked to activation of mTOR (the "mammalian target of rapamycin"), which has long been known to be involved in myelination. During OPC differentiation, mTOR regulates GRK-mediated desensitization of GPR17 by promoting the nuclear translocation of the ubiquitin ligase MDM2, which had been previously only involved in cancer via regulation of p53 activity and now emerges as a new interesting actor in oligodendrogenesis (Fumagalli et al. 2015). Specifically, treatment of OPCs with either the mTOR inhibitor rapamycin, or with nutlin-3, a small molecule inhibitor of Mdm2-p53 interactions, was shown to keep MDM2 in the cytosol, where it could bind to GRK2 and sustain its degradation, thus impairing the physiological desensitization of GPR17 (Fumagalli et al. 2015). Important, prevention of GPR17 desensitization was also associated to a defect of OPC maturation, confirming that aberrantly elevated GPR17 levels in late stage OPCs blocks cells at immature stages (Fumagalli et al. 2015).

In another study, GPR17 plasma membrane recycling and stability was shown to be also modulated by SNX27, a recently identified protein of the endosome-associated retromer complex, whose functions in oligodendrocytes had never been studied. It was found that, after endocytosis, GPR17 is either sorted into lysosomes for degradation or recycled to the plasma membrane. Balance between degradation and recycling was important for modulation of receptor levels at the cell surface, and thus for the silencing or maintenance of GPR17-signaling pathways, that, in turn, affect OPC differentiation (see also Sect. 3.2). The endocytic trafficking of GPR17 was mediated by interaction of SNX27 with a type I PDZ-binding motif located at the C-terminus of the receptor. Of note, SNX27 knock-down reduced GPR17 plasma membrane recycling in differentiating oligodendrocytes while accelerating terminal cell maturation. Interestingly, trisomy-linked down-regulation of SNX27 in the brain of Ts65Dn mice, a model of Down syndrome, correlated with a dysfunction in $GPR17^{+}$ cells and an increase in mature oligodendrocytes, which, however, failed in reaching full maturation, eventually leading to hypomyelination (Meraviglia et al. 2016). Thus, disruption of SNX27/GPR17 interactions leading to alterations of GPR17 membrane trafficking might contribute to pathological oligodendrocyte differentiation and myelination defects present in Down syndrome (Meraviglia et al. 2016).

3 Role of GPR17 in Central Nervous System Pathophysiology

3.1 GPR17 Specific Roles in Oligodendroglial Functions and Myelination

3.1.1 Physiological Roles

In the healthy intact brain, GPR17 expression is predominantly in oligodendrocyte (OL) cells. The very first demonstration that, in the adult brain, GPR17 is highly expressed by a sub-population of endogenous quiescent parenchymal OPCs dates back to 2008 (Lecca et al. 2008) and has sparked a lot of interest on GPR17 role in CNS myelination. Specifically, GPR17 was shown to be present in ramified early neural cell precursors dispersed throughout brain's gray and white matter that also positively stained for typical early OPC markers. Since then, increasing evidence has progressively accumulated to show a pivotal role of GPR17 in OPC maturation, with different and apparently paradoxical effects during different phases of the maturation process (Chen et al. 2009; Fumagalli et al. 2011a) (see also below).

In vitro studies on purified rat postnatal OPC cultures showed that GPR17 expression coincides with a specific temporal window of the OL differentiation process. It covers two distinct phases: a first phase, during which early differentiation markers like NG2, A2B5, PDGF receptor-alpha and the immature PLP isoform DM-20 are still present (early stage 2 OPCs in Fig. 4), and a subsequent phase characterized by more ramified, still immature pre-oligodendrocytes (stages 3 and 4 in Fig. 4), where NG2 has been downregulated and more advanced markers like O4, O1 and the proteolipid myelin protein PLP are present (Fumagalli et al. 2011a). Based on these data, GPR17 is currently utilized by other independent scientists to specifically label pre-immature OLs at these two transition stages (Mitew et al. 2013; Nakatani et al. 2013; Crociara et al. 2013; Ferrara et al. 2016).

Of note, GPR17 expression progressively increases during the transition of OPCs to pre-OLs (when it is maximally expressed in cellular processes), but is then gradually silenced and never found in fully morphologically mature OLs (Fumagalli et al. 2011a) (see Fig. 4). Accordingly, *in vivo*, GPR17 is present in a subset of NG2/Olig2-positive OPCs expressing the first myelin proteins, but not in more mature cells expressing myelin basic protein (MBP). Also during rodent brain development, GPR17 expression in OPCs precedes myelin production. Interestingly, GPR17 immunoreactivity appears first

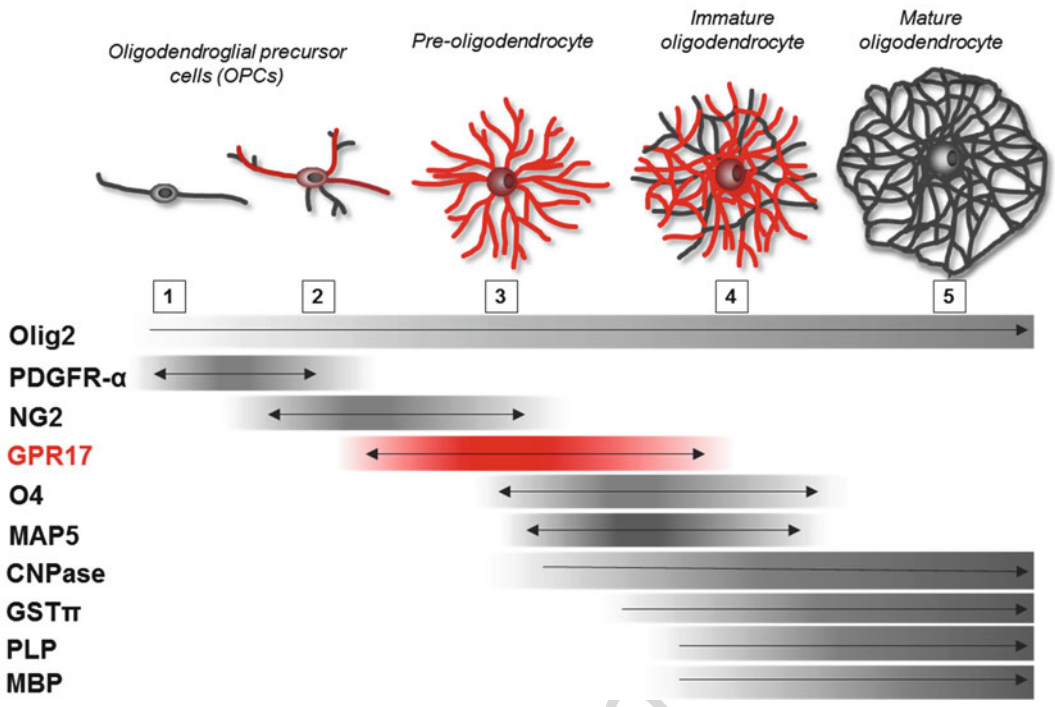

Fig. 4 Transient GPR17 expression during oligodendroglial differentiation. The expression pattern of GPR17 (in *red*) during oligodendroglial differentiation is shown in parallel to other known oligodendroglial markers (other colours). Progressive differentiation stages are indicated with numbers from 1 to 5. From a functional point of view, GPR17 exerts opposing stage-specific roles: a positive role for differentiation in early OPCs and a negative function for OL maturation in late OPCs. In late OPCs, gradual silencing of GPR17 is needed to allow OPCs to complete their maturation (see text for more details)

in the cell body, partially coinciding with markers of the Golgi apparatus, and then gradually extends to cellular processes (Boda et al. 2011). Early after birth, the expression of the receptor is low, but progressively expands to cover the 80% of OPCs at the end of the third week of life. Afterwards, GPR17 is down-regulated while myelination proceeds (Boda et al. 2011).

