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APPROACHES TO G-PROTEIN COUPLED RECEPTORS DEORPHANIZATION: THE GPR32 CASE STUDY

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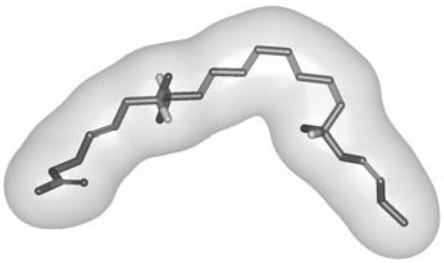
The D-series resolvins are known as endogenous lipid mediators biosynthesized from docosahexaenoic acid, and play an important role in numerous pharmacological actions like limiting the neutrophil migration, increasing the resolution rate of the acute inflammation, restoration of tissue homeostasis, etc. Recently, Resolvin D1 was proposed to interact to an orphan G-protein coupled receptor (GPCR) – GPR32, being a potential candidate for the natural ligand of this receptor. This new findings are analyzed and discussed in the present work by comparing the protein distances and similarities between GPR32 and characterized GPCRs using computational chemistry techniques based on phylogeny, amino acid conservation and diversity, ligand similarity, etc. Small variation regarding the topology of the phylogenetic trees were noticed when receptors were cluster based on sequence or sequence fragments. In contrast, ligand similarities induce considerable changes in phylogenetic topologies and protein distances but despite this aspect, FPR2 receptor still shows the strongest relatedness with GPR32 orphan.



INTRODUCTION

G protein coupled receptors (GPCRs) are a large protein family of receptors well known for their important role in signal transmission to the cell and for their structural architecture characterized by seven *alpha*-helices connected by three intracellular (IL-1 to IL-3) and three extracellular loops (XL-1 to XL-3). Despite the fact that GPCRs are the most studied biological targets due to their relevance for drug discovery process, for about 100 receptors the natural ligand and function are still unknown. These receptors are known as orphan GPCRs (oGPCRs).

G protein-coupled receptor 32, known as GPR32, is a human gene which encodes an orphan member of the class A GPCRs.[1] Basic information about its pharmacological function or about its endogenous or other active ligands was missing until recently,[2,3] when resolvin D1 was reported to bind at the active site of GPR32. This seemed to be one important step in the deorphanization process of GPR32, since pairing of an orphan GPCR with a potential ligand remains a reasonable solution to elucidate the regulation mechanism of cellular signaling and biological significance for the receptor. However, the same compound, resolving D1, could not be confirmed as active against GPR32 receptor in a high-throughput screening assay which aimed to identify the natural ligands for a set of 82 oGPCRs,[4] putting on hold the GPR32 deorphanization.

In this work, we have investigated the relatedness between GPR32 orphan receptor and human characterized class A GPCRs using several computational chemistry techniques based on phylogeny, amino acid conservation, and ligands similarity in order to identify potential new relatives for orphan GPR32.

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METHODS

Sequence alignment

The sequences of GPR32 and 194 human characterized class A GPCRs have been automatically downloaded in Fasta format from Uniprot database.[5] The sequence alignment was performed with the T-Coffee package[6] using a multistep alignment procedure. Characterized receptors of the same sub-family were aligned separately and each resulting alignment was manually refined in order to preserve the highly-conserved amino acid motifs specific for each transmembrane domain[7] and to avoid deletions or insertions in the *alpha*-helices. Next, the alignments for each sub-family were merged successively until all the class A characterized GPCRs were included. In the final step, the sequence of GPR32 was added to the final alignment and the output was refined manually.

