PHYLOGENETIC ANALYSIS OF LIPID MEDIATOR GPCRS

SAYAKA MIZUTANI[1] smizutan@kuicr.kyoto-u.ac.jp
MICHIHIRO TANAKA[1] mtanaka@kuicr.kyoto-u.ac.jp
CRAIG E. WHEELOCK[2] craig.wheelock@ki.se
MINORU KANEHISA[1,3] kanehisa@kuicr.kyoto-u.ac.jp
SUSUMU GOTO[1] goto@kuicr.kyoto-u.ac.jp

[1] *Bioinformatics Center, Institute for Chemical Research, Kyoto University, Gokasho, Uji, Kyoto 611-0021, Japan*
[2] *Department of Medical Biochemistry and Biophysics, Division of Physical Chemistry II, Karolinska Institutet, Stockholm, 171-77, Sweden*
[3] *Human Genome Center, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan*

Lipid mediators is the collective term for prostanoids, leukotrienes, lysophospholipids, platelet-activating factor, endocannabinoids and other bioactive lipids, that are involved in various physiological functions including inflammation, immune regulation and cellular development. They act autocrinically and paracrinically by binding to their ligand-specific G-protein coupled receptors (GPCRs). Since 1990's a number of lipid GPCRs have been cloned in humans, with a few more identified in other vertebrates. However, the conservation of these receptors has been poorly investigated in other eukaryotes. Herein we performed a phylogenetic analysis by collecting their orthologs in 13 eukaryotes with complete genomes. The analysis shows that orthologs for prostaglandin receptors are likely to be conserved in the 13 eukaryotes. In contrast, those for lysophospholipid and cannabinoid receptors appear to be conserved in only vertebrates and chordates. Receptors for leukotrienes and other bioactive lipids are limited to vertebrates. These results indicate that the lipid mediators and their receptors have coevolved with the development of highly modulated physiological functions such as immune regulation and the formation of the central nervous system. Accordingly, examining the presence and role of lipid mediator GPCR orthologs in invertebrate species can provide insight into the development of fundamental biological processes across diverse taxa.

Keywords: lipid mediators, eicosanoids, GPCRs, phylogenetic analysis, eukaryotes, invertebrates

1. Introduction

Lipid mediators is a collective term for prostanoids, leukotrienes (LTs), lysophospholipids, platelet-activating factor (PAF), endocannabinoids (CB), and other bioactive lipids. These mediators have a range of biological activities and are important in multiple fundamental biological processes including reproduction, signaling processes, immune function and disease etiology and pathogenesis to name a few. Lipid mediators are synthesized from precursor membrane lipids (*e.g.,* arachidonic acid) and have relatively short half-lives, which limits their signaling functions to be autocrinic and paracrinic. These mediators act on Class I G protein-coupled receptors (GPCRs) [18].

Eicosanoids are a major class of lipid mediators that are the oxygenated products of arachidonic acid [17, 5]. Following its release from membrane phospholipids, arachidonic acid can be modified by 3 distinct enzymatic pathways including cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (P450) pathways. Eicosanoids are further divided into subgroups based upon these pathways, with prostaglandins (PGs) being synthesized via COX-dependent pathways in combination with terminal synthases and LTs being synthesized via LOX-dependent pathways. The different eicosanoids bind to distinct GPCRs that demonstrate receptor-specific binding affinities. There are currently 5 known prostanoid receptors: EP FP, DP, IP and TP, which preferentially respond to PGE_2, $PGF2\alpha$, PGD_2 and PGI_2 and thromboxane A_2 (TXA_2), respectively. The EP group is further divided into 4 subtypes termed EP1-4. The physiological effects of each PG have been well-studied in mammals [5]. PGE_2 and PGI_2 induce several inflammatory responses including fever, pain, and vasodilatation.. $PGF2\alpha$ and PGE_2 have essential roles in reproduction including oocyte maturation and uterine contraction in mammals [22]. TXA_2 acts in platelet aggregation.