The transient nature of GPR17 expression in OPCs suggests that the receptor may display stage-specific roles during OL development. Intriguingly, as already reported for the Wnt/β-catenin pathway (Fancy et al. 2009; Ye et al. 2009) and more recently proposed for the transcription factor Olig2 (Mei et al. 2013), GPR17 exhibits opposing functions on OL differentiation in relation to its expression stage. In cultured cortical postnatal rat OPCs, early receptor obliteration with small interfering RNAs profoundly affected their ability to generate mature OLs, suggesting that cells are retained at a less differentiated stage (Fumagalli et al. 2011a) (Fig. 4). Although the molecular mechanisms at the basis of these events have not been yet investigated, these data highlight a pivotal role of GPR17 in the initial phases of the differentiation process. They support the hypothesis that, at these stages, GPR17 may be important to keep cells at an immature state which may, in turn, be necessary to prepare them for myelination (Fumagalli et al. 2011a). In contrast, cultured cortical progenitors from GPR17 knock-out E15.5 mouse embryos differentiated earlier toward mature OLs compared to control cells (Chen et al. 2009). The reasons for these discrepancies remain unknown, although it may be hypothesized that compensatory mechanisms are activated as a result of early embryonic GPR17 knock out. Of course, the generation of

conditional transgenic mice in which deletion of GPR17 in OPCs could be induced under controlled conditions at specific ages will help clarifying this issue.

Lecca and coworkers also clearly showed that GPR17 is no longer present in morphologically mature MBP-positive cells (Lecca et al. 2008), raising for the first time the possibility that loss of GPR17 at advanced differentiation stages is a prerequisite to allow cells to complete terminal maturation. Subsequent *in vivo* data showed that myelinogenesis is indeed defective in transgenic mice overexpressing GPR17 under the promoter of 2′,3′-Cyclic-nucleotide 3′-phosphodiesterase (CNPase), a relatively advanced OL marker (Chen et al. 2009). These animals exhibited motor disabilities, tremors and precocious death within the second week of life. The forced and un-timely expression of GPR17 at a maturation stage (i.e., in $CNPase^+$ cells), at which GPR17 is normally already downregulated, might have created conflicting signals leading to defective terminal maturation. Thus, interference with the stage-restricted expression of GPR17 resulting in un-programmed receptor expression in late OPCs completely alters the differentiation program of these cells. This hypothesis is fully in line with the demonstration that OPCs incorporating a vector for GPR17 overexpression maintained an immature morphological phenotype and never expressed the mature marker CNPase, and with data showing that, under conditions where terminal OPC maturation is impaired, such as demyelinating diseases (see Sect. 3.1.2) or treatment with the mTOR inhibitor rapamycin, that reduces OPC maturation, GPR17 is markedly up-regulated (Fumagalli et al. 2015; Tyler et al. 2011).

Both intrinsic and extrinsic mechanisms could contribute to GPR17 stage-specific functions during oligodendrocyte differentiation. GPR17 can be extrinsically regulated by physiological ligands accumulating in the extracellular milieu: activation of early OPCs (stages 2 and 3 in Fig. 4) with GPR17 endogenous putative ligands (i.e., UDP-glucose or LTD_4) indeed promoted conversion to more mature cells expressing myelin markers (Lecca et al. 2008; Fumagalli et al. 2011a; Ceruti et al. 2011). Consistent with these data, GPR17 antagonists like cangrelor (Fig. 3) delayed the ability to generate mature cells (Lecca et al. 2008; Fumagalli et al. 2011a), suggesting that GPR17 endogenous ligands are basally released in culture and are responsible for the observed spontaneous OPC *in vitro* maturation. In another independent study, while not modifying the potential of adult multipotent neural stem cells, montelukast, which also acts as a GPR17 antagonist (Ciana et al. 2006; Benned-Jensen and Rosenkilde 2010; Lecca et al. 2008), markedly increased their proliferation rate, suggesting that GPR17 antagonism induces retention of cells at a more undifferentiated stage (Huber et al. 2011).

As already mentioned, besides cAMP inhibition, GPR17 has been also shown to specifically mediate activation of delayed rectifier K^+ currents (Table 1) in a sub-population of OPCs and $O4^+$ pre-OLs, but not in mature OLs. This effect was shown to contribute to the terminal maturation of OPCs and to their migratory abilities.

In contrast with the above studies, MDL29,951, the new putative GPR17 agonist mentioned above, was reported to inhibit, rather than stimulate, OL maturation (Hennen et al. 2013). However, it is worth to note that, due to the *transient* expression of GPR17 in culture, the timing of OPC manipulation and treatment is crucial for obtaining comparable results. On the other hand, as already mentioned, MDL29,951 is not a selective ligand for this receptor, and independent effects could be due to its antagonistic activity at the glycinergic site of the glutamate NMDA receptor (Salituro et al. 1992), which has been indeed reported to promote OPC differentiation (Li et al. 2013).

Globally, these findings suggest that GPR17 exerts opposing stage-specific roles: a positive role for differentiation in early OPCs and a negative function for OL maturation in late OPCs. They also suggest that, in late OPCs, physiological GPR17 silencing is needed to allow cells to complete their maturation program. The latter

may occur via either GPR17 desensitization/internalization by endogenous agonists or by GPR17-mediated engagement of intracellular pathways culminating in nuclear events, or both. Blockade of GPR17 mRNA translation into the receptor protein a specific microRNA has been also recently reported to contribute to GPR17 regulation during OPC maturation (Lecca et al. 2016).

3.1.2 Dysregulation in Demyelinating Neurodegenerative Diseases

The demonstration that levels of endogenous nucleotides and cysLTs are massively increased upon CNS trauma and ischemia and their hypothesized roles as danger signals after injury (Davalos et al. 2005; Haynes et al. 2006) has raised the hypothesis that GPR17 may act as a crucial mediator of reactivity to acute injury. While physiologically GPR17 is mostly an oligodendroglial receptor, after acute injury, GPR17 is sequentially induced in dying neurons inside and at the borders of the ischemic/traumatic lesion, in infiltrating microglia/macrophages and in activated parenchymal OPCs in the lesion's surrounding areas, with similar expression patterns in different models of pathology. In more detail, in both rats and mice, 24 h after permanent middle cerebral artery occlusion (MCAo), GPR17 is up-regulated in neurons damaged by the ischemic insult inside the ischemic core (Ciana et al. 2006; Lecca et al. 2008). When the penumbra area is well visible and most of the neurons in the core are dead, GPR17 appears on highly activated microglia and blood-borne macrophages at the borders of the lesion (Lecca et al. 2008). This has been independently confirmed to also occur in a transient MCAo rodent model, where the number of GPR17 expressing cells was significantly upregulated in two distinct phases, 24 h and 7 days after reperfusion, consistent with an early acute neuronal injury followed by a late microgliosis (Zhao et al. 2012). It is known that OPCs are extremely sensitive to the pathophysiological state of the brain, and that they react to many different types of experimentally induced insults. Starting from 72 h after the insult, in the regions surrounding the ischemic area and in the ipsilateral corpus striatum of MCAo mice, a higher number of GPR17-expressing OPCs was indeed found compared to contralateral hemisphere (Lecca et al. 2008), suggesting an increased proliferation rate in response to demyelination.