Phylogenetic trees generation

The degree of relatedness between GPR32 and class A GPCRs was evaluated by clustering sequences based on the resulted alignments when criteria regarding the sequence similarity, conservation, diversity, binding site location and ligands' 2D similarity were applied. Sequence clustering was performed with Neighbor-Joining method from ClustalX[8] software and was visualized as circular trees by using the iTOL software package.[9,10]

Ligands setup

Ligands active on characterized GPCRs and orphan GPR32 were downloaded from IUPHAR database,[11] which is publicly available database containing pharmacological information manually curated regarding a large variety of drug targets. Ligands were subjected to several filter criteria which referred mainly to the ligand type, molecular weight and unique compound per each receptor. Therefore the final set of compounds contained a number of 1674 agonists, non-peptide molecules-type. Additionally, duplicates molecules and salts were eliminated. Ligands' similarity was estimated using the Extended-Connectivity Fingerprint (ECFP) method.[12]

RESULTS AND DISCUSSIONS

The sequence similarity and protein distances between orphan GPR32 receptor and characterized class A GPCRs were estimated using six criteria defined below:

C1: sequence similarity between GPR32 and characterized class A GPCRs using the complete amino acid sequences;

C2: sequence similarity between GPR32 and characterized class A GPCRs using only the fragments containing the seven *alpha*-helices and the second extracellular loop (XL2) which covers the binding site;

C3: binding site similarity between GPR32 and characterized class A GPCRs using the Gloriam's classification;[13]

C4: sequence similarity of fragments containing the amino acids conserved in class A GPCR;[7]

C5: sequence similarity of fragments containing the amino acids non-conserved in class A GPCR;

C6: 2D similarity between ligands reported to be active on GPR32 and active ligands on characterized class A GPCR.

Formyl-peptide receptors (FPRs) are the closest homologues of orphan GPR32 when C1 criterion was used. GPR32 shows a sequence similarity around 56% and a sequence identity between 30-34% with FPRs family. The results are in agreement with data reported previously.[1] Others receptors which showed significant sequence similarity (more than 40%) with GPCR were members of chemokine, opioid or prostaglandins families (Fig. 1).

For an easier visualization, in Fig. 1, branches of hormone receptors (a) and aminergic, melatonin, melanocortin, cannabinoid and S1Ps (b) were clashed because of their reduced similarity to GPR32. For the same reasons, the branch lengths were ignored and only the topological information is shown.

The second criterion, C2, does not provide any supplementary information as the similarity of GPR32 with class A GPCRs increased with an average of 20% for each characterized receptor and therefore the topology between GPR32 and characterized class A GPCR is similar for C1 and C2 criteria (Fig. 2A). When only fragments containing the binding site were considered, sequence similarities with GPR32 ranges approximately in the same interval as in the case of C1 criterion with the specification that in some cases the similarity (or dissimilarity) with GPCR32 are accentuated. For example, the FPR receptors are still the closest homologues for orphan GPR32 also when binding site similarity was evaluated, but the sequence similarity and implicitly the relatedness degree are higher. Similar observations were obtained when protein distances and phylogenetic trees were generated according to C1-C3 criteria.