To date, four different LT receptors have been identified: cysteinyl leukotriene receptor 1 and 2 (CysLT1/2) and leukotriene B4 (LTB_4) receptor 1 and 2 (BLT1/2). The primary ligands for CysLT1/2 are the glutathione peptide conjugated LTs (LTC_4, LTD_4 and LTE_4) and the main ligand for the BLT1/2 is LTB_4. However, recently, a COX-catalyzed product 12-HHT (12S-hydroxy-5Z,8E,10E-heptadecatrienoic acid) was found to be an endogenous ligand of BLT2 [13]. Leukotriene receptors are highly expressed in neutrophils and eosinophils, and are involved in the chemotasis of these cells to the inflamed site. Recently it has been elucidated that they are also expressed in immune cells, regulating immune responses [24]. Lysophopholipids are structurally categorized into lysophosphatidic acids (LPAs) and sphingosine 1-phosphates (S1Ps), both of which generate a large number of species depending on the *sn-1* and *sn-2* positions of the lipid architecture and the positions and the number of double bonds in the acyl chains. Recently 6 distinct receptors for LPAs, LPA1-6, and five for S1Ps, S1P1-5, have been identified. Lysophopholipids are involved in the regulation of cellular responses including cell proliferation, differentiation, migration, adhesion and morphogenesis. Several pathological studies have revealed their essential roles in the formation of the central nervous system, angiogenesis and the regulation of vascular contraction [2, 11, 19]. S1Ps are also involved in the regulation of lymphocyte migration [14]. Finally, two CB receptors, CB1 and CB2, have been identified; CB1 is expressed mainly in the central nervous system and CB2 in immune cells [18].

Most GPCR-based studies to date have been mostly limited to humans and rodents. Accordingly, the conservation of these receptor genes and their functions are poorly understood in other eukaryotes. To address this point, we performed a phylogenetic analysis to investigate the variation of lipid GPCRs in 13 eukaryotic organisms in an attempt to correlate the emergence of lipid signaling functions to the development of physiological activities in which they are involved.

2. Materials and Methods

2.1. *Obtaining experimentally characterized lipid mediator GPCRs*

46 amino acid sequences (26 in *H. sapiens*, 10 in *M. musculus*, seven in *R. norvegicus*, two in *X .laevis* and one in *D. rerio*) of experimentally annotated lipid mediator GPCRs were obtained from the UniProt database [25]. The UniProt IDs and annotations of the query sequences are listed in Table 1.

2.2. *BLAST search for lipid mediator GPCR candidates*

Using the query sequences we performed a BLAST search [1] for 16 eukaryotic organisms with complete genomes, *H. sapiens* (hsa), *M. musculus* (mmu), *R. norvegicus* (rno), *X. laevis* (xla), *D. rerio* (dre), *C. intestinalis* (cin), *B. floridae* (bfo), *S. purpuratus* (spu), *D. melanogaster* (dme), *C. elegans* (cel), *B. malayi* (bmy), *N. vectensis* (nve), *T. adhaerens* (tad), *A. thaliana* (ath), *S. cerevisiae* (sce) and *M. brevicollis* (mbr). Amino acid sequences of the 16 organisms were downloaded from the KEGG GENES database [9]. Since a large part of the search results consisted of olfactory receptor sequences, each of the 46 search results was given an E-value optimally small enough to remove all known olfactory receptors, based on an assumption that candidate lipid GPCRs have higher degrees of similarity with the queries than olfactory receptors.

2.3. *Identifying ortholog candidates using phylogenetic trees*

For each of the organisms the refined search results were merged with the 46 lipid query sequences. The merged sequences were used to perform a multiple sequence alignment with the program E-INS-I in MAFFT version 5.8 [10] and to construct a rooted phylogenetic tree using the neighbor-joining (NJ) method [16] using the program QuickTree [7] with bootstrap values. Nine human olfactory receptor sequences were used as an outgroup in order to identify the root of the tree. In order to identify ortholog candidates, query sequences were grouped into seven classes, prostanoid receptors (PG), leukotriene receptors (LT), fatty acid receptors (FA), lysophospholipid receptors (LPA & S1P), cannabinoid receptors (CB), platelet-activating factor receptors (PAF), bile acid receptors (BA) and non-lipid GPCRs. In this process subsets that belong to the same class, such as receptors for PGE_2 and $PGF2\alpha$ in the prostanoid receptor class (PG), were not distinguished from each other. Since the BLAST search results contained many non-lipid GPCRs whose similarities are higher than olfactory receptors, we also considered 16 known human non-lipid receptors, which were also in the BLAST hits, so that the annotated part of the search results were increased. On the constructed trees ortholog candidates of the query sequences were manually extracted by the procedures (i) and (ii).