Dysregulated expression of GPR17 has been described also after traumatic injury, in both brain (Boda et al. 2011) and spinal cord (Ceruti et al. 2009). In stab wound, a model of cortical trauma, early after lesion, the density of GPR17-expressing OPCs in gray matter was reduced compared to contralateral cortex, consistent with a global oligodendroglial loss. At later times, $GPR17^+$ cells increased significantly in number around the lesion in both gray and white matter, likely due to the expansion of the NG2 cell pool, which, in turn, reflects an attempt to replace dead OPCs. This reactivity lasted up to 7 days and then declined over time, going back to basal levels at 14 days after lesion. This pattern has been confirmed in human samples from patients with traumatic brain injury (Franke et al. 2013). In both neurosurgical and autopsy specimens, GPR17 expression was evident inside the contused core and progressively declined distally according to a spatio-temporal gradient. Inside and around the core, GPR17 labeled dying neurons, reactive astrocytes, and activated microglia/macrophages. In peri-contused parenchyma, GPR17 was found on OPCs, some of which had proliferated, indicating re-myelination attempts. In agreement with the above data, in a double transgenic model of Alzheimer's disease (the APPPS1 mouse) a high number of GPR17-positive cells accumulated close to amyloid plaques in gray matter, revealing receptor up-regulation as a feature of oligodendroglial reactivity also in this pathological condition (Boda et al. 2011).

Similar GPR17 changes have been reported also in typically de-myelinating diseases such as in models of multiple sclerosis (MS). In this disease, remyelination occurs after the initial myelin damage, but it fails after multiple demyelination episodes, which eventually leads to axonal degeneration and progressive disability

(Franklin and Ffrench-Constant 2008). Interestingly, synthesis of cysLTs is increased in MS plaques and in the spinal cord of mice subjected to experimental autoimmune encephalomyelitis (EAE), an immune-mediated model of demyelination (Whitney et al. 2001). Of note, montelukast, an antagonist at both CysLT1 and GPR17, attenuated CNS infiltration of inflammatory cells and the clinical symptoms of EAE (Wang et al. 2011). However, the exact contribution of GPR17 to these effects has not been investigated in detail. Overexpression of the GPR17 transcript has been observed in both EAE mice and in a cohort of human MS tissues (Chen et al. 2009). GPR17 expression was significantly increased in MS plaques as compared with white matter from non-neurological donor samples and normal-appearing white matter from MS donors. In a similar way, acute damage to myelin induced by lysolecithin (Lys) injection in corpus callosum induced a strong overexpression of GPR17 at the lesion site 10 days after injury (Boda et al. 2011). Thus, independently of the original cause, GPR17 is abnormally up-regulated in MS and some models of neurodegenerative conditions characterized by myelin disruption (Fumagalli et al. 2016).

On this basis, it could be hypothesized that, after damage, GPR17 is initially induced to promote the growth and differentiation of OPCs; however, at later stages, due to lack of appropriate environmental stimuli, presence of inflammatory signals and/or intrinsic factors, physiological GPR17 downregulation is impeded, thus freezing cells at a stand-by stage, where they are neither proliferating nor differentiating. When this happens, interventions targeting GPR17 may help bypassing this checkpoint and facilitate terminal maturation. Since GPR17 is a *membrane* receptor that, at variance from other intrinsic regulators of oligodendrogenesis, can be easily targeted and manipulated with pharmacological agents, it is envisaged that agents counteracting GPR17 aberrant expression under these conditions could induce OPCs to resume myelination and promote neurorepair. To support this hypothesis, in MCAo animals, administration of GPR17 antagonists such as cangrelor or montelukast (Ciana et al. 2006; Lecca et al. 2008), or GPR17 silencing due to *in vivo* delivery of specific antisense oligonucleotides (Ciana et al. 2006; Lecca et al. 2008) or small interfering RNAs (Zhao et al. 2012) resulted in a significant reduction in brain's ischemic volume. Use of GPR17 anti-sense oligonucleotides also reduced damage and improved functional recovery in a model of spinal cord injury, in line with the hypothesis that GPR17 is aberrantly overexpressed as a consequence of damage (Ceruti et al. 2009).

In contrast to what observed in MCAo, in a rat *neonatal* model of ischemic periventricular leukomalacia (PVL), a common cerebral white matter injury, the GPR17 agonist UDP-glucose (and not an antagonist) significantly contributed to myelin sheaths recovery and improved motor functions, learning and coordination in PVL pups (Mao et al. 2012). The reason for this discrepancy may reside in the different outcome of the ischemic insult in neonatal brain compared to adults. It could be hypothesized that, in neonatal pups, existing OPCs, which are very sensitive to ischemic death, are immediately killed by the ischemic insult, with no obvious GPR17 upregulation; conversely, being these cells generated at distinct waves during the first weeks of life at much higher rates compared to adulthood, a GPR17 agonist (instead of an antagonist) would allow to properly activate newborn OPCs, thus favouring the formation of myelin sheaths and neurological recovery.

Several of the still obscure aspects of GPR17 pathophysiology have been linked to the difficulty of establishing a causal relationship between GPR17 expression and myelination *in vivo*. Since GPR17 is no longer expressed in mature myelinating OLs (Lecca et al. 2008; Fumagalli et al. 2015), it was impossible to demonstrate that cells that have expressed GPR17 in their earlier life can indeed myelinate. Only recently, the generation of the first GPR17iCreER^{T2}-GFP reporter mouse line for fate mapping studies has allowed to follow the final destiny of GPR17^{+} cells during both physiological differentiation and in disease, thanks to

the inducible expression of the green fluorescence protein (GFP). In these mice, upon tamoxifen induced recombination, OPCs expressing GPR17 at that very specific moment, become green and can be traced as such for the entire animal's like. Use of these mice has allowed to show that, in normal brain, GFP^+ cells differentiate very slowly (needing about 3 months to reach maturity), but after acute insults, they rapidly reacted to damage with proliferation and migration toward the injured site, thus representing a 'reserve pool' of adult quiescent progenitors maintained for repair purposes (Vigano et al. 2016). A full characterization of the long-term events occurring in the brain of ischemic MCAo GPR17iCreER^{T2}-GFP mice has shown that, despite massive recruitment of GFP^+ green OPCs at the ischemic site, only a few percentage of these cells become mature myelinating OLs, likely due to local unfavourable inflammatory milieu (Vigano et al. 2016; Bonfanti et al. 2017).