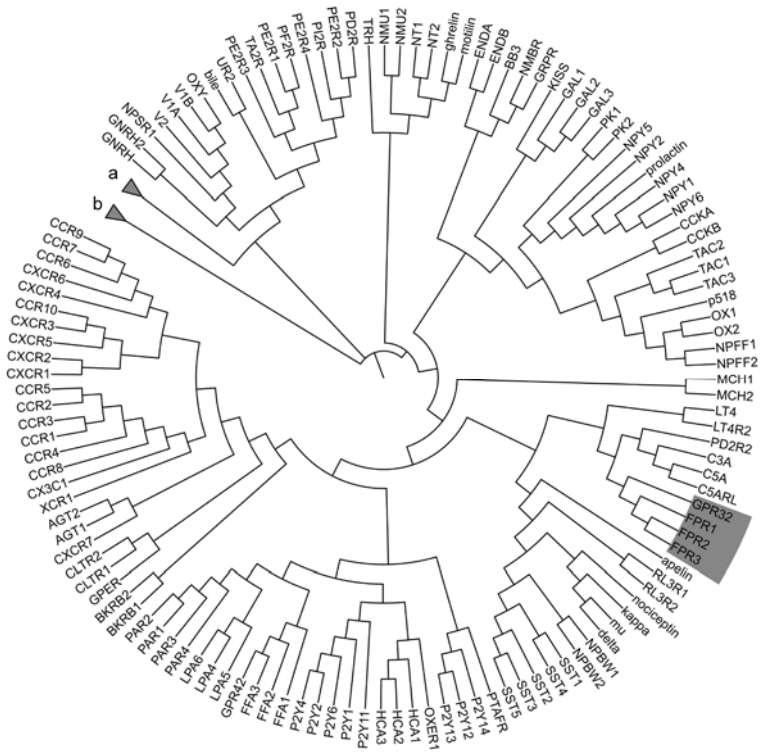

Fig. 1 – Phylogenetic tree of human class A characterized GPCRs and orphan GPR32 receptor based on sequence alignment. GPR32 and its closest homologues, FPRs, are highlighted in gray.

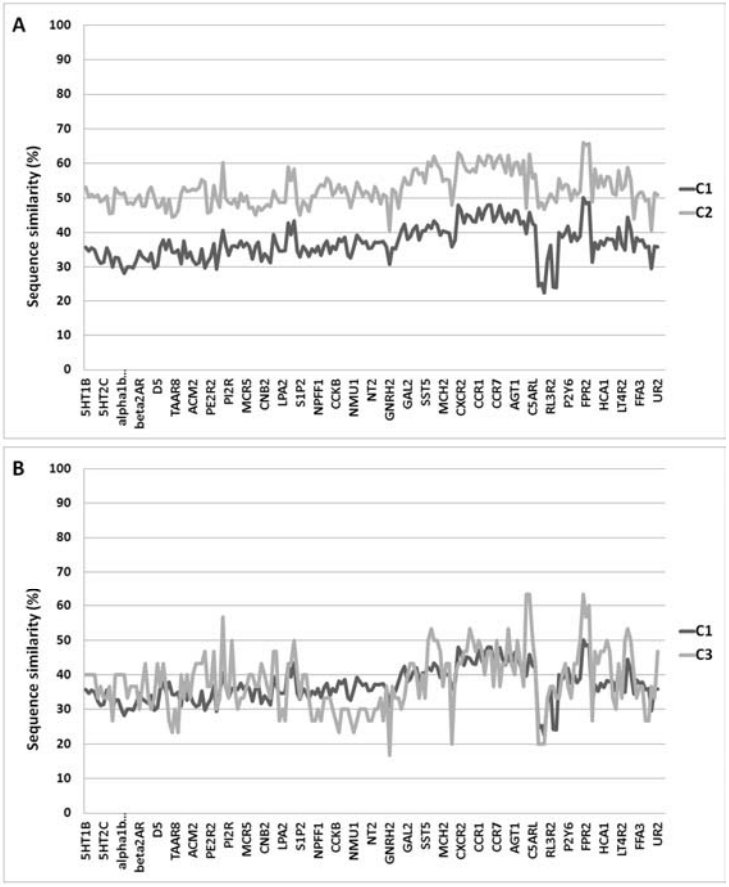

Fig. 2 – Sequence similarity (%) between orphan GPR32
and characterized class A GPCRs according to transmembrane fragments and XL2 (A) and binding site fragments (B).

Neither amino acid conservation or diversity did not induce considerably changes between the closer homologues of GPR32. Although the tree topology is modified, the interval containing the protein distances is similar. As expected, the fragments containing only the class A conserved amino acids[7] showed a high similarity between aligned fragments and slight modification in topology of the closer homologues of GPR32 characterized class A receptors.

A number of 1674 agonists of 134 class A characterized GPCRs has been selected from IUPHAR[11] database to evaluate the relationship degree with orphan GPR32. For GPR32, three active ligands previously reported as agonists[2,3] (Fig. 3) have been used as query structures.

Clustering based on the 2D similarity of the active agonists determined significant changes in the overall association of the receptors. However, FPR2 receptor is the closest relative of GPR32 receptor even when active ligands were used to establish the homologues found in the immediate neighborhood of GPR32 in the protein space (Fig. 4).