(i) For each class of queries, define ortholog cluster(s) via the following two steps;

Step 1. For each query, if the nearest query node(s) belongs to the same class, all the internal candidates are considered as orthologs.

Step 2. Repeat Step 1 until no such nearest queries are found.

(ii) To expand each ortholog cluster defined in (i), candidates in the sibling cluster are tested for validity by BLAST search against the human genome; if queries in the defined ortholog cluster are listed as the top hits, the candidates are considered as orthologs.

Fig. 1 shows the overall flow chart of the methods described in the sections 2.1-2.3.

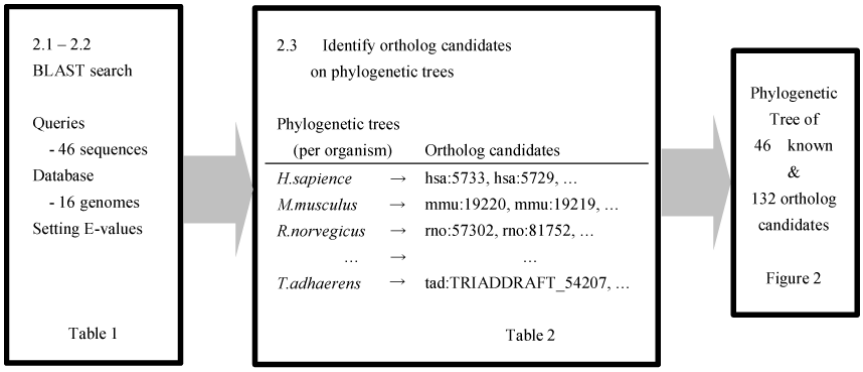

Figure 1. Flow chart of the overall method.

3. Results

3.1. *BLAST search results*

The total of 3134 hits was obtained from the BLAST search results with selective E-values. The E-values and the number of BLAST hits for each query are listed in the following table below.

Table 1. The number of BLAST hits for 46 query sequences with selective E-values

Uniprot ID	KEGG ID[a]	Uniprot annotation	E-val[b]	# of BLAST hits	Pubmed ID
Prostanoid receptors					
P34995	hsa:5731	Prostaglandin E2 receptor EP1 subtype	-	38	8253813
P35375	mmu:19216	Prostaglandin E2 receptor EP1 subtype	-	41	7690750
P43116	hsa:5732	Prostaglandin E2 receptor EP2 subtype	-	65	8078484
Q62053	mmu:19217	Prostaglandin E2 receptor EP2 subtype	-	42	7556658
P35408	hsa:5734	Prostaglandin E2 receptor EP4 subtype	10^{-1}	829	8163486