More recently, it has been demonstrated that GPR17 over-activation inhibited oligodendrocyte survival by reducing intracellular cAMP levels and inducing expression of the pro-apoptotic gene *Xaf1*. GPR17 overactivation also negatively regulated protein kinase A signaling pathway and expression of the transcription factor c-Fos. In line with these data, in the lysolecithin-mediated demyelination injury model, the pharmacological inhibition of GPR17 with pranlukast increased oligodendrocyte survival and promoted immature oligodendrocyte differentiation through the upregulation of Epac1, the exchange factor directly activated by cAMP (Ou et al. 2016). These data are fully consistent with our results in other injury models characterized by demyelination and abnormal GPR17 upregulation (summarized in Fumagalli et al. 2016), suggesting that under these conditions GPR17 inhibition has potential for treatment of demyelinating diseases (Ou et al. 2016).

3.2 GPR17 in Brain Rejuvenation

A recent report has investigated the roles of GPR17 in age-associated cognitive decline (Marschallinger et al. 2015). Authors have first shown that oral administration of montelukast (an antagonist of both CysLTR1 and GPR17, see above), for 6 weeks to moderately old rats (20 months old) resulted in structural and functional rejuvenation of aged brains, as demonstrated by restoration of blood brain barrier integrity, reduced microglia activation in the brain, increased levels of hippocampal neurogenesis and significantly improved learning and memory tasks. Important, montelukast had no effects on the behaviour and cognitive abilities of young animals, suggesting that its actions specifically target an aging associated defect (see also below). Regression and correlation analyses showed that montelukast-induced learning improvement in the old animals was independent of the changes in microglia morphology but rather depended on the rate of neurogenesis measured as increased number of proliferating neuroblasts in hippocampal dentate gyrus. Interestingly, authors also provided immunohistochemical evidence for the presence of GPR17, but not CysLTR1, in a subset of doublecortin $(DCX)^+$ newborn neurons in hippocampal dentate gyrus, suggesting a role in the proliferation and specification of these cells. Studies on neurospheres obtained from mice lacking FOXO1, a GPR17 regulating transcription factor, and from GPR17$^{-/-}$ mice indeed confirmed montelukast effects be due to action on GPR17/DCX^+ neuroblasts in hippocampal dentate gyrus, leading to increased neurogenesis. Globally these data suggest that, under normal conditions, GPR17 exerts a negative control on the proliferation of neural progenitors in the hippocampus; in aged animals, due to the overall decrease of neurogenesis, GPR17 inhibition of proliferation becomes detrimental and contributes to memory impairment. Under such pathological conditions, montelukast can restore neurogenesis by alleviating GPR17 inhibitory effect (Marschallinger et al. 2015).

3.3 GPR17 in Gliomas

OL markers such as Olig2, PDGFRα and NG2 are often expressed in glioma cells. Little is known about the origin of these tumors, but it is possible that they arise from dysregulated OPCs (Liu et al. 2011b). Considering that OPCs are the only proliferating population in the adult brain, defects in differentiation mechanisms favouring cell proliferation could be a primary cause of gliomas. A complementary strategy for tumor treatment is to promote pathways for maintaining quiescence and/or driving terminal differentiation of the tumoral progenitors. In this respect, a recent microarray analysis of mouse and human gliomas aimed at unveiling new candidates promoting differentiation or quiescence has highlighted GPR17 as a new potential target (Dougherty et al. 2012). In glioma cells, treatment with UDP, UDP-glucose or LTD_4 indeed reduced the formation of glioma spheres suggesting that GPR17 stimulation can represent a good strategy to drive the differentiation of highly proliferative uncommitted tumor cells to the oligodendroglial fate, negatively affecting both tumor cell proliferation and self-renewal (Dougherty et al. 2012). These data are in line with the fact that most of the OPCs expressing GPR17 in brain are quiescent (Lecca et al. 2008), and support the pro-differentiative effects of its putative endogenous ligands (see also Sects. 2.1 and 3.2).

4 Conclusions

GPR17 has emerged as a new GPCR of great interest for drug development. It is almost exclusive localization to OPCs, the myelin forming cells and the only (slowly) proliferating cell population in the intact brain, has highlighted GPR17 as a novel pharmacological target for demyelinating diseases. At variance from other myelinating genes, GPR17 is a membrane receptor, thus amenable for pharmacological modulation, which has attracted a lot of interest for the development of new therapeutic approaches to MS and other neurodegenerative diseases characterized by myelin disruption. The recent demonstration that GPR17 is also expressed by a subset of hippocampal neural progenitors involved in cognitive functions does not detract from the potential interest of GPR17 ligands in neurodegenerative diseases, since, as demonstrated by the montelukast study (Marschallinger et al. 2015), these ligands may be active only when specific pathological GPR17 changes are present.

The recent studies on GPR17 revealed its transient expression in OPCs and a more complex role than expected: a pro-differentiating role in early OPCs and a negative function on maturation in late stage OPCs. Thus, the apparently contrasting *in vitro* data obtained with different GPR17 stimulatory agents (Lecca et al. 2008; Fumagalli et al. 2011a; Hennen et al. 2013) may depend on the specific differentiation stage at which these compounds have been added to cultured OPCs. It may well be that the function of GPR17 is different in the intact and diseased brain, based on the availability of its endogenous ligands. If uracil nucleotides, cysLTs, oxysterols and chemokines like SDF-1 are indeed among the signaling molecules able to activate GPR17 *in vivo* (see also below), we envisage that their role would be more likely unveiled under pathological conditions, where these ligands massively accumulate at lesion sites inside the CNS.

Experiments in a wide variety of rodent models of neurodegeneration have shown that, independently of the nature of the insult (ischemic, traumatic or toxic) and of the presence of any concomitant neuronal pathology, demyelinating conditions invariably led to GPR17 upregulation. We believe that this dysregulation reflects an initial attempt to repair the lesion by stimulating OPCs differentiation via GPR17, but that this attempt is later invalidated by the inability of maturing cells to downregulate/internalize the receptor, which, in turn, leads a differentiation blockade. On this basis, it is envisaged that *GPR17 antagonists* would be useful in MS and neurodegenerative diseases. By counteracting GPR17 aberrant dysfunction, antagonists would help OPCs to complete their maturation, thus

re-establishing endogenous remyelination, as recently also confirmed (Ou et al. 2016).

Due to the still ambiguous state of the pharmacology for this receptor, the Nomenclature Committee of the International Union of Pharmacology (NC-IUPHAR) has not yet officially de-orphanized this GPCR (Davenport et al. 2013). However, as also emphasized by NC-IUPHAR, much of the work in this area has been based on recombinant expression systems using different host cells and transfection methodologies compared to data derived from native cells. In recombinant "artificial" cell systems, activity tests are highly dependent on the experimental conditions utilized and subject to several artifacts, especially in the case of receptors' constitutive activation, a typical feature of several GPCRs including GPR17 (Benned-Jensen and Rosenkilde 2010; Maekawa et al. 2009; Qi et al. 2013; Eggerickx et al. 1995; Uhlenbrock et al. 2002; Rosenkilde et al. 2006; Qin et al. 2011; Im 2004) that can profoundly alter ligand behavior (Kenakin 2001; Davenport et al. 2013).