Other receptors which share ligands with similar chemical core with GPR32 are free fatty acid and prostanoid receptors. The first 10 relatives according to sequence and ligand similarity are presented in Table 1.

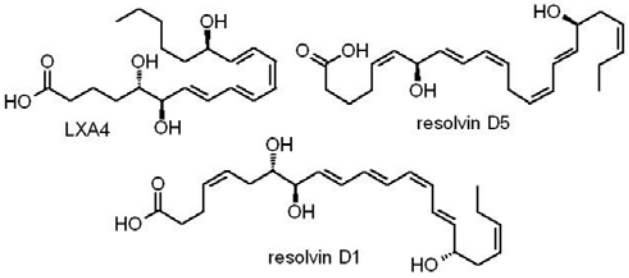

Fig. 3 – Chemical structures of active GPR32 agonists.

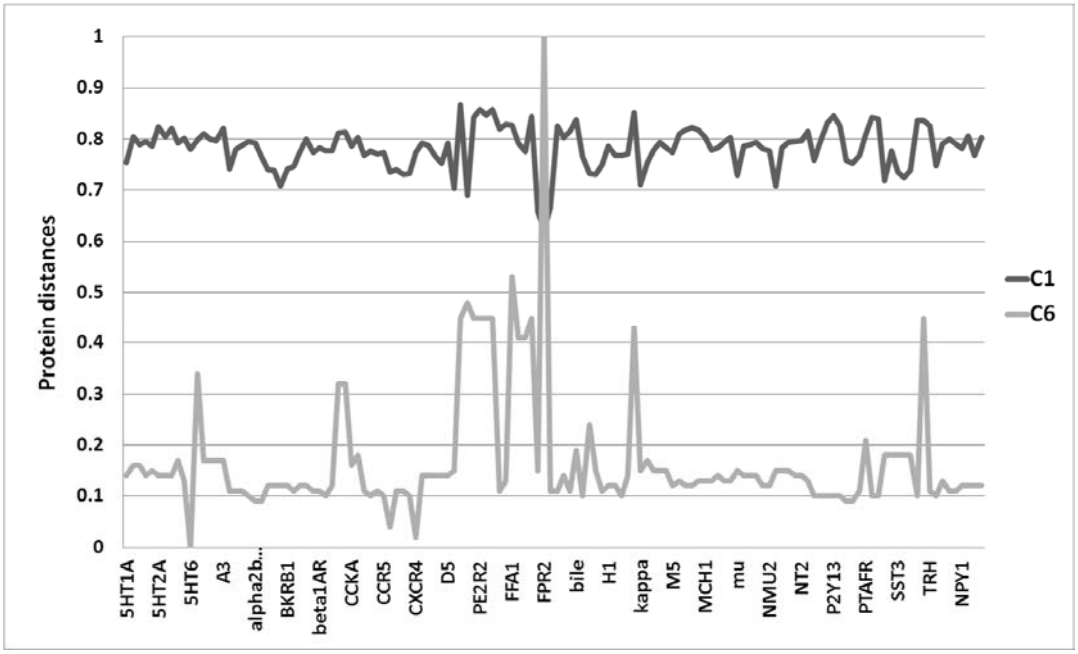

Fig. 4 – Protein distance variation between GPR32 and characterized class A receptors according to ligands' similarities.

Table 1

The closest homologues of orphan GPR32 receptor resulted from sequence clusterization (C1) and ligands similarities (C6)

C1		C6	
Receptor	Family	Receptor	Family
FPR2	Formyl-peptide receptors	FPR2	Formyl-peptide receptors
FPR1	Formyl-peptide receptors	FFA1	Free fatty acid receptors
FPR3	Formyl-peptide receptors	PD2R2	Prostanoid receptor

Table 1 (continued)