Table 1 cont'd

Prostanoid receptors					
P43114	rno:84023	Prostaglandin E2 receptor EP4 subtype	-	942	8185583
Q9R261	rno:498475	Prostaglandin D2 receptor	10^{-1}	81	10448933
P43118	rno:25652	Prostaglandin F2-alpha receptor	-	124	7972878
P43119	hsa:5739	Prostacyclin receptor	10^{-2}	73	7512962
P43252	mmu:19222	Prostacyclin receptor	10^{-2}	91	7511597
P43253	rno:292661	Prostacyclin receptor	10^{-2}	96	7803522
P21731	hsa:6915	Thromboxane A2 receptor	-	55	1825698
P30987	mmu:21390	Thromboxane A2 receptor	10^{-1}	45	1375456
P34978	rno:24816	Thromboxane A2 receptor	-	56	7635958
Leukotriene receptors					
Q15722	hsa:1241	Leukotriene B4 receptor 1	10^{-1}	1116	8702478
Q9NPC1	hsa:56413	Leukotriene B4 receptor 2	-	237	10913346
Q9Y271	hsa:10800	Cysteinyl leukotriene receptor 1	10^{-3}	1225	10462554
Q99JA4	mmu:58861	Cysteinyl leukotriene receptor 1	10^{-2}	1956	11705452
Q9NS75	hsa:57105	Cysteinyl leukotriene receptor 2	10^{-6}	923	11093801
Fatty acid receptors					
O14842	hsa:2864	Free fatty acid receptor 1	-	237	12496284
Q76JU9	mmu:233081	Free fatty acid receptor 1	10^{-2}	67	12629551
O15552	hsa:2867	Free fatty acid receptor 2	10^{-3}	418	12684041
Q8VCK6	mmu:233079	Free fatty acid receptor 2	10^{-2}	474	12684041
O14843	hsa:2865	Free fatty acid receptor 3	10^{-2}	868	12711604
Q8TDS5	hsa:165140	Oxoeicosanoid receptor 1	10^{-3}	589	12065583
Lysophospholipid receptors					
Q92633	hsa:1902	Lysophosphatidic acid receptor 1	10^{-7}	351	9070858
Q9PU17	xla:373591	Lysophosphatidic acid receptor 1	10^{-6}	295	11278944
Q9PU16	xla:379403	Lysophosphatidic acid receptor 1	10^{-6}	286	11278944
Q9HBW0	hsa:9170	Lysophosphatidic acid receptor 2	10^{-1}	611	15143197
Q9UBY5	hsa:23566	Lysophosphatidic acid receptor 3	10^{-3}	704	10488122
Q99677	hsa:2846	Lysophosphatidic acid receptor 4	10^{-6}	1331	12724320
P43657	hsa:10161	Lysophosphatidic acid receptor 6	10^{-4}	1375	18297070
P21453	hsa:1901	Sphingosine 1-phosphate receptor 1	10^{-6}	486	9488656
Q9DDK4	dre:64617	Sphingosine 1-phosphate receptor 1	10^{-3}	655	11112429
O95136	hsa:9294	Sphingosine 1-phosphate receptor 2	10^{-7}	279	10617617
Q99500	hsa:1903	Sphingosine 1-phosphate receptor 3	10^{-10}	166	10617617
O95977	hsa:8698	Sphingosine 1-phosphate receptor 4	10^{-1}	244	10753843
Q9JKM5	rno:60399	Sphingosine 1-phosphate receptor 5	-	252	10799507
Cannabinoid receptors					
P21554	hsa:1268	Cannabinoid receptor 1	10^{-7}	382	15620723
P47746	mmu:12801	Cannabinoid receptor 1	10^{-7}	372	8777318
P34972	hsa:1269	Cannabinoid receptor 2	10^{-6}	405	10051546
P47936	mmu:12802	Cannabinoid receptor 2	10^{-7}	264	8679694
	PAF receptors				
P25105	hsa:5724	Platelet-activating factor receptor	10^{-1}	1109	1656963
Q62035	mmu:19204	Platelet-activating factor receptor	10^{-1}	997	8670084
P46002	rno:58949	Platelet-activating factor receptor	10^{-2}	1249	8168510
Bile acid receptor					
Q8TDU6	hsa:151306	G-protein coupled bile acid receptor 1	-	8	12419312

[a] hsa: *H. sapiens*, mmu: *M. musculus*, rno: *R. norvegicus*, xla: *X. laevis*, dre: *D. rerio*, cin: *C. intestinalis*, bfo: *B. floridae*, spu: *S. purpuratus*, dme: *D. melanogaster*, cel: *C. elegans*, bmy: *B. malayi*, nve: *N. vectensis*, tad: *T. adhaerens*.

[b] Hyphens represents no E-values set.