In terms of drug development, neither uracil nucleotides nor CysLTs are suitable to this purpose, because neither ligand class is competent to discriminate between the functions of purinergic receptors, CysLT receptors, and GPR17 *in vivo*, where multiple receptors are often co-expressed. Nevertheless, the already available *in vivo* rodent data reporting positive neuro-reparative effects induced by commercially available montelukast or pranlukast (Yu et al. 2005a, b), which are potent (although non selective) GPR17 antagonists, foster the search for further GPR17 ligands (Eberini et al. 2011; Hennen et al. 2013) and may represent an important advancement for patients with neurodegenerative diseases.

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References

Abbracchio MP, Burnstock G, Boeynaems JM, Barnard EA, Boyer JL, Kennedy C, Knight GE, Fumagalli M, Gachet C, Jacobson KA, Weisman GA (2006) International Union of Pharmacology LVIII: update on the P2Y G protein-coupled nucleotide receptors: from molecular mechanisms and pathophysiology to therapy. Pharmacol Rev 58(3):281–341

Benned-Jensen T, Rosenkilde MM (2010) Distinct expression and ligand-binding profiles of two constitutively active GPR17 splice variants. Br J Pharmacol 159(5):1092–1105

Blasius R, Weber RG, Lichter P, Ogilvie A (1998) A novel orphan G protein-coupled receptor primarily expressed in the brain is localized on human chromosomal band 2q21. J Neurochem 70(4):1357–1365

Boda E, Vigano F, Rosa P, Fumagalli M, Labat-Gest V, Tempia F, Abbracchio MP, Dimou L, Buffo A (2011) The GPR17 receptor in NG2 expressing cells: focus on in vivo cell maturation and participation in acute trauma and chronic damage. Glia 59(12):1958–1973

Bonfanti E, Gelosa P, Fumagalli M, Dimou L, Viganò F, Tremoli E, Cimino M, Sironi L, Abbracchio MP (2017) The role of oligodendrocyte precursor cells expressing the GPR17 receptor in brain remodelling after stroke. Cell Death Dis [in press]

Brink C, Dahlen SE, Drazen J, Evans JF, Hay DW, Nicosia S, Serhan CN, Shimizu T, Yokomizo T (2003) International Union of Pharmacology XXXVII. Nomenclature for leukotriene and lipoxin receptors. Pharmacol Rev 55(1):195–227

Buccioni M, Marucci G, Dal Ben D, Giacobbe D, Lambertucci C, Soverchia L, Thomas A, Volpini R, Cristalli G (2011) Innovative functional cAMP assay for studying G protein-coupled receptors: application to the pharmacological characterization of GPR17. Purinergic Signal 7(4):463–468

Calleri E, Ceruti S, Cristalli G, Martini C, Temporini C, Parravicini C, Volpini R, Daniele S, Caccialanza G, Lecca D, Lambertucci C, Trincavelli ML, Marucci G, Wainer IW, Ranghino G, Fantucci P, Abbracchio MP, Massolini G (2010) Frontal affinity chromatography-mass spectrometry useful for characterization of new ligands for GPR17 receptor. J Med Chem 53 (9):3489–3501

Capra V, Ravasi S, Accomazzo MR, Citro S, Grimoldi M, Abbracchio MP, Rovati GE (2005) CysLT1 receptor is a target for extracellular nucleotide-induced heterologous desensitization: a possible feedback mechanism in inflammation. J Cell Sci 118(Pt 23):5625–5636

Carbajal KS, Miranda JL, Tsukamoto MR, Lane TE (2011) CXCR4 signaling regulates remyelination by endogenous oligodendrocyte progenitor cells in a viral model of demyelination. Glia 59(12):1813–1821

Ceruti S, Villa G, Genovese T, Mazzon E, Longhi R, Rosa P, Bramanti P, Cuzzocrea S, Abbracchio MP (2009) The P2Y-like receptor GPR17 as a sensor of

damage and a new potential target in spinal cord injury. Brain 132(Pt 8):2206–2218

Ceruti S, Vigano F, Boda E, Ferrario S, Magni G, Boccazzi M, Rosa P, Buffo A, Abbracchio MP (2011) Expression of the new P2Y-like receptor GPR17 during oligodendrocyte precursor cell maturation regulates sensitivity to ATP-induced death. Glia 59(3):363–378

Chang JM, Di Tommaso P, Taly JF, Notredame C (2012) Accurate multiple sequence alignment of transmembrane proteins with PSI-Coffee. BMC Bioinf 13(Suppl 4):S1

Chen Y, Wu H, Wang S, Koito H, Li J, Ye F, Hoang J, Escobar SS, Gow A, Arnett HA, Trapp BD, Karandikar NJ, Hsieh J, Lu QR (2009) The oligodendrocyte-specific G protein-coupled receptor GPR17 is a cell-intrinsic timer of myelination. Nat Neurosci 12(11):1398–1406

Ciana P, Fumagalli M, Trincavelli ML, Verderio C, Rosa P, Lecca D, Ferrario S, Parravicini C, Capra V, Gelosa P, Guerrini U, Belcredito S, Cimino M, Sironi L, Tremoli E, Rovati GE, Martini C, Abbracchio MP (2006) The orphan receptor GPR17 identified as a new dual uracil nucleotides/cysteinyl-leukotrienes receptor. EMBO J 25(19):4615–4627

Coppi E, Maraula G, Fumagalli M, Failli P, Cellai L, Bonfanti E, Mazzoni L, Coppini R, Abbracchio MP, Pedata F, Pugliese AM (2013) UDP-glucose enhances outward K(+) currents necessary for cell differentiation and stimulates cell migration by activating the GPR17 receptor in oligodendrocyte precursors. Glia 61(7):1155–1171

Crociara P, Parolisi R, Conte D, Fumagalli M, Bonfanti L (2013) Cellular and molecular characterization of multipolar Map5-expressing cells: a subset of newly generated, stage-specific parenchymal cells in the mammalian central nervous system. PLoS One 8(5): e63258

Daniele S, Lecca D, Trincavelli ML, Ciampi O, Abbracchio MP, Martini C (2010) Regulation of PC12 cell survival and differentiation by the new P2Y-like receptor GPR17. Cell Signal 22(4):697–706

Daniele S, Trincavelli ML, Gabelloni P, Lecca D, Rosa P, Abbracchio MP, Martini C (2011) Agonist-induced desensitization/resensitization of human G protein-coupled receptor 17: a functional cross-talk between purinergic and cysteinyl-leukotriene ligands. J Pharmacol Exp Ther 338(2):559–567

Daniele S, Trincavelli ML, Fumagalli M, Zappelli E, Lecca D, Bonfanti E, Campiglia P, Abbracchio MP, Martini C (2014) Does GRK-beta arrestin machinery work as a "switch on" for GPR17-mediated activation of intracellular signaling pathways? Cell Signal 26 (6):1310–1325

Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, Littman DR, Dustin ML, Gan WB (2005) ATP mediates rapid microglial response to local brain injury in vivo. Nat Neurosci 8(6):752–758

Davenport AP, Alexander SP, Sharman JL, Pawson AJ, Benson HE, Monaghan AE, Liew WC, Mpamhanga CP, Bonner TI, Neubig RR, Pin JP, Spedding M, Harmar AJ (2013) International union of basic and clinical pharmacology. LXXXVIII G protein-coupled receptor list: recommendations for new pairings with cognate ligands. Pharmacol Rev 65(3):967–986

