Significance of G-X-W motif in the myocilin olfactomedin domain

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Introduction

Myocilin, trabecular meshwork inducible glucocorticoid response, also known as MYOC, is encoded by the MYOC gene in humans. The myocilin protein is a secreted 55–57-kDa glycoprotein whose known structural features include a myosin-like domain, a leucine zipper region, and an olfactomedin domain [1]. Most of the diseases causing mutations lie on the olfactomedin domain, which is highly conserved among species. This suggests that the domain may play an important role in myocilin function. The olfactomedin domain was first identified in extracellular matrix protein of the olfactory neuroepithelium [2]. A number of metazoans are known to have this extracellular protein domain. Some of the olfactomedin containing metazoan proteins are latrophilins, myocilins, optimedins, and noelins [3].

The mouse and human genomes encode at least 12 olfactomedin-related gene products. These proteins have a variable N-terminal domain and a conserved olfactomedin C-terminal domain [4]. The initial member of the olfactomedin family was identified as a secreted glycoprotein in the bullfrog olfactory neuroepithelium [2]. Olfactomedin

family members are expressed in many tissues and exhibit tissue-specific expression patterns [5]. Several olfactomedin proteins are expressed in the nervous system. They include olfactomedin-1 (noelin-1) in brain and retina [6], olfactomedin–noelin–tiarin protein 1 (ONT1) in midbrain and hindbrain roof plate and axial and paraxial mesoderm [7], tiarin in non-neural ectoderm [8], olfactomedin-2 in brain and retina, optimedins (olfactomedin-3) in brain, and retina [9], myocilin in the eye [10], and gliomedin in Schwann cells [11].

Retinoic acid (RA), a metabolite of vitamin A, profoundly affects cell proliferation, cell differentiation, and morphogenesis in several vertebrate embryonic tissues including the neural crest [12–15]. RA receptors and cellular RA binding proteins both are expressed in neural crest derivatives [16, 17]. RA treatment alters the expression pattern of homeobox (Hox)-containing genes, which are being implicated in segmental specification in various embryonic tissues including the neural crest [18].

Some of the olfactomedins are shown to have key roles in the development of nervous system. Noelins are needed for development of the neural crest and regulation of neuronal fate [19, 20]. Tiarin affects dorsalization in the developing spinal cord [7, 8]. ONT1, another member of the olfactomedin family, is responsible for development of neural crest cells [7]. Optimedins is a downstream target of Pax6 [21], a transcription factor that is critical for central nervous system development. Gliomedin, a member of the olfactomedin protein family, is expressed by Schwann cells and is required for molecular assembly of developing nodes of Ranvier in the peripheral nervous system [11]. Olfactomedin domain-containing proteins play important roles in neurogenesis and dorsal ventral patterning.

Lipocalins transport small hydrophobic molecules such as steroids, bilins, retinoids, and lipids. The members of the family show high affinity and selectivity for hydrophobic molecules. The presence of six- or eight-stranded β -barrel and highly conserved motifs/short conserved region in their amino acid sequences is a common feature of lipocalin [22].

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Q5F0G5_DANRE/216-472
Q4TBM2_TETNG/191-447
F1NJM9_CHICK/110-365
D2I0I8_AILME/244-500
F1ST70_PIG/246-502
Q8HWZ2_PIG/232-488
Q3SX06_BOVIN/233-489
F1MP43_BOVIN/247-503
MYOC_BOVIN/233-489
MYOC_MACFA/234-490
B4DV60_HUMAN/1-233
Q8N854_HUMAN/200-456
B4DVZ2_HUMAN/204-460
MYOC_HUMAN/247-503
B4DV44_HUMAN/189-445
B6V6K6_HUMAN/247-503
Q6PKX1_HUMAN/3-259
B6ECI7_HUMAN/247-342
B6V6K7_HUMAN/247-342
MYOC_RABIT/233-489
Q924K5_RAT/232-488
MYOC_RAT/245-501
Q05AC1_MOUSE/233-489
MYOC_MOUSE/233-489
Q924K4_MOUSE/233-489
Q3UN79_MOUSE/233-489
MYOC_FELCA/233-489
MYOC_CANFA/226-842
F1PC52_CANFA/240-496

database (Pfam ID: PF02191). Pfam is a database of protein families that includes their annotations and multiple sequence alignments generated using hidden Markov models. With the multiple sequence alignment in Pfam, the 218 members were analyzed for the existence of the conserved glycine-X-tryptophan (G-X-W) motif, a signature for the lipocalin family.