3.2. *Frequency of candidate lipid mediator GPCRs in 13 eukaryotic genomes*

132 ortholog candidates were identified from the 13 constructed phylogenetic trees. No BLAST search hits were obtained for *A. thaliana*, *S. cerevisiae* and *M. brevicollis*. Table 2 shows the number of orthologs for each class of lipid mediator GPCR. Ortholog candidates for PG GPCRs were detected in all organisms except for nematodes. Those for LPA receptors and CB receptors were extracted in vertebrates and chordates. Ortholog candidates for other receptors including LT receptors and FA receptors were obtained only in vertebrates.

Table 2. Conservation of lipid mediator GPCRs.

Organism categories	Species	Prostanoid receptors	Leukotriene receptors	Fatty acid receptors	Lysophospholipid receptors	Cannabinoid receptors	PAF receptor	Bile acid receptor
Vertebrata	*H. sapiens* (hsa)	8 (5)	4 (4)	4 (4)	10 (9)	2 (2)	1 (1)	1 (1)
	M. musculus (mmu)	8 (4)	4 (1)	3 (2)	10	2 (2)	1(1)	0
	R. norvegicus (rno)	9 (5)	4	3	11 (1)	2	1 (1)	0
	X. laevis (xla)	4	0	0	4 (2)	2	1	0
	D. rerio (dre)	13	12	10	14 (1)	3	2	1
Chordata	*C. intestinalis* (cin)	2	0	0	2	1	0	0
	B. floridae (bfo)	11	0	0	0	1	0	0
Echinodermata	*S. purpuratus* (spu)	3	0	0	0	0	0	0
Arthropoda	*D. melanogaster* (dme)	1	0	0	0	0	0	0
Nematoda	*C. elegans* (cel)	0	0	0	0	0	0	0
	B. malayi (bmy)	0	0	0	0	0	0	0
Cnidaria	*N. vectensis* (nve)	1	0	0	0	0	0	0
Placozoa	*T. adhaerens* (tad)	2	0	0	0	0	0	0

Numbers include both the newly detected ortholog candidates and query sequences. Queries, if any, are indicated in parentheses.

3.3. *Proposed phylogenetic tree of query sequences and detected candidates in 13 eukaryotic organisms.*

We constructed a phylogenetic tree using the 46 query sequences and 132 newly detected ortholog candidates (Fig. 2). It mostly agreed with the classification of lipid GPCR

orthologs, which means that members of the same class had higher sequence similarities with each other than to members of other groups. Lysophospholipid receptors were separated into two groups: 1) LPA1-3 and S1P and 2) other LPA receptors. This distinct separation pattern has been observed in previous studies with phylogenetic trees of experimentally annotated human lipid GPCRs [18].