Dougherty JD, Fomchenko EI, Akuffo AA, Schmidt E, Helmy KY, Bazzoli E, Brennan CW, Holland EC, Milosevic A (2012) Candidate pathways for promoting differentiation or quiescence of oligodendrocyte progenitor-like cells in glioma. Cancer Res 72 (18):4856–4868

Eberini I, Daniele S, Parravicini C, Sensi C, Trincavelli ML, Martini C, Abbracchio MP (2011) In silico identification of new ligands for GPR17: a promising therapeutic target for neurodegenerative diseases. J Comput Aided Mol Des 25(8):743–752

Eggerickx D, Denef JF, Labbe O, Hayashi Y, Refetoff S, Vassart G, Parmentier M, Libert F (1995) Molecular cloning of an orphan G-protein-coupled receptor that constitutively activates adenylate cyclase. Biochem J 309(Pt 3):837–843

Fancy SP, Baranzini SE, Zhao C, Yuk DI, Irvine KA, Kaing S, Sanai N, Franklin RJ, Rowitch DH (2009) Dysregulation of the Wnt pathway inhibits timely myelination and remyelination in the mammalian CNS. Genes Dev 23(13):1571–1585

Ferrara G, Errede M, Girolamo F, Morando S, Ivaldi F, Panini N, Bendotti C, Perris R, Furlan R, Virgintino D, Kerlero de Rosbo N, Uccelli A (2016) NG2, a common denominator for neuroinflammation, blood-brain barrier alteration, and oligodendrocyte precursor response in EAE, plays a role in dendritic cell activation. Acta Neuropathol 132(1):23–42

Franke H, Parravicini C, Lecca D, Zanier ER, Heine C, Bremicker K, Fumagalli M, Rosa P, Longhi L, Stocchetti N, De Simoni MG, Weber M, Abbracchio MP (2013) Changes of the GPR17 receptor, a new target for neurorepair, in neurons and glial cells in patients with traumatic brain injury. Purinergic Signal 9(3):451–462

Franklin RJ, Ffrench-Constant C (2008) Remyelination in the CNS: from biology to therapy. Nat Rev Neurosci 9 (11):839–855

Fratangeli A, Parmigiani E, Fumagalli M, Lecca D, Benfante R, Passafaro M, Buffo A, Abbracchio MP, Rosa P (2013) The regulated expression, intracellular trafficking and membrane recycling of the P2Y-like receptor GPR17 in Oli-neu oligodendroglial cells. J Biolog Chem 288(7):5241–5256

Fredriksson R, Lagerstrom MC, Lundin LG, Schioth HB (2003) The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. Mol Pharmacol 63(6):1256–1272

Fumagalli M, Trincavelli L, Lecca D, Martini C, Ciana P, Abbracchio MP (2004) Cloning, pharmacological characterisation and distribution of the rat G-protein-coupled P2Y(13) receptor. Biochem Pharmacol 68 (1):113–124

Fumagalli M, Daniele S, Lecca D, Lee PR, Parravicini C, Fields RD, Rosa P, Antonucci F, Verderio C, Trincavelli ML, Bramanti P, Martini C, Abbracchio MP (2011a) Phenotypic changes, signaling pathway, and functional correlates of GPR17-expressing neural precursor cells during oligodendrocyte differentiation. J Biol Chem 286(12):10593–10604

Fumagalli M, Lecca D, Abbracchio MP (2011b) Role of purinergic signalling in neuro-immune cells and adult neural progenitors. Front Biosci 16:2326–2341

Fumagalli M, Bonfanti E, Daniele S, Lecca D, Martini C, Trincavelli ML, Abbracchio MP (2015) The ubiquitin ligase Mdm2 controls oligodendrocyte maturation by intertwining mTOR with G protein-coupled receptor kinase 2 in the regulation of GPR17 receptor desensitization. Glia in press. doi:10.1002/glia.22896

Fumagalli M, Lecca D, Abbracchio MP (2016) CNS remyelination as a novel reparative approach to neurodegenerative diseases: The roles of purinergic signaling and the P2Y-like receptor GPR17. Neuropharmacology 104:82–93

Garenc C, Julien P, Levy E (2010) Oxysterols in biological systems: the gastrointestinal tract, liver, vascular wall and central nervous system. Free Radic Res 44(1):47–73

Hannedouche S, Zhang J, Yi T, Shen W, Nguyen D, Pereira JP, Guerini D, Baumgarten BU, Roggo S, Wen B, Knochenmuss R, Noel S, Gessier F, Kelly LM, Vanek M, Laurent S, Preuss I, Miault C, Christen I, Karuna R, Li W, Koo DI, Suply T, Schmedt C, Peters EC, Falchetto R, Katopodis A, Spanka C, Roy MO, Detheux M, Chen YA, Schultz PG, Cho CY, Seuwen K, Cyster JG, Sailer AW (2011) Oxysterols direct immune cell migration via EBI2. Nature 475(7357):524–527

Haupt VJ, Daminelli S, Schroeder M (2013) Drug promiscuity in PDB: protein binding site similarity is key. PLoS One 8(6):e65894

Haynes SE, Hollopeter G, Yang G, Kurpius D, Dailey ME, Gan WB, Julius D (2006) The P2Y12 receptor regulates microglial activation by extracellular nucleotides. Nat Neurosci 9(12):1512–1519

Hennen S, Wang H, Peters L, Merten N, Simon K, Spinrath A, Blattermann S, Akkari R, Schrage R, Schroder R, Schulz D, Vermeiren C, Zimmermann K, Kehraus S, Drewke C, Pfeifer A, Konig GM, Mohr K, Gillard M, Muller CE, Lu QR, Gomeza J, Kostenis E (2013) Decoding signaling and function of the orphan G protein-coupled receptor GPR17 with a small-molecule agonist. Sci Signal 6 (298):ra93

Huber C, Marschallinger J, Tempfer H, Furtner T, Couillard-Despres S, Bauer HC, Rivera FJ, Aigner L (2011) Inhibition of leukotriene receptors boosts neural progenitor proliferation. Cell Physiol Biochem 28(5):793–804

Im DS (2004) Discovery of new G protein-coupled receptors for lipid mediators. J Lipid Res 45 (3):410–418

Im DS (2013) Intercellular lipid mediators and GPCR drug discovery. Biomol Ther 21(6):411–422

Jiang Q, Guo D, Lee BX, Van Rhee AM, Kim YC, Nicholas RA, Schachter JB, Harden TK, Jacobson KA (1997) A mutational analysis of residues essential for ligand recognition at the human P2Y1 receptor. Mol Pharmacol 52(3):499–507

Kanaoka Y, Maekawa A, Austen KF (2013) Identification of GPR99 protein as a potential third cysteinyl leukotriene receptor with a preference for leukotriene E4 ligand. J Biol Chem 288(16):10967–10972

Kenakin T (2001) Inverse, protean, and ligand-selective agonism: matters of receptor conformation. FASEB J 15(3):598–611

Kotani M, Mollereau C, Detheux M, Le Poul E, Brezillon S, Vakili J, Mazarguil H, Vassart G, Zajac JM, Parmentier M (2001) Functional characterization of a human receptor for neuropeptide FF and related peptides. Br J Pharmacol 133(1):138–144