PD2R2	Prostanoid receptor	TA2R	Prostanoid receptor
C3A	Complement peptide receptor	PE2R1	Prostanoid receptor
delta	Opioid receptor	PF2R	Prostanoid receptor
PAR4	Protease-activated receptors	PE2R3	Prostanoid receptor
nociceptin	Opioid receptor	PE2R2	Prostanoid receptor
AGT2	Angiotensin receptors	PE2R4	Prostanoid receptor
C5A	Complement peptide receptor	PD2R	Prostanoid receptor

CONCLUSIONS

Formyl peptide receptors and FPR2 especially, proved to be the closest relative of orphan GPR32 when sequence and ligand similarities were analyzed. The overall architecture of the phylogenetic trees generated based on sequence or sequence fragments is similar and only small variations regarding the receptors topology in the phylogenetic trees are noticed, while the protein distances are slightly modified. Ligands similarities estimated at two dimensional level determine considerable changes in phylogenetic topologies and protein distances. Even so, FPR2 receptor shows the strongest relatedness with GPR32 orphan by all used criteria.

REFERENCES

1. A. Marchese, T. Nguyen, P. Malik, S. Xu, R. Cheng, Z. Xie, H.H. Heng, S.R. George, L.F. Kolakowski Jr, and B.F. O'Dowd, *Genomics*, **1998**, *50*, 281-6.
2. N. Chiang, G. Fredman, F. Bäckhed, S.F. Oh, T. Vickery, B.A. Schmidt and C.N. Serhan, *Nature*, **2012**, *484* 524-528.
3. S. Krishnamoorthy, A. Recchiuti, N. Chiang, S. Yacoubian, C.H. Lee, R. Yang, N.A. Petasis and C.N. Serhan. *Proc. Natl. Acad. Sci. U.S.A*., **2010**, *107*, 1660-1665.
4. C. Southern, J.M. Cook, Z. Neetoo-Isseljee, D.L. Taylor, C.A. Kettleborough, A. Merritt, D.L. Bassoni, W.J.Raab, E. Quinn, T.S. Wehrman, A.P. Davenport, A.J. Brown, A. Green, M.J. Wigglesworth, S. Rees, *J Biomol Screen*, **2013**, *18*, 599-609.
5. The UniProt Consortium. *Nucleic Acids Res*., **2012**, *40*, D71-D75.
6. C. Notredame, D.G. Higgins and J. Heringa, *J. Mol. Biol.*, **2000**, *302*, 205-217.
7. J.M. Baldwin, G.F. Schertler,V.M. Unger, *J. Mol. Biol.*, **1997**, *272*, 144-164.
8. M.A. Larkin, G. Blackshields, N.P. Brown, R. Chenna,, P.A. McGettigan, H. McWilliam, F. Valentin, I.M. Wallace, A. Wilm, R. Lopez, J.D. Thompson, T.J. Gibson and D.G. Higgins, *Bioinformatics,* **2007**, *23*, 2947-2948.
9. I. Letunic and P. Bork, *Bioinformatics*, **2006**, *23*, 127-128.
10. I. Letunic and P. Bork, *Nucleic Acids Res*., **2011**, doi: 10.1093/nar/gkr201.
11. A.J. Harmar, R. Hills, E.M. Rosser, M. Jones, O.P. Buneman, D.R. Dunbar, S.D Greenhill, V.A. Hale, J.L. Sharman, T.I. Bonner, W.A. Catterall, A.P. Davenport AP, P. Delagrange, C.T. Dollery, S.M. Foord,G.A Gutman, V. Laudet, R.R. Neubig, E.H. Ohlstein, R.W. Olsen, J. Peters, J.P. Pin, R.R. Ruffolo, D.B. Searls, M.W. Wright and M. Spedding, *Nucleic Acids Res*., **2009**, *37*, D680–5.
12. SIM2D-Translational Informatics Web Apps; http://pasilla.health.unm.edu/tomcat/biocomp/sim2d.
13. D.E. Gloriam, S.M. Foord, F.E Blaney, S.L. Garland, *J. Med. Chem.,* **2009**, *52*, 4429-4442.