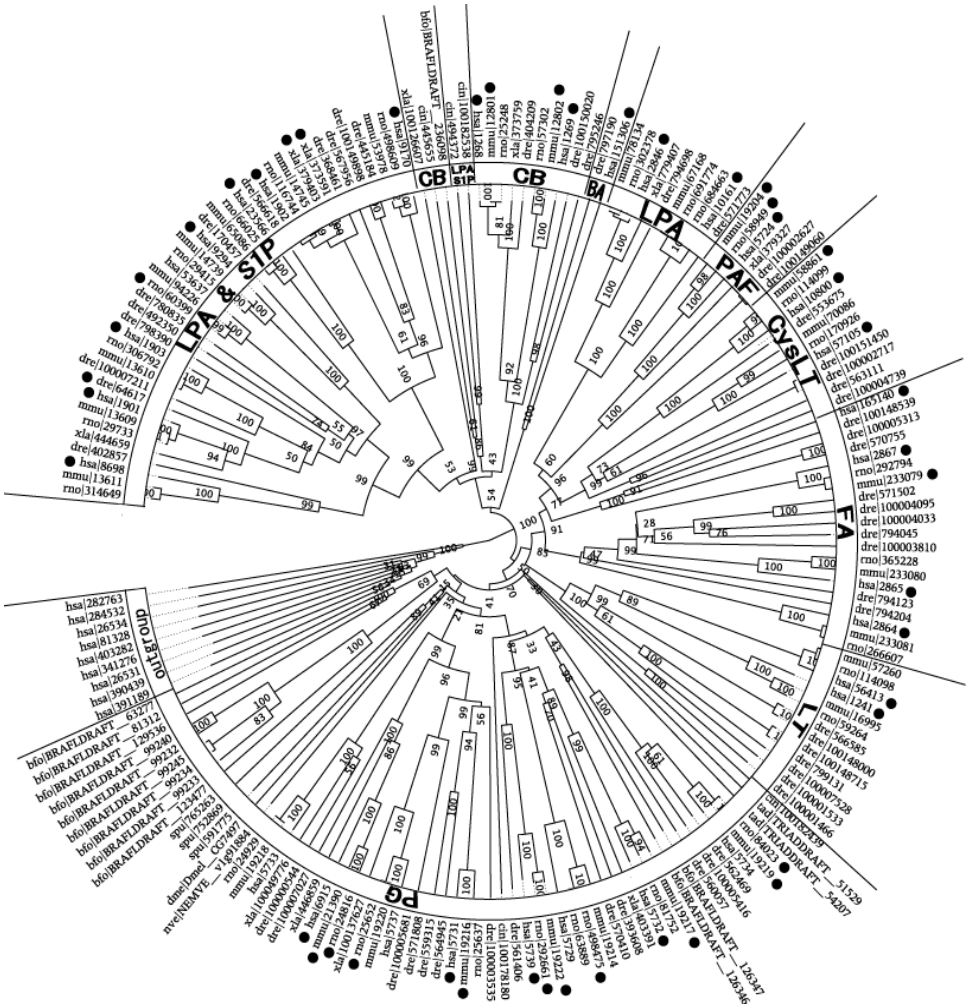

Figure 2. Proposed phylogenetic tree of lipid mediator GPCRs in 13 eukaryotes. Queries are indicated with solid circles. Sequences are represented by KEGG IDs using three letter organism codes used in the KEGG database (see Table 2). PG: prostanoid receptors, LT: leukotriene receptors, FA: fatty acid receptors, LPA: lysophosphatidic acid receptors, S1P: sphingosine 1-phosphate receptors, CB: cannabinoid receptors, PAF: platelet-activating factor receptor, BA: bile acid receptors.

4. Discussions

Table 2 shows an asymmetric conservation pattern of lipid mediator GPCRs in 13 eukaryotic organisms. Prostanoid receptors were conserved in all eukaryotes except for in nematodes. In contrast a limited conservation in other lipid mediator receptors was observed. Lysophosphospholipid and CB receptors appear to be conserved in vertebrates and chordates. Other receptors including LT, FA, PAF and BA receptors were only found in vertebrates. Herein we discuss this tendency in a functional aspect focusing on the asymmetric conservation of these mediator molecules in invertebrates.

4.1. *Conservation of prostanoid receptors in invertebrates*

Prostanoid receptors are conserved widely in the eukaryotic genomes. This conservation can be explained considering their roles in reproduction and fundamental immune systems in invertebrates. Synthesis and biological effects of prostanoids have been reported in *C. intestinalis* [8]. The detected candidates would be potential receptors for the *Ciona* prostanoid molecules. Generally the roles of lipid mediators are largely unknown in non-chordate invertebrates. However, there is a fair amount of research that supports the signaling functions of PGs and other eicosanoids in invertebrates. In insects detailed analyses have revealed that PGs and other eicosanoids play essential roles in egg laying and in cellular immunity against microbial invasions [20]. These compounds have also been shown to have a role in reproduction in non-insectan invertebrates [21]. However, most of the ortholog candidates detected in *C. intestinalis*, *B. floridae*, *S. purpuratus*, *D. melanogaster*, *N. vectensis* and *T. adhaerens* form independent clusters on the phylogenetic tree (Fig. 2). This may be due to the diversification of PGs in invertebrates. In fact PGs that have been found in invertebrates include invertebrate specific forms in addition to the classic vertebrate forms (PGE_2, PGD_2, etc.) [15]. It is remarkable that PG receptors were not found in *C. elegans*, a free living worm, and *B. malayi*, a parasitic worm. It has been reported that *B. malayi* releases PGs, which causes the inhibition of platelet aggregation in the host blood cells [12]. The absence of PG receptors in these organisms can be understood by their usage in the host cells, but not in their innate physiology. Collectively, these results suggest a hypothesis regarding the functional roles of PG receptors in invertebrates. However, in order to fully understand the conservation and evolution of PG and other eicosanoid functions, the variation in their biosynthetic pathways, in particular the genomic conservation of COX and LOX, should also be investigated.