Lecca D, Abbracchio MP (2008) Deorphanisation of G protein-coupled receptors: a tool to provide new insights in nervous system pathophysiology and new targets for psycho-active drugs. Neurochem Int 52 (3):339–351

Lecca D, Ceruti S (2008) Uracil nucleotides: from metabolic intermediates to neuroprotection and neuroinflammation. Biochem Pharmacol 75 (10):1869–1881

Lecca D, Trincavelli ML, Gelosa P, Sironi L, Ciana P, Fumagalli M, Villa G, Verderio C, Grumelli C, Guerrini U, Tremoli E, Rosa P, Cuboni S, Martini C, Buffo A, Cimino M, Abbracchio MP (2008) The recently identified P2Y-like receptor GPR17 is a sensor of brain damage and a new target for brain repair. PLoS One 3(10):e3579

Lecca D, Marangon D, Coppolino GT, Mendez AM, Finardi A, Costa GD, Martinelli V, Furlan R, Abbracchio MP (2016) MiR-125a-3p timely inhibits oligodendroglial maturation and is pathologically up-regulated in human multiple sclerosis. Sci Rep 6:34503

Levit A, Beuming T, Krilov G, Sherman W, Niv MY (2014) Predicting GPCR promiscuity using binding site features. J Chem Inf Model 54(1):184–194

Li M, Hale JS, Rich JN, Ransohoff RM, Lathia JD (2012) Chemokine CXCL12 in neurodegenerative diseases: an SOS signal for stem cell-based repair. Trends Neurosci 35(10):619–628

Li C, Xiao L, Liu X, Yang W, Shen W, Hu C, Yang G, He C (2013) A functional role of NMDA receptor in regulating the differentiation of oligodendrocyte precursor cells and remyelination. Glia 61(5):732–749

Liu C, Yang XV, Wu J, Kuei C, Mani NS, Zhang L, Yu J, Sutton SW, Qin N, Banie H, Karlsson L, Sun S,

Lovenberg TW (2011a) Oxysterols direct B-cell migration through EBI2. Nature 475(7357):519–523

Liu C, Sage JC, Miller MR, Verhaak RG, Hippenmeyer S, Vogel H, Foreman O, Bronson RT, Nishiyama A, Luo L, Zong H (2011b) Mosaic analysis with double markers reveals tumor cell of origin in glioma. Cell 146(2):209–221

Maekawa A, Balestrieri B, Austen KF, Kanaoka Y (2009) GPR17 is a negative regulator of the cysteinyl leukotriene 1 receptor response to leukotriene D4. Proc Natl Acad Sci U S A 106(28):11685–11690

Mamedova L, Capra V, Accomazzo MR, Gao ZG, Ferrario S, Fumagalli M, Abbracchio MP, Rovati GE, Jacobson KA (2005) CysLT1 leukotriene receptor antagonists inhibit the effects of nucleotides acting at P2Y receptors. Biochem Pharmacol 71(1–2):115–125

Mao FX, Li WJ, Chen HJ, Qian LH, Buzby JS (2012) Periventricular leukomalacia long-term prognosis may be improved by treatment with UDP-glucose, GDNF, and memantine in neonatal rats. Brain Res 1486:112–120

Marschallinger J, Schaffner I, Klein B, Gelfert R, Rivera FJ, Illes S, Grassner L, Janssen M, Rotheneichner P, Schmuckermair C, Coras R, Boccazzi M, Chishty M, Lagler FB, Renic M, Bauer HC, Singewald N, Blumcke I, Bogdahn U, Couillard-Despres S, Lie DC, Abbracchio MP, Aigner L (2015) Structural and functional rejuvenation of the aged brain by an approved anti-asthmatic drug. Nat Commun 6:8466

Marteau F, Le Poul E, Communi D, Labouret C, Savi P, Boeynaems JM, Gonzalez NS (2003) Pharmacological characterization of the human P2Y13 receptor. Mol Pharmacol 64(1):104–112

Marucci G, Dal Ben D, Lambertucci C, Santinelli C, Spinaci A, Thomas A, Volpini R, Buccioni M (2016) The G protein-coupled receptor GPR17: overview and update. ChemMedChem 11(23):2567–2574

Mei F, Wang H, Liu S, Niu J, Wang L, He Y, Etxeberria A, Chan JR, Xiao L (2013) Stage-specific deletion of Olig2 conveys opposing functions on differentiation and maturation of oligodendrocytes. J Neurosci 33(19):8454–8462

Meraviglia V, Ulivi AF, Boccazzi M, Valenza F, Fratangeli A, Passafaro M, Lecca D, Stagni F, Giacomini A, Bartesaghi R, Abbracchio MP, Ceruti S, Rosa P (2016) SNX27, a protein involved in down syndrome, regulates GPR17 trafficking and oligodendrocyte differentiation. Glia 64 (8):1437–1460

Mitew S, Hay CM, Peckham H, Xiao J, Koenning M, Emery B (2013) Mechanisms regulating the development of oligodendrocytes and central nervous system myelin. Neuroscience 276:29–47

Nakatani H, Martin E, Hassani H, Clavairoly A, Maire CL, Viadieu A, Kerninon C, Delmasure A, Frah M, Weber M, Nakafuku M, Zalc B, Thomas JL, Guillemot F, Nait-Oumesmar B, Parras C (2013) Ascl1/Mash1 promotes brain oligodendrogenesis during myelination and remyelination. J Neurosci 33 (23):9752–9768

Ou Z, Sun Y, Lin L, You N, Liu X, Li H, Ma Y, Cao L, Han Y, Liu M, Deng Y, Yao L, Lu QR, Chen Y (2016) Olig2-targeted G-protein-coupled receptor Gpr17 regulates oligodendrocyte survival in response to lysolecithin-induced demyelination. J Neurosci 36 (41):10560–10573

Parravicini C, Ranghino G, Abbracchio MP, Fantucci P (2008) GPR17: molecular modeling and dynamics studies of the 3-D structure and purinergic ligand binding features in comparison with P2Y receptors. BMC Bioinf 9:263

Parravicini C, Abbracchio MP, Fantucci P, Ranghino G (2010) Forced unbinding of GPR17 ligands from wild type and R255I mutant receptor models through a computational approach. BMC Struct Biol 10:8

Parravicini C, Daniele S, Palazzolo L, Trincavelli ML, Martini C, Zaratin P, Primi R, Coppolino G, Gianazza E, Abbracchio MP, Eberini I (2016) A promiscuous recognition mechanism between GPR17 and SDF-1: molecular insights. Cell Signal 28(6):631–642

Paruchuri S, Tashimo H, Feng C, Maekawa A, Xing W, Jiang Y, Kanaoka Y, Conley P, Boyce JA (2009) Leukotriene E4-induced pulmonary inflammation is mediated by the P2Y12 receptor. J Exp Med 206 (11):2543–2555

Patel JR, McCandless EE, Dorsey D, Klein RS (2010) CXCR4 promotes differentiation of oligodendrocyte progenitors and remyelination. Proc Natl Acad Sci U S A 107(24):11062–11067