Lipocalin prediction

The members of the olfactomedin family were analyzed using the LipocalinPred database (<http://bioinfo.icgeb.res.in/lipocalinpred/lipohome.html>). The support vector machine (SVM)-based prediction software was used with

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S. no.	Species	Sequence length	Swissprot ID	SVM score	Decision	Protein name
1	Amphioxus	1–202	C3ZUA6	0.42	Lipocalin	Uncharacterized
2	Mouse	212–468	Q8BU90	0.05	Lipocalin	Uncharacterized
3	Human	139–394	A8MW65	0.34	Lipocalin	Uncharacterized
4	Deer tick	460–707	B7Q3V3	0.39	Lipocalin	Colmedin, putative
5	Ciona	135–349	Q69HN9	0.30	Lipocalin	Lectomedin 1 alpha-like
6	Rat	143–398	O88923	0.22	Lipocalin	Latrophilin-2
7	Bovine	206–461	O97827	0.30	Lipocalin	Latrophilin-3
8	Mouse	206–461	Q80TS3	0.30	Lipocalin	Latrophilin-3
9	Zebra fish	46–301	A8E7K0	0.27	Lipocalin	Latrophilin 3-similar
10	Sea urchin	229–477	Q86RA9	0.12	Lipocalin	Amassin
11	Rat	138–401	B0BNI5	0.04	Lipocalin	Olfactomedin-like protein 3
12	<i>Caenorhabditis</i>	233–483	Q8IG71	0.27	Lipocalin	Collagen/olfactomedin domain-containing protein 2
13	Mouse	242–499	B7ZNU5	0.02	Lipocalin	Olfm4 protein
14	Green puffer	143–366	Q4T0H5	0.37	Lipocalin	Whole genome sequencing
15	Brugia	256–524	A8QAV3	0.18	Lipocalin	predicted
16	Green puffer	219–474	Q4SAP8	0.41	Lipocalin	Uncharacterized

Fig. 2 **a** Secondary structure of human myocilin olfactomedin domain predicted by psipred. **b** Secondary structure of bovine β -lactoglobulin from lipocalin family predicted by psipred. Both the sequences are rich in β -sheets

default values of position-specific score matrix (PSSM) and secondary structure composition (SSC) hybrid to identify lipocalins using features like amino acid composition, dinucleotide composition, PSSM profile, and secondary structure composition.

Secondary structure prediction

The secondary structure of all the sequences was derived using PSIPRED tool. PSIPRED (<http://bioinf.cs.ucl.ac.uk>) is a protein structure prediction server, which carries out a reliable secondary structure prediction on a protein.

Result

Olfactomedin and G-X-W motif

The sequences of the olfactomedin family of proteins were retrieved from Pfam. Multiple sequence alignment of the olfactomedin family protein sequence shows the conserved G-X-W motif. The alignment is shown in Fig. 1.

Lipocalin prediction

The sequences retrieved from Pfam were predicted for their lipocalin nature using lipocalin prediction. The results of prediction are shown in Table 1.

Secondary structure prediction

The secondary structure analysis of most of the sequences of olfactomedin family showed maximum similarities among themselves with most of the sequences containing β -sheets. The similarities are depicted in Fig. 2a, b.

Physicochemical nature of olfactomedin and lipocalin

It is seen that both the lipocalins and olfactomedins have similar aggregation abilities. Both can form oligomers. They are secretory in nature. Other similarities like presence of disulfide bonds and expression modulation in the presence of steroid are also observed. The physicochemical similarities are shown in Table 2.