4.2. *Leukotriene, Lysophospholipid and cannabinoid receptors in invertebrates*

Leukotriene receptors appear to be conserved only in vertebrates. In mammals the well-known function of LT receptors is the inflammation responses in leukocytes. Thus the LT signaling may have evolved with the development of leukocytes. Interestingly, although the origin of the leukocyte can be traced to chordates, no LT receptors were detected in their genomes. Lysophispholipid receptor orthologs were detected in vertebrates and in *C. intestinalis*, but not in other invertebrates. One of the two candidates detected in *C.*

intestinalis (hsa:494372 on Fig. 2) has been previously annotated as EDG-3-like protein [6, 23]. Cannabinoid receptor orthologs were also detected in the chordates. One ortholog (cin:445655) had previously been reported [4] with equal similarities to the mammalian CB1 and CB2. Interestingly, the one we detected in *B. floridae* was different from what has previously identified by other group [3]. In mammals, lysophospholipids are known to be involved in the formation of the central nervous system, angiogenesis and the regulation of the vascular and the immune systems. This information may collectively explain the coevolution of lysophospholipid and cannabinoid signaling functions with the development of highly modulated nervous, immune and vascular systems in vertebrates as the origin of these systems are thought to be traced back into Chordates.

4.3. *Possible improvements on our method*

Our method searches for sequences with higher similarities to the queries than to olfactory receptor sequences in each genome. This is based on the assumption that if orthologs of queries had been emerged in the common ancestor, their sequence similarities could be conserved with a higher degree to the query sequences than to other GPCRs in a genome. Although, this process successfully reduces the number of false positives in the BLAST search hits, which makes the following phylogenetic analyses much easier, it also limits the possibility of detecting candidate genes that may have been evolved independently of the queries. In addition, in the obtained BLAST search hits there were many sequences that remained unannotated especially in the invertebrates. Annotation of these sequences by known non-lipid GPCRs such as amine receptors and peptide receptors should help in characterizing these elements.

5. Conclusions

We performed a phylogenetic analysis to search for mammalian homologues of lipid mediator GPCRs. Our results show that orthologs for the PG receptors are conserved widely in all tested eukaryotic organisms except in nematodes. Lysophospholipid receptors and CB receptors are observed in vertebrates and chordates. Leukotriene receptors, PAF receptors and BA receptors appear to be vertebrate-specific. The eukaryotic conservation of PG receptors can be explained by their reproductive function, which has been demonstrated by previous investigations in insects and other invertebrates. The absence of other receptors in non-chordate invertebrates indicates that lipid signaling via GPCRs may have evolved with the development of highly modulated vascular, immune and nervous systems in vertebrates. These results can give new insight into our understanding of lipid signaling in invertebrates.

Acknowledgements

This research was partly funded by a joint Japan Society for the Promotion of Science (JSPS) and VINNOVA scientific co-operation grant. CEW was supported by a Centre for Allergy Research (CfA) research fellowship.

References

[1] Altschul, S.F. et al., Gapped BLAST and PSI-BLAST: a new generation of protein database search programs, *Nucleic Acids Res.*, 25:3389-3402, 1997.

[2] Contos, J.J. et al., Requirement for the lpA1 lysophosphatidic acid receptor gene in normal suckling behavior, *Proc. Natl. Acad. Sci.*, 97:13384-13389, 2000.

[3] Elphick, M.R., BfCBR: a cannabinoid receptor ortholog in the cephalochordate *Branchiostoma floridae* (Amphioxus), *Gene,* 399:65-71, 2007.