Pugliese AM, Trincavelli ML, Lecca D, Coppi E, Fumagalli M, Ferrario S, Failli P, Daniele S, Martini C, Pedata F, Abbracchio MP (2009) Functional characterization of two isoforms of the P2Y-like receptor GPR17: [35S]GTPgammaS binding and electrophysiological studies in 1321N1 cells. Am J Physiol Cell Physiol 297(4):C1028–C1040

Qi AD, Harden TK, Nicholas RA (2013) Is GPR17 a P2Y/leukotriene receptor? examination of uracil nucleotides, nucleotide sugars, and cysteinyl leukotrienes as agonists of GPR17. J Pharmacol Exp Ther 347(1):38–46

Qin Y, Verdegaal EM, Siderius M, Bebelman JP, Smit MJ, Leurs R, Willemze R, Tensen CP, Osanto S (2011) Quantitative expression profiling of G-protein-coupled receptors (GPCRs) in metastatic melanoma: the constitutively active orphan GPCR GPR18 as novel drug target. Pigment Cell Melanoma Res 24 (1):207–218

Raccosta L, Fontana R, Maggioni D, Lanterna C, Villablanca EJ, Paniccia A, Musumeci A, Chiricozzi E, Trincavelli ML, Daniele S, Martini C, Gustafsson JA, Doglioni C, Feo SG, Leiva A, Ciampa MG, Mauri L, Sensi C, Prinetti A, Eberini I, Mora JR, Bordignon C, Steffensen KR, Sonnino S, Sozzani S, Traversari C, Russo V (2013) The oxysterol-CXCR2

axis plays a key role in the recruitment of tumor-promoting neutrophils. J Exp Med 210(9):1711–1728

Rajagopalan L, Rajarathnam K (2004) Ligand selectivity and affinity of chemokine receptor CXCR1. Role of N-terminal domain. J Biol Chem 279 (29):30000–30008

Rosenkilde MM, Benned-Jensen T, Andersen H, Holst PJ, Kledal TN, Luttichau HR, Larsen JK, Christensen JP, Schwartz TW (2006) Molecular pharmacological phenotyping of EBI2. An orphan seven-transmembrane receptor with constitutive activity. J Biol Chem 281(19):13199–13208

Salituro FG, Harrison BL, Baron BM, Nyce PL, Stewart KT, Kehne JH, White HS, McDonald IA (1992) 3-(2-Carboxyindol-3-yl)propionic acid-based antagonists of the N-methyl-D-aspartic acid receptor associated glycine binding site. J Med Chem 35 (10):1791–1799

Sensi C, Daniele S, Parravicini C, Zappelli E, Russo V, Trincavelli ML, Martini C, Abbracchio MP, Eberini I (2014) Oxysterols act as promiscuous ligands of class-A GPCRs: in silico molecular modeling and in vitro validation. Cell Signal 26(12):2614–2620

Temporini C, Ceruti S, Calleri E, Ferrario S, Moaddel R, Abbracchio MP, Massolini G (2009) Development of an immobilized GPR17 receptor stationary phase for binding determination using frontal affinity chromatography coupled to mass spectrometry. Anal Biochem 384(1):123–129

Tyler WA, Jain MR, Cifelli SE, Li Q, Ku L, Feng Y, Li H, Wood TL (2011) Proteomic identification of novel targets regulated by the mammalian target of rapamycin pathway during oligodendrocyte differentiation. Glia 59(11):1754–1769

Uhlenbrock K, Gassenhuber H, Kostenis E (2002) Sphingosine 1-phosphate is a ligand of the human gpr3, gpr6 and gpr12 family of constitutively active G protein-coupled receptors. Cell Signal 14(11):941–953

Vigano F, Schneider S, Cimino M, Bonfanti E, Gelosa P, Sironi L, Abbracchio MP, Dimou L (2016) GPR17 expressing NG2-Glia: oligodendrocyte progenitors serving as a reserve pool after injury. Glia 64 (2):287–299

Wang L, Du C, Lv J, Wei W, Cui Y, Xie X (2011) Antiasthmatic drugs targeting the cysteinyl leukotriene receptor 1 alleviate central nervous system inflammatory cell infiltration and pathogenesis of experimental autoimmune encephalomyelitis. J Immunol 187(5):2336–2345

Wheatley M, Wootten D, Conner MT, Simms J, Kendrick R, Logan RT, Poyner DR, Barwell J (2012) Lifting the lid on GPCRs: the role of extracellular loops. Br J Pharmacol 165(6):1688–1703

Whitney LW, Ludwin SK, McFarland HF, Biddison WE (2001) Microarray analysis of gene expression in multiple sclerosis and EAE identifies 5-lipoxygenase as a component of inflammatory lesions. J Neuroimmunol 121(1–2):40–48

Wu B, Chien EY, Mol CD, Fenalti G, Liu W, Katritch V, Abagyan R, Brooun A, Wells P, Bi FC, Hamel DJ, Kuhn P, Handel TM, Cherezov V, Stevens RC (2010) Structures of the CXCR4 chemokine GPCR with small-molecule and cyclic peptide antagonists. Science 330(6007):1066–1071

Ye F, Chen Y, Hoang T, Montgomery RL, Zhao XH, Bu H, Hu T, Taketo MM, van Es JH, Clevers H, Hsieh J, Bassel-Duby R, Olson EN, Lu QR (2009) HDAC1 and HDAC2 regulate oligodendrocyte differentiation by disrupting the beta-catenin-TCF interaction. Nat Neurosci 12(7):829–838

Yu GL, Wei EQ, Wang ML, Zhang WP, Zhang SH, Weng JQ, Chu LS, Fang SH, Zhou Y, Chen Z, Zhang Q, Zhang LH (2005a) Pranlukast, a cysteinyl leukotriene receptor-1 antagonist, protects against chronic ischemic brain injury and inhibits the glial scar formation in mice. Brain Res 1053(1–2):116–125

Yu GL, Wei EQ, Zhang SH, Xu HM, Chu LS, Zhang WP, Zhang Q, Chen Z, Mei RH, Zhao MH (2005b) Montelukast, a cysteinyl leukotriene receptor-1 antagonist, dose- and time-dependently protects against focal cerebral ischemia in mice. Pharmacology 73 (1):31–40

Zhang K, Zhang J, Gao ZG, Zhang D, Zhu L, Han GW, Moss SM, Paoletta S, Kiselev E, Lu W, Fenalti G, Zhang W, Muller CE, Yang H, Jiang H, Cherezov V, Katritch V, Jacobson KA, Stevens RC, Wu B, Zhao Q (2014) Structure of the human P2Y12 receptor in complex with an antithrombotic drug. Nature 509 (7498):115–118

Zhang D, Gao ZG, Zhang K, Kiselev E, Crane S, Wang J, Paoletta S, Yi C, Ma L, Zhang W, Han GW, Liu H, Cherezov V, Katritch V, Jiang H, Stevens RC, Jacobson KA, Zhao Q, Wu B (2015) Two disparate ligand-binding sites in the human P2Y1 receptor. Nature 520(7547):317–321

Zhao B, Zhao CZ, Zhang XY, Huang XQ, Shi WZ, Fang SH, Lu YB, Zhang WP, Xia Q, Wei EQ (2012) The new P2Y-like receptor G protein-coupled receptor 17 mediates acute neuronal injury and late microgliosis after focal cerebral ischemia in rats. Neuroscience 202:42–57

Author Queries

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