Discussion

Diverse biological functions of lipocalins

Lipocalins participate in distributing and transporting small, hydrophobic molecules, due to their ability bind them. The physiological significance of lipocalins is not limited to transfer processes. They play an important role in the regulation of immunological and developmental processes. Lipocalins are also involved in the reactions of organisms to stress factors and in signal transduction pathways [23]. The enzymatic activity found in some members of the lipocalin family, as well as the interaction with natural membranes,

Table 2 Similar features of lipocalins and olfactomedins

Physical properties	Lipocalin	Olfactomedin domain
pI	4.5 to 5.9	4.8–8.0
Aggregation	Dimer to oligomer	Dimer to oligomer
Secretory proteins	Yes	Yes
Disulfide bond	Yes	Yes
Expression modulation	Steroid hormone	Steroid hormone
beta-Sheet nature	Yes	Yes
Retinoic acid bind	Yes	Yes
G-X-W motif	Yes	Yes

both directly with lipids, steroids (palmitic acid) and through membrane-localized protein receptors are of special interest.

Regulation of olfactomedin in presence of retinoic acid

Retinoic acid, both in its all-*trans* and 9-*cis* form, is shown to upregulate the olfactomedin mRNA expression [24]. Retinoic acid, in combination with demethylation agents like 5-aza-2'-deoxycytidine, also showed a positive effect on upregulation of olfactomedin. The *cis*-form of retinoic acid shows a higher induction effect than the all-*trans* form. The level of olfactomedin increases with the amount of retinoic acid binding protein (RABP). The upregulation of olfactomedin is shown to be higher than that of RABP. The RABP upregulates the retinoic acid biosynthesis enzymes, retinoic acid receptor, and retinoic acid metabolism enzyme. The positive coregulation with RABP suggests that olfactomedin may play a role in the function of RA by associating with it. Thus, olfactomedin family members may transport lipids or act as a carrier protein for lipids in signal transduction pathways [24].

In vitro evidence of myocilin interaction with palmitic acid

Canine myocilin is associated with lipid modified by palmitic acid [25]. Protein palmitoylation is a reversible, regulated, posttranslational modification that can regulate conformation, membrane association, protein–protein interactions, and intracellular localization of the target protein. The leucine heptad repeat of myocilin can serve as a source of hydrophobic behavior. Pulse chase experiments with ³H palmitic acid detected myocilin [25], reflecting a possible function of myocilin as a lipid receptor that binds oxidized low-density lipoprotein or free fatty acids.

Olfactomedin and lipocalins

Sequences of both lower and higher order metazoans were considered for classification. The results showed that this varied range of organisms contained olfactomedin protein that fall under different categories like latrophilin-2, latrophilin-3, olfactomedin domain-containing protein 2, olfactomedin 3 protein, olfactomedin 4 protein, lectomedin 1 alpha-like protein, and amassin.

The G-X-W motif is highly conserved in all the members of lipocalin family. The multiple sequence alignment of the olfactomedin family of proteins shows that the G-X-W motif is found to be conserved. Thus, it may be inferred that the olfactomedin domain-containing proteins is likely to be involved in fatty acid or retinol binding.

Myocilin olfactomedin domain protein circular dichroism shows the significance of beta-sheet and beta-turn

secondary structure [26]. The structure of lipocalins is characterized by the presence of six or eight β -strands that are connected by loops to form β -barrels [27].

Conclusion

Outlier lipocalins share several common features with olfactomedin domain of myocilin and other related olfactomedin domains. The olfactomedin domain harbors the G-X-W motif that is conserved in all lipocalin proteins. This motif has been experimentally proved to interact with retinol in human retinol serum binding protein. The interaction of the olfactomedin domain of myocilin with palmitic acid has been found in animal models. Lipocalins and olfactomedins also share similar structural features. The physical nature of olfactomedins is similar with those of the lipocalins. An upregulation in the synthesis of olfactomedin in the presence of retinoic acid shows a possible functional similarity between the lipocalins and olfactomedins. In combination, these suggest that myocilin, an olfactomedin family protein, may also be placed under the family of lipocalins.

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