[4] Elphick, M.R., Satou, Y., Satoh, N., The invertebrate ancestry of endocannabinoid signaling: an orthologue of vertebrate cannabinoid receptors in the urochordate *Ciona intestinalis*, *Gene,* 302:95-101, 2003.

[5] Funk, C.D., Prostaglandins and leukotrienes: advances in eicosanoid biology, *Science*, 294:1871-1875, 2001.

[6] Hla, T., Genomic insights into mediator lipidomics, *Prostaglandins & other Lipid Mediators*, 77:197-209, 2005.

[7] Howe K, Bateman A, Durbin R, QuickTree: building huge Neighbour-Joining trees of protein sequences, *Bioinformatics,* 18:1546-1547, 2002.

[8] Jarving, R, On the evolutionary origin of cyclooxygenase (COX) isozymes: characterization of marine invertebrate *COX* genes points to independent duplication events in vertebrate and invertebrate lineages, *J. Biol. Chem.,* 279:13624-13633, 2004.

[9] Kanehisa, M et al., KEGG for linking genomes to life and the environment. *Nucleic Acids Res.,* 36:D480-D484, 2008.

[10] Katoh, K., Toh, H., Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics,* 9:286-298, 2008.

[11] Kingsbury, MA et al., Non-proliferative effects of lysophosphatidic acid enhance cortical growth and folding, *Nature Neurosci.,* 6:1292-1299, 2003.

[12] Liu L.X. and Weller P.F., Intravascular Filarial parasites inhibit platelet aggregation. Role of parasite-derived prostanoids, *J. Clin. Invest.*, 89:1113-1120, 1992.

[13] Okuno, T. et al., 12(S)-hydroxyheptadeca-5Z, 8E, 10E–trienoic acid is a natural ligand for leukotriene B4 receptor 2, *J Exp Med,* 205:759-766, 2008

[14] Rosen, H and Goetzl, E, Sphingosine 1-phosphate and its receptors: an autocrine and paracrine network, *Nature Rev. Immunol.,* 5:560-570, 2005.

[15] Rowley, A.F. et al., Prostaglandin in non-insectan invertebrates: recent insights ad unsolved problems, *J.Exp.Biol.,* 208:3-14, 2005.

[16] Saitou N and Nei M, The neighbor-joining method: a new method for reconstructing phylogenetic trees, *Mol. Biol. Evol.* 4:406-425, 1987.
[17] Samuelsson, B et al., Leukotrienes and lipoxins: structures, biosynthesis, and biological effects, *Science* 237:1171-1176, 1987.
[18] Shimizu, T., Lipid mediators in health and disease: enzymes and receptors as therapeutic targets for the regulation of immunity and inflammation, *Annu. Rev. Pharmacol. Toxicol*, 49:123–150, 2009.
[19] Spiegel, S and Milstien, S, Sphingosine-1-phosphate: an enigmatic signaling lipid, *Nature Rev. Mol. Cell. Biol.,* 4:397-407, 2003.
[20] Stanley, D., Prostaglandins and other eicosanoids in insects: biological significance, *Annu. Rev. Entomol.,* 51:25-44, 2006.
[21] Stanley, D.W. and Miller, J.S., Eicosanoids in animals reproduction: what can we learn from invertebrates? In *Eicosanoids and Related Compounds in Plants and Animals* (ed. Rowley, A.F., Kuhn, H and Schewe, T), pp. 183-196, 1998.
[22] Tanaka, C and Kato R, *New Pharmacology*, 4th edition, Nankodo, Japan, 2002.
[23] Terajima, D, Identification and sequence of seventy-nine new transcripts expressed in hemocytes of *Ciona intestinalis*, three of which may be involved in characteristic cell-cell communication, *DNA research,* 10:203-212, 2003.
[24] Terawaki, K. et al., Absence of leukotriene B4 receptor 1 confers resistance to airway hyperresponsiveness and Th2-type immune responses, *J. Immunol.* 175:4217-4225, 2005.
[25] The UniProt Consortium, The Universal Protein Resource (UniProt) in 2010, *Nucleic Acids Research* 38:D142-D148, 